

## Separation of growth-stimulating peptides for *Bifidobacterium* from soybean conglycinin

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### Abstract

**AIM:** To isolate and identify the soybean conglycinin peptides that selectively stimulates the growth of bifidobacteria *in vitro*, and to investigate the effect of soybean conglycinin peptides on intestinal ecosystem *in vivo*.

**METHODS:** Soybean conglycinin was purified from soybean seeds by gel filtration (Sephacrose-CL-6B). These proteins were submitted to hydrolysis by pepsin. Several growth-stimulating peptides for bifidobacteria were isolated chromatographically from pepsin hydrolysis of soybean conglycinin and identified by means of matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF-MS). Parallel to *in vitro* study, *in vivo* experiments with soybean conglycinin peptides were performed in mice. Ninety male KM mice were randomly assigned into five groups of 16 mice each, and each group was administered for 21d intragastrically with physiological saline (control), conglycinin, pepsin-treated conglycinin (PTC), the most active fraction which isolated from pepsin-treated conglycinin (P2-PTC) and HCl-full hydrolysis of conglycinin (HCl-FHC), respectively. Intestinal microflora were evaluated by standard microbiologic methods and biochemical assays of cecal content samples after treatment.

**RESULTS:** The results showed that the peptides which were isolated from soybean conglycinin could stimulate the growth of bifidobacteria *in vitro*, and the molecular mass of purified peptides with MALDI-TOF-MS ranged from 693.32 to 1829.55. Compared with control group, *in vivo* experiments showed that P2-PTC group decreased cecal pH ( $7.08 \pm 0.08$  vs  $7.21 \pm 0.09$ ,  $P < 0.05$ ) and enterococci counts ( $5.38 \pm 0.26 \log_{10} \text{CFU/g}$  vs  $5.78 \pm 0.19 \log_{10} \text{CFU/g}$ ,  $P < 0.05$ ), significantly increased sIgA level ( $172.08 \pm 35.40 \text{ ng/g}$  vs  $118.27 \pm 33.93 \text{ ng/g}$ ,  $P < 0.01$ ) and  $\beta$ -galactosidase activity

( $1.28 \pm 0.23 \text{ U/g}$  vs  $1.82 \pm 0.58 \text{ U/g}$ ,  $P < 0.05$ ).

**CONCLUSION:** The results have shown that conglycinin is good source for enzyme-mediated production of peptides which stimulate the growth of bifidobacteria. These peptides are inactive within the sequence of the parent protein but can be released during enzymatic hydrolysis, and *in vivo* experiments demonstrate that conglycinin peptides may be beneficial for improving gastrointestinal health.

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**Key words:** Conglycinin pepsin peptides bifidobacteria

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### INTRODUCTION

In recent years, a large number of biologically active peptides have been isolated from bacterial, fungal, plant, and animal sources. The concept that peptides with potential biological activity might be present in food is not new. Soybean storage proteins are composed mainly of two major components, conglycinin and glycinin<sup>[1]</sup>. Many studies have demonstrated that the enzymatic hydrolysis of conglycinin improved its functional properties and had various physiological activities such as antihypertensive<sup>[2]</sup>, immunostimulating<sup>[3]</sup> and antioxidant activities<sup>[4]</sup>.

It is now generally accepted that the human gastrointestinal tract is host to various species of microorganisms and these bacteria play a significant role in health and disease<sup>[5]</sup>. Bifidobacteria are gram-positive anaerobic bacteria, which normally inhabit the gut of human beings and animals, the presence of a great deal of bifidobacteria has been considered essential to promote intestinal health and to strengthen the local immune response<sup>[6]</sup>. The use of bifidobacteria as food supplements and therapeutical pharmaceuticals has been limited because of their slow growth. Several growth stimulating substances have been investigated, most of which derived from human and bovine milk<sup>[7,8]</sup>, however, the effect of peptides isolated from soybean conglycinin has not been understood well.

In this paper, we report that peptides obtained from the digestion of conglycinin with pepsin are effective growth factors for bifidobacteria, based on experiments *in vitro*. We

also evaluated the *in vivo* effects of soybean conglycinin peptides on gut ecosystem in mice.

