Development of Techniques To Genetically Manipulate Members of the Genera *Cytophaga*, *Flavobacterium*, *Flexibacter*, and *Sporocytophaga*

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The *Bacteroides*-*Cytophaga*-*Flavobacterium* branch of the eubacterial phylogenetic tree contains a diverse group of bacterial species. Techniques for the genetic manipulation of *Bacteroides* spp. are well developed (A. A. Salyers, N. B. Shoemaker, and E. P. Guthrie, Crit. Rev. Microbiol. 14:49–71, 1987). Recently we developed techniques to genetically manipulate the gliding bacterium *Cytophaga johnsonae* (M. J. McBride and M. J. Kempf, J. Bacteriol. 178:583–590, 1996). We now demonstrate that some of these techniques allow genetic manipulation of a number of environmentally or medically significant bacteria in this group. The *Bacteroides* transposon Tn4351 was introduced into *Cytophaga hutchinsonii*, *Cytophaga succinicans*, *Flavobacterium meningosepticum*, *Flexibacter canadensis*, *Flexibacter* sp. strain FS1, and *Sporocytophaga myxococcoides* by conjugation. Tn4351 integrated itself into the host chromosomes and conferred erythromycin resistance. We isolated several auxotrophic mutants of *Flavobacterium meningosepticum* following Tn4351 mutagenesis. The *C. johnsonae*-*Escherichia coli* shuttle vector pCP11 functioned in *C. succinicans* but not in the other bacteria. pLYL03 did not replicate in any of these bacteria and should function as a convenient suicide vector. The identification of a system of gene transfer, a selectable marker, a suicide vector, and a transposon that functions in these diverse bacteria allows genetic manipulations to be performed.

The *Cytophaga*-*Flavobacterium*-*Bacteroides* subphylum of the eubacterial phylogenetic tree is composed of a large and diverse group of organisms (13). Members of this group include nonmotile bacteria belonging to genera such as *Bacteroides* and *Flavobacterium* and motile gliding bacteria belonging to genera such as *Cytophaga*, *Flexibacter*, *Sporocytophaga*, and *Capnocytophaga*. These bacteria are of interest because of their biodegradative abilities (14, 21, 29, 39), their ability to produce bioactive compounds (10, 12, 16, 18, 19, 38, 40), and their capacity to cause disease in animals and humans (3, 6–8, 23, 24, 33). *Cytophagas* and related bacteria are also of interest because they are among the most common of the bacteria that display gliding motility, the movement of cells over surfaces without the aid of flagella. The mechanism of bacterial gliding motility is not known.

Until recently, techniques for genetic manipulation of bacteria in the *Cytophaga*-*Flavobacterium*-*Bacteroides* group were available only for members of the genus *Bacteroides* (31) and related bacteria in the genera *Prevotella* (34) and *Porphyromonas* (11). We developed techniques to genetically manipulate the gliding bacterium *Cytophaga johnsonae* (25). We found that the *Bacteroides* transposon Tn4351 functions in *C. johnsonae* and constructed several *C. johnsonae* cloning vectors. In this study we extend the usefulness of some of these genetic tools to other members of the genus *Cytophaga* and to members of the related genera *Flexibacter*, *Flavobacterium*, and *Sporocytophaga*.

Bacterial strains and growth conditions. The organisms and plasmids used in this study are listed in Table 1. *Escherichia coli* strains were routinely grown in Luria-Bertani (LB) me-

