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Diversity and Characteristics of Terrestrial Cyanobacteria near Gimhae City, Korea

Shachindra Nath Tripathi[1]*, **Ik Kyo Chung**[2], **and Jin Ae Lee**[3]*
[1]Department of Botany, Banaras Hindu University, Varanasi 221005, India
[2]Division of Earth Environmental System, Pusan National University, Busan 609-735, Korea
[3]School of Environmental Science and Engineering, Inje University, Gimhae 621-749, Korea

Cyanobacteria samples were collected from smears and mats growing on the surfaces of buildings, roadside walls, rocks, and monuments near Gimhae City, Korea. These populations were not as thick and dense, nor as diverse, as those found in temperate and tropical regions. Crusts at Gimhae City were dominated by colonial forms of species from *Chroococcus, Aphanothece, Aphanocapsa, Gloeothece, Gloeocapsa,* and *Chroococcidiopsis,* as well as by filamentous forms from species of *Scytonema* and *Tolypothrix.* The colonial forms appeared primarily as smears, especially for species of *Gloeocapsa* and *Chroococcidiopsis,* whereas filamentous forms were the major constituents of mat populations. Invariably, the cells and trichomes of these colonial and filamentous forms were enclosed with a thick, firm, multi-colored laminar sheath, as seen by transmission electron microscopy. Such an association between cell walls and trichomes was stronger in the colonial forms, particularly for *Gloeocapsa,* compared with the filamentous forms, in which the sheath did not detach from the wall even under arid conditions. The organization of cytoplasmic structures, particularly the thylakoid membranes, was least affected by acute dryness.

Keywords: diversity, sheath, terrestrial cyanobacteria, ultrastructure

Over the past 3.5 billion years, the nearly ubiquitous cyanobacteria have tolerated a wide range of environmental stresses, e.g., heat and cold shock, anaerobiosis and oxygeny, photooxidation, nitrogen starvation, salinity, osmotic stress, and drought (Schopf, 2000). These terrestrial cyanobacteria are found primarily on the surfaces of bare rocks, building materials, soil, and tree bark, all areas where little or no precipitation may occur. Such adaptability allows these organisms to survive without irreversible damage when moisture is insufficient for most other life forms. Not only are these habitats subject to extremes of desiccation but also to temperatures that may be several degrees higher than ambient. For example, cyanobacteria are found on rocks in the Swiss Alps where annual temperatures can vary by 73°C (Jaag, 1945), as well as in the tropics with temperatures ranging from 20°C to 50°C (Zehnder, 1953), and atop exposed buildings at 70°C (Tripathi and Talpasayi, 1980). The flora at these sites are primarily comprised of cyanobacterial species. Jaag (1945) reported the widespread occurrence of such communities, which were dominated by *Gloeocapsa, Scytonema,* and *Stigonema.* Storm (1920) described three different communities in Norway: 1) reddish-black crusts dominated by *Gloeocapsa,* 2) the brown, cushion-like jelly masses of *Scytonema,* and 3) *Stigonema.* Golubic (1967) observed grey felt masses of *Hapalosiphon intricatus* with an undergrowth of *Aphanocapsa* in the darkest portions of a cave in Yugoslavia, where the outer community consisted of *Scytonema myochrous* and the intermediate position contained *Aphanocapsa* and *Chroococcus.* Golubic (1967) found *Gloeocapsa sanguinea, Gloeocapsa kuetzingiana, Gloeocapsa compacta,* and *S. myochrous* on old, drying church walls. Rishbeth (1948), Brook (1968), and Schlichting (1975) also demonstrated the dominance of cyanobacterial flora on building surfaces and materials that received little water.

In the Tropics, Fritsch (1907) made extensive studies of sub-aerial algae and showed that the cyanobacteria comprised mainly the species of *Aphanocapsa, Gloeocapsa, Chroococcus, Nostoc, Tolypothrix, Scytonema, Hapalosiphon,* and *Stigonema.* Subsequent research on the Indian continent also revealed an abundance of cyanobacterial growth in habitats that underwent frequent wetting and drying (Biswas, 1924; Ghose, 1927; Bharadwaja, 1933, 1940; Rao, 1936, 1937). On the campus of the Banaras Hindu University, India, where tree bark and the surfaces of buildings are exposed to wide fluctuations in humidity (20% to 85% RH), temperature (6°C to 44°C), and light intensity (250 to 436 cal cm^{-2} day^{-1}), and where summertime mid-day surface temperatures can reach 60°C to 70°C, the flora mainly include non-spore-forming species of *Chroococcus, Gloeocapsa, Gloeothece, Aphanocapsa, Aphanothece, Lyngbya, Microcoleus, Symploca, Tolypothrix,* and *Scytonema* (Tripathi et al., 1991). In desert settings, Shields and Drouet (1962) have demonstrated the presence and survival of non-spore-forming algae, especially cyanobacteria, in the soils rather than on rocks, bark, and buildings. Davis (1972) has compiled a list of nearly 105 algal species, including desiccation-tolerant cyanobacteria, and have described their habitats and survivability. Furthermore, Metting (1981) has completed a detailed review of the systematics and ecology of soil algae.

Nevertheless, little information is available about the diversity and distribution of cyanobacteria that inhabit the surfaces of buildings, rocks, or trees in Korea. It is important to understand the significance of these organisms while gaining knowledge about the structural, physiological, and molecular components of their mechanisms for desiccation tolerance, as well as their potential for societal, commer-

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*Corresponding author; fax +91-542-236-8174, +82-55-334-7092
e-mail tns46@yahoo.com, envjal@inje.ac.kr

cial, and biotechnological applications. Therefore, we investigated the diversity and structural characteristics of terrestrial cyanobacteria near Gimhae City, Korea.

