NOTES

Survey of Extrachromosomal DNA Found in the Filamentous Cyanobacteria

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Cleared lysates of 13 species of filamentous cyanobacteria were examined for the presence of extrachromosomal DNA by using agarose gel electrophoresis and ethidium bromide staining. Seven of the 13 species contained extrachromosomal covalently closed circular DNA, and all but 1 species contained multiple elements. There was no correlation between the presence of extrachromosomal DNA and either the range of metabolic activities found in the cyanobacteria or the differentiated cell types or structures elaborated by the morphologically complex filamentous cyanobacteria.

There has been no systematic survey of the extrachromosomal (EC) DNA in cyanobacteria, and as yet there has been no direct information on the occurrence of such DNA in the morphologically complex filamentous cyanobacteria. At least two species of unicellular cyanobacteria have been shown to contain EC DNA in the form of supertwisted covalently closed circles (CCC). Anacystis nidulans (IU 625) contains an EC DNA element of approximately $35 \times 10^6$ to $39 \times 10^6$ daltons (D. Heaton and E. W. Frampton, Abstr. Annu. Meet. Am. Soc. Microbiol. 1976, 1117, p. 131), whereas the marine cyanobacterium Agmenellum quadruplicatum contains five discrete classes of CCC DNA ranging from $3 \times 10^6$ daltons to $65 \times 10^6$ to $80 \times 10^6$ daltons (11). The information coded for by these EC DNA elements is not known.

As part of a study on the metabolism of DNA during cell differentiation in filamentous cyanobacteria, a survey was carried out to identify and characterize the EC DNA elements found in a group of 13 species representing all of the morphological classes of cyanobacteria. Anacystis nidulans (IU 625) was also examined as a control since this strain harbors EC DNA (Heaton and Frampton, Abstr. Annu. Meet. Am. Soc. Microbiol. 1976, 1117, p. 131). Because of the need to examine EC DNA from a large number of organisms, agarose gel electrophoretic techniques were used to identify and determine the size and conformation of isolated DNA elements. In this paper evidence is presented that EC CCC DNA elements commonly occur in filamentous cyanobacteria.

The cyanobacterial strains examined are listed in Table 1 and are catalogued according to their taxonomic position (14). All organisms examined either were obtained as axenic cultures or were made axenic by the procedure of Wolk (18). Cultures were maintained on slants of BG-11 mineral medium containing 1% purified agar (Difco, no. 0560-01), and samples for analysis were grown in 100-ml batches in 250-ml flasks on a rotary shaker as previously described (12). Species capable of aerobically fixing molecular nitrogen were grown on an eightfold dilution of the nitrogen-free medium of Allen and Arnon (1), and non-nitrogen fixers were grown on BG-11 medium (15). Because the studies were aimed at examining minor DNA components, it was critical that the cultures used were axenic. Before the start of the experiments, cultures were monitored for the presence of contaminating organisms and were discarded if not axenic. The monitoring program consisted of microscopic examination before and after inoculation into enriched media (6).

The general procedure used for isolation of EC DNA was that described by Myers et al. (10), except that the cells were lysed by the detergent procedure of Clewell and Helinski (4). In this procedure, cells are gently lysed under conditions in which the chromosomal DNA remains membrane bound and can be selectively removed by centrifugation. EC DNA is concentrated and then separated and identified by agarose gel electrophoresis. The methods were tested by analyzing the EC DNA in Anacystis nidulans IU 625 because it had been previously
shown that this strain contains a CCC DNA element with a molecular weight between 35 x 10^6 and 39 x 10^6 (Heaton and Frampton, Abstr. Annu. Meet. Am. Soc. Microbiol. 1976, 1117, p. 131). However, two classes of CCC DNA were identified: the expected EC DNA of 37 x 10^6 daltons and an additional EC DNA of 4.7 x 10^6 daltons. The smaller EC DNA had not been previously described, and its resolution suggests that the survey methods used in this work are at least as sensitive as those previously employed for identifying EC DNA in cyanobacteria. There are variations in the ability to gently lyse cells of filamentous cyanobacteria by the method of Clewell and Helinski (4). The majority of species examined showed significant lysis as judged by examination under a phase-contrast microscope and from the amount of chromosomal fragments and/or EC DNA released. Several species, such as Anabaena variabilis, Anabaena dolium, Fremeylea diplophion, and Nostoc muscorum, were completely lysed under the conditions used. Disruption of the remaining species was aided by the modification of Witholt et al. (17), in which a mild osmotic shock is applied after the addition of lysozyme, but even under these conditions total lysis was never achieved. Two species, Calothrix desertica and Gloeotrichia, were not lysed under any of the mild conditions used in this study and were not further characterized.

