ISSN(Print) : 1738-7248, ISSN(Online) : 2287-7428
Korean J. Food Preserv.
28(3), 313-317 (2021)
https://doi.org/10.11002/kjfp.2021.28.3.313

사단법인 한국식품저장유통학회
The Korean Society of Food Preservation

연구노트

Evaluation of randomly amplified polymorphic DNA-polymerase chain reaction method for the identification of eighteen lyophilized probiotics commercially available

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제품화된 18종 동결건조 프로바이오틱스의 동정을 위한 RAPD-PCR 분석법 평가

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Abstract

Identification of probiotics is crucial to ensure the quality of food products manufactured at the industrial scale. Although various techniques have been introduced for bacterial identification, randomly amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) is generally accepted as a conventional method for bacterial identification. In this study, RAPD-PCR method was evaluated for the identification of eighteen commercial probiotic strains. In results, cellular and product (lyophilized) forms of each probiotic strain were successfully identified based on band intensities and size of the amplified genes. Therefore, RAPD-PCR method may be applied for the identification of lyophilized probiotics commercially available.

Key words : probiotics, lactic acid bacteria, randomly amplified polymorphic DNA, RAPD

Introduction

Due to the recent escalating interest in health foods, considering their health benefits, such as immune-boosting, cholesterol-lowering, and anti-inflammatory effects (Granato et al., 2010), the probiotics market has gradually expanded (de Simone, 2019; Kechagia et al., 2013). Quality control of probiotics produced on a commercial scale is important to ensure that safe and reliable products are provided to the consumers. Typically, this includes identification of probiotic bacteria, and detection of contaminants and toxic compounds. Although various techniques have been introduced for bacterial identification (e.g., 16S rRNA sequencing, restriction fragment length polymorphism, rep-PCR, and ribotyping) (Mohania et al., 2008; Yu et al., 2009), randomly amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) analysis is

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Received 11 November 2020; Revised 21 December 2020; Accepted 08 April 2021.

regarded as the standard method (Booysen et al., 2002; Temmerman et al., 2004). The principle of RAPD-PCR is that oligonucleotide primers bind to different loci in the genome of a bacterium and randomly amplify unique fragments (Williams et al., 1990). This method is used to reveal the overall differences or similarities among interspecies or intraspecies (Jarocki et al., 2020; Sharma et al., 2020).

In this study, RAPD-PCR was applied for the rapid identification of eighteen commercial probiotic strains. First, the most suitable primer was screened among three universal primers (5'-ACGCAGGCA-3', 5'-ACGAGGCAC-3', and 5'-ACGCGCCCT-3'). Then, RAPD-PCR patterns of cellular and product (lyophilized) forms were compared to determine uniformity. Results from this study suggest that the present RAPD-PCR method may be applied for the identification of lyophilized probiotics commercially available.

Materials and methods

Bacterial strains and culture conditions

The bacterial strains used in this study are listed in Table 1. The bacterial cells were incubated in de Man, Rogosa, and Sharp (MRS) broth (BD Difco, Franklin Lakes, NJ, USA) at 37℃ aerobically in a static incubator. *Bacillus coagulans* IDCC 1201 and *Lactococcus lactis* IDCC 2301 were incubated in a shaking incubator at 200 rpm and 45℃ and 37℃, respectively.

Randomly amplification polymorphic DNA

Genomic DNA from cellular and lyophilized form of probiotics was isolated using the Wizard™ genomic DNA purification kit (Promega, Madison, USA). Briefly, lyophilized probiotics were washed twice with ddH2O to remove excipients prior to DNA isolation. PCR was performed with the universal primers: 5'-ACGCAGGCA-3' (Sesena et al., 2004); 5'-ACGAGGCAC-3' (Tilsala-Timisjarvi and Alatossava, 1998); and 5'-ACGCGCCCT-3' (Johansson et al., 1995). Randomly amplified PCR products were analyzed and visualized using a GelDoc imaging system (Bio-Rad, Hercules, CA). Finally, the products were quantified using Image Lab software (Bio-Rad, version 6.1) for further analysis.

