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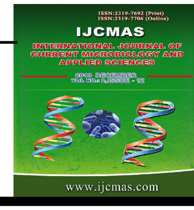

Review Article

Rock-dwelling cyanobacteria: survival strategies and biodeterioration of monuments

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A B S T R A C T

Cyanobacteria (Blue-green algae) are a morphologically diverse and widely distributed group of photosynthetic prokaryotes endowed with remarkable adaptability to varying environmental conditions and effective protective mechanisms against various abiotic stresses (e.g., desiccation, high light intensity, solar UV radiation and oxidative stress) which enable them to colonize and inhabit almost all kinds of terrestrial and aquatic habitats, including extreme lithic habitats, such as rocks and walls of monuments and buildings. The rock-dwelling (lithobiontic) cyanobacteria grow both as epiliths and endoliths. As a major and dominant component of biofilms or crusts on exposed surfaces, they are responsible for the biodeterioration and aesthetically unacceptable discoloration of buildings and monuments of historical, cultural or religious importance. The biodeterioration of monuments caused by cyanobacteria and other microbes constitute a major problem of world-wide concern, posing a challenge to their conservation and restoration.

Introduction

Cyanobacteria (Blue-green algae) are an ancient, morphologically diverse, metabolically versatile and widely distributed group of oxygenic photosynthetic prokaryotes (Stanier and Cohen-Bazire, 1977). With long evolutionary history of approximately 2.4 billion years, they are among the ancient life forms on earth (Fischer, 2008). The ability of many cyanobacteria to perform both photosynthesis and nitrogen fixation together with their efficient nutrient uptake mechanisms and adaptation to low light intensity makes them highly productive and efficient biological system. Comprising about 150 genera with more than 2000 species, they exhibit remarkable diversity in their morphology, ranging from simple unicellular and colonial to complex filamentous forms with or without branching (Van den Hoek *et al*., 1995). Endowed with effective protective or defence mechanisms against various abiotic stresses and remarkable adaptability to varying environmental conditions, they successfully colonize and inhabit almost all kinds of terrestrial and aquatic habitats, including extreme lithic habitats, such as rocks and walls of monuments and buildings (Tandeau de Marsac and Houmard, 1993; Budel, 1999). Although the potential and/or actual applications of a large number of cyanobacterial species in diverse areas, such as agriculture, aquaculture, pollution control (bioremediation), bioenergy and biofuels, nutraceuticals, food industries, and pharmaceuticals have been well-documented (Patterson, 1996; Pandey, 2010, Tan, 2007; Skulberg, 2000; Sekar and Chandramohan, 2008), the rock-dwelling (lithobiontic) cyanobacteria have gained notoriety as potent biodeterogens, causing aesthetically unacceptable discoloration and biodeterioration of colonized surfaces of walls of monuments and buildings of historical, cultural or religious importance (Crispim and Gaylarde, 2005; Crispim *et al*., 2003; Crispim *et al*., 2006; Crispim *et al*., 2004; Gaylarde and Gaylarde, 1999; Gaylarde and Gaylarde, 2005). Globally, there is increasing concern regarding the restoration and conservation of monuments deteriorated by various physical, chemical and biological agents, including cyanobacteria and algae.

