

Characterization of a Cryptic Plasmid Isolated from the Antilisterial *Bifidobacterium longum* A24

Ki-Hwan Kim · Soon-Yong Youn · Ji-Eun Kan and Sung-Sik Yoon¹

Department of Biological Resources and Technology, 1Institute of Functional Biomaterials and Biotechnology, Yonsei University, Korea

Introduction

Bifidobacterium is gram-positive, non-spore-forming, nonmotile, irregular rod-shaped bacteria that often resemble Y or V shapes. They are strictly anaerobic fermentative organisms that utilize glucose by a very characteristic shunt pathway typified by the enzyme fructose-6-phosphate phosphoketolase and produce primarily acetic and lactic acids. Bifidobacteria is inhabited in the intestines of humans, some animals, and insects, where they are considered as beneficial organism, with a large number of potential health benefits attributed to them. The benefits include prevention and treatment of diarrhea, establishment of a healthy flora in premature infants, alleviation of constipation and symptoms of lactose intolerance, enhancement of immune function, suppression of tumorigenesis, and cholesterol reduction. The wide probiotic activities of bifidobacteria point to their vast potential for improving human health. Because of this potential they are frequently included in fermented dairy products as probiotic adjuncts. However, the lack of molecular tools for studying this group of high G+C gram-positive bacteria has limited our ability to understand what characteristics of these bacteria are important for probiotic activities. To date, plasmids have only been detected in the five species as such as *B. longum* and *B. breve* (human), *B. globosum* (pig), and *B. indicum* and *B. asteroides* (honeybee). Some plasmids have been characterized at the sequence level, and two plasmids were reported to replicate via a rolling circle replication mechanism. Recently, the complete genome sequence of a strain of *B. longum* was deciphered.

In this study, we isolated and analyzed a cryptic plasmid from the antilisterial *B. longum* A24 from infants feces. The plasmid was named pBIFA24 and compared its nucleotide sequence with NCBI database after sequenced using the primer walking approach. Secondary structure of 3 ORF was analyzed using both PHD and prosite program.

Materials and Methods

1. Plasmid Isolation and Purification

Plasmid DNA preparations from *E. coli* and *Bifidobacterium longum* A24 were carried out using Miniprep kit(Qiagen, Valencia, CA) with slight modification according to the manufacturer's recommendations.

2. Southern Hybridization and Single-Stranded DNA Detection

Southern hybridization was performed using a DIG DNA Labeling and Detection kit (Roche, Indianapolis, IN) according to the manufacturer's instructions. The accumulation of single-stranded DNA intermediates, indicative of rolling circle replication, was evaluated by the effect of S1 nuclease digestion on *B. longum* A24 plasmid, according to Leenhouts (1991).

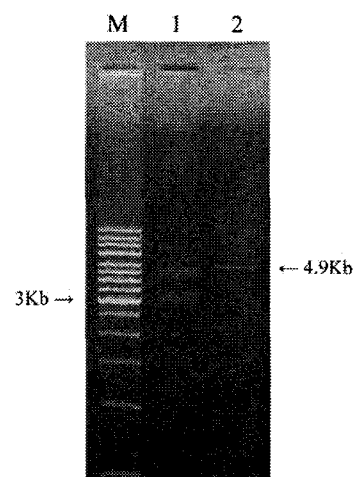
3. DNA Sequencing and Analysis

DNASTar (DNASTar, Inc., Madison, WI) was used for assembly of contigs, and primer walking was used to fill in gaps in the plasmid sequences. Basic DNA and amino acid sequence analyses were performed using DNASTar and PHD (http://www.public.iastate.edu/~pedro/pprotein_query.html) programs. The BLAST server at the National Center for Biotechnology Information was used for sequence similarity searches and open reading frame (ORF) predictions. The PROSITE at the SWISS-PROT server were used for conserved domain searches. Multiple sequence alignments were performed using CLUSTAL W of DNASTar.

Results and Discussion

1. Plasmid DNA of *Bifidobacterium longum* A24

The plasmid DNA of *B. longum* A24 verified one band when treatment as *Kpn* I and size was about 4.9kb.



References

1. Leenhouts, L. J., B. Tolner, S. Bron, J. Kok, G. Venema, and Seegers, J. F. M. L. 1991. Nucleotide sequence and characterization of the broad host range lactococcal plasmid pWV01. *Plasmid* 26: 55–66.
2. O'Sullivan, D. J. 2001. Screening of intestinal microflora for effective probiotic bacteria. *J. Agric. Food Chem.* 49:1751–1760.
3. O'Riordan, K., and Fitzgerald, G. F. 1999. Molecular characterisation of a 5.75-kb cryptic plasmid from *Bifidobacterium breve* NCFB 2258 and determination of mode of replication. *FEMS Microbiol. Lett.* 174:285–294.
4. Park, M. S., Shin, D. W. Lee, K. H. and Ji, G. E. 1999. Sequence analysis of plasmid pKJ50 from *Bifidobacterium longum*. *Microbiology* 145:585–592.
5. Scardovi, V. 1986. The genus *Bifidobacterium* Orla-jensen 1924, 472AL, pp. 1418–1434. In Sneath, P. H. A., N. S. Mair, M. E. Sharpe, and J. G. Holt (Ed.), *Bergey's Manual of Systematic Bacteriology*, vol. 2. Williams & Wilkins, Baltimore, Md.