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BIOCOLONIZATION AND BIODETERIORATION OF MONUMENTS BY CYANOBACTERIA

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ABSTRACT

Cyanobacteria are a morphologically diverse, remarkably adaptable and widely distributed group of photosynthetic prokaryotes, many of which colonize, grow and survive on/in water-limited and nutrient-poor lithic substrates, such as natural rocks/stones and walls of historic monuments and buildings as lithobionts or lithobiontic organisms. They are often the primary colonizers of lithic substrates. They possess protective mechanisms against various abiotic stresses, such as desiccation, high irradiance, high levels of UV-radiation and high temperature which are often encountered on exposed rock surfaces and external walls of lithic monuments and buildings. The biocolonization and growth of cyanobacteria as lithobionts are influenced by various propertiesof monuments, buildings or buildingstones as well as by environmental factors. As photoautotrophs and primary colonizers of lithic substrates, they facilitate and promote the growth and development of heterotrophic microbes, such as bacteria and fungi.The production of extracellular polymeric substances (EPS) leads to the formation of cyanobacteria-dominated phototrophic biofilms or sub-aerial biofilms on exposed surfaces of monuments, buildings and rocks/stones. Globally, thebiocolonization, growth and activities of lithobiontic cyanobacteria and other organisms cause unpleasant discoloration, biodeterioration (bioweathering) of monuments and buildings of historical, cultural or religious importance, leading to the aesthetic damage, structural damage and economic loss.These constitute serious problems world-wide. The article provides an overview of the processes of biocolonization and biodeterioration of monuments by lithobiontic(rock-inhabiting) cyanobacteria, and their survival as lithobionts.

Keywords: Cyanobacteria, Lithobiontic, Bioreceptivity, Biofilms, Biocolonization, Biodeterioration

INTRODUCTION

Cyanobacteria, which are also called blue-green algae, area widely distributed group of oxygenic photosynthetic prokaryoteshaving remarkable ecological and economic importance (Stanier and Cohen-Bazire, 1977; Vincent, 2000; Bullerjahnand Post, 2014; Patterson, 1996; Pandey, 2017; Skulberg, 2000).Their morphology is diverse, comprisingunicellular, colonial and filamentous (branched or unbranched and heterocystous or non-heterocystous) forms, andtheir size range from microscopic picoplankton to macroscopic colonies. The presence of oxygenic photosynthesis andthe occurrence of diazotrophy (nitrogen fixation) inmany genera make them unique among prokaryotes. Their metabolic versatility, adaptability to varying environmental conditions and protective mechanisms against various abiotic stresses enable them to colonize, grow and survive in a wide range of terrestrial and aquatic habitats, including those with extreme conditions as encountered on/in lithic habitats, such as natural rocks, monuments and buildings (Tandeau de Marsac and Houmard, 1993; Budel, 1999; Potts, 1999; Ehling-Schulz and Scherer, 1999). The lithobiontic (rock-inhabiting) cyanobacteria, which include epilithic (growing on the external surface of rocks) and endolithic (growing inside the rocks) genera, cause unpleasant discoloration and contribute significantly, along with other organisms(e.g. algae, bacteria, fungi and lichens), to the biodeterioration or bioweathering of monuments and buildings of historical, cultural or religious importance, leading to the decrease in their aesthetic value, structural damage and economic loss (Crispim and Gaylarde, 2005; Crispim *et al*., 2003; Crispim *et al*., 2006; Crispim *et al*., 2004; Gaylarde and Gaylarde, 1999; Gaylarde and Gaylarde, 2005). Monuments and architectural buildings contribute significantly to the tourism and economy of a country. The restoration and conservation of important stone-made monuments, buildings and artefacts deteriorated by various physical, chemical and biological factors/agents are serious issues world-wide.

