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Research Article

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Detection of vitamins produced by plant-isolated *Lactobacillus rhamnosus* PN04

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ABSTRACT

Lactic acid bacteria (LAB) are useful microbes for producing food and pharmaceutical science. To investigate the potency for the production of vitamins by lactic acid bacteria are necessary. In this study, vitamins B6 and B9 produced by Lactobacillus rhamnosus PN04. By high perform liquid chromatography analysis, Lactobacillus rhamnosus PN04 could produce vitamin B6, vitamin B9, equaled to 0.062 mg/ml, 0.002 mg/ml, respectively. The results were also confirmed using thin-layer chromatography analysis. Only vitamin B6 (Pyridoxine) was detected due to vitamin B9 was low. As a result, Lactobacillus rhamnosus PN04 could be the vitamin source for pyridoxine and folic acid.

Keywords: *Lactobacillus rhamnosus* PN04, vitamin, thin layer chromatography, high performance liquid chromatography

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INTRODUCTION

Lactic acid bacteria (LAB) are used widely in our life such as food, pharmaceutical fields. They are able to synthesize many kinds of products including, exopolysaccharides, vitamin, bacteriocins or bioactive peptides, cyclodipeptides [1]. Lactic acid bacteria (LAB) can be used in food fermentation. Their products are important in preserve food as well as contribute to quality of food products [2-3]. Some of LAB can be important probiotic for prevention infant diseases and used in dairy supplement [4-5]. Intestinal LAB also produce many vitamins and essential amino acids required for the growth development of their hosts.

There are two types of vitamins including fat (vitamin A, D, E and K) and water soluble (B-group vitamin and vitamin C). Each vitamin in B-group shows different actions and sometimes and acts together to maintain metabolic processes in human such as energy supplement, memmory improvement. Normally, B-group vitamins are found in cereals-based products [3]. Otherwise, humans and animals can not produce vitamins by theirselves. However some bacteria, yeasts, fungi and algae may produce B9 (folic acid), vitamin B12 (cobalamin), vitamin K2 (menaguino), riboflavin, thiamine (B1), and other essential vitamins [6-7]. LAB are now known that certain strains can synthesize water-soluble vitamins [8-11].

Up to now, *Lactobacillus rhamnosus* PN04 was isolated in vegetables [12], being a interesting source to exploit whether this strain can produce vitamin or not. Therefore, the study tried to perform a primary detection of vitamin production in *Lactobacillus rhamