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## **A Survey of Growth Stimulators in Natural Rubber Serum Powder for *Bifidobacterium bifidum***

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Natural rubber serum powder, rich in crude protein and carbohydrates, had a strong growth-stimulating activity for *Bifidobacterium bifidum*, which was unable to grow in a fully synthetic medium, B<sub>12</sub> assay medium. Natural rubber serum powder was fractionated by ultrafiltration (molecular weight cut off 1000). The active ultrafiltrate was furthermore concentrated and desalted with an adsorptive microconcentrator, which adsorbs virtually all amino acids and peptides. Through this purification step, it was found that the adsorbed fraction obtained could not exhibit a growth-stimulating activity independently but acted complementarily with ammonium sulfate contaminated in the ultrafiltrate. Furthermore, ammonium sulfate could be substituted with other ammonium salts, ammonium chloride and ammonium nitrate. The adsorbed fraction was subsequently analyzed on reversed-phase high performance liquid chromatography, and the activities of the eluates were measured on B<sub>12</sub> assay medium supplemented with ammonium sulfate. Consequently, it was proved that several ingredients in the adsorbed fraction enhanced the growth of *B. bifidum*.

### INTRODUCTION

Bifidobacteria are gram-positive, saccharolytic, anaerobic bacteria that are major components of the human gastrointestinal tract. The most important benefits on the host health are inhibition or displacement of undesirable microorganisms, elimination of procarcinogens, immunomodulation, and vitamin production (Gibson and Wang, 1994; Kado-oka *et al.*, 1991; Modler *et al.*, 1990; Noda *et al.*, 1994; Wilhelm *et al.*, 1987). As a result, they are often used as food supplements and in milk fermentation (Ishibashi, 1990). However, biomass production is low because these microorganisms require a growth-stimulating factor, bifidogenic growth stimulator (BGS) which is usually absent even in fully synthetic media composed of sugars, vitamins, nucleic and amino acids. Therefore, several investigations have been reported on a number of potential sources of BGSs. Poch and Bezkorovainy (1988) showed the availability of bovine casein digest and yeast extract as BGSs in a fully synthetic medium similar to the well-known Norris medium (Poupard *et al.*, 1973). Moreover, Ibrahim and Bezkorovainy (1994) investigated the growth-stimulating activity of various organic compounds for *B. longum* by using B<sub>12</sub> assay medium, which alone permitted only limited bifidobacterial growth. However, most of them are costly and/or have some other drawback. For example, *B. bifidum* cannot utilize many kinds of oligosaccharides, such as fructooligosaccharide (Hidaka, 1993) and soybean oligosaccharide (Masai, 1988), which are well known as BGSs. Except these oligosaccharides, there are a few reports on completely identified BGSs to date (Mori *et al.*, 1997).

Natural rubber serum powder (NRSP) is a natural rubber waste, rich in crude protein and carbohydrates, produced during the manufacturing process of latex rubber (Ishizaki, 1989). Several attempts to utilize NRSP as a newly fermentable substrate have been carried out in our laboratory as means of reducing its polluting effect on the environment (Tripetchkul *et al.*, 1992; Chinachoti *et al.*, 1997). We have observed that NRSP has a strong growth-stimulating activity for a wide range of bifidobacteria of human origin (Ishizaki, 1995). In addition, Oiki *et al.* (1996a) investigated the effect of NRSP on *B. bifidum* in detail using bifidobacterium medium and/or the minimal medium without organic nutrient. NRSP showed synergistic effects with various organic nutrients in a similar manner to casein, which is composed of many kinds of peptides. However, both nutrients had different concentration-related effects on the growth of *B. bifidum* (Oiki *et al.*, 1996b). Further investigating into the nature of NRSP, the complexity of the experimental media appears to make it difficult to clearly understand the nutritional effects of NRSP on *B. bifidum*, and to distinguish whether the effect of NRSP is attributed to a simple nutritional source or BGS. Furthermore, we have observed a lower sensitivity of cells to the growth-stimulating activity of BGSs when partially purified BGSs from NRSP was added to bifidobacterium medium which alone could easily lead to much cell proliferation. To solve these problems, the use of a chemically defined medium mentioned above is necessary. In this paper, we attempted to prove the growth-stimulating effects of NRSP by using B<sub>12</sub> assay medium, and to isolate the active ingredients from NRSP that act as BGSs.

