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Cyanomatrix and Cyanofilm

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Abstract

Cyanobacteria are diverse in their habitats, structure, and metabolism that can grow as unicellular or long filaments and secreting high molecular mass polymers (extracellular polymer substances, EPS), which can either be released into the surrounding environment or remain attached to the cell surface. Four different cyanobacterial environmental samples (soil crust, microbial mats, pond water and marine bloom) were studied to compare between the cyanobacteria species that grow and form cyanomatrix and cyanofilms in those environmental habitats using light and scanning electron microscope (SEM). Diatoms were also detected with cyanobacteria at microbial mats and pond water samples. All samples had variable concentrations and compositions of extracellular matrix and biofilm. Microscopic observation and analyses also revealed complex network of filamentous cyanobacteria and extracellular polymer secretions, which binds and traps particles of soil and minerals.

Keywords: Cyanobacteria; Extracellular polymer substances (EPS); Biofilm; Matrix; Biological soil crust; Microbial mats; Pond and marine bloom water

Introduction

Cyanobacteria are prokaryotic microorganisms, unicellular and filaments cells, synthesis carbohydrates through their photosynthetic mechanism. When the growth conditions are not suitable to survive, cyanobacteria are adapted to secrete high molecular mass polymers, which released into the surrounding environment as extracellular polymeic substances (EPS) [1]. EPS are formed of polysaccharides, proteins, humic substances, nucleic acids, and lipids [2] and play a major role in the protection of cells against environmental conditions (desiccation, extreme temperature, light intensity, salinity, metallic trace elements or other environmental stresses) thus tolerate the cyanobacteria cells to colonize special ecological niches.

Ecologically, Cyanobacteria are common in many habitats of soil (soil crust, microbial mats, sands and rocks) and water (lakes, ponds, springs, wetlands, streams, rivers, and marine) these environmental communities play a significant ecological role in the cycling of carbon, nitrogen, oxygen, and other nutrients [3,4]. Biological soil crusts are a common feature of all dry land soils form at the soil surface by an intimate association between mineral grains and organic matter with varying proportions of cyanobacteria, algae, lichens and mosses. Because dry land soils contain only small amounts of organic C, particularly humic substances, EPS is often a major source of C [1]. EPS secreted by cyanobacteria are recognized as agents of soil particle aggregation, improve the fertility of the soils, immobilize nutrients in the surface that would otherwise be loss by leaching also reported to be associated with colony development [5]. The production and biochemical composition of EPS varies with soil type, environmental conditions and nutritional levels.

Microbial mats often support abundant populations of phototrophic microorganisms (cyanobacteria and microalgae) and chemo heterotrophs microorganisms (archaea, bacteria, fungi and protozoa). The cyanobacterial diversity in microbial mats with *Microcoleus* sp., *Oscillatoria* sp., *Lyngbya* sp., *Pseudanabaena* sp., *Phormidium* sp. *and Spirulina* sp. as the most abundant among the filamentous type and *Chroococcus* sp., *Gloeopcasa* sp., *Synechocystis* sp. *and Mixosarcina* sp. among the unicellular type. Structures of these mats are often embedded in a thick mucilaginous matrix of EPS, which may consist of 90% or more of polysaccharides and implicated in the adhesion of microorganisms to sediment particles, thereby influencing the stability of the sediment bed [4]. Hu et al. [6] reported that the number of constitutive monosaccharides of the EPS samples ranged, in various combinations, from 6 to 12 monosaccharides in the EPS produced by four cyanobacteria (*Microcoleus vaginatus, Scytonema javanicum, Phormidium tenue and Nostoc* sp.).

Cyanobacteria possess structures and skills that support their proliferation in aquatic ecosystems. They flourish in water that is salty, brackish or fresh, in cold and hot springs, and in environments where no other microalgae can exist. The cyanobacteria comprise a large component of marine plankton with global distribution. Many bloom-forming cyanobacterial genera exist as macroscopic colonies that are present in buoyant surface blooms or dispersed throughout the water column. *Microcystis*, *Anabaena, Aphanizomenon* and *Trichodesmium* are common and often dominant colonial, bloom-forming cyanobacteria responsible for harmful (toxic, food-web disrupting, and hypoxia generating) blooms in nutrient-enriched in aquatic ecosystems worldwide [7] . Under suitable meteorological and hydrological conditions, EPS-producing cyanobacterial colonies in lakes aggregate and float up to form the mucilaginous cyanobacterial blooms.

