Homology of Plasmids in Strains of Unicellular Cyanobacteria

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Six strains of unicellular cyanobacteria were examined for the presence of plasmids. Analysis of lysates of these strains by CsCl-ethidium bromide density centrifugation yielded a major chromosomal DNA band and a minor band containing covalently closed circular plasmid DNA, as shown by electron microscopy and agarose gel electrophoresis. The sizes of the various plasmid species were determined; in each of the *Synechococcus* strains 6301, 6707, and 6908 two plasmid species were found with molecular weights of 5.3×10^6 and 32.7×10^6 . *Synechococcus* strain 7425 had two plasmids of molecular weight 5.4×10^6 and 24×10^6 . *Synechococcus* strain 6312 and *Synechocystis* strain 7005 each contained one plasmid species with molecular weight of 15.9×10^6 and 2.0×10^6 , respectively. Restriction enzyme analysis revealed identical cleavage patterns for the plasmids of identical molecular weight.

The cyanobacteria, traditionally known as the blue-green algae, are photoautotrophic organisms with a number of interesting properties which make them very attractive for molecular biological studies (for reviews, see Carr and Whitton, 1973; Fogg *et al.*, 1973; Stanier *et al.*, 1971; Wolk, 1973). For instance, the cyanobacteria are the only prokaryotic organisms in which an oxygenic type of photosynthesis very similar to that in plants is present (Stanier, 1977). In addition, their molecular mechanism of oxidative metabolism is interesting because of the similarity with that of the mitochondria of eukaryotic cells (Dickerson *et al.*, 1976). Furthermore, some of the cyanobacteria are able to fix nitrogen, a process which is of increasing scientific and agricultural interest (Stewart, 1969; Rippka *et al.*, 1971; Fogg *et al.*, 1973). However, the molecular biology of these processes is still poorly understood.

Little is known about the properties and organization of the DNA of the cyanobacteria, although recently a few studies have

been published on the chromosomal DNA of some unicellular cyanobacteria (Herdmann, 1976; Herdmann and Carr, 1974; Mann and Carr, 1974; Roberts *et al.*, 1977). Of particular interest is the observation that some unicellular cyanobacteria contain extra-chromosomal (plasmid) DNA (Asato and Ginoza, 1973; Restaino and Frampton, 1975; Roberts and Koths, 1976). Plasmids have proven extremely useful in the genetic analysis of various bacteria. Therefore we were encouraged to analyze a number of unicellular cyanobacteria for the presence of plasmids.

In this paper we present evidence for the presence of covalently closed circular (CCC) plasmid DNA in six strains of unicellular cyanobacteria, *Synechococcus* 6301, S.6312, S.6707, S.6908, S.7425 and *Synechocystis* 7005. The sizes and molecular weights of the various plasmids present in these strains were determined by agarose gel electrophoresis and electron microscopy. Digestion of the two plasmid species of *Synechococcus* 6301, S.6707, and S.6908 with different re-

striction enzymes showed identical cleavage patterns on both agarose and polyacrylamide gels.

We have developed a fast screening method for determining the number of plasmid species in the plasmid band of CsCl–ethidium bromide gradients. The method involves comparing heat-treated samples to unheated samples on agarose gels.

MATERIALS AND METHODS

Cyanobacterial strains. The cyanobacterial strains used were obtained from the collection of Dr. R. Y. Stanier at the Pasteur Institute, Paris. Strains 6301, 6312, 6707, 6908, 7002, and 7425 are unicellular cyanobacteria assigned to the typological group *Synechococcus* (R. Rippka, personal communication). Strain *Anacystis nidulans* 0100, obtained from Dr. N. G. Carr, Liverpool, and strain *Anacystis nidulans* IU625, obtained from Dr. R. Safferman, Cincinnati, which were also analyzed during this study, differ in no way from strain 6301 and are therefore referred to as 6301; strain *Synechococcus cedrorum* IU1911 (obtained from Dr. R. Safferman, Cincinnati) is identical to strain 6908 and is referred to as 6908 (Stanier *et al.*, 1971). Strain 7005 is a unicellular cyanobacterium assigned to the typological group *Synechocystis* (R. Rippka, personal communication) and classified as *Microcystis aeruginosa* (Stanier *et al.*, 1971). Strain 7002, also known as *Agmenellum quadruplicatum* strain PR6 (Stanier *et al.*, 1971), was used for the isolation of marker plasmids, since during the progress of the present study it was shown that this strain contains plasmids of six different molecular weights (Roberts and Koths, 1976).

