Physical mapping of the *Myxococcus xanthus* genome by random cloning in yeast artificial chromosomes

(DNA/restriction mapping/contiguous clones/ordered clone bank/mapping strategy)

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ABSTRACT Random segments of *Myxococcus xanthus* DNA were cloned in yeast artificial chromosomes (YACs) to construct a physical map of the genome. EcoRI restriction maps of 409 YAC clones with inserts averaging 111 kilobase pairs (kb) were determined. Comparison to the map of a 300-kb region of *M. xanthus* obtained from clones in *Escherichia coli* indicates that segments of DNA cloned in YACs are stably maintained in yeast and that their sequences accurately reflect the structure of the *Myxococcus* genome. The 409 YAC inserts were ordered within 60 map segments (contigs) by aligning their EcoRI restriction maps and by hybridization with 18 gene-specific DNA probes. These 60 map segments may represent the entire *Myxococcus* genome and could be used to organize its genetic information. This study illustrates the utility of YACs for cloning large segments of DNA and for reliable long-range genomic mapping.

The study of many organisms is hindered by the absence of a long-range genetic map. A physical map can substitute for a genetic map, providing a long-range structural framework on which genetic loci can be positioned. A physical map consists of landmarks such as restriction sites, transposon insertion sites, or of partial DNA sequences that have been physically ordered.

Several strategies for constructing physical genomic maps have been described that utilize a large number of randomly cloned segments of DNA to create a continuous array of overlapping segments (1–3). Random segments are ordered by finding regions that overlap, a process that ultimately requires comparing every segment with every other segment. This ordering step is difficult because an entire genome must be covered by mapping a large number of cloned fragments, each of which constitutes a small fraction of the genome. One way around this difficulty is to start with a smaller number of larger clones, thereby decreasing the number of comparisons required. Since DNA fragments as large as 800 kilobase pairs (kb) can be cloned in yeast artificial chromosomes (YACs; refs. 4 and 5), they should permit mapping a genome with fewer clones than would be required with *Escherichia coli* vectors. Although such an approach has been proposed (4), it remains to be demonstrated that accurate genomic maps can be constructed from information obtained with YAC clones. Before YACs can be confidently used for mapping in general and for the random cloning approach in particular, it must be shown that genomic DNA can be maintained in YACs without deletion or rearrangement and is selected with little bias favoring one genomic region over another.

*Myxococcus xanthus* is a microbe whose genetic character is poorly understood, yet is of interest because it is the most primitive organism known that exhibits multicellular development with cellular differentiation (6). Many mutations that alter its development have been isolated and studied genetically. Both specialized and generalized transduction are available to transfer genomic segments of <50 kb, but no reliable method for long-range genetic mapping yet exists.

To correct this deficiency, we have begun to construct a physical map of the *M. xanthus* genome by random cloning into YACs. By identifying those YACs that contain contiguous pieces of *Myxococcus* DNA, comparing some particular sequences with established maps, and testing related YACs for internal consistency, we show that physical maps produced by YACs faithfully represent the native genome. No rearrangements or deletions were evident in any of the clones examined in detail. We describe mapping with a single restriction enzyme and many YAC clones. A set of 409 YACs, possibly containing the entire *M. xanthus* genome, was ordered into 60 groups of contiguous clones (contigs) by comparing their restriction maps.

MATERIALS AND METHODS

Plasmids. The plasmids containing specific *Myxococcus* genes (and their sources) are: p104 (7); pJR167, containing tgl (provided by J. Rodriguez); pKNS264, containing mgl (8); pYLC72, containing dsg (9); pKAM019, containing asgB (10); pHBK209 (provided by H. Kaplan), containing DNA adjacent to Tn5 lac insertion Ò4521 (11); pDAH244, containing carB (provided by D. Hodgson); pREG1255, containing bsgA (12); pLJS43, containing csgA (13); pJDK1, containing tps ops (14); pLJS61 (provided by L. Shimkets), containing DNA adjacent to Tn5 lac insertion Ò4435 (11); pKO23, containing tag (15); pRB12, containing the frz genes (16); pJR103, containing mbhA (17); and pMxY62, pMxH1, pMxH8, and pMxH16, which contain random cloned fragments of the *Myxococcus* genome (provided by H. Kimsey). Appropriate fragments containing only *Myxococcus* DNA were excised from agarose gels after electrophoresis, purified, 32P-labeled, and used as hybridization probes (see below).

