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The Red Microalga *Galdieria* as a Promising Organism for Applications in Biotechnology

Mária Čížková, Milada Vítová and Vilém Zachleder

Abstract

The genus *Galdieria* refers to red algae and includes microscopic inhabitants of highly acidic (pH 1–2), often volcanic habitats. They are thermophilic or thermo-tolerant organisms, some of them surviving temperatures up to 56°C. As other extremophilic microorganisms, they exhibit unique features derived from their modified metabolisms. In this chapter, we will review the special abilities of *Galdieria* species such as metabolic flexibility to grow photoautotrophically, heterotrophically or mixotrophically, ability to utilize a whole range of unusual carbon sources, capability of surviving extreme environments or their extremely high resistance to metals. We will discuss the potential of *Galdieria* for applications in biotechnology, for example, phycocyanin production, nutrient removal from urban wastewaters, bio-mining, treatment of acidic mine drainage, selective metal precipitation, bioremediation of acidic metal-contaminated areas or recovery of critical and scarce metals from secondary sources.

Keywords: *Galdieria*, red algae, extremophiles, metals, biotechnology, recovery

1. Introduction

The alga *Galdieria* belongs to the class *Cyanidiophyceae*, which are unicellular red algae classified into three genera *Cyanidium*, *Cyanidioschyzon*, and *Galdieria*. They have a relatively simple morphology consisting of spherical thick-walled cells containing one chloroplast, 1–3 mitochondria, a nucleus, a vacuole, and storage products. They inhabit diverse environments including hot sulfur springs, streams, mud, rock walls, endolithic habitats, etc. *Galdieria* is an extremophilic organism surviving in acidic geothermal environments with very low pH and high temperatures. It exhibits a unique metabolism enabling auto-, mixo-, and heterotrophic growth. Versatility in the utilization of carbon sources for heterotrophic growth is enormous including a large range of sugars and alcohols. Besides low pH and high temperature, the *Galdieria* have several unique mechanisms for metal tolerance, enabling them to grow in environments rich in toxic metals [1, 2], where other organisms cannot grow at all or with difficulties.

For its unique extremophilic properties, the alga *Galdieria* is an unrivaled organism for biotechnological applications in surroundings with high temperatures, low pH, and high concentrations of heavy metals and organic compounds, particularly sugars. It can be cultivated under conditions where other microalgae often become contaminated with microorganisms, which is a major problem for their

application in biotechnology [3]. The autotrophic cultivation of *Galdieria* follows predominant research trends in microalgal pigments, β carotene, astaxanthin, and phycocyanin used in feed, in foods, in health applications or biofuels production. The composition of storage glycogen and lipids for biofuels can be largely changed depending on the growth conditions. *Galdieria* biomass has potential for use as food ingredients, both for protein-rich or insoluble dietary fiber-rich diets and for its low concentration of lipids.

2. Taxonomy and biodiversity

The order Cyanidiales from the class Cyanidiphyceae are a group of asexual, unicellular organisms that diverged from ancestral red algae around 1.3 billion years ago [4, 5]. These unicellular red algae were classified into three genera, *Cyanidium*, *Cyanidioschyzon*, and *Galdieria*. *Cyanidioschyzon merolae* is clearly recognizable thanks to its characteristic size and shape, but the other two algae are morphologically very similar. Until 1981 *Cyanidium caldarium* was used as a synonym for *Galdieria sulphuraria*, however, this species can grow only autotrophically while *G. sulphuraria* is also able to grow heterotrophically [6, 7]. Based on morphological, ultrastructural and ecophysiological studies the class Cyanidiphyceae was therefore instituted, containing the family Cyanidiaceae, including *Cyanidium caldarium* and the family Galdieriaceae, including *Galdieria sulphuraria* [8].

Different Cyanidiphycean species, including *Galdieria*, are found all over the world; however, their distribution is discontinuous as they are restricted to hot springs and geothermal habitats. This is related to the discontinuity of geothermal environments. Two decades ago, little was known about their biodiversity, their population structures, and the phylogenetic relationships of Cyanidiales.