Media and buffers. Cells were grown in BG11 medium (Stanier *et al.*, 1971), except those of strain 7002 which were grown in ASN III medium supplemented with 0.1 µg/ml vitamin B12 (R. Rippka, personal communication). ASN III medium contains per liter: NaCl, 25 g; MgSO₄·7H₂O, 3.5 g; MgCl₂·6H₂O, 2 g; KCl, 0.5 g; CaCl₂·2H₂O,

0.5 g; citric acid, 0.003 g; ferric ammonium citrate, 0.003 g; EDTA, 0.0005 g; K₂HPO₄·3H₂O, 0.029 g; Na₂CO₃, 0.02 g; NaNO₃, 0.75 g, and 1 ml of the trace metal mix of BG11 medium. The medium was sterilized 20 min at 121°C. SE buffer contains 0.12 M NaCl and 0.05 M EDTA, pH 8.0, TE buffer contains 0.01 M tris(hydroxymethyl)aminomethane and 0.001 M EDTA, pH 7.6, and lysis buffer contains 25% sucrose, 0.05 M tris(hydroxymethyl)aminomethane, and 0.1 M EDTA, pH 8.0.

Culture conditions. Stock cultures were grown in 50 ml of the appropriate medium in 250-ml Erlenmeyer flasks in a light cabinet at a temperature of 26–28°C. Cultures of large volume (4 liter) were grown in 5-liter Erlenmeyer flasks equipped with magnetic stirrers and incubated in water baths provided with lateral illumination from banks of two white fluorescent tubes. The light intensity was in the range of 4000 to 6000 lux at the surface of the Erlenmeyer flask. The 4-liter cultures were gassed with N₂ containing 5% (v/v) CO₂. They were inoculated with 5–10 ml of liquid stock cultures of cells in exponential growth phase (for the strains 7005 and 7425, that show very long lag times) or late-exponential growth phase (for all other strains). The following growth temperatures proved to be optimal: 37°C for the strains 6301, 6707, 6908, and 7002, 34°C for strains 6312 and 7425, and 30°C for strain 7005. Under these conditions the doubling time during exponential growth was 5 ± 1 h for 6301, 6707, and 6908, 14 ± 2 h for 6312, 7005, and 7425, and 9 ± 1 h for 7002. The density of the cultures at the end of the exponential phase, when the cells were harvested for plasmid isolation, was 2–4 × 10⁸ cells/ml for 6301, 6707, 6908, and 7002, 1–2 × 10⁸ cells/ml for 6312, and 2–5 × 10⁷ cells for 7005 and 7425.

Isolation of plasmid DNA. The cells of a 4-liter culture were harvested by centrifugation, washed once in SE buffer, resuspended in 68 ml lysis buffer, and transferred into four Beckman R30 tubes (17 ml in each tube). After incubating the cells with lysozyme

(final concentration 2 mg/ml) for 1 h at 37°C, a clear lysate was prepared by a modification of the method described by Guerrey *et al.* (1973): to each of the four R30 tubes 3 ml of 10% sodium dodecyl sulfate (SDS) was added and mixed carefully, and the 20-ml mixture was incubated for 1 h at 37°C. Then 5 ml of 5 M NaCl was added gently and mixed, and the tubes were kept in ice for 2 h. Most of the chromosomal DNA was removed by centrifugation at 25,000 rpm for 30 min at 4°C in a Beckman type 30 rotor. (Occasionally SDS was substituted by Sarkosyl or Brij; this yielded the same number of plasmid species.) The supernatant (clear lysate) of each R30 tube was concentrated by polyethylene glycol (PEG) precipitation (Humphreys *et al.*, 1975) to 7.15 ml. This concentrated clear lysate was transferred into a cellulose nitrate tube (Beckman 50Ti) and incubated with RNase (0.05 ml of 10 mg/ml, heated for 10 min at 80°C) for 30 min at 37°C. CsCl and 0.8 ml ethidium bromide (5 mg/ml) were added, the CsCl was dissolved carefully, and the solution was centrifuged at 37,500 rpm for 60 h at 15°C. 7.8 g CsCl was added in the case of strains 6301, 6312, 6908, and 7002, 7.7 g CsCl in the case of strain 7425, 8.1 g CsCl in the case of strain 6707, and 7.6 g CsCl in the case of strain 7005. After centrifugation the gradients were screened with ultraviolet light and the plasmid and chromosomal DNA bands were collected by puncturing the bottom of the tube. The DNA was precipitated with ethanol, dissolved in TE buffer, and analyzed by electron microscopy and agarose gel electrophoresis. For electron microscopic analysis the ethanol precipitation was preceded by an isoamyl alcohol extraction (3×).

