Isolation and characterization of epilithic chlorophytes and cyanobacteria from two Spanish cathedrals (Salamanca and Toledo)

by

J.J. Ortega-Calvo[1], P.M. Sanchez-Castillo[2], M. Hernandez-Marine[3] and C. Saiz-Jimenez[1]

With 4 plates and 2 tables

Ortega-Calvo, J.J., P.M. Sanchez-Castillo, M. Hernandez-Marine & C. Saiz-Jimenez (1993): Isolation and characterization of epilithic chlorophytes and cyanobacteria from two Spanish cathedrals (Salamanca and Toledo).

Abstract: The chlorophytes and the cyanobacteria present in samples from the outer zones of the cathedrals of Salamanca and Toledo (Spain) were isolated and determined. A total of 21 taxa were studied, 8 belonging to Cyanobacteria and 13 to Chlorophyta. The most common species within the samples studied are the chlorophytes *Klebsormidium flaccidum* and *Muriella terrestris,* and the cyanobacteria *Microcoleus vaginatus* and *Phormidium autumnale.* Species distribution is discussed in relation to microclimatic conditions. It is found that in some cases, samples taken near ground level are characterized by the absence of cyanobacteria, which are however present in samples taken from places exposed to sunlight, at 50 m height. The possible biodeterioration mechanisms in which these chlorophytes and cyanobacteria could be involved are discussed.

Introduction

The weathering of building stones is a natural process in which physical, chemical and biological agents are involved. The microbial communities developed in and on stone walls are of extreme importance in biological deterioration. These communities include bacteria, cyanobacteria, algae, fungi, etc. Their deterioration ability has been reported several times (Krumbein & Pochon 1964; Grant 1982; Anagnostidis et al. 1983; Grilli-Caiola et al. 1987, etc.). These communities can change the chemical and mineralogical composition of the stone surface, decreasing its stability. They make the colonization by mosses and vascular plants possible, similarly to soil formation processes. Within these microbial communities, the algae and cyanobacteria

[1]Instituto de Recursos Naturales y Agrobiologia, C.S.I.C., Apartado 1052, 41080 Sevilla (Spain).
[2]Departamento de Biologia Vegetal, Facultad de Ciencias, Universidad de Granada, 18001 Granada (Spain).
[3]Departamento de Botanica, Facultad de Farmacia, Universidad de Barcelona, 08028 Barcelona (Spain).

are the primary colonizers due to their phototrophism and low nutrient requirements. They grow on cornices, in holes and fissures or beneath crusts, where water is retained, and often form rather apparent biofilms. The aim of this work was to characterize the most representative species of chlorophytes and cyanobacteria present in surface layers of the cathedral walls of Salamanca and Toledo (Spain), in order to relate them with stone weathering processes.

Materials and methods

Salamanca cathedral was constructed mainly in the 16th century. Its architectural features belong to the late gothic style. It was constructed with Villamayor sandstone, characterized by a gold-like colour ("golden sandstone") produced by open air oxidation after its extraction from the quarry. This sandstone is the typical building stone of the historic monuments of Salamanca. It is composed mainly of quartz and felspar, and has a porosity of 32% (Vicente 1983). The Salamancan climate is typically continental.

Toledo cathedral was constructed from the 13th to the 16th centuries, and it is the largest gothic church in Spain after Sevilla cathedral. Different materials were used in its construction, granite and limestone being predominant. They show different characteristics, depending on the age and quarry of origin. The climate is continental.

A description of sampling sites is given in Table I. Samples were taken by scraping the stones' surface. Enrichment cultures were made in parafilm-sealed Petri dishes with BG11 (Rippka et al. 1979) or BBM (Chantanachat & Bold 1962) solid medium, by placing on the agar surface small fragments of stones containing visible growth of algae or cyanobacteria. Cultures were kept at room temperature and in continuous light (provided by fluorescent lamps giving a light intensity of 400 lux), and were checked weekly with a dissecting microscope to check the growth of microorganisms. Colony-forming chlorophytes and

Table I. Description of samples

CATHEDRAL	SAMPLE	CHARACTERISTICS
Salamanca	1	North facade, 0-20 cm height. Substrate: sandstone.
	2	North wall, at 50 m. High humidity. Substrate: sandstone.
	3	Cornice from north facade, at 50 m. Substrate: sandstone.
	4	North facade roof. Substrate: roof tile.
Toledo	1	Ajacent wall to "Hombre de Palo" street, at 1.6 m. Substrate: granite.
	2	Location: the same as 1. Substrate: mortar.
	3	Clock Gate, floor. Substrate: marble.
	4	Access to the Clock Gate, left side wall, 1.20 m height. Substrate: granite.
	5	Clock Gate, left side wall, at 1 m height, crevice. Substrate: granite.
	6	The same as 5, but out of the crevice. Substrate: granite.
	7	Clock Gate, at 1.70 m height, growth beneath black sulphated crusts. Substrate: granite.

cyanobacteria were isolated by transferring colonies to fresh media using a steel loop. Filamentous cyanobacteria that did not form colonies were isolated by transferring either single filaments or agar pieces that contained filaments using a sterile Pasteur pipette that was pulled out to a very thin capillary. After several transfers uni-algal or uni-cyanobacterial isolates were obtained. As soon as each isolate was identified as containing algae or cyanobacteria, it was maintained in a BBM or BG11 medium, respectively. In total 80 isolates were obtained. For morphology and life cycle observations with an optical microscope, the isolates were incubated at 25°C under a light-dark cycle (12L-12D) at 400 lux.

At present, all taxa described in this paper are maintained as uni-algal or uni-cyanobacterial cultures. The culture collection is maintained on agar slants in test tubes at 4°C with a 12L-12D cycle (light intensity about 100 lux). It is inoculated every four months with excellent results in regeneration of the isolates.