MATERIALS AND METHODS

Description of Study Area

We examined the sub-aerial growth patterns of cyanobacteria species on the surfaces of buildings, rocks, and monuments (e.g., the Tomb of King Suro) near Gimhae City, Korea (35° 15'N; 178° 54'E; elevation ca. 50 m). These sites were constructed primarily from stone. The local climate manifests features of a sub-temperate region. Meteorological data collected between 1998 to 2003 (Fig. 1A-D), showed an average ambient temperature ranging from -2°C to +27°C; from May to October, temperatures stayed above 20°C, and reached a maximum in June and July. However, minimum and maximum temperatures were -5°C and +32°C. Maximum precipitation was received between June and September (400 to 500 mm), but an exceptionally high amount (755 mm) was recorded in August of 2002. In the drier months of April and May, some sporadic rains (up to 300 mm) also occurred. The average RH ranged from 50% to 90%, being higher from June to September, and reaching maximum values in August. Irradiation was measured in a range of 130 to 250 cal cm^{-2} h^{-1}, but a maximum of 300 cal cm^{-2} h^{-1} was recorded in May 1999 and a minimum of 60 cal cm^{-2} h^{-1} occurred in July 1998.

Field Work

Algal samples were collected aseptically by scraping crusts from the surfaces of buildings, rocks, and monuments. Their cyanobacterial forms were identified both by direct exami-

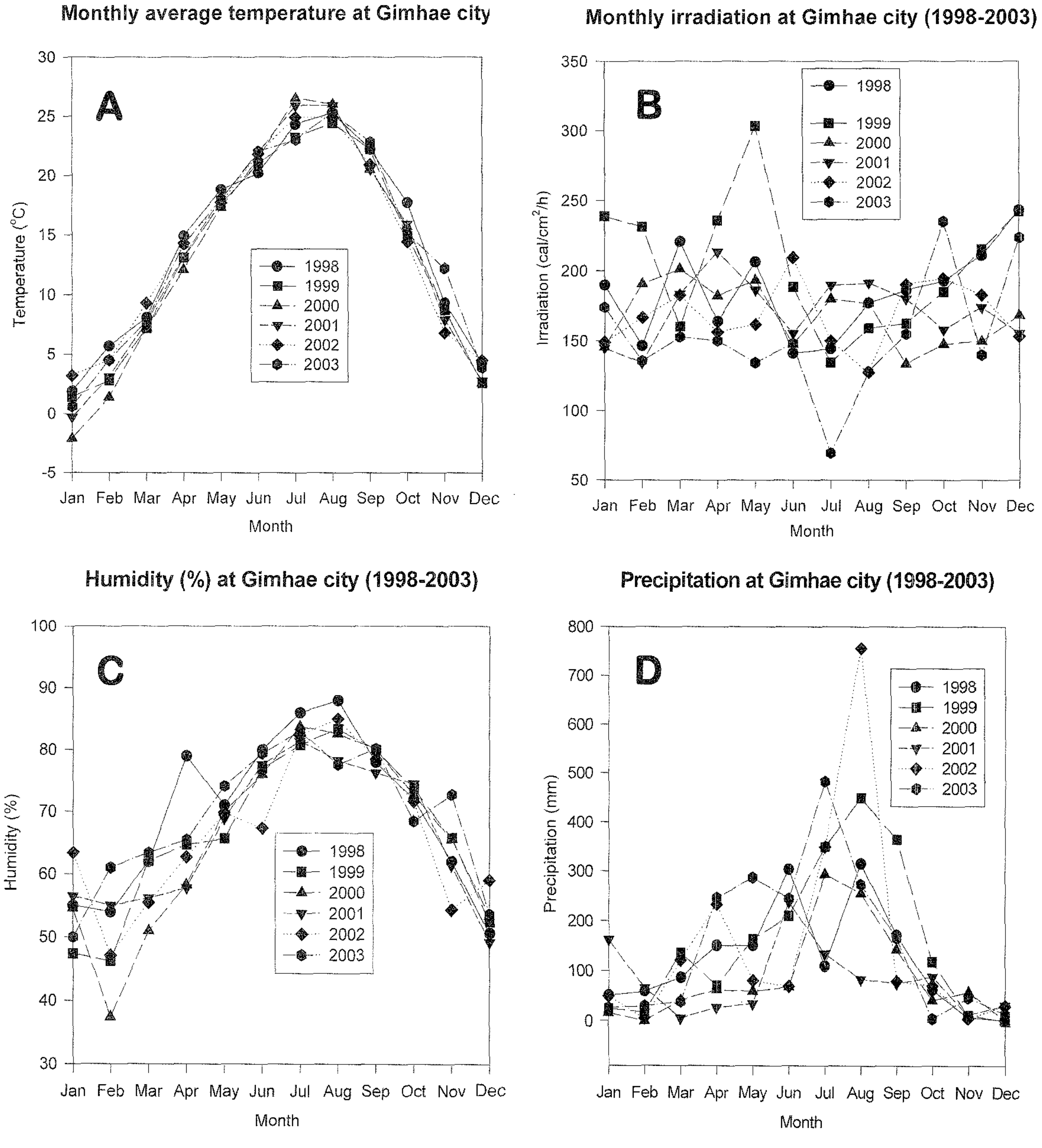

Figure 1. Climatic conditions at Gimhae city (1998-2003). (**A**) Monthly average temperature; (**B**) Monthly average irradiation; (**C**) Monthly average humidity (%); (**D**) Monthly average precipitation.

nation under a microscope as well as by consulting the taxonomic monographs of Desikachary (1959) and Geitler (1932). When identification proved difficult at the species level, the genera were simply numbered. The terms 'colonial' and 'filamentous' are used here to describe the predominant cyanobacterial crusts.

Transmission Electron Microscopy

TEM was performed with an electron microscope (JEM 1200EX-II; JEOL, Japan), according to the methods of Rippka et al. (1974), at the Histology Laboratory, Inje University. Algal samples were collected from their natural habitats under dry conditions. Before pre-fixing with 0.5% W/V glutaraldehyde, we used an air-blower at room temperature (RT; 25°C) to remove as many soil particles as possible from the sample surfaces. We then increased the glutaraldehyde concentration to 3% for 4 h at RT. After rinsing six times with sodium cacodylate buffer (0.1 M pH 7.2), we post-fixed these materials with osmium tetroxide (1%) in the above buffer for 30 min at RT. After the materials were washed five times with buffer and once with distilled water, they were dehydrated by washing with ethanol and acetone.