In those cases where the different plasmid species of one host strain were separated, the CsCl-ethidium bromide plasmid fraction was sedimented through a 10–30% sucrose gradient in 0.1 M NaCl, 10 mM Tris-HCl, 1 mM EDTA, 0.1% sarkosyl, pH 7.6, in a Spinco SW27 rotor at 23,000 rpm at 10°C for 16 h.

Restriction endonucleases. EndoR·*Pst*I was isolated as described by Smith *et al.* (1976). EndoR·*Hae*III and endoR·*Hind*II were gifts of Dr. P. D. Baas, Utrecht. EndoR·*Eco*RI was a gift of Dr. J. Sussenbach, Utrecht. EndoR·*Bam*HI was purchased from New England, Biolabs, Beverly, Mass.

Cleavage of plasmid DNA by restriction endonucleases. Plasmid DNA (1.0 to 2.0 µg) was incubated with the amount of restriction endonuclease appropriate to achieve a complete digestion in 2 h. The reaction volume was 0.04 ml. The reaction conditions for the different enzymes were: 10 mM Tris-HCl, pH 7.6, 7 mM MgCl₂, 7 mM mercaptoethanol, at 30°C for endoR·*Pst*I and at 37°C for endoR·*Hae*III and endoR·*Hind*II; 10 mM Tris-HCl, pH 7.6, and 25 mM MgCl₂ at 37°C for endoR·*Eco*RI; and 10 mM Tris-HCl, pH 7.6, 7 mM MgCl₂, 7 mM mercaptoethanol, 150 mM NaCl, 0.01% BSA at 37°C for endoR·*Bam*HI.

Gel electrophoresis of DNA. Electrophoresis of plasmid DNA was performed on horizontal slab gels of 0.6% agarose (3 mm thick, 19 cm wide, and 19 cm long). Ethidium bromide was present both in the gel and in the electrophoresis buffer at a concentration of 2 µg/ml. The electrophoresis buffer was 0.04 M Tris, 0.01 M sodium acetate, 0.001 M EDTA, pH 7.8. The gels were run at 150–200 V for 2–6 h. Photographs of the gels were made under long wave ultraviolet light.

To calculate the molecular weights of the plasmid DNAs, preparations of different standard reference DNAs were run on the same gel. These standard reference DNAs were the plasmids isolated from strain M46 (*E. coli* W3110N carrying three independent plasmids derived from *Salmonella panama*) with molecular weights of 2.7×10^6 , 9.3×10^6 , and 20.6×10^6 (Van Embden *et al.*, 1976) obtained from Dr. J. D. A. van Embden, the CCC double-stranded (DS) DNAs of bacteriophages M13, ϕ X174, and PM2 with molecular weights of 4.0×10^6 , 3.54×10^6 , and 6.3×10^6 , respectively (Brack

et al., 1975; Van den Hondel and Schoenmakers, 1976; Sanger *et al.*, 1977), and the plasmids isolated from *Synechococcus* strain 7002 with molecular weights of 3.0×10^6 , 6.5×10^6 , 10.3×10^6 , 20.1×10^6 , 24.7×10^6 , and 75×10^6 (Roberts and Koths, 1976).