dium (32) at 37°C. *Cytophaga succinicans*, *Flavobacterium meningosepticum*, *Flexibacter canadensis*, and *Flexibacter* sp. strain FS1 were grown in CYE broth (25). For solid CYE or LB medium 15 g of agar was added per liter of medium. Cultures of *C. succinicans*, *Flexibacter canadensis*, and *Flexibacter* sp. strain FS1 were incubated at 30°C. Cultures of *Flavobacterium meningosepticum* were incubated at 37°C unless indicated otherwise. *Cytophaga* minimal medium (CMM) was used to identify auxotrophic mutants of *Flavobacterium meningosepticum*. CMM consisted of 22 mM glucose, 7.6 mM (NH₄)₂SO₄, 0.8 mM MgSO₄, and 15 g of agar per liter. A trace salts solution (27 mM CaCl₂, 11.8 mM MnCl₂, 7.2 mM FeSO₄, and 0.174 mM ZnSO₄, all in 0.1 N HCl) (2.5 ml) was added per liter after autoclaving. *Flavobacterium meningosepticum* was incubated at 30°C on CMM because of its poor growth on this minimal medium at 37°C. *C. hutchinsonii* and *Sporocytophaga myxococcoides* were grown on a medium (DMA filter paper) that consisted of 5.8 mM NaNO₃, 2.0 mM MgSO₄, 6.7 mM KCl, 36 µM FeSO₄, 0.6 mM NaHCO₃, 1.0 mM KPO₄, and 10 g of agar per liter. The pH of this medium was adjusted to 7.3. A piece of sterile Whatman filter paper was placed on each plate to supply the carbon and energy source for these cellulolytic bacteria. *C. hutchinsonii* and *S. myxococcoides* were incubated at 26°C. Antibiotics when needed were used at the following concentrations (in micrograms per milliliter): ampicillin, 100; tetracycline, 15; streptomycin, 50; and rifampin, 25. Because of differences in sensitivity to erythromycin, we used this antibiotic at different concentrations for the different bacterial strains. Erythromycin was used at the following concentrations (in micrograms per milliliter): *Cytophaga hutchinsonii* and *S. myxococcoides* (30 to 60), *C. succinicans* (60), *Flexibacter canadensis*, 100; *Flexibacter* sp. strain FS1 (100 to 200); and *Flavobacterium meningosepticum*, 200.

Conjugation. Rifampin resistance was used as a counter-selectable marker for many conjugation experiments. Rifam-

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TABLE 1. Organisms and plasmids used in this study

Strain or plasmid	Genotype or description	Reference or source
Bacterial strains		
<i>E. coli</i>		
S17-1	<i>hsdR17</i> ($r_K^- m_K^-$) <i>recA</i> RP4-2 (Tc ^r ::Mu-Km ^r ::Tn7 Str ^r)	37
HB101	λ^- <i>recA13 proA2 leu lacY1 galK2 xyl-5 mtl-1 ara-14 F^- hsdS20</i> ($r_B^- m_B^+$) <i>supE44 rpsL20</i>	5
<i>C. succinicans</i>		
RL-8	Wild type	2
CSR1	Rifampin-resistant mutant of <i>C. succinicans</i> RL-8	This study
<i>C. hutchinsonii</i> ATCC 33406	Wild type	ATCC ^a
<i>Flexibacter canadensis</i>		
ATCC 29591	Wild type	ATCC
FCR1	Rifampin-resistant mutant of <i>Flexibacter canadensis</i> ATCC 29591	This study
<i>Flexibacter</i> sp.		
FS1	Wild type	36
FSR1	Rifampin-resistant mutant of <i>Flexibacter</i> sp. strain FS1	This study
FSR3D	Tn4351 transconjugant of <i>Flexibacter</i> sp. strain FSR1	This study
FSR3F	Tn4351 transconjugant of <i>Flexibacter</i> sp. strain FSR1	This study
FSR3H	Tn4351 transconjugant of <i>Flexibacter</i> sp. strain FSR1	This study
FSR3L	Tn4351 transconjugant of <i>Flexibacter</i> sp. strain FSR1	This study
FSR3O	Tn4351 transconjugant of <i>Flexibacter</i> sp. strain FSR1	This study
<i>Flavobacterium meningosepticum</i>		
ATCC 13253	Wild type	ATCC
FMR1	Rifampin-resistant mutant of <i>Flavobacterium meningosepticum</i> ATCC 13253	This study
FMR3I	Tn4351 transconjugant of <i>Flavobacterium meningosepticum</i> FMR1	This study
FMR3J	Tn4351 transconjugant of <i>Flavobacterium meningosepticum</i> FMR1	This study
FM-Aux1	Tn4351-induced auxotrophic mutant of <i>Flavobacterium meningosepticum</i> ATCC 13253	This study
FM-Aux2	Tn4351-induced auxotrophic mutant of <i>Flavobacterium meningosepticum</i> ATCC 13253	This study
FM-Aux3	Tn4351-induced auxotrophic mutant of <i>Flavobacterium meningosepticum</i> ATCC 13253	This study
<i>S. myxococcoides</i> ATCC 10010	Wild type	ATCC
Plasmids		
R751	IncP; Tp ^r	26
R751::Tn4351 Ω 4	IncP; Tp ^r ::Em ^{tb} Tc ^r	35
pCP11	<i>E. coli</i> - <i>C. johnsonae</i> shuttle plasmid; Ap ^r Em ^{tb}	25
pVOH1	ColE1; Ap ^r Cm ^r Tc ^r Em ^{tb}	15
pLYL03	ColE1; Ap ^r Em ^{tb}	22