## MATERIALS AND METHODS

### Microorganism

*B. bifidum* JCM 1254, of human origin, was purchased from the Japan Collection of Microorganisms, Wako.

### Natural rubber serum powder (NRSP)

NRSP was provided by Nakanihon Air Service Co., Ltd. (Nagoya). NRSP used in this study was the spray-dried product of natural rubber serum imported from Malaysia. It was composed of many kinds of amino acids, peptides, inorganic salts and so on. The detailed composition of NRSP was described previously (Ishizaki, 1989).

### Bioassay

The microorganism was grown in anaerobic jars (GasPak, BBL Microbiology Systems, Cockeysville, MD, U.S.A.) in thioglycolate (TGC) medium without dextrose (Difco Laboratories, Detroit, MI, U.S.A.) and stored at 5°C. The stored culture was precultured in the bifidobacterium medium (Ishizaki, 1995) at 37°C for 24 h. The culture broth was centrifuged at 8,000 × g for 10 min. The precipitate cells were washed two times and suspended with sterile solution of KCl (1.08%). The basal medium used for a bioassay was B<sub>12</sub> assay medium (Difco). The assay medium (3.6 ml) was mixed with 0.4 ml of samples and then inoculated with 5% of the prepared inoculum. The control contained 0.4 ml of water instead of the samples. Culture was carried out under an anaerobic condition at 37°C. The extent of growth was determined by measuring the absorbance at

562 nm ( $A_{562}$ ) at various time intervals. The growth experiments were performed in duplicate or more each time, and resulting values were averaged in each experiment.

### Purification of BGSSs

NRSP was applied to ultrafiltration with a membrane of molecular weight cut off (MWCO) 1000 (Amicon, Inc., Beverly, MA, U.S.A.). The ultrafiltrate obtained was transferred into an adsorptive microconcentrator (MICROCON-SCX, Amicon) and centrifuged at  $1,200\times g$  for 1 min to remove low-molecular-weight contaminants from the samples. The components adsorbed on the column were eluted with 1.4 N  $\text{NH}_4\text{OH}/\text{MeOH}$  desorption reagent by centrifugation at  $14,000\times g$  for 15 sec. The eluate was dried with Speed Vac Concentrator (Savant Instruments, Inc., Holbrook, NY, U.S.A.) and dissolved in water. The prepared samples were subjected to a reversed-phase high performance liquid chromatography (HPLC) on a DAISOPAK SP-120-5-ODS-BP column (Daiso Co., Ltd., Osaka; 25 cm  $\times$  4.6 mm inner diameter) and eluted with a linear  $\text{CH}_3\text{CN}$  gradient (5–40%, 35 min) in water at a flow rate of 0.5 ml/min, while monitoring the absorbance at 210 nm. The isolated fractions were then collected and dried with Speed Vac Concentrator.

## RESULTS AND DISCUSSION

### Effect of NRSP in $\text{B}_{12}$ assay medium

Although *B. bifidum* could not be grown in  $\text{B}_{12}$  assay medium at all, the addition of 1% (w/v) NRSP to the medium remarkably enhanced its growth (Fig. 1). This result

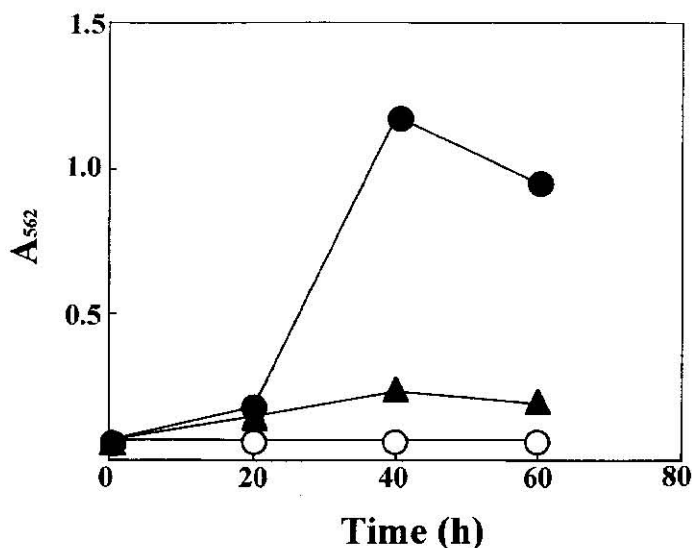


Fig. 1. Effects of NRSP and ammonium sulfate on *Bifidobacterium bifidum* JCM 1254 in  $\text{B}_{12}$  assay medium.  $\text{B}_{12}$  assay medium (○) was supplemented with 10 g/l NRSP (●) and 2.5 g/l ammonium sulfate (▲).

indicates the higher sensitivity of cells to the growth-stimulating activity of NRSP on B<sub>12</sub> assay medium, the cell growth being slower compared to it in the bifidobacterium medium. NRSP contains ammonium sulfate (Ishizaki, 1989), the concentration of which was found to be ca. 25% (w/w-NRSP). Since ammonium sulfate is known to be one of the major nitrogen sources of bifidobacteria (Tanaka and Mutai, 1980), this inorganic salt in NRSP was considered to exhibit some of the growth-stimulating activity of NRSP as a contaminant. Actually, ammonium sulfate aided the growth of *B. bifidum* on a nutritional medium, which was inferior to the effect of NRSP (Oiki *et al.*, 1996b). Therefore, the effect of ammonium sulfate at the concentration corresponding to that contained in NRSP on *B. bifidum* was assessed in this experiment. As shown in Fig. 1, the growth of the test strain was slightly affected by the addition of 0.25% ammonium sulfate to the medium. These results indicate that biologically active ingredients other than ammonium sulfate, namely BGSs, would probably be the main active principles of NRSP and that B<sub>12</sub> assay medium was useful for evaluating the growth-stimulating activity of BGSs. In the process of purifying the BGSs from NRSP, thus, the growth-stimulating activity was defined as the absorbance of 562 nm after cultivation for 40 h.

#### Partial purification of BGSs from NRSP

As a first purification step, we performed fractionation of NRSP by using ultrafiltration (MWCO 1000) to estimate the molecular size of the BGSs in NRSP. As shown in Fig. 2, the ultrafiltrate obtained had an activity almost equal to that of untreated NRSP, but the retained fraction hardly showed any growth-stimulating effects. This suggested that a low-molecular-weight material less than 1000 Da would be responsible for the growth-stimulating activity of NRSP.

Adsorptive microconcentrator contains a strong cation exchange membrane which adsorbs virtually all amino acids and peptides. This device was used to concentrate peptides and to desalt the ultrafiltrate. The eluate containing ammonium sulfates and so on was not found to have any growth-stimulating activity. However, the adsorbed fraction obtained also did not entirely exhibit growth-stimulating effects, even when the concentration of the fraction in the medium was increased (Fig. 3). Thus, despite a lack of the growth-stimulating activity in ammonium sulfate (Fig. 1), the activity of the ultrafiltrate was completely lost by desalting. To solve this contradiction, we assumed that ammonium sulfate removed could be one of the key components for the expression of the activity of the adsorbed fraction. Figure 3 shows that the adsorbed fraction expressed a growth-stimulating activity for *B. bifidum* in a dose-dependent manner in the presence of 0.25% ammonium sulfate. This result revealed the presence of certain growth stimulators in the adsorbed fraction. The activity of the adsorbed fraction, which was supplemented with ammonium sulfate, at the concentration corresponding to that contained in the ultrafiltrate was a little lower than that of the ultrafiltrate itself (The former value was 0.55 in Fig. 3, and the latter was 0.92 in Fig. 2). Thus, we established a successful bioassay system to isolate the desired BGSs by completely desalting the ultrafiltrate and then supplementing a small amount of ammonium sulfate to B<sub>12</sub> assay medium.

