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Molecular Characterization of the Region Encoding Integrative Functions from Enterococcal Bacteriophage ϕFC1

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Abstract: Bacteriophage ϕFC1 is a temperate phage which was identified as a prophage in the *Enterococcus faecalis* KBL703 chromosome. Phage ϕFC1 integrates into the host chromosome by site-specific recombination. The phage attachment site P (attP) was localized within the 0.65-kb *XhoI-HindIII* fragment and the nucleotide sequence of the region was determined. An open reading frame (*mj1*) which adjoined the phage attachment site encoded a deduced protein related to the site-specific recombinase family. The organization of this region was comparable to other site-specific recombination systems. The molecular weight of the expressed MJ1 in *E. coli* was in good agreement with the predicted 53,537 Da of the *mj1* gene product. Elucidation of the phage-specific integration process in this study would provide useful genetic tools such as a chromosomal integration system.
Key words: attachment site P (attP), site-specific recombinase, site-specific recombinase family, site-specific recombination.

Enterococcus faecalis is a Gram-positive facultatively anaerobic coccoid bacteria and a normal component of the human intestinal flora. Enterococci are relatively avirulent in healthy persons, but they are opportunistic pathogens in hospitalized or immunocompromised patients. Recently the pathogenicity of enterococci has received increasing attention because of its high prevalence in nosocomially infected patients. Enterococci are now among the top three nosocomial bacterial pathogens in the United States (Olmsted *et al.*, 1994).

E. faecalis KBL703 strain and its temperate phage ϕFC1 has been identified and characterized by Kim, Y. W. *et al.* (Kim *et al.*, 1994). In this report, the site-specific recombination system of phage ϕFC1 was analyzed. The 650 bp fragment carrying phage ϕFC1 attachment sites was mapped and sequenced. The putative site-specific recombinase gene of ϕFC1 was also identified, and the amino acid sequence of the deduced protein was compared with those of other site-specific recombinases. The organization of this region is well consistent with the fact that the functions required for integration (i.e., attP and integrase) are tightly clustered in all the known examples of site-specific recombination processes (Raya *et al.*, 1992). The ORF, named *mj1*, encodes a 464 amino acid peptide whose molecular weight is 53 kDa when expressed in *E. coli*.

There have been well-characterized examples of site-specific recombination in gram-negative bacteriophages, of which the best-studied system is that of bacteriophage λ (Landy, 1989). But the integration system of gram-positive phages is less well documented, especially in *E. faecalis*. There is a marked dearth of reported investigations into the molecular aspects of the life cycle of enterococcal bacteriophages. The resulting paucity of information in this area is in sharp contrast to our detailed knowledge about temperate phages in *E. coli* and *Bacillus*, and must act as a barrier to the use of temperate phages as tools for studying enterococci. The characterization of site-specific recombination in enterococcal phage ϕFC1 can provide many useful tools for introducing and stabilizing desirable genes in the bacterial chromosome. For these reasons, enterococcal bacteriophage ϕFC1 has been studied in our laboratory and this report forms a part of ongoing efforts.

Materials and Methods

Bacteria, phage, and plasmids

Bacteria and plasmids used in this study are listed in Table 1. *E. faecalis* KBL703 was propagated at 37°

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Table 1. Bacteria and plasmids

Strain or plasmid	Relevant characteristics
Enterococcus faecalis	
KBL703	ϕFC1 lysogen
Plasmids	
pFE1	pUC19 carrying 7.7-kb *Eco*RI ϕFC1 fragment
pFX4(+/−)	pUC19 carrying 3.3-kb *Xba*I ϕFC1 fragment
pFX5	pUC19 carrying 3.1-kb *Xba*I ϕFC1 fragment
pFX8	pUC19 carrying 0.6-kb *Xba*I ϕFC1 fragment
pFE1/900H	pUC19 carrying 0.9-kb *Hin*dIII fragment of pFX5
pFX5/H1	pUC19 carrying 1.5-kb *Hin*dIII fragment of pFX5
pFX5/P1	pUC19 carrying 2.0-kb *Xba*I-*Pst*I fragment of pFX5
pFX5/P2	pUC19 carrying 1.1-kb *Xba*I-*Pst*I fragment of pFX5
pFX5/P2:650*Hin*dIII-*Xho*I	pUC19 carrying 0.65-kb *Hin*dII-*Xho*I fragment of pFX5/P2
pFX5/P2:800*Hin*dIII-*Xho*I	pUC19 carrying 0.8-kb *Hin*dIII-*Xho*I fragment of pFX5/P2
pFX4(+)Δa~p	pFX4(+) deletion derivatives
pFX4(−)Δ1~18	pFX4(−) deletion derivatives
pFX5/P2:650*Hin*dIII-*Xho*IΔ1~2	pFX5/P2:650*Hin*dIII-*Xho*I deletion derivatives
pTMJ1	pT7Blue(R) carrying *mj1* at *Eco*RV site
pETMJ1	pET14b carrying *Nde*I-*Bam*HI fragment of pTMJ1