Biocolonization and Growth of Cyanobacteria as Lithobionts

Microbial populations occurring on a stone substratum are due to the successive colonization by different microorganisms over the years. Cyanobacteria are often the primary colonizers of nutrient-poor and water-limited rock surfaces in light-exposed environments (Budel, 1999). They can colonize and affect rocks or stones in their natural state as well as when they are used as building stones in monuments and buildings. Due to the photoautotrophy along with diazotrophy (nitrogen fixation) in many cases, they have simple nutritional requirements which enable them to grow and survive on nutrient-poor lithic substrates. The total properties of a substrate that determine its susceptibility to colonization by microorganisms or other organisms is known as bioreceptivity (Guillitte and Dreesen, 1995). Due to microscopic size and abundant population, cyanobacterial cells, filaments, propagules and resting spores can be transported or dispersed passively by wind to varying distances and heights. The colonization process starts when an organism (or its propagules and resting spores) settle down on a suitable substratum and finds the optimal and conducive ecological conditions for growth and multiplication. The nature and properties of rocks as well as environmental conditions determine the type of lithophytic community colonizing the rocks. Different lithotypes used in the construction of monuments and buildings, such marble, sandstone limestone, travertine, dolomite, and granite are susceptible to colonization by cyanobacteria at varying degree (Macedo *et al*., 2009). Cyanobacteria can grow on the stone surface as epiliths (epilithobionts) oras endoliths(endolithobionts) in the pores, fissures, cracks and cavities of the stone (Crispim and Gaylarde, 2005; Gaylarde *et al*., 2012).They are adaptable to grow at low light intensity and, therefore, can occur deeper in the porous and translucent stone/ rocks as well as on the internal wall surface of monuments and buildings (Lyalikova and Petushkova, 1991; Saiz-Jiminez, 1990). The microhabitat inside the rocks protects the endolithic cyanobacteria from intense solar radiation, temperature fluctuationsand desiccation, and provides them retained moisture, mineral nutrients and growth surfaces (Friedmann, 1982; Friedmann and Ocampo-Friedmann, 1984; Bell, 1993; Walker *et al*., 2005).

Thecolonization and growth of cyanobacteria and other microorganisms on exposed surfaces of lithic monuments and buildings are influenced by various physical and chemical properties of monuments and buildings, such as surface roughness, texture, hardness, mineralogical composition, moisture retention, pH, porosity and permeability as well as by the ambient environmental factors, such as temperature, relative humidity, rainfall, wind and light (Guillitte and Dreesen, 1995; Gaylarde*et al*., 2003; Miller, 2012). Exposed lithic habitats present extreme or harsh conditions, such as frequent and prolonged desiccation, high and variabletemperature, highirradiance, high levels of ultraviolet (UV) radiation and nutrient scarcity, posing multipleabiotic stresses for the growth and survival of cyanobacteria. Moreover, many abiotic stresses act as factors to induce oxidative stress in the organisms. Cyanobacteria are known to possess effective mechanisms for desiccation tolerance, photoprotection against high light intensities and UV-radiation, protection against high temperature and protection against oxidative stress (Potts, 1994, 1999; Groniger *et al*., 2000; Ehling-Schulz and Scherer,1999; Adhikary, 2003, 2004; Qiu *et al*., 2003). The protective mechanisms against various abiotic stresses constitute eco-physiological adaptation or survival strategies of cyanobacteria to grow on exposed surfaces of rocks and buildings. The protection of cyanobacteria against the frequently encountered dessiccation stress is attributed to the production of extracellular polymeric substances (EPS) which regulate loss and uptake of water from cells (De Philippis and Vincenzini, 1998; Adhikary, 1998; Caiola *et al*., 1993, 1996). The mucilaginous EPS provide hydrated microenvironment to cyanobacterial cells (Rossiand De Philippis, 2015).The extracellular polymeric substances, often called exopolysaccharides, consist of various sugars, such as glucose, galactose, rhamnose, fucose, arbinose, xylose, mannose, and acidic residues of glucuronic acid and galacturonic acid (Drewsand Weckesser, 1982; De Philippis and Vincenzini, 1998, 2003; Adhikary, 1998). The EPS are also known as slimes, sheath or capsule.In addition to the protection against dessiccation, cyanobacterial EPS play crucial roles in biocolonization, cell aggregation and biofilm formation on lithic surfaces.

