Natural Rubber Serum that Contains a Special Growth Promoter for *Bifidobacterium*

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Natural rubber serum (NRS), was found to have a remarkable growth-promoting effect on various kinds of microorganisms, in particular, anaerobes. NRS stimulated the growth of all the species of *Bifidobacterium* tried and synergism was noted between the effects of NRS and nutrients in media that contained yeast extract, meat extract, and the casein as nutrients.

There is a 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. Newborn infants are devoid of intestinal flora, but as a result 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 found in infants, but gradual changes in the intestinal flora occur with aging such that they become the third largest group in healthy adults. 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. Subsequent studies have focused on various kinds of oligosaccharides. 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 Bezkorovainy reported that trypsin-digested $\kappa$-casein was an extremely potent growth enhancer. We reported in previous papers that natural rubber serum (NRS), 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 microorganisms. We report here that NRS that has been digested by a protease has very strong growth-promoting effects on a wide range of bifidobacteria.

The NRS used this research was the spray-dried product of natural rubber serum imported from Malaysia (NRSRP), provided by Nakanihon Air Service Co., Ltd. (Nagoya). It was digested with papain before drying under the conditions prescribed by the manufacturer.

Microorganisms used were *Bifidobacterium* *brave* JCM 1192$^T$, *B. breve* 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, and *B. adolescentis* JCM 1275. Microorganisms were stored in thioglycolate (TG) medium without dextrose (Difco U.S.A.) at 5°C and transplanted to the complete medium for pre-culture. The pre-culture was done at 37°C for 24 h for preparation of inocula.

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$_2$PO$_4$, 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 a disposable filtration unit (syringe), DISMIC-25, (Toyo Roshi Co., Ltd, Tokyo) before inoculation of cells.

The minimal medium for measurement 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.

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 measured at 562 nm and the dry cell weight (DCW) was calculated from a previously constructed standard curve.

![Fig. 1. Effects of NRS in Minimal Medium](image)

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

![Fig. 2. Effects of NRS in Complete Medium](image)

Growth of microorganism is expressed as in Fig. 1. Black bars indicate growth in complete medium without NRS while dotted bars indicate growth in complete medium with 1% NRS.
To find whether or not NRS 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) NRS had been added was compared. The results are shown in Fig. 1. From the results, it is clear that NRS had a strong growth-promoting effect on all species of *Bifidobacterium* tested.

To obtain further information about the effects of NRS, the growth of bifidobacteria in complete medium and in NRS-supplemented complete medium was compared. The results are shown in Fig. 2. As shown in this Figure, the growth rate of all the bifidobacteria tested in NRS-supplemented complete medium was higher than that in complete medium alone. Thus, NRS stimulated the growth of all species of *Bifidobacterium* tested and NRS enhanced the effects of the complete nutritional medium. Thus, NRS appears to contain a growth-promoting factor that is absent or present at insufficient levels in nutrient-containing complete medium.

For further understanding of the growth-promoting effect of NRS, *B. infantis* JCM 1222T was selected among eight strains used in the previous experiments because this is a type strain and the growth of this strain was almost the average of the growth of all strains tested. The growth of *B. infantis* JCM 1222T 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 with yeast extract, meat extract, and 0.5% casein, which is half the amount of casein used to prepare the complete medium. The results are shown in Fig. 3. As seen from this Figure, NRS had a strong effects on the growth of the microorganism with all combinations of the nutrients in the medium tested in this series of experiments. Note that 0.5% NRS in medium that contained yeast extract only, meat extract only, or casein only as nutrients stimulated the growth of the microorganism to a great extent. This effect was also observed in medium that contained two of the three in combination. From these results, it is clear that NRS has a synergistic effect when added to the conventional complete medium that is used for standard cultured of *Bifidobacterium*.

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References