## MATERIALS AND METHODS

### *Separation of conglycinin from soybean*

The conglycinin was prepared from soybean seeds by the procedure of Iwabuchi<sup>[9]</sup> and Lovati<sup>[10]</sup>, as reported briefly below. Soybeans were finely ground and defatted with hexane at room temperature. Ground meals, extracted with 30 mmol/L Tris-HCl buffer (pH 8.0) containing 0.01 mol/L  $\beta$ -mercaptoethanol for 1 h at room temperature, were spun by centrifugation (20 min at 4 000 r/min, 20 °C). The supernatant (adjusted to pH 6.4) was spun by centrifugation (15 000 r/min, 20 min, 4 °C), the precipitates were discarded, and the supernatant was adjusted to pH 4.8. The crude conglycinin was collected by the same centrifugation procedure as described above. The precipitated conglycinin was dissolved in 30 mmol/L Tris-HCl buffer (pH 8.0) containing 0.01 mol/L  $\beta$ -mercaptoethanol. The supernatant proteins were fractionated by ammonium sulphate precipitation. The 51-100% saturation fraction was dialyzed against water and applied to a Sepharose-CL-6B column (Pharmacia). Elution was performed with the phosphate buffer (2.6 mmol/L  $\text{KH}_2\text{PO}_4$ , 32.5 mmol/L  $\text{K}_2\text{HPO}_4$ , 0.4 mol/L NaCl, 10 mmol/L  $\beta$ -mercaptoethanol, pH 7.6). Finally the purified conglycinin was dialyzed against water. After readjusted to pH 7.0 and analyzed for protein concentration by the method of micro-Kjeldahl, the protein solutions were freeze-dried and stored at 4 °C. These materials were used as protein samples.

### *Sodium dodecyl sulfate gel electrophoresis (SDS-PAGE)*

SDS-PAGE was performed according to Laemmli<sup>[11]</sup>, using 10% polyacrylamide gels. The protein was stained with Coomassie brilliant blue (R-250), scanned, and the profiles of each lane were analyzed by densitometry in the Kodak Digital Science Software 1DTM.

### *Hydrolysis of conglycinin by pepsin*

Conglycinin was hydrolyzed by pepsin (Amersico) using a 1:30 enzyme: substrate ratio. Enzymatic hydrolysis was performed after acidification to pH 1.4 with 1 mol/L HCl, and mixture of pepsin and conglycinin was incubated for 2 h at 37 °C. The reaction was terminated by heating at 70 °C for 30 min, and the solution was adjusted to pH 7 with 1 mol/L NaOH. After centrifugation (20 min, 3 000 r/min, 4 °C), the supernatant was collected and lyophilized. The nitrogen concentrations of the conglycinin digestion were evaluated by the method of micro-Kjeldahl.

### *Full hydrolysis of conglycinin by hydrochloric acid*

The conglycinin was fully hydrolyzed with 6 mol/L HCl at 110 °C for 24 h. The solution was composed of amino acid composition of conglycinin. The hydrolysates were neutralized and lyophilized.

### *Separation of peptides*

Components of pepsin-treated conglycinin were separated using Sephadex-G15 (Pharmacia) gel filtration chromatography,

tracking the maximum growth stimulatory activity of the resulting fractions on *Bifidobacterium longum* (FCM1192) which was endorsed by Professor Ming-Sheng Dong (Nanjing Agricultural University). The elution was carried out with 0.05 mol/L acetate, and the absorbance of the column effluent was monitored at 280 nm. The molecular mass of the most active fraction was identified by means of MALDI-TOF-MS (BIFLEX<sup>TM</sup> III, BRUKER). In this study,  $\alpha$ -cyano-4-hydroxy-cinnamic acid solution was used as matrix. Peptide concentrations in a sample throughout the purification were determined with the method of micro-Kjeldahl.

### *Bacterial growth assays in vitro*

The basal medium used for a bioassay *in vitro* was a fully synthetic medium as described by Hassinen<sup>[12]</sup>. For growth assays, the assay medium (5 mL) was mixed with 0.15 mg/mL (nitrogen concentration) samples and inoculated with 100  $\mu\text{L}$  of bacteria culture. The control contained ammonium acetate which has equal nitrogen concentration with samples. Culture was done under anaerobic conditions at 37 °C. The extent of growth was measured by the absorbance at 460 nm after 48 h of cultivation. The growth experiments were done in triplicates.

