PLASMIDS IN TWO CYANOBACTERIAL STRAINS

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1. Introduction

The cyanobacteria are an heterogenous group of photosynthetic prokaryotes [1]. An extremely wide variation of DNA base composition and genome size was shown among cyanobacteria of different subgroups or within the same subgroup [2–4]. The presence of extrachromosomal DNA elements may contribute even more to this divergence.

Although such DNA elements have been encountered in several cyanobacteria [5–7], until recently, only in the case of cyanobacteria *Agmenellum quadruplicatum*, these elements were shown to be covalently closed circular (CCC) DNA (plasmids) [6]. While the present study was in progress, the presence of two different plasmids in several closely related cyanobacteria species was reported [8].

Characterization of the cyanobacterial plasmids may provide useful information regarding:

(a) The heterogeneity between and within the subgroups;
(b) The understanding of the intermediary position that this group occupies in the evolution of the photosynthetic world between the prokaryotes and eukaryotic plants [1];
(c) Development of genetic tools, as most genetic techniques proved to be inadequate in cyanobacteria [9].

Here two different cyanobacterial strains were found to harbor plasmids. One is the unicellular strain *Anacystis* sp. 6311 which possesses a plant type, oxygen evolving photosynthetic system [1] and the other is the filamentous *Oscillatoria limnetica* which in addition to the oxygen evolving system, displays also the bacterial type anoxygenic photosynthesis [10]. Both plasmids are presented by electron micrographs. The *Anacystis* plasmid is further characterized by restriction endonuclease analysis.

2. Materials and methods

Anacystis sp. 6311 (Department of Bacteriology and Immunology, University of California at Berkeley) was maintained and grown in a Chu 11 mineral salt medium [11] with $NaNO_3$ concentration modified to 1.5 g/l. *Oscillatoria limnetica* was grown aerobically photoautotrophically as in [12].

The CCC DNA was isolated, using the sodium dodecyl sulphate (SDS) NaCl lysis procedure [13], with modified incubation conditions for lysozyme: 2.5 mg/ml for 3 h at 37°C. Further steps for its purification are as follows: KCl was added to 0.5 M final conc. and the mixture was incubated at 68°C for 10 min, cooled to 4°C and spun at 12 000 × *g* for 2 min. The CCC DNA was precipitated from the clear supernatant by ethanol and then chloroform and phenol extracted. After a second ethanol precipitation the DNA was treated with RNase (50 μg/ml) and subsequently by proteinase K (50 μg/ml), each for 1 h at 37°C. The above procedure of chloroform, phenol extractions and ethanol precipitation was repeated and the DNA precipitate dissolved in TE buffer (pH 7.4) was used for structural analysis.

Electrophoresis was performed on 1% agarose slab gels (150 × 150 × 3 mm) in TAE buffer (Tris 40 mM, sodium acetate 20 mM, acetic acid 26 mM, sodium EDTA 1 mM, pH 8.0). Conditions for electrophoresis were: 20 V, 17 mA, 18 h. The gels were stained with ethidium bromide (5 μg/ml) for 10 min and photographed with short ultraviolet using Polaroid film type 677.

Reaction mixtures for restriction endonucleases (Bethesda Res. Labs. and New England Biolabs) were performed in a buffer containing Tris 10 mM (pH 7.5), MgCl 7 mM, NaCl 50 mM, dithiothreitol (DTT) 1 mM and 2–5 units enzyme/μg DNA, incubated at 37°C for 1–2 h and heated for 10 min at 65°C.

3. Results

An extrachromosomal DNA of *Anacystis sp.* 6311 was first observed as a distinct band by isopycnic centrifugation of a crude extract in CsCl–ethidium bromide. In this band circular forms of DNA were visualized by electron microscope. Electron micrographs of the purified extrachromosomal DNA of the cyanobacteria *Anacystis sp.* 6311 and *O. limnetica,* revealed supercoiled and relaxed circular forms, the latter are presented in fig.1. The contour lengths of the relaxed molecules were compared with reference pBR322 standard molecules [14] and their molecular weights were calculated to be 5.0 and 5.1 megadelatons for *Anacystis* and *O. limnetica,* respectively (table 1). Since *O. limnetica* is relatively resistant to lysozyme on the one hand, and very sensitive to low osmotic pressures on the other, the yields of purified plasmid DNA obtained hitherto, were insufficient for further analysis.