Isolation of DNA. Large DNA was isolated from *M. xanthus* DK1622 (18) by the procedure of Olson et al. (19) as follows. Cells were grown in CTT broth (20) and harvested at 4 × 10^8 cells per ml and resuspended to 2.5 × 10^8 cells per ml in prelysis buffer [0.1 M Tris-HCl, pH 7.6/15% (wt/vol) sucrose/10 mM EDTA]. For each centrifuge tube, 4 ml of this suspension was allowed to drip slowly (0.5–1 ml/min) down the side of a 250-ml flask containing 7 ml of lysing buffer [0.4 M Tris-HCl, pH 9/0.2 M EDTA/3% sodium N-lauroylsarcosine].

Abbreviations: CHEF, contour-clamped homogeneous electric field; FIGE, field-inversion gel electrophoresis; YAC, yeast artificial chromosome consisting of DNA inserted into the pYAC4 vector; kb, kilobase pairs of DNA; Mb, megabase pairs of DNA; contig, a set of contiguous cloned segments of a genome.

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cosinate (sarkosyl) while the flask was rocked in a circle at 20–30 rpm. The lysed cell slurry was incubated at 65°C for 15 min, cooled, and poured onto a single sucrose gradient. After centrifugation as described by Olson et al. (19), large DNA was collected from the bottom of the gradient with the open end of a 10-ml glass pipet, concentrated to 1–2 mg/ml, and dialyzed against TE buffer (10 mM Tris·HCl/1 mM EDTA, pH 8) in a collodion bag (UH 100/1; Schleicher & Schuell).

**YAC Construction.** *Myxococcus* DNA was partially digested with *EcoRI* endonuclease. The extent of digestion was monitored by pulsed-field electrophoresis in 1% agarose gels by using a contour-clamped homogeneous electric field (CHEF) device with a hexagonal electrode configuration (21), a field strength of 6.7 V/cm, and an 80-s switch time for 20 hr at 9–10°C. The pYAC4 vector, which contains an *EcoRI* cloning site, was prepared as described (4). YACs were constructed by ligation of a mixture of 100 µg of partially digested *Myxococcus* DNA and 200 µg of prepared pYAC4 “chromosome arms” (4), followed by separation of large DNA from unreacted vector fragments by sucrose gradient sedimentation. Fractions containing DNA > 50 kb were pooled, concentrated, and dialyzed against TE in a collodion bag. This DNA was used to transform yeast strain AB1380 as described (4, 22) except that spheroplasts were plated on regeneration/selection plates without uracil and with 10 µg of adenine per ml. Primary transformants were picked to yeast minimal plates containing 10 µg of adenine per ml and lacking uracil and tryptophan (23); colonies that grew within 3 days at 30°C were saved.

**Analysis of YAC Clones.** Large DNA was prepared in agarose as described (24) from YAC-containing strains. Each yeast strain saved (above) was grown to saturation in 1.5 ml of yeast minimal medium lacking uracil and tryptophan at 30°C and was cast in a 75-µl mold. The size of each YAC was determined by pulsed-field electrophoresis of the DNA in a CHEF device with a 20-s switch time, followed by transfer to Nytran and hybridization with 32P-labeled *EcoRI*-digested pBR322 DNA. Concatamers of phage λ DNA (c1857Samp7) made as described (25) were used as standards. YACs 100–160 kb in size (468 total) were analyzed further. To identify YACs that contained specific cloned *M. xanthus* genes, YAC DNA was separated from yeast chromosomal DNA by field-inversion gel electrophoresis (FIGE) (26), transferred to Nytran, and probed with DNA fragments from cloned genes. The FIGE was run in a standard 20-cm bed agarose gel box with a pulse controller (PC750; Hoefer). The electric field strength was 3 V/cm, switched 2.1 s in the forward direction and 0.7 s in the reverse direction with a ramping factor of 2, in a 1% agarose gel in 0.25× TBE buffer (27) run for 12 hr at 27°C. Under these conditions, YAC DNA (100–160 kb) migrates 3–4 cm, allowing 80 samples to be run (in four rows) per gel. DNA was blotted and hybridized to labeled cloned *Myxococcus* DNA as described below.