C in THB (Difco Co.) supplemented with 2% glycine. Bacteriophage ϕFC1 was induced from KBL703 cells with UV treatment as described previously (Kim *et al.*, 1994).

Southern hybridization

E. faecalis chromosomal DNA and bacteriophage ϕFC1 DNA were isolated by the methods of Jos *et al.* (1987) and Hill *et al.* (1991), respectively. DNA fragments cleaved with *Eco*RI and/or *Bam*HI were transferred from agarose gels to nylon membrane, Hybond N^+ (Amersham, USA) as described by Sambrook *et al.* (1989). The hybridization probes were labeled with Dig-oxygenin-11-dUTP (DIG) by the random primer extension method (Sambrook *et al.*, 1989). The color reaction was developed with NBT (nitroblue tetrazolium salt) and X-phosphate solution in the dark.

Sequence analysis

A sequence analysis of the region encoding integrative functions was performed by the dideoxynucleotide chain termination method (Sanger *et al.*, 1977) using the Sequenase Version 2.0 sequencing kit (USB). In order to construct clones for nucleotide sequence analysis, nested sets of deletion mutants were generated *via* the exonuclease III strategy outlined by Sambrook *et al.* (1989).

Construction of pETMJ1

A 1.5-kb fragment containing *mj1* was amplified by polymerase chain reaction (PCR) with primers P1 (5'-CACGTGCAGCATTGTATATAC-3') and P2 (5'-ACCGAATGCATGTTCGTATTG-3'). Amplification reaction was performed in 25 μl volume containing 10 mM of Tris-Cl, pH 8.3, 50 mM of KCl, 1.5 mM of $MgCl_2$, 0.4 mM of dNTP (Promega), 100 pmol of each primer, 200 ng of pFE1 DNA, and 2.5 units of Taq DNA polymerase (Boehringer Mannheim) using the Erichom double block programmed for 30 cycles of 30 sec at 90℃, 30 sec at 58℃, and 1.5 min at 72℃.

The amplified 1.5-kb fragment was cloned in pT7Blue vector (Novagene) at the *Eco*RV site to make pTMJ1. The pTMJ1 was digested with *Nde*I and *Bam*HI, and the fragment carrying the entire coding region of *mj1* was inserted downstream from the lacUV5 promoter of pET14b (Novagene). This plasmid was designated as pETMJ1.

Production of the *mj1* gene product in *E. coli*

For culture and isopropyl-β-D-thiogalactopyranoside (IPTG) induction, LB broth supplemented with 100 μg/ml of ampicillin was used. Overnight cultures of BL 21 (DE3) harboring pETMJ1 were inoculated into a 5 ml LB broth. When the optical density of the culture at 600 nm reached 0.6, IPTG was added to a final concentration of 0.4 mM and incubation was continued for an additional 3 h. The cells were harvested and analyzed by SDS- 10% polyacrylamide gel electrophoresis.

