Distribution of Multicopy Single-Stranded DNA among Myxobacteria and Related Species

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Multicopy single-stranded DNA (msDNA) is a short single-stranded linear DNA originally discovered in Myxococcus xanthus and subsequently found in Stigmatella aurantiaca. It exists at an estimated 500 to 700 copies per chromosome (T. Yee, T. Furuichi, S. Inouye, and M. Inouye, Cell 38:203-209, 1984). We found msDNA in other myxobacteria, including Myxococcus coralloides, Cystobacter violaceus, Cystobacter ferrugineus (Cbf17), Nannocystis exedens, and nine independently isolated strains of M. xanthus. The presence of msDNA in N. exedens would extend its phylogenetic distribution into another family of myxobacteria. Flexibacter elegans, a Cytobapha-like gliding bacteria which may be even more distinctly related, also contained an msDNA but at a much lower copy number. msDNA was not detected in closely related strains of the myxobacteria Cystobacter fuscus and C. ferrugineus (Cbf16 and Cbf18) and the more distantly related eu细菌 Herpetosiphon giganteus, Taxeobacter ocellatus, Lysobacter antibiotics, Lysobacter enzymogenes, Cytobapha johnsonae, Rhodopseudomonas sphaeroides, and Rhodospirillum rubrum. Thus far, msDNA has been found in certain gliding bacteria but not in others.

Myxobacteria are gram-negative bacteria that can be found in soil or on decaying organic matter (2). Upon starvation, cells aggregate and form fruiting bodies. Because of this unique behavior, myxobacteria systems have been studied as models for differentiation (for a review, see reference 7). In particular, various aspects of Myxococcus xanthus development have been studied extensively (7). Recently, we have found that M. xanthus contains 500 to 700 copies of a multicopy single-stranded DNA (msDNA) per chromosome (9). This msDNA, which includes 163 bases of single-stranded DNA and at least one ribonucleotide (adenosine) on its 5' end, was found to hybridize with a unique chromosomal region. The DNA sequence of msDNA and the unique chromosomal region (msd) has been determined and found to have exact sequence correspondence. Examination of this sequence shows that msDNA has an extensive secondary structure (9). An msDNA was also found in another myxobacterium, Stigmatella aurantiaca, whereas various other gram-negative and gram-positive bacteria unrelated to myxobacteria did not contain msDNA (9).

In the present report, we examined how widely msDNA exists in various bacteria closely and distantly related to M. xanthus. We first tested eight strains of M. xanthus independently isolated from various regions of the United States, one strain of M. xanthus from the Fiji Islands, one strain of Myxococcus coralloides, together with M. xanthus DZF1, and S. aurantiaca (Table 1). The existence of msDNA was determined by subjecting a total DNA preparation to electrophoresis on a 5% acrylamide gel followed by ethidium bromide staining as described previously (9). All of these strains had an msDNA (Fig. 1). As shown previously (9), the msDNA in S. aurantiaca (lane 3) migrates slightly more slowly than the other msDNAs, indicating a structural variance among these DNAs.

To test for homology between M. xanthus DZF1 msDNA and the other msDNAs, Southern blot analysis was done (8) by using a clone of the M. xanthus msd region. DNA from these various strains was prepared (1), glyoxylated (4), electrophoresed on a 1.5% agarose gel, and transferred to a Biodyne A nylon membrane filter (SD 939; Pall Ultrafine Filtration Corp., Glen Cove, N.Y.) by the RNA transfer protocol of the manufacturer. A clone, pMgG3, which contains 0.7 kilobase of M. xanthus DZF1 chromosomal DNA including the msDNA chromosomal region (msd), was nick translated (6) and hybridized with this blot. Prehybridization was done (essentially as described in reference 4) in 50% (vol/vol) formamide-5× Denhardt solution-5× SSPE (1× SSPE is 0.18 M NaCl, 0.01 M sodium phosphate [pH 6.3], 0.001 M EDTA)-0.3% sodium dodecyl sulfate, and sonicated DNA at 42°C for 3 h. This was followed by replacement with 2× 10⁹ cpm of nick-translated probe per ml in fresh hybridization solution containing the same ingredients as described above and then by incubation overnight at 42°C. The incubation was followed by three washings at room temperature with 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)-0.1% sodium dodecyl sulfate and two washings at 50°C with 0.1× SSC-0.1% sodium dodecyl sulfate. Autoradiography was done overnight at −70°C with Kodak XAR-5 film. The efficiencies of hybridization with all of the M. xanthus strains (including DZF1) and M. coralloides were approximately equal (Fig. 2). However, hybridization with S. aurantiaca DWF4 was significantly less (lane 2), indicating that it is less homologous with the M. xanthus DZF1 msDNA than with any of the other msDNAs tested (Fig. 2). Recently, the chromosomal region from S. aurantiaca, which contains the msd region, has been cloned and sequenced (T. Furuichi, S. Inouye and M. Inouye, manuscript in preparation). The region which encodes the msDNA was found to be approximately 80% homologous with M. xanthus msDNA, which is consistent with the results of the hybridization experiments.