### *Animals and diets*

Ninety male KM mice (21 d old, body weight 10-13 g, Shanghai SLAC Experimental Animals Co.Ltd, China) were housed in a room with controlled lighting (12 h/d), and constant temperature. During the first week, all mice had a commercially available basal diet and then randomly assigned into five groups of 16 mice each. Each group was administered for 21 d intragastrically with physiological saline (control), conglycinin, pepsin-treated conglycinin (PTC), the most active fraction which was isolated from pepsin-treated conglycinin (P2-PTC), and HCl-full hydrolysis of conglycinin (HCl-FHC), respectively. The volume of every treatment for the latter four groups was 0.5 mL with equal amount of nitrogen (0.3 mg/mL).

### *Preparation of sample and enumeration of bacteria*

At the end of the experimental period, mice were killed by decapitation. Blood samples were collected and spun by centrifugation at 3 000 r/min for 10 min to obtain sera. Serum level of IL-2 was assayed by radioimmunoassay (Beijing North Institute of Biotechnology) using RIA methods. Cecal samples were collected and immediately frozen and stored at -30 °C until analysis and subsequent testing for microbial composition. The following media were used: MacConkey agar for *E.coli*, bile esculine azide agar for *enterococci*. Cecal contents were cultured with triplicate plates for the microbial colony count. After incubation at 37 °C for 24 h, results were expressed as colony-forming units per gram of cecal contents. The pH of the cecal contents was measured using a pH meter. Contents of small intestine were also collected and diluted by water a ratio of 1:10. After centrifugation at 3 000 r/min for 10 min, sIgA level of supernatant was assayed by radioimmunoassay (Beijing North Institute of Biotechnology) using RIA methods.

### Enzyme assays

A protocol for detecting fructose-6-phosphate phosphoketolase (F-6-PPK) activity in the extracts from cecum samples was employed based on the assay as previously described by Orban *et al.*<sup>[13]</sup>. A positive reaction was recorded if an immediate red-violet color change was visible, and color formation was recorded spectrophotometrically at 505 nm. Quantitative determinations of  $\beta$ -galactosidase enzyme activities were performed in cecal contents suspensions. The method was used for measuring  $\beta$ -galactosidase activity described by Brigidi *et al.*<sup>[14]</sup>. One unit of  $\beta$ -galactosidase activity was defined as the amount which released 1  $\mu$ mol of *p*-nitrophenol per minute and results were expressed as Units/g of wet sample.

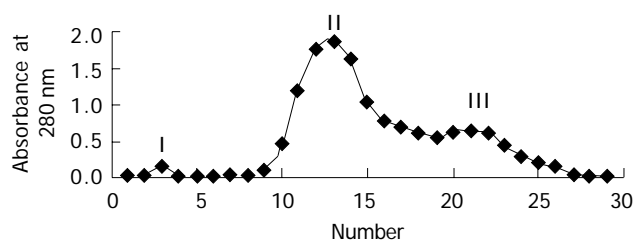
### Statistical analysis

Data were expressed as mean  $\pm$  SD. Statistical significance was determined by applying one-way analysis of variance (ANOVA).  $P < 0.05$  was considered statistically significant.

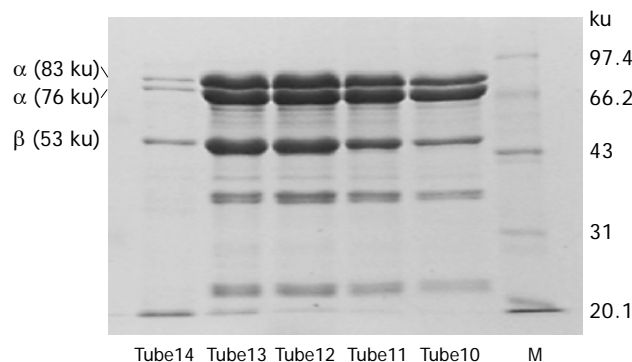
## RESULTS

### Soybean conglycinin separation

The procedure for fractionation of conglycinin was based on the isoelectric precipitation and size exclusion chromatography. The elution profile of crude conglycinin on sepharose-CL-6B is shown in Figure 1. The conglycinin was mainly detected in peak II. The gel electrophoretic pattern of purified conglycinin is illustrated in Figure 2. The major bands in the conglycinin were the  $\alpha$ ,  $\alpha'$  and  $\beta$  subunits, the purification rate of conglycinin was 90.13%.



**Figure 1** Chromatography of soybean conglycinin on sepharose-CL-6B. Bed size, 1 cmx100 cm, flow rate 30 mL/h, absorbance at 280 nm.



