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Isolation and characterization of non-heterocystous tropical cyanobacteria growing on nitrogen-free medium

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Introduction

Cyanobacteria, also called blue-green algae, are oxygen-evolving photosynthetic prokaryotes (Stanier & Cohen-Bazire 1977). They have long been classified according to the botanical system (Bourrelly 1979), but following a study of 178 strains of cyanobacteria cultured in axenic conditions Rippka *et al.* (1979) proposed a new classification of these organisms. The unicellular forms are divided into two sections; the first includes strains that reproduce by binary fission or budding, and the second, strains that reproduce by multiple fission. The third section of cyanobacteria includes filamentous, non-heterocystous cyanobacteria. The heterocyst-forming strains are assigned to section IV when the cells divide in one plane, and to section V when they divide in many planes.

The ability to reduce dinitrogen to ammonia, or nitrogen fixation, is encountered in filamentous and in unicellular cyanobacteria (Rippka *et al.* 1979). However, the most important group of nitrogen-fixing blue-green algae comprises the heterocystous strains of sections IV and V according to Rippka *et al.* The heterocysts are thick-walled cells differentiated by the strains in conditions of nitrogen starvation; their main function is to protect the nitrogenase complex from oxygen damage and to ensure nitrogenase activity in aerobiosis (Wolk 1982).

In 1970, Stewart and Lex demonstrated that a non-heterocystous filamentous strain in section III could fix dinitrogen anaerobically. Since then, an active nitrogenase has been also found in various strains of this group under micro-aerobic or anaerobic conditions (Rippka & Waterbury 1977; Stewart 1980). Nitrogen fixation by unicellar cyanobacteria of section I appears exceptional; in section II, half of the strains can express nitrogenase activity anaerobically (Rippka & Waterbury 1977; Rippka *et al.* 1979).

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Tropical soils are considered to be relatively rich in cyanobacteria (Mague 1977). This is due to the ability of these strains to resist large climatic variations: such as relative humidity in the daily range of 30–100% and light intensity as high as 100 klux at 13·00 h. Adaptation of cyanobacteria to such variations is due to the fact that as there are many strains in a biotope, each strain reaches its optimum activity in a certain range of conditions. Although the procedure of isolation and the relative biomass may make the heterocystous strains more obvious, the presence of non-heterocystous strains is also of real importance, particularly in biotopes newly colonized.

The study of the colonization by algae of a highly saline sandy soil, progressively washed by seepage water, has shown that nitrogen-fixing activity can occur even when heterocystous strains are scarce (Reynaud & Roger 1981a, b). Thus, possibility of isolating new nitrogen-fixing cyanobacteria assigned to sections I, II or III of the cyanobacteria from such a biotope was investigated.

In this paper, we describe the isolation of 14 non-heterocystous nitrogen-fixing cyanobacteria. Their morphological characteristics are determined and phenotypic properties such as photoheterotrophy, pigment content, drug resistance and toxin production are studied. The plasmid content of these strains was also investigated.

Materials and methods

Sampling site

Ten cores of algal mat were sampled on the freshwater waterlogged bank of Retba lake located on the north coast of Cap Vert (Senegal) at 40 km from Dakar (Reynaud & Roger 1981a).

Isolation procedure

Strains were isolated on BG-11_0 medium (Allen & Stanier 1968) by the serial dilution method (Reynaud & Laloë 1985). Aerobic nitrogen-fixing cyanobacteria were maintained in the mineral medium BG-11_0; anaerobic or micro-aerophilic nitrogen-fixing strains were usually cultivated in BG-11 medium (Rippka *et al.* 1979). The cultures, which were grown at 30°C without shaking, were illuminated with white light at an intensity of 1000 lux.

Cyanophages

Cyanophages LPP-1 (Schneider *et al.* 1964), LPP-2 (Safferman *et al.* 1969), AS-1 (Safferman *et al.* 1972) and N1 (Adolph & Haselkorn 1971) were provided kindly by R. Rippka, Institut Pasteur, France. N(S)1 was isolated in our laboratory (Franche 1984).

Purification of the strains

Several methods of purification were employed according to the degree of contamination of the cyanobacteria.

When the strain was only slightly contaminated or harboured motile cells, standard platings on Petri dishes and micromanipulation of the colonies (Rippka *et al.* 1981) were sufficient to obtain a pure culture.

If this procedure failed, attempts were made to prevent growth of contaminating bacteria with antibiotics (Stewart 1980). Each cyanobacterial strain was cultured in

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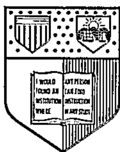

Cornell University
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Ithaca 2/11/87

Mon cher Huguenin,

Tu trouveras ci-joint 3 publications qui font partie de mon cyanotestament; à ce propos je me demande ce que devient l'impression de ma thèse ?.

C'est actuellement l'époque des trappages et des baguages ; je ne suis pas souvent dans mon bureau mais j'ai réservé cette semaine pour la rédaction du rapport d'activité.