Research based on environmental PCR studies revealed an unexpected level of genetic diversity among Cyanidiales. It was demonstrated that the Cyanidiales comprise a species-rich branch of red algae [9]. The high divergence rates in the Cyanidiales could be possibly explained by an elevated mutation rate in these taxa, resulting potentially from DNA damage in their extreme environments. The analyses also reject the putative mesophilic origin of Cyanidiales and suggest ancestral thermo-acidotolerancy of this lineage [9].

Sequencing of the *rbcL* gene with high sequence divergence within the genus has contributed to the taxonomy of *Galdieria* [10–12]. A cladogram defining molecular relationships among these algae shows that *Cyanidium caldarium* and *Cyanidioschyzon merolae* form a sister group relationship with *Galdieria* [11]. The genus *Galdieria* is divided into two clades, one of which includes *G. sulphuraria* accessions from Naples (Italy), California, and Yellowstone and the other one includes *G. sulphuraria* accessions from Java (Indonesia) and Russian species [11].

Based on molecular phylogenetics, three well supported *Galdieria* species exist: *G. maxima* Sentsova, *G. sulphuraria* (Galdieri) Merola (including two Russian species *G. partita* Sentsova and *G. daedala* Sentsova, described based on morphological differences) and *G. phlegrea* Pinto, Ciniglia, Cascone et Pollio [9, 13, 14].

Consequently, the main lineages were identified: *G. phlegrea* [14] comprising strains thriving in acidic non-thermophilic Italian sites; *G. sulphuraria*, a group that is geographically dispersed worldwide, including *G. sulphuraria* strains as well as *G. partita* and *G. daedala*, isolated from acid-thermal springs in Russia [15]. A final lineage contains the cosmopolitan species *G. maxima* [15], which is clustered independently from the *Galdieria* clade (*G. sulphuraria* + *G. phlegrea*) and shows an unexplained sister group relationship with the morphologically distinct *Cyanidioschyzon merolae* [5, 9, 10, 12, 16].

Generally, *Galdieria* (Cyanidiales) is well known from Italy [5, 9, 14, 17] Yellowstone National Park, USA [18, 19], New Zealand [20], Iceland [10], and recently from the Czech Republic [21, 22].

Phylogenetic analyses of the *rbcL* gene also showed that *Galdieria* from the coal mining site at Ostrava, Czech Republic, belongs to the cosmopolitan species *G. sulphuraria*, for now the only eukaryotic organism forming visible biomass on a burning coal-waste heap [10]. This was the first evidence of this species growing in central Europe, and isolates were closely related to the Italian strains, together forming the continental European lineage of *G. sulphuraria* [21]. Another, non-thermophilic strain of *Galdieria*, also found in the Czech Republic [22], referred to as CCALA 965 (Culture Collection of Autotrophic Organisms, Institute of Botany, CAS, Třeboň, Czech Republic) was found to belong to the species *G. phlegrea*, so far known only from Italy [13, 14].

3. Morphology and extremophile properties

Morphology of the unicellular taxa Cyanidiales is relatively simple. Thick-walled cells are of a spherical shape and usually contain one chloroplast, 1–3 mitochondria, a nucleus, a vacuole, and energy reserve products [8, 12, 15, 23, 24].

Representatives of the order Cyanidiales are unparalleled among phototrophic microorganisms (eukaryotes) in their ability to thrive in acidic (pH 0.5–3.5) and high temperature (38–56°C) geothermal environments. Soils, sediments, and endolithic habitats around hot springs, boiling mud pools, and steaming fumaroles are typical for these extremophiles, which are dominant in local microbial communities [5, 9, 10, 14, 17–20, 25–30]. They are the principal photosynthetic organisms found in hot acidic waters [31], where even photosynthetic prokaryotes, such as the cyanobacteria, are completely absent [32–34].

