

Diversity of algae and cyanobacteria growing on buildings in France

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Abstract

Algae and cyanobacteria in biofilms cause discolouration of the building surfaces they colonise. In France, a comprehensive study of the diversity of the species involved in this phenomenon is lacking. 73 samples were therefore realised around France and analysed for their micro-organisms composition. Green algae were the most frequent organisms encountered, with *Klebsormidium flaccidum* (53%), *Trebouxia* spp. (51%), *Stichococcus bacillaris* (45%), *Chlorosarcinopsis minor* (27%) and *Chlorella* cf. *mirabilis* (26%) being the most abundant ones. Colonial cyanobacteria were then mainly represented by *Cyanosarcina parthenonensis* (27%), *Chroococcus lithophilus* (21%), *Gloeocapsa sanguinea* (15%) and filamentous cyanobacteria by *Calothrix pulvinata* (21%), *Leptolyngbya foveolarum* (18%) and *Phormidium corium* (12%).

Keywords: Algae; Cyanobacteria; Biofilms; Buildings; Mortar; Concrete; Paint; France

Introduction

Algae and cyanobacteria are able to adapt their morphology and their physiology to colonize different habitats, as long as illumination, humidity and nutrients are provided. Some representatives of these organisms may grow in extreme conditions, such as deserts, stones or walls. They are notably found on edified structures, as buildings, where architectural defaults such as exposed frontages, leaking gutters, air-conditioner evacuation or water rejection on basis of walls will fulfil the necessary conditions of dampness. Associated with other microorganisms, they will form a biofilm causing primarily discolouration of the external facade of buildings, and then possible physico-chemical deterioration of the substrate (SAND 1997).

Some literature has been published concerning the identification of epilithic algae and cyanobacteria in South America (CRISPIM et al. 2004, GAYLARDE & GAYLARDE 2005, GAYLARDE & GAYLARDE 1999, 2000, VIDELA et al. 2000), Asia (JOSHI & MUKUNDAN 1997, TRIPATHI et al. 1991, WEE 1988, WEE & LEE 1980), and Europe (ANAGNOSTIDIS et al. 1983, BELLINZONI et al. 2003, FLORES et al. 1997, GAYLARDE & GAYLARDE 2005, GÓMEZ-ALARCÓN et al. 1995, NOGUEROL-SEOANE & RIFÓN-LASTRA 1997, ORTEGA-CALVO et al. 1993, PALMER & HIRSCH 1991, RIFÓN-LASTRA & NOGUEROL-SEOANE 2001, RINDI & GUIRY 2003, RINDI & GUIRY 2004, SCHLICHTING 1975, TOMASELLI et al. 2000), but most of them deal with historic monuments colonisation. In France, a few works are known

(DUBOSC et al. 2001, GROSSIN & DUPUY 1978, RINDI & GUIRY 2004, RINDI et al. 2003), but no comprehensive study at the scale of the country has been published yet.

Therefore, we aimed to furnish a more complete investigation of the algae and cyanobacteria established on buildings in diverse regions of France, in relation with the materials they were growing on.

Materials and methods

Sampling sites and methods

In 2004 and 2005, 73 samples of biofilms were collected randomly from buildings in 18 departments of France (map 1). The surfaces, composed of diverse substrates (mortar, cement, paint, organic finish) showed green, red or blackish discolouration. Local informations (such as geographical exposition, nature of the substrate) were noted for further statistical treatment. The samples were collected by scraping the surface with a sterile scalpel, ensuring to remove a portion of the substrate to allow an exhaustive study of the biofilm. They were stored in polyethylene containers until identification.

Microbiological analysis

Identification of algae and cyanobacteria was achieved according to their morphological characteristics. A portion of the material was placed for 1-2 h in water to make the removing of the biofilm easier, thus allowing a primary study of the populations in their natural state. Another portion of material was placed into Petri dishes with agar Bold Basal medium to promote culture growth. Cultures were grown in a chamber with a photoperiod of 16h/8h light/dark, light intensity of $15 \pm 3 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPF (Photosynthetic Photon Flux) and a temperature of $20 \pm 0,5$ °C. The primary cultures were used to obtain unialgal replicates using the streaking plate method. This culture state favoured the observation of relevant characters, often not detectable in field material, leading to the definitive identification of the strain. In order to highlight some of the traits used in taxonomy, lugol's solution and India ink were employed. Identifications were based on taxonomic criteria established by: (BOURRELLY 1966, 1985, Ettl & GÄRTNER 1995, GEITLER 1932, JOHN et al. 2002, KOMÁREK & ANAGNOSTIDIS 1999, KOMÁREK & FOTT 1983). Drawings were realised using a drawing tube and were taken using a numerical camera, both attached to an optical microscope.

