JOURNAL OF BACTERIOLOGY, Apr. 2002, p. 1859–1864 Vol. 184, No. 7
0021-9193/02/$04.00+0 DOI: 10.1128/JB.184.7.1859–1864.2002

Construction of an Integration-Proficient Vector Based on the Site-Specific Recombination Mechanism of Enterococcal Temperate Phage ϕFC1

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The genome of temperate phage ϕFC1 integrates into the chromosome of *Enterococcus faecalis* KBL 703 via site-specific recombination. In this study, an integration vector containing the *attP* site and putative integrase gene *mj1* of phage ϕFC1 was constructed. A 2,744-bp fragment which included the *attP* site and *mj1* was inserted into a pUC19 derivative containing the *cat* gene to construct pEMJ1-1. *E. faecalis* KBL 707, which does not contain the bacteriophage but which has a putative *attB* site within its genome, could be transformed by pEMJ1-1. Southern hybridization, PCR amplification, and DNA sequencing revealed that pEMJ1-1 was integrated specifically at the putative *attB* site within the *E. faecalis* KBL 707 chromosome. This observation suggested that the 2,744-bp fragment carrying *mj1* and the *attP* site of phage ϕFC1 was sufficient for site-specific recombination and that pEMJ1-1 could be used as a site-specific integration vector. The transformation efficiency of pEMJ1-1 was as high as 6×10^3 transformants/μg of DNA. In addition, a vector (pATTB1) containing the 290-bp *attB* region was constructed. pATTB1 was transformed into *Escherichia coli* containing a derivative of the pET14b vector carrying *attP* and *mj1*. This resulted in the formation of chimeric plasmids by site-specific recombination between the cloned *attB* and *attP* sequences. The results indicate that the integration vector system based on the site-specific recombination mechanism of phage ϕFC1 can be used for genetic engineering in *E. faecalis* and in other hosts.

Enterococci are gram-positive anaerobic bacteria that normally occur in the intestines of most animals from cockroaches to humans. In humans, the typical concentration of enterococci in stool can be up to 10^8 CFU per g (9, 29). Enterococci tolerate a variety of growth conditions, including hypotonic, hypertonic, acidic, and alkaline environments, and can survive at temperatures ranging from 10 to 45°C. In addition, they are intrinsically resistant to many antibiotics and can become opportunistic pathogens in hospitalized and immunocompromised patients. Acquired resistance to antibiotics is also of great concern. Enterococci are now among the most common members of the nosocomial group of bacterial pathogens (16, 24, 31, 35).

Bacteriophage ϕFC1 was first isolated in our laboratory from a culture of the lysogenic strain of *Enterococcus faecalis* (KBL 703) following induction by UV irradiation. Bacteriophage ϕFC1 has a double-stranded DNA genome of approximately 40.5 kbp, an icosahedral head, and a sheathless noncontractile tail. It has been classified into Bradley's group B or *Siphoviridae* according to the International Committee on Taxonomy of Viruses classification system (12, 13).

Phage ϕFC1 integration into the host chromosome occurs by a site-specific mechanism. A gene that encodes a putative site-specific recombinase and that is upstream of the *attP* site has been identified. The gene, *mj1*, encodes a 465-amino-acid polypeptide with similarity in its N-terminal domain to site-specific recombinases (10). The MJ1 integrase displays significant overall homology (57%) with the integrases of listerial phage A118 and lactococcal bacteriophage TP901-1. Analysis of the DNA sequences around the *attP* region identified two predicted bacterium-phage junction regions (*attL* and *attR*). The corresponding bacterial attachment site (*attB*) was deduced from the sequences of these regions (11).

In this study, a vector system based on the site-specific recombination apparatus of temperate bacteriophage ϕFC1 of *E. faecalis* KBL 703 was constructed. We wanted to determine whether putative integrase gene *mj1* could serve as an efficient mediator of integration and if the *mj1* and *attP* sites were sufficient for site-specific integration into the *attB* site. The vector we constructed, which included the *attP* site and *mj1*, could integrate into the *attB*-like site on chromosomal DNA of *E. faecalis* strain KBL 707, which contains no bacteriophage. This strain also was easily lysogenized by phage ϕFC1, produced from *E. faecalis* KBL 703 after UV irradiation. In addition, a 290-bp fragment containing the *attB* site was cloned into vector pACYC184 for use in intermolecular integration assays to determine the minimum size of the *attB* site and the eventual necessity for other host factors required for site-specific recombination. Our results showed that the phage ϕFC1 integrase could function in *Escherichia coli* as well as in *E. faecalis* strains and that a 290-bp fragment containing the *attB* site was the only host factor required to produce a functional target site for the vector containing the *attP* site and the integrase gene.

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MATERIALS AND METHODS

Bacterial strains, bacteriophage, and plasmids. *E. faecalis* strains were propagated at 37°C in Todd-Hewitt broth (THB; Difco Laboratories, Detroit, Mich.) without shaking. *E. coli* was grown with agitation at 37°C in Luria-Bertani broth.

TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Relevant characteristics	Reference or source
Bacterial strains		
E. faecalis		
KBL 703	Original lysogenic strain for phage ΦFC1	8, 35
KBL 707	Indicator strain for phage ΦFC1	29
E. coli JM109	*recA1 endA1 gyrA96 thi hsdR17 supE44 relA1* (*lac-proAB*) F *traD36 proAB lacI*^{q}*ZM15*	38
Plasmids		
pESH2.8	Amp^r Cm^r; replicative ori of ColE1; pEK104 carrying a 2.8-kbp *Hin*dIII fragment of pS3.6	25
pCONT3.7	Amp^r Cm^r; replicative ori of ColE1	This study
pEMJ1-1	pCONT3.7 carrying a 2,744-bp *attP* fragment and *int* fragment of phage ΦFC1	This study
pATTB1	pACYC carrying 290-bp *attB* fragment	This study
pETMJ1	pET14b carrying *int* and *attP*	23
pREC1	Plasmid by recombination of pETMJ1 and pATTB1	This study
pT7BlueR	Amp^r; T cloning vector	Novagen
pATTR	pT7BlueR carrying the *attR* region	This study
pATTL	pT7BlueR carrying the *attL* region	This study

The plasmids used in this study are listed in Table 1. Temperate phage ϕFC1 was purified after induction by UV irradiation from *E. faecalis* KBL 703, and its DNA was extracted in accordance with standard procedures (32).

UV inducibility of bacteriophage ϕFC1. Bacteriophage ϕFC1 was induced from the *E. faecalis* lysogenic strain by UV irradiation (12, 13). Active strains were incubated at 37°C in THB until the optical density at 600 nm reached approximately 0.4. Cells were then harvested and resuspended in 50 ml of sterile 0.1 M $MgSO_4$, and, after UV irradiation with a 15-W germicidal lamp emitting 16 ergs/mm^2 for 15 s, they were transferred to double-strength THB and incubated at 37°C again. Changes in the turbidity of the cultures were recorded every 20 min.

Amplification of the *attB*, *attL*, *attR*, and *attP* regions. The putative integrase gene and the *mj1* and *attP* sites were amplified from phage ϕFC1 DNA with primers PHY-1 (5′-AAC TGC AGG GCG CAA GAA ACA ACT GCT T-3′) and PHY-2 (5′-GAA GAT CTT GTT CTC GAG CAT AGT CTC C-3′). The *attL* region was amplified from the genomic DNA of *E. faecalis* KBL 707 transformants with primers ON-2 (5′-CGG ATT GCC AGA TGG ATG AT-3′) and PHY-2. The *attR* region was amplified with primers ON-1 (5′-CGG CCA TTG AAT TAG GGT GT-3′) and PHY-1. A 290-bp fragment containing *attB* was amplified from *E. faecalis* KBL 703 genomic DNA with intermolecular integration assay primers PATB-1 (5′-CCC TCG GGC GGA TTG CCA GAT GGA TGA T-3′) and PATB-2 (5′-CCC CCG AGC GGC CAT TGA ATT AGG GTG T-3′). A junction region of pREC1 was amplified from plasmid DNA, which was extracted from *E. coli* cotransformed with pATTB1 and pETMJ1, with primer set PATB-1 and PHY-2. PCR was conducted as follows: 94°C for 5 min, and then 30 cycles of 94°C for 1 min, 60°C for 1 min, and 72°C for 1 to 3 min. This was followed by a final extension period of 72°C for 10 min. PCR products were analyzed on 0.8 to 1.2% agarose gels in Tris-acetate-EDTA buffer.

For transformation, 20 μl of frozen cells was thawed on ice, mixed with plasmid DNA, and transferred to the electrode pin. A single electric pulse (PG 240 bacterial electrode and Progenitor II; Hoefer Co.) with the following parameters was applied: 12,000 V/cm peak voltage, 4.7 ms, and 100 μF. The cell suspension was mixed with ice-cold SGM17MC (4) and left on ice for 5 min. After incubation at 37°C for 2 h, transformed cells were spread on a streptococcal regeneration plate containing 5 μg of chloramphenicol per ml.

Southern blotting. DNA restriction fragments separated on an agarose gel were transferred to a Hybond-N membrane (Amersham) by the capillary method (32). Appropriate probes were labeled with digoxigenin-11-dUTP, and prehybridizations and hybridizations were performed as recommended by the supplier (Boehringer-Mannheim). Digoxigenin-11-dUTP-labeled λ DNA digested with *Dra*I was used as a molecular marker for the hybridization.

DNA sequencing. DNA cycle sequencing was done using the ABI PRISM dye terminator cycle sequencing ready reaction kit (Perkin-Elmer Co.). Double-stranded template DNA (0.5 μg) and 3.5 pmol of primer were mixed with 8 μl of terminator ready reaction mixture, and cycle sequencing was carried out with a GeneAmp PCR system 9600 (Perkin-Elmer Co.). The amplified DNA was purified with Centri-Sep spin columns (Applied Biosystems), and the nucleotide sequence was analyzed with an ABI PRISM 310 genetic analyzer.

The left junction region, *attL,* which lies between the *attB* site and pEMJ1-1 on a 617-bp DNA fragment, was amplified from the genomic DNA of the *E. faecalis* KBL 707 transformant by PCR using primers ON-2 and PHY-2 and sequenced. *attR*, the other junction region of the 400-bp fragment, was amplified from the genomic DNA of the *E. faecalis* KBL 707 transformant by PCR using primers ON-1 and PHY-3 (5′-GCG TTA ACT GCC AAT ATA GC-3′) and then sequenced.