Table 1
Sizes of cyanobacterial CCC DNA

Source of CCC DNA	Size of CCC DNA (megadaltons)
PBR322 (14)	2.87 ± (0.13)
O. limnetica	5.16 ± (0.31)
Anacystis sp. 6311	5.0 ± (0.36)

Contour lengths of the cyanobacteria and a reference DNA molecule (PBR322) were measured in the same electron micrographs. 15 molecules of each DNA type were measured, each 5 times and averaged. The molecular weights of the cyanobacterial DNA molecules were calculated from the ratio of the contour lengths of the cyanobacterial to the reference DNA. Numbers in parenthesis are the standard deviation values. The DNA was spread according to [17] and examined in electron microscope, Philips IL EM300

The electrophoretic mobility of *Anacystis* purified DNA on a 1% agarose gel revealed three distinct bands: chromosomal (c), open circular (OC) and covalently closed circular (CCC) DNA (fig.1A) [15].

The restriction cleavage pattern of the *Anacystis* plasmid DNA produced by restriction endonucleases, which cut the *Anacystis* sp. plasmid infrequently, is presented in fig.1. The sizes of the apparent fragments were calculated using as size markers λ *Eco* RI and $\phi\chi$ *Hpa* II fragments and presented in table 2.

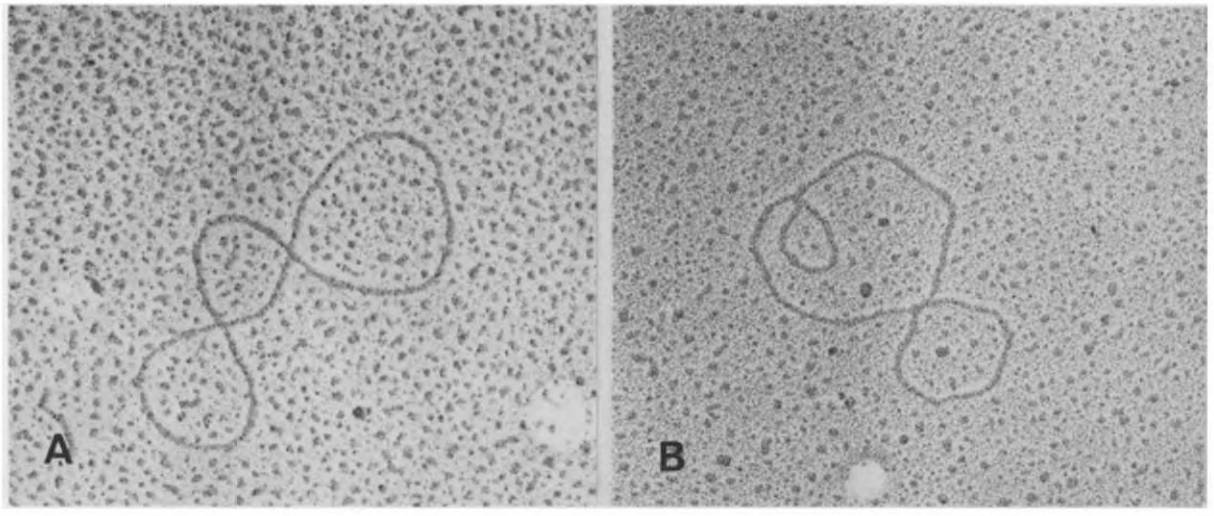

Fig.1. Relaxed forms of cyanobacterial plasmids present in purified plasmid DNA preparations. (A) *Anacystis sp.* 6311, (B) *O. limnetica.* The DNA was spread according to [17], and viewed in an Philips IL EM300. Magnification × 64 250.

