Talanta 153 (2016) 372–380

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Rapid identification of osmolytes in tropical microalgae and cyanobacteria by ^{1}H HR-MAS NMR spectroscopy

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ARTICLE INFO

Article history:
Received 13 November 2015
Received in revised form
5 February 2016
Accepted 10 February 2016
Available online 12 February 2016

Keywords:
Tropical
Microalgae
Cyanobacteria
HR MAS
NMR
Glucosylglycerol

ABSTRACT

In this study, we report the chemical characterization of 47 tropical microalgae and cyanobacteria by HR-MAS. The generated data confirm the interest of HR-MAS as a rapid screening technique with the major advantage of its easiness. The sample is used as powder of freeze-dried microalgae without any extraction process before acquisition. The spectral fingerprints of strains are then tested as variables for a chemotaxonomy study to discriminate cyanobacteria and dinoflagellates. The individual factor map generated by PCA analysis succeeds in separating the two groups, essentially thanks to the presence of specific carbohydrates. Furthermore, more resolved signals enable to identify many osmolytes. More precisely the characteristics δ of 2-*O*-alpha-*D*-glucosylglycerol (GG) are observed in all 21 h-MAS spectra of tropical cyanobacteria. After specific extraction, complementary analysis by 1D and 2D-NMR spectroscopies validates the identification of this osmolyte.

1. Introduction

The world is projected to hold 9.6 billion people by 2050. Sustainably feeding this exploding population requires meeting great different needs simultaneously in terms of energy or food for example. Our society has to look for new resources to answer all these needs. In this context, microalgae constitute a particularly interesting resource in comparison with conventional feedstock. They are characterized by a fast growth with few nutritional demands, the ability to fix atmospheric CO_2 and to use it as carbon source, a high photosynthetic efficiency and a variety of metabolites produced that could be used in various industrial sectors [1].

Among all photosynthetic organisms, cyanobacteria inhabit the widest range of ecological habitats. They are found in cold and hot, alkaline and acidic, marine, freshwater, saline, terrestrial and symbiotic environments. The distribution of cyanobacteria stretches from polar to equatorial latitudes [2]. Cyanobacteria are able to establish competitive growth in almost any environment that has at least liquid water and sunlight [3]. They appeared approximatively 2600–3500 million years ago, based on fossil records, biomarker analysis and phylogenetic relationships with other living forms [4]. Architects of our atmosphere, these photosynthetic organisms still play an essential role in biogeochemical transformations. Indeed, great interest is being focused on cyanobacteria. Many species are capable of not only surviving but thriving in conditions thought to be inhabitable, tolerating desiccation, high temperature, extreme pH and high salinity, illustrating their capacity to acclimate to extreme environments. Cyanobacteria provide an excellent platform for the production of renewable biofuels and other products. They are characterized by containing carotenoids, chlorophyll a and phycobiliproteins [5,6]. More specially, in stress conditions as high salt concentration, cyanobacteria are able to synthesize their own compatible solutes, as well as uptake them from the surroundings [7]. Osmolytes are low-molecular mass, uncharged, hydrophilic molecules that do not interfere with cellular physiology even if they are accumulated in high amounts. These compatible solutes help restore osmotic balance with surroundings and maintain membrane integrity and protein stability [8]. These substances include several chemical groups such as carbohydrates (trehalose, sucrose), polyols (glycerol), heterosids (2-*O*-alpha-*D*-glucosylglycerol (GG)), amino acids and derivatives (proline, glycine betaine) [9,10]. While trehalose is a common compatible solute in many bacteria, plants and yeast, GG has been regarded as typical for cyanobacteria. On the other hand, the commercial interest for these osmolytes is based on

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http://dx.doi.org/10.1016/j.talanta.2016.02.024
0039-9140/

their potential medical applications. Indeed, it can be included in the development of moisturisers, skin care products and possibly play the role of protective compounds for healthy cells during chemotherapy [11].

Few data are available on the tropical microalgal biodiversity and a better knowledge of the chemical composition of these microalgae presents a major scientific and biotechnological interest. Many of these microalgae remain unknown to science, giving logical heed to explore this realm for potential application. Then, the obtained chemical finger printings for every strain were used to carry out a preliminary chemotaxonomic study; this technique was already used on various marine models to point out the differences between bacterial strains or algal species [12].

The purpose of this work is firstly to obtain an overview of the global biochemical composition of microalgae and cyanobacteria from the Southwest Indian Ocean using a non-invasive technique: the *in vivo* ^{1}H HR-MAS NMR (High Resolution Magic Angle Spinning Nuclear Magnetic Resonance). The second objective is to identify molecules of interest and more precisely osmoprotective compounds such as glucosylglycerol (GG) in the case of cyanobacteria. The last objective is to perform statistical analysis to study the correlation between their spectral print and their taxonomic group. The analyzed zone is between 3 and 4 ppm corresponding to carbohydrates. Sure enough, from the work of Chauton et al. [13], there seems to be a relation corresponding to the shifts of the carbohydrates (3–4 ppm) and the taxonomy of microalgae.

