

## Complete DNA Sequence and Analysis of a Cryptic Plasmid Isolated from *Lactobacillus bif fermentans* in Kimchi

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**Abstract** The complete 1,486 nucleotide sequence of a cryptic plasmid separated from *Lactobacillus bif fermentans* strain A02 isolated from Kimchi has been determined. The plasmid, designated as pA021, encodes a 33,488 Da putative Rep protein. Based on the sequence similarity, the protein shows homology with coding protein of pRS1, a previously reported plasmid of *Oenococcus oeni* and the replication initiation protein (Rep) of the Staphylococcal pT181 plasmid family.

**Key words:** *Lactobacillus bif fermentans*, plasmid, Kimchi

Lactic acid bacteria are known to participate as starter microorganisms in Kimchi fermentation [6, 7, 9]. Many *Lactobacillus* species possess one or more natural resident plasmids of various sizes. We isolated *Lactobacillus bif fermentans* strain A02 from Kimchi. *L. bif fermentans* is known as one of the major lactic acid bacteria that grow in Kimchi [11]. In this paper, we report the cloning and sequence analysis of a plasmid, named pA021, that is present in this strain.

*L. bif fermentans* were cultured at 30°C on MRS [8] solid or liquid media without agitation. Plasmids were extracted from the culture following a published method [12]. The plasmid DNA was excised from 0.8% agarose gel and recovered by conventional electrophoretic elution method, followed by ethanol precipitation. Purified plasmid pA021 was linearized with *EcoRI* (single site) or *HindIII* (single site), and fragments were then separately cloned into pUC19. Complete nucleotide sequence of the plasmid was determined by the sequencing with universal M13/pUC19 sequencing primer and specific primers derived from sequences of the cloned plasmid. DNA sequencing was carried out by Korea Basic Science Institute (Gwangju, Korea) using an Automatic DNA Sequencer (ABI<sup>PRISM</sup>



**Fig. 1.** Linear map of pA021. The putative *rep* gene is shown as a boxed arrow.

Selected restriction enzyme cleavage, promoter binding site (Promoter), and ribosome binding sequence (RBS) are shown.

Model 377, Perkin Elmer, U.S.A.). PC/GENE program (Intelligenetics, Switzerland) was used to analyze and align sequences and to determine the putative open reading frames (ORFs). Sequence-similarity searching of the current version of GenBank was accomplished with the BLAST [2].

The total DNA sequence of pA021 consists of 1,486 bp with a GC content of 42.4 mol%. The entire plasmid pA021 with selected features is shown in Fig. 1. The first GC pair of the unique *EcoRI* site was arbitrarily designated as bp 1. The major ORF extends over 852 bp (from positions 396 to 1247), encoding a putative polypeptide of 283 amino acids with a molecular mass of 33,488 Da and isoelectric point of 5.97, as shown in Fig. 2. Putative ribosome binding site seems to be typical AGGA, and the starting nucleotide of mRNA is predicted as C, depicted with an underlined bold character in Fig. 2. Promoter binding site consists of 3 regions, -10, -16, and -35, and the general nucleotide sequences for *Lactobacillus* species are TA<sup>(-10)</sup>TAAT, T<sup>(-16)</sup>RTG, and TT<sup>(-35)</sup>GACA [15]. Percent occurrence of bases in each promoter regions (-10, -16, and -35 regions) is 95–81–52–67–71–100, 52–38–57–57, 95–86–52–48–57–57%, respectively. A putative promoter binding site of pA021 is assigned as nucleotide sequences starting position 169, shown on Fig. 2. The consensus -10, -16, and -35 promoter sites, TA<sup>(-10)</sup>TTAT, C<sup>(-16)</sup>GTG, and TT<sup>(-35)</sup>GTGA, are highly conserved, showing 75% identity.

Comparison of many sequences deposited in GenBank with that of pA021 suggests that the deduced protein of the