Analyses of restriction endonuclease digests of plasmid DNA were carried out by electrophoresis either on horizontal 2% agarose slab gels as described above or on discontinuous polyacrylamide slab gels as described previously (Van den Hondel and Schoenmakers, 1976).

Heat treatment of plasmid DNA. To determine whether the DNA bands on the agarose gels contain CCC DS DNA, 50- μ l samples of DNA in TE buffer containing 0.1% Sarkosyl were heated for 2 min at 100°C followed by cooling in ice-water, and immediately applied to the gel in order to start the electrophoresis before renaturation takes place. The CCC DS and single-stranded (SS) DNAs from the bacteriophages M13 and ϕ X174 (Van den Hondel *et al.*, 1975; Weisbeek *et al.*, 1976) were heat treated in the same way.

Electron microscopy. Plasmid DNA was spread for electron microscopy by the formamide modifications of a protein monolayer technique (Davis *et al.*, 1971). The spreading conditions used were: 0.5 μ g plasmid DNA/ml, 0.2 μ g open circular (OC) DS ϕ X174 DNA/ml, 50% (v/v) formamide, 0.1 M ammonium acetate, pH 8.0, 10 mM EDTA, and 0.02% (w/v) cytochrome *c*. The sub-phase was triple-distilled water. The cytochrome *c*-nucleic acid film was picked up on carbon-coated copper grids. The specimens were stained with alcoholic uranyl acetate and rotary shadowed with platinum (Keegstra *et al.*, 1977). The specimens were examined and photographed in a Philips EM 301 at different (film) magnifications. The magnification was calibrated on each film with a carbon grating replica (2160 lines per mm). Length measurements were carried out with a Hewlett-Packard 9864A digitizer connected to a 9820A calculator. The molecu-

lar weights were calculated from the internal standard OC DS ϕ X174 DNA [molecular weight 3.54×10^6 (Sanger *et al.*, 1977)].

RESULTS

Analysis for the Presence of Plasmid DNA

A general method to analyze bacterial strains for the presence of covalently closed circular (CCC) plasmid DNA is the lysis of the bacterial cells, followed by an analysis of the cell content in CsCl-ethidium bromide gradients. Since CCC DNA bands at a heavier position than relaxed chromosomal DNA (due to a restrictive uptake of ethidium bromide), the presence of two DNA bands in a CsCl-ethidium bromide gradient indicates that the cells contain plasmid DNA. In this way six lysozyme-sensitive strains of unicellular cyanobacteria were tested, namely, *Synechococcus* strains 6301 (*Anacystis nidulans*), 6908 (*Synechococcus cedrorum*), 6312, 6707, 7425, and *Synechocystis* strain 7005 (*Microcystis aeruginosa*). CsCl-ethidium bromide density gradient centrifugation of the PEG-concentrated clear lysates of cultures of each of these strains resulted in gradients with two DNA bands. The minor band present on the dense side of the major, chromosomal, DNA band indicates the presence of plasmid DNA in a CCC form. A similar result was found for *Anacystis nidulans* by Asato and Ginoza (1973) and Restaino and Frampton (1975). The DNA present in the minor band was analyzed both by agarose gel electrophoresis and electron microscopy, which confirmed the CCC conformation and revealed the number and the size of the different plasmid species.

Plasmid Species

To determine the number of the different plasmid species from the agarose gel patterns (Fig. 1), it was necessary to verify the CCC character of the DNA bands. Instead of the usual methods (Meyers *et al.*, 1976; Van Embden *et al.*, 1976) we have used another, based on the property of CCC DNA to main-

FIG. 1. Agarose gel electrophoresis of plasmid DNA isolated from the unicellular cyanobacterial strains *Synechococcus* 6301, 6312, 6707, 6908, and 7425 and *Synechocystis* 7005. The plasmid DNAs were purified by CsCl-ethidium bromide centrifugation and analyzed by gel electrophoresis on a 0.6% horizontal agarose slab gel in the presence of 2 μ g ethidium bromide per ml. Bands were visualized under ultraviolet light. Migration was from top (cathode) to bottom (anode). Marker plasmid DNA (M) from *Synechococcus* 7002 and *E. coli* M46 were run on the same gel and the molecular weights of the CCC form of these marker DNAs are indicated on the left and the right hand side of the gel, respectively.

tain its CCC character after heat treatment (2 min at 100°C, followed by fast cooling), whereas open circular (OC) or linear DNA is converted into single-stranded (SS) DNA. Since SS DNA has a higher mobility than OC DNA, electrophoresis of heat-treated OC DNA will result in the simultaneous disappearance of an OC DNA band and appearance of a faster migrating SS DNA band. Electrophoresis of heat-treated CCC DNA will not lead to a change in the position of the DNA band.