Phototrophic Biofilm Formation

As photoautotrophs andprimary colonizers of light-exposed lithic habitats or substrates, the growth of cyanobacteriaprovides a significant input of organic matter to the stone substrates, facilitating and promoting the growth of heterotrophicor chemoorganotrophic microbes like bacteria and fungi. This leads to the formation of laminated subaerial biofilms (SAB) on exposed surfaces of rocks, monuments and buildings. The microbial communities in biofilms are held together and attached to underlying surfaces by EPS (Gorbushina, 2007; Gaylarde and Morton, 1999; Crispim and Gaylarde, 2005; Tomaselli *et al*., 2000). Phototrophic biofilms refer to the light-driven and surface-attached microbial communities characterized by the obvious presence of photosynthetic organisms (e.g. cyanobacteria, microalgae and diatoms) which produce and provide organic substrates and oxygen to heterotrophic microbial components. Thick multilayered phototrophic biofilms are usually referred to as microbial mats or phototrophic mats (Stal, 1995; Stal *et al*., 1985; Ward *et al*., 1998). Pollutants, dusts and other particulate matter from the environment can be trapped by the biofilms developed on the lithic surfaces. Epilithic cyanobacterial assemblages or biofilms are commonly referred to as crusts and patinas in cultural heritage studies. The formation of biofilms or crusts results in higher and longer moisture retention at the stone surface, increasing the susceptibility of the surface for further colonization.

Lithobiontic Cyanobacteria as Agents of Biodeterioration

Biodeterioration, also called bio weathering or biogenic weathering, can be defined as the undesirable change in the properties, qualities or value of a material or structure due to the growth and activities of living organisms, such as bacteria, fungi, cyanobacteria, algae, lichens, mosses and liverworts. The organisms capable of causing biodeterioration are known as biodeteriogens**.** In case stone or stone-made monuments, buildings and artefacts, it is geophysical and geochemical processes mediated by biological agents, leading to undesirable changes in mechanical (physical) and chemical properties which ultimately results in structural and aesthetic damage. Bio deterioration caused by microorganisms is a complex process that involves the interaction of various microbes with the stones or building materials of monuments/ buildings and the ambient environment.

Biodeterioration of lithic (stone-made) monuments, buildings and artefacts induced by various microorganisms, including cyanobacteria is a serious problem all over the world as it results not only in considerable reduction in aesthetic value and structural deformities but also in economic loss due to high expenses involved in their restoration and conservation. In addition to directly causing biodeterioration of monuments and buildings, cyanobacteriacan indirectly damage them by promoting and facilitating the growth of heterotrophic microorganisms like bacteria and fungi which can show stronger deteriorating activity (Tiano, 1993; Tomaselli *et al*., 2000; Crispim *et al*., 2003; Zurita *et al*., 2005). The co-existing heterotrophs can utilize the photosynthetic products, such as sugars and polysaccharides produced and secreted by cyanobacteria. The lithobiontic cyanobacteria and heterotrophs can act synergistically in the process of biodeterioration. Heterotrophs (bacteria and fungi) are known to release various inorganic and organic acids which weaken the mineral matrix of stone substratum by solubilizing or chelating the minerals (Warscheid and Braams, 2000; Gaylarde *et al*., 2003; Dakaland Cameotra, 2012; Wakefield and Jones, 1998).

Lithobiontic cyanobacteria implicated in biodeterioration of a wide range of lithic (stone-made) monuments, architectural structures and artefacts, such as cathedral, chapel, church, monastery, mosque, temples, palace, pyramids, historical buildings, statues, tombs and towers have been reported from various countries, lying in tropical, subtropical and temperate regions, of the world. They constitute major or dominant component of epilithic biofilms. The lithobiontic cyanobacteriamost widespread and commonly reported belong to both coccoid (unicellular and colonial) and filamentous forms, andinclude the genera *Synechococcus*, *Synechocystis*, *Chroococcus*, *Gloeocapsa*, *Gloeothece, Chroococcidiopsis*, *Xenococcus, Myxosarcina*, *Phormidium*, *Lyngbya*, *Leptolyngbya*, *Calothrix*, *Plectonema*, *Pleurocapsa*, *Chlorogloeopsis*, *Nostoc*, *Microcoleus*, *cytonema*, *Tolypothrix, Hapalosiphon* (Macedo *et al*., 2009; Ortega-Calvo *et al*., 1995; Ortega-Morales, 2006; Lewin, 2006; Keshari and Adhikary, 2013; Tripathi *et al*., 1997; Gaylarde *et al*., 2012). Many cyanobacteria growing epilithically can assume endolithic mode of life by subsequent colonization of interior of natural or constructional stones through pores, fissures or cracks. Both epilithic and endolithic organisms can cause biodeterioration of natural or building stone.The endolithic cyanobacterium *Chroococcidiopsis* is found in rocks in hot and cold deserts with extreme aridity, and is tolerant to extreme cold, heat and arid conditions (Wierzchos *et al*., 2006; Friedmann, 1980).

