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Physical Map of the Genome of *Rhodobacter capsulatus* SB 1003

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A map of the chromosome of *Rhodobacter capsulatus* was constructed by overlapping the large restriction fragments generated by endonucleases *Ase*I and *Xba*I. The analyses were done by hybridization of single fragments with the restriction fragments blotted from pulsed-field gels and by grouping cosmids of a genomic library of *R. capsulatus* into contigs, corresponding to the restriction fragments, and further overlapping of the contigs. A technical difficulty due to a repeated sequence made it necessary to use hybridization with cloned genes and prior knowledge of the genetic map in order to close the physical circle in a unique way. In all, 41 restriction sites were mapped on the 3.6-Mb circular genome and 22 genes were positioned at 26 loci of the map. Cosmid clones were grouped in about 80 subcontigs, forming two groups, one corresponding to the chromosome of *R. capsulatus* and the other corresponding to a 134-kb plasmid. *cos* site end labeling and partial digestion of cosmids were used to construct a high-resolution *Eco*RV map of the 134-kb plasmid. The same method can be extended to the entire chromosome. The cosmid clones derived in this work can be used as a hybridization panel for the physical mapping of new genes as soon as they are cloned.

Rhodobacter capsulatus is a purple, nonsulfur photosynthetic bacterium that is widely used in studies of carotenoid biosynthesis, photochemical reaction centers, and nitrogen fixation. Its popularity is justly based on the availability of several convenient systems for genetic analysis (5). The first to be discovered was a small transducing phage called the gene transfer agent, which packages 4.6-kb fragments of chromosomal or plasmid DNA in donor strains and delivers them to *R. capsulatus* recipients (17). The gene transfer agent can be used for insertional mutagenesis, gene replacement, and linkage analysis (23). Subsequently, by use of an integrated R′ factor to mobilize the bacterial chromosome (24), it was shown that linkage could be measured by cotransfer frequency in conjugational crosses. This work suggested that there is a single circular linkage group in *R. capsulatus*. Finally, the development of shuttle vectors based on broad-host-range plasmids made it possible to do efficient random and site-specific mutagenesis with transposons and to clone genes by complementation of mutants (13).

The method of pulsed-field gel electrophoresis (PFGE) of large fragments of DNA (19), generated by using rarely cutting restriction endonucleases, has made it possible to map megabase regions of eukaryotes (8) and whole genomes of prokaryotes (2, 22). Approximately 40 bacterial genome maps (15) have been constructed this way. These maps provide low physical resolution, so their utility has been primarily to demonstrate the mapping strategy and to confirm existing genetic maps (21).

Recently, a fine-structure physical map and an ordered hybridization panel of the *Escherichia coli* genome (12) have proved to be extraordinarily useful tools in genetic studies. The hybridization panel, consisting of about 400 recombinant lambda phages of known location on the *E. coli* map, was used for precise mapping of many newly cloned genes by a simple procedure (13). The existence of such a map and hybridization panel for *R. capsulatus* would facilitate genetic studies of this organism. A step towards this map is the creation of a low-resolution physical map, which can itself be used for hybridization of cloned genes, adding them to the existing genetic map of *R. capsulatus* (24). The next step would be the construction of a cosmid encyclopedia (an ordered gene library), and finally the physical map of these cosmids would make up the high-resolution map of the *R. capsulatus* chromosome. This two-step construction of the fine-structure map will simplify alignment of the cosmid maps because the whole bacterial genome will be split into cosmid subcontigs that can be analyzed and mapped separately.

In this paper, we describe the construction of a restriction map of the chromosome of *R. capsulatus*, the mapping of 22 cloned genes, and the creation of a gene encyclopedia for this organism. A cryptic plasmid was also found and mapped. An end-labeling procedure suitable for fine-structure mapping was applied to the cosmids derived from the cryptic plasmid, allowing its *Eco*RV map to be determined.

MATERIALS AND METHODS

Strains, plasmids, and culture conditions. Bacterial strains, plasmids, and cloned genes of *R. capsulatus* are listed in Table 1. *R. capsulatus* SB1003 was grown in the light in RCVB medium (8) for 36 h. *E. coli* DH5α containing the cosmid clones was grown in microtiter plates in 100 μl of SOC medium (16) per well (each well contained 30 μg of kanamycin per ml). All other manipulations of *E. coli* cells were done with LB medium (16).

DNA preparation. Alkaline plasmid extractions for mapping and labeling were done as previously described (16). Genomic DNA of *R. capsulatus* was prepared by lysis with sodium dodecyl sulfate in the presence of proteinase K followed by phenol extraction. Samples of *R. capsulatus* DNA for PFGE were prepared by embedding bacterial cells in 0.6% agarose blocks (19) at a final concentration of 5×10^9 cells per ml, which is equivalent to 1 μg of DNA per 10 μl of

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TABLE 1. Strains and plasmids used