Figure 1 shows a typical electrophoretic separation of EC DNA isolated by the method of Myers et al. (10). Preparations from all species examined showed a band of ethidium bromide-staining material (DNA) which migrated with a mobility of approximately 0.33 relative to the dye front. Often a diffuse band of DNA extended from this band to the dye marker. These bands were due to the presence of chromosomal fragments in the preparation and are similar to those in the gels of Myers et al. (10). The chromosomal bands were present for all species examined and showed no change in electrophoretic mobility when run in gels with various concentrations of ethidium bromide, a condition in which the mobility of CCC DNA should change (1, 7). The amount of chromosomal fragments can be greatly decreased if care is taken in handling (pouring and mixing) the preparation at stages before removal of the membrane-bound chromosomal DNA.

Over half of the preparations examined contained narrow ethidium bromide-staining bands in addition to the chromosomal band. Table 2 lists the number of such bands for each species. The number and position of these bands were strain specific, and showed little variation between preparations even though cultures of different ages with varying growth rates were analyzed.

Separation of the isolated EC DNA by electrophoresis in agarose gels before and after treatments known to nick CCC DNA (8, 14) and in agarose gels containing increasing concentrations of ethidium bromide reveals that these additional bands are composed of right-hand-supertwisted DNA (5, 13).

It is possible to determine the molecular weight of an EC DNA element by comparing its electrophoretic mobility with that of CCC DNA of known size from characterized strains of Esch-
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Figure 2 shows a relationship be-

tween relative electrophoretic mobility and

the molecular weight for a set of plasmids isolated

from strains of E. coli which is similar to that

shown for a different set of plasmids by Myers

et al. (10). By using Fig. 2 as a standard curve,

the molecular weights of all but one of the

cyanobacterial EC CCC DNA elements

determined (Table 2). The molecular weight

of the small CCC DNA from Phormidium luridum

could not be determined because it migrated

ahead of the smallest standard.

The present study defines the minimum
distribution of CCC DNA in filamentous

cyanobacteria. Seven of 13 species examined contained

CCC DNA, and all but 1 contained multiple

elements. The presence of more than one type

of CCC DNA per cell is apparently a common

phenomenon in cyanobacteria. Characterization

of the relationships between the multiple forms

of CCC DNA within a single species will require

further analysis. However, from a comparison of

the molecular weight of each of the multiple

types, it is unlikely they are multimers of a single

CCC DNA form. The same argument has been

made for the five species of CCC DNA found in

Agmenellum quadruplicatum (11).

The smallest CCC DNA seen was less than

2.1 \times 10^6 daltons, whereas the largest was

approximately 74 \times 10^6 daltons. Over one-half

of the CCC DNAs found were in the range of 24

\times 10^6 to 48 \times 10^6 daltons. The error in determin-
ing the molecular weight is approximately 15%

and results from the facts that the absolute

position of a band on a gel is not independent of

the amount of DNA loaded on the gel and that

small errors in measuring the position of the

DNA-containing band on the gel may result in

large differences in the molecular weight because

electrophoretic mobility is a logarithmic func-
tion of molecular weight (10).

In assessing the results reported here, one

should place emphasis on the identification of

strains containing CCC DNA rather than on the

subjected to electrophoresis at room temperature for

3 h at 2.0 V/cm. The gel slab was then removed from

the apparatus and stained in the dark with a solution

of ethidium bromide (1 \mu g/ml) for 1 h. Fluorescent

DNA-ethidium bromide complexes were then ob-
served by transmitted UV light, and the positions of

bands on the gel were either measured directly or

photographed through a Wratten no. 25 filter by

using either Polaird positive type 107 film or Pola-
r oid positive/negative type 665 film. “O” indicates

the starting origin for electrophoresis, and “C” indicates

the separated band of chromosomal DNA fragments.