Nucleotide sequence accession numbers. The nucleotide sequence data shown in Fig. 3 have been deposited in GenBank under accession no. AY026043 and AY026044.

RESULTS

Characterization of *E. faecalis* KBL 707. Induction of prophage is usually initiated by exposure of cells to UV radiation or DNA-damaging chemicals such as mitomycin C (22). In this study, we monitored changes in turbidity in cultures of *E. faecalis* KBL 703 and KBL 707 after UV irradiation (Fig. 1).

E. faecalis KBL 703 strain carries three plasmids (p703/9, p703/5, and p703/4) and temperate bacteriophage ϕFC1, whereas *E. faecalis* KBL 707 strain contains neither plasmids nor bacteriophage. Previous studies have shown that among several *E. faecalis* strains, *E. faecalis* KBL 707 was the most easily lysogenized by infection with phage ϕFC1, produced from *E. faecalis* KBL 703 after UV irradiation (12, 13). In addition, when the *attB* site was amplified from *E. faecalis* KBL 707 with primers that amplify the *attB* site of *E. faecalis* KBL 703, the expected amplification product, with a size of 300 bp, was obtained (data not shown). This suggests that phage ϕFC1 was integrated into the *attB*-like site of the genome of *E. faecalis* KBL 707. For this reason, we tested the proficiency of putative integrase gene *mj1* and the *attP* region of phage ϕFC1 for site-specific recombination in *E. faecalis* KBL 707.

Construction of site-specific integration plasmid pEMJ1-1. A 3.7-kbp *Hin*dIII fragment from pESH2.8, a pUC19 derivative that contains the enterococcal replication origin from enterococcal plasmid p703/5 and the chloramphenicol acetyltransferase gene, was subjected to self-ligation and was named pCONT3.7. Since the vector was constructed as a control vec-

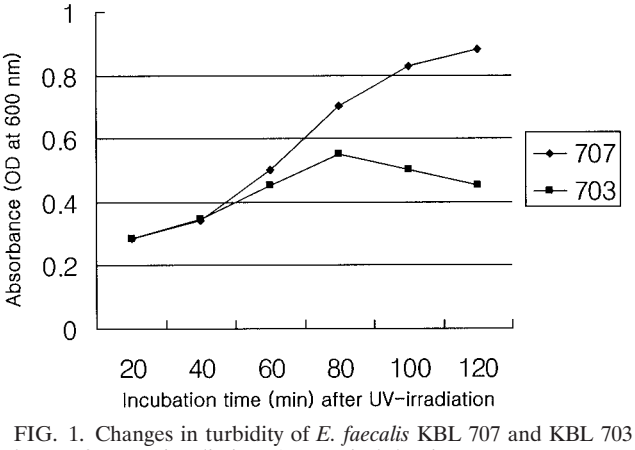

FIG. 1. Changes in turbidity of *E. faecalis* KBL 707 and KBL 703 cultures after UV irradiation. OD, optical density.

