Transposon mutagenesis of Nostoc sp. strain ATCC 29133, a filamentous cyanobacterium with multiple cellular differentiation alternatives

Michael F. Cohen, James G. Wallis, Elsie L. Campbell and John C. Meeks

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Nostoc sp. strain ATCC 29133 (PCC 73102; Nostoc 29133) is a symbiotically-competent, facultatively heterotrophic, diazotrophic cyanobacterium with the capacity to differentiate specialized cells such as heterocysts, akinetes and hormogonial filaments. We have optimized several methods for physiological and molecular genetic analysis of Nostoc 29133. By use of a Tn5 derivative, Tn5-1063 (Kmr Bmr Smr), delivered by conjugation from Escherichia coli, antibiotic-resistant mutants of Nostoc 29133 were generated at a frequency of approximately 1 x 10^-4, 0.4% of which expressed a nitrogen fixation (heterocyst) defective phenotype. Mutant strain UCD 328 was isolated after co-culture of 86 Nostoc 29133::Tn5-1063 clones with the symbiotic plant partner, Anthoceros punctatus; strain UCD 328 expressed a symbiotic phenotype of increased frequency of hormogonia-dependent infection. The transposon and flanking genomic DNA was recovered from strain UCD 328, the mutation and phenotype reconstructed by homologous recombination in Nostoc 29133, and the transposition site identified from a Nostoc 29133 genomic library. Transposon mutagenesis has thus provided the means for isolation and identification of developmental and symbiotic-specific genes of Nostoc 29133.

Keywords: Nostoc sp. ATCC 29133, diazotrophic cyanobacterium, symbiotic cyanobacterium, transposon mutagenesis, homologous recombination

INTRODUCTION

Cyanobacteria are a morphologically diverse group of eubacteria, united by their characteristic oxygenic photosynthetic mode of growth. In certain filamentous cyanobacterial genera classified in Sections IV and V (sensu Rippka et al., 1979), such as Nostoc, vegetative cells can mature in four developmental directions, depending upon the environmental growth conditions. First, they can divide upon reaching a critical size and perpetuate the vegetative growth cycle. Second, the vegetative cells can divide uncoupled from biomass increase or DNA replication to form transient hormogonial filaments (Damerval et al., 1991). Hormogonia formation can be induced by environmental conditions which include light quality and excess nutrients (Herdman & Rippka, 1988). Third, a few or the entire population of vegetative cells can differentiate into akinetes (spores) which function in survival under environmental extremes (Herdman, 1987). A variety of environmental conditions, including nutrient (especially phosphate) deprivation, and autogenic production of a sporulation factor (Hirosawa & Wolk, 1979), stimulate their formation. Fourth, under conditions of combined nitrogen deprivation a small fraction (3-10%) of the vegetative cells will, in a defined spatial pattern along the filaments, differentiate into heterocysts which are specialized for nitrogen fixation in an oxic environment (Wolk, 1982). Thus, these filamentous cyanobacteria provide an experimental system for the study of how cells perceive, integrate and respond to environmental signals in the context of differentiation processes.

Techniques have been developed for genetic analysis of heterocyst differentiation and function in Anabaena sp.
### Table 1. Bacterial strains and plasmids

