

Stress Tolerance Effect of Three Different Sub-aerial *Cyanobacteria* sp. Isolated from Exteriors of Buildings under Adverse Environmental Conditions

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Abstract

The current interest of scientific study aims at survival mechanisms of the cyanobacteria on the extreme habitats (i.e. building facades and monuments) growing under adverse conditions. The present investigation points towards finding out indigenous which are tolerant of conflicting environmental conditions, such as pH, temperature and calcium carbonate. Three isolates of cyanobacteria *Scytonema coactile*, *Scytonema geitleri* and *Lyngbya aerugineo-coerulea* from a cave, building façade, and temple, Orissa respectively were examined. Tolerance to stress at different pH and temperature were evaluated by quantifying cyanobacteria growth at different time intervals. Tolerance to CaCO₃ was studied by subjecting the isolates to the desired concentration 0.0001 - 1% w/v. Each organism was grown for 15 days at 25°C ± 1°C under continuous light intensity (7.5 W/m²) and then harvested, succeeded by SDS gel-electrophoresis protein analysis. Results revealed that three isolated cyanobacteria species from different sub-aerial habitats responded in a specific manner to different stress conditions and to various concentration of CaCO₃ concerning protein synthesis. A 30 and 38 kDa protein was overproduced by all isolates under pH and temperature stress, whereas for CaCO₃ stress, the protein of 16 and 22 kDa was overproduced by *Lyngbya aerugineo-coerulea* respectively which concluded that the survival of the isolates under stress conditions depends on specific protein synthesis. Generally, isolates tolerant to different stress may be due to specific protein synthesis for their survival to extreme habitats.

Keywords: Cyanobacteria, environmental stress, extreme habitats, tolerance, adaptation (CaCO₃- Calcium Carbonate)

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INTRODUCTION

Very few studies focus on phototrophic biofilms which are the important colonizers in a wide range of extreme environments including soils, rocks, modern and ancient building facades (Gaylarde *et al.*, 2001; Garcia-Pichel *et al.*, 2001; Hoffmann, 2002). Such Biofilms occurring as greenish, blackish or blackish-brown mat are mostly dominated by oxygenic phototrophic Cyanobacteria, particularly sheath forming ones, and produce extracellular polymeric substances (EPS) (Wimpenny *et al.*, 2000; Jiao *et al.*, 2010) suiting to the environmental conditions prevailing on the surfaces (Saarela *et al.*, 2004). Several cyanobacteria forming such phototrophic mats have been found on the building facades and monuments in different locations of eastern India exposed to intense solar radiation and extreme dryness. In such habitats, the organisms receive water from leakage area, roof guttering, and inadequate drainage of flat areas or adjacent water courses. During the rainy season, the average value of rainfall in Orissa state, on the east coast of India is 100mm to 330mm between mid months June to August followed by a long dry season of winter. The air temperature ranges from 15°C to 28°C between September to January and summer air temperature ranges from 28°C to 45°C between February to mid-June. In such a situation, they have to withstand not only long periods of matrix water stress but also extremes of prolonged desiccation, high temperature, and solar radiation. Reports are supporting that several microorganisms, biofilms, and microbial mats growing on and within different substratum, composed of K, Na, Ca, Fe, Ag, Pb, and P contribute to the selective bioleaching of the component material. It has also been found out that precipitation of calcium salt by cyanobacterial cells growing on limestone and migration of calcium from and to neighboring sites is an important mechanism in stone degradation by these organisms (Schultze-Lam and Beveridge, 1994; Ascaso *et al.*, 1998; Ortega-Morales *et al.*, 2000). They deposit CaCO₃ in the presence of light and solubilize it at night due to a change in carbonate concentrations. Such mobilization of calcium ions and the trapping of released particles of calcite in the gelatinous sheath of cyanobacterial cells lead to degradation of calcareous stone (Pentecost, 1988; Warschied,

1991; Rossi *et al.*, 2002; Crispim *et al.*, 2006). Hence studying the metabolic activities of such organisms to various environmental regimes, e.g. pH and temperature prevailing on the facades, would throw light on their survival mechanism under extreme environmental conditions.

In the present study, the effect of stress, pH, and the temperature was evaluated on three cyanobacteria species isolated from three different habitats. To find out the cyanobacterial species those were associated on the building facades and stone monuments, the present investigation was also undertaken to examine growth behavior of three cyanobacteria species *Scytonema coactile*, *Scytonema geitleri* and *Lyngbya aerugineo-coerulea* isolated from sub-aerial habitats to various concentrations of Calcium carbonate commonly found in various concentrations on the lime-washed building facades. In addition to these, the further objective was to find out the protein profile of each organism in the control condition and the change, if any, that occur when they are exposed to different environmental variables especially looking at the protein profile which was repressed, induced or overproduced under the stress conditions.

MATERIALS AND METHODS

Cyanobacterial isolates

Microbial crusts were collected from a blackish-brown crust on the limestone surface in the twilight zone of Gupteswar cave, Odisha; from the blackish crust on the lime-washed building facade of Utkal University campus, Bhubaneswar and the brownish crust on the stone surface of Bhaskareswar temple, Bhubaneswar, Odisha (Fig. 8a-c). Scrapped crust samples were collected and stored in pre-sterilized screwcapped Tarson bottles and transported to the laboratory for analysis. The samples were wetted with sterile water for 12 h and examined under the light microscope. A pinch of the pre-wetted crust was transferred to the BG-11 medium (Rippka *et al.*, 1979) with or without combined nitrogen and to agarplates (1.2 % w/v) and incubated at 25 ± 1°C under illumination with continuous light from fluorescent tubes at an intensity of 7.5 W/m². The organisms appearing in the plates were isolated, made axenic with repeated sub-culturing following Venkataraman (1969), and were identified using