Results

Integration of phage ϕFC1 DNA into the *E. faecalis* genome

To establish lysogeny, recombination of the viral DNA and the host chromosomal DNA occurs *via* a Campbell type of integration (Campbell, 1982) at the specific viral attachment site (attP) and the host chromosomal site (attB). Southern blotting analysis was done to confirm integration of phage ϕFC1 DNA into *E. faecalis* genome. *Eco*RI-*Bam*HI digested DNAs of phage ϕFC1 and KBL703 were separated on a 0.8% agarose gel, transferred to a nylon membrane, and hybridized to the digoxygenin-labeled probe of the total phage (lane 3-4, Fig. 1). Comparison of the Southern blot of genomic DNA from KBL703 to that of phage FC1 DNA revealed two junction fragments of 10.5 and 5.5 kb, designated as the attachment site L (attL) and the attachment site R (attR), respectively. A 7.7-kb *Eco*RI fragment of free phage ϕFC1 DNA hybridized with two *Eco*RI junction fragments of the chromosomal digest of KBL703 (lane 1-2, Fig. 1). Therefore, the attP site is located within the 7.7-kb *Eco*RI fragment. This 7.7-kb fragment was still detected at a reduced intensity in the chromosomal digests of KBL703, suggesting that a small population of phage ϕFC1 was replicating lytically within this strain or was induced spontaneously while DNA preparation occurred.

Location and cloning of the attP site

The 7.7-kb *Eco*RI fragment of the phage ϕFC1-containing attP site was cloned in pUC19 and the restriction map of resulting plasmid pFE1 was determined (Fig. 2). To localize the attP site, various restriction fragments were generated, cloned in pUC19, and used as probes for southern hybridization (Fig. 3). When 0.6-kb, 3.3-kb and 3.1-kb *Xba*I-digested fragments of pFE1 were used as probes respectively, the first two detected only the attR 5.5-kb band (lane 2, 4, Fig. 3). But the 3.1-kb fragment hybridized to both the attL and attR bands, though the attR band was faint (lane 3, Fig. 3). Therefore, the attP sequence could be located at one end of the 3.1-kb *Xba*I fragment, which was next to the 3.3-kb *Xba*I fragment. When *Eco*RI-digested KBL703 chromosomal DNA was probed with various restriction sub-fragments of the 3.1-kb *Xba*I fragment, such as the 0.65-kb *Hin*dIII-*Xho*I, 0.8-kb *Xho*I-*Hin*dIII, 2.0-kb *Pst*I-*Xba*I, and 1.5-kb *Hin*dIII fragments, only the 1.5-kb *Hin*dIII and 0.65-kb *Hin*dIII-*Xho*I fragments could detect both the attR and attL bands. The results were consistent with the attP being near one end of the 3.1-kb *Xba*I fragment. Since the 0.65-kb fragment was the shortest fragment to hybridize with both bands,

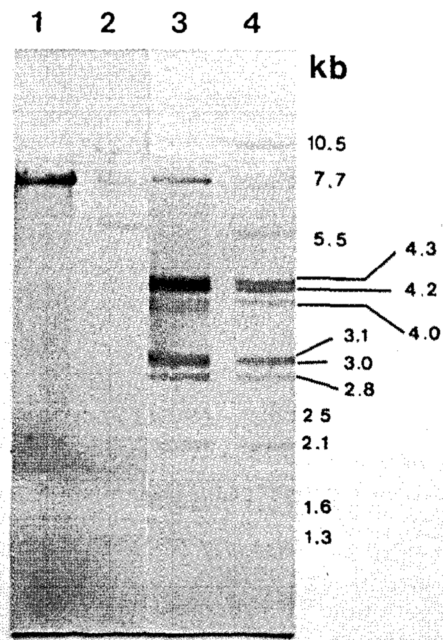

Fig. 1. Site-specific integration of phage ϕFC1 into the host chromosome. *Eco*RI-*Bam*HI digested phage (lane 1, 3) or KBL703 chromosomal DNA (lane 2, 4) hybridized to the free phage ϕFC1 (lane 3, 4) or the 7.7-kb *Eco*RI fragment of ϕFC1 (lane 1, 2).

