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Physical Map of the Genome of *Planctomyces limnophilus*, a Representative of the Phylogenetically Distinct Planctomycete Lineage

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A physical map of the chromosome of *Planctomyces limnophilus* DSM 3776^T was constructed by pulsed-field gel electrophoresis techniques. A total of 32 cleavage sites for the rare-cutting restriction endonucleases *Pac*I, *Pme*I, and *Swa*I were located on the chromosome, which was shown to be circular and approximately 5.2 Mbp in size. An extrachromosomal element was detected but was found not to be cleaved by any of the enzymes used in the analysis of the chromosome. The order of the fragments on the chromosome was determined by hybridization of excised, labelled restriction fragments to Southern blots of pulsed-field gel electrophoresis-separated restriction digests. Seven genetic markers, *rrs*, *rrl*, *atpD*, *tuf*, *gyrB*, *rpoD*, and *dnaK*, on the chromosome were located by hybridization. Probes for all genetic markers were obtained by PCR. For five of these markers, probes were constructed by PCR with degenerate primers targeting conserved sequences. The arrangement of the genetic markers was compared with that found in other bacteria.

Planctomyces limnophilus is a member of the order *Planctomycetales*, a group of budding microorganisms lacking peptidoglycan and representing a phylogenetically distinct lineage within the domain *Bacteria*, as defined by rRNA sequence analysis (3, 23, 31). The ecology, phylogeny, and cell biology of the planctomycetes have been recently reviewed (11). Members of the order *Planctomycetales* have been observed in and isolated from aquatic habitats. The inability to recover some species, including the type species of the genus *Planctomyces*, *P. bekefii*, as pure and viable cultures led to the belief that the organisms are rare and relatively limited in distribution. However, recent studies using new cultivation methods (30), molecular identification of new isolates (36), and 16S rRNA gene (rDNA)-based molecular ecology studies (7, 21) have shown that planctomycetes are much more ubiquitous than previously thought and suggest that they may be essential contributors to nutrient cycling processes.

The planctomycetes share a number of morphological features, having spherical or ovoid cells which bear crateriform structures and, in some cases, nonprosthecate appendages. The presence of a membrane-bounded nuclear body, previously unknown in bacteria, has been demonstrated in the planctomycete *Gemmata obscuriglobus* (12). A number of unusual molecular features have been observed, including unlinked *rrn* operon organization in some species (20, 24) and a short 5S rRNA molecule consisting of 109 to 111 nucleotides (3).

Despite their unique properties, the planctomycetes are not well characterized genetically, and no information on the general genomic organization of planctomycete chromosomes has been reported. Physical genome maps have been constructed for representatives of most other bacterial phyla, and a conserved "backbone" structure of certain key housekeeping genes, to which most bacterial genomes conform, has been proposed (6). In this work, we present a physical map of the genome of *P. limnophilus* and show the locations of some housekeeping genes.

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MATERIALS AND METHODS

Preparation of DNA in agarose plugs. *P. limnophilus* DSM 3776^T was grown in PYGV medium (32) at 28°C with moderate shaking for 6 days. The cell material was recovered by centrifugation and resuspended in cell suspension buffer (CSB) (10 mM Tris [pH 7.2], 20 mM NaCl, 50 mM EDTA). Cell density was determined spectrophotometrically, and the suspension was diluted, with CSB, to an optical density at 600 nm of 0.8. The diluted cell suspension was mixed with an equal volume of 2% (wt/vol) low-melting-point agarose (Sigma, St. Louis, Mo.) and pipetted into plug molds (Bio-Rad, Hercules, Calif.) which were chilled at 4°C for 15 min. After removal from the molds, the plugs were incubated in five times their own volume of proteinase K reaction buffer (PKRB; 100 mM EDTA, pH 8.0; 0.2% [wt/vol] sodium deoxycholate; 1% [wt/vol] sodium lauryl sulfate; proteinase K [1 mg/ml; Boehringer, Mannheim, Germany]) at 50°C overnight. Residual proteinase K was removed by five successive washes, each of 1-h duration, of the plugs in wash buffer (20 mM Tris, pH 8.0; 50 mM EDTA). The plugs were stored in this buffer at 4°C.

