KKU Res.j. 2016; 21(2) : 110-115
http://resjournal.kku.ac.th

The Potential of Prebiotic production from wild yam as *Lactobacillus casei* growth promoter

Phanrawi Muadsri[*]

Department of Biology and Biotechnology, Faculty of Science and Technology, NakhonSawanRajabhat University, Thailand
[*]*Corresponding author : Peerawon@gmail.com*

Abstract

The infant intestinal tract is essentially sterile part and experiences a period of steady colonization over the following weeks and months as it is exposed to microorganisms from the environment. It is generally accepted that these microorganisms have a major impact on the overall development and function of the gastrointestinal mucosa and immune system. This research aims at prebiotic extraction from wild yam which is a plant in the local area. The study found that prebiotic extracted from wild yam soaked for 24 hours and 3 days gave the prebiotic concentration of 1,437 µg/ml and 1,141 µg/ml, respectively. The optimum conditions for prebiotic extraction consisted of the extraction ratio, temperature and soaking time of 1:30 (w/v), 85°C and 30 minutes, respectively. The growth of *Lactobacillus casei* subsp. *rhamnosus* in fermented milk supplemented with wild yam extract was investigated. Result showed that the fermented milk with prebiotic extract (3 days soaking wild yam) was able to promote growth of *L. casei* subsp. *rhamnosus*.
Keywords : *wild yam, prebiotic, probiotic, fermented milk, extract*

1. Introduction

Nowadays, people play much attention on health care by healthy food consumption, food that contains prebiotic. It is an alternative for anyone who takes an interest in health (1). Probiotic is beneficial microorganism in human digestive system. They cause a several positive effect in our body such as reducing the amount of pathogenic microorganisms and reduce the risk of colon cancer (2). Moreover, our body is also able to absorb more available calcium in condition of calcium deficiency which is cause of bone decay reduction. Finally, probiotic could well increase immunity in the body (3) .

The increasing level of bifidobacteria and lactobacilli should be regarded as effective prebiotic condition; prebiotic substances are able to display several characteristics. First, they should be neither hydrolysis nor absorption of prebiotic substances in the upper part of the gastrointestinal tract. Second, the compound should be fermented selectively by the gut flora. Third, it should stimulate the growth and/or activity of a limited number of beneficial bacteria in the colon in such a way that the composition of the

intestinal microorganism is altered towards a healthier one and, as such, induces effects beneficial to health (4). In a recent review of the evidence for the prebiotic nature of several compounds inulin and oligofructose were confirmed as the most extensively studied prebiotic compounds with major prebiotic efficacy (5).

Lactobacillus casei subsp. *rhamnosus* has been successfully exploited commercially as a pharmaceutical product for more than 20 years. Its beneficial effects include treatment and prevention of nonorganic diarrhea (6). We recently showed *in vitro* that this strain has probiotic activities such as the ability to adhere to intestinal cells and antibacterial activity against several pathogens. Previous study determined the ability of probiotic group to survive passage through the gastrointestinal tract and to evaluate the persistence of the strain after discontinuation of its administration. The minimum effective dose is not precisely known, but the usual recommended oral administration is in excess of 10^9 cfu/day (7).

This study recognizes the importance of prebiotic substances produced from natural raw materials that are used locally to reduce cost of production and adding value to existing local plants. In our experiment, wild yam was extracted and measured the prebiotic substances. Extract substances were then studied for their effect on *L. casei* subsp. *rhamnosus* growth and survival in fermented milk.

2. Material and methods

2.1 Raw material preparation

Fresh wild yam was washed and sliced crosswise into small pieces. They were treated by soaking (water flow) for 24 hours and 3 days before drying at 70 °C for 24 hours. Dry samples were then ground and filtered through a sieve with a pore size of 75 microns. Dry powder samples were stored and used in the next step.

2.2 The wild yam powder extraction

Two treatment of wild yam powders were conducted at ratio of 1:30 % (w/v) with distilled water at the temperature of 85 °C for 30minutes. The suspension was then centrifuged at 5,000 rpm. The supernatant was sterilized at 121 °C for 5 minutes and stored in the refrigerator at 4°C.

2.3 Quantification of prebiotic

The wild yam extract was analyzed to measure prebiotic substances using following equation.(8)

Non-reducing sugar = total sugar(µg/ml) – reducing sugar (µg/ml)

2.4 The growth of probiotic bacteria

The fermented milk was prepared by addition of milk powder(12 g) mixed with 88 ml of wild yam extract (2 treatment; soaked in water for 24 hours and 3 days). The solution was then pasteurized at 63 °C for 15 minutes and inoculated with 2% (v/v) *L.casei* subsp. *rhamnosus*. Afterthat, fermented milk was incubated at 37 °C for 48 hrs and collected sample every 2 hrs for 24 hrs. The last sample was finally recollected at 48 hrs. The growth numbers of *L. casei* subsp. *rhamnosus* in each sample was measured by pour plate method. After incubating at 37 °C for 48 hrs; the survival numbers of probiotic bacteria was also studied in fermented milk which stored at 4 °C. The sample was collected every 7 day for 21 day.