Survival strategies of rock-dwelling Cyanobacteria

The rock-dwelling organisms are called lithobionts or lithobiontic organisms. Organisms growing attached to the external surfaces of the rocks are termed epiliths, while those inside or in the interior of rocks are termed endoliths. Further, the endoliths are called chasmoendoliths if they inhabit fissures and cracks in rocks, cryptoendoliths if they dwell within structural cavities or natural pore spaces of rocks and euendoliths if they actively penetrate the rocks (Golubic *et al*., 1981). Both surface and interior of rocks provides environment for the growth of various microorganisms, including cyanobacteria. Lithobiontic (epilithic and endolithic) cyanobacteria, which include coccoid as well as filamentous forms, have been recorded from various regions or countries of the world (Büdel, 1999; Adhikary, 2000; Friedmann and Ocampo-Friedmann, 1984; Tripathi *et al*., 2007). Büdel (1999) studied the ecology and diversity of rock-inhabiting cyanobacteria in tropical regions, particularly in Africa and South America. The frequently recorded lithobiontic cyanobacteria comprise manly the species of *Aphanocapsa*, *Aphanothece*, *Chroococcus*, *Gloeocapsa*, *Gloeothece*, *Chroococcidiopsis*, *Lyngbya*, *Phormidium*, *Oscillatoria*, *Nostoc*, *Calothrix*, *Scytonema* and *Tolypothrix*. Epilithic cyanobacteria successfully colonize and inhabit the rock walls of temples and monuments (Adhikary and Satapathy, 1996; Tripathi *et al*.,1997; Adhikary 2000; Pattanaik and Adhikary, 2002). The endolithic microhabitats provide protection from desiccation, temperature fluctuations, intense solar radiation or UV radiation and wind, while allowing penetration of photosynthetically active radiation, due to the translucent property of rocks, which is used by phototrophs like cyanobacteria (Friedmann and Ocampo-Friedmann, 1984). Cyanobacteria in biofilms, the assemblages of various groups of microorganisms, on open rock surfaces and external walls of monuments or buildings frequently experience water stress and high light intensities combined with increased levels of ultraviolet (UV) radiation (Büdel, 1999). Available reports suggest that cyanobacteria possess effective and efficient mechanisms for desiccation tolerance (Potts, 1994,1999) and photoprotection against high light intensities and UV-radiation (Groniger *et al*.,2000; Ehling-Schulz and Scherer,1999) as survival strategies on rocks and buildings. The mechanisms of desiccation tolerance are not well understood despite the fact that numerous prokaryotic and eukaryotic organisms are capable of surviving more or less complete dehydration. Desiccation tolerance constitutes the sum of various simple and complex interactions at the structural, physiological and molecular levels (Potts, 1999). In many cyanobacteria, desiccation tolerance has been attributed to the production of extracellular polysaccharides (Philippis and Vincenzini, 1998; Adhikary, 1998). They regulate loss and uptake of water from cyanobacterial cells and provide protection to cell walls from damage during swelling and shrinkage (Caiola *et al*., 1993, 1996). The presence of UV-absorbing pigments in epilithic cyanobacteria constitutes a photoprotective mechanism against harmful solar UV-radiation (Roy *et al*., 1997; Pattanaik and Adhikary, 2001). Mycosporine-like amino acids (MAAs) and scytonemin are well characterized UV-photoprotective compounds/pigments reported in many cyanobacteria (Ehling-Schulz and Scherer,1999; Groniger *et al*.,2000). MAAs, which exhibit absorption maxima in the range 310 to 360 nm, are intracellular water-soluble substances containing cyclohexenone or cyclohexenimine chromophores conjugated with the nitrogen substituent of an amino acid or its imino alcohol (Ehling-Schulz and Scherer, 1999; Sinha *et al*., 2003). Scytonemin is a yellow-brownish, lipid-soluble pigment located in the extracellular sheaths of many cyanobacteria. With molecular mass of 544 Da, it contains indolic and phenolic subunits. It exhibits *in vivo* absorption maximum at 370 nm (Ehling-Schulz and Scherer, 1999). The synthesis of scytonemin in cyanobacteria is known to be induced by UV radiation. Some studies have shown that both desiccation and UV stress in cyanobacteria leads to oxidative stress i.e., the accumulation of reactive oxygen species (ROS), such as singlet oxygen ($^1O_2$), superoxide radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\cdot}$),which damage cellular structures. Cyanobacteria possess antioxidative defence system, which includes both antioxidant enzymes, such as superoxide dismutase (SOD) and reactive oxygen (RO)-quenching pigments, such as carotenoids for protection against oxidative stress (Qiu *et al*.,2003; Ehling-Schulz and Scherer,1999). Cyanobacteria growing on exposed rock surface or walls of monuments have been reported to contain higher amount of superoxide dismutase (SOD) enzyme as desiccation tolerance mechanism (Sahu and Sabat, 2002). The role of carotenoids in photoprotection against high light intensities is well known in all photosynthetic organisms, including cyanobacteria. An increase in the carotenoids/Chlorophyll *a* ratio has been reported in response to UV-radiation (Quesada and Vincent, 1997), supporting an additional role of carotenoids as reactive-oxygen quenching pigments.