Restriction endonuclease digestion of DNA in agarose plugs. Each DNA plug (75-μl volume) was equilibrated twice in 300 μl of 1× restriction endonuclease buffer (supplied with the enzyme by the manufacturer) at 4°C for 30 min. The buffer was replaced, and the DNA was incubated with 20 to 50 U of enzyme in a total volume of 200 μl at 4°C overnight. The reaction mixture was then incubated at the recommended temperature for digestion (usually 37°C) for 5 h, after which the buffer was removed and replaced with 0.5× TBE (1× TBE is 0.09 M Tris-borate–0.002 M EDTA). The restriction endonucleases used in these experiments were obtained from New England Biolabs (Beverly, Mass.) or Boehringer.

PFGE. For clamped homogeneous electric field (CHEF) electrophoresis, one-half of a digested plug was loaded onto the agarose gel and electrophoresis was performed in 0.5× TBE at 14°C with a Bio-Rad CHEF DRIII system. For transverse alternating-field electrophoresis, one-quarter of a digested plug was loaded onto the agarose gel and electrophoresis was performed in 1× TAFE buffer (0.2 M Tris-HCl; 10 mM EDTA [free acid], pH 8.3) at 8°C with a GeneLine system (Beckman, Palo Alto, Calif.). *Hanensula wingei* chromosomal pulsed-field gel electrophoresis (PFGE) marker (Bio-Rad), *Saccharomyces cerevisiae* chromosomal PFGE marker, lambda ladder PFGE marker, and low-range PFGE marker (all from New England Biolabs) were used as size standards. Specific electrophoresis conditions are described in the figure legends. DNA bands were visualized by staining gels with SYBR-Green (Molecular Probes, Eugene, Oreg.) and exposure to UV transillumination. Fragment sizes were

determined by comparison with the size standards. Bands were sized only in the linear portion of the calibration curve, and the electrophoresis conditions were adjusted to shift different bands into the linear range.

PCR amplification of genes used as probes in hybridization. PCR was performed with a model 480 thermal cycler (Perkin-Elmer, Norwalk, Conn.). All amplifications were performed in a total volume of 100 μl with template genomic DNA from *P. limnophilus*, with *Taq* polymerase, PCR buffer and deoxynucleoside triphosphates from Boehringer. Oligonucleotide primers were synthesized by Pharmacia (Roosendaal, The Netherlands). A summary of other PCR conditions is provided in Table 1.

Southern blotting of PFGE gels. PFGE-separated restriction fragments were transferred from the gels to nylon membranes (Boehringer) with a VacuGene vacuum blotter (Pharmacia, Uppsala, Sweden) by an alkaline transfer protocol. The gel was stained with SYBR-Green for 1 h, and the fragments were nicked by UV irradiation. Prior to fragment transfer, the gel was soaked in transfer buffer (0.4 M NaOH, 1.5 M NaCl) for 15 min. Transfer was performed for 1.5 h in transfer buffer. The membrane was neutralized by washing in 0.5 M Tris-HCl, pH 7.0, rinsed with 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 sodium citrate), and air dried, and the DNA was fixed by UV irradiation.

Preparation of labelled probes. Prior to being labelled, PCR products were purified by agarose gel electrophoresis, excision of the desired band, and recovery of the DNA with the Prep-A-Gene kit (Bio-Rad). Restriction fragments were purified by the following protocol. PFGE gels were stained with SYBR-Green for 30 min. The desired band was excised and recovered from the agarose with the Prep-A-Gene kit. Template DNAs for the *P. limnophilus* genomic DNA and lambda DNA probes were obtained by using Prep-A-Gene for the recovery of DNA from *P. limnophilus* agarose plugs and the lambda ladder PFGE marker, respectively. In all cases, the recovered DNA was digested with 10 U of *Sal*I for 1 h at 37°C and purified by successive extractions with phenol and chloroform and sodium acetate-ethanol precipitation. DIG-labelled probes were generated by random-prime labelling with the HighPrime DNA labelling kit (Boehringer), according to the manufacturer's instructions.

Hybridization. All hybridizations were performed at 68°C with "high-SDS" hybridization buffer (7% [wt/vol] sodium dodecyl sulfate [SDS]; 5× SSC; 50 mM sodium phosphate, pH 7.0; 0.1% [wt/vol] *N*-lauroyl sarcosine; 2% [wt/vol] blocking reagent) in a Hybaid hybridization oven. All hybridization, washing, and chemiluminescence detection steps were performed as recommended by Boehringer (2a).