Results

The samples investigated were generally composed by bacteria, algae, cyanobacteria, fungi, mosses and some grazers such as rotifers. We focus this paper on algae and cyanobacteria specimens, as they are primary colonisers after the ubiquitous bacteria. In rare cases, diatoms were present but not identified.

A total of 22 taxa of Chlorophyta and 25 taxa of Cyanobacteria were identified among the 73 samples, which emphasizes the low diversity of the communities developing on buildings (table 1). Effectively, the mean specific richness for each sample was quite low, as we identified an average of 5 species per sample, the more diversified sample being composed of 12 species. For each specimen identified, a short description and a drawing (figure 1) will be provided, as well as a photography when it completes the data (figure 2).

Chlorophyta

The taxa are hereby described following an alphabetical order, but their drawings will be classified as a function of their degree of organisation. Otherwise stated, the colour of those algae is green.

Apatococcus lobatus (Chodat) J.B. Petersen

Cells spherical to somewhat compressed, grouped in irregular clusters, up to 50 µm in diameter; one chloroplast parietal, sometimes observed as bilobed, without pyrenoid; 5-7 µm in diameter.

Bracteacoccus sp.

Cells spherical; many polygonal parietal chloroplasts, without pyrenoid; 5-9 µm in diameter. We could not identify the specimens to the species level, as it requires the observation of zoospores.

Chlorella ellipsoidea Gerneck

Cells ellipsoidal; one parietal chloroplast, cup- to band-shaped, with one pyrenoid; autospores ellipsoidal, usually a big one with the others smaller; L=4-12 µm, w=2-8 µm.

Chlorella homosphaera Skuja

Cells spherical; one chloroplast parietal and cup-shaped without pyrenoid; 4-7 µm in diameter.

Chlorella minutissima Fott et Nováková

Cells spherical; one chloroplast parietal and cup-shaped without pyrenoid; 3-4 µm in diameter.

Chlorella cf. *mirabilis* Andreeva

Cells spherical; one chloroplast parietal and cup-shaped with one pyrenoid; 3-4,5 µm in diameter. Differs from *C. vulgaris* in the diameter of cells (7,5-10 µm).

Choricystis chodatii (Jaag) Fott

Cells ellipsoidal ; one rounded end, the other pointed ; one chloroplast parietal and cup-shaped without pyrenoid ; L=5-12 µm, w=2-4,5 µm.

Choricystis minor (Skuja) Fott

Differs from *C. chodatii* in the dimensions of cells ; L=4-6 µm, w=2-3,5 µm.

Chlorosarcinopsis eremi Chantanachat et Bold

Cubical packs with subspherical cells; one parietal and cup-shaped chloroplast, covering approximately ½ of the cells, with one pyrenoid; 3-5 µm in diameter.

Chlorosarcinopsis minor (Gerneck) Herndon

Cubical packs with subspherical cells; one parietal and cup-shaped chloroplast, covering approximately ½ of the cells, with one pyrenoid; cell walls becoming thicker in old specimens; 9-13 µm in diameter.

Coccobotrys verrucariae Chodat 1913 em. Vischer

Cells globulous, grouped in cubical packs or in short filaments; one parietal chloroplast without pyrenoid; 5-10 µm in diameter.

Coccomyxa olivacea Petersen

Cells ovoid to ellipsoidal, grouped within a mucilaginous envelope with concentric striations surrounding cells, forming colonies up to 50 µm in diameter; one parietal and cup-shaped chloroplast without pyrenoid; 3-5 µm in diameter. The dimensions are close to those of *Coccomyxa subglobosa*, but the shape of the chloroplasts and the aspect of the colony led us think about *C. olivacea*.

Desmococcus olivaceus (Persoon ex Acharius) Laundon

Cells subspherical forming short filaments; one parietal and cup-shaped chloroplast with one pyrenoid; 5-9 µm in diameter.

Geminella terricola Petersen

Cells subspherical to cylindrical, solitary in cultures or forming short filaments, enclosed within a mucilage; one parietal and cup-shaped chloroplast with one pyrenoid; 5-12 µm in diameter.

Hormotila mucigena Borzí

Cells spherical to oval, solitary along mucilaginous branches and at branch apices, forming colonies up to 50 µm in diameter; one chloroplast with a pyrenoid, hardly visible in our sample; 5-8 µm in diameter.