Cyanobacteria in biodeterioration of monuments

The biodeterioration of rock-made monuments, buildings and artefacts caused by the colonization, growth and activities of various microorganisms, such as cyanobacteria, algae, fungi, actinomycetes and bacteria is a global problem, resulting in damage of our cultural heritage as well as in economic loss due to high expenses involved in their restoration and conservation. In broad perspective, biodeterioration can be defined as the undesirable change in the properties or qualities of a material or a structure by the vital activities of organisms. The natural weathering or decay of stone or stone-made buildings and monuments is caused by a combination of physical, chemical and biological processes. The relative significance of these processes in weathering depends on environmental and biological conditions. The role of various groups of microorganisms in weathering of rocks in nature and their involvement in the biodeterioration of historic monuments and buildings are reportedly well-known (Gaylarde and Gaylarde, 2005; Gaylarde *et al*., 2003). The mechanisms involved in microbes-mediated biodeterioration include production and secretion of acid and alkali, alteration of surface properties (e.g., wettability, heat absorption, water retention) of constructional rocks and direct penetration of cells or filaments of microorganisms in to the rocks (Allsopp and Seal, 1986). As photoautotrophic organisms and primary colonisers or pioneer microorganisms of lithic substrates, cyanobacteria facilitate and promote the growth of heterotrophic microorganisms, like bacteria, fungi and actinomycetes, resulting in the formation of well-organised and laminated phototrophic biofilms or crusts on walls. As a major and dominant component of biofilms, they are directly or indirectly responsible for decay, biodeterioration, bioweathering and aesthetically unacceptable discoloration of buildings and monuments. Colonization and growth of cyanobacteria on monuments/buildings is controlled by both constructional properties of monuments/buildings and ambient environmental or climatic conditions (Guillitte and Dreesen, 1995; Gaylarde *et al*., 2003; Crispim and Gaylarde, 2005). The bioreceptivity of building materials for a particular group of microorganisms is controlled by surface conditions, mineral composition, porosity and permeability (Guillitte and Dreesen, 1995; Gaylarde *et al*., 2003). The resistance of cyanobacteria to desiccation and their tolerance to high levels of light intensities and ultraviolet (UV) radiation provide them a distinct advantage over many other organisms on exposed surfaces. They produce extracellular polymeric substances (EPSs) which play crucial roles in surface colonisation, cell aggregation and biofilm formation and stabilization. Biogenic pigments, both photosynthetic and non-photosynthetic, produced by cyanobacteria cause discoloration of colonized monuments/ buildings, whereas production of EPSs, siderophores and osmolytes, and acid or alkaline secretion are primarily responsible for weathering and decay of their constructional rocks due to chelating and solubilising effects on rock minerals (Gaylarde and Gaylarde,1999; Ortega-Morales *et al*.,2000; Wessels and Büdel, 1995). EPSs cause chemical damage because of the negatively charged polysaccharides are able to chelate cations present in rock minerals and, being organic matter, support microbial growth. Internationally, many studies have confirmed cyanobacteria-induced biogenic weathering and biodeterioration of historic buildings and monuments (Crispim and Gaylarde, 2005; Crispim *et al*., 2003; Crispim *et al*., 2006; Crispim *et al*., 2004; Gaylarde and Gaylarde,1999; Gaylarde and Gaylarde, 2005). The available reports are mostly from European and Latin American countries, which are based on the studies focussing on colonization and biodeterioration of external walls of churches and other monuments by cyanobacteria. These studies revealed the presence of a wide variety of coccoid and filamentous cyanobacteria on external walls.

Monuments, besides being cultural or national heritage, are important in tourism and economy of a country. Their biodeterioration due to the attack of various microorganisms constitutes a major problem of world-wide concern. In order to develop effective control and restoration strategies for monuments, knowledge of diversity and activity of various microbes in the biofilms formed on the surface is essential. Efforts to conserve monuments would be futile unless the cause and mechanism of their deterioration is known. The complex problem of biodeterioration should be investigated in holistic and comprehensive manner with interdisciplinary and inter-factorial approach. Knowledge of various environmental/climatic and building factors promoting the bioremediation of monuments is important.

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