RESULTS

Size and number of restriction fragments. Twenty restriction endonucleases were screened for their ability to cleave *P. limnophilus* DNA into a suitable number of resolvable restriction fragments. The enzymes chosen for screening were mostly those with AT-rich recognition sequences, because the DNA base composition of *P. limnophilus* is 53 mol% GC (13). The majority of enzymes produced fragments which were too numerous (>30) and too small (<150 kb) for use in the construction of a physical map. Digestion with the enzymes *Xba*I, *Spe*I, and *Nhe*I, which contain in their recognition sequences the tetranucleotide CTAG, rare in bacterial genomes, produced about 30 fragments in each case. Of these, three or four fragments were between 200 and 500 kb in size. The remainder of the fragments (20 to 30) were smaller than 150 kb and could not be accurately sized. The only enzymes which cleaved the DNA of *P. limnophilus* into fragments suitable for mapping were the 8-bp AT cutters *Pac*I (TTAATTAA), *Pme*I (GTTTA AAC), and *Swa*I (ATTTAAAT), which produced 5, 10, and 17 fragments, respectively. Fig. 1 shows banding patterns obtained after *Pac*I, *Pme*I, and *Swa*I digests were electrophoresed under different PFGE conditions. The use of a number of different conditions was necessary because of the large range in size of the fragments (10 to 1,890 kb) and the presence of some fragments that were very close in size (in one case there was only a 4-kb difference between fragments).

The estimated mean sizes of the fragments produced by each enzyme are listed in Table 2. The total size of the genome, derived by addition of the fragments produced by *Pme*I is about 200 kb smaller than that obtained by adding the fragments produced by *Pac*I and *Swa*I. The genome size of *P. limnophilus* was determined in this study to be 5.204 Mbp, the average of the sizes obtained from the three enzymes.

TABLE 1. PCR conditions used to amplify gene fragments for use as genetic markers

Gene	Gene function	Primer pair (5′→3′)[a]	Amt of primer (μg)	Buffer	dNTP[b] concn (mM)	Amt of *Taq* polymerase (U)	Description of cycles[c]	Approx fragment size (bp)	Reference for primers
rrs	16S rRNA	GAGTTTGATCCTGGCTCAG and AGAAAGGAGG TGATCCAGCC	0.5	1× PCR	0.2	2	HS + 28 cycles: 52°C 1 min, 72°C 2 min, 93°C 1 min	1,400	18
rl	23S rRNA	AGTAGYGGCGAGCGAA and TTCGGRGAGAACS AGMTA	0.5	1× PCR	0.2	2	HS + 28 cycles: 52°C 1 min, 72°C 2 min, 93°C 1 min	550	18
atpD	Membrane-bound ATP synthase, F_1 sector, β subunit	CCGTTYGTYCGYGGTGGT and TTCSAGRTARAT GAAYGCGTC	0.5	1× PCR	0.4	2.5	HS + 35 cycles: 57°C 2 min, 72°C 2 min, 94°C 2 min	550	This study[d]
tuf	Protein chain elongation factor, EF-Tu	ATATGCGGCCGCCATYGGHCACGTBGACCA and AAAATATGCGGCCGCTCNCCNGGCATNACC	0.5	1× PCR	0.2	2.5	HS + 35 cycles: 57°C 2 min, 72°C 2 min, 94°C 2 min	1,350	22
gyrB	DNA gyrase, subunit B	GAAGTCATCATGACCGTTCTGCAYGCNGGNGGN AARTTYGA and AGCAGGGTACGGATGTGCGAG CCRTCNACRTCNGCRTCNGTCAT	0.5	1× PCR	0.2	2.5	HS + 35 cycles: 57°C 2 min, 72°C 2 min, 94°C 2 min	1,300	40
rpoD	RNA polymerase, σ^{70} subunit	GGCTCGAGATHGCNAARMGNTA and CCGAATTC GCYTGNCKDATCCACC	0.8	1× PCR + 0.01% gelatin	0.4	2.5	HS + 35 cycles: 53°C 2 min, 72°C 2 min, 94°C 2 min	170	8
dnaK	Heat shock protein, DNA synthesis	CGGGATCCCGACNGTRCCRGCNTAYTTYAATGAY and GGAATTCCNGCNACNGCYTCRTCNGGRTT	5	1× PCR	0.2	2.5	HS + 30 cycles: 94°C, 1 min 30 s, 55°C 2 min, 72°C 1 min	700	35