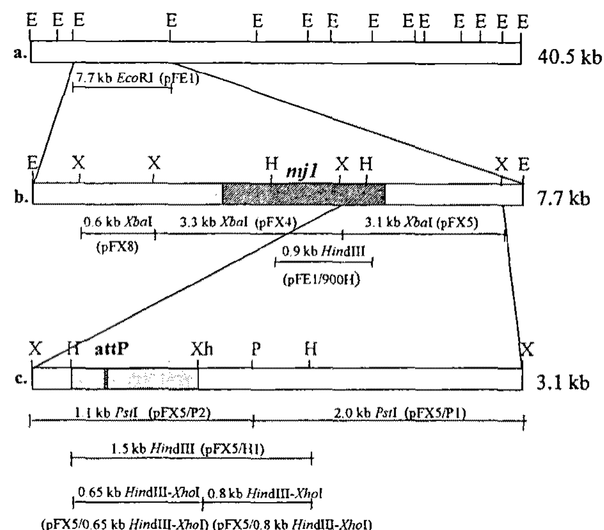

Fig. 2. Restriction map of phage ϕFC1 genome. a: *Eco*RI restriction map of the 40.5-kb ϕFC1 genome. b: *Xba*I restriction map of the 7.7-kb *Eco*RI fragment. c: Restriction map of the 3.1-kb *Xba*I fragment. Each fragment shown in this figure was ligated to pUC19 to generate the plasmid named in the parenthesis. A shaded portion in b is the region encoding a putative site-specific recombinase, *mj1*, and in c is the shortest fragment carrying attP site (E: *Eco*RI, X: *Xba*I, H: *Hin*dIII, P: *Pst*I, Xh: *Xho*I).

attP was assigned to the 0.65-kb *Hin*dIII-*Xho*I fragment. The schematic representation of the predicted Campbell-like integration of phage ϕFC1 into the bacterial attB site is shown in Fig. 4.

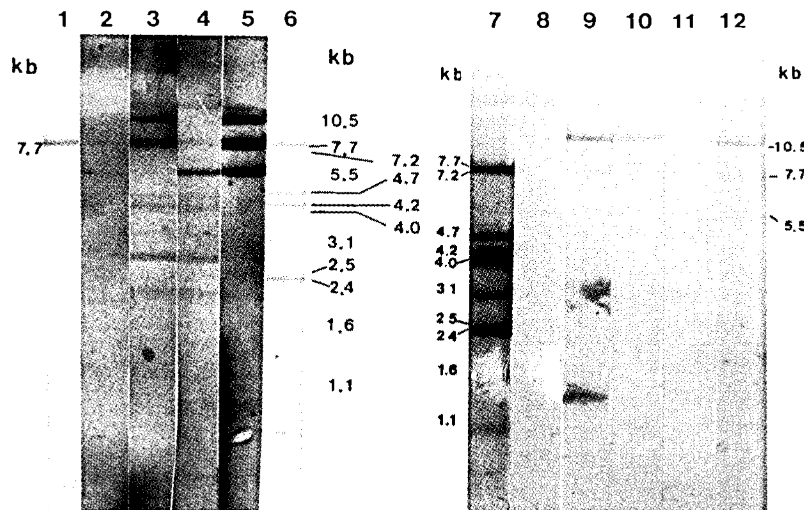

Fig. 3. Identification of the attP-bearing restriction fragments. Southern hybridization analysis of *Eco*RI digested phage (lane 1, 7) or KBL703 chromosomal DNA (lane 2 to 6, 8 to 12) was performed. Various restriction fragments shown in Fig. 2 were used as probes separately. These are: 0.6-kb (lane 2), 3.3-kb (lane 4), or 3.1-kb (lane 1, 3) *Xba*I fragments of pFE1, 0.65-kb *Hin*dIII-*Xho*I (lane 9), 0.8-kb *Xho*I-*Hin*dIII (lane 10), 2.0-kb *Pst*I-*Xba*I (lane 11), or 1.5-kb *Hin*dIII (lane 12) fragments of pFX5. The 0.65-kb *Hin*dIII-*Xho*I fragment was the shortest fragment which could hybridize to both bands. Total phage ϕFC1 in lane 6, 7, and 7.7-kb *Eco*RI fragment in lane 5,8 were used as probes for size reference.

Sequence analysis of the attP-containing region

The nucleotide sequence of the ca. 2-kb region containing the 0.65-kb *Hin*dIII-*Xho*I fragment was determined. To determine nucleotide sequences, the 3.3-kb *Xba*I fragment was prepared from ϕFC1 and inserted into pUC19 in both directions to generate pFX4(+) and pFX4(−), and was treated with exonuclease III, nuclease S1, and the Klenow fragment. pFX5/P2:650 *Xho*I-*Hin*dIII containing the 0.65-kb fragment was also deleted with exonuclease III for sequence analysis. The sequencing strategy is shown in Fig. 5. The sequences of 2,350 nucleotides containing the mj1 and 650-bp *Xho*I-*Hin*dIII fragment is shown in Fig. 6. The 0.65-kb fragment of the attP site is AT rich (72%), and this feature is typical of other site-specific systems of recombination (Raya *et al.*). An open reading frame was found near the attP site. Since all the lysogenic phages examined to date showed the clustering of phage-encoded recombination functions, the open reading frame was supposed to be related to the site-specific recombination event. The initiation codon ATG, stop codon UAG, and putative ribosome-binding site AGGAGC of MJ1 are indicated.