To rapidly isolate the BGSs from NRSP, the adsorbed fraction was directly analyzed on reversed-phase HPLC. The chromatogram is shown in Fig. 4. The eluates were

broadly fractionated into four fractions, and the growth-stimulating activity of each fraction was measured on B<sub>12</sub> assay medium supplemented with 0.25% ammonium sulfate. Consequently, each fraction except for fraction A showed a growth-stimulating activity which was unique to that fraction (Table). The activity of the mixture of all the HPLC fractions was nearly identical with that of the adsorbed fraction (data not shown). Since all the active fractions showed a positive ninhydrin-reaction test, the active ingredients in these fractions appeared to be amino acids and/or peptides. However, the addition of a mixture of 20 kinds of amino acids could not stimulate the growth of the test strain on B<sub>12</sub> assay medium (data not shown). This indicates that the peptide structure of the substances could be solely responsible for their functional activity.

Some of growth stimulators for other microorganisms, for example, *Klebsiella*, *Lactobacillus*, have been isolated from proteinaceous compounds as a single compound (Berg *et al.*, 1981; Zhao *et al.*, 1996). However, in this study, composite growth factors existed in the adsorbed fraction. Interestingly, the high-molecular-weight fraction obtained by ultrafiltration also exhibited remarkable growth-stimulating effects when

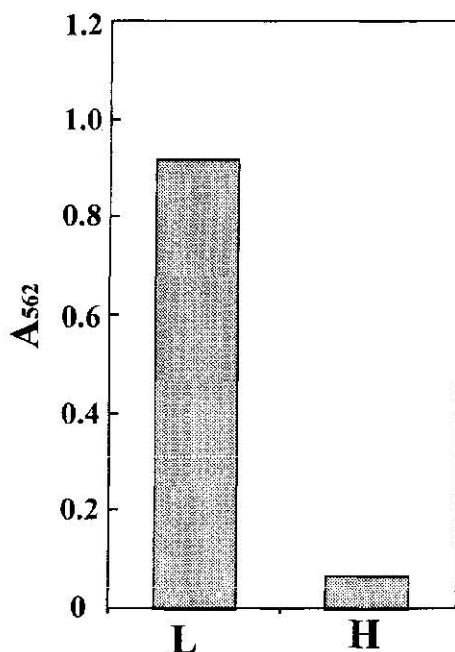
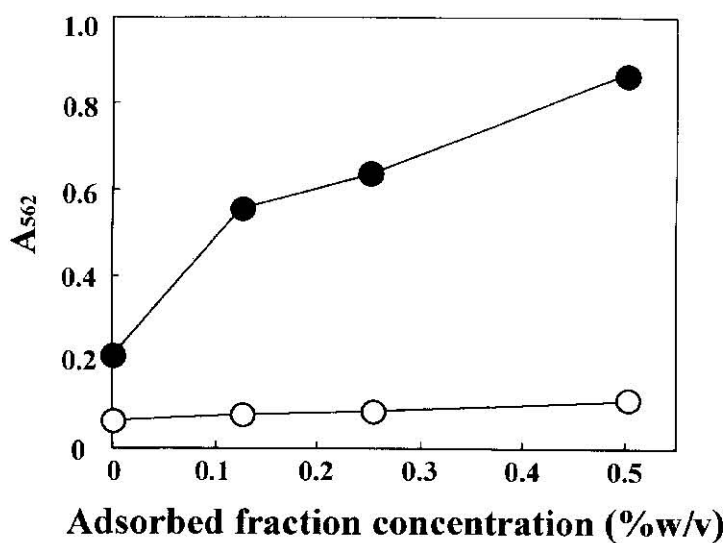