Literature used for determinations and for taxonomic purposes included the following: Butcher 1952; Fott & Novakova 1969; Gärtner 1985; Geitler 1932; Handa & Nakano 1988; Komárek & Comas 1984; Komárek & Fott 1983; Petersen 1932; Philipose 1967; Rieth 1972; Silva et al. 1972. Some other specialized papers have been cited in the following text.

Results

A total of 21 taxa were obtained, 8 of them belonging to Cyanobacteria and 13 to Chlorophyta. Neither Bacillariophyta nor Xanthophyta were isolated, although they have been described in similar habitats (Grant 1982; Anagnostidis et al. 1983). The species distribution is shown in Table II. The description of the taxa is given below:

Table II. Species distribution within the samples studied

	SALAMANCA				TOLEDO						
	1	2	3	4	1	2	3	4	5	6	7
CYANOBACTERIA											
Microcoleus vaginatus		+	+				+				
Nostoc sp.						+					
Phormidium autumnale		+	+	+			+				
Phormidium fragile			+								
Phormidium subfuscum			+				+				
Phormidium tenue							+		+		
Plectonema sp.					+						
Plectonema boryanum						+		+			
CHLOROPHYTA											
Bracteacoccus sp.							+				
Chlorella sp.	+					+					
Chlorella homosphaera					+						
Chlorella reisiglii						+					
Chlorella vulgaris			+	+							
Chlorokybus atmophyticus	+										
Ecdysichlamys obliqua		+	+								
Friedmannia israeliensis						+					
Klebsormidium flaccidum	+			+	+	+	+		+	+	
Muriella terrestris	+					+				+	
Scenedesmus quadricauda			+								
Stichococcus bacillaris	+										+
Trebouxia decolorans	+										

Microcoleus vaginatus (Vauch.) Gom. Fig. 1

Filaments single or forming a coiled thallus, sparsely branched; sheaths colourless, diffluent, not lamellated and enclosing many densely aggregated and contorted trichomes; trichomes 5.5-7 μm broad, dirty blue or olive green, attenuated, not constricted at the cross walls; the cross walls are often granulated; cells 3-6 μm long; end cells capitate, with a very variable calyptra.

Nostoc sp. Fig. 2

Thallus gelatinous, mostly proliferating, globose, olivaceous or blue-green; filaments flexuous, variously entangled; barrel-shaped cells or nearly spherical, 4-6 μm in diameter; heterocyst nearly spherical, 6-7 μm in diameter.

Phormidium autumnale (Ag.) Gom. Figs. 3 & 4

Thallus expanded, dark blue-green or brownish-green; filaments straight, rarely flexuous, variously entangled; sheath firm, mucilaginous, distinct or diffluent in an amorphous mucilaginous matrix; trichomes blue-green, not constricted at the cross-walls, 5.5-7 μm broad, ends briefly attenuated, straight or scarcely curved, prominently capitate; cells 3-5 μm long, septa frequently granulated, end cell with a rounded or truncated conical calyptra.

Phormidium fragile (Menegh.) Gom. Fig. 5

Sheath diffluent; filaments more or less flexuous, entangled or nearly parallel, trichomes distinctly constricted at the cross-walls, septa granulated, attenuated at the ends, 2 μm broad, pale blue-green; cells nearly quadrate, 1.5-2 μm long; end cell acute-conical, calyptra absent.

Phormidium subfuscum Kütz. Fig. 6

Filaments straight, fragile, short, parallel; sheath diffluent into a lamellose mucus; trichomes pale blue-green, 6-8 μm broad, not constricted at the cross-walls; cells up to 4 μm long, cross-walls often granulated; ends more or less briefly attenuated, capitate; end cell straight acute-conical.

This isolate was differentiated from *P. autumnale* after Kann & Komárek (1970).

Phormidium tenue (Menegh.) Gom. Fig. 7

Thallus pale blue-green, thin, expanded; trichome straight or slightly bent, densely entangled, slightly constricted at the cross-walls, attenuated at the ends, 1.5-2 μm