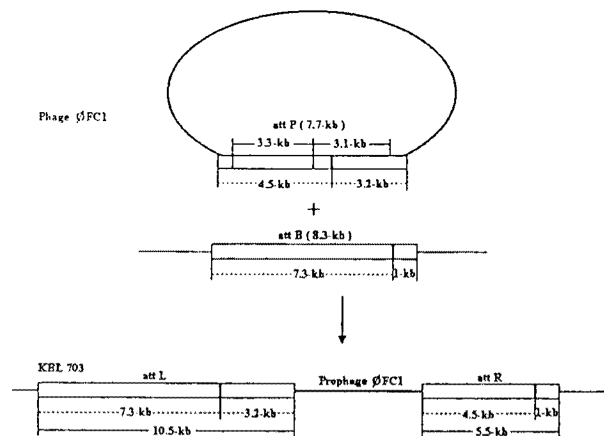

Fig. 4. Schematic representation of the Campbell type integration of phage ϕFC1 into bacterial attP site.

Amino acid sequence homology of MJ1

Comparison of the deduced amino acid sequence of MJ1 with all Genbank proteins was carried out using the BLAST algorithm. The N-terminal half of the MJ1 showed significant homology with other site-specific recombinases, such as invertases, resolvases, and the CisA of *Bacillus subtilis* (Fig. 7). Amino acids of MJ1

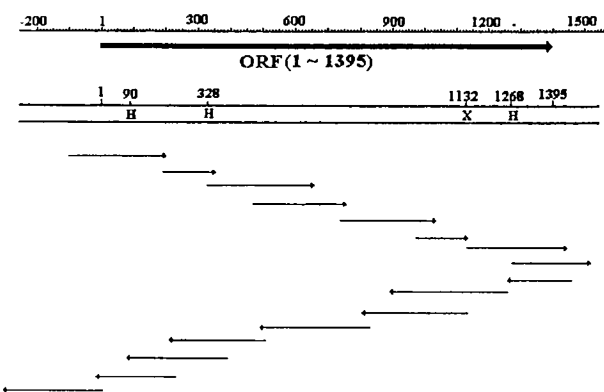

Fig. 5. Sequencing strategy of the region encoding *mj1*: Each arrow is an independent clone analyzed.