G. sulphuraria is a unicellular, spherical, spore-forming red alga. In addition to acidophilic and thermophilic properties, it has the ability to grow phototrophically, mixotrophically and moreover heterotrophically while utilizing sugars, alcohols or amino acids [7, 35, 36]. *G. sulphuraria* cells are morphologically indiscernible from cells of *Cyanidium caldarium*, however, are well recognizable thanks to their ready ability to grow heterotrophically in the dark [16, 18]. In its natural environment, *G. sulphuraria* has a yellow to green color; however, when heterotrophically grown in liquid medium, it looks like yellow-green to dark blue-green. The cell size of *G. sulphuraria* is larger than that of *C. caldarium*. It reproduces by endospore formation ranging from four to thirty-two. As originally described, *G. sulphuraria* has a single, cup-shaped, parietally localized chloroplast [8] and includes a vacuole and mitochondria [11].

Morphological similarities between *G. sulphuraria* and *G. phlegrea* are so high that methods for recognition of these species, their habitats, and growth requirements, together with molecular analyses, are used.

G. phlegrea (Figure 1) prefers a relatively low temperature (25–38°C) for growth and inhabits rather dry endolithic sites with high acidity (pH 0.5–1.5) [9, 14, 22]. The Latin word *phlegreus* means volcanic, what is consistent with the specific locality, (Campi Flegrei, Naples, Italy), where the alga was found [14]. The locality provides diverse environmental conditions in the form of hot springs, streams, mud, rock walls, with different pHs and temperature ranges, producing the different microhabitats occurring in that site. [14].

Strain DB01 of *G. phlegrea* from the Tinto River (Spain) has typical coccoid cells with thick smooth cell walls. Mature cells reach the average size of 6.4 µm. The cell possesses a blue-green chloroplast without pyrenoids. Typical for the alga is asexual

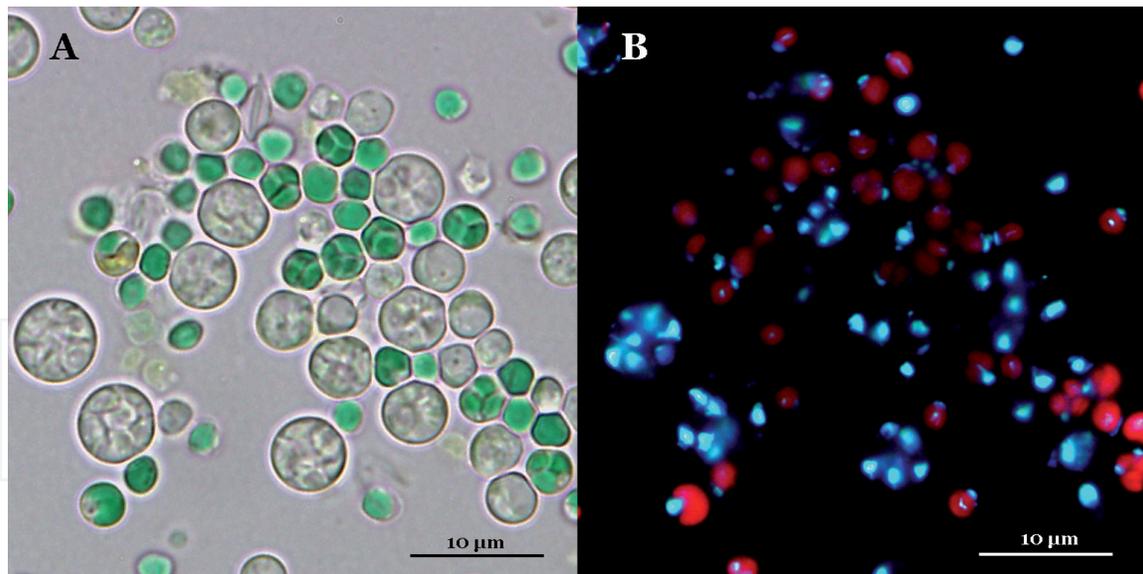


Figure 1. Microphotographs of *Galdieria phlegrea* in bright field (A) and fluorescence (B). Nuclei in the panel B are in blue (stained by DAPI) and chloroplasts are in red (autofluorescence). The chloroplasts of large mother dividing cells are not visible. The bar is 10 μm .

reproduction by autospores originating in the parental cell and resulting in auto-sporangia with 2–8 daughter cells. Testing the culture conditions of the isolate DB01 showed that the algae were not strictly thermophilic [13].