the monographs (Desikachary, 1959; Komarek and Hauer, 2015). The organisms at their exponential growth phase were used in all the experiments. Three cyanobacteria species *Scytonema coactile*, isolated from the blackish-brown crust on the limestone surface in the twilight zone of Gupteswar cave, Odisha; *Scytonema geitleri*, from the blackish crust on the lime-washed building facade of Utkal University campus, Bhubaneswar and *Lyngbya aerugineo-coerulea*, from the brownish crust on the stone surface of Bhaskareswar temple, Bhubaneswar, Odisha were isolated and used in the experiments to understand their metabolic activity under various environmental variables. Growth, chlorophyll *a* and carotenoid (Mackinney, 1941; Jessen, 1978), cell-protein (Lowry *et al.*, 1951) and extracellular carbohydrate content (Herbert *et al.*, 1971) and changes in the protein profile of the organisms in response to the environmental variables were estimated following.

Effect of pH and temperature stress in cyanobacteria growth

Tolerance of cyanobacterial isolates were evaluated by quantification of cyanobacteria growth (optical density at 750nm) in BG - 11 medium with different pH of 4, 5, 6, 7, 8, 9 and 10 respectively after 15 days of Cyanobacteria growth. Tolerance of isolates to different temperatures (35°C, 45°C, 55°C and 65°C) was also evaluated simultaneously. The control conditions were considered to be pH 7 and 25°C.

Effect of Calcium carbonate in cyanobacteria growth

Tolerance to various concentrations of Calcium carbonate was evaluated. The stock solution of Calcium carbonate was prepared with distilled water, sterilized separately from the mineral salts and added aseptically to the culture medium to obtain the desired concentration (0.0001, 0.0005, 0.001, 0.005, 0.01, 0.05, 0.1, 0.5 and 1%, w/v). The organisms were grown up to 15 days at 25 ± 1°C under a continuous light intensity of 2000lux and then harvested.

Analysis of Protein profiling

Changes in the protein patterns induced by pH, temperature, and calcium carbonate were detected by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS – PAGE) analysis of total cell proteins. Cell treatment and electrophoresis

were done as described by Lowry *et al.*, 1951.

Statistical analysis

To compare tolerance of different isolates to distinct conditions, the optical density obtained for each isolate after 15 days at 25°C, pH 7 was considered as 100% growth. Data represent the average of three replicates per treatment. The mean value of 3 replicates of each experiment ± S.D has been presented.

RESULTS

The analysis of biochemical constituents of the three selected sub-aerial Cyanobacteria expressed in µg/ml were given in (Fig. 1a-c; 2a-c; 3a-c). Increase or decrease of growth, chlorophyll-*a*, carotenoids, soluble cell-protein content of the cells as well as quantity of extracellular carbohydrates released by these organisms to different conditions of pH and temperature stress and in different concentration of calcium carbonate over their respective control was examined after incubating for 15 days at 25°C under 2000lux light intensity. All these three isolated, purified from the different substratum of a cave, building facades and temple from the eastern region of India is shown in (Fig. 8a-c). The results in respect to different conditions of pH and changes in pH medium after 15 days of incubation of culture which is represented in (Fig. 1a-c). *S. coactile* grew over a wide range of pH, mostly at acidic range from 4 to 7 whereas on other hand, two species isolates *S. geitleri* and *Lyngbya aerugineo-coerulea* from exteriors of building and temple showed their maximum growth at pH 7 or 8, but pH above 9 was found to be detrimental or lethal. None of the pHs, however, encouraged the synthesis of carotenoid in its cells and the pH which was adverse to the growth triggered the release of extracellular carbohydrate into the medium. However, the chlorophyll-*a* and soluble cell protein content were increased significantly at pH 7. Interestingly the range of pH from 9 and 10 encouraged the release of extracellular carbohydrate significantly revealed maximum as compared to control (*S. coactile* and *Lyngbya aerugineo-coerulea*).

Analysis of tolerance of isolates to different temperature stress, 35°C, 45°C, 55°C for 8 hours and at 65°C for 3 hours revealed that three isolates endure the 35°C - 55°C better than 65°C temperature after subsequently

cultivating for 15 days at 25°C under 2000lux light intensity presented in (Fig. 2a-c). As expected for cyanobacteria (Haubner *et al.*, 2006), most isolates grew better at 25°C. However, with the increase of duration of exposure to a higher temperature, showed significantly decrease on growth, chlorophyll-a, soluble cell protein, and extracellular carbohydrate content in all three isolates as compared to control which is depicted in (Fig. 2a-c). However, at a higher temperature beyond 35°C maybe not favorable for extracellular

carbohydrate liberation by any of the test organisms. It was also found that all three isolates grew slowly with a pre-exposure to 65°C for up to 3 hours. However, prolonged incubation beyond 3 hours was detrimental to the growth of all the three experimental isolates.