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GAATTCGTACACATTTTAAAGCACAAAAACCGCCAATGGGGAGTGGTGTGAAGTGCG 60  
 CTGTGCAGAAATTTTAAAGCAACAGCGCTTTGAAAAATTCACAAACTTTCGACCGGCCCT 120  
 CTAACAGCGCGTCAAGGTGTACCAAAAATCGTACCTTTTCGATTTTCTT**TGTGAAATCCA** 180  
**GTATATTCGTGATATTA**CTCGATCGCTAAGATACAAAAATACAAAAGAAAAACCCG 240  
 AAGTGCAGTTCCTAAGTTTGGCGACCCCGGAACACITCGAGTATCAATAACTTTTAAAG 300  
 TTCGTGGATTATATAGCCGTAATAGCATTATCAAAAATTTAAAAAGTACACGATAAC 360  
 TCCTAATCGCACTAGGGCGGATAGGAGTTTTATAT**start ORF (Rep)**  
 RBS → **ATG**CCAACTACCAAGTATCGATTG 420  
 M P T N Q V S I  
 ATCGAATCACTGTTCTGGGSAACTTAAAGCTGAATATCGGGAGTGCACGTTGATGA 480  
 D R I T V S G E L K A E Y R E L Q R V M  
 ATGCCCTAGGAAGCTCGTGGGAACCTCTCGACGGCGTTTTAGGTTAATTCGGGAATATC 540  
 N A L G S S W E L S D G V F R L I R E Y  
 CTAACGGGACTACTGAAATGTTGCCATTACCGCTGAGAAATGCCGTTTCAAGCTGGTTCTT 600  
 P N G D T E N V A Y Y A E N A F Q A G S  
 GCGGGCTGGACTTAAACCGGAATAAATAGCTGAACGAAAGCTTGAATGAAACGGG 660  
 W R L D F N P N K L A E R E K L E M K R  
 CGATTGATTACTGACTGATGTGCATTTTACCCGCTTGGTTTGGCGTTTGAAGCTCTTA 720  
 A I D L L T D V H F T R L D L A F D V F  
 ACAACGAATGGGTATGAAATACCGTATTATCGTCCAAACGCTCACAGCGCAATATG 780  
 N N E L G M K Y R I Y R P N V S Q R E Y  
 GCGTTTATACGGCAACATGGCAAAAGCGGTGGAGACGATATATACGGGCTCAACAGTT 840  
 G V Y T A Q W T K A V E T I Y Y G S N S  
 CCGAGCAACAAATTCGGCAATATAACAAGCTAGTGAACAACTAAGAAAAATATGCCG 900  
 S E Q Q I R Q Y N K L V E Q T K K N M P  
 TCCAGAGCGGTGTTGAACATGGATGCCTTAGAATTCGAATTAAGAGGTCGGAAACCTA 960  
 L P D G V E H W M R L E L Q L R G R K P  
 CGGAATGGGTAGAGCGTGTCAAGGATATGCTGATGATTTTCGATTGCCAAACTATGATC 1020  
 T E W V E R A K G M L D F R L P N Y D  
 GAATTAATAAATAAAGTACAGAAATGGCTCTTTCGCTTGAAGAGGCTGCTCTTGGT 1080  
 R I K N K N D R M A L F A L E K G L L D  
 GGTCTGATTCAGTGATAAAAAATAAAGACCGTCTTCGTAATTCAGAAAGAACAGT 1140  
 W S D F S D K N K K A R L R K L Q K E Q  
 ACCGATGACACACTGGCCCGAGAATGTTGATTGCTGATTCACAGCAAGAACGATTGC 1200  
 Y D D T L A R E L F D L L I A Q Q E R L  
 end ORF ←  
 GGGGAGAACTTGCAAGCTACTTAGCGGAATTTGATATTACAGGAGTAGTCGGCAGCATGTG 1260  
 R G E L A S Y L A E F D I Q E \*  
 GAGACAAGGGTCAAGTTGCTTGAACCTGCCTGCAAGCGGTTGCCCTTTTCGGAATAAAA 1320  
 CAACCCAAAACCTGCCACGGCAAAAAAGCTAC TGCATTTTTCGTTGGCCACGGGCGA 1380  
 GTGATTAGGTGTTCAAGCGAAGCTTGGCTTGGGTGGGTGAAACCCACGGTTTAAAAA 1440  
 AAGGGGATCAACAGCTTGTTCGCCCTTTTGTAGTTTGAAGTGAAC 1486

**Fig. 2.** Complete DNA sequence of pA021 and the deduced product of the ORF.

The numbering of the DNA sequence is indicated. The sequences of putative promoter and ribosome binding sites (RBS) are depicted in bold. The start and end codons of the ORF are indicated as a box. Putative transcription initiation nucleotide is represented as underlined bold character. Sequences of hairpin loop structures are underlined.

ORF is a replication initiation protein (Rep). The deduced protein showed 57% homology to Rep of pRS1 in *Oenococcus oeni* and 48% homology to the replicase of *Leuconostoc mesenteroides* [1, 5]. The sequence of the putative ribosome binding site of the ORF was the same as that of ORF1 of pRS1 in *O. oeni*. Sequence between ribosome binding site (RBS) and initiation codon ATG of the two ORFs was GTTTTTATT and CTTTTTTTT, respectively, with 78% identity.

