

Note

Effect of Depolymerized Alginites on the Growth of Bifidobacteria

Hisayoshi Akiyama, Taeko Endo, Ryo Nakakita, Kousaku Murata,*,† Yoshimasa Yonemoto, and Kenichi Okayama

Otsuka Chemical Co., Ltd., Division of Food and Beverage Research, Kawachi, Tokushima 771-01, Japan
*Research Institute for Food Science, Kyoto University, Uji, Kyoto 611, Japan

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Bifidobacterium, a Gram-positive anaerobe first isolated from a breast-fed infant by Tissier,11 is the predominant microorganism in the intestinal tract of humans and some animals.21 The organism is known to decrease intestinal pH by producing such organic acids as acetic, propionic, butyric, and lactic acids from certain sugars. Such acids are thought to be involved in reducing the population of putrefactive bacteria in the intestine25 and in eliciting the defense mechanism against gastrointestinal disorders.26 The low pH caused by the acids also inhibits the uptake of amines and promotes intestinal peristalsis, leading to a repression of unusual fermentation or constipation.27 Through these properties, bifidobacteria are believed to maintain the intestinal milieu and to prevent the senility of hosts. From the standpoint of better human health, methods are now being sought to increase the bifidobacterial population in intestinal flora, and several kinds of sugars such as fructo-, galacto-, xylono-, and agarooligosaccharides have been confirmed to enhance the growth of bifidobacteria.28 30 We found that the growth of bifidobacteria in skim milk was also accelerated in the presence of alginites depolymerized with bacterial alginate lyases.

Alginate is a polymer comprising of residues of β-D-mannuronic and α-L-guluronic, and these residues are arranged in a block structure of homopolymeric (poly β-D-mannuronic and α-L-guluronic) or heteropolymeric (a random sequence of these two residues) regions.12 The depolymerization of sodium alginate (from Kimita Chemical Industries Co., Tokyo, Japan), which was prepared by alkaline extraction from a sea-weed Lentinia spp. and had a molecular weight of 49,500, was carried out in a mixture (20 ml) containing 4.0% sodium alginate and cell extracts (0.6 mg/ml of protein) prepared from bacterium strain A-2, a potent alginate lyase producer.44 The conditions for cultivating the strain and preparing cell extracts have been described previously.52 Protein was determined by the method of Lowry et al.13 After incubating at 45°C for 2 hr, the mixture was heated at 100°C for 5 min. Depolymerization of the alginate by the alginate lyase reaction produced a mixture of oligomers having an average molecular weight of 2,000 (data not shown). Sodium alginate was also similarly treated with heat-inactivated (100°C for 5 min) cell extracts and used as a control. To cultivate the bifidobacteria, all of which were obtained from Japan Bifidus Foundation, Tokyo, Japan, 1.0 ml of an appropriately diluted alginate solution and 0.2 ml (2 × 10⁷ cells) of a cell suspension were successively added to 9.0 ml (in a 1.0 cm diameter × 16 cm test tube) of 10% skim milk (Takanashi Milk Products Co., Yokohama, Japan) that had been previously sterilized at 115°C for 10 min. The tubes were put into an anaerobic jar (BBI, Gaspack Anaerobic System; Nippon Becton Dickinson Co., Tokyo, Japan) and incubated at 37°C. Growth was determined by either a colony count or pH titration of the cultures. The cell numbers were counted by streaking the cultures onto plates of BL-agar (1.5%); (Niissui Seiyaku Co., Tokyo, Japan) with a spiral plater (Spiral System Instruments, Cincinnati, Ohio, U.S.A.). Each culture (10 ml) was titrated with 0.1 M NaOH, and the growth is expressed in terms of the volume of NaOH required.

The effect of depolymerized alginites on the growth of Bifidobacterium breve I-53-8 was examined and compared with that of yeast extracts (Difco Laboratories, Detroit, Michigan, U.S.A.) or alginate before depolymerization (Fig. 1). Alginate before depolymerization showed little effect on the growth of the bacterium, the growth being almost the same as that of the control. On the other hand, the addition of depolymerized alginites markedly accelerated the growth of B. breve I-53-8, although the effect was somewhat lower than that of yeast extracts.

The effect of depolymerized alginites was further confirmed by using various kinds of bifidobacteria (B. adolescentis M101-4, B. bifidum A234-4, B. breve I-53-8, B. infantis I-10-5, and B. longum M101-2) and by comparing with that of lactosucrose and galactooligosaccharides (Fig. 2). Lactosucrose (Lactosucrose LS-35; Esumi Sugar Refining Co., Yokohama, Japan) had a structure of O-β-D-Gal-(1→4)-O-α-D-Glc-(1→2)-β-D-Fru, where Gal, Glic, and Fru denote galactose, glucose, and fructose, respectively.51 Galactooligosaccharides (Oligomates 50; Yakult Pharmaceutical Ind. Co., Tokyo, Japan) was a mixture of various

Fig. 1. Effect of Depolymerized Alginites on the Growth of B. breve I-53-8.

Depolymerized alginate (□), yeast extracts (●), and alginate before depolymerization (○) were used at 0.4%, and the growth was determined by a colony count. The growth in skim milk (■) with no additives was taken as a control.

Fig. 2. Effect of Depolymerized Alginites on the Growth of Various Bifidobacteria.

Bifidobacteria were grown in skim milk supplemented with none (A), depolymerized alginate (0.04%, B-I), depolymerized alginate (0.4%, B-II), alginate before depolymerization (0.4%, C), lactosucrose (0.04%, D) and galactooligosaccharides (0.08%, E) at 35°C for 48 hr, and the cultures were titrated with 0.1 M NaOH. The numbers below the bars represent the strains used (I, B. adolescentis M101-4; 2, B. bifidum A234-4; 3, B. breve I-53-8; 4, B. infantis I-10-5; and 5, B. longum M101-2).
oligosaccharides with a structure of Gal-(Gal)nGlu (n=1—4). The detailed structure and properties of the saccharides have been described by Tanaka et al.\textsuperscript{15} When depolymerized alginites were used at 0.04%, the growth of all bacteria was slightly enhanced, the effect being almost comparable with that of lactosucrose (0.04%) or galactooligosaccharides (0.08%). However, when the dose of depolymerized alginites was increased to 0.4%, the growth of all bifidobacteria was markedly enhanced, although the effect varied depending on the strains used. On the other hand, alginate before depolymerization showed an inhibitory effect on the growth of all bifidobacteria.

Thus, enzymatically depolymerized alginites were found to promote the growth of bifidobacteria in skim-milk. At present we have no idea why and by which means depolymerized alginites can enhance the growth of bifidobacteria. Since alginate is a heteropolymer of β-D-mannuronate, and the C5 epimer of α-L-guluronate and depolymerized alginites are inert as a carbon source for the growth of bifidobacteria (data not shown), we are investigating the effect of alginate oligomers containing mannnuronate and guluronate in various proportions on the growth of bifidobacteria.

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References