RESULTS

Algal Community

Algal communities appeared as black-brown streaks or patches (Fig. 2A-D, 3A-D) on the surfaces of buildings, rocks and monuments. These were dominated by colonial and filamentous cyanobacterial forms belonging to the Orders Chroococcales, Chamaesiphonales, Nostocales, and Scytonematales. The greenish black-brown smears were categorized as colonial crusts; the other crusts were termed filamentous.

These cyanobacterial taxa were examined directly under a microscope, and are described in Tables 1 and 2 and in Figure 4A-L. Species of *Chroococcidiopsis* and *Gloeocapsa* were the dominant constituents of the colonial crusts. The former were found on terrestrial surfaces that maintained wet conditions for longer durations while the latter were more commonly distributed on dry surfaces that were moist for only brief periods. These two genera were intermingled with other colonial forms, primarily species of *Chroococcus*, *Aphanothece*, *Aphanocapsa*, and *Gloeothece*. The Chroococcales members included *Chroococcus various*, *Chroococcus motanus*, *Chroococcus coharens*, *Aphanothece naegellii*, *Aphanothece nidulans*, *Aphanocapsa montana*, *Aphanocapsa brunnea*, *Gloeothece rupestris*, and *Gloeothece fusco-lutea*. *Gloeocapsa pleurocapsoides*, *Gloeocapsa polydermatica*, *G. kuetzingiana*, *Gloeocapsa aeruginosa*, *Gloeocapsa atrata*, and *Gloeocapsa montana* were the most frequently encountered Gloeocapsean forms, particularly on building surfaces. Older, wet structures also showed occasional occurrences of *Entophysalis granulosa*.

In contrast, older building, rocks, and monuments were colonized by much thicker, blackish-brown algal growth that

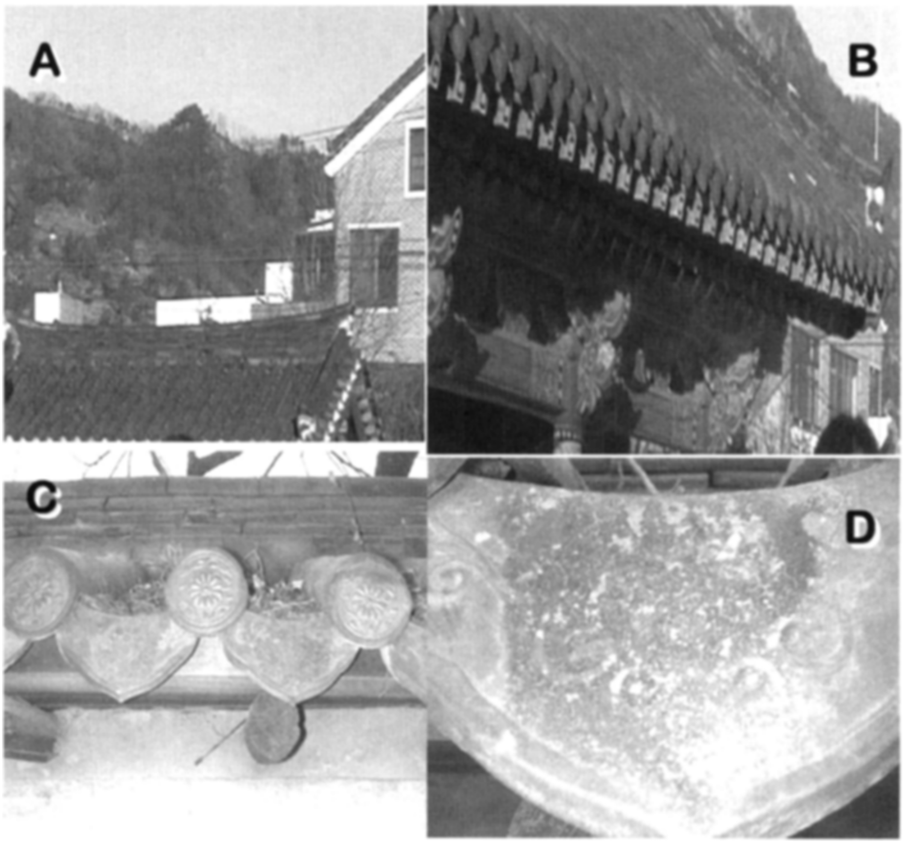

Figure 2. Building surfaces showing cyanobacterial growth. (**A**) and (**B**) Building surfaces showing growth of subaerial cyanobacteria; (**C**) Panels on wall of a monument for water run-off showing cyanobacterial growth; (**D**) A closure view of the panels on wall of the monument.