**Figure 2** SDS-PAGE pattern of purified conglycinin.  $\alpha$ ,  $\alpha'$  and  $\beta$  indicate subunits of conglycinin. M: standard molecular weight proteins.

### Growth-stimulating activity of conglycinin hydrolysates *in vitro*

The results of the *in vitro* experiments of the effect of soybean conglycinin hydrolysates on the regulation of growth activity for *Bifidobacterium longum* is shown in Table 1. Compared with control, pepsin-treated conglycinin (PTC) could significantly promote the growth of *Bifidobacterium longum* ( $P < 0.01$ ), while HCl-full hydrolysis of conglycinin (HCl-FHC) had no significant effect on the growth of *Bifidobacterium longum* after 48 h.

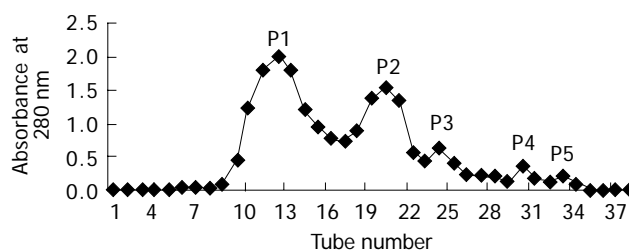
**Table 1** Effect of soybean conglycinin hydrolysates on the growth of *bifidobacterium longum* ( $n = 9$ )

Group	Control	Conglycinin	PTC	HCl-FHC
$A_{460}$	0.11 $\pm$ 0.04	0.31 $\pm$ 0.11	0.66 $\pm$ 0.07 <sup>bd</sup>	0.12 $\pm$ 0.02

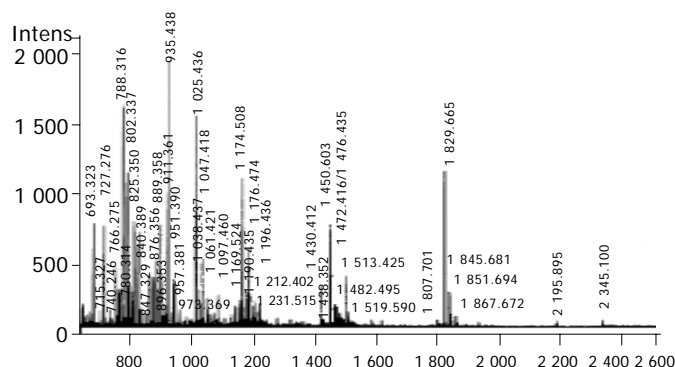
Values are expressed as the mean  $\pm$  SD. <sup>b</sup> $P < 0.01$  vs control group, <sup>d</sup> $P < 0.01$  vs HCl-FHC group.

### Separation of peptides

Five fractions (P1-P5) were separated from pepsin-treated conglycinin using Sephadex-G15 gel filtration column chromatography based on absorbance at 280 nm (Figure 3). Results of bacterial growth assays showed that P2 had the maximum growth stimulatory activity (Table 2). MALDI-TOF-MS analysis of P2 is shown in Figure 4. The molecular mass ranged from 693.32 to 1829.55. It demonstrated that the fraction P2 was comprised of peptides with short lengths.



**Figure 3** Chromatography of soybean conglycinin hydrolysates on sephadex-G15. Bed size, 1.0 cmx100 cm, flow rate 15 mL/h, absorbance at 280 nm.



**Figure 4** MALDI-TOF-MS analysis of fraction P2.

**Table 2** Growth-stimulating activity of the isolated fractions from pepsin-treated conglycinin on bifidobacteria ( $n = 9$ )

	Control	P1	P2	P3	P4	P5	PTC	Conglycinin	HCl-FHC
A <sub>460 nm</sub>	0.35±0.05	0.38±0.05	0.50±0.10 <sup>a</sup>	0.45±0.09 <sup>a</sup>	0.33±0.08	0.36±0.07	0.42±0.06	0.37±0.05	0.36±0.08

Values are expressed as the mean±SD, <sup>a</sup> $P < 0.05$  vs control group.

