Exploiting *Galdieria sulphuraria* Genetic Resources for the Biorecovery of Rare Earth Elements (REEs)

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ABSTRACT

Exploration of life in extreme environments allows the discovery of intriguing organisms with extraordinary biotechnological potential. Example of extreme environments is represented by hot springs, where harsh conditions ($pH < 1$; $T > 50°C$; high concentrations of metals) are prohibitive for most living organisms, except for archaea, bacteria and a few eukaryotes like the unicellular alga *Galdieria sulphuraria*. In the present project, I used *G. sulphuraria* to explore its potentiality in recovering Rare Earth Elements (REEs). Firstly, I studied its phylogeny and evolution using the complete plastid and mitochondrial genomes. Results from the organelle phylogenies showed the subdivision of the species into 6 sublineages. The influence of selective pressure could mainly explain divergent evolution since high variability of the dS-dN values was shown when comparing each pair of sublineages. Secondly, I used *G. sulphuraria*, strain SAG 107.79, to perform exposition tests to the REEs cerium. Evaluation of the growth rate and cell vitality demonstrated the absence of significant effects on the microalgal physiology. Transcriptomic data highlighted the activation of pathways probably involved in metal homeostasis. Among the Biological processes, transcripts involved in Localization and Transport were subjected to a higher increase in expression following cerium treatments. I performed phylogenetic analysis using the amino acid sequences of these transcripts from the representative members of the 6 lineages, integrated with the orthologous sequences from the NCBI non-redundant database. All phylogenies identified a close relationship of these transcripts with archaea, bacteria, or fungi, thus suggesting the intervention of Horizontal Gene Transfer (HGT) events in the biorecovery potential of *G. sulphuraria*. The biotechnological application of *G. sulphuraria* was also evaluated at strain level. Two genetically distant strains, SAG 107.79 and ACUF 427, were selected to test the different performances in the bioremoval of yttrium, cerium, europium and terbium from single- and quaternary-metal solutions. From both test solutions, ACUF 427 was the better biosorbent in acidic conditions, while SAG 107.79 better bio removed metal ions from subneutral solutions.

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DECLARATION

I declare that this thesis is a presentation of original work and I am the sole author. This work has not previously been presented for an award at this, or any other, University. All sources are acknowledged as references.

CHAPTER 1: INTRODUCTION

1.1. EXTREME ENVIRONMENTS

Exploration of life on Earth pushed researchers to study a great variety of environments dispersed worldwide. So far, the choice of the areas to be studied relied on the idea that life was possible just in “neutral” environments. The anthropocentric consideration of the term “neutral” considers mainly the temperature, which has to be between 4 and 40°C, pH between 5 and 8.5, and salinity between that of freshwater and seawater, which are the main features affecting the life cycle of organisms (Oarga 2009). In particular, the instability of biological molecules over 70°C tricked researchers into thinking that this was the highest temperature compatible with life (Zierenberg et al. 2000). Indeed, in vitro experiments demonstrated the loss of the three-dimensional structure of proteins and enzymes when the temperature exceeds 70°C (Zierenberg et al. 2000). In 1978, however, Brock et al. discovered the first organism (*Thermus acquaticus*) able to live at 50-80°C and get energy from the symbiotic relationship with cyanobacteria (Brock 1978). The discovery of life in environments characterised by extremes of these parameters opened up a new vision of panspermia and plausible life outside the Earth (Rothschild and Mancinelll 2001). The definition of “extreme environment” is quite complex because of the high number of variables that must be considered to define it. Some researchers coined the term “Extremobiosphere” as the biome characterised by extreme boundaries of the known physicochemical world (Bull and Goodfellow 2019). Generally, any habitat is defined “extreme” when it is characterised by high or low parameters such as pH, temperature, salinity, hydrostatic pressure, electromagnetic, ionizing radiations and desiccation (Oarga 2009).

1.1.1. pH

One of the most critical factors affecting organisms' life is pH, which determines the availability of inorganic ions and metabolites (Oarga 2009). Protoplasts' pH should remain close to neutrality ($5 \leq pH \leq 8.5$) to make sure that all the metabolic activities of the cells are active (Oarga 2009). When pH reaches extremely low values, proteins denature (Rothschild and Mancinelll 2001), making life impossible for most living organisms. Exceptional organisms that are able to thrive in acidic environments, such as hot springs, active or abandoned mines, and hydrothermal vents ($pH < 4$), are called acidophiles (Dhakar and Pandey 2016). This group of organisms includes archaea, bacteria and a few eukaryotes, which developed efficient systems to maintain the intracellular pH close to neutrality (Oarga 2009; Iovinella et al. 2020; Dhakar and Pandey 2016; Seckbach 2017). Reduction of the membrane permeability allows microorganisms to resist acidity and reduce the influx of H^+ inside the protoplast. Acidophilic archaea membranes, for example, are composed of glycerol ethers instead of glycerol esters, less susceptible to acid hydrolysis (Baker-Austin and Dopson 2007). Substitution of negatively-charged channels with positively-charged ones and the accumulation of monovalent cations inside the protoplast also reduce the influx of protons (Baker-Austin and Dopson 2007). In the worst case of protoplast acidification, repair mechanisms are activated to reduce minor proteomic and genomic damages (Baker-Austin and Dopson 2007).

At the opposite extreme ($pH > 8.5$), organisms called alkaliphiles succeed in colonizing and thriving in alkaline environments (Rothschild and Mancinelll 2001), where the paucity of H^+ creates harsh conditions for aerobic prokaryotes with a membrane-bound ATP synthase (Krulwich et al. 1998; Rothschild and Mancinelll 2001). The influx of H^+ is also affected by acidic cell walls and low-PI proteins inside the protoplasts (Mesbah and Wiegel 2011). The problem could be solved by sequestration of H^+ within the membrane and modification of the alpha-subunit of F_1F_0-ATPase to increase protons capture and prevent their loss (Krulwich et al. 1998).

1.1.2. TEMPERATURE

Living organisms are subjected to diverse environmental challenges, and one of the most important is the temperature. High temperatures increase the fluidity of membranes to a lethal level, as well as the denaturation of biomolecules (Rothschild and Mancinelll 2001). Proteins and nucleic acids lose their structure at 100 degrees, while photosynthesis is already inhibited at 75 degrees because of chlorophyll denaturation. Temperature also influences the solubility of gases in water, such as O_2 and CO_2, making them unavailable for aquatic organisms (Rothschild and Mancinelll 2001). On the other hand, low temperatures highly influence water availability for the organisms. Water molecules often underlie enzymatic reactions, making them indispensable for metabolic processes. When they are unavailable, for example, because they are stuck in ice crystals, many biochemical reactions stop working, thus leading to cell death (Oarga 2009). Ice crystals in the external environment and inside the protoplast also create the mechanical breaking of cell membranes (Rothschild and Mancinelll 2001; Oarga 2009).

Organisms that are able to cope with the powerful effects of temperature are called hyperthermophiles (optimal growth temperature > 80°C) and psychrophiles (optimal growth temperature < 15°C). Some hyperthermophile enzymes are stable even at higher temperatures, such as the amylopullulanase at 142°C (Schuliger et al. 1993).The upper limit for temperature in eukaryotes registered so far is more or less 60 degrees (Oarga 2009). Psychrophile organisms, thriving with temperatures just below 0 degrees, belong to all the major taxa, and in particular active microbial communities were found at -18°C, which is the lowest temperature ever recorded for life (Clarke 2014; Rothschild and Mancinelll 2001).

1.1.3. PRESSION

Pressure affects living organisms because it can change the fluidity of cellular membranes due to volume changes (Rothschild and Mancinelll 2001). Intense pressure on cells induces compression of lipids resulting in the decrease of membrane fluidity. Further consequences of the high pressure include the dissociation of multimeric complexes, such as ribosomes and variations of diverse mechanisms involved in the metabolism of the organisms. Many organisms adapted to high pressure, but sudden changes of it result, anyway, in lethal consequences. Organisms that can survive at a pressure that is 1000 times higher than 1 standard atmosphere (0.1 MPa) are called piezophiles. These organisms were mainly discovered in deep-sea environments where the pressure can reach 110MPa (Abe and Horikoshi 2001). Adaptation to high pressure includes the incorporating monounsaturated and polyunsaturated fatty acids in the cell membranes to reduce the loss of their fluidity (Casadei et al. 2002). Accumulation of organic compounds called piezolytes (e.g. β-hydroxybutyrate) was also demonstrated in response to high pressure (Singleton et al. 2002).

On the other hand, little is known about life at low pressure. A microbial community was discovered on Mount Everest, where the pressure is 0.03 MPa, but no growth was observed below 10^{-6} MPa (Liu et al. 2007). Diverse organisms, exposed to Low Earth Orbit (LEO) and high vacuum, were able to survive at that pressure, but, at the same time, they confirmed the impossibility of growing (Saffary et al. 2002; Jönsson et al. 2008; Olsson-Francis et al. 2010).

1.1.4. RADIATION

Radiation is an important parameter affecting the mutagenic events in organisms. The term “radiation” includes the transit of particles, such as neutrons, electrons, protons, alpha particles and heavy ions, or electromagnetic waves, like gamma rays, X-rays, ultraviolet (UV) radiation, visible light, infrared, microwaves or radio waves (Rothschild and Mancinelll 2001; Oarga 2009). Effects of radiations include the reduction of motility, inhibition of photosynthesis and the more severe mutation of nucleic acid (Rothschild and Mancinelll 2001). In the latter case, the damage could be directly on the filaments of DNA and RNA or indirect through the production and intervention of reactive oxygen species, ROS (Rothschild and

MancinellI 2001). ROS are also involved in the lethal proteomic damage, which includes mainly proteins containing cysteine residues, iron-sulphur or heme groups (Daly et al. 2007). Mechanisms that living organisms execute against the harmful effects of radiation include the activation of nucleic acids repair mechanisms and the intracellular accumulation of protecting substances (Oarga 2009). Small molecules identified in *Deinococcus radiodurans* can protect enzymatic activity at 50 kiloGrey (kGy) radiation. Their protective activity is possible thanks to the presence of Mn^{2+} that binds phosphate to reduce reactive superoxides to peroxides (Daly et al. 2010).

1.1.5 DESICCATION

Presence of water inside the cell is essential for the regular performance of metabolic processes. The absence of water induces the collapse of cell membranes and proteins and the subsequent metabolism ceasing. Organisms that survive for long periods in desiccating environments are called xerophiles. When intracellular water weakens, xerophiles activate the state of anhydrobiosis, during which metabolic activity is inhibited (Rothschild and MancinellI 2001; Oarga 2009). The ability of xerophiles to resist desiccation lies in the high capacity of these organisms to prevent water loss through morphological changes (Wharton and Marshall 2002) and accumulation of lyoprotectant polyols (e.g. trehalose) before activating the anhydrobiosis (Dose et al. 1992). Survival strategies of xerophiles also include single- and double-strand repair mechanisms, as DNA damage usually occurs during desiccation (Torsvik and Øvreås 2008). Anyhow, when the water level reduces to a critical level, cellular death can occur during anhydrobiosis, and it is due to protein and nucleic acid denaturation, structural breakage through Maillard reactions, and accumulation of reactive oxygen species (Rothschild and MancinellI 2001).

1.1.6 SALINITY

Environmental high salt concentration affects the osmotic state of organisms and induces effects such as desiccation, turgor pressure and cellular dehydration (Rothschild and Mancinelll 2001). The organisms must adapt to the osmotic alterations, as uptake of nutrients, proteins biosynthesis and enzymatic reactions are highly affected by them (Oarga 2009). Representatives from both bacteria, archaea and eukarya, called halophiles, can survive in environments characterized by high salinity (Oarga 2009). Halophiles thriving in marine environments must counteract high concentrations of NaCl, while those living in athalassohaline waters (saline lakes not of marine origin) developed different mechanisms to survive with high quantities of more than one salt (Kanekar et al. 2012). Adaptations to desiccation, induced by high salinity, include the accumulation of inorganic ions, such as Na^+, k^+ and Cl^- to prevent water loss. (Paul et al. 2008). Furthermore organic osmolytes (e.g. glutamate, glycerol, trehalose), accumulated in the cytoplasm, can modulate the osmotic equilibrium between free and bound water molecules (Kanekar et al. 2012; Oren 2002).

1.2. HOT SPRINGS ENVIRONMENTS

Hot springs are considered extreme environments because they are characterized by more than one feature described above (Figure 1.1). They are the result of secondary volcanic activity and are defined by extremely high temperatures (100°C) and low pH (pH = 0). They have a worldwide distribution, but at the same time, they are limited to narrow zones of the Earth’s surface, including Yellowstone National Park in the USA (Toplin et al. 2008; Skorupa et al. 2013), Iceland (Ciniglia et al. 2014), Japan (Toplin et al. 2008), Russia (Sentsova 1991), New Zealand (Toplin et al. 2008), Italy (Yoon et al. 2004), Turkey (Iovinella et al. 2018; 2020) and Taiwan (Hsieh et al. 2015).

Figure 1.1. Examples of hot springs in Yellowstone National Park (USA) and Dyadin (Turkey). a, b Morning Glory Pool, Yellowstone National Park, Wyoming, USA; c, e Grand Prismatic Spring, Yellowstone National Park, Wyoming, USA; d, f Dyadin thermal bath, Turkey; Green arrow = Cyanobacteria and *Chromatiaceae*; black arrow = Cyanobacteria; red arrow = Cyanobacteria and *Chloroflexus*; blue arrow = protozoa, bacteria; orange arrow = Quartz, pyroxenes, dolomites; **a, b c, e** (Nugent et al. 2015); **d, f** (Iovinella et al. 2018)

Typical habitats of hot springs are characterized by hot sulphurous mines, fumaroles, hot mud and geysers, which are created as a consequence of magma cooling when it comes to contact with rainwater infiltrated deep within the rocks. Hot spring soils are characterized by two layers based on pH values and redox state (Stetter et al. 1986;

Segerer et al. 1993). The superficial layer is composed of 30 cm acidic and oxidized soil full of ferric iron compounds; the lower layer contains reducing compounds (e.g. H_2S), has a subneutral pH (4-8) and is mainly an anaerobic area due to the stream of volcanic exhalations (Stetter et al. 1986; Segerer et al. 1993). The acidic condition is caused by the oxidizing power of the atmosphere together with the activity of sulfur-oxidizing bacteria (*Thiobacillus thiooxidans*). Briefly, gases such as hydrogen sulfide (H_2S) and pyrite (FeS_2) come out from the cracks of the rock oxidase, naturally or through the action of bacteria, first to elemental sulphur and then sulphuric acid, H_2SO_4 (Johnson 1998; Crognale et al. 2018). The lowering of the environmental pH in geothermal sites, up to values close to 0, is directly correlated to the concentration of sulphuric acid (Crognale et al. 2018).

A further extreme peculiarity of hot springs is the presence of high quantities of toxic and heavy metals, precious and rare earth metals. The severe acidity of hot springs helps solubilize the metals in the form of sulfides (Johnson 1998). Moreover, when rainwater percolates inside the cracks of the rock, reaching great depths and warmed by Earth's natural heating, becomes saturated with minerals and metals and forms what is generally called "geothermal fluids (Lo et al. 2014).

1.3. HOT SPRINGS POLYEXTREMOPHILIC MICROALGAE

Inhabitants of hot springs must tolerate all the extreme conditions just described simultaneously, and for this reason, they are identified as "polyextremophiles". As exceptionally versatile organisms that cope with more than one harsh condition, polyextremophiles are deeply studied in biotechnological fields (Dhakar and Pandey 2016).

Most of the polyextremophiles from hot springs consist of prokaryotes, while few representatives of eukaryotes can tolerate environmental conditions so extreme. In these environments, microalgae live at the limit of their potentiality, adapting their metabolism and biological processes to extreme life (Elster 1999). Concerning the ability to thrive in such acidic hot springs, microalgae can be classified as acido-tolerants and acidophiles. Acido-tolerant microalgae are able to adapt to a wide range of pH ($2 \leq pH \leq 8$), even if their best metabolic performances were registered with neutral pH. Acido-tolerant microalgae belong to the group of Heterokontophyta as well. Examples include the genus *Pinnularia*, a diatom typical of acidic soils next to hot springs and mines (Ciniglia et al. 2007), and *Ochromonas vulcanica*, a Chrysophyceae firstly isolated in the Russian region of Karelia (Gromov et al. 1990) and then also identified in the Ansanto valley, Avellino, Italy (Albertano et al. 1994). Many green microalgae are acido-tolerants, like the genus *Chlamydomonas*, commonly found in Italian hot springs (Pollio et al. 2005). Some members of the genus *Chlorella* can thrive in moderately acidic conditions (*Chlorella saccharophila* var. *Saccharophila*, Chloroccoccales), while others are adapted to more acidic environments (*Chlorella saccharophila* var. *ellipsoidea* and *Chlorella protothecoides* var. *acidicola*) (Huss et al. 2002). We could also mention *Viridiella fridericiana* (Albertano et al. 1991) and *Pseudococcomyxa simplex* (Albertano et al. 1990). Acidophilic microalgae have optimal growth at $pH < 3$. Among the Volvocales (Chlorophyta), we can mention the genus *Dunaliella*, with the only species Dunaliella acidophila as acidophilic microalga, which was previously classified as *Spermatozopsis acidophila* (Albertano et al. 1981). Further acidophilic microalgae are represented by four genera belonging to the class of Cyanidiophyceae (Yoon et al. 2006).

1.4. CYANIDIOPHYCEAE (RHODOPHYTA)

Cyanidiophyceae are members of an ancient (1.3 billion years) unicellular microalgae group thriving in geothermal sites (Figure 1.2), where the ecological conditions are very extreme, such as low values of pH (pH 0.5-3.0), high temperature (50°C-55°C) and huge amounts of heavy metals (Pinto et al. 2003). The first taxonomic analyses were done employing morphological and physiological tools (Albertano et al. 2000;

Sentsova 1991; Pinto et al. 2003). Based on a few characteristics, such as the shape of the cell, number and shape of the plastid, presence-absence of the cell wall, modality of cell division and the number of endospores, three genera (*Galdieria*, *Cyanioschyzon* and *Cyanidium*) and eight species (*G. sulphuraria*, *G. daedala*, *G. partita*, *G. phlegrea*, *G. maxima*, *C. merolae*, *C. caldarium* and *C. chilense*) were established (Albertano et al. 2000; Sentsova 1991; Pinto et al. 2003).

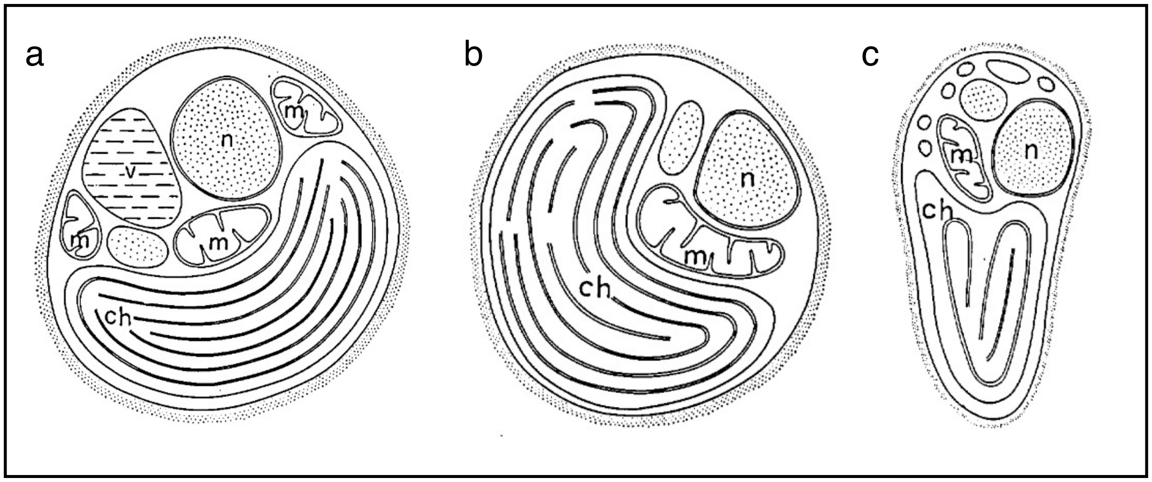

Figure 1.2. Sketch of the ultrastructure of *G. sulphuraria* (a), *C. caldarium* (b) and *C. merolae* (c); ch = chloroplast; m = mitochondrium; n = nucleus; v = vacuole (Merola et al. 1981).

The genus *Cyanidium* comprises a polyextremophilic species, *Cyanidium caldarium* (Merola et al. 1981) and a mesophilic one, *Cyanidium chilense* (Ciniglia et al. 2019). A very simple morphology characterizes them: round shape, absence of vacuole and only 1 mitochondrion (figure 1.2b). Cell's dimensions vary between 2 and 6 *µ*m (Merola et al. 1981); they divide asexually through the production of 4 endospores, and their typical pigments are chlorophyll a, C-phycocyanin, allophycocyanin and carotenoids (Allen 1959). *Cyanidioschyzon merolae* is the only species belonging to the genus *Cyanidioschyzon*; cellular dimensions are lower than those of *Cyanidium* (1,4 *µ*m x 3-4 *µ*m), indicating an oblong shape; they have 1 mitochondrion and 1 plastid with the same pigments of *Cyanidium* (figure 1.2c). An important feature of *C. merolae* is the absence of the cell wall and the binary fission as the modality of cellular division, differing from the other genus of Cyanidiophyceae (Albertano et al. 2000).

Species of the Genus *Galdieria* are facultative heterotrophic microalgae, able to utilize both ammonium and nitrate as nitrogen sources (Albertano et al. 2000). They are furnished with 1 or more vacuoles, 1 mitochondrion and a multilobed plastid (figure 1.2a). So far, 4 species were established in this genus based on morphological features: *G. sulphuraria* (Merola et al. 1981), *G. partita*, *G. daedala* and *G. maxima* (Sentsova 1991). In the last years, the employment of molecular tools allowed researchers to discover more genera and species, such as *Galdieria phlegraea* (Ciniglia et al. 2004; Qiu et al. 2013) and the newest genus *Cyanidiococcus* with the species of *Cyanidiococcus yangmingshanensis* (Liu et al. 2020). The discovery of these new genera and species brought the number of identified Cyanidiophyceae to 4 genera and 9 species.

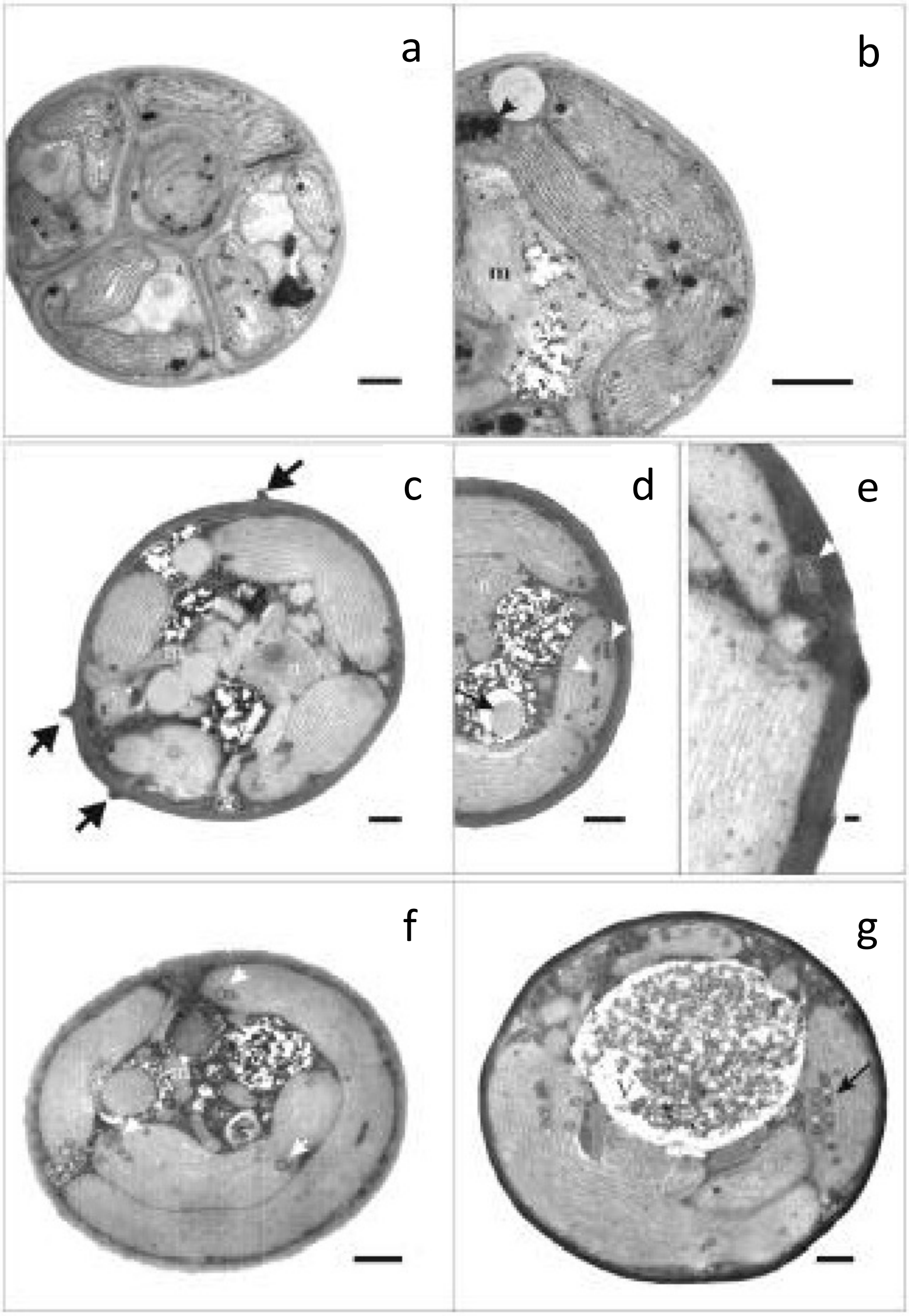

Figure 1.3. TEM micrograph observations of *G sulphuraria*; a = sporangium; b, black arrowhead = plastoglobuli; c, black arrow = conical protrusions; d, black arrow = spherical inclusion; d, white arrowheads = plastoglobules; e, white arrow = Golgi; f, white arrowheads = plastoglobules; g, plastoglobules;(Pinto et al. 2003)

1.5. PHYLOGENY OF CYANIDIOPHYCEAE

Physiological and morphological techniques are inadequate tools to establish the taxonomy of Cyanidiophyceae. Small cellular dimensions and the presence of similar diagnostic criteria between the species make establishing a new genus or species quite complex (Albertano et al. 2000; Merola et al. 1981; Sentsova 1991; Pinto et al. 2003). Given their long evolutionary history (Yoon et al. 2004), it is intriguing that only a handful of recognized species have survived in this lineage and it is more likely that the genetic diversity of Cyanidiophyceae is underestimated. Over the years, many authors discussed the vast biodiversity of the Cyanidiophyceae based on the plastidial and nuclear genes (Ciniglia et al. 2004; Toplin et al. 2008; Hsieh et al. 2015). Sampling campaigns in different geothermal stations throughout the world increased the Cyanidiophyceae molecular background, leading to the general idea that in this microalgal group, there are more lineages than known based on morphological and physiological tools. The latest phylogenetic analysis on Cyanidiophyceae populations, based on the partial rbcL gene, highlighted that the genetic structures of *Galdieria sulphuraria* and *Galdieria maxima* are much more complex than what could be expected from an ancient unicellular alga (Iovinella et al. 2018). Based on phylogeny and genetic diversity estimation, the authors hypothesized the subdivision of *G. sulphuraria* in 5 different subclades supported by bootstrap and posterior probability values (Figure 1.4).

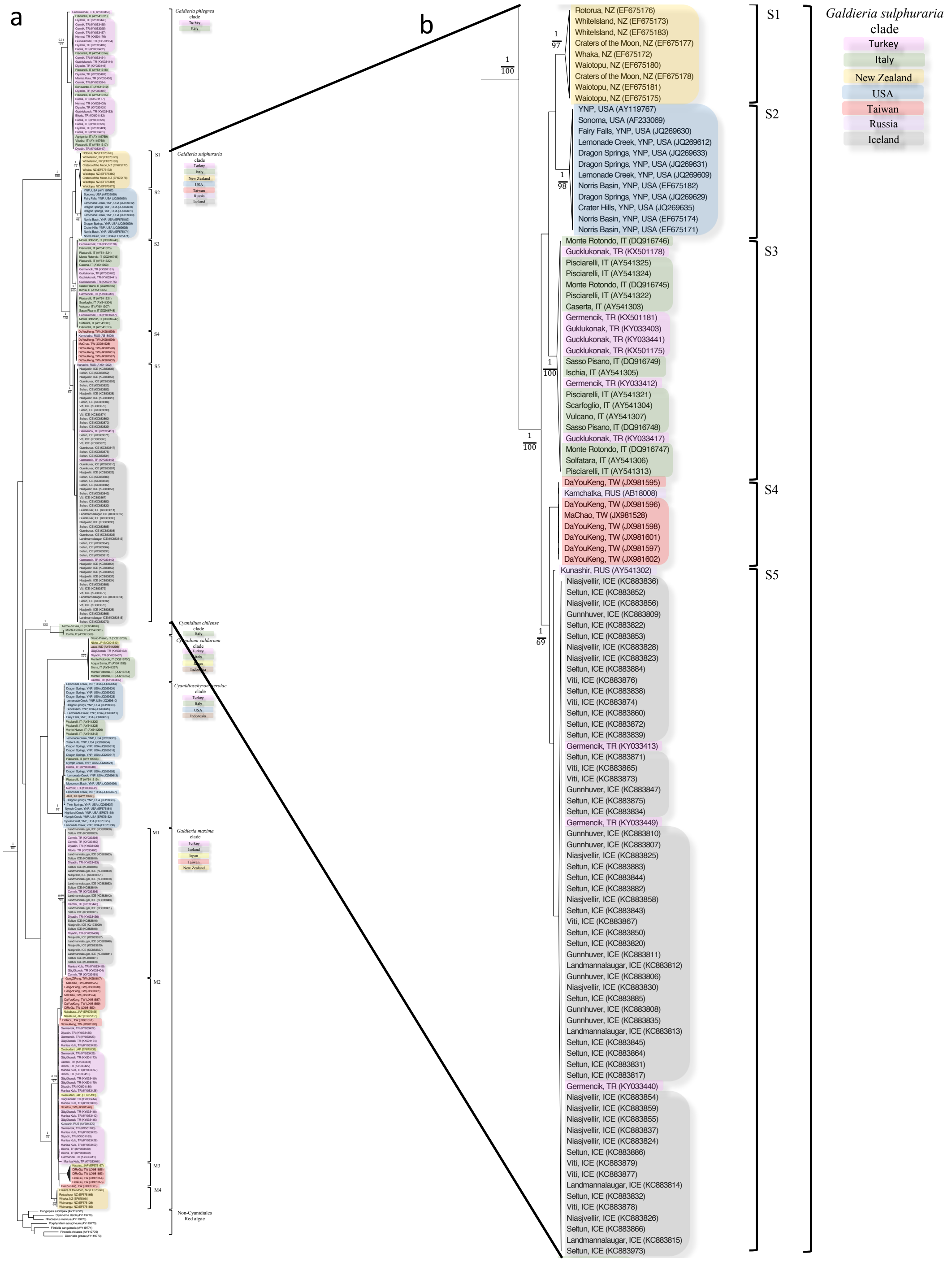

Figure 1.4. Maximum likelihood tree of Cyanidiophyceae inferred from RAxML analysis based on partial rbcL sequences (a); zoom in the *G. sulphuraria* clade (b); strains grouped in the squares of the same colour were originally collected from the same geothermal region. Codes S1-5 represent the subclades established by the authors (Iovinella et al. 2018)

The phylogenetic tree highlighted the clear separation and isolation by the geographic distance of the populations from New Zealand (subclade S1) and USA (subclade S2). Taiwanese population (subclade S4), along with one strain (IPPAS P500) originally isolated in a geothermal area of the Kamchatka peninsula (Russia), grouped all together in the same subclade distinct from the others. Subclades S3 and S5, unlike the previous ones, grouped strains from different worldwide geothermal regions. In particular, subclade S3 grouped Italian strains with some Turkish isolates, while in subclade S5, there were all the Icelandic strains, some isolates from the Turkish area and one strain (IPPAS P508) from Kunashir, Russia (Figure 1.4). This subdivision within the single species of *G. sulphuraria* could result from genetic drift. As stated by Gross and Oesterelt 1999, Cyanidiophyceae could not survive when the environmental conditions differ from those typical of geothermal sites. This feature would cause a failure in the exchange of isolates between different hot springs because they are too far from each other and, therefore, a lack of gene flow between populations (Gross and Oesterhelt 1999). The groupings shown in Figure 1.5, drawn based on the results of Iovinella et al. 2018, however, show that some populations of *G. sulphuraria* do not cluster together based on geographical origin, thus indicating an intrinsic genetic structure. There is the hypothesis that the Icelandic population, for example, originated by the diffusion of a few isolates from extreme eastern Russia, presuming the possible movement of Cyanidiophyceae from one side of the globe to another. However, how these microalgae can move are still to be clarified (Ciniglia et al. 2014).

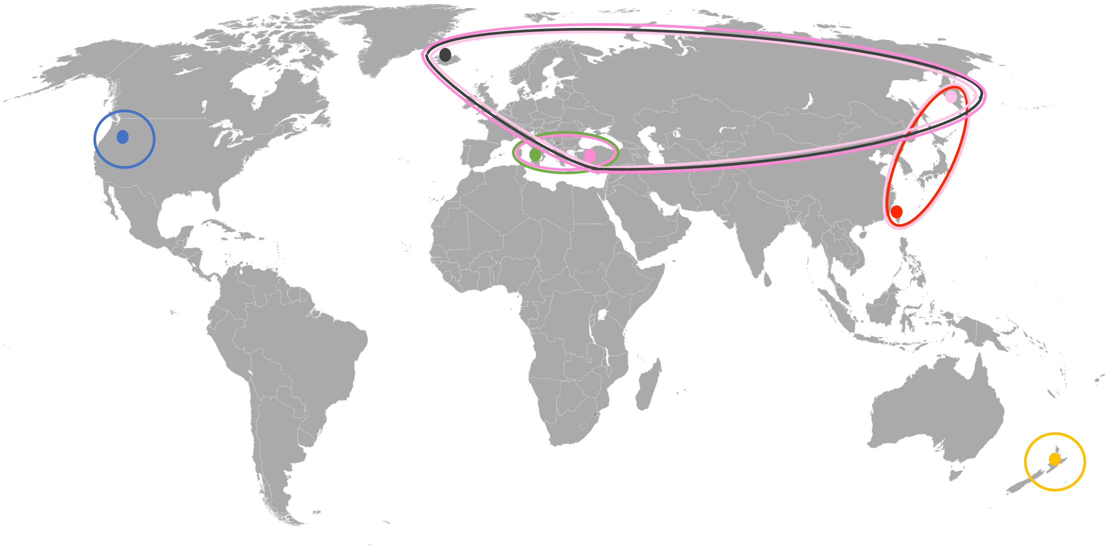

Figure 1.5. Worldwide distribution of *G. sulphuraria* strains based on data from Iovinella et al. 2019; geographical populations are identified by colour circles; different populations grouped together represent the subclades identified by the phylogenetic analysis (Iovinella et al. 2018)

1.5. POTENTIALITY OF CYANIDIOPHYCEAE IN METAL BIOREMEDIATION

The presence of heavy, precious and rare earth metals in hot springs suggests that organisms thriving in these environments are not inhibited by large amounts of metals and could accumulate them without suffering their toxic effects. Indeed *G. sulphuraria* and *C. merolae* were employed several times for the biotechnological remediation of metals (Misumi et al. 2008; Osaki et al. 2009; Minoda et al. 2015; Ju et al. 2016; Fukuda et al. 2018; Jalali et al. 2018;Čížková et al. 2019; Cho et al. 2020).

The first evidence of the great resistance of *C. merolae* toward metals came from Misumi et al. in 2008. The authors uncovered a higher metal resistance of the microalgae than *Arabidopsis thaliana* and hypothesised the possible mechanisms for modifying the concentration of intracellular metal ions (Misumi et al. 2008). A study of the *C. merolae* genome identified the gene coding for the phytochelatin synthase involved in synthesising phytochelatin. These molecules play an essential role in forming metal complexes, kidnapping the metal ions from the cytoplasm, thus reducing their toxic effects on the microalgae (Osaki et al. 2009). In the last decade, the interest in using Cyanidiophyceae species, and in particular *G. sulphuraria*, in the bioremediation of metal is growing, thanks to the promising results and the increasingly comprehensive knowledge of their genomes. Japanese researchers focused their studies on the employment of *G. sulphuraria* in the recovery of Rare Earth Elements (REEs). They first analysed the potential of *G. sulphuraria*, strain ACUF 074, in bioaccumulating various REEs, changing different parameters, such as the concentration of metal ions, pH of the experimental media, preincubation of the microalgal stock solutions (Minoda et al. 2015). After that, they efficiently recovered gold and palladium (over 80%) from aqua regia-based metal wastewater, using 1.4 mg/mL of biomass (dry weight) and an exposition time of 30 minutes. They also identified a suitable modality for the desorption of metal ions from the biomass, using a solution of 1 M thiourea and 0.1 M HCl (Ju et al. 2016). *G. sulphuraria* biomass grown under mixotrophic conditions recovered the radionuclide Cesium as well, reaching a percentage of biorecovery of 52 ± 15% after 10 days of exposition to the metal (Fukuda et al. 2018).

A new strain of *G. sulphuraria*, isolated from the Qotur Suie acidic spring, was used for the biorecovery of Vanadium (III), Titanium (IV) and Uranium (VI), important metals used in industry as a source of energy (Jalali et al. 2018). They studied the sorption process pattern applying the Langmuir, Freundlich, and Dubinin–Radushkevich (D-R) isotherm models. Moreover, they explored the kinetics and thermodynamics of the metal biosorption mechanism (Jalali et al. 2018). The last study on the employment of *G. sulphuraria* in metal bioremediation was reported in 2020. Mechanisms of resistance of *G. sulphuraria* to lead treatment were deeply studied by Cho et al in 2020. They applied different techniques and discovered that lead tolerance could be ascribed to four mechanisms: 1) intervention of polysaccharide; 2) precipitation of Pb-PO_4; 3) transport of organic Pb to cell vacuoles; 4) chelation of thiol-Pb involved in the disruption of protein secondary structures (Cho et al. 2020).

1.6. THE DIFFICULT SUPPLY OF RARE EARTH ELEMENTS (REEs)

Rare Earth Elements (REEs) are a group of metallic elements that include the lanthanides, plus scandium and yttrium. They have unique magnetic and catalytic properties and are, up to now, irreplaceable materials in numerous technologies, such as wind turbines, solar panels, batteries, fluorescent lamps, computer and mobile monitors, TV screens etc. They are also used as fertilizers in agriculture, aquaculture, or as animal growth enhancers (Pang et al 2002; Binnemans et al. 2013; Castor and Hedrick 2006).

The high popularity of technologies requiring REEs is causing these metals' increasing demand and price (Binnemans et al. 2013). Nowadays, the principal source of REEs comes from China, which is not only the owner of the largest ore in the world but it is also specialized in the separation of the individual elements, as well as in the production of rare-earth permanent magnets and phosphors lamps (Binnemans et al. 2013). This monopoly is inducing the other countries to rely their REEs economy on the possible exploitation of geothermal plants to extract metals from geothermal fluids (Bourcier et al. 2005) or on the recycling of End-of-Life products (Binnemans et al. 2013). One possible way is the development of a system that allows double exploitation of geothermal fluids (Figure 1.6): on the one hand, the use of the heat released by the water to generate electricity, on the other hand, the production of constituents dissolved in it (Bourcier et al. 2005). Generation of geothermal fluids occurs when groundwater percolates inside the rocks until reaching great depths nearby the magma chamber. The close contact with the heat produced by the magma generates superheated and pressurized water, which then returns to the surface, cooling down along the way and generating geysers and hot springs (Figure 1.6). During the water flow, compounds from the rock cracks' surface are solubilized, enriching the composition of the geothermal fluids, especially with minerals and metals (Bourcier et al. 2005). This allows their extraction without adding costs to their solubilisation (Gallup 1998; Bourcier et al. 2005).

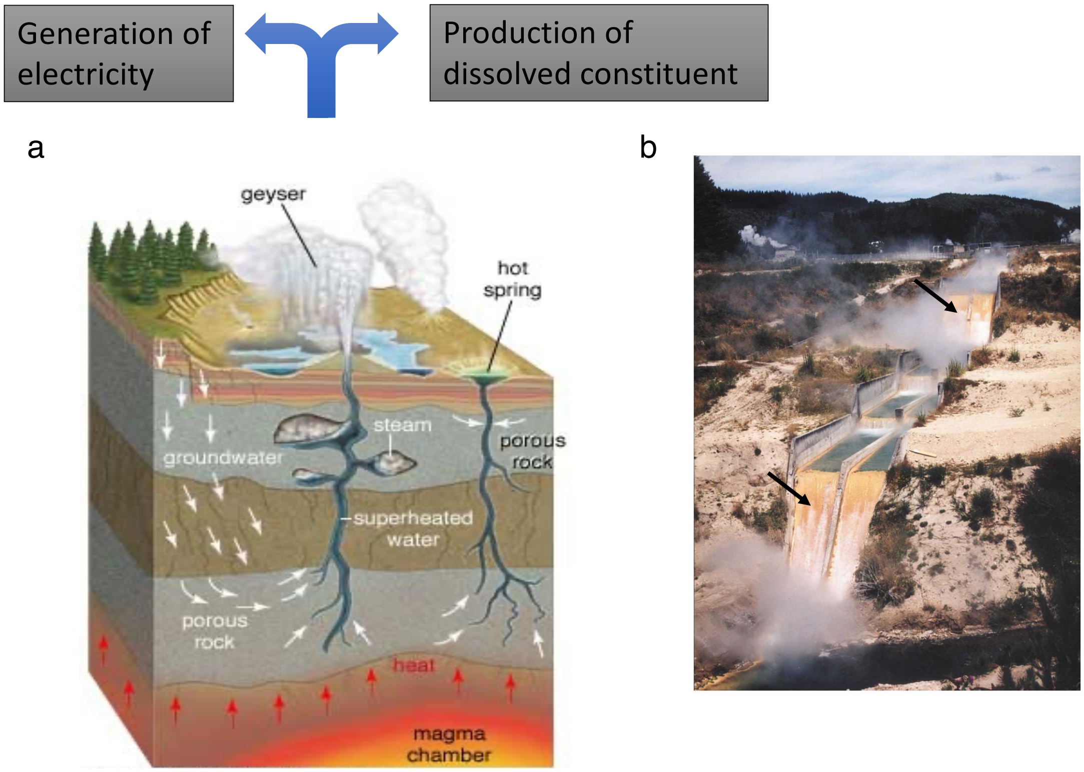

Figure 1.6. Representation of the groundwater flowing inside the rock (white arrows) and the generation of geothermal fluids (a), Encyclopædia Britannica, Inc.); Geothermal fluid drain at Wairakei in New Zealand (b); the pilot plant was designed to extract colloidal silica from geothermal fluids. Black arrows indicate the silica precipitates; bluish color of the water indicates presence of colloidal silica (Bourcier et al. 2005).

Many works analysed metals extraction from geothermal fluids, such as zinc, lead, copper, lithium, arsenic, precious and rare earth metals. Physicochemical methods are often used to recover of high-value metals from geothermal fluids, such as chemical precipitation, chemical coagulation, ion exchange, membrane technologies, electrochemical technologies, and adsorption using activated carbon (Dhankhar and Hooda 2011; Farooq et al. 2010). Some weaknesses exist with these techniques: 1) they are ineffective when the metal concentration is very low; 2) a large amount of toxic waste will be produced, which requires further treatments, increasing the operating costs and the negative effects on the environment; 3) some physicochemical methods are very expensive, such as ion exchange and membrane technologies. 4) Incomplete metal adsorption (Lo et al. 2014).

Biosorption and bioaccumulation (Figure 1.7) are new biological approaches for extracting resources from geothermal fluids; being greener and cheaper methods, they could replace the physiochemical ones for the recovery of high-value metals (Das 2010). During the biosorption, metal particles are absorbed on the cell's surface, which could be viable and not (microbial and algal biomass, proteins, and other biomaterials), forming small agglomerates. The reactions that allow the sequestration of the metal ions are based on physiochemical interactions, such as electrostatic interactions, ion exchange and metal ion chelation or complexation. Important roles are played by the functional groups on the cell surface (e.g. hydroxyl, carbonyl, carboxylate, sulfhydryl, amine, amide, imine, phosphodiester; Figure 1.7), which are able to bind the metal ion based on their protonation state (Lo et al. 2014). Control of the experimental pH is crucial as the protonation or deprotonation of the functional groups is highly affected by the pH: the higher is pH, and therefore also the deprotonation of the functional groups, the more metal ions recovery would occur (Pacheco et al. 2011). Bioaccumulation occurs when cells actively transport the metals inside the cell through an energy (ATP)-driven process. Operating conditions (e.g. temperature, pH and nutrients) need to be strictly controlled to maintain cell viability, but the selectivity of the process is higher than that of the biosorption and the accumulation is not affected by pH (Lo et al. 2014).

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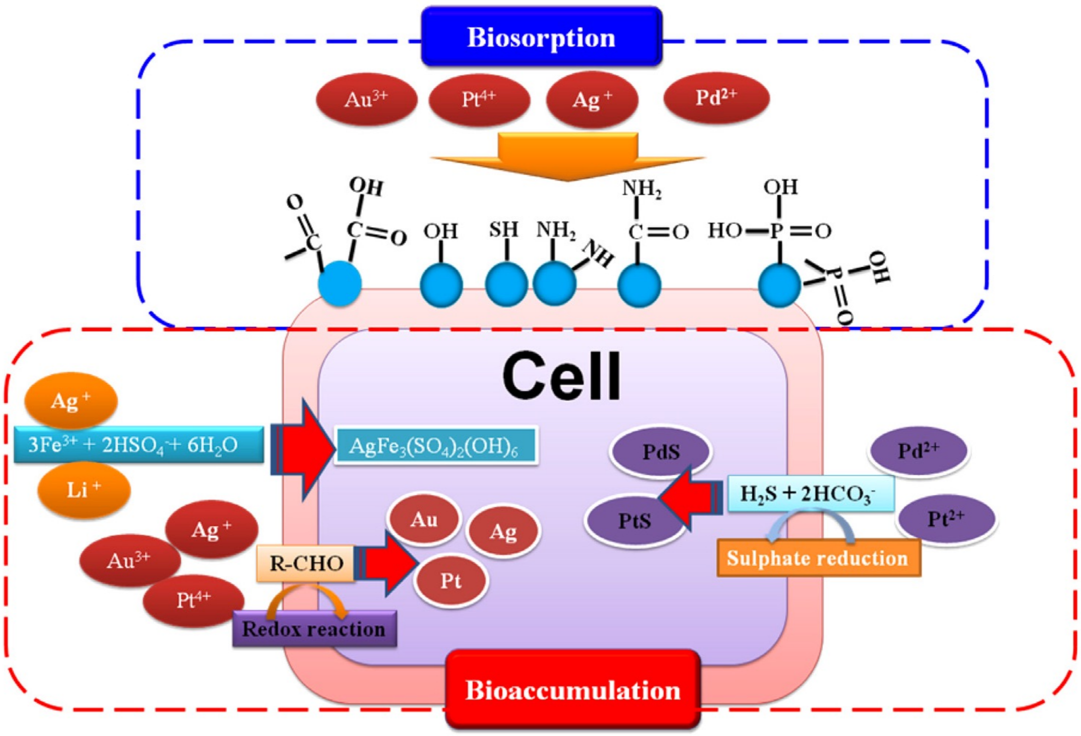


Figure 1.7. Schematic representation of biosorption and bioaccumulation mechanisms (Lo et al. 2014).

Few data were published on the quality and quantity of REEs in geothermal environments (Lo et al. 2014). Concentrations of cerium (Ce), neodymium (Nd), samarium (Sm), europium (Eu), gadolinium (Gd), dyprosium (Dy), erbium (Er) and ytterbium (Yb) were published from California, New Mexico and Italy (Michard 1989). In the last years, these metals were used in technology (lasers, camera lenses, x-ray machines), energy (batteries, lamps), and industry (fluorescent glass, cleaning agents). All rare metals have great economic value because they are essential to the functionality of some modern technologies, and at the same time, they are hardly available and difficult to isolate.

Recycling of End-Of-Life products could represent an alternative way for the supply of REEs, even if, at the moment, only 1% of these products are recycled due to collection and technological problems (Castor and Hedrick 2006). At the same time, these processes have an important environmental benefit since products to be recycled miss the radioactive thorium, naturally present in REE ores (Castor and Hedrick 2006). To date, hydrometallurgical and pyrometallurgical techniques are the most popular methods employed in recycling REEs. Even for recycling, lately, biological approaches are widely employed thanks to the high benefit in terms of efficiency, costs and environmental impacts.

1.7. AIMS OF THE PROJECT

The project's overall aim was to evaluate the potential application of *G. sulphuraria* species for the biorecovery of Rare Earth Elements. Despite being an ancient unicellular organism, previous molecular studies demonstrated the complex taxonomy and biology of *G. sulphuraria*. Although its genome is highly reduced and simplified, it comprises countless genes that provide *G. sulphuraria* the proper attributes to survive several stressors. Since the evolution of a single gene is not necessarily representative of the species' evolution, the project's first aim was to evaluate the molecular evolution and phylogeny within the species of *G. sulphuraria*, using the mitochondrial and plastid genomes from different strains (Chapter 2). After identifying more molecular lineages, one lineage was chosen to analyse the physiological and transcriptomic response to the Rare Earth Element Ce^{3+} to understand the potential of *G. sulphuraria* in the biorecovery of REEs and identifying the potential activated mechanisms in the presence of metal (Chapter 3). Finally, phylogenetic analysis of genes that increased transcription with Ce^{3+} treatments were done. Results of the phylogenetic analysis from the genomic phylogenies were used as starting point to conduct an NCBI search of the orthologous genes in the other identified lineages and other taxa using the non-redundant (nr) database (Chapter 4).

CHAPTER 2: ORGANELLE PHYLOGENOMICS UNVEIL THE DIVERGING EVOLUTION OF 6 LINEAGES IN GALDIERIA SULPHURARIA (CYANIDIOPHYCEAE).

2.1. INTRODUCTION

Taxonomists successfully used morphological traits to estimate species delimitation in families of macroorganisms. The application of morphology to uncover the biodiversity of microorganisms could be challenging because of the paucity of diagnostic characters. The described ultrastructure of *G. sulphuraria* was used by previous researchers to classify the strains collected in different worldwide geothermal sites (Merola et al. 1981; Albertano et al. 2000; G. Pinto et al. 2003; Ciniglia et al. 2014; Sentsova 1991). Lately, however, it was observed that the elementary shape (small round ball) and the simple ultrastructure that characterize *G. sulphuraria* cells do not match the diversity observed at the molecular, biochemical and physiological level (Ciniglia et al. 2004). Plastidial and nuclear phylogenies increased the molecular knowledge of *G. sulphuraria* and provided the basis for the idea that these ancient microalgae have been evolving into more genetic lineages than thought (Del Mondo et al. 2019; Iovinella et al. 2020; Toplin et al. 2008; Hsieh et al. 2015).

The latest reported phylogenetic analysis, based on the partial Ribulose-1,5-bisphosphate carboxylase/oxygenase gene (*rbcL*), highlighted the intricate genetic structure of *G. sulphuraria* and the hypothesis of diverging clades within the species (Toplin et al. 2008; Hsieh et al. 2015; Iovinella et al. 2018). The subdivision of the species into more subgroups reflected the geographical position of the populations and thus their isolation caused by not-acidothermal environments and the impossibility of long-distance dispersion. As a result, speciation events have been taking place over thousands or millions of years, and more strains, species, or ecotypes might be discovered (Toplin et al. 2008). With the increased amount of molecular data, Hsieh et al. 2015 established hypothetical species, identified as Operational Taxonomic Units (OTUs), and confirmed the involvement of the habitat heterogeneity in incrementing genetic diversity within *G. sulphuraria* (Hsieh et al. 2015). Genetic distance between the subgroups was confirmed by analysing the Inter-Populational Pairwise Genetic Differentiation, F_{ST}. High values of F_{ST} indicated a low level of gene flow between *G. sulphuraria* populations and thus a diverging and isolated evolution (Iovinella et al. 2018).

In the middle 1990s, a new technology for high throughput genome sequencing was developed and called "next-generation sequencing (NGS)" (Ronaghi et al. 1996). The NGS technology produces big amount of data, useful to infer robust phylogenomic and understand the evolutionary history of microorganisms, such as *G. sulphuraria*. Organelle genomes significantly contribute to organisms'biology since they contain important genes coding for photosynthesis and respiration functionality (Herrmann 2003; Martin and Schnarrenberger 1997). Moreover, they are characterised by low mutation rates and conserved gene content, which make them attractive for phylogenetic and evolutionary inferences (Kim et al. 2015; Yang et al. 2015; Lee et al. 2016).

My present study aimed to better understand the phylogenetic relationship among different *G. sulphuraria* strains, improving the analysis from a gene-level to an organelle-genome one. The NGS data from 44 *Galdieria* species were used to retrieve the plastid and mitochondrial genomes and reconstruct their structure. From the assembled genomes, coding sequences (CDSs) were extracted, concatenated in a single alignment and used to infer the species phylogeny. To further understand the evolutionary history of *G. sulphuraria*, plastid and mitochondrial genes were analysed separately (gene phylogeny) and compared to the species ones. Finally, an overall and a lineage-level analysis of the synonymous and non-synonymous substitutions were performed to understand if natural selection forces have been affecting the divergence and the paraphyletic evolution of the species.

2.2. MATERIALS AND METHODS

2.2.1. STRAINS ISOLATION

Galdieria strains were obtained from the Algal Collection of University of Naples (www.acuf.net), the Culture Collection of Autotrophic Organisms (https://ccala.butbn.cas.cz/), the Collection of Microorganisms from Extreme Environments, the Institute of Plant Physiology, Russian Academy of Sciences (http://en.cellreg.org/Collection-IPPAS.php), the Culture Collection of Algae at Göttingen University (https://www.uni-goettingen.de/en/culture+collection+of+algae+%28sag%29/184982.html), the Tung-Hai Algal Lab Culture Collection (http://algae.thu.edu.tw/lab/?page_id=42). New samples were collected from different microenvironments, being part of the acido-thermal area of Campi Flegrei, Naples, Italy (Figure 2.1; Table S2.1). Samples were collected by scraping the mineral substratum and stored in sterile falcon tubes. Cultures were then serially diluted and the strains were isolated by streaking the diluted solutions across Allen's agar plates. The obtained colonies were inoculated in Allen medium pH 1.5 (Allen and Stanier 1968) and cultivated at 37°C under continuous fluorescent illumination of 45 μmol photons·m^{-2}·s^{-1}.