Meilleur souvenir aux membres de la commission et au secrétariat,

Clt Reynaud

New York State
College of Agriculture and Life Sciences

College of Arts and Sciences

New York State
College of Veterinary Medicine

5 ml of liquid BG-11_0 medium containing 5–20 μg of one of the following antibiotics: streptomycin, gentamicin, kanamycin, spectinomycin, ampicillin and nalidixic acid. When positive growth was observed, the cyanobacterium was reisolated on solid medium as previously described (Rippka *et al.* 1981). The penicillin selection method of Rippka *et al.* (1981) was applied to very badly contaminated strains.

Presence of contaminants in the cyanobacterial cultures was checked by microscopic examination and by plating one drop of the culture on a plate containing nutrient agar (Difco) or BG-11 medium with 0.5% (w/v) glucose and 0.05% (w/v) casamino acids (Rippka *et al.* 1979).

Morphological characterization

The morphology of the colonies was studied after two to three weeks of growth at 30°C on BG-11 or BG-11_0 plates. The structural properties of the cyanobacterial cells were determined using a Zeiss light microscope. Presence of motile endospores (baeocytes) was checked on agar plates as described by Waterbury & Stanier (1978).

Growth rates

Strains were inoculated into 100 ml amounts of liquid BG-11 and BG-11_0 medium, in 500 ml nephelo-culture flasks. Optical absorbance at 650 nm was measured daily with a Bausch and Lomb spectrophotometer. Generation rates were calculated from the mean of measurements on three cultures.

Acetylene reduction activity (ARA)

ARA was tested in dense culture growing on BG-11_0 medium under aerobic, microaerophilic or anaerobic conditions (Rippka & Waterbury 1977).

Photoheterotrophy

Photoheterotrophy was demonstrated by growth of cyanobacterial strains in 5 ml liquid BG-11 medium supplemented with an organic substrate and with 10^{-5} mol/l dichlorophenyldimethyl urea (Rippka 1978). Organic substrates tested were D-glucose, sucrose, D-fructose, D-mannose, D-galactose, D-arabinose, D-xylose (0.5% w/v); glycerol, D-mannitol, inositol (0.2% w/v); acetate and glycollate (0.1% w/v).

Drug resistance

Drug resistances of the strains were determined on agar plates and in liquid BG-11 medium. Concentrations of 5, 10, 20 or 50 μg/ml of the following antibiotics (Sigma) were tested: streptomycin, kanamycin, gentamicin, tetracycline, spectinomycin, ampicillin, penicillin G, carbenicillin and chloramphenicol.

Phycobiliprotein content

Each cyanobacterial strain was grown in 100 ml of BG-11 medium under low light (500 lux). At the end of exponential growth, cells were harvested and phycobiliproteins extracted and identified according to the methods described by Tandeau de Marsac (1977) and Bryant (1982). The absorbance of the phycobiliprotein extract was scanned between 350 and 750 nm using a spectrophotometer (Beckman Model 25).

Sensitivity to cyanophages

A drop of cyanophage stock (2×10^8 plaque-forming units/ml) was spotted onto lawns

of cyanobacteria containing 10^8 cells in a 0.5% agar overlay. The plates were examined after seven to ten days of incubation at a light intensity of 1000 lux.

Cyanobacterin production
Production of toxin was determined on agar plates by testing each strain against all the others. A drop of a middle-phase exponential culture of the cyanobacterium tested was spotted on to lawns of all the other cyanobacterial strains. Inhibition growth was checked after 10–15 days.

Plasmid extraction
Plasmid DNA was partially purified using a modification of the technique of Meyers *et al.* (1976) previously described (Franche & Reynaud 1986). Molecular weights of the plasmids were determined by comparing their migration in agarose gel electrophoresis to those of the plasmids extracted from the unicellular cyanobacteria PCC 7002 (Roberts & Kotz 1976).

Agarose gel electropheresis
Electrophoresis was carried out in 0.7% (w/v) agarose (Sigma, Type I) slab gels using *Tris*-borate buffer (89 mmol/l *Tris*-base, 2.5 mmol/l EDTA, 89 mmol/l boric acid, pH 8). Gels ($22 \times 14 \times 0.4$ cm) were run vertically for 5 h at 120 v. After migration, gels were stained with ethidium bromide (2 μg/ml) for 1 h and photographed with a Polaroid MP4 apparatus.

Results and discussion

Cyanobacteria isolation
From the samples collected in the algal mat and treated by the serial dilution method, a macroscopic (stereomicroscope Wild M5) and a microscopic examination was conducted to look for cyanobacterial colonies corresponding to non-heterocystous filamentous or unicellular strains growing on mineral medium without nitrogen.

Fourteen obviously different strains, including 13 unicellular and one filamentous forms, were identified and further purified. Some problems were encountered with two unicellular cyanobacteria which failed after axenization to grow on mineral medium without nitrogen. Consequently, these two strains, 83U3 and 83U4, were maintained in BG-11 medium. The filamentous cyanobacterium was difficult to maintain in pure culture. Rapid death was observed on agar plates when the trichomes were freed of contaminants; however, it was possible to obtain a good development in liquid medium without nitrogen source.