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      10         20         30         40         50
5' CCGAA GAAGA ATATC AGGAG TTGAA ATCCG ACTAC GCCAA ATTGT TTTTA
      60         70         80         90         100
   CAGTT AGAAA ATATT GATGA AAACA ACTAG TATTT ATTTT GAAAG GAGCA
                                                      RBS
      110        120        130        140        150
   ATTTT ATATG AAACG TGCAG CATTG TATAT ACGTG TATCC ACAAT GGAAC
          5'-CACG TGCAG CATTG TATAT AC-3' (Primer 1)
      160        170        180        190        200
   AAGCC AAGGA AGGAT ACAGC ATTCC CGCAC AAACA GATAA ACTAA AAGCT
                                                      HindIII
      210        220        230        240        250
   TTTGC AAAAG CAAAA GATAT GGCAG TTGCA AAAGT ATATA CTGAT CCAGG
      260        270        280        290        300
   GTTTT CAGGA GCAAA AATGG AGCGC CCTGC ATTAC AAGAA ATGAT ATCTG
      310        320        330        340        350
   ATATT CAAAA TAAAA AAATT GATGT GGTTC TAGTC TACAA ATTAG ACAGG
      360        370        380        390        400
   CTTTC ACGTT CACAA AAGAA TACAT TGTAT TTAAT TGAAG ATGTA TTTCT
      410        420        430        440        450
   AAAAA ATAAT GTAGA CTTTA TCAGC ATGCA AGAAA GCTTT GACAC ATCAA
                                        HindIII
      460        470        480        490        500
   CACCT TTTGG CCGTG CGACG ATAGG AATGT TATCC GTTTT TGCAC AATTA
      510        520        530        540        550
   GAGCG AGACA CAATT ACAGA AAGAA TGCAC ATGGG AAGAA CAGAA CGTGC
      560        570        580        590        600
   AAAAC AAGGA TACTA TCACG GAAGT GGCAT TGTTC CCTTA GGTTA CGATT
      610        620        630        640        650
   ATGTG CATGG AGAAT TAATT ATCAA TGATT ACGAG GCGCA AATTA TTCAA
      660        670        680        690        700
   GAAAT CTATG ATTTA TATGT GAACC AAGGT AAAGG ACAGC AATAT ATAAC
      710        720        730        740        750
   AAAAC GTATG GTTGC AAAAT ACCCA GATAA GGTAA AAACA TTAAC CATAG
      760        770        780        790        800
   TAAAG TATGC CTTAA CCAAT CCATT ATATA TTGGC AAAAT AAGTT GGGAC
      810        820        830        840        850
   GGCAA AGTGT ATGAT GGCCA TCACT CACCT ATAAT TGATA AATCT ATGTA
      860        870        880        890        900
   CGATA AAGCT CAAGA AATTA TTGCC AGAAT GGCTC AAAAA GGTGG CGAAC
      910        920        930        940        950
   AGCAT GGAAA TCAAT TAGGG CTTTT ATTAG GGATT ACTTA TTGTG GTAAA
      960        970        980        990        1000
   TGCGG AGCTA AAGTA TTTCG TTATG TATCA GGAGG CAAAA AATAT CGATA
      1010       1020       1030       1040       1050
   TAATT ATTAT ATGTG TAGAT CAGTA AAGAA AATGC TACCT TCGCT AGTAA
      1060       1070       1080       1090       1100
   AAGAT TGGAA CTGCA AACAA CCTAG TCTCA GACAA GAAGT AGTTG AAAAG
      1110       1120       1130       1140       1150
   AAAGT AATAG ATTCA CTTAA ATCAT TGGAC TTCAA AAAAA TCGAA CGTGA
      1160       1170       1180       1190       1200
   ATTAA AACAA GTTGA AAATA AAACA AAATC AAAAA TCACC ACTAT TAATA