### Effect of conglycinin peptides on serum IL-2 levels and sIgA concentrations of small intestine in mice

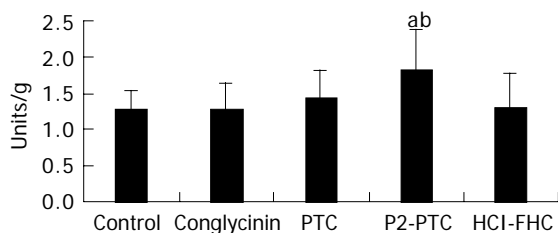
Serum IL-2 levels and small intestine sIgA concentrations are presented in Table 3. Level of sIgA in P2-PTC group was higher than that in control group ( $P < 0.05$ ) and HCl-FHC group ( $P < 0.01$ ). The level of serum IL-2 showed no significant difference among the five treated groups.

### F-6-PPK activities in cecal contents

The detection of F-6-PPK was considered to be the most reliable indicator that the bacteria belong to the genus *Bifidobacterium*<sup>[15]</sup>. The results in Table 4 showed that the enzyme F-6-PPK was detected in all samples. Activities of F-6-PPK in the PTC and P2-PTC groups were significantly higher than that in the control group ( $P < 0.01$ ) and HCl-FHC group.

### β-galactosidase activities in cecal contents

Compared with control group, β-galactosidase activities in cecal contents of P2-PTC group were significantly increased by 42.2% ( $1.82 \pm 0.58$  U/g vs  $1.28 \pm 0.23$  U/g,  $P < 0.05$ ). The β-galactosidase activities of conglycinin group were significantly lower than that in P2-PTC group ( $1.27 \pm 0.34$  U/g vs  $1.82 \pm 0.58$  U/g,  $P < 0.05$ ). But no significant difference was found between P2-PTC and PTC groups, and between P2-PTC and HCl-FHC group ( $1.82 \pm 0.58$  U/g vs  $1.43 \pm 0.38$  U/g,  $1.82 \pm 0.58$  U/g vs  $1.30 \pm 0.47$  U/g,  $P > 0.05$ ). The result is shown in Figure 5.



**Figure 5** Effect of conglycinin peptides on β-galactosidase activities in cecal contents ( $n = 8$ ). Values are expressed as the mean±SD, <sup>a</sup> $P < 0.05$  vs control group, <sup>b</sup> $P < 0.01$  vs conglycinin group.

**Table 3** Effect of conglycinin peptides on levels of serum IL-2 and small intestinal sIgA in mice ( $n = 8$ )

Group	Control	Conglycinin	PTC	P2-PTC	HCl-FHC
sIgA (ng/g)	118.27±33.93	133.17±36.15	139.01±32.65	172.08±35.40 <sup>ab</sup>	104.43±30.75
IL-2 (ng/mL)	0.602±0.255	0.445±0.112	0.602±0.351	0.693±0.305	0.618±0.141

Values are expressed as the mean±SD, <sup>a</sup> $P < 0.05$  vs control group, <sup>b</sup> $P < 0.01$  vs HCl-FHC group.

**Table 4** F-6-PPK activities in cecal contents ( $n = 8$ )

Group	Control	Conglycinin	PTC	P2-PTC	HCl-FHC
A <sub>505 nm</sub>	0.19±0.03	0.36±0.06 <sup>ac</sup>	0.56±0.08 <sup>bd</sup>	0.44±0.12 <sup>bd</sup>	0.22±0.10

Values are expressed as mean±SD, <sup>a</sup> $P < 0.05$ , <sup>b</sup> $P < 0.01$  vs control group, <sup>c</sup> $P < 0.05$ , <sup>d</sup> $P < 0.01$  vs HCl-FHC group.

### Bacterial concentrations and cecal pH

As shown in Table 5, compared with control, cecal pH level and the population of *Enterococci* in P2-PTC group were significantly decreased ( $P < 0.05$ ).

## DISCUSSION

In recent years, modification of the human intestinal microbiota has become an important objective of dietetics<sup>[16]</sup>. This goal can be achieved in two ways: (1) administration of beneficial bacteria with the expectation that they will be able to colonize the intestinal tract; (2) providing prebiotics, which have shown an ability to promote the growth of desirable bacteria<sup>[17-19]</sup>. Bifidobacteria are part of the beneficial microbiota of the human intestine<sup>[20]</sup>, and they are considered to be important in maintaining intestinal microbiota balance<sup>[21]</sup>. Therefore, many attempts have been made to increase the number of bifidobacteria in the intestine.