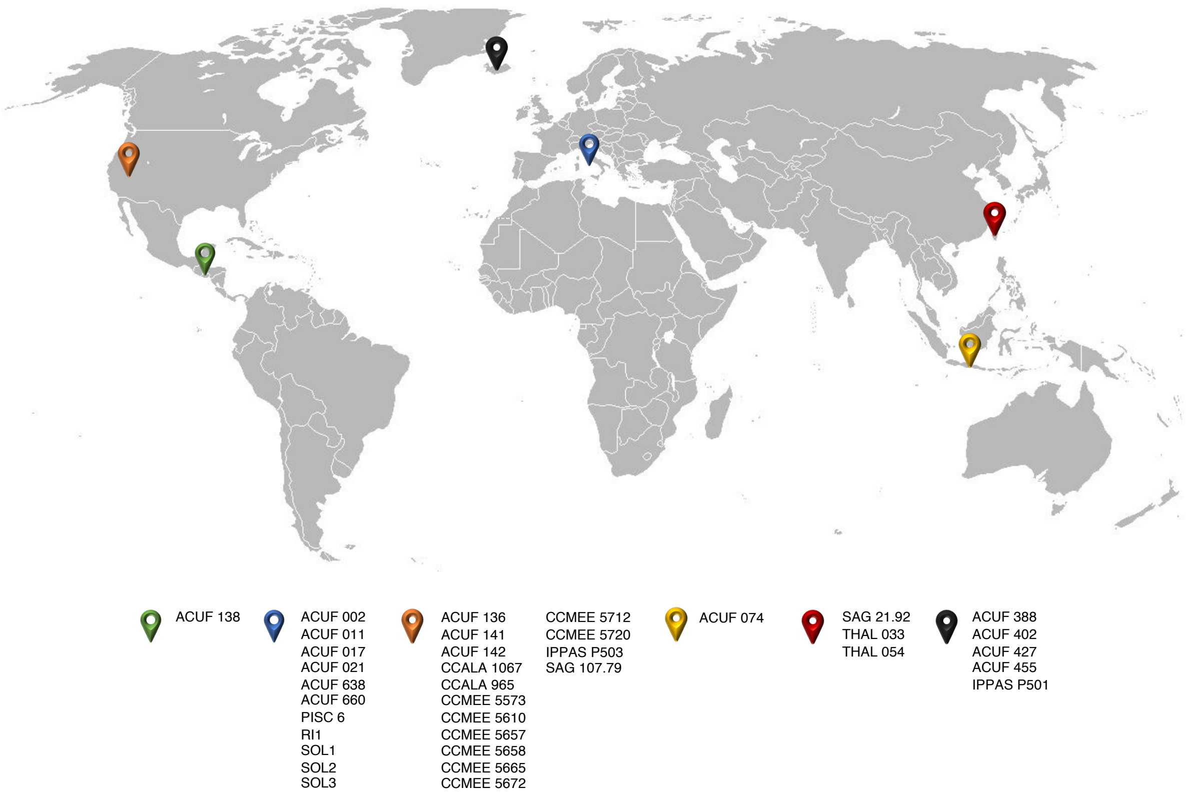


Figure 2.1. Worldwide distribution of *G. sulphuraria* strains used in this study. Details of the collection sites, along with the sample source and corresponding reference are listed in Table S2.1.

2.2.2. DNA EXTRACTION, SEQUENCING AND CONTIGS ASSEMBLY

DNA was extracted using a mixed SDS-CTAB protocol. Briefly microalgal pellets were harvested by centrifugation at 13200 rpm for 5 minutes, resuspended in 40 µl of PBS pH 7.5 and 500 µl of DNA extraction buffer 1 (Table S2.2), and incubated at 55˚C for 30 minutes, mixing by inverting every 10 minutes. Then 150 µl of DNA extraction buffer 2 (Table S2.2) was added and incubated for a further 10 minutes at 65˚C. DNA was extracted by adding and gently mixing 690 µl of Phenol:Chloroform:Isoamyl Alcohol 25:24:1 to the mixture. Samples were then centrifuged at 13200 rpm for 5 minutes to collect the aqueous phase, which was then incubated with 0.8% of isopropanol at -20˚C for 2 hours to precipitate the DNA. Next, samples were centrifuged at 15000 g for 30 minutes at 4˚C and the supernatant was then discarded. Pellets were washed with 200 µl of 70% ethanol and then centrifuged to discard the supernatant. Finally, pellets were air dried, resuspended in 40 µl of TE buffer and incubated with 1 µl of RNAse A and 1 µl of Proteinase K for 2 hours at 37˚C. A following clean-up step was done using Qiagen DNeasy Plant Mini Kit and DNA quality and concentration was assessed using a Nanodrop photospectrometer ND-1000.

Library preparations were done using NEBNext® Ultra™ II DNA Library Prep Kit for Illumina Sequencing according to the manufacturer’s instructions. Libraries were then sequenced with Illumina MiSeq (Illumina, San Diego, CA) and the resulted reads were trimmed with Trimmomatic (Bolger et al. 2014) and assembled using Spades v3.1 (Bankevich et al. 2012).

2.2.3. IDENTIFYING ORGANELLE GENOME CONTIGS, GENE PREDICTION AND ANNOTATION

Mitochondrial and plastid contigs were identified by comparing known proteins from the reference genome, strain 074W (KJ700460 for mitochondria and NC_024665 for plastid) using TBLASTN from BLAST+ version 2.2.27 (Camacho et al. 2009). The extracted contigs were re-assembled into 1 single scaffold using Ragout v. 2.3 and employing the organelle genomes from strain 074W as the references (Kolmogorov et al. 2018). Scaffolds were annotated with GeSeq (Tillich et al. 2017) using *G. sulphuraria*, strain 074W, chloroplast and mitochondrial genomes of the Organelle Genome Resources of the NCBI Reference Sequence Database (RefSeq) as the BLAT reference sequences (Kent 2002). Ribosomal RNAs (rRNAs) and transfer RNAs (tRNAs) were annotated using ARAGORN (Laslett and Canback 2004) and tRNAscan-SE v2.0 (Lowe and Chan 2016), while RNAweasel server (http://megasun.bch.umontreal.ca/cgi-bin/RNAweasel/RNAweaselInterface.pl) was used to determine intron types in the tRNAs. Finally, the annotated genomes were visualized using OrganellarGenomeDraw v1.2 (Lohse et al. 2013). All the annotated chloroplast and mitochondrial genomes were deposited into the GenBank database (Table S2.3-S2.4).

2.2.4. PLASTID AND MITOCHONDRIAL SPECIES PHYLOGENY

Mitochondrial and plastid CDSs present in 80% of the *G. sulphuraria* strains were retrieved from the assemblies using Geseq and Blast+ setting the e-value threshold of e-06. The resulted genes, 18 for mitochondria and 126 for plastid, were aligned separately using MAFFT v7.453 and the resulting alignments, , were uploaded to Gblocks version 0.91 b (Castresana 2000) to remove poorly aligned regions and to concatenate the reduced alignments. The resulting multigene mitochondrial and plastid sequences were aligned again using MAFFT to produce the final alignments 13609 bp and 116936 bp long, respectively. Four red algal taxa belonging to Florideophyceae (*Chondrus crispus*, *Gracilariopsis chorda*) and Bangiophyceae (*Porphyra umbilicalis*, *Pyropia Haitanensis*) along with *Cyanidioschyzon merolae*, strain 10D (Cyanidiophyceae) were chosen as outgroup taxa (Table S2.1). Maximum likelihood (ML) analyses were performed with IQ-Tree v. 2.0.3 (Nguyen et al. 2015), using the best substitution model estimated under the partition scheme selected by the program (- spp, -m TEST). Phylogenetic trees were inferred applying 10000 ultrafast bootstrap replicates, UFBoot (Minh et al. 2013) and 1000 replicates of the approximate likelihood ratio test [aLRT] and Shimodaira-Hasegawa, SH-aLRT (Anisimova et al. 2011) for the branch statistical support.

2.2.5. PLASTID AND MITOCHONDRIAL GENE PHYLOGENY AND ESTIMATION OF SELECTION

Gene phylogenies were performed on all the mitochondrial and plastid genes using IQ-Tree v. 2.0.3 (Nguyen et al. 2015). The same options were applied as described above for the branch statistical support (10000 replicates UFBoot and 1000 replicates SH-aLRT). The gene trees were then compared to the species trees to calculate the Gene Concordance factor, gCF, and the Site concordance factor, sCF (Minh et al. 2020).

Alignments used to infer the gene phylogenies were also employed to calculate the overall synonymous and non-synonymous substitutions rates using Mega X v. 10.1.8 (Kumar et al. 2018). For every gene finally, evolutionary pairwise rates were calculated among *Galdieria sulphuraria* lineages using the Codon-based Z-test of Selection implemented in Mega X v. 10.1.8 (Kumar et al. 2018).

2.2.6. ORGANELLE GENOMES CO-LINEAR ANALYSIS

Plastid and mitochondrial scaffolds obtained from the re-assemble step were aligned using the ProgressiveMauve algorithm (Darling et al. 2010) and applying the default settings.

2.3. RESULTS

2.3.1. GENERAL FEATURES OF PLASTID AND MITOCHONDRIAL GENOMES

Comparing the *Galdieria* organelle genomes with those of the non-*Galdieria* red algae, *G. sulphuraria* organelle genomes are conserved and smaller (13% of reduction for plastid genome and 40% for mitochondrial one). Moreover, plastid genomes show a reduced GC content, while the mitochondrial ones show an increase of it.

Plastid genomes vary between 159.544 and 167.984 bp and are constituted by a large single copy (LSC) region, a small single copy (SSC) region and two repeats inverted to each other (IRs, Figure 2.2). LSC regions show a variation in length (123.686-133.376 bp) and GC content (27.60-31.02%) among the different isolates. SSC regions also demonstrate a variation in length (16.083-33.121 bp), but a consistent GC percentage (27.97-26.50%). The two regions were separated by the two IRs ones, which comprised 3 rRNA and 2 tRNA sequences. These repeats varied from 2.894 to 5.077 bp and showed a high GC content (44.74-46.34%). Most of the genomes contained 39 intronless tRNA and 182 genes (CDSs) coding for diverse functions connected with the production of pigments, metabolism, photosynthesis, biosynthesis, transcription and translation machinery (Table S2.3).

Mitochondrial genomes showed a discrete GC content variability ranging from 41.83 to 44.90%. Likewise, genome sizes varied between 20,554 bp and 21,787 bp. An extensive non-coding and variable region is present in all the *G. sulphuraria* mitochondrial genomes. In the same area, but on the other strand, some isolates revealed an open reading frame (ORF181), which was extremely variable among them (Figure 2.3). Commonly, all the genomes comprised genes coding for 2 rRNA, 7 tRNA and 19 CDS coding for proteins involved in the respiration system (Table S2.4).

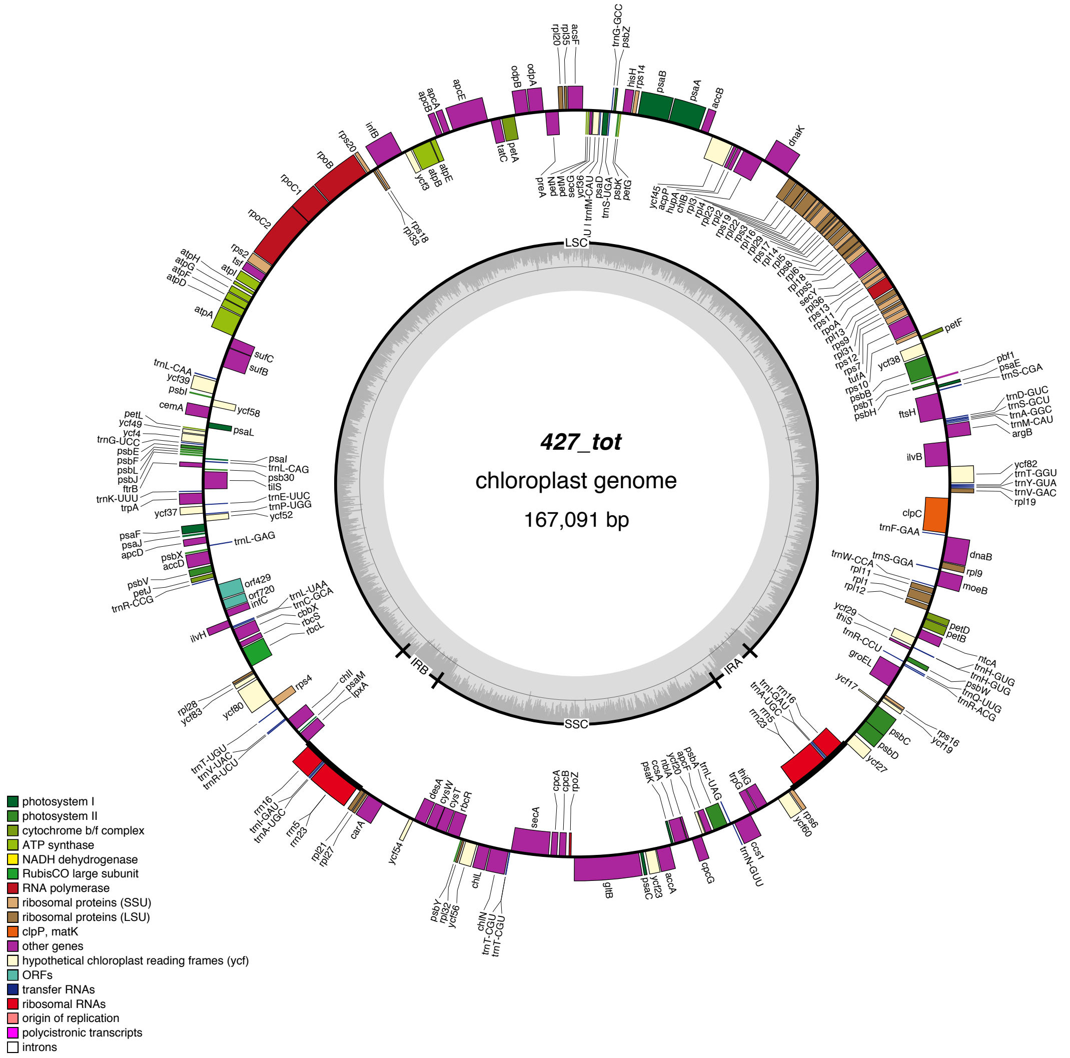


Figure 2.2. Map of *G. sulphuraria,* strain ACUF 427*,* plastid genome. Functional categories of the genes are identified with the colored blocks labelled in the legend.

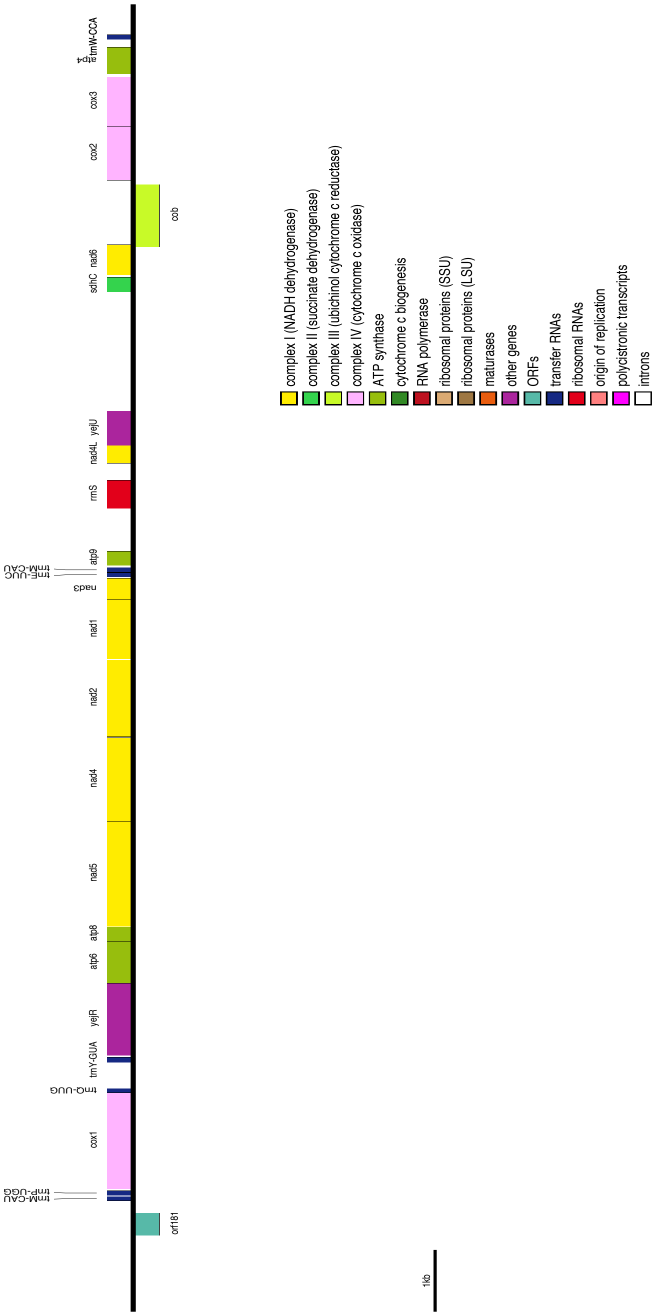


Figure 2.3. Map of *G. sulphuraria*, strain ACUF 427, mitochondrial genome. Functional categories of the genes are identified with the colored blocks labelled in the legend.

2.3.2. PLASTID AND MITOCHONDRIAL SPECIES PHYLOGENIES

Plastid phylogenomic, based on 126 concatenated genes, strongly supports the monophyletic origin of the Cyanidiophyceae class (100% UFBoot, 100% SH-aIRT). *G. sulphuraria* lineage originated from one single ancestor confirming the monophyly of the species, but it has been undergoing a divergence evolution into smaller sublineages and to a paraphyletic development: each *G. sulphuraria* group forms a small monophyletic population separated from the others so far and keep evolving separately (100% UFBoot; Figure 2.4a).

The ancestor organism that originated the *G. sulphuraria* lineage mutated, separating the strain ACUF 138 (100% UFBoot and SH-aIRT). The early divergence of this strain, collected from a San Salvador site, caused a high accumulation of mutations, which represent 10-13% of the total alignment length compared with the other strains (Table 2.1). A following diverging event (100% UFBoot and SH-aIRT) generated the microalgae that will then colonize the acido-thermal areas surrounding the Mediterranean sea (Rio Tinto, Italy and Turkey). Strains belonging to this clade are derived from a common ancestor and diverge from each other by up to 5%. The whole sublineage, instead, is separated from the others by more than 12000 bp, which is 11% of the total bp (Table 2.1).

Alongside the evolution of the above-mentioned lineages, further diverging events led to the origin of more separated subgroups in *G. sulphuraria*. A big clade includes all the strains from the Culture Collection of Microorganisms from Extreme Environments (CCMEE) and the Culture Collection of Autotrophic Organisms (CCALA). The Mexican strain ACUF 136 also groups with all these taxa, but it slightly diverged from them, becoming a sister taxon (100% UFBoot and SH-aIRT). Even this sublineage is characterized by low intrapopulation genetic dissimilarity (0-2%) and a high percentage of divergence (8-11%) with the strains of the other sublineages. Strains CCMEE 5610 and CCMEE 5657 slightly diverged from the other ones (7% of dissimilarity) and form a separated sister clade (100% UFBoot and SH-aIRT; Table 2.1, Figure 2.4a).

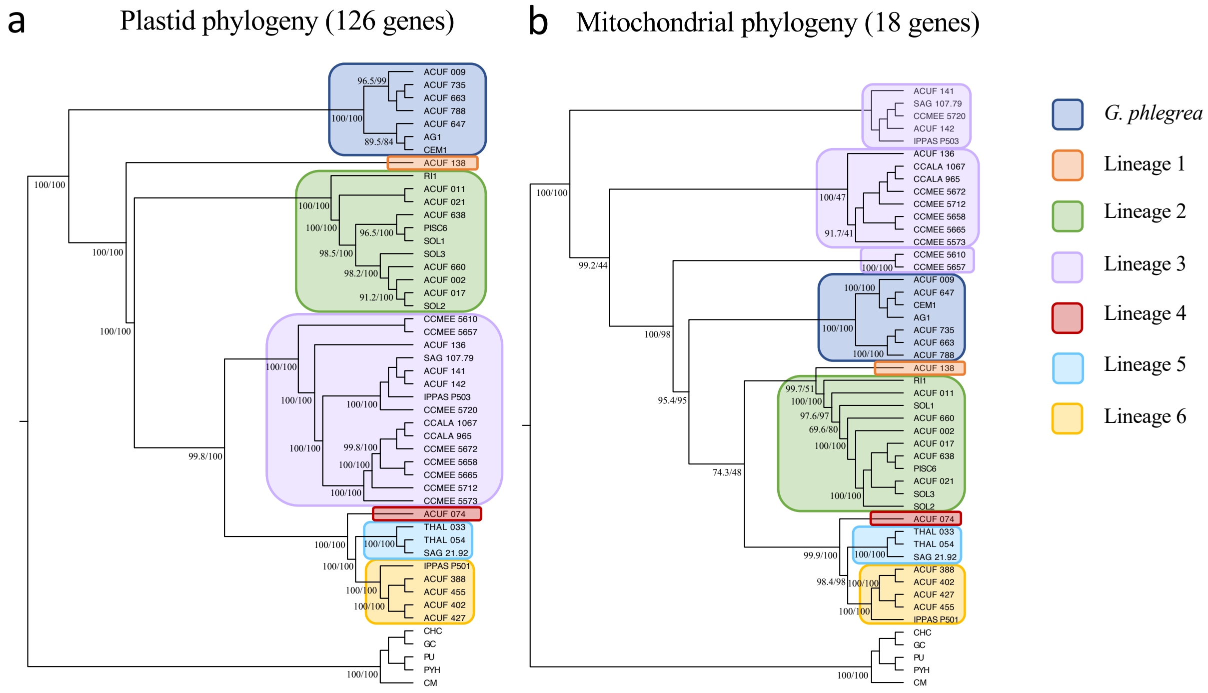


Figure 2.4. Comparison of the plastid and mitochondrial Species trees of Cyanidiophyceae. The phylogenies were inferred from Maximum Likelihood (ML) analysis using the concatenated plastid and mitochondrial CDSs, and the partition scheme for the best substitution model. Ultrafast bootstrap (UFBoot) and the Approximate Likelihood Ratio Test [aLRT] and Shimodaira-Hasegawa (SH-aLRT) support values are indicated near nodes; different color boxes represent *G. phlegrea* and *G. sulphuraria* lineages and include strains from different geographic locations: blue box = *G. phlegrea*; orange box = San Salvador strain; green box = Mediterranean strains; purple box = strains from the Atlantic region plus US; red box = Java island strain; light blue box = Taiwanese strains; Yellow box = Icelandic and Russian strains; CHC = *C. crispus*; GC = *G. chorda*; PU = *P. umbilicalis*; PYH = *P. haitanensis*; CM = *C. merolae;* detailed collection site information are listed in Table S2.1.

Concurrently, another ancestral strain, separated from the latter population, led to the paraphyletic development of strains from Iceland, Taiwan and the Indonesian island of Java (100% UFBoot and SH-alRT). Even if the plastid phylogeny highlights the separation of these strains in three main groups (100% UFBoot and SH-alRT, Figure 2.4a, red, light blue and yellow boxes), the percentage of the mutations among them reaches only 6%, while the separation of the whole clade from the other lineages confirmed the percentage of diverging rate of 8-11% (Table 2.1).

	Lin. 1	Lin. 2	Lin. 3	Lin. 4	Lin. 5	Lin. 6
Lin. 1	-					
Lin. 2	10%	-				
Lin. 3	9-10%	8-9%	-			
Lin. 4	9%	8-9%	8%	-		
Lin. 5	9%	8-9%	8%	5%	-	
Lin. 6	10%	8-9%	8%	6%	3%	-

Table 2.1. Hamming dissimilarity percentage among the *G. sulphuraria* lineages. The analysis was performed over the total length of the concatenated alignment used to infer the plastid species phylogeny.

Mitochondrial phylogenomics inferred with 18 concatenated genes also confirms the monophyly of Cyanidiophyceae (100% UFBoot and SH-aIRT), but at the same time highlights different phylogenetic relationships of *G. sulphuraria* sublineages compared to the plastid one (Figure 2.4b). Three different diverging events resulted in an early separation of all the strains from the American areas. The first segregation occurrence gave rise to one monophyletic subgroup well supported by the statistical analysis (100% UFBoot and SH-aIRT, Figure 2.4b). Phylogenetic tree highlights the subsequent separation of other strains (CCMEE, CCALA and ACUF 136) that usually cluster with the above subgroup in plastid analyses. This separation, anyway, is supported only by SH-aIRT statistic (44% UFBoot, 99.2% SH-aIRT); neither the percentage of dissimilarity among them (1%) confirmed the separation of the lineage in two different groups (Table 2.2). The third diverging moment separated the remaining two strains from the same lineage (CCMEE 5610 and CCMEE 5657), whose differences represent 12% of the whole mitochondrial genome (Table 2.2) compared with the above-mentioned strains.

Remaining *G. sulphuraria* strains group in a large clade and cluster as a sister taxa of the other species *G. phlegrea,* which usually happens with plastid markers (95% UFBoot, 95.4 % SH-aIRT; Figure 2.4b). Within this group, populations from the Mediterranean areas clustered all together in a monophyletic clade, even if small divergences were highlighted at an intrapopulation level. Separation of these strains was supported by 100% of UFBoot and 100% SH-aIRT and the mean percentage of nucleotide divergence is 10%, with higher values (14%) when comparing it with the early diverged American strains (Table 2.2). Surprisingly, the mitochondrial phylogeny groups the above population with the strain ACUF 138 from San Salvador. It is to be noted that this unusual association is supported only by SH-aIRT (51% UFBoot, 99.7% SH-aIRT) and that the two lineages differed by 10% of their whole mitochondrial genomes. On the other branch of the big clade, the paraphyletic evolution of the strains from Iceland and Eastern Asia, already highlighted by the plastid genome, was confirmed by the mitochondrial one (100% UFBoot, 99.9% SH-aIRT). Strains ACUF 074 confirms a slight separation from the others (7-8%) and in the meantime, the phylogenetic relationship of strains from Taiwan and Iceland was confirmed by both statistics. These strains are characterized by a very low intrapopulation variation (≤ 1 %) and differ by 7% from each other (Table 2.2).

	Lin. 3/A	Lin. 3/B	Lin. 3/C	Lin. 1	Lin. 2	Lin. 4	Lin. 5	Lin. 6
Lin. 3/A	-							
Lin. 3/B	1%	-						
Lin. 3/C	12%	12%	-					
Lin. 1	14%	15%	11%	-				
Lin. 2	14%	14-15%	10%	10%	-			
Lin. 4	13%	13%	9%	10%	9%	-		
Lin. 5	13%	13%	9%	10%	9%	7%	-	
Lin. 6	13%	13%	9%	10%	9-10%	8%	7%	-

Table 2.2. Hamming dissimilarity percentage among the *G. sulphuraria* lineages. The analysis was performed over the total length of the concatenated alignment used to infer the mitochondrial species phylogeny.

2.3.3. PLASTID AND MITOCHONDRIAL GENE PHYLOGENIES

Phylogenetic relationship among *G. sulphuraria* strains was assessed in every plastid and mitochondrial gene. These were compared with the concatenated one in order to understand the contribution of every single gene to the evolution of *G. sulphuraria* organelle genomes. Divergence of the species in lineages was confirmed by almost all the single gene phylogenies (Figures 2.5-2.6).

Phylogenetic trees from all plastid genes (126/126) highlighted the paraphyletic evolution of *Galdieria sulphuraria* Lin. 1, 2, 4, 5 and 6 as already revealed by the species phylogeny, except for Lin. 3. Despite the monophyletic origin of this lineage, strains belonging to this group were subjected to further diversification into small groups. One of them, which comprises strains CCMEE 5610 and CCMEE 5657 (lineage 3C), is quite separated from the others and this divergence is more evident in the mitochondrial phylogeny (M = 17/18; P= 124/126), where it is not part of the big monophyletic lineage (Figure 2.5). The strict relationship between lineages 5 and 6 is confirmed by most of the plastid genes (110/126) and half of mitochondrial ones (9/18), while just 91 plastid genes over 126 and 6 mitochondrial genes over 18 support the phylogenetic relationship of these clades with lineage 4 (Figure 2.5) More ancient diverging events, which gave rise to lineage 3 as a monophyletic group, are supported only by 29 plastid genes. Separation of lineages 1 and 2 has a discrete support from the plastid genes (89/126 and 42/126), but any of the mitochondrial genes supports it (Figure 2.5).

Monophyletic clustering of the Asian and Icelandic strains is fully supported by all the mitochondrial genes (18 genes over 18 ones used in the concatenated alignment phylogeny (Figure 2.6). Divergence of the American populations in three different clades, which were statistically supported in the plastid phylogeny as a single monophyletic group, is partly highlighted by the gene phylogenies (18/18 for lineage 3/A, 2/18 for lineage 3/B and 15/18 for lineage 3/C). On the contrary, most of the plastid genes don’t support this further subdivision of the American strains, except lineage 3/A (126/126; Figure 2.6). Analysing the inter-lineages relationships in the species phylogeny, there is a clear increase in the number of mitochondrial genes that support them starting from oldest to newest diverging events (Figure 2.6). Subsequent diverging events, which were highlighted by internal nodes, are confirmed by a higher number of genes than the oldest ones and they concerned about the clustering of the strain ACUF 138 with the Mediterranean ones (4/18 genes), the close relationship of the Asian and Icelandic populations (8/18 genes) and the one that related the sister group of the latter two with the strain ACUF 074 (6/18 genes).

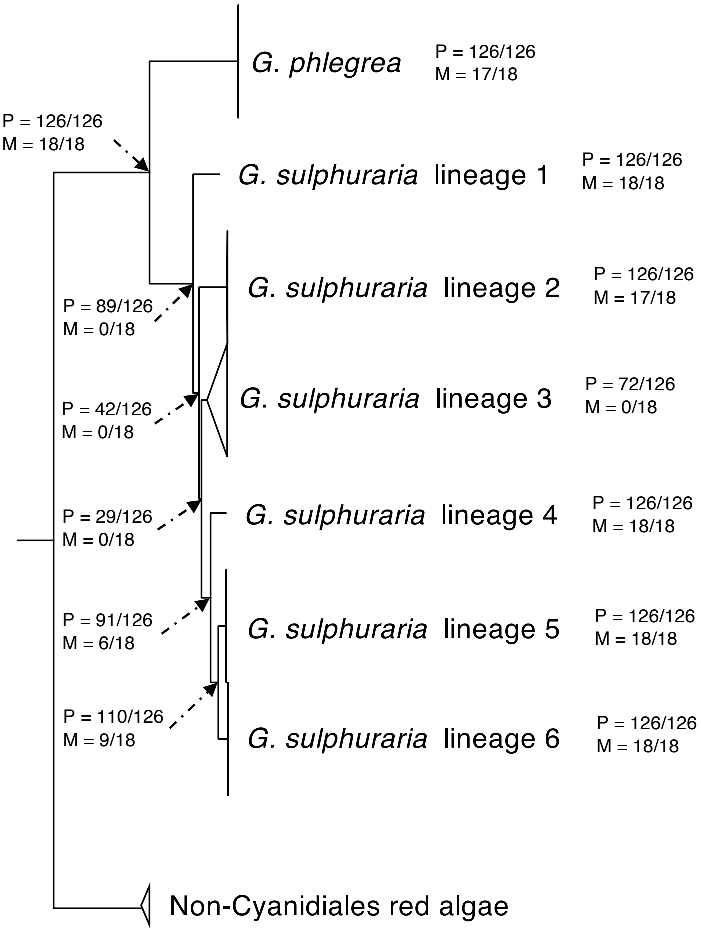


Figure 2.5. Simplified plastid Species tree of Cyanidiophyceae. Number of plastid and mitochondrial genes supporting the Species tree topology are indicated near the lineages and by the arrows near the nodes.

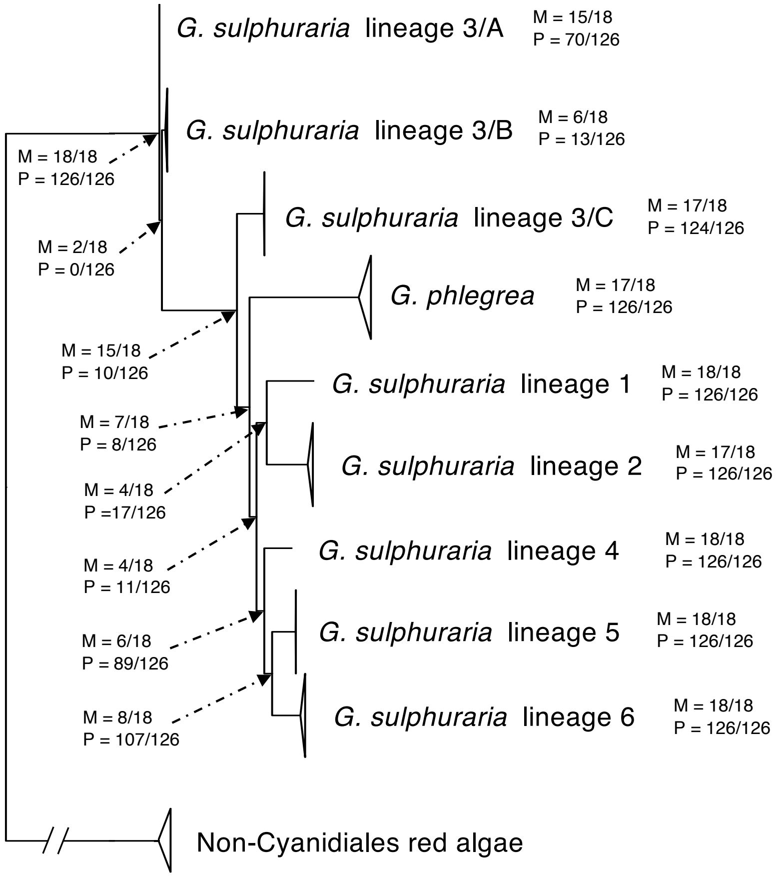


Figure 2.6. Simplified mitochondrial Species tree of Cyanidiophyceae. Number of plastid and mitochondrial genes supporting the Species tree topology are indicated near the lineages and by the arrows near the nodes.

2.3.4. ESTIMATION OF SELECTION

A comparison of the synonymous (dS) and non-synonymous (dN) substitutions was used to evaluate the degree of selective pressure on the evolution of plastid and mitochondrial genes. Non-synonymous substitution is a nucleotide substitution that changes in the encoded amino acid, while synonymous one doesn't produce any changes. When a DNA sequence is undergoing a process of molecular adaptation more amino acid alterations should take place, Therefore the ratio between dN and dS is commonly used to identify sequences that are undergoing adaptation. Overall, both plastid and mitochondrial genes showed a positive correlation between synonymous and non-synonymous substitutions (Plastid genes correlation = 0.920, p-value < 0.05; Mitochondrial genes correlation = 0.710, p-value < 0.05; Figure 2.7), even though they revealed different evolutionary rates.

Most of the plastid genes were found to be evolving at a very slow rate. They showed similar non-synonymous substitutions (dN < 0.4, Figure 2.7, Table S2.5). Faster evolving genes, such as, *accB*, *chlI*, *ftrB*, *ntcA*, *odpB*, *petA*, *rpoC2*, *tatC*, accumulate higher numbers of mutations with a consistent increment of both mutations toward a positive selection of the genes. Indeed non-synonymous substitutions were found to range between 0.00 and 1.48, values much higher of those of synonymous sites (from 0.13 to 0.65, Figure 2.7, Table S2.5).

Mitochondrial genes revealed more variable substitution rates, showing a higher increment of synonymous mutations compared to plastid ones. Evolutionary rates of some mitochondrial genes were found to be comparable with the slowest plastid genes; for instance, gene *nad6* has the slowest rate (dS = 0.05, dN = 0.14; Table S2.6) among both the plastid and mitochondrial genes. Increment of the non-synonymous substitutions revealed by some of the plastid genes is absent in the mitochondrial ones. Conversely, the evolution of the latter is toward a purifying selection, since the increment of the synonymous substitutions is higher than the non-synonymous ones (dN < 0.75, dS < 0.88). The only exceptions found are genes *sdhC*, *cox3* and *nad6*, showing a slightly positive selection (Table S2.6). The overall mean of synonymous and non-synonymous substitutions in the mitochondrial gene *sdhC* reveals a comparable evolution of this gene with the fastest rate in plastid genes (p-value < 0.05), even though the statistical evidence for a selective pressure is not highlighted (Table S2.6). Two mitochondrial genes, *cox3* and *yejR*, evolve at faster selective rates and differently either from the plastid genes, or the other mitochondrial ones (Table S2.6).

Study of the evolutionary pairwise rates among *G. sulphuraria* lineages was used to understand if selective forces are affecting the population structure of the species at a gene level. Even if all *G. sulphuraria* lineages revealed an overall purifying selection for both plastid and mitochondrial genomes (Table S2.7-S2.8), pairwise comparisons show a slightly different substitution rate at the inter-lineage level, demonstrating the possibility of selective forces affecting and shaping *G. sulphuraria* populations evolution (Figure 2.8a-b). The dN-dS average values of all the plastid genes vary between -0.132 (Lineage 6) to -0.191 (Lineage 1), while the mitochondrial genes vary between -0.202 (Lineage 4) to -0.302 (Lineage 3/A). Lineage more closely related from a phylogenetic point of view, demonstrate similar evolutionary rates. Lineage 5 and 6 are sister clades in both plastid and mitochondrial phylogenies and share similar substitution rates, -0.135/-0.132 for plastid genome and -0.255/-0.253 for the mitochondrial one (Table S7-S8). The difference between plastids and mitochondria evolution varies between the lineages and is highlighted by the mtDNA/ptDNA dN-dS ratio (Figure 2.8c). Lineage 5 and 6 mtDNA evolves almost 2 times faster than the ptDNA (ratios = 1.89/1.92), while lineage 1 and 3/C organelle genomes are subjected to more similar evolutionary forces, as demonstrated by the lower mtDNA/ptDNA ratios (1.37/1.23).

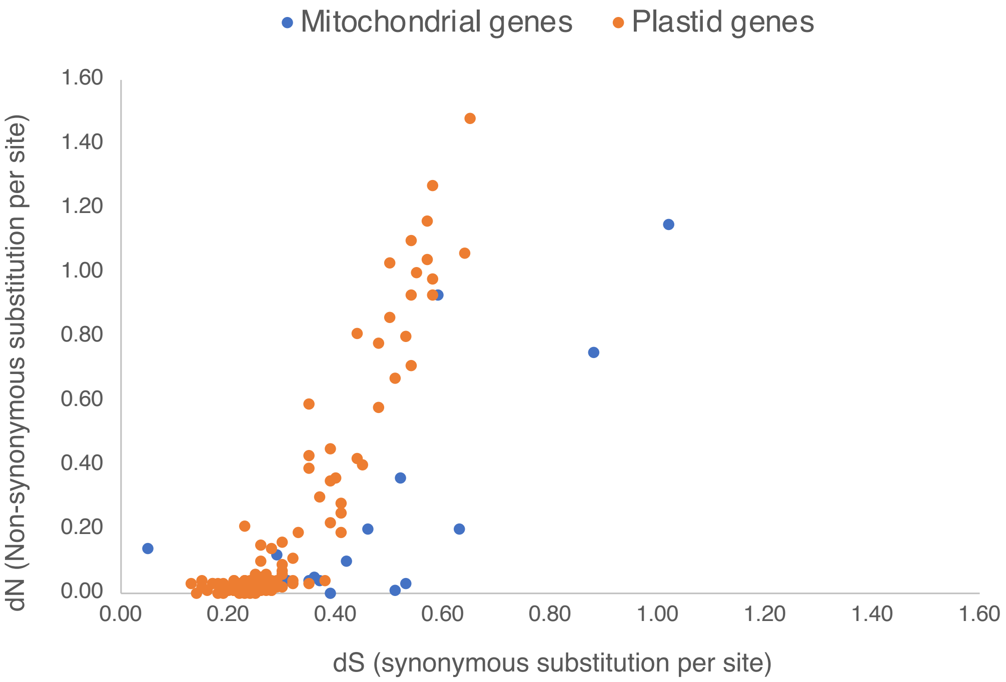
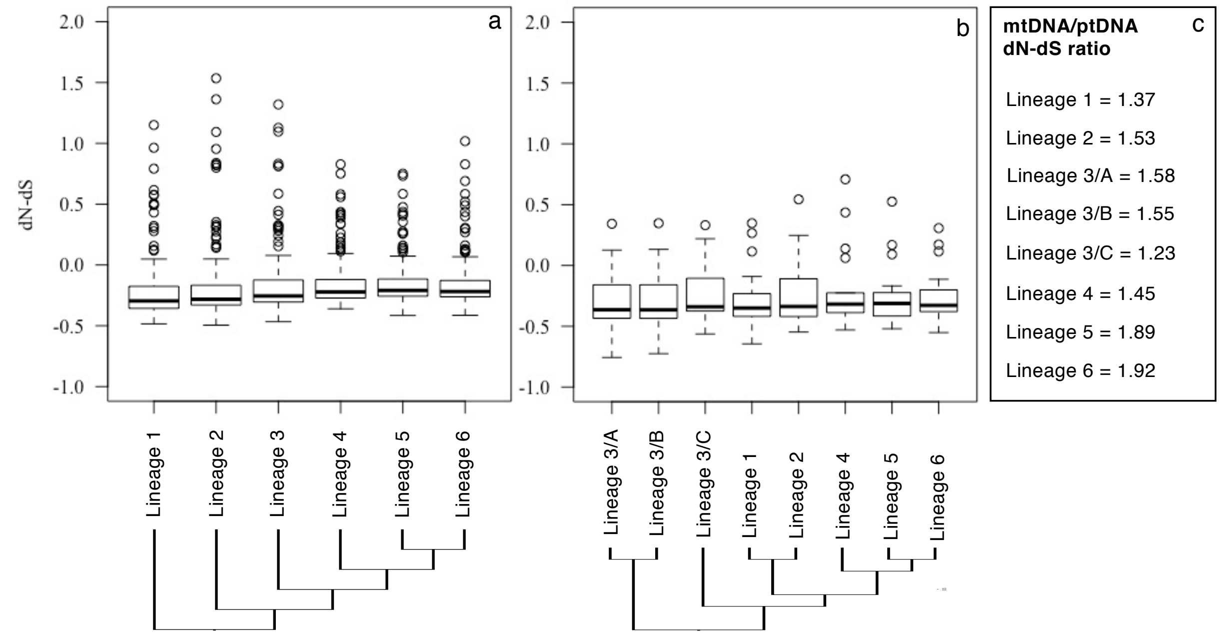


Figure 2.7. Relationship between Synonymous (dS) and non-synonymous (dN) substitutions per site for all the mitochondrial and plastid genes in *G. sulphuraria* strains. Plastid genes correlation = 0.920, p-value < 0.05; Mitochondrial genes correlation = 0.710, p-value < 0.05.



mtDNA/ptDNA dN-dS ratio

Lineage 1 = 1.37

Lineage 2 = 1.53

Lineage 3/A = 1.58

Lineage 3/B = 1.55

Lineage 3/C = 1.23

Lineage 4 = 1.45

Lineage 5 = 1.89

Lineage 6 = 1.92

Figure 2.8. dN-dS average values calculated for each pair of Lineages for plastid (a) and mitochondrial (b) genomes. Data are expressed as the mean of all single organelle genes dN-dS values, calculated with the Nei-Gojobori method implemented in MEGA X (Kumar 2018). Details of the dN-dS data, along with p-value for null hypothesis of strict neutrality (dN = dS), are listed in table S2.7-S2.8. Ratios between mitochondrial and plastid dN-dS for all the identified lineages are indicated in the third section of the figure (c).

2.3.5. COMPARATIVE GENOME ANALYSIS

Both plastid and mitochondrial genomes were conserved among *G. sulphuraria* species (ACUF 138, ACUF 017, SAG 107.79, ACUF 074, THAL 033 and ACUF 427) representative of the six lineages with some small variations. Plastid genome synteny using the ProgressiveMauve algorithm identified 23 locally collinear blocks (LCBs) with no or little inter-block spacers. LCBs identify conserved segments that appear to be internally free from genome rearrangements. Comparing the genome synteny of *Galdieria* strains with that of *Cyanidioschyzon merolae*, there is a massive rearrangement of the gene clusters including translocation and inversion (Figure S2.1). Inverted gene clusters mainly contain iron-sulfur and sulphate transporters (*sufB*, *sufC*), ribosomal proteins (*rpl*, *rps*), photosystem II and I proteins (*psa*, *psb*). Mitochondrial genomes syntenic analysis identifies mainly 3 LCBs, which are consistent in all the *G. sulphuraria* lineages (Figure S2.2). The arrangement of the LCBs is also uniform with that of *C. merolae*, except for block containing the nad6 and *cytb* genes, which is inverted.

2.4. DISCUSSION

The simple morphology of *Galdieria* species makes molecular tools indispensable to thoroughly understand the taxonomy and biology of these organisms. With the advent of NGS technology and the sequencing of the whole genome, phylogenomics is widely used to achieve these purposes, and one of the remarkable profits from these data is to use organelle genomes to infer robust phylogenies. Mitochondrial and plastid genomes contain essential genetic information connected to many vital functionalities and were already used to understand the evolution of brown algal plastids (Le Corguillé et al. 2009) or find evidence for organelle genome reduction (Yang et al. 2015) and rearrangement (Kim et al. 2018).

Plastid genomes of all the strains analysed in this study were consistent and congruent with the findings of previous studies (Jain et al. 2014). All of them demonstrated a partition in four distinct zones (LSC, SSC and two inverted repeats) and the presence of the second inverted region (Figure 2.2), reflecting a similar structure with the genomes of land plants, green algae, glaucophytes and eukaryotes with red algal-derived plastids genomes (Jain et al. 2014). Thus, it is quite possible that this region was present in the common ancestor and was then lost or rearranged in those lineages that diverged afterwards (Jain et al. 2014).

Mitochondrial genomes were found to be congruent and consistent across all the strains. The main interesting feature of the mitogenome is the high GC content, shared by all the isolates, confirming the result of previous work (Jain et al. 2014). They hypothesized the high GC skew as a consequence of the heterotrophic life of *G. sulphuraria*, which requires an increase of the energy requested by mitochondria when it finds itself living endolithically and in the dark (Jain et al. 2014). All the mitochondrial genomes diverged only by a long non-coding sequence of which is not known the exact functionality. Non-coding DNA may have important functions in transcriptional and translational regulation or may be the original site of DNA replication (Bronstein et al. 2018). For instance, the longest non-coding and most variable region in animal mitochondrial DNA is identified as Control Region (CR) and comprises a third strand of DNA, creating a D-loop (Bronstein et al. 2018). In vertebrates this region has strand-specific bias and is involved in the asymmetric DNA replication mechanism (Fonseca et al. 2014). This kind of mechanism was also hypothesised by Cho et al. (2020) for the mitochondrial replication in *Galdieria maxima* strains as the main mechanism causing the high mutation rate in their mitochondrial genomes (Cho et al. 2020).

Plastid and mitochondrial phylogenomics confirm the monophyly of *Cyanidiophyceae* species, supporting the phylogenies already stated in other studies (Ciniglia et al. 2004; Yoon et al. 2006; Iovinella et al. 2018). Analysing the phylogenetic relationships within the species of *G. sulphuraria*, an incongruent evolution is revealed by the two organelle genomes. Plastid and Mitochondria originated as a result of two separate events of absorption of cyanobacteria and α-proteobacteria from unicellular eukaryotes (Leblanc et al. 1997). The close relationship of a symbiont with the host gave new functionalities to the latter, such as aerobic respiration and/or photosynthesis. The two genomes could have evolved differently; it is well established that mitochondrial genomes, for example, have been widely evolving as a consequence of gene transfer from the symbiont to the nucleus and the loss of redundant genes (Leblanc et al. 1997).

Both phylogenies identify the subdivision of *G. sulphuraria* in more diverging lineages. The six lineages are: 1) ACUF 138 from San Salvador (orange box), 2) Mediterranean clade (green box), 3) strains from the Atlantic region plus Yellowstone National Park, US (purple box), 4) ACUF 074 from Java, Indonesia (red box), 5) Taiwanese strains (light blue box) and 6) Icelandic and Russian strains (yellow box) (Figure 2.4). Terminal branches of both trees, leading to the single strains, are always very short, while the branches leading to the different lineages are always very long, suggesting a low genetic diversity within each lineage but a high variability among them (Iovinella et al. 2018). This divergence is also highlighted by the percentage of the single nucleotide polymorphisms (SNPs) among the genomes (Table 2.1-2.2). Most of the lineages diverged from each other by 11% of both genomes and this percentage fell into the range of 8-11% identified as the common threshold level for genus assignment in Rhodophyta (Cassano et al. 2012; Liu et al. 2020).

Phylogenetic relationships between the 6 lineages were assessed in every plastid and mitochondrial gene to understand their contribution to the genetic evolution of *G. sulphuraria*. All the organelle genes highlight the divergence of the species into the 6 lineages, although not all of them support the phylogenetic relationship among them as stated by the species phylogenies described above. So far, plastid genes were considered a single unit and, for this reason, to be evolving in the same way (Doyle 1992; Gitzendanner et al. 2018). Lately data demonstrated that the plastid genes are not so linked to each other and they are subjected to different evolutionary forces (Gonçalves et al. 2019; Ruhlman et al. 2017). The high variability of the dS/dN ratios across both the plastid and mitochondrial genes identified in this work, confirms that the evolution of the organelle genes could be subjected to different forces (Figure

2.7). Moreover, dN-dS values, calculated for each pair of lineages and expressed as the mean values of all the genes (Figure 2.8; Table S2.7-S2.8), demonstrate a stronger purifying selection for mitochondrial genomes ($-0.202 < dN\text{-}dS < -0.302$) than the plastid ones ($-0.191 < dN\text{-}dS < -0.132$). In both genomes, the early diverged strains are those subjected to the higher evolutionary pressure, while lineages closely related to each other from a phylogenetic point of view share similar purifying selection rates (Table S2.7-S2.8).

Besides the differential evolution of the organelle genes, incongruence between Species and Gene trees could be explained with further biological processes. Hybridization and recombination are important events contributing to the genetic variation in populations (Maddison 1997; Som 2014). Plastid recombination was confirmed in diatoms and cyanobacteria (D'Alelio et al. 2013; D'Alelio and Ruggiero 2015) using the Pairwise Homoplasy Index test, PHI-test (Bruen et al. 2006).

Further biological mechanisms inducing phylogenetic incongruence are the incomplete lineage sorting and the horizontal gene transfer (Maddison 1997; Som 2014). The first one implies the persistence of ancestral polymorphisms during subsequent speciation events in a short period of time (Galtier and Daubin 2008). Rapid species radiation and the sharing of the ancestral polymorphisms across the strains could result in phylogenetic discordance (Galtier and Daubin 2008; Tang et al. 2012).

Lastly, the exchange of genetic material between organisms, known as horizontal gene transfer, deeply influences the phylogenetic relationship among strains (Galtier and Daubin 2008; Maddison 1997; Som 2014). The occurrence of horizontal gene transfer events in *G. sulphuraria* was widely confirmed in the last years (Rossoni et al. 2019; Schönknecht et al. 2013; Jain et al. 2014). Acquired genes are mainly involved in the adaptation of the species to harsh environments, including metal and xenobiotic resistance/detoxification, cellular oxidant reduction, carbon and aminoacid metabolism, osmotic resistance and salt tolerance, non-metabolic and uncertain functions (Rossoni et al. 2019).

Thanks to the extreme versatility of *G. sulphuraria* to cope with high range of temperature, 28-42°C (Rossoni et al. 2019), high range of pH, 1-6.5 (Iovinella et al. 2020), high concentrations of heavy and rare earth elements (Cho et al. 2020; Minoda et al. 2015; Čížková et al. 2019), the interest of using this microalga in biotechnology is growing and with it also the knowledge of its biology. Even if morphological traits slightly vary between the species belonging to *Galdieria*, molecular tools identify a huge variability even within the single species of *G. sulphuraria*. Although the general structure of the plastid and the mitochondrial genomes is consistent across the strains, phylogenetic analysis identifies the divergence of the species in more lineages that have been evolving separately. Yang et al (2015) hypothesized the rapid radiation of the species followed by strong constraints on gene content and genome evolution as possible explanations of this feature (Yang et al. 2015). Synonymous and non-synonymous substitutions, analysed in this study, confirmed the differential evolutionary pressure between the strains. High mutation rates along with events such as horizontal gene transfer could be the main evolutionary forces shaping the genetic structure of the population of *G. sulphuraria*.

CHAPTER 3: PHYSIOLOGICAL AND TRANSCRIPTOMIC RESPONSE OF *GALDIERIA SULPHURARIA* TO THE RARE EARTH METAL Ce^{3+}

3.1. INTRODUCTION

Cerium is the most abundant metal among the members of Rare Earth Elements (REEs), so called because the majority of them were originally isolated from rare minerals (Castor and Hedrick 2006). In the last years, these elements received more attention thanks to their applications in medical, agricultural and technological fields. Historically Ce^{3+} oxalate was used to relieve vomiting in case of sea sickness, pregnancy and gastrointestinal disorders (Dahle and Arai 2015). Cerium compounds were also employed as anticoagulants thanks to their property to replace Ca^{2+} in biomolecules, as well as a bacteriostatic and bactericidal drug in topical burn treatments (Dahle and Arai 2015; Schmidlin et al. 2012). Moreover, recent studies demonstrated the possibility of combining Cerium Chloride with fluoride solution to enhance protective effects during erosion of treated dentine (Wegehaupt et al. 2010).

In China, where there is the largest reserve of Rare Earth Metal in the world (Pang et al. 2002), low concentrations of these elements are used as fertilisers. Enhancement of radical growth in Chinese cabbage was especially demonstrated in plants treated with 1mg/L of Ce, while tomato plants showed an increased P and K metabolization when seedlings were sprayed with 5mg/L of $CeCl_3$ (Tang and Tong 1988). Evidence on the positive effect of $CeCl_3$ on photosynthesis was also demonstrated by Chu et al (1996), who showed that 0.5mg/L of $CeCl_3$ could accelerate the synthesis of chlorophyll a, resulting in a photosynthesis improvement (Chu et al. 1996). However, the biggest application of Cerium is in the new green and low-carbon technology, where it is indispensable for batteries alloys, automotive catalysts, Nickel metal-hybride batteries and lamp phosphors (Binnemans et al. 2013; Castor and Hedrick 2006). All these applications require huge amounts of Cerium that cannot be supplied exclusively by mines; a valid alternative to obtain further quantities of this metal could be achieved thanks to the use of biotechnological methods.

REEs recycle from Nickel metal-hybride batteries and lamp phosphors is often made through the application of expensive hydrometallurgical and pyrometallurgical methods. These methods are simple and easy to operate, but at the same time require large amounts of chemical to further process the metal mixture extracted and obtain all the single elements (Binnemans et al. 2013). Biological approaches are emerging as alternative ways thanks to their high efficiency, low cost and environmental impact. In particular, bioaccumulation is the active transport of metal ions inside the cell through an energy (ATP)-driven process (Lo et al. 2014). Microorganisms are usually used for this purpose, such as bacteria, fungi, yeasts and microalgae (Tsuruta 2007; Sadovsky et al. 2016).

Extremophilic life of *Galdieria sulphuraria* in the harsh conditions induced by geothermal site, makes this microalga one of the strongest organisms among the unicellular eukaryotes (Sentsova 1991; G. Pinto et al. 2003; Ciniglia et al. 2004; 2014). Actually, high temperatures (50°C-55°C), extremely acidic pH (pH ≤ 0.0-0.5) and high amounts of minerals and heavy metals make geothermal sites hostile for the majority of living organisms except for some, archea, bacteria, fungi and algae (Brock 1978; Albertano et al. 1994) and, among the latter ones, genus *Galdieria* represents 90% of the thermoacidophilic eukaryotic biomass (Schönknecht et al. 2013). The coexistence of huge *Galdieria* biomass together with huge amounts of metals in extremely acidic geothermal sites makes this microalga more interesting because it could be one of the best candidates for the biological recovery of metals. Schönknecht et al. in 2013 hypothesized the capacity of *G. sulphuraria*, strain ACUF 074, to accumulate metals inside the cell, thanks to the huge variety of genes in its genome coding for metals transporters and plasma membrane uptake (Schönknecht et al. 2013).

In this study, a first evaluation of the potential metal recovery activity of *Galdieria sulphuraria* was performed by analysing the physiological response of the strain SAG 107.79 to a wide range of the Ce^{3+}. Strain SAG 107.79 was chosen because of the availability of its genome and transcriptome. Changes in growth rate and cell vitality experiments were analysed for 14 days to assess the general state of health of the microalgae. Moreover, the amount of metal recovered by the biomass was measured after 24 hours of contact with 25 mg/L of Ce^{3+} dissolved in water pH 2.5, 3.5, 4.5 and 5.5. The measurements of the metal were done after 24h hours of treatment and expressed as μmol of Ce^{3+} per gram of dried matter (μmol/g dm). Finally, the transcriptomic response of *G. sulphuraria* to Ce^{3+} was analysed through RNA-seq data.