Table 1. The amount of prebiotic extract from wild yam

prebiotic compounds (µg/ml)	
24 hours soaking	3 days soaking
1,437	1,141

3. Result

The result showed the amount of prebiotic in wild yam from two treatments by soaking at 24 hours and 3 days were 1,437 µg/ml and 1,141 µg/ml, respectively (Table 1). However, the condition of 24 hours soaking provided the extract which stimulated the bacterial growths lower than the extract from wild yam soaking for 3 days (data not shown). Therefore, the fermented milk should be produced from wild yam extract treated by 3 days soaking. Milk powder with the addition of wild yam extract (soaked for 3 day) was able to encourage the growth of *L. casei* subsp. *rhamnosus* higher than control (without wild yam extract). The fermented milk with prebiotic extract (3 days soaking wild yam) was able to promote the growth of *L. casei* subsp. *rhamnosus*. The maximum cell obtained was 7.6×10^9 cfu/ml (Figure 1).

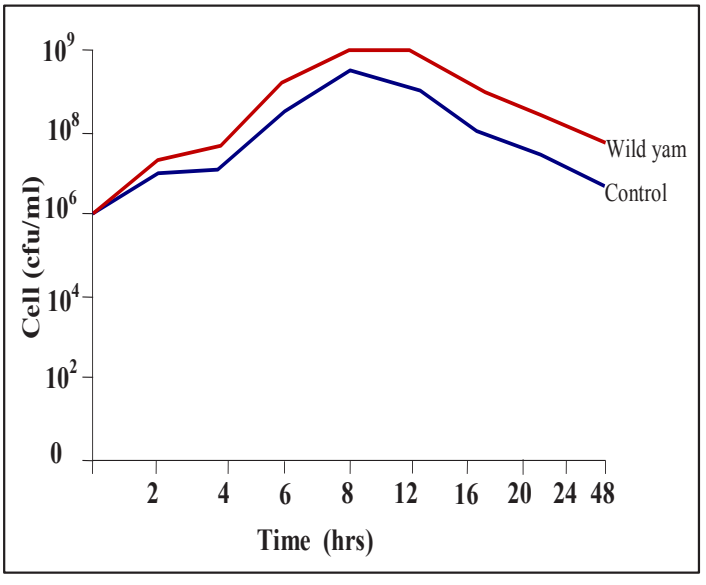

Figure 1. Effect of wild yam extract on *L. casei* subsp. *rhamnosus* growth (cfu/ml) in the fermented milk.

After 4 weeks; it was found that survival of probiotic in fermented milk with the addition of wild yam extract could increase the probiotic survival more than fermented milk without addition of wild yam extract (Figure 2). Prebiotic substances of post-fermentation in fermented milk were lower than pre-fermentation. This might be resulted from the growth of probiotic bacteria in fermented milk (Figure 3).

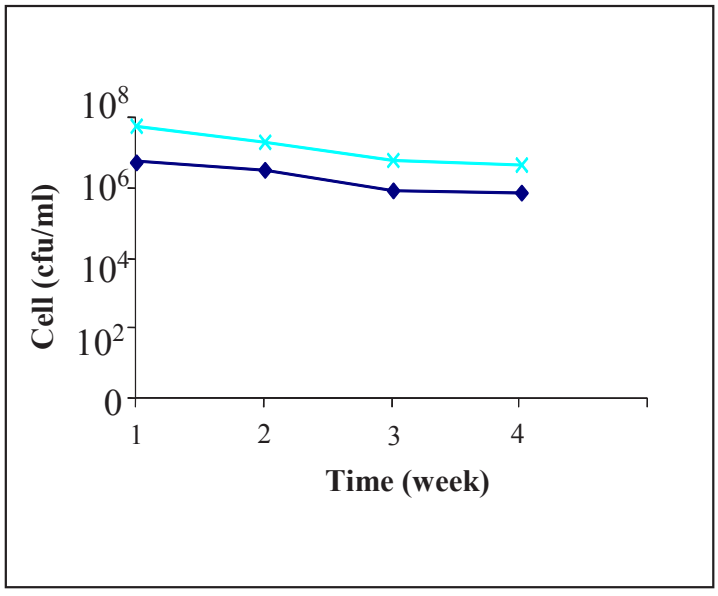

Figure 2. The survival of *L. casei* subsp. *rhamnosus*. in fermented milk by storage at 4°C (×) represent wild yam and (◇) represent control.