To evaluate the stress tolerance of three isolates were exposed to different concentrations of Calcium carbonate (CaCO₃) compared to control (Fig. 3a-c). Analysis results showed that upon treatment with calcium carbonate, growth,

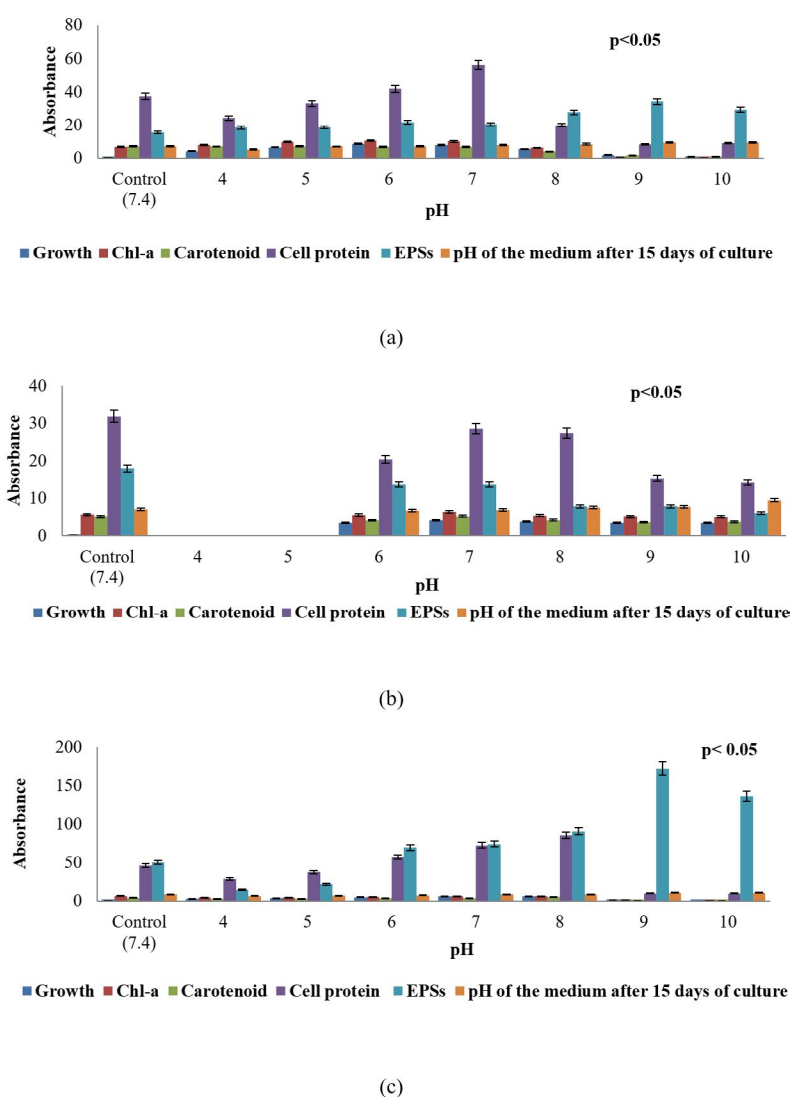


Fig. 1. Growth response, chlorophyll – a, carotenoid, soluble cell-protein, and extracellular polysaccharides content of three sub-aerial cyanobacteria, (a) *Scytonema coactile* (limestone, Gupteswar cave), (b) *Scytonema geitleri* (lime-washed wall, Building) and (c) *Lyngbya aeruginosa-coerulea* (Stone carving, Bhaskareswar Temple) submitted to different conditions of pH and changes in pH medium after 15 days of incubation of culture.

chlorophyll-*a* as well as cell-protein content of all the three isolates decreased and the decrease was proportionate with increasing concentration of the chemical in the medium. *S. coactile* tolerated up to 0.1% of CaCO₃ and beyond leading to cell bleaching and lethal of the organism whereas, other isolates, *S. geitleri* from the lime-washed

wall of the building could survive up to 0.05% of CaCO₃ which was almost 10 times less in concentration which *S. coactile*, isolated from a cave could tolerate. However, on the other hand, the cyanobacterium *L. aerugineo-coerulea* from exteriors stone carving of the temple was able to tolerate and grew even in presence of 1.5%