The lithobiontic cyanobacteria through different mechanisms can cause aesthetic damage and biodeterioration of stone monuments, buildings and artworks (Dakal and Cameotra, 2012; Macedo *et al*., 2009; Crispim and Gaylarde, 2005; Gaylarde and Morton, 1999). Aesthetic damage results from the discoloration or disfigurement of the stone surface due to the cyanobacterial photosynthetic pigments and formation of pigmentedbiofilms, patinas or crusts. The structural damage of monument or building stone due to biodeterioration is manifested as cracking, exfoliation, pitting, textural changes, crumbling and appearance of uneven surfaces. Cyanobacteria-induced biodeterioration involves both physical (geophysical) and chemical (geochemical) processes (Macedo, 2009). The physical or geophysical process cause mechanical damage to the building stone due to the pressure or stress exerted by the growth of cyanobacterial cells, filaments, biofilms inside the pores and fissures of stones. Due to the hygroscopic nature of EPS, they undergo large volume changes by shrinking and swelling cycles, resulting in considerable mechanical stress that leads to the alteration of pore size and distribution, and loosening and detachment of grains of the building stone (Saiz-Jimenez, 1999; Griffin *et al*., 1991). Geophysical process of biodeterioration is mostly regulated by the porosity of the rocks/stone (Warscheidand Braams, 2000).Moreover, the discolored areas on the stone surface due to the growth of cyanobacteria or development of biofilms absorb more solar radiation than the normal areas, leading to the thermal gradient which induces physical stress by expansion and contraction (Warscheid and Braams, 2000).

The chemical or geochemical process involves the chemical substances or metabolic products produced and secreted by organisms which have direct action on stones, weakening the mineral lattice. The water absorbed by biofilms is retained for longer duration which may facilitate aqueous chemical reactions responsible for stone weathering (Gorbushina, 2007).The production of EPS, siderophores or other chelating agents, and acid or alkaline secretion are implicated in bioweathering of natural and building stones by chelating and solubilising effects on rock minerals (Gaylarde and Gaylarde,1999; Ortega- Morales *et al*., 2000; Wessels and Büdel, 1995). Due to anionic nature, EPScan sequester cationic elements of minerals from the lithic substrates (Welch and Vandevivere, 1994; Rossi *et al*., 2012).

CONCLUSION

The biocolonization and biodeterioration of historically and culturally important stone-made monuments, buildings and artworksby various microorganisms are serious problemsworld-wide, and their restoration and conservation are difficult task.Globally, the increasing concernover their biodeterioration and the efforts directed towards their restoration and conservation brought about a greater interest in the biology, eco-physiology and activities of lithobiontic microorganisms as well as their activities responsible for biodeterioration. A multidisciplinary approach, integrating the concepts from biology, microbiology, ecology, physiology, geology, chemistry, building science and engineering, and material science is important in order to gain a holistic understanding of biodeterioration as well as for devising the effective methods for their control. Knowledge of various climatic or microclimatic and building factors promoting the bioremediation of monuments is equally important. The direct and indirect role of cyanobacteria, which readily colonize the external surfaces of historic monuments and buildings can not be neglected. The detection and identification of lithobiontic cyanobacteria colonizing historic monuments and buildings built of different stone types or building materials and located under different climatic conditions is extremelysignificant for the future study of the biodeteriogenic process and the development of effective control methods.

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