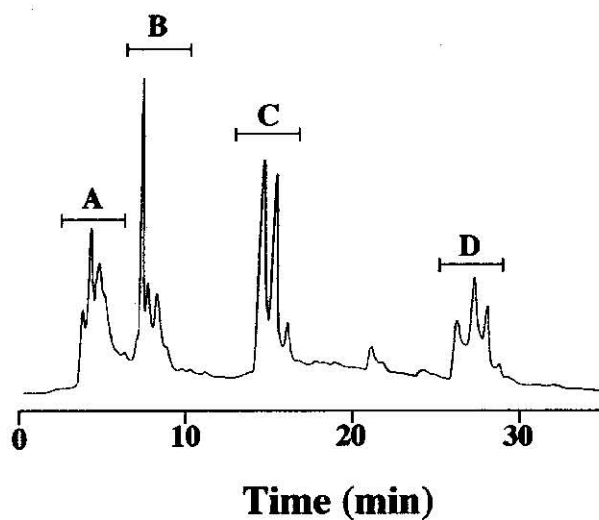
Fig. 2. Effects of ultrafiltrated NRSP on *Bifidobacterium bifidum* JCM 1254 in B<sub>12</sub> assay medium.

NRSP was fractionated into high- and low-molecular weight fractions (H and L, respectively) by ultrafiltration (MWCO 1000). Each fraction was added at the concentration corresponding to that contained in 10 g/l NRSP.



**Fig. 3.** Effects of concentrations of adsorbed fraction on *Bifidobacterium bifidum* JCM 1254.

Adsorbed fraction was obtained by treatment of the ultrafiltrate (MWCO 1000) of NRSP with adsorptive microconcentrator. B<sub>12</sub> assay medium was supplemented with the adsorbed fraction in the presence of 2.5 g/l ammonium sulfate (●) and without ammonium sulfate (○). The concentration of the adsorbed fraction (0.12%) corresponded to that contained in 10 g/l NRSP.



**Fig. 4.** Reversed-phase HPLC analysis of adsorbed fraction. The crude BGS from adsorptive microconcentrator was subjected to HPLC.

**Table.** Effects of fractions from reversed-phase HPLC on *Bifidobacterium bifidum* JCM 1254

Sample <sup>a</sup>	Activity (A <sub>540</sub> ) <sup>b</sup>
Adsorbed fraction	0.387
Fraction A	0.015
Fraction B	0.241
Fraction C	0.188
Fraction D	0.115

<sup>a</sup> Each sample was added at the concentration corresponding to that contained in 10 g/l NRSP.

<sup>b</sup> The activity of the sample was defined as the difference in cell growth of *B. bifidum* observed after 40 h in B<sub>12</sub> assay medium containing 2.5 g/l ammonium sulfate, with and without the sample.

0.25% ammonium sulfate was added (data not shown). Poch and Bezkorovainy (1991) have tried to isolate BGSs from casein enzymatic hydrolyzates. Although they found that sulfhydryl groups were indispensable for the expression of the activity of casein enzymatic hydrolyzates, the several synthetic peptides including cysteine and/or cystine had no growth-stimulating activity. Furthermore, dispersal over several fractions and decrease in the growth-stimulating activity were observed during the purification of BGSs from casein enzymatic hydrolyzates. These results were similar to those obtained in our experiment. Possibly, BGSs derived from proteinaceous compounds could stimulate the growth of *Bifidobacterium* synergistically. Regardless of the number of active peptides in the compounds, the importance of peptide structure has been emphasized because of the unique action mechanism which can not be explained as the effect of simple amino acid suppliers. Zhao *et al.* (1996) have supposed that the peptides target periplasmic binding proteins or their peptide permeases of gram-negative bacteria, resulting in the stimulation of a particular peptide transport system dependent on peptide structure. The activities of four fractions obtained with reversed-phase HPLC were different to some extent. This result indicates the preferential utilization of the BGSs by *B. bifidum* as the specificity of milk peptide utilization observed in *Lactococcus lactis* (Juillard *et al.*, 1998). Further investigation into the structure-activity relationships of the BGSs should give some clues to clarify the complexity of demand for nutrients, which is responsible for the limited bifidobacterial growth.