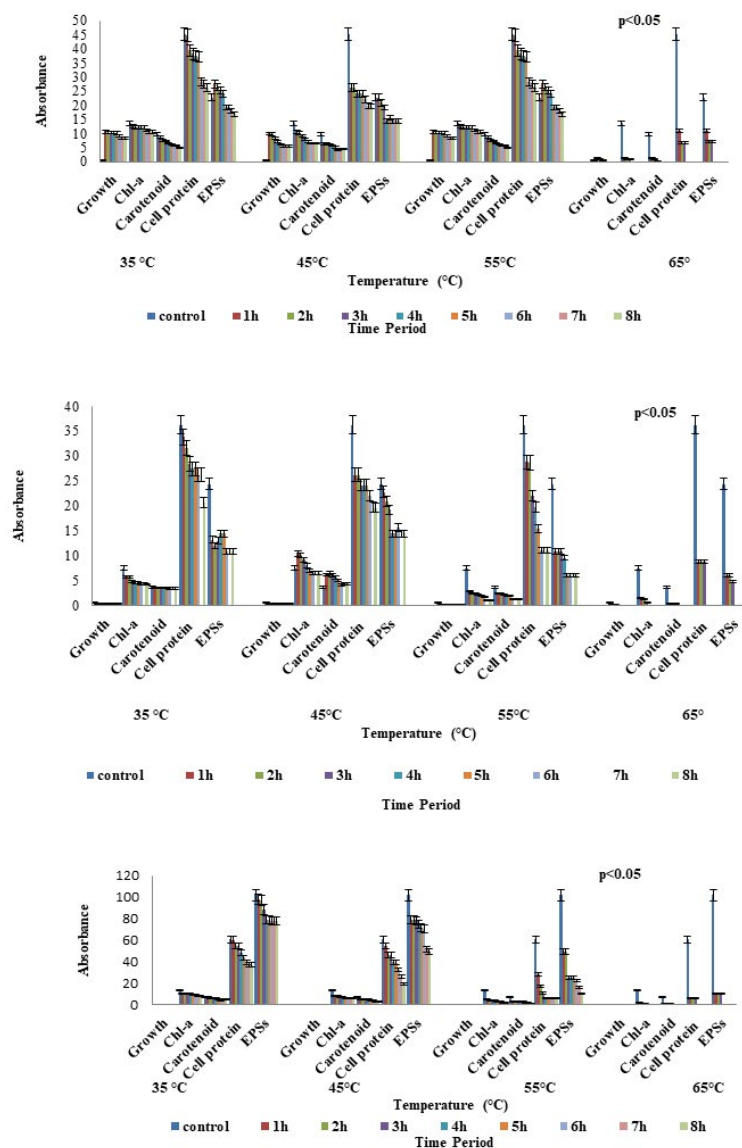


Fig. 2. Growth response, chlorophyll-*a*, carotenoid, soluble cell-protein, and extracellular polysaccharides content of three sub-aerial cyanobacteria, (a) *Scytonema coactile* (limestone, Gupteswar cave), (b) *Scytonema geitleri* (lime-washed wall, Building) and (c) *Lyngbya aerugineo-coerulea* (Stone carving, Bhaskareswar Temple) upon exposure to various temperature. Homogenized suspension of the organism was subjected to heat treatment (Temp. 35°C-65°C for up to 8h) and then cultured at 25 ± 1°C for 15 days.

CaCO₃ in the medium. It was also observed that exposure to the low concentration of CaCO₃ up to 0.005% in *S. coactile* and up to 0.05% in the culture of *L. aerugineo-coerulea* showed an increased in extracellular carbohydrate content but was not observed in isolates (*S. geitleri*). These findings indicated that cyanobacterium occurring in the caves and on the stone carving of the temple

possesses the capability to utilize the salt at low concentrations which triggered liberation EPS leading to its binding on the exposed surfaces on which they occur. Each isolates are having its uniqueness showed specific under adverse environmental stress.

Further analyses were carried out based on the results concerning the effect of

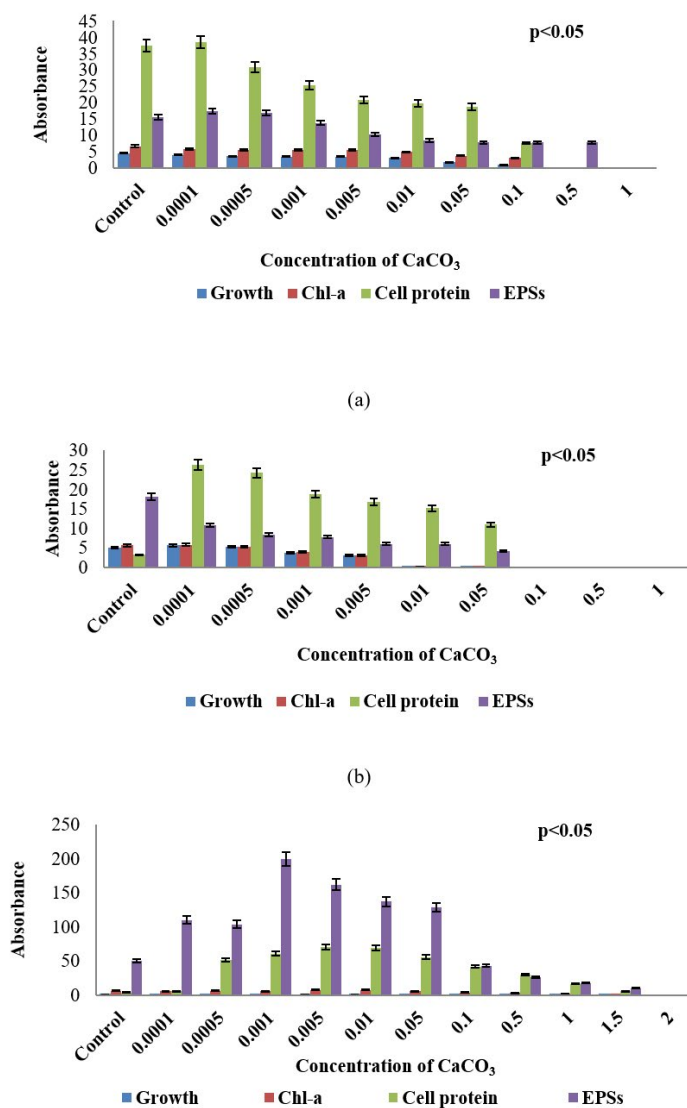


Fig. 3. Growth response, chlorophyll – a, carotenoid, soluble cell-protein, and extracellular polysaccharides content of three sub-aerial cyanobacteria, (a) *Scytonema coactile* (limestone, Gupteswar cave), (b) *Scytonema geitleri* (lime-washed wall, Building) and (c) *Lyngbya aerugineo-coerulea* (Stone carving, Bhaskareswar Temple) upon treatment to different concentrations of Calcium carbonate. Cultures were incubated at 25 ± 1°C under 7.5 W/m² light intensity up to 15 days.

pH, temperature and calcium carbonate on metabolic activities; three isolates were chosen for SDS-PAGE analysis of the protein profiles. SDS-PAGE analysis of isolates generated reproducible protein profiles is represented in Table 1- 3 and Fig. 4-7. Qualitative and quantitative differences in the protein patterns of isolates were detected upon their growth at acidic/alkaline of pH 6 and 9 respectively, when compared with the control conditions (pH 7.4) were represented in (Table - 1; Fig. 4), several proteins were repressed e.g. 18 kDa proteins in all the isolates and besides low molecular weight protein-like 58, 44, 38 and 28kDa proteins in these two isolates, *S. coactile* and *L. aerugineo-coerulea* were repressed. None

of the proteins were induced in *S. geitleri* and *L. aerugineo-coerulea* when cultured on acidic and alkaline pH, whereas *S. coactile* induce the 30kDa protein at pH 6 and 102, 54 and 48 kDa proteins at pH 9 which possibly are the key factors allowing the organism to tolerate a wide range of pH unlike the other two isolates. Both the *Scytonema* species also overproduced certain proteins when grown at acidic/alkaline pH. In *S. coactile* the low and high molecular weight proteins (102 and 30 kDa) were overproduced whereas, in *S. geitleri* similarly 16 kDa protein was overproduced in acidic pH including several low molecular weight proteins e.g. 48, 38, 36, 30 and 28 kDa and one chaperonin 62 kDa were overproduced when the isolates

Table 1. Changes in the protein profile of three sub-aerial cyanobacteria strains, *Scytonema coactile*, *S. geitleri* and *Lyngbya aerugineo-coerulea* has grown at specific pH 6 and 9 under continuous illumination (2000 lux) at $28\pm 2^\circ\text{C}$ up to 15 days

Organisms	a pH of the culture	Repression of protein (kDa)	Induction of protein(kDa)	Over production of protein (kDa)
<i>Scytonema coactile</i>	6	18	30	30
	9	18, 28, 38	48, 54, 102	30, 102
<i>Scytonema geitleri</i>	6	18		16
	9	18		16, 28, 30, 36,
<i>Lyngbya aerugineo-coerulea</i>	6	38		38,48, 62
	9	18, 28, 38, 44, 58		

Table 2. Changes in the protein profile of three sub-aerial cyanobacteria strains, *Scytonema coactile*, *S. geitleri* and *Lyngbya aerugineo-coerulea*, after exposure to different duration of temperature (35°C , 45°C , 55°C) for 8 hours and 3 hours at (65°C), and then incubated under continuous illumination (2000 lux) at $28\pm 2^\circ\text{C}$ up to 15 days

Organisms	Growth condition (Temp.)	Repression of protein (kDa)	Induction of protein(kDa)	Over production of protein (kDa)
<i>Scytonema coactile</i>	35°C	18	48	
	45°C	18, 28	48	
	55°C	22, 28, 36, 40, 78,99	48	
	65°C	12,18, 36, 40, 62,68, 78, 96, 99	48	30
<i>S.geitleri</i>	35°C	38		18
	45°C	38		18, 36
	55°C	38		16, 18, 36
	65°C	16, 38	36	18, 30, 36
<i>Lyngbya aerugineo-coerulea</i>	35°C			
	45°C			
	55°C			
	65°C	18, 22, 34, 44, 58,62	42, 66, 78	38

were cultured at pH 9 which possibly allowed the organism to thrive at the alkaline pH. However, the detected changes are distinct, depending on the isolate and growth conditions.

In different isolates, proteins with the same molecular weight were overproduced under stress temperature shown in (Table - 2; Fig. 5-6). For example, isolates *S. coactile*, only one 30 kDa protein was overproduced and *S. geitleri* revealed the simultaneous overproduction of three proteins (36, 30 and 18 kDa) upon exposure to higher temperatures at 65°C. However, other isolates, *L. aerugineo-coerulea* 38 kDa protein was overproduced which possibly confers protection and supports their important cellular functions. Some proteins were detected after submitting the three isolates to temperature stress at 35 - 65°C possibly were synthesized de novo, suggesting their importance in the survival and growth of sub-aerial cyanobacteria in stress conditions. For instance, with *S. coactile*, 48 kDa proteins were detected or induced after exposure to a temperature at 35 - 65°C whereas, isolate *S. geitleri* showed a 34 kDa proteins and other three proteins (78, 66 and 42 kDa) were detected. High tolerance to temperature stress (65°C) was not related to the number of induced proteins.

Upon exposure to different concentrations of calcium carbonate, however, several proteins

were repressed and the number increased with increasing concentration in three isolates (Table -3; Fig. 7). However, showed one 22 kDa and 24 kDa protein were selectively induced in two isolates, *S. coactile* as well as *L. aerugineo-coerulea* respectively, and in the other species, two proteins 16 kDa and 22 kDa were overproduced in presence of higher concentration of CaCO₃. On the contrary, none of the proteins were overproduced in either of the *Scytonema* species when grown in presence of CaCO₃ in the culture. These results showed that the selected strains growing under similar cultural conditions expressed a specific protein either repressed or induced or overproduced in response to a specific stressor conferring protection to the organisms to overcome the stress in terms of their growth pattern and metabolic activities.

DISCUSSIONS

Cyanobacteria strains isolated from exteriors surface of buildings and monuments were examined for growth and biochemical contents about pH, temperature and different concentrations of CaCO₃ stress tolerance. Our results showed remarkable variation concerning these attributes amongst the three isolates studied. All the three isolates isolated from different sites did not show uniform growth responses when cultivated under defined pH regimes varying from

Table 3. Changes in the protein profile of three sub-aerial cyanobacteria strains, *Scytonema coactile*, *S. geitleri* and *Lyngbya aerugineo-coerulea* grown in presence of different concentrations of calcium carbonate (%) under continuous illumination (2000 lux) at 28±2°C up to 15 days

Organisms	Concen. of calcium carbonate (%)	Repression of protein (kDa)	Induction of protein (kDa)	Over production of protein (kDa)
<i>Scytonema coactile</i>	0.001	48, 54, 90, 96, 99	22	
	0.01	8, 28, 34, 36, 38, 42, 48, 62, 68, 78, 90, 96, 112	22	
	0.1	8, 18, 28, 30, 34, 36, 38, 42, 48, 54, 62, 66, 68, 78, 90, 96, 99, 102	22	
<i>Scytonema geitleri</i>	0.001	12, 18, 62		
	0.01	12, 18, 34, 62		
	0.1	12, 18, 30, 34, 36, 38, 40, 42, 62		
<i>Lyngbya aerugineo-coerulea</i>	0.001	28, 34, 38, 40, 42, 58, 62	24	
	0.1	28, 34, 36, 38, 42, 58, 62	24	
	1	28, 34, 36, 38, 42, 58, 62	24	16, 22