Soybean proteins and their derived foods have been consumed for a long time in the diets of the people in China and other Asian countries. The health promoting properties of soybean proteins have been accepted worldwide. Peptides derived from soybean proteins with short lengths, produced during *in vitro* and *in vivo* digestion, are considered to have pharmacological and physiological activities<sup>[22]</sup>. However, the effect of conglycinin peptides on growth of bifidobacteria had not been reported yet. In this study, we used pepsin to hydrolyze the conglycinin *in vitro* and results demonstrated that conglycinin was a good raw material for enzyme-mediated production of growth-stimulating peptides for bifidobacteria. The peptides, which were obtained as a consequence of screening for highly active growth stimulators during the separation, were highly hydrophilic and comprised of 6-10 amino acid residues, and these peptides were inactive within the sequence of the parent protein, but could be released during enzymatic hydrolysis. Compared with the HCl-full hydrolysis of conglycinin, the results also indicated that the function of conglycinin peptides was not nutritional but it had physiological properties. Different enzymes in the gastrointestinal tract have different effects on various kinds

**Table 5** Effect of conglycinin peptides on pH and total number of *enterococci* and *E.coli* of cecum in mice ( $n = 8$ )

Group	Control	Conglycinin	PTC	P2-PTC	HCL-FHC
pH	7.21±0.09	7.19±0.06	7.17±0.09	7.08±0.08 <sup>a</sup>	7.18±0.09
<i>E.coli</i> log <sub>10</sub> CFU/g	6.32±0.34	6.21±0.5	6.19±0.16	6.14±0.22	6.27±0.30
<i>Enterococci</i> log <sub>10</sub> CFU/g	5.78±0.19	5.55±0.29	5.42±0.37	5.38±0.26 <sup>a</sup>	5.66±0.14

Values are expressed as the mean±SD, <sup>a</sup> $P < 0.05$  vs control group.

of foods, which contain numerous sorts of protein. The process above may release prolific bioactive peptides, which have physiological and metabolic programming function on the body.

In conjunction with *in vitro* studies, pepsin-treated conglycinin was administered to mice in an effort to determine the effects of these peptides on intestinal ecosystem. The results showed that administering these peptides could significantly increase  $\beta$ -galactosidase enzyme activity. It had been reported that the increase in galactosidase was presumably a consequence of elevated numbers of bifidobacteria, which have high levels of this enzyme<sup>[13]</sup>. This suggests that conglycinin peptides could induce the change of bifidobacteria in the intestine. From our results, conglycinin peptides could lower cecal pH markedly and decrease the population of *E.coli*. We also observed that conglycinin peptides decreased the population of *enterococci*, which not only appears to share the main characteristics of lactic acid bacteria (LAB), but also comprises pathogenic species. *Enterococci* show an extensive range of resistance to various antibiotics, and the evolution of its virulence has been documented<sup>[23]</sup>. In the present study, the result indicated that conglycinin peptides did not promote the growth of all species of LAB, especially pathogenic species. It had been reported that bifidobacteria could prevent colonization of pathogens in gastrointestinal tract by lowering gut pH, and have health-promoting effects such as enhancement of immune system<sup>[24,25]</sup>. Gibson *et al.*<sup>[26]</sup> suggested that the higher bifidobacteria population, detrimental to other anaerobic bacteria species, may lead to changes in the microbial ecology of the colon. The accepted mechanism that bifidobacteria are inhibitory is related to the higher production of acetic and lactic acids. Increased acid production resulted in a lower pH which prevented enteric colonization of potentially pathogenic microorganisms and growth of putrefactive bacteria<sup>[27]</sup>. Furthermore, bifidobacteria stimulate immune function. There is considerable evidence from animal studies that probiotic organisms could modulate the mucosal and systemic immune systems<sup>[28]</sup>. This stimulation of host immunity is thought to relate to the ability of microorganisms to adhere to intestinal cells and interact with the gut-associated lymphoid tissue (GALT)<sup>[29,30]</sup>. This ability can increase immunoglobulin output into the intestinal lumen<sup>[31–33]</sup>. sIgA is one of the principal factors preventing bacterial translocation, which can result in sepsis and death of the host. The classic view is that sIgA exerts its effect by aggregating bacteria, thereby mediating clearance of those bacteria from the gut and preventing invasion of bacteria to the body<sup>[34]</sup>. Some bifidobacteria strains have recently shown to stimulate sIgA production in intestine<sup>[35]</sup>. Therefore, bifidobacteria may stimulate active IgA production, thereby reducing infections. Our study also showed that local production of sIgA in the

small intestine increased significantly, suggesting that the higher amount of bifidobacteria in the cecum of mice caused by ingestion of conglycinin peptides might produce beneficial effects within the gastrointestinal tract. Thus, the health-promoting effect of conglycinin peptides in human beings may have relationship with bifidobacteria of gastrointestinal tract.