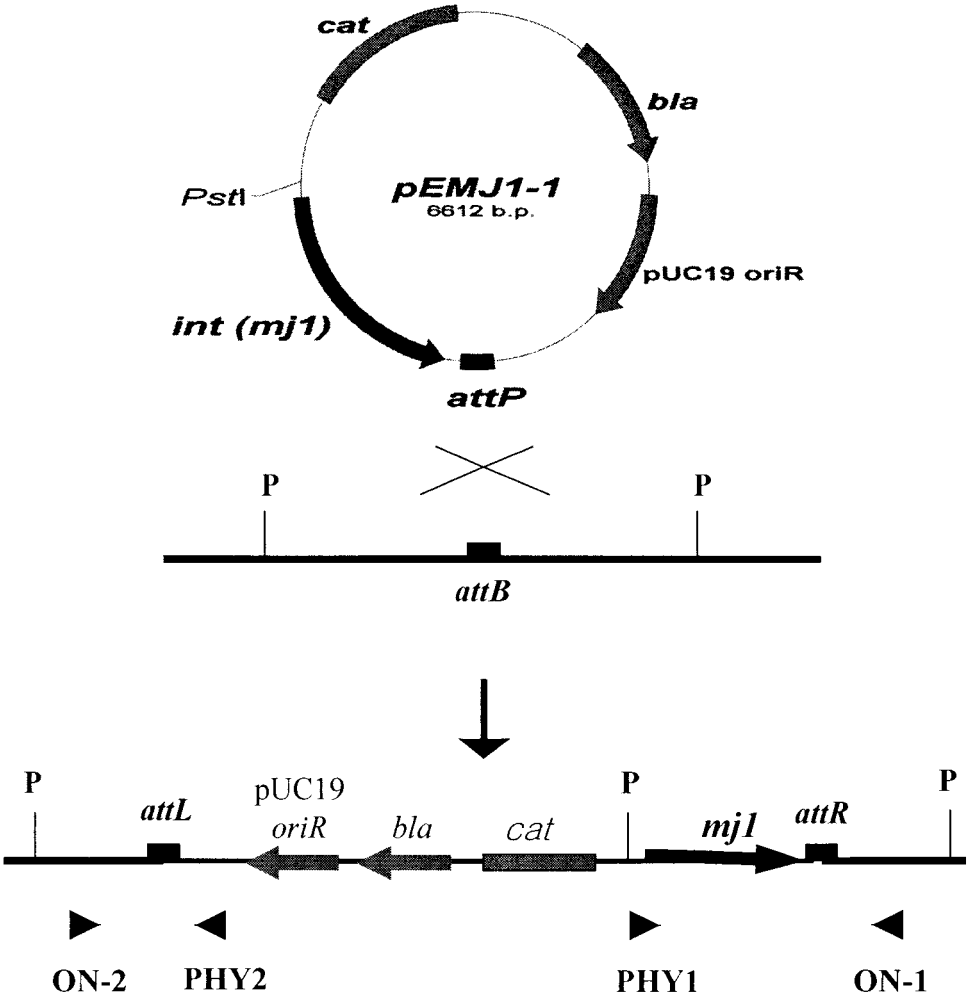

FIG. 2. Schematic representation of the site-specific integration of pEMJ1-1 into the *attB* locus of the *E. faecalis* KBL 707 chromosome. P, *Pst*I site; arrowheads, primers.

tor for site-specific integration vector pEMJ1-1, the possibility that pCONT3.7 could exist in *E. faecalis* was tested. No colonies were obtained when *E. faecalis* KBL 707 was transformed with pCONT3.7 and grown on a THB plate containing 5 μg of chloramphenicol per ml. This result showed that pCONT3.7, which is derived from pESH2.8, could not replicate in *E. faecalis* KBL 707 because it lacked an enterococcal replication origin.

A 2,744-bp fragment containing putative integrase gene *mj1* and the *attP* site from phage ϕFC1 DNA was ligated to pCONT3.7 to create pEMJ1-1, which was then transformed into *E. faecalis* KBL 707. The electroporation efficiency was 6×10^3 colonies/μg of DNA. Although colonies were obtained, no plasmid DNA could be isolated from the *E. faecalis* KBL 707 transformant because pEMJ1-1 does not have an enterococcal replication origin. The result suggested that putative integrase MJ1 had elicited recombination between the *attP* site on pEMJ1-1 and the putative *attB* site in *E. faecalis* KBL 707 chromosomal DNA (Fig. 2).

Site-specific integration of pEMJ1-1 into the *E. faecalis* chromosome. Five colonies of *E. faecalis* KBL 707 transformants were randomly selected and subjected to Southern blotting with a fragment containing *mj1* and the *attP* site as the probe. Intact *E. faecalis* KBL 707 DNA was used as a control. If strand exchange between the *attB*-like site on the chromosomal DNA of *E. faecalis* KBL 707 and the *attP* site on pEMJ1-1 occurred, the probe containing the *attP* site and *mj1* should hybridize to both ends of the vector that had been integrated into the host genome. Two signals, approximately 7 and 5.5 kbp, were detected by Southern blotting, which confirmed that pEMJ1-1 had integrated into the host genome as a single copy (data not shown). If pEMJ1-1 used the *attP* site to integrate into the putative *attB* site on the chromosomal DNA of *E. faecalis* KBL 707, transformants would have junction regions *attL* and *attR*. We were able to amplify the *attL* and *attR* regions as 617- and 2,417-bp fragments, respectively, from the genomic DNA of *E. faecalis* KBL 707 transformants. These fragments had the predicted sizes (data not shown). The results verified our findings that two junction regions were generated by integration of pEMJ1-1 into the putative *attB* locus of *E. faecalis* KBL 707.

To determine whether the *attB* site on the chromosomal DNA of *E. faecalis* KBL 703 and the *attB*-like site of *E. faecalis* KBL 707 had the same sequence or not and to confirm that

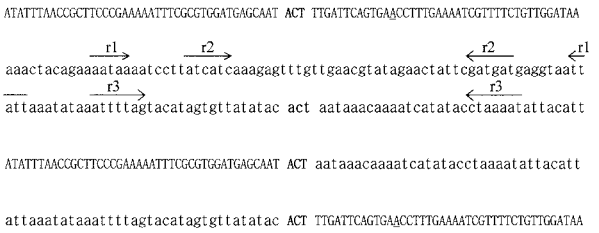

FIG. 3. Nucleotide sequences of the *attB*, *attP*, *attL*, and *attR* regions. *attL* and *attR* were sequenced after pEMJ1-1 integrated into the *E. faecalis* KBL 707 chromosome. Uppercase, *E. faecalis* KBL 707 chromosomal DNA sequences; lowercase, phage ϕFC1 DNA sequences; boldface, 3-bp core. In the *attB* sequence, the single nucleotide difference in the real *attB* nucleotide sequence of *E. faecalis* KBL 703 is underlined (T in place of A in *E. faecalis* KBL 703). Arrows (*attP* sequence), inverted repeats r1, r2, and r3.

pEMJ1-1 was integrated into the chromosome of *E. faecalis* KBL 707 via site-specific recombination, the junction regions were sequenced. The 617- and 400-bp fragments were used to analyze the left and right junction regions, respectively, and the sequences were compared to those of the natural junction regions between *E. faecalis* KBL 703 and phage ϕFC1 (Fig. 3). The sequence of the *attB*-like site of *E. faecalis* KBL 707 was identical to that of the original *attB* site of *E. faecalis* KBL 703 except for 1 nucleotide. The results also showed that pEMJ1-1 was integrated into the *attB*-like site in a manner similar to that for phage ϕFC1, through strand exchange between the *attP* and the *attB* sites mediated by the integrase. In addition, the core sequences (ACT) on the *attP* and *attB* sites, in which direct recombination occurred, were conserved in the *attL* and *attR* regions after strand exchange between the *attP* and *attB* sites. These results confirmed that pEMJ1-1 was integrated into the *attB*-like site through the action of integrase MJ1 and that the 2,744-bp fragment containing the *attP* site and *mj1* was sufficient to allow site-specific recombination of pEMJ1-1, as well as phage ϕFC1.