<table>
<thead>
<tr>
<th>Strain or plasmid</th>
<th>Relevant characteristics*</th>
<th>Reference(s)</th>
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<tbody>
<tr>
<td><em>E. coli</em></td>
<td></td>
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<tr>
<td>DH5α</td>
<td><em>recA1 hsdR17 (rK mK) endA1</em></td>
<td>Grant et al. (1990)</td>
</tr>
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<td>DH5α-MCR</td>
<td>Genotype of DH5α and <em>merA</em> Δ(<em>merA hsdMRS merBC</em>)</td>
<td>Grant et al. (1990)</td>
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<tr>
<td>HB101</td>
<td><em>recA1 hsdS20 (rK mK)</em></td>
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<td><em>Nostoc</em></td>
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<tr>
<td>ATCC 29133 (PCC 73102)</td>
<td>Fix⁺ Sym⁺</td>
<td>Rippka et al. (1979); Enderlin &amp; Meeks (1983)</td>
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<tr>
<td>UCD 7801</td>
<td>Fix⁺ Sym⁺</td>
<td>Enderlin &amp; Meeks (1985)</td>
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<tr>
<td>UCD 328</td>
<td>Superinfective <em>Nostoc</em> 29133::Tn5-1063 strain, Fix⁺</td>
<td>This study</td>
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<tr>
<td>UCD 339</td>
<td>pSCR3 integrated by single recombination in the genome</td>
<td>This study</td>
</tr>
<tr>
<td>UCD 340</td>
<td>Reconstruction of the UCD 328 mutation</td>
<td>This study</td>
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<tr>
<td><strong>Plasmids</strong></td>
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<tr>
<td>pDUCA7</td>
<td>Cosmid shuttle vector with RK2 and pDU1 oriV's, Km⁺</td>
<td>Buikema &amp; Haselkorn (1991)</td>
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<tr>
<td>pRK212.1</td>
<td>RK2 Mu phage insertion/deletion derivative, Km⁺</td>
<td>Figurski et al. (1976)</td>
</tr>
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<td>pRK2013</td>
<td>RK2 derivative with ColE1 oriV', Km⁺</td>
<td>Figurski &amp; Helinski (1979)</td>
</tr>
<tr>
<td>pRK2073</td>
<td>pRK2013::Tn7, Km⁺ Sp⁺</td>
<td>Better &amp; Helinski (1983)</td>
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<tr>
<td>pRL6</td>
<td>Shuttle vector with pMB1 and pDU1 oriV's</td>
<td>Wolk et al. (1984)</td>
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<td>pRL479</td>
<td>Recovered 19 kb <em>EcoRV</em> Tn5-1063 bearing fragment from UCD 328, Bm⁺ Km⁺ Sm⁺</td>
<td>Elhai &amp; Wolk (1988b)</td>
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<td>pRL1063a</td>
<td>Tn5-1063, RK2 oriT, Bm⁺ Km⁺ Sm⁺</td>
<td>Wolk et al. (1991)</td>
</tr>
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<td>pRL1075</td>
<td>Used for reconstructing Tn5-1063 insertions, Cm⁺ Em⁺ Suc⁺</td>
<td>Black et al. (1993)</td>
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<td>pSCR1 with NotI site filled in, Bm⁺ Km⁺ Sm⁺</td>
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<td>pSCR3</td>
<td>pSCR2 with positive selection cassette from pRL1075, Bm⁺ Cm⁺ Em⁺ Km⁺ Sm⁺ Suc⁺</td>
<td>This study</td>
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<td>pSCR4</td>
<td>Subclone of 11 kb <em>EcoRV</em> fragment into pRL479</td>
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<td>pSCR5</td>
<td>Subclone of 6.2 kb SpeI fragment into pUC18</td>
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<tr>
<td>pUC18</td>
<td>pBR322-derived vector</td>
<td>Vieira &amp; Messing (1982)</td>
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strain PCC 7120 (Buikema & Haselkorn, 1993; Wolk, 1991). However, *Anabaena* 7120 has limitations as an experimental system in developmental processes since it does not differentiate hormogonia or akinetes (Rippka et al., 1979).