Strain or plasmid	Description	Gene probe used for mapping	Source or reference	No. of cosmids identified (no. of locations)
R. capsulatus SB1003	Prototroph		27	
E. coli DH5α	*recA hsdR mcrB*		14	
pLorist 6	λ-*ori* cosmid		6	
pRCN106	Ammonia-dependent growth	*adgA*	1	11
Unnamed	Reaction center H subunit	*puhA*	28	12
p118-1005	Cytochrome *c* biosynthesis	*ccl1* and *2*	R. G. Kranz	10
pBc2	Cytochrome c_2	*cycA*	3	12
pDCT215	C_4-dicarboxylate transport	*dctP*	7	11
p245	Fructose-phosphate kinase	*fruK*	L. F. Wu and M. Saier	12
Unnamed	Glutamine synthetase	*glnA*	20	8
Unnamed	DNA gyrase B	*gyrB*	L. Sveen	7
Unnamed	Topoisomerase IV subunit B	*parE*	L. Sveen	9
p26helAB	Cytochrome *c* biosynthesis	*helAB*	R. G. Kranz	7
pTZ18::1.5E/E	Hydrogenase	*hupSL*	26	4
pUCA6nifAB	Mo metabolism	*nifB*	11	26 (2)
pRbnifR1	Regulator of N fixation	*nifR1*	14	3
pRCN200	Regulator of N fixation	*nifR4*	14	9
p17-4	bc_1 complex	*petCBA*	4	8
pJAj9	Chlorophyll biosynthesis	*pufQ*	G. Drews	9
Unnamed	Ribosomal DNA (16S)	*rrn*	L. Sveen	3 (4)
pRCR2	β subunit of RNA polymerase	*rpoB*	B. Abella	11 (2)
Unnamed	α-Ketoglutarate dehydrogenase	*sucA*	10	6
pMBR440	Tryptophan biosynthesis	*trpC*	B. Marrs	8
pUL4I	Alternative nitrogenase	*anfGDHA*	W. Klipp	9 (2)

agarose matrices. Lysis was achieved by using 3% lauryl sarcosine–1 mg of proteinase K per ml in 0.2 M EDTA (pH 8.0) at 55°C for 14 to 36 h.

Enzymatic manipulation of DNA. Restriction endonucleases, Klenow fragment of DNA polymerase, calf intestinal phosphatase, and T4 DNA ligase were purchased from New England BioLabs and used according to their instructions. For cleavage of embedded samples of DNA used in PFGE, 5 to 20 U of enzyme and 3-h incubations at 37°C were used. For random primed labeling of DNA bands, bands were cut out of 1.4% low-melting-point agarose gels and heated at 95°C for 5 min. Five microliters of melted gel containing 5 to 10 μg of DNA was added to the labeling reaction (16), and the entire mixture was used as a hybridization probe. Extracts for in vitro packaging in λ heads and enzymes for SP6 or T7 in vitro transcription were obtained from Promega Corp. Dephosphorylation, ligation, and packaging in λ extracts were done as described previously (16). In vitro transcription was done according to the manufacturer's instructions (Promega Corp.). Terminase for *cos* cleavage of cosmids was obtained from Takaba Corp. and used in accordance with the manufacturer's instructions. Cosmid mapping was performed by using the λ mapping system produced by Amersham.

Electrophoresis. PFGE was performed with the Bio-Rad system. Standard running conditions were as follows: 24 h, 0.5× Tris-borate-EDTA buffer (16), 1% Bethesda Research Laboratories agarose, a running temperature of 8 to 40°C, and a field strength of 10 V/cm. To resolve restriction fragments between 40 and 700 kb, a gradual change of switching intervals from 5 to 30 s was used. To resolve 8- to 90-kb fragments, the switching gradient was from 0.3 to 3 s. After electrophoresis, gels were stained with ethidium bromide and photographed with Polaroid 667 film.

Gene library construction. A λ-*ori* cosmid, Lorist 6, a derivative of Lorist 2 (6), was used for the gene library construction. Cloning of size-fractioned, *Sau*3A-cut DNA of *R. capsulatus* was achieved by using *Hin*dIII-*Bam*HI and *Eco*RV-*Bam*HI "arms" of the cosmid (16). With cosmid arm cloning, it is possible to use dephosphorylated inserts and thus avoid cloning nonneighboring DNA fragments in the same cosmid. Size fractionation was done with a 10 to 40% sucrose gradient, and fractions were analyzed by conventional electrophoresis and PFGE. Fractions with mean fragment sizes of 35 to 45 kb were used for cloning. Purified dephosphorylated inserts (300 ng of DNA) were ligated with an equimolar amount of cosmid arms in 10 μl of ligation mixture and packaged in vitro into λ heads. After infection of *E. coli* DH5α and plating on LB medium containing 30 μg of kanamycin per ml, a total of approximately 4,000 cosmid clones were recovered. Individual colonies were picked and grown in the wells of 18 96-well microtiter plates and stored in 40% glycerol at −70°C.

Hybridization analysis of cosmid library. A device that made it possible to print 96 *E. coli* cultures from each of nine microtiter plates on one 150-mm petri dish in an ordered way and to prepare six such plates at once was constructed. The device has two plastic components, a lower one that holds the petri dish and an upper one with fixed posts that guide a 96-prong printer. The upper part can be translated precisely to three positions left-to-right and three positions north-to-south with respect to the lower part, resulting in a densely packed grid (9 by 96 dots). After overnight growth on Colony Screen DuPont membranes, such replicas were picked up, processed according to the instructions of the manufacturer, and hybridized with different DNA probes. Each hybridization gave a pattern of cosmids in the primary microtiter plates corresponding to the probe used, providing an opportunity to group the initial set of cosmids into contigs.