Generic assignment
Generic assignment of the strains was based on the classification of Rippka *et al.* (1979) and was determined following microscopic examination of the young axenic cultures. The morphological characteristics of the 14 isolates are summarized in Table 1. Figures 1a–e illustrate five typical shapes of cyanobacterial colonies and the microscopic appearance of each strain is shown in Figs 2a–n.

Four unicellular strains which divided by binary fission were assigned to section I of the cyanobacteria. When a sheath enclosed the cells, cyanobacteria were identified as *Gloeothece* sp. (division in one plane, strain 83U1, Fig. 2a) or as *Gloeocapsa* sp.

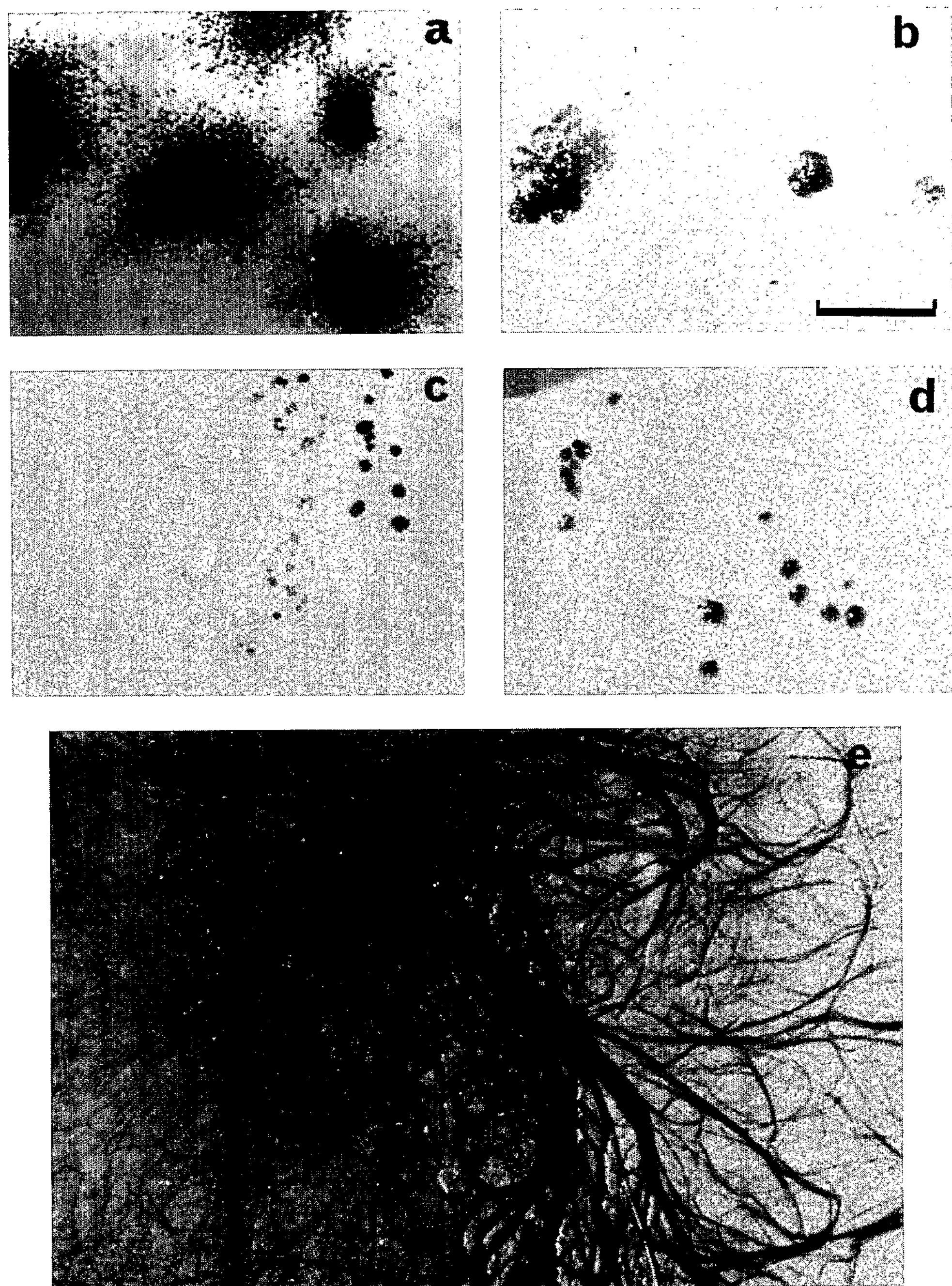


Fig. 1. Morphology of colonies produced by strains *Gloeothece* 83U1 (a), *Dermocarpa* 83U6 (b), *Dermocarpella* 83U7 (c), *Chroococcidiopsis* 83U10 (d) and LPP group A 83U14 (e). Photographs were taken after 15 days of incubation on agar plates containing BG-11_0 medium. Colonies were noted rounded (a), broken (b), rough (c), smooth (d) and diffuse (e). Bar marker represents 0.5 cm.

Table 1 Morphological characteristics of the fourteen cyanobacteria

Section	Genus	Strain	Colony morphology		Vegetative cells		Presence of a sheath	Reproduction by		Division of cells in		Presence of baeocytes	
			Shape	Size (diam mm)	Shape	Size (μm)		binary fission	multiple fission	one plane	many planes	motile	non-motile
I	*Gloeothece*	83U1	Rounded	0.5	Hemispherical	4.6w,8 l*	+	+	–	+	–	–	–
	Gloeocapsa	83U2	Smooth	0.3	Spherical	6	+	+	–	–	+	–	–
	Synechococcus	83U3	Discoïdal	0.2	Cylindrical	1.5w,4 l	–	+	–	+	–	–	–
	Synechocystis	83U4	Discoïdal	0.2	Hemispherical	2.5w,3 l	–	+	–	–	+	–	–
II	*Dermocarpa*	83U5	Broken	1.0	Spherical	9	+	–	+	–	+	+	–
		83U6	Smooth	0.2	Spherical	4–7	±	–	+	–	+	+	–
	Dermocarpella	83U7	Rough	0.1	Spherical	4–9	+	+	+	–	+	+	–
	Myxosarcina	83U8	Broken	0.5	Spherical	6–8	–	+	+	–	+	+	–
	Chroococcidiopsis	83U9	Smooth	0.4	Spherical	4–5	±	+	+	–	+	–	+
		83U10	Smooth	0.3	Spherical	4–6	+	+	+	–	+	–	+
		83U11	Smooth	0.3	Spherical	4–6	+	+	+	–	+	–	+
		83U12	Smooth	0.2	Spherical	4–6	+	+	+	–	+	–	+
	Pleurocapsa group	83U13	Broken	0.8	Spherical	4–9	+	+	+	–	+	+	–
III	LPP group A	83F1	Diffuse	—	Discoïdal	12w,2 l	+	+	–	+	–	–	–

* w, width: l, length.

Table 2 Comparison of the generation times of the strains in the mineral medium with or without nitrogen

Strain	Mean generation time (h)	
	BG-11 medium	BG-11_0 medium
Gloeothece 83U1	25	140
Gloeocapsa 83U2	29	84
Synechococcus 83U3	52	*
Synechocystis 83U4	32	*
Dermocarpa 83U5	72	170
83U6	97	340
Dermocarpella 83U7	100	174
Myxosarcina 83U8	126	130
Chroococcidiopsis 83U9	106	247
83U10	ND	ND
83U11	ND	ND
83U12	103	133
Pleurocapsa group 83U13	ND	ND
LPP group A 83F1	109	123

Strains were cultured aerobically without shaking.
*No growth in BG-11 medium aerobically.

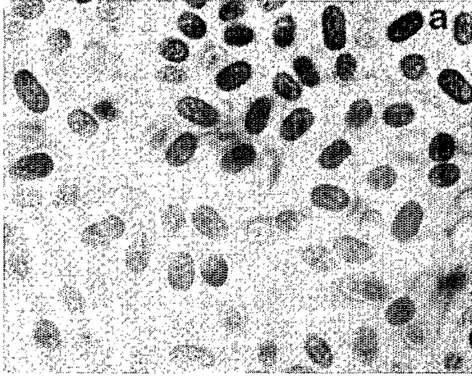
(division in two planes, strain 83U2, Fig. 2b). When the cells were not surrounded by a sheath, strains were identified as *Synechococcus* sp. (division in one plane, strain 83U3, Fig. 2c) or as *Synechocystis* (division in many planes, strain 83U4, Fig. 2d).

The nine unicellular strains assigned to section II of the cyanobacteria were characterized by multiple fission of the cells. The strains 83U5 and 83U6 shown in Figs. 2e–f were identified as *Dermocarpa*; they harboured motile typical baeocytes. The cyanobacterium 83U7 (Fig. 2g) has the characteristic structure of the genus *Dermocarpella*, since pear-shaped structures and motile baeocytes were observed. The formation of cubic cell agregates was a characteristic encountered in the genus *Myxosarcina* (motile baecocytes, strain 83U8, Fig. 2h) and in the genus *Chroococcidiopsis* (immotile baecocytes, strains 83U9-83U12, Figs. 2j–m). The irregular cellular agregates observed in the culture of 83U13 (Fig. 2i) permitted this strain to be placed in the *Pleurocapsa* group.

The filamentous non-heterocystous strain named 83F1 belonged to section III of the classification of Rippka *et al.* (1979). As the trichomes were surrounded by a heavy sheath (Fig. 2n), the strain was identified as a LPP group A cyanobacterium; however, the motility of the trichomes observed on agar plates was characteristic of the genus *Oscillatoria*.

Phenotypic properties

Growth rates. The growth rates of the 14 cyanobacterial strains are shown in Table 2; the rates were 25–109 h in BG-11 medium, and between 84–340 h in mineral medium without nitrogen source. Such values, which corresponded to slow-growing cyanobacteria, have already been observed with a number of other coccoid cyanophyceae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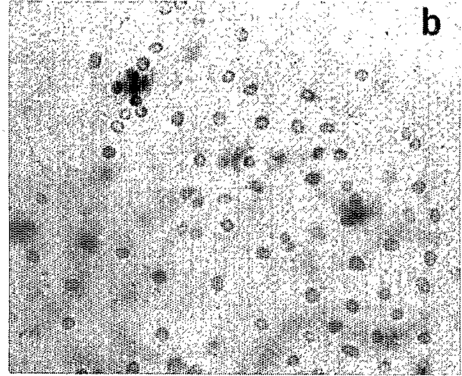
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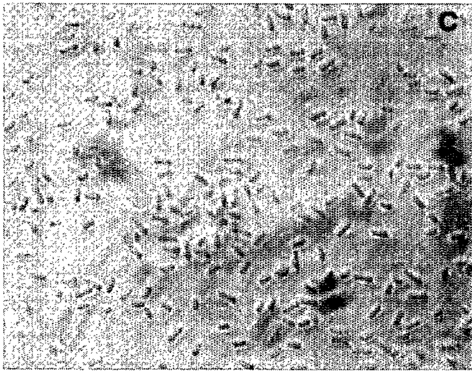
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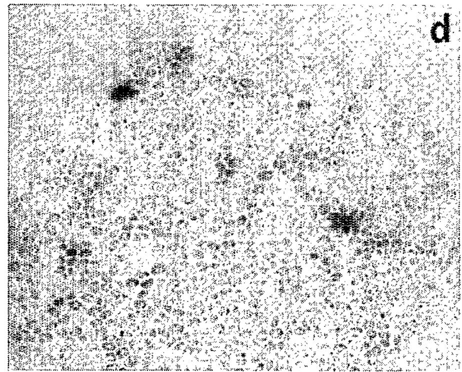
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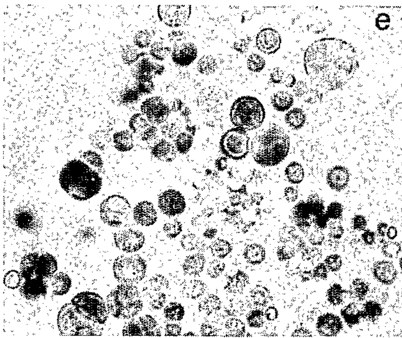
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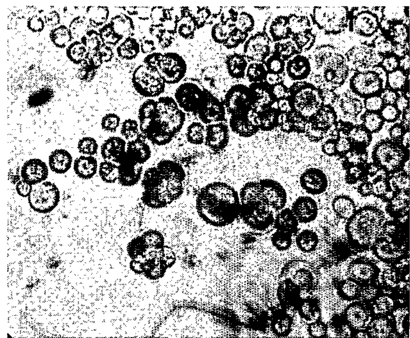

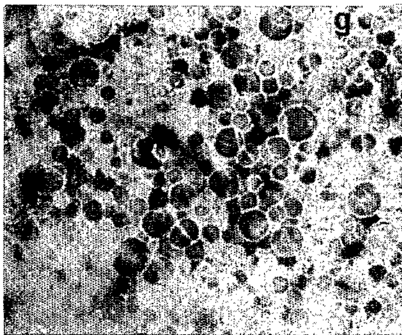
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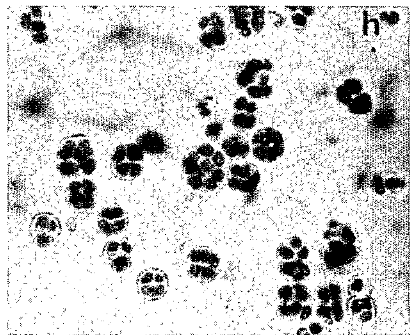
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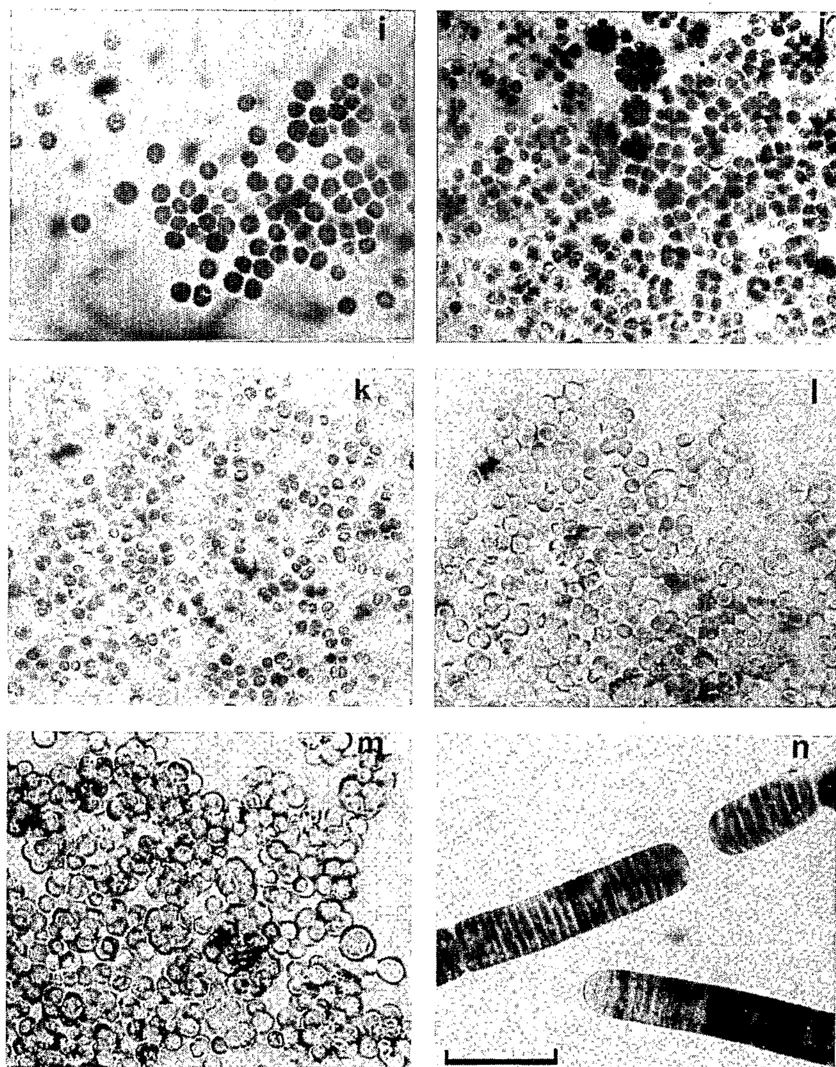