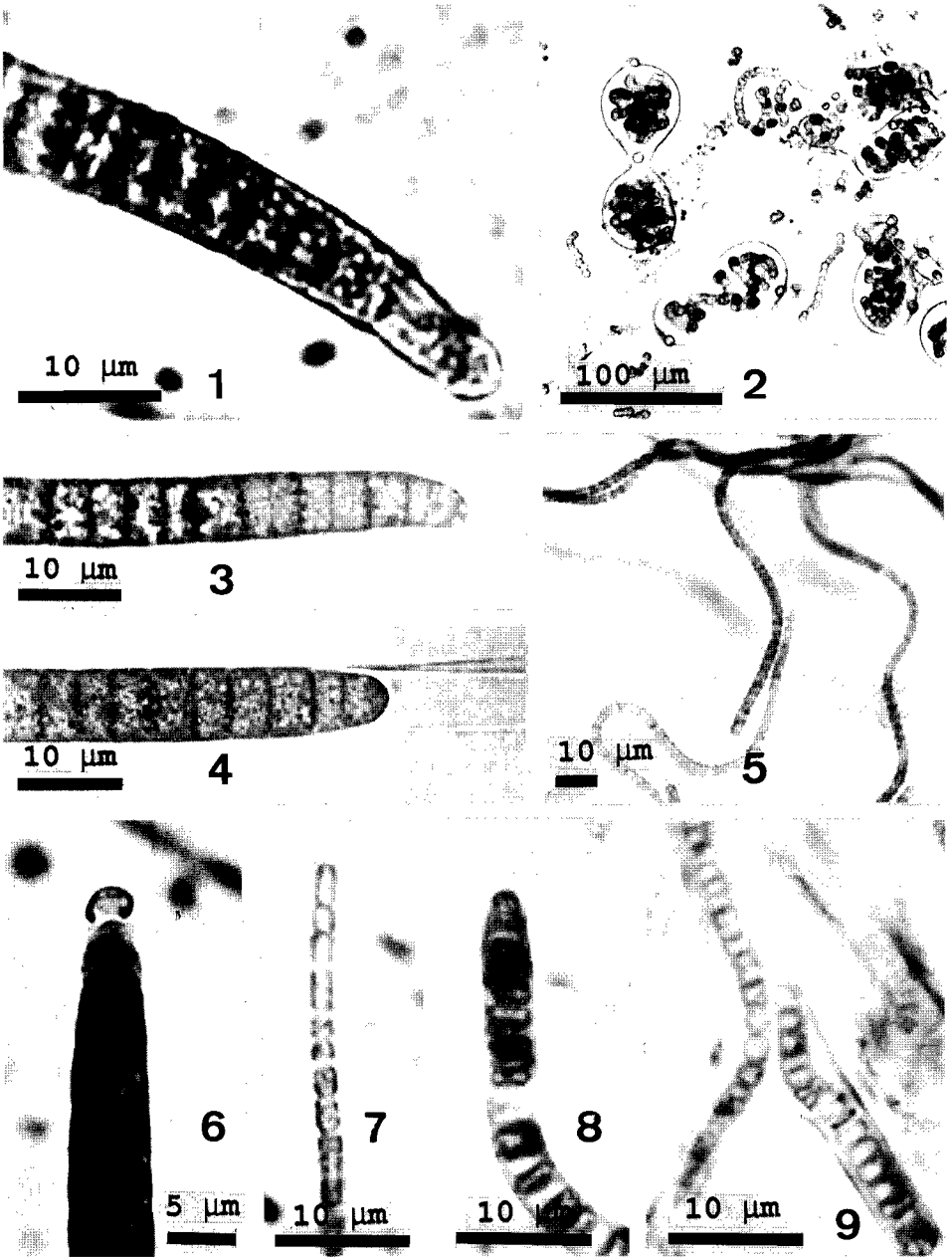


Fig. 1. Apex of trichome of *Microcoleus vaginatus* Fig. 2. Proliferating thalli of *Nostoc* sp. Figs. 3 & 4. *Phormidium autumnale.* Fig. 3. Apex of trichome. Fig. 4. Filament showing sheath and trichome. Fig. 5. *Phormidium fragile.* Fig. 6. *Phormidium subfuscum.* Fig. 7. *Phormidium tenue.* Fig. 8. *Plectonema* sp. Fig. 9. False branching of *Plectonema boryanum.*

broad, pale blue-green; sheath thin, diffluent; cells up to 2 times longer than broad, septa not granulated, cross-walls not commonly visible; end cell acute-conical, calyptra absent.

Plectonema sp. Fig. 8

Gelatinous sheath; cells quadrate, or slightly broader than long, 2-3 μm broad in young cultures, and up to 4 μm in old ones; frequent false branching, sometimes the new filament does not go through the sheath, producing in old cultures the coexistence of two trichomes in one sheath; apical cells rounded.

Plectonema boryanum Gom. Fig. 9

Trichomes with constrictions at the cross-walls, especially in younger zones; cells 3-4 μm broad, shorter than broad, up to quadrate.

CHLOROPHYTA

Bracteacoccus sp. Figs. 10 & 11

Spherical cells up to 25 μm in diameter; abundant small chloroplasts without pyrenoid; some mature cells have small bubble-like protuberances; reproduction with 4 to 8 autospores, 5-6 μm in diameter (the isolates were assigned to this genus and not to *Muriella* because of the greater cell size and number of chloroplasts).

