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Antilisterial Activity by Human *Bifidobacterium* spp. Isolated from Infant Stools

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Introduction

Bifidobacteria were first isolated from the faeces of breast-fed infants by Tissier (1990), who used the name *Bacillus bifidus communis*. Bifidobacteria are Gram-positive, non-gas-producing, anaerobes with bifid morphology, and Important constituents of the intestinal microflora of humans and animals. They have led to their use as probiotics in microbial adjunct nutrition and the management of gastrointestinal infection and antibiotic-associated diarrhoea. Recently, The anbimicrobial peptides are of great interest in food industry as natural preservatives and possible substitutes for chemical preservation. In this study, we investigated that the inhibitory effect of bifidobacteria to the production of antimicrobial proteinaceous compounds against the food pathogens. In addition, antimicrobial substances produced by bifidobacteria that were purified and characterized.

Materials and Methods

1. Microorganism

Faecal samples from breast fed infants were suspended and diluted with peptone water. It was inoculated on BL agar and incubated in anaerobic system. After incubation at 37℃ for 36h, the colonies were selected. Pathogens as an indicator strain was *Listeria monocytogenes* KCCM 40307. It was incubated in BHI broth at 37℃ for 12h.

2. Antilisterial Activity Assay

The isolated strains were inoculated into MRS broth with 0.05% L-cysteine. The cultured supernatants were separated, neutralized, and filter-sterilized. *Listeria monocytogenes* was inoculated into tryptic soy broth along with the pH-controlled filtered

supernatant (PCFS) and the saline as the control. After incubation at 37℃ for 12h, the absorbance was measured at 600nm.

3. Identification of Isolates

The isolated strain was identified by using the morphological property of Bergey's manual, the scanning electron micrograph, the API 50 CHL kit, and the PCR with a species-specific primer designed from the 16S rRNA sequence.

4. Isolation of Plasmid DNA

Plasmid DNA was isolated by using mini-prep method (Sambrook, 1989). The plasmid DNA was used for agarose gel electrophoresis and restriction enzyme digestion (RNase).

Properties of Antilisterial substance. the thermal stability (60~121 ℃), the pH stability (pH 3~11), and the sensitivity to pronase E and proteinase (10mg/ml) of the PCFS were tested by antilisterial activity assay.

5. Isolation and Purification of Antilisterial Substance

The antilisterial substance was concentrated by using the ultrafiltration (cut-off 3,000 Da) and 50% ammonium sulfate precipitation. The extracts were subjected to ion exchange chromatography (DEAE sepharose CL-6B) and reversed phase HPLC with the symmetry C18 column.

6. SDS-PAGE Analysis

The antilisterial substance was analyzed by SDS-PAGE. The sample was heated at 100℃ for 3min and applied to a SDS-polyacrylamide (20%) gel for electrophoresis.

Conclusion

1. The strain A24, which showed the highest antilisterial activity among 52 isolates from infants feces, was identified as *Bifidobacterium longum* A24.

2. *Bifidobacterium* A24 had three plasmids; 37.2kb, 3.5kb, 2.3kb in size.

3. Optimized medium condition of *Bifidobacterium* A24 was 2% (w/v) glucose and 3% (w/v) beef extract as carbon and nitrogen source, respectively.

4. The antilisterial activity remained stable at pH 5.0~7.0 and by heat treatments(60~121℃), but it decreased significantly by the two protease, acid (HCl), alkali (NaOH), and TCA treatments.

5. The antilisterial substance finally purified by reverse phase HPLC analysis.

6. Molecular weight was estimated as 9.5kDa on 20% SDS-PAGE.

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