The characteristics of EPS produced by cyanobacteria from a soil crust, microbial mats, fresh water ponds and marine bloom water were investigated in this work. Light and scanning electron microscopy (SEM) with energy dispersive X-ray spectroscopy (EDS) analysis were employed in order to observe the cyanomatrix and cyanofilms formed in those environmental samples.

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Materials and Methods

Sample sites

The formation of cyanomatrix and cyanofilm were studied in four cyanobacterial environmental habitats (soil crusts, microbial mats, pond and marine bloom water). All samples were collected in plastic containers from those habitats of Qatar.

Microscopy

Light and scanning electron microscope (SEM) were used to study and analyzed the present of cyanobacteria and the production of extracellular polysaccharides (EPS). Energy dispersive X-ray spectroscopy (EDS) was performed to detect elements and its compounds in the (EPS). Operation conditions were 30 kV

Results and Discussion

Figure 1 show tow of the environmental samples, microbial mats (A and B) and pond water (C) that after 3 weeks form a transparent biofilm, green color in the center indicated the growth of cyanobacteria and oxygen bubbles also present 9 Figure 4) because of photosynthesis of cyanobacteria cells. Water was required for full recovery of photosynthesis. Garcia-Pichel and Pringault [8] showed how cyanobacteria were able to migrate to the soil surface when wet and move back down the subsoil upon subsequent drying in order to avoid desiccation and photo-damage. Between rainfall events cyanobacteria can remain indefinitely in a desiccated state, partly because of the protection afforded to the cells by the EPS. Satoh et al. [9] suggested that the physical structure of the EPS is the main contributing factor to the amount and absorption of moisture allowing cyanobacteria to absorb many times their dry weight in water. Otero and Vincenzini [10] also evaluated the effect of N source and light intensity on the synthesis of EPS with three strains of the genus *Nostoc* under laboratory conditions. High light intensities enhanced total carbohydrate synthesis and led to some structural changes of the EPS. The ability of cyanobacterial filaments to create surface aggregates and production of EPS is closely associated with water and light, if the light is low, the red color appear in biofilm (Figure 4). Pond (Figures 1C and 4) and marine bloom water after 3 weeks incubation at room temperature revealed that cyanobacteria firmly grow and anchored to the wall of glass bottles, probably by secreted adhesive mucilage. The cell monolayer developed outwardly from the surface, becoming a complex biofilm. Scanning electron microscopy has shown that cyanobacterial biofilm occurs as a matrix or network of strands (Figures 3A-3C). The EPS contribute directly to the properties of biofilms in that they normally permit considerable amounts of water to be bound. The Light and environmental scanning electron microscopy(SEM) images (Figures 2 and 3) showing dominance of cyanobacteria (*Oscillatoria*, *Lyngbya*, *Phormidium*and *Microcoleus*) and diatoms in all samples except for water bloom just present of cyanobacteria (*Trichodesmium*). SEM is mostly used for imaging of exopolysaccharide and has been reported by many researchers [11,12]. SEM results also performed the present of EPS from mucilaginous cyanobacterial in all analyzed samples. The accumulation of cells and associated polysaccharide can be seen in Figure 3 formed a microbial aggregate surrounded by a numerous matrix of polysaccharide material.

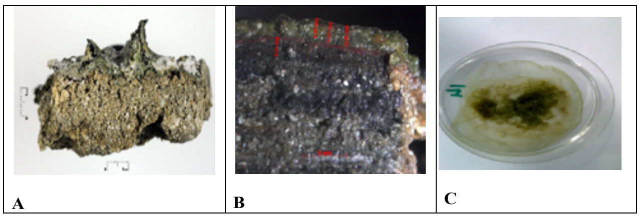

Figure 1: Tow types of environmental samples, microbial mats (A, B). Pond water (C).