*Nostoc* sp. strain ATCC 29133 (PCC 73102; *Nostoc* 29133) displays all of the cellular differentiation processes described above (Rippka et al., 1979). Unlike *Anabaena* 7120, *Nostoc* 29133 is also capable of photosynthetic and dark-heterotrophic growth (Rippka et al., 1979). Moreover, *Nostoc* 29133 was originally isolated from symbiotic association with the cycad *Macrozamia* sp. (Rippka et al., 1979) and, via infection by hormogonia, can reconstitute a symbiotic...
association with the bryophyte *Anthoceros punctatus* (Campbell & Meeks, 1989; Enderlin & Meeks, 1983) and the angiosperm *Gunnera manicata* (Johansson & Bergman, 1994). When in association with *A. punctatus*, *Nostoc* spp. differentiate heterocysts at a frequency of about 45% of the total cells (Enderlin & Meeks, 1983; Meeks, 1990), fix N₂ under photo- and dark-heterotrophic conditions at a rate that is at least three- to fivefold higher than free-living cultures (Steinberg & Meeks, 1991) and release approximately 80% of the fixed nitrogen as ammonium for growth of the associated eukaryotic tissue (Meeks et al., 1985).

We are interested in the interactions between *Nostoc* spp. and *Anthoceros punctatus* that regulate symbiotic cyanobacterial gene expression, enzyme activity and cellular differentiation, and whether such response systems differ from those operative in the free-living growth state. A screen to identify mutants with altered symbiotic competence, for which there is no positive selection, requires a population composed only of clones already known to harbour a mutation. Transposon mutagenesis has an advantage over chemical and UV mutagenesis since it was packaged into *E. coli* DH5α-MCR. Restriction analysis of 23 randomly chosen clones showed an average insert size of 28 kb. A total of 2112 clones were precipitated with 0.6 vol. of 2-propanol followed by centrifugation at 12000g for 10 min. The pellet was washed before, washed twice with 5 M NaCl and then resuspended in 0.5 ml TE and extracted one to three times with phenol/ chloroform as above. Nucleic acids in the final aqueous phase were precipitated with ethanol. The pellet was resuspended in 0.5 ml TE and extracted one to three times with phenol/ chloroform until the interface was clear. The aqueous phase was precipitated and the pellet washed once with 70% (v/v) ethanol and dried under vacuum. The resulting pellet was resuspended in 50 μl TE containing 1 μM RNase T₁ (Bethesda Research Laboratories).

Plasmids were isolated from 50 ml liquid cultures of *Nostoc* 29133 grown under N₃ selection and having Chl a concentrations of approximately 5 μg ml⁻¹. *Nostoc* cells were harvested as before, washed twice with 5 M NaCl and then resuspended in 2 ml TE. Lysozyme was added to a final concentration of 5 mg ml⁻¹. After mixing, the tube was incubated with gentle shaking for 2 h at 37 °C then Triton X-100 (0.5 ml of a 20%, v/v, solution) was added to lyse the cells. The resulting solution was made up to a concentration of 1 M NaCl by the addition of cold 5 M NaCl and the suspension was stored overnight at 4 °C. The suspension was then centrifuged at 12000g for 10 min and the supernatant was extracted with phenol/ chloroform as above. Nucleic acids in the final aqueous phase were precipitated with 0.6 vol. of 2-propanol followed by centrifugation at 12000g for 10 min. The pellet was washed twice with 70% ethanol, vacuum dried, and resuspended in glass-distilled water.

**METHODS**

**Cultures and media.** The bacterial strains and plasmids used in this study are listed in Table 1. *Escherichia coli* strains were routinely cultured in Luria-Bertani (LB) broth (Sambrook et al., 1989) and cell concentrations determined by measuring the optical density of cultures at 600 nm (OD₆₀₀). Sources and culture conditions for sibroant-free and *Nostoc*-associated *Anthoceros punctatus*, and all free-living *Nostoc* strains, were described previously (Enderlin & Meeks, 1983). The basal medium for growth of *Nostoc* was that of Allen & Amon (1955) (AA) supplemented with 5 mM MOPS, pH 7.8 (−N); and, when specified, 25 mM NH₄Cl (+N); it was used at full strength when solidified with 1% (w/v) agar (purified using the methods of Bruat & Wood, 1962) or diluted fourfold in liquid culture (AA/4). Chlorophyll a (Chl a) in methanolic extracts was quantified as described previously (Meeks et al., 1983). To select for resistance conferred by the npt gene, kanamycin (Km; 12.5 or 25 μg ml⁻¹) was used for *E. coli* and neomycin (Nm; 10 μg ml⁻¹) was used for *Nostoc* 29133.