Intermolecular integration assay with *E. coli*. A 290-bp fragment containing bacterial attachment site *attB*, amplified from *E. faecalis* KBL 703 genomic DNA, was ligated into the *Ava*I site of pACYC184 to create pATTB1 (Fig. 4). The *mj1* expression plasmid pETMJ1, which is a pET14b derivative carrying integrase gene *mj1* and the *attP* site (11, 23), and pATTB1 were cotransformed into *E. coli* JM 109. Restriction enzyme digestion of plasmid DNA isolated from the transformed cells indicated that, in addition to the original plasmids, a chimeric plasmid (pREC1) had formed through an intermolecular integration reaction between the *attB* site on pATTB1 and the *attP* site on pETMJ1 (Fig. 4 and 5). To determine more accurately if site-specific recombination between pATTB1 and pETMJ1 had occurred, plasmid DNA from *E. coli* JM 109 cotransformed with pATTB1 plus pETMJ1 was analyzed by junction amplification and sequencing. The junction was amplified as expected, and the *attL* and *attR* sequences were identical to those determined for *E. faecalis* (Fig. 3 and 5). The results demonstrated that site-specific recombination resulting in formation of the chimeric plasmid had occurred, that plasmids pATTB1 and pETMJ1 carried functional *attB* and *attP* sites, respectively, and that the phage ϕFC1 integrase could function in *E. coli* as well as in *E. faecalis* without the need for any additional cofactors.

DISCUSSION

Characteristic features of the sequences of numerous *attP* sites include (i) a central core sequence, where synapsis and strand exchange occur, which is also present in the three other attachment sites (*attB*, *attL*, and *attR*), and (ii) two unique elements termed the P and P′ arm sequences, which surround the core region. Both arm and core DNA sequences contain inverted and direct repeats that act as the binding site for the specific integrase (13, 18, 20, 29). The *attP* site of phage ϕFC1 contains a core sequence (5′-ACT-3′), which is relatively short compared to those of other bacteriophages, and two inverted repeats surrounding it. The site of specific recombination is within the core sequence (5′-ACT-3′), as determined by comparison of the *attL*, *attR*, *attB*, and *attP* regions. This recombination event thus occurs without DNA synthesis or sequence duplication, a typical feature of other temperate phages (26).

Identification of the locations of *attB* sites shows that integration into tRNA genes, especially at their 3′ ends, is a common occurrence among gram-negative phages such as P22, P4, 186, HP1, 16-3, and ϕCTX and in actinomycete integrative plasmids such as SLP1, pSAM2, pMEA100, and pSE211 (1, 27). One explanation for this target site preference is that the integrase uses the symmetrical sequence in the tRNA gene as a recognition site (1). Studies of phages that have inserted their genomes into a tRNA gene have shown that the length of the homologous region between *attB* and *attP* is usually large, ranging from 17 bp for mv4 (5) to 182 bp for HP1 (average of 40 to 60 bp) (36). Insertion of phage into the tRNA gene does not cause disruption because of the presence of a truncated tRNA gene. This insertion is therefore not lethal. In contrast, phages of gram-positive bacteria integrate either into a gene coding for a protein (L54a [17], ϕ13 [3], ϕLC3 [21], or TP901-1 [2]) or into an intergenic region (ϕ11 [18]), except for mycobacteriophage L5 and *Lactobacillus delbrueckii* subsp. *bulgaricus* bacteriophage mv4, which integrate into tRNA genes (15, 18). From analyses of DNA sequence data, it appears that the *attB* site for phage ϕFC1 is a part of an open reading frame which encodes a protein with 43% identity to the C-terminal end of *E. coli* RadC. RadC is a protein involved in DNA repair following damage by UV or ionizing radiation or alkylating agents (6).

The integration vectors based on the site-specific recombination of temperate phages that we have developed offer a number of advantages. They could be used to introduce a single copy of heterologous DNA into the *attB* site on the bacterial chromosome. It is possible that large fragments of DNA could be integrated, because this recombination system should be able to accommodate a fragment at least as large as the phage genome. For a system using sequences from phage ϕFC1, this could be up to 40.5 kbp. In a system that does not necessitate packaging of DNA in the phage head, such as the one described here, there is no theoretical upper size limit for the passenger DNA. Because a single copy is integrated, expression studies may be performed under conditions that mimic those for chromosomal genes or operons present in only one copy. The specific integration of such vectors should not