G. maxima is characterized by facultative heterotrophy; however, *G. maxima* strains grow very poorly when cultivated under dark conditions. Spherical cells are significantly larger (10–16 μm diameter) compared with other thermoacidophilic algal species [18, 23]. Cell size is thus used as the main character to distinguish one from the other. Inside the cell are at least two parietal plastids, lobe or oval shaped [37].

4. Genomes

Genetic information for the red algae *Galdieria* (*G. maxima*, *G. partita*, and *G. sulphuraria*) is located in the nucleus, in two small chromosomes, which differ in length. The smaller chromosome ranges from 0.8 to 1.8 μm and the larger one from 1.2 to 2.3 μm . The genome is characterized by an unusually high gene density, small or absent introns, and very few repetitive sequences. A genome size of 10.8 Mbp was estimated for *G. sulphuraria* [38]. In other strains of *G. sulphuraria*, genome sizes were found between 9.8 and 14.2 Mbp [39]. These genome characteristics refer to the smallest known genomes of all free living eukaryotes [38, 40].

The mitochondrial genome is extremely small in size with a very low genetic content. It is characterized by the highest guanine-cytosine content among all red algae.

The plastid genome contains a large number of intergenic stem-loop structures but is otherwise rather typical in size, structure, and content in comparison with other red algae. It is assumed that the unique genomic characteristic resulted from both the harsh conditions in which *Galdieria* lives and its unusual ability to grow mixotrophically, heterotrophically, and endolithically. The authors [41] suggested that “these conditions place additional mutational pressures on the mitogenome due to the increased reliance on the mitochondrion for energy production, whereas the decreased reliance on photosynthesis and the presence of numerous stem-loop structures may shield the plastome from similar genomic stress.”

5. Biotechnological applications

Galdieria with its extensive extremophilic properties, which are unique not only among all eukaryotic organisms but even in extremophilic prokaryotes, has a broad utilization in biotechnology. It is the only algae that can grow photo-, mixo-, and heterotrophically to biomass concentrations above 100 g/L dry weight [42]. For heterotrophic growth, it can use over 27 different kinds of sugars and polyols to produce a huge biomass and beneficial compounds [7, 43, 44]. It tolerates concentrations of glucose and fructose up to 166 g/L, salt concentrations up to 1–2 M, and pH values below pH 1 [42, 45] and can attain 80–110 g L⁻¹ biomass in continuous flow cultures [43]. The ability of *Galdieria* to grow under conditions intolerable for other organisms, even prokaryotic ones, predetermines its biotechnical applications in such surroundings as different, often toxic, wastewaters, treatment of acid mine drainage, selective metal precipitation, bioremediation of acidic metal-contaminated areas, or recovery of critical and scarce metals from secondary sources.

5.1 Wastewaters

Recycling of valuable components and nutrients from wastewaters using algae has recently been studied extensively. But only limited types of wastewaters can be treated because wastewaters are generally acidic and most algal species grow with difficulty at low pH, and absorption rates that can be achieved by bioaccumulation decrease substantially [46–49]. The acidophilic alga *G. sulphuraria* is the only alga that has commercial potential for remediation of these wastewaters [50, 51]. Nutrient removal from municipal wastewater by the alga *G. sulphurea* was found to be very efficient for ammoniacal-nitrogen (88.3%) and phosphate (95.5%) in large scale outdoor bioreactors [51]. Additionally, many crucial elements, including phosphate and rare earth elements from wastewater were successfully bio-sorbed [51, 52]. It can be concluded that *G. sulphuraria* can be applied as a preferred strain for energy-efficient nutrient removal from urban wastewaters [51], achieving higher nutrient removal efficiencies and removal rates than other strains. This alga can also be used for bio-sorption of precious metals from metal-containing wastewaters. The great advantage is that precious metals can be efficiently bio-sorbed by *Galdieria* cells even if they are present in very low concentrations. Over 90% of gold and palladium were recovered from aqua-regia-based metallic wastewater where metal concentrations were so low that they could not be recycled chemically or pyro-metallurgically. Because the entire process could be completed within 1 h, the use of *G. sulphuraria* has promising applications in metal recovery [53], particularly where Pt and Au could be selectively re-eluted from cells into a solution containing 0.2 M ammonium salts without other contaminating metals [54].