**DNA isolations and manipulations.** Small-scale preparations of plasmid DNA from *E. coli* were done using standard methods. Large-scale plasmid purifications from *E. coli* were carried out using a commercial kit (Qiagen). DNA restriction enzymes were obtained from New England Biolabs. Hybridization of radiolabelled probes to DNA immobilized on nylon membranes (Gene Screen Plus; Dupont NEN Products) was done as described by Sambrook et al. (1989) using 50% (v/v) formamide.
genesis of Anabaena 7120 (Wolk et al., 1991) was adapted for use with Nostoc 29133. Tri-parental plate matings were conducted on sterile conjugation filters (Millipore HATF 082) lying on agar plates containing AA+N + 0.5% (v/v) LB broth. In preparation for conjugation, Nostoc filaments were fragmented by sonic cavitation to a length of four to six cells per filament, allowed to recover by incubation under growth conditions for at least 6 h in 50 ml AA/4+N and were concentrated to approximately 75 µg Chl a ml⁻¹ immediately before mating. E. coli was prepared for mating by combining in a centrifuge tube equal numbers of the donor strain (DH5α containing the transposon-bearing plasmid pRL1063a) and the conjugal strain (HB101 containing the conjugal plasmid pRK2013) both grown at 28 °C and low light intensity (1.5 W m⁻²) in air plus 1 ml⁻¹ N₂ gas for 24-26 h to allow for conjugal mating. To allow the expression of antibiotic resistance, the filters were incubated in selective media, and colonies counted after 2 weeks. In addition to the three developmental alternatives, heterotrophic growth and the ability to establish a symbiotic association with A. punctatus, two other considerations influenced our choice of Nostoc 29133 as an experimental strain. First, Nostoc 29133 is reported to have no type II endonuclease activity (Lambert & Carr, 1984; Shestakov & Reaston, 1987). This deficiency is an advantage when attempting to transfer DNA into the strain, obviating the in vivo methylation used in Anabaena strains (Elhai & Wolk, 1988b). Second, the lack of endogenous plasmids in Nostoc 29133 (Houmand & Tandeau de Marsac, 1988) eases the identification and recovery of vector plasmids introduced into the strain.

**RESULTS AND DISCUSSION**

**Nostoc 29133 as an experimental strain**

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**Conjugation procedures**

Initial attempts at introducing the transposon Tn*5*-1063 into *Nostoc 29133*, using the conjugation protocol designed for use in *Anabaena 7120* (Elhai & Wolk, 1988b), yielded only one mutant out of three filter matings. Moreover, conjugation frequencies of only about 5 x 10⁻⁷ were observed using this same protocol to introduce a pDU1-based shuttle vector, pRL6, into *Nostoc 29133* (Wallis, 1993). When *Nostoc 29133* cell suspensions were mixed with *E. coli* suspensions at the concentration used with *Anabaena 7120* (Elhai & Wolk, 1988b), the *Nostoc 29133* bleached rapidly on non-selective medium; *Nostoc 29133* by itself did not bleach. For conjugal transfer of pRL1063a, an approximately 20-fold reduction in the reported *E. coli* concentration gave the highest frequency of resultant *Nostoc 29133::Tn*5*-1063* colonies. Control matings transferring pDUCA7 from *E. coli* donors to *E. coli* recipients showed that exponential cultures of *E. coli*...
mated at a nearly 10-fold higher frequency than stationary-phase cultures (data not shown); the growth phase of *Nostoc* 29133 had no influence on the frequency of conjugation (Wallis, 1993). Therefore, for conjugal matings between *Nostoc* 29133 and *E. coli*, exponential-phase *E. coli* was used and the cells plated immediately after mixing.