Keratococcus bicaudatus (A. Braun) Petersen

Cells solitary, ellipsoidal to crescent-shaped, sometimes asymmetrical, with apices acute and sometimes prolonged into a spine; one parietal and cup-shaped chloroplast with one pyrenoid; L=8-16 µm, w=3-5,5 µm.

Klebsormidium flaccidum (Kützing) P.C. Silva, Mattox et W.H. Blackwell

Cells cylindrical to barrel-shaped, forming long filaments slightly constricted at cross-walls; one parietal and cup-shaped chloroplast, covering approximately 2/3 of the cells, with one clearly visible pyrenoid; L=5-20 (25) µm, w=4-9 (12) µm.

Klebsormidium pseudostichococcus (Heering) nov. comb.

Cells cylindrical, solitary or arranged in short filaments composed of up to 20 cells; one parietal chloroplast with one pyrenoid; L=5-10 µm, w=3,5 µm.

Palmellopsis gelatinosa Korschikoff

Cells spherical to ellipsoidal, grouped within a mucilaginous envelope, forming colonies of up to 100 µm in diameter; one parietal and cup-shaped chloroplast with one pyrenoid; 5-9 µm in diameter.

Stichococcus bacillaris Nägeli

Cells cylindrical, always observed solitary; one parietal and cup-shaped chloroplast without pyrenoid; L=5-10 µm, w=2-4,5 µm.

Trebouxia spp.

Cells spherical, usually solitary but sometimes observed in groups; one axile chloroplast, massive, star-shaped, with numerous short outgrowths and one central pyrenoid; 9-23 µm in diameter. We could not identify the specimens to the species level, as it requires the observation of zoospores, but we think that our samples include different species.

Trentepohlia iolithus (Linnaeus) Wallroth

Cells cylindrical, becoming globular or swollen with age, with thick cell wall, and forming red to orange irregularly branched filaments; L=15-30 µm, w=14-25 µm.

Cyanobacteria

Otherwise stated, the colour of cyanobacterial cells is blue-green.

Aphanocapsa fusco-lutea Hansgirg

Cells spherical, sparsely distributed in a formless mucilage, forming colonies up to 30 µm in diameter; 1-2 µm in diameter.

Aphanothece saxicola Nägeli

Cells ovoid to cylindrical; sparsely distributed in a translucent and formless mucilage, forming colonies up to 50 µm in diameter; L=2-2,5 µm, w=1 µm.

Calothrix pulvinata (C. Agardh) Bornet et Flahault

Cells cylindrical, forming trichomes deeply constricted at cross-walls, swollen at the base and slightly attenuated at the end, surrounded by a firm sheath, colourless to yellowish; heterocyst basal, apart from the sheath; filament 7-12 µm wide, up to 1 mm long; L=3-6 µm, w=5-10 µm.

Chroococcus limneticus Lemmermann

Cells spherical, surrounded by individual mucilaginous envelopes, distributed in a colourless and spherical gelatinous slime; 4,5-5,5 µm in diameter.

Chroococcus lithophilus Ercegović

Cells spherical, hemispherical after division, surrounded by a thin, colourless to yellowish brown mucilage; 4-8 µm in diameter.

Cyanosarcina parthenonensis Anagnostidis in Anagnostidis et Pantazidou

Cells subspherical, olive-green, arranged in more or less cubic and dense aggregates up to 20 µm in diameter; 2-4 µm in diameter.

Gloeocapsa aeruginosa Kützing

Cells spherical, surrounded by colourless mucilaginous envelopes, more or less spherical; many subcolonies assembled to form an amorphous colony up to 50 µm in diameter; 2,5-3 µm in diameter.

Gloeocapsa atrata Kützing

Cells spherical, densely distributed in colourless mucilaginous subcolonies, hardly visible in our samples; 4-5 µm in diameter.

Gloeocapsa biformis Ercegović

Cells spherical, distributed in yellow-brown, lamellated mucilaginous subcolonies; 2-3 µm in diameter.

Gloeocapsa decorticans (A. Braun) Richter in Wille

Cells spherical to slightly oval, forming subcolonies envelopped by yellow-brown mucilage; 4-5 µm in diameter.

Gloeocapsa kuëtzingiana Nägeli

Cells spherical, forming subspherical subcolonies envelopped by a colourless mucilage; 3-5 µm in diameter.

Gloeocapsa sanguinea (Agardh) Kützing

Cells spherical to oval, surrounded by distinctly stratified envelopes and distributed in intensely red, spherical mucilaginous colonies; 3-6 µm in diameter.

Gloeocapsa violascea (Corda) Rabenhorst

Cells spherical to oval, surrounded by distinctly stratified envelopes and distributed in violet to blackish, spherical mucilaginous colonies; 5-7 µm in diameter.

Gloeothece cf. *incerta* Skuja

Cells oval-cylindrical, surrounded by individual envelopes, and sparsely distributed within a yellow mucilage; L=2-3 µm, w=2 µm.

Gloeothece palea (Kützing) Rabenhorst

Cells oval to cylindrical, surrounded by individual envelopes, and sparsely distributed in a yellow mucilage; L=2-2,5 µm, w=1 µm.

Leptolyngbya foveolarum (Montagne ex Gomont) Anagnostidis et Komárek

Filament straight; sheath not lamellated; trichomes constricted at the cell wall; rounded end; L=1-3 µm, w=1,5-2 µm.

Leptolyngbya tenue (Gomont) Anagnostidis et Komárek

Filament straight; sheath not lamellated; ; trichomes constricted at the cell wall; rounded end; L=3-6 µm, w=1-1,5 µm.

Microcoleus vaginatus (Vaucher) Gomont

Filaments formed by a mat of interwoven trichomes surrounded by a sheath, not constricted at the cross-walls and provided with a calyptra.; L=2,5-4,5 µm, w=5-7 µm.

Nostoc commune (Vaucher 1803) Bornet et Flahault

Trichomes composed by short barrel-shaped cells, deeply constricted at the cross-walls, surrounded by an amorphous and colourless mucilage; an heterocyst often observed outside the colony; cells 3,5-4 µm in diameter.

Nostoc microscopicum (Carmichael in Hooker) Bornet et Flahault

Trichomes composed by short barrel-shaped cells, deeply constricted at the cross-walls, surrounded by a spherical, firm yellow mucilage; heterocyst subspherical, intercalated in the trichomes; colonies up to 200 µm in diameter; cells 4-5 µm in diameter.

Phormidium ambiguum (Kützing) Gomont

Filament straight; cell wall not constricted; rounded end, not attenuated; L=1,5-3 µm, w=4-6 µm.

Phormidium corium (C. Agardh) Gomont

Filament straight; sheath not lamellated; cell wall not constricted; rounded end; L=2-5 µm, w=3-7 µm.

Phormidium molle Gomont

Filament straight; cell wall not constricted; L=3-6 µm, w=2-3 µm.

Phormidium cf. usterii Schmidle 1904

Filament straight; cell wall not constricted; rounded end; L=1-2,5 µm, w=3-4 µm.

Scytonema mirabile (Dillwyn 1809) Bornet 1889

Cells cylindrical forming entangled, mostly false-branched filaments; heterocysts spherical intercalated; filaments surrounded by yellow-brown sheaths, strongly lamellated; L=2,5-6,5 µm, w=7-8 µm.

Most of the species found are widespread subaerial algae and cyanobacteria, reported from many geographical areas. The predominant green algae were *Klebsormidium flaccidum* (53%), *Trebouxia* spp. (51%), *Stichococcus bacillaris* (45%), *Chlorosarcinopsis minor* (27%) and *Chlorella cf. mirabilis* (26%). Among cyanobacteria colonial forms were the most frequently observed with *Cyanosarcina parthenonensis* (27%), *Chroococcus lithophilus* (21%), *Gloeocapsa sanguinea* (15%) and *G. violascea* (8%). They were always identified directly from the samples, as they did not grow in culture. Some filamentous cyanobacteria were also identified commonly as *Calothrix pulvinata* (21%), *Leptolyngbya foveolarum* (18%) and *Phormidium corium* (12%). Contrary to the colonial forms, they were observed as short filaments in the samples but they developed abundantly in culture, which made easier their identification (table 2).

Green algae	%	Cyanobacteria	%
<i>Klebsormidium flaccidum</i>	53	<i>Cyanosarcina parthenonensis</i>	27
<i>Trebouxia</i> spp.	51	<i>Chroococcus lithophilus</i>	21
<i>Stichococcus bacillaris</i>	45	<i>Calothrix pulvinata</i>	21
<i>Chlorosarcinopsis minor</i>	27	<i>Leptolyngbya foveolarum</i>	18
<i>Chlorella cf. mirabilis</i>	26	<i>Gloeocapsa sanguinea</i>	15
<i>Chlorella ellipsoidea</i>	12	<i>Phormidium corium</i>	12
<i>Chlorella minutissima</i>	12	<i>Gloeocapsa violascea</i>	8
<i>Geminella terricola</i>	12	<i>Chroococcus limneticus</i>	7
<i>Bracteacoccus</i> sp.	12		

Table 2: Frequency occurrences (%) of main algae and cyanobacteria from a total of 73 samples.

Discussion

Three kinds of discolorations were observed on buildings: the green ones, mainly composed of green algae; the blackish ones, composed of green algae and cyanobacteria; and the red ones, due to the green alga, *Trentepohlia iolithus*.

Among the green algae, *Klebsormidium flaccidum* is of first importance, for due to its frequency of occurrence, and because it was often present as the dominant alga in the investigated biofilms. This filamentous green alga is a common coloniser of various substrates, and it knows a widespread distribution in temperate regions (GAYLARDE & GAYLARDE 2005, JOHN 1988, ORTEGA-CALVO et al. 1991, ORTEGA-CALVO et al. 1993, RIFÓN-LASTRA & NOGUEROL-SEOANE 2001, RINDI & GUIRY 2004) and in tropical ones (CRISPIM et al. 2004, GAYLARDE & GAYLARDE 1999, 2000). This occurrence can be attributed to the capacity of species of *Klebsormidium* to survive in highly dehydrated state (RINDI & GUIRY 2004), even if they generally grow in places of high humidity. *Trebouxia* spp., *Stichococcus bacillaris* and *Chlorella* cf. *mirabilis* are common subaerial algae but they were rarely encountered as the dominant specimens of the biofilms. They were identified on all kind of substrates. This was not the case for *Chlorosarcinopsis minor* which was quite exclusively identified on painted substrates, where the alga was always the dominant one. This ecology has seemingly never been noted.

The extensive and conspicuous red stainings observed on buildings could be attributed to the green alga *Trentepohlia iolithus*. The red colour is due to β -carotene pigments contained in lipid droplets, their characteristic orange colour being brought out when one pass a finger on the algae mat. When encountered, this alga is often dominant and associated with a few other green algae, in most samples *Stichococcus bacillaris* and *Chlorella* cf. *mirabilis* and fungi. It was never observed in our samples together with cyanobacteria. The substrates colonised were found to be one-coat rendering mortars, classical mortars and concrete, which appears to be in good agreement with the ecology of specimens observed previously in France and in western Ireland (RINDI & GUIRY 2002, RINDI et al. 2003). *Trentepohlia iolithus* is known to be widespread in Europe, especially in humid places (RINDI et al. 2003). After our observations, *T. iolithus* is very common in Brittany, where it is the main alga source of discolorations on buildings in the countryside, and along the Manche and Atlantic coast. Indeed, these regions subjected to the oceanic climate experience high humidity. However, red colonizations attributed to *T. iolithus* are also sometimes encountered in places hundred kilometers far from the sea (samples 7711, 7715, 451, 191 and observations in Grenoble), under drier climates. Species of the genus *Trentepohlia* are widespread under tropical climates where one of the predominant algae together with cyanobacteria (CHEW & PING 2003, CRISPIM et al. 2004, GAYLARDE & GAYLARDE 2005, GAYLARDE & GAYLARDE 1999, 2000, JOHN 1988, WEE & LEE 1980).

Most papers dealing with cyanobacteria growing on buildings, present identifications at the generic level, so we lack information to compare those data to our results. Thus, although being ones of the most abundant cyanobacteria in our samples, two of our specimens were apparently never described on buildings substrates: *Cyanosarcina parthenonensis*, which was only mentioned from marbles of the Parthenon (ANAGNOSTIDIS et al. 1983), and *Calothrix pulvinata*, which was never described as a subaerial specimen, even if some descriptions of the genus *Calothrix* were already published (CRISPIM et al. 2004, JOHN 1988, WEE & LEE 1980).

Lastly, one can note that green algae were more frequently present in our samples than cyanobacteria (table 2). Indeed, green algae are known to be usually dominant in temperate regions, as they need humidity and moderate light to develop, whereas cyanobacteria are dominant in tropical ones (GAYLARDE & GAYLARDE 2005, JOHN 1988). This is due to the ability of cyanobacteria to withstand high temperatures and dessication, thanks to their sheaths or mucilage. They can also protect themselves from high solar irradiation with pigments. That was the case in our samples with the sheath of *Calothrix pulvinata* being sometimes shaded in orange, or the slime of *Gloeocapsa sanguinea*, G.

violascea, *Chroococcus lithophilus* or *Nostoc microscopicum* colored in red, violet or yellow-orange, those colour otherwise being often responsible of the blackish aspect of some samples.

Concluding remarks

Most of algae and cyanobacteria species described here are widespread and well-known from building substrates. To complete this work, a forthcoming article will investigate the preference of those specimens for the building substrates sampled and the impact of environmental conditions on their establishment.

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