[a] Abbreviations: B = C, G, or T; D = A, G, or T; H = A, C, or T; K = G or T; M = A or C; N = A, C, G, or T; R = A or G; S = C or G; Y = C or T.
[b] dNTP, deoxynucleoside triphosphate.
[c] HS, hot-start PCR. HS for *dnaK* was 94°C for 5 min prior to addition of *Taq* polymerase. HS for all other genes was 98°C for 3 min prior to addition of *Taq* polymerase.
[d] Based on the sequence data presented in reference 25.

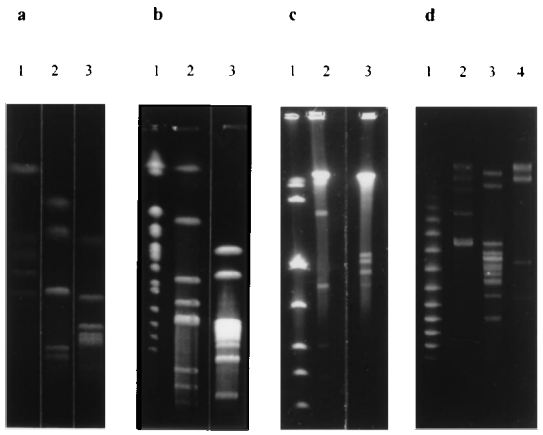

FIG. 1. The chromosome of *P. limnophilus* was analyzed by digestion with *Pac*I, *Pme*I, and *Swa*I followed by PFGE. IST, initial switch time; FST, final switch time. (a) Run parameters: CHEF; 0.8% (wt/vol) gel; run time, 48 h; field strength, 3 V/cm; IST, 300 s; FST, 400 s; included angle, 106°. Lanes: 1, *Hanensula wingei* chromosomal PFGE marker; 2, *Pac*I digest; 3, *Pme*I digest. (b) Run parameters: CHEF; 1% (wt/vol) agarose gel; run time, 22 h; field strength, 6 V/cm; IST, 30 s; FST, 120 s; included angle, 120°. Lanes: 1, *S. cerevisiae* chromosomal PFGE marker; 2, *Pme*I digest; 3, *Swa*I digest. (c) Run parameters: CHEF; 1% (wt/vol) agarose gel; run time, 15 h; field strength, 6 V/cm; IST, 2 s; FST, 4 s; included angle, 120°. Lanes: 1, Low-range PFGE marker; 2, *Pme*I digest; 3, *Swa*I digest. (d) Run parameters: transverse alternating-field electrophoresis 1% (wt/vol) agarose gel; run time, 22 h; field strength, 300 V; Switch time, 22 s. Lanes: 1, Lambda ladder PFGE marker; 2, *Pme*I digest; 3, *Swa*I digest; 4, *Pac*I digest.

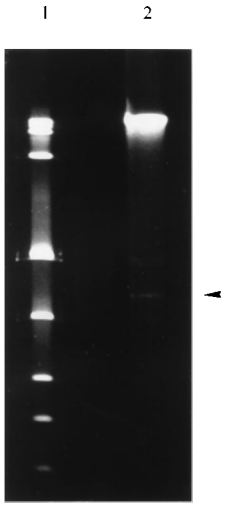

FIG. 2. PFGE of undigested *P. limnophilus* DNA. The presence of an extrachromosomal element is indicated by the arrowhead. Run parameters: CHEF; 1% (wt/vol) agarose gel; run time, 15 h; field strength, 6 V/cm; IST, 2 s; FST, 4 s; included angle, 120°. Lanes: 1, Low-range PFGE marker; 2, *P. limnophilus* DNA.

Electrophoresis of undigested chromosomal DNA with short pulse times revealed the presence of an extrachromosomal element (Fig. 2). This band was shown to be intact after digestion of the total DNA with each of the three mapping enzymes, demonstrating that the plasmid contains no cleavage sites for these enzymes and hence does not contribute to the cleavage patterns obtained from the chromosomal DNA.

