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COMPARISON OF PLASMIDS FROM THE CYANOBACTERIUM *NOSTOC* PCC 7524 WITH TWO MUTANT STRAINS UNABLE TO FORM HETEROCYSTS

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1. Introduction

Cyanobacteria (blue-green bacteria) are O_2-evolving photosynthetic prokaryotes some species of which fix N_2 in air because the nitrogenase is protected from O_2 inactivation by being localized in differentiated cells called heterocysts [1]. Recently much attention has been paid to the possible role of plasmids as vehicles for the transfer of *nif* genes and other genetic determinants associated with N_2-fixation in prokaryotes [2,3]. This paper characterizes the extrachromosomal DNA of the N_2-fixing heterocystous cyanobacterium *Nostoc* 7524 and two non-heterocystous deletion mutants of this organism using gel electrophoresis, electron microscopy and restriction enzyme analysis. Three species of covalently closed circular (CCC) DNA of different molecular weight were found. It is also concluded that the failure of the non-heterocystous mutants to differentiate heterocysts cannot be readily ascribed to a loss of plasmid-borne genetic material.

2. Materials and Methods

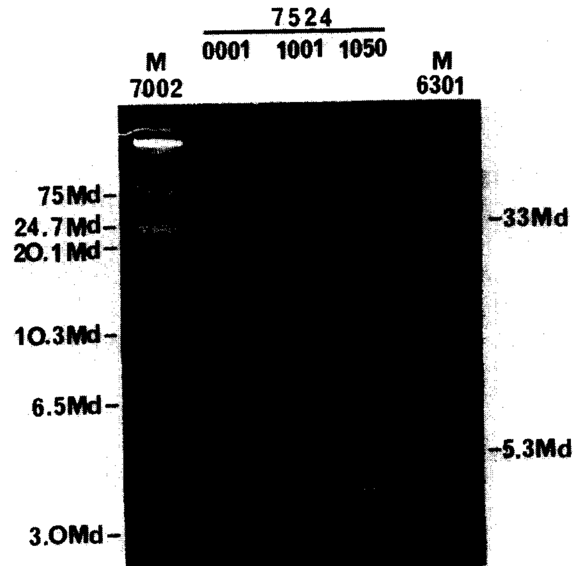
Nostoc PCC 7524, *Synechococcus* PCC 6301 and PCC 7002 were obtained from the Pasteur Culture Collection, Paris [4]. The culture conditions and media used were as previously described [5,6]. The non-heterocystous strains of *Nostoc* 7524, ND1001 and ND1050, which have short and long trichome morphologies, respectively, were spontaneous mutants which arose independently during prolonged maintenance of the wild-type heterocystous strain ND0001 in medium BG-11_0 + $NaNO_3$ (1.5 g/l). *Synechococcus* 6301 and 7002 were used for the isolation of marker plasmid DNA since they harbour plasmids of known molecular weights [6,7]. Checks on bacterial contamination were carried out routinely [6].

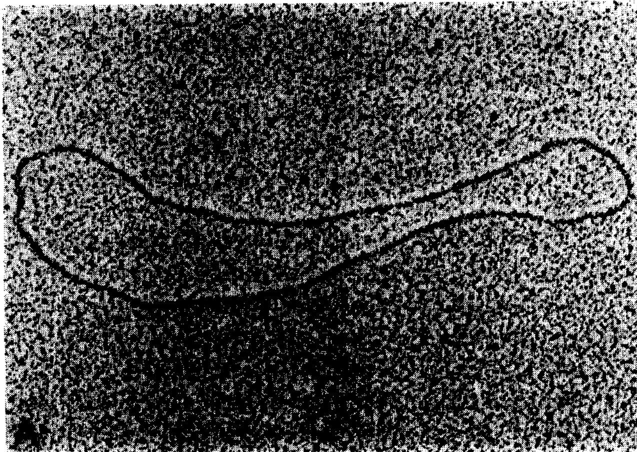
Plasmid DNA was isolated by CsCl-ethidium bromide density gradient centrifugation as before [6] except that after concentrating the cleared lysate with polyethylene glycol, the precipitate was dissolved in 26.7 g TE buffer. Thereafter 28.4 g (for *Nostoc* 7524) or 28.7 g (for *Synechococcus* 6301 and 7002) CsCl was added and dissolved carefully. 3 ml of ethidium bromide solution (5 mg/ml) was then added and the solution was centrifuged at 45 000 rev./min for 20 h at 15°C in a Beckman VTi 50 vertical rotor. Gel electrophoresis, heat treatment, and restriction enzyme analysis of plasmid DNA were performed as before [6]. Electron microscopy was carried out according to Davis et al. [8]. The spreading solution contained 60% formamide, 0.4 M ammonium acetate, 10 mM EDTA, pH 7.8. The hypophase was distilled H_2O, redistilled twice over quartz. The restriction endonuclease endoR*Hin*dII was a kind gift from Dr. P.D. Baas, State University of Utrecht. Other restriction enzymes were purchased from Bethesda Research Laboratories, Rockville, MD or New England Biolabs, Beverly, MA.