To demonstrate the validity of this method we compared heat-treated CCC and OC DNA of the bacteriophages M13 and ϕ X174 with unheated DNA on agarose gels. As shown in Fig. 2 the OC DNA band of M13 and ϕ X174 disappears after heat treatment, whereas a new faster migrating band appears. This new band has the same mobility as SS DNA of M13 and ϕ X174, indicating that it consists of SS DNA. As a control the OC and CCC DNA bands of unheated M13 and ϕ X174 DNA were checked for their OC and CCC character by alkaline sucrose gradient centrifugation (data not shown). It is clear from the gels that the CCC DNA band does not change its position after heat treatment.

With this method we analyzed the plasmid

DNA of the six cyanobacterial strains. The results are shown in Fig. 2. Each plasmid species should appear in one or two bands on the gel, in the unheated samples as CCC or CCC and OC DNA, in the heated samples as CCC or CCC and SS DNA, respectively. We found plasmid DNA of two discrete sizes in strains 6301, 6707, 6908, and 7425 and of only one size in strains 6312 and 7005.

It is interesting to note that the OC form of the larger plasmids of strains 6301, 6707, and 6908 (all with a molecular weight of 33×10^6 , see below) show a greater mobility than their corresponding CCC forms, whereas generally OC DNA migrates slower than CCC DNA. A combined analysis of these larger plasmids in neutral sucrose gradients and agarose gel electrophoresis showed that the DNA form with the higher S value in the sucrose gradient migrates slower on the agarose gels and behaves like CCC DNA after heat treatment. A similar observation has been made by Van Embden *et al.* (1976) with a plasmid of MW 54×10^6 from *Salmonella*.

Molecular Weights

The molecular weight of the plasmids present in the six strains were determined

FIG. 2. Agarose gel electrophoresis of heat-treated and unheated plasmid DNAs from the cyanobacterial strains studied together with heat-treated and unheated double-stranded (DS) and heat-treated single-stranded (SS) DNA of the bacteriophages M13 and ϕ X174. Heat-treated samples of DNA (Δ) were obtained by heating the samples for 2 min at 100°C followed by quick cooling. Conditions of electrophoresis were those described in the legend to Fig. 1. The positions of the OC and CCC form of bacteriophage M13 and the CCC form of the cyanobacterial plasmid DNAs are indicated. The other bands in the gel represent OC form DNA except the upper band of the sample of M13 DNA which contains OC dimeric M13 DNA, and the upper band of the sample of strain 7005 which is probably chromosomal DNA.

TABLE 1
MOLECULAR WEIGHT DETERMINATIONS BY AGAROSE GEL ELECTROPHORESIS AND CONTOUR LENGTH
MEASUREMENTS OF PLASMIDS FROM SIX UNICELLULAR CYANOBACTERIA

Strain	Plasmid designation	Molecular weights from contour length		Molecular weights from gel electrophoresis	
		Number of molecules measured	MW ^a ($\times 10^6$)	Number of determinations	MW ($\times 10^6$)
6301	pUH1	35	5.28 ± 0.24	3	5.1–5.4
	pUH2	35	32.7 ± 1.3	3	32–34
6707	pUH3		n.d. ^b	3	5.1–5.4
	pUH4		n.d.	3	32–34
6908	pUH5	36	5.31 ± 0.17	3	5.1–5.4
	pUH6	25	32.4 ± 1.2	3	32–34
7425	pUH7	76	5.43 ± 0.22	3	5.2–5.5
	pUH8	17	23.7 ± 0.9	3	23–25
6312	pUH9	24	15.9 ± 0.5	3	15.5–16.5
7005	pUH10	78	2.01 ± 0.05	3	1.9–2.1

^a Molecular weights were calculated from OC DS ϕ X174 DNA (molecular weight 3.5×10^6) as an internal standard.