5.2 Rare earth elements

Lanthanides [Rare Earth Elements (REEs)] have unique magnetic and catalytic properties and are, up to now, irreplaceable materials in numerous technologies, for example, wind turbines, solar panels, batteries, fluorescent lamps, computer and mobile monitors, TV screens etc. They are also used as fertilizers in agriculture, in aquaculture, or as animal growth enhancers.

Methods for extraction of lanthanides from ores, including pyro-metallurgy and hydro-metallurgy, have severe negative environmental impacts, as well as being expensive. Currently, industrial extraction of lanthanides from monazite involves either a basic process that uses concentrated sodium hydroxide or an acidic process that uses concentrated sulfuric acid. These processes generate large amounts of

hazardous waste containing thorium and uranium [55]. Moreover, requirements for REEs are continually increasing, becoming critical due to risks of reduced availability of resources and their possible exhaustion.

One way to solve the problem would be efficient, sustainable, and cheap recycling of REE-containing wastewaters and others industrial wastes.

Considerable research efforts have been directed toward the development of efficient biological methods for recovering small amounts of these materials from wastewater systems [48, 49]. Research has recently focused on environmentally friendly technologies of metal recovery, including REEs, from secondary resources [56, 57] including bio-sorption by algae or cyanobacteria [52, 58]; for review, see [59, 60].

However, if REEs were present in an aquatic environment, together with other metals, most algae could not accumulate high concentrations of REEs [61, 62] due to metal-inhibited growth. The extraction of REEs or other metals have now been simplified by the use of *Galdieria* cells, which were effective in the recovery of many crucial elements, including phosphates and REEs [51, 52].

Similar to its relative *Cyanidium caldarium*, *G. sulphuraria* is resistant to high concentrations of metals in solution, including Al^{3+} . Moreover, it could be used to selectively recover lanthanides and Cu^{2+} ions from water containing various kinds of metals at a pH of 2.5. The concentration of soluble metals in solution remained unchanged at pH values within the range 0.5–5.0 [52]. In contrast, this process is usually difficult to achieve by bacterial bio-sorption. Lowering of pH to 1.0–1.5 enabled the recovery of lanthanides from cells whereas Cu^{2+} ions remained dissolved in aqueous acid. The use of *G. sulphuraria* also allowed recovery of over 90% of low levels of metals (0.5 ppm) from solution by cell fractionation at pH values in the range of 1.5–2.5. This system did not require any genetic manipulation or treatment of the cells for the efficient recovery of lanthanides [52].

Recycling from different mineral ores and electronic wastes (luminophores) often meets difficulties in that REEs are not suitable for bio-sorption because they are present in solid forms and are almost insoluble in nutrient solutions for algal cultivation. The material can be readily dissolved in aqueous acid, but the efficiency of metal bio-sorption for most algae is usually decreased under acidic conditions or the algae cannot grow at a low pH. Application of extremophilic red alga *Galdieria* would therefore be an advantageous solution to this problem and seems to be the aim of future research. The species *Galdieria phlegrea* has already been used to test the bio-accumulation of REEs from luminophores added into the medium in the form of a powder. Algal cells were cultured mixotrophically in a liquid medium with the addition of glycerol as a source of carbon. Luminophores from two different sources (fluorescence lamps and energy saving light bulbs) were tested. In spite of the low solubility of luminophores, *G. phlegrea* could grow in the presence of luminophores and accumulate REEs [63, 64].

Another rich source of lanthanides is bauxite residue, called red mud, which is a by-product of the production of alumina (aluminum oxide) from bauxite. However, less than 2% of the residue produced annually is currently being reused [65], due to difficulties related to high pH, salinity, low solid content, size of fine particles, and the leaching of metals [66]. The ability to grow in the presence of red mud and accumulate REEs was successfully tested with *G. phlegrea* (Figure 2) [67].