^a ATCC, American Type Culture Collection, Rockville, Md.^b Em^r not expressed in *E. coli*.

pin-resistant mutants of *C. succinicans*, *Flavobacterium meningosepticum*, *Flexibacter canadensis*, and *Flexibacter* sp. strain FS1 were obtained by plating approximately 10^9 cells on CYE medium containing 25 μ g of rifampin per ml. *E. coli* S17-1 (37) was used to mobilize pCP11, and HB101 was used to mobilize R751::Tn4351 Ω 4. Donor strains of *E. coli* were grown to mid-exponential phase (approximately 2×10^8 cells per ml) in LB. Recipient strains of *C. succinicans* CSR1, *Flavobacterium meningosepticum* FMR1, *Flexibacter canadensis* FCR1, and *Flexibacter* sp. strain FSR1 were grown to mid-exponential phase (approximately 2×10^8 cells per ml) in CYE at 30°C. Cells were harvested by centrifugation and mixed together (1:1 ratio), and approximately 10^9 cells were plated on nonselective CYE medium solidified with 20 g of agar per liter as previously described (25). After incubation overnight at 30°C, cells were scraped off of the plates, diluted in CYE, and plated on CYE agar containing 25 μ g of rifampin per ml and 60 to 200 μ g of erythromycin per ml to select for plasmid (pCP11) or transposon (Tn4351) transfer. Colonies were counted after 2 to 4 days of incubation at 30°C.

Conjugation experiments with the cellulolytic bacteria *C. hutchinsonii* and *S. myxococcoides* were performed somewhat differently. Rifampin-resistant mutants of *C. hutchinsonii* and *S. myxococcoides* were not isolated. We relied instead on the inability of the *E. coli* donor cells to grow with filter paper as a carbon and energy source and on the sensitivity of *E. coli* to erythromycin. (The donor cells carry the *ermF* gene on Tn4351, but this gene is not expressed in *E. coli*.) We did not work with liquid cultures of *C. hutchinsonii* and *S. myxococcoides* because of their poor growth on soluble substrates such as glucose and cellobiose. Instead, cells were spotted onto DMA filter paper medium and incubated for 2 days at 26°C to allow visible growth to appear. *E. coli* donor cells were prepared as described above, and approximately 10^9 donor cells were spotted on the *C. hutchinsonii* or *S. myxococcoides* cells. Plates were incubated for 18 h at 26°C to allow conjugative transfer. The filter paper was then removed from the nonselective DMA agar and transferred to DMA agar containing 30 to 60 μ g of erythromycin per ml. Transconjugants were observed after 5 or 6 days as bright yellow spots of growth on the filter paper and

TABLE 2. Transfer of Tn4351 into members of the genera *Cytophaga*, *Flavobacterium*, *Flexibacter*, and *Sporocytophaga*^a

Recipient	Efficiency of transfer (transconjugants/ recipient cell)	No. of transconjugants that contained R751 in addition to Tn4351/ total no. of trans- conjugants ^b
<i>Flexibacter canadensis</i> FCR1	3.6×10^{-9}	0/5
<i>Flexibacter</i> sp. strain FSR1	3.3×10^{-6}	11/17
<i>C. succinicans</i> CSR1	3.2×10^{-5}	15/15
<i>Flavobacterium meningosepticum</i> FMR1	7.0×10^{-6}	6/12
<i>C. hutchinsonii</i>	ND ^c	8/10
<i>S. myxococcoides</i>	ND	6/6

^a The *Bacteroides* transposon Tn4351 was transferred from *E. coli* by conjugation on the plasmid R751.