In conclusion, our work placed emphasis on the activity (*in vitro* and *in vivo*), physiological and biochemical properties of the mixture peptides. The proteolysis of soybean conglycinin with the gastrointestinal protease pepsin results in the generation of growth-promoting peptides for bifidobacteria. Using the MALDI-TOF-MASS strategy, we were able to identify several low-molecular-mass peptides responsible for this activity. The digestion of conglycinin represents an important mechanism to obtain peptides which exhibit significant physiological roles in addition to their nutritional importance. Pepsin is an endopeptidase responsible for the hydrolysis of a wide range of proteins, we may infer that the mixture is a group of bioactive peptides that have identical C-terminus or N- terminus. Further characterization of the composition of the conglycinin hydrolysates, elucidation of the relationship between peptide structure and activity awaits future study.

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## REFERENCES

- 1 Maruyama N, Fukuda T, Saka S, Inui N, Kotoh J, Miyagawa M, Hayashi M, Sawada M, Moriyama T, Utsumi S. Molecular and structural analysis of electrophoretic variants of soybean seed storage proteins. *Phytochemistry* 2003; **64**: 701–708
- 2 Kuba M, Tanaka K, Tawata S, Takeda Y, Yasuda M. Angiotensin I-converting enzyme inhibitory peptides isolated from tofuyo fermented soybean food. *Biosci Biotechnol Biochem* 2003; **67**: 1278–1283
- 3 Tsuruki T, Kishi K, Takahashi M, Tanaka M, Matsukawa T, Yoshikawa M. Soymetide, an immunostimulating peptide derived from soybean beta-conglycinin, is an fMLP agonist. *FEBS Lett* 2003; **540**: 206–210
- 4 Chen HM, Muramoto K, Yamauchi F. Structural analysis of antioxidative peptides from Soybean  $\beta$ -conglycinin. *J Agric Food Chem* 1995; **43**: 574–578
- 5 Kaur IP, Chopra K, Saini A. Probiotics: potential pharmaceutical applications. *Eur J Pharm Sci* 2002; **15**: 1–9
- 6 Arunachalam KD. Role of Bifidobacteria in nutrition, medicine and technology. *Nutrition Research* 1999; **19**: 1559–1597
- 7 Gomes AM, Malcata FX, Klaver FA. Growth enhancement of *Bifidobacterium lactis* Bo and *Lactobacillus acidophilus* Ki by milk hydrolyzates. *J Dairy Sci* 1998; **81**: 2817–2825
- 8 Liepke C, Adermann K, Raida M, Magert HJ, Forssmann