Fig. 2. Light micrographs of the fourteen nitrogen-fixing cyanobacteria. (a), *Gloeothece* 83U1; (b), *Gloeocapsa* 83U2; (c), *Synechococcus* 83U3; (d), *Synechocystis* 83U4; (e), *Dermocarpa* 83U5; (f), *Dermocarpa* 83U6; (g), *Dermocarpella* 83U7; (h), *Myxosarcina* 83U8; (i), *Pleurocapsa* 83U13; (j), *Chroococcidiopsis* 83U9; (k), *Chroococcidiopsis* 83U10; (l), *Chroococcidiopsis* 83U11; (m), *Chroococcidiopsis* 83U12; (n), LPP A 83F1. Bar marker (on Plate n) represents 20 μm.

(Potts *et al.* 1983). The possibility of particular nutritional requirements of the very slow-growing strains and the optimal growth temperatures were not further investigated.

Nitrogen fixation activity. Two of the unicellular cyanobacteria of section I, *Gloeothece* 83U1 and *Gloeocapsa* 83U2, were found to fix nitrogen aerobically,

whereas *Synechococcus* sp. 83U3 and *Synechocystis* sp. 83U4 synthesized nitrogenase only anaerobically. A screening of 52 unicellular cyanobacteria of section I shows that only 20% of these strains have nitrogenase activity, mostly in anaerobic conditions (Rippka *et al.* 1979).

The nine cyanobacteria of section II were able to grow in aerobic BG-11 medium without a nitrogen source. This property appeared uncommon in this section since a screening of 31 unicellular strains that reproduced by multiple fission revealed that none of them fixed N aerobically; however, 61% of them fixed N anaerobically (Rippka & Waterbury 1977).

Since the discovery of nitrogenase activity in *Plectonema boryanum* (Stewart & Lex 1970), about half of the cyanobacteria of section III have been shown to fix nitrogen in anaerobic conditions (Rippka & Waterbury 1977; Stewart *et al.* 1979). Aerobic nitrogen fixers remain infrequent in this group (Stewart 1980). The strain 83F1 identified as LPP group A was found to have a good growth rate in mineral medium without nitrogen source. The mechanism of nitrogenase protection in such strains remains to be elucidated.

Photoheterotrophic growth. Eight of the 14 cyanobacterial isolates were facultative photoheterotrophic strains: they could grow in the light on an organic substrate in the absence of CO_2 fixation (Table 3). As already observed by other workers (Rippka *et al.* 1979; Smith 1982), the carbon sources most frequently used by these strains were D-glucose and sucrose. D-arabinose, D-galactose, D-xylose, glycerol, acetate and glycollate did not support growth of any strains.

Drug resistance. The standard methods used to assess antibiotic sensitivity of bacteria were difficult to apply to cyanobacteria because of their slow growth rates; a degradation of the antibiotics after a period of two weeks of incubation in the light at 30°C was sometimes observed. Nevertheless, significant levels of resistance to kanamycin, gentamicin and to antibiotics of the β-lactam family (ampicillin, carbenicillin and penicillin), were observed in some strains (Table 4). Growth in the presence of the latter antibiotics suggests the synthesis of a β-lactamase. Such an activity has already been encountered in various cyanobacteria (Kushner & Breuil 1977).

Phycobiliprotein. Phycobiliproteins are the major photosynthetic accessory pigments of cyanobacteria and red algae (Stanier & Cohen-Bazire 1977; Tandeau de Marsac 1983). They are identified on the basis of their long-wavelength visible absorption properties. Phycocyanin (λ max 620 nm), allophycocyanin (λ max 650 nm) and allophycocyanin B (λ max 670 nm) have been encountered in all cyanobacteria so far examined (Tandeau de Marsac 1983). C-phycoerythrin (λ max 550–570 nm) and phycoerythrocyanin (λ max 565 nm, shoulder at 590 nm) are two pigments synthesized only by some cyanobacteria (Bryant 1982).

