# *BIFIDOBACTERIUM* GROWTH-PROMOTING PEPTIDE ISOLATED FROM PAPAIN-DIGESTED MEAT PROTEINS

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**Abstract – This paper describes meat protein-derived prebiotic peptides which promote the growth of *Bifidobacterium* bacteria. Enzymatic hydrolyzates of porcine muscle actomyosin promoted the growth of *Bifidobacterium bifidum* in skim milk. Of five proteases tested, papain was the most suitable enzyme for induction of high growth-promoting activity. The peptide corresponding to the *Bifidobacterium* growth-promoting activity was purified and identified as a tripeptide Glu-Leu-Met. Although Glu-Leu-Met showed growth-promoting activity, neither the dipeptides (Glu-Leu, Leu-Met) nor amino acids (Glu, Leu, Met), which are parts of the tripeptides, showed activity. Therefore, the sequence of the tripeptide is critical for its growth-promoting activity. From the results of this study, enzymatic hydrolyzates of meat proteins and corresponding active peptides would be useful for the novel prebiotic material for developing functional foods.**

**Key Words – Actomyosin, Prebiotics, Probiotics**

## I. INTRODUCTION

Probiotics can be considered to be 'live microorganisms which, when administered in adequate amounts (as part of food), confer a health benefit on the host [1]. Probiotic foods are regarded as physiologically functional if these products have been satisfactorily demonstrated to beneficially affect one or more target functions in the body, beyond adequate nutritional effects, in a way that is relevant to either an improved state of health and well-being or a reduction of the risk of disease. Probiotic bacteria are mainly human intestinal strains of *Lactobacillus* and *Bifidobacterium*.

Much attention has also been paid to prebiotics in the food industry. Prebiotics was initially defined as 'non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon and thus improve the health of the host' [2]. Later, this definition was updated as 'a selectively fermented ingredient that allows specific changes both in the composition and/or activity in the gastrointestinal microflora that confers benefits' [3]. As representative prebiotic substances, oligo-saccharides and dietary fibers have been utilized to enhance the growth of probiotic bacteria [4].

In addition to probiotics and prebiotics, the concept of synbiotics [2] has been proposed (Figure 1). Synbiotics are foods containing both probiotic bacteria and prebiotic substances to provide a diet in which the growth of the probiotic bacteria is enhanced by the prebiotics, thus promoting the chance of the probiotic bacteria becoming established in the gut and conferring a health benefit [5]. This combination would result in improvement of intestinal microflora.

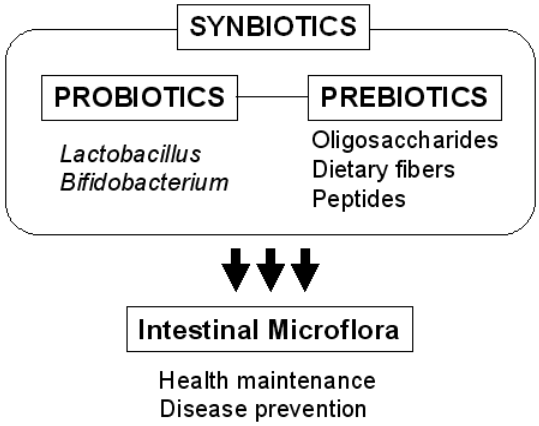


Figure 1. Concept of probiotics, prebiotics and synbiotics.

Utilization of probiotics and prebiotics is one of main trends for developing functional foods, such as functional dairy products. However, they are relatively new concept in the meat industry [6]. In addition to oligosaccharides and dietary fibers, the presence of prebiotic peptides has been reported [7]. Liepke et al. [8] reported peptides that stimulate the growth of *Bifidobacterium*. These peptides (5584 and 5801 Da) were isolated from pepsin-treated human milk. Apart from protein-derived peptides, Etoh et al. [9] discovered a growth-stimulating peptide (Ala-Thr-Pro-Glu-Lys-Glu-Glu-Pro-Thr-Ala) for *Bifidobacterium bifidum* from natural rubber serum.

In this study, efforts were directed to investigated meat protein-derived novel probiotic substances. We found that the hydrolyzate of porcine skeletal muscle protein (actomyosin) enhanced the growth of *Bifidobacterium* strains. Also, one of the corresponding prebiotic peptides was purified and identified as Glu-Leu-Met.

## II. MATERIALS AND METHODS

*Materials and Reagents*

Actomyosin was prepared from fresh pork trim (ham). Papain, ficin and trypsin were purchased from Wako Chemical (Tokyo, Japan). Proteinase K and pronase E were obtained from Merk (Darmstadt, Germany). Other chemicals were from Wako Chemical Co.

*Digestion of Actomyosin with Proteases*

Five kinds of proteases (papain, ficin, trypsin, proteinase K, pronase E) were used for the digestion of actomyosin. Actomyosin (100mg) was suspended in distilled water (10ml) and one of five proteases (10mg) was added. After 3 h of digestion at 37°C, each solution was heated at 95C for 10 min to inactivate the protease. After removal of insoluble materials by centrifugation, the supernatant solution was used for the measurement of Bifidobacterium growth-promoting activity and purification of peptides with activity.