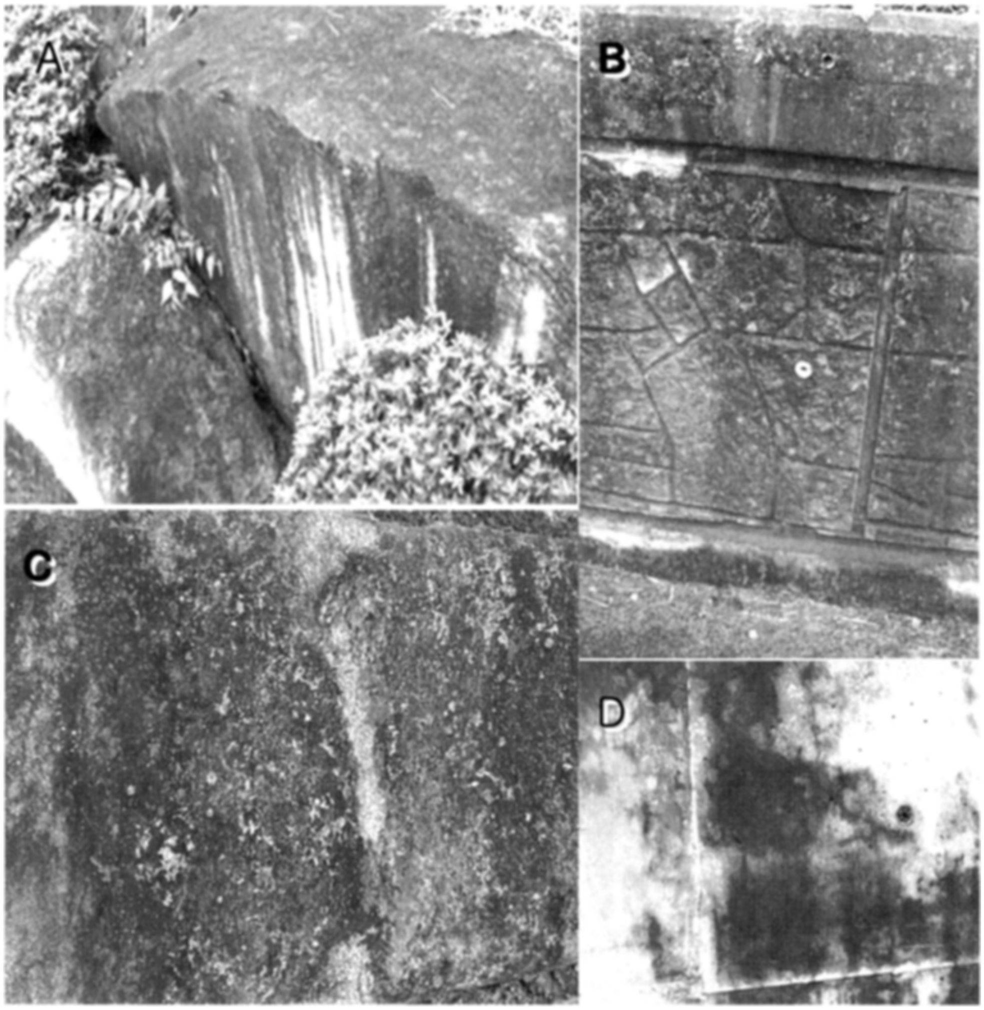

Figure 3. Cyanobacterial growth on rocks and roadside walls at the Inje University campus. (**A**) Rock surfaces showing colonial cyanobacterial crusts; (**B**) Road side wall surfaces showing growth of subaerial cyanobacteria; (**C**) Building surfaces showing filamentous cyanobacterial crusts; (**D**) Building surfaces showing colonial cyanobacterial smears.

comprised mainly the tufted mats of filamentous cyanobacteria. These mats had three layers: upper, middle, and lower. The middle portion contained compact and prostrate filaments that were the main component of those mats. The upper layer was formed by aerial growth of the prostrate filaments while the lowest portion held the empty sheath, and dead and decaying mat filaments. Surfaces that tended to retain water for longer periods were most commonly inhabited by non-heterocystous filamentous cyanobacteria, such as species of *Microcoleus* and *Phormidium*, that were intermixed with the colonial forms of *Chroococcus*, *Aphanothece*, *Aphanocapsa*, *Gloeothece*, and *Gloeocapsa*. In the older mats, species of *Lyngbya* were the major component. Surfaces with less water-holding capacity supported a higher population of species of *Scytonema* and *Tolypothrix*. Colonial forms made up most of the lower strata in those filamentous mats. Under uni-algal conditions, the most common species were *Tolypothrix byssoidea*, *Tolypothrix fragilis*, *Scytonema schmidtii*, *Scytonema dilatatum*, and *Scytonema guyanense*. Most building surfaces with mat-forming cyanobacteria were dominated by species of *Scytonema* mixed with *Tolypothrix*. Under wet conditions, these mats were sometimes composed of non-heterocystous filamentous forms, such as *Phormidium* sp.

Structural Characteristics

Cyanobacteria that inhabited areas exposed to direct sunlight were invariably characterized by firm, thick, and sometimes lamellated sheaths of various hues. Individual cells (e.g., species of *Gloeocapsa*), trichomes (e.g., *Scytonema*/*Tolypothrix*), and entire colonies (e.g., *Chroococcidiopsis* and *Gloeocapsa*) were enclosed by these sheaths. In contrast, species of *Chroococcus*, *Aphanothece*, *Aphanocapsa*, *Microcoleus*, *Lyngbya*, and *Phormidium*, which either occupied shady environments or inhabited other colonial or filamentous crusts tended to have colorless and, sometimes, diffluent sheaths.

As has been observed with certain aquatic species, some of our terrestrial cyanobacteria that were found within other forms or on wet surfaces showed complex, Gram-negative cell walls. There, the cytoplasmic membrane was covered by a peptidoglycan layer, and an outer membrane with an additional extracellular layer(s) i.e., the S layer(s), was

Table 1. Dominant cyanobacterial flora in colonial crusts on buildings, rocks, and monuments.

Taxon	Habits and Habitats
Chroococcus various	2- to 6-µm globular cells occur mainly in groups as part of algal smears and crusts, or intermingled in the lower portions of filamentous crusts (attached with substrata). Indistinctly lamellated sheaths are thick (up to 6 µm), colorless, yellowing, or dirty-brown (when dry).
C. motanus	Brownish-black gelatinous colonies (up to 30-µm diam.) with 4- to 6-µm cells (single or in groups of 2 to 4) as part of colonial and filamentous crusts
C. coharens	5- to 20-µm, bluish-black colonies comprising single cells or groups of 2 to 10 cells (2- to 7-µm diam.) are common in all terrestrial habitats when exposed to sun, or within other colonial or filamentous forms.
Aphanothece naegellii	Colonies are yellow to olive-green gelatinous masses with oval cells (size 3 to 6 µm X 5 to 8 µm) with diffluent sheaths. Predominantly single or as a part of filamentous crusts, and also within colonies of colonial forms.
A. nidulans	Small, irregularly shaped colonies comprising mostly cylindrical cells (1 to 2 µm X 4 to 5 µm), with hyaline or yellow diffluent sheath common to colonial forms.
Aphanocapsa montana	Colonies consist of yellow-green or colorless, irregular gelatinous masses containing spherical cells (2- to 5-µm diam.), with mucilaginous and diffluent sheaths. Forms are both colonial and filamentous.
A. brunnea	Spherical cells (4- to 5-µm diam.) embedded within membranous, diffluent, and gelatinous sheath are distributed mainly within filamentous crusts.
Gloeothece rupestris	Oval to subglobose colonies comprising ellipsoidal-cylindrical cells (4 to 5 µm X 8 to 16 µm), with thick, diffluent sheath (colorless to brownish). Occurs primarily in colonial crusts.
G. fusco-lutea	Colonial structure similar to *G. rupestris*, but with smaller cells. Colonies are larger, containing 16 to 32 cells. Distribution is confined mainly to colonial crusts.
Gloeocapsa pleurocapsoides	Cells (5- to 10-µm diam.) are enclosed with thin and firm, variously colored 3- to 4-µm lamellated sheath, predominant in colonial crusts.
G. polydermatica	Spherical cells (3- to 5-µm diam.) are contained within compact colonies with very thick, distinct, firm, lamellated sheath, predominant in colonial crusts.
G. kuetzingiana	Densely aggregated spherical cells (4- to 8-µm diam.) develop thin, soft, brownish or blackish colonies of nearly 150-µm diam. Firm and lamellated sheaths (4- to 5-µm-thick) are major component of colonial crusts.
G. aeruginosa	Spherical cells (2- to 4-µm diam.) form small, spherical colonies (15- to 50-µm diam.) that are crustaceous, granulose, or cartilaginous. Sheath of cells is firm and indistinctly lamellated. Mainly observed in filamentous crusts.
G. atrata	Colorless, indistinctly lamellated, firm sheath bearing colonies of cells (3- to 5-µm diam.) are common in filamentous and colonial crusts as crustaceous thalli.
G. montana	Broad, mucilaginous, light bluish-green to dirty-yellow colonies (15- to 30-µm diam.) with spherical cells (2- to 5-µm diam.) and brownish homogeneous sheaths are common in colonial crusts, and occur occasionally in filamentous crusts.
Chroococcidiopsis sp.	Colonies with broad, spherical cells (5- to 7-µm diam.) and firm sheath and varying numbers of cells/endospores. Most common on surfaces exposed to sun, and also with filamentous crusts.
Entophysalis granulosa	Colonies more or less like *Gloeocapsa* sp. Cells are arranged in straight rows. Distribution is similar to *Chroococcidiopsis* sp. and *Gloeocapsa* sp.

attached to the cell wall (Fig. 5). These S layers (extracellular polysaccharides) comprised three layers. The inner layer (S_1), attached to the cell wall, was electron-translucent, following another much more electron-dense layer (S_2). The third-outermost layer was highly electron-dense (S_3). Likewise, some of the cyanobacteria from dry surfaces, such as species of *Gloeocapsa*, *Chroococcidiopsis*, *Lyngbya*, *Scytonema*, and *Tolypothrix*, had several additional S layers (S_1, S_2, and S_3) with electron-dense margins (Fig. 5A-F). These individual cells of the colonial forms also were surrounded by several additional S layers (Fig. 5C-E). The sheath appeared to have initially formed around a single cell or trichome, then expanded through continual deposition of sheath material to accommodate the synchronously dividing cells of the colony or filament (marked with arrows in Fig. 5A, F). Invariably, an electron-translucent layer (S_1) was found between the intact cell envelopes and those electron-dense layers (S_2 and S_3). This was especially true for the dry colonies or filaments, while such an intermediate layer was either negligible or absent in some forms found in either wet (Fig. 5A, B, D) or very dry conditions (not shown).

Whether examined in a dry or re-hydrated state, no major ultrastructural variations were seen in the cellular organizations of these terrestrial cyanobacteria, and their cell walls, cell membranes, and thylakoid membranes appeared intact. Nevertheless, the composition of dry cyanobacterial cells, based primarily on the appearance of the thylakoid membranes, was more electron-dense than in the re-hydrated cells. Several inclusion bodies also were more common in the dry than in the re-hydrated cells. Furthermore, in some cases, mainly in the filamentous forms, dehydration revealed a distinct discontinuity (S_1 layer) between the sheath and the cell wall/cell membrane.

DISCUSSION

Flora compositions of the algal populations examined

Table 2. Dominant cyanobacterial flora in filamentous crusts on buildings, rocks and monuments.

Taxon	Habits and Habitats
Phormidium sp.	Trichomes cylindrical, constricted with rectangular cells (1- to 5-µm-long) and round apices. Filaments are attached to substratum. Colorless, firm, lamellated sheath (0.5- to 2-µm-thick) with ends open. Always encountered in filamentous crusts.
P. ambiguum	Blue-green to dark, variously entangled filaments with slightly constricted trichomes at cross walls, where cells are shorter than broad, 1.5- to 3-µm-long. Sheath is thin. Filaments are observed within filamentous crusts dominated by *Scytonema* spp.
P. calcicola	Thick mats forming thallus with densely entangled filaments, mainly found with other filamentous forms and occasionally with colonial crusts. Filaments and trichomes are 6 to 8 µm and 5 to 7 µm, respectively. Sheath is very thick, colorless, and unlamellated.
Lyngbya sp.	Thallus is caespitose, extensive, dense floccose, lubricous, purple-violet when dried, often becoming dull yellow to colorless. Sheath is thin, trichomes are constricted at cross walls (5- to 8-µm-wide). Filaments occasionally intervene with other filamentous forms.
Microcoleus chithonoplastes	Dirty- to dark-green lamellated thallus comprising single, coiled, but seldom-branched filaments with trichome bundles, observed with other filamentous forms. Trichomes are constricted at the cross walls, 2- to 6-µm-wide with 3- to 10-µm-long cells.
Scytonema schmidtii	Abundant and repeatedly false-branched filament-bearing thallus is common to filamentous crusts. Filaments (10- to 12-µm diam.) are extremely and irregularly intricate, and bear a yellowish-brown, thick, firm sheath. Trichomes (9- to 12-µm-wide) are extremely torulose.
S. bohneri	Filaments (10- to 12-µm-wide) have mainly single or occasionally double false branches, cells rectangular, short at the apices, thick sheathed (1 to 3 µm) and possessing thalli, are predominantly observed under uni-algal states.
S. dilatatum	Filaments 8- to 12-µm-wide, with single and double false branches, sheath colorless to yellowish-brown and 1- to 2-µm-thick; trichomes (5- to 9-µm-wide), with bluish-green thallus; are more often found with other filamentous forms.
S. guyanense	Thick, blackish-green mats with 12- to 20-µm-wide filaments in bundles and mostly false double branches. Thick, firm, dark-yellow to brown sheath is most abundant form in filamentous crusts.
Tolypothrix byssoidea	Brown to blackish cushion-like thallus with filaments (10- to 15-µm diam.) and irregularly branched trichomes (9- to 11-µm-wide) and thin, firm sheath are most common with filamentous crusts.
T. fragilis	Trichomes (4- to 6-µm-wide) with lamellated yellow to brown sheath, forming short, straight filaments (5- to 7-µm-wide) form thin mats on terrestrial surfaces.

near Gimhae City, Korea, were similar to the terrestrial cyanobacteria reported by Fritsch (1907), Storm (1920), Jaag (1945), Rishbeth (1948), Golubic (1967), Brook (1968), Schlichting (1975), and Tripathi et al. (1991). The main components of our algal populations were species of *Gloeocapsa*, *Scytonema*, and *Tolypothrix*, which are also observed in tropical regions (Tripathi et al., 1991). However, unlike in the Tropics, we did not find that species of *Chroococcus*, *Aphanothece*, *Aphanocapsa*, *Lyngbya*, *Phormidium*, and *Symploca* were the dominant forms in cyanobacterial crusts, but were instead part of the population that grew within or below those crusts. Species of *Chroococcidiopsis*, commonly recorded as endolithic cyanobacteria in deserts and Antarctic soils (Broady, 1981; Potts, 1994) were also dominant at Gimhae City. None of our collected samples contained *Nostoc*, *Calothrix*, *Hyperthrix*, *Stigonema*, and *Hapalosiphon*, even though these have been reported from tropical and subtropical habitats (Fritsch, 1907; Jaag, 1945). In a study of wall flora, Rishbeth (1948) described the widespread growth of green algal forms for *Chlorococcum* sp., *Cladophora glomerata*, *Oedogonium* sp., *Hormidium flaccidum*, *Prasiola crispa*, and *Trentepholia aurea*. To these, Schlichting (1975) added other green forms of *Bracteococcus* sp., *Chlorella* sp., *Gloeocystis* sp., *Pleurococcus* sp., *Stichococcus* sp., *Ulothrix* sp., and several cyanobacterial forms, including *Agmenellum quadruplicatum*, *Coccochloris* sp., *Coelosphaericum* sp., *Gomphoshaeria* sp., *Nomoeothrix janthina*, *Pelogloea protuberans*, *Plectonema* sp., and *Schizothrix* sp. These algal forms were apparently inconspicuous in the terrestrial habitats near Gimhae City. Therefore, we can conclude that our terrestrial sampling sites were not very favorable to the growth of either green or blue-green algae.

The diversity of species possible at a particular site depends upon environment. Under extreme conditions, that range is greatly reduced and some types of organisms may exist only as pure cultures (Brock, 1978). Tropical regions are characterized by high values for temperature (25°C to 35°C), relative humidity (>90%), moisture retention, and light intensity, all parameters that are favorable to cyanobacterial growth. In tropical urban areas, more extreme temperatures are possible for ambient layers (45°C) and building surfaces (70°C). At Gimhae City, however, temperatures normally ranged between 20°C and 28°C, with solar radiation values of 150 to 200 cal cm^{-2} h^{-1}, relative humidity <90% during the rainy season (June to October), and a maximum annual precipitation of 500 to 750 mm. Therefore, these moderate local conditions were more similar to those found in sub-temperate zones. Moreover, the topography of the Gimhae City region meant that less water was retained on terrestrial surfaces. All of these characteristics may explain the lower diversity of taxa and smaller populations of cyanobacteria in this area of Korea. In addition, the sporadic occurrence of cyanobacterial growth was confined mainly to surfaces that did not remain wet for long periods.