Blot hybridization. DNA fragments from pulsed-field gels were transferred onto GeneScreen Plus nylon membranes by the standard capillary procedure (16) and hybridized with 0.5 × 10^6 to 2 × 10^6 cpm of randomly labeled probe per ml. Hybridization, washing, and removal of probe were done

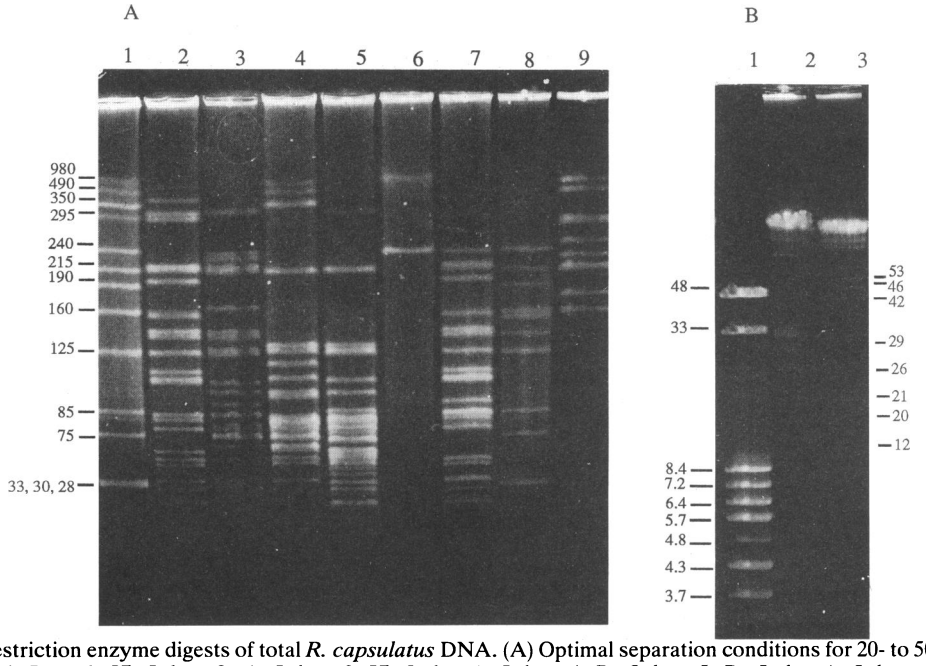

FIG. 1. PFGE of restriction enzyme digests of total *R. capsulatus* DNA. (A) Optimal separation conditions for 20- to 500-kb fragments (see Materials and Methods). Lane 1, *Xba*I; lane 2, *Ase*I; lane 3, *Xba*I plus *Ase*I; lane 4, *Dra*I; lane 5, *Dra*I plus *Ase*I; lane 6, *Spe*I; lane 7, *Spe*I plus *Ase*I; lane 8, *Xba*I plus *Spe*I; lane 9, *Sca*I. (B) Optimal conditions for separating 5- to 80-kb fragments. Lane 1, lambda DNA size standards; lane 2, *Xba*I; lane 3, *Ase*I. Fragment sizes (in kilobases) shown on the left, based on a lambda ladder, are the averages measured in several gels. These sizes are repeated in the *Xba*I column of Table 2.

according to the membrane manufacturer's protocol. The only exception was that the final wash in 0.1× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) was done at 65 to 75°C. Filters were exposed to Kodak X-ray film from 2 to 48 h. By keeping filters wet, each filter could be used for five to seven hybridizations, the previous probe being removed each time.

RESULTS

Choice of optimal enzyme for long-range mapping. For optimal physical mapping, a restriction endonuclease should cut the bacterial genome into 10 to 30 pieces. This number of fragments can be well separated and mapped by conventional PFGE. Unfortunately, most known 8-base cutters have high-GC recognition sequences and cut the high-GC genome of *R. capsulatus* too often. *Xba*I has previously been shown to provide a convenient set of fragments from *R. capsulatus* DNA (18). Among the 6-base cutters with high-AT recognition sequences, we found *Xba*I (15 fragments), *Ase*I (25 fragments), *Sca*I (14 fragments), *Dra*I (25 fragments), and *Spe*I (3 fragments) to be useful (Fig. 1). The first two were chosen for mapping because they yielded a good separation of fragments with uniform distribution in the gels. The sizes of restriction fragments generated by these enzymes are shown in Table 2. These sizes were measured in seven independent experiments by using a λ concatemer ladder as the standard. The largest *Xba*I fragment was measured by using a switching gradient of 20 to 50 s, the three smallest *Xba*I and *Ase*I fragments were measured with a 0.3- to 3-s switching gradient, and the other fragments were measured under standard conditions (see Materials and Methods).