3.2. MATERIAL AND METHODS

3.2.1. MICROALGAL CULTURES PREINCUBATION

G. sulphuraria strain SAG 107.79 (http://sagdb.uni-goettingen.de/) was isolated by streaking the culture three times across the agar plates, and colonies were inoculated in Allen medium (Allen 1959) supplied with sucrose (5 mg/L) at pH 2.5. The culture was then grown on the orbital shaker (150 rpm) at 37° C, under continuous fluorescent light (50 *μ*mol photons·m–2·s–1), and weekly refreshed with new medium until it reached the late logarithmic phase. The growth was monitored by measuring the optical density of the culture with a spectrophotometer.

3.2.2. EXPOSITION TESTS

A Ce^{3+} stock solution was prepared dissolving 2 grams of $CeCl_3.H_2O$ (Alfa Aesar, USA) in 1 litre of Milli-Q water and sterilized by passing through a 0.44*μ*m filter. The analysis to measure and compare the growth rate and the cell vitality in samples treated with metal were performed as follows: The same quantity of microalgal cells (OD = 0.2; 6×10^6 cells/mL) was collected from the culture, washed twice with sterilized Milli-Q water and incubated in 50 mL flasks with Allen medium, pH 2.5, enriched with increasing concentrations of Ce^{3+} (10, 25, 50, 75, 100, 125 and 150 mg/L). Samples were then cultivated with constant light (50 *μ*mol photons·m–2·s–1), at 37° C and 150 rpm for 14 days. Negative controls were grown in Allen medium setting up the same experimental conditions described above.

The following exposition tests were performed in order to measure the quantity of Ce^{3+} bio-recovered by *G. sulphuraria*. Since Ce^{3+} reacts with phosphate groups present in the Allen medium, these experiments were done in ultrapure water to avoid any kind of precipitation. The experiments were conducted in a final volume of 2 mL, containing 3 x 10^8 *G. sulphuraria* cells and 25 mg/L of Ce^{3+} dissolved in ultrapure water pH 2.5, 3.5, 4.5 and 5.5. Control tests were performed in Ce^{3+} -free water solutions (negative control) and in Ce^{3+} solutions without *G. sulphuraria* cells (positive control) both at the same pHs. All the exposition and control tests were performed in triplicates.

The experimental design just described was also used to analyse the transcriptomic response of *G. sulphuraria* to Ce^{3+}. Samples were collected from Ce^{3+} -treated and untreated cultures at pH 2.5 and 5.5 after one hour of treatment; six biological replicates were performed for each culture.

3.2.3. GROWTH RATE AND CELL VITALITY MONITORING

Samples were collected from each flask (Day 0, 3, 6, 10 and 14) to measure the cells/*μ*l using a haemocytometer and a light microscope (Nikon Optiphot-2).

The percentage of the viable cells was assessed via Trypan Blue assay by mixing 500 *μ*l of the cultures with the same volume of Trypan Blue (0.4% w/v). After 5 minutes at room temperature, cultures were washed twice with Allen pH 2.5, and dead cells were measured using the haemocytometer and the optical microscope. The vitality of the cultures was assessed applying the following formula:

$$\text{\% of viable cells} = \frac{Number\ of\ viable\ cells}{Number\ of\ total\ cells} \text{ x } 100$$

3.2.4. Ce^{3+} QUANTIFICATION BY ICP-MS

Samples were centrifuged after 24 hours of treatment to separate the pellets from the supernatants. Supernatants were filtered with 0.2 μm filters and were used to measure Ce^{3+} concentration through inductively coupled plasma mass spectrometry, using an ICP-MS PerkinElmer (PerkinElmer, Waltham, MA, USA). The evaluation of the metal uptake was conducted by measuring the total metal removed using the following formula:

$$\text{Total metal removed } (\mu\text{mol/g dm}) = (C_{\text{biomass}} \times V/M)/\text{metal molecolar weight}$$

where C_{biomass} is the metal concentration measured in the biomass fraction; V is the volume of the test solutions; and M is microalgal biomass (g, dry matter).

3.2.5. TRANSCRIPTOMIC ANALYSIS

3.2.5.1 EXTRACTION OF RNA, ILLUMINA LIBRARY CONSTRUCTION AND SEQUENCING

Samples were centrifuged after 1 hour of Ce^{3+} treatment, washed with 50mM KH_2PO_4 pH 7 and frozen in liquid nitrogen. Total RNA was isolated using RNeasy Kit (Qiagen) after the frozen biomass was mechanically disrupted with a pestle. RNA integrity was determined using an Agilent BioAnalyzer 2100 (Agilent Technologies).

RNA library preparation and sequencing were performed at Novogene (UK) Company Limited (Cambridge). Library preparation was performed using NEB Next® Ultra™ RNA Library Prep Kit (NEB, San Diego, CA, USA), employing AMPure XP Beads to purify the products of the reactions during the library prep. Poly-a mRNA was isolated using poly-T oligo-attached magnetic beads, then fragmented through sonication and enriched into 250-300bp fragments. The purified mRNA was converted to cDNA and subjected to the adaptor ligation. The barcoded fragments were finally multiplexed and ran on the Illumina Novaseq 6000 (s4 flow cell) to acquire 20 million read pairs per sample, using the 150bp PE sequencing mode.

3.2.5.2. DETECTION OF DIFFERENTIALLY EXPRESSED TRANSCRIPTS

Transcripts quantifications were done using the software Salmon v 1.4.0 (Patro et al. 2017), aligning the RNA-seq reads to the annotated and indexed transcriptome of *G. sulphuraria*, strain SAG 107.79 (The transcriptome was kindly provided by Jessica Downing). Differential expression analysis were performed with R Sleuth package and transcripts with a FDR < 0.01 were considered as differentially expressed.

3.2.5.3. FUNCTIONAL ANNOTATION AND ENRICHMENT ANALYSES

Functional annotation and enrichment analyses were performed using OmicsBox software v 1.4.11 (https://www.biobam.com/omicsbox). To annotate the differentially expressed genes, they were mapped to the NCBI non-redundant (nr) database using BLASTx algorithm setting the p-value threshold 10^{-5} (Altschul et al. 1990). Concurrently, genes were also mapped to InterProScan, https://www.ebi.ac.uk/interpro/about/interproscan (Quevillon et al. 2005). The annotated genes were then associated to the Gene Ontology (GO) terms and mapped to the main functional categories biological process, molecular function and cellular component. When the annotated genes resulted in an enzyme, the enzyme commission numbers (EC) were also identified. Annotated and mapped transcripts were used for GO enrichment analysis applying the p-value correction of the Benjamini-Hochberg method (Ferreira and Nyangoma 2008; Wang et al. 2009). The GO term with an FDR value <0.01 was considered significantly enriched.

3.2.6. MICROSCOPIC OBSERVATIONS

3.2.6.1. LIGHT MICROSCOPE OBSERVATION

Alizarin Red was used to visualize the bioaccumulated Ce^{3+}, following the method described in Minoda et al. (2015). Briefly, samples were washed with Milli-Q water at pH 2.5, and stained with 1% of Alizarin for 30 minutes at 37°C. Samples were then washed again with Milli-Q water and observed at microscope (Nikon Optiphot-2).

3.2.6.2. SERIAL BLOCK FACE SCANNING ELECTRON MICROSCOPE (SBF-SEM) OBSERVATIONS

Samples were also prepared for the SBF-SEM observation using the high pressure freezing protocol. Briefly, samples were harvested by centrifugation, washed twice with distilled water and finally centrifuged to discard the supernatants. Pellets were pipetted into 0.1 mm deep cavities of the planchettes and closed with the flat side previously dipped in Hexadecene. Samples were soaked in 0.1% Tannic Acid for 18 hours at -90°C and then washed with acetone. They were then incubated with 1% Osmium for 12 hours, slowly increasing the temperature to -60°C. After adding fresh 1% Osmium, samples were slowly (101 hours) warmed up to -20°C, then 4°C to finally room temperature. They were then incubated in 0.1% Tannic Acid for 2 hours and 1% Osmium for further 2 hours and finally incubated with Spurr resin, gradually increasing the percentage of the resin. In between the incubation steps, samples were washed with acetone.

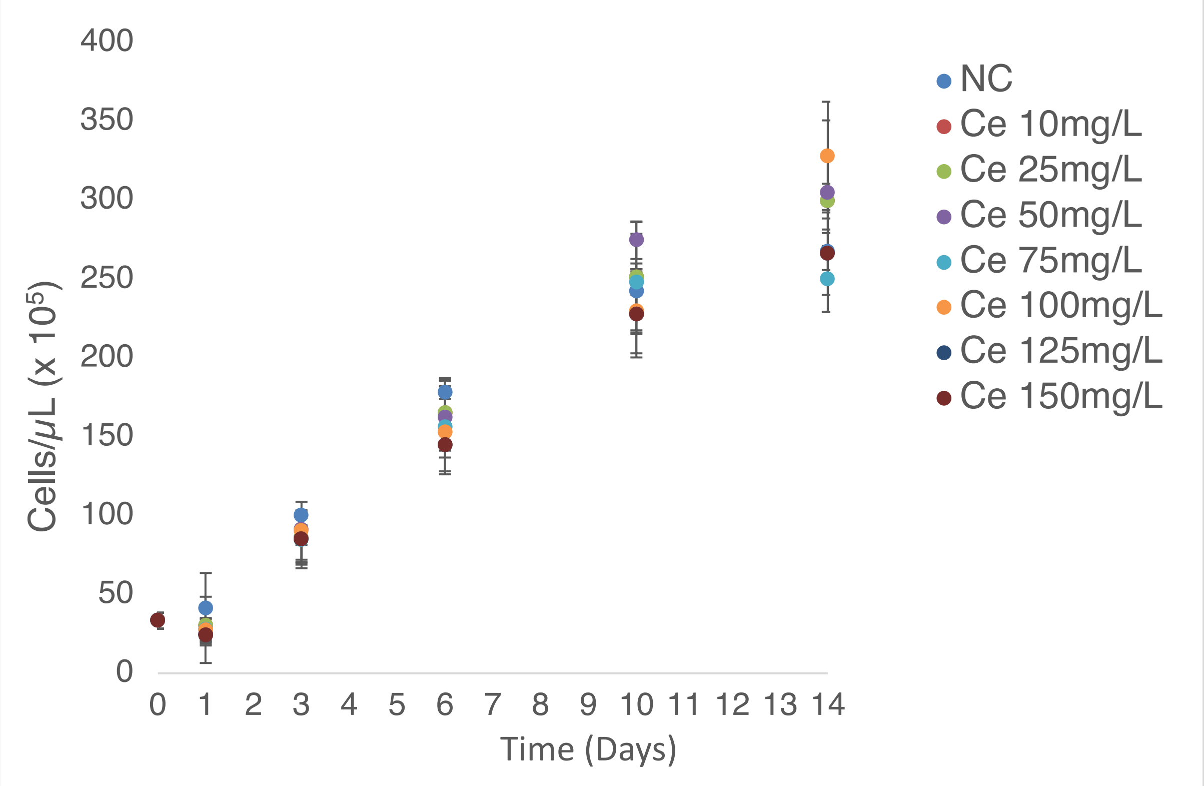
3.2.7. STATISTICAL ANALYSIS

All the experiments were performed in triplicates and the data were expressed as mean ± standard deviation. Cell density, percentage of viable cells and metal recovery percentage values were analysed through one-way analysis of variance (ANOVA). A multiple comparison Tukey test was then used to evaluate the significance of the differences among the treatments.

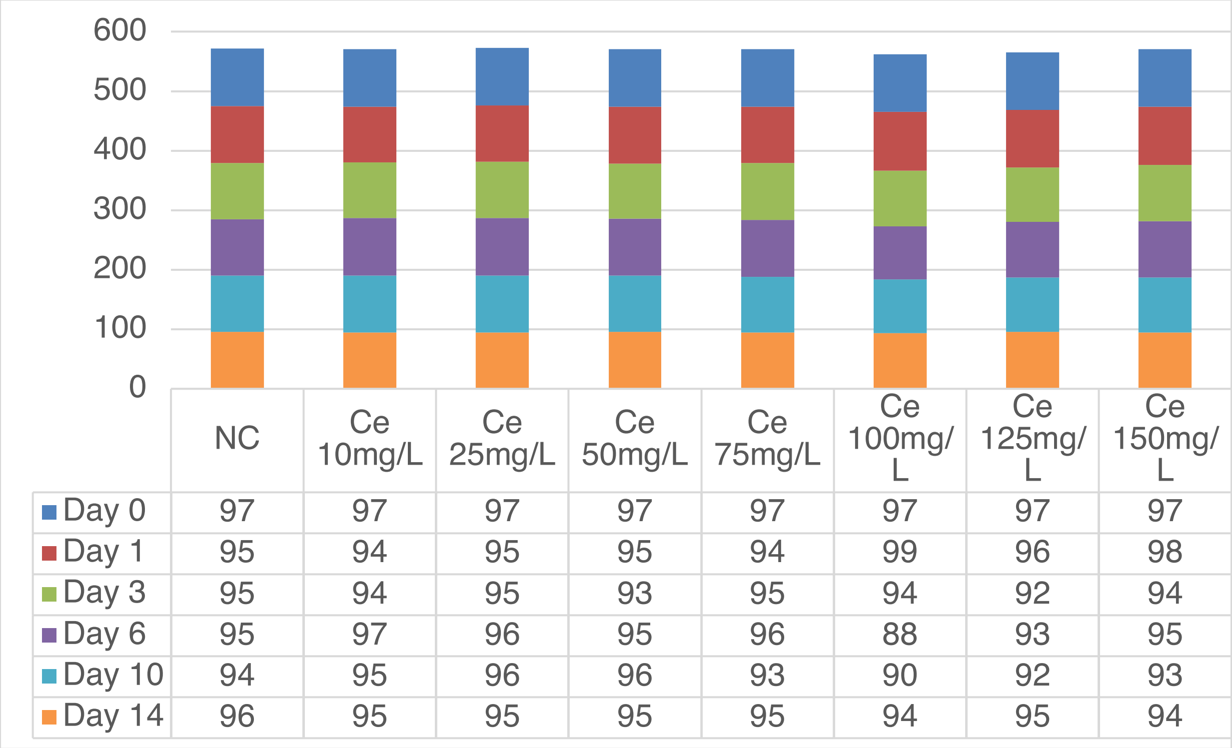
3.3. RESULTS

3.3.1. EFFECTS OF Ce^{3+} ON GROWTH RATE

Growth rate is one the parameters used to understand the effects of external factors on the state of health of microorganisms. *G. sulphuraria*, strain SAG107.79, could tolerate treatments with the Rare Earth Metal Ce^{3+}, since no negative growth rate was registered throughout the experiments. Metal treatments did not accelerate growth, thus suggesting a null influence of Ce^{3+} on the growth performance of *G. sulphuraria* (Figure 3.1). As shown in figure 3.1, the increasing concentration of the metal did not result in a associated increment of the cell, even if significant differences were registered only on the last day of treatments (14th day, p-value < 0.05). The cultures started the assay from the same cell density (3 x 10^6 cells/μL), and the highest biomass yield (33 x 10^6 cells/μL) was reached by the sample treated with Ce^{3+} 125mg/L, while the lowest one (25 x 10^6 cells/μL) was reached by 75mg/L. Variations of the cell vitality induced by the Ce^{3+} treatments were evaluated measuring the percentage of viable cells in both treated and untreated samples throughout the experiments. Data showed no significant differences between all the samples ($p < 0.05$; Figure 3.2).



3.1. Growth rate of *G. sulphuraria,* strain SAG 107.79, treated with increasing concentrations of Ce^{3+} . Data are shown as mean value (± S.D.; *n* = 3). Days marked with asterisk are significantly different from the control (Tukey test; p-value < 0.05).



	NC	Ce 10mg/L	Ce 25mg/L	Ce 50mg/L	Ce 75mg/L	Ce 100mg/L	Ce 125mg/L	Ce 150mg/L
🟦 Day 0	97	97	97	97	97	97	97	97
🟥 Day 1	95	94	95	95	94	99	96	98
🟩 Day 3	95	94	95	93	95	94	92	94
🟪 Day 6	95	97	96	95	96	88	93	95
🟦 Day 10	94	95	96	96	93	90	92	93
🟧 Day 14	96	95	95	95	95	94	95	94

Figure 3.2. Percentage of *G. sulphuraria* viable cells calculated with the method of Trypan blue. Data are shown as mean value (± S.D..; *n* = 3). No significant differences were calculated among the treatments and the days of the experiments.

3.3.2 METAL BIOACCUMULATION ASSAY

In the present chapter, Ce^{3+} recovery capacity of *G. sulphuraria* was evaluated measuring Ce^{3+} concentration in the microalgal biomass and expressed as μmol/g dm using test solutions with different initial pH. Increments of the pH highly affected the removal capacity of SAG 107.79; indeed, in acidic conditions (pH 2.5) the microalga was able to remove low amounts of Ce^{3+} (< 2.5 *μ*mol/g, Figure 3.3). Increasing the medium pH to 3.5, a light increase of the metal removed was observed (5.39 ± 0.40 *μ*mol/g). Significant higher quantities of the metal being removed were registered when subneutral test solutions were used ($p\text{-value} < 0.05$). Specifically, Ce^{3+} recovered reached the value of 17.82 ± 4.21 *μ*mol/g at pH 4.5 and 32.91 ± 1.87 *μ*mol/g at pH 5.5 (Figure 3.3),

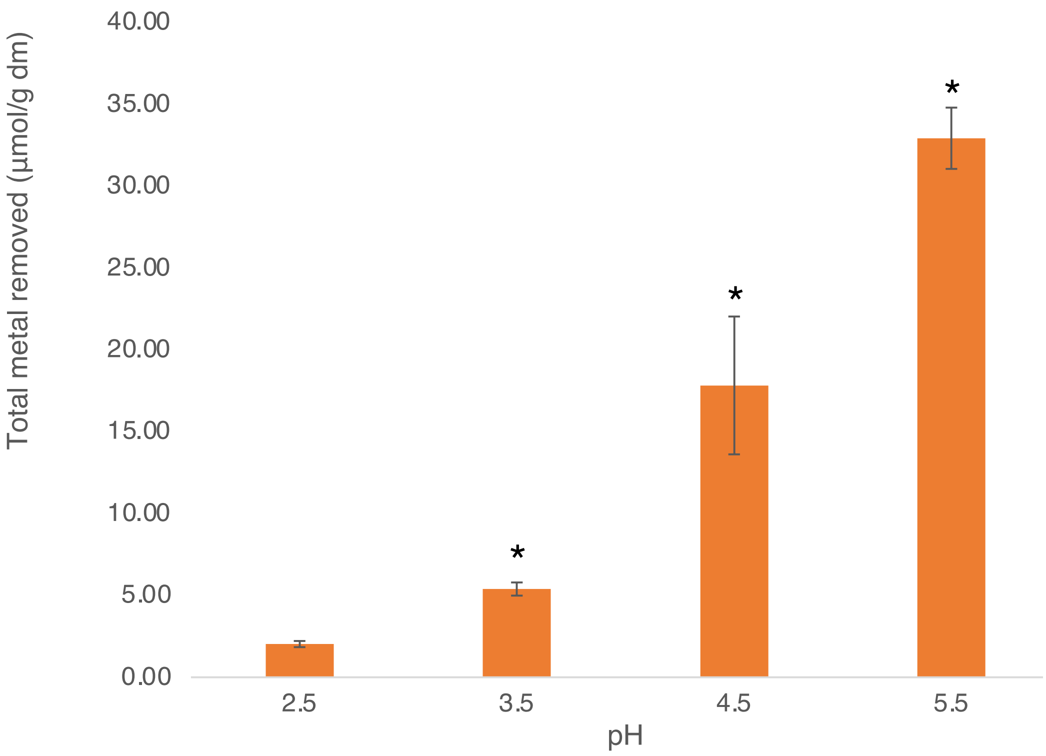


Figure 3.3. Total Ce^{3+} measured in G. sulphuraria biomass and expressed as *μ*mol/g dm. Data are shown as mean value (□ S.D.; n = 3); pH columns marked with asterisk are significantly different from the sample tested at pH 2.5 ($p\text{-value} < 0.05$).

3.3.3 TRANSCRIPTOMIC ANALYSIS

3.3.3.1 GENERAL PATTERN OF GENE EXPRESSION

In order to characterize the physiological response of *G. sulphuraria* to Ce^{3+} and understand the molecular mechanisms involved in metal homeostasis, differentially expressed genes were characterised in response to acute metal application.

As a result of the Ce^{3+} treatment, 887 transcripts were identified as differentially expressed (DE; $FDR < 0.01$), 459 of which were increased and 428 were decreased (Figure 3.4). Only the genes which increased their expression following Ce^{3+} treatment were considered for the subsequent analysis. Among the biological process, the most representative Gene Ontology term (#183 sequences) regarded the organic substance metabolic process (GO:0071704), followed by the cellular metabolic process (#172 sequences, GO:0044237) and the primary metabolic one (#168 sequences; GO:0044238; Table S3.1). A lower number of sequences represented the processes of transmembrane transport (#40 sequences, GO:0055085) and cellular component organization (#23 sequences, GO:0071840; Table S3.1). Among the molecular function, the transmembrane transporter activity remained the less represented with 40 sequences (GO:0022857), while the highest number of sequences regarded the ion (#125 sequences, GO:0043167) and the heterocyclic compound (#114 sequences, GO:1901363) binding (Table S3.1). The "intracellular anatomical structure" and the "membrane" GO terms (GO:0005622, go:0016020) are the most represented among the cellular component, with 129 and 105 sequences, respectively (Table S3.1).

An Enrichment analysis (Fisher's Exact Test) was performed to identify transcripts that were over-represented. The annotated transcripts were enriched in 44 GO terms belonging to the three functional categories, biological process (BP), molecular function (MF), and cellular component (CC), in metal homeostasis ($FDR < 0.05$). The enriched GO terms were ulteriorly reduced to the most specific ones, resulting in 17 GO ones ($FDR < 0.05$), using the option "Reduce to Most Specific" that removed more general GO terms and got only the most specific ones, which are the lowest in the GO directed acyclic graph.

Among the biological processes, the highest number of sequences was recorded for the oxidation-reduction process (GO:0055114), carboxylic acid (GO:0019752), carbohydrate (GO:0005975) and purine ribonucleotide metabolic processes (GO:0009150). Moreover, the biosynthetic processes of ribonucleotide (GO:0009260), amide (GO:0043604) and organonitrogen compounds (GO:1901566) were also significantly enriched. The GO terms regarding the generation of precursor metabolites (GO:0006091) and transmembrane transport (GO:0055085) were also increased (Figure 3.5). The functional category “cytoplasm (GO:0005737)” was the only most represented among the cellular component one (FDR < 0.05).GO categories significantly enriched (FDR < 0.05) among the molecular functions regarded the nucleotide binding (GO:0000166), and oxidoreductase (GO:0016491), transmembrane transporter (GO:0022857), ligase activity (GO:0016874), hydrolase, acting on glycosyl bonds (GO:0016798) activities (FDR < 0.05).

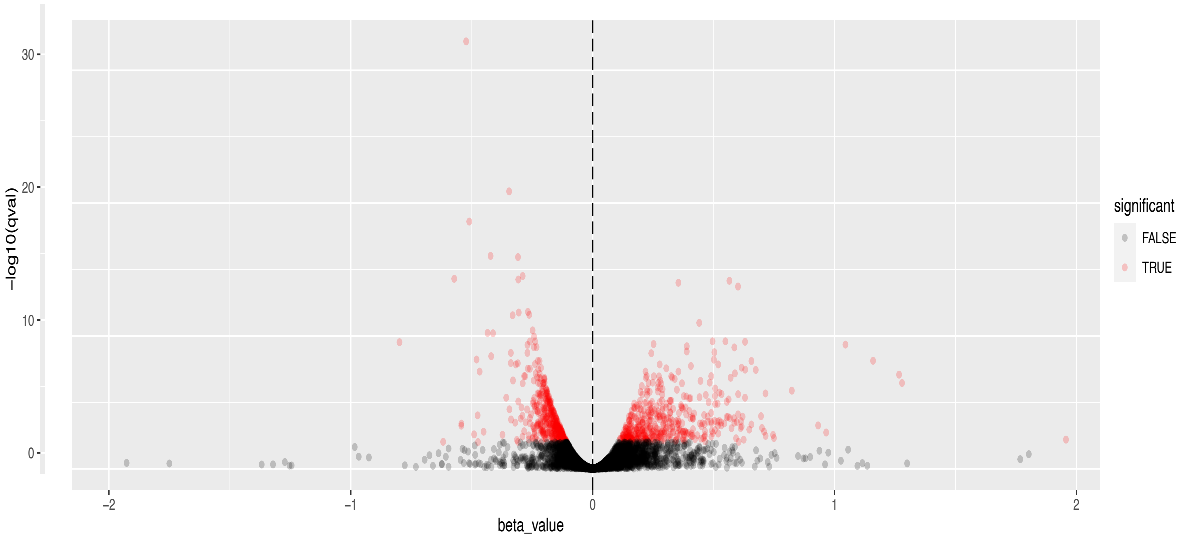


Figure 3.4. Volcano plot representing the up and down regulated genes in *G. sulphuraria* treated with 25 mg/L of Ce^{3+} . The adjusted p-value (FDR) was set up at 0.01 and the red dots were considered statistically differential expressed. The x-label represents the log2FC, which is positive for gene up-regulated and negative for gene down-regulated; on the y-label is the FDR as -log10; data points that are more far away from the central location of the volcano have the highest log2FC and the lower p-value; black dots are not considered statistically differential expressed.

3.3.3.2 OXIDATION-REDUCTION REACTIONS

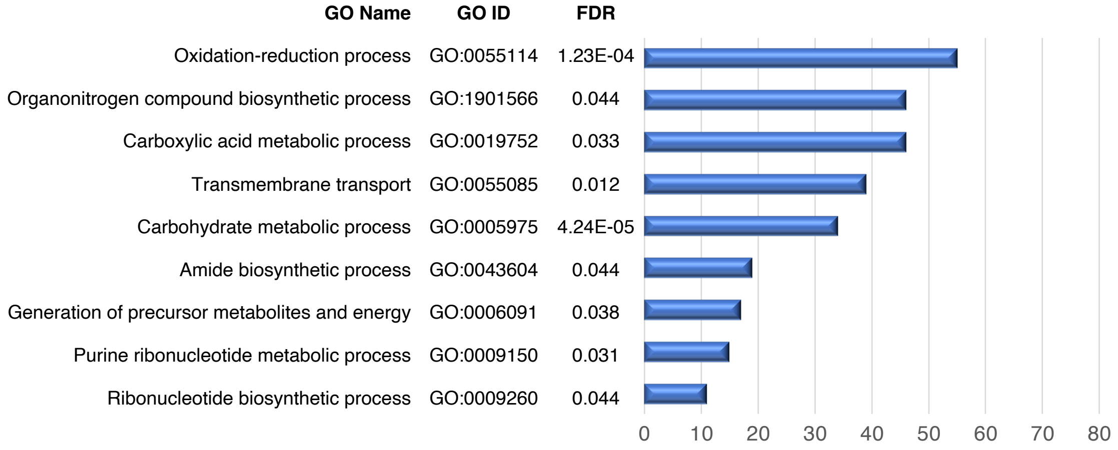
The oxidation-reduction reactions are one of the most up-represented GO terms following Ce^{3+} treatments. They comprise 55 up-regulated transcripts belonging to the oxidation-reduction process (GO:0055114) and 51 transcripts increased in abundance belonging to oxidoreductase activity (GO:0016491). Most of them are dehydrogenase, like 3-hydroxybutyryl-CoA dehydrogenase, alcohol dehydrogenase, aldehyde dehydrogenase (NAD+) and myo-inositol 2-dehydrogenase. Remarkably, among the oxidation-reduction reactions, is the transcript increase of peroxiredoxin, involved in the control of peroxide levels inside the cells and the increase of the phytoene desaturase involved in the carotenoids biosynthetic pathway. The last interesting enzyme is the mercuric reductase that converts the toxic form of the mercury Hg^{2+} in less toxic one (Figure 3.5).

3.3.3.3 CELLULAR METABOLIC PATHWAYS

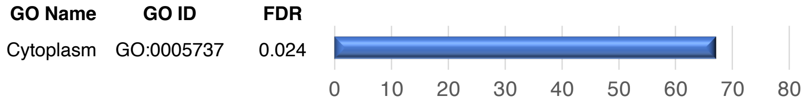
Ce^{3+} treatments affected diverse processes that bring energy to the organism. One of the up-represented GO terms regarded the generation of metabolite precursors (17 transcripts, GO:0006091; FDR < 0.05; Figure 3.5). The ribonucleotide biosynthetic process (GO:0009260) and the child term purine ribonucleotide metabolic process (GO:0009150) were also significantly enriched with 11 and 15 up-regulated transcripts (FDR < 0.05), which were also involved in the ADP and ATP biosynthetic processes. Strictly correlated to the previous functional categories was the ligase activity (GO:0016874), molecular function that includes transcripts (23 transcripts; FDR < 0.05) involved in reactions of joining two molecules within a single one, using the energy from the hydrolysis of ATP, or similar molecules (Figure 3.5).

Further GO terms that are involved in the metabolism of *G. sulphuraria* are the carbohydrate (34 transcripts; GO:0005975) and carboxylic acid (GO:0019752) metabolic process (FDR < 0.05). An extensive variety of transcripts belonging to the first GO term were increased, for example galactosidase, glucosidase, pyrophosphatase and kinase. The enriched term regarding the hydrolase activity, acting on glycosyl bonds (GO:0016798), which belongs to the molecular function category was also up-represented and involved in the carbohydrate metabolic reactions (14 transcripts; FDR < 0.05). Also the carboxylic acid metabolic process term was significantly up-represented including 46 up-regulated transcripts, among which are the 2-isopropylmalate synthase and the acetolactate synthase large subunit (Figure 3.5). GO terms significantly enriched were also the amide (19 transcripts; GO:0043604) and the organonitrogen compound biosynthetic process (46 transcripts; GO:1901566).

a Biological processes



b Cellular components



c Molecular Functions

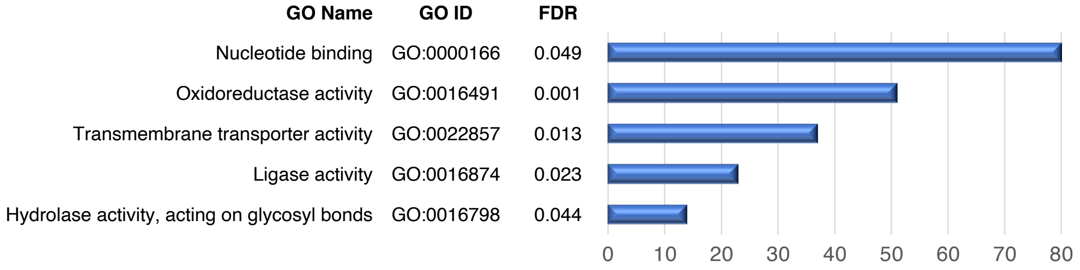
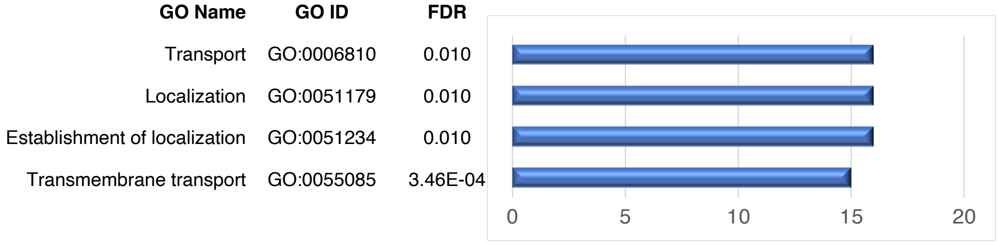


Figure 3.5. Distribution of enriched Gene Ontology (GO) terms belonging to the functional categories biological processes (a), cellular component (b) and molecular function (c) (FDR < 0.05).

3.3.3.4 LOCALIZATION

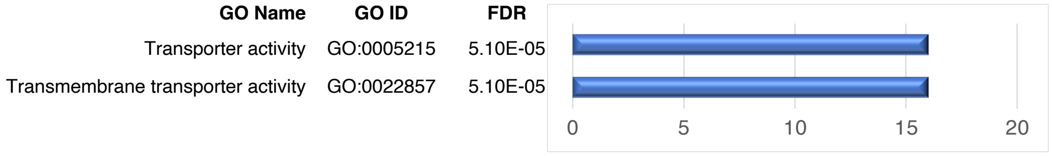
Genes that are most differentially expressed (65 transcripts; FDR < 0.01), resulting from the Ce^{3+} treatments, were enriched in the GO term of localization (GO:0051179; Figure 3.6). These biological processes regard the transport, tether and maintenance of substances or cellular entities in a specific location of the cell. Strictly correlated to the above-mentioned process, is that one regarding the capacity of the organism to localize the substance or a cellular component in the cell (16 transcripts; establishment of localization, GO:0051234). The movement of these substances inside, outside or within the cell mediated by a transporter, a pore or a motor protein are identified with the enriched GO term "transport" (GO:0006810; GO:0006810) and the subsequent child term of the transmembrane transport (15 transcripts; GO:0055085). The molecular function categories regarding the transporter activity (GO:0005215) and transmembrane transporter activity (GO:0022857) were also significantly enriched (FDR < 0.05), both with 16 transcripts increased (GO:0051179; Figure 3.6).

a Biological processes



GO Name	GO ID	FDR
Transport	GO:0006810	0.010
Localization	GO:0051179	0.010
Establishment of localization	GO:0051234	0.010
Transmembrane transport	GO:0055085	3.46E-04

b Cellular components



GO Name	GO ID	FDR
Transporter activity	GO:0005215	5.10E-05
Transmembrane transporter activity	GO:0022857	5.10E-05

Figure 3.6. Distribution of enriched Gene Ontology (GO) terms belonging to the functional categories of biological processes (a) and cellular component (b); these GO terms included genes that most increased their transcription (log2FC > 0.5; FDR < 0.05).

3.3.3.5 EFFECT OF PH ON THE PROTEIN POST-TRANSLATIONAL MODIFICATION (PTM)

Samples that were treated with Ce^{3+} dissolved in a test solution at pH 5.5 showed a new set of differentially expressed genes compared with those samples treated with the metal at pH 2.5 (Figure 3.7). The genes that increased their transcription were 141 ($FDR < 0.01$) and they were enriched in 6 GO functional categories ($FDR < 0.05$). The highest number of sequences (#17) were involved in the phosphate-containing compound metabolic process (GO:0006796) followed by 11 sequences that are part of the GO term “Cellular protein modification process” (GO:0006464; $FDR < 0.05$). Among the molecular function category, 6 up-regulated transcripts were enriched in the GO term regarding the helicase activity (GO:0004386), and 23 transcripts regarded the ATP binding, as well (GO:0005524; $FDR < 0.05$).

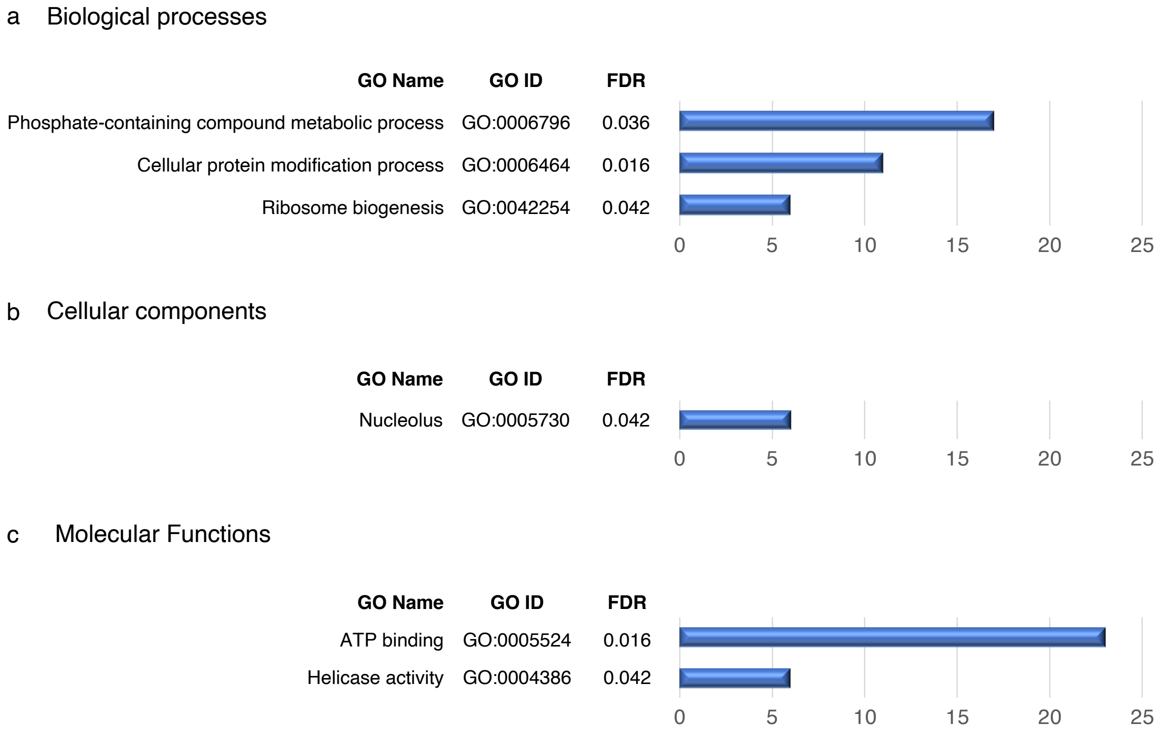


Figure 3.7. Distribution of most enriched Gene ontology (GO) terms, resulting from the interaction of Ce^{3+} treatments and medium pH ($FDR < 0.05$).

3.4. DISCUSSION

In this study, the absence of negative effects of the Rare Earth Element Ce^{3+} on the physiology of the extremophilic red microalga *G. sulphuraria* confirmed the possibility of using it to recover and recycle this metal. Enrichment of the culture medium with increasing concentrations of Ce^{3+} did not reduce the microalgal growth since the final biomass densities were comparable. The ineffective influence of metal ions on the physiological health of *G. sulphurari*a was also confirmed by the percentage of viable cells when comparing the treated samples with the untreated ones. These results were the first evidence of such a strong capacity of a microorganism to tolerate concentrations of Ce^{3+} ions up to 150mg/L. Previous studies highlighted the stimulating effects of low concentrations of Cerium (1.7 and 0.1mg/L) on the growth of *Chlorophyceae* and *Cyanophyceae* species, whereas high concentrations induced significant inhibitory effects (Goecke et al. 2017). Chinese researchers also studied the effects of Cerium on plants since, in their country, this element is widely used as a fertiliser. The chemical properties of Cerium allow it to substitute calcium in biomolecules, improving photosynthesis, nitrogen metabolism and consequently also growth in calcium-deficient conditions (Chao et al. 2008).

Data from the bioaccumulation assays confirmed the capacity of *G. sulphuraria* to remove Ce^{3+} ions from the surrounding medium, which was directly correlated to the pH of the medium itself (up to pH 4.5). Metal recovery rate is strictly correlated to extrinsic factors such as pH, temperature, metal ion concentration and biomass concentration (Franklin et al. 2002; Monteiro et al. 2011). The influence of the medium pH could be correlated to charged groups of the cell wall. Protonation and deprotonation of carboxyl, phosphate, hydroxyl or amine create plausible binding sites for metal ions (Monteiro et al. 2011).

Transcriptomic data obtained from the RNA-seq analysis highlighted an increment of diverse reactions to boost cellular energy. This is confirmed by the identification of genes involved in the ADP and ATP biosynthetic process that increased their transcription. The energy derived from these reactions is used in diverse metabolic pathways, such as the carbohydrate biosynthesis processes. Genes involved in carbohydrate metabolism were often identified in previous studies on the effects of heavy metals on microalgae (Olsson et al. 2015; Puente-Sánchez et al. 2016; Simon et al. 2013). The activation of this metabolic pathway, regardless of the treatment analysed, suggests it as an occurring response of microorganisms to metal ions.

Genes involved in carbohydrate biosynthetic processes could also increase their expression for the production of cell wall hemicellulose and the Extracellular Polymeric Substances (EPS). The EPSs consist of exopolysaccharides, uronic acids, aromatic, acetate and sulphate groups, exoproteins, lipids and nucleic acids, which role is to protect the culture from potentially stressful environmental factors (Miazek et al. 2015). EPSs production as a strategy to protect cells from metal ions and metal nanoparticles was already demonstrated in Cyanobacteria. For instance, Synechocystis, strain PCC6803, produced high quantities of EPSs attached to the cell's surface (CPS) and also released in the culture medium (RPS) when the microalgae were treated with cadmium and cobalt (Jittawuttipoka et al. 2013).

Among the up-represented GO terms, carboxylic acid and amide biosynthetic processes could lead to the biosynthesis of EPS and their involvement in the bio-uptake of Ce^{3+}. Indeed, exopolysaccharides are full of hydroxyl (-OH) and carboxyl (C-O) functional groups, along with C = O group of the amide, which as active binding sites for heavy metals (e.g., Pb^{2+}, Cu^{2+}, and Zn^{2+}) through complexation, ion exchange, surface precipitation and electrostatic attraction (Zhao et al. 2019).

The above-mentioned metabolic pathways suggested that the bio-recovery of Ce^{3+} could be ascribed mainly to the molecular bond of metal ions on the surface of *G. sulphuraria* cell walls. Microscope observations of samples stained with the Alizarin Red confirmed the presence of a mantle of Extracellular Polymeric Substances (EPS), whose thickness was greater in samples grown with higher concentrations of Ce^{3+} (Figure 3.8).

Nevertheless, the genes most increased were associated with the metabolic pathways of localization and transmembrane transport. These reactions regarded the transport, tether and maintenance of substances in a specific cell location. Identifying these GO terms, so specific and interconnected between them, could represent a way to understand mechanisms that the microalga uses when metal ions accumulate inside the protoplast (Figure 3.8).

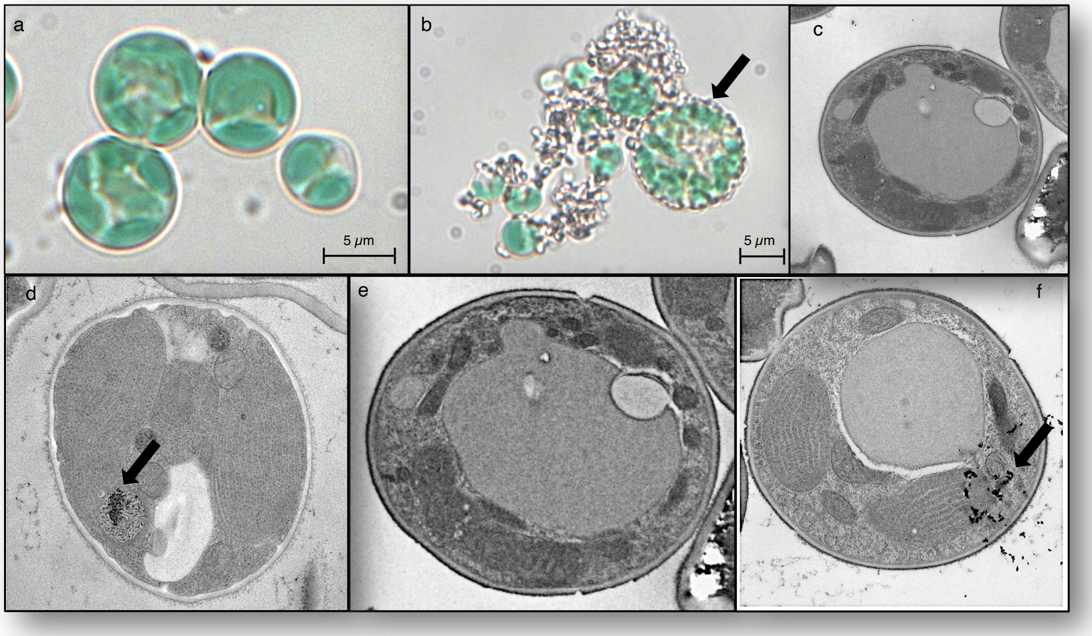


Figure 3.8. Pictures of untreated and treated cells of *G. sulphuraria*, strain SAG 107.79. (a) Light microscopy observation of cells in Allen medium pH 2.5; (b) Light microscopy observation of cells treated with Ce^{3+} 10 mg/L in Allen medium pH; (c) SBF-SEM observation of cells in Allen medium pH 2.5; (d) SBF-SEM observations of cells in Allen medium pH 2.5, treated with 10 mg/L of Ce^{3+}; (e) SBF-SEM observations of cells in ultrapure H_2O pH 2.5; (f) SBF-SEM observations of cells in ultrapure H_2O pH 2.5, treated with 10 mg/L of Ce^{3+}; black arrows indicate accumulation of Ce^{3+}

The transcriptomic response of *G. sulphuraria* to Ce^{3+} was also evaluated in subneutral experimental conditions. The interactive effects of metal ions and medium pH mainly affected the GO term regarding the proteins' post-translational modification

(PTM). The hypothesis in a major involvement of the EPS could be a reasonable explanation of the results here as well. Medium pH is the primary cause for the ionization of the functional groups being part of the exopolysaccharides. Examples include carboxylate, phosphate and amino groups, whose negative charges are all feasible binding sites for metal cations (Comte et al. 2008).

CHAPTER 4: PHYLOGENETIC CHARACTERIZATION OF THE OVEREXPRESSED GENES RELATED TO THE BIOLOGICAL PROCESS OF LOCALIZATION (GO:0051179)

4.1. INTRODUCTION

Transcriptomic analysis of *G. sulphuraria*, strain SAG 107.79, treated with Ce^{3+}, identified a discrete number of overexpressed transcripts involved in the Biological Process of Localization, GO:0051179 and Transmembrane Transport, GO:0006810 (Chapter 3). When metal ions are accumulated inside the protoplast, it's plausible that the organism tries to maintain metal homeostasis activating enzymatic machinery regarding the transport, tether and maintenance of substances in a specific cell location.

Previous studies used bioinformatic tools to predict transmembrane transporters in *G. sulphuraria* (Schönknecht et al. 2013; Lee et al. 2017). Lee et al. (2017) analysed the genomes of *Chondrus crispus*, *G. sulphuraria* and *Cyanidioschyzon merolae* to identify and compare the transport proteins in the three species. They found a total of 477 transporter proteins in *G. sulphuraria* involved in the movement of diverse substrates, such as cations, anions, carbon sources, amino acids, macromolecules and others (Lee et al. 2017). Secondary carriers were the most represented (#252 proteins), followed by primary active transporters (#98 proteins). Among the secondary carriers, *G. sulphuraria* showed the highest number of MFS transporter, sugar porter (SP) family and H+:phosphate symporters, PHS (Lee et al. 2017). Transcriptomic analysis allowed me to determine a discrete number of genes whose expression was most affected by Ce^{3+} treatments (log2FC > 0.5). Blast search identified them as part of the Major Facilitator Superfamily (MFS), the Amino acid permease, APC family and the Metal ion (Mn^{2+}-iron) transporter, Nramp family.

Cells' inward or outward movement of solutes can occur through simple diffusion across the membrane, endocytosis and exocytosis, or selective transport mediated by transmembrane proteins (Yan 2013). In the latter case, Major Facilitator Superfamily (MFS) represents the most famous protein family involved in the translocation of solutes across the double lipid layer. The reaction could involve two or more substrates, which can move in the same direction (MFS symporters) or the opposite one (MFS antiporters). Until now, the crystal structure of only seven MFS proteins belonging to six subfamilies was characterized (Yan 2013). Bioinformatic tools were also widely employed to predict the structure of these proteins. The predictions identified 12 transmembrane helices (TMs) or more (Yan 2013) organized in two distinct and folded domains, the N and C ones, whose termini are located on the cytoplasmic side of the membrane (Yan 2013). This structure, called the MFS fold, is shared by all the six subfamilies, even if the proteins widely differ in the amino acid sequence, the substrate specificities, and the transport coupling mechanisms (Yan 2013).

Transport Classification Database is a specific database for transport protein research where structural, functional, mechanistic, evolutionary and medical information for transporters from all kingdoms of life is annotated, http://www.tcdb.org/ (Saier et al. 2016). In this database, there are 10,000 ubiquitous proteins ascribed to the MFS family (Yan 2013). This number is still growing thanks to the increase of data from genomes sequencing (Yan 2013; Saier et al. 2016). Based on their function and substrate specificity, proteins belonging to this big family were classified into 76 subfamilies, Transporter Classification Database, http://www.tcdb.org/ (Saier et al. 2016, Yan 2013).

The Amino acid-Polyamine-organoCation (APC) superfamily is the second largest superfamily of secondary carriers after the Major Facilitator Superfamily (MFS) and includes proteins acting as solute:cation symporters or solute:solute antiporters (Jack et al. 2000; Vastermark et al. 2014). Some APC proteins allow the transmembrane movement of a wide range of amino acids or related compounds, while others grant the translocation of only some of them (Jack et al. 2000). Farcasanu et al. in 1998 described the involvement of a high-affinity permease for histidine in the transport of Mn^{2+}. The protein showed the new functionality due to a mutation in the coding gene, which produced a frameshift and a protein truncation (Farcasanu et al. 1998).

The family of natural resistance-associated macrophage protein (Nramp) metal ion transporters comprises important proteins related to metal ion transport across cellular membranes and metal ion homeostasis (Nevo and Nelson 2006; Lanquar et al. 2004). Trace metals like Cu^{2+}, Mn^{2+}, Fe^{2+} and Zn^{2+}, when present in low concentration, are essential cofactors of many enzymes (Nevo and Nelson 2006). In plants, they are mainly required in chloroplasts, where they play essential roles in photosynthesis (Lanquar et al. 2004). The Nramp proteins also intervene if an excess of heavy metals, such as cadmium, lead, and mercury, occurs, extruding them at the root level or sequestering them in cell compartments such as the vacuole (Lanquar et al. 2004).

In the previous chapter, transcriptomic data identified the increased expression of ~40 transcripts regarding the MFS protein superfamily. Part of these transcripts (# 14) also falls in those most affected by metal treatment (Log2FC > 0.5). Among them, functional annotation identified the sugar porter (SP) subfamily (# 9) that comprises important proteins mostly, but not exclusively, involved in the uptake of mono and disaccharides (Yan 2013; Pao et al. 1998). The second identified subfamily was the phosphate:H^+ symporter, PHS (# 5), whose protein members slightly differ in amino acid sequences. The level of similarity is so high that three of the four identified plants' PHS proteins strictly cluster together in the phylogenetic tree (Pao et al. 1998). Amino acid permease, APC family and the Metal ion (Mn^{2+}-iron) transporter, Nramp family were represented by just one overexpressed gene each.

So far, investigations on the phylogeny of the above-mentioned transcripts in *G. sulphuraria* were not been thoroughly performed (Schönknecht et al. 2013). In light of the subdivision of *G. sulphuraria* in six sublineages, as highlighted by the organelle phylogenomic analysis, the present chapter aimed at the understand what are the phylogenetic relationships among the most affected transcripts from Ce^{3+}. Starting from these premises, amino acid sequences were retrieved from the transcriptome of *G. sulphuraria*, strain SAG 107.70, exposed to Ce^{3+}, and used as queries to find orthologous sequences and perform phylogenetic analysis.

4.2 METHODS

Derived amino-acid sequences, corresponding to the highest increased expressed transcripts (log2FC > 2), were retrieved from the transcriptome of the strain SAG 107.79 and blasted again 5 strains belonging to the lineages identified in the organelle phylogenomic analysis (ACUF 017, ACUF 074, ACUF 427, ACUF 138, THAL 033), whose genomic and transcriptomic data are available, applying the p-value threshold of 10^{-6}. At the same time, sequences were blasted against the non-redundant (nr) database using BLASTP from BLAST+ version 2.2.27 (Camacho et al. 2009) to find the orthologous sequences in Archaea (taxid:2157), Bacteria (taxid:2), Streptophyta (taxid:35493), Chlorophyta (taxid:3041), Rhodophyta (taxid:2763), Stramenopiles (taxid:33634), Alveolata (taxid:33630), Amoebozoa (taxid:554915), Fungi (taxid:4751), Choanoflagellates (taxid:28009) and Animals (taxid:33208). For a more stringent search, the following settings were applied: p-value threshold of 10^{-6}, minimum identity percentage of 30% and minimum query coverage of 80%. Sequences were aligned using MAFFT v7.453 (Katoh and Toh 2008),and the resulting alignments were uploaded to Gblocks version 0.91b (Castresana 2000) to remove poorly aligned regions.

Maximum likelihood (ML) analyses were performed with IQ-Tree v. 2.0.3 (Nguyen et al. 2015), using the best substitution model selected by the program (-m TEST). Phylogenetic trees were inferred applying 10000 ultrafast bootstrap replicates, UFBoot (Minh et al. 2013) and 1000 replicates of the approximate likelihood ratio test [aLRT] and Shimodaira-Hasegawa, SH-aLRT (Anisimova et al. 2011) for the branch statistical support.

4.3. RESULTS

4.3.1. MFS SUPERFAMILY CLUSTERED WITH FUNGI

The phylogenetic tree of the MFS superfamily, sugar porter (SP) subfamily showed a polyphyletic origin for this superfamily (Figure 4.1). Sequences extracted from the transcriptomes of the six sublineages produced a monophyletic clade, fully supported by statistical analysis (100% UFBoot, 100% SH-aIRT). Inside this clade, sequences from different strains slightly differed from each other: sister clades are those formed by strains SAG 107.79 and ACUF 017 from one side (99% UFBoot, 94.6% SH-aIRT) and strains ACUF 074 and ACUF 427 from the other side (98% UFBoot, 78.9% SH-aIRT). THAL 033 slightly diverged from the latter two strains even if not fully supported by the statistic (85% UFBoot, 67.6% SH-aIRT), while ACUF 138 represented the more dissimilar strain, being clustered alone outside of the other 5 strains (100% UFBoot, 100% SH-aIRT), as demonstrated already in the organelle phylogenomics. The whole clade formed a sister group with the majority of *G. sulphuraria* orthologous genes retrieved from NCBI database through the blast search (100% UFBoot, 100% SH-aIRT). Amino acid sequences from *G. sulphuraria*, newly transcriptomes and those already published in NCBI, formed a supported clade with sequences from Fungi (100% UFBoot, 100% SH-aIRT). Surprisingly, two plants species fell in this clade (*Carpinus fangiana* and *Quercus suber*) and also further *G. sulphuraria* sequences retrieved from NCBI, whose position in the phylogenetic tree would suggest polyphyletic origin of the MFS, SP (Figure 4.1).

Unlike the SP subfamily, transcripts belonging to the Phosphate:H^+ symporter (PHS), showed a monophyletic origin (Figure 4.2). Even if *G. sulphuraria* sequences showed small differences as happened for the SP subfamily, all the sequences from the new transcriptomes and those retrieved from NCBI clustered all together in the same clade, fully supported by bootstrap and by the approximate likelihood ratio test (100% UFBoot, 100% SH-aIRT). *G. sulphuraria* sequences formed a sister clade with Fungi (Figure 4.2), as demonstrated already for the SP subfamily, but Fungi species that were part of this clade differed from those present in the SP subfamily phylogenetic tree (Figures 4.1-4.2).

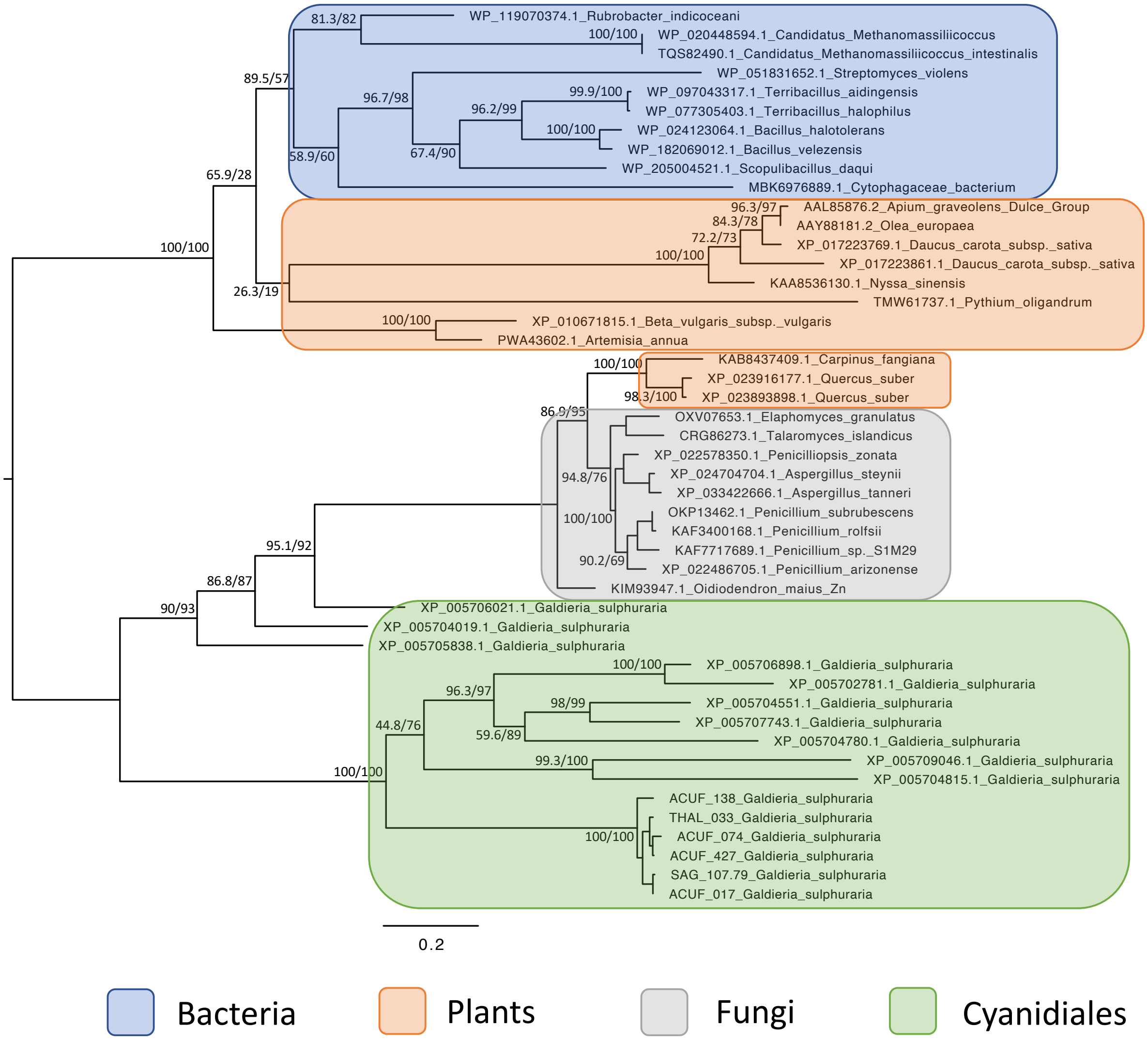


Figure 4.1. Maximum likelihood tree of the Major Facilitator Superfamily (MFS), sugar porter (SP) family. Ultrafast bootstrap (UFBoot) and the Approximate Likelihood Ratio Test [aLRT] and Shimodaira-Hasegawa (SH-aLRT) support values are indicated near nodes.

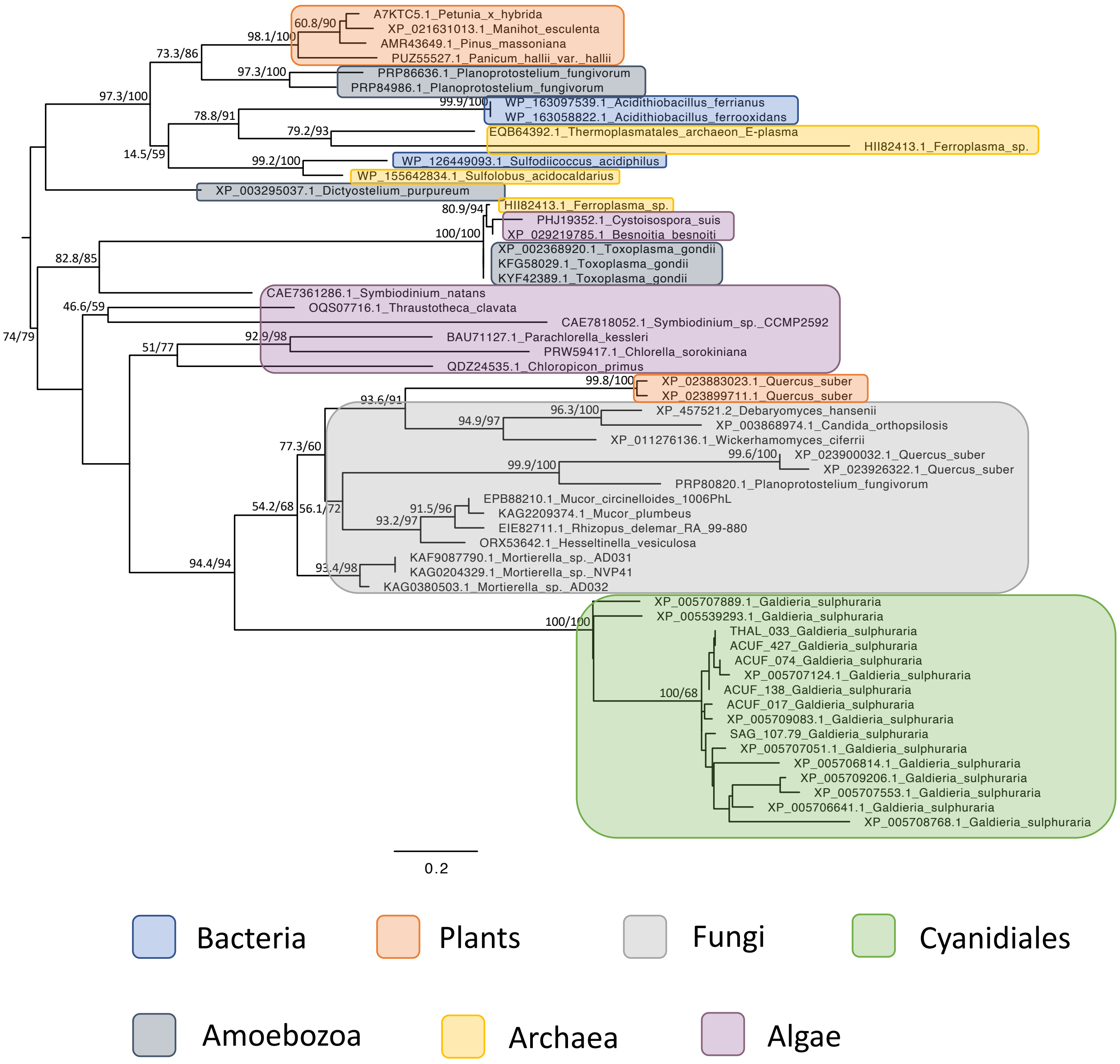


Figure 4.2. Maximum likelihood tree of the Major Facilitator Superfamily (MFS), Phosphate:H+ symporter (PHS) family. Ultrafast bootstrap (UFBoot) and the Approximate Likelihood Ratio Test [aLRT] and Shimodaira-Hasegawa (SH-aLRT) support values are indicated near nodes.

4.3.2. APC FAMILY TRANSCRIPT WAS RELATED EXCLUSIVELY TO EXTREMOPHILIC BACTERIA AND ARCHAEA

Genes belonging to the Amino Acid Permease, APC family that increased their expression in response to Ce^{3+} showed a clear relationship with extremophilic bacteria (Figure 4.3). Also this phylogeny demonstrated a monophyletic origin of the transcript (100% UFBoot, 100% SH-alRT), and little variations among the strains, some of which were totally supported by UFBoot and SH-alRT (99% UFBoot, 99.5% SH-alRT). The grouping of *G. sulphuraria* sequences with extremophilic bacteria, anyway, is only moderately supported (79% UFBoot, 78.4% SH-alRT). Conversely, the whole clade clustered undoubtedly with archaea (Figure 4.3; 98% UFBoot, 98.6% SH-alRT).

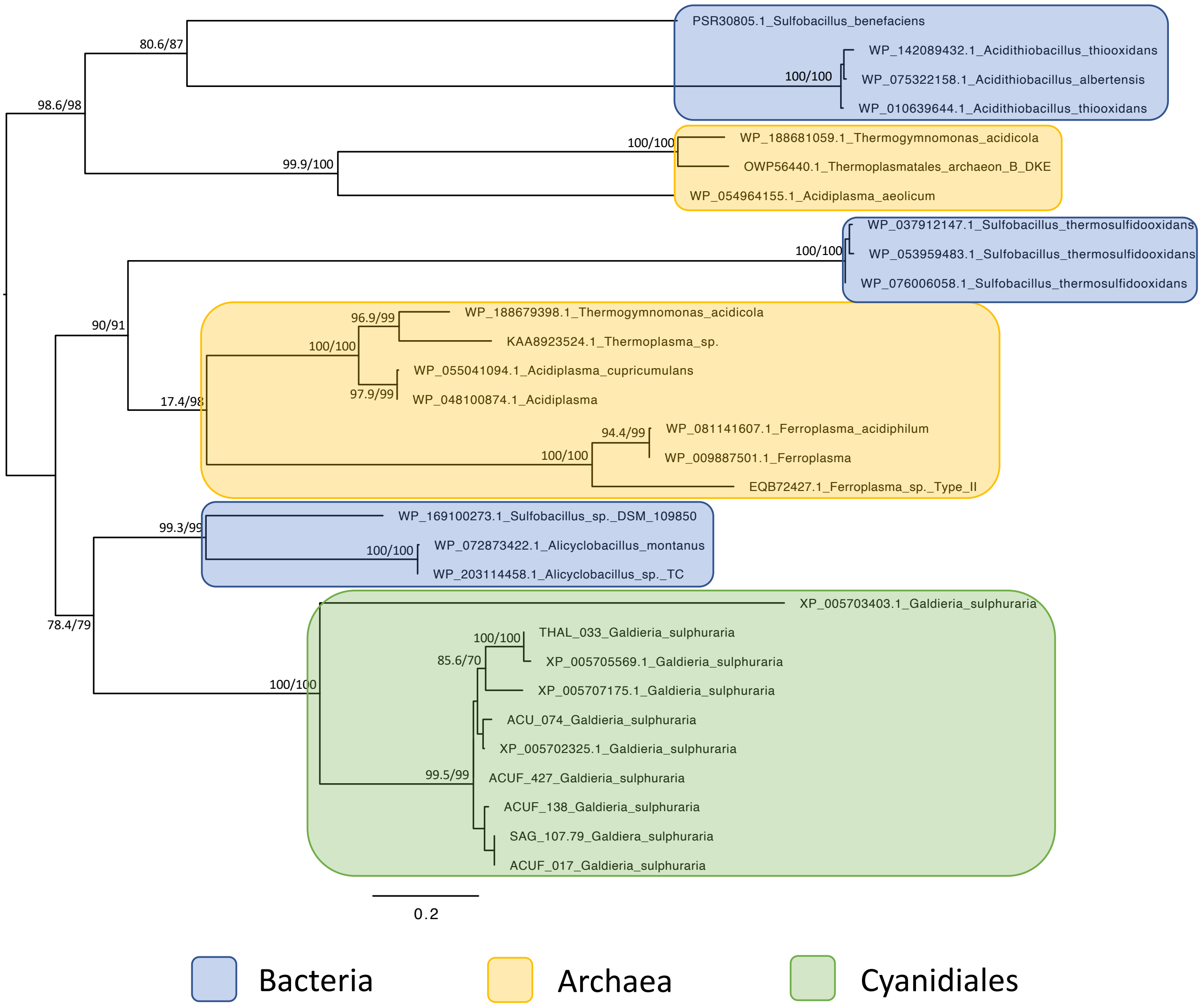


Figure 4.3. Maximum likelihood tree of the Amino acid-Polyamine-Organocation (APC) superfamily. Ultrafast bootstrap (UFBoot) and the Approximate Likelihood Ratio Test [aLRT] and Shimodaira-Hasegawa (SH-aLRT) support values are indicated near nodes.

4.3.3. METAL ION (MN2+-IRON) TRANSPORTER, NRAMP FAMILY CLUSTERED WITH A HIGH RANGE OF ORGANISMS.

Metal ion (Mn^{2+}-iron) transporter, Nramp family, includes genes whose expression was increased by Ce^{3+} treatment. Blast search identified these transporters in Bacteria, Chlorophyta, Archaea, Fungi, Amoebozoa, Streptophyta, Stramenopiles, Alveolata, Choanoflagellates and Animals (Figure 4.4). In the clade of Cyanidiales, one branch grouped all the sequences extracted from the recent transcriptomes (100% UFBoot, 100% SH-alRT), while the other branch comprised further *G. sulphuraria* sequences, along with those from *Cyanidioschyzon merolae* and *Cyanidiococcus yangmingshanensis* (green boxes; Figure 4.4;100% UFBoot, 100% SH-alRT). Altogether, they formed a well-supported clade (100% UFBoot, 95.3% SH-alRT) and strictly correlated with the Stramenopiles group (Figure 4.4; 100% UFBoot, 98.8% SH-alRT).

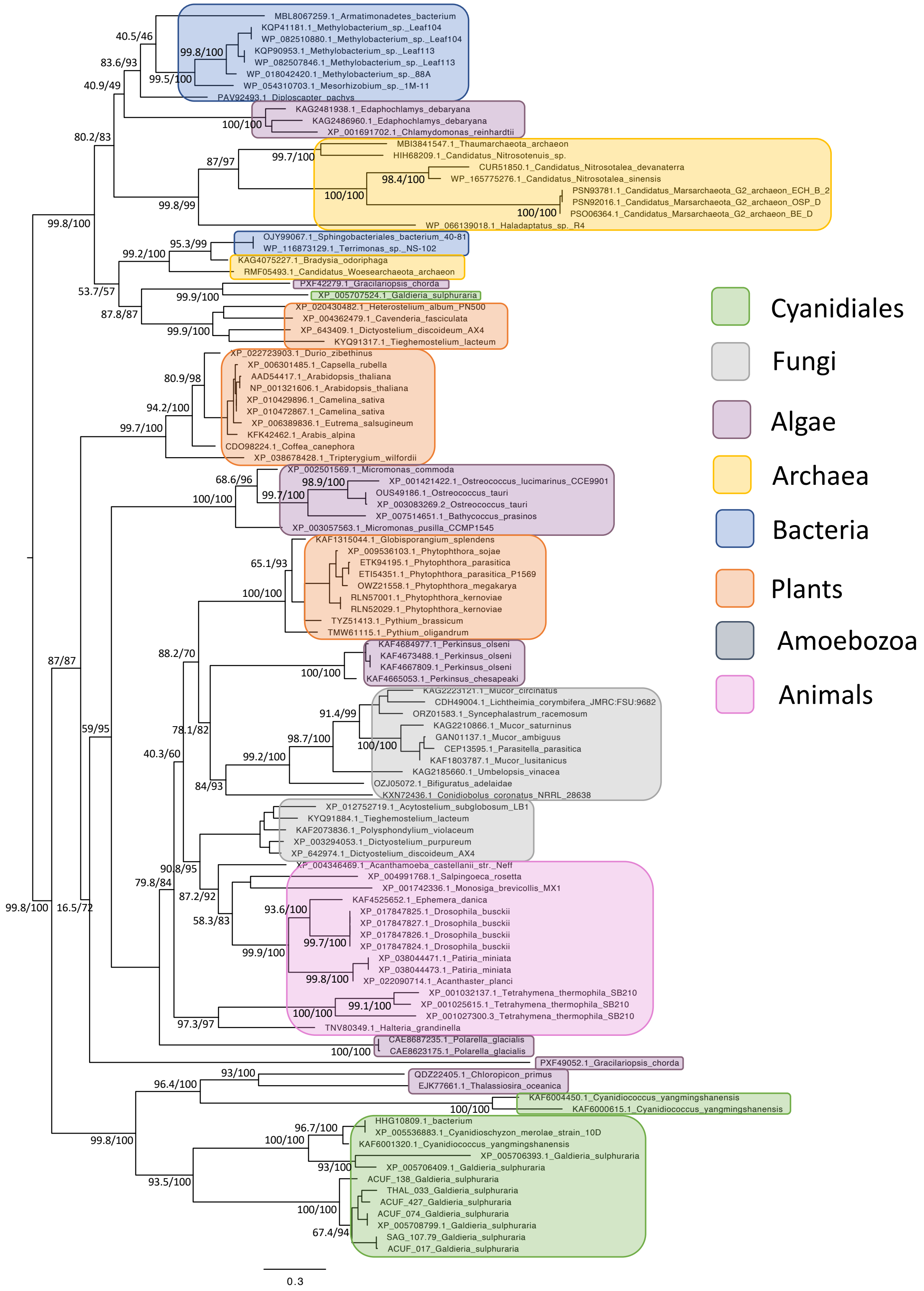


Figure 4.4. Maximum likelihood tree of the Metal ion (Mn2+-iron) transporter, Nramp family. Ultrafast bootstrap (UFBoot) and the Approximate Likelihood Ratio Test [aLRT] and Shimodaira-Hasegawa (SH-aLRT) support values are indicated near nodes.

4.4. DISCUSSION

Phylogenetic characterization of transmembrane transporters is still poorly investigated. Evidence of the involvement of the Horizontal Gene Transfer (HGT) in the phylogenetic relationship of these proteins was analysed in a previous study (Schönknecht et al. 2013). Phylogenetic tree regarding the MFS superfamily, SP family identified a strict relationship with Fungi, while the Acid-Polyamine-Organocation (APC) superfamily clustered with taxa from Archaea (Schönknecht et al. 2013).

Phylogenetic investigations in the present study confirmed the strict relationship of transcripts from the MFS, SP family to the sequences retrieved from diverse taxa of Fungi (Figure 4.1). Moreover, it is intriguing the finding of some NCBI retrieved sequences of *G. sulphuraria*, grouped with all the Fungi sequences and separated from the other *G. sulphuraria* sequences. This phylogenetic separation corroborated the polyphyletic origin of the MFS, SP family and the involvement of HGT events.

Hypothetical origin from Fungi through horizontal gene transfer was also highlighted for the MFS, PHS family, even if the taxa from Fungi slightly differed from the previous ones (Figure 4.2). Monophyletic origin of these sequences must be underlined as an important difference with the SP family. Indeed, all *G. sulphuraria* transcripts, newly sequences and those retrieved from NCBI, clustered all together in the same clade and shared the same common ancestor (Figure 4.2).

A different origin could be hypothesized for the Amino Acid Permease, APC family. Blast search in the NCBI non-redundant (nr) database did not find any hits in most of the taxa used for the other phylogenetic analysis (Figure 4.3). Even when increasing the threshold of the identity percentage and the query coverage for the blast search, no significant hits were found. The presence of these proteins in just *G.sulphuraria*, extremophilic archaea and bacteria lead to the hypothesis that this function is specific to these taxa and is shared among them through HGT events.

Unlike the previous protein families, metal ion (Mn^{2+}-iron) transporter, Nramp family members did not cluster with any of fungi or extremophilic organisms. *G. sulphuraria* sequences strictly correlated only with the Stramenopiles group (Figure

4.4). These proteins, anyway, are widely distributed, being present in all the taxa considered for the Blast search, but they were all phylogenetically distant from the clade *G.sulphuraria*-Stramenopiles (Figure 4.4).

CHAPTER 5 (PUBLISHED PAPER IN PLANTS, 2022): BIOREMOVAL OF YTTRIUM (III), CERIUM (III), EUROPIUM (III), AND TERBIUM (III) FROM SINGLE AND QUATERNARY AQUEOUS SOLUTIONS USING THE EXTREMOPHILE *GALDIERIA SULPHURARIA* (GALDIERIACEAE, RHODOPHYTA)

ABSTRACT

The lanthanides are among the rare earth elements (REEs), which are indispensable constituents of modern technologies and are often challenging to acquire from natural resources. The demand for REEs is so high that there is a clear need to develop efficient and environmental-ly-friendly recycling methods. In the present study, living cells of the extremophile *Galdieria sulphuraria* were used to remove four REEs, yttrium, cerium, europium, and terbium, from single- and quaternary-metal aqueous solutions. Two different strains, SAG 107.79 and ACUF 427, were exposed to solutions buffered at pH 2.5, 3.5, 4.5, and 5.5. Our data demonstrated that the removal performances were strain and pH dependent for all metal ions. At lower pH, ACUF 427 outperformed SAG 107.79 considerably. By increasing the pH of the solutions, there was a significant surge in the aqueous removal performance of both strains. The same trend was highlighted using quaternary-metal solutions, even if the quantities of metal removed were significantly lower. The present study provided the first insight into the comparative removal capacity of the Galdieria sulphuraria strains. The choice of the appropriate operational conditions such as the pH of the metal solutions is an essential step in developing efficient, rapid, and straightforward biological methods for recycling REEs.

5.1. INTRODUCTION

The successful application of metals in a great variety of fields such as machinery, energy, transportation, building and construction relies on their characteristic features such as high robustness, thermal and electrical conductivity, and great performance at high temperatures (Graedel et al. 2011). Metals can be repeatedly recycled, decreasing the necessity to extract them from mines (Graedel et al. 2011). According to the report of the Working Group on the Global Metal Flows to UNEP's International Resource Panel, the recycling rates of “base metals” (iron, copper, zinc, etc.) are above 50%. In contrast, a large number of metals used in small amounts in new technologies such as red phosphorus, permanent magnets, solar cells, and computer chips are rarely (<1%) recycled (Graedel et al. 2011; Reck and Graedel 2012). At the same time, however, the high popularity of these machineries is causing the demand and price increment of their components, especially of the irreplaceable rare earth elements, REEs (Binnemans et al. 2013).

REEs, which comprise 17 metallic elements (15 lanthanides, plus Scandium and Yttrium) with the same chemical properties, have been classified as “critical raw materials” by the European Commission because of their high-supply risk (European Commission 2010). In many cases, obsolete electrical and electronic equipment can be re-used for advanced technological applications. In these cases, the equipment can be resold or donated to schools or charities without further modifications; alternatively, in particular computers, they can be regenerated or disassembled into different components, cleaned, repaired and reassembled and put back on the market, in order to prolong their “life cycle” and reduce the amount of WEEE. When this is not possible, the recycling and recovery of WEEE components have become an essential process to reduce the costs of the disposal and production of the equipment and to minimize the environmental and health risks connected to them.

Physicochemical methods are often used for the recycling and recovery of REEs (Dhankhar and Hooda 2011; Farooq et al. 2010)(Dhankhar and Hooda 2011; Farooq et al. 2010), even if some weaknesses exist with these techniques: (1) they are ineffective when the metal concentration is deficient; (2) a large amount of toxic waste will be produced, requiring further treatments, which increases the operating costs and environmental responsibility; (3) some physicochemical methods are costly such as ion exchange and membrane technologies; and (4) incomplete metal adsorption (Lo et al. 2014). New methods are thus warranted.

Biosorption and bioaccumulation are biological approaches emerging as promising methods in replacing the physicochemical ones, being eco-friendly and cheaper methods (Das 2010). Biosorption is a metabolism-independent mechanism involving chemical or physical interactions between the metal ions and biosorbent. Despite the high selectivity, metal uptake capacity and reduced sludge production, biosorption is highly dependent on the pH value. This is related to the protonation/deprotonation of the functional groups involved in the binding of the metal ions (Zeraatkar et al. 2016; Pacheco et al. 2011). Bioaccumulation is the intracellular accumulation of metals, and it occurs when cells actively transport them inside the protoplast through an energy (ATP)-driven process (Lo et al. 2014). The process is highly economically feasible and selective, even so, the operating conditions (e.g., temperature, pH, and nutrients) need to be strictly controlled to maintain the cell viability (Lo et al. 2014).

Modern technological components and the contaminated effluents used for their production are often made of multiple rare earth elements (Menad and Seron 2017). Most scientific studies have regarded the bioremoval efficiency in a single-metal system, and little has been examined in a multiple-metal system (Monteiro et al. 2011). The lack of data produces an uncertain estimation of the actual capacity of organisms to recover metals. Mixed-metals effects cannot be predictable from the effects of the sin-gle-metals since they depend on extrinsic factors such as the used organism, temperature, pH, metal ion, and biomass concentration (Franklin et al. 2002; Monteiro, Castro, and Malcata 2011). In general, a mixture of metals can induce three kinds of behaviour: (1) synergism, when the effect of the mixture is greater than the sum of the individual metal; (2) antagonism, when the effect of the mixture is less than the sum of the individual effects; and (3) lack of interaction when the effect of the mixture is equivalent to the sum of the individual effects (Qi and Aldrich 2008; Monteiro et al. 2011).

Based on these premises, in the present study, two different strains of the extremophile *Galdieria sulphuraria* were tested for the aqueous bioremoval of yttrium

(Y^{3+}), cerium (Ce^{3+}), europium (Eu^{3+}), and terbium (Tb^{3+}), important REE constituents of phosphorus lamps, in a single- and quaternary-system. *G. sulphuraria* species are unicellular microalgae strains thriving in geothermal sites, where the ecological conditions are very extreme such as low pH (0.5–3.0), high temperature (50 °C – 55 °C), and vast amounts of heavy, precious, and rare earth metals (Del Mondo et al. 2019; Iovinella et al. 2020; Eren et al. 2018; Ciniglia et al. 2014). The coexistence of *G. sulphuraria* and huge amounts of metals in their natural environments makes this microalga one of the best candidates for the biological recovery of metals. Indeed, in the last decade, the interest in using *G. sulphuraria* in the bio-uptake of metals has rapidly grown, thanks to the promising results and the increasingly comprehensive knowledge of their genomes (Minoda et al. 2015; Ju et al. 2016; Fukuda et al. 2018; Sirakov et al. 2021). Data produced in this study primarily aimed to highlight the comparative evaluation of two *G. sulphuraria* strains in terms of the total metal removed in a single-metal system. Quaternary-metal solutions were then used to analyse the influence of the mixed metals on the removal capacity for each metal.

5.2. MATERIALS AND METHODS

5.2.1. METAL STOCK SOLUTIONS

In this study, the removal capacity of Ce^{3+}, Eu^{3+}, Y^{3+}, and Tb^{3+} as single- and quaternary-systems was studied using the living algal biomass of *G. sulphuraria* at a constant and equimolar concentration of 178 μmol/L. Y^{3+}, Ce^{3+}, Eu^{3+}, and Tb^{3+} were acquired from Alfa Aesar (USA) in the form of chloride salt monohydrate ($MetalCl_3.H_2O$, 99.9%). Stock solutions were prepared, dissolving 2 g of each metal salt in 1 litre of Milli-Q water and acidified at pH 2.5, 3.5, 4.5, and 5.5 using sulphuric acid (98%). All of the solutions were then sterilised with a 0.45 μm filter. To prevent interferences with the chemical analyses, all materials were previously rinsed with nitric acid and deionised water prior to use. In addition, the initial concentration (Ci) of the pH adjusted REE solutions were verified by ICP-MS before the experiments to ensure that there was no precipitation involved for the tested REE concentration (178 *µ*mol/L). The pH of the metal solution was measured before and after sterilisation (pHmeter Mettler-Toledo GmbH Process, Switzerland).

5.2.2. MICROALGAL CULTURE PREINCUBATION

Two *G. sulphuraria* strains, genetically distant from each other, were used for this study. Strain ACUF 427 was obtained from the algal collection of the University of Naples "Federico II" (www.acuf.net) (accessed on 15 April 2022) and was formerly collected from the acidic soil of the thermal station in Gunnuhver, Southwest Iceland. Strain SAG 107.79, originally sampled from a very hot acidic water in Sonoma, California, was obtained from the Culture Collection of Algae at Göttingen University (https://www.uni-goettingen.de/en/culture+collection+of+algae+%28sag%29/184982.html) (accessed on 15 April 2022). Both algal cultures were further isolated by streaking the colonies three times across Allen agar plates, starting from a diluted solution of the cultures. The ultimate colonies were eluted in Allen medium at pH 2.5 and grown at a temperature of 37 °C and constant light intensity (50 *µ*mol photons/m2 s). The cultures were refreshed weekly with a new medium until the microalgae reached the logarithmic growth phase.

5.2.3. EXPERIMENTAL DESIGN

The metal uptake experiments were performed in triplicate, in 24-well plates (Thermo Fisher Scientific, Waltham, Massachusetts, US) 2 mL solutions, stirred on a tilting shaker (MR-1 Biosan, BioScientifica, Rome, Italy), and kept in a climatic chamber (ThermoFisher Scientific, Waltham, Massachusetts, US) at 37 °C. A defined volume of microalgal culture was centrifuged at 13.000 rpm for 2 min at 4 °C; the supernatant was discarded, and the algal pellet was washed twice with autoclaved deionised water and then added to the experimental metal solution (water + metal), in order to achieve an initial optical cell density of 5 OD (ƛ = 550 nm; Secomam spectrophotometer Prim light). Positive controls (metal solutions without microalgal biomass) and negative controls (algal biomass without metals) were also considered.

pH of each metal solution was measured at the beginning and at the end of exposure (24 h) (pHmeter Mettler-Toledo GmbH Process, Greifensee, Switzerland).

After 24 h of treatment, the samples were collected and centrifuged at 10,000 rpm to separate the biomass fractions from the supernatant ones. The supernatant samples were filtered with a 0.2 μm filter, while the biomass samples were washed twice with H2O at the corresponding pH and digested using aqua regia (HNO3:HCl = 3:1 v/v). Digestion was conducted in a microwave oven (Milestone OneTouch, Bergamo, Italy) at 175 °C for 10 min, following the U.S. Standard recommendations (US-EPA 3051A). Metal concentrations were finally measured in the supernatant and the digested samples through inductively coupled plasma mass spectrometry using an ICP-MS (Aurora M90 Bruker Daltonics). The evaluation of the metal uptake was conducted by measuring the total metal removed and the removal efficiency. The first measure was carried out using the following formula:

$$\text{Total metal removed } (\mu\text{mol/g dm}) = (C_{\text{biomass}} \times V/M)/\text{metal molecular weight}$$

where Cbiomass is the metal concentration measured in the biomass fraction; V is the volume of the test solutions; and M is microalgal biomass (g, dry matter).

5.2.4. STATISTICAL ANALYSIS

All of the experiments were performed in triplicate and the data were expressed as mean ± standard deviation. Metal recovery values were analysed through one-way analysis of variance (ANOVA). A multiple comparison Tukey test was then used to evaluate the significance of the differences among the treatments.

5.3. RESULTS

5.3.1. REMOVAL OF Y^{3+}, CE^{3+}, EU^{3+} AND TB^{3+} IN THE SINGLE-METAL SYSTEM: THE INFLUENCE OF THE INITIAL pH

In the present paper, the bioremoval of rare metals by two different *G. sulphuraria* strains was assessed at different initial pH. The pH values were monitored for 24 h both in the control and in the treated samples; a slight decrease (less than 0.5 ± 0.02) was recorded only in the treated samples and remained constant until the end of the experiments (data not shown).

Both *G. sulphuraria* strains SAG 107.79 and ACUF 427 were able to extract the solute metals from the surrounding medium even if the extent of the ability was strain-dependent and metal-dependent. Tests performed in acidic conditions (pH 2.5) highlighted a significant difference in the removal performance amongst the two strains for all of the treatments. While strain SAG 107.79 was able to remove low amounts of each metal (< 2.5 μmol/g, Figure 5.1a), strain ACUF 427 removed 22.43 ± 2.05 μmol/g of Y^{3+}, 20.98 ± 0.72 μmol/g of Ce^{3+}, 23.49 ± 0.55 μmol/g of Eu^{3+}, and 22.26 ± 2.42 μmol/g of Tb^{3+} (Figure 5.1b). Increasing the medium pH, a gradual rise in the metal removed was observed in SAG 107.79. At a pH of 3.5, 4.13 ± 0.13 μmol/g of Y^{3+}, 5.39 ± 0.40 μmol/g of Ce^{3+}, 7.18 ± 0.19 μmol/g of Eu^{3+}, and 6.60 ± 0.10 μmol/g of Tb^{3+} were removed from the test solutions (Figure 5.1a). The improved removal performances induced by pH, just described for SAG 107.79, were not highlighted in ACUF 427, which indeed accumulated a comparable metal quantity to those at pH 2.5 (Y^{3+} = 20.13 ± 1.48 μmol/g, Ce^{3+} = 25.08 ± 0.83 μmol/g, Eu^{3+} = 24.66 ± 1.78 μmol/g and Tb^{3+} = 24.14 ± 1.08 μmol/g; Figure 5.1b). A further increase in pH induced an appreciable boost toward significant quantities of metals being removed for both strains, also showing significant differences among the metals (p-value < 0.05). At a pH of 4.5, SAG 107.79 was able to recover 14.26 ± 2.23 μmol/g of Y^{3+}, 17.82 ± 4.21 μmol/g of Ce^{3+}, 32.45 ± 7.23 μmol/g of Eu^{3+}, and 35.21 ± 6.56 μmol/g of Tb^{3+} (Figure 5.1a), while ACUF 427 removed 28.36 ± 3.68 μmol/g of Y^{3+}, 29.82 ± 1.90 μmol/g of Ce^{3+}, 36.78 ± 5.95 μmol/g of Eu^{3+}, and 40.58 ± 1.47 μmol/g of Tb^{3+} (Figure 5.1b). The increment of the metals removed from the solutions became even more evident when the tests were performed at the initial pH of 5.5. Indeed, the removed quantities for SAG 107.79 were 31.31 ± 3.28 μmol/g of Y^{3+}, 32.91 ± 1.87 μmol/g of Ce^{3+}, 43.02 ± 0.32 μmol/g of Eu^{3+}, and 36.12 ± 2.26 μmol/g of Tb^{3+} (Figure 5.1a) and for ACUF 427, they were 25.25 ± 5.87 μmol/g of Y^{3+}, 42.60 ± 4.28 μmol/g of Ce^{3+}, 42.91 ± 6.80 μmol/g of $Eu^{3+,}$ and 34.24 ± 3.13 μmol/g of Tb^{3+} (Figure 5.1b).

The influence of the pH on the removal efficiency of each metal species is highlighted in Figure 5.2a,b. Using SAG 107.79, the Y^{3+} removal increments were a 2.1-fold increase (FC) at pH 3.5, 7.5 FC at pH 4.5, and 16.3 at pH 5.5 (Figure 5.2a). Similar increments were also obtained with Ce^{3+} (2.7 FC at pH 3.5, 8.9 FC at pH 4.5, and 16.3 at pH 5.5). Higher pH remarkably affected the Eu^{3+} removal from the solutions, as the data demonstrated an increase in the metal removed of 3.4 FC at pH 3.5 and 15.3 FC at pH 4.5 until it reached the increment of 20.3 FC at the highest pH tested (Figure 2a). Similar to the Eu3+, the Tb3+ removal was hugely affected from pH 4.5, while at pH 5.5, there was no further increase (3.1 FC at pH 3.5, 16.3 FC at pH 4.5, and 16.9 at pH 5.5; Figure 5.2a).

Unlike SAG 107.79, pH affected the removal efficiency to a lesser extent when using the strain ACUF 427. The Y^{3+} results obtained at pH 3.5 were highly comparable with those at pH 2.5, demonstrating a reduction in the uptake, even if not significant (p-value > 0.05). Higher pH induced a slight increase, but this was again not significant (FC < 1.27 for pH 3.5, 4.5, and 5.5; Figure 5.2b). Smaller increments were observed instead for the other metal species: Ce^{3+} removal showed an FC = 1.20 at pH 3.5, 1.42 at pH 4.5, and 2.03 at pH 5.5 (Figure 5.2b).

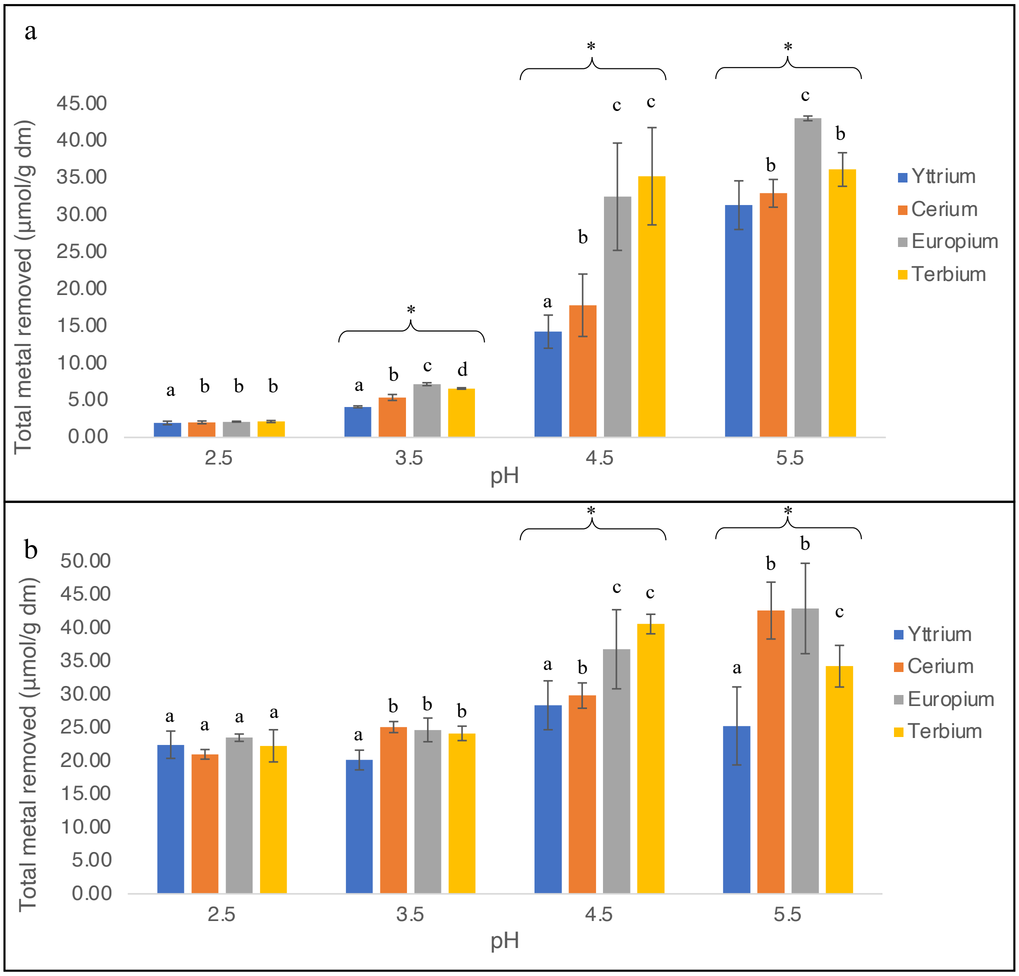


Figure 5.1. Metal removed from the single-metal aqueous solutions by *G. sulphuraria*, strains SAG 107.79 (a) and ACUF 427 (b). Data were divided based on the pH of the solutions. Different letters in the same experiment indicate a significant difference, $p < 0.05$; Symbol (*) indicates a $p < 0.05$ significant difference compared to the pH 2.5.

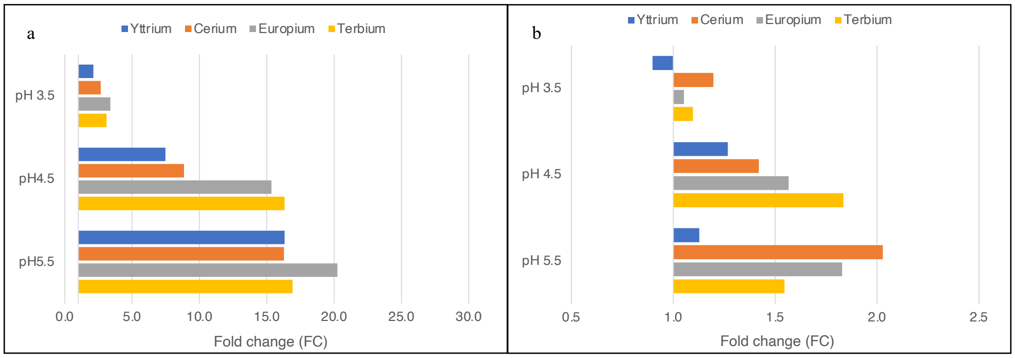


Figure 5.2. Fold change of the total metal removed from the single-metal aqueous solutions by *G. sulphuraria*, strains SAG 107.79 (a) and ACUF 427 (b). The fold change was calculated by comparing the µmol/g dm of every metal obtained at pH 3.5, 4.5, and 5.5 with the µmol/g dm measured at pH 2.5.

5.2.2. Removal of Y^{3+}, Ce^{3+}, Eu^{3+} and Tb^{3+} in Quaternary-Metal System: The Combined Effect of the pH and the Simultaneous Presence of the Metals

The removal capacity of both strains was also evaluated using quaternary solutions in which the metals were present in equimolar quantities. The total metals removed from the solutions were lower than the single-metal systems, but still, significant differences were highlighted among the metal species and the pH. At pH 2.5, strain SAG 107.79 confirmed the inability to recover metals in appreciable quantities (less than 1.03 *µ*mol/g, Figure 5.3a), while the ACUF 427 strain reached more significant values (Y^{3+} = 5.08 *µ*mol/g, Ce^{3+} = 5.16 *µ*mol/g, Eu^{3+} = 10.01 *µ*mol/g, Tb^{3+} = 11.47 *µ*mol/g; Figure 5.3b). Similar to the single solutions, increments in the pH increased the metal bioremoval when SAG 107.79 was used for the assays. Indeed, at pH 3.5, 1.14 ± 0.06 *µ*mol/g of Y^{3+}, 1.65 ± 0.15 *µ*mol/g of Ce^{3+}, 3.79 ± 0.14 *µ*mol/g of Eu^{3+}, and 3.35 ± 0.14 μmol/g of Tb^{3+} were measured in the biomass (Figure 5.3a). The same increments were not registered for ACUF 427 at the same pH (Y^{3+} = 3.56 ± 0.66 μmol/g, Ce^{3+} = 3.92 ± 0.88 μmol/g, Eu^{3+} = 8.47 ± 2.07 μmol/g, Tb^{3+} = 9.02 ± 2.20 μmol/g; Figure 5.3b).

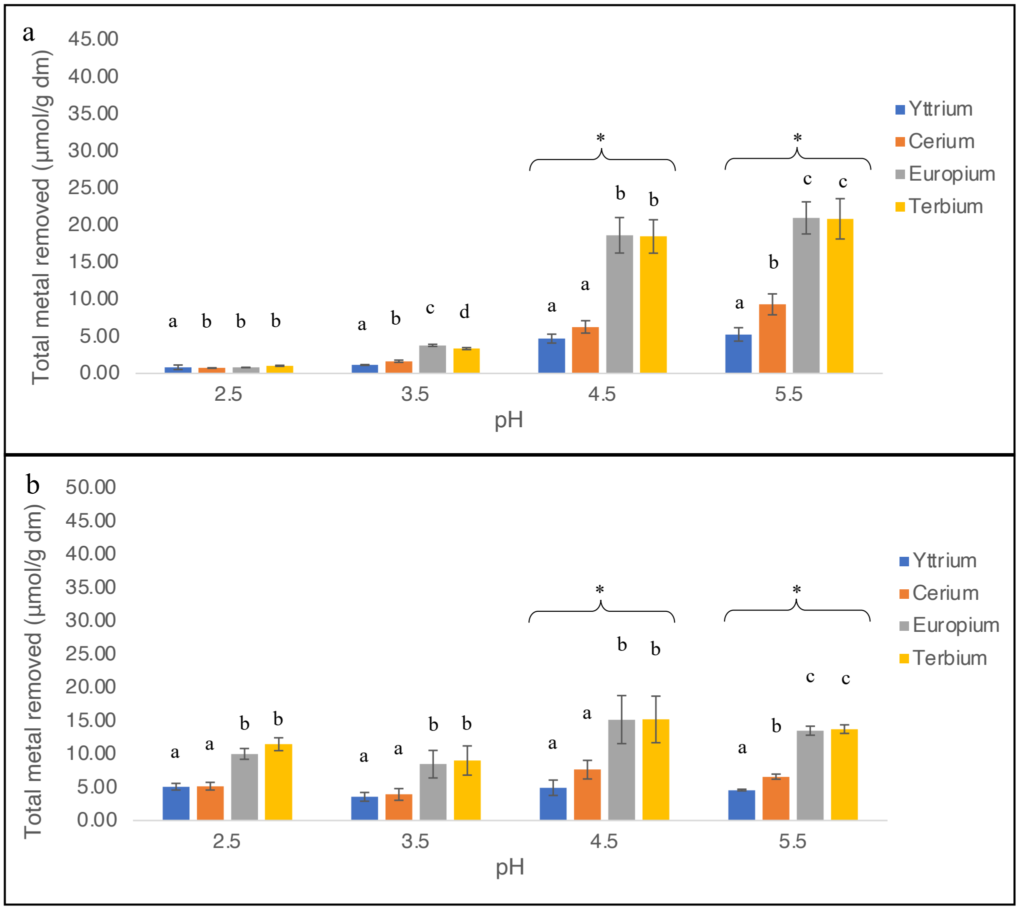


Figure 5.3. The metal removed from the quaternary-metal aqueous solutions by *G. sulphuraria*, strains SAG 107.79 (**a**) and ACUF 427 (**b**). Data were divided based on the pH of the solutions. Different letters in the same experiment indicate a significant difference, $p < 0.05$; Symbol (*) indicates a $p < 0.05$ significant difference compared to the pH 2.5.

A further increase in the pH at 4.5 deeply influenced the removal rates for both strains as already shown in the single-metal systems, even if to a lesser extent. Metal quantities removed by SAG 107.79 at pH 4.5 were 4.69 ± 0.60 μmol/g of Y^{3+}, 6.28 ± 0.84 μmol/g of Ce^{3+}, 18.63 ± 2.39 μmol/g of Eu^{3+}, and 18.48 ± 2.26 μmol/g of Tb^{3+} (Figure 5.3a). Comparable quantities were also registered in ACUF 427 (Y^{3+} = 4.92 ± 1.15 μmol/g, Ce^{3+} = 7.64 ± 1.40 μmol/g, Eu^{3+} = 15.17 ± 3.61 μmol/g, Tb^{3+} = 15.20 ± 3.50 μmol/g; Figure 5.3b). Finally, at pH 5.5, a slight increase in the metals accumulated was recorded, even if not significantly compared to the previous pH. The total metals removed were 5.26 ± 0.91 μmol/g of Y^{3+}, 9.32 ± 1.41 μmol/g of Ce^{3+}, 20.98 ± 2.17 μmol/g of Eu^{3+}, and 20.85 ± 2.72 μmol/g of Tb^{3+} for SAG 107.79 (Figure 5.3a) and 4.58 ± 0.12 μmol/g of Y^{3+}, 6.59 ± 0.38 μmol/g of Ce^{3+}, 13.50 ± 0.68 μmol/g of Eu^{3+}, and 13.74 ± 0.64 μmol/g of Tb^{3+} for ACUF 427 (Figure 5.3b).

Using the SAG 107.79 strain, the comparison of the metal quantities at pH 3.5, 4.5, and 5.5 with those at pH 2.5 highlighted a significant increase in the Yttrium removal at pH 4.5 (FC = 6.3), and it remained almost unchanged at pH 5.5 (FC = 6.6). Unlike Yttrium, the ulterior increase in the pH to 5.5 more deeply affected the removal of Ce^{3+}, Eu^{3+}, and Tb^{3+}, even if the rates changed among the metal species (Figure 5.4a). Indeed, the Ce^{3+} quantities increased 2.3 FC at pH 3.5, 8.6 FC at pH 4.5, and 12.8 FC at pH 5.5. Significantly higher quantities were registered for Europium, whose removal rates increased 4.7 times at pH 3.5, 23.3 times at pH 4.5, and up to 26.1 times at pH 5.5 (Figure 5.4a). As shown for the other metals, the pH solution of 4.5 most influenced the removal of Tb^{3+}, increasing the metal quantity of 18.1 FC, while the removed Terbium at pH 5.5 was comparable to that at the previous pH (20.3 FC; Figure 5.4a).

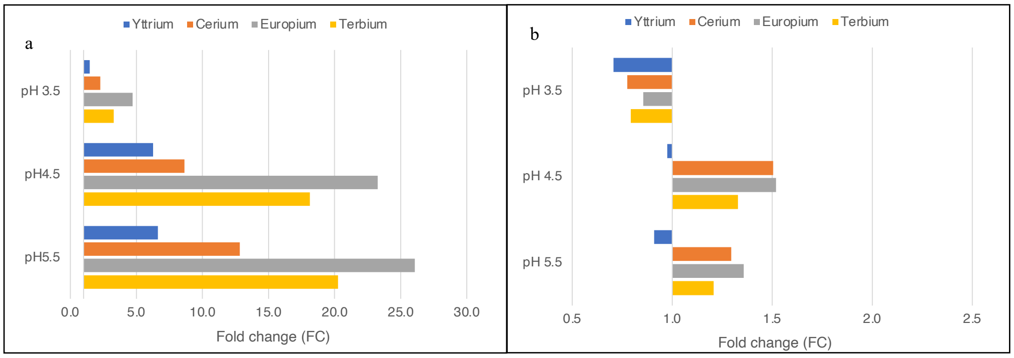


Figure 5.4. Fold change of the total metal removed from the quaternary-metal aqueous solutions by *G. sulphuraria*, strains SAG 107.79 (**a**) and ACUF 427 (**b**). The fold change was calculated by comparing the µmol/g dm of every metal obtained at pH 3.5, 4.5, and 5.5 with the µmol/g dm measured at pH 2.5.

A different performance was highlighted when ACUF 427 was used for the assays, demonstrating a weak influence of the pH solution on the metal removal rates. Among the metal species, Yttrium was removed from the microalgae in the same way regardless of the pH (pH 3.5 = 0.7; pH 4.5 = 1.0; pH 5.5 = 0.9; *p*-value > 0.05; Figure 5.4b). In contrast, the Ce^{3+}, Eu^{3+}, and Tb^{3+} removal rates were slightly affected by the increase in the pH to 4.5 (Ce^{3+} = 1.5 FC, Eu^{3+} = 1.52 FC, and Tb^{3+} = 1.33 FC). Small increments in the metal quantities were also registered at pH 5.5, reaching an FC of 1.29, 1.36, and 1.21, respectively, for Ce^{3+}, Eu^{3+} and Tb^{3+} (Figure 5.4b).

To analyse the effect of the simultaneous presence of the four metals in equimolar quantities on the bioremoval capacity of both strains of *G. sulphuraria*, the total amounts of removed metals were calculated and compared with those obtained with the single-metal solutions. The total metals removed by SAG 107.79 were 148.08 *µ*mol/g at pH 4.5 and 56.41 *µ*mol/g at pH 5.5 (Table S5.1). In contrast, the total metal quantities calculated in ACUF 427 at different pH did not always exceed those of every single metal. In particular, at pH 2.5, the total amount (31.72 *µ*mol/g) was slightly higher than the single-metal quantities; at pH 3.5, the total amount (24.96

μmol/g) was comparable to the single-metal (Ce^{3+}, Eu^{3+} and Tb^{3+}; Table S5.2). At pH 4.5, the total amount of metals removed from the quaternary solution was 42.93 μmol/g, which was statistically higher when compared to the Y^{3+} and Ce^{3+} quantities at the same pH from the single-metal system (Table S5.2). Finally, at pH 5.5, the total metal removed was 38.41 μmol/g, which did not reach the Eu^{3+} and Ce^{3+} quantities removed from the single-metal system (Table S5.2).

5.4. DISCUSSION

The ability of algae in recovering REEs from e-waste has been widely examined and assessed in the literature (Cao et al. 2021). The removal of REEs is strictly correlated to the physical, chemical, and biological characteristics of the algae. Namely, the algal cell wall is rich in functional groups, which contribute to the high-removal performances, as assessed for different microalgae such as *Desmodesmus multivariabilis*, *Chlorella vulgaris*, and *Chlamydomonas reinhardii* (Birungi and Chirwa 2014) as well as for macroalgae (Pinto et al. 2020; Jacinto et al. 2018). REEs after binding on the cell wall can be transported into cells by carrier proteins and stored in different cell compartments (Cao et al. 2021).

The utility of microalgae for the biorecycling of REEs from end-of-life products has been predominantly evaluated in a single-metal context. However, in light of a scale-up application of these systems, the main practical interest is the assessment of the microalgal biorecovery capacity in a multi-metal system, which is more likely to be the scenario of industrial effluents (Monteiro et al. 2011; Birungi et al. 2017). In the present study, we investigated the ability of *G. sulphuraria* strains SAG 107.79 and ACUF 427 to accumulate four rare earth metals, Y^{3+}, Ce^{3+}, Eu^{3+}, and Tb^{3+}, from aqueous solutions at a wide range of pH (from 2.5 to 5.5) in single- and quaternary-metal systems. We found that the ACUF 427 strain was superior in highly acidic conditions and that both strains performed well under more weakly acidic contexts.

When single-metal solutions were used, significant differences were highlighted between the two strains (p-value < 0.05). At pH 2.5, ACUF 427 outperformed SAG 107.79 as it removed metal quantities 10-fold higher than those of the other strain

(Figure 5.1a,b). Higher pH induced a remarkable increase in the metal removal, especially for the SAG 107.79 strain. The fold change, calculated by comparing the quantity of the metals per grams of dried biomass at the chosen pH and pH 2.5, highlighted significant differences among the metal species removed from the solutions. In contrast, increments of the metals removed by ACUF 427, based on the pH solutions, were much lower than those by SAG 107.79, thus hypothesising that the capacity of the metal removal of this strain was less affected by the pH (Figure 5.2a,b). Few data have been produced in the research on *G. sulphuraria* to study the accumulation of different kinds of metals in single-metal solutions. The *G. sulphuraria* strain ACUF 074 was used for the recovery of a great variety of REEs by varying various parameters such as the concentration of the metal ions, the pH of the experimental solutions, and the preincubation of the microalgal stock solutions (Minoda et al. 2015). In particular, the authors described a reduction in the REE uptake with increasing pH of the solutions, which is in contrast to the results of the present study. Afterward, they efficiently recovered gold and palladium (over 80%) from aqua regia-based metal wastewater, using 1.4 mg/mL of biomass (dry matter) and an exposition time of 30 min. They also identified a suitable modality for the desorption of metal ions from the biomass using a solution of 1 M thiourea and 0.1 M HCl (Ju et al. 2016). *G. sulphuraria* biomass grown under mixotrophic conditions was also able to recover the radionuclide cesium, reaching a recovery percentage of 52 ± 15%, after 10 days of exposition to the metal (Fukuda et al. 2018).

From the quaternary solutions of the metals, a significant reduction in the bioremoval was highlighted for both strains (*p*-value < 0.05), but SAG 107.79 still outperformed ACUF 427 when considering the calculated fold change (Figure 4a). Unlike the results of SAG 107.79, in ACUF 427, the simultaneous presence of four metals at pH 3.5 resulted in a reduction in all the metals, even if not significant. ACUF 427 reached values significantly lower at higher pH than those calculated for SAG 107.79 (Figure 4b). Data from the present study confirmed the results obtained by Čížková et al. (2021), who used *G. phlegrea* to evaluate the simultaneous removal of REEs using luminophores. The concentration of the included metals was not in equimolar quantities, but it precisely reflected the measured metal of the luminophores (Čížková et al. 2021). Considering the results of every single metal, the authors measured up to 1.98 mg/g, except for Yttrium (11.14 mg/g), which was already present in high concentration in the initial solution of 178.65 mg/g (Čížková et al. 2021).

When the experimental design mainly includes a biosorption approach, a high variety of physicochemical and biological parameters such as metal ionic characteristics (e.g., atomic weight, ion radius, valence, etc.), the nature of the biosorbents (e.g., cell age), and the biosorption conditions (e.g., pH, temperature, contact time, etc.) must be considered. These represent the main reasons for a different removal rate by biomass (Chen and Wang 2007). Among the metal ion characteristics, the most important are the atomic weight, electronegativity, ionic radius, and covalent index (Prasher et al. 2004; Chen and Wang 2007). Previous data have demonstrated the positive correlation between the biosorption rates and the atomic weight, being larger ions capable of binding sites with two distant functional groups (Prasher et al. 2004; Haug and Smidsrod 1970). An increased biosorption was also observed when more electronegative metal ions were used (Chong and Volesky 1995). Different biosorption rates for a high variety of metals were explained by the influence of the covalent index ($X^2{}_mr$) with the freeze-dried cells of *Rhizopus arrhizus* (Brady and Tobin 1995), and *Saccharomyces cerevisiae* (Chen and Wang 2007).