      1210       1220       1230       1240       1250
   ACCAA ATTTC CAAGA AGCAT AACGA AAAAC AAAAA ATTCT AGATT TGTAT
                                              XbaI
      1260       1270       1280       1290       1300
   CAATA TGGTA CATTT GATGT CACAA TGCTT AATGA ACGTA TGAAA AAAAT
      1310       1320       1330       1340       1350
   TGATA ATGAA ATAAA TGCGT TAACT GCCAA TATAG CAAAC TTAGA AGGTA
                                                      KpnI
      1360       1370       1380       1390       1400
   CCAAA AGTGA GTCAT TAATT AATAA GCTTG AAACG TTAAA AACTT TTAAT
                           HindIII
      1410       1420       1430       1440       1450
   TGGGA AACTG AAACT ACAGA AAATA AAATC CTTAT CATCA AAGAG TTTGT
      1460       1470       1480       1490       1500
   TGAAC GTATA GAACT ATTTG ATGAT GAGGT AATTA TTAAA TATAA ATTTT
      1510       1520       1530       1540       1550
   AGGTA CATAG TGTTA TTTAC ACTAA TAAAC AAAAT CATAT ACCTA AAATA
      1560       1570       1580       1590       1600
   TTACA TTTAT ACAAA CCTAT AGACA ATACG AACAT GCATT CGGTA TAATT
              (Primer 2) 3'-GT TATGC TTGTA CGTAA GCCA-5'
      1610       1620       1630       1640       1650
   GTATT ACTAG GAGGC CGATA TTATG AAAAC TAATT ATGTA GGAGT AGTTG
      1660       1670       1680       1690       1700
   AAAAG ATTAG AATGT TAAGT ATGTA CCCAA AAATG ATCCT AGTTC GATTC
      1710       1720       1730       1740       1750
   TCATT TAGTA ACACA GGACG AAACT ATAAA CTGTA TCGTC TCCAA ATACG
      1760       1770       1780       1790       1800
   AATTA GCAAT ATGTT ACTAA TGCTA CCCGG AAAAA TCTGA ACTAG CTGTC
      1810       1820       1830       1840       1850
   TATGG TCATT TGAAT AAAAG AAATC AAACT TGTAA TTGAA AAAAT GCTTG
      1860       1870       1880       1890       1900
   TAAGG AAAAC TTTGA TTAGT GCAAT AAAAA GATAT TTAAA TTTGG TNATT
      1910       1920       1930       1940       1950
   TTACT GTTCT TACCT ATAGA ATGGA TAATT AAACC AATAT TAATA ATATA
      1960       1970       1980       1990       2000
   TTTTA AGGAG GACCA GTTTG TCAAT CAATA GTTTA CAACA AAAAG AAAAG
      2010       2020       2030       2040       2050
   TCTCT ATTAT CAGAT ATAAC AAAAC TTGAA GGAGN CTATG CTCGA GAACA
                                                  XhoI
      2060       2070       2080       2090       2100
   AAAGA AAATA GCTAA TTCAG AGAAA AAGAT AGCTG ATAGT TCTAA AAAAA
      2110       2120       2130       2140       2150
   TAGAA AGTTC TAAGT CAATA TCCAC ATAAG ATCTC AAAGC AGAAT CAAAG
      2160       2170       2180       2190       2200
   AATCA GAAAC TAAAA AATCT TTAGC ATCTA AAGAA AAATC AGCTA CTATT
      2210       2220       2230       2240       2250
   TCTTC TAAGT TAGCA AAAAA AAGAA AAGAA TTAGG TGATA TTCAA GTAAA
      2260       2270       2280       2290       2300
   GCTAA GTAAA CAGNG TACTA TAGAA AATAC ACAGT TCCAA AAGAA TCTAA
      2310       2320       2330       2340       2350
   AAAAA ACTTA CGACA CTCAG ATAAT GAAGA TAAAA AGACC CAGAC TGCAG 3'
                                                      PstI
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Fig. 6. Nucleotide sequences of the bacteriophage ϕFC1 integration region: Relevant restriction sites of the 0.65-kb *Xho*I-*Hin*dIII fragment (bases 1374 to 2041) are indicated. The *mj1* gene (bases 108 to 1502) is oriented from left to right. The presumptive RBS (ribosome binding site) AGGAGC is found 8-bp upstream of mj1 initiation codon ATG. The sequences and annealing positions of PCR primer 1 and 2 for mj1 amplification are also shown.

from 50 to 155 were be aligned with those of invertases or resolvases from 36 to 138. In this range, most amino acids were highly conserved. MJ1 also showed a high homology with the *cis*A cistron of the *Bacillus subtilis* sporulation gene *spoIVC* which encodes a site-specific recombinase protein (Sato *et al.*, 1990). CisA and MJ1 had similarities in their C-terminal halves, which showed no homology with other site-specific recombinases, and the size of proteins were about 55-kDa. The homology extends (amino acids 3~153, 298~330 of CisA with 5~235, 272~304 of MJ1, respectively) much longer than that of any other site-specific recombinases.

A) *E. faecalis* bacteriophage ϕFC1 putative recombinase MJ1 (464 aa)
B) *Basillus subtilis* DNA recombinase Cis A (500 aa)
C) Bacteriophage P1 recombinase Cin (186 aa)
D) *Escherichia coli* DNA invertase Pin (184 aa)
E) Bacteriophage Mu DNA invertase Gin (193 aa)
F) *Enterococcus faecalis* transposon Tn917 Reslovase (183 aa)
G) Transposon Tn5422 Resolvase (184 aa)
H) *Staphylococcus aureus* transposon Tn552 Resolvase (197 aa)

Fig. 7. Alignment of the amino acid sequences of MJ1 and other site-specific recombinases: The amino acids are designated by the standard one-letter symbols. Highly conserved amino acids are shown in the black background, and less well conserved amino acids are shaded. A blank is an empty space for alignment and a dot is an amino acid which shows no homology. The number in the parenthesis means total length, and the starting and the ending position of each alignment was shown. Comparison of the deduced amino acid sequence of MJ1 with all Genbank proteins was carried out by using the BLAST algorithm.

Fig. 8. Construction of *mj1* expression plasmid pETMJ1.