An increase in conjugation frequency was also obtained by use of a different conjugal plasmid. The plasmid, pRK2013, contains the conjugal transfer functions of RK2 inserted into a derivative of plasmid CoEl1, and is dependent on the CoEl1 origin of replication (Figurski & Helinski, 1979). Control mating experiments between *E. coli* strains demonstrated that pDUCA7 was mobilized 50-fold more effectively by pRK2073, a Km<sup>a</sup> derivative of pRK2013, than by plasmids pRL443 and pRK212.1, which use the RK2/RP4 origin of replication (data not shown). When *Nostoc* 29133 was the recipient, mating via pRK2073 yielded a frequency of exconjugants per viable c.f.u. (5·5 × 10<sup>-4</sup> Km<sup>a</sup> per c.f.u.) that was at least 50-fold higher than the frequency using pRL443 (<9·1 × 10<sup>-6</sup> Km<sup>a</sup> per c.f.u.). The improved mobilization may be explained by the higher copy number of pRK2073 compared to the RK2/RP4-based conjugal plasmids, perhaps producing more of the proteins necessary for the conjugation process. Compatibility between the CoEl1 and the RK2/RP4 origins of replication allows both the conjugal plasmid and the mobilized vector plasmid to reside in the same *E. coli* cell, which may also contribute to the observed increase in the frequency of transfer.

### *Nostoc* 29133 DNA isolation and library construction

To obtain DNA for the construction of a genomic library and for the recovery of Tn5-1063 transposition sites, a method of total DNA isolation from *Nostoc* 29133 was devised that reliably gave high yields. The major modification of the DNA isolation method of Ausubel *et al.* (1987) was the introduction of two preliminary washes with 5 M NaCl to remove *Nostoc* exopolysaccharides, which appear to inhibit the extent of cell lysis in subsequent steps. The exopolysaccharide content of the starting cellular material can be reduced by harvesting dilute cultures (<5 μg Chl a ml<sup>-1</sup>) grown in ammonium-supplemented medium. This method routinely yielded approximately 1 μg DNA per 2 μg Chl a, and has been applied to some other *Nostoc* strains with similar success (Wallis, 1993).

Before introducing *Nostoc* 29133 DNA into *E. coli*, restriction of the DNA by the methylation-dependent restriction systems (MDRS) which are present in many *E. coli* K 12 strains was examined; MDRS restrict DNA introduced into *E. coli* cells only if that DNA is methylated at specific sequences (Raleigh, 1992). A method for isolating plasmid DNA from *Nostoc* 29133 was necessary to perform this experiment. Several published methods specific to cyanobacteria (Felkner & Barnum, 1988; Potts, 1989; Stutzman & Gendel, 1985) failed to yield usable DNA, principally because few *Nostoc* 29133 cells were lysed. As with the total DNA isolation protocol, preliminary 5 M NaCl washes in the plasmid preparation protocol improved subsequent cell lysis. This protocol yielded 17 μg pDUCA7 DNA per μg Chl a.

For a comparison of transformation efficiencies, *E. coli* strains DH5<sup>x</sup> and DH5<sup>x</sup>-MCR were transformed with vector pDUCA7 isolated from *Nostoc* 29133. These *E. coli* strains are isogenic except that the loci coding for the MDRS have been deleted from strain DH5<sup>x</sup>-MCR (Grant *et al*., 1990). In control experiments plasmid DNA isolated from *E. coli* HB101 transformed the two DH5<sup>x</sup> strains at nearly equal efficiencies. However, plasmid DNA isolated from *Nostoc* 29133 transformed DH5<sup>x</sup>-MCR with at least a 200-fold greater frequency than it transformed DH5<sup>x</sup> (Table 2). To bypass this apparent restriction barrier, a *Nostoc* 29133 genomic DNA library, constructed in pDUCA7 and packaged in λ phage, was transfected into *E. coli* strain DH5<sup>x</sup>-MCR. This genomic library has served as a source for wild-type DNA corresponding to the transposition sites of all *Nostoc* 29133::Tn5-1063 mutants so far recovered from the genome (unpublished observations), including that of strain UCD 328 discussed below.