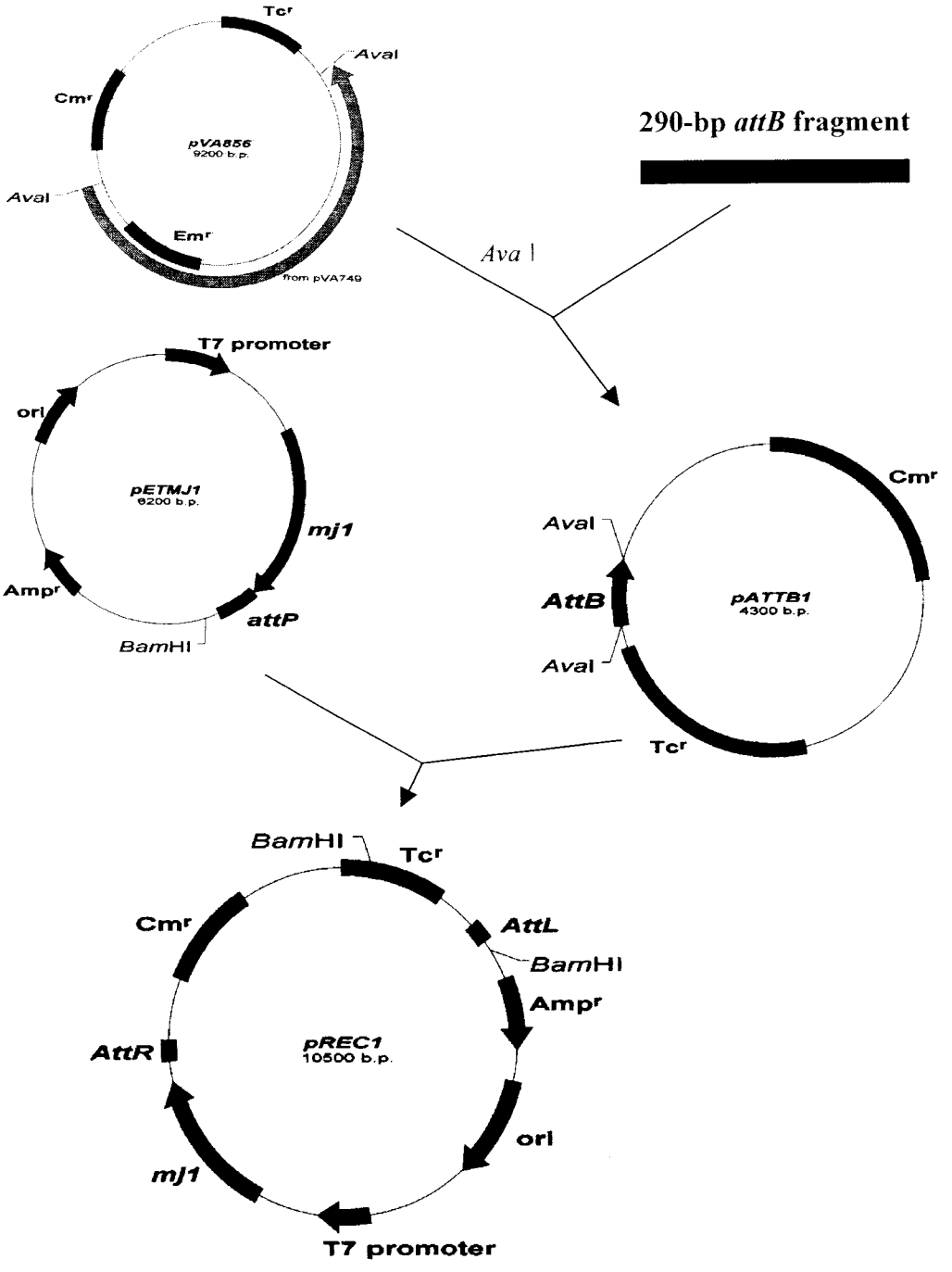

FIG. 4. Schematic representation of the construction of *attB*-containing plasmid pATTB1 and chimeric plasmid pREC1. pVA856 is a shuttle vector constructed by joining pACYC184 and pVA749 at the *Ava*I site. The pACYC184 fragment from pVA856 was used for construction of pATTB1. A 290-bp *attB* fragment was amplified from the *E. faecalis* KBL 703 chromosome by PCR. pATTB1 and pETMJ1 were cotransformed into *E. coli* JM 109 for the intermolecular integration assay.

affect the viability of the transformants, since integration occurs in nonessential sites on the genome. Moreover, easy modification of these new vectors will allow us to extend the use of this methodology to other biotechnologically important bacteria and even to mammalian cells (7, 14).