^b Colony blots were performed to determine whether individual erythromycin-resistant transconjugants carried R751 integrated into their chromosomes. All transconjugants carried Tn4351.

^c ND, not determined. Efficiency of transfer could not be calculated because the experimental protocol did not allow us to determine the number of recipient cells for conjugation experiments with *C. hutchinsonii* and *S. myxococcoides*.

were streaked on fresh DMA filter paper medium containing erythromycin.

Colony hybridizations and Southern blots. Colony hybridizations were used to verify the presence or absence of the transposon Tn4351 in the transconjugants. Cells were spotted onto Magna NT nylon membranes (Micron Separations Inc.) with toothpicks. The membranes were incubated in 0.5 M NaOH for 5 min to lyse the cells. Next they were washed two times for 3 min each with the following series of solutions: (i) 0.5 M Tris (pH 7.4), (ii) 2× SSPE, (iii) 95% ethanol, and (iv) 2× SSPE. 1× SSPE consisted of 1 mM EDTA and 180 mM NaCl in 10 mM sodium phosphate buffer (pH 7). Nucleic acid was cross-linked to the membranes with a UV crosslinker (Ultra-Lum Inc., Carson, Calif.). For Southern blots, chromosomal DNA was isolated, digested with restriction enzymes, separated by gel electrophoresis, and transferred to nylon membranes by standard procedures (32). Colony blot or Southern blot hybridizations were performed in a solution containing 50% formamide, 0.25% skim milk, and 6× SSPE for 18 h at 42°C. Duplicate blots were probed separately with radiolabeled pVOH1 (to detect Tn4351 sequences) and R751 (to detect the transposon delivery vector). pVOH1 is a derivative of pBR328 that carries Tn4351 but has no sequences in common with our transposon delivery vector, R751 (15). The radiolabeled probes were prepared by using the Prime-a-Gene random labeling kit (Promega). Following hybridization, blots were washed at 55°C in 10 mM sodium phosphate buffer (pH 7.0) containing 10 g of sodium dodecyl sulfate per liter.

Tn4351 confers erythromycin resistance on members of the genera *Cytophaga*, *Flavobacterium*, *Flexibacter*, and *Sporocytophaga*. Since Tn4351 functions in *Bacteroides* spp. (31) and in three different isolates of *C. johnsonae* (25), we suspected that it might be generally useful in other members of this large group of bacteria. We introduced Tn4351 into *C. hutchinsonii*, *C. succinicans* CSR1, *Flavobacterium meningosepticum* FMR1, *Flexibacter canadensis* FCR1, *Flexibacter* sp. strain FSR1, and *S. myxococcoides* by conjugation with *E. coli* as described above. Erythromycin-resistant colonies were obtained in each case (Table 2). We attempted to isolate plasmid DNA from four transconjugants of each strain by standard procedures (32). Free plasmid was not found, indicating that R751 does not replicate stably in any of them (data not shown). It has

previously been demonstrated that R751 is not maintained by *Bacteroides* spp. (1, 35) or by *C. johnsonae* (25).

Colony blot hybridizations confirmed that the transconjugants contained Tn4351 (Fig. 1A). Note that DNA from every transconjugant hybridized with radiolabeled pVOH1, indicating that Tn4351 was present in each case (Fig. 1A, spots 3 to 6). pVOH1 did not hybridize with DNA from any of the recipient strains before conjugation (Fig. 1A, spots 2).