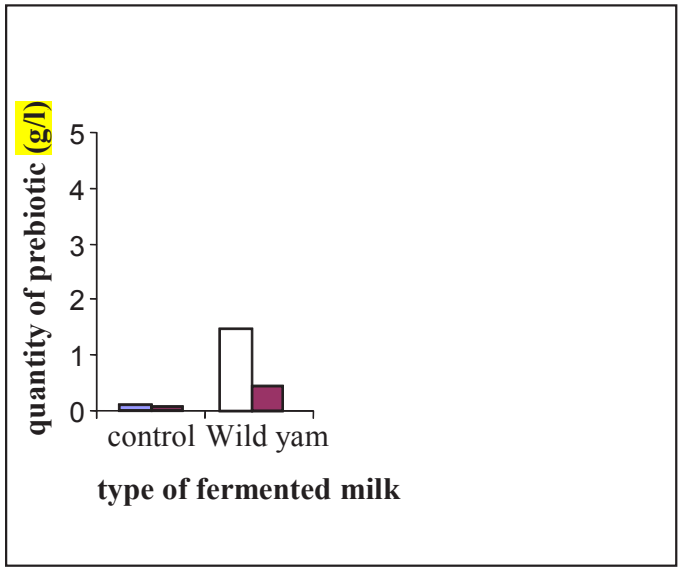

Figure 3. Quantification of prebiotic in fermented milk from pre – fermentation and post – fermentation. (□) represent pre- and (■) represent post – fermentation.

4. Discussion

The wild yam extract was 3 days soaking. This condition showed the highest stimulation for the cell concentration of *L. casei* subsp. *rhamnosus*. It was found that this wild yam extract could promote the growth of probiotic better than control. The highest cell concentration of *L. casei* subsp. *rhamnosus* was 7.6×10^9 cfu/ml. This result suggested that prebiotic substances in wild

yam properly supported the growth and survival of *L. casei* subsp. *rhamnosus*. Since, lactobacilli could produce fructosidases which was able to hydrolyze fructose moieties and metabolise inulin-type fructans efficiently (9). The previous study confirmed the better ability of bifidobacteria populations in prebiotic substances fermentation when compared to glucose. The other factor that influenced to growth rate of probiotics in fermented milk is residual dioscorine in wild yam. Dioscorine is an alkaloid toxin which could inhibit the growth of bacteria (10). In this research, the residual dioscorine in 3 days soaking treatment. Is lower than 24 hrs soaking treatment because more water and more time are needed to remove the poison contain in wild yam tuber (11). This optimal treatment of wild yam also demonstrated the highest growth rate of probiotic in fermented milk. The result of survival experiment showed that the highest survival rate of probiotic occurred in fermented milk supplemented with wild yam. The unique characteristic of probiotic for inulin and oligofructose can utilization was described by the presence of an inducible β – fructofuranosidase. This enzyme is able to hydrolyse the β (2–1) glycosidic linkages between the fructose moieties (12). This ability of prebiotic is important cause of survival of probiotic in fermented milk which supplemented with wild yam. Therefore, wild yam extract should be further used in making fermented dairy products for health because it can promote the growth and survival of the probiotic.

5. References

(1) Fuller R. Probiotics in man and animals. J. Applied Bacteriology. 1989 ; 66 : 365-378.

(2) Ingrid W, Gerhard R, Beatrice LP. Protective Role of Probiotic and Prebiotic in Colon Cancer. American Journal of Clinical Nutrition. 2001 ; 73 : 451- 455.

(3) Fuller R. and Gibson GR. Modification of the intestinal microfolra using Probiotic and Prebiotic, Scand J Gastroenterol. 1997 ; 32 : 28-31.

(4) Gibson GR. and Roberfroid MB. Dietary modulation of the human colonic microbiota : Introducing the concept of Prebiotic. Journal of nutrition. 1995 ; 125 : 1401-1412.

(5) Handan K and Robert W. Fermentation of fructooligosaccharide by Lactic acid bacteria and Bifidobacteria. Applied and Environmental Microbiology. June 2000 ; 66(6) : 2682-2684 .

(6) Niness KR. Inulin and oligofructose : what are they?. Journal of Nutrition. 1999 ; 129(7) : 1402-1406.

(7) Bandaru SR. Possible mechanisms by which pro- and prebiotic influence colon carcinogenesis and tumor growth. American Society for Nutritional Sciences. 1999 ; 129: 1478-1482.

(8) Santad W, Paiboon T, Akkasit J, Worrapanit C, Preeya H, Tipparat H, Arunporn I, Buncha O. Extraction and analysis of prebiotics from selected plantsfrom southern Thailand. Songklanakarin J. Sci. Technol. Sep. - Oct. 2011 : 33 (5), 517-523.

(9) Barrangou R, Klaenhammer TR, Altermann E. Functional and comparative genomic analyses of an operon involved in fructooligo-saccharide utilization by Lactobacillus acidophilus.Proc Natl Acad Sci U S A. 2003 ;100(15): 8957- 62.

(10) Megh RB and Jun K. Bitterness and Toxicity in Wild Yam (Dioscorea spp.) Tubers of Nepal. Plant Foods for Human Nutrition. 2005; 60: 129 – 135.

(11) Mohd H, Haji R, Mohd. Rizuwan Y, Md. SJ, Mohd SS, Nurul SM, Asma AZ. An Overview on Mechatronic Application and Effectiveness of Developed Stand Alone Dioscorine Removal System. Journal of Automation and Control, 2013: Vol. 1 : No. 1 : 1-5.

(12) Gibson, GR, Beatty EB, Wang X, Cummings JH. Selective stimulation of bifidobacteriaIn the human colon by oligofructose and inulin.Gastroenterology. 1995 ; 108 : 975-982.