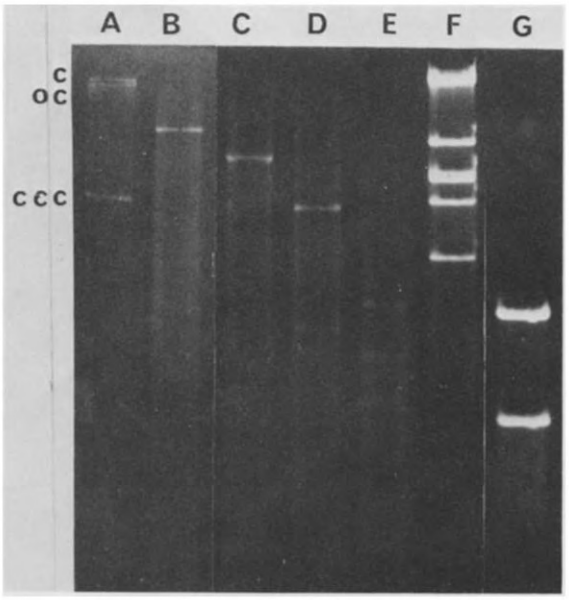

Fig.2. Restriction cleavage pattern of *Anacystis sp.* 6311 plasmid. (A) The untreated plasmid; (B–E) treatments by restriction endonucleases *Bam* HI, *Hin*dIII, *Bgl* I and *Hin*cII, respectively; F, λ *Eco* RI fragments; G, H1 and H2 fragments of ϕX *Hpa* II digest. For experimental conditions see section 2.

The enzymes *Bam* HI and *Hin*dIII seem to cut once and twice, respectively. The size of the resulting *Bam* HI fragment (7800 basepairs) and the sum of two fragments produced by cleavage with *Hin*dIII (6400, 1700 basepairs) are compatible with the contour length value (5.0 megadaltons) of the intact plasmid. The plasmid possesses probably more than two and three sites for *Bgl* I and *Hin*cII, respectively, since the sizes of the apparent fragments add up to values lower (by 900 and 1000 basepairs, respectively) than that of the intact plasmid. Preliminary results of experiments with other restriction enzymes revealed the presence of numerous *Hae* III, *Hpa* II, *Hha* I and *Hin*fI sites (data not shown).

Table 2
The sizes of the restriction fragments of *Anacystis sp.* 6311 plasmid

Kilobase pairs

Bam H1	*Hin*dIII	*Bgl* I	*Hin*cII
7.8	6.4	4.45	2.75
	1.7	2.45	2.20
			1.85

The fragment sizes were calculated from the ratios of the gel mobility of cyanobacterial to the size markers DNA (see fig.1)

The *Hin*dIII and *Bgl* I fragments that we observed in *Anacystis sp.* 6311 plasmid digests, are very similar in size to those observed in digests of one of the two plasmids, described in *Anacystis nidulans* IU625 and *Synecoccus cedrorum* [8]. It is thus possible that the plasmid that we describe in *Anacystis sp.* 6311 and one of the plasmids found in *Anacystis nidulans* IU625 [8] are identical, in spite of the fact that *Anacystis nidulans* IU625 and *Anacystis sp.* 6311 were isolated in different locations and kept independently [4]. It should be noted that *Anacystis sp.* 6311 and not IU625 has been maintained in our laboratory for the last decade.

The implication of the presence of the same plasmid in several cyanobacterial strains is still obscure. No phenotypic expression has been correlated as yet to plasmid function in cyanobacteria. *Anacystis sp.* 6311 has been found in our laboratory to be sensitive to a wide range of antibiotics; a function of antibiotic resistance is therefore quite unlikely. If the plasmid observed by us is indeed identical to that present in *A. nidulans* IU625, it probably does not encode for resistance to heavy metals as well [8]. This aspect is now under study.

The observation that *O. limnetica* harbors a plasmid, supports the suggestion that the occurrence of plasmids in cyanobacteria might be a general phenomenon [7]. This strain has been isolated from a unique ecological niche, the Solar Lake of Elat [16] and its structural and physiological features are markedly different than those of *Anacystis* [10].

Experiments are designed now for further characterization of the plasmid and its cloning, in order to study its phenotypic expression and develop an efficient transformation system in cyanobacteria.

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