^b n.d., not determined.

by agarose gel electrophoresis of the plasmid DNAs together with marker CCC DNAs of known molecular weight. Since the mobility of CCC DNA is proportional to the logarithm of its molecular weight in the range of 0.6×10^6 to 95×10^6 (Meyers *et al.*, 1976), the molecular weights of the various cyanobacterial CCC DNA species were calculated from the plot of the logarithms of the molecular weights of the marker CCC DNAs against their position on the gel (Table 1).

The plasmid DNA samples were also analyzed in the electron microscope. The same number of plasmid species per cyanobacterial strain was found as indicated by the agarose gels. The molecular weights, as calculated from the contour lengths (Table 1), were in good agreement with those determined from the electrophoretic mobilities.

Homology of Plasmids from Various Strains

Since the plasmid species present in 6301, 6707, and 6908 were similar in size, we considered the possibility that they are related. One test of relationship is to compare their

restriction enzyme fragment patterns (Thompson *et al.*, 1974). Therefore the two plasmid species from 6301, 6707, and 6908 were separated by sucrose centrifugation, digested separately with various restriction endonucleases and analyzed on gels.

Digestion with the restriction enzymes endoR·*Hind*II and endoR·*Hae*III yielded identical fragment patterns for the 5.3×10^6 plasmids pUH1, pUH3, and pUH5, and for the 33×10^6 plasmids pUH2, pUH4, and pUH6 (Figs. 3 and 4). Indistinguishable patterns were also observed after digestion with the restriction endonucleases endoR·*Eco*RI, endoR·*Bam*HI, and endoR·*Pst*I (results not shown). The number of fragments produced by each enzyme are given in Table 2. The results indicate that the plasmid species with corresponding sizes present in the strains 6301, 6707, and 6908 contain very homologous DNA sequences. A further biochemical study is needed, however, to determine whether these plasmids are identical.

Analysis of the 5.4×10^6 plasmid pU7 from 7425 with the restriction enzymes endoR·*Hind*II and endoR·*Hae*III revealed

FIG. 3. Cleavage patterns of the plasmids pUH1 through pUH6 after digestion with endoR·*Hind*II. DNA samples (1 to 2 μ g) were treated with endoR·*Hind*II for 2 h prior to stopping with EDTA. The reaction mixtures were analyzed on a horizontal 2% agarose slab gel.

FIG. 4. Cleavage patterns of the plasmids pUH1 through pUH6 with endoR·*Hae*III. Digests were fractionated by gel electrophoresis (a) on a horizontal 2% agarose gel, (b) on a discontinuous polyacrylamide slab gel, consisting of a 3% polyacrylamide gel on top of a 10% polyacrylamide gel layer. DNA samples (1 to 2 μ g) were treated with endoR·*Hae*III for 2 h prior to stopping with EDTA.

restriction patterns which are different from the patterns of the 5.3×10^6 plasmids pUH1, pUH3, and pUH5 (results not shown), suggesting that pUH7 is not related to these plasmids.

Check on Bacterial Contaminants

To exclude the possibility of contamination both the stock cultures and the 4-liter cultures were checked routinely for the presence of any bacterial contaminant in the following way. A sample of the cultures was (i) spread on agar-solidified bacterial growth medium supplemented with casamino acids (0.5 g/liter) and sucrose (0.5 g/liter) and incubated under conditions that enable only bacteria to grow (in the dark), (ii) subjected to Gram staining (cyanobacteria are Gram-negative) as a control on the uniformity of the culture, (iii) transferred to standard liquid medium and to the same medium supplemented with casamino acids (0.5 g/liter) and glucose (0.5 g/liter), incubated for 1 week, and monitored for contamination by microscopic examination, and (iv) examined in thin section in the electron microscope for the presence of cells without thylakoid membranes. If these checks gave no indication of contamination, we concluded that the isolated plasmid(s) originated from the cyanobacterial strain under study.