We have previously observed that Tn4351 frequently causes insertions of the vector, R751, into the genome of *C. johnsonae* (25). Similar results were obtained with *Bacteroides* spp. (1, 31, 35, 41). Such events probably occur by transposition of IS4351 (or Tn4351) followed by recombination with R751:Tn4351, resulting in a single large insertion in the chromosome. Insertions of R751 were also observed with most of the strains of *Cytophaga*, *Flavobacterium*, *Flexibacter*, and *Sporocytophaga* tested in this study (Fig. 1B and Table 1). The frequency of R751 insertions among transconjugants was strain dependent. All 15 of the *C. succinicans* transconjugants that we examined carried R751 DNA along with Tn4351 inserted in the host genome. At the other extreme, none of the five Tn4351 transconjugants of *Flexibacter canadensis* that we tested carried vector sequences. For most of the other strains, we found some transconjugants that carried just Tn4351 and others that carried Tn4351 plus R751.

Introduction of Tn4351 into *Flavobacterium meningosepticum* results in mutations. The results presented above indicate that DNA can be transferred from *E. coli* into members of the genera *Cytophaga*, *Flavobacterium*, *Flexibacter*, and *Sporocytophaga*. They also demonstrate that the *ermF* gene of Tn4351 functions in distantly related bacteria belonging to these genera. Tn4351 should be a useful tool to generate mutations in these bacteria. We used Tn4351 mutagenesis to isolate auxotrophic mutants of *Flavobacterium meningosepticum*. We transferred 200 erythromycin-resistant transconjugants to CYE and CMM media containing erythromycin and identified three auxotrophic mutants. These mutants grew well on the complex medium (CYE) but failed to grow on the minimal medium (CMM). In a control experiment, 300 colonies of *Flavobacterium meningosepticum* that did not carry Tn4351 were transferred to CMM, and all exhibited growth. This suggests that the Tn4351 insertions were responsible for the phenotypes of the three auxotrophic mutants described above.

Southern blot analyses of Tn4351 insertions. We examined the randomness of Tn4351 insertions by performing Southern blot analyses of transconjugants of *Flexibacter* sp. strain FSR1 and *Flavobacterium meningosepticum*. For these analyses we selected five transconjugants of *Flexibacter* sp. strain FSR1 which contained Tn4351 but did not harbor R751 sequences. We also selected five transconjugants of *Flavobacterium meningosepticum*, including the three auxotrophic mutants described above. Colony blot hybridization indicated that all of the transconjugants contained Tn4351 and that *Flavobacterium meningosepticum* FM-Aux3 also contained R751 DNA, whereas the other transconjugants did not (data not shown). Chromosomal DNA isolated from each transconjugant of *Flexibacter* sp. strain FSR1 and *Flavobacterium meningosepticum* was digested separately with restriction enzyme *EcoRI* or *EcoRV*. Fragments were separated by gel electrophoresis, transferred to membranes, and probed with radiolabeled pVOH1 to detect Tn4351 sequences. Digestion with *EcoRI* should give rise to two bands that hybridize with radiolabeled Tn4351 if a simple insertion of Tn4351 (either as a single Tn4351 unit or in tandem as shown in Fig. 2A) had occurred. One band should migrate at 3.9 kb, and the size of the other band should depend on the site of insertion in the chromo-

FIG. 1. Detection of Tn4351 sequences in transconjugants by colony blot hybridizations. Tn4351 was transferred on R751 from *E. coli* to each of the recipients. DNA from four erythromycin-resistant transconjugants of each strain was transferred to nylon membranes (in duplicate) and probed with radiolabeled pVOH1 (A) to detect Tn4351 sequences and with radiolabeled R751 (B) to detect delivery vector sequences. Spots 1, R751::Tn4351Δ4 in *E. coli* (positive control for transposon and delivery vector); spots 2, wild-type recipient cells before conjugation (negative control); spots 3 to 6, erythromycin-resistant transconjugants.