We searched for msDNA in 14 other species of myxobacteria and bacteria shown to be less related to M. xanthus (3, 5, 7; Table 1). These include myxobacteria within the same family (Cystobacteraceae) as M. xanthus and S. aurantiaca and a myxobacterium in another family (Sorangiaceae) but within the same order (Myxobacterales) (3). Examples of more distantly related bacteria were also...
### TABLE 1. Bacterial strains checked for msDNA

<table>
<thead>
<tr>
<th>Bacterial strain</th>
<th>Source*</th>
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<tbody>
<tr>
<td><strong>Myxococcus xanthus</strong></td>
<td></td>
</tr>
<tr>
<td>DZF1</td>
<td>Hans Reichenbach (HR2)</td>
</tr>
<tr>
<td>DK823</td>
<td>Hans Reichenbach (HR3)</td>
</tr>
<tr>
<td>DK829</td>
<td>Hans Reichenbach (HR1)</td>
</tr>
<tr>
<td>DK843</td>
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<td>DK862</td>
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<td>DK870</td>
<td>Hans Reichenbach (HR4)</td>
</tr>
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<td>DK898</td>
<td>Hans Reichenbach (HR1)</td>
</tr>
<tr>
<td><strong>Myxococcus coralloides</strong></td>
<td></td>
</tr>
<tr>
<td>DK817</td>
<td>Yosemite, Calif.; Dale Kaiser</td>
</tr>
<tr>
<td><strong>Stigmatella aurantiaca</strong></td>
<td></td>
</tr>
<tr>
<td>DW4</td>
<td>Minneapolis, Minn.; David White</td>
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<tr>
<td><strong>Cystobacter fuscus</strong></td>
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<tr>
<td>Cbf16</td>
<td>Hans Reichenbach (HR2)</td>
</tr>
<tr>
<td>Cbf15</td>
<td>Hans Reichenbach (HR3)</td>
</tr>
<tr>
<td><strong>Cystobacter violaceus</strong></td>
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</tr>
<tr>
<td>Cbf17</td>
<td>Hans Reichenbach (HR1)</td>
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<tr>
<td><strong>Cystobacter ferrugineus</strong></td>
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</tr>
<tr>
<td>Cbf610</td>
<td>Hans Reichenbach (HR1)</td>
</tr>
<tr>
<td>Cbf16</td>
<td>Hans Reichenbach (M1, HR1)</td>
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<td>Cbf18</td>
<td>Hans Reichenbach (HR2)</td>
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<td><strong>Nannocystis exedens</strong></td>
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<tr>
<td>Nae465</td>
<td>Hans Reichenbach (HR1)</td>
</tr>
<tr>
<td><strong>Herpetosiphon giganteus</strong></td>
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</tr>
<tr>
<td>Hpa2</td>
<td>Hans Reichenbach (HR1)</td>
</tr>
<tr>
<td>Hpg12</td>
<td>Hans Reichenbach (Herpetosiphon aurantiacus ATCC 23779)</td>
</tr>
<tr>
<td><strong>Taxeobacter ocellatus</strong></td>
<td></td>
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<tr>
<td>Txol</td>
<td>Hans Reichenbach (M1, HR1)</td>
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<tr>
<td><strong>Lysobacter antibioticus</strong></td>
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<td><strong>Lysobacter enzymogenes</strong></td>
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<tr>
<td>Lyel</td>
<td>Hans Reichenbach (495 from P. Christensen, Edmonton; ATCC 29487)</td>
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<tr>
<td><strong>Cytophaga johnsonae</strong></td>
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</tr>
<tr>
<td>Cyj1</td>
<td>Hans Reichenbach (NCIB 1050)</td>
</tr>
<tr>
<td><strong>Flexibacter elegans</strong></td>
<td></td>
</tr>
<tr>
<td>Fxe1</td>
<td>Hans Reichenbach (HR1; Cytophaga-like)</td>
</tr>
<tr>
<td><strong>Rhodopseudomonas sphaeroides</strong></td>
<td>Nonsulphur purple; Duane Yoch</td>
</tr>
<tr>
<td><strong>Rhodospirillum rubrum</strong></td>
<td></td>
</tr>
<tr>
<td>S1</td>
<td>Nonsulphur purple; Duane Yoch</td>
</tr>
</tbody>
</table>

* Alternative strain names are in parentheses.