**Mapping YACs with EcoRI.** For mapping the *EcoRI* sites of individual YACs, two cubes of YAC DNA embedded in agarose (~10-µl volumes) were cut and rinsed twice with 50 µl of permeation buffer [50 mM Tris·HCl, pH 7.5/100 mM NaCl/2 mM dithiothreitol/0.1 mg/ml bovine serum albumin (fraction V; Sigma)] for a total of 1 hr at 37°C. The rinsed blocks were placed in 0.5-ml microfuge tubes containing 20 µl of permeation buffer and one unit of *EcoRI* (New England Biolabs), then incubated at 4°C for 16–20 hr to allow the enzyme to permeate the blocks. Digestion with *EcoRI* was started by raising the temperature to 37°C and adding 2 µl of 200 mM MgCl2. Digestion was stopped by addition of 50 µl of 500 mM EDTA (pH 8) and storage on ice. Twenty digested samples were loaded across the narrow dimension of a 13 × 18 cm 1% agarose gel (100 ml in 0.5× TBE buffer) and electrophoresed for 20 hr in 0.5× TBE buffer at 9–10°C by CHEF at 6.7 V/cm with an 8-s switch time. A mixture of λ phage DNA high molecular weight fragments (Bethesda Research Laboratories) and concatamers of λ phage DNA were used as molecular weight standards. DNA was visualized with ethidium bromide, transferred (28) to Nytran (Schleicher & Schuell), and hybridized (28) to 32P-labeled (29) DNA probes.

**RESULTS AND DISCUSSION**

Constructing a YAC Library of *M. xanthus* DNA. Published estimates of the genome size for *M. xanthus* range from 5.7 to 11.5 megabase pairs (Mb) (30, 31). For a genome of this size, a library that contains 50 Mb of random DNA fragments has a high probability of containing the entire genome (32). Accordingly, high molecular weight DNA from *M. xanthus* was cleaved with *EcoRI* under conditions that would cut only a fraction of the total sites, joined to pYAC4 artificial chromosome ends, and introduced into Saccharomyces cerevisiae by DNA transformation, after which 1840 individual transformed yeast colonies were selected. The size of the YAC from each transformant was determined with a CHEF electrophoresis apparatus, followed by Southern blot hybridization to 32P-labeled pBR322, which is part of the pYAC4 vector. Most of the transformants contained YAC DNA molecules that migrated as a single band in the 40- to 180-kb range. Yeast clones containing the largest YACs (468 of the 1840) were chosen to constitute the library. Their average insert size is 111 kb; the whole library comprises a total of 32 Mb of *M. xanthus* DNA. Clones that had two or more bands hybridizing to the vector probe (65 of the 1840), indicating that they contained multiple YACs, were not included.