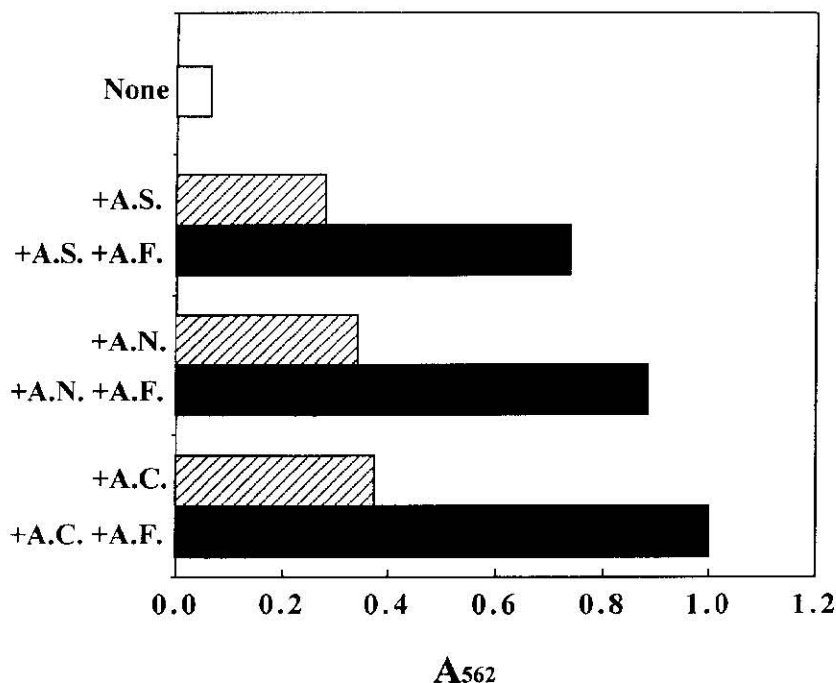
#### Effects of several ammonium salts on the expression of activity of the BGSs

We found in the course of this purification step that a small amount of ammonium sulfate was required for the expression of the activity of the BGSs on B<sub>12</sub> assay medium. Since ammonium sulfate is one of ammonium salts which are major nitrogen sources for *Bifidobacterium* (Matteuzzi *et al.*, 1978), we focused on the role of ammonium sulfate as an ammonium salt in the complementary effect with the BGSs. Ammonium nitrate and ammonium chloride were tested as other ammonium salts. As shown in Fig. 5, both



ammonium salts also aided the expression of the activity of the BGSs although their complementary effects on the growth were different.

Oiki *et al.* (1996a) showed that NRSP stimulated the growth of *B. bifidum* in a similar manner to casein on the basis that both could be utilized not only as a growth stimulator but also as a source of nitrogen by *B. bifidum* on the minimal bifidobacterium medium. However, the complete removal of ammonium sulfate from NRSP revealed that the BGSs in NRSP could not act as a simple nitrogen supplier independently but express the activity through the complementary effects with inadvertently contaminated ammonium sulfate on a synthetic medium such as B<sub>12</sub> assay medium. At present, we can not clearly understand the role of ammonium sulfate in the expression of the activity of the BGSs. It is considered that ammonium sulfate would support the expression of the activity of the BGSs either directly or indirectly. Poch and Bezkorovainy (1991) have proposed a carrier for common metals, vitamins, or lipids, as one of the action mechanisms of  $\kappa$ -casein. On the other hand, Juillard *et al.* (1998) investigated the specificity of milk peptide utilization by *Lactococcus lactis*. They also found that the



**Fig. 5.** Effects of several ammonium salts on the expression of the activity of the BGSs. B<sub>12</sub> assay medium containing the indicated ammonium salts was supplemented with (black bars) and without (hatched bars) 0.24% (w/v) adsorbed fraction (A.F.). Ammonium salts were added as follows: +A.S., 18.9mM ammonium sulfate; +A.N., 37.8mM ammonium nitrate; +A.C., 37.8mM ammonium chloride.