In the present study, a correlation approach was used to understand the influence of the above-mentioned physiochemical properties on the bioremoval degree of *G. sulphuraria*. A positive correlation with the covalent index and the electronegativity was registered at pH 2.5 when the ACUF 427 strain was used ($R^2 = 0.93$, *p*-value < 0.05), while no significant correlations were found with the other metal properties and at higher pH (data not shown). Different results were obtained with the SAG 107.79 strain, where only positive correlations were obtained between the metal removal and the ionic radius at a higher pH ($R^2 > 0.91$, *p*-value < 0.05; data not shown). The incongruent results obtained from the correlation analysis suggested a more complicated system than the easier biosorption approach. The uptake level also depends on the composition and the specific properties of the cell wall of the microalgae. Important microalgal cell wall components are peptidoglycan, polysaccharides, and proteins (Bailey and Staehelin 1968). Most of these molecules carry charged groups such as carboxyl, phosphate, hydroxyl, or amine, which could be protonated or deprotonated, depending on the media pH. Oxygen, sulphur, and phosphorus atoms present in these groups perfectly react with rare earth trivalent ions, with the last classified as a Pearson hard acid (Jacinto et al. 2018). This electrostatic attraction plays an essential role in the recovery process and could explain the differential uptake of the two strains of *G. sulphuraria*.

The recovery of rare earth can also be achieved by bioprecipitation, thanks to the release of organic phosphates, which cause the precipitation of the metal in the form of phosphate (Dev et al. 2020). Moreover, ions could either be attached to the cell surface or transported and accumulated inside the cell, in various contexts (Jacinto et al. 2018). Bioaccumulation is generally enhanced by specific cysteine-rich peptides such as glutathione, metallothioneins, lipopolysaccharides, and phytochelatins (Zeraatkar et al. 2016). The different removal rates amongst the two strains, especially at pH 2.5, could also be ascribed to the different transportation systems activated by the microalgae. In general, the transport across the membrane is also affected by the physiochemical parameters (e.g., molecular size and polarity) [26,36,37]. Inside the cell, metal ions could accumulate in the vacuoles or be bound by specific molecules for storage or detoxification. In this regard, the genomic analyses identified in *G. sulphuraria* enzymes such as arsenite methyl transferases and mercury reductase employed in the biotransformation into less toxic and metal derivates (Schönknecht et al. 2013); these findings provide a reasonable explanation of the high-metal resistance by this extremophilic alga.

5.5. CONCLUSIONS

Knowing the effect of the pH is one step towards fully understanding the mechanisms involved in the bioremoval of rare earth metals, and the findings of this study represent an added value for developing an efficient system. Based on the data produced and our willingness to speculate on the best conditions in terms of the pH solution and chosen strain of *G. sulphuraria* for each metal and using equimolar quaternary solutions, all four metal species were best removed by SAG 107.79 at pH 5.5. If there is a necessity to use more acidic solutions to remove these metals, the best choice lies in ACUF 427. Further data on the cellular localisation of the metal ions could represent an important step in understanding the contribution of biosorption and bioaccumulation to the bioremoval of REEs using *G. sulphuraria* biomass.

CHAPTER 5: GENERAL DISCUSSION

In the present project, molecular and physiological evaluation of *G. sulphuraria* was done in view of utilizing these microalgae for the biorecovery of Rare Earth Elements (REEs). Metabolic flexibility and the capacity of *Galdieria* to survive with huge quantities of metals make this microalga one of the best candidates for the biological recovery of metals. This hypothesis is corroborated by the interesting findings in its genome of several genes coding for a huge variety of metal transporters and plasma membrane uptake systems, which should confer *G. sulphuraria* the ability to bioaccumulate metals inside the cell protoplast (Schönknecht et al. 2013). To successfully use these microalgae in the biorecovery of REEs, it is crucial to have a thorough knowledge of their biology, taxonomy and genetic structure. Plastid and mitochondrial phylogenomic analysis in this project identified the subdivision of *G. sulphuraria* species in 6 sublineages with a percentage of nucleotide differences up to 10% for plastid sequences and 15% for mitochondrial ones (Fig. 2.4). These values fall in the range used in past studies as the threshold for establishing the new genus *Cyanidiococcus* and the new species *C. yangmingshanensis* (Cho et al. 2020). Genetic variation of *G.sulphuraria* species was confirmed by dN-dS mean values calculated for each pair of lineages (Fig. 2.8a-b). In general, plastid and mitochondrial genomes demonstrated different evolutionary rates between the sublineages (Fig. 2.8c), with the mitochondrial genomes always evolving faster than the plastid ones, as demonstrated by the mtDNA/ptDNA ratios (Fig. 2.8c).

The divergent genome evolution of *G. sulphuraria* is so evident to split the species into 6 sublineages and make intriguing the comparative biology and biotechnology of the 6 sublineages when employed for the biorecovery of REEs. Starting from the physiological and transcriptomic evaluation of strain SAG 107.79 following Ce^{3+} treatments, genes that mostly changed their expression were then employed in phylogenetic analysis to understand the molecular relationship of the hypothetical mechanisms involved in metal homeostasis within the Cyanidiophyceae.

The physiological state of the strain SAG 107.79 under Ce^{3+} treatments was firstly evaluated, measuring the growth rate and the percentage of viable cells. Data demonstrated that Ce^{3+} up to 150 mg/L did not significantly affect the life of *G. sulphuraria* since treated samples behaved exactly in the same way as the untreated ones. Several studies were made on the positive effects of Cerium on plants (Yuguan et al. 2009; Zhou et al. 2011; Zhao et al. 2012). Wang et al. in 2012 demonstrated a growth enhancement of the cyanobacterium *Anabaena flosaquae* treated with a low concentration of Cerium (0.05 and 0.1 mg/L of Cerium). At the same time, they demonstrated growth inhibition with higher concentrations of the metal (5 and 10 mg/L). *G. sulphuraria* insensitivity toward quantities of Cerium 10 times higher than those provided to *A. flosaquae* makes it a good competitor in the biorecovery of Cerium.

Results of metal bioaccumulation assays also confirmed the capacity to recover a high quantity of Ce^{3+} (up to 32.91 ± 1.87 *µ*mol/g) from the surrounding media when the pH of the solution was 5.5. Minoda et al. (2015) used *G. sulphuraria*, strain 074, to estimate the capacity of this organism to bioaccumulate three lanthanides (neodymium, dysprosium and lanthanum) and Copper, using a wide range of metal quantities (0.5, 5 and 25 ppm). These experiments assessed the capacity of the strain to uptake 90% of the metals from the external environment when they were grown in distilled water and a metal concentration of 0.5 ppm (Minoda et al. 2015), but the recovery rate decreased with an increased quantity of metals; when the experiment was conducted with the concentration of 25 ppm, 50% of the metals were recovered by the microalgal biomass (Minoda et al. 2015). They finally suggested the great potential of *G. sulphuraria* to biorecovery metals, even if the recovery rate decreases with the increasing amount of metals.

It is possible that the efficiency of recovery is affected by the concentration of the metal in the external environment because a massive quantity of metals that enter the cells could generate the overproduction of Reactive Oxygen Species (ROS), and therefore, cell death (Ma et al. 2015). Transcriptomic data from this project did not highlight any significant increase in expression of the main genes related to the oxidative stress (superoxide dismutase, ascorbate peroxidase and catalase), except for peroxiredoxin, which is involved in the control of peroxide levels inside the cells.

Several genes related to the metabolic pathway of carbohydrates and proteins increased their expression following Ce^{3+} treatments. this increment may be related to the basic metabolism of *G. sulphuraria*, or they could be connected to the involvement of the cell wall and the extracellular polymeric substances (EPS). Indeed, *G. sulphuraria* cell wall consists of a layer of hemicellulose (galactose, glucose, mannose, xylose, arabinose and uronic) and a layer of proteins (Bailey and Staehelin 1968). Production of EPS could be an additional strategy that these microalgae enact to cope with the high quantities of metals, as happens in numerous microalgal taxa (Ozturk et al. 2010; Xiao and Zheng 2016; Zhao et al. 2019; Xie et al. 2020; Naveed et al. 2020).

A small group of genes that showed the highest fold change in their expression were related to the biological process of localization and specifically to the transmembrane transport. According to the phylogenetic analysis, *G. sulphuraria* acquired these genes from other taxa, such as fungi, bacteria and archaea, through the Horizontal Gene Transfer (HGT). Data demonstrated that in most of the genes, all the *G. sulphuraria* strains grouped in the same clade, thus identifying their monophyletic origin through one single event of HGT. *G. sulphuraria* clade was separated into different branches corresponding to the 6 lineages previously identified with the phylogenomic, thus confirming the parallel evolution of the lineages. The sole exceptions are represented by the Major Facilitator Superfamily (MFS), sugar porter (SP) family, where all the sequences of *G. sulphuraria*, including the newly sequenced genes from my genomics, clustered all together, except for three strains that formed a sister clade with fungi (Fig. 4.1).

Horizontal gene transfer (HGT) events are an indispensable mechanism for archaea and bacteria to adapt to changing environments (Schönknecht et al. 2013). Indeed, HGT from other taxa underlies the extreme metabolic versatility of *G. sulphuraria* by conferring it the capacity to thrive in harsh environments typical of geothermal sites (Qiu et al. 2013). Remarkably, Ce^{3+} treatments induced the overexpression of transcripts originating from HGT from fungi, bacteria and Archaea. The intervention of these particular transcripts, in the presence of the metal, confirmed the hypothesis by which *G. sulphuraria* versatility is mainly due to acquired abilities through HGT events.

The employment of two genetically distant strains of *G. sulphuraria*, SAG 107.79 and ACUF 427, demonstrated that Rare Earth Elements were removed from the microalgal biomass at different rates (Iovinella et al. 2022). Significant differences were highlighted at acidic pH (2.5), where ACUF 427 outperformed SAG 107.79. When the pH of the metal solution was subneutral (5.5), results were reversed, demonstrating SAG 107.79 a better accumulator of REEs. Why 2 strains of the same species demonstrated different capabilities in removing metal ions from the media still needs to be clarified. It is plausible that different characteristics of the cell wall or transmembrane transporters are at the base of these differences (Iovinella et al. 2022)

All the data produced in the present project confirmed the natural capacity of *G. sulphuraria* to live in extremely metal-rich environments without suffering any toxic effect. Bio-uptake of metals from the surrounding environments could be related to the molecular interaction of metal ions to the cell wall and EPS residues. At the same time, they should be able to transport the ions inside the protoplast, but what happens inside it is still under investigation. Anyway, the high capacity of *G. sulphuraria* to recover a significant quantity of metal ions from the culture solution and the possibility to produce high quantities of EPSs, which have huge biotechnological applications, make this extremophilic microorganism one of the best candidates to achieve this purpose.

APPENDIX

SUPPLEMENTARY TABLES

Table S2.1. Strain voucher, collection site information and source for every sample used in the phylogenomic analysis.

Taxa	Lineage (Lin.)	Strain voucher	Sampling site (Country)	Habitat	pH	Temperature (°C)	Source (Reference)
Cyanidiophyceae							
G. sulphuraria	Lin. 1	138	San Salvador (SV)	N/A	N/A	N/A	ACUF (Gross et al., 2001)
	Lin. 2	002	Piscarelli (IT)	Dry cripto-endolithic site	1.0	18-30	ACUF (Pinto et al., 2003)
		011	Caserta (IT)	Acidic rock	0.8	15	ACUF (Ciniglia et al., 2004)
		017	Solfatara (IT)	Fumarols	1.0	38	ACUF (Ciniglia et al., 2004)
		021	Vulcano Island (IT)	N/A	N/A	N/A	ACUF (Ciniglia et al., 2004)
		638	Güglükonak (TR)	Thermal bath	1.0	54	ACUF (Iovinella et al., 2018)
		660	Güglükonak (TR)	Thermal bath	1.0	54	ACUF (Iovinella et al., 2018)
		PISC 6	Piscarelli (IT)	Dry cripto-endolithic site	1.0	18-30	This study
		RI1	Rio tinto (ES)	Acidic periodic water flow	0.85-1.55	12.2-19.4	N/A (Aguilera et al., 2007)
		SOL1	Solfatara (IT)	Fumarols	1.0	38	This study
		SOL2	Solfatara (IT)	Fumarols	1.0	38	This study
		SOL3	Solfatara (IT)	Fumarols	1.0	38	This study
	Lin. 3	136	Mexicali (MX)	N/A	N/A	N/A	ACUF (Gross et al., 2001)
		141	Yellostone National Park (US)	Acidic hot spring	3-4	N/A	ACUF (Pinto et al., 2003)
		142	N/A (IS)	N/A	N/A	N/A	ACUF (Gross et al., 2001)
		1067	Azores (PT)	Porous sandstone, endolithic	2.1	N/A	CCALA (Gross et al., 2001)
		965	Soos (CZE)	Diatom field	0.8-2.0	<30	CCALA (Gross et al., 2002)
		5573	Yellostone National Park (US)	Acidic soil	1.0	55	CCMEE (Toplin et al., 2008)
		5610	Yellostone National Park (US)	Acidic crust	4.0	40	CCMEE (Toplin et al., 2008)
		5657	Owakudani (JP)	Acidic pool edge	2.5	>45	CCMEE (Toplin et al., 2008)
		5658	Owakudani (JP)	Acidic pool edge	2.5	>45	CCMEE (Toplin et al., 2008)

		5665	Kusatsu (JP)	Acidic pool	2.0	49	CCMEE (Toplin et al., 2008)
		5672	Owakudani (JP)	Acidic pool edge	3.0	42-55	CCMEE (Toplin et al., 2008)
		5712	Craters of the Moon (NZ)	Acidic steam hole	N/A	N/A	CCMEE (Toplin et al., 2008)
		5720	White Island (NZ)	Acidic stream	2.5-3.0	45	CCMEE (Toplin et al., 2008)
		P503	Kamchatka (RU)	N/A	N/A	N/A	IPPAS (Sentsova 1991)
		107.79	California (US)	Acidic hot water	1.0	70-75	SAG (Allen 1959)
	Lin. 4	074	Java (ID)	Fumarols	N/A	35	ACUF (Pinto et al., 2003)
	Lin. 5	21.92	Yangmingshan National Park (TW)	Hot spring	N/A	N/A	SAG (Gross et al., 2001)
		033	GengZiPeng (TW)	Acidic Stream	2.6	45	THAL (Hsieh et al., 2015)
		054	DaYouKeng (TW)	Acidic pool	2.2	54	THAL (Hsieh et al., 2015)
	Lin. 6	388	Landmannalaugar (IS)	Acidic soil	1.0	42-47	ACUF (Ciniglia et al., 2014)
		402	Niasjvellir (IS)	Acidic soil and stream	0.0-4.5	31.2-47	ACUF (Ciniglia et al., 2014)
		427	Gunnhuver (IS)	Fumarole, acidic soil and mud	0.0-1.0	25-29	ACUF (Ciniglia et al., 2014)
		455	Viti (IS)	Acidic soil and mud	1.0-1.5	25-40	ACUF (Ciniglia et al., 2014)
		P501	Kamchatka (RU)	N/A	N/A	N/A	IPPAS (Sentsova 1991)
G. phlegrea		009	Nepi (IT)	Sulphur spring	0.8	12	ACUF (Pinto et al., 2003)
		647	Çermik (TR)	Thermal bath	7.0	24.6	ACUF (Ciniglia et al., 2018)
		663	Güglükonak (TR)	Thermal bath	1.0	54	ACUF (Ciniglia et al., 2018)
		735	Biloris (TR)	Thermal bath	7.0	25.8	ACUF (Ciniglia et al., 2018)
		788	Diyadin (TR)	Hot spring, pool and soil	6.5	45	ACUF (Ciniglia et al., 2018)
		AG1	Rio tinto (ES)	Acidic stream	2.32-2.88	12.3-27.3	N/A (Aguilera et al., 2007)
		CEM1	Rio tinto (ES)	Acidic stream coming from flooded mine	2.38-2.62	12.4-22.6	N/A (Aguilera et al., 2007)
C. merolae		10D	Sardinia (IT)	Acidic hot spring	1.5	45	ATCC (Kuroiwa et al., 1994)
Floridophyceae							
C. crispus		PCG	Nova Scotia (CA)	N/A	N/A	N/A	N/A (Janouškovec et al., 2013)
G. chorda		N/A	N/A	N/A	N/A	N/A	N/A (Lee et al., 2016)
Bangiophyceae							
P. umbilicalis		LB 2951	Schoodic Point, Maine (US)	N/A	N/A	N/A	N/A (Brawley et al., 2017)

P. haitanensis	PH-38	N/A	N/A	N/A	N/A	N/A (Wang et al., 2013)

Table S2.2. DNA extraction buffers.

Buffer 1.1	**Buffer 2.2**
200mM Tris-HCl pH8	100mM Tris-HCl pH8
200mM NaCl	700mM NaCl
100mM LiCl	20mM EDTA pH8
25mM EDTA pH8	2% CTAB
1M Urea	0.0125mM PVP-40
1% SDS	
1% NP-40	

Table S2.3. Plastid Genomes general features.

Lineages	**Strain voucher**	**General features**				
		Total bp	**GC%**	**CDS**	**tRNA**	**rRNA**
Lineage 1	ACUF 138	163,901	28.64	157	28	3
Lineage 2	ACUF 002	162,675	28.28	182	34	1
	ACUF 011	148,238	29.05	163	30	3
	ACUF 017	162,286	28.17	182	36	3
	ACUF 021	170,655	31.52	170	33	1
	ACUF 638	155,810	29.69	170	35	3
	ACUF 660	147,713	28.62	169	35	3
	PISC 6	175,108	31.89	171	35	3
	RI1	148,273	29.62	159	27	1
	SOL1	152,291	30.32	155	31	3

	SOL2	156,020	28.19	176	36	3
	SOL3	153,283	28.26	171	32	3
Lineage 3	ACUF 136	168,425	28.93	182	36	3
	ACUF 141	161,728	30.25	170	33	0
	ACUF 142	183,802	30.76	182	34	0
	CCALA 1067	169,260	29.07	182	34	1
	CCALA 965	169,260	29.07	182	34	1
	CCMEE 5573	176,807	29.85	182	36	3
	CCMEE 5610	190,930	31.99	166	33	0
	CCMEE 5657	158,684	32.67	149	29	0
	CCMEE 5658	156,251	31.5	152	20	0
	CCMEE 5665	187,312	29.93	182	34	0
	CCMEE 5672	195,936	30.83	182	34	0
	CCMEE 5712	167,903	28.83	182	36	3
	CCMEE 5720	181,646	30.43	182	36	3
	IPPAS P503	181,572	30.43	182	36	3
	SAG 107.79	157,839	29.98	161	32	3
Lineage 4	ACUF 074	162,912	27.96	182	36	3
Lineage 5	SAG 21.92	163,887	27.05	182	36	3
	THAL 033	162,804	27.89	182	36	3
	THAL 054	163,029	27.89	182	36	3
Lineage 6	ACUF 388	170,805	28.69	182	36	3
	ACUF 402	170,803	28.7	182	36	3
	ACUF 427	163,732	28.25	182	34	0
	ACUF 455	163,772	28.25	182	34	0
	IPPAS P501	163,843	28.27	182	34	0
	ACUF 009	163,081	30.56	167	32	0
	ACUF 647	149,849	30.41	154	25	0
	ACUF 663	163,201	30.56	167	32	0
	ACUF 735	163,124	30.55	167	32	0
	ACUF 788	126,866	32.68	117	19	0
	AG1	147,116	31.05	148	26	0
	CEM1	180,839	30.26	179	34	0

Table S2.4. Mitochondrial Genomes general features.

Lineages	Strain voucher	General features				
		Total bp	**GC%**	**CDS**	**tRNA**	**rRNA**
Lineage 1	ACUF 138	20,983	44.76	19	7	0
Lineage 2	ACUF 002	21,661	44.85	19	7	1
	ACUF 011	20,649	44.57	19	7	2
	ACUF 017	21,878	44.9	19	7	2
	ACUF 021	21,807	44.83	19	7	1
	ACUF 638	21,458	44.92	19	7	1
	ACUF 660	21,657	44.89	19	7	1
	PISC 6	21,712	44.87	19	7	1
	RI1	21,804	44.77	19	7	1
	SOL1	21,265	44.66	19	7	2
	SOL2	20,881	44.74	19	7	1
	SOL3	21,688	44.82	19	7	2
Lineage 3	ACUF 136	21,071	41.31	19	7	0
	ACUF 141	21,688	41.79	18	7	2
	ACUF 142	21,233	41.53	18	7	1
	CCALA 1067	21,772	41.44	19	7	1
	CCALA 965	20,965	41.08	19	7	0
	CCMEE 5573	21,626	41.27	19	7	1
	CCMEE 5610	22,694	43.00	19	6	2
	CCMEE 5657	19,007	42.57	18	6	1
	CCMEE 5658	21,882	41.40	19	7	0
	CCMEE 5665	21,882	41.43	19	7	1
	CCMEE 5672	21,790	41.48	19	7	0
	CCMEE 5712	21,869	41.47	19	7	1
	CCMEE 5720	21,688	41.78	18	7	0
	IPPAS P503	21,622	41.75	18	7	1
	SAG 107.79	21,738	41.83	18	7	2
Lineage 4	ACUF 074	21,437	44.00	19	7	2
Lineage 5	SAG 21.92	19,538	43.24	18	6	2
	THAL 033	20,554	43.42	19	6	2
	THAL 054	20,805	43.57	19	6	2
Lineage 6	ACUF 388	21,650	43.76	19	6	1
	ACUF 402	21,479	43.71	19	6	1
	ACUF 427	21,150	43.75	19	6	1

ACUF 455	20,586	43.59	19	6	1
IPPAS P501	21,870	43.95	19	6	2
ACUF 009	22,255	41.31	18	5	1
ACUF 647	22,399	41,18	18	4	1
ACUF 663	22,303	41,25	18	4	1
ACUF 735	22,417	40,98	18	5	1
ACUF 788	23,427	40,82	18	5	1
AG1	23,321	41,04	18	4	1
CEM1	23,754	40,88	18	4	1

Table S2.5. Evolutionary rates for all the plastid genes and all the sublineages.

	Lin. 1	**Lin. 2**	**Lin. 3**	**Lin. 4**	**Lin. 5**	**Lin. 6**
accA	-0.072	-0.080	-0.077	-0.119	0.121	0.117
accB	0.431	0.329	0.298	0.338	0.585	0.435
accD	-0.349	-0.315	-0.268	-0.236	-0.216	-0.243
acsF	-0.400	-0.408	-0.344	-0.338	-0.287	-0.307
apcA	-0.276	-0.257	-0.239	-0.188	-0.221	-0.201
apcB	-0.324	-0.316	-0.337	-0.254	-0.219	-0.217
apcD	-0.325	-0.264	-0.223	-0.283	-0.200	-0.215
apcE	-0.081	0.800	-0.038	-0.011	0.011	-0.008
apcF	-0.290	-0.261	-0.246	-0.267	-0.177	-0.176
argB	-0.384	-0.290	-0.312	-0.268	-0.277	-0.291
atpB	-0.359	-0.369	-0.334	-0.269	-0.236	-0.260
atpD	-0.252	-0.242	-0.154	-0.137	-0.148	-0.173
atpE	-0.276	-0.287	-0.230	-0.248	-0.197	-0.323
atpF	-0.203	-0.145	-0.156	-0.173	-0.126	-0.173
atpG	-0.049	0.254	0.667	0.128	0.141	0.161
atpI	-0.284	-0.253	-0.274	-0.218	-0.195	-0.189
carA	0.156	0.150	0.444	0.133	0.136	0.131
cbbX	-0.342	-0.324	-0.305	-0.243	-0.247	-0.242
ccs1	-0.305	-0.333	-0.271	-0.270	-0.211	-0.221
ccsA	-0.337	-0.335	-0.272	-0.246	-0.240	-0.255
cemA	-0.383	-0.328	-0.338	-0.271	-0.252	-0.281
chlB	-0.315	-0.318	-0.281	-0.256	-0.236	-0.270

chlI	0.118	0.218	1.320	0.260	0.256	1.018
chlL	-0.332	-0.291	-0.286	-0.222	-0.210	-0.223
chlN	-0.383	-0.312	-0.321	-0.283	-0.284	-0.272
clpC	-0.461	-0.399	-0.352	-0.325	-0.322	-0.329
cpcA	-0.176	-0.207	-0.161	-0.134	-0.151	-0.153
cpcB	-0.314	-0.291	-0.278	-0.229	-0.227	-0.210
cpcG	-0.301	-0.252	-0.248	-0.217	-0.197	-0.206
cysT	0.792	-0.002	0.032	0.112	0.104	0.107
cysW	0.321	0.839	0.242	0.559	0.276	0.297
desA	-0.341	-0.305	-0.285	-0.275	-0.244	-0.247
dnaB	-0.325	-0.303	-0.288	-0.273	-0.218	-0.275
dnaK	-0.366	-0.338	-0.323	-0.284	-0.281	-0.285
ftrB	0.964	0.168	0.154	0.581	0.262	0.268
ftsH	-0.104	-0.085	-0.059	-0.059	-0.047	0.828
gltB	-0.291	-0.259	-0.245	-0.222	-0.198	-0.213
groEL	-0.398	-0.315	-0.320	-0.265	-0.262	-0.262
hisH	-0.190	-0.183	-0.124	-0.142	-0.116	-0.125
hupA	-0.484	-0.495	-0.466	-0.358	-0.393	-0.393
ilvB	-0.355	-0.293	-0.304	-0.260	-0.241	-0.271
ilvH	-0.399	-0.405	-0.300	-0.314	-0.284	-0.274
infB	0.126	0.049	0.011	0.164	0.026	0.024
lpxA	-0.383	-0.352	-0.311	-0.297	-0.265	-0.259
moeB	0.037	-0.172	-0.146	-0.120	-0.111	-0.128
ntcA	0.616	0.275	0.813	0.409	0.354	0.584
odpA	1.150	-0.062	0.020	0.094	0.073	0.066
odpB	0.278	0.316	0.312	0.828	0.750	0.302
petA	0.492	0.311	0.586	0.338	0.406	0.690
petB	-0.400	-0.315	-0.294	-0.284	-0.248	-0.260
petD	-0.438	-0.355	-0.301	-0.360	-0.308	-0.322
petF	-0.251	-0.283	-0.220	-0.152	-0.191	-0.216
preA	-0.256	-0.248	-0.233	-0.200	-0.219	-0.216
psaA	-0.340	-0.365	-0.279	-0.272	-0.256	-0.247
psaB	-0.404	-0.366	-0.323	-0.282	-0.265	-0.278
psaD	-0.281	-0.329	-0.287	-0.208	-0.200	-0.228
psaF	-0.268	-0.278	-0.044	-0.210	-0.166	-0.211
psaL	-0.451	-0.342	-0.320	-0.283	-0.262	-0.269
psbA	-0.266	-0.261	-0.224	-0.218	-0.203	-0.186
psbB	-0.354	-0.326	-0.278	-0.257	-0.252	-0.250
psbC	-0.394	-0.355	-0.343	-0.259	-0.263	-0.243
psbD	-0.392	-0.337	-0.297	-0.308	-0.276	-0.282

psbV	-0.034	0.826	-0.055	-0.023	-0.054	-0.054
psbW	-0.247	-0.225	-0.201	-0.182	-0.188	-0.196
rbcL	-0.280	-0.297	-0.248	-0.231	-0.220	-0.219
rbcR	-0.357	-0.302	-0.294	-0.239	-0.228	-0.221
rbcS	-0.330	-0.390	-0.322	-0.249	-0.337	-0.259
rpl1	-0.432	-0.340	-0.308	-0.310	-0.254	-0.302
rpl2	-0.432	-0.356	-0.331	-0.300	-0.288	-0.295
rpl3	-0.279	-0.254	-0.253	-0.210	-0.182	-0.237
rpl4	-0.228	-0.155	0.127	-0.049	-0.121	-0.178
rpl5	-0.303	-0.358	-0.332	-0.322	-0.288	-0.256
rpl6	-0.199	-0.292	-0.247	-0.199	-0.141	-0.164
rpl9	-0.278	-0.286	-0.230	-0.204	-0.179	-0.230
rpl11	-0.289	-0.293	-0.255	-0.209	-0.213	-0.177
rpl12	-0.334	-0.250	-0.203	-0.180	-0.164	-0.183
rpl13	0.507	1.362	1.128	0.410	0.476	0.520
rpl14	-0.361	-0.317	-0.284	-0.227	-0.228	-0.263
rpl16	-0.474	-0.407	-0.418	-0.310	-0.401	-0.378
rpl18	-0.281	-0.264	-0.294	-0.194	-0.201	-0.218
rpl19	-0.330	-0.226	-0.328	-0.281	-0.171	-0.230
rpl20	-0.300	-0.209	-0.199	-0.169	-0.193	-0.203
rpl21	-0.274	-0.262	-0.211	-0.202	-0.177	-0.203
rpl22	-0.288	-0.300	-0.323	-0.214	-0.256	-0.216
rpoA	-0.346	-0.314	-0.336	-0.304	-0.275	-0.278
rpoB	0.004	1.535	0.064	0.143	0.159	0.127
rpoC1	-0.132	-0.088	-0.068	-0.056	-0.040	-0.024
rpoC2	0.573	0.819	0.833	0.436	0.436	0.489
rps2	-0.326	-0.296	-0.290	-0.292	-0.240	-0.254
rps3	-0.381	-0.350	-0.307	-0.301	-0.284	-0.275
rps4	-0.451	-0.439	-0.336	-0.286	-0.278	-0.301
rps5	-0.401	-0.367	-0.395	-0.335	-0.314	-0.333
rps6	-0.340	-0.390	-0.280	-0.309	-0.256	-0.246
rps7	-0.215	-0.220	-0.255	-0.213	-0.207	-0.242
rps8	-0.373	-0.346	-0.274	-0.279	-0.415	-0.414
rps9	-0.065	0.000	0.413	-0.043	-0.032	-0.027
rps10	-0.219	-0.160	-0.170	-0.201	-0.203	-0.222
rps11	-0.308	-0.264	-0.325	-0.241	-0.301	-0.262
rps12	-0.401	-0.270	-0.301	-0.278	-0.246	-0.212
rps13	-0.351	-0.285	-0.255	-0.257	-0.246	-0.231
rps14	-0.278	-0.221	-0.179	-0.134	-0.193	-0.214
secA	-0.043	0.009	0.078	-0.035	-0.021	-0.049

secY	-0.406	-0.334	-0.322	-0.268	-0.288	-0.302
sufB	-0.355	-0.332	-0.312	-0.267	-0.263	-0.255
sufC	-0.376	-0.323	-0.288	-0.268	-0.272	-0.244
tatC	0.495	0.353	0.323	0.427	0.419	0.399
thiG	-0.322	-0.386	-0.297	-0.287	-0.249	-0.267
tilS	-0.054	0.186	0.371	-0.028	-0.002	0.006
trpA	-0.187	-0.176	0.281	-0.180	-0.080	-0.104
tsf	-0.292	-0.290	-0.232	-0.203	-0.209	-0.250
tufA	-0.404	-0.378	-0.347	-0.309	-0.283	-0.307
ycf3	-0.375	-0.281	-0.334	-0.251	-0.267	-0.265
ycf4	0.049	0.141	0.193	0.751	0.066	0.105
ycf23	0.578	-0.046	-0.051	0.013	0.011	-0.014
ycf27	-0.428	-0.352	-0.307	-0.294	-0.286	-0.309
ycf29	-0.299	-0.257	-0.234	-0.199	-0.183	-0.234
ycf37	-0.056	0.235	-0.082	-0.077	-0.070	-0.087
ycf38	-0.312	-0.292	-0.257	-0.264	-0.255	-0.246
ycf39	-0.251	-0.255	-0.283	-0.191	-0.213	-0.168
ycf45	-0.306	-0.231	-0.203	-0.224	-0.225	-0.235
ycf56	-0.205	-0.219	-0.230	-0.225	-0.198	-0.218
ycf58	-0.163	-0.181	-0.179	-0.134	-0.150	-0.164
ycf60	0.019	0.954	0.042	0.055	0.045	0.067
ycf80	0.304	1.094	0.328	0.224	0.732	0.303
ycf82	-0.275	-0.284	-0.192	0.186	-0.167	-0.151
ycf83	-0.353	-0.381	-0.284	-0.269	-0.281	-0.216

Table S2.6. Evolutionary rates for all the mitochondrial genes and all the sublineages.

	Lin. 3/A	**Lin. 3/B**	**Lin. 3/C**	**Lin. 1**	**Lin. 2**	**Lin. 4**	**Lin. 5**	**Lin. 6**
atp6	-0.159	-0.159	-0.105	-0.091	-0.110	0.709	-0.168	-0.114
atp8	-0.486	-0.507	-0.365	-0.329	-0.451	-0.525	-0.482	-0.451
atp9	-0.482	-0.486	-0.375	-0.480	-0.505	-0.378	-0.416	-0.405
cob	-0.706	-0.725	-0.563	-0.645	-0.548	-0.506	-0.497	-0.552
cox1	-0.291	-0.293	-0.270	-0.265	-0.301	-0.235	-0.223	-0.279
cox2	-0.410	-0.419	-0.416	-0.475	-0.420	-0.459	-0.416	-0.372
cox3	0.095	0.114	0.220	0.266	0.247	0.139	0.169	0.172
nad1	-0.434	-0.435	-0.301	-0.350	-0.015	-0.293	-0.298	-0.293
nad2	-0.364	-0.365	-0.450	-0.367	-0.341	-0.350	-0.341	-0.369
nad3	-0.429	-0.410	-0.352	-0.419	-0.368	-0.318	-0.318	-0.341
nad4	-0.357	-0.360	-0.366	-0.356	-0.337	-0.326	-0.303	-0.328
nad4L	-0.756	-0.610	-0.433	-0.409	-0.472	-0.530	-0.514	-0.534
nad5	-0.324	-0.335	-0.009	-0.329	-0.298	-0.249	-0.306	-0.270
nad6	0.126	0.132	0.102	0.115	0.105	0.062	0.091	0.115
sdhC	0.343	0.347	0.331	0.347	0.544	0.436	0.525	0.307
yejR	-0.096	-0.096	-0.295	-0.232	-0.167	-0.225	-0.521	-0.201
yejU	-0.408	-0.417	-0.340	-0.426	-0.352	-0.388	-0.312	-0.380

Table S2.7. Evolutionary pairwise rates among *G. sulphuraria* lineages using the Codon-based Z-test of Selection. Data are expressed as mean of all the plastid genes substitution rate.

	Lin. 1	**Lin. 2**		**Lin. 3**		**Lin. 4**		**Lin. 5**		**Lin. 6**		**dN-dS**	**p-value**
		dN-dS	**p-value**	**dN-dS**	**p-value**	**dN-dS**	**p-value**	**dN-dS**	**p-value**	**dN-dS**	**p-value**	**mean**	**median**
Lin. 1	/	-0.173	0.00	-0.163	0.00	-0.204	0.00	-0.202	0.00	-0.212	0.00	-0.191	0.00
Lin. 2		/		-0.126	0.00	-0.149	0.00	-0.152	0.00	-0.141	0.00	-0.146	0.00
Lin. 3				/		-0.107	0.00	-0.129	0.00	-0.144	0.00	-0.147	0.00
Lin. 4						/		-0.120	0.00	-0.092	0.00	-0.139	0.00
Lin. 5								/		-0.071	0.00	-0.135	0.00
Lin. 6										/		-0.132	0.00

Table S2.8. Evolutionary pairwise rates among *G. sulphuraria* lineages using the Codon-based Z-test of Selection. Data are expressed as mean of all the mitochondrial genes substitution rates.

	Lin. 3/A	**Lin. 3/B**		**Lin. 3/C**		**Lin. 1**		**Lin. 2**		**Lin. 4**		**Lin. 5**		**Lin. 6**		**dN-dS**	**p-value**
		dN-dS	**p-value**	**dN-dS**	**p-value**	**dN-dS**	**p-value**	**dN-dS**	**p-value**	**dN-dS**	**p-value**	**dN-dS**	**p-value**	**dN-dS**	**p-value**	**mean**	**median**
Lin. 3/A	/	-0.026	0.06	-0.257	0.00	-0.430	0.00	-0.362	0.00	-0.284	0.00	-0.345	0.00	-0.413	0.00	-0.302	0.00
Lin. 3/B		/		-0.261	0.00	-0.397	0.00	-0.348	0.00	-0.277	0.00	-0.349	0.00	-0.411	0.00	-0.296	0.00
Lin. 3/C				/		-0.251	0.00	-0.161	0.00	-0.219	0.00	-0.251	0.00	-0.242	0.00	-0.235	0.00
Lin. 1						/		-0.177	0.00	-0.131	0.00	-0.210	0.00	-0.235	0.00	-0.261	0.00
Lin. 2								/		-0.149	0.00	-0.220	0.00	-0.142	0.00	-0.223	0.00
Lin. 4										/		-0.219	0.00	-0.136	0.00	-0.202	0.00
Lin. 5												/		-0.189	0.00	-0.255	0.00
Lin. 6														/		-0.253	0.00

TABLE S3.1. Distribution of the 10 most abundant Gene Ontology (GO) terms assigned to *G. sulphuraria* for biological process, molecular function and cellular component

Biological process

GO-id	GO-term	#Seqs
GO:0071704	organic substance metabolic process	183
GO:0044237	cellular metabolic process	172
GO:0044238	primary metabolic process	168
GO:0006807	nitrogen compound metabolic process	126
GO:0009058	biosynthetic process	90
GO:0044281	small molecule metabolic process	81
GO:0055114	oxidation-reduction process	58
GO:0051234	establishment of localization	51
GO:0055085	transmembrane transport	40
GO:0071840	cellular component organization or biogenesis	23

Molecular function

GO-id	GO-term	#Seqs
GO:0043167	ion binding	125
GO:1901363	heterocyclic compound binding	114
GO:0097159	organic cyclic compound binding	114
GO:0016740	transferase activity	90
GO:0036094	small molecule binding	89
GO:0016787	hydrolase activity	85
GO:0097367	carbohydrate derivative binding	64
GO:0016491	oxidoreductase activity	54
GO:0005515	protein binding	43
GO:0022857	transmembrane transporter activity	40

Cellular component

GO-id	GO-term	#Seqs
GO:0005622	intracellular anatomical structure	129
GO:0016020	membrane	105
GO:0043226	organelle	95
GO:0005737	cytoplasm	93
GO:0031224	intrinsic component of membrane	83
GO:0005829	cytosol	13
GO:1902494	catalytic complex	13

GO:0012505	endomembrane system	13
GO:0031975	envelope	9
GO:0098796	membrane protein complex	8

Table S5.1. Total metal removed from single and quaternary metal aqueous solutions by *G. sulphuraria*, strain SAG 107.79. Data are expressed as µmol/g dry matter. The total metal removed quantities were calculated by adding the amount of every metal component (Y^{3+} + Ce^{3+} + Eu^{3+} + Tb^{3+}).

		pH 2.5		pH 3.5		pH 4.5		pH 5.5	
	Metal system	**Single**	**Quaternary**	**Single**	**Quaternary**	**Single**	**Quaternary**	**Single**	**Quaternary**
Total metal removed (µmol/g dm)	Y^{3+}	0.39	0.17	0.83	0.23	4.75	1.56	10.44	1.75
	Ce^{3+}	0.41	0.15	1.08	0.33	5.94	2.09	10.97	3.11
	Eu^{3+}	0.42	0.16	1.44	0.76	10.82	6.21	14.34	6.99
	Tb^{3+}	0.40	0.19	1.22	0.62	10.85	5.69	11.13	6.43
	Total metals	/	0.66	/	1.93	/	15.56	/	18.28

Table S5.2. Total metal removed from single and quaternary metal aqueous solutions by *G. sulphuraria*, strain ACUF 427. Data are expressed as µmol/g dry matter. The total metal removed quantities were calculated by adding the amount of every metal component (Y^{3+} + Ce^{3+} + Eu^{3+} + Tb^{3+}).

		pH 2.5		pH 3.5		pH 4.5		pH 5.5	
	Metal system	**Single**	**Quaternary**	**Single**	**Quaternary**	**Single**	**Quaternary**	**Single**	**Quaternary**
metal removed	Y^{3+}	22.43	5.08	20.13	3.56	28.36	4.92	25.25	4.58

Ce^{3+}	20.98	5.16	25.08	3.92	29.82	7.64	42.60	6.59
Eu^{3+}	23.49	10.01	24.66	8.47	36.78	15.17	42.91	13.50
Tb^{3+}	22.26	11.47	24.14	9.02	40.58	15.20	34.24	13.74
Total metals	/	31.72	/	24.96	/	42.93	/	38.41

SUPPLEMENTARY FIGURES

Figure S2.1. Synteny comparison of *G. sulphuraria* plastid genomes. One representative of each lineage was analysed and compared with the reference genome of *C. merolae*. Homologous gene clusters are identified as Locally Collinear Blocks (LCBs) and visualized by the colored boxes.

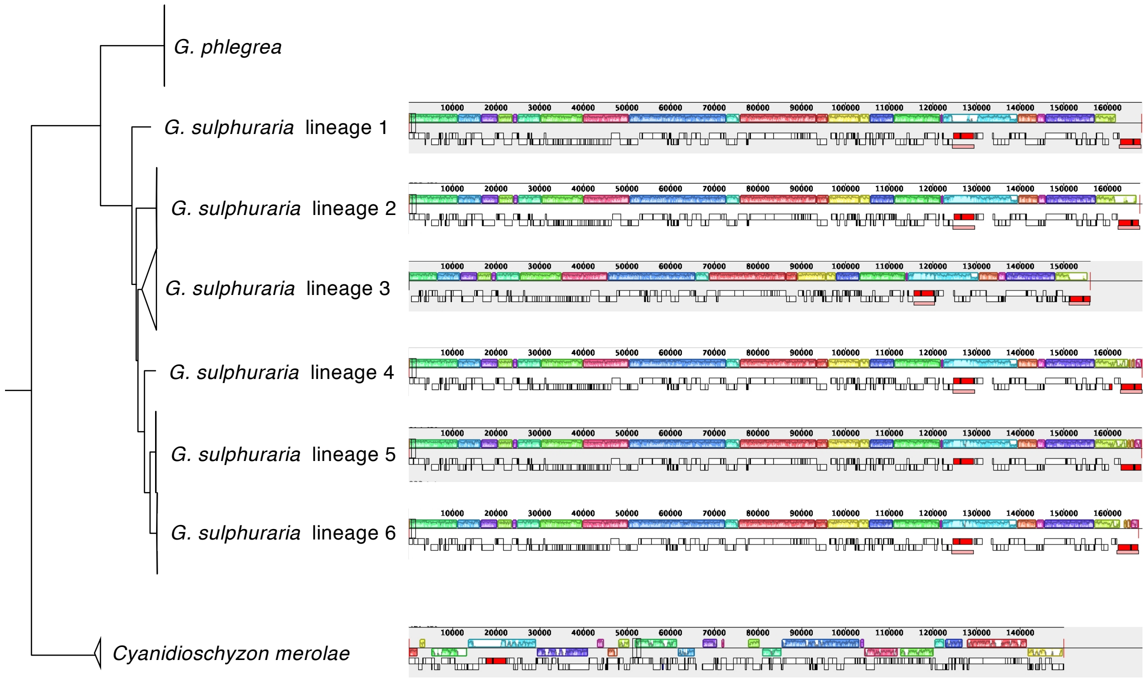
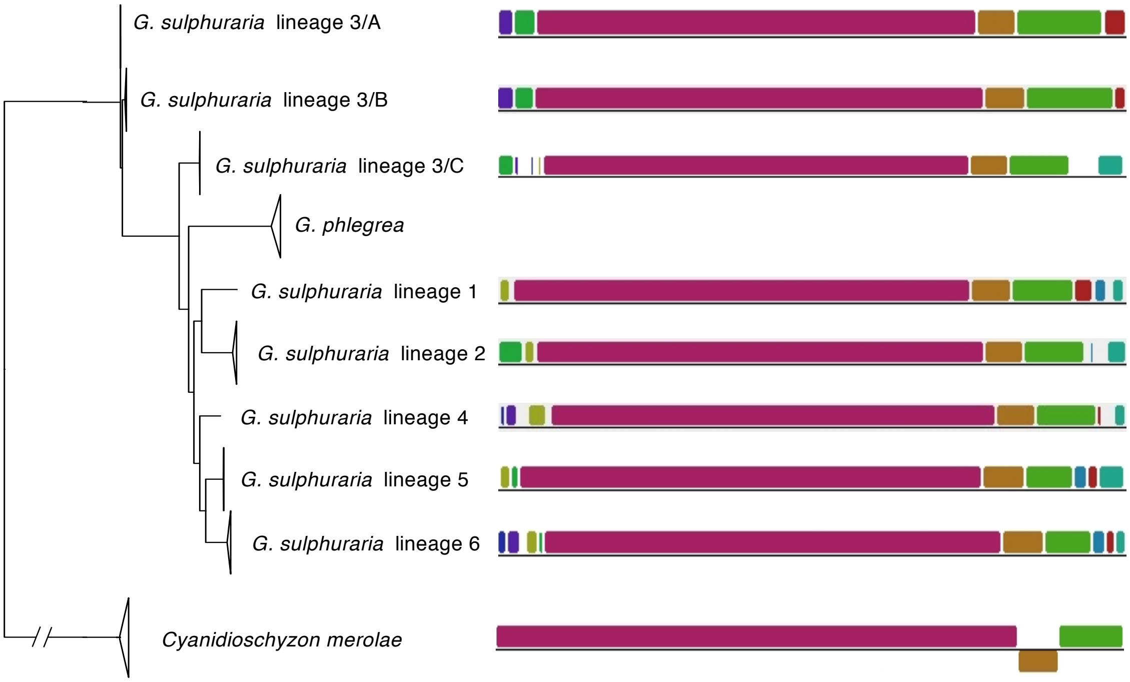


Figure S2.2. Synteny comparison of *G. sulphuraria* mitochondrial genomes. One representative of each lineage was analysed and compared with the reference genome of *C. merolae*. Homologous gene clusters are identified as Locally Collinear Blocks (LCBs) and visualized by the colored boxes.



REFERENCES

Abe, Fumiyoshi, and Koki Horikoshi. 2001. “The Biotechnological Potential of Piezophiles.” *Trends in Biotechnology* 19 (3): 102–8. https://doi.org/10.1016/S0167-7799(00)01539-0.

Albertano, P., C. Ciniglia, G. Pinto, and A. Pollio. 2000. “The Taxonomic Position of Cyanidium, Cyanidioschyzon and Galdieria: An Update.” *Hydrobiologia* 433: 137–43. https://doi.org/10.1023/A:1004031123806.

Albertano, P., G. Pinto, and A. Pollio. 1994. “Ecophysiology and Ultrastructure of an Acidophilic Species of Ochromonas (Chrysophyceae, Ochromonadales).” *Archiv Für Protistenkunde* 144 (1): 75–82. https://doi.org/10.1016/S0003-9365(11)80226-5.

Albertano, P, G Pinto, A Pollio, and R Taddei. 1990. “Morphology, Ultrastructure and Ecology of an Acidophilic Alga Pseudococcomyxa Simplex (Mainx) Fott (Chlorococcales).” *Arch. Hydrobiol., Algol. Stud.* 37: 401–8.

Albertano, P, G Pinto, S Santisi, and R Taddei. 1981. “Spermatozopsis Acidophila Kalina (Chlorophyta, Volvocales), a Little Known Alga from Highly Acidic Environments.” *Giorn. Bot. Ital.* 115: 65–76.

Albertano, P, A Pollio, and R Taddei. 1991. “Viridiella Fridericiana (Chlorococcales, Chlorophyta), a New Species Isolated from Extremely Acid Environments.” *Phycologia* 30 (4): 346–54.

Allen, M. M., and R. Y. Stanier. 1968. “Selective Isolation of Blue-Green Algae from Water and Soil.” *Journal of General Microbiology* 51 (2): 203–9. https://doi.org/10.1099/00221287-51-2-203.

Allen, Mary Belle. 1959. “Studies with Cyanidium Caldarium, an Anomalously Pigmented Chlorophyte.” *Archiv Für Mikrobiologie* 32 (3): 270–77. https://doi.org/10.1007/BF00409348.

Altschul, Stephen F., Warren Gish, Webb Miller, Eugene W. Myers, and David J. Lipman. 1990. “Basic Local Alignment Search Tool.” *Journal of Molecular Biology* 215 (3): 403–10. https://doi.org/10.1016/S0022-2836(05)80360-2.

Anisimova, Maria, Manuel Gil, Jean Franois Dufayard, Christophe Dessimoz, and Olivier Gascuel. 2011. “Survey of Branch Support Methods Demonstrates Accuracy, Power, and Robustness of Fast Likelihood-Based Approximation Schemes.” *Systematic Biology* 60 (5): 685–99. https://doi.org/10.1093/sysbio/syr041.

Bailey, R W, and L. A. Staehelin. 1968. “The Chemical Composition of Isolated Cell Walls of Cyanidium Caldarium.” *Journal of General Microbiology* 54 (2):

269–76. https://doi.org/10.1099/00221287-54-2-269.

Baker-Austin, Craig, and Mark Dopson. 2007. “Life in Acid: PH Homeostasis in Acidophiles.” *Trends in Microbiology* 15 (4): 165–71. https://doi.org/10.1016/j.tim.2007.02.005.

Bankevich, Anton, Sergey Nurk, Dmitry Antipov, Alexey A. Gurevich, Mikhail Dvorkin, Alexander S. Kulikov, Valery M. Lesin, et al. 2012. “SPAdes: A New Genome Assembly Algorithm and Its Applications to Single-Cell Sequencing.” *Journal of Computational Biology* 19 (5): 455–77. https://doi.org/10.1089/cmb.2012.0021.

Binnemans, Koen, Peter Tom Jones, Bart Blanpain, Tom Van Gerven, Yongxiang Yang, Allan Walton, and Matthias Buchert. 2013. “Recycling of Rare Earths: A Critical Review.” *Journal of Cleaner Production* 51: 1–22. https://doi.org/10.1016/j.jclepro.2012.12.037.

Birungi, Z. S., and E. M.N. Chirwa. 2014. “The Kinetics of Uptake and Recovery of Lanthanum Using Freshwater Algae as Biosorbents: Comparative Analysis.” *Bioresource Technology* 160: 43–51. https://doi.org/10.1016/j.biortech.2014.01.033.

Birungi, Z. S., E. M.N. Chirwa, and O. J. Botai. 2017. “Competitive Adsorption in a Ternary System of Toxic Metals and Rare Earth Elements Using Desmodesmus Multivariabilis: Empirical and Kinetic Modelling.” *Journal of Applied Phycology* 29 (6): 2899–2910. https://doi.org/10.1007/s10811-017-1197-0.

Bolger, Anthony M, Marc Lohse, and Bjoern Usadel. 2014. “Genome Analysis Trimmomatic: A Flexible Trimmer for Illumina Sequence Data.” *BIOINFORMATICS* 30 (15): 2114–20. https://doi.org/10.1093/bioinformatics/btu170.

Bourcier, W. L., M. Lin, and G. Nix. 2005. “Recovery of Minerals and Metals from Geothermal Fluids.” *2003 SME Annual Meeting*, 19.

Brady, Joseph M., and John M. Tobin. 1995. “Binding of Hard and Soft Metal Ions to Rhizopus Arrhizus Biomass.” *Enzyme and Microbial Technology* 17 (9): 791–96. https://doi.org/10.1016/0141-0229(95)00142-R.

Brock, T. D. 1978. “The Genus Cyanidium.” In *Thermophilic Microorganisms and Life at High Temperatures*, 255–302.

Bronstein, Omri, Andreas Kroh, and Elisabeth Haring. 2018. “Mind the Gap! The Mitochondrial Control Region and Its Power as a Phylogenetic Marker in Echinoids.” *BMC Evolutionary Biology* 18 (1).

https://doi.org/10.1186/s12862-018-1198-x.

Bruen, Trevor C, Hervé Philippe, and David Bryant. 2006. “A Simple and Robust Statistical Test for Detecting the Presence of Recombination.” *Genetics* 172 (4): 2665–81. https://doi.org/10.1534/genetics.105.048975.

Bull, Alan T., and Michael Goodfellow. 2019. “Dark, Rare and Inspirational Microbial Matter in the Extremobiosphere: 16 000 m of Bioprospecting Campaigns.” *Microbiology (United Kingdom)* 165 (12): 1252–64. https://doi.org/10.1099/mic.0.000822.

Camacho, Christiam, George Coulouris, Vahram Avagyan, Ning Ma, Jason Papadopoulos, Kevin Bealer, and Thomas L Madden. 2009. “BLAST+: Architecture and Applications.” *BMC Bioinformatics* 10 (1): 421. https://doi.org/10.1186/1471-2105-10-421.

Cao, Ying, Penghui Shao, Yidi Chen, Xiaoyu Zhou, Liming Yang, Hui Shi, Kai Yu, Xianxin Luo, and Xubiao Luo. 2021. “A Critical Review of the Recovery of Rare Earth Elements from Wastewater by Algae for Resources Recycling Technologies.” *Resources, Conservation and Recycling* 169 (November 2020): 105519. https://doi.org/10.1016/j.resconrec.2021.105519.

Casadei, M. A., P. Mañas, G. Niven, E. Needs, and B. M. Mackey. 2002. “Role of Membrane Fluidity in Pressure Resistance of Escherichia Coli NCTC 8164.” *Applied and Environmental Microbiology* 68 (12): 5965–72. https://doi.org/10.1128/AEM.68.12.5965-5972.2002.

Cassano, Valeria, Mariana C. Oliveira, María Candelaria Gil-Rodríguez, Abel Sentíes, Jhoana Díaz-Larrea, and Mutue T. Fujii. 2012. “Molecular Support for the Establishment of the New Genus Laurenciella within the Laurencia Complex (Ceramiales, Rhodophyta).” *Botanica Marina* 55 (4): 349–57. https://doi.org/10.1515/bot-2012-0133.

Castor, S, and J Hedrick. 2006. “Rare Earth Elements.” In *Society for Mining, Metallurgy, and Exploration: Littleton, Colorado*, 769–92.

Castresana, J. 2000. “Selection of Conserved Blocks from Multiple Alignments for Their Use in Phylogenetic Analysis.” *Molecular Biology and Evolution* 17 (4): 540–52. https://doi.org/10.1093/oxfordjournals.molbev.a026334.

Chao, Liu, Pan Bofu, Cao Weiqian, Lu Yun, Huang Hao, Chen Liang, Liu Xiaoqing, Wu Xiao, and Hong Fashui. 2008. “Influences of Calcium Deficiency and Cerium on Growth of Spinach Plants.” *Biological Trace Element Research* 121 (3): 266–75. https://doi.org/10.1007/s12011-007-8054-y.

Chen, Can, and Jianlong Wang. 2007. “Influence of Metal Ionic Characteristics on Their Biosorption Capacity by Saccharomyces Cerevisiae.” *Applied Microbiology and Biotechnology* 74 (4): 911–17. https://doi.org/10.1007/s00253-006-0739-1.

Cho, Chung Hyun, Seung In Park, Claudia Ciniglia, Eun Chan Yang, Louis Graf, Debashish Bhattacharya, and Hwan Su Yoon. 2020. “Potential Causes and Consequences of Rapid Mitochondrial Genome Evolution in Thermoacidophilic Galdieria (Rhodophyta).” *BMC Evolutionary Biology* 20 (112): 1–15. https://doi.org/10.21203/rs.3.rs-36820/v1.

Cho, Yao Chang Lee, Liang Ching Hsu, Chun Chieh Wang, Pin Chen Chen, Shao Lun Liu, Heng Yi Teah, Yu Ting Liu, and Yu Min Tzou. 2020. “Molecular Mechanisms for Pb Removal by Cyanidiales: A Potential Biomaterial Applied in Thermo-Acidic Conditions.” *Chemical Engineering Journal* 401 (December): 125828. https://doi.org/10.1016/j.cej.2020.125828.