4 to 10. The isolates, *S. geitleri* from cave tolerated and grew exclusively at acidic range (pH 4 -7) and whereas another species under the same genus, *S. geitleri* from the lime-washed wall of building grew in near neutral range, however, survived well up to pH 9, and further only former species was capable of excreting extracellular carbohydrate which is one of the primary substances might be

helping the organisms to adhere the substratum and grow under the adverse environment of the caves. On the contrary, other species, *L. aerugineo-coerulea* grew mostly at the neutral range, produced copious mucilaginous substances at ranging pH 8-10 which possibly suggesting that these substances help the organism to thrive on the exposed surfaces of the stone carving of the

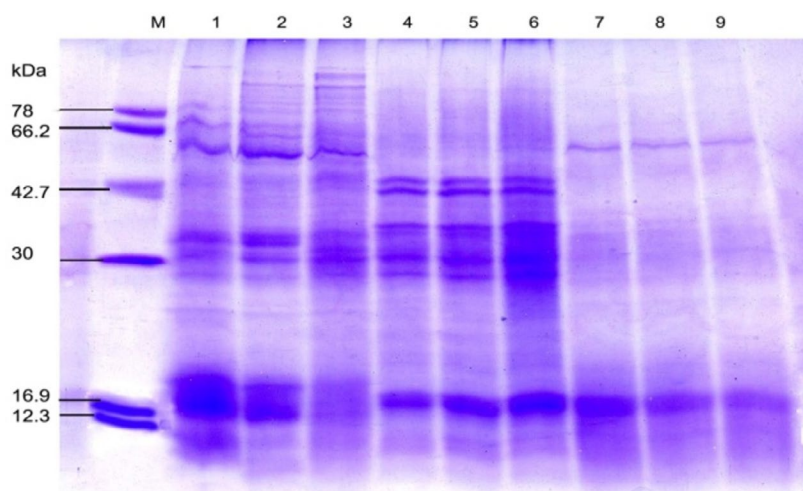


Fig. 4. SDS-PAGE of the protein profiling of three sub-aerial cyanobacteria isolates *Scytonema coactile*, Lane 2-3; *S. geitleri*, Lane 5-6 and *Lyngbya aerugineo-coerulea*, Lane 8-9 grown at pH 6 and 9 up to 15 days. An equal amount of proteins were loaded into each well. Control (pH - 7.4), Lane 1,4 and 7; M. Molecular mass of standard proteins in kDa.

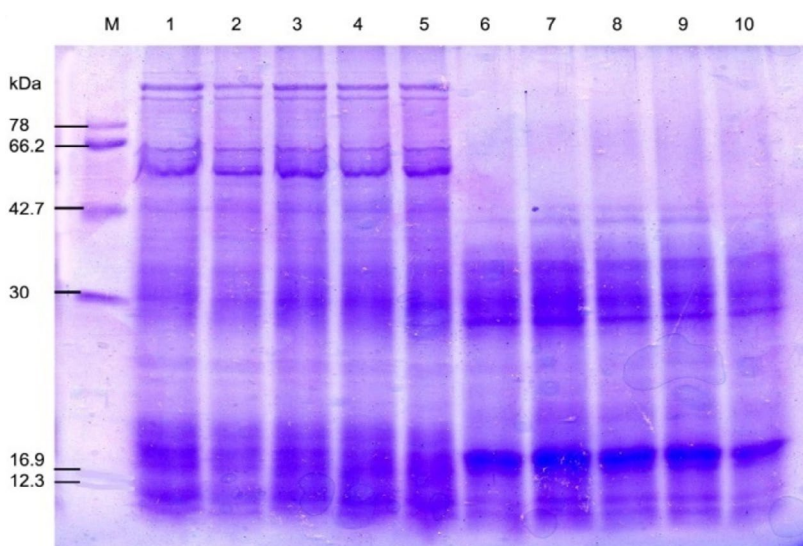


Fig. 5. SDS-PAGE of the protein profiling of three sub-aerial cyanobacteria isolates: *Scytonema coactile* Lane 2-5 and *S. geitleri* Lane 7-10 after exposure to different duration of temperature for 8 hours (35°C, 45°C, 55°C) and 3 hours (65°C), then incubated for 15 days under continuous illumination (2000 lux) at 25±2°C. An equal amount of proteins were loaded into each well. Control (25°C), Lane 1, 6; M. Molecular mass of standard proteins in kDa.

temple from where it was isolated. Further, the tendency to change the media pH towards acidic range upon growth by the two *Scytonema* species indicates their biodeteriogenic potential. There are reports that the pH of the culture medium always tends to change to alkaline range. Such a change

in attributed to the influence of extracellular products of cyanobacteria which are composed of amino acids, polypeptides, carbohydrates, hormones and vitamins (Whitton, 1965; Singh, 1974; Adhikary and Pattnaik, 1981; Grant, 1982; Stranger-Johannessen, 1988; Paul and Rout, 2017).