oligopeptide fraction in milk required some essential amino acids contaminated for the expression of the growth-stimulating activity. Thus, it has been observed that peptide fraction expresses the activity together with additional nitrogen sources. Generally, some nutrients such as peptides and sugars are incorporated into a cell through various accumulated energy forms, such as proton motive force and ATP-driven force (Poolman, 1993). In our case, no cell-growth was observed in B<sub>12</sub> assay medium alone (Fig. 1). This result indicates that intracellular metabolic flux would not be fully activated. As a result, intracellular ATP production and formation of pH gradient between inner and outer membrane did not occur. According to this hypothesis, the addition of ammonium sulfate, which was easily incorporated into a cell, triggered the cell growth as an additional nitrogen source, then the BGSs were subsequently utilized by the metabolically activated cells. Oiki *et al.* (1996b) actually found the metabolic activation of cells in the presence of NRSP. If there were nitrogen sources in the medium enough to grow cells even slightly, the BGSs might be effectively utilized by the test strain. Figure 5 demonstrates the expression of the activity of the BGSs by several ammonium salts which induced a little cell growth as nitrogen sources on B<sub>12</sub> assay medium. Especially, ammonium chloride exhibited a complementary effect more remarkably than other ammonium salts. The addition of ammonium chloride instead of ammonium sulfate to B<sub>12</sub> assay medium would lead to the establishment of a more excellent bioassay system for the further purification of the BGSs. At the industrial level, it is expected that a higher cell density culture should be done by the combination of the BGSs derived from NRSP with relatively cheap ammonium salts instead of expensive organic nitrogen sources. Although the studies on the uptake of sugars by *Bifidobacterium* have been reported by several investigators (Degnan and Macfarlane, 1993; Krzewinski *et al.*, 1996, 1997), no publications on the peptide transport system are available. It is true that the BGSs would exhibit a higher activity owing to the peptide structure, but the extent of energy demand for the uptake of the peptides might characterize the limited bifidobacterial growth compared to other microorganisms.

Furthermore, the peptide content in the medium used has been found to affect the regulation of proteolytic activity in *Lactococcus lactis* (Meijer *et al.*, 1996). On the other hand, it has been reported that *Bifidobacterium* utilizes peptides produced by the proteinase of lactic acid bacteria, which is employed as a mixed starter in milk, due to its relatively low proteolytic activity (Taminc *et al.*, 1995). In this view point, the relationship between proteolytic activity of *Bifidobacterium* and the content of nitrogen sources in a medium seems to be interesting. Thus, the presence of nitrogen sources in a medium could play a significant role in the process of the utilization of peptides in these microorganisms.

## REFERENCES

- Berg, R. W., W. E. Sandine and A. W. Anderson 1981 Identification of a growth stimulator for *Lactobacillus sanfrancisco*. *Appl. Environ. Microbiol.*, **42**: 786–788
- Chinachoti, N., H. Matsusaki, K. Sonomoto and A. Ishizaki 1997 Utilization of xylose as an alternative carbon source for nisin Z production by *Lactococcus lactis* IO-1. *J. Fac. Agr., Kyushu Univ.*, **42**: 171–181
- Degnan, B. A. and G. T. Macfarlane 1993 Transport and metabolism of glucose and arabinose in *Bifidobacterium breve*. *Arch. Microbiol.*, **160**: 144–151