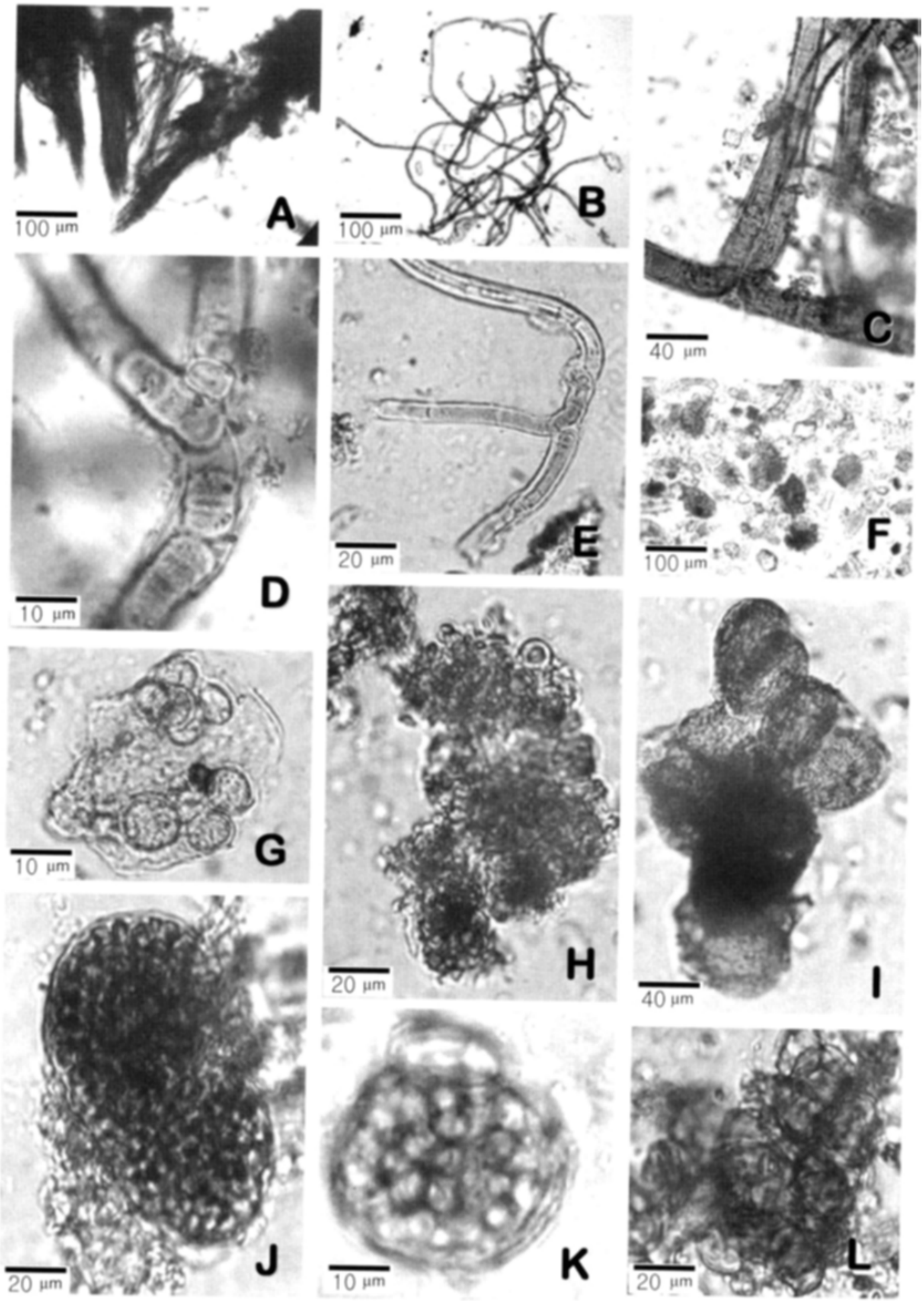

Figure 4. Some of the dominant filamentous and colonial cyanobacterial forms. (**A**) *Scytonema* sp. filaments in bundles; (**B**) Sparsely dispersed filaments of *Scytonema* sp.; (**C**) *Scytonema* sp. with double false branching; (**D**) *Tolypothrix* sp., with hormogonia, and showing single false branching adjacent to heterocyst; (**E**) *Tolypothrix* sp. filament with single false branching; (**F**) Cyanobacetrial components colonial crusts; (**G**) Nanocysts of *Chroococcidiopsis* sp.; (**H**) Intermingled colonies *Aphanothece* sp. and *Gloeocapsa* sp.; (**I**) Colonies of *Chroococcidiopsis* sp.; (**J**) Single colony (enlarged) of *Chroococcidiopsis* sp; (**K**) *Chroococcidiopsis* sp colony (dry); (**L**) Thick, firm, colored sheath bearing *Gloeocapsa* sp. colonies.

This availability of water is an important determinant of the distribution and activity of microbial communities (Potts, 1994, 1995). Thus, we could consider the Gimhae terrestrial habitats to be extreme environments, particularly because those surfaces remained dry for seven to eight months each year, and the inhabiting algal forms were

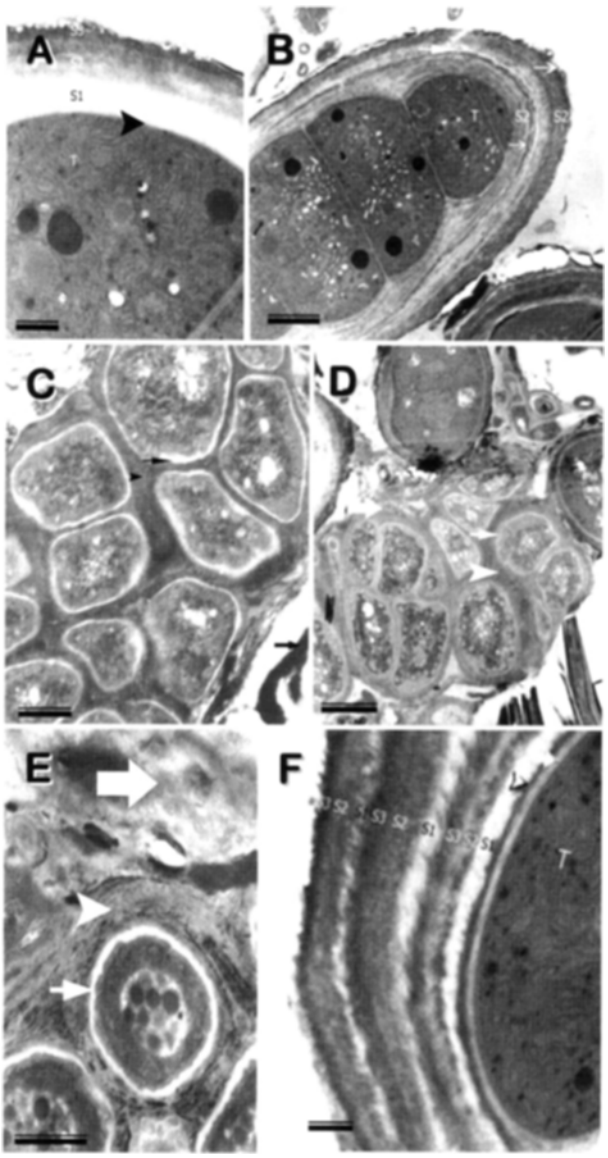

Figure 5. TEM of some colonial and filamentous cyanobacteria. (**A**) *Chroococcus* sp. (rehydrated) showing S1, S2 and S3 layers of sheath, and intact cell (marked arrow) and thylakoid (T) membranes (scale bar, 500 nm); (**B**) A portion of *Scytonema* sp. (rehydrated) filament (scale bar, 2 μm), and (**D**) *Gloeocapsa* sp. (rehydrated) colony showing indistinct S1 and distinct S2 and S3 layers of sheath (scale bar, 4 μm); (**C**) Nanocysts of *Chroococcidiopsis* sp. (dry) with distinct S1, S2 and S3 layers (marked with different arrow types) of sheath, and dense cytoplasm (scale bar, 500 nm); (**E**) *Gloeocapsa* sp. (dry) with different distinct layers (marked arrows) of sheath and dense cytoplasm (scale bar, 500 nm); (**F**) *Scytonema* sp. (dry, apical portion) showing distinct multiple layers (S1, S2 and S3) of sheath (scale bar, 500 nm).