*Bifidobacterium Growth-Promoting Activity*

A sample solution of protease-digested acto-myosin or respective peptides (50 µl) was added to 5 ml of sterilized skim milk. After inoculation of *Bifidobacterium bifidum* JCM1254, they were incubated at 37°C for 48 h. After incubation, the pH of skim milk was measured with a combination electrode and pH meter. Since *Bifidobacterium* produces organic acids (e.g., lactic acid and acetic acid), the pH decrease of skim milk indicates the growth level of *Bifidobacterium*.

*Purification of Peptides*

Various peptides generated in protease-digested actomyosin solution was fractionated by gel filtration chromatography (column: Sephadex G25 fine, 26mm x 100mm). After fractionation by gel filtration, further fractionation was carried out by high-performance liquid chromatography (HPLC) with reversed –phase mode (column: CAPCELL PAK C18 UG120, 4.6 x 150mm; Shiseido, Tokyo, Japan). Elution was performed with a linear gradient system (solvent A: 0.1% TFA in distilled water, solvent B: 0.1% TFA in $CH_3CN$), and absorbance was detected at 215 nm (first HPLC run). The fraction with Bifidobacterium growth-promoting activity was rechromatographed under the same conditions as described above (second HPLC run). The purified peptide with activity was lyophilized and dissolved with distilled water.

*Analysis of Purified Peptide*

The molecular formula of purified peptide was confirmed from its mass spectrum obtained using LCMS-QP8000α (Shimadzu, Kyoto, Japan). The sequence of peptide was analyzed by automated Edman degradation using a PPSQ31A Protein Sequencer (Shimadzu).

*Synthesis of Peptides*

The tripeptide with the sequence of Glu-Leu-Met and two kinds of dipeptides (Glu-Leu and Leu-Met) were synthesized by the solid phase method with 430A Peptide Synthesizer (Applied Biosystems, Inc.).

## III. RESULTS AND DISCUSSION

Addition of enzymatic hydrolyzates of porcine actomyosin to skim milk promoted the growth of *Bifidobacterium bifidum* JCM1254. Of five proteases, papain was the most suitable enzyme for induction of high growth-promoting activity.

The peptide corresponding to the *Bifidobacterium* growth-promoting activity was purified from the papain-hydrolyzed porcine actomyosin by the combination of gel filtration chromatography and HPLC with reversed-phase mode. Tripeptide with the growth-promoting activity was purified and its amino acid sequence was determined as Glu-Leu-Met. Although Glu-Leu-Met showed growth-promoting activity of *Bifidobacterium bifidum*, neither the dipeptides (Glu-Leu, Leu-Met) nor amino acids (Glu, Leu, Met), which are parts of the tripeptides, showed growth-promoting activity (Figure 2). Therefore, the sequence of the tripeptide is critical for its growth-promoting activity.

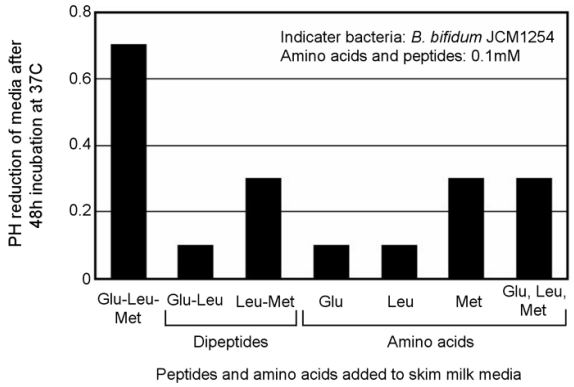


Figure 2. Bifidobacterium growth - promoting activities of Glu-Leu-Met and related dipeptides and amino acids.

Glu-Leu-Met promoted the growth of 11 strains (3 *B. bifidum*, 3 *B. breve*, 1 *B. adolescentis*, 1 *B. infantis*, 3 *B. longum*) of 26 *Bifidobacterium* strains (7 *B. adolescentis*, 5 *B. bifidum*, 5 *B. breve*, 5 *B. infantis*, 3 *B. longum*, 1 *B. catenulatum*) in skim milk. On the other hand, Glu-Leu-Met did not promoted the growth of pathogenic bacteria (*Escherichia coli*, *Salmonella enteritidis*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Yersinia enterocolitica*, *Clostridium perfringens*, *Bacillus cereus*).

In recent years, meat protein-derived peptides with bioactivities, such as anti-hypertensive and anti-oxidative activities, have been reported [10, 11]. Such peptides have a great possibility for developing functional meat products. Prebiotic peptide would be also useful for such purpose. Combination use of probiotic bacteria (e.g., *Bifidobacterium*) and prebiotic peptides in fermented meat products is an interesting direction. Traditional fermented meat products would be reborned as new functional meat products.

## IV. CONCLUSION

Enzymatic hydrolyzates of meat proteins and corresponding *Bifidobacterium* growth-promoting peptides (e.g. Glu-Leu-Met) would be utilized as the prebiotic material for developing novel functional foods including functional meat products.

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