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Environmental parameters, and not phylogeny, determine the composition of extracellular polymeric substances in microbial mats from extreme environments

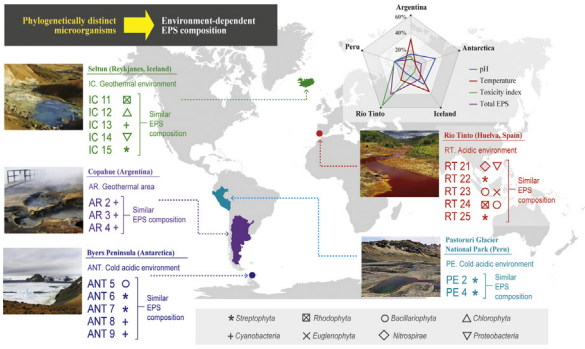
Yolanda Blanco [a], Luis Alfonso Rivas [a,1], Elena González-Toril [a], Marta Ruiz-Bermejo [a], Mercedes Moreno-Paz [a], Víctor Parro [a], Arantxa Palacín [a,2], Ángeles Aguilera [a], Fernando Puente-Sánchez [b,*]

[a] Centro de Astrobiología (INTA-CSIC), Instituto Nacional de Técnica Aeroespacial, Carretera de Ajalvir Km 4, Torrejón de Ardoz, 28850 Madrid, Spain
[b] Systems Biology Program, Centro Nacional de Biotecnología, C/Darwin n° 3, Campus de Cantoblanco, 28049 Madrid, Spain

HIGHLIGHTS

- Biofilm formation provides stress resistance in extreme environments.
- We analyzed 20 biofilms taken *in situ* from five contrasting extreme environments.
- Environment, rather than phylogeny, determined exopolymeric substances composition.
- Abundance of particular sugars could be traced to different environmental stressors.
- Optimal biofilm composition is a hallmark of the adaptation to novel environments.

GRAPHICAL ABSTRACT



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ABSTRACT

The ability to establish biofilms is a key trait for microorganisms growing in extreme environments. The extracellular polymeric substances (EPS) present in biofilms provide not only surface attachment, but also protection against all kinds of environmental stressors, including desiccation, salinity, temperature or heavy metal pollution. The acquisition of suitable biofilm characteristics might thus be an important process mediating the adaptation of microorganisms to novel environmental conditions. In this work we have characterized the EPS of 20 phylogenetically diverse biofilms collected *in situ* from five contrasting extreme environments, including two geothermal areas (Copahue, Argentina; Seltun, Iceland), two cold areas (Pastoruri glacier, Peru; Byers Peninsula, Antarctica) and one extremely acidic river (Río Tinto, Spain). Biofilms were subjected to biochemical characterization, glycan profiling and immunoprofiling with an antibody microarray. Our results showed that environmental conditions strongly influence biofilm characteristics, with microorganisms from the same environment achieving similar EPS compositions regardless of the phylogeny of their main species. The concentration of some monosaccharides in the EPS could be related to environmental conditions such as temperature or heavy metal toxicity, suggesting that in some cases stress resistance can be mediated by specific sugars. Overall, our results highlight the existence of conserved EPS compositional patterns for each extreme environment, which could in turn be exploited to engineer ecological adaptations in genetically modified microorganisms.

* Corresponding author at: Centro Nacional de Biotecnología, C/Darwin n° 3, Campus de Cantoblanco, 28049 Madrid, Spain.
E-mail address: fpuente@cnb.csic.es (F. Puente-Sánchez).
[1] Current address: Inmunología y Genética Aplicada, INGENASA, C/Hermanos García Noblejas, 41, 28037 Madrid, Spain.
[2] Independent researcher.

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1. Introduction

Exploration of extreme terrestrial environments has turned up a diverse assortment of microorganisms that not only live, but also thrive, at environmental extremes previously thought to be inhospitable to terrestrial life. These conditions include extremes of pH, temperature, pressure, radiation, water availability, salinity or nutrient concentration (Rothschild and Mancinelli, 2001). However, in spite of the apparent hostility of such habitats, they contain a higher level of biodiversity and biomass than expected. Additionally, most of the microbial communities found in extreme environments are distributed and assembled in extensive biofilms and microbial mats. Although the development of these structures in aquatic and terrestrial environments has been well documented (reviewed by Bolhuis et al., 2014), the distribution pattern and factors controlling their development in extreme environments are not yet well understood.

The growth of microbial mats and biofilms is promoted by the excretion of extracellular polymeric substances (EPS) by the cells, which serves as an adhesive agent enabling cellular attachment and form the biofilm matrix embedding the cells (Wolf et al., 2007). EPS are a complex mixture of biomolecules, *e.g.*, proteins, humic-like substances, polysaccharides, uronic acids, nucleic acids, lipids, and glycoproteins, surrounding the cells (Sheng et al., 2010). EPS make up the intercellular space of microbial aggregates and form the structure and architecture of the biofilm matrix. The key functions of EPS comprise the mediation of the initial attachment of cells to different substrates and protection against environmental stress and dehydration (Sutherland, 2001). Besides, EPS can afford a stable environment, promoting the growth of organisms. Thus, it prevents the losses of organisms and retains diversity over a long period of time, allowing the development of synergistic relationships among species (Ras et al., 2011).

These EPS secretions may constitute up to 60% of the dry biomass (Hill et al., 1997), and their presence is considered tightly related to the capability of the organisms to successfully cope with environmental constraints and with the formation of complex microbial mats on a great variety of substrates. Thus, since the extracellular matrix is a vital and complex component of all biofilms, it has to play an even more important role in the development of microbial communities in extreme environments by providing a suitable architectural structure, mechanical stability and protection against external conditions, which are incompatible with most life forms.