Table 1. Bacterial strains used in this study

Strain	Origin	ATCC accession no.
Enterococcus faecium IDCC 2102	Infant feces	BAA-3146
Streptococcus thermophilus IDCC 2201	Homemade yogurt	BAA-3150
Lactococcus lactis IDCC 2301	Homemade cheese	BAA-2834
Lactobacillus gasseri IDCC 3101	Breast milk	BAA-2841
Lactobacillus rhamnosus IDCC 3201	Infant feces	BAA-2836
Lactobacillus acidophilus IDCC 3302	Infant feces	BAA-2845
Lactobacillus casei IDCC 3451	Infant feces	BAA-2843
Lactobacillus plantarum IDCC 3501	Kimchi (fermented food)	BAA-2838
Lactobacillus salivarius IDCC 3551	Healthy child saliva	BAA-2835
Lactobacillus reuteri IDCC 3701	Breast milk	BAA-2837
Lactobacillus helveticus IDCC 3801	Infant feces	BAA-2840
Lactobacillus fermentum IDCC 3901	Homemade cheese	BAA-2842
Bifidobacterium longum IDCC 4101	Infant feces	BAA-2847
Bifidobacterium bifidum IDCC 4201	Infant feces	BAA-2850
Bifidobacterium lactis IDCC 4301	Infant feces	BAA-2848
Bifidobacterium breve IDCC 4401	Infant feces	BAA-2849
Lactobacillus johnsonii IDCC 9203	Infant feces	BAA-3147
Bacillus coagulans IDCC 1201	Green malt	BAA-3143

Results and discussion

Selection of a suitable primer for RAPD-PCR analysis

Prior to identification using the RAPD-PCR analysis, identification of eighteen probiotic strains was verified by 16S rRNA sequence analysis (data not shown). Next, three universal primers were evaluated and the most suitable primer was selected. Unique and distinctive band patterns of the eighteen species were clearly observed based on band intensities and gene size (Fig. 1). However, when performing PCR using P1 primer, faint bands were observed for