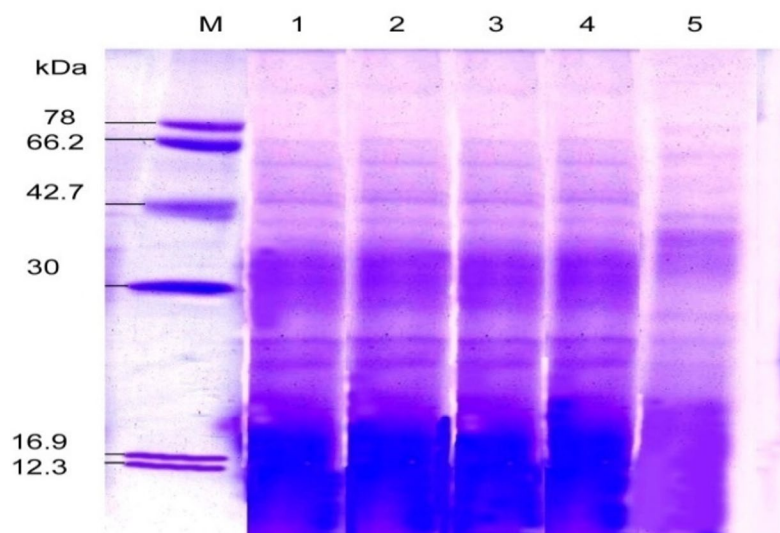


Fig. 6. SDS-PAGE of the protein profile of sub-aerial cyanobacteria isolate: *Lyngbya aerugineo-coerulea* Lane 2-5 after exposure to different duration of temperature for 8 hours (35°C, 45°C, 55°C) and 3 hours (65°C), and then incubated for 15 days under continuous illumination (2000 lux) at 25±2°C. An equal amount of proteins were loaded into each well. Control (25°C), Lane 1; M. Molecular mass of standard proteins in kDa.

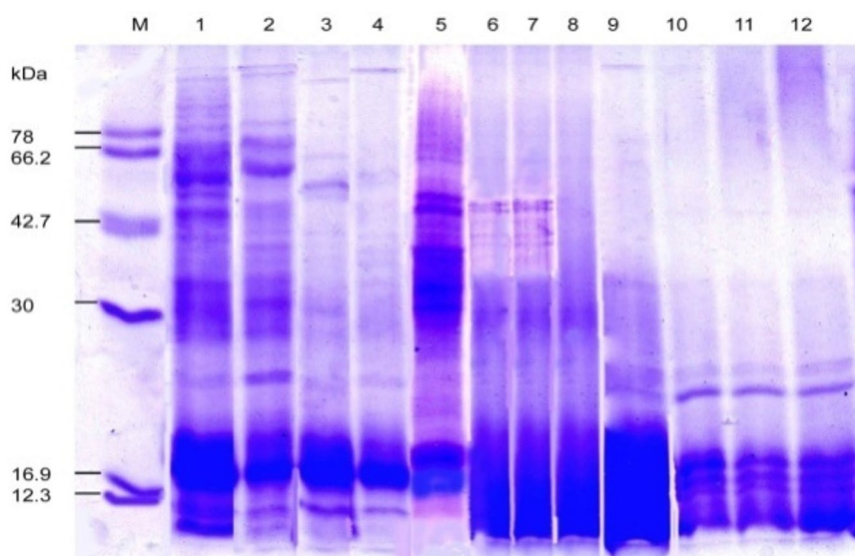


Fig. 7. SDS-PAGE of the protein profiling of three sub-aerial cyanobacteria isolates: *Scytonema coactile*, Lane 2-4 *S. geitleri*, Lane 6-8, and *Lyngbya aerugineo-coerulea*, Lane 10-12 grown in presence of different concentration of calcium carbonate (%) in the medium up to 15 days. An equal amount of proteins were loaded into each well. Control (Lane 1, 5, 9); Molecular mass of standard proteins in kDa.

However, upon the growth of both the *Scytonema* species in culture, the pH of the medium did not change to the alkaline range, rather decreased to the acidic range after 15 days of culture and the organism continued to grow in an acidic environment. Previous studies have shown that the biocorrosion process on stones can also be established by alkaline reaction (Philippis and Vincenzini, 1998). The major damaging agents for deterioration are the microorganisms like green algae, fungi, chemo-organotrophic bacteria, cyanobacteria and litho-autotrophic bacteria found on the building walls made of concrete as well as of stones cause damage by excretion of organic acids like oxalic and glucouronic acid which degrade the carbonaceous material of the stone (Gaylarde and Morton, 1999; Krumbein and Jens, 1981; Krumbein, 1988; Marthe and Choudhury, 1975; Diereks *et al.*, 1991). Several nitrifying microorganisms have also been found on historic sandstone buildings secrete nitric acids as metabolic end products also cause corrosion of the rocks (Bock *et al.*, 1988; Sand, 1987).

Generally, isolates irrespective of the habitats from where they were isolated, all were sensitive to higher temperature >35°C through there exists a variation in the degree of tolerance from species to species. Even exposure to a temperature up to 55°C up to 8 hours did not destroy these organisms, as they revived upon subsequent culture showed their tolerance capacity to elevated temperature. All the three

species of cyanobacteria showed healthy filaments enclosed by a distinct sheath, however, could survive and grow at a reduced rate in comparison to control might be their capacity to survive the adverse conditions of the tropics during summer months. The cyanobacterial forms belonging to genus *Scytonema* and *Lyngbya*, which forms dominant crust /mats on the exposed substratum of stone and facades of buildings, possessed well defined colored sheath layers around their trichome. Such layer has been shown to retain moisture (Bravery, 1988; Hoffmann, 1989; Wessels and Büdel, 1995) and allow the organisms to lose or gain water slowly in a controlled way from their cells which probably is an important factor in preventing damage to the cells during dehydration and/or desiccation period (Tripathi and Talpasayi, 1980; Grili-Caiola *et al.*, 1996), which might also be a critical factor allowing these organisms to tolerate and survive after exposure to a higher temperature. Differential mechanisms of tolerance towards the stressed environment have been reported in the literature, which might be responsible for the variability observed in the parameters examined amongst the isolates.