Although literature on biofilm characterization is abundant, a large part of it is focused on either clinical isolates (Hall-Stoodley et al., 2008; Gunn et al., 2016; Zhang et al., 2018) or man-made systems such as water treatment plants or microbial fuel cells (Miqueleto et al., 2010; Sheng et al., 2010; Read et al., 2010; Salama et al., 2016). On the other hand, studies focusing on natural environments are often performed on environmental isolates rather than in biofilms collected *in situ* from their native habitats (Nichols et al., 2005; Ortega-Morales et al., 2007; Ren et al., 2015). Finally, studies often focus on a single species and/or on a single environment (Nichols et al., 2005; Goltsman et al., 2015; Caruso et al., 2018), which precludes the detection of phylogenetic or environmentally determined patterns in biofilm composition.

In this work we have characterized the microbial EPS composition in 20 phylogenetically distinct benthic biofilms from different extreme environments. Since temperature and pH are among the most restrictive environmental parameters for the presence of microorganisms, five different extreme environments were selected taking into consideration these parameters, (i) two extreme geothermal areas, the Copahue Volcano region (Neuquen, Argentina) and the Seltun area (Reykjanes, Iceland), where water temperature can reach up to 77 °C and pH range from 3 to 7 (Aguilera et al., 2010; Urbieta et al., 2014, 2015), (ii) Pastoruri Glacier area (Huascarán National Park, Perú), an extreme cold acidic ecosystem (pH 2–5, T^a 4–10 °C) (González-Toril et al., 2015), (iii) Río Tinto (Huelva, Spain), a temperate extremely acidic river (pH 1.5–3, 20–25 °C) showing high levels of heavy metals (mg/L in most cases) (Aguilera et al., 2007; Aguilera, 2013), (iv) Byers Peninsula (Livingston Island, Antarctica), an extreme cold natural environment (pH 6–7, T^a 4–5 °C) (Quesada et al., 2009).

The environmental parameters (pH, temperature, conductivity, and heavy metal content) were measured at each sampling point, and the biofilms were collected and further analyzed in the laboratory. The biofilms were classified based on their phylogeny, using a combination of microscopic and molecular techniques, and their EPS were extracted and characterized by a mixture of biochemical techniques, glycan profiling and immunoprofiling with an antibody microarray. Our results highlight the existence of conserved EPS compositional patterns for each extreme environment, which are independent of the phylogenetic composition of the biofilm. This suggests that the acquisition of optimal biofilm characteristics is a hallmark of microbial adaptation to novel environments.

2. Material and methods

2.1. Field sites and sample collection

Twenty microbial mats and biofilms from five different extreme environments were analyzed (Fig. S1). *In situ* measurements of water pH, temperature, redox potential and conductivity, were carried out as described previously (Santofimia et al., 2013) (Table 1). Water samples were filtered through 0.45 μm Millipore membranes. Total concentrations of nine recoverable metals were measured for each water sample (Zn, Cu, Fe, Co, Ni, As, Cd, Cr and Pb) using X-ray Fluorescence Reflection (TXRF) and Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) (Santofimia et al., 2013). Microbial mats and biofilms were taken using a sterile plastic spatula and then placed in 50 mL Falcon tubes. The samples were immediately frozen in dry ice, freeze-dried and kept in the laboratory at −20 °C until the analyses were carried out.

2.2. Morphotype and molecular identification

Since eighteen samples were mainly formed by photosynthetic species (algae and cyanobacteria), microscopic identification and quantification of species were carried out. Thus, subsamples of *ca.* 2 cm^2 from each mat or biofilm were taken, resuspended in 5 mL of BG11 media (Rippka et al., 1979) at the different environmental pH and preserved at 4% formaldehyde. Species identification was carried out by direct microscopic observation down to the lowest possible taxonomic level using different phenotypic features based on previous studies of these communities (Aguilera et al., 2007; Souza-Egipsy et al., 2008; Aguilera et al., 2010; González-Toril et al., 2015; Urbieta et al., 2014; Urbieta et al., 2015). Cell counts were performed in triplicate in a Sedwerick-Rafter chamber. A Zeiss Axioscope 2 microscope equipped with phase-contrast was used in this work.

Biofilms IC14 and RT21 were predominantly composed by bacteria. In these cases, DNA extraction, PCR amplification, cloning and sequencing of the 16S RNA gene were carried out in order to identify the prokaryotic microbial diversity, following previously reported methodology (Souza-Egipsy et al., 2008; González-Toril et al., 2015; Urbieta et al., 2014; Urbieta et al., 2015). Briefly, Fast DNA Spin kit for soil (Q-Bio Gene Inc., CA, USA) was used for DNA extraction according to the manufacturer's instructions. DNA obtained was purified by passage through a GeneClean Turbo column (Q-Bio Gene Inc., CA, USA). The 16S rRNA genes were amplified according to previously described methodologies (Souza-Egipsy et al., 2008; García-Moyano et al., 2012) using the universal Bacteria-specific primers 27f and 1492r (Achenbach and Woese, 1995; Lane, 1991). Archaea-specific primers 21f and 1492r (DeLong, 1992) were also assayed. PCR amplified genes were purified by GeneClean Turbo Column (Q-Bio Gene Inc., CA, USA) and cloned using the Topo TA Cloning Kit (Invitrogen, CA, USA). Primers used for sequencing were primers M13f and M13r recommended by