Determination of fragment order. Hybridization of blotted PFGE gels with isolated restriction fragments was used to determine the order of the fragments produced by the three enzymes. The results of these hybridizations are listed in Table 3, and an example is provided in Fig. 3. The deduced physical map of the *P. limnophilus* genome is depicted in Fig. 4. Most of the fragments produced by *Pac*I, *Pme*I, and *Swa*I could be ordered by hybridization with probes derived from digestion with these three enzymes. The exceptions were the fragments SwC, SwF, and SwI, which could not be ordered because they coincided with the large fragments PaA and PmA. The order of these three *Swa*I fragments was determined by hybridization with probes derived from digestion with the enzymes *Xba*I, *Spe*I, and *Nhe*I.

The band hybridization experiments also demonstrated the circularity of the chromosome, which was finally revealed by the linkage of PaA and PaE, PmB and PmJ, and SwE2 and SwP.

Location of genetic markers of the physical map. Seven genes or gene fragments could be assigned to the physical map of *P. limnophilus* by hybridization experiments. The gene probes used are listed in Table 1, and the locations of the genes are provided in Table 3 and depicted in Fig. 4.

For the *rrs* genes, amplification of the entire gene was performed with conserved primers that are now almost routinely used in phylogenetic studies. Fragments of the *rrl* genes were amplified with the use of published primers targeting universally conserved regions (18). A PCR product of a portion of the *atpD* gene was generated with specific primers designed on the basis of previously determined sequence data (25). The remaining genetic markers, *tuf*, *gyrB*, *rpoD*, and *dnaK*, were created by PCR with published degenerate primers which bind to relatively conserved portions of these genes.

Each gene probe was assigned to a maximum-length region of the chromosome because of the present limited resolution of the physical map. These intervals ranged in size from approximately 10 kb (one *rrs* locus and *rrl*) to about 400 kb (one *dnaK* locus).

Two copies of the 16S (*rrs*) and 23S (*rrl*) rRNA genes are present on the physical map, and the genes coding for 16S rRNA are physically separate from those coding for 23S rRNA. The two *rrs* genes are separated from each other by a distance of approximately 1,400 kb, whereas the two *rrl* genes are located on the same 10-kb fragment. The 5S rRNA (*rrf*) gene(s) is separated from the *rrl* genes by an intergenic spacer region of

TABLE 2. Sizes of fragments obtained from digestion of *P. limnophilus* genomic DNA[a]

Fragment	Fragment size (kb)		
	*Pac*I	*Pme*I	*Swa*I
A	1,890	1,810	795
B	1,810	1,008	640
C	1,004	630	388
D	344	500	366
E1	250	431	351
E2			351
F		415	337
G		148	322
H		86	315
I		33	300
J		10	293
K			257
L			199
M			195
N			50
O			48
P			40

[a] Total sizes for *Pac*I, *Pme*I, and *Swa*I fragments were 5,298, 5,068, and 5,247 kb, respectively.

TABLE 3. DNA bands identified by isolated restriction fragments or genetic markers used as hybridization probes

Band or marker used as probe	Hybridizing target band(s)		
	*Pac*I	*Pme*I	*Swa*I
*Pac*I bands			
A	A	A, B, J	C, E, F, I, J, K, P
B	B	A, C, E, F, H, I	A, E, G, H, M, N
C	C	B, C, D, G	B, D, H, L
D	D	A	G, J, O
E	E	B	B
*Pme*I bands			
A	A, B, D	A	C, F, G, I, J, O
B	A, C, E	B	B, E, K, P
C	B, C	C	A, H
D	C	D	D, H, L
E	B	E	A, M, N
F	B	F	E, M
H	B	H	E
I	B	I	E, G
*Swa*I bands			
A	B	C, E	A
B	C, E	B, G	B
O	D	A	O
P	A	B	P
*Xba*I bands[a]			
A	B, C	C	A, H
B	A	A	F, I
1	A	A	C
2	A	B	E
*Spe*I bands			
A	C	D, G	B, L
B	B	E, F	A, M, N
C	A	A	C
E	A	A	F
G	A	B	E, K, P
*Nhe*I bands			
A	A	B	E, K, P
B	A	A	F, I
Genetic markers			
rrs	A, B	A, E	I, M
rrl rrf	A	J	E
atpD	B, D	A, F	J, M
gyrB	B	E	A
rpoD	B	F	E
dnaK	A, C	A, B	B, C
tuf	A	B	E, K

[a] *Xba*I bands 1 and 2 were obtained after hybridization of *Xba*I digests with SpC. (These probes demonstrate the linkage of SwC and SwE.)

approximately 300 bp, as indicated by PCR amplification using primers targeting the 3′ end of *rrl* and the 5′ end of *rrf* (data not shown). The distances between the *rrl-rrf* gene cluster and the two *rrs* genes are approximately 900 and 2,400 kb.