**Fidelity and Stability of the YAC Clones.** By using a technique originally developed by Burke et al. (4), the size and order of *EcoRI* fragments from each YAC were deduced from its partial digestion pattern (Fig. 1). We have modified

![FIG. 1. Mapping the EcoRI sites of YAC DNA. The DNA of a representative Myxococcus YAC (clone 921) was digested in an agarose block with EcoRI endonuclease for 10 min (left lane) or 50 min (right lane) under conditions designed for partial digestion. The digested samples were electrophoresed in a CHEF device device to separate DNA molecules in the range of 1–150 kb. DNA fragments were transferred from the gel to Nytran and probed with 32P-labeled DNA derived from the centromeric end of the YAC vector (4). An autoradiogram exposed for 3 days at −70°C with an intensifying screen is shown. To its immediate left are the measured sizes of each DNA fragment, and to its right is the deduced map of EcoRI sites (short horizontal lines) on the YAC (thick vertical line). The position of each DNA size standard is indicated at the extreme left.
the Burke technique to start with yeast cells in agarose, which simplifies the DNA preparation and digestion procedure. Fig. 1 shows that the same (complete) set of partial digestion fragments was produced after 10 and 50 min of digestion. By digesting each new cell sample for 10 min and 50 min, variations in strain growth could be accommodated, and it becomes practical to analyze many strains in one experiment. We have also used CHEF electrophoresis (21), which can resolve bands that differ by >2 kb and, under the particular conditions employed, in which mobility is a linear function of molecular size for molecules of 15–150 kb. Because the same filter could be probed sequentially with DNA specific for the centromeric and noncentromeric ends of a YAC (4), two measurements of the size of each EcoRI fragment were obtained, which were then averaged.

Before attempting to order the YAC clones, we tested the extent to which M. xanthus DNA that had been selected in a YAC and replicated in yeast accurately represents the M. xanthus genome. An extensively mapped region of the M. xanthus genome that surrounds the putative origin of replication was compared to YACs derived from that region. This region extends over 330 kb and has been isolated in a set of overlapping cosmid clones in E. coli (7). One of these clones (p104) was used as a probe to identify YACs from this region. Four YACs hybridized with p104; eight others could be assigned to the region by their fragment patterns. Fig. 2 compares the EcoRI fragment sizes and order from YACs with the cosmid map of Komano et al. (7). The agreement is good, with an average difference of 13% in corresponding fragment sizes between the two maps. No deletions or rearrangements of M. xanthus DNA in the YACs were evident. To test stability, DNA was isolated from a particular yeast transformant and again from 20 single colony isolates of the same transformant after 27 generations of growth. The same YAC fragment sizes and fragment order were found in all these colonies; no deletions or rearrangements were observed.

Identification of Related YACs by Matching Their Ordered Fragment Sizes. Restriction mapping by the method described in Fig. 1 was attempted for the 468 large YACs: 409 maps were completed; the remaining 57 clones yielded incomplete or ambiguous maps. The 409 YACs constitute what we will call the mapping set. They contain a total of 45 Mb of M. xanthus DNA.

To identify YACs with contiguous DNA segments, the 409 maps were screened pairwise for overlaps. In the first stage of comparison, YACs which had at least four pairs of fragments that matched in order and size within 10% were assigned to the same group. Exceptions to the four-fragment minimum were made for those 83 of the 409 YACs whose overlaps contained an EcoRI fragment of 60 kb or more, because such large fragments were rare and the probability of a false overlap was correspondingly low. From the 409 members of the mapping set, 78 groups of overlapping YACs were evident. These groups contained an average of 5.2 (409/78) YACs each, and the average total length of a group, from the sum of its constituent fragments, was 150 kb. Since all but three groups had two or more fragment members, errors arising from the measurement of fragment length could be reduced by averaging, and maps corresponding to each of the 78 groups were obtained. The distribution of numbers of YACs per group approximates a Poisson distribution with a mean of 5.2 (Fig. 3), consistent with the hypothesis that the M. xanthus cloned segments were drawn at random from the whole M. xanthus genome. By this analysis, <1 group is expected to have no YAC representative; if all M. xanthus sequences can survive in yeast, the library may be complete.

In a second stage, the ends of different groups from the first stage were scanned for overlaps. Many inconsistent overlaps of three fragments were seen, so it was again required that at least four fragments of overlap, allowing fragment errors to vary ±10% in size. This second stage is not logically independent of the first since similar criteria were applied, but comparing one whole group with another provided greater sensitivity and reliability than individual YAC-to-YAC comparisons. Indeed, 12 new overlaps were picked up.