- Gibson, G. R., and X. Wang 1994 Regulatory effects of bifidobacteria on the growth of other colonic bacteria. *J. Appl. Bacteriol.*, **77**: 412-420
- Hidaka, H. 1993 Functional sugar to improve human health, fructooligosaccharide. *Gendai Kagaku*, **268**: 64-68 (In Japanese)
- Ibrahim, S. A. and A. Bezkorovainy 1994 Growth-promoting factors for *Bifidobacterium longum*. *J. Food Sci.*, **59**: 189-191
- Ishibashi, N. 1990 Application of *Bifidobacterium* for yogurt production. *Biseibutsu*, **6**: 66-73 (in Japanese)
- Ishizaki, A. 1989 Utilization of natural rubber waste as medium ingredients for fermentation process. *Microb. Utiliz. Renewable Res.*, **6**: 235-241
- Ishizaki, A. 1995 Natural rubber serum which contains a special growth promoter for *Bifidobacterium*. *Biosci. Biotech. Biochem.*, **59**: 1150-1151
- Juillard, V., A. Guillot, D. L. Bars and J.-C. Gripon 1998 Specificity of milk peptide utilization by *Lactococcus lactis*. *Appl. Environ. Microbiol.*, **64**: 1230-1236
- Kado-oka, Y., S. Fujiwara and T. Hirota 1991 Effects of bifidobacteria cells on mitogenic response of splenocytes and several functions of phagocytes. *Milchwissenschaft*, **46**: 626-630
- Krzewinski, F., C. Brassart, F. Gavini and S. Bouquelet 1996 Characterization of the lactose transport system in the strain *Bifidobacterium bifidum* DSM 20082. *Curr. Microbiol.*, **32**: 301-307
- Krzewinski, F., C. Brassart, F. Gavini and S. Bouquelet 1997 Glucose and galactose transport in *Bifidobacterium bifidum* DSM 20082. *Curr. Microbiol.*, **35**: 175-179
- Masai, T. 1988 Characteristics and application of soy-bean oligosaccharide. *Syokuhin to Kagaku*, **30**: 98-103
- Matteuzzi, D., F. Crociani and O. Emaldi 1978 Amino acids produced by bifidobacteria and some clostridia. *Ann. Microbiol.*, **129B**: 175-181
- Meijer, W., J. D. Marugg and J. Hugenholtz 1996 Regulation of proteolytic enzyme activity in *Lactococcus lactis*. *Appl. Environ. Microbiol.*, **62**: 156-161
- Modler, H. W., R. C. McKellar and M. Yaguchi 1990 Bifidobacteria and bifidogenic factors. *Can. Inst. Food Sci. Technol. J.*, **23**: 29-41
- Mori, H., Y. Sato, N. Taketomo, T. Kaniyama, Y. Yoshiyama, S. Meguro, H. Sato and T. Kaneko 1997 Isolation and structural identification of bifidogenic growth stimulator produced by *Propionibacterium freudenreichii*. *J. Dairy Sci.*, **80**: 1959-1964
- Noda, H., N. Akasaka and M. Ohsugi 1994 Biotin production by bifidobacteria. *J. Nutr. Sci. Vitaminol.*, **40**: 181-188
- Oiki, H., K. Sonomoto and A. Ishizaki 1996a Stimulation by natural rubber serum powder of the growth of *Bifidobacterium bifidum*. *J. Fac. Agr., Kyushu Univ.*, **40**: 271-277
- Oiki, H., K. Sonomoto and A. Ishizaki 1996b Growth-stimulating effects of natural rubber serum on *Bifidobacterium bifidum*. *J. Ferment. Bioeng.*, **82**: 165-167
- Pock, M. and A. Bezkorovainy 1988 Growth-enhancing supplements for various species of the genus *Bifidobacterium*. *J. Dairy Sci.*, **71**: 3214-3221
- Poch, M. and A. Bezkorovainy 1991 Bovine milk  $\kappa$ -casein trypsin digest is a growth enhancer for the genus *Bifidobacterium*. *J. Agric. Food Chem.*, **39**: 73-77
- Poolman, B. 1993 Energy transduction in lactic acid bacteria. *FEMS Microbiol. Rev.*, **12**: 125-148
- Poupard, J. A., I. Husain and R. F. Norris 1973 Biology of the bifidobacteria. *Bacteriol. Rev.*, **37**: 136-165
- Tamine, A. Y., V. M. E. Marshall and R. K. Robinson 1995 Microbiological and technological aspects of milks fermented by bifidobacteria. *J. Dairy Res.*, **62**: 151-187
- Tanaka, R. and M. Mutai 1980 Improved medium for selective isolation and enumeration of *Bifidobacterium*. *Appl. Environ. Microbiol.*, **40**: 866-869
- Tripathi, S., M. Tonokawa and A. Ishizaki 1992 Ethanol production by *Zymomonas mobilis* using natural rubber waste as a nutritional source. *J. Ferment. Bioeng.*, **74**: 384-388
- Wilhelm, H. P., D. T. Lee and J. E. Rosenblatt 1987 Bacterial interference by anaerobic species isolated from human feces. *Eur. J. Clin. Microbiol.*, **6**: 266-270
- Zhao, Q. Y., J. M. Piot, V. Gautier and G. Cottenceau 1996 Isolation and characterization of a bacterial growth-stimulating peptide from a peptic bovine hemoglobin hydrolysate. *Appl. Microbiol. Biotechnol.*, **45**: 778-784