The genome of *P. limnophilus* contains two loci for each of the genetic markers *atpD*, *tuf*, and *dnaK*. The two *tuf* genes are quite closely linked (not more than 600 kb apart), whereas the *atpD* and *dnaK* genes are separated by about 1,000 and 900 kb, respectively. There is only one locus for *rpoD* and *gyrB*. The copy number of each of these genetic markers was confirmed by digestion of *P. limnophilus* genomic DNA with frequently cutting restriction endonucleases such as *Hin*dIII and *Eco*RI followed by hybridization with each marker (data not shown).

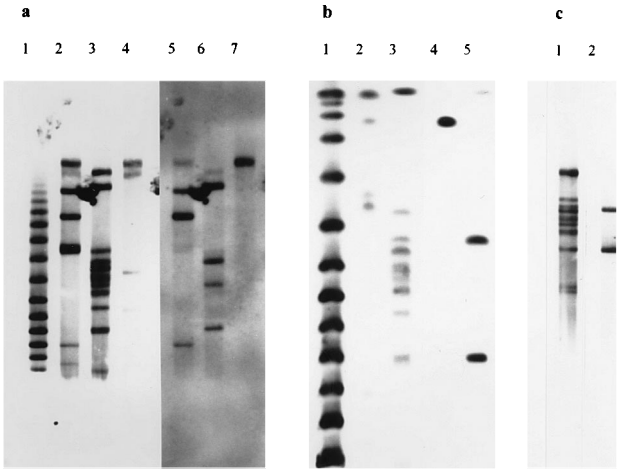

FIG. 3. Southern hybridization of digested *P. limnophilus* DNA with excised, labelled restriction fragments. (a) Lanes 1 to 4, *P. limnophilus* genomic DNA/lambda DNA probe; lanes 5 to 7, PaC probe. Hybridizations with lambda ladder PFGE marker (lane 1), *Pme*I digest (lanes 2 and 5), *Swa*I digest (lanes 3 and 6), and *Pac*I digest (lanes 4 and 7) are shown. (b) Lanes 1 to 3, *P. limnophilus* genomic DNA/lambda DNA probe; lanes 4 to 5, PmD probe. Hybridizations with lambda ladder PFGE marker (lane 1), *Pme*I digest (lanes 2 and 4), and *Swa*I digest (lanes 3 and 5) are shown. (c) Lane 1, *P. limnophilus* genomic DNA/lambda DNA probe; lane 2, NhA probe. Hybridizations with *Swa*I digest are shown.

DISCUSSION

A physical map of the genome of *P. limnophilus* DSM 3776^T with sites for three restriction endonucleases has been constructed. To our knowledge this is the first genome restriction map of a member of the order *Planctomycetales*. The chromosome of *P. limnophilus* has been shown to be circular, as is the case with most of the bacterial genomes previously investigated (6, 10). *P. limnophilus* contains an extrachromosomal element;

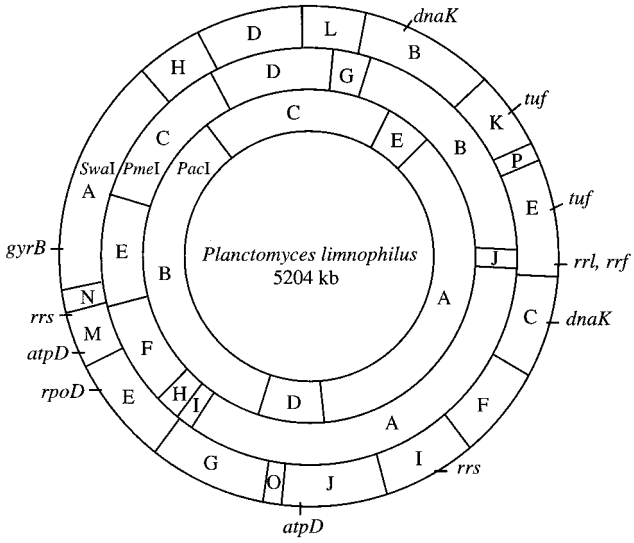

FIG. 4. Deduced physical map of the chromosome of *P. limnophilus* DSM 3776^T, showing restriction sites for enzymes *Pac*I, *Pme*I, and *Swa*I. The locations of several genetic markers are also shown.