exposed to desiccation, albeit at temperatures lower than those common in tropical regions.

Desiccation-tolerant cyanobacteria commonly synthesize copious amounts of an extracellular polysaccharide (EPS), which undergoes striking changes in its rheological properties in response to water availability (Tripathi et al., 1991; Potts, 1994, 1997; Hill et al., 1997). The release of exopolysaccharides regulates the loss and uptake of water (Philippis and Vincentini, 1998; Potts, 2000). Moreover, an increase in the amount of acid-, sulfate-, and beta-linked polysaccharides is related to water deficits (Grilli et al., 1996). At Gimhae City, the cyanobacteria, either colonial or filamentous, typically showed thick, firm, variously hued sheaths that were sometimes lamellated. For example, ultrathin sections of cryo-substituted old-aged filaments of *Phormidium uncinatum* revealed that the sheath consisted of a single layer of fine fibrillar material, with a total thickness of up to 0.3 mm. This sheath was directly attached to a complex extracellular wall layer that was formed by an S layer and oscillin surface fibrils (Hoiczyk, 1998). An examination of cells from the desiccation-tolerant *Nostoc commune* revealed an electron-translucent layer (S_1) attached to an intact cell membrane and an extracellular glycan layer (S_3 i.e., bulk EPS). This S_1, which was discontinuous when cells were dry, was found, after 15 min of re-hydration, to be attached to the intact cell membrane (Potts, 1994). Nevertheless, Hill et al. (1997) have reported that this discontinuity corresponds precisely to the electron translucent layer (layer S_1), which appears as a white, unstained area around the desiccated cells when they are then post-stained for examination. It is possible that the material(s) present in S_1 are low-molecular-mass carbohydrates that retain dye and have different pH characteristics in relation to the bulk EPS when sections are prepared and stained for light-microscopic analysis (Hill et al., 1994). Here, the secreted EPS of *N. commune* constituted about 60% of the colony's dry weight. This organism (primarily the S_3 layer) harbors water-stress proteins (Wsp) that account for >70% of the total soluble protein in desiccated cells, as well as UV-absorbing pigments that represent approximately 10% of the colony dry weight (Hill et al., 1994; Böhm et al., 1995). Ultrastructurally, the sheaths of cyanobacteria collected from Gimhae City could be classified into three groups: 1) species of *Chroococcus*, *Aphanocapsa*, *Microcoleus*, and *Phormidium*; 2) species of *Gloeocapsa*, *Chroococcidiopsis*, and *Entophysalis*, which are characterized by sheath structures more similar to those found with *P. uncinatum* (Hoiczyk, 1998) and *N. commune* (Potts, 1994; Hill et al., 1997); and 3) species of *Scytonema* and *Tolypothrix*, and some also of *Gloeocapsa*, which contain three multiple layers (S_1, S_2, and S_3). The S_2 appeared to be amorphous and granular while the S_3 was fibrous and more electron-dense, requiring more detailed examination. The S_1 layer was least prominent in our Gloeocapsean colonies. Noticeably, almost all the cyanobacterial forms, except for the Oscillatorean members, showed cells that were completely enclosed by the sheath, as well as intact cellular components. The cell wall, cell membrane, thylakoid membranes, cyanophycin granules, and carboxysomes also were intact in the desiccated cells of *Chroococcus* sp., *Chroococcidiopsis* sp., and *N. commune* (Potts et al., 1983; Peat et al., 1988; Potts, 1994, 1999).

In addition to understanding the structure, physiology, and molecular mechanisms necessary for the growth and survival of terrestrial-desiccation organisms, it is important for societal and commercial reasons that researchers determine the best methods for drying cells (or their components) and storing them long-term (Potts et al., 2005). Oxidative stresses that are generated during the processes of re-hydration and dehydration may possibly be overcome through the use of antioxidants and antioxidative enzymes (Potts, 1999; Tripathi and Srivastava, 2001). Although no specific proteins have been identified as water-stress proteins, a group of acidic proteins (32- to 39-kDa) earlier treated as Wsps have been shown to accumulate in extracellular glycan and share homologies with carbohydrate-modifying enzymes (Scherer and Potts, 1989; Hill et al., 1994). The accumulation of two non-reducing sugars -- trehalose and sucrose -- in several cyanobacteria (Hershkovitz et al., 1991; Potts, 1994) has been proposed as a means for circumventing lethal damage by replacing structural water during the drying period (Crowe et al., 1997). Brockbank (2000) has reported that the stabilization of cells and cell products has a market value of several billion US dollars. In addition to their applications in exobiological studies, many desiccation-tolerant pathogenic bacteria, e.g., *Staphylococcus aureus*, are now considered useful as biowarfare agents.

Overall, the extremely low diversity in cyanobacterial populations near Gimhae City, Korea, demonstrates that an environment characterized by lower values for temperatures, light intensity, precipitation, and water retention is not as conducive to cyanobacterial growth as that found in tropical regions. However, the relatively unexplored species at these Korean sites are structurally similar to other desiccation-tolerant species, and may provide a good source for researchers attempting to understand possible mechanisms of desiccation tolerance as well as those developing new biotechnological applications.

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