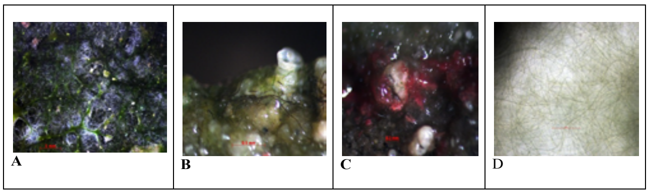

Figure 2: Light microscope micrographs of the microbial mats surfacewith filamentous cyanobacteria forming a dense network on the sand grains with different scale 1, 0.5, 0.2 mm and 500 μm (A-D).

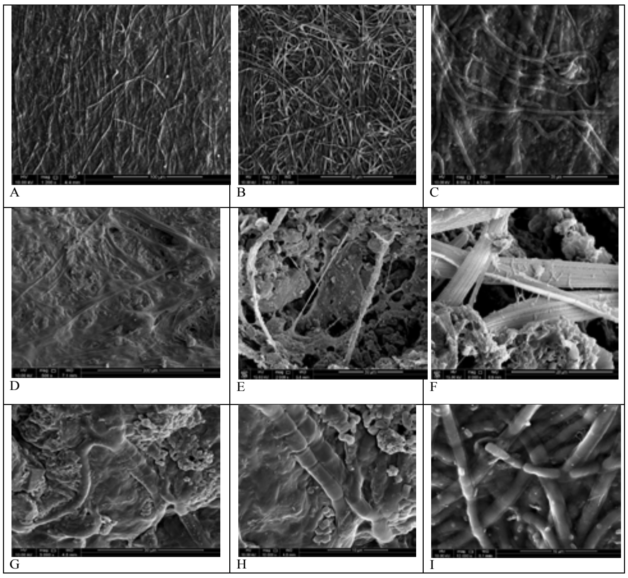

Figure 3: Environmental scanning electron microscopy images showing: cyanobacerial filaments within the pond water samples (A,B, C) and microbial mat, cyanobacerial filaments and diatoms (D, E, F) with extracellular polymeric substances (EPS). Precipitation of minerals(mainly halite and calcite) on EPS (G, H).Thick biofilm from pond water bindingand trapping bacterial cells (I). Cyanobacterial filaments all embedded in a massive biofilm.

The EPS in biofilms have protective effects to the cells by maintaining a highly hydrated layer surrounding the biofilm, the EPS will prevent lethal desiccation in some natural biofilms and may thus protect against diurnal variations in humidity light intensity, high and low temperatures, high salinity, UV radiation, heavy metals, changes in pH or ionic strength of the suspending fluid, conservation of extracellular enzyme activities, colony or aggregation of cyanobacteria and shelter from predation [13,14]. In medicine EPS interact with antimicrobial agents to protect the microbial cells, either by preventing access of the compounds or by effectively reducing their concentration [15]. EPS may offer little protection against bacteriophage or bacteriocins when these are present in appreciable concentrations. The association of EPS with cyanobacteria has been demonstrated by both electron microscopy and light microscopy (Figure 3) in all samples.

Cyanobacteria are ubiquitous microorganisms that occur naturally

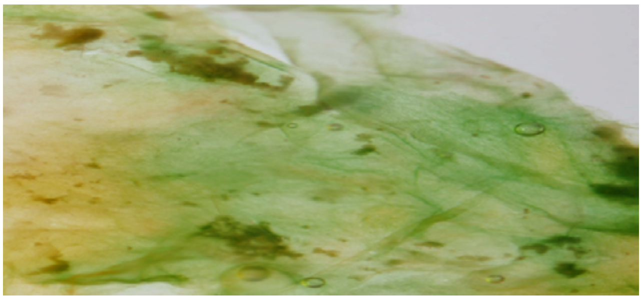

Figure 4: Growth of pond water biofilm after 3 weeks, oxygen bubbles and two different colors, green and red were appeared.

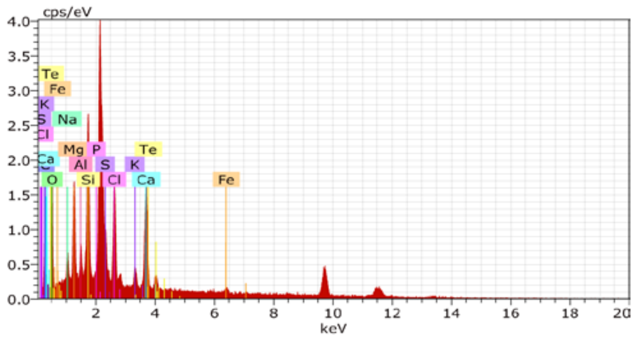

Figure 5: EDS analysesof microbial mat.