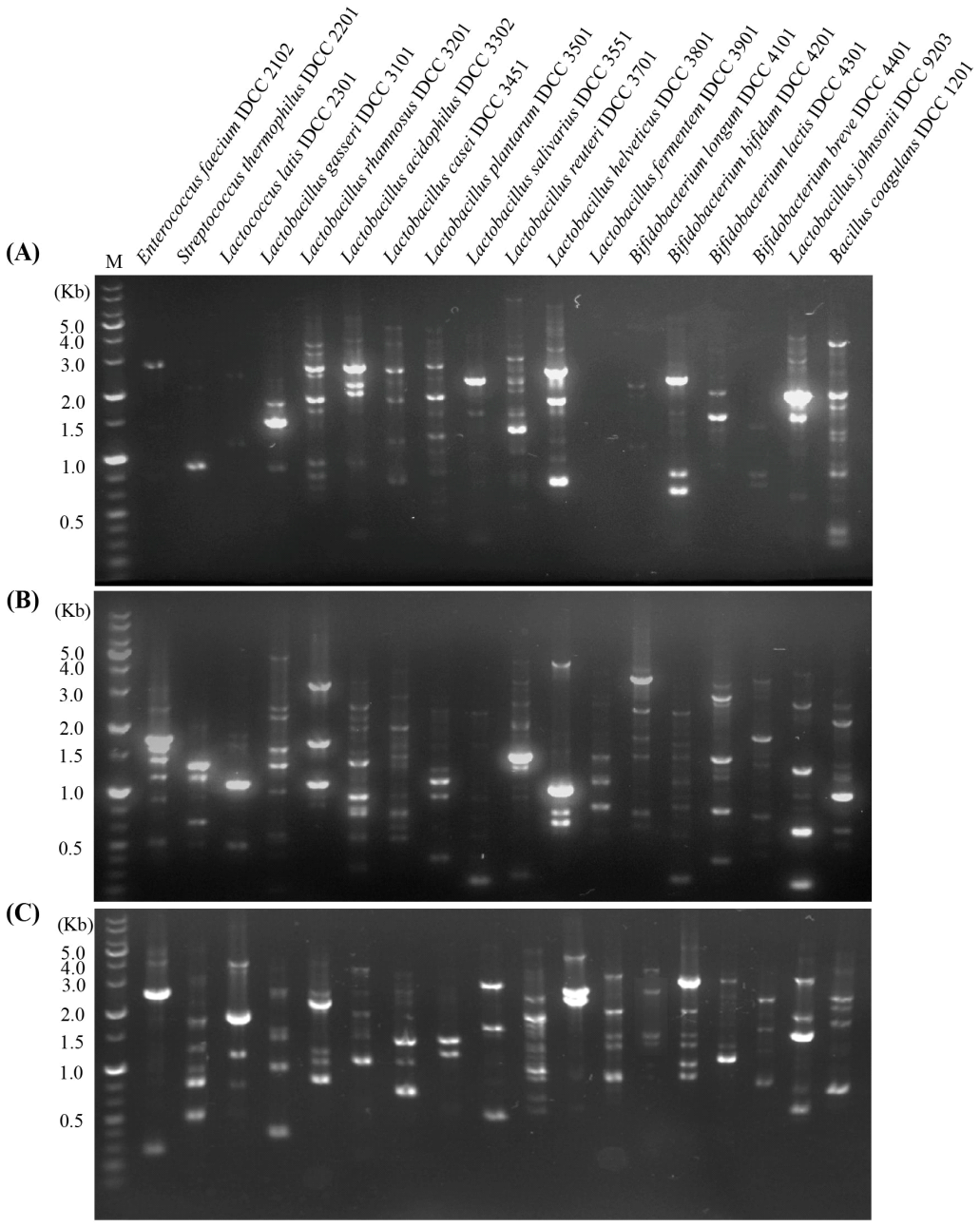

Fig. 1. Evaluation of three universal primers for RAPD-PCR analysis.

A, P1 (5'-ACGCAGGCA-3'); B, P2 (5'-ACGAGGCAC-3'); C, P3 (5'-ACGCGCCCT-3') primers. The figure is the representative from three independent experiments.

Enterococcus faecium IDCC 2102, *Streptococcus thermophilus* IDCC 2201, *L. lactis* IDCC 2301, *Bifidobacterium longum* IDCC 4101, *B. breve* IDCC 4401, and *Lactobacillus fermentum* IDCC 3901 (Fig. 1A). When performing the analysis using P2 primer, it was difficult to distinguish *L. salivarius* IDCC 3551 from *B. bifidum* IDCC 4201 due to the absence of a major band. Finally, P3 primer was selected as the suitable primer as it showed significant differences between the eighteen probiotic strains tested in the present study (Fig. 1C). These results are consistent with previous reports which showed differences or similarities among interspecies through RAPD-PCR analysis (Jarocki et al., 2020; Sharma et al., 2020).

Rapid identification of eighteen lyophilized probiotics commercially available

Based on the RAPD-PCR patterns using primer P3, a blind test for the rapid identification of lyophilized eighteen probiotics was performed. The RAPD-PCR profiles of the lyophilized form of probiotics were consistent with those of the cellular form (Fig. 1C and 2). For example, the two bands (~2.5 kb and ~0.3 kb) in lane 3 of sample No. 3 were identified as *E. faecium* IDCC 2102. One major band (~1.8 kb) and three minor bands (~4.0 kb, ~1.2 kb, and ~0.8 kb) in lane 12 were identified as *L. lactis* IDCC 2301. Two major bands (~3.0 kb and ~2.7 kb) and one minor band (~5.0 kb) in lane 16 were identified as *L. helveticus* IDCC 3801. Finally, the correlation between cellular and lyophilized forms of probiotics was determined bassed on the intensities of the major bands observed only for a specific strain (Fig. 1C and 2) using Image Lab software (Bio-Rad, version 6.1). The coefficient of determination (R^2) was 0.91, indicating the RAPD-PCR method used in this study was reliable for the identification of the eighteen lyophilized probiotic strains commercially available (Fig. 3).

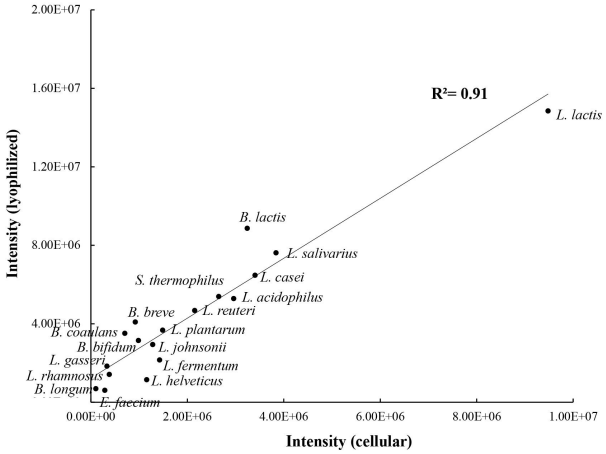

Fig. 3. Correlation of RAPD-PCR patterns between cellular and lyophilized forms of probiotics.