Chong, K. H., and B. Volesky. 1995. “Description of Two-metal Biosorption Equilibria by Langmuir-type Models.” *Biotechnology and Bioengineering* 47 (4): 451–60. https://doi.org/10.1002/bit.260470406.

Chu, ZX, MH Mu, FZ Wang, and HX Shao. 1996. “Effect of CeCl3 on the Photosynthesis of Plants Relation to Fe2+.” In *Environmental Behavior and Ecotoxicology of Rare Earth Elements.*, 75–81.

Ciniglia, P Cennamo, A De Natale, M De Stefano, M Sirakov, M Iovinella, HS Yoon, and A Pollio. 2019. “Cyanidium Chilense (Cyanidiophyceae, Rhodophyta) from Tuff Rocks of the Archeological Site of Cuma, Italy.” *Phycological Research*, no. November 2018. https://doi.org/10.1111/pre.12383.

Ciniglia, Paola Cennamo, Mario De Stefano, Gabriele Pinto, Paolo Caputo, and Antonino Pollio. 2007. “Pinnularia Obscura Krasske (Bacillariophyceae, Bacillariophyta) from Acidic Environments: Characterization and Comparison with Other Acid-Tolerant Pinnularia Species.” *Fundamental and Applied Limnology* 170 (1): 29–47. https://doi.org/10.1127/1863-9135/2007/0170-0029.

Ciniglia, Claudia, Eun Chan Yang, Antonino Pollio, Gabriele Pinto, Manuela Iovinella, Laura Vitale, and Hwan Su Yoon. 2014. “Cyanidiophyceae in Iceland: Plastid Rbc L Gene Elucidates Origin and Dispersal of Extremophilic Galdieria Sulphuraria and G. Maxima (Galdieriaceae, Rhodophyta).” *Phycologia* 53 (6): 542–51. https://doi.org/10.2216/14-032.1.

Ciniglia, Claudia, Hwan Su Yoon, Antonino Pollio, Gabriele Pinto, and Debashish Bhattacharya. 2004. “Hidden Biodiversity of the Extremophilic Cyanidiales Red Algae.” *Molecular Ecology* 13 (7): 1827–38. https://doi.org/10.1111/j.1365-294X.2004.02180.x.

Čížková, M, K Bišová, V Zachleder, D Mezricky, M Rucki, and M Vítová. 2019. “Recovery of Rare Earth Elements from Luminophores Using the Red Alga Galdieria,” no. September: 2018–19. http://www.acuf.net/index.php?lang=en.

Čížková, Mária, Pauline Mezricky, Dana Mezricky, Marian Rucki, Vilém Zachleder, and Milada Vítová. 2021. “Bioaccumulation of Rare Earth Elements from Waste Luminophores in the Red Algae, Galdieria Phlegrea.” *Waste and Biomass Valorization* 12 (6): 3137–46. https://doi.org/10.1007/s12649-020-01182-3.

Clarke, Andrew. 2014. “The Thermal Limits to Life on Earth.” *International Journal of Astrobiology* 13 (2): 141–54. https://doi.org/10.1017/S1473550413000438.

Comte, S., G. Guibaud, and M. Baudu. 2008. “Biosorption Properties of Extracellular Polymeric Substances (EPS) towards Cd, Cu and Pb for Different PH Values.” *Journal of Hazardous Materials* 151 (1): 185–93. https://doi.org/10.1016/j.jhazmat.2007.05.070.

Corguillé, Gildas Le, Gareth Pearson, Marta Valente, Carla Viegas, Bernhard Gschloessl, Erwan Corre, Xavier Bailly, et al. 2009. “Plastid Genomes of Two Brown Algae, Ectocarpus Siliculosus and Fucus Vesiculosus: Further Insights on the Evolution of Red-Algal Derived Plastids.” *BMC Evolutionary Biology* 9 (1): 253. https://doi.org/10.1186/1471-2148-9-253.

Crognale, Simona, Stefania Venturi, Franco Tassi, Simona Rossetti, Heba Rashed, Jacopo Cabassi, Francesco Capecchiacci, et al. 2018. “Microbiome Profiling in Extremely Acidic Soils Affected by Hydrothermal Fluids: The Case of the Solfatara Crater (Campi Flegrei, Southern Italy).” *FEMS Microbiology Ecology* 94 (12). https://doi.org/10.1093/femsec/fiy190.

D’Alelio, Domenico, and Maria Valeria Ruggiero. 2015. “Interspecific Plastidial Recombination in the Diatom Genus Pseudo-Nitzschia.” *Journal of Phycology* 51 (6): 1024–28. https://doi.org/10.1111/jpy.12350.

D’Alelio, Domenico, Nico Salmaso, and Andrea Gandolfi. 2013. “Frequent Recombination Shapes the Epidemic Population Structure of Planktothrix (Cyanoprokaryota) in Italian Subalpine Lakes.” *Journal of Phycology* 49 (6):

1107–17. https://doi.org/10.1111/jpy.12116.

Dahle, Jessica T., and Yuji Arai. 2015. “Environmental Geochemistry of Cerium: Applications and Toxicology of Cerium Oxide Nanoparticles.” *International Journal of Environmental Research and Public Health* 12 (2): 1253–78. https://doi.org/10.3390/ijerph120201253.

Daly, Michael J., Elena K. Gaidamakova, Vera Y. Matrosova, Juliann G. Kiang, Risaku Fukumoto, Duck Yeon Lee, Nancy B. Wehr, Gabriela A. Viteri, Barbara S. Berlett, and Rodney L. Levine. 2010. “Small-Molecule Antioxidant Proteome-Shields in Deinococcus Radiodurans.” *PLOS ONE* 5 (9): e12570. https://doi.org/10.1371/JOURNAL.PONE.0012570.

Daly, Michael J., Elena K. Gaidamakova, Vera Y. Matrosova, Alexander Vasilenko, Min Zhai, Richard D. Leapman, Barry Lai, et al. 2007. “Protein Oxidation Implicated as the Primary Determinant of Bacterial Radioresistance.” *PLOS Biology* 5 (4): e92. https://doi.org/10.1371/JOURNAL.PBIO.0050092.

Darling, Aaron E, Bob Mau, and Nicole T Perna. 2010. “Progressivemauve: Multiple Genome Alignment with Gene Gain, Loss and Rearrangement.” *PLoS ONE* 5 (6). https://doi.org/10.1371/journal.pone.0011147.

Das, Nilanjana. 2010. “Recovery of Precious Metals through Biosorption - A Review.” *Hydrometallurgy* 103 (1–4): 180–89. https://doi.org/10.1016/j.hydromet.2010.03.016.

Dev, Subhabrata, Ankur Sachan, Fahimeh Dehghani, Tathagata Ghosh, Brandon R. Briggs, and Srijan Aggarwal. 2020. “Mechanisms of Biological Recovery of Rare-Earth Elements from Industrial and Electronic Wastes: A Review.” *Chemical Engineering Journal* 397 (February): 124596. https://doi.org/10.1016/j.cej.2020.124596.

Dhakar, Kusum, and Anita Pandey. 2016. “Wide PH Range Tolerance in Extremophiles: Towards Understanding an Important Phenomenon for Future Biotechnology.” *Applied Microbiology and Biotechnology* 100 (6): 2499–2510. https://doi.org/10.1007/s00253-016-7285-2.

Dhankhar, Rajesh, and Anju Hooda. 2011. “Fungal Biosorption-an Alternative to Meet the Challenges of Heavy Metal Pollution in Aqueous Solutions.” *Environmental Technology* 32 (5): 467–91. https://doi.org/10.1080/09593330.2011.572922.

Dose, K, A Bieger-Dose, M Labusch, and M Gill. 1992. “Survival in Extreme Dryness and DNA-Single-Strand Breaks.” *Advances in Space Research* 12

(4): 221–29. https://doi.org/10.1006/enrs.1994.1018.

Doyle, Jeff J. 1992. "Gene Trees and Species Trees : Molecular Systematics as One-Character Taxonomy." *Systematic Botany* 17 (1): 144–63.

Elster, Josef. 1999. "Enigmatic Microorganisms and Life in Extreme Environments." In *Origin - Evolution and Versatility of Microorganisms (Phylogeny, Structure, Physiology and Extreme Environments*, edited by Joseph Seckbach. Kluwer Academic Publishers. https://doi.org/10.1007/978-94-011-4838-2.

Farcasanu, I. C., M. Mizunuma, D. Hirata, and T. Miyakawa. 1998. "Involvement of Histidine Permease (Hip1p) in Manganese Transport in Saccharomyces Cerevisiae." *Molecular and General Genetics* 259 (5): 541–48. https://doi.org/10.1007/s004380050846.

Farooq, Umar, Janusz A. Kozinski, Misbahul Ain Khan, and Makshoof Athar. 2010. "Biosorption of Heavy Metal Ions Using Wheat Based Biosorbents - A Review of the Recent Literature." *Bioresource Technology* 101 (14): 5043–53. https://doi.org/10.1016/j.biortech.2010.02.030.

Ferreira, J. A., and S. O. Nyangoma. 2008. "A Multivariate Version of the Benjamini-Hochberg Method." *Journal of Multivariate Analysis* 99 (9): 2108–24. https://doi.org/10.1016/j.jmva.2008.02.013.

Fonseca, Miguel M., D. James Harris, and David Posada. 2014. "The Inversion of the Control Region in Three Mitogenomes Provides Further Evidence for an Asymmetric Model of Vertebrate MtDNA Replication." Edited by Dan Mishmar. *PLoS ONE* 9 (9): e106654. https://doi.org/10.1371/journal.pone.0106654.

Franklin, Natasha M, Jennifer L Stauber, Richard P Lim, and P Petocz. 2002. "Toxicity of Metal Mixtures to a Tropical Freshwater Alga (Chlorella Sp.): The Effect of Interactions between Copper, Cadmium, and Zinc on Metal Cell Binding and Uptake." *Environmental Toxicology and Chemistry* 21 (11): 2412–22.

Fukuda, Shin ya, Rie Yamamoto, Koji Iwamoto, and Ayumi Minoda. 2018. "Cellular Accumulation of Cesium in the Unicellular Red Alga Galdieria Sulphuraria under Mixotrophic Conditions." *Journal of Applied Phycology* 30 (6): 3057–61. https://doi.org/10.1007/s10811-018-1525-z.

Gallup, Darrell L. 1998. "Geochemistry of Geothermal Fluids and Well Scales, and Potential for Mineral Recovery." *Ore Geology Reviews* 12 (4): 225–36. https://doi.org/10.1016/S0169-1368(98)00004-3.

Galtier, Nicolas, and Vincent Daubin. 2008. “Dealing with Incongruence in Phylogenomic Analyses.” *Philosophical Transactions of the Royal Society B: Biological Sciences*. Royal Society. https://doi.org/10.1098/rstb.2008.0144.

Gitzendanner, Matthew A., Pamela S. Soltis, Gane K.S. Wong, Brad R. Ruhfel, and Douglas E. Soltis. 2018. “Plastid Phylogenomic Analysis of Green Plants: A Billion Years of Evolutionary History.” *American Journal of Botany* 105 (3): 291–301. https://doi.org/10.1002/ajb2.1048.

Goecke, Franz, Milada Vítová, Jaromír Lukavský, Linda Nedbalová, Tomáš Řezanka, and Vilém Zachleder. 2017. “Effects of Rare Earth Elements on Growth Rate, Lipids, Fatty Acids and Pigments in Microalgae.” *Phycological Research* 65 (3): 226–34. https://doi.org/10.1111/pre.12180.

Gonçalves, Deise J.P., Beryl B. Simpson, Edgardo M. Ortiz, Gustavo H. Shimizu, and Robert K. Jansen. 2019. “Incongruence between Gene Trees and Species Trees and Phylogenetic Signal Variation in Plastid Genes.” *Molecular Phylogenetics and Evolution* 138 (September): 219–32. https://doi.org/10.1016/j.ympev.2019.05.022.

Graedel, T. E., Julian Allwood, Jean Pierre Birat, Matthias Buchert, Christian Hagelüken, Barbara K. Reck, Scott F. Sibley, and Guido Sonnemann. 2011. “What Do We Know about Metal Recycling Rates?” *Journal of Industrial Ecology* 15 (3): 355–66. https://doi.org/10.1111/j.1530-9290.2011.00342.x.

Gromov, B.V., O.V. Gavrilova, and A.V. Pljusch. 1990. “Ultrastructure of Ochromonas Globosa (Chrysophyceae).” *Archiv Für Protistenkunde* 138 (4): 291–97.

Gross, W., and Christine Oesterhelt. 1999. “Ecophysiological Studies on the Red Alga Galdieria Sulphuraria Isolated from Southwest Iceland.” *Plant Biology* 1 (6): 694–700. https://doi.org/10.1111/j.1438-8677.1999.tb00282.x.

Haug, Arne, and Olav Smidsrod. 1970. “Selectivity of Some Anionic Polymers for Divalent Metal Ions.” *Acta Chemica Scandinavia*, 843–54.

Herrmann, Johannes M. 2003. “Converting Bacteria to Organelles: Evolution of Mitochondrial Protein Sorting.” *Trends in Microbiology* 11 (2): 74–79. https://doi.org/10.1016/S0966-842X(02)00033-1.

Hsieh, Chia Jung, Shing Hei Zhan, Yiching Lin, Sen Lin Tang, and Shao Lun Liu. 2015. “Analysis of RbcL Sequences Reveals the Global Biodiversity, Community Structure, and Biogeographical Pattern of Thermoacidophilic Red Algae (Cyanidiales).” *Journal of Phycology* 51: 682–94.

https://doi.org/10.1111/jpy.12310.

Huss, Volker A R, Claudia Ciniglia, Paola Cennamo, Salvatore Cozzolino, Gabriele Pinto, and Antonino Pollio. 2002. "Phylogenetic Relationships and Taxonomic Position of Chlorella-like Isolates from Low PH Environments (PH < 3.0)." *BMC Evolutionary Biology* 9: 13.

Iovinella, Manuela, Dora Allegra Carbone, Diana Cioppa, Seth J. Davis, Michele Innangi, Sabrina Esposito, and Claudia Ciniglia. 2020. "Prevalent PH Controls the Capacity of Galdieria Maxima to Use Ammonia and Nitrate as a Nitrogen Source." *Plants* 9 (2): 232. https://doi.org/10.3390/plants9020232.

Iovinella, Manuela, Ayla Eren, Gabriele Pinto, Antonino Pollio, Seth J Davis, Paola Cennamo, and Claudia Ciniglia. 2018. "Cryptic Dispersal of Cyanidiophytina (Rhodophyta) in Non-Acidic Environments from Turkey." *Extremophiles* 22: 713–23. https://doi.org/10.1007/s00792-018-1031-x.

Iovinella, Manuela, Francesco Lombardo, Claudia Ciniglia, Maria Palmieri, Maria Rosa di Cicco, Marco Trifuoggi, Marco Race, et al. 2022. "Bioremoval of Yttrium (III), Cerium (III), Europium (III), and Terbium (III) from Single and Quaternary Aqueous Solutions Using the Extremophile Galdieria Sulphuraria (Galdieriaceae, Rhodophyta)." *Plants* 11 (10): 1376. https://doi.org/10.3390/plants11101376.

Jacinto, Jéssica, Bruno Henriques, A. C. Duarte, Carlos Vale, and E. Pereira. 2018. "Removal and Recovery of Critical Rare Elements from Contaminated Waters by Living Gracilaria Gracilis." *Journal of Hazardous Materials* 344 (February): 531–38. https://doi.org/10.1016/j.jhazmat.2017.10.054.

Jack, D. L., I. T. Paulsen, and Jr Saier. 2000. "The Amino Acid/Polyamine/Organocation (APC) Superfamily of Transporters Specific for Amino Acids, Polyamines and Organocations." *Microbiology* 146 (8): 1797–1814. https://doi.org/10.1099/00221287-146-8-1797.

Jain, Kanika, Kirsten Krause, Felix Grewe, Gaven F. Nelson, Andreas P.M. Weber, Alan C. Christensen, and Jeffrey P. Mower. 2014. "Extreme Features of the Galdieria Sulphuraria Organellar Genomes: A Consequence of Polyextremophily." *Genome Biology and Evolution* 7 (1): 367–80. https://doi.org/10.1093/gbe/evu290.

Jalali, F., J. Fakhari, and A. Zolfaghari. 2018. "Investigation on Biosorption of V (III), Ti(IV), and U(VI) Ions from a Contaminated Effluent by a Newly Isolated Strain of Galdieria Sulphuraria." *Separation Science and*

Technology (Philadelphia) 54 (14): 2222–39. https://doi.org/10.1080/01496395.2018.1543323.

Jittawuttipoka, Thichakorn, Mariane Planchon, Olivier Spalla, Karim Benzerara, François Guyot, Corinne Cassier-Chauvat, and Franck Chauvat. 2013. "Multidisciplinary Evidences That Synechocystis PCC6803 Exopolysaccharides Operate in Cell Sedimentation and Protection against Salt and Metal Stresses." *PLoS ONE* 8 (2). https://doi.org/10.1371/journal.pone.0055564.

Johnson, D. Barrie. 1998. "Biodiversity and Ecology of Acidophilic Microorganisms." *FEMS Microbiology Ecology* 27 (4): 307–17. https://doi.org/10.1016/S0168-6496(98)00079-8.

Jönsson, K. Ingemar, Elke Rabbow, Ralph O. Schill, Mats Harms-Ringdahl, and Petra Rettberg. 2008. "Tardigrades Survive Exposure to Space in Low Earth Orbit." *Current Biology* 18 (17). https://doi.org/10.1016/j.cub.2008.06.048.

Ju, Xiaohui, Kensuke Igarashi, Shin ichi Miyashita, Hiroaki Mitsuhashi, Kazumi Inagaki, Shin ichiro Fujii, Hitomi Sawada, Tomohiko Kuwabara, and Ayumi Minoda. 2016. "Effective and Selective Recovery of Gold and Palladium Ions from Metal Wastewater Using a Sulfothermophilic Red Alga, Galdieria Sulphuraria." *Bioresource Technology* 211: 759–64. https://doi.org/10.1016/j.biortech.2016.01.061.

Kanekar, P.P., S.P. Kanekar, A.S. Kelkar, and P.K. Dhakephalkar. 2012. "Halophiles – Taxonomy, Diversity, Physiology and Applications." In *Microorganisms in Environmental Management: Microbes and Environment*, edited by T. Satyanarayana, B.N. Johri, and A. Prakash, 1–34. Dordrecht: Springer. https://doi.org/10.1007/978-94-007-2229-3.

Katoh, Kazutaka, and Hiroyuki Toh. 2008. "Recent Developments in the MAFFT Multiple Sequence Alignment Program." *Briefings in Bioinformatics* 9 (4): 286–98. https://doi.org/10.1093/bib/bbn013.

Kent, W. J. 2002. "BLAT---The BLAST-Like Alignment Tool." *Genome Research* 12 (4): 656–64. https://doi.org/10.1101/gr.229202.

Kim, Jong Im, Hwan Su Yoon, Gangman Yi, Woongghi Shin, and John M. Archibald. 2018. "Comparative Mitochondrial Genomics of Cryptophyte Algae: Gene Shuffling and Dynamic Mobile Genetic Elements." *BMC Genomics* 19 (1): 275. https://doi.org/10.1186/s12864-018-4626-9.

Kim, Kyeong Mi, Eun Chan Yang, Jeong Ha Kim, Wendy A Nelson, and Hwan Su Yoon. 2015. "Complete Mitochondrial Genome of a Rhodolith, Sporolithon Durum (Sporolithales, Rhodophyta).” *Mitochondrial DNA* 26 (1): 155–56. https://doi.org/10.3109/19401736.2013.819500.

Kolmogorov, Mikhail, Joel Armstrong, Brian J. Raney, Ian Streeter, Matthew Dunn, Fengtang Yang, Duncan Odom, et al. 2018. “Chromosome Assembly of Large and Complex Genomes Using Multiple References.” *Genome Research* 28 (11): 1720–32. https://doi.org/10.1101/gr.236273.118.

Krulwich, Terry Ann, Masahiro Ito, David B. Hicks, Raymond Gilmour, and Arthur A. Guffanti. 1998. “PH Homeostasis and ATP Synthesis: Studies of Two Processes That Necessitate Inward Proton Translocation in Extremely Alkaliphilic Bacillus Species.” *Extremophiles* 2 (3): 217–22. https://doi.org/10.1007/s007920050063.

Kumar, Sudhir, Glen Stecher, Michael Li, Christina Knyaz, and Koichiro Tamura. 2018. “MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms.” *Molecular Biology and Evolution* 35 (6): 1547–49. https://doi.org/10.1093/molbev/msy096.

Lanquar, Viviane, Françoise Lelièvre, Hélène Barbier-Brygoo, and Sébastien Thomine. 2004. “Regulation and Function of AtNRAMP4 Metal Transporter Protein.” *Soil Science and Plant Nutrition* 50 (7): 1141–50. https://doi.org/10.1080/00380768.2004.10408587.

Laslett, Dean, and Bjorn Canback. 2004. “ARAGORN, a Program to Detect TRNA Genes and TmRNA Genes in Nucleotide Sequences.” *Nucleic Acids Research* 32 (1): 11–16. https://doi.org/10.1093/nar/gkh152.

Leblanc, C., O. Richard, B. Kloareg, S. Viehmann, K. Zetsche, and C. Boyen. 1997. “Origin and Evolution of Mitochondria: What Have We Learnt from Red Algae?” *Current Genetics* 31 (3): 193–207. https://doi.org/10.1007/s002940050196.

Lee, Jun Mo, Chung Hyun Cho, Seung In Park, Ji Won Choi, Hyun Suk Song, John A. West, Debashish Bhattacharya, and Hwan Su Yoon. 2016. “Parallel Evolution of Highly Conserved Plastid Genome Architecture in Red Seaweeds and Seed Plants.” *BMC Biology* 14 (1): 75. https://doi.org/10.1186/s12915-016-0299-5.

Lee, Justin, Shounak Ghosh, and Milton H. Saier. 2017. “Comparative Genomic Analyses of Transport Proteins Encoded within the Red Algae Chondrus Crispus, Galdieria Sulphuraria, and Cyanidioschyzon Merolae11.” *Journal of Phycology* 53 (3): 503–21. https://doi.org/10.1111/jpy.12534.

Liu, Shao-Lun, Yin-Ru Chiang, Hwan Su Yoon, and Han-Yi Fu. 2020.

"Comparative Genome Analysis Reveals Cyanidiococcus Gen. Nov., a New Extremophilic Red Algal Genus Sister to Cyanidioschyzon (Cyanidioschyzonaceae, Rhodophyta)." *Journal of Phycology*, 0–1. https://doi.org/10.1111/jpy.13056.

Liu, Yongqin, Tandong Yao, Shichang Kang, Nianzhi Jiao, Yonghui Zeng, Sijun Huang, and Tingwei Luo. 2007. "Microbial Community Structure in Major Habitats above 6000 m on Mount Everest." *Chinese Science Bulletin* 52 (17): 2350–57. https://doi.org/10.1007/s11434-007-0360-4.

Lo, Yung Chung, Chieh Lun Cheng, Yin Lung Han, Bor Yann Chen, and Jo Shu Chang. 2014. "Recovery of High-Value Metals from Geothermal Sites by Biosorption and Bioaccumulation." *Bioresource Technology* 160 (November 2016): 182–90. https://doi.org/10.1016/j.biortech.2014.02.008.

Lohse, Marc, Oliver Drechsel, Sabine Kahlau, and Ralph Bock. 2013. "OrganellarGenomeDRAW--a Suite of Tools for Generating Physical Maps of Plastid and Mitochondrial Genomes and Visualizing Expression Data Sets." *Nucleic Acids Research* 41 (Web Server issue). https://doi.org/10.1093/nar/gkt289.

Lowe, Todd M, and Patricia P Chan. 2016. "TRNAscan-SE On-Line: Integrating Search and Context for Analysis of Transfer RNA Genes." *Web Server Issue Published Online* 44. https://doi.org/10.1093/nar/gkw413.

Ma, Ning, Chunsheng Li, Xiaoyan Dong, Dongfeng Wang, and Ying Xu. 2015. "Different Effects of Sodium Chloride Preincubation on Cadmium Tolerance of Pichia Kudriavzevii and Saccharomyces Cerevisiae." *Journal of Basic Microbiology* 55 (8): 1002–12. https://doi.org/10.1002/jobm.201400847.

Maddison, Wayne P. 1997. "Gene Trees in Species Trees." *Systematic Biology* 46 (3): 523–36. https://doi.org/10.1093/sysbio/46.3.523.

Martin, W., and Claus Schnarrenberger. 1997. "The Evolution of the Calvin Cycle from Prokaryotic to Eukaryotic Chromosomes: A Case Study of Functional Redundancy in Ancient Pathways through Endosymbiosis." *Current Genetics* 32 (1): 1–18. https://doi.org/10.1007/s002940050241.

Merola, Aldo, Rosa Castaldo, Paolo De Luca, Raffaele Gambardella, Aldo Musacchio, and Roberto Taddei. 1981. "Revision of Cyanidium Caldarium. Three Species of Acidophilic Algae." *Giornale Botanico Italiano* 115 (4–5): 189–95. https://doi.org/10.1080/11263508109428026.

Mesbah, Noha M, and Juergen Wiegel. 2011. "Halophiles Exposed Concomitantly to Multiple Stressors: Adaptive Mechanisms of Halophilic

Alkalithermophiles.” In *Halophiles and Hypersaline Environments*, edited by A Ventosa, A Oren, and Y Ma, 249–73. Springer, Berlin. https://doi.org/10.1007/978-3-642-20198-1.

Miazek, Krystian, Waldemar Iwanek, Claire Remacle, Aurore Richel, and Dorothee Goffin. 2015. “Effect of Metals, Metalloids and Metallic Nanoparticles on Microalgae Growth and Industrial Product Biosynthesis: A Review.” *International Journal of Molecular Sciences* 16 (10): 23929–69. https://doi.org/10.3390/ijms161023929.

Michard, Annie. 1989. “Rare Earth Element Systematics in Hydrothermal Fluids.” *Geochimica et Cosmochimica Acta* 53 (3): 745–50. https://doi.org/10.1016/0016-7037(89)90017-3.

Minh, Bui Quang, Matthew W. Hahn, and Robert Lanfear. 2020. “New Methods to Calculate Concordance Factors for Phylogenomic Datasets.” *Molecular Biology and Evolution* 37 (9): 2727–33. https://doi.org/10.1093/molbev/msaa106.

Minh, Bui Quang, Minh Anh Thi Nguyen, and Arndt Von Haeseler. 2013. “Ultrafast Approximation for Phylogenetic Bootstrap.” *Molecular Biology and Evolution* 30 (5): 1188–95. https://doi.org/10.1093/molbev/mst024.

Minoda, Ayumi, Hitomi Sawada, Sonoe Suzuki, Shin ichi Miyashita, Kazumi Inagaki, Takaiku Yamamoto, and Mikio Tsuzuki. 2015. “Recovery of Rare Earth Elements from the Sulfothermophilic Red Alga Galdieria Sulphuraria Using Aqueous Acid.” *Applied Microbiology and Biotechnology* 99 (3): 1513–19. https://doi.org/10.1007/s00253-014-6070-3.

Misumi, Osami, Takayuki Sakajiri, Syunsuke Hirooka, Haruko Kuroiwa, and Tsuneyoshi Kuroiwa. 2008. “Cytological Studies of Metal Ion Tolerance in the Red Algae Cyanidioschyzon Merolae.” *Cytologia* 73 (4): 437–43. https://doi.org/10.1508/cytologia.73.437.

Mondo, Angelo Del, Manuela Iovinella, Milena Petriccione, Angelina Nunziata, Seth J. Davis, Diana Cioppa, and Claudia Ciniglia. 2019. “A Spotlight on Rad52 in Cyanidiophytina (Rhodophyta): A Relic in Algal Heritage.” *Plants* 8 (2): 1–26. https://doi.org/10.3390/PLANTS8020046.

Monteiro, Cristina M., Susana C. Fonseca, Paula M.L. Castro, and F. Xavier Malcata. 2011. “Toxicity of Cadmium and Zinc on Two Microalgae, Scenedesmus Obliquus and Desmodesmus Pleiomorphus, from Northern Portugal.” *Journal of Applied Phycology* 23 (1): 97–103. https://doi.org/10.1007/s10811-010-9542-6.

Monteiro, Cristina M, Paula ML Castro, and Xavier F Malcata. 2011. “Capacity of Simultaneous Removal of Zinc and Cadmium from Contaminated Media, by Two Microalgae Isolated from a Polluted Site.” *Environmental Chemistry Letters* 9 (4): 511–17. https://doi.org/10.1007/s10311-011-0311-9.

Naveed, Sadiq, Chonghua Li, Jinyu Zhang, Chunhua Zhang, and Ying Ge. 2020. “Sorption and Transformation of Arsenic by Extracellular Polymeric Substances Extracted from Synechocystis Sp. PCC6803.” *Ecotoxicology and Environmental Safety* 206 (April): 111200. https://doi.org/10.1016/j.ecoenv.2020.111200.

Nevo, Yaniv, and Nathan Nelson. 2006. “The NRAMP Family of Metal-Ion Transporters.” *Biochimica et Biophysica Acta - Molecular Cell Research* 1763 (7): 609–20. https://doi.org/10.1016/j.bbamcr.2006.05.007.

Nguyen, Lam Tung, Heiko A Schmidt, Arndt Von Haeseler, and Bui Quang Minh. 2015. “IQ-TREE: A Fast and Effective Stochastic Algorithm for Estimating Maximum-Likelihood Phylogenies.” *Molecular Biology and Evolution* 32 (1): 268–74. https://doi.org/10.1093/molbev/msu300.

Nugent, Paul W., Joseph A. Shaw, and Michael Vollmer. 2015. “Colors of Thermal Pools at Yellowstone National Park.” *Applied Optics* 54 (4): B128. https://doi.org/10.1364/ao.54.00b128.

Oarga, Andreea. 2009. “Life in Extreme Environments.” *Revista de Biologia e Ciências Da Terra* 9 (1): 1–10.

Olsson-Francis, Karen, Rosa De La Torre, and Charles S. Cockell. 2010. “Isolation of Novel Extreme-Tolerant Cyanobacteria from a Rock-Dwelling Microbial Community by Usine Exposure to Low Earth Orbit.” *Applied and Environmental Microbiology* 76 (7): 2115–21. https://doi.org/10.1128/AEM.02547-09.

Olsson, Sanna, Fernando Puente-Sánchez, Manuel J. Gómez, and Angeles Aguilera. 2015. “Transcriptional Response to Copper Excess and Identification of Genes Involved in Heavy Metal Tolerance in the Extremophilic Microalga Chlamydomonas Acidophila.” *Extremophiles* 19 (3): 657–72. https://doi.org/10.1007/s00792-015-0746-1.

Oren, Aharon. 2002. “Biotechnological Applications and Potentials of Halophilic Microorganisms.” In *Halophilic Microorganisms and Their Environments*, edited by Aharon Oren, 357–88. New York: Springer.

Osaki, Yuko, Tomoo Shirabe, Hiromi Nakanishi, Takayoshi Wakagi, and Etsuro Yoshimura. 2009. “Characterization of Phytochelatin Synthase Produced by the Primitive Red Alga Cyanidioschyzon Merolae.” *Metallomics* 1 (4): 353–58. https://doi.org/10.1039/b823013g.

Ozturk, Sahlan, Belma Aslim, and Zekiye Suludere. 2010. “Cadmium(II) Sequestration Characteristics by Two Isolates of Synechocystis Sp. in Terms of Exopolysaccharide (EPS) Production and Monomer Composition.” *Bioresource Technology* 101 (24): 9742–48. https://doi.org/10.1016/j.biortech.2010.07.105.

Pacheco, Pablo H., Raúl A. Gil, Soledad E. Cerutti, Patricia Smichowski, and Luis D. Martinez. 2011. “Biosorption: A New Rise for Elemental Solid Phase Extraction Methods.” *Talanta* 85 (5): 2290–2300. https://doi.org/10.1016/j.talanta.2011.08.043.

Pang, Xin, Decheng Li, and An Peng. 2002. “Application of Rare-Earth Elements in the Agriculture of China and Its Environmental Behavior in Soil.” *ESPR* 9 (2): 143–48.

Pao, Stephanie S., Ian T. Paulsen, and Milton H. Saier. 1998. “Major Facilitator Superfamily.” *Microbiology and Molecular Biology Reviews* 62 (1): 1–34.

Patro, Rob, Geet Duggal, Michael I. Love, Rafael A. Irizarry, and Carl Kingsford. 2017. “Salmon Provides Fast and Bias-Aware Quantification of Transcript Expression.” *Nature Methods* 14 (4): 417–19. https://doi.org/10.1038/nmeth.4197.

Paul, Sandip, Sumit K. Bag, Sabyasachi Das, Eric T. Harvill, and Chitra Dutta. 2008. “Molecular Signature of Hypersaline Adaptation: Insights from Genome and Proteome Composition of Halophilic Prokaryotes.” *Genome Biology* 9 (4): 1–19. https://doi.org/10.1186/GB-2008-9-4-R70/FIGURES/7.

Pinto, G, P Albertano, Claudia Ciniglia, Salvatore Cozzolino, Antonino Pollio, Hwan S. Yoon, and D. Bhattacharya. 2003. “Comparative Approaches to the Taxonomy of the Genus Galdieria Merola (Cyanidiales, Rhodophyta).” *Cryptogamie, Algol.* 24 (November 2015): 13–23. http://cat.inist.fr/?aModele=afficheN&cpsidt=14626326.

Pinto, João, Bruno Henriques, José Soares, Marcelo Costa, Mariana Dias, Elaine Fabre, Cláudia B. Lopes, Carlos Vale, José Pinheiro-Torres, and Eduarda Pereira. 2020. “A Green Method Based on Living Macroalgae for the Removal of Rare-Earth Elements from Contaminated Waters.” *Journal of Environmental Management* 263 (January). https://doi.org/10.1016/j.jenvman.2020.110376.

Pollio, Antonino, Paola Cennamo, Claudia Ciniglia, Mario De Stefano, Gabriele

Pinto, and Volker A.R. Huss. 2005. “Chlamydomonas Pitschmannii Ettl, a Little Known Species from Thermoacidic Environments.” *Protist* 156 (3): 287–302. https://doi.org/10.1016/j.protis.2005.04.004.

Prasher, S. O., M. Beaugeard, J. Hawari, P. Bera, R. M. Patel, and S. H. Kim. 2004. “Biosorption of Heavy Metals by Red Algae (Palmaria Palmata).” *Environmental Technology* 25 (10): 1097–1106. https://doi.org/10.1080/09593332508618378.

Puente-Sánchez, Fernando, Sanna Olsson, and Angeles Aguilera. 2016. “Comparative Transcriptomic Analysis of the Response of Dunaliella Acidophila (Chlorophyta) to Short-Term Cadmium and Chronic Natural Metal-Rich Water Exposures.” *Microbial Ecology* 72 (3): 595–607. https://doi.org/10.1007/s00248-016-0824-7.

Qi, B C, and C Aldrich. 2008. “Biosorption of Heavy Metals from Aqueous Solutions with Tobacco Dust.” *Bioresource Technology* 99 (13): 5595–5601. https://doi.org/10.1016/j.biortech.2007.10.042.

Qiu, Huan, Dana C. Price, Andreas P.M. Weber, Valérie Reeb, Eun Chan Yang, Jun Mo Lee, Su Yeon Kim, Hwan Su Yoon, and Debashish Bhattacharya. 2013. “Adaptation through Horizontal Gene Transfer in the Cryptoendolithic Red Alga Galdieria Phlegrea.” *Current Biology* 23 (19): R865–66. https://doi.org/10.1016/j.cub.2013.08.046.

Quevillon, E., V. Silventoinen, S. Pillai, N. Harte, N. Mulder, R. Apweiler, and R. Lopez. 2005. “InterProScan: Protein Domains Identifier.” *Nucleic Acids Research* 33 (SUPPL. 2): 116–20. https://doi.org/10.1093/nar/gki442.

Ronaghi, Mostafa, Samer Karamohamed, Bertil Pettersson, Mathias Uhlén, and Pål Nyrén. 1996. “Real-Time DNA Sequencing Using Detection of Pyrophosphate Release.” *Analytical Biochemistry* 242 (1): 84–89. https://doi.org/10.1006/ABIO.1996.0432.

Rossoni, Alessandro W., Dana C. Price, Mark Seger, Dagmar Lyska, Peter Lammers, Debashish Bhattacharya, and Andreas P.M. Weber. 2019. “The Genomes of Polyextremophilic Cyanidiales Contain 1% Horizontally Transferred Genes with Diverse Adaptive Functions.” *ELife* 8 (May). https://doi.org/10.7554/eLife.45017.

Rossoni, Alessandro W, Gerald Schönknecht, Hyun Jeong Lee, Ryan L Rupp, Samantha Flachbart, Tabea Mettler-Altmann, Andreas P.M. Weber, and Marion Eisenhut. 2019. “Cold Acclimation of the Thermoacidophilic Red Alga Galdieria Sulphuraria: Changes in Gene Expression and Involvement of Horizontally Acquired Genes." *Plant and Cell Physiology* 60 (3): 702–12. https://doi.org/10.1093/pcp/pcy240.

Rothschild, LJ, and RL Mancinelll. 2001. "Life in Extreme Environments." *Nature* 409: 1092–1101. https://doi.org/10.1007/978-1-4020-6285-8.

Ruhlman, Tracey A., Jin Zhang, John C. Blazier, Jamal S. M. Sabir, and Robert K. Jansen. 2017. "Recombination-Dependent Replication and Gene Conversion Homogenize Repeat Sequences and Diversify Plastid Genome Structure." *American Journal of Botany* 104 (4): 559–72. https://doi.org/10.3732/ajb.1600453.

Sadovsky, David, Asher Brenner, Boaz Astrachan, Boaz Asaf, and Raphael Gonen. 2016. "Biosorption Potential of Cerium Ions Using Spirulina Biomass." *Journal of Rare Earths* 34 (6): 644–52. https://doi.org/10.1016/S1002-0721(16)60074-1.

Saffary, Roya, Renu Nandakumar, Dennis Spencer, Frank T Robb, Joseph M Davila, Marvin Swartz, Leon Ofman, Roger J Thomas, and Jocelyne DiRuggiero. 2002. "Microbial Survival of Space Vacuum and Extreme Ultraviolet Irradiation: Strain Isolation and Analysis during a Rocket Flight." *FEMS Microbiology Letters* 215 (1): 163–68. https://doi.org/10.1111/J.1574-6968.2002.TB11386.X.

Saier, Milton H., Vamsee S. Reddy, Brian V. Tsu, Muhammad Saad Ahmed, Chun Li, and Gabriel Moreno-Hagelsieb. 2016. "The Transporter Classification Database (TCDB): Recent Advances." *Nucleic Acids Research* 44 (D1): D372–79. https://doi.org/10.1093/nar/gkv1103.

Schmidlin, Patrick R., Alexandre Tchouboukov, Florian J. Wegehaupt, and Franz E. Weber. 2012. "Effect of Cerium Chloride Application on Fibroblast and Osteoblast Proliferation and Differentiation." *Archives of Oral Biology* 57 (7): 892–97. https://doi.org/10.1016/j.archoralbio.2012.01.010.

Schönknecht, Gerald, Wei-hua Chen, Chad M Ternes, Guillaume G Barbier, Roshan P Shrestha, Mario Stanke, Andrea Bräutigam, et al. 2013. "Gene Transfer from Bacteria and Archaea Facilitated Evolution of an Extremophilic Eukaryote." *Science* 339: 1207–10.

Schuliger, J.W, SH Brown, JA Baross, and RM Kelly. 1993. "Purification and Characterization of a Novel Amylolytic Enzyme from ES4, a Marine Hyperthermophilic Archaeum." *Molecular Marine Biology and Biotechnology* 2: 76–87.

Seckbach, Joseph. 2017. *Algae and Cyanobacteria in Extreme Environments*.

Springer. Vol. 11.

Segerer, Andreas H, Sigfried Burggraf, Gerhard Fiala, Gertrud Huber, Robert Huber, Ursula Pley, and Karl O Stetter. 1993. “Life in Hot Springs and Hydrothermal Vents.” *Origins of Life and Evolution of the Biosphere* 23 (1): 77–90. https://doi.org/10.1007/BF01581992.

Sentsova, U. 1991. “On the Diversity of Acido-Thermophilic Unicellular Algae of the Genus Galdieria (Rhodophyta, Cyanidiophyceae).” *Botaničeskij Žurnal* 76 (1): 69–78. https://ci.nii.ac.jp/naid/10003747751/.

Simon, Dana F., Rute F. Domingos, Charles Hauser, Colin M. Hutchins, William Zerges, and Kevin J. Wilkinson. 2013. “Transcriptome Sequencing (RNA-Seq) Analysis of the Effects of Metal Nanoparticle Exposure on the Transcriptome of Chlamydomonas Reinhardtii.” *Applied and Environmental Microbiology* 79 (16): 4774–85. https://doi.org/10.1128/AEM.00998-13.

Singleton, Martin R, Lois M Wentzell, Yilun Liu, Stephen C West, and Dale B Wigley. 2002. “Structure of the Single-Strand Annealing Domain of Human RAD52 Protein.” *Proceedings of the National Academy of Sciences* 99 (21): 13492–97. https://doi.org/10.1073/pnas.212449899.

Sirakov, Maria, Maria Palmieri, Manuela Iovinella, Seth J Davis, Milena Petriccione, Maria Rosa, Di Cicco, Mario De Stefano, and Claudia Ciniglia. 2021. “Cyanidiophyceae (Rhodophyta) Tolerance to Precious Metals : Metabolic Response to Cl 4 K 2 Pd and AuCl 4 K.” *Plants*, 1–8.

Skorupa, D. J., V. Reeb, R. W. Castenholz, D. Bhattacharya, and T. R. Mcdermott. 2013. “Cyanidiales Diversity in Yellowstone National Park.” *Letters in Applied Microbiology* 57 (5): 459–66. https://doi.org/10.1111/lam.12135.

Som, Anup. 2014. “Causes, Consequences and Solutions of Phylogenetic Incongruence.” *Briefings in Bioinformatics* 16 (3): 536–48. https://doi.org/10.1093/bib/bbu015.

Stetter, K. O., G. Fiala, R. Huber, G. Huber, and A. Segerer. 1986. “Life above the Boiling Point of Water?” *Experientia* 42 (11–12): 1187–91. https://doi.org/10.1007/BF01946389.

Tang, Qiong Ying, Si Qing Liu, Dan Yu, Huan Zhang Liu, and Patrick D Danley. 2012. “Mitochondrial Capture and Incomplete Lineage Sorting in the Diversification of Balitorine Loaches (Cypriniformes, Balitoridae) Revealed by Mitochondrial and Nuclear Genes.” *Zoologica Scripta* 41 (3): 233–47. https://doi.org/10.1111/j.1463-6409.2011.00530.x.

Tang, XK, and Z Tong. 1988. “Effects of Rare Earth Elements on Plant Root Growth and Activity.” *Chinese Rare Metal* 5: 22–24.

Tillich, Michael, Pascal Lehwark, Tommaso Pellizzer, Elena S Ulbricht-Jones, Axel Fischer, Ralph Bock, and Stephan Greiner. 2017. “GeSeq - Versatile and Accurate Annotation of Organelle Genomes.” *Nucleic Acids Research* 45 (W1): W6–11. https://doi.org/10.1093/nar/gkx391.

Toplin, J. A., T. B. Norris, C. R. Lehr, T. R. McDermott, and R. W. Castenholz. 2008. “Biogeographic and Phylogenetic Diversity of Thermoacidophilic Cyanidiales in Yellowstone National Park, Japan, and New Zealand.” *Applied and Environmental Microbiology* 74 (9): 2822–33. https://doi.org/10.1128/AEM.02741-07.

Torsvik, Vigdis, and Lise Øvreås. 2008. “Microbial Diversity, Life Strategies, and Adaptation to Life in Extreme Soils.” In *Microbiology of Extreme Soils*, edited by P Dion and CS Nautiyal, 15–43. Berlin: Springer.

Tsuruta, Takehiko. 2007. “Accumulation of Rare Earth Elements in Various Microorganisms.” *Journal of Rare Earths* 25 (5): 526–32. https://doi.org/10.1016/S1002-0721(07)60556-0.

Vastermark, Ake, Simon Wollwage, Michael E. Houle, Rita Rio, and Milton H. Saier. 2014. “Expansion of the APC Superfamily of Secondary Carriers.” *Proteins: Structure, Function and Bioinformatics* 82 (10): 2797–2811. https://doi.org/10.1002/prot.24643.

Wang, Likun, Zhixing Feng, Xi Wang, Xiaowo Wang, and Xuegong Zhang. 2009. “DEGseq: An R Package for Identifying Differentially Expressed Genes from RNA-Seq Data.” *Bioinformatics* 26 (1): 136–38. https://doi.org/10.1093/bioinformatics/btp612.

Wegehaupt, Florian J., Beatrice Sener, Thomas Attin, and Patrick R. Schmidlin. 2010. “Application of Cerium Chloride to Improve the Acid Resistance of Dentine.” *Archives of Oral Biology* 55 (6): 441–46. https://doi.org/10.1016/j.archoralbio.2010.03.016.

Wharton, D A, and A T Marshall. 2002. “Changes in Surface Features during Desiccation of the Anhydrobiotic Plant Parasitic Nematode Ditylenchus Dipsaci.” *Tissue and Cell* 34 (2): 81–87. https://doi.org/10.1016/S0040-8166(02)00011-3.

Xiao, Rui, and Yi Zheng. 2016. “Overview of Microalgal Extracellular Polymeric Substances (EPS) and Their Applications.” *Biotechnology Advances* 34 (7): 1225–44. https://doi.org/10.1016/j.biotechadv.2016.08.004.

Xie, Qiting, Na Liu, Daohui Lin, Ruohua Qu, Qiongzhi Zhou, and Fei Ge. 2020. “The Complexation with Proteins in Extracellular Polymeric Substances Alleviates the Toxicity of Cd (II) to Chlorella Vulgaris.” *Environmental Pollution* 263: 114102. https://doi.org/10.1016/j.envpol.2020.114102.

Yan, Nieng. 2013. “Structural Advances for the Major Facilitator Superfamily (MFS) Transporters.” *Trends in Biochemical Sciences* 38 (3): 151–59. https://doi.org/10.1016/j.tibs.2013.01.003.

Yang, Eun Chan, Kyeong Mi Kim, Su Yeon Kim, Jung Mo Lee, Ga Hun Boo, Jung Hyun Lee, Wendy A Nelson, et al. 2015. “Highly Conserved Mitochondrial Genomes among Multicellular Red Algae of the Florideophyceae.” *Genome Biology and Evolution* 7 (8): 2394–2406. https://doi.org/10.1093/gbe/evv147.

Yoon, Hwan Su, Claudia Ciniglia, Min Wu, Josep M. Comeron, Gabriele Pinto, Antonino Pollio, and Debashish Bhattacharya. 2006. “Establishment of Endolithic Populations of Extremophilic Cyanidiales (Rhodophyta).” *BMC Evolutionary Biology* 6: 78. https://doi.org/10.1186/1471-2148-6-78.

Yoon, Hwan Su, Jeremiah D. Hackett, Claudia Ciniglia, Gabriele Pinto, and Debashish Bhattacharya. 2004. “A Molecular Timeline for the Origin of Photosynthetic Eukaryotes.” *Molecular Biology and Evolution* 21 (5): 809–18. https://doi.org/10.1093/molbev/msh075.

Yuguan, Ze, Zhou Min, Luo Luyang, Ji Zhe, Liu Chao, Yin Sitao, Duan Yanmei, Li Na, and Hong Fashui. 2009. “Effects of Cerium on Key Enzymes of Carbon Assimilation of Spinach under Magnesium Deficiency.” *Biological Trace Element Research* 131 (2): 154–64. https://doi.org/10.1007/s12011-009-8354-5.

Zeraatkar, Amin Keyvan, Hossein Ahmadzadeh, Ahmad Farhad Talebi, Navid R. Moheimani, and Mark P. McHenry. 2016. “Potential Use of Algae for Heavy Metal Bioremediation, a Critical Review.” *Journal of Environmental Management* 181: 817–31. https://doi.org/10.1016/j.jenvman.2016.06.059.

Zhao, Haiquan, Qiuping Zhou, Min Zhou, Chunxiao Li, Xiaolan Gong, Chao Liu, Chunxiang Qu, Ling Wang, Wenhui Si, and Fashui Hong. 2012. “Magnesium Deficiency Results in Damage of Nitrogen and Carbon Cross-Talk of Maize and Improvement by Cerium Addition.” *Biological Trace Element Research* 148 (1): 102–9. https://doi.org/10.1007/s12011-012-9340-x.

Zhao, Jinfeng, Shixiang Liu, Na Liu, Han Zhang, Qiongzhi Zhou, and Fei Ge.

2019. “Accelerated Productions and Physicochemical Characterizations of Different Extracellular Polymeric Substances from Chlorella Vulgaris with Nano-ZnO.” *Science of the Total Environment* 658: 582–89. https://doi.org/10.1016/j.scitotenv.2018.12.019.

Zhou, Min, Xiaolan Gong, Wang Ying, Liu Chao, Mengmeng Hong, Ling Wang, and Hong Fashui. 2011. “Cerium Relieves the Inhibition of Chlorophyll Biosynthesis of Maize Caused by Magnesium Deficiency.” *Biological Trace Element Research* 143 (1): 468–77. https://doi.org/10.1007/s12011-010-8830-y.

Zierenberg, Robert A, Michael W Adams, and Alissa J Arp. 2000. “Life in Extreme Environments: Hydrothermal Vents.” *Proceedings of the National Academy of Sciences USA* 97 (24): 12961–62.

Abe, Fumiyoshi, and Koki Horikoshi. 2001. “The Biotechnological Potential of Piezophiles.” *Trends in Biotechnology* 19 (3): 102–8. https://doi.org/10.1016/S0167-7799(00)01539-0.

Albertano, P., C. Ciniglia, G. Pinto, and A. Pollio. 2000. “The Taxonomic Position of Cyanidium, Cyanidioschyzon and Galdieria: An Update.” *Hydrobiologia* 433: 137–43. https://doi.org/10.1023/A:1004031123806.

Albertano, P., G. Pinto, and A. Pollio. 1994. “Ecophysiology and Ultrastructure of an Acidophilic Species of Ochromonas (Chrysophyceae, Ochromonadales).” *Archiv Für Protistenkunde* 144 (1): 75–82. https://doi.org/10.1016/S0003-9365(11)80226-5.

Albertano, P, G Pinto, A Pollio, and R Taddei. 1990. “Morphology, Ultrastructure and Ecology of an Acidophilic Alga Pseudococcomyxa Simplex (Mainx) Fott (Chlorococcales).” *Arch. Hydrobiol., Algol. Stud.* 37: 401–8.

Albertano, P, G Pinto, S Santisi, and R Taddei. 1981. “Spermatozopsis Acidophila Kalina (Chlorophyta, Volvocales), a Little Known Alga from Highly Acidic Environments.” *Giorn. Bot. Ital.* 115: 65–76.

Albertano, P, A Pollio, and R Taddei. 1991. “Viridiella Fridericiana (Chlorococcales, Chlorophyta), a New Species Isolated from Extremely Acid Environments.” *Phycologia* 30 (4): 346–54.

Allen, M. M., and R. Y. Stanier. 1968. “Selective Isolation of Blue-Green Algae from Water and Soil.” *Journal of General Microbiology* 51 (2): 203–9. https://doi.org/10.1099/00221287-51-2-203.

Allen, Mary Belle. 1959. “Studies with Cyanidium Caldarium, an Anomalously

Pigmented Chlorophyte.” *Archiv Für Mikrobiologie* 32 (3): 270–77. https://doi.org/10.1007/BF00409348.

Altschul, Stephen F., Warren Gish, Webb Miller, Eugene W. Myers, and David J. Lipman. 1990. “Basic Local Alignment Search Tool.” *Journal of Molecular Biology* 215 (3): 403–10. https://doi.org/10.1016/S0022-2836(05)80360-2.

Anisimova, Maria, Manuel Gil, Jean Franois Dufayard, Christophe Dessimoz, and Olivier Gascuel. 2011. “Survey of Branch Support Methods Demonstrates Accuracy, Power, and Robustness of Fast Likelihood-Based Approximation Schemes.” *Systematic Biology* 60 (5): 685–99. https://doi.org/10.1093/sysbio/syr041.

Bailey, R W, and L. A. Staehelin. 1968. “The Chemical Composition of Isolated Cell Walls of Cyanidium Caldarium.” *Journal of General Microbiology* 54 (2): 269–76. https://doi.org/10.1099/00221287-54-2-269.

Baker-Austin, Craig, and Mark Dopson. 2007. “Life in Acid: PH Homeostasis in Acidophiles.” *Trends in Microbiology* 15 (4): 165–71. https://doi.org/10.1016/j.tim.2007.02.005.

Bankevich, Anton, Sergey Nurk, Dmitry Antipov, Alexey A. Gurevich, Mikhail Dvorkin, Alexander S. Kulikov, Valery M. Lesin, et al. 2012. “SPAdes: A New Genome Assembly Algorithm and Its Applications to Single-Cell Sequencing.” *Journal of Computational Biology* 19 (5): 455–77. https://doi.org/10.1089/cmb.2012.0021.

Binnemans, Koen, Peter Tom Jones, Bart Blanpain, Tom Van Gerven, Yongxiang Yang, Allan Walton, and Matthias Buchert. 2013. “Recycling of Rare Earths: A Critical Review.” *Journal of Cleaner Production* 51: 1–22. https://doi.org/10.1016/j.jclepro.2012.12.037.

Birungi, Z. S., and E. M.N. Chirwa. 2014. “The Kinetics of Uptake and Recovery of Lanthanum Using Freshwater Algae as Biosorbents: Comparative Analysis.” *Bioresource Technology* 160: 43–51. https://doi.org/10.1016/j.biortech.2014.01.033.

Birungi, Z. S., E. M.N. Chirwa, and O. J. Botai. 2017. “Competitive Adsorption in a Ternary System of Toxic Metals and Rare Earth Elements Using Desmodesmus Multivariabilis: Empirical and Kinetic Modelling.” *Journal of Applied Phycology* 29 (6): 2899–2910. https://doi.org/10.1007/s10811-017-1197-0.

Bolger, Anthony M, Marc Lohse, and Bjoern Usadel. 2014. “Genome Analysis Trimmomatic: A Flexible Trimmer for Illumina Sequence Data.”

BIOINFORMATICS 30 (15): 2114–20. https://doi.org/10.1093/bioinformatics/btu170.

Bourcier, W. L., M. Lin, and G. Nix. 2005. “Recovery of Minerals and Metals from Geothermal Fluids.” *2003 SME Annual Meeting*, 19.

Brady, Joseph M., and John M. Tobin. 1995. “Binding of Hard and Soft Metal Ions to Rhizopus Arrhizus Biomass.” *Enzyme and Microbial Technology* 17 (9): 791–96. https://doi.org/10.1016/0141-0229(95)00142-R.

Brock, T. D. 1978. “The Genus Cyanidium.” In *Thermophilic Microorganisms and Life at High Temperatures*, 255–302.

Bronstein, Omri, Andreas Kroh, and Elisabeth Haring. 2018. “Mind the Gap! The Mitochondrial Control Region and Its Power as a Phylogenetic Marker in Echinoids.” *BMC Evolutionary Biology* 18 (1). https://doi.org/10.1186/s12862-018-1198-x.

Bruen, Trevor C, Hervé Philippe, and David Bryant. 2006. “A Simple and Robust Statistical Test for Detecting the Presence of Recombination.” *Genetics* 172 (4): 2665–81. https://doi.org/10.1534/genetics.105.048975.

Bull, Alan T., and Michael Goodfellow. 2019. “Dark, Rare and Inspirational Microbial Matter in the Extremobiosphere: 16 000 m of Bioprospecting Campaigns.” *Microbiology (United Kingdom)* 165 (12): 1252–64. https://doi.org/10.1099/mic.0.000822.

Camacho, Christiam, George Coulouris, Vahram Avagyan, Ning Ma, Jason Papadopoulos, Kevin Bealer, and Thomas L Madden. 2009. “BLAST+: Architecture and Applications.” *BMC Bioinformatics* 10 (1): 421. https://doi.org/10.1186/1471-2105-10-421.