This study demonstrated that plasmid pEMJ1-1, containing integrase gene *mj1* and the *attP* region of the enterococcal bacteriophage ϕFC1, could integrate into the chromosomal DNA of *E. faecalis* KBL 707 via a site-specific recombination process that obeys Campbell's classic model of integration for phage lambda. Integrative recombination took place precisely between the *attP* and *attB* sites. Sequence data from the junctions confirmed that the *attB* region of *E. faecalis* KBL 707 was similar to that of *E. faecalis* KBL 703. It also showed that specific interaction between the *attP* and the *attB* sequences occurred during integration of pEMJ1-1 and that the core

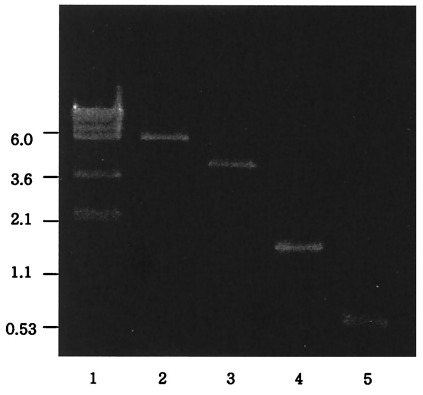

FIG. 5. Intermolecular integration assay with *E. coli*. The presence of a *Bam*HI fragment of approximately 1.3 kbp and a 600-bp PCR product is indicative of site-specific recombination between pATTB1 and pETMJ1. Lane 1, λ DNA digested with *Dra*I as a size marker; lane 2, pETMJ1 digested with *Bam*HI; lane 3, pATTB1 digested with *Bam*HI; lane 4, the 1.3-kbp *Bam*HI fragment from *E. coli* cotransformed with pATTB1 and pETMJ1; lane 5, the 600-bp PCR product from the junction on chimeric plasmid pREC1.

sequence was preserved during this process. All of the genes required for integration of phage ϕFC1 could be localized within a 2,744-bp DNA fragment which contained 1.4-kbp integrase gene *mj1* and the *attP* site. In addition, a 290-bp fragment containing the *attB* sequence could serve as the *attB* locus for the *attP* site in recombination mediated by the integrase. Use of the enterococcal *attB* site as an integration target enables plasmids containing the *attP* site, such as pEMJ1-1 and pETMJ1, to transform not only *E. faecalis* strains containing the *attB* site within their genomes but also other cells that have been engineered to carry the *attB* sequence. Further studies will enable development of vectors derived from phage ϕFC1, which are expected to provide powerful tools for the stable, single-copy gene insertion of DNA into industrial and laboratory strains of bacteria, and even mammalian cells.