As part of the search for overlaps between groups, 18 different DNA hybridization probes obtained from specific M. xanthus genes were used to identify related YACs. Southern blots of YAC DNA separated from the normal yeast chromosomes by FIGE electrophoresis were used for hybridization with the probes. All probes hybridized with at least 2 YACs, the average being 5.4 YACs per probe. The fact that all probes hybridized with some YACs supports the assertion that all M. xanthus sequences clonable in E. coli are clonable in YACs. Assuming a genome size of 9–10 Mb, the fact that, on average, 5.4 YACs hybridized to a given probe argues that the mapping set may be a complete library, with an average segment represented, as expected, five times. Of the YAC groups, there were 10 in which all members of each group identified in the first stage hybridized with the same probe in the second. No group hybridizing to a probe failed

![Fig. 2. Comparison of a related set of YACs to the physical map based on E. coli cosmids from the same part of the M. xanthus genome. YAC DNA was separated from yeast chromosomal DNA by FIGE electrophoresis, transferred to nitrocellulose membranes, and exposed to 32P-labeled p104 probe. YACs 1387, 838, 419, and 890 hybridized with the probe. YACs 1186 and 1503 were not tested with the p104 probe but were assigned to this contig (number 3 of Table 1) on the basis of matching restriction maps, as were 440, 660, 775, 898, 1308, and 1619. Vertical lines represent EcoRI restriction sites, and the one or two-digit numbers above each map are distances in kb between adjacent sites. The bottom line is the EcoRI restriction map derived from a set of E. coli cosmid clones by Komano et al. (7). EcoRI sites closer than 2 kb can be resolved by the method used (Fig. 1) only under special conditions. Accordingly, they are not included in the comparison with the E. coli map.](image-url)

![Fig. 3. Distribution of number of YACs per group. The observed numbers of YACs per group are shown by the solid bars. The expected numbers of YACs are calculated for a Poisson distribution of 409 YACs among 78 groups with an average of 5.2 (409/78) YACs per group. See the text for the description of a group.](image-url)
this test. This check on the internal consistency of the groups confirms the fidelity and genetic stability of YACs as carriers of segments of the *M. xanthus* genome and the validity of the overlap criteria that were used to define a group.

Six probes hybridized to subsets of YACs drawn from two different groups, establishing the contiguity of those pairs of groups. The tag and csgA probes actually linked three groups together. Given the hybridization to these probes, there

Table 1. *Myxococcus* YAC contigs

<table>
<thead>
<tr>
<th>Contig</th>
<th>Size*</th>
<th>YACs†</th>
<th>Minimal YAC set‡</th>
<th>Markers§</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>450</td>
<td>21</td>
<td>803, 276, 1808, 1416, 1468, 1109</td>
<td>tag, csgA</td>
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<tr>
<td>2</td>
<td>410</td>
<td>18</td>
<td>423, 1690, 1419, 1899, 1409, 441</td>
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</tr>
<tr>
<td>3</td>
<td>380</td>
<td>12</td>
<td>1619, 1378, 890, 440</td>
<td>p104</td>
</tr>
<tr>
<td>4</td>
<td>380</td>
<td>13</td>
<td>1038, 812, 128, 897, 1368</td>
<td>ω432, pMXH8</td>
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<td>380</td>
<td>19</td>
<td>883, 1569, 649, 948, 906</td>
<td>ω4435, pMXY62</td>
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<td>320</td>
<td>14</td>
<td>263, 665, 799, 1142</td>
<td>argB</td>
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<td>13</td>
<td>1080, 1683, 436</td>
<td>dxg</td>
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<td>8</td>
<td>260</td>
<td>13</td>
<td>847, 557, 1183</td>
<td>tgl (csgB, rif)</td>
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<td>230</td>
<td>6</td>
<td>968, 535, 1400</td>
<td>—</td>
</tr>
<tr>
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<td>220</td>
<td>8</td>
<td>1305, 797, 1167</td>
<td>curR, frz</td>
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<td>11</td>
<td>220</td>
<td>9</td>
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<td>—</td>
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<td>4</td>
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<td>5</td>
<td>617, 1304</td>
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<td>896, 461</td>
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<td>29</td>
<td>160</td>
<td>10</td>
<td>1566, 1306</td>
<td>pMXH1</td>
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<tr>
<td>30</td>
<td>150</td>
<td>7</td>
<td>929, 486</td>
<td>—</td>
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</table>