Cao, Ying, Penghui Shao, Yidi Chen, Xiaoyu Zhou, Liming Yang, Hui Shi, Kai Yu, Xianxin Luo, and Xubiao Luo. 2021. “A Critical Review of the Recovery of Rare Earth Elements from Wastewater by Algae for Resources Recycling Technologies.” *Resources, Conservation and Recycling* 169 (November 2020): 105519. https://doi.org/10.1016/j.resconrec.2021.105519.

Casadei, M. A., P. Mañas, G. Niven, E. Needs, and B. M. Mackey. 2002. “Role of Membrane Fluidity in Pressure Resistance of Escherichia Coli NCTC 8164.” *Applied and Environmental Microbiology* 68 (12): 5965–72. https://doi.org/10.1128/AEM.68.12.5965-5972.2002.

Cassano, Valeria, Mariana C. Oliveira, María Candelaria Gil-Rodríguez, Abel Sentíes, Jhoana Díaz-Larrea, and Mutue T. Fujii. 2012. “Molecular Support for the Establishment of the New Genus Laurenciella within the Laurencia

Complex (Ceramiales, Rhodophyta).” *Botanica Marina* 55 (4): 349–57. https://doi.org/10.1515/bot-2012-0133.

Castor, S, and J Hedrick. 2006. “Rare Earth Elements.” In *Society for Mining, Metallurgy, and Exploration: Littleton, Colorado*, 769–92.

Castresana, J. 2000. “Selection of Conserved Blocks from Multiple Alignments for Their Use in Phylogenetic Analysis.” *Molecular Biology and Evolution* 17 (4): 540–52. https://doi.org/10.1093/oxfordjournals.molbev.a026334.

Chao, Liu, Pan Bofu, Cao Weiqian, Lu Yun, Huang Hao, Chen Liang, Liu Xiaoqing, Wu Xiao, and Hong Fashui. 2008. “Influences of Calcium Deficiency and Cerium on Growth of Spinach Plants.” *Biological Trace Element Research* 121 (3): 266–75. https://doi.org/10.1007/s12011-007-8054-y.

Chen, Can, and Jianlong Wang. 2007. “Influence of Metal Ionic Characteristics on Their Biosorption Capacity by Saccharomyces Cerevisiae.” *Applied Microbiology and Biotechnology* 74 (4): 911–17. https://doi.org/10.1007/s00253-006-0739-1.

Cho, Chung Hyun, Seung In Park, Claudia Ciniglia, Eun Chan Yang, Louis Graf, Debashish Bhattacharya, and Hwan Su Yoon. 2020. “Potential Causes and Consequences of Rapid Mitochondrial Genome Evolution in Thermoacidophilic Galdieria (Rhodophyta).” *BMC Evolutionary Biology* 20 (112): 1–15. https://doi.org/10.21203/rs.3.rs-36820/v1.

Cho, Yao Chang Lee, Liang Ching Hsu, Chun Chieh Wang, Pin Chen Chen, Shao Lun Liu, Heng Yi Teah, Yu Ting Liu, and Yu Min Tzou. 2020. “Molecular Mechanisms for Pb Removal by Cyanidiales: A Potential Biomaterial Applied in Thermo-Acidic Conditions.” *Chemical Engineering Journal* 401 (December): 125828. https://doi.org/10.1016/j.cej.2020.125828.

Chong, K. H., and B. Volesky. 1995. “Description of Two-metal Biosorption Equilibria by Langmuir-type Models.” *Biotechnology and Bioengineering* 47 (4): 451–60. https://doi.org/10.1002/bit.260470406.

Chu, ZX, MH Mu, FZ Wang, and HX Shao. 1996. “Effect of CeCl3 on the Photosynthesis of Plants Relation to Fe2+.” In *Environmental Behavior and Ecotoxicology of Rare Earth Elements.*, 75–81.

Ciniglia, P Cennamo, A De Natale, M De Stefano, M Sirakov, M Iovinella, HS Yoon, and A Pollio. 2019. “Cyanidium Chilense (Cyanidiophyceae, Rhodophyta) from Tuff Rocks of the Archeological Site of Cuma, Italy.” *Phycological Research*, no. November 2018.

https://doi.org/10.1111/pre.12383.

Ciniglia, Paola Cennamo, Mario De Stefano, Gabriele Pinto, Paolo Caputo, and Antonino Pollio. 2007. “Pinnularia Obscura Krasske (Bacillariophyceae, Bacillariophyta) from Acidic Environments: Characterization and Comparison with Other Acid-Tolerant Pinnularia Species.” *Fundamental and Applied Limnology* 170 (1): 29–47. https://doi.org/10.1127/1863-9135/2007/0170-0029.

Ciniglia, Claudia, Eun Chan Yang, Antonino Pollio, Gabriele Pinto, Manuela Iovinella, Laura Vitale, and Hwan Su Yoon. 2014. “Cyanidiophyceae in Iceland: Plastid Rbc L Gene Elucidates Origin and Dispersal of Extremophilic Galdieria Sulphuraria and G. Maxima (Galdieriaceae, Rhodophyta).” *Phycologia* 53 (6): 542–51. https://doi.org/10.2216/14-032.1.

Ciniglia, Claudia, Hwan Su Yoon, Antonino Pollio, Gabriele Pinto, and Debashish Bhattacharya. 2004. “Hidden Biodiversity of the Extremophilic Cyanidiales Red Algae.” *Molecular Ecology* 13 (7): 1827–38. https://doi.org/10.1111/j.1365-294X.2004.02180.x.

Čížková, M, K Bišová, V Zachleder, D Mezricky, M Rucki, and M Vítová. 2019. “Recovery of Rare Earth Elements from Luminophores Using the Red Alga Galdieria,” no. September: 2018–19. http://www.acuf.net/index.php?lang=en.

Čížková, Mária, Pauline Mezricky, Dana Mezricky, Marian Rucki, Vilém Zachleder, and Milada Vítová. 2021. “Bioaccumulation of Rare Earth Elements from Waste Luminophores in the Red Algae, Galdieria Phlegrea.” *Waste and Biomass Valorization* 12 (6): 3137–46. https://doi.org/10.1007/s12649-020-01182-3.

Clarke, Andrew. 2014. “The Thermal Limits to Life on Earth.” *International Journal of Astrobiology* 13 (2): 141–54. https://doi.org/10.1017/S1473550413000438.

Comte, S., G. Guibaud, and M. Baudu. 2008. “Biosorption Properties of Extracellular Polymeric Substances (EPS) towards Cd, Cu and Pb for Different PH Values.” *Journal of Hazardous Materials* 151 (1): 185–93. https://doi.org/10.1016/j.jhazmat.2007.05.070.

Corguillé, Gildas Le, Gareth Pearson, Marta Valente, Carla Viegas, Bernhard Gschloessl, Erwan Corre, Xavier Bailly, et al. 2009. “Plastid Genomes of Two Brown Algae, Ectocarpus Siliculosus and Fucus Vesiculosus: Further Insights on the Evolution of Red-Algal Derived Plastids.” *BMC Evolutionary*

Biology 9 (1): 253. https://doi.org/10.1186/1471-2148-9-253.

Crognale, Simona, Stefania Venturi, Franco Tassi, Simona Rossetti, Heba Rashed, Jacopo Cabassi, Francesco Capecchiacci, et al. 2018. “Microbiome Profiling in Extremely Acidic Soils Affected by Hydrothermal Fluids: The Case of the Solfatara Crater (Campi Flegrei, Southern Italy).” *FEMS Microbiology Ecology* 94 (12). https://doi.org/10.1093/femsec/fiy190.

D’Alelio, Domenico, and Maria Valeria Ruggiero. 2015. “Interspecific Plastidial Recombination in the Diatom Genus Pseudo-Nitzschia.” *Journal of Phycology* 51 (6): 1024–28. https://doi.org/10.1111/jpy.12350.

D’Alelio, Domenico, Nico Salmaso, and Andrea Gandolfi. 2013. “Frequent Recombination Shapes the Epidemic Population Structure of Planktothrix (Cyanoprokaryota) in Italian Subalpine Lakes.” *Journal of Phycology* 49 (6): 1107–17. https://doi.org/10.1111/jpy.12116.

Dahle, Jessica T., and Yuji Arai. 2015. “Environmental Geochemistry of Cerium: Applications and Toxicology of Cerium Oxide Nanoparticles.” *International Journal of Environmental Research and Public Health* 12 (2): 1253–78. https://doi.org/10.3390/ijerph120201253.

Daly, Michael J., Elena K. Gaidamakova, Vera Y. Matrosova, Juliann G. Kiang, Risaku Fukumoto, Duck Yeon Lee, Nancy B. Wehr, Gabriela A. Viteri, Barbara S. Berlett, and Rodney L. Levine. 2010. “Small-Molecule Antioxidant Proteome-Shields in Deinococcus Radiodurans.” *PLOS ONE* 5 (9): e12570. https://doi.org/10.1371/JOURNAL.PONE.0012570.

Daly, Michael J., Elena K. Gaidamakova, Vera Y. Matrosova, Alexander Vasilenko, Min Zhai, Richard D. Leapman, Barry Lai, et al. 2007. “Protein Oxidation Implicated as the Primary Determinant of Bacterial Radioresistance.” *PLOS Biology* 5 (4): e92. https://doi.org/10.1371/JOURNAL.PBIO.0050092.

Darling, Aaron E, Bob Mau, and Nicole T Perna. 2010. “Progressivemauve: Multiple Genome Alignment with Gene Gain, Loss and Rearrangement.” *PLoS ONE* 5 (6). https://doi.org/10.1371/journal.pone.0011147.

Das, Nilanjana. 2010. “Recovery of Precious Metals through Biosorption - A Review.” *Hydrometallurgy* 103 (1–4): 180–89. https://doi.org/10.1016/j.hydromet.2010.03.016.

Dev, Subhabrata, Ankur Sachan, Fahimeh Dehghani, Tathagata Ghosh, Brandon R. Briggs, and Srijan Aggarwal. 2020. “Mechanisms of Biological Recovery of Rare-Earth Elements from Industrial and Electronic Wastes: A

Review." *Chemical Engineering Journal* 397 (February): 124596. https://doi.org/10.1016/j.cej.2020.124596.

Dhakar, Kusum, and Anita Pandey. 2016. "Wide PH Range Tolerance in Extremophiles: Towards Understanding an Important Phenomenon for Future Biotechnology." *Applied Microbiology and Biotechnology* 100 (6): 2499–2510. https://doi.org/10.1007/s00253-016-7285-2.

Dhankhar, Rajesh, and Anju Hooda. 2011. "Fungal Biosorption-an Alternative to Meet the Challenges of Heavy Metal Pollution in Aqueous Solutions." *Environmental Technology* 32 (5): 467–91. https://doi.org/10.1080/09593330.2011.572922.

Dose, K, A Bieger-Dose, M Labusch, and M Gill. 1992. "Survival in Extreme Dryness and DNA-Single-Strand Breaks." *Advances in Space Research* 12 (4): 221–29. https://doi.org/10.1006/enrs.1994.1018.

Doyle, Jeff J. 1992. "Gene Trees and Species Trees : Molecular Systematics as One-Character Taxonomy." *Systematic Botany* 17 (1): 144–63.

Elster, Josef. 1999. "Enigmatic Microorganisms and Life in Extreme Environments." In *Origin - Evolution and Versatility of Microorganisms (Phylogeny, Structure, Physiology and Extreme Environments*, edited by Joseph Seckbach. Kluwer Academic Publishers. https://doi.org/10.1007/978-94-011-4838-2.

Farcasanu, I. C., M. Mizunuma, D. Hirata, and T. Miyakawa. 1998. "Involvement of Histidine Permease (Hip1p) in Manganese Transport in Saccharomyces Cerevisiae." *Molecular and General Genetics* 259 (5): 541–48. https://doi.org/10.1007/s004380050846.

Farooq, Umar, Janusz A. Kozinski, Misbahul Ain Khan, and Makshoof Athar. 2010. "Biosorption of Heavy Metal Ions Using Wheat Based Biosorbents - A Review of the Recent Literature." *Bioresource Technology* 101 (14): 5043–53. https://doi.org/10.1016/j.biortech.2010.02.030.

Ferreira, J. A., and S. O. Nyangoma. 2008. "A Multivariate Version of the Benjamini-Hochberg Method." *Journal of Multivariate Analysis* 99 (9): 2108–24. https://doi.org/10.1016/j.jmva.2008.02.013.

Fonseca, Miguel M., D. James Harris, and David Posada. 2014. "The Inversion of the Control Region in Three Mitogenomes Provides Further Evidence for an Asymmetric Model of Vertebrate MtDNA Replication." Edited by Dan Mishmar. *PLoS ONE* 9 (9): e106654. https://doi.org/10.1371/journal.pone.0106654.

Franklin, Natasha M, Jennifer L Stauber, Richard P Lim, and P Petocz. 2002. “Toxicity of Metal Mixtures to a Tropical Freshwater Alga (Chlorella Sp.): The Effect of Interactions between Copper, Cadmium, and Zinc on Metal Cell Binding and Uptake.” *Environmental Toxicology and Chemistry* 21 (11): 2412–22.

Fukuda, Shin ya, Rie Yamamoto, Koji Iwamoto, and Ayumi Minoda. 2018. “Cellular Accumulation of Cesium in the Unicellular Red Alga Galdieria Sulphuraria under Mixotrophic Conditions.” *Journal of Applied Phycology* 30 (6): 3057–61. https://doi.org/10.1007/s10811-018-1525-z.

Gallup, Darrell L. 1998. “Geochemistry of Geothermal Fluids and Well Scales, and Potential for Mineral Recovery.” *Ore Geology Reviews* 12 (4): 225–36. https://doi.org/10.1016/S0169-1368(98)00004-3.

Galtier, Nicolas, and Vincent Daubin. 2008. “Dealing with Incongruence in Phylogenomic Analyses.” *Philosophical Transactions of the Royal Society B: Biological Sciences*. Royal Society. https://doi.org/10.1098/rstb.2008.0144.

Gitzendanner, Matthew A., Pamela S. Soltis, Gane K.S. Wong, Brad R. Ruhfel, and Douglas E. Soltis. 2018. “Plastid Phylogenomic Analysis of Green Plants: A Billion Years of Evolutionary History.” *American Journal of Botany* 105 (3): 291–301. https://doi.org/10.1002/ajb2.1048.

Goecke, Franz, Milada Vítová, Jaromír Lukavský, Linda Nedbalová, Tomáš Řezanka, and Vilém Zachleder. 2017. “Effects of Rare Earth Elements on Growth Rate, Lipids, Fatty Acids and Pigments in Microalgae.” *Phycological Research* 65 (3): 226–34. https://doi.org/10.1111/pre.12180.

Gonçalves, Deise J.P., Beryl B. Simpson, Edgardo M. Ortiz, Gustavo H. Shimizu, and Robert K. Jansen. 2019. “Incongruence between Gene Trees and Species Trees and Phylogenetic Signal Variation in Plastid Genes.” *Molecular Phylogenetics and Evolution* 138 (September): 219–32. https://doi.org/10.1016/j.ympev.2019.05.022.

Graedel, T. E., Julian Allwood, Jean Pierre Birat, Matthias Buchert, Christian Hagelüken, Barbara K. Reck, Scott F. Sibley, and Guido Sonnemann. 2011. “What Do We Know about Metal Recycling Rates?” *Journal of Industrial Ecology* 15 (3): 355–66. https://doi.org/10.1111/j.1530-9290.2011.00342.x.

Gromov, B.V., O.V. Gavrilova, and A.V. Pljusch. 1990. “Ultrastructure of Ochromonas Globosa (Chrysophyceae).” *Archiv Für Protistenkunde* 138 (4): 291–97.

Gross, W., and Christine Oesterhelt. 1999. “Ecophysiological Studies on the Red Alga Galdieria Sulphuraria Isolated from Southwest Iceland.” *Plant Biology* 1 (6): 694–700. https://doi.org/10.1111/j.1438-8677.1999.tb00282.x.

Haug, Arne, and Olav Smidsrod. 1970. “Selectivity of Some Anionic Polymers for Divalent Metal Ions.” *Acta Chemica Scandinavia*, 843–54.

Herrmann, Johannes M. 2003. “Converting Bacteria to Organelles: Evolution of Mitochondrial Protein Sorting.” *Trends in Microbiology* 11 (2): 74–79. https://doi.org/10.1016/S0966-842X(02)00033-1.

Hsieh, Chia Jung, Shing Hei Zhan, Yiching Lin, Sen Lin Tang, and Shao Lun Liu. 2015. “Analysis of RbcL Sequences Reveals the Global Biodiversity, Community Structure, and Biogeographical Pattern of Thermoacidophilic Red Algae (Cyanidiales).” *Journal of Phycology* 51: 682–94. https://doi.org/10.1111/jpy.12310.

Huss, Volker A R, Claudia Ciniglia, Paola Cennamo, Salvatore Cozzolino, Gabriele Pinto, and Antonino Pollio. 2002. “Phylogenetic Relationships and Taxonomic Position of Chlorella-like Isolates from Low PH Environments (PH < 3.0).” *BMC Evolutionary Biology* 9: 13.

Iovinella, Manuela, Dora Allegra Carbone, Diana Cioppa, Seth J. Davis, Michele Innangi, Sabrina Esposito, and Claudia Ciniglia. 2020. “Prevalent PH Controls the Capacity of Galdieria Maxima to Use Ammonia and Nitrate as a Nitrogen Source.” *Plants* 9 (2): 232. https://doi.org/10.3390/plants9020232.

Iovinella, Manuela, Ayla Eren, Gabriele Pinto, Antonino Pollio, Seth J Davis, Paola Cennamo, and Claudia Ciniglia. 2018. “Cryptic Dispersal of Cyanidiophytina (Rhodophyta) in Non-Acidic Environments from Turkey.” *Extremophiles* 22: 713–23. https://doi.org/10.1007/s00792-018-1031-x.

Iovinella, Manuela, Francesco Lombardo, Claudia Ciniglia, Maria Palmieri, Maria Rosa di Cicco, Marco Trifuoggi, Marco Race, et al. 2022. “Bioremoval of Yttrium (III), Cerium (III), Europium (III), and Terbium (III) from Single and Quaternary Aqueous Solutions Using the Extremophile Galdieria Sulphuraria (Galdieriaceae, Rhodophyta).” *Plants* 11 (10): 1376. https://doi.org/10.3390/plants11101376.

Jacinto, Jéssica, Bruno Henriques, A. C. Duarte, Carlos Vale, and E. Pereira. 2018. “Removal and Recovery of Critical Rare Elements from Contaminated Waters by Living Gracilaria Gracilis.” *Journal of Hazardous Materials* 344 (February): 531–38. https://doi.org/10.1016/j.jhazmat.2017.10.054.

Jack, D. L., I. T. Paulsen, and Jr Saier. 2000. “The Amino Acid/Polyamine/Organocation (APC) Superfamily of Transporters Specific for Amino Acids, Polyamines and Organocations.” *Microbiology* 146 (8): 1797–1814. https://doi.org/10.1099/00221287-146-8-1797.

Jain, Kanika, Kirsten Krause, Felix Grewe, Gaven F. Nelson, Andreas P.M. Weber, Alan C. Christensen, and Jeffrey P. Mower. 2014. “Extreme Features of the Galdieria Sulphuraria Organellar Genomes: A Consequence of Polyextremophily.” *Genome Biology and Evolution* 7 (1): 367–80. https://doi.org/10.1093/gbe/evu290.

Jalali, F., J. Fakhari, and A. Zolfaghari. 2018. “Investigation on Biosorption of V (III), Ti(IV), and U(VI) Ions from a Contaminated Effluent by a Newly Isolated Strain of Galdieria Sulphuraria.” *Separation Science and Technology (Philadelphia)* 54 (14): 2222–39. https://doi.org/10.1080/01496395.2018.1543323.

Jittawuttipoka, Thichakorn, Mariane Planchon, Olivier Spalla, Karim Benzerara, François Guyot, Corinne Cassier-Chauvat, and Franck Chauvat. 2013. “Multidisciplinary Evidences That Synechocystis PCC6803 Exopolysaccharides Operate in Cell Sedimentation and Protection against Salt and Metal Stresses.” *PLoS ONE* 8 (2). https://doi.org/10.1371/journal.pone.0055564.

Johnson, D. Barrie. 1998. “Biodiversity and Ecology of Acidophilic Microorganisms.” *FEMS Microbiology Ecology* 27 (4): 307–17. https://doi.org/10.1016/S0168-6496(98)00079-8.

Jönsson, K. Ingemar, Elke Rabbow, Ralph O. Schill, Mats Harms-Ringdahl, and Petra Rettberg. 2008. “Tardigrades Survive Exposure to Space in Low Earth Orbit.” *Current Biology* 18 (17). https://doi.org/10.1016/j.cub.2008.06.048.

Ju, Xiaohui, Kensuke Igarashi, Shin ichi Miyashita, Hiroaki Mitsuhashi, Kazumi Inagaki, Shin ichiro Fujii, Hitomi Sawada, Tomohiko Kuwabara, and Ayumi Minoda. 2016. “Effective and Selective Recovery of Gold and Palladium Ions from Metal Wastewater Using a Sulfothermophilic Red Alga, Galdieria Sulphuraria.” *Bioresource Technology* 211: 759–64. https://doi.org/10.1016/j.biortech.2016.01.061.

Kanekar, P.P., S.P. Kanekar, A.S. Kelkar, and P.K. Dhakephalkar. 2012. “Halophiles – Taxonomy, Diversity, Physiology and Applications.” In *Microorganisms in Environmental Management: Microbes and Environment*, edited by T. Satyanarayana, B.N. Johri, and A. Prakash, 1–34. Dordrecht: Springer. https://doi.org/10.1007/978-94-007-2229-3.

Katoh, Kazutaka, and Hiroyuki Toh. 2008. “Recent Developments in the MAFFT Multiple Sequence Alignment Program.” *Briefings in Bioinformatics* 9 (4): 286–98. https://doi.org/10.1093/bib/bbn013.

Kent, W. J. 2002. “BLAT---The BLAST-Like Alignment Tool.” *Genome Research* 12 (4): 656–64. https://doi.org/10.1101/gr.229202.

Kim, Jong Im, Hwan Su Yoon, Gangman Yi, Woongghi Shin, and John M. Archibald. 2018. “Comparative Mitochondrial Genomics of Cryptophyte Algae: Gene Shuffling and Dynamic Mobile Genetic Elements.” *BMC Genomics* 19 (1): 275. https://doi.org/10.1186/s12864-018-4626-9.

Kim, Kyeong Mi, Eun Chan Yang, Jeong Ha Kim, Wendy A Nelson, and Hwan Su Yoon. 2015. “Complete Mitochondrial Genome of a Rhodolith, Sporolithon Durum (Sporolithales, Rhodophyta).” *Mitochondrial DNA* 26 (1): 155–56. https://doi.org/10.3109/19401736.2013.819500.

Kolmogorov, Mikhail, Joel Armstrong, Brian J. Raney, Ian Streeter, Matthew Dunn, Fengtang Yang, Duncan Odom, et al. 2018. “Chromosome Assembly of Large and Complex Genomes Using Multiple References.” *Genome Research* 28 (11): 1720–32. https://doi.org/10.1101/gr.236273.118.

Krulwich, Terry Ann, Masahiro Ito, David B. Hicks, Raymond Gilmour, and Arthur A. Guffanti. 1998. “PH Homeostasis and ATP Synthesis: Studies of Two Processes That Necessitate Inward Proton Translocation in Extremely Alkaliphilic Bacillus Species.” *Extremophiles* 2 (3): 217–22. https://doi.org/10.1007/s007920050063.

Kumar, Sudhir, Glen Stecher, Michael Li, Christina Knyaz, and Koichiro Tamura. 2018. “MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms.” *Molecular Biology and Evolution* 35 (6): 1547–49. https://doi.org/10.1093/molbev/msy096.

Lanquar, Viviane, Françoise Lelièvre, Hélène Barbier-Brygoo, and Sébastien Thomine. 2004. “Regulation and Function of AtNRAMP4 Metal Transporter Protein.” *Soil Science and Plant Nutrition* 50 (7): 1141–50. https://doi.org/10.1080/00380768.2004.10408587.

Laslett, Dean, and Bjorn Canback. 2004. “ARAGORN, a Program to Detect TRNA Genes and TmRNA Genes in Nucleotide Sequences.” *Nucleic Acids Research* 32 (1): 11–16. https://doi.org/10.1093/nar/gkh152.

Leblanc, C., O. Richard, B. Kloareg, S. Viehmann, K. Zetsche, and C. Boyen. 1997. “Origin and Evolution of Mitochondria: What Have We Learnt from Red Algae?” *Current Genetics* 31 (3): 193–207.

https://doi.org/10.1007/s002940050196.

Lee, Jun Mo, Chung Hyun Cho, Seung In Park, Ji Won Choi, Hyun Suk Song, John A. West, Debashish Bhattacharya, and Hwan Su Yoon. 2016. “Parallel Evolution of Highly Conserved Plastid Genome Architecture in Red Seaweeds and Seed Plants.” *BMC Biology* 14 (1): 75. https://doi.org/10.1186/s12915-016-0299-5.

Lee, Justin, Shounak Ghosh, and Milton H. Saier. 2017. “Comparative Genomic Analyses of Transport Proteins Encoded within the Red Algae Chondrus Crispus, Galdieria Sulphuraria, and Cyanidioschyzon Merolae11.” *Journal of Phycology* 53 (3): 503–21. https://doi.org/10.1111/jpy.12534.

Liu, Shao-Lun, Yin-Ru Chiang, Hwan Su Yoon, and Han-Yi Fu. 2020. “Comparative Genome Analysis Reveals Cyanidiococcus Gen. Nov., a New Extremophilic Red Algal Genus Sister to Cyanidioschyzon (Cyanidioschyzonaceae, Rhodophyta).” *Journal of Phycology*, 0–1. https://doi.org/10.1111/jpy.13056.

Liu, Yongqin, Tandong Yao, Shichang Kang, Nianzhi Jiao, Yonghui Zeng, Sijun Huang, and Tingwei Luo. 2007. “Microbial Community Structure in Major Habitats above 6000 m on Mount Everest.” *Chinese Science Bulletin* 52 (17): 2350–57. https://doi.org/10.1007/s11434-007-0360-4.

Lo, Yung Chung, Chieh Lun Cheng, Yin Lung Han, Bor Yann Chen, and Jo Shu Chang. 2014. “Recovery of High-Value Metals from Geothermal Sites by Biosorption and Bioaccumulation.” *Bioresource Technology* 160 (November 2016): 182–90. https://doi.org/10.1016/j.biortech.2014.02.008.

Lohse, Marc, Oliver Drechsel, Sabine Kahlau, and Ralph Bock. 2013. “OrganellarGenomeDRAW--a Suite of Tools for Generating Physical Maps of Plastid and Mitochondrial Genomes and Visualizing Expression Data Sets.” *Nucleic Acids Research* 41 (Web Server issue). https://doi.org/10.1093/nar/gkt289.

Lowe, Todd M, and Patricia P Chan. 2016. “TRNAscan-SE On-Line: Integrating Search and Context for Analysis of Transfer RNA Genes.” *Web Server Issue Published Online* 44. https://doi.org/10.1093/nar/gkw413.

Ma, Ning, Chunsheng Li, Xiaoyan Dong, Dongfeng Wang, and Ying Xu. 2015. “Different Effects of Sodium Chloride Preincubation on Cadmium Tolerance of Pichia Kudriavzevii and Saccharomyces Cerevisiae.” *Journal of Basic Microbiology* 55 (8): 1002–12. https://doi.org/10.1002/jobm.201400847.

Maddison, Wayne P. 1997. “Gene Trees in Species Trees.” *Systematic Biology*

46 (3): 523–36. https://doi.org/10.1093/sysbio/46.3.523.

Martin, W., and Claus Schnarrenberger. 1997. “The Evolution of the Calvin Cycle from Prokaryotic to Eukaryotic Chromosomes: A Case Study of Functional Redundancy in Ancient Pathways through Endosymbiosis.” *Current Genetics* 32 (1): 1–18. https://doi.org/10.1007/s002940050241.

Merola, Aldo, Rosa Castaldo, Paolo De Luca, Raffaele Gambardella, Aldo Musacchio, and Roberto Taddei. 1981. “Revision of Cyanidium Caldarium. Three Species of Acidophilic Algae.” *Giornale Botanico Italiano* 115 (4–5): 189–95. https://doi.org/10.1080/11263508109428026.

Mesbah, Noha M, and Juergen Wiegel. 2011. “Halophiles Exposed Concomitantly to Multiple Stressors: Adaptive Mechanisms of Halophilic Alkalithermophiles.” In *Halophiles and Hypersaline Environments*, edited by A Ventosa, A Oren, and Y Ma, 249–73. Springer, Berlin. https://doi.org/10.1007/978-3-642-20198-1.

Miazek, Krystian, Waldemar Iwanek, Claire Remacle, Aurore Richel, and Dorothee Goffin. 2015. “Effect of Metals, Metalloids and Metallic Nanoparticles on Microalgae Growth and Industrial Product Biosynthesis: A Review.” *International Journal of Molecular Sciences* 16 (10): 23929–69. https://doi.org/10.3390/ijms161023929.

Michard, Annie. 1989. “Rare Earth Element Systematics in Hydrothermal Fluids.” *Geochimica et Cosmochimica Acta* 53 (3): 745–50. https://doi.org/10.1016/0016-7037(89)90017-3.

Minh, Bui Quang, Matthew W. Hahn, and Robert Lanfear. 2020. “New Methods to Calculate Concordance Factors for Phylogenomic Datasets.” *Molecular Biology and Evolution* 37 (9): 2727–33. https://doi.org/10.1093/molbev/msaa106.

Minh, Bui Quang, Minh Anh Thi Nguyen, and Arndt Von Haeseler. 2013. “Ultrafast Approximation for Phylogenetic Bootstrap.” *Molecular Biology and Evolution* 30 (5): 1188–95. https://doi.org/10.1093/molbev/mst024.

Minoda, Ayumi, Hitomi Sawada, Sonoe Suzuki, Shin ichi Miyashita, Kazumi Inagaki, Takaiku Yamamoto, and Mikio Tsuzuki. 2015. “Recovery of Rare Earth Elements from the Sulfothermophilic Red Alga Galdieria Sulphuraria Using Aqueous Acid.” *Applied Microbiology and Biotechnology* 99 (3): 1513–19. https://doi.org/10.1007/s00253-014-6070-3.

Misumi, Osami, Takayuki Sakajiri, Syunsuke Hirooka, Haruko Kuroiwa, and Tsuneyoshi Kuroiwa. 2008. “Cytological Studies of Metal Ion Tolerance in the Red Algae Cyanidioschyzon Merolae.” *Cytologia* 73 (4): 437–43. https://doi.org/10.1508/cytologia.73.437.

Mondo, Angelo Del, Manuela Iovinella, Milena Petriccione, Angelina Nunziata, Seth J. Davis, Diana Cioppa, and Claudia Ciniglia. 2019. “A Spotlight on Rad52 in Cyanidiophytina (Rhodophyta): A Relic in Algal Heritage.” *Plants* 8 (2): 1–26. https://doi.org/10.3390/PLANTS8020046.

Monteiro, Cristina M., Susana C. Fonseca, Paula M.L. Castro, and F. Xavier Malcata. 2011. “Toxicity of Cadmium and Zinc on Two Microalgae, Scenedesmus Obliquus and Desmodesmus Pleiomorphus, from Northern Portugal.” *Journal of Applied Phycology* 23 (1): 97–103. https://doi.org/10.1007/s10811-010-9542-6.

Monteiro, Cristina M, Paula ML Castro, and Xavier F Malcata. 2011. “Capacity of Simultaneous Removal of Zinc and Cadmium from Contaminated Media, by Two Microalgae Isolated from a Polluted Site.” *Environmental Chemistry Letters* 9 (4): 511–17. https://doi.org/10.1007/s10311-011-0311-9.

Naveed, Sadiq, Chonghua Li, Jinyu Zhang, Chunhua Zhang, and Ying Ge. 2020. “Sorption and Transformation of Arsenic by Extracellular Polymeric Substances Extracted from Synechocystis Sp. PCC6803.” *Ecotoxicology and Environmental Safety* 206 (April): 111200. https://doi.org/10.1016/j.ecoenv.2020.111200.

Nevo, Yaniv, and Nathan Nelson. 2006. “The NRAMP Family of Metal-Ion Transporters.” *Biochimica et Biophysica Acta - Molecular Cell Research* 1763 (7): 609–20. https://doi.org/10.1016/j.bbamcr.2006.05.007.

Nguyen, Lam Tung, Heiko A Schmidt, Arndt Von Haeseler, and Bui Quang Minh. 2015. “IQ-TREE: A Fast and Effective Stochastic Algorithm for Estimating Maximum-Likelihood Phylogenies.” *Molecular Biology and Evolution* 32 (1): 268–74. https://doi.org/10.1093/molbev/msu300.

Nugent, Paul W., Joseph A. Shaw, and Michael Vollmer. 2015. “Colors of Thermal Pools at Yellowstone National Park.” *Applied Optics* 54 (4): B128. https://doi.org/10.1364/ao.54.00b128.

Oarga, Andreea. 2009. “Life in Extreme Environments.” *Revista de Biologia e Ciências Da Terra* 9 (1): 1–10.

Olsson-Francis, Karen, Rosa De La Torre, and Charles S. Cockell. 2010. “Isolation of Novel Extreme-Tolerant Cyanobacteria from a Rock-Dwelling Microbial Community by Usine Exposure to Low Earth Orbit.” *Applied and Environmental Microbiology* 76 (7): 2115–21.

https://doi.org/10.1128/AEM.02547-09.

Olsson, Sanna, Fernando Puente-Sánchez, Manuel J. Gómez, and Angeles Aguilera. 2015. “Transcriptional Response to Copper Excess and Identification of Genes Involved in Heavy Metal Tolerance in the Extremophilic Microalga Chlamydomonas Acidophila.” *Extremophiles* 19 (3): 657–72. https://doi.org/10.1007/s00792-015-0746-1.

Oren, Aharon. 2002. “Biotechnological Applications and Potentials of Halophilic Microorganisms.” In *Halophilic Microorganisms and Their Environments*, edited by Aharon Oren, 357–88. New York: Springer.

Osaki, Yuko, Tomoo Shirabe, Hiromi Nakanishi, Takayoshi Wakagi, and Etsuro Yoshimura. 2009. “Characterization of Phytochelatin Synthase Produced by the Primitive Red Alga Cyanidioschyzon Merolae.” *Metallomics* 1 (4): 353–58. https://doi.org/10.1039/b823013g.

Ozturk, Sahlan, Belma Aslim, and Zekiye Suludere. 2010. “Cadmium(II) Sequestration Characteristics by Two Isolates of Synechocystis Sp. in Terms of Exopolysaccharide (EPS) Production and Monomer Composition.” *Bioresource Technology* 101 (24): 9742–48. https://doi.org/10.1016/j.biortech.2010.07.105.

Pacheco, Pablo H., Raúl A. Gil, Soledad E. Cerutti, Patricia Smichowski, and Luis D. Martinez. 2011. “Biosorption: A New Rise for Elemental Solid Phase Extraction Methods.” *Talanta* 85 (5): 2290–2300. https://doi.org/10.1016/j.talanta.2011.08.043.

Pang, Xin, Decheng Li, and An Peng. 2002. “Application of Rare-Earth Elements in the Agriculture of China and Its Environmental Behavior in Soil.” *ESPR* 9 (2): 143–48.

Pao, Stephanie S., Ian T. Paulsen, and Milton H. Saier. 1998. “Major Facilitator Superfamily.” *Microbiology and Molecular Biology Reviews* 62 (1): 1–34.

Patro, Rob, Geet Duggal, Michael I. Love, Rafael A. Irizarry, and Carl Kingsford. 2017. “Salmon Provides Fast and Bias-Aware Quantification of Transcript Expression.” *Nature Methods* 14 (4): 417–19. https://doi.org/10.1038/nmeth.4197.

Paul, Sandip, Sumit K. Bag, Sabyasachi Das, Eric T. Harvill, and Chitra Dutta. 2008. “Molecular Signature of Hypersaline Adaptation: Insights from Genome and Proteome Composition of Halophilic Prokaryotes.” *Genome Biology* 9 (4): 1–19. https://doi.org/10.1186/GB-2008-9-4-R70/FIGURES/7.

Pinto, G, P Albertano, Claudia Ciniglia, Salvatore Cozzolino, Antonino Pollio, Hwan S. Yoon, and D. Bhattacharya. 2003. “Comparative Approaches to the Taxonomy of the Genus Galdieria Merola (Cyanidiales, Rhodophyta).” *Cryptogamie, Algol.* 24 (November 2015): 13–23. http://cat.inist.fr/?aModele=afficheN&cpsidt=14626326.

Pinto, João, Bruno Henriques, José Soares, Marcelo Costa, Mariana Dias, Elaine Fabre, Cláudia B. Lopes, Carlos Vale, José Pinheiro-Torres, and Eduarda Pereira. 2020. “A Green Method Based on Living Macroalgae for the Removal of Rare-Earth Elements from Contaminated Waters.” *Journal of Environmental Management* 263 (January). https://doi.org/10.1016/j.jenvman.2020.110376.

Pollio, Antonino, Paola Cennamo, Claudia Ciniglia, Mario De Stefano, Gabriele Pinto, and Volker A.R. Huss. 2005. “Chlamydomonas Pitschmannii Ettl, a Little Known Species from Thermoacidic Environments.” *Protist* 156 (3): 287–302. https://doi.org/10.1016/j.protis.2005.04.004.

Prasher, S. O., M. Beaugeard, J. Hawari, P. Bera, R. M. Patel, and S. H. Kim. 2004. “Biosorption of Heavy Metals by Red Algae (Palmaria Palmata).” *Environmental Technology* 25 (10): 1097–1106. https://doi.org/10.1080/09593332508618378.

Puente-Sánchez, Fernando, Sanna Olsson, and Angeles Aguilera. 2016. “Comparative Transcriptomic Analysis of the Response of Dunaliella Acidophila (Chlorophyta) to Short-Term Cadmium and Chronic Natural Metal-Rich Water Exposures.” *Microbial Ecology* 72 (3): 595–607. https://doi.org/10.1007/s00248-016-0824-7.

Qi, B C, and C Aldrich. 2008. “Biosorption of Heavy Metals from Aqueous Solutions with Tobacco Dust.” *Bioresource Technology* 99 (13): 5595–5601. https://doi.org/10.1016/j.biortech.2007.10.042.

Qiu, Huan, Dana C. Price, Andreas P.M. Weber, Valérie Reeb, Eun Chan Yang, Jun Mo Lee, Su Yeon Kim, Hwan Su Yoon, and Debashish Bhattacharya. 2013. “Adaptation through Horizontal Gene Transfer in the Cryptoendolithic Red Alga Galdieria Phlegrea.” *Current Biology* 23 (19): R865–66. https://doi.org/10.1016/j.cub.2013.08.046.

Quevillon, E., V. Silventoinen, S. Pillai, N. Harte, N. Mulder, R. Apweiler, and R. Lopez. 2005. “InterProScan: Protein Domains Identifier.” *Nucleic Acids Research* 33 (SUPPL. 2): 116–20. https://doi.org/10.1093/nar/gki442.

Ronaghi, Mostafa, Samer Karamohamed, Bertil Pettersson, Mathias Uhlén, and Pål Nyrén. 1996. “Real-Time DNA Sequencing Using Detection of

Pyrophosphate Release." *Analytical Biochemistry* 242 (1): 84–89. https://doi.org/10.1006/ABIO.1996.0432.

Rossoni, Alessandro W., Dana C. Price, Mark Seger, Dagmar Lyska, Peter Lammers, Debashish Bhattacharya, and Andreas P.M. Weber. 2019. "The Genomes of Polyextremophilic Cyanidiales Contain 1% Horizontally Transferred Genes with Diverse Adaptive Functions." *ELife* 8 (May). https://doi.org/10.7554/eLife.45017.

Rossoni, Alessandro W, Gerald Schönknecht, Hyun Jeong Lee, Ryan L Rupp, Samantha Flachbart, Tabea Mettler-Altmann, Andreas P.M. Weber, and Marion Eisenhut. 2019. "Cold Acclimation of the Thermoacidophilic Red Alga Galdieria Sulphuraria: Changes in Gene Expression and Involvement of Horizontally Acquired Genes." *Plant and Cell Physiology* 60 (3): 702–12. https://doi.org/10.1093/pcp/pcy240.

Rothschild, LJ, and RL Mancinelll. 2001. "Life in Extreme Environments." *Nature* 409: 1092–1101. https://doi.org/10.1007/978-1-4020-6285-8.

Ruhlman, Tracey A., Jin Zhang, John C. Blazier, Jamal S. M. Sabir, and Robert K. Jansen. 2017. "Recombination-Dependent Replication and Gene Conversion Homogenize Repeat Sequences and Diversify Plastid Genome Structure." *American Journal of Botany* 104 (4): 559–72. https://doi.org/10.3732/ajb.1600453.

Sadovsky, David, Asher Brenner, Boaz Astrachan, Boaz Asaf, and Raphael Gonen. 2016. "Biosorption Potential of Cerium Ions Using Spirulina Biomass." *Journal of Rare Earths* 34 (6): 644–52. https://doi.org/10.1016/S1002-0721(16)60074-1.

Saffary, Roya, Renu Nandakumar, Dennis Spencer, Frank T Robb, Joseph M Davila, Marvin Swartz, Leon Ofman, Roger J Thomas, and Jocelyne DiRuggiero. 2002. "Microbial Survival of Space Vacuum and Extreme Ultraviolet Irradiation: Strain Isolation and Analysis during a Rocket Flight." *FEMS Microbiology Letters* 215 (1): 163–68. https://doi.org/10.1111/J.1574-6968.2002.TB11386.X.

Saier, Milton H., Vamsee S. Reddy, Brian V. Tsu, Muhammad Saad Ahmed, Chun Li, and Gabriel Moreno-Hagelsieb. 2016. "The Transporter Classification Database (TCDB): Recent Advances." *Nucleic Acids Research* 44 (D1): D372–79. https://doi.org/10.1093/nar/gkv1103.

Schmidlin, Patrick R., Alexandre Tchouboukov, Florian J. Wegehaupt, and Franz E. Weber. 2012. "Effect of Cerium Chloride Application on Fibroblast and Osteoblast Proliferation and Differentiation." *Archives of Oral Biology* 57 (7): 892–97. https://doi.org/10.1016/j.archoralbio.2012.01.010.

Schönknecht, Gerald, Wei-hua Chen, Chad M Ternes, Guillaume G Barbier, Roshan P Shrestha, Mario Stanke, Andrea Bräutigam, et al. 2013. "Gene Transfer from Bacteria and Archaea Facilitated Evolution of an Extremophilic Eukaryote." *Science* 339: 1207–10.

Schuliger, J.W, SH Brown, JA Baross, and RM Kelly. 1993. "Purification and Characterization of a Novel Amylolytic Enzyme from ES4, a Marine Hyperthermophilic Archaeum." *Molecular Marine Biology and Biotechnology* 2: 76–87.

Seckbach, Joseph. 2017. *Algae and Cyanobacteria in Extreme Environments*. *Springer*. Vol. 11.

Segerer, Andreas H, Sigfried Burggraf, Gerhard Fiala, Gertrud Huber, Robert Huber, Ursula Pley, and Karl O Stetter. 1993. "Life in Hot Springs and Hydrothermal Vents." *Origins of Life and Evolution of the Biosphere* 23 (1): 77–90. https://doi.org/10.1007/BF01581992.

Sentsova, U. 1991. "On the Diversity of Acido-Thermophilic Unicellular Algae of the Genus Galdieria (Rhodophyta, Cyanidiophyceae)." *Botaničeskij Žurnal* 76 (1): 69–78. https://ci.nii.ac.jp/naid/10003747751/.

Simon, Dana F., Rute F. Domingos, Charles Hauser, Colin M. Hutchins, William Zerges, and Kevin J. Wilkinson. 2013. "Transcriptome Sequencing (RNA-Seq) Analysis of the Effects of Metal Nanoparticle Exposure on the Transcriptome of Chlamydomonas Reinhardtii." *Applied and Environmental Microbiology* 79 (16): 4774–85. https://doi.org/10.1128/AEM.00998-13.

Singleton, Martin R, Lois M Wentzell, Yilun Liu, Stephen C West, and Dale B Wigley. 2002. "Structure of the Single-Strand Annealing Domain of Human RAD52 Protein." *Proceedings of the National Academy of Sciences* 99 (21): 13492–97. https://doi.org/10.1073/pnas.212449899.

Sirakov, Maria, Maria Palmieri, Manuela Iovinella, Seth J Davis, Milena Petriccione, Maria Rosa, Di Cicco, Mario De Stefano, and Claudia Ciniglia. 2021. "Cyanidiophyceae (Rhodophyta) Tolerance to Precious Metals : Metabolic Response to Cl 4 K 2 Pd and AuCl 4 K." *Plants*, 1–8.

Skorupa, D. J., V. Reeb, R. W. Castenholz, D. Bhattacharya, and T. R. Mcdermott. 2013. "Cyanidiales Diversity in Yellowstone National Park." *Letters in Applied Microbiology* 57 (5): 459–66. https://doi.org/10.1111/lam.12135.

Som, Anup. 2014. “Causes, Consequences and Solutions of Phylogenetic Incongruence.” *Briefings in Bioinformatics* 16 (3): 536–48. https://doi.org/10.1093/bib/bbu015.

Stetter, K. O., G. Fiala, R. Huber, G. Huber, and A. Segerer. 1986. “Life above the Boiling Point of Water?” *Experientia* 42 (11–12): 1187–91. https://doi.org/10.1007/BF01946389.

Tang, Qiong Ying, Si Qing Liu, Dan Yu, Huan Zhang Liu, and Patrick D Danley. 2012. “Mitochondrial Capture and Incomplete Lineage Sorting in the Diversification of Balitorine Loaches (Cypriniformes, Balitoridae) Revealed by Mitochondrial and Nuclear Genes.” *Zoologica Scripta* 41 (3): 233–47. https://doi.org/10.1111/j.1463-6409.2011.00530.x.

Tang, XK, and Z Tong. 1988. “Effects of Rare Earth Elements on Plant Root Growth and Activity.” *Chinese Rare Metal* 5: 22–24.

Tillich, Michael, Pascal Lehwark, Tommaso Pellizzer, Elena S Ulbricht-Jones, Axel Fischer, Ralph Bock, and Stephan Greiner. 2017. “GeSeq - Versatile and Accurate Annotation of Organelle Genomes.” *Nucleic Acids Research* 45 (W1): W6–11. https://doi.org/10.1093/nar/gkx391.

Toplin, J. A., T. B. Norris, C. R. Lehr, T. R. McDermott, and R. W. Castenholz. 2008. “Biogeographic and Phylogenetic Diversity of Thermoacidophilic Cyanidiales in Yellowstone National Park, Japan, and New Zealand.” *Applied and Environmental Microbiology* 74 (9): 2822–33. https://doi.org/10.1128/AEM.02741-07.

Torsvik, Vigdis, and Lise Øvreås. 2008. “Microbial Diversity, Life Strategies, and Adaptation to Life in Extreme Soils.” In *Microbiology of Extreme Soils*, edited by P Dion and CS Nautiyal, 15–43. Berlin: Springer.

Tsuruta, Takehiko. 2007. “Accumulation of Rare Earth Elements in Various Microorganisms.” *Journal of Rare Earths* 25 (5): 526–32. https://doi.org/10.1016/S1002-0721(07)60556-0.

Vastermark, Ake, Simon Wollwage, Michael E. Houle, Rita Rio, and Milton H. Saier. 2014. “Expansion of the APC Superfamily of Secondary Carriers.” *Proteins: Structure, Function and Bioinformatics* 82 (10): 2797–2811. https://doi.org/10.1002/prot.24643.

Wang, Likun, Zhixing Feng, Xi Wang, Xiaowo Wang, and Xuegong Zhang. 2009. “DEGseq: An R Package for Identifying Differentially Expressed Genes from RNA-Seq Data.” *Bioinformatics* 26 (1): 136–38. https://doi.org/10.1093/bioinformatics/btp612.

Wegehaupt, Florian J., Beatrice Sener, Thomas Attin, and Patrick R. Schmidlin. 2010. “Application of Cerium Chloride to Improve the Acid Resistance of Dentine.” *Archives of Oral Biology* 55 (6): 441–46. https://doi.org/10.1016/j.archoralbio.2010.03.016.

Wharton, D A, and A T Marshall. 2002. “Changes in Surface Features during Desiccation of the Anhydrobiotic Plant Parasitic Nematode Ditylenchus Dipsaci.” *Tissue and Cell* 34 (2): 81–87. https://doi.org/10.1016/S0040-8166(02)00011-3.

Xiao, Rui, and Yi Zheng. 2016. “Overview of Microalgal Extracellular Polymeric Substances (EPS) and Their Applications.” *Biotechnology Advances* 34 (7): 1225–44. https://doi.org/10.1016/j.biotechadv.2016.08.004.

Xie, Qiting, Na Liu, Daohui Lin, Ruohua Qu, Qiongzhi Zhou, and Fei Ge. 2020. “The Complexation with Proteins in Extracellular Polymeric Substances Alleviates the Toxicity of Cd (II) to Chlorella Vulgaris.” *Environmental Pollution* 263: 114102. https://doi.org/10.1016/j.envpol.2020.114102.

Yan, Nieng. 2013. “Structural Advances for the Major Facilitator Superfamily (MFS) Transporters.” *Trends in Biochemical Sciences* 38 (3): 151–59. https://doi.org/10.1016/j.tibs.2013.01.003.

Yang, Eun Chan, Kyeong Mi Kim, Su Yeon Kim, Jung Mo Lee, Ga Hun Boo, Jung Hyun Lee, Wendy A Nelson, et al. 2015. “Highly Conserved Mitochondrial Genomes among Multicellular Red Algae of the Florideophyceae.” *Genome Biology and Evolution* 7 (8): 2394–2406. https://doi.org/10.1093/gbe/evv147.

Yoon, Hwan Su, Claudia Ciniglia, Min Wu, Josep M. Comeron, Gabriele Pinto, Antonino Pollio, and Debashish Bhattacharya. 2006. “Establishment of Endolithic Populations of Extremophilic Cyanidiales (Rhodophyta).” *BMC Evolutionary Biology* 6: 78. https://doi.org/10.1186/1471-2148-6-78.

Yoon, Hwan Su, Jeremiah D. Hackett, Claudia Ciniglia, Gabriele Pinto, and Debashish Bhattacharya. 2004. “A Molecular Timeline for the Origin of Photosynthetic Eukaryotes.” *Molecular Biology and Evolution* 21 (5): 809–18. https://doi.org/10.1093/molbev/msh075.

Yuguan, Ze, Zhou Min, Luo Luyang, Ji Zhe, Liu Chao, Yin Sitao, Duan Yanmei, Li Na, and Hong Fashui. 2009. “Effects of Cerium on Key Enzymes of Carbon Assimilation of Spinach under Magnesium Deficiency.” *Biological Trace Element Research* 131 (2): 154–64. https://doi.org/10.1007/s12011-009-8354-5.

Zeraatkar, Amin Keyvan, Hossein Ahmadzadeh, Ahmad Farhad Talebi, Navid R. Moheimani, and Mark P. McHenry. 2016. “Potential Use of Algae for Heavy Metal Bioremediation, a Critical Review.” *Journal of Environmental Management* 181: 817–31. https://doi.org/10.1016/j.jenvman.2016.06.059.

Zhao, Haiquan, Qiuping Zhou, Min Zhou, Chunxiao Li, Xiaolan Gong, Chao Liu, Chunxiang Qu, Ling Wang, Wenhui Si, and Fashui Hong. 2012. “Magnesium Deficiency Results in Damage of Nitrogen and Carbon Cross-Talk of Maize and Improvement by Cerium Addition.” *Biological Trace Element Research* 148 (1): 102–9. https://doi.org/10.1007/s12011-012-9340-x.

Zhao, Jinfeng, Shixiang Liu, Na Liu, Han Zhang, Qiongzhi Zhou, and Fei Ge. 2019. “Accelerated Productions and Physicochemical Characterizations of Different Extracellular Polymeric Substances from Chlorella Vulgaris with Nano-ZnO.” *Science of the Total Environment* 658: 582–89. https://doi.org/10.1016/j.scitotenv.2018.12.019.

Zhou, Min, Xiaolan Gong, Wang Ying, Liu Chao, Mengmeng Hong, Ling Wang, and Hong Fashui. 2011. “Cerium Relieves the Inhibition of Chlorophyll Biosynthesis of Maize Caused by Magnesium Deficiency.” *Biological Trace Element Research* 143 (1): 468–77. https://doi.org/10.1007/s12011-010-8830-y.

Zierenberg, Robert A, Michael W Adams, and Alissa J Arp. 2000. “Life in Extreme Environments: Hydrothermal Vents.” *Proceedings of the National Academy of Sciences USA* 97 (24): 12961–62.

Abe, Fumiyoshi, and Koki Horikoshi. 2001. “The Biotechnological Potential of Piezophiles.” *Trends in Biotechnology* 19 (3): 102–8. https://doi.org/10.1016/S0167-7799(00)01539-0.

Albertano, P., C. Ciniglia, G. Pinto, and A. Pollio. 2000. “The Taxonomic Position of Cyanidium, Cyanidioschyzon and Galdieria: An Update.” *Hydrobiologia* 433: 137–43. https://doi.org/10.1023/A:1004031123806.

Albertano, P., G. Pinto, and A. Pollio. 1994. “Ecophysiology and Ultrastructure of an Acidophilic Species of Ochromonas (Chrysophyceae, Ochromonadales).” *Archiv Für Protistenkunde* 144 (1): 75–82. https://doi.org/10.1016/S0003-9365(11)80226-5.

Albertano, P, G Pinto, A Pollio, and R Taddei. 1990. “Morphology, Ultrastructure and Ecology of an Acidophilic Alga Pseudococcomyxa Simplex (Mainx) Fott (Chlorococcales).” *Arch. Hydrobiol., Algol. Stud.* 37: 401–8.

Albertano, P, G Pinto, S Santisi, and R Taddei. 1981. “Spermatozopsis Acidophila Kalina (Chlorophyta, Volvocales), a Little Known Alga from Highly Acidic Environments.” *Giorn. Bot. Ital.* 115: 65–76.

Albertano, P, A Pollio, and R Taddei. 1991. “Viridiella Fridericiana (Chlorococcales, Chlorophyta), a New Species Isolated from Extremely Acid Environments.” *Phycologia* 30 (4): 346–54.

Allen, M. M., and R. Y. Stanier. 1968. “Selective Isolation of Blue-Green Algae from Water and Soil.” *Journal of General Microbiology* 51 (2): 203–9. https://doi.org/10.1099/00221287-51-2-203.

Allen, Mary Belle. 1959. “Studies with Cyanidium Caldarium, an Anomalously Pigmented Chlorophyte.” *Archiv Für Mikrobiologie* 32 (3): 270–77. https://doi.org/10.1007/BF00409348.

Altschul, Stephen F., Warren Gish, Webb Miller, Eugene W. Myers, and David J. Lipman. 1990. “Basic Local Alignment Search Tool.” *Journal of Molecular Biology* 215 (3): 403–10. https://doi.org/10.1016/S0022-2836(05)80360-2.

Anisimova, Maria, Manuel Gil, Jean Franois Dufayard, Christophe Dessimoz, and Olivier Gascuel. 2011. “Survey of Branch Support Methods Demonstrates Accuracy, Power, and Robustness of Fast Likelihood-Based Approximation Schemes.” *Systematic Biology* 60 (5): 685–99. https://doi.org/10.1093/sysbio/syr041.

Bailey, R W, and L. A. Staehelin. 1968. “The Chemical Composition of Isolated Cell Walls of Cyanidium Caldarium.” *Journal of General Microbiology* 54 (2): 269–76. https://doi.org/10.1099/00221287-54-2-269.

Baker-Austin, Craig, and Mark Dopson. 2007. “Life in Acid: PH Homeostasis in Acidophiles.” *Trends in Microbiology* 15 (4): 165–71. https://doi.org/10.1016/j.tim.2007.02.005.