- WG, Zucht HD. Human milk provides peptides highly stimulating the growth of bifidobacteria. *Eur J Biochem* 2002; **269**: 712-718
- 9 **Iwabuchi S**, Yamauchi F. Determination of Glycinin and  $\beta$ -conglycinin in soybean proteins by Immunological methods. *J Agric Food Chem* 1987; **35**: 200-205
  - 10 **Lovati MR**, Mnzoni C, Corsini A, Fumagalli R, Sirtori CR. 7S globulin from soybean is metabolized in human cell cultures by a specific uptake and degradation system. *J Nutr* 1996; **126**: 2871-2873
  - 11 **Laemmli UK**. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970; **227**: 680-685
  - 12 **Hassinen JB**, Durbin GT, Tomarelli RM. The minimal nutritional requirements of *Lactobacillus Bifidus*. *J Bacteriol* 1951; **62**: 771-777
  - 13 **Orban JL**, Patterson JA. Modification of the phosphoketolase assay for rapid identification of bifidobacteria. *J Microbiol Methods* 2000; **40**: 221-224
  - 14 **Brigidi P**, Vitali B, Swennen E, Bazzocchi G, Matteuzzi D. Effects of probiotic administration upon the composition and enzymatic activity of human fecal microbiota in patients with irritable bowel syndrome or functional diarrhea. *Res Microbiol* 2001; **152**: 735-741
  - 15 **Gavini F**, Van Esbroeck M, Touzel JP, Fourment A, Goossens H. Detection of Fructose-6-phosphate Phosphoketolase (F6PPK), a Key Enzyme of the Bifid-Shunt, in *Gardnerella vaginalis*. *Anaerobe* 1996; **2**: 191-193
  - 16 **Ziemer CJ**, Gibson GR. An overview of probiotics, prebiotics and synbiotics in the functional food concept: perspectives and future strategies. *Int Dairy J* 1998; **8**: 473-479
  - 17 **Saarela M**, Lahteenmaki L, Crittenden R, Salminen S, Mattila-Sandholm T. Gut bacteria and health foods--the European perspective. *Int J Food Microbiol* 2002; **78**: 99-117
  - 18 **Sullivan A**, Nord CE. The place of probiotics in human intestinal infections. *Int J Antimicrob Agents* 2002; **20**: 313-319
  - 19 **Zubillaga M**, Weill R, Postaire E, Goldman C, Caro R, Boccio J. Effect of probiotics and functional foods and their use in different diseases. *Nutrition Res* 2001; **21**: 569-579
  - 20 **Favier CF**, Vaughan EE, De Vos WM, Akkermans ADL. Molecular monitoring of succession of bacterial communities in human neonates. *Appl Environ Microbiol* 2002; **68**: 219-226
  - 21 **O'Sullivan D**, Kullen MJ. Tracking of probiotic bifidobacteria in the intestine. *Int Dairy J* 1998; **8**: 513-525
  - 22 **Liu K**. Expanding soybean food utilization. *Food Technol* 2000; **54**: 46-58
  - 23 **Aguirre M**, Collins MD. Lactic acid bacteria and human clinical infection. *J Appl Bacteriol* 1993; **75**: 95-107
  - 24 **Tuohy KM**, Probert HM, Smejkal CW, Gibson GR. Using probiotics and prebiotics to improve gut health. *DDT* 2003; **8**: 692-700
  - 25 **McNaught CE**, MacFie J. Probiotics in clinical practice: a critical review of the evidence. *Nutrition Res* 2001; **21**: 343-353
  - 26 **Gibson GR**, Wang X. Regulatory effects of bifidobacteria on the growth of other colonic bacteria. *J Appl Bacteriol* 1994; **77**: 412-420
  - 27 **Servin AL**. Antagonistic activities of Lactobacilli and bifidobacteria against microbial pathogens. *FEMS Microbiol Rev* 2004; **28**: 405-440
  - 28 **Meydani SN**, Ha WK. Immunological effects of yoghurt. *Am J Clin Nutr* 2000; **71**: 861-872
  - 29 **McGhee JR**, Mestecky J, Dertzbaugh MT, Eldridge JH, Hirasawa M, Kiyono H. The mucosal immune system: from fundamental concepts to vaccine development. *Vaccine* 1992; **10**: 75-88
  - 30 **Herias MV**, Hesse C, Telemo E, Midtvedt T, Hanson LA, Wold AE. Immunomodulatory effects of *Lactobacillus plantarum* colonizing the intestine of gnotobiotic rats. *Clin Exp Immunol* 1999; **116**: 283-290
  - 31 **Fukushima Y**, Kawata Y, Mizumachi K, Kurisaki J, Mitsuoka T. Effect of bifidobacteria feeding on fecal flora and production of immunoglobulins in lactating mouse. *International J Food Microbiol* 1999; **46**: 193-197
  - 32 **Gorbach SL**. Probiotics and gastrointestinal health. *Am J Gastroenterol* 2000; **95**: S2-S4
  - 33 **Vitini E**, Alvarez S, Medina M, Medici M, de Budeguer MV, Perdigon G. Gut mucosal immunostimulation by lactic acid bacteria. *Biocell* 2000; **24**: 223-232
  - 34 **Everetta ML**, Palestranta D, Millerb SE, Bollingera RR, Parkera W. Immune exclusion and immune inclusion: A new model of host-bacterial interactions in the gut. *Clin Applied Immunol Rev* 2004; **4**: 321-332
  - 35 **Xiang MJ**, Ni YX, Li YZ, Yu SC. The effect of drinking living Bifidobacterial juice on intestinal bacteria flora and SIgA. *Shanghai Dier Yike Daxue Xuebao* 1996; **15**: 220-221