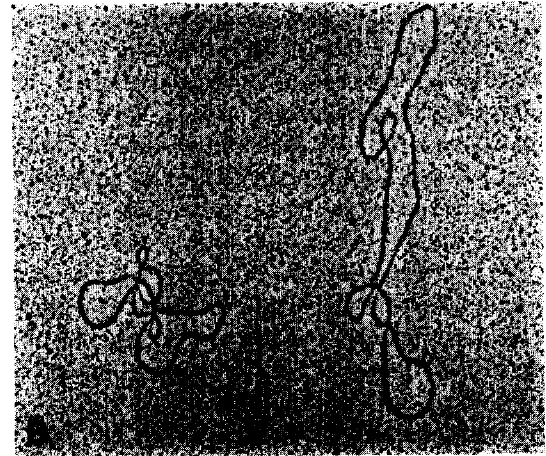
3. Results and Discussion

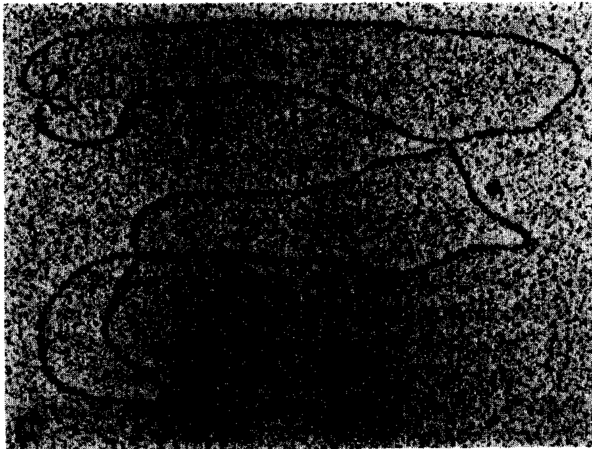
Nostoc 7524 (wild-type strain ND0001) and the two non-heterocystous mutants (ND1001 and ND1050) all contained extrachromosomal DNA which was observed as a satellite band after dye-buoyant-density gradient centrifugation. This band was collected and subjected to further investigations.

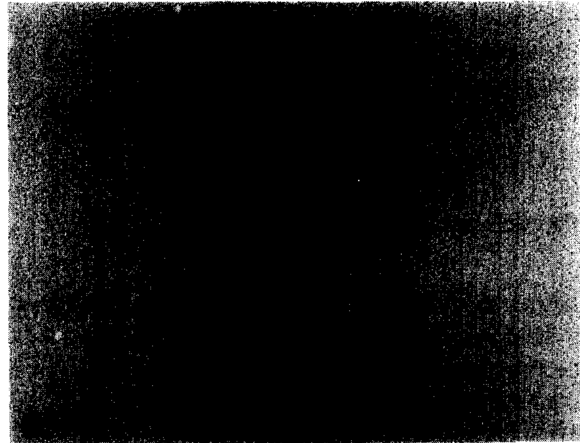
Gel electrophoresis of the CsCl-ethidium bromide fraction was performed on 0.6% horizontal agarose gels to separate the plasmid components present and 4 distinct bands were routinely observed in material from all 3 strains (Fig. 1). To determine whether these bands had a CCC or an open circular (OC) conformation two methods were used – heat treatment and sucrose-gradient centrifugation. Both methods