Chlorella sp. Fig. 12

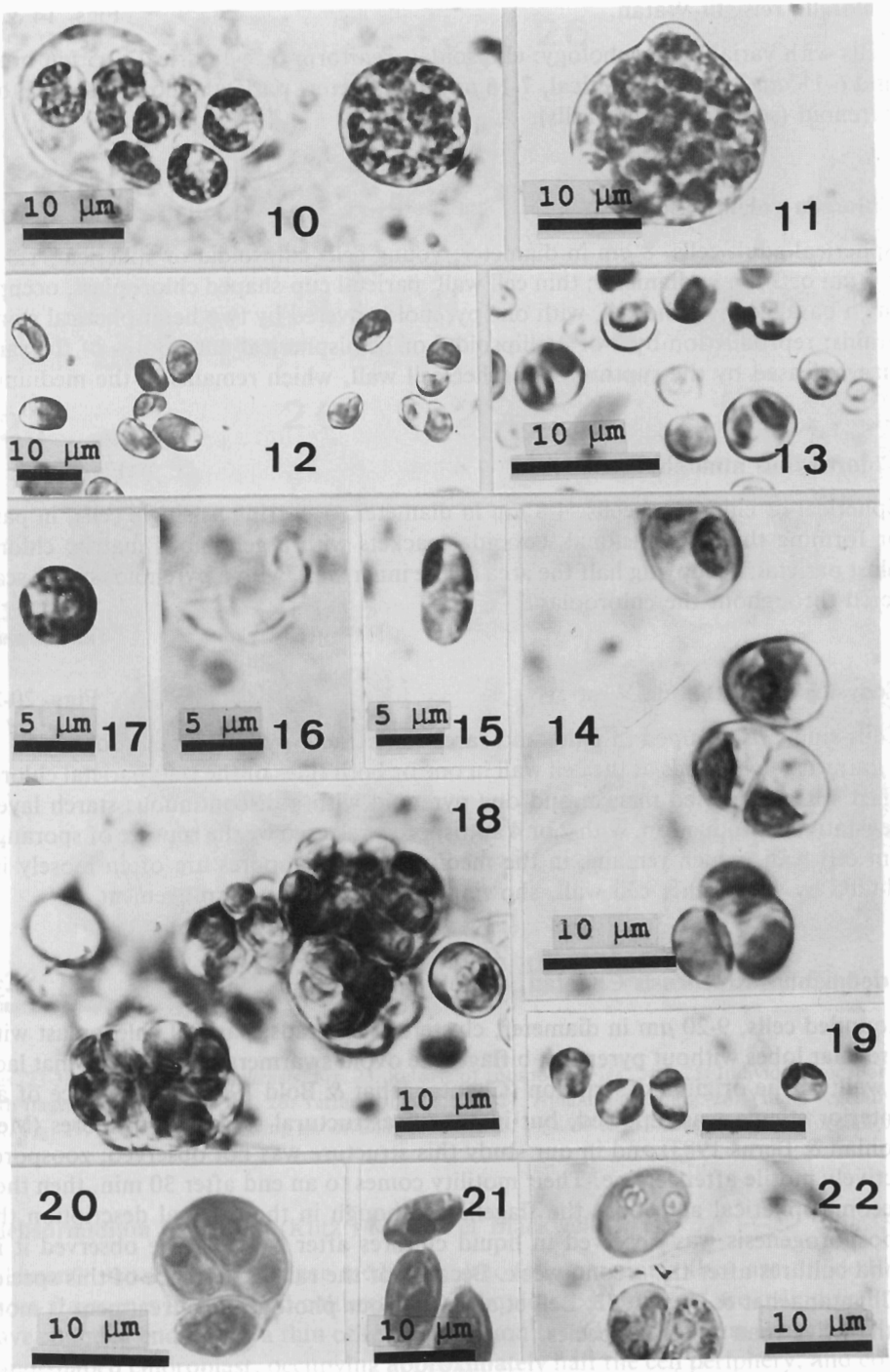
Rod-shaped cells, 3-5 μm long and 2-3 μm broad; cell division resulted in two daughter cells, the mother cell wall is hardly seen; one chloroplast occupying most of the cell volume, with starch spread over the plastid.

Chlorella homosphaera Skuja Fig. 13

(Syn.: *Palmellococcus homosphaera* (Skuja) Handa et Nakano).

Rounded cells, 2.5 μm in diameter; thin cell wall throughout culture period; parietal chloroplast covering most of the cell periphery, without pyrenoid.

Figs. 10 & 11. *Bracteacoccus* sp. Fig. 10. Release of aplanospores. Fig. 11. Mature vegetative cell. Fig. 12. *Chlorella* sp. Fig. 13. *Chlorella homosphaera.* Note cells with dividing chloroplast prior to autospore formation. Figs. 14 & 15. *Chlorella reisiglii.* Fig. 14. Rounded vegetative cells and autospore formation. Fig. 15. Ellipsoidal cell. Figs. 16 & 17. *Chlorella vulgaris.* Fig. 16. Remains of the mother cell-wall. Fig. 17. Vegetative cell. Figs. 18 & 19. Typical clusters and single cells of *Chlorokybus atmophyticus.* Figs. 20-22. *Ecdysichlamys obliqua.* Fig. 20. Tetrad. Fig. 21. Ovoidal cells. Fig. 22. Sporangium enclosing two autospores and sporangium cell wall.


10 µm
10
11
12
13
5 µm
17
5 µm
16
5 µm
15
14
18
19
20
21
22

Chlorella reisiglii Watan. Figs. 14 & 15

Cells with variable morphology: ellipsoidal, reniform or cylindrical, 2-5 μm broad and 6-11 μm long, or spherical, 7-16 μm in diameter; parietal chloroplast with one pyrenoid (several in adult cells).

Chlorella vulgaris Beij. Figs. 16 & 17

Spherical adult cells, 8 μm in diameter; young cells ellipsoidal or spherical, 1.5 $\times$ 2.5 μm or 3 μm in diameter; thin cell wall; parietal cup-shaped chloroplast, occupying a basal zone of the cell, with one pyrenoid covered by two hemispherical starch grains; reproduction by 2 or 4 ellipsoidal or hemispherical autospores of the same size, released by the rupture of mother cell wall, which remains in the medium.