Arrowheads (<) indicate the positions of plasmid

DNA.
Table 2. EC DNA in selected strains of cyanobacteria

<table>
<thead>
<tr>
<th>Family</th>
<th>Species</th>
<th>EC DNA (no.)</th>
<th>Mol wt* (×10^6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chroococcales</td>
<td>Anacystis nidulans</td>
<td>2</td>
<td>4.7, 37</td>
</tr>
<tr>
<td>Oscillatoriales</td>
<td>Phormidium luridum</td>
<td>2</td>
<td>&lt;2.1, 9.0</td>
</tr>
<tr>
<td></td>
<td>Plectonema boryanum</td>
<td>1</td>
<td>9.4</td>
</tr>
<tr>
<td>Nostocae</td>
<td>Anabaena cylindrica</td>
<td>5</td>
<td>3.4, 24, 40, 53, 74</td>
</tr>
<tr>
<td></td>
<td>A. doliium</td>
<td>3</td>
<td>5.7, 26, 36</td>
</tr>
<tr>
<td></td>
<td>A. variabilis</td>
<td>2</td>
<td>25, 32</td>
</tr>
<tr>
<td></td>
<td>Cylindrosporum sp.</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C. licheniforme</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nodularia sphaerocarpa</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nostoc muscorum</td>
<td>4</td>
<td>3.3, 33, 42, 74</td>
</tr>
<tr>
<td>Scytonemataceae</td>
<td>Fremyella diploisiphon</td>
<td>4</td>
<td>24, 32, 40, 48</td>
</tr>
<tr>
<td></td>
<td>Tolypothrix tenuis</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Stigonemataceae</td>
<td>Fischerella sp.</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>F. musicola</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Rivulariacae</td>
<td>Calothrix desertica</td>
<td>IL,</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gloeotrichia sp.</td>
<td>IL</td>
<td></td>
</tr>
</tbody>
</table>

* The total number of CCC forms in each strain is given. No forms other than CCC were observed.
* The molecular weight was determined by comparing the electrophoretic mobility of the EC DNA with the mobility of standard plasmids shown in Fig. 2. The error for each weight as explained in the text is ±15%.
* IL, Insufficient lysis.

The absence of such DNA in other strains. At least a 0.2-ml packed-cell volume was used for each analysis. Simple calculations (not shown) suggest that this amount of cell material should provide 80 to 320 times the EC DNA required to obtain a visible band on an agarose slab gel for CCC DNA present at one copy per genome copy.

This study only defines the minimum number of CCC DNA types actually present in a strain. It is possible that certain classes of EC DNA were not recovered. Thus, in Anagenium quadruplicatum five size classes of CCC DNA can be seen if preparations of cesium chloride-ethidium bromide-purified CCC DNA are spread by the Kleinschmidt technique (8) of electron microscopy, whereas only three of the size classes can be seen upon agarose gel electrophoresis of the same preparations (11).

There are no correlations between the presence of CCC DNA and either the range of metabolic activities found in filamentous cyanobacteria or the differentiated cell types and structures which can be produced by certain species.

**Fig. 2.** Relative electrophoretic mobility of plasmids of known molecular weight isolated from strains of E. coli grown in L-broth (16). Where the plasmid marker of interest coded for drug resistance, anti-biotics were added to the growth medium. Cleared lysates of all bacteria were prepared and subjected to electrophoresis as described in the legend to Fig. 1. Mobility was measured relative to that of the plasmid pVHS1. The plasmids and their E. coli strains included: pVHS1 from strain C600, monomer (m) = 2.2 × 10^6 daltons, dimer (d) = 4.4 × 10^6 daltons; pSC101 from strain C600, 5.8 × 10^6 daltons; pMB9 from strain HB129, dimer (d) = 7.3 × 10^6 daltons; Sa from strain J53, 25 × 10^6 daltons; RK2 from strain J53, 40 × 10^6 daltons; RP4 from strain J53, 40 × 10^6 daltons; and R64 from strain J55, 72 × 10^6 daltons.
The forms did not show any extrachromosomal elements.

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LITERATURE CITED