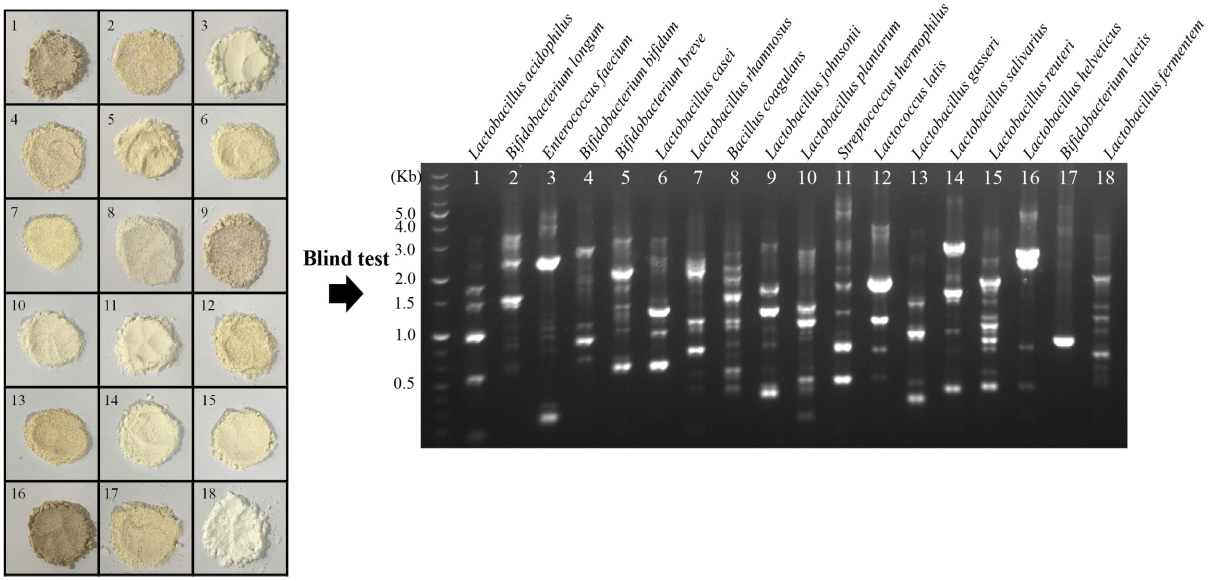

Fig. 2. Blind test for identification of eighteen lyophilized probiotics commercially available using RAPD-PCR analysis.
The figure is the representative from three independent experiments.

요 약

제품화를 위한 프로바이오틱스의 신속한 동정은 품질보증을 위한 중요한 단계이다. 이를 위해 다양한 동정 방법들이 시도되고 있으나, RAPD-PCR 분석법은 편리성 등의 장점으로 널리 오랫동안 사용되어 왔다. 본 연구에서는 18종 프로바이오틱스의 동정을 위하여 RAPD-PCR 분석법을 적용하였고, 이를 기반으로 제품형태를 블라인트 테스트한 결과, 18종 균주별로 정확히 동정됨을 확인할 수 있었다. 그리하여, 본 연구에서 적용된 RAPD-PCR 분석법은 제품화된 프로바이오틱스의 품질표준을 위한 적용이 가능함을 시사한다.

Acknowledgments

This work was supported by Korea Institute of Planning and Evaluation for Technology in Food, Agriculture and Forestry (IPET) through High Value-added Food Technology Development Program, funded by Ministry of Agriculture, Food and Rural Affairs (MAFRA) (321033-3) and a National Research Foundation of Korea (NRF) funded by the Korea government (MSIT) (No. 2020R1C1C1005251). This work was performed at the Ildong Bioscience Research Center and School of Food Science and Biotechnology in Kyungpook National University.

Conflict of interests

The authors declare no potential conflict of interest.

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