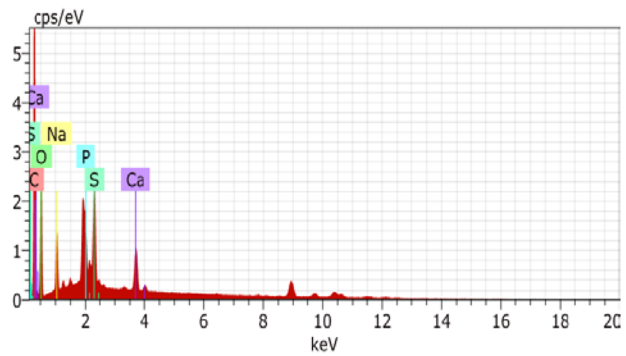

Figure 6: EDS analysesof pond water biofilm.

and serve as one of the biomaterials with a high capacity for removing metallic trace elements from contaminated waters. The cell surface of cyanobacteria consists of polysaccharides, proteins and lipids, which act as a basic binding site for metallic trace elements. Therefore, it is the most important organism for environment in terms of removing wastes from the water. Exudates from several species of cyanobacteria are reported to act as strong complexion agents for some metals Ozturk et al., [16] investigated cadmium (II) resistance and its association with exopolysaccharide (EPS) production in cyanobacteria. Scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDS) analysis confirmed that a considerable amount of metals had precipitated on the cell surface. Fourier transform infrared (FT-IR) spectrum analysis of EPSs belonging to both isolates indicated the presence of C–H and C–O group, which may serve as binding sites for divalent cations. Figures 5 and 6 representative EDS spectra for microbial mats and pond water respectively the Comparison of EDS spectra of cyanobacterial mats and the pond water cyanofilm EDX analyses detected carbon(C) and oxygen (O) in high quantity in all samples. A mixture of minerals was also detected such as calcium (Ca), phosphorus (P), sodium (Na) and sulfur (S) in low quantity. Silicon (Si), Magnesium (Mg), chloride (Cl) were also detected in microbial mats and soil crust with other trace minerals (Figures 5 and 6). It has been shown that bacterial surfaces are good sorption interfaces for Al-K-Fe silicate ions [17].

Figures 3D, 3E and 3F demonstrate microbial mats and soil crust SEM images which are widely colonized by cyanobacteria and diatoms. The abundance of a biofilm of EPS reflects the behavior developed by microbial mats living in such hyper saline systems. EPS layers allow cyanobacteria to increase their fossilization potential through early diagenesis of crystallization and cementation by gypsum and halite of these soft shaped layers. Taher [18] investigated microbial mats and biofilms on the coastal Sabkha in Ras Gemsa, Red Sea, using scanning electron microscopy and found the sediment surface colonized by cyanobacteria revealed that sand grains of the studied samples are incorporated into the biofilm by trapping and binding processes. Filamentous cyanobacteria and their EPS found in the voids in and between the particles construct a network that effectively interweaves and stabilizes the surface sediments. In advanced stages, the whole surface is covered by a spider web-like structure of biofilm, leading to a planar surface morphology.

Filamentous cyanobacteria, particularly *Microcoleus chthonoplastes*, have great effect in modifying the sediment surface as they form thick sheaths consisting of EPS which is very tough and resistant to degradation. In addition, Gerdes et al. [19] in their study of the tidal flats of the North Sea have revealed that the biomass production is increased in places characterized by the predominance of *M. chthonoplastes*. Accumulation of sedimentary particles by binding and trapping is a typical feature associated with this species. Besides this, the binding meshwork of thick EPS effectively interweaves and stabilizes the surface sediments.EPS of the common *M. chthonoplastes* (Figures 3G and 3H) was seen to bind sediment grains in the microbial mats and soil crust samples. This stabilizes the topsoil, reduces soil erosion, and enhances the organic matter in the first millimeter of the topsoil [20,21] also investigated the compressive strength of three cyanobacteria in the field and found a higher compressive strength with increasing cyanobacterial biomass. As the cyanobacterial biomass increased gradually, more EPS was released forming more soil aggregates.

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