Phycoerythrin was detected in two of the 14 isolates: *Gloeothece* sp. 83U1 and LPP group A 83F1. Bryant (1982), following a survey of 240 strains, observed that this phycobiliprotein was widely distributed among all taxonomic sections of cyanobacteria whereas C-phycoerythrocyanin was synthesized mainly by filamentous heterocystous cyanobacteria (sections IV and V according to Rippka *et al.* 1979). Synthesis of C-

Table 3 Organic compounds utilized by facultative photoheterotrophic strains

Strain	Organic substrate					
	D-glucose	Sucrose	D-fructose	D-ribose	Inositol	D-mannitol
Gloeothece 83U1	−	−	−	−	−	−
Gloeocapsa 83U2	−	−	−	−	−	−
Synechococcus 83U3	−	−	−	−	−	−
Synechocystis 83U4	+	+	−	−	−	+
Dermocarpa 83U5	−	+	+	−	+	−
83U6	−	−	−	−	−	−
Dermocarpella 83U7	(+)	−	−	−	−	−
Myxosarcina 83U8	+	+	(+)	(+)	−	−
Chroococcidiopsis 83U9	+	+	−	−	−	−
83U10	−	−	−	−	−	−
83U11	(+)	−	−	−	−	−
83U12	−	−	(+)	−	+	−
Pleurocapsa group 83U13	−	−	−	−	−	−
LPP group A 83F1	+	+	−	−	−	−

+, good growth, (+), weak growth; −, no growth.

Table 4 Main phenotypic properties and plasmid content of the strains

Strain	Pigment synthesis		Antibiotic resistance	Toxin	Efficiency of lysis	Plasmids	
	PE	PEC		production		Number of plasmids	Molecular weight ($\times 10^6$)
Gloeothece 83U1	+	–	–	–	ϵ	0	–
Gloeocapsa 83U2	–	–	Amp, Pen, Car	–	+	0	–
Synechococcus 83U3	–	–	Gen, Tet, Pen	–	+++	4	24–18–15–3
Synechocystis 83U4	–	–	–	–	+	0	–
Dermocarpa 83U5	–	–	Km	–	–	0	–
83U6	–	+	–	–	+++	1	10
Dermocarpella 83U7	–	–	Km, Tet	–	+	1	15
Myxosarcina 83U8	–	–	Pen$_i$	–	ϵ	0	–
Chroococcidiopsis 83U9	–	–	–	–	ϵ	0	–
83U10	–	–	–	–	+	0	–
83U11	–	–	Km, Amp$_i$, Pen$_i$	–	ϵ	0	–
83U12	–	+	Amp, Pen, Car	+	+	0	–
Pleurocapsa group 83U13	–	–	Km, Amp, Pen, Car	–	ϵ	0	–
LPP group A 83F1	+	–	–	–	ϵ	0	–

PE, C-phycoerythrin; PEC, phycoerythrocyanin.
Amp, ampicillin; Car, carbenicillin; Gen. gentamicin; Kan, kanamycin; Pen, penicillin; Tet. tetracycline.
Antiobiotics were employed at a concentration of 10 μg/ml.
i (subscript), resistance intermediate as growth rate of strain was slow.
Following microscopic examination of the cells after lysozyme treatment, lysis was noted as: +++, good; +, slight; ϵ, almost no lysis; –, no lysis.

phycoerythrocyanin was, however, observed in two unicellular strains: *Dermocarpa* 83U6 and *Chroococcidiopsis* 83U12.

Sensitivity to cyanophages., Several cyanophages of filamentous and unicellular cyanobacteria have been isolated and characterized by many workers (Padan & Shilo 1973; Gromow 1983; Safferman *et al.* 1983). Some strains of the genera *Anacystis* and *Synechococcus* are sensitive to the phage named AS-1 (Safferman *et al.* 1972); LPP-1 and LPP-2 infect some filamentous non-heterocystous cyanobacteria of the genera *Lyngbia, Plectonema* and *Phormidium* (Johnson & Potts 1985). N-1 and N(S)1 are two cyanophages which lyse some filamentous heterocystous cyanobacteria of the genus *Anabaena* (Adolph & Haselkorn 1971; Franche 1984).

None of the 14 strains was found to be sensitive to one of the five phages previously described. This result confirms the very narrow host range of phages of cyanobacteria (Padan & Shilo 1973; Safferman *et al.* 1983).

Cyanobacterin production. Some cyanobacteria produce metabolic compounds called cyanobacterins which inhibit the growth of other blue-green or eukaryotic algae (Srivastava 1972; Gleason & Paulson 1984). The strain *Chroococcidiopsis* 83U12 is likely to synthesize such an algicide because the axenic culture of this strain inhibited the growth of *Dermocarpa* 83U5. The mechanism of this inhibition was not further investigated. A cyanobacterin produced by a filamentous heterocystous cyanobacterium, *Scytonema hofmanni*, has been recently characterized; it was identified as a low molecular weight metabolite which affected thylakoid membrane structure and inhibited photosynthetic electron transport (Gleason & Paulson 1984).

