International Biodeterioration & Biodegradation 123 (2017) 236–243

Contents lists available at ScienceDirect

International Biodeterioration & Biodegradation

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

Heat shock treatments for the control of lithobionts: A case study with epilithic green microalgae

Stefano Bertuzzi [a], Lydia Gustavs [b], Gaia Pandolfini [a], Mauro Tretiach [a, *]

[a] Department of Life Sciences, University of Trieste, Italy
[b] Department of Applied Ecology and Phycology, Institute of Biological Sciences, University of Rostock, Germany

ARTICLE INFO

Article history:
Received 2 March 2017
Received in revised form
29 June 2017
Accepted 29 June 2017
Available online 18 July 2017

Keywords:
Biocides
Biodeteriogens
Chlorophyll *a* fluorescence
Hydration
Polyols
Non-reducing sugars

ABSTRACT

Heat shock treatments are an innovative and eco-friendly method to devitalize biodeteriogens of outdoor stone monuments. They consist in short (up to 6 h), mild (40–60 °C) thermal treatments applied to artificially wet surfaces, and are effective against lichens and bryophytes. Epilithic green algae, a polyphyletic and diversified group of photoautotrophs, are among the most important colonizers of stone monuments, forming conspicuous biofilms which cause chemical, physical and aesthetical damage to the substratum. Like lichens and bryophytes, they are able to face the extreme conditions of a stone surface but their resistance mechanisms are only partially known. The present study aims to test the applicability of heat shock treatments to six morphologically and phylogenetically distant green microalgae. Their survival mechanisms have been investigated in relation to photosynthesis and content of selected polyols and non-reducing sugars. Chlorophyll *a* fluorescence measurements and observations at the epifluorescence microscope demonstrate that the thermal treatments cause negative effects on all the species, although they do not necessarily kill the whole populations, as observed in all the lichens and bryophytes tested so far. The survival capability is discussed in relation to the production of extracellular polymeric substances and non-reducing sugars.

1. Introduction

An important goal in the field of stone surface restoration is the control of biodeteriogens (Warscheid and Braams, 2000). This is commonly achieved by using chemical products containing one or more active principles that can be dangerous for the workers, the environment and the substrata (Caneva et al., 1996, 2008). Several less invasive techniques have been proposed, from the use of nanosilver (Essa and Khallaf, 2014) and TiO_2 particles (Graziani and D'Orazio, 2015) as nano antifoulant coatings, to physical methods (Salvadori and Charola, 2011). Tretiach et al. (2012) and Bertuzzi et al. (2013) proposed a new devitalization technique, which is simple to apply, cheap and eco-compatible, because it consists in the warming at 40–60 °C of artificially hydrated surfaces colonized by the biodeteriogens. The heat shock treatments (HSTs) may be very effective. Treatments of 6–12 h devitalized all the lichens and bryophytes tested so far (Tretiach et al., 2012; Bertuzzi et al., 2013). These poikilohydrous organisms are generally considered as stress-tolerant, but this is true only when they are dry, when their metabolism is in a quiescent state. Inversely, they are highly sensitive to the increase of their temperature if partially or fully hydrated, because they are metabolically active (Lange, 1955; Nörr, 1974; Meyer and Santarius, 1998; Glime, 2007).

HSTs can be used alone or in combination with traditional chemical applications: in this case the devitalization effects of the biocides are increased also at low concentrations, and application periods are shortened (Tretiach et al., 2012; Bertuzzi et al., 2013). Most probably, the new technique might be extended to other poikilohydrous organisms, as literature data suggest, but further experimental work is badly needed (Tretiach et al., 2012).