To conclude, the alga *G. sulphuraria* offers great potential for the direct recovery of REEs from metal-containing wastewaters (even if present at very low concentrations) or from solid waste material (luminophores) as well as for bio-mining from bauxite ore residue (red mud).

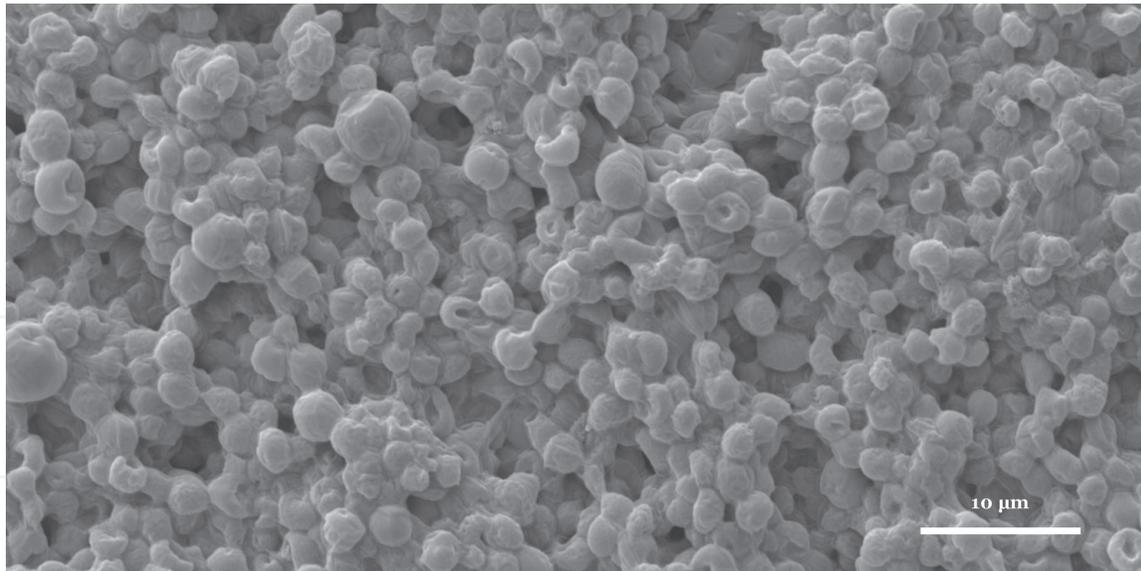


Figure 2.
Electron microphotograph of the dense freeze-dried culture of *Galdieria phlegrea* used for REEs recovery. The bar is 10 μm . (provided by Dr. Jens Hartmann).

5.3 Phycocyanin

Phycocyanin is an important compound that can be obtained from microalgal and cyanobacterial cultures. It is used as a fluorescent marker in diagnostic histochemistry [68, 69] and as a dye in foods and cosmetics [70] or as a therapeutic agent [71, 72]. Production of phycocyanin as a photosynthetic pigment in most microalgae grown heterotrophically is low and not suitable for biotechnological applications [73]. In contrast, phycocyanin as a major pigment of *G. sulphuraria* can be produced even under heterotrophic conditions in darkness [7, 74]. Due to superior biomass productivity, the productivity of phycocyanin in cultures of *G. sulphuraria* was 1–2 orders of magnitude greater than in *Arthrospira (Spirulina) platensis*, which was used recently for commercial production of phycocyanin [43] and was dependent on sunlight and climatic conditions. Besides light independence, *G. sulphuraria* tolerates a concentrated medium and can metabolize many different compounds as a source of carbon and energy [7, 75].

For example, *G. sulphuraria* could grow in restaurant and bakery waste hydrolysates in which sugars and free amino acids were utilized as substrates. Ammonium and inorganic nutrients were, however, needed in order to maximize phycocyanin synthesis [76].

The feasibility of utilizing crude glycerol (a major waste by-product of biofuel production from oilseed rape) as a carbon source for heterotrophic growth of green microalgae [54] was confirmed for *Chlorella* and also for *G. sulphuraria*.