Total genomic size of *R. capsulatus*. The total genomic size of the *R. capsulatus* chromosome was estimated by summation of the individual fragment lengths represented in each digest. Those sums for *Xba*I and *Ase*I are 3,679 and 3,639 kb, respectively. This difference is explained in part by the presence of a large plasmid consisting of fragments Xba-11, Xba-12′, and Xba-13. Its size is 134 kb. This plasmid has no *Ase*I sites, so the sum of the sizes of *Xba*I-generated fragments should differ from the sum of the sizes of *Ase*I-generated fragments by the size of this plasmid. The observed difference of 40 kb is less than 134 kb; this discrepancy may be due to the error in estimating the sizes of the largest fragments.

PFGE mapping of fragments generated by *Ase*I and *Xba*I. All *Ase*I and *Xba*I fragments were cut out of low-melting-point agarose gels run under standard conditions. Most fragments were resolved as single bands, but Xba-3, Xba-3′, Xba-12, Xba-12′, Ase-5, Ase-6, Ase-13, and Ase-14 were cut out as doublets. These DNA fragments were labeled by random priming and used as probes for blot hybridization. Groups of *Xba*I-, *Ase*I-, and *Xba*I-plus-*Ase*I-generated fragments were separated by PFGE under standard conditions and transferred to nylon membranes. Filters corresponding to each group were hybridized with a single fragment probe. For example, if an *Xba*I fragment used as a probe identifies two *Ase*I fragments, it will link them. Using one of those *Ase*I fragments as a second probe, one can map the whole genome step by step. The results of typical hybridizations are shown in Fig. 2. Troubles arise when an *Xba*I fragment overlaps more than three *Ase*I fragments or when this overlapping is significantly unequal. In the latter case, the mapping sequence of steps would be interrupted.

Blot hybridization with Xba-11 and Xba-13 revealed positive bands of the same size in the *Xba*I and *Xba*I-plus-*Ase*I lanes but nothing (except weakly positive cross-reacting bands) in the *Ase*I lane (Fig. 3). This probably means that Xba-11 and Xba-13 are contained in a large plasmid which

TABLE 2. Sizes of restriction fragments generated by *Ase*I and *Xba*I cleavage of the *R. capsulatus* genome and corresponding numbers of hybridizing cosmids

Band no.	Size (kb) of fragment generated with:		No. of cosmids hybridizing to each *Ase*I fragment	
	*Ase*I	*Xba*I	Actual[a]	Expected[b]
1	650	980	99	145
2	370	490	69	86
3	320	350, 350	76	76
4	305	295	58	72
5	220	240	56	54
6	210	215	56	52
7	195	190	64	49
8	155	160	45	41
9	145	125	42	39
10	138	85	45	37
11	126	75	48	35
12	110	33, 33	65	31
13	105	30	28	30
14	102	28	28	30
15	84		20	26
16	80		23	25
17	75		19	24
18	53		19	19
19	46		22	18
20	42		17	18
21	29		29	15
22	26		16	14
23	21		19	13
24	20		19	13
25	12		7	11
Total	3,639	3,679	989	989

[a] The actual number of hybridizing cosmids is higher than 864 (the size of the analyzed set) because one cosmid can hybridize with more than one probe.

[b] The expected number of cosmids was calculated on the assumption that the number is proportional to the length of the fragment. Departures from expectation could be due to selection against some inserts or to overrepresentation of fragments near the origin of replication of the bacterial chromosome.

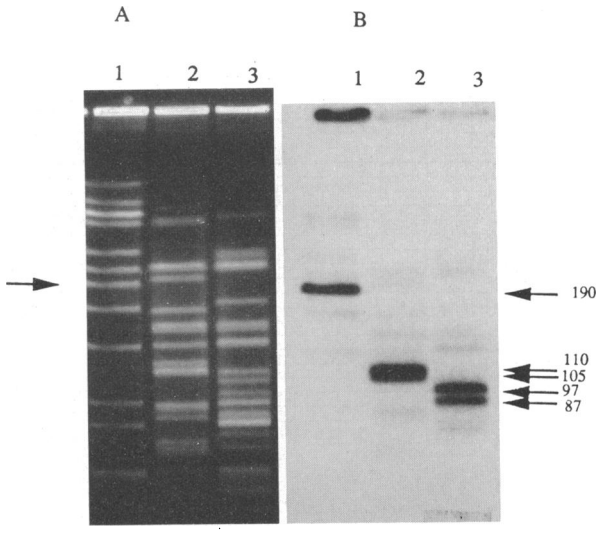

FIG. 2. Physical map construction with Southern blots with fragments from the pulsed-field gels. In this example, fragment Xba-7 (arrow) was eluted, labeled, and used to probe a gel containing *Xba*I (lane 1), *Ase*I (lane 2), and *Xba*I-plus-*Ase*I (lane 3) digests of total *R. capsulatus* DNA. (A) Ethidium bromide-stained gel; (B) autoradiogram. Sizes (in kilobases) of the detected fragments are indicated.

lacks *Ase*I sites. The plasmid did not enter the gel in the *Ase*I lane under the conditions used (9). Because of the limitations of the mapping method mentioned above, the partial physical map can be improved only by using another mapping strategy, which is described next.

