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Natural Rubber Serum Powder, an Enhancer for the Growth of *Bifidobacterium*

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There is great interest in the possibility of increasing numbers of bifidobacteria in the intestine from the standpoint of nutrition and health in various mammals including humans. Natural rubber serum powder (NRSP), was found to have a remarkable growth-promoting effect on various kinds of microorganism, in particular, anaerobes. NRSP stimulated the growth of all the species of *Bifidobacterium* employed and synergism was noted between the effects of NRSP and nutrients in media that contained yeast extract, meat extract, and casein as nutrients.

INTRODUCTION

Bifidobacteria are nonmotile, Gram-positive bacteria that are natural inhabitants of the gut in warm-blooded animals. The most common species in human infants are *Bifidobacterium infantis*, *B. brave* and *B. longum*. Newborn infants are devoid of intestinal flora but, as a results of breast-feeding, the bifidobacterial population rapidly increases in size and cell counts of 10^{10} to 10^{11} per g of feces are common in infants. Bifidobacteria form the largest group of bacteria formed in infants but gradual changes in the intestinal flora occur with aging such that they become the third largest group in healthy adults (Modler et al., 1990). For this reason, various attempts have been made to increase levels of bifidobacteria in the intestine, in particular through the use of possible growth-promoting factors. At the initial stage of such studies, compounds found in mother's milk were reported to have the ability to promote growth of bifidobacteria (Gyorgy et al., 1954). Subsequent studies have focused on various kinds of oligosaccharide (Yazawa et al., 1978, Hidaka et al., 1986, Kohmoto et al., 1988, and Hayakawa et al., 1990). Moreover, bifidobacteria cannot grow in fully synthetic media and require undefined biological products, such as those found in bovine casein digest, bovine milk whey, hog gastric mucin or yeast extract, for growth. Although the active ingredients in these complex biological mixtures have not been identified (Pock and Bezkarovainy 1988), Pock and Bezkarovainy reported that trypsin-digested κ -casein was as extremely potent growth enhancer (Pock and Bezkarovainy 1991). We reported in previous papers that natural rubber serum powder (NRSP), the spray-dried product of the serum obtained during the process for separation of natural rubber latex, had strong growth-enhancing effects on various kinds of anaerobic microorganism (Ishizaki, 1989 and Triptetchkul 1992). We report here that NRSP that has been digested by a protease has very strong growth

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-promoting effects on a wide range of bifidobacteria.

MATERIALS AND METHODS

Microorganisms

Microorganisms used were *Bifidobacterium* brave JCM 1192^T, *B. brave* JCM 1273, *B. pseudolongum* JCM 1205^T, *B. longum* JCM 1217^T, *B. infantis* JCM 1222^T, *B. bifidum* JCM 1255^T, *B. bifidum* JCM 1254, *B. adolescentis* JCM 1275.

Medium

The complete nutritional medium for bifidobacteria was prepared by mixing of solutions A and B. Solution A consisted of 10 g of casein, 5 g of meat extract, 5 g of yeast extract, 10 g of glucose, 3 g of KH_2PO_4 , and 1 ml of Tween 80 in 1 liter of distilled water. The pH of solution A was adjusted to 6.8 and the solution was sterilized by autoclaving for 10 min at 110°C. Solution B contained 10 g of sodium ascorbate and 0.5 g of cysteine hydrochloride in 1 liter of distilled water (adjusted to pH 6.8) and it was added to solution A aseptically via disposable filtration unit (syringe), DISMIC-25, (Toyo Roshi Kaisha Ltd, Tokyo) before inoculation of cells.

The minimal medium for determination of the effects of nutrients on the growth of the various microorganisms was prepared by selective omission of organic nutrients, namely, casein, meat extract and yeast extract, from the complete medium.

Natural rubber serum powder (NRSP)

NRSP was provided by Nakanihon Koku Co., Ltd. (Nagoya). NRSP used in this research was the spray-dried product of natural rubber serum imported from Malaysia (Ishizaki, 1989) and it was digested with papain prior to drying under the conditions prescribed by the manufacturer.

Culture and analysis

Microorganisms were stored in thioglycolate (TGC) medium without dextrose (Difco USA) at 5°C and transplanted to the complete medium for pre-culture. The pre-culture was carried out at 37°C for 24 h for preparation of inocula. One drop of the inoculum was added to 10 ml of the experimental medium and cultured at 37°C for 24 h. The optical absorbance of the culture broth was determined at 562 nm and the dry cell weight (DCW) was calculated from a previously constructed standard curve.

RESULTS AND DISCUSSION

Effects of NRSP in minimal medium

To determine whether or not natural rubber serum powder (NRSP) might have a growth-promoting effect on bifidobacteria, the growth of bifidobacteria in minimal medium, as a control, and in medium to which 1% (w/v) NRSP had been added was compared. The results are shown in Fig. 1, in which black bars indicate the growth in minimal medium and dotted bars indicate growth in the medium to which 1% (w/

v) NRSP has been added. From the results it is clear that NRSP had a remarkable growth-promoting effect on all species of *Bifidobacterium* tested.