Fig. 9. Overexpression of *mj1* gene product in *E. coli*. The total cellular proteins of BL21(DE3) harboring pET14b (lane 2, 3) or pETMJ1 (lane 4, 5), with (+) or without (−) IPTG induction (final concentration, 0.4 mM), were analyzed by SDS-10% Polyacrylamide gel electrophoresis. A new 53-kDa band was observed in the IPTG induced cells carrying pETMJ1. Lane 1 is the molecular weight standard (Gibco BRL).

Overexpression of MJ1 in *E. coli*

We constructed pETMJ1 to determine the molecular weight of the *mj1* gene product as described in Materials and Methods (Fig. 8). A protein band of approximately 53 kDa, which was in good agreement with the predicted 53,537 Da of the *mj1* gene product, was observed after IPTG induction in Fig. 9.

Discussion

This study was aimed to find functions related to bacteriophage ϕFC1 integration into the host chromosome. In this study, we localized the attP site of phage ϕFC1 within the 650-bp *Hin*dIII-*Xho*I fragment, and sequenced the putative site-specific recombinase gene (*mj1*) which adjoined the phage attachment site. The

size of the expressed MJ1 in *E. coli* was in good agreement with the predicted 53,537 Da of the *mj1* gene product. MJ1 encoded a deduced protein related to the site-specific recombinase family.

The 650-bp *Hin*dIII-*Xho*I fragment carrying the attP site was AT rich (72%) when compared to other regions of phage ϕFC1. A random 3-kb region analyzed by Kim, Y. W. (unpublished data) has 64% AT contents and the ORF *mj1* in this study shows 67%. The significance of its high AT content is related to the fact that negatively supercoiled DNA, required as a substrate for integrative recombination, tends to partially denature in regions of high AT content, and local instability facilitates the integration of phage DNA (Landy, 1989). An inverted repeat (26 bases) spaced by 14 bp was found near, but within the *mj1* gene (bases 1222 to 1487 in Fig. 6). It formed a hairpin secondary structure (−25.45 kcal/mol), though its role was not investigated.

All the site-specific recombination proteins characterized thus far fall into either the Hin-related or the Int-related families (Argos *et al.*, 1986). The Int-related family is divided into major groups: integrases of bacteriophages; the FLP protein of *Saccharomyces cerevisiae*; Int of conjugative transposons Tn916 and Tn 1545; plasmid integrases from the *Streptomyces* and *E. coli*; and the Cre protein of phage P1. Alignment of proteins from the Int-related family showed extensive diversity and the homologies are located only in the C terminus part where His (family position 396), Arg (399), and Tyr (433) are highly conserved (Argos *et al.*, 1986). These residues play an important role in the DNA cleavage reaction catalyzed by these enzymes. The Hin-related family, however, includes resolvase proteins of several transposons (e.g., Tn3 and γδ) as well as the invertase proteins that mediate phenotypic variations (e.g., Salmonella Hin, phage Mu Gin and phage P1 Cin). All the members of the Hin-related family are so homologous that all genes catalyzing inversions in prokaryotes can complement each other (Plasterik *et al.*, 1983). When the Hin-related family and the Int-related family were compared, neither global nor local homologies were detected (Argos *et al.*, 1986). The N-terminal half of MJ1 has a significant homology with almost all of the Hin-related site-specific recombinase. But MJ1 do not show a homology with the Int-related family. This suggests that a recombination between enterococcal bacteriophage ϕFC1 and its host *E. faecalis* may occur by a different mechanism from other bacteriophages. Or there may be another site-specific recombinase protein. But it seems unplausible, since the phage-encoded integration elements are always clustered.

Although the region carrying the attP site was sequenced, and fragments containing attL and attR sites were determined, the absence of a phage-cured strain prevented us from determining attB on bacterial chromosome. Nucleotide sequence determination of attB, attR, attL, and attP will enable us to find the core sequences, the exact site of the recombination. The characterization of a site-specific recombination system from an enterococcal bacteriophage ϕFC1 may provide a useful tool for introducing and stabilizing desirable genes into the bacterial chromosome. Integration systems based on bacteriophage sequences, attP and an integrase offer a number of advantages. Even a 40-kb DNA may be integrated into the nonessential specific site. Since a single copy is integrated, expression studies may be performed under conditions that mimic chromosomal genes, or operons present with only one copy. Therefore, elucidation of the phage-specific integration process will make a significant advance in our knowledge of *E. faecalis* and provide us with useful genetic tools for treatment of some nosocomial diseases.

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