Chlorokybus atmophyticus Geitler Figs. 18 & 19

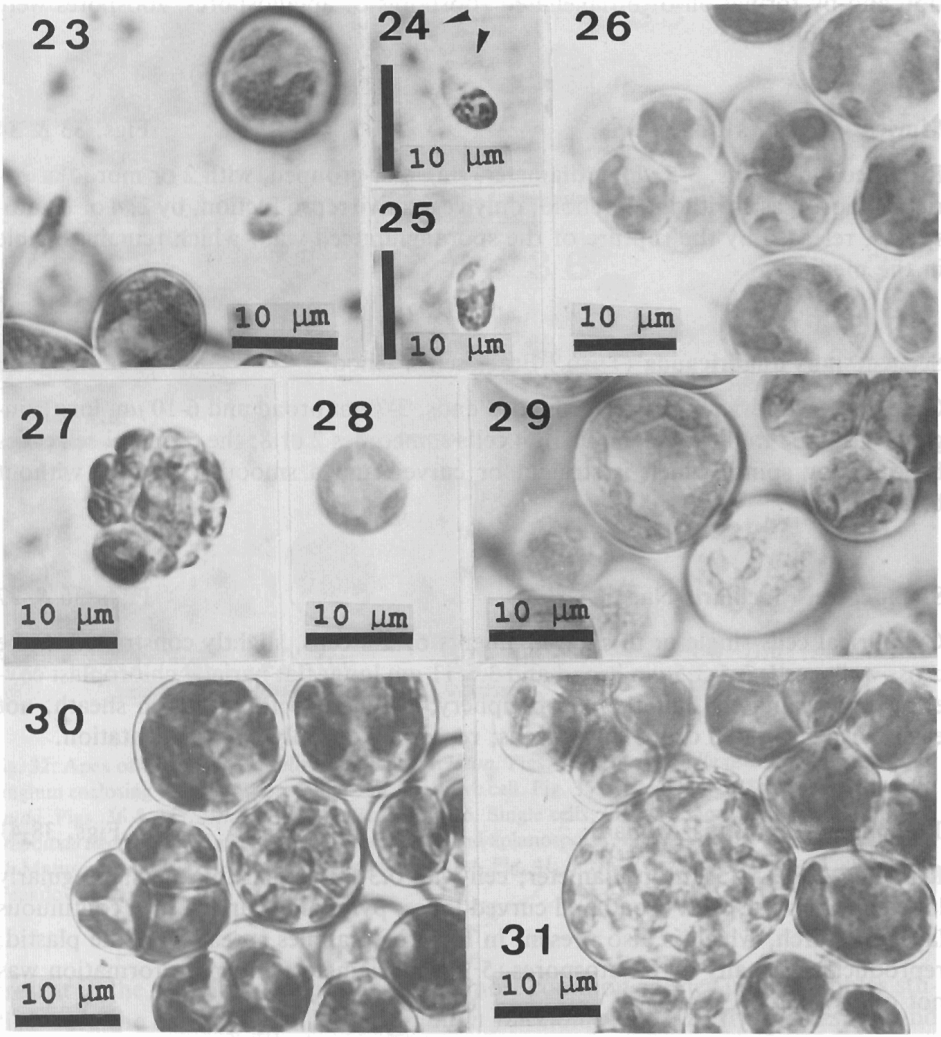
Spherical or ellipsoidal cells, 4-8 μm in diameter, occurring as single cells, in pairs or forming three-dimensional, cuboidal packets with a gelatinous matrix; chloroplast parietal, occupying half the area of the inner wall, with a pyrenoid; starch scattered throughout the chloroplast.

Ecdysichlamys obliqua West Figs. 20-22

Cells single or grouped in pairs and rarely in tetrads, ovoidal or ellipsoidal, 12 $\times$ 5 μm; with polar buds at the cell wall in one or both sides of the cell; parietal chloroplast with undulated margin and one pyrenoid with a discontinuous starch layer; vegetative reproduction, with 2 or 4 autospores, released by the rupture of sporangium cell wall, which remains in the medium; the autospores are often loosely included by the mother cell wall, showing a typical oblique arrangement.

Friedmannia israeliensis Chantan. et Bold Figs. 23-31

Rounded cells, 9-20 μm in diameter, clustered in tetrads; parietal chloroplast with irregular lobes without pyrenoid; biflagellate ovoid swarmers of 3 $\times$ 8 μm that lack a wall; in the original description (Chantanachat & Bold 1962) the presence of an anterior stigma was reported, but in the ultrastructural study of zoospores (Melkonian & Berns 1983) and in our study this structure was not observed; zoospores actively motile after release. Their motility comes to an end after 30 min. then they become spherical and loose the flagella. Although in the original description the zoosporogenesis was observed in liquid cultures after 6 weeks, we observed it in solid cultures after the second week. Because of the rare occurrence of this species (Chantanachat & Bold 1962; Bell et al. 1986), our photographic treatment is more exhaustive than in other species.



Figs. 23-32. *Friedmannia israeliensis*. Fig. 23. Release of zoospores. Figs. 24 & 25. Individual zoospores with flagella (arrowheads). Fig. 26. Tetrads of cells. Fig. 27. Aplanospores. Fig. 28. Young vegetative cell. Fig. 29. Isolated cells showing the dividing chloroplast. Figs. 30 & 31. Zoosporangium formation.

Klebsormidium flaccidum (Kütz.) Mattox et Blackwell Fig. 32

Filaments usually long in agar medium, although sometimes they are divided in short fragments in old solid cultures and in liquid medium; cells are cylindrical or have rounded ends, with a thin cell wall, 6-11 µm broad and 6-15 µm long; parietal band-shaped chloroplast, occupying approximately half the cell periphery, and containing one pyrenoid with a discontinuous starch layer; reproduction by fragmenta-

tion and by formation of biflagellated zoospores or aplanospores; zoospores were observed only once.

Muriella terrestris Boye-Pet Figs. 33 & 34

Rounded adult cells, 3-5 μm in diameter, single or grouped, with 2 or more flat parietal chloroplasts without pyrenoid; only vegetative reproduction, by 2, 4 or 8 autospores, released by the rupture of the sporangium cell wall, which remains visible in the medium.

Scenedesmus quadricauda (Turp.) Bréb. sensu Chod. Fig. 35

Oblong or cylindrical cells with rounded ends, 3-7 μm broad and 6-10 μm long, single or grouped in linear coenobia of 4 cells sometimes 2 or 8; the terminal cell poles have a long spine, which is straight or curved and a smooth cell wall, without bridges.

Stichococcus bacillaris Naeg. Figs. 36 & 37

Cylindrical cells single or in short filaments of 2-4 cells, slightly constricted at the cross walls; cells 2.5-3.5 μm broad and 3.5-11 μm long; flat parietal chloroplast covering approximately half the cell periphery; pyrenoid without starch sheath, not easily visible; lipidic drops in old cells; reproduction only by fragmentation.

Trebouxia decolorans Ahmadj. Figs. 38-41

Rounded cells, 5-15 μm in diameter; cell wall 0.3-0.8 μm in thickness; irregularly lobed chloroplast, with broad and curved lobes; pyrenoid rounded by a continuous layer of starch, which is also present in form of granules spread over the plastid; reproduction usually by 8 autospores, 5 μm in diameter; zoospore formation was not observed.