Plasmid content

Cyanobacterial plasmid DNA was first observed by Asato & Ginosa (1973) in a unicellular strain of *Anacystis nidulans*. Since then, many species of blue-green algae have been found to contain endogenous plasmids whose molecular weights ranged from $1.5–75 \times 10^6$ (Lau *et al.* 1980; Simon 1978; Potts 1984).

Three unicellular strains were found to harbour one to four small plasmids (Table 4, Fig. 3). Nevertheless, the possibility that extrachromosomal DNA was absent in the other strains cannot be excluded since most of them were almost insensitive to lysozyme. The standard plasmid extraction procedure (Franche & Reynaud 1986) appeared unsatisfactory because of the presence of extracellular material surrounding the cells. Specific washing of the cyanobacteria such as those described for marine Gram negative bacteria (Laddaga & MacLeod 1981) or sheathed enterobacteria (Meynell 1971), might increase the sensitivity of the cells to lysozyme.

A correlation between the presence of a plasmid in cyanobacteria and phenotypic properties such as carbon source utilization, antibiotic resistance, heavy-metal resistance, toxin production or gas-vacuolation has not yet been established, though these properties seem likely to be plasmid determined. Nevertheless, small extra-chromosomal DNA fragments have been useful in the development of shuttle vectors in unicellular cyanobacteria (Buzby *et al.* 1983; Gendel *et al.* 1983; Kuhlemeier *et al.* 1983).

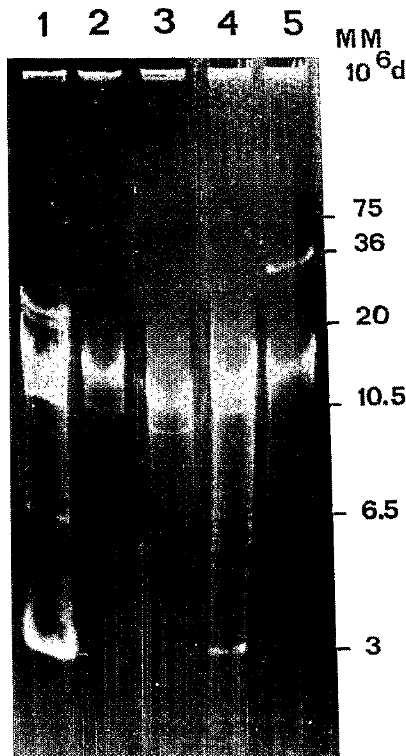

Fig. 3. Agarose gel electrophoresis of plasmid DNA extracted according to Franche & Reynaud (1985). Plasmid DNA was extracted from strains: PCC7002 (1), 83U7 (2), 83U6 (3), 83U3 (4) and *E. coli* (RP4) (5).

Conclusion

The nitrogen-fixing cyanobacteria are the free-living nitrogen fixers which play the most important role in the maintenance of fertility of tropical soils. Many species of cyanobacteria are found in such biotopes (Mague 1977). From a waterlogged soil in West Africa, 14 non-heterocystous nitrogen-fixing strains have been isolated, purified and characterized. Though aerobic nitrogen fixation is a property infrequent in cyanobacteria of sections I, II and III, 12 of them have nitrogenase activity when aerobic. It should be of interest to study the oxygen protection mechanisms of nitrogenase in these strains.

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Summary

Fourteen non-heterocystous cyanobacteria were selected from a continuous waterlogged biotope on a mineral medium free of combined nitrogen. Following morphological characterization, four, nine and one of the strains respectively, were assigned to sections I, II and III of a new cyanobacterial classification. Their phenotypic properties, including growth rates, photoheterotrophy, phycobiliprotein content, antibiotic resistance and toxin production were determined. Three strains were found to harbour one to three plasmids of low molecular weight.

Résumé

Isolement et caractérisation de cyanobactéries non-hétérocystées tropicales cultivées sur un milieu dépourvu d'azote

Quatorze souches de cyanobactéries non-hétérocystées ont été isolées d'un biotope inondé sur un milieu minéral dépourvu d'azote combiné. Après détermination de leurs caractères morphologiques, respectivement quatre, neuf et une des souches ont été assignées aux sections I, II et III de la classification des cyanobactéries. Leurs propriétés phénotypiques telles que taux de croissance, photohétérotrophie, contenu en phycobiliprotéines, résistance aux antibiotiques et la production de toxine ont été déterminées. Trois des souches contiennent de un à trois plasmides de faible masse moléculaire.

Resumen

Aislamiento y caracterización de cianobacterias tropicales no heterocísticas en un medio sin nitrogéno

Mediante un medio mineral sin nitrogéno combinado se seleccionaron catorce cepas de cianobacterias no heterocísticas a partir de un biotopo permanentemente inundado. Apartir de sus carácterísticas morfológicas cuatro, nueve y una de las cepas fueron asignadas respectivamente a las secciones I, II y III de un nuevo sistema para la clasificación de cianobacterias. Se determinaron sus principales características fenotípicas, incluyendo tasas de crecimiento, fotoheterotrofia, contenido en ficobiliproteína, resistencia a antibióticos y producción de toxinas. Tres de las cepas contenían de uno a tres plásmidos de bajo peso molecular.