Many of the facades of buildings and monuments showed a characteristic form of biodeterioration caused by cyanobacteria and algae on the exposed rocks, stones, and limestone surfaces. They affect the stability of the limestone matrix, and further, through chemical corrosion of the stone forming minerals such as oxidation

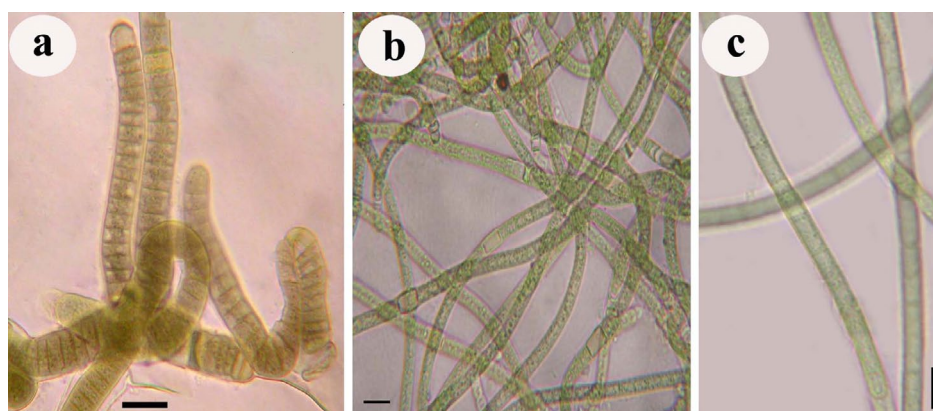


Fig. 8. Photograph showing three different sub-aerial cyanobacteria species isolates, a. *Scytonema coactile* (blackish-brown crust on the limestone surface in the twilight zone of Gupteswar cave), b. *Scytonema geitleri* (blackish crust on the lime-washed building facade) and c. *Lyngbya aerugineo-coerulea* (brownish crust on the stone surface of Bhaskareswar temple) from the eastern region of India.

and hydration reactions as well as dissolution of carbonates and solubilization of some elements from silicate bearing minerals, enhance the deterioration process. The constructed materials of buildings with calcareous substratum are usually found covered by biofilms. In such environments, the physical parameters, among which the more important are high levels of humidity and light intensity, promote the growth of some species of sub-aerial calciphytic cyanobacteria that can precipitate calcium carbonate forming extracellular encrustations. Previous studies have reported that precipitation of extra-cellular calcium carbonate is a common phenomenon due to algal growth in caves and therefore it represents a serious threat to these structures due to calcium deposition (Ortega-Calvo *et al.*, 1995, Pitri and Ricci, 1993). Another reason is most of the building walls and even ancient stone monuments are periodically washed by lime to clear the greenish/blackish mats temporarily from the exposed surfaces. Some evidence suggested that speleothem carbonate formation comprised mineralization of microbes in their cells and sheath possible on the exposed surfaces of stone sculptured monuments (Baskar *et al.*, 2007).

It has been documented that most organisms including cyanobacteria and algae investigated to date respond to stress by synthesizing a new set of proteins (Bhagawat and Apte, 1989; Adhikary, 2003; Ahikary *et al.*, 2013). The results obtained in this work showed that three different species of cyanobacteria *S. coactile*, *S. geitleri* and *L. aeruginoso-coerulea* isolated from different sub-aerial habitats responded similar pattern to pH and temperature stress, and when exposed to various concentrations of CaCO₃ concerning protein synthesis. Upon subjected to either of these stressors, specific proteins were repressed, induced, or overproduced in the cells conferring protection to the organism to cope with the stressor. When a protein is repressed, it is assumed that the organism is susceptible to injury. Similarly, when any protein is overproduced or induced it might be enabling the organism to tolerate stress, adapt, and grow normally performing usual physiological functions. Despite the report on the occurrence of stress proteins very little is known about their precise physiological function. Specific stress

polypeptides were detected in *Phormidium autumnale* and *Chroococciopsis* sp. when exposed to drought stress. Also when exposed to salt stress polypeptide of similar size were induced suggesting that both salt stress and drought stress cause similar response (Hershkovitz *et al.*, 1991). In *Synechococcus* sp. PCC 6301, several proteins with an apparent molecular weight of 91, 79, 74, 65, 61, 49, 45, 24, 22, 18, 16, 14, and 12 kDa were affected by temperature shift (Barbelyet *et al.*, 1985). Similar repression of several proteins was observed in the experimental organisms isolated from the twilight zone of a cave, building facades and surface of a stone monument in response to various environmental variables. Also, induction and accumulation of proteins have been demonstrated to be closely correlated with the development of stress tolerance (Potts, 1994). However, might be changed in protein expression contributes to the survival process. Changes in the expression of a large number of proteins occur due to a particular stress, yet only some of these proteins are probably involved in stress tolerance. It may also be possible that the synthesis of a protein indicates sensitivity to a stressor rather than being a part of the tolerance mechanism. In general, the results of the present work indicate that protein synthesis and modification in all these experimental organisms from three different sub-aerial habitats is an adaptive behavior so far as they can tolerate a specific pH level, temperature, or calcium carbonate concentration for their survival.

CONCLUSION

The present study focused on the survival strategy of cyanobacteria which mainly depends on the nature of the substratum and environment. These factors allow the micro-organisms to form sub-aerial biofilms on extreme habitats. This leads to the characterization of species on different habitats based on ecological requirements. Moreover, it was observed that the most representative genera occurring in unfavorable environments develop different strategies to survive in such conditions due to the production of sheath composed of extracellular polymeric substances as a protection against desiccation, ensuring the maintenance of moisture by balancing changes in humidity and temperature

which permits cyanobacteria to resist drought periods. Due to the presence of these colloidal polymeric substances, the cyanobacteria biofilms cause various changes in the substratum by biogeophysical and biogeochemical processes and leads to biodeterioration.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTIONS

Both the authors have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

ETHICS STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

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