some. More-complicated insertions involving integration of R751 could give rise to three or more bands after digestion with *Eco*RI. Most of the samples digested with *Eco*RI gave rise to only two bands (Fig. 2B, lanes 2 to 6; Fig. 2C, lanes 2 to 5). The restriction pattern was different for each transconjugant, indicating that Tn4351 had inserted itself at different locations in the chromosome. There are no *Eco*RV sites in Tn4351. Simple random insertions of Tn4351 should give rise to single *Eco*RV fragments that hybridize with Tn4351. The sizes of the fragments should depend on the sites of insertions. In contrast, transposition of Tn4351 followed by recombination to integrate R751 could result in additional fragments. Of the 10 *Eco*RV-digested samples in Fig. 2, 9 produced single bands, as expected of simple insertions (Fig. 2B, lanes 7 to 11; Fig. 2C, lanes 7 to 10). As indicated above, colony blot hybridization had determined that *Flavobacterium meningosepticum* FM-Aux3 had R751 integrated into the chromosome. As expected, digestion of *Flavobacterium meningosepticum* FM-Aux3 DNA with *Eco*RI or *Eco*RV produced additional bands (Fig. 2C, lanes 6 and 11).

We have previously reported that Tn4351 inserts at a number of different locations in the *C. johnsonae* genome and causes a variety of different mutations (25). Tn4351 has also been used to mutagenize *Bacteroides thetaiotaomicron* (1, 9, 30). Insertions of the transposon alone and of the transposon with vector DNA both result in mutations in *C. johnsonae* and in *B. thetaiotaomicron* (25, 31, 35). Tn4351 mutagenesis will probably be useful for isolating a variety of different types of mutants of members of the genera *Cytophaga*, *Flavobacterium*, *Flexibacter*, and *Sporocytophaga*. Tn4351 contains two antibiotic resistance genes: *ermF* is expressed in *Bacteroides* spp. and in *C. johnsonae*, but not in *E. coli*, while *tetX* is expressed in

E. coli but not in *Bacteroides* spp. or in *C. johnsonae* (25, 35). *Cytophaga*, *Flexibacter*, *Flavobacterium*, or *Sporocytophaga* DNA containing Tn4351 insertions can thus be cloned in *E. coli* using tetracycline as a selective marker. We have used this strategy to clone regions of *C. johnsonae* DNA containing Tn4351 insertions (20).

pCP11 functions in *C. johnsonae* and *C. succinicans*. We have previously reported the development of a *C. johnsonae*-*E. coli* shuttle vector, pCP11 (25). pCP11 contains the *ermF* gene of Tn4351 and thus confers erythromycin resistance on *C. johnsonae*. pCP11 also contains features that facilitate propagation and selection in *E. coli* and conjugative transfer from *E. coli* into *C. johnsonae*. We tested the usefulness of pCP11 in other members of the genus *Cytophaga* and in related bacteria. pCP11 was introduced into each strain by conjugation with *E. coli*. pCP11 was transferred to *C. succinicans* CSR1, resulting in erythromycin-resistant colonies at a frequency of 10^{-6} per recipient cell. We isolated pCP11 from *C. succinicans* and demonstrated that it had the predicted *Eco*RI restriction pattern (data not shown). We also attempted to transfer pCP11 into *C. hutchinsonii*, *Flavobacterium meningosepticum* FMR1, *Flexibacter canadensis* FCR1, *Flexibacter* sp. strain FSR1, and *S. myxococcoides*. We did not obtain any erythromycin-resistant transconjugants from these matings. Since we know that the *ermF* gene functions in all of these bacteria and that DNA can be efficiently transferred by conjugation from *E. coli* into all of these bacteria, our inability to obtain erythromycin-resistant colonies following conjugative transfer of pCP11 is probably due to lack of replication or plasmid instability. pCP11 was constructed from a cryptic plasmid (pCP1) isolated from *C. psychrophila* (25). On the basis of 16S rRNA sequences, *C. psychrophila* is closely related to *C. johnsonae* and