G. sulphuraria has also been grown on sugar beet molasses [42]. Under heterotrophic conditions, phycocyanin synthesis depends mostly on available ammonium ions [77]. Ammonium sulphate was tolerated in higher molar concentrations than glucose, fructose, or sodium chloride [22, 23].

G. sulphuraria is well suited for heterotrophic growth to an extremely high cell density, which is among the highest biomass concentrations ever reported for microalgal cultures. Nearly 5% of sugar is employed for biomass yield, which is comparable to the biomass yields in industrially important heterotrophic microorganisms [78].

The high tolerance of *Galdieria* species to concentrated substances is probably an adaptation to the high concentrations of sulfuric acid and other salts present

in acidic springs. *G. sulphuraria* tolerated and grew well concentrations of glucose and fructose of up to 166 g/L (0.9 M) and an ammonium sulphate concentration of 22 g/L (0.17 M) without negative effects on specific growth rate. In carbon-limited fed-batch cultures, biomass dry weight concentrations of 80–120 g/L were obtained while phycocyanin accumulated to concentrations between 250 and 400 mg/L [42].

The ability of *G. sulphuraria* to accumulate high levels of phycocyanin in heterotrophic or mixotrophic cultures compete with or at least represents an alternative to the cyanobacterium, *Arthrospira (Spirulina) platensis* that is currently used for synthesis of phycocyanin [77].

Since a number of positive health effects have been associated with phycocyanin [79], and phycocyanin from *A. platensis* has been approved for food use in the USA and EU in 2013 and 2014, respectively, interests in applications of phycocyanin have increased substantially over recent years [80].

In addition to phycocyanin, *G. sulphuraria* could also provide floridosides suggested as a commercial products [81–83]. Its biomass was also tested and found to be a suitable and safe component in foods, as well as a dietary supplement [84].

5.4 Biofuels

The world-wide and continuous increase in fossil fuel consumption, leading probably in the relatively near future to an exhaustion of resources, has led to increased research for alternative energy sources. Production of biofuels by algae might provide a viable alternative to fossil fuels; however, this technology must overcome a number of serious obstacles before it could compete in the fuel market and be broadly deployed. Application of remarkably extremophilic *G. sulphuraria* could overcome at least some of these.

Microalgae often become contaminated with other microorganisms in large-scale outdoor cultivations, which is a major problem that inhibits algal growth and decreases the quality of biofuel and high-value products. A lack of resistance to these factors could be catastrophic for future algae farmers. The red alga *G. sulphuraria* and other species of the same genus have great potential to produce large quantities of biofuel [53] and other beneficial compounds without becoming contaminated with other microorganisms, under both mixotrophic and heterotrophic conditions. Furthermore, the algae are tolerant of pH and temperature extremes that offer a reliable means of controlling the composition of large-scale cultures.

5.5 Glycogen

The extremophilic red algae, similarly to other Rhodophyta, produce glycogen as energy and carbon reserves, instead of starch, which is characteristic of other microalgae and higher plants [81]. Glycogen, in contrast to starch, is readily soluble in cold water and more accessible by enzymes. In red algae, glycogen accumulates in a lower molecular weight form than glycogen from other microalgae and is a highly branched (higher than any other glycogen) glucose polymer [81]. Amylopectin, as a highly branched glucose polymer in starch, is used in various products such as peritoneal dialysis solutions and sports drinks. However, it is costly to prepare because of its insoluble, granular nature. The application of glycogen offers a cheaper alternative.

The alga *G. sulphuraria* can grow to a very high biomass concentration [42], accumulating glycogen up to 50% of the dry cell weight. Another advantage of this alga is that it can grow heterotrophically using many organic sources and also very cheap waste glycerol [84].

Apart from *G. sulphuraria*, the production of glycogen by most other microorganisms is too low for biotechnological applications because it is produced only under growth limiting conditions [85]. Thus, *G. sulphuraria* can be used as a cheap and efficient producer of glycogen, which could be applied as an alternative to starch in several fields [83].