A key group of litho-biodeteriogens is represented by epilithic green microalgae (EGM), for their important role in the early colonization of stone surfaces (Rindi, 2007). With a strong tendency to be cosmopolitan and with a relatively low substrate specificity, EGM can flourish as soon as light and water are available, often forming biological patinas (biofilms). These soon include other deteriogenic organisms such as bacteria, fungi and protozoa (Gaylarde and Morton, 1999), and promote the later colonization by lichens, bryophytes and vascular plants. Biofilms have attracted particular attention when growing on anthropogenic substrata

* Corresponding author.
E-mail address: tretiach@units.it (M. Tretiach).

http://dx.doi.org/10.1016/j.ibiod.2017.06.023
0964-8305/

such as stone heritage (Cutler et al., 2013; Polo et al., 2012), building facades and roof tiles (Häubner et al., 2006; Cutler and Viles, 2010; Shirakawa et al., 2011; Hallmann et al., 2013; Gladis-Schmacka et al., 2014). Besides the aesthetical damage, phototrophic biofilms can cause physical damage to the substratum (Cutler and Viles, 2010; Hallmann et al., 2013; Caneva et al., 2008), whereas the associated fungal and bacterial components may have harmful consequences for humans for the release of allergens (Karsten et al., 2007). Moreover, algal biofilms change the physicochemical properties of the substratum: they increase water absorption, slow evaporation, favour heat retention and accumulation of pollutants (Steiger et al., 1993; Decho, 2000). Furthermore, several EGM excrete chelating agents and/or organic acids, that sequester metal cations (Young and Urquhart, 1998) and cause bio-corrosion of the substratum (Ortega-Calvo et al., 1995). The latter phenomenon is further enhanced by the release of carbon dioxide derived from respiration, which readily dissolves carbonate-containing minerals through the formation of carbonic acid in aqueous solution (Griffin et al., 1991).

The major part of EGM can survive harsh, hostile, rapidly changing conditions: many of them withstand UV-radiation, temperature extremes and prolonged periods without liquid water (Potts, 1994; Yancey et al., 1982). The survival to the fluctuating water availability typical of terrestrial habitats is often a matter of avoidance vs tolerance mechanisms, when not a mixture of these two strategies. If colony formation, excretion of extracellular polymeric substances (EPSs), and thick cell walls, often containing sporopollenin and cellulose, avoid or delay water loss, the biosynthesis and accumulation of organic compounds known as “compatible solutes” represent a key factor in desiccation-tolerant EGM (Karsten et al., 2007; Oren, 2007; Gustavs et al., 2010). Polyols in particular increase significantly under osmotic stress conditions in several terrestrial algae. These sugar-alcohols have generally been interpreted as organic osmolytes, thus causing water reflux and balancing osmotic pressure (Yancey, 2005). Polyols are soluble at high concentrations in water and uncharged at physiological pH values, and they exert multiple functions in metabolism. Besides their role as organic osmolytes, polyols can also act as compatible solutes, antioxidants, rapidly available respiratory substrates, and heat protectants (through the stabilization of proteins and other macromolecules) (Eggert and Karsten, 2010 and references therein). Non-reducing sugars, on the contrary, contribute to the transient “vitrification” of the cytoplasm, i.e. the formation of a glassy state, from which the normal status of the cytosol can quickly be recovered as soon as water becomes available. In addition, non-reducing sugars appear to substitute for water, thus maintaining the structure of membranes when water leaves a dehydrating cell (Crowe et al., 1998, 2005; Fernández-Marín et al., 2013).

Since not all EGM are desiccation-tolerant (Büdel, 2011; Holzinger and Karsten, 2013), it could be questioned whether HSTs are really effective against algal biofilms, because some taxa might reveal unsuspected heat tolerance also in the wet state in relation to their peculiar biology and evolutionary history.

This study was therefore designed *(i)* to test the applicability of HSTs on a group of EGMs selected for their contrasting morphology and different phylogenetical position, growth habitat preference and resistance mechanisms, and *(ii)* to investigate to which extend their response is related to selected low molecular weight carbohydrates, namely polyols and non-reducing sugars.