*To estimate the contig size, the fragment sizes were obtained by averaging the measurements from all YACs for each fragment, and then all fragment sizes along the contig were added together.

†The number of YACs were assigned to each contig on the basis of matching EcoRI-site patterns or by mutual hybridization of one or more probes made from marker clones listed in the last column.

‡The minimal set of overlapping YACs (designated by a three- or four-digit number) that cover the entire contig are listed in order from one end of the contig to the other. The total at the bottom is the minimal number of YAC clones predicted to cover all of the DNA represented by the library.

§*Myxococcus* genes or random cloned fragments that were assigned to a particular contig (by hybridization of a 32P-labeled fragment of that clone to the YAC set) are indicated by their common designation. (See Materials and Methods for the origin of each marker.) Genes in parentheses are inferred to lie on a contig because of genetic linkage to a gene that was assigned by hybridization to that contig. Tn5 lac insertions are designated by their F number, and plasmids are designated with a "p" prefix.
appears to be only one way to order the three groups (Fig. 4). Overall, the groups joined into contigs by hybridization to the same probe overlapped by an average of 2.7 EcoRI fragments and 15% of the original group length, too small to have allowed joining by direct matching.

In all, 18 groups were linked by the combined use of specific DNA probes and four-fragment overlaps; the 78 groups merged into the 60 contigs shown in Table 1. End-to-end, these contigs include 10.8 Mb of _M. xanthus_ DNA, but since some of this DNA is likely to be overlapping, the total genome length may be less. Although the 60 contigs have not yet been joined into a complete physical map of _M. xanthus_ DNA, the data do map 16 previously known genes, indicated in the last column of Table 1, onto their corresponding contigs.

Because the contigs linked by probes overlapped by an average of only 2.7 EcoRI fragments, it is unlikely that the 60 contigs of Table 1 can be further ordered by requiring four EcoRI fragment overlaps. More data, and perhaps additional restriction enzymes, would seem to be required to complete the physical map of _M. xanthus_. To identify overlaps between two contigs, single-copy probes specific for the ends of each could be generated and hybridized to the entire YAC library. In principle, this approach would be limited only by completeness of the YAC library. Gaps, if they existed, might be filled with other types of clones (33). A more efficient way to complete the map is suggested by the discovery of Keseler et al. (34) that _Ase I_ cleaves _M. xanthus_ DNA into 16 fragments. The YAC contigs could be correlated to the fragments produced by _Ase I_. Selected YACs could be used as linking clones to span _Ase I_ restriction enzyme sites. Moreover, the large _Ase I_ fragments are likely to span any regions that may be missing from the YAC library.

The results reported here encourage the use of YAC libraries for genome mapping, illustrating its feasibility on the 10-Mb scale. Although the statistical data do not rule out the possibility that some _M. xanthus_ DNA was not cloned in YACs, and despite the high (68%) G+C content of _M. xanthus_ compared to yeast, no deletions or rearrangements were evident in Fig. 2. YACs hybridizing to each of 18 single-copy DNA probes were found at the expected frequency and gave mutually consistent EcoRI fragment patterns in every case. Moreover, no EcoRI sites that demarcate fragments > 2 kb were present in some YACs but absent in others. Strategies that combine the use of YACs with other long-range physical mapping techniques could prove to be a universal approach for mapping large genomes.

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