Large amounts of glycogen were obtained in mixotrophic cultures, [53] where the maximum glycogen content per mL of culture was almost 10- and 2-fold greater than those of autotrophic and heterotrophic cultures, respectively. The accumulation of glycogen was enhanced by the addition of glucose, and the amount and composition of glycogen were determined by growth conditions. It is assumed that in addition to glycogen, other forms of carbon may be stored, although pathways are, as yet, unknown [75, 86].

5.6 Nutritional applications

Because of their high content of protein, algal biomass, in general, and green algae particularly, have been used in many foods, mostly in the form of dried biomass.

Difficulties in introducing microalgal-based ingredients into foods are technological and include sensorial obstacles such as its unattractive green-brownish color and unpleasant fishy smell increasing after longer storing [87]. Another problem is bacterial contamination, which decreases the commercial quality of algal biomass. Such disadvantages of green algae are not encountered using *Galdieria* species. *G. sulphuraria* can grow heterotrophically even in large-scale bioreactors under so extreme conditions that contamination by other organisms is not likely. Biomass is colorless, has a low lipid content, mainly of monounsaturated fatty acids, and oxidation during shelf life is negligible. Heterotrophic growth enables high cell densities to be achieved using cheap glycerol as a source of carbon. Consequently, in addition to other specific applications, red algal biomass can be used as a source of protein and other macronutrients. *G. sulphuraria* is rich in proteins (26–32%) and polysaccharides (63–69%), and poor in lipids. Under heterotrophic cultivation conditions, the lipid moiety mainly contained monounsaturated fatty acids. Nutritional applications of red algae were firstly suggested by Bailey and Staehelin [87], who found very high levels of protein in their cell walls.

G. sulphuraria proteins are strictly associated with polysaccharide components and therefore not digestible. However, a commercial enzyme preparation containing a mixture of polysaccharidases was developed, and *G. sulphuraria* proteins were good substrates for human gastrointestinal enzymes. *G. sulphuraria* biomass therefore has the potential to be used either for protein-rich or for insoluble dietary fiber-rich applications. Among micronutrients, some B group vitamins and pigments are present. Carotenoids are minor pigments in *G. sulphuraria*, detected only in the autotrophic algae, the main ones being astaxanthin and lutein. The absence of carotenoids under heterotrophic growth conditions is due to the lack of photosynthesis. Phycobiliproteins are present under heterotrophic and mixotrophic cultivation conditions. The cells grown on organic source of carbon frequently lose their photosynthetic antenna undermining the accumulation of the phycobilins. In *G. sulphuraria*, allophycocyanin is the dominant form in the autotrophic algae, while phycoerythrin was the main phycobiliprotein in the heterotrophic algae.

G. sulphuraria can therefore be used to develop new food ingredients, including preparations that are rich in bioavailable proteins and dietary fiber [84].

6. Conclusions

The unicellular red alga *Galdieria* is an amazing organism, not only because of its ability to live in extreme habitats, but also because of its metabolic flexibility to grow photo-autotrophically, heterotrophically or mixotrophically on diverse carbon sources. These properties make it a suitable candidate for biotechnological applications, including those inappropriate for other organisms. The biotechnological potential of *Galdieria* lies in the tremendous variability of cultivation conditions under which it is able to grow, including the ability to accumulate toxic heavy metals as well as rare ones such as Au, Pt or lanthanides. This alga can also produce biotechnologically attractive products such as phycocyanin, glycogen, protein-rich or insoluble dietary fiber-rich additives for nutritional applications. This opens up fields for its use in a wide range of industries such as the food industry, pharmaceuticals, healthcare, waste management, the metal recycling industry, bioremediation, wastewater management, etc. *Galdieria* is certainly worth the effort to research it for future uses.

Acknowledgements

We thank prof. J. D. Brooker for critical reading and language corrections of the manuscript. We are grateful to Dr. Jens Hartmann, Donau University, Krems, Austria, for the electron-microscopy photograph.

This work was supported by the National Program of Sustainability I, ID: LO1416 and by the Program Interreg V-A Austria-Czech Republic, project ATCZ172 REEgain.

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