Discussion

Several conclusions arise from this taxonomic enumeration in relation to the stone biodeterioration process. Most of the species found in this study are common in soils and corticolous habitats (Metting 1981; Hernandez-Marine 1984; Handa & Nakano 1988; Cambra & Hernandez-Marine 1989). They are rather simple forms, with a difficult taxonomy often requiring the help of a culture for the observation of relevant characters, which are often not detectable in field material. The isolation procedure facilitated the taxonomic work in that sense. The media used, with proved suitability for use in general culture collections (Rippka et al. 1979; Chantanachat & Bold 1962), and the number of isolates, suggest that most of the species

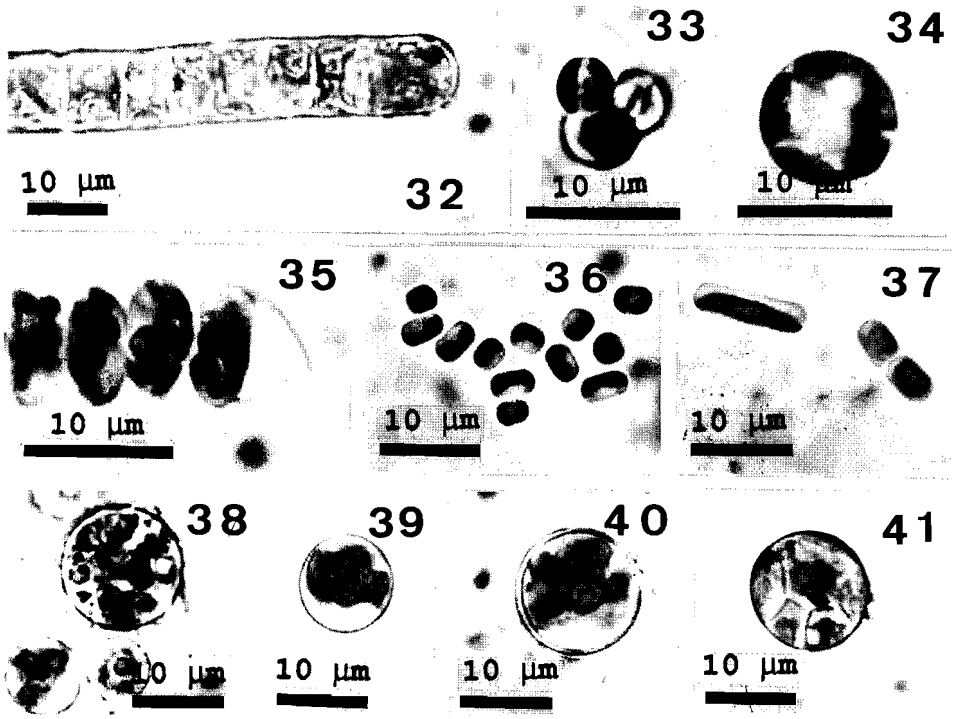


Fig. 32. Apex of filaments of *Klebsormidium flaccidum*. Figs. 33 & 34. *Muriella terrestris*. Fig. 33. Sporangium enclosing four autospores. Fig. 34. Vegetative cell. Fig. 35. Coenobium of *Scenedesmus quadricauda*. Figs. 36 & 37. *Stichococcus bacillaris*. Fig. 36. Single cells. Fig. 37. Short filament. Figs. 38-41. *Trebouxia decolorans*. Fig. 38. Aplanosporangium and aplanospores. Fig. 39. Young vegetative cell. Fig. 40. Mature vegetative cell, note axile lobed chloroplast. Fig. 41. Surface view of a mature cell undergoing breakdown.

present in the samples were isolated. The most common species in the samples studied were the chlorophytes *Klebsormidium flaccidum* and *Muriella terrestris,* and the cyanobacteria *Microcoleus vaginatus* and *Phormidium autumnale.* Only *M. vaginatus* and *Plectonema boryanum* were present amongst 20 species of cyanobacteria detected by Anagnostidis et al. (1983) in marbles of the Parthenon. They, however, also found unicellular cyanobacteria, which we did not observe in our samples. Probable differences in climate, substrate and pollution levels have an effect on the set of species detected. Wee & Lee (1980) also found different species of algae and cyanobacteria on buildings of Singapore, the algae *Trentepohlia odorata* (Wiggers) Wittrock and *Chlorococcum* sp. and the cyanobacteria *Anacystis montana* (Lightf.) Dr. et Daily and *A. thermale* (Kütz.) Dr. et Daily being the most common species.

De Winder et al. (1989b), studying the cyanobacterial-algal crusts from the coastal dunes of the Netherlands, found an ecological succession where an initial colonization by cyanobacteria was followed by the establishment of a population in which

Klebsormidium flaccidum was the dominant species. This succession was explained in terms of differences in tolerance to water stress, where filamentous cyanobacteria showed a higher resistance to drought than *K. flaccidum* (De Winder et al. 1989a). It was concluded that the green alga became dominant only after the improvement of water retention properties of the substrate by the pioneering cyanobacteria. Certainly water availability problems also have to be faced by the organisms growing on stone surfaces, such as the chlorophytes and cyanobacteria found in our study, and the differences in their response to these problems also have to influence their distribution. Although the number of samples taken in this study makes it difficult to draw clear-cut conclusions on species distribution, there are clear differences that correlate with differences in microclimate. Sample 1 from Salamanca, taken from ground level, showed a complete absence of cyanobacteria, while samples 2 and 3 situated at 50 m height, which probably suffered longer periods of dessication due to sunlight, showed the presence of cyanobacteria. Furthermore, the higher humidity of sample 1 allowed the development of more complex microbial communities, where fungi degrading the organic matter produced by algae may have an important role in the biodeterioration process.