The first studied taxon is the cryptophyta. About 20 genera with 60 freshwater and marine species form the cryptophytes. Members of this group generally dominate in cool, oligotrophic waters and often form blooms in winter and early spring. Most species are photosynthetically active motile single cells. In addition to chlorophyll, a number of carotenoids and phycobiliproteins are present in the plastids [14]. The second taxon is rhodophyta. The red color of these algae is due the presence of high amounts of the biliprotein phycoerythrin in addition to the blue phycocyanin and chlorophyll a. Up to now only Porphyridium species are used biotechnologically for the production of arachidonic acid, pigments (phycocyanin, phycoerythrin) and extra-cellular polysaccharides [15]. The third taxon is dinoflagellata. Although the

Table 1
Presentation of the strains according to the taxonomic group and sampling place and growth medium.

Taxonomic group	Family	Genus	Species	Site of origin	CM	Code
Rhodophyta	ND	*cf Porphyridium*	sp.	Glorieuses	BG11	C-64
	Stylonemataceae	*Choodactylon*	*cf. ornatum*	Mayotte	BG11	C-03
Dinoflagellata	Gymnodiniaceae	*Amphidinium*	sp.	Reunion	F2	P-38
		Amphidinium	sp.	Maurice	F2	P-41
		Amphidinium	sp.	Europa	F2	P-42
		Amphidinium	sp.	Europa	F2	P-43
		Amphidinium	*massartii*	Glorieuses	F2	P-44
		Amphidinium	sp.	Glorieuses	F2	P-45
		Amphidinium	sp.	Glorieuses	F2	P-46
		Amphidinium	sp.	Madagascar	F2	P-59
		Amphidinium	*operculatum*	Madagascar	F2	P-60
		Amphidinium	sp.	Madagascar	F2	P-63
		Amphidinium	*massartii*	Reunion	F2	P-80
	Prorocentraceae	*Prorocentrum*	*lima*	Reunion	F2	P-04
		Prorocentrum	*lima*	Reunion	F2	P-08
		Prorocentrum	*lima*	Reunion	F2	P-37
	Symbiodiniaceae	*Symbiodinium*	sp. (Clade F)	Reunion	F2	P-72
		Symbiodinium	sp. (Clade B)	Reunion	F2	P-73
		Symbiodinium	sp.	Reunion	F2	P-76
		Symbiodinium	sp. (Clade D)	Reunion	F2	P-78
		Symbiodinium	sp. Y6-1	Reunion	F2	P-79
Diatoms	Bacillariaceae	*Navicula*	sp1	Reunion	F2+Si	P-91
		Navicula	sp1	Reunion	F2+Si	P-92
		Nitzschia	sp. cf. *coarctata*	Reunion	F2+Si	P-89
		Nitzschia	sp. cf. *distans*	Reunion	F2+Si	P-90
Cyanobacteria	*ND*	*Cyanothece*	sp.	Glorieuses	BG11	C-58
		Cyanobacteria	sp1	Reunion	BG11	C-18
		Cyanobacteria	sp2	Reunion	BG11	C-23
		Cyanobacteria	sp3	Reunion	BG11	C-24
		Cyanobacteria	sp4	Reunion	BG11	C-30
		Cyanobacteria	sp5	Glorieuses	BG11	C-59
		Cyanobacteria	sp6	Glorieuses	BG11	C-61
		Limnothrix	sp.	Reunion	BG11	C-33
	Pseudanabaenaceae	*Leptolyngbya*	sp1	Mayotte	BG11	C-10
		Leptolyngbya	sp2	Mayotte	Z8	C-13
		Leptolyngbya	sp. *RS01*	Mayotte	BG11	C-16
		Spirulina	sp. *cf.subsalsa*	Reunion	BG11	C-17
		Spirulina	*sp.*	Reunion	BG11	C-27
	LPP-group	LPP-group	sp. LPP1	Mayotte	BG11	C-09
		LPP-group	sp. LPP1	Mayotte	BG11	C-12
		LPP-group	sp. LPP1	Mayotte	BG11	C-14
	Ocillatoriaceae	*Roseofilum*	sp1	Reunion	BG11	C-07
		Roseofilum	sp1	Reunion	BG11	C-32
	Synechococcaceae	*Synechococcus*	*elongatus*	Madagascar	F2	C-01
		Synechococcus	*elongatus*	Glorieuses	BG11	C-60
	Merismopediaceae	*Synechocystis*	sp.	Madagascar	F2	C-02
Cryptophyta	ND	Cryptophyta	sp.	Glorieuses	F2	P-67

CM: culture medium, F2: Gillard's medium and Si: silica, BG11: BlueGreen Medium, Code: identification PHYTOBANK collection; ND: not determined at the moment.