REFERENCES

1. **Campbell, A.** 1992. Chromosomal insertion sites for phages and plasmids. J. Bacteriol. **174:**7495–7499.
2. **Christiansen, B., L. Brondsted, F. K. Vogensen, and K. Hammer.** 1996. A resolvase-like protein is required for the site-specific integration of the temperate lactococcal bacteriophage TP901–1. J. Bacteriol. **178:**5164–5173.
3. **Coleman, C., J. Knights, R. Russel, D. Shanley, T. H. Birkbeck, G. Dougan, and I. Charles.** 1991. Insertional inactivation of the Staphylococcus aureus β-toxin by bacteriophage phi-13 occurs by site- and orientation-specific integration of the phi-13 genome. Mol. Microbiol. **5:**933–939.
4. **Cruz-Rodz, A. L., and M. S. Gilmore.** 1990. High efficiency introduction of plasmid DNA into glycine treated *Enterococcus faecalis* by electroporation. Mol. Gen. Genet. **224:**152–154.
5. **Dupont, L., B. Boizet-Bonhoure, M. Coddeville, F. Auvray, and P. Ritzenthaler.** 1995. Characterization of genetic elements required for site-specific integration of *Lactobacillus delbrueckii* subsp. *bulgaricus* bacteriophage mv4 and construction of an integration-proficient vector for *Lactobacillus plantarum*. J. Bacteriol. **177:**586–595.
6. **Felzenszwalb, I., S. Boiteux, and J. Laval.** 1992. Identification of the radC102 mutant. Order of the genes in the 81.5–82.0 min region of the *Escherichia coli* chromosome. Nucleic Acids Res. **20:**366.
7. **Groth, A. C., E. C. Olivares, B. Thyagarajan, and M. P. Calos.** 2000. A phage integrase directs efficient site-specific integration in human cells. Proc. Natl. Acad. Sci. USA **97:**5995–6000.
8. **Jeon, Y. W., S. Y. Jeong, Y. W. Kim, and H. I. Chang.** 1994. Cloning of replication origin from enterococcal plasmid p703/5. Kor. J. Appl. Microbiol. Biotechnol. **22:**18–22.
9. **Jett, B. D., and M. S. Gilmore.** 1994. Virulence of enterococci. Clin. Microbiol. Rev. **7:**462–478.
10. **Kim, M.-J.** 1995. Sequencing and expression of a putative site-specific recombinase gene (*mj1*) of an enterococcal bacteriophage ϕFC1. M.S. thesis. Korea University, Seoul, Korea.
11. **Kim, M.-J., J. Y. Lee, Y. W. Kim, and H. I. Chang.** 1996. Molecular characterization of the region encoding integrative functions from enterococcal temperate phage ϕFC1. J. Biochem. Mol. Biol. **29:**448–454.
12. **Kim, Y. W.** 1999. Genetic studies of bacteriophage ϕFC1. from *Enterococcus faecalis*. Ph.D. thesis. Korea University, Seoul, Korea.
13. **Kim, Y. W., and H. I. Chang.** 1994. Isolation and molecular characterization of ϕFC1, a new temperate phage from *Enterococcus faecalis*. Mol. Cell **4:**155–158.
14. **Kolot, M., N. Silberstein, and E. Yagil.** 1999. Site-specific recombination in mammalian cells expressing the Int recombinase of bacteriophage HK022. Mol. Biol. Rep. **26:**207–213.
15. **Landy, A.** 1989. Dynamic, structural, and regulatory aspects of site-specific recombination. Annu. Rev. Biochem. **58:**913–949.
16. **Leclercq, R., E. Derlot, J. Duval, and P. Courvalin.** 1988. Plasmid-mediated resistance to vancomycin and teicoplanin in *Enterococcus faecium*. N. Engl. J. Med. **319:**157–161.
17. **Lee, C. Y., and J. J. Iandolo.** 1986. Integration of staphylococcal phage L54a occurs by site-specific recombination: structural analysis of the attachment sites. Proc. Natl. Acad. Sci. USA **83:**5474–5478.
18. **Lee, C. Y., and J. J. Iandolo.** 1988. Structural analysis of staphylococcal bacteriophage ϕ11 attachment sites. J. Bacteriol. **170:**2409–2411.
19. **Lee, M. H., L. Pascopella, W. R. Jacobs, and G. H. Hatfull.** 1991. Site-specific integration of mycobacteriophage L5. Proc. Natl. Acad. Sci. USA **88:**3111–3115.
20. **Leong, J. M., S. Nunes-Düby, C. F. Lesser, P. Youderian, M. M. Susskind, and A. Landy.** 1985. The phi-80 and P22 attachment sites. J. Biol. Chem. **260:**4468–4477.
21. **Lillehaug, D., and N. K. Birkeland.** 1993. Characterization of genetic elements required for site-specific integration of the temperate lactococcal bacteriophage ϕLC3 and construction of integration-negative ϕLC3 mutants. J. Bacteriol. **175:**1745–1755.
22. **Matsushiro, A., K. Sato, H. Miyamoto, T. Yamamura, and T. Honda.** 1999. Induction of prophages of enterohemorrhagic *Escherichia coli* O157:H7 with norfloxacin. J. Bacteriol. **181:**2257–2260.
23. **Moitoso de Vargas, L., C. A. Pargellis, N. M. Hansan, E. W. Bushman, and A. Landy.** 1988. Autonomous DNA binding domains of lambda integrase recognize two different sequence families. Cell **54:**923–929.
24. **Murray, B. E., K. V. Singh, S. M. Markowitz, H. A. Lopardo, J. E. Patterson, and M. J. Zervos.** 1991. Evidence for clonal spread of a single strain of β-lactamase-producing *Enterococcus* (*Streptococcus*) *faecalis* to six hospitals in five states. J. Infect. Dis. **163:**780–785.
25. **Park, J.-H.** 1997. Cloning of replication origin of enterococcal plasmid p703/5 and construction of a promoter probe vector for *Enterococcus faecalis*. M.S. thesis. Korea University, Seoul, Korea.
26. **Raya, R. R., C. Fremaux, G. L. de Antoni, and T. R. Klaenhammer.** 1992. Site-specific integration of the temperate bacteriophage ϕadh into the *Lactobacillus gasseri* chromosome and molecular characterization of the phage (*attP*) and bacterial (*attB*) attachment sites. J. Bacteriol. **174:**5584–5592.
27. **Reiter, W., P. Palm, and S. Yeats.** 1989. Transfer RNA genes frequently serve as integration sites for prokaryotic genetic elements. Nucleic Acids Res. **17:**1907–1914.
28. **Rew, J.** 1991. Catalogue of strains, 4th ed., p. 23. Korean Culture Center of Microorganisms, Yonsei University, Seoul, Korea.
29. **Rice, E. W., J. W. Messer, C. H. Johnson, and D. J. Reasoner.** 1995. Occurrence of high-level aminoglycoside resistance in environmental isolates of enterococci. Appl. Environ. Microbiol. **61:**374–376.
30. **Romeo, D. A., and T. R. Klaenhammer.** 1992. IS *946*-mediated integration of heterologous DNA into the genome of *Lactococcus lactis* subsp. *lactis*. Appl. Environ. Microbiol. **58:**699–702.
31. **Sahm, D. F., J. Kissinger, M. S. Gilmore, P. R. Murray, R. Mulder, and J. Solliday.** 1989. In vitro susceptibility studies of vancomycin-resistant *Enterococcus faecalis*. Antimicrob. Agents Chemother. **33:**1588–1591.
32. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
33. **Segall, A. M., and H. A. Nash.** 1993. Synaptic intermediates in bacteriophage lambda site-specific recombination: integrase can align pairs of attachment sites. EMBO J. **12:**4567–4576.
34. **Song, J. S., J. H. Park, C. W. Kim, Y. W. Kim, W. J. Lim, I. Y. Kim, and H. I. Chang.** 1999. Characterization of the replication region of the *Enterococcus faecalis* plasmid p701/5. J. Microbiol. Biotechnol. **9:**91–97.
35. **Uttley, A. H. C., C. H. Collins, J. Naidoo, and R. C. George.** 1988. Vancomycin-resistant enterococci. Lancet **i:**57–58.
36. **Waldman, A. S., S. D. Goodman, and J. J. Scocca.** 1987. Nucleotide sequences and properties of the sites involved in lysogenic insertion of the bacteriophage HPc1 genome into the *Haemophilus influenzae* chromosome. J. Bacteriol. **169:**238–246.
37. **Yanisch-Perron, C., J. Vieira, and J. Messing.** 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene **33:**103–119.