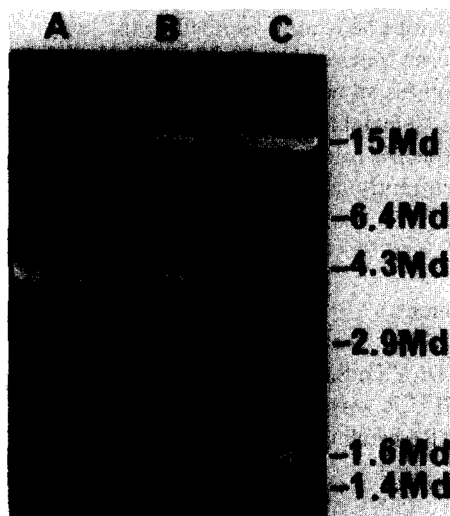


Fig. 3. Cleavage pattern of plasmid pDU1 with endoR.*BglI*. (A) pDU1 digested with endoR.*BglI*; (B) pDU1 after digestion with endoR.*BglI* plus marker fragments of endoR.*Hin*dIII digest of λ DNA; (C) marker fragments of endoR.*Hin*dIII digest of λ DNA. The molecular weights of the λ *Hin*dIII fragments are indicated on the right hand side of the gel. The reaction mixtures were analysed on a 1% horizontal agarose slab gel as previously described [6].

revealed the presence of 3 distinct CCC plasmid species (data not shown). This accords with the findings of CCC DNA in a variety of cyanobacteria [6].

The molecular weights of the three plasmids were calculated by reference to marker CCCDNA isolated from *Synechococcus* 6301 and 7002 (Fig. 1). The *Nostoc* 7524 plasmids designated pDU1, pDU2 and pDU3 were found to have molecular weights of 4 ± 0.1, 8 ± 0.2 and 28 ± 3.0 megadaltons, respectively.

Electron microscopy of extrachromosomal DNA from *Nostoc* 7524 revealed the presence of 3 plasmids of different contour lengths. These contour lengths were measured using plasmid pBR322 [9] as internal standard and molecular weights of 4.1 ± 0.4, 7.9 ± 0.6 and 25 ± 2 were calculated for plasmids pDU1 (Fig. 2A, B, D), pDU2 (Fig. 2C) and pDU3 (Fig. 2D), respectively.

Plasmid pDU1, which may be useful as a cloning vehicle due to its small size and ease of isolation, was characterized further by restriction enzyme analysis.

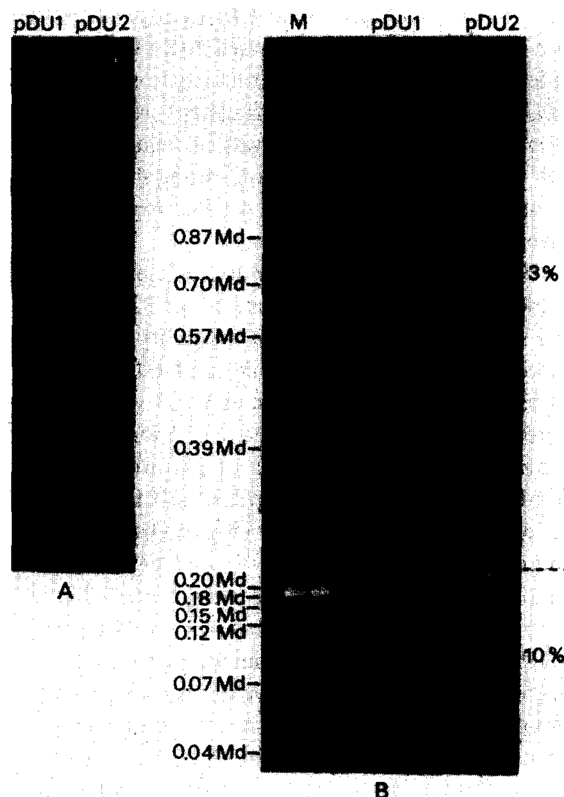


Fig. 4. Cleavage patterns of plasmids pDU1 and pDU2 with endoR.*Hin*dII. Digests were fractionated by gel electrophoresis on (A) a 1% horizontal agarose gel, and (B) a discontinuous polyacrylamide slab gel, consisting of a 3% polyacrylamide gel on top of a 10% polyacrylamide gel layer. Marker fragments (M) from endoR.*Hae*III digestion of ϕX174 RF DNA were also run on the same polyacrylamide gel. The molecular weights of these are indicated on the left hand side of the gel.

Fig. 1. Agarose gel electrophoresis of covalently closed circular (C) and open circular (O) plasmid DNA isolated from *Nostoc* 7524 strains ND0001, ND1001 and ND1050. Marker plasmid DNA (M) isolated from *Synechococcus* 7002 and 6301 were run on the same gel and the molecular weights of the CCC form of the marker DNAs are indicated on the left and right hand sides of the gel, respectively. Gel electrophoresis was performed on a 0.6% horizontal agarose slab gel as previously described [6].