### Mutagenesis and mutant phenotypes

Tri-parental filter matings using the conjugal plasmid pRK2013 to introduce Tn5-1063 into *Nostoc* 29133 were conducted on AA+N to allow the growth of any resulting nitrogen fixation and nitrate assimilation mutants. The procedure resulted in a frequency of 1·0 ppm (±0·8) × 10<sup>-6</sup> [mean (±sd), n = 7] *Nostoc* 29133::Tn5-1063 colonies per viable *Nostoc* cell in the mating mixture (based on a value of 1·75 × 10<sup>-13</sup> g Chl a per viable cell). The yield of resultant colonies per filter is about five times lower than that reported for Tn5-1063 mutagenesis of *Anabaena* 7120 (Wolk *et al*., 1991).

Of 5·1 × 10<sup>8</sup> mutant colonies screened, 14 Fox<sup>−</sup> mutants, all with discernible heterocyst defects, were identified. Two Fox<sup>−</sup> strains had a fragmented, short-filament phenotype in the presence or absence of combined nitrogen. Symbiotic competence was retained in four Fox<sup>−</sup> strains; it has been shown previously that for strain UCD 223, a *Nostoc* 29133 Fox<sup>−</sup> mutant generated by chemical mutagenesis, the microaerobic symbiotic cavities

<p>| Table 2. Effect of methylation dependent restriction systems on transformation frequency |
|-------------------------------------|------------------|------------------|------------------|</p>
<table>
<thead>
<tr>
<th>Source of pDUCA7</th>
<th><em>E. coli</em> strain</th>
<th>Km&lt;sup&gt;a&lt;/sup&gt; colonies</th>
<th>Ratio DH5&lt;sup&gt;x&lt;/sup&gt;/DH5&lt;sup&gt;x&lt;/sup&gt;-MCR</th>
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<td>pDUCA7</td>
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<tr>
<td></td>
<td>DH5&lt;sup&gt;x&lt;/sup&gt;</td>
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<td>7·9 × 10&lt;sup&gt;-2&lt;/sup&gt;</td>
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<td><em>Nostoc</em> 29133</td>
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<td>&lt;1·0 × 10&lt;sup&gt;-5&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DH5&lt;sup&gt;x&lt;/sup&gt;-MCR</td>
<td>0·2 × 10&lt;sup&gt;-2&lt;/sup&gt;</td>
<td>&lt;0·005</td>
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of Anthoceros punctatus can replace the physiological function of the heterocyst envelope (Campbell & Meeks, 1992). The heterocyst envelope polysaccharide of two Anabaena strains is apparently the same as that found in their respective akinetes (Cardemil & Wolk, 1976, 1981). We examined the akinetes of Fox- Nostoc 29133::Tn5-1063 strains which had microscopically observable defects in their heterocyst envelopes, but found no corresponding defects in the continuity of their akinete envelopes.

The promoterless bacterial luciferase genes, luxAB, of Tn5-1063 can form transcriptional fusions to the Nostoc 29133 genome upon transposition. To screen for transpositions within transcribed regions of the genome, several filters containing mutant colonies were assayed for luciferase activity by exposure to X-ray film. Light emission was detected from 30% (313/1063) of the mutant colonies when grown on minimal medium with or without NH4+. The 14 Fox- mutant colonies were separately screened for changes in luciferase activity after 2 d of ammonium deprivation; one group of seven mutants showed increased levels of expression, a second group of five mutants showed no change and a third group of two mutants showed a decrease.