Also samples 1 and 2 from Toledo presented an interesting difference in species distribution, in spite of their being from the same place. There is clearly a higher number of species in the mortar than in the granite. Microclimatic differences, probably including pH, porosity (allowing a higher degree of water retention) and richer substrate, seem to produce a species segregation. This was also reported by Schlichting (1975), who found growth of *Chlorella* sp. on bricks, while *Chroococcus* sp. and *Schizothrix* sp. only appeared on the mortar between them. He also noted that algae may grow in abundance on a limestone or granite block and yet be totally absent on a sandstone block of the same age directly beside it.

The species *Stichococcus bacillaris,* found exclusively in sample 7 from Toledo, has also been found in archaeological remains in Italy, even in zones protected with a plastic film, probably due to its low nutrient requirement and the relatively high humidity of the environment (Favali et al. 1978). It was also found to be the cause of the "maladie verte" of Lascaux (Lefevre & Laporte 1969), and of the biological agression to Roman frescoes (Grilli-Caiola et al. 1987). This is not surprising because it is one of the most common algae on walls, soils and tree bark. The growth of this species in our sample was cryptoendolithic, colonizing the cavities beneath the black sulphated crusts (Saiz-Jimenez & Garcia del Cura 1991).

The active boring or passive endolithic algae and/or cyanobacteria reported in other habitats (Golubić 1969; Schneider 1976; Saiz-Jimenez et al. 1990) were not observed in this study. All samples presented epilithic growth (with the exception of sample 7 of Toledo). The possible deterioration mechanisms in which the species detected could be involved are direct as well indirect ones. All cyanobacteria identified and the chlorophyte *Klebsormidium flaccidum* presented a developed sheath, which has been reported to be a possible factor in the gradual destruction of rocks, by loosening particles with their swelling and shrinking after wetting and dessication periods, respectively (Friedmann 1971; Golubić 1973; Anagnostidis et al. 1983). The retention of water and the subsequent delay of drying which in turn exacerbate

water-induced damage of the underlying material is another possible mechanism of degradation caused by algae (Grant 1982).

On the other hand, algae and cyanobacteria may play an indirect role by supporting the growth of other organisms such as fungi or bacteria, with higher destructive potential. They can utilize organic matter, for example carbohydrates, with production of organic acids. This has been found in sample 1 from Salamanca (De la Torre et al. 1991). This material also may contribute to the formation of biofilm glycocalyx constituted mainly by extracellular polymeric substances (Characklis & Cooksey 1983). The polyanionic nature of the glycocalyx dictates that it functions as an ion exchange matrix, serving to concentrate organic nutrients and at the same time to limit the penetration of charged molecules such as cationic biocides (Costerton & Lashen 1984). However, the interactions between the individual components of biofilms are still not understood (Stal et al. 1989). The possibly relevant role of such interactions in the stone biodeterioration process suggests the need to study these microbial communities as a whole after their dissection and characterization of individual components.

Acknowledgement

This research has been supported by the C.E.C. through contract EV4V 0061 E and C.I.C.Y.T. (PAT89-0767-C04), Spain.

References

ANAGNOSTIDIS, K., A. ECONOMOU-AMILLI & M. ROUSSOMOUSTAKAKI (1983): Epilithic and chasmolithic microflora (Cyanophyta, Bacillariophyta) from marbles of the Parthenon (Acropolis Athens, Greece). - Nova Hedwigia **38:** 227-287.

BELL, R.A., P.V. ATHEY & M.R. SOMMERFELD (1986): Cryptoendolithic algal communities of the Colorado Plateau. - J. Phycol. **22:** 429-435.

BUTCHER, R.W. (1952): Contributions to our knowledge of the smaller marine algae. - J. Mar. Biol. Ass. U.K. **31:** 175-191.

CAMBRA, J. & M.C. HERNANDEZ-MARINE (1989): Observaciones sobre las algas corticicolas del nordeste y sudeste de España. - Anales Jard. Bot. Madrid. **46:** 115-126.

CHARACKLIS, W.G. & K.E. COOKSEY (1983): Biofilms and microbial fouling. - Adv. Appl. Microbiol. **29:** 93-138.

CHANTANACHAT, S. & H.C. BOLD (1962): Phycological studies II. Some algae from arid soils. - Univ. Texas Publ. no. 6218. Austin, Texas. pp. 74.

COSTERTON, J.W. & E.S. LASHEN (1984): The inherent biocide resistance of corrosion-causing biofilm bacteria. - Mat. Perform. **23:** 13-16.

DE LA TORRE, M.A., G. GOMEZ-ALARCON, P. MELGAREJO & C. SAIZ-JIMENEZ (1991): Fungi in weathered sandstone from Salamanca cathedral, Spain. - Sci. Total Environ. **107:** 159-168.

DE WINTER, B., H.C.P. MATTHIJS & L.R. MUR (1989a): The role of water retaining substrata on the photosynthetic response of three drought tolerant phototrophic micro-organisms isolated from a terrestrial habitat. - Arch. Microbiol. **152:** 458-462.

DE WINDER, B., J. PLUIS, L. DE REUS & L.R. MUR (1989b): Characterization of a cyanobacterial, algal crust in the coastal dunes of the Netherlands. - In: COHEN, Y. & E. ROSENBERG (eds.): Microbial Mats. Physiological Ecology of Benthic Microbial Communities: 77-83. Am. Soc. Microbiol., Washington.

FAVALI, F.A., N. BARBIERI & M. BASSI (1978): A green alga growing on a plastic film used to protect archaeological remains. - Int. Biodet. Bull. **14:** 89-93.