Effects of NRSP in complete medium

To obtain further information about the effect of NRSP, the growth of bifidobacteria in complete medium and in NRSP-supplemented complete medium was compared. The results are shown in Fig. 2. To our surprise, the growth rate of all the bifidobacteria tested in NRSP-supplemented complete medium was higher than

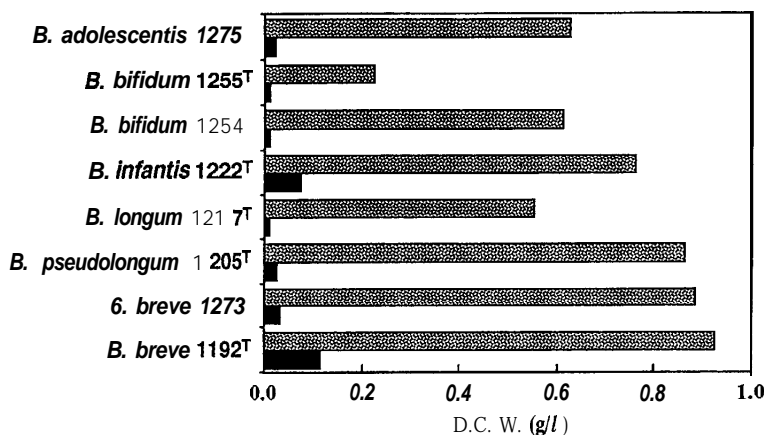


Fig. 1. Effects of NRSP in minimal medium. Growth of microorganisms is expressed in terms of dry cell weight (D. C. W.) per liter. Black bars indicate growth in minimal medium without NRSP while dotted bars indicate growth in minimal medium with 1% NRSP.

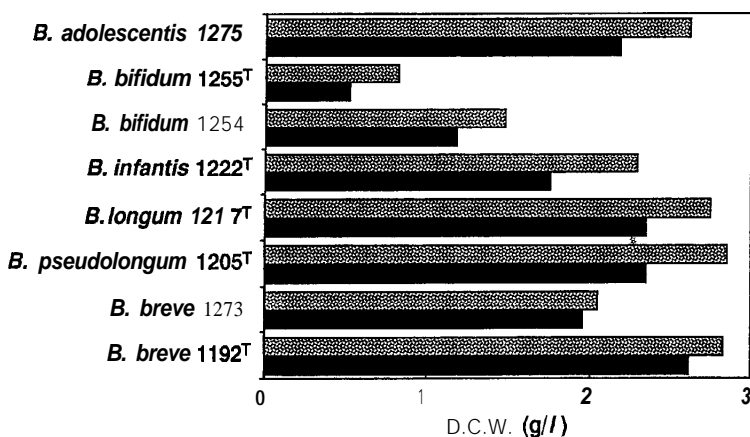


Fig. 2. Effects of NRSP in complete medium. Growth of microorganism is expressed as in Fig. 1. Black bars indicate growth in complete medium without NRSP while dotted bars indicate growth in complete medium with 1% NRSP.

that in complete medium alone. Thus, NRSP stimulated the growth of all species of *Bifidobacterium* tested and NRSP enhance the effects of the complete nutritional medium. Thus, NRSP appears to contain a growth-promoting factor that is absent or present or insufficient levels in nutrient-containing complete medium.

Effects of NRSP compared to most of other nutrients

For a further understanding of the growth-promoting effect of NRSP, growth of *B. infantis* JCM 1222 in minimal medium was compared with that in medium from which various nutrients had been omitted, namely, yeast extract, meat extract, and casein. Effects of several combinations of these nutrients, namely, yeast extract plus meat extract, meat extract plus casein, and yeast extract plus casein, were also investigated. In addition, we tested a medium supplemented with yeast extract, meat extract and 0.5% casein, namely, half the amount of casein used to prepare the complete medium. The results are shown in Fig. 3. As seen from this Figure, NRSP had a remarkable effect on the growth of the microorganism with all combinations of the nutrients in the medium tested in this series of experiments. Note that 1% NRSP in medium that contained yeast extract only, meat extract only, or casein only as nutrients stimulated the growth of the microorganism to a remarkable extent. This effect was also observed in medium that contained two of the three in combination. From these results, it is clear that NRSP has a synergistic effect when added to the conventional complete medium that is used for standard cultures of *Bifidobacterium*.

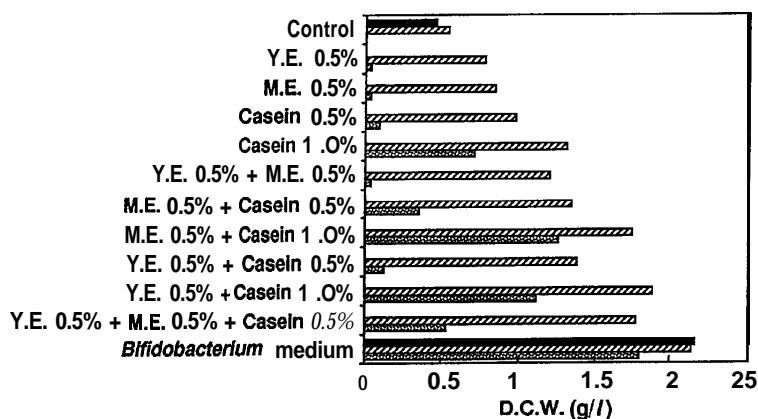


Fig. 3. Effects of NRSP with various combinations of nutrients. Growth of *Bifidobacterium infantis* JCM 1222 is expressed in terms of dry cell weight (D. C. W.) per liter. Dotted bars indicate the growth in medium without NRSP, hatched bars indicate the growth in medium with 0.5% NRSP and black bars indicate the growth in medium with 1% NRSP. Y. E., yeast extract, M. E., meat extract. All percentages represent w/v.

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