Cosmid library analysis. Cosmid clones (864 in all) from nine plates of the stored library were taken for further analysis. By using the printing device described in Materials and Methods, those clones were replicated on nylon filters as an ordered set. Such sets of cosmid clones were hybridized with all individual *Xba*I and *Ase*I fragments, except for the six which were available only as doublets. The results of a typical hybridization are shown in Fig. 4. All of the clones hybridized with at least one *Xba*I and one *Ase*I fragment, except for clones corresponding to Xba-11, Xba-13, and Xba-12′, which did not hybridize with any *Ase*I fragment. Some groups of cosmids hybridize with two fragments generated by one enzyme, thus linking those two fragments. Most groups of cosmids corresponding to one fragment (subcontig) harbor only two such sets of linking cosmids, or "ends," allowing the construction of a unique sequence of fragments. In other cases, a third end caused by internal homologies between fragments can be discriminated by the overlapping of contigs generated by a different enzyme or by using restriction analysis to directly check for the presence of the expected site.

Mapping of fragments Ase-18, Ase-11, and Ase-15. One end of Ase-18 can be linked with both Ase-2 and Ase-6 by the methods mentioned above; therefore, they cannot determine a unique map position for this fragment. To eliminate this uncertainty, the ends of the insert of a single cosmid that hybridized with Ase-18, Ase-6, and Ase-2 probes and carried an *Ase*I restriction site were transcribed separately by using SP6 and T7 RNA polymerases in vitro. After hybridization

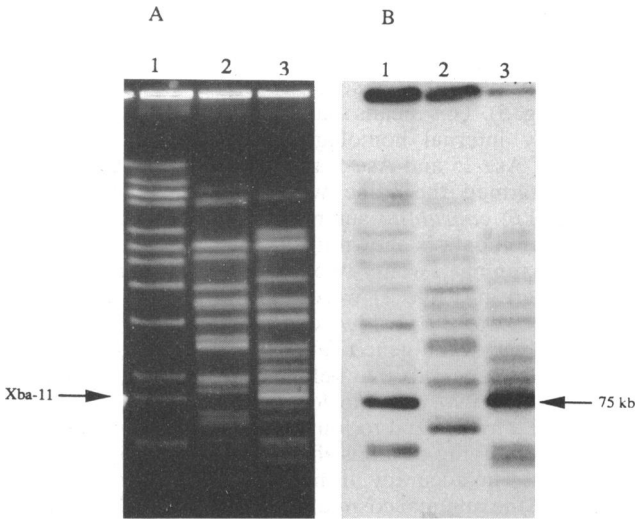

FIG. 3. Southern blot of total *R. capsulatus* DNA with fragment Xba-11 as the probe. Lane 1, *Xba*I; lane 2, *Ase*I; lane 3, *Xba*I plus *Ase*I. Xba-11 hybridizes only with itself in lanes 1 and 3. All of the significant hybridization in lane 2 is to undigested DNA in the well. Xba-11 is derived from a large plasmid that has no *Ase*I sites.

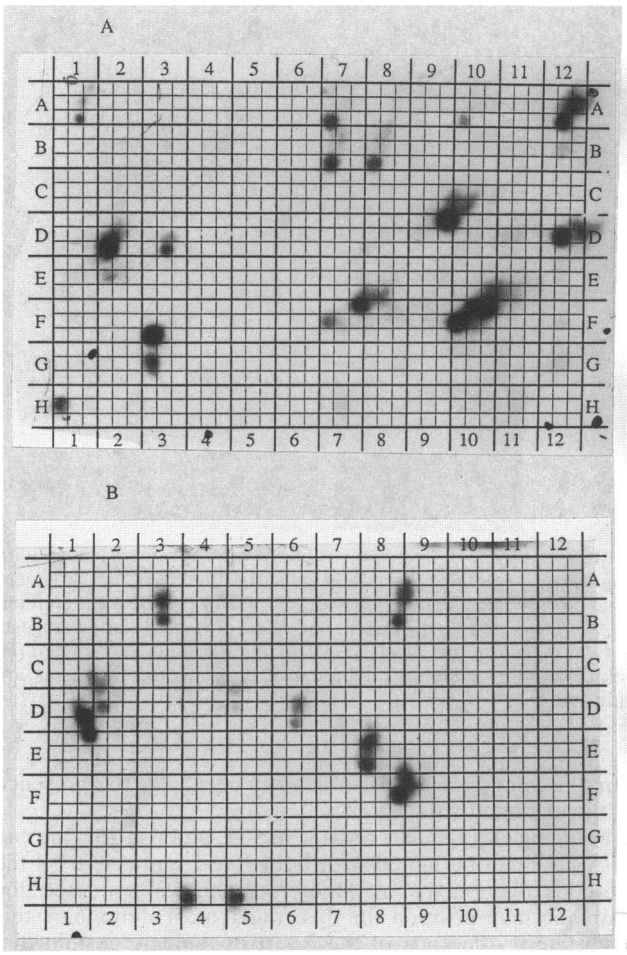

FIG. 4. Detection of cosmid sets by Southern hybridization with individual fragments from pulsed-field gels. (A) Grid of 864 cosmids hybridized with Xba-11. All of these cosmids are derived from the large plasmid. (B) Cosmids detected by fragment Ase-20.