there have been no previous reports of plasmids in planctomycetes. The deduced size of the *P. limnophilus* genome is 5.204 Mb. The discrepancy between the total genome sizes obtained by summation of the *Pme*I fragments and those of the other two enzymes may arise from the presence of comigrating bands, from anomalous migratory behavior of some *Pme*I fragments, or from the loss of fragments smaller than 20 kb by diffusion from the stored agarose plugs. The presence of comigrating bands in the *Pme*I digest was checked by a *Pme*I-*Swa*I double digest, followed by two dimensional PFGE; no such doublet bands were found (data not shown).

The deduced mean genome size, 5.204 Mb, is significantly larger than the 4.2 Mb previously determined by thermal renaturation methods (15). A similar phenomenon has been reported in studies of *Mycoplasma* genome size (28).

The genome size of *P. limnophilus* is positioned midway in the scale of bacterial genome sizes, which range from 0.6 Mb for *Mycoplasma genitalium* to 9.5 Mb in *Myxococcus xanthus* (6, 10). A relatively large genome, facilitating adaptation to changing conditions, has been suggested to be necessary for bacteria which live in nutrient-poor or fluctuating environments (29). Eutrophic lakes, such as that from which *P. limnophilus* was isolated (13), could be considered to constitute such a demanding environment.

In the majority of bacterial genome studies, the markers used have been cloned and characterized genes from the organism under investigation. Such genes are not currently available for *P. limnophilus*. An alternative is the use of heterologous probes from other bacteria. However, it was anticipated that the large phylogenetic distance between the planctomycetes and other bacteria could hinder the use of heterologous probes, which might not contain sufficient homology to the target sequences. Therefore, genetic markers were generated from *P. limnophilus* genomic DNA by PCR amplification of the target genes. The use of degenerate primers to obtain sequence data of housekeeping genes for use in phylogenetic studies is becoming more common (40). However, this approach has not been previously used for the generation of genetic markers for use in genome mapping, although it has recently been suggested (10). The ability of these primers to bind to the DNA of planctomycetes, which are phylogenetically distant from the bacteria whose sequences were used as the basis for primer design, suggests a high degree of conservation of parts of these genes. The rapidly growing database of sequence data generated from genome sequencing projects with *Escherichia coli*, *Bacillus subtilis*, *Haemophilus influenzae*, and *Mycoplasma* species will provide a valuable resource for the design of PCR primers for other conserved genes. The use of homologous PCR-generated probes such as those described here has the added advantage of avoiding the tedious optimization of hybridization conditions required when heterologous probes are used. In addition, the PCR-amplified genes are available for further study such as microrestriction analysis or sequencing.

The number and distribution of rRNA genes and the unlinked nature of these genes found in this study are in agreement with the results of the ribotyping study of *P. limnophilus* (24). This rRNA gene organization contrasts with that of some other organisms reported to have unlinked rRNA genes and for which genomic maps have been constructed. Borges and Bergquist (4) reported that in *Thermus thermophilus*, which carries two copies of each of the rRNA genes, one *rrs* gene is linked to an *rrl* and an *rrf* gene while the other is distantly located from the second *rrl-rrf* cluster. In *Campylobacter jejuni*, two of three *rrs* genes are proximal to one *rrl* gene while the third *rrs* gene is linked to another *rrl* gene and a third *rrl* gene stands alone (34).

The genome of *P. limnophilus* contains two loci for the *tuf* genes, as has been found in studies of some other bacteria (4). Duplication of the *tuf* gene in gram-negative bacteria has been previously reported (9).

The location of these genetic markers in the chromosome of *P. limnophilus* can be compared with their organization in other bacteria for which physical and/or genetic maps have been constructed. In a recent review of bacterial genomics, Cole and Saint Girons (6) demonstrated the presence of a backbone of key housekeeping genes, the position of which is conserved in the genomes of most bacteria investigated so far. When the maps are aligned in a linear form around the origin of replication (defined by *gyrB* or *dnaA*, linked to a *rrn* operon), it is seen that the rRNA genes are located on either side of the origin, usually with unequal distribution, and transcribed in the direction away from the origin. If the location of *gyrB* is assumed to represent the origin of replication for *P. limnophilus*, then it can be seen that the rRNA genes are located on only one side of the origin instead of being flanking (Fig. 4). One *rrs* locus is quite closely linked to the origin. It appears that this arrangement has not been found in the bacterial genomes investigated so far.