Fig. 2. Electron micrographs of plasmids from *Nostoc* 7524 strain ND0001. (A) pDU1; (B) internal marker DNA of pBR322 (left) and pDU1 (right); (C) pDU2; (D) pDU1 (left) and pDU (right). The plasmid DNA was purified by CsCl-ethidium bromide density gradient centrifugation and spread for electron microscopy as previously described [8].

The restriction enzyme endoR.*Bgl*I cut pDU1 at one site to produce a double stranded linear DNA molecule. When run on a 1% horizontal agarose gel using λ DNA endoR.*Hin*dIII marker fragments as standard (Fig. 3) its molecular weight was calculated to be 4.1 ± 0.1 megadaltons thus confirming the value obtained by contour length and gel electrophoresis investigations.

From electron microscopy and gel electrophoresis evidence it was found that plasmid pDU2 was twice the size of plasmid pDU1; the possibility that it may therefore be a dimeric molecule of pDU1 was then investigated. Plasmids pDU2 and pDU1 were incubated separately with the same restriction enzyme in order to compare their restriction patterns. Digestion of pDU2 and pDU1 with endoR.*Hin*dII followed by gel electrophoresis on agarose (Fig. 4A) and discontinuous polyacrylamide (Fig. 4B) slab gels revealed similar restriction patterns for each plasmid. Thus, plasmid pDU2 appears to be a dimer of plasmid pDU1. There are reports of dimeric plasmids in other bacteria [10,11] but they have not hitherto been reported in cyanobacteria. The restriction enzyme patterns of plasmids pDU1 and pDU3 were investigated after incubation with endoR.*Xba*I and were found to be different (data not shown). From this evidence we conclude that pDU3 is unlikely to be derived from a single plasmid DNA form.

Collectively, the data provide evidence for the presence of 3 species of CCCDNA in the heterocystous cyanobacterium *Nostoc* 7524. This compares with plasmid numbers ranging from 0–5 reported for various other heterocystous cyanobacteria [12]. Neither the plasmid content nor plasmid size were different in the wild type and in the non-heterocystous mutants. The latter appear to be deletion mutants, since wild-type revertants have never been detected in repeated selection experiments on medium lacking a source of combined nitrogen. The results indicate that the failure of the mutants to differentiate heterocysts cannot be readily ascribed to the loss of plasmid-borne genes coding for heterocyst differentiation unless, of course, the deletion is so small as to make no detectable change in plasmid molecular weight. The specific roles of such plasmids in cyanobacteria are currently under investigation.

Acknowledgments

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References

[1] Stewart, W.D.P. (1980) Annu. Rev. Microbiol. 34, 497–536.
[2] Hollaender, A. et al. (Eds.) (1977) Genetic Engineering for Nitrogen Fixation. Plenum, New York.
[3] Ausubel, F.M., Janssen, K.A., Ow, D.W., Riedel, G.E., Ruvkun, G.B., Cannon, F.C. and Cannon, M.C. (1980) in: Nitrogen Fixation (Stewart, W.D.P. and Gallon, J.R., Eds.) Academic Press, London pp. 395–421.
[4] Rippka, R., Deruelles, J., Waterbury, J.B., Herdman, M. and Stanier, R.Y. (1979) J. Gen. Microbiol. 111, 1–41.
[5] Sutherland, J.M., Herdman, M. and Stewart, W.D.P. (1979) J. Gen. Microbiol. 115, 273–287.
[6] Van Den Hondel, C.A.M.J.J., Keegstra, W., Borrias, W.E. and Van Arkel, G.A. (1979) Plasmid 2, 323–333.
[7] Roberts, T.M. and Koths, K.E. (1976) Cell 9, 551–557.
[8] Davis, R., Simon, M. and Davidson, N. (1971) in: Methods in Enzymology Vol. 21 (Grossman, L. and Moldave, K., Eds.), Academic Press, New York, pp. 413–428.
[9] Sutcliffe, J.G. (1978) Nucl. Acids Res. 5, 2721–2728.
[10] Bedbrook, J.R. and Ausubel, F.M. (1976) Cell 9, 707–716.
[11] Chang, A.C.Y. and Cohen, S.N. (1978) J. Bacteriol. 134, 1141–1156.
[12] Simon, R.D. (1978) J. Bacteriol. 136, 414–418.