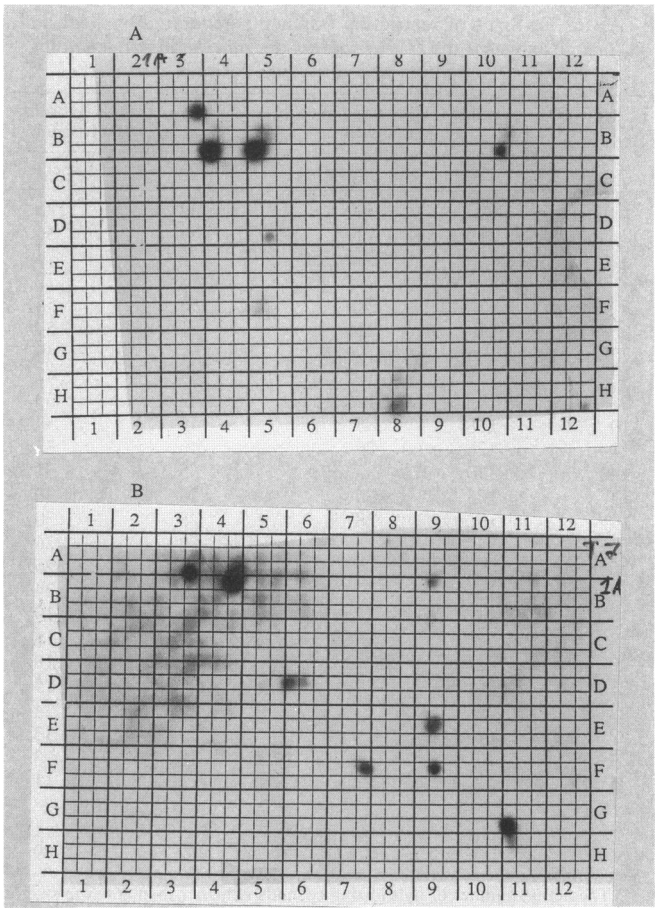

FIG. 5. Use of the cosmid set to link restriction fragments. Cosmid 1A3 was transcribed in vitro by SP6 RNA polymerase and separately by T7 RNA polymerase to provide probes complementary to each end of the cosmid insert. The labeled SP6 transcripts (A) and labeled T7 transcripts (B) were hybridized to the full cosmid set. The cosmids identified in panel A correspond to fragment Ase-2, and those in panel B correspond to fragment Ase-18. Therefore, fragments Ase-2 and Ase-18 are linked by cosmid 1A3.

with a full set of cosmid clones, one RNA probe revealed a set of eight cosmids belonging to the Ase-2 contig, and the other RNA hybridized to seven cosmid clones of the Ase-18 contig (Fig. 5). This means that hybridization with Ase-6 was caused by internal homologies between fragments. The linkage of Ase-15 and Ase-5 and the sequence Ase-19-11-14 were confirmed the same way. A physical map of the genome of *R. capsulatus* summarizing the PFGE and cosmid hybridization data is shown in Fig. 6.

Gene mapping. Twenty-two genes cloned from *R. capsulatus* (listed in Table 1) were hybridized with the cosmid array. Three to 30 positive cosmid clones were found to hybridize with each gene (Table 1; Fig. 6). Eighteen genes hybridized to cosmids that belong to one contig, while three hybridized to two possible locations each. The ribosomal DNA probe hybridized to four regions, explaining the presence of mystifying cosmids that link all four corresponding fragments. The accuracy of mapping, which corresponds to the size of the minimal cosmid subcontig, is roughly 80 kb, or 1.5 min, of the *E. coli* map (21).

Circularity of the physical map of *R. capsulatus*. All the methods of physical mapping used gave us two partial sequences for the *Ase*I fragments: 20-12-13-10-4-17-1-8 and 5-15-16-23-7-2-18-19-11-14-3-24-9-22-21-6. Our attempts to connect these sets by using physical mapping were unsuccessful. The riboprobe generated by the distal end of the most distal cosmid belonging to the Ase-8 contig revealed repeated sequences that exist on both ends of the other (Ase-5-6) cosmid set. Moreover, in the cosmid contig corresponding to Ase-20, there is a gap in cloned material corresponding to the distal 10 kb of Ase-20 (data not shown). The linkage of these two sets of contigs must therefore rely on a comparison of our map with the existing genetic map (24). There are five genetic loci on both maps: *nifHDK ... hup*, *glnA*, *trp*, and *nifABSE*. To maintain this order of genes on the physical map, our two sets of cosmid contigs have to be connected as follows: Ase-8 to Ase-6 and Ase-20 to Ase-5.

Mapping of the cryptic plasmid of *R. capsulatus*. It was mentioned above that cosmids corresponding to Xba-11, Xba-12′, and Xba-13 did not hybridize to any *Ase*I fragment. One possible explanation is that these clones are derived from a plasmid consisting of the three *Xba*I fragments and lacking an *Ase*I site. To check this possibility, 23 cosmids corresponding to Xba-11, Xba-11-12′, Xba-12′-13 and Xba-13-11 subcontigs were extracted and cut by *Eco*RV, *Hin*dIII, *Xba*I, and *Ase*I independently and in pairs. Direct fragment

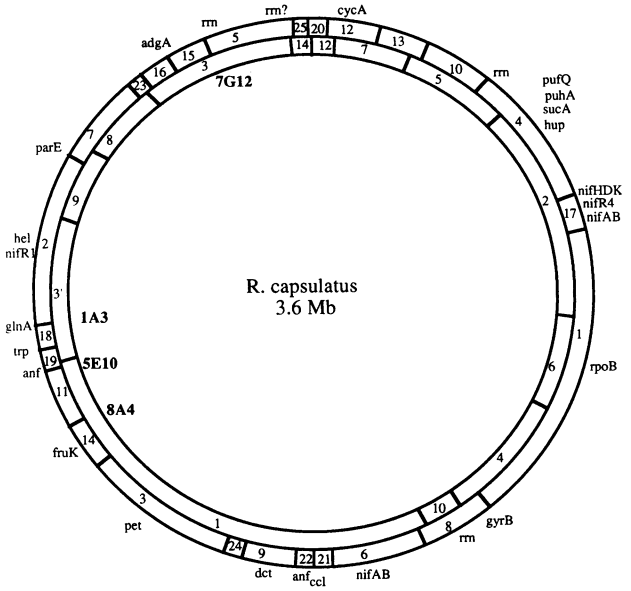

FIG. 6. Combined physical and genetic map of the *R. capsulatus* chromosome. The inner circle shows the *Xba*I sites; the outer circle shows the *Ase*I sites. Designations inside the inner circle indicate the cosmids used for linking the *Ase*I restriction fragments. The assignment of the fourth *rrn* operon to Ase-5 is tentative. See the text for further discussion of the gene locations.

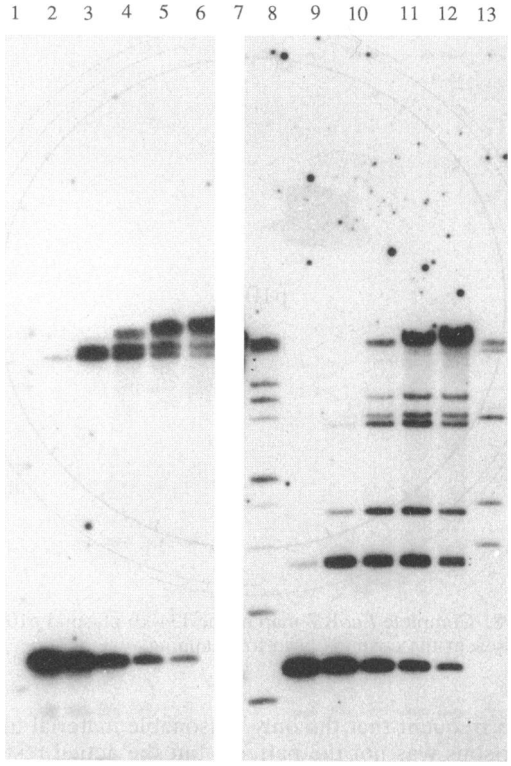

FIG. 7. Physical mapping of cosmids by partial digestion of *cos*-site-labeled fragments. Cosmid 3A10 DNA (0.5 μg) was linearized with purified lambda terminase, digested with various amounts (1, 0.5, 0.15, and 0.05 U) of *Eco*RV, and then annealed with either a labeled *cos*L (lanes 2 through 6) or a labeled *cos*R (lanes 8 through 12) fragment to provide a nested set of end-labeled, partially digested fragments. These were separated by electrophoresis with lambda DNA size standards (lanes 1, 7, and 13) and autoradiographed. The *Eco*RV restriction map of the original cosmid, in both directions from the *cos* site, was determined by measuring the fragment sizes.

alignment did not produce a unique circular restriction map. The ends of the inserts of cosmids 3A10, 1D7, 3F3, 6H1, 2A1, and 2B1 were therefore transcribed in vitro, and the RNA was used as probe for hybridization with other cosmids belonging to the group. This way, a minimum set of overlapping cosmids covering the presumable plasmid was established. Individual cosmids were cleaved with λ terminase, partially digested with *Eco*RV, annealed independently to labeled oligonucleotides complementary to *cos*L and *cos*R, and separated on 0.3% agarose gels. The extent of cleavage generating optimal intensity of all bands was achieved by titrating *Eco*RV, and the optimal amount of enzyme was found to be 0.1 to 0.03 U/μg of DNA (Fig. 7). The gels were dried and exposed to X-ray film, revealing the sequence of DNA fragments from each *cos* site to any *Eco*RV site of the cosmid. The *Eco*RV restriction map of the 134-kb plasmid in *R. capsulatus* SB1003 is presented in Fig. 8. The size of the plasmid agrees with that reported in reference 25.

DISCUSSION

The elements of the mapping strategy chosen were previously used in the construction of chromosomal maps of various microorganisms. Blot hybridization with eluted restriction fragments was used, for example, in mapping the chromosome of the cyanobacterium *Anabaena* sp. strain PCC 7120 (2). Linking hybrid molecules were first used to resolve uncertainties generated by other mapping techniques with *E. coli* (21). Our way, though similar in principle, differed in detail. The blot hybridization with restriction fragments produces clear results only if the overlap of restriction fragments generated by different enzymes is roughly equal (see Results for details). In some cases, it does not give a unique sequence for the restriction fragments. The hybridization of the cosmid library to all the restriction fragments gives an independent way of verifying the chromosomal map. The main source of uncertainties in both types of hybridizational mapping is internal homologies between different restriction fragments. Labeling the whole restriction fragments, we see all the cosmids or restriction fragments, not only those overlapping the studied region of the chromosome, but everything that carries any repeat that exists in the probe. If the repeat is a substantial portion of a restriction fragment, which in practice may happen with restriction fragments smaller than 50 kb, uncertainty in the map will result. The use of RNA transcripts of the ends of the inserts of a cosmid, giving us smaller probes and reducing the probability of labeling repeats, helped us solve this problem. In addition to confirmation and/or correction of the chromosomal map, the cosmid library produced 80 sets of cosmids corresponding to the restriction fragments and the restriction sites between them. The sequence of these sets was established by comparison with the existing physical map. These sets provide the first step towards the fine-structure map of the chromosome of *R. capsulatus*. Our preliminary plans were to establish the order of cosmids and in that way to generate at least a partial fine-structure map within the sets by comparing restriction patterns of cosmids. Unfortunately, even with the uncertainty in size measurements less than 2%, coincidences between fragment sizes

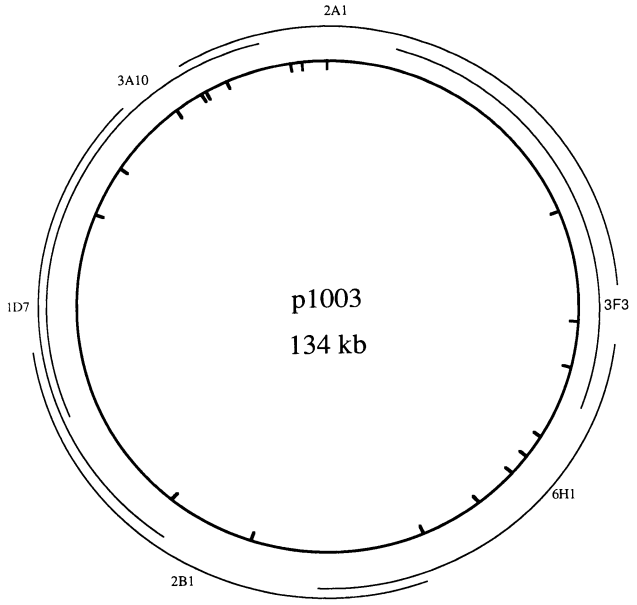

FIG. 8. Complete *Eco*RV map of the 134-kb plasmid p1003. The arcs represent the cosmids used for mapping.

were so frequent that the only reasonable material for such comparisons was not the patterns but the actual restriction maps of the cosmids. The most efficient way to construct such maps is "*cos* labeling." This logical development of the Smith-Birnstiel mapping procedure was proposed in the middle of the 1980s but was used successfully in only a few cases (29), probably because of the lack (until recently) of commercially available λ terminase of proper quality. Using this method, we determined the restriction map of the 134-kb plasmid of *R. capsulatus* relatively easily and are now mapping the chromosome.

A very important factor for such work is the representativity of the gene library consisting of 864 clones, which is equal to 10 genome equivalents for *R. capsulatus*. All 22 genes tested were found in the library, and the number of cosmids hybridizing to each fragment is in good correlation with the size of the fragment (Table 2). This confirms the absence of large gaps in the library. These results support the choice of cosmid Lorist 6 as the cloning vector. Unlike many ColE1 *ori* cosmids, the copy number of Lorist 6 depends very little on the insert sequences (6). Comparing restriction maps of 23 cosmids corresponding to the 134-kb plasmid, we found no rearrangements that characterize the ColE1 *ori* cosmid libraries. Therefore, the gene library, grouped into sets, gives us good starting material to generate a high-resolution map of the chromosome of *R. capsulatus*.

The total size of the genome (3.6 Mb) is less than that of *Rhodobacter sphaeroides* (4.5 Mb) (22) or of *E. coli* (4.7 Mb) (21). By means of physical mapping, we generated two stretches of mapped genes that were connected by comparison with the existing genetic map (24). Six genetic markers that existed on both maps are located in the same order and roughly separated by the same distances. Sixteen genes not mapped earlier were added to the existing genetic map by our study. What is more important, the hybridization panel of cosmids with both the location and the map position of each cosmid already known provides a way for easy mapping of any newly cloned gene.

The limited number of mapped genes makes it impossible to compare the genetic map of *R. capsulatus* with the map of its closest relative, *R. sphaeroides*, recently constructed by Suwanto and Kaplan (22). The striking difference between the two species is the presence in *R. sphaeroides* of a megaplasmid (or, as it was called, a second chromosome) carrying two *rrn* operons. The only plasmid found in our organism as an independent set of cosmids was p1003. The absence of smaller plasmids was confirmed by conventional electrophoresis (data not shown).

The existence of a correlated physical and genetic map and especially of a cosmid panel of *R. capsulatus* DNA will simplify mapping and cloning in that system. It will make possible the generation of functional (transcriptional) maps of gene families responding to general signals such as SOS or nitrogen starvation. The resolution of such studies can be improved by the construction of the higher-resolution physical map of the chromosome of *R. capsulatus*, which is now in progress.

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