Included (Table 1). Strains were harvested from liquid or solid medium depending on which yielded a higher density of cells. Analysis of *M. xanthus* DZF1 indicated that cells harvested from liquid or solid medium contain approximately the same amount of msDNA (data not shown). The total cellular DNA isolated from *M. xanthus* DZF1 is shown in Fig. 3, lane i. The band at the top of the lane is the chromosomal DNA, and approximately halfway down is the previously characterized msDNA (9; Fig. 1). There is also a faint band of RNase-resistant material farther down from the msDNA band. At present, the nature of this band is unknown except that it is assumed to be DNA. At the bottom of the lane, one can see incompletely degraded RNA. The following lanes in Fig. 3 contained preparations which had msDNAs: h, *Nannocystis exedens*; j, *Flexibacter elegans*; m, *Cystobacter violaceus*, and o, *Cystobacter ferrugineus*. All but one of these preparations had two bands present; *F. elegans* had only one faint band. It should be noted that if the cells had contained less than one-tenth the concentration of msDNA in these preparations, the msDNA would not have been detectable under the conditions used. We cannot conclude with certainty that these RNase-resistant bands were single-stranded DNA like msDNA. We have found that *M. xanthus* msDNA migrates more slowly in the absence of RNase treatment (unpublished data). Consistent with this, we have found that all of the msDNA-like species examined in this report migrate more slowly in the absence of RNase treatment (data not shown). Therefore, we believe that these previously unidentified DNAs are probably msDNAs.

Based on analysis of 16S rRNA, it has recently been shown that *S. aurantiaca* is closely related to *Myxococcus* species and can be reclassified within the same family of...
Cystobacteraceae (3). *M. xanthus, C. violaceus, and C. ferrugineus* are within this same family. *N. exedens* has been found to be more distantly related and placed in the family Sorangiaceae. The finding of msDNA in another family of myxobacteria indicates that msDNA may have originated in an ancestral myxobacteria. An msDNA is also present in *F. elegans*, which is a Cytophaga-like bacteria. Biochemical studies had originally supported a taxonomic scheme where the Cytophaga-like bacteria and myxobacteria form a common phylogenetic branch (7). Recent 16S rRNA studies indicate that there is little relationship between these groups (3). It is also interesting to note that some closely related myxobacteria did not have an msDNA. Only three strains of *C. ferrugineus* showed an msDNA (Fig. 3, lane o, strain Cbfel17, versus lanes n and p, strains Cbfel16 and Cbfel18).

We thank Dale Kaiser for *M. coralloides* and for the nine *M. xanthus* strains. David White for *S. aurantiaca*, Duane Yoch for *Rhodospseudomonas sphaeroides* and *Rhodospirillum rubrum*, and Hans Reichenbach for all the other myxobacteria and related bacteria.

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**LITERATURE CITED**


FIG. 1. Presence of msDNA in *M. coralloides*, *S. aurantiaca*, and various strains of *M. xanthus*. Total cellular DNA was purified and electrophoresed on a 5% acrylamide gel followed by staining with ethidium bromide. Lanes: 1, molecular weight standard (pBR322 digested with *HaeIII*); 2, *M. xanthus DZF1*; 3, *S. aurantiaca* DW4; 4, *M. coralloides* DK817; 5 through 13, *M. xanthus* DK818, DK823, DK829, DK843, DK851, DK853, DK862, DK870, and DK898, respectively. The arrow indicates the position of msDNA.

FIG. 2. Hybridization of the *M. xanthus DZF1* ms region with msDNA from *S. aurantiaca*, *M. coralloides*, and 10 strains of *M. xanthus*. pMG03, a plasmid containing 0.7 kilobase of chromosomal DNA from DZF1 and including the msd region, was nick translated and hybridized with msDNAs from various strains. Lanes: 1, *M. xanthus* DZF1; 2, *S. aurantiaca* DW4; 3, *M. coralloides* DK817; 4 through 12, *M. xanthus* DK818, DK823, DK829, DK843, DK851, DK853, DK862, DK870, and DK898, respectively.

FIG. 3. Presence of msDNA in myxobacteria and in other less-related bacteria. Total nucleic acid was isolated from bacteria grown in liquid medium or scraped from solid medium. Samples were treated with RNase A, electrophoresed on a 5% acrylamide gel, stained with ethidium bromide, and photographed. Lane i contains total cellular DNA isolated from *M. xanthus DZF1*. Approximately halfway down this lane is a major band which is the msDNA previously characterized (9; Fig. 1). In addition, there is a minor band below it which is also resistant to RNase digestion (data not shown) and therefore probably is also DNA. Lanes: a, pBR322 digested with *HaeIII*; b, *Herpetosiphon giganteus* Hpa2; c, *H. giganteus* Hpg12; d, *Taxobacter ocellatus* Tox1; e, *Lysobacter antibioticus* Lyal; f, *Lysobacter enzymogenes* Lyel; g, *Cytophaga johnsonae* Cyj1; h, *N. exedens* Nae655; i, *M. xanthus* DZF1; j, *F. elegans* Fxel; k, *Cystobacter fuscus* Cbf16; l, *C. fuscus* Cbf15; m, *C. violaceus* Cbf17; n through p, *C. ferrugineus* Cbf16, Cbf17, and Cbf18, respectively; q, *Rhodospseudomonas sphaeroides*; r, *Rhodospirillum rubrum* S1.