Of 86 randomly chosen Fix+ Nostoc 29133::Tn5-1063 strains tested for symbiotic competence, one (strain UCD 328) was found to have a high initial frequency of infection. After 2 weeks of co-culture, strain UCD 328 infected Anthoceros punctatus at about a 50-fold higher frequency than the parental Nostoc 29133 strain and fivefold higher than Nostoc sp. strain UCD 7801 (Fig. 1), our original isolate from A. punctatus (Enderlin & Meeks, 1983). Strain UCD 328 was found to be more sensitive than wild-type Nostoc 29133 to a hormogonia-inducing factor in A. punctatus growth-conditioned medium (M. F. Cohen & J. C. Meeks, unpublished). Furthermore, unlike other Nostoc strains, which may grow to a high density in the medium surrounding the plant (Enderlin & Meeks, 1983), epiphytic growth of strain UCD 328 was severely reduced; strain UCD 328 showed no obvious growth defect in the free-living state.

Reconstruction of the strain UCD 328 mutation

Southern blots of total EcoRV-digested DNA from strain UCD 328, probed with HincII-digested pRL1063a, showed a single 19 kb band of hybridizing DNA (data not shown), implying that the genome bears only one copy of the transposon. The mutation was reconstructed by insertion mutagenesis to confirm that the phenotype of strain UCD 328 was a result of the Tn5-1063 insertion. The positive selection plasmid, pSCR3, containing Tn5-1063 with an inactivated transposase gene in a 6.2 kb Nostoc 29133 SpeI fragment (Fig. 2), was introduced into Nostoc 29133 by conjugation. Single recombination of pSCR3 into the Nostoc 29133 genome gave rise to a Nmr Emr Sucs phenotype. A second recombination on the opposite side of the transposon resulted in a Nmr Emr Sucs' phenotype and was selected by plating on sucrose-containing medium (Cai & Wolk, 1990). Southern analysis confirmed single recombination (strain UCD 339) and double recombination (strain UCD 340) of pSCR3 at the same locus as the transposition site in strain UCD 328 (Fig. 3). Strain UCD 340 was co-cultured with Anthoceros punctatus and found to have an initial infection frequency near that of strain UCD 328 (Fig. 1). A 2 kb fragment of DNA flanking the transposition site of strain UCD 328
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**Fig. 3.** Southern analysis of *Nostoc* 29133 strains. Markers indicate size of DNA in kb. A blot of total DNA (3-4 μg per lane) digested with *NheI* from wild-type *Nostoc* ATCC 29133, *Nostoc* 29133::*Tn5-1063* (strain UCD 339); and a *Nostoc* 29133::pSCR3 double recombinant (strain UCD 340) was probed with a 660 bp *EagI--NheI* fragment from pSCR1 having homology both to the transposon ends and to the *Nostoc* DNA flanking the right side of the transposon (Fig. 2). The uninterrupted 2.2 kb band has 540 bp of direct homology to the probe. Two bands, each containing a transposon end with flanking DNA, can be seen in strains UCD 328 and UCD 340; the 1.5 kb and the 2.6 kb bands respectively have 660 bp and 53 bp of direct homology to the probe. Strain UCD 339 contains both a wild type 2.2 kb band and the two bands resulting from the transposon insertion.

hybridized strongly to three cosmid clones out of 2112 clones from the genomic library. Southern analysis revealed the hybridizing stretch of DNA to be on an 11 kb *EcoRV* fragment common to all three cosmids. Restriction mapping of a 6.2 kb *SpeI* fragment (Fig. 2), internal to the 11 kb *EcoRV* fragment, showed it to be identical to the *Nostoc* DNA flanking the transposition site in pSCR3.

This study demonstrates that transposon and insertion mutants of *Nostoc* 29133 can be generated. Such mutants will be useful in investigations of its developmental alternatives and its symbiotic interactions with plant tissues. We are currently characterizing the physiology and molecular genetics of strain UCD 328.

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