DISCUSSION

The results described in this paper reveal that the unicellular cyanobacterial strains *Synechococcus* 6301, 6312, 6707, 6908, 7425, and *Synechocystis* 7005 contain plasmid DNA of sizes ranging in molecular weight from 2×10^6 to 33×10^6 . All the plasmids were isolated under rigorous lysis conditions. It may be possible that under these conditions very large plasmid species remain unnoticed. Therefore, we tested our lysis procedure on *Synechococcus* strain 7002, from which Roberts and Kothe (1976) isolated several plasmid species, the largest having a molecular weight of 75×10^6 . We were able to isolate the same number of

TABLE 2
NUMBER OF FRAGMENTS AFTER DIGESTION OF
THE PLASMIDS pUH1 THROUGH pUH6 BY
VARIOUS ENDONUCLEASES

	pUH1	pUH3	pUH5	pUH2	pUH4	pUH6
<i>EcoRI</i>	0	0	0	9	9	9
<i>BamHI</i>	1	1	1	7	7	7
<i>PstI</i>	8	8	8	18 ^a	18 ^a	18 ^a
<i>HindIII</i>	8	8	8	25 ^a	25 ^a	25 ^a
<i>HaeIII</i>	22 ^a	22 ^a	22 ^a	>36 ^a	>36 ^a	>36 ^a

^a Determination of the exact number of DNA fragments becomes inaccurate in the case of a high number of restriction enzyme cleavage sites. In these cases only a minimal estimate of the number of fragments is given.

plasmid species, thus our lysis procedure is suitable for the isolation of plasmids up to a molecular weight of at least 75×10^6 .

Three of the strains contain plasmids of similar sizes, (Table 1), namely, the plasmids of 5.3×10^6 and 33×10^6 MW in strains 6301, 6707, and 6908. Analysis of these plasmids with five different restriction endonucleases reveal identical cleavage patterns for the 5.3×10^6 plasmids pUH1, pUH3, and pUH5, and for the 33×10^6 plasmids pUH2, pUH4, and pUH6. Since similar cleavage patterns are strong indications of DNA sequence homologies (Thompson *et al.*, 1974), these results suggest that the strains 6301, 6707, and 6908 carry homologous plasmid species, though these strains are different in size and base composition of their genomes (Herdmann *et al.*, 1978). This may be an indication that a mechanism for the transfer of plasmids exists between these three strains. A similar relationship has been shown between plasmids of different *Haemophilus influenzae* strains (Elwell *et al.*, 1977), *Thermus flavus* strains (Hishinuma *et al.*, 1978), *Pseudomonas puta* strains (Duggleby *et al.*, 1977), and *Streptomyces coelicolor* strains (Schrempf and Goebel, 1977).

It is worth noting that the plasmids pUH1, pUH3, and pUH5, contain one cleavage site for the restriction enzyme endoR·*BamHI*. This enzyme produces cohesive ends, which is helpful for the preparation of recombinant DNA *in vitro* (Roberts, 1976). This property

together with the relatively small size of these plasmids may facilitate molecular genetics in some of the unicellular cyanobacteria.

At the moment nothing is known about the function of the plasmids found in the different cyanobacterial strains. It is reasonable to assume that these plasmids have functions comparable to those of plasmids in other bacterial strains, e.g., resistance to heavy metals and drugs, sexuality, biodegradation of aromatic compounds, and bacteriocin production (for a list of functions, see Novick *et al.*, 1976). At the moment we are testing for toxin production and resistance to heavy metals and antibiotics.

Another function that often has been postulated for plasmids in cyanobacteria and other photosynthetic bacteria is involvement in photosynthesis (Asato and Ginoza, 1973; Gibson and Niederman, 1970; Roberts and Koths, 1976; Saunders *et al.*, 1976). However, we could find no plasmids in *Synechococcus* strain 6715, so the presence of plasmids may not be an absolute requirement for photosynthesis in cyanobacteria. On the other hand the possibility cannot be excluded that some plasmids provide additional photosynthetic properties, for instance the ability to function better under difficult photosynthetic conditions such as high light intensities. Elimination of plasmids from the cyanobacteria with the aid of a curing agent may give information on this matter.

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