FIG. 2. Analyses of Tn4351 insertions in *Flexibacter* sp. strain FSR1 and *Flavobacterium meningosepticum*. (A) Partial map of R751::Tn4351Ω4 constructed from published data (35). Restriction enzyme sites: E, *Eco*RI; H, *Hind*III. R751::Tn4351Ω4 contains a tandem insertion of Tn4351 as indicated. (B) Autoradiogram of Southern blot of wild-type *Flexibacter* sp. strain FSR1 and of five different erythromycin-resistant transconjugants. Chromosomal DNA was digested with *Eco*RI (lanes 1 to 6) or *Eco*RV (lanes 7 to 11). Lane 1 contains DNA from wild-type *Flexibacter* sp. strain FSR1. The five transconjugants shown are FSR3D (lanes 2 and 7), FSR3F (lanes 3 and 8), FSR3H (lanes 4 and 9), FSR3L (lanes 5 and 10), and FSR3O (lanes 6 and 11). (C) Autoradiogram of Southern blot of wild-type *Flavobacterium meningosepticum* and of five different erythromycin-resistant transconjugants. Chromosomal DNA was digested with *Eco*RI (lanes 1 to 6) or *Eco*RV (lanes 7 to 11). Lane 1 contains DNA from wild-type *Flavobacterium meningosepticum*. The five transconjugants shown are FMR3I (lanes 2 and 7), FMR3J (lanes 3 and 8), FMR-Aux1 (lanes 4 and 9), FMR-Aux2 (lanes 5 and 10), and FMR-Aux3 (lanes 6 and 11). The numbers to the left of each autoradiogram are the sizes (in kilobases) of the molecular size markers.

C. succinicans but more distantly related to the other bacteria used in this study (27). pCP1 is probably a narrow- to moderate-host-range plasmid that is able to replicate in a limited number of *Cytophaga* species.

We attempted to identify a convenient suicide vector for construction of gene disruptions in members of the genera *Cytophaga*, *Flavobacterium*, *Flexibacter*, and *Sporocytophaga*. pLYL03 (22) carries the *ermF* gene and thus should confer erythromycin resistance on the bacteria described above. This plasmid also carries an origin of transfer allowing conjugative transfer from *E. coli*. We transferred pLYL03 into *C. hutchinsonii*, *C. succinicans* CSR1, *Flavobacterium meningosepticum* FMR1, *Flexibacter canadensis* FCR1, *Flexibacter* sp. strain FSR1, and *S. myxococcoides* but did not obtain any erythromycin-resistant colonies. Since pLYL03 apparently does not replicate in these bacteria, it should be an ideal suicide vector. Small internal fragments of a gene of interest could be cloned into the polycloning site of pLYL03, propagated in *E. coli*, and transferred to the appropriate cytophaga, flavobacterium, flexibacter, or sporocytophaga by conjugation. Erythromycin-resistant mutants with disruptions in the gene of interest should arise as a result of homologous recombination.

Members of the genera *Cytophaga*, *Flavobacterium*, *Flexibacter*, and *Sporocytophaga* are important for a number of reasons. *C. hutchinsonii* and *S. myxococcoides* efficiently degrade crystalline cellulose (21, 29). The enzymes of cellulose degradation

have not been studied, but development of techniques for genetic manipulation (Tn4351 mutagenesis, pLYL03-mediated gene disruption) should make cloning and analyses of the genes coding for these enzymes possible. Cytophagas and related bacteria that degrade hemicelluloses (14) and recalcitrant molecules, such as pesticides (39) and halogenated aromatic compounds (4), have also been identified. *Flexibacter canadensis* is a common denitrifying bacterium that has an impact on global cycling of nitrogen compounds (17). A number of cytophagas, and related bacteria, including *Flavobacterium meningosepticum* from this study, can cause disease in humans and animals (3, 6–8, 23, 24, 33). The virulence mechanisms of these bacteria are not understood. Finally, a number of new antibiotics (10, 18, 19, 38), antitumor drugs (28), and other bioactive compounds (12, 16, 40) have recently been isolated from cytophagas, flavobacteria, and flexibacters. Techniques to genetically manipulate these bacteria may allow us to utilize them to our benefit and limit their potential detrimental effects.

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