The similarity of the deduced protein to the Rep of pCW7 in *Staphylococcus aureus*, pT181 plasmid family, and to the Rep of pTZ12 in *Bacillus subtilis* was 48% and 47%, respectively [3, 4]. It is of interest to note that all the plasmids, pRS1, pT181, pCW7, and pTZ12, are replicated by the rolling circle replication (RCR) mechanism [1, 3–5, 14]. Thus, the homology results suggest that pA021 is also replicated by the RCR mechanism. DNA sequence alignment among Reps of pA021, pRS1, and pT181 are shown in Fig. 3. The deduced protein has low homology to

|       |  |                                   |                 |    |
|-------|--|-----------------------------------|-----------------|----|
| pA021 | M-----P  | TN-----                           | qVSiDRIT        | 12 |
| pRS1  | M-----   | nnLd-kKraPSnStLtpisgikkeyr        | VSVDRIT         | 35 |
| pT181 | Myknhanhnsnh                                       | lenhLdnfSktySnsRldah              | vcisdp-kIstDamT | 49 |
|       | *  | **                                | *** **          |    |
| pA021 | vsGe-Lkaeyre-LqRvMnalgss--                         | WeLsdSvFrIireyngdt                | EnVA            | 57 |
| pRS1  | ltadcpLevidgk-LSRwMkethlf--                        | ipLSsGgFKvIEDNI                   | AdtpEqVA        | 82 |
| pT181 | ivGn-LnrnagalSkfmsvepqr                            | IwdilqtKFK-----                   | Akalqekv        | 91 |
|       | ** ** *  | * * * * *                         | * * * * *       |    |
| pA021 | YyaenaFQAgSW-----                                  | RIDFNPNK-LaerEkIemKRAI-dILtDvhFTR | 100             |    |
| pRS1  | fvEYtqFQknri-----                                  | RIDFNPNhsmTkgqgwIlskI-atLknkkfSR  | 126             |    |
| pT181 | YIEYdkvkAdSwdrrnmRleFNPNK-L                        | ITrdEmiWIKqnlisymeDdgFTR          | 140             |    |
|       | * * * * *  | ***** * * *                       | * * * * *       |    |
| pA021 | LDLAFDvFNneLgmKYRiYRPNVsqreygyvtaqwkavETIYYGsnSSE  | 150                               |                 |    |
| pRS1  | cDvAFDiFNspieKYRiFRPgtSskyfIsaaGK-----             | PETIYYGaqkS                       | 172             |    |
| pT181 | LDLAFD-FeedLsdyYamsdkaVkkTiFYgrnGK-----            | PETkyfGvrdSn                      | 185             |    |
|       | ***** * * *  | * * * * *                         | ***** * * *     |    |
| pA021 | QQIRQYNKLEVEQTKKmpIPDgv-EHwMRELLQLRGRKptewverakGmL | 199                               |                 |    |
| pRS1  | aQIRQYNKLEVEQTKKrtiPDni-knWRRELLQLRGRKisDypqkvKSaL | 221                               |                 |    |
| pT181 | rFIRiYNK--kQerKdnadaevmsEHIWRvEieLk-RdmvDYwncdfSdL | 232                               |                 |    |
|       | * * * * *  | ***** * * * *                     | ***** * * * *   |    |
| pA021 | DdFrIPnydrIkknDRmaIFaLekglIdWsdfsdkNkkarIrkLqKEgy  | 249                               |                 |    |
| pRS1  | DnF  | 224                               |                 |    |
| pT181 | hiliq-PdwtIqrtaDRaiVfMLIsdooewgkI-hrNsrykyknlIkeis | 280                               |                 |    |
|       | * *  |                                   |                 |    |
| pA021 | ddtLareIfdILiAqqePLrgeIasyIaEFdiqe                 | 283                               |                 |    |
| pRS1  | -----iIlgiddRf-----                                | 233                               |                 |    |
| pT181 | pydLtdImkstLkAnekalQkqidfwhqEFkfwk                 | 314                               |                 |    |
|       | * * * * *  |                                   |                 |    |

**Fig. 3.** Multiple alignment of the predicted amino acid sequence of replication proteins from pA021, pRS1, and pT181.

The number at the end of every line indicates the last amino acid noted. Underlined sequences in pT181 Rep protein indicate the four divergent domains reported by Projan and Novick [14]. Identical amino acids in a same column are shown as capital letters. Asterisks indicate identical or similar amino acids in proteins.

four divergent domains [14] of pT181, while sequences of the center region are highly conserved. This result supports that the central region of the Rep protein is responsible for the protein's active site and divergent domains play a role of plasmid-specific origin recognition or binding, as previously reported [1, 14].

In this study, we have reported the complete nucleotide sequence of a small cryptic plasmid from *L. bifementans* isolated from Kimchi. The plasmid harbors a putative *rep* gene, which can be applicable to construction of a stably maintained cloning vector for genetically improved Kimchi-fermenting *Lactobacillus* species [10, 13].

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