2. Materials and methods

2.1. Organisms and culture conditions

Six microalgae were investigated, five isolated from natural or artificial stone substrata and one isolated from the lichen *Flavoparmelia caperata* (Supplementary Information S1). *Coccomyxa subellipsoidea* Acton (NIES 2166), *Coenochloris* sp., *Pleurochloris* sp. and *Stichococcus bacillaris* Nägeli were grown on solid 3NBBM + V (http://www.uni-goettingen.de/en/list-of-media-and-recipes/186449.html); *Apatococcus lobatus* (Chodat) J.B.Petersen (SAG, 2096) and *Trebouxia gelatinosa* Archibald were grown on solid 3NTM (3 Nitrogen Trebouxia Medium; 1.5% agar) obtained by adding to the liquid BBM 15 g l^{-1} bacteriologic agar (gel strength 845 g cm^{-2}), glucose (20 g l^{-1}), casein peptone (10 g l^{-1}) and sodium nitrate (0.5 g l^{-1}). All strains were cultured in a growth chamber at 20 ± 1 °C, with a light/darkness regime of 14/10 h, and 20 ± 1 μmol photons m^{-2} s^{-1}. Light was provided by neon lamps (Sylvania Grolux T8F36W/GROG13F36W), and was checked with a Micro-Quantum 2060-M Sensor (Walz, Effeltrich, DE). The cultures for the experiments were prepared by making suspensions of algae in tubes containing 4 ml of distilled, sterilized water and by inoculating 50 μl of a cell water suspension on hand-cut sterile filter paper discs (Whatman, 60 ± 5 g m^{-2}, diam. 15 mm). The discs were laid on solid 3NBBM + V or TOM inside Petri dishes; 6 discs were placed in each Petri dish. The cultures were grown at the culture conditions mentioned above for 4 weeks before exposure.

For the quantification of polyols, algae were pre-cultivated at 20 °C and 40 μmol photons m^{-2} s^{-1} (L 36W/954 Lumilux de lux daylight, Osram, Munich, DE) at a light-dark cycle of 16:8 h for two weeks on solid 3NBBM + V (medium 26a in Schlösser, 1997). Biomass was harvested with a sterile loop and diluted into 1 ml of liquid 3 NBBM + V. For each species, two Petri dishes were equipped with solid 3 NBBM + V and four sterile cellulose acetate (CA) filter discs (Roth, Karlsruhe, DE). Each disc was inoculated with 100 μl of the algal suspension, dried for 5 min under a sterile air laminar flow and the Petri dishes were sealed with parafilm.

2.2. Desiccation and heat shock treatments

Twelve hours before the beginning of HSTs, 12 culture discs for each species for each treatment [DRY and WET (see below), at 20 °C, 40 °C and 60 °C] were laid on a glass sterilized Petri dish (15 cm in diam.) in the space between the wall and a smaller open Petri dish (5 cm in diam.) positioned at the centre, containing silica gel (DRY treatments) or distilled water (WET treatments). The bigger Petri dishes were closed and sealed with alimentary cling film and left in the growth chamber overnight (c. 14 h). Then the Petri dishes were introduced in an oven at 20 ± 1, 40 ± 1, 60 ± 1 °C for 6 h. After this exposure, the Petri dishes were returned to the original growth chamber at c. 20 °C for acclimation, and finally the samples were processed for the standard Chl_aF measurements and for the epifluorescence observations, that were carried out after 2 h and after 2 weeks of recovery. Photographs of the algal cultures were taken before and after exposure, and after recovery with a Nikon Coolpix 8400 (Chiyoda, Tokyo, JP).

To monitor the polyols concentration in response to a decreased cell water content, four of eight discs per species were placed in a polyethylene humidity chamber filled with saturated KCl solution which created a relative humidity (RH) of 70% in the headspace (Häubner et al., 2006; Karsten and Holzinger, 2012). The chambers were equipped with four ca. 3 cm long glass columns carrying a perforated metal grid above the saturated solution. The discs with algal biomass were placed on the top of the metal grid and the whole chamber was closed air-tightly to facilitate the development of a stable relative air humidity while the remaining four discs were kept as a control in the Petri dishes. The conditions inside one chamber were continuously checked using a combined humidity/temperature sensor (MSR 145, Seuzach, CH). The humidity chambers were kept under the standard cultivation conditions and daily