FOTT, B. & M. NOVAKOVA (1969): A monograph of the genus *Chlorella.* The freshwater species. - In: FOTT, B. (ed.): Studies in Phycology: 10-74. Academia, Praga.

FRIEDMANN, E.I. (1971): Light and scanning electron microscopy of the endolithic desert algal habitat. - Phycologia **10:** 411-428.

GÄRTNER, G. (1985): Die Gattung *Trebouxia* Puymaly (Chlorellales, Chlorophyceae). - Arch. Hydrobiol. Suppl. 71, **4:** 495-548.

GEITLER, L. (1932): Cyanophyceae. - In: Rabenhorst's Kryptogamenflora vol. 14. Akad. Verlag, Leipzig. pp. 1196.

GOLUBIĆ, S. (1969): Distribution, taxonomy and boring patterns of marine endolithic algae. - Am. Zool. **9:** 747-751.

GOLUBIĆ, S. (1973): The relationship between blue-green algae and carbonate deposits. - In: CARR, N.G. & WHITTON)eds.): The Biology of Blue-Green Algae, Bot. Monogr. **9:** 434-472. Blackwell Sci. Pub., London.

GRANT, C. (1982): Fouling of terrestrial substrates by algae and implications for control - review. - Int. Biodet. Bull. **18:** 57-65.

GRILLI-CAIOLA, M.G., C. FORNI & P. ALBERTANO (1987): Characterization of the algal flora growing on ancient Roman frescoes. - Phycologia **26:** 387-396.

HANDA, S. & T. NAKANO (1988): Some corticolous algae from Miyajima Island, Western Japan. - Nova Hedwigia **46:** 165-186.

HERNANDEZ-MARINE, M.C. (1984): Algas edaficas (Delta del rio Ebro). - Anales Biol. (Secc. Espec.) Murcia **2:** 119-125.

KANN, E. & J. KOMÁREK (1970): Systematisch-ökologische Bemerkungen zu den Arten des Formenkreises *Phormidium autumnale.* - Schweiz. Z. Hydrol. **32:** 495-516.

KOMÁREK, J. & A. COMAS (1984): The genus *Ecdysichlamys* (Chlorellales). - Preslia **56:** 13-28.

KOMÁREK, J. & B. FOTT (1983): Das Phytoplankton des Süsswassers. Systematik und Biologie. Vol. 7(1). Chlorophyceae, Chlorococcales. - E. Schweiz. Verlag., Stuttgart. pp. 1044.

KRUMBEIN, W.E. & J. POCHON (1964): Ecologie bacteriènne des pierres alterées des monuments. - Ann. Inst. Pasteur **107:** 724-732.

LEFEVRE, M. & G.S. LAPORTE (1969): The "maladie verte" of Lascaux. Diagnosis and treatment. - Stud. Speleol. **2:** 35-44.

MELKONIAN, M. & B. BERNS (1983): Zoospore ultrastructure in the green alga *Friedmannia israelensis:* an absolute configuration analysis. - Protoplasma **114:** 67-84.

METTING, B. (1981): The systematics and ecology of soil algae. - Bot. Rev. **47:** 195-312.

PETERSEN, J.B. (1932): Einige neue Erdalgen. - Arch. Protistenk. **76:** 395-408.

PHILIPOSE, M.T. (1967): *Chlorococcales.* - Indian Council Agricultural Research, New Delhi. pp. 365.

RIETH, A. (1972): Über *Chlorokybus atmophyticus* Geitler 1942. - Arch. Protistenk. **114:** 330-342.

RIPPKA, R., J. DERUELLES, J.W. WATERBURY, M. HERDMAN & R.G. STANIER (1979): Generic assignments, strain histories and properties of pure cultures of cyanobacteria. - J. Gen. Microbiol. **111:** 1-61.

SAIZ-JIMENEZ, C. & M.A. GARCIA DEL CURA (1991): Sulfated crusts: a microscopic, inorganic and organic analysis. - In: BAER, N.S., C. SABBIONI & A.I. SORS (eds.): Science, Technology and European Cultural Heritage: 527-530. Butterworth-Heinemann Ltd., Oxford.

SAIZ-JIMENEZ, C., J. GARCIA-ROWE, M.A. GARCIA DEL CURA, J.J. ORTEGA-CALVO, E. ROEKENS & R. VAN GRIEKEN (1990): Endolithic cyanobacteria in Maastricht limestone. - Sci. Total Environ. **94:** 209-220.

SCHLICHTING, H.E., Jr. (1975): Some subaerial algae from Ireland. - Br. Phycol. J. **10:** 257-261.

SCHNEIDER, J. (1976): Biological and inorganic factors in the destruction of inorganic coasts. - Contr. Sed. **6:** 1-112.

SILVA, P.C., K.R. MATTOX & W.H. BLACKWELL (1972): The generic name *Hormidium* as applied to green algae. - Taxon **21:** 639-645.

STAL, L.J., E. BOCK, E.J. BOUWER, L.J. DOUGLAS, D.L. GUTNIK, K.D. HECKMANN, P. HIRSCH, J.M. KOLBEL-BOELKE, K.C. MARSHALL, I.J. PROSSER, C. SCHUTT & Y. WATANABE (1989): Cellular physiology and interactions of biofilm organisms. - In: CHARAKLIS, W.G. & P.A. WILDERER (eds.): Structure and Function of Biofilms: 269-286. John Wiley, Chichester.

VICENTE, M.A. (1983): Clay mineralogy as the key factor in weathering of arenisca dorada (golden sandstone) of Salamanca, Spain. - Clay Min. **18:** 215-217.

WEE, Y.C. & K.B. LEE (1980): Proliferation of algae on surfaces of buildings in Singapore. - Int. Biodet. Bull. **16:** 113-117.