Bankevich, Anton, Sergey Nurk, Dmitry Antipov, Alexey A. Gurevich, Mikhail Dvorkin, Alexander S. Kulikov, Valery M. Lesin, et al. 2012. “SPAdes: A New Genome Assembly Algorithm and Its Applications to Single-Cell Sequencing.” *Journal of Computational Biology* 19 (5): 455–77. https://doi.org/10.1089/cmb.2012.0021.

Binnemans, Koen, Peter Tom Jones, Bart Blanpain, Tom Van Gerven, Yongxiang Yang, Allan Walton, and Matthias Buchert. 2013. “Recycling of Rare Earths: A Critical Review.” *Journal of Cleaner Production* 51: 1–22. https://doi.org/10.1016/j.jclepro.2012.12.037.

Birungi, Z. S., and E. M.N. Chirwa. 2014. “The Kinetics of Uptake and Recovery of Lanthanum Using Freshwater Algae as Biosorbents: Comparative Analysis.” *Bioresource Technology* 160: 43–51. https://doi.org/10.1016/j.biortech.2014.01.033.

Birungi, Z. S., E. M.N. Chirwa, and O. J. Botai. 2017. “Competitive Adsorption in a Ternary System of Toxic Metals and Rare Earth Elements Using Desmodesmus Multivariabilis: Empirical and Kinetic Modelling.” *Journal of Applied Phycology* 29 (6): 2899–2910. https://doi.org/10.1007/s10811-017-1197-0.

Bolger, Anthony M, Marc Lohse, and Bjoern Usadel. 2014. “Genome Analysis Trimmomatic: A Flexible Trimmer for Illumina Sequence Data.” *BIOINFORMATICS* 30 (15): 2114–20. https://doi.org/10.1093/bioinformatics/btu170.

Bourcier, W. L., M. Lin, and G. Nix. 2005. “Recovery of Minerals and Metals from Geothermal Fluids.” *2003 SME Annual Meeting*, 19.

Brady, Joseph M., and John M. Tobin. 1995. “Binding of Hard and Soft Metal Ions to

Rhizopus Arrhizus Biomass.” *Enzyme and Microbial Technology* 17 (9): 791–96. https://doi.org/10.1016/0141-0229(95)00142-R.

Brock, T. D. 1978. “The Genus Cyanidium.” In *Thermophilic Microorganisms and Life at High Temperatures*, 255–302.

Bronstein, Omri, Andreas Kroh, and Elisabeth Haring. 2018. “Mind the Gap! The Mitochondrial Control Region and Its Power as a Phylogenetic Marker in Echinoids.” *BMC Evolutionary Biology* 18 (1). https://doi.org/10.1186/s12862-018-1198-x.

Bruen, Trevor C, Hervé Philippe, and David Bryant. 2006. “A Simple and Robust Statistical Test for Detecting the Presence of Recombination.” *Genetics* 172 (4): 2665–81. https://doi.org/10.1534/genetics.105.048975.

Bull, Alan T., and Michael Goodfellow. 2019. “Dark, Rare and Inspirational Microbial Matter in the Extremobiosphere: 16 000 m of Bioprospecting Campaigns.” *Microbiology (United Kingdom)* 165 (12): 1252–64. https://doi.org/10.1099/mic.0.000822.

Camacho, Christiam, George Coulouris, Vahram Avagyan, Ning Ma, Jason Papadopoulos, Kevin Bealer, and Thomas L Madden. 2009. “BLAST+: Architecture and Applications.” *BMC Bioinformatics* 10 (1): 421. https://doi.org/10.1186/1471-2105-10-421.

Cao, Ying, Penghui Shao, Yidi Chen, Xiaoyu Zhou, Liming Yang, Hui Shi, Kai Yu, Xianxin Luo, and Xubiao Luo. 2021. “A Critical Review of the Recovery of Rare Earth Elements from Wastewater by Algae for Resources Recycling Technologies.” *Resources, Conservation and Recycling* 169 (November 2020): 105519. https://doi.org/10.1016/j.resconrec.2021.105519.

Casadei, M. A., P. Mañas, G. Niven, E. Needs, and B. M. Mackey. 2002. “Role of Membrane Fluidity in Pressure Resistance of Escherichia Coli NCTC 8164.” *Applied and Environmental Microbiology* 68 (12): 5965–72. https://doi.org/10.1128/AEM.68.12.5965-5972.2002.

Cassano, Valeria, Mariana C. Oliveira, María Candelaria Gil-Rodríguez, Abel Sentíes, Jhoana Díaz-Larrea, and Mutue T. Fujii. 2012. “Molecular Support for the Establishment of the New Genus Laurenciella within the Laurencia Complex (Ceramiales, Rhodophyta).” *Botanica Marina* 55 (4): 349–57. https://doi.org/10.1515/bot-2012-0133.

Castor, S, and J Hedrick. 2006. “Rare Earth Elements.” In *Society for Mining, Metallurgy, and Exploration: Littleton, Colorado*, 769–92.

Castresana, J. 2000. “Selection of Conserved Blocks from Multiple Alignments for Their Use in Phylogenetic Analysis.” *Molecular Biology and Evolution* 17 (4): 540–52. https://doi.org/10.1093/oxfordjournals.molbev.a026334.

Chao, Liu, Pan Bofu, Cao Weiqian, Lu Yun, Huang Hao, Chen Liang, Liu Xiaoqing, Wu Xiao, and Hong Fashui. 2008. “Influences of Calcium Deficiency and Cerium on Growth of Spinach Plants.” *Biological Trace Element Research* 121 (3): 266–75. https://doi.org/10.1007/s12011-007-8054-y.

Chen, Can, and Jianlong Wang. 2007. “Influence of Metal Ionic Characteristics on Their Biosorption Capacity by Saccharomyces Cerevisiae.” *Applied Microbiology and Biotechnology* 74 (4): 911–17. https://doi.org/10.1007/s00253-006-0739-1.

Cho, Chung Hyun, Seung In Park, Claudia Ciniglia, Eun Chan Yang, Louis Graf, Debashish Bhattacharya, and Hwan Su Yoon. 2020. “Potential Causes and Consequences of Rapid Mitochondrial Genome Evolution in Thermoacidophilic Galdieria (Rhodophyta).” *BMC Evolutionary Biology* 20 (112): 1–15. https://doi.org/10.21203/rs.3.rs-36820/v1.

Cho, Yao Chang Lee, Liang Ching Hsu, Chun Chieh Wang, Pin Chen Chen, Shao Lun Liu, Heng Yi Teah, Yu Ting Liu, and Yu Min Tzou. 2020. “Molecular Mechanisms for Pb Removal by Cyanidiales: A Potential Biomaterial Applied in Thermo-Acidic Conditions.” *Chemical Engineering Journal* 401 (December): 125828. https://doi.org/10.1016/j.cej.2020.125828.

Chong, K. H., and B. Volesky. 1995. “Description of Two-metal Biosorption Equilibria by Langmuir-type Models.” *Biotechnology and Bioengineering* 47 (4): 451–60. https://doi.org/10.1002/bit.260470406.

Chu, ZX, MH Mu, FZ Wang, and HX Shao. 1996. “Effect of CeCl3 on the Photosynthesis of Plants Relation to Fe2+.” In *Environmental Behavior and Ecotoxicology of Rare Earth Elements.*, 75–81.

Ciniglia, P Cennamo, A De Natale, M De Stefano, M Sirakov, M Iovinella, HS Yoon, and A Pollio. 2019. “Cyanidium Chilense (Cyanidiophyceae, Rhodophyta) from Tuff Rocks of the Archeological Site of Cuma, Italy.” *Phycological Research*, no. November 2018. https://doi.org/10.1111/pre.12383.

Ciniglia, Paola Cennamo, Mario De Stefano, Gabriele Pinto, Paolo Caputo, and Antonino Pollio. 2007. “Pinnularia Obscura Krasske (Bacillariophyceae, Bacillariophyta) from Acidic Environments: Characterization and Comparison with Other Acid-Tolerant Pinnularia Species.” *Fundamental and Applied Limnology* 170 (1): 29–47. https://doi.org/10.1127/1863-9135/2007/0170-0029.

Ciniglia, Claudia, Eun Chan Yang, Antonino Pollio, Gabriele Pinto, Manuela Iovinella, Laura Vitale, and Hwan Su Yoon. 2014. “Cyanidiophyceae in Iceland: Plastid Rbc L Gene Elucidates Origin and Dispersal of Extremophilic Galdieria Sulphuraria and G. Maxima (Galdieriaceae, Rhodophyta).” *Phycologia* 53 (6): 542–51. https://doi.org/10.2216/14-032.1.

Ciniglia, Claudia, Hwan Su Yoon, Antonino Pollio, Gabriele Pinto, and Debashish Bhattacharya. 2004. “Hidden Biodiversity of the Extremophilic Cyanidiales Red Algae.” *Molecular Ecology* 13 (7): 1827–38. https://doi.org/10.1111/j.1365-294X.2004.02180.x.

Čížková, M, K Bišová, V Zachleder, D Mezricky, M Rucki, and M Vítová. 2019. “Recovery of Rare Earth Elements from Luminophores Using the Red Alga Galdieria,” no. September: 2018–19. http://www.acuf.net/index.php?lang=en.

Čížková, Mária, Pauline Mezricky, Dana Mezricky, Marian Rucki, Vilém Zachleder, and Milada Vítová. 2021. “Bioaccumulation of Rare Earth Elements from Waste Luminophores in the Red Algae, Galdieria Phlegrea.” *Waste and Biomass Valorization* 12 (6): 3137–46. https://doi.org/10.1007/s12649-020-01182-3.

Clarke, Andrew. 2014. “The Thermal Limits to Life on Earth.” *International Journal of Astrobiology* 13 (2): 141–54. https://doi.org/10.1017/S1473550413000438.

Comte, S., G. Guibaud, and M. Baudu. 2008. “Biosorption Properties of Extracellular

Polymeric Substances (EPS) towards Cd, Cu and Pb for Different PH Values." *Journal of Hazardous Materials* 151 (1): 185–93. https://doi.org/10.1016/j.jhazmat.2007.05.070.

Corguillé, Gildas Le, Gareth Pearson, Marta Valente, Carla Viegas, Bernhard Gschloessl, Erwan Corre, Xavier Bailly, et al. 2009. "Plastid Genomes of Two Brown Algae, Ectocarpus Siliculosus and Fucus Vesiculosus: Further Insights on the Evolution of Red-Algal Derived Plastids." *BMC Evolutionary Biology* 9 (1): 253. https://doi.org/10.1186/1471-2148-9-253.

Crognale, Simona, Stefania Venturi, Franco Tassi, Simona Rossetti, Heba Rashed, Jacopo Cabassi, Francesco Capecchiacci, et al. 2018. "Microbiome Profiling in Extremely Acidic Soils Affected by Hydrothermal Fluids: The Case of the Solfatara Crater (Campi Flegrei, Southern Italy)." *FEMS Microbiology Ecology* 94 (12). https://doi.org/10.1093/femsec/fiy190.

D'Alelio, Domenico, and Maria Valeria Ruggiero. 2015. "Interspecific Plastidial Recombination in the Diatom Genus Pseudo-Nitzschia." *Journal of Phycology* 51 (6): 1024–28. https://doi.org/10.1111/jpy.12350.

D'Alelio, Domenico, Nico Salmaso, and Andrea Gandolfi. 2013. "Frequent Recombination Shapes the Epidemic Population Structure of Planktothrix (Cyanoprokaryota) in Italian Subalpine Lakes." *Journal of Phycology* 49 (6): 1107–17. https://doi.org/10.1111/jpy.12116.

Dahle, Jessica T., and Yuji Arai. 2015. "Environmental Geochemistry of Cerium: Applications and Toxicology of Cerium Oxide Nanoparticles." *International Journal of Environmental Research and Public Health* 12 (2): 1253–78. https://doi.org/10.3390/ijerph120201253.

Daly, Michael J., Elena K. Gaidamakova, Vera Y. Matrosova, Juliann G. Kiang, Risaku Fukumoto, Duck Yeon Lee, Nancy B. Wehr, Gabriela A. Viteri, Barbara S. Berlett, and Rodney L. Levine. 2010. "Small-Molecule Antioxidant Proteome-Shields in Deinococcus Radiodurans." *PLOS ONE* 5 (9): e12570. https://doi.org/10.1371/JOURNAL.PONE.0012570.

Daly, Michael J., Elena K. Gaidamakova, Vera Y. Matrosova, Alexander Vasilenko, Min Zhai, Richard D. Leapman, Barry Lai, et al. 2007. "Protein Oxidation Implicated as the Primary Determinant of Bacterial Radioresistance." *PLOS Biology* 5 (4): e92. https://doi.org/10.1371/JOURNAL.PBIO.0050092.

Darling, Aaron E, Bob Mau, and Nicole T Perna. 2010. "Progressivemauve: Multiple Genome Alignment with Gene Gain, Loss and Rearrangement." *PLoS ONE* 5 (6). https://doi.org/10.1371/journal.pone.0011147.

Das, Nilanjana. 2010. "Recovery of Precious Metals through Biosorption - A Review." *Hydrometallurgy* 103 (1–4): 180–89. https://doi.org/10.1016/j.hydromet.2010.03.016.

Dev, Subhabrata, Ankur Sachan, Fahimeh Dehghani, Tathagata Ghosh, Brandon R. Briggs, and Srijan Aggarwal. 2020. "Mechanisms of Biological Recovery of Rare-Earth Elements from Industrial and Electronic Wastes: A Review." *Chemical Engineering Journal* 397 (February): 124596. https://doi.org/10.1016/j.cej.2020.124596.

Dhakar, Kusum, and Anita Pandey. 2016. "Wide PH Range Tolerance in Extremophiles: Towards Understanding an Important Phenomenon for Future Biotechnology." *Applied*

Microbiology and Biotechnology 100 (6): 2499–2510. https://doi.org/10.1007/s00253-016-7285-2.

Dhankhar, Rajesh, and Anju Hooda. 2011. "Fungal Biosorption-an Alternative to Meet the Challenges of Heavy Metal Pollution in Aqueous Solutions." *Environmental Technology* 32 (5): 467–91. https://doi.org/10.1080/09593330.2011.572922.

Dose, K, A Bieger-Dose, M Labusch, and M Gill. 1992. "Survival in Extreme Dryness and DNA-Single-Strand Breaks." *Advances in Space Research* 12 (4): 221–29. https://doi.org/10.1006/enrs.1994.1018.

Doyle, Jeff J. 1992. "Gene Trees and Species Trees : Molecular Systematics as One-Character Taxonomy." *Systematic Botany* 17 (1): 144–63.

Elster, Josef. 1999. "Enigmatic Microorganisms and Life in Extreme Environments." In *Origin - Evolution and Versatility of Microorganisms (Phylogeny, Structure, Physiology and Extreme Environments*, edited by Joseph Seckbach. Kluwer Academic Publishers. https://doi.org/10.1007/978-94-011-4838-2.

Farcasanu, I. C., M. Mizunuma, D. Hirata, and T. Miyakawa. 1998. "Involvement of Histidine Permease (Hip1p) in Manganese Transport in Saccharomyces Cerevisiae." *Molecular and General Genetics* 259 (5): 541–48. https://doi.org/10.1007/s004380050846.

Farooq, Umar, Janusz A. Kozinski, Misbahul Ain Khan, and Makshoof Athar. 2010. "Biosorption of Heavy Metal Ions Using Wheat Based Biosorbents - A Review of the Recent Literature." *Bioresource Technology* 101 (14): 5043–53. https://doi.org/10.1016/j.biortech.2010.02.030.

Ferreira, J. A., and S. O. Nyangoma. 2008. "A Multivariate Version of the Benjamini-Hochberg Method." *Journal of Multivariate Analysis* 99 (9): 2108–24. https://doi.org/10.1016/j.jmva.2008.02.013.

Fonseca, Miguel M., D. James Harris, and David Posada. 2014. "The Inversion of the Control Region in Three Mitogenomes Provides Further Evidence for an Asymmetric Model of Vertebrate MtDNA Replication." Edited by Dan Mishmar. *PLoS ONE* 9 (9): e106654. https://doi.org/10.1371/journal.pone.0106654.

Franklin, Natasha M, Jennifer L Stauber, Richard P Lim, and P Petocz. 2002. "Toxicity of Metal Mixtures to a Tropical Freshwater Alga (Chlorella Sp.): The Effect of Interactions between Copper, Cadmium, and Zinc on Metal Cell Binding and Uptake." *Environmental Toxicology and Chemistry* 21 (11): 2412–22.

Fukuda, Shin ya, Rie Yamamoto, Koji Iwamoto, and Ayumi Minoda. 2018. "Cellular Accumulation of Cesium in the Unicellular Red Alga Galdieria Sulphuraria under Mixotrophic Conditions." *Journal of Applied Phycology* 30 (6): 3057–61. https://doi.org/10.1007/s10811-018-1525-z.

Gallup, Darrell L. 1998. "Geochemistry of Geothermal Fluids and Well Scales, and Potential for Mineral Recovery." *Ore Geology Reviews* 12 (4): 225–36. https://doi.org/10.1016/S0169-1368(98)00004-3.

Galtier, Nicolas, and Vincent Daubin. 2008. "Dealing with Incongruence in Phylogenomic Analyses." *Philosophical Transactions of the Royal Society B: Biological Sciences*. Royal

Society. https://doi.org/10.1098/rstb.2008.0144.

Gitzendanner, Matthew A., Pamela S. Soltis, Gane K.S. Wong, Brad R. Ruhfel, and Douglas E. Soltis. 2018. “Plastid Phylogenomic Analysis of Green Plants: A Billion Years of Evolutionary History.” *American Journal of Botany* 105 (3): 291–301. https://doi.org/10.1002/ajb2.1048.

Goecke, Franz, Milada Vítová, Jaromír Lukavský, Linda Nedbalová, Tomáš Řezanka, and Vilém Zachleder. 2017. “Effects of Rare Earth Elements on Growth Rate, Lipids, Fatty Acids and Pigments in Microalgae.” *Phycological Research* 65 (3): 226–34. https://doi.org/10.1111/pre.12180.

Gonçalves, Deise J.P., Beryl B. Simpson, Edgardo M. Ortiz, Gustavo H. Shimizu, and Robert K. Jansen. 2019. “Incongruence between Gene Trees and Species Trees and Phylogenetic Signal Variation in Plastid Genes.” *Molecular Phylogenetics and Evolution* 138 (September): 219–32. https://doi.org/10.1016/j.ympev.2019.05.022.

Graedel, T. E., Julian Allwood, Jean Pierre Birat, Matthias Buchert, Christian Hagelüken, Barbara K. Reck, Scott F. Sibley, and Guido Sonnemann. 2011. “What Do We Know about Metal Recycling Rates?” *Journal of Industrial Ecology* 15 (3): 355–66. https://doi.org/10.1111/j.1530-9290.2011.00342.x.

Gromov, B.V., O.V. Gavrilova, and A.V. Pljusch. 1990. “Ultrastructure of Ochromonas Globosa (Chrysophyceae).” *Archiv Für Protistenkunde* 138 (4): 291–97.

Gross, W., and Christine Oesterhelt. 1999. “Ecophysiological Studies on the Red Alga Galdieria Sulphuraria Isolated from Southwest Iceland.” *Plant Biology* 1 (6): 694–700. https://doi.org/10.1111/j.1438-8677.1999.tb00282.x.

Haug, Arne, and Olav Smidsrod. 1970. “Selectivity of Some Anionic Polymers for Divalent Metal Ions.” *Acta Chemica Scandinavia*, 843–54.

Herrmann, Johannes M. 2003. “Converting Bacteria to Organelles: Evolution of Mitochondrial Protein Sorting.” *Trends in Microbiology* 11 (2): 74–79. https://doi.org/10.1016/S0966-842X(02)00033-1.

Hsieh, Chia Jung, Shing Hei Zhan, Yiching Lin, Sen Lin Tang, and Shao Lun Liu. 2015. “Analysis of RbcL Sequences Reveals the Global Biodiversity, Community Structure, and Biogeographical Pattern of Thermoacidophilic Red Algae (Cyanidiales).” *Journal of Phycology* 51: 682–94. https://doi.org/10.1111/jpy.12310.

Huss, Volker A R, Claudia Ciniglia, Paola Cennamo, Salvatore Cozzolino, Gabriele Pinto, and Antonino Pollio. 2002. “Phylogenetic Relationships and Taxonomic Position of Chlorella-like Isolates from Low PH Environments (PH < 3.0).” *BMC Evolutionary Biology* 9: 13.

Iovinella, Manuela, Dora Allegra Carbone, Diana Cioppa, Seth J. Davis, Michele Innangi, Sabrina Esposito, and Claudia Ciniglia. 2020. “Prevalent PH Controls the Capacity of Galdieria Maxima to Use Ammonia and Nitrate as a Nitrogen Source.” *Plants* 9 (2): 232. https://doi.org/10.3390/plants9020232.

Iovinella, Manuela, Ayla Eren, Gabriele Pinto, Antonino Pollio, Seth J Davis, Paola Cennamo, and Claudia Ciniglia. 2018. “Cryptic Dispersal of Cyanidiophytina (Rhodophyta) in Non-

Acidic Environments from Turkey." *Extremophiles* 22: 713–23. https://doi.org/10.1007/s00792-018-1031-x.

Iovinella, Manuela, Francesco Lombardo, Claudia Ciniglia, Maria Palmieri, Maria Rosa di Cicco, Marco Trifuoggi, Marco Race, et al. 2022. "Bioremoval of Yttrium (III), Cerium (III), Europium (III), and Terbium (III) from Single and Quaternary Aqueous Solutions Using the Extremophile Galdieria Sulphuraria (Galdieriaceae, Rhodophyta)." *Plants* 11 (10): 1376. https://doi.org/10.3390/plants11101376.

Jacinto, Jéssica, Bruno Henriques, A. C. Duarte, Carlos Vale, and E. Pereira. 2018. "Removal and Recovery of Critical Rare Elements from Contaminated Waters by Living Gracilaria Gracilis." *Journal of Hazardous Materials* 344 (February): 531–38. https://doi.org/10.1016/j.jhazmat.2017.10.054.

Jack, D. L., I. T. Paulsen, and Jr Saier. 2000. "The Amino Acid/Polyamine/Organocation (APC) Superfamily of Transporters Specific for Amino Acids, Polyamines and Organocations." *Microbiology* 146 (8): 1797–1814. https://doi.org/10.1099/00221287-146-8-1797.

Jain, Kanika, Kirsten Krause, Felix Grewe, Gaven F. Nelson, Andreas P.M. Weber, Alan C. Christensen, and Jeffrey P. Mower. 2014. "Extreme Features of the Galdieria Sulphuraria Organellar Genomes: A Consequence of Polyextremophily." *Genome Biology and Evolution* 7 (1): 367–80. https://doi.org/10.1093/gbe/evu290.

Jalali, F., J. Fakhari, and A. Zolfaghari. 2018. "Investigation on Biosorption of V (III), Ti(IV), and U(VI) Ions from a Contaminated Effluent by a Newly Isolated Strain of Galdieria Sulphuraria." *Separation Science and Technology (Philadelphia)* 54 (14): 2222–39. https://doi.org/10.1080/01496395.2018.1543323.

Jittawuttipoka, Thichakorn, Mariane Planchon, Olivier Spalla, Karim Benzerara, François Guyot, Corinne Cassier-Chauvat, and Franck Chauvat. 2013. "Multidisciplinary Evidences That Synechocystis PCC6803 Exopolysaccharides Operate in Cell Sedimentation and Protection against Salt and Metal Stresses." *PLoS ONE* 8 (2). https://doi.org/10.1371/journal.pone.0055564.

Johnson, D. Barrie. 1998. "Biodiversity and Ecology of Acidophilic Microorganisms." *FEMS Microbiology Ecology* 27 (4): 307–17. https://doi.org/10.1016/S0168-6496(98)00079-8.

Jönsson, K. Ingemar, Elke Rabbow, Ralph O. Schill, Mats Harms-Ringdahl, and Petra Rettberg. 2008. "Tardigrades Survive Exposure to Space in Low Earth Orbit." *Current Biology* 18 (17). https://doi.org/10.1016/j.cub.2008.06.048.

Ju, Xiaohui, Kensuke Igarashi, Shin ichi Miyashita, Hiroaki Mitsuhashi, Kazumi Inagaki, Shin ichiro Fujii, Hitomi Sawada, Tomohiko Kuwabara, and Ayumi Minoda. 2016. "Effective and Selective Recovery of Gold and Palladium Ions from Metal Wastewater Using a Sulfothermophilic Red Alga, Galdieria Sulphuraria." *Bioresource Technology* 211: 759–64. https://doi.org/10.1016/j.biortech.2016.01.061.

Kanekar, P.P., S.P. Kanekar, A.S. Kelkar, and P.K. Dhakephalkar. 2012. "Halophiles – Taxonomy, Diversity, Physiology and Applications." In *Microorganisms in Environmental Management: Microbes and Environment*, edited by T. Satyanarayana, B.N. Johri, and A. Prakash, 1–34. Dordrecht: Springer. https://doi.org/10.1007/978-94-007-2229-3.

Katoh, Kazutaka, and Hiroyuki Toh. 2008. "Recent Developments in the MAFFT Multiple Sequence Alignment Program." *Briefings in Bioinformatics* 9 (4): 286–98. https://doi.org/10.1093/bib/bbn013.

Kent, W. J. 2002. "BLAT---The BLAST-Like Alignment Tool." *Genome Research* 12 (4): 656–64. https://doi.org/10.1101/gr.229202.

Kim, Jong Im, Hwan Su Yoon, Gangman Yi, Woongghi Shin, and John M. Archibald. 2018. "Comparative Mitochondrial Genomics of Cryptophyte Algae: Gene Shuffling and Dynamic Mobile Genetic Elements." *BMC Genomics* 19 (1): 275. https://doi.org/10.1186/s12864-018-4626-9.

Kim, Kyeong Mi, Eun Chan Yang, Jeong Ha Kim, Wendy A Nelson, and Hwan Su Yoon. 2015. "Complete Mitochondrial Genome of a Rhodolith, Sporolithon Durum (Sporolithales, Rhodophyta)." *Mitochondrial DNA* 26 (1): 155–56. https://doi.org/10.3109/19401736.2013.819500.

Kolmogorov, Mikhail, Joel Armstrong, Brian J. Raney, Ian Streeter, Matthew Dunn, Fengtang Yang, Duncan Odom, et al. 2018. "Chromosome Assembly of Large and Complex Genomes Using Multiple References." *Genome Research* 28 (11): 1720–32. https://doi.org/10.1101/gr.236273.118.

Krulwich, Terry Ann, Masahiro Ito, David B. Hicks, Raymond Gilmour, and Arthur A. Guffanti. 1998. "PH Homeostasis and ATP Synthesis: Studies of Two Processes That Necessitate Inward Proton Translocation in Extremely Alkaliphilic Bacillus Species." *Extremophiles* 2 (3): 217–22. https://doi.org/10.1007/s007920050063.

Kumar, Sudhir, Glen Stecher, Michael Li, Christina Knyaz, and Koichiro Tamura. 2018. "MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms." *Molecular Biology and Evolution* 35 (6): 1547–49. https://doi.org/10.1093/molbev/msy096.

Lanquar, Viviane, Françoise Lelièvre, Hélène Barbier-Brygoo, and Sébastien Thomine. 2004. "Regulation and Function of AtNRAMP4 Metal Transporter Protein." *Soil Science and Plant Nutrition* 50 (7): 1141–50. https://doi.org/10.1080/00380768.2004.10408587.

Laslett, Dean, and Bjorn Canback. 2004. "ARAGORN, a Program to Detect TRNA Genes and TmRNA Genes in Nucleotide Sequences." *Nucleic Acids Research* 32 (1): 11–16. https://doi.org/10.1093/nar/gkh152.

Leblanc, C., O. Richard, B. Kloareg, S. Viehmann, K. Zetsche, and C. Boyen. 1997. "Origin and Evolution of Mitochondria: What Have We Learnt from Red Algae?" *Current Genetics* 31 (3): 193–207. https://doi.org/10.1007/s002940050196.

Lee, Jun Mo, Chung Hyun Cho, Seung In Park, Ji Won Choi, Hyun Suk Song, John A. West, Debashish Bhattacharya, and Hwan Su Yoon. 2016. "Parallel Evolution of Highly Conserved Plastid Genome Architecture in Red Seaweeds and Seed Plants." *BMC Biology* 14 (1): 75. https://doi.org/10.1186/s12915-016-0299-5.

Lee, Justin, Shounak Ghosh, and Milton H. Saier. 2017. "Comparative Genomic Analyses of Transport Proteins Encoded within the Red Algae Chondrus Crispus, Galdieria Sulphuraria, and Cyanidioschyzon Merolae11." *Journal of Phycology* 53 (3): 503–21. https://doi.org/10.1111/jpy.12534.

Liu, Shao-Lun, Yin-Ru Chiang, Hwan Su Yoon, and Han-Yi Fu. 2020. “Comparative Genome Analysis Reveals Cyanidiococcus Gen. Nov., a New Extremophilic Red Algal Genus Sister to Cyanidioschyzon (Cyanidioschyzonaceae, Rhodophyta).” *Journal of Phycology*, 0–1. https://doi.org/10.1111/jpy.13056.

Liu, Yongqin, Tandong Yao, Shichang Kang, Nianzhi Jiao, Yonghui Zeng, Sijun Huang, and Tingwei Luo. 2007. “Microbial Community Structure in Major Habitats above 6000 m on Mount Everest.” *Chinese Science Bulletin* 52 (17): 2350–57. https://doi.org/10.1007/s11434-007-0360-4.

Lo, Yung Chung, Chieh Lun Cheng, Yin Lung Han, Bor Yann Chen, and Jo Shu Chang. 2014. “Recovery of High-Value Metals from Geothermal Sites by Biosorption and Bioaccumulation.” *Bioresource Technology* 160 (November 2016): 182–90. https://doi.org/10.1016/j.biortech.2014.02.008.

Lohse, Marc, Oliver Drechsel, Sabine Kahlau, and Ralph Bock. 2013. “OrganellarGenomeDRAW--a Suite of Tools for Generating Physical Maps of Plastid and Mitochondrial Genomes and Visualizing Expression Data Sets.” *Nucleic Acids Research* 41 (Web Server issue). https://doi.org/10.1093/nar/gkt289.

Lowe, Todd M, and Patricia P Chan. 2016. “TRNAscan-SE On-Line: Integrating Search and Context for Analysis of Transfer RNA Genes.” *Web Server Issue Published Online* 44. https://doi.org/10.1093/nar/gkw413.

Ma, Ning, Chunsheng Li, Xiaoyan Dong, Dongfeng Wang, and Ying Xu. 2015. “Different Effects of Sodium Chloride Preincubation on Cadmium Tolerance of Pichia Kudriavzevii and Saccharomyces Cerevisiae.” *Journal of Basic Microbiology* 55 (8): 1002–12. https://doi.org/10.1002/jobm.201400847.

Maddison, Wayne P. 1997. “Gene Trees in Species Trees.” *Systematic Biology* 46 (3): 523–36. https://doi.org/10.1093/sysbio/46.3.523.

Martin, W., and Claus Schnarrenberger. 1997. “The Evolution of the Calvin Cycle from Prokaryotic to Eukaryotic Chromosomes: A Case Study of Functional Redundancy in Ancient Pathways through Endosymbiosis.” *Current Genetics* 32 (1): 1–18. https://doi.org/10.1007/s002940050241.

Merola, Aldo, Rosa Castaldo, Paolo De Luca, Raffaele Gambardella, Aldo Musacchio, and Roberto Taddei. 1981. “Revision of Cyanidium Caldarium. Three Species of Acidophilic Algae.” *Giornale Botanico Italiano* 115 (4–5): 189–95. https://doi.org/10.1080/11263508109428026.

Mesbah, Noha M, and Juergen Wiegel. 2011. “Halophiles Exposed Concomitantly to Multiple Stressors: Adaptive Mechanisms of Halophilic Alkalithermophiles.” In *Halophiles and Hypersaline Environments*, edited by A Ventosa, A Oren, and Y Ma, 249–73. Springer, Berlin. https://doi.org/10.1007/978-3-642-20198-1.

Miazek, Krystian, Waldemar Iwanek, Claire Remacle, Aurore Richel, and Dorothee Goffin. 2015. “Effect of Metals, Metalloids and Metallic Nanoparticles on Microalgae Growth and Industrial Product Biosynthesis: A Review.” *International Journal of Molecular Sciences* 16 (10): 23929–69. https://doi.org/10.3390/ijms161023929.

Michard, Annie. 1989. “Rare Earth Element Systematics in Hydrothermal Fluids.” *Geochimica* *et Cosmochimica Acta* 53 (3): 745–50. https://doi.org/10.1016/0016-7037(89)90017-3.

Minh, Bui Quang, Matthew W. Hahn, and Robert Lanfear. 2020. “New Methods to Calculate Concordance Factors for Phylogenomic Datasets.” *Molecular Biology and Evolution* 37 (9): 2727–33. https://doi.org/10.1093/molbev/msaa106.

Minh, Bui Quang, Minh Anh Thi Nguyen, and Arndt Von Haeseler. 2013. “Ultrafast Approximation for Phylogenetic Bootstrap.” *Molecular Biology and Evolution* 30 (5): 1188–95. https://doi.org/10.1093/molbev/mst024.

Minoda, Ayumi, Hitomi Sawada, Sonoe Suzuki, Shin ichi Miyashita, Kazumi Inagaki, Takaiku Yamamoto, and Mikio Tsuzuki. 2015. “Recovery of Rare Earth Elements from the Sulfothermophilic Red Alga Galdieria Sulphuraria Using Aqueous Acid.” *Applied Microbiology and Biotechnology* 99 (3): 1513–19. https://doi.org/10.1007/s00253-014-6070-3.

Misumi, Osami, Takayuki Sakajiri, Syunsuke Hirooka, Haruko Kuroiwa, and Tsuneyoshi Kuroiwa. 2008. “Cytological Studies of Metal Ion Tolerance in the Red Algae Cyanidioschyzon Merolae.” *Cytologia* 73 (4): 437–43. https://doi.org/10.1508/cytologia.73.437.

Mondo, Angelo Del, Manuela Iovinella, Milena Petriccione, Angelina Nunziata, Seth J. Davis, Diana Cioppa, and Claudia Ciniglia. 2019. “A Spotlight on Rad52 in Cyanidiophytina (Rhodophyta): A Relic in Algal Heritage.” *Plants* 8 (2): 1–26. https://doi.org/10.3390/PLANTS8020046.

Monteiro, Cristina M., Susana C. Fonseca, Paula M.L. Castro, and F. Xavier Malcata. 2011. “Toxicity of Cadmium and Zinc on Two Microalgae, Scenedesmus Obliquus and Desmodesmus Pleiomorphus, from Northern Portugal.” *Journal of Applied Phycology* 23 (1): 97–103. https://doi.org/10.1007/s10811-010-9542-6.

Monteiro, Cristina M, Paula ML Castro, and Xavier F Malcata. 2011. “Capacity of Simultaneous Removal of Zinc and Cadmium from Contaminated Media, by Two Microalgae Isolated from a Polluted Site.” *Environmental Chemistry Letters* 9 (4): 511–17. https://doi.org/10.1007/s10311-011-0311-9.

Naveed, Sadiq, Chonghua Li, Jinyu Zhang, Chunhua Zhang, and Ying Ge. 2020. “Sorption and Transformation of Arsenic by Extracellular Polymeric Substances Extracted from Synechocystis Sp. PCC6803.” *Ecotoxicology and Environmental Safety* 206 (April): 111200. https://doi.org/10.1016/j.ecoenv.2020.111200.

Nevo, Yaniv, and Nathan Nelson. 2006. “The NRAMP Family of Metal-Ion Transporters.” *Biochimica et Biophysica Acta - Molecular Cell Research* 1763 (7): 609–20. https://doi.org/10.1016/j.bbamcr.2006.05.007.

Nguyen, Lam Tung, Heiko A Schmidt, Arndt Von Haeseler, and Bui Quang Minh. 2015. “IQ-TREE: A Fast and Effective Stochastic Algorithm for Estimating Maximum-Likelihood Phylogenies.” *Molecular Biology and Evolution* 32 (1): 268–74. https://doi.org/10.1093/molbev/msu300.

Nugent, Paul W., Joseph A. Shaw, and Michael Vollmer. 2015. “Colors of Thermal Pools at Yellowstone National Park.” *Applied Optics* 54 (4): B128. https://doi.org/10.1364/ao.54.00b128.

Oarga, Andreea. 2009. “Life in Extreme Environments.” *Revista de Biologia e Ciências Da Terra* 9 (1): 1–10.

Olsson-Francis, Karen, Rosa De La Torre, and Charles S. Cockell. 2010. “Isolation of Novel Extreme-Tolerant Cyanobacteria from a Rock-Dwelling Microbial Community by Usine Exposure to Low Earth Orbit.” *Applied and Environmental Microbiology* 76 (7): 2115–21. https://doi.org/10.1128/AEM.02547-09.

Olsson, Sanna, Fernando Puente-Sánchez, Manuel J. Gómez, and Angeles Aguilera. 2015. “Transcriptional Response to Copper Excess and Identification of Genes Involved in Heavy Metal Tolerance in the Extremophilic Microalga Chlamydomonas Acidophila.” *Extremophiles* 19 (3): 657–72. https://doi.org/10.1007/s00792-015-0746-1.

Oren, Aharon. 2002. “Biotechnological Applications and Potentials of Halophilic Microorganisms.” In *Halophilic Microorganisms and Their Environments*, edited by Aharon Oren, 357–88. New York: Springer.

Osaki, Yuko, Tomoo Shirabe, Hiromi Nakanishi, Takayoshi Wakagi, and Etsuro Yoshimura. 2009. “Characterization of Phytochelatin Synthase Produced by the Primitive Red Alga Cyanidioschyzon Merolae.” *Metallomics* 1 (4): 353–58. https://doi.org/10.1039/b823013g.

Ozturk, Sahlan, Belma Aslim, and Zekiye Suludere. 2010. “Cadmium(II) Sequestration Characteristics by Two Isolates of Synechocystis Sp. in Terms of Exopolysaccharide (EPS) Production and Monomer Composition.” *Bioresource Technology* 101 (24): 9742–48. https://doi.org/10.1016/j.biortech.2010.07.105.

Pacheco, Pablo H., Raúl A. Gil, Soledad E. Cerutti, Patricia Smichowski, and Luis D. Martinez. 2011. “Biosorption: A New Rise for Elemental Solid Phase Extraction Methods.” *Talanta* 85 (5): 2290–2300. https://doi.org/10.1016/j.talanta.2011.08.043.

Pang, Xin, Decheng Li, and An Peng. 2002. “Application of Rare-Earth Elements in the Agriculture of China and Its Environmental Behavior in Soil.” *ESPR* 9 (2): 143–48.

Pao, Stephanie S., Ian T. Paulsen, and Milton H. Saier. 1998. “Major Facilitator Superfamily.” *Microbiology and Molecular Biology Reviews* 62 (1): 1–34.

Patro, Rob, Geet Duggal, Michael I. Love, Rafael A. Irizarry, and Carl Kingsford. 2017. “Salmon Provides Fast and Bias-Aware Quantification of Transcript Expression.” *Nature Methods* 14 (4): 417–19. https://doi.org/10.1038/nmeth.4197.

Paul, Sandip, Sumit K. Bag, Sabyasachi Das, Eric T. Harvill, and Chitra Dutta. 2008. “Molecular Signature of Hypersaline Adaptation: Insights from Genome and Proteome Composition of Halophilic Prokaryotes.” *Genome Biology* 9 (4): 1–19. https://doi.org/10.1186/GB-2008-9-4-R70/FIGURES/7.

Pinto, G, P Albertano, Claudia Ciniglia, Salvatore Cozzolino, Antonino Pollio, Hwan S. Yoon, and D. Bhattacharya. 2003. “Comparative Approaches to the Taxonomy of the Genus Galdieria Merola (Cyanidiales, Rhodophyta).” *Cryptogamie, Algol.* 24 (November 2015): 13–23. http://cat.inist.fr/?aModele=afficheN&cpsidt=14626326.

Pinto, João, Bruno Henriques, José Soares, Marcelo Costa, Mariana Dias, Elaine Fabre, Cláudia B. Lopes, Carlos Vale, José Pinheiro-Torres, and Eduarda Pereira. 2020. “A

Green Method Based on Living Macroalgae for the Removal of Rare-Earth Elements from Contaminated Waters." *Journal of Environmental Management* 263 (January). https://doi.org/10.1016/j.jenvman.2020.110376.

Pollio, Antonino, Paola Cennamo, Claudia Ciniglia, Mario De Stefano, Gabriele Pinto, and Volker A.R. Huss. 2005. "Chlamydomonas Pitschmannii Ettl, a Little Known Species from Thermoacidic Environments." *Protist* 156 (3): 287–302. https://doi.org/10.1016/j.protis.2005.04.004.

Prasher, S. O., M. Beaugeard, J. Hawari, P. Bera, R. M. Patel, and S. H. Kim. 2004. "Biosorption of Heavy Metals by Red Algae (Palmaria Palmata)." *Environmental Technology* 25 (10): 1097–1106. https://doi.org/10.1080/09593332508618378.

Puente-Sánchez, Fernando, Sanna Olsson, and Angeles Aguilera. 2016. "Comparative Transcriptomic Analysis of the Response of Dunaliella Acidophila (Chlorophyta) to Short-Term Cadmium and Chronic Natural Metal-Rich Water Exposures." *Microbial Ecology* 72 (3): 595–607. https://doi.org/10.1007/s00248-016-0824-7.

Qi, B C, and C Aldrich. 2008. "Biosorption of Heavy Metals from Aqueous Solutions with Tobacco Dust." *Bioresource Technology* 99 (13): 5595–5601. https://doi.org/10.1016/j.biortech.2007.10.042.

Qiu, Huan, Dana C. Price, Andreas P.M. Weber, Valérie Reeb, Eun Chan Yang, Jun Mo Lee, Su Yeon Kim, Hwan Su Yoon, and Debashish Bhattacharya. 2013. "Adaptation through Horizontal Gene Transfer in the Cryptoendolithic Red Alga Galdieria Phlegrea." *Current Biology* 23 (19): R865–66. https://doi.org/10.1016/j.cub.2013.08.046.

Quevillon, E., V. Silventoinen, S. Pillai, N. Harte, N. Mulder, R. Apweiler, and R. Lopez. 2005. "InterProScan: Protein Domains Identifier." *Nucleic Acids Research* 33 (SUPPL. 2): 116–20. https://doi.org/10.1093/nar/gki442.

Ronaghi, Mostafa, Samer Karamohamed, Bertil Pettersson, Mathias Uhlén, and Pål Nyrén. 1996. "Real-Time DNA Sequencing Using Detection of Pyrophosphate Release." *Analytical Biochemistry* 242 (1): 84–89. https://doi.org/10.1006/ABIO.1996.0432.

Rossoni, Alessandro W., Dana C. Price, Mark Seger, Dagmar Lyska, Peter Lammers, Debashish Bhattacharya, and Andreas P.M. Weber. 2019. "The Genomes of Polyextremophilic Cyanidiales Contain 1% Horizontally Transferred Genes with Diverse Adaptive Functions." *ELife* 8 (May). https://doi.org/10.7554/eLife.45017.

Rossoni, Alessandro W, Gerald Schönknecht, Hyun Jeong Lee, Ryan L Rupp, Samantha Flachbart, Tabea Mettler-Altmann, Andreas P.M. Weber, and Marion Eisenhut. 2019. "Cold Acclimation of the Thermoacidophilic Red Alga Galdieria Sulphuraria: Changes in Gene Expression and Involvement of Horizontally Acquired Genes." *Plant and Cell Physiology* 60 (3): 702–12. https://doi.org/10.1093/pcp/pcy240.

Rothschild, LJ, and RL Mancinelll. 2001. "Life in Extreme Environments." *Nature* 409: 1092–1101. https://doi.org/10.1007/978-1-4020-6285-8.

Ruhlman, Tracey A., Jin Zhang, John C. Blazier, Jamal S. M. Sabir, and Robert K. Jansen. 2017. "Recombination-Dependent Replication and Gene Conversion Homogenize Repeat Sequences and Diversify Plastid Genome Structure." *American Journal of Botany* 104 (4): 559–72. https://doi.org/10.3732/ajb.1600453.

Sadovsky, David, Asher Brenner, Boaz Astrachan, Boaz Asaf, and Raphael Gonen. 2016. “Biosorption Potential of Cerium Ions Using Spirulina Biomass.” *Journal of Rare Earths* 34 (6): 644–52. https://doi.org/10.1016/S1002-0721(16)60074-1.

Saffary, Roya, Renu Nandakumar, Dennis Spencer, Frank T Robb, Joseph M Davila, Marvin Swartz, Leon Ofman, Roger J Thomas, and Jocelyne DiRuggiero. 2002. “Microbial Survival of Space Vacuum and Extreme Ultraviolet Irradiation: Strain Isolation and Analysis during a Rocket Flight.” *FEMS Microbiology Letters* 215 (1): 163–68. https://doi.org/10.1111/J.1574-6968.2002.TB11386.X.

Saier, Milton H., Vamsee S. Reddy, Brian V. Tsu, Muhammad Saad Ahmed, Chun Li, and Gabriel Moreno-Hagelsieb. 2016. “The Transporter Classification Database (TCDB): Recent Advances.” *Nucleic Acids Research* 44 (D1): D372–79. https://doi.org/10.1093/nar/gkv1103.

Schmidlin, Patrick R., Alexandre Tchouboukov, Florian J. Wegehaupt, and Franz E. Weber. 2012. “Effect of Cerium Chloride Application on Fibroblast and Osteoblast Proliferation and Differentiation.” *Archives of Oral Biology* 57 (7): 892–97. https://doi.org/10.1016/j.archoralbio.2012.01.010.

Schönknecht, Gerald, Wei-hua Chen, Chad M Ternes, Guillaume G Barbier, Roshan P Shrestha, Mario Stanke, Andrea Bräutigam, et al. 2013. “Gene Transfer from Bacteria and Archaea Facilitated Evolution of an Extremophilic Eukaryote.” *Science* 339: 1207–10.

Schuliger, J.W, SH Brown, JA Baross, and RM Kelly. 1993. “Purification and Characterization of a Novel Amylolytic Enzyme from ES4, a Marine Hyperthermophilic Archaeum.” *Molecular Marine Biology and Biotechnology* 2: 76–87.

Seckbach, Joseph. 2017. *Algae and Cyanobacteria in Extreme Environments*. *Springer*. Vol. 11.

Segerer, Andreas H, Sigfried Burggraf, Gerhard Fiala, Gertrud Huber, Robert Huber, Ursula Pley, and Karl O Stetter. 1993. “Life in Hot Springs and Hydrothermal Vents.” *Origins of Life and Evolution of the Biosphere* 23 (1): 77–90. https://doi.org/10.1007/BF01581992.

Sentsova, U. 1991. “On the Diversity of Acido-Thermophilic Unicellular Algae of the Genus Galdieria (Rhodophyta, Cyanidiophyceae).” *Botaničeskij Žurnal* 76 (1): 69–78. https://ci.nii.ac.jp/naid/10003747751/.

Simon, Dana F., Rute F. Domingos, Charles Hauser, Colin M. Hutchins, William Zerges, and Kevin J. Wilkinson. 2013. “Transcriptome Sequencing (RNA-Seq) Analysis of the Effects of Metal Nanoparticle Exposure on the Transcriptome of Chlamydomonas Reinhardtii.” *Applied and Environmental Microbiology* 79 (16): 4774–85. https://doi.org/10.1128/AEM.00998-13.

Singleton, Martin R, Lois M Wentzell, Yilun Liu, Stephen C West, and Dale B Wigley. 2002. “Structure of the Single-Strand Annealing Domain of Human RAD52 Protein.” *Proceedings of the National Academy of Sciences* 99 (21): 13492–97. https://doi.org/10.1073/pnas.212449899.

Sirakov, Maria, Maria Palmieri, Manuela Iovinella, Seth J Davis, Milena Petriccione, Maria Rosa, Di Cicco, Mario De Stefano, and Claudia Ciniglia. 2021. “Cyanidiophyceae (

Rhodophyta) Tolerance to Precious Metals : Metabolic Response to Cl 4 K 2 Pd and AuCl 4 K." *Plants*, 1–8.

Skorupa, D. J., V. Reeb, R. W. Castenholz, D. Bhattacharya, and T. R. Mcdermott. 2013. "Cyanidiales Diversity in Yellowstone National Park." *Letters in Applied Microbiology* 57 (5): 459–66. https://doi.org/10.1111/lam.12135.

Som, Anup. 2014. "Causes, Consequences and Solutions of Phylogenetic Incongruence." *Briefings in Bioinformatics* 16 (3): 536–48. https://doi.org/10.1093/bib/bbu015.

Stetter, K. O., G. Fiala, R. Huber, G. Huber, and A. Segerer. 1986. "Life above the Boiling Point of Water?" *Experientia* 42 (11–12): 1187–91. https://doi.org/10.1007/BF01946389.

Tang, Qiong Ying, Si Qing Liu, Dan Yu, Huan Zhang Liu, and Patrick D Danley. 2012. "Mitochondrial Capture and Incomplete Lineage Sorting in the Diversification of Balitorine Loaches (Cypriniformes, Balitoridae) Revealed by Mitochondrial and Nuclear Genes." *Zoologica Scripta* 41 (3): 233–47. https://doi.org/10.1111/j.1463-6409.2011.00530.x.

Tang, XK, and Z Tong. 1988. "Effects of Rare Earth Elements on Plant Root Growth and Activity." *Chinese Rare Metal* 5: 22–24.

Tillich, Michael, Pascal Lehwark, Tommaso Pellizzer, Elena S Ulbricht-Jones, Axel Fischer, Ralph Bock, and Stephan Greiner. 2017. "GeSeq - Versatile and Accurate Annotation of Organelle Genomes." *Nucleic Acids Research* 45 (W1): W6–11. https://doi.org/10.1093/nar/gkx391.

Toplin, J. A., T. B. Norris, C. R. Lehr, T. R. McDermott, and R. W. Castenholz. 2008. "Biogeographic and Phylogenetic Diversity of Thermoacidophilic Cyanidiales in Yellowstone National Park, Japan, and New Zealand." *Applied and Environmental Microbiology* 74 (9): 2822–33. https://doi.org/10.1128/AEM.02741-07.

Torsvik, Vigdis, and Lise Øvreås. 2008. "Microbial Diversity, Life Strategies, and Adaptation to Life in Extreme Soils." In *Microbiology of Extreme Soils*, edited by P Dion and CS Nautiyal, 15–43. Berlin: Springer.

Tsuruta, Takehiko. 2007. "Accumulation of Rare Earth Elements in Various Microorganisms." *Journal of Rare Earths* 25 (5): 526–32. https://doi.org/10.1016/S1002-0721(07)60556-0.

Vastermark, Ake, Simon Wollwage, Michael E. Houle, Rita Rio, and Milton H. Saier. 2014. "Expansion of the APC Superfamily of Secondary Carriers." *Proteins: Structure, Function and Bioinformatics* 82 (10): 2797–2811. https://doi.org/10.1002/prot.24643.

Wang, Likun, Zhixing Feng, Xi Wang, Xiaowo Wang, and Xuegong Zhang. 2009. "DEGseq: An R Package for Identifying Differentially Expressed Genes from RNA-Seq Data." *Bioinformatics* 26 (1): 136–38. https://doi.org/10.1093/bioinformatics/btp612.

Wegehaupt, Florian J., Beatrice Sener, Thomas Attin, and Patrick R. Schmidlin. 2010. "Application of Cerium Chloride to Improve the Acid Resistance of Dentine." *Archives of Oral Biology* 55 (6): 441–46. https://doi.org/10.1016/j.archoralbio.2010.03.016.

Wharton, D A, and A T Marshall. 2002. "Changes in Surface Features during Desiccation of the Anhydrobiotic Plant Parasitic Nematode Ditylenchus Dipsaci." *Tissue and Cell* 34 (2): 81–87. https://doi.org/10.1016/S0040-8166(02)00011-3.

Xiao, Rui, and Yi Zheng. 2016. "Overview of Microalgal Extracellular Polymeric Substances

(EPS) and Their Applications." *Biotechnology Advances* 34 (7): 1225–44. https://doi.org/10.1016/j.biotechadv.2016.08.004.

Xie, Qiting, Na Liu, Daohui Lin, Ruohua Qu, Qiongzhi Zhou, and Fei Ge. 2020. "The Complexation with Proteins in Extracellular Polymeric Substances Alleviates the Toxicity of Cd (II) to Chlorella Vulgaris." *Environmental Pollution* 263: 114102. https://doi.org/10.1016/j.envpol.2020.114102.

Yan, Nieng. 2013. "Structural Advances for the Major Facilitator Superfamily (MFS) Transporters." *Trends in Biochemical Sciences* 38 (3): 151–59. https://doi.org/10.1016/j.tibs.2013.01.003.

Yang, Eun Chan, Kyeong Mi Kim, Su Yeon Kim, Jung Mo Lee, Ga Hun Boo, Jung Hyun Lee, Wendy A Nelson, et al. 2015. "Highly Conserved Mitochondrial Genomes among Multicellular Red Algae of the Florideophyceae." *Genome Biology and Evolution* 7 (8): 2394–2406. https://doi.org/10.1093/gbe/evv147.

Yoon, Hwan Su, Claudia Ciniglia, Min Wu, Josep M. Comeron, Gabriele Pinto, Antonino Pollio, and Debashish Bhattacharya. 2006. "Establishment of Endolithic Populations of Extremophilic Cyanidiales (Rhodophyta)." *BMC Evolutionary Biology* 6: 78. https://doi.org/10.1186/1471-2148-6-78.

Yoon, Hwan Su, Jeremiah D. Hackett, Claudia Ciniglia, Gabriele Pinto, and Debashish Bhattacharya. 2004. "A Molecular Timeline for the Origin of Photosynthetic Eukaryotes." *Molecular Biology and Evolution* 21 (5): 809–18. https://doi.org/10.1093/molbev/msh075.

Yuguan, Ze, Zhou Min, Luo Luyang, Ji Zhe, Liu Chao, Yin Sitao, Duan Yanmei, Li Na, and Hong Fashui. 2009. "Effects of Cerium on Key Enzymes of Carbon Assimilation of Spinach under Magnesium Deficiency." *Biological Trace Element Research* 131 (2): 154–64. https://doi.org/10.1007/s12011-009-8354-5.

Zeraatkar, Amin Keyvan, Hossein Ahmadzadeh, Ahmad Farhad Talebi, Navid R. Moheimani, and Mark P. McHenry. 2016. "Potential Use of Algae for Heavy Metal Bioremediation, a Critical Review." *Journal of Environmental Management* 181: 817–31. https://doi.org/10.1016/j.jenvman.2016.06.059.

Zhao, Haiquan, Qiuping Zhou, Min Zhou, Chunxiao Li, Xiaolan Gong, Chao Liu, Chunxiang Qu, Ling Wang, Wenhui Si, and Fashui Hong. 2012. "Magnesium Deficiency Results in Damage of Nitrogen and Carbon Cross-Talk of Maize and Improvement by Cerium Addition." *Biological Trace Element Research* 148 (1): 102–9. https://doi.org/10.1007/s12011-012-9340-x.

Zhao, Jinfeng, Shixiang Liu, Na Liu, Han Zhang, Qiongzhi Zhou, and Fei Ge. 2019. "Accelerated Productions and Physicochemical Characterizations of Different Extracellular Polymeric Substances from Chlorella Vulgaris with Nano-ZnO." *Science of the Total Environment* 658: 582–89. https://doi.org/10.1016/j.scitotenv.2018.12.019.

Zhou, Min, Xiaolan Gong, Wang Ying, Liu Chao, Mengmeng Hong, Ling Wang, and Hong Fashui. 2011. "Cerium Relieves the Inhibition of Chlorophyll Biosynthesis of Maize Caused by Magnesium Deficiency." *Biological Trace Element Research* 143 (1): 468–77. https://doi.org/10.1007/s12011-010-8830-y.

Zierenberg, Robert A, Michael W Adams, and Alissa J Arp. 2000. "Life in Extreme

Environments: Hydrothermal Vents." *Proceedings of the National Academy of Sciences USA* 97 (24): 12961–62.