Comparing the genomic organizations for genes other than *rrn* between bacteria that are only distantly related is difficult. This is because the choice of genes for mapping studies usually reflects genetically well-studied auxotrophic markers and/or characteristic properties of the organism, such as virulence factors, degradative enzymes, or unusual metabolic pathways rather than key housekeeping genes (10). The absence of a putative origin of replication in many maps further hinders comparative analysis. For this reason, it is not presently possible to determine a universal organization for genes such as *atpD*, *tuf*, *rpoD*, and *dnaK*. However, some comparisons between the genome of *P. limnophilus* and those of selected other bacteria can be drawn. For example, in *P. limnophilus*, *rpoD* is located close to the putative origin, as in *E. coli* (1), whereas *rpoD* in *B. subtilis* (41), *Rhodobacter sphaeroides* (33), and *Clostridium perfringens* (14) is found almost opposite the origin. *rpoD* in *P. limnophilus* is closely linked to *rrl*, as is the case with *Campylobacter* species (34). One of the *atpD* loci in *P. limnophilus* is close to the origin, as in *E. coli* (1), *Mycoplasma mycoides* strains (28), *Mycoplasma pneumoniae* (38), and *Mycoplasma genitalium* (27). It is interesting that in *P. limnophilus*, each *atpD* gene is near an *rrs* locus, suggesting a possible duplication of the whole chromosomal segment containing these two genes. The large repeated elements of the *rrn* loci have been suggested as good candidates for intrachromosomal homologous recombination, and many intraspecies chromosomal variations are found between *rrn* loci (5, 19, 29). In addition, extra copies of the region of the chromosome close to the origin of replication have been reported (16). Linkage of *atpD* and *rrs* is also seen in *Campylobacter* species (34) and *Salmonella typhimurium* (39).

In *B. subtilis* (41) and *Borrelia* species (26), *dnaK* is closely linked to the origin, while in *P. limnophilus* it is found directly opposite the origin, as it is in *M. genitalium* (27). Although the reported physical map of *Chlamydia trachomatis* does not show a putative origin of replication (2), if the origin is tentatively placed at the single *rrn* locus (the two are often linked in organisms with only one *rrn* operon), then the *dnaK* gene appears to also be opposite to the origin. The *tuf* genes of *E. coli* (1), *B. subtilis* (41), *R. sphaeroides* (33), *M. pneumoniae* (38), *M. genitalium* (27), and *C. perfringens* (14) are similarly centrally located, whereas they are distal in *P. limnophilus*, as is also found for *Mycoplasma hominis* strains (17).

There is an apparent similarity in some features of genome

architecture between *P. limnophilus*, *C. trachomatis*, and some *Mycoplasma* species. These organisms share a common feature: the lack of a true (peptidoglycan-containing) cell wall and, in the case of *Mycoplasma* spp., complete absence of a cell wall. In addition, on the basis of 16S rDNA sequence analysis, the planctomycetes and chlamydiae are phylogenetically each other's closest neighbors (37). However, comparative genome analysis of members of these three taxa can only be done with confidence when a greater number of genetic markers have been assigned to the maps. Alternatively, on the basis of the comparisons provided here and elsewhere (6) it could be predicted that, with the exception of the *rrn* genes, there may be no genomic organization conserved between phylogenetically unrelated bacteria for the genes used in this study. Many inter- and intraspecies studies have revealed conserved organization of these genes. It is therefore possible that comparison of genomic organization is only worthwhile for taxa below the level of species.

The results of this study have provided a physical map of the genome of *P. limnophilus*, to which several genetic markers have been assigned. This map will serve as a tool for the future study of genomic organization in this organism. In addition, similar studies of the architecture of other planctomycete genomes could reveal whether some of the features described here for *P. limnophilus* are shared by other planctomycetes. As the field of bacterial genomics expands and more data become available for comparison, it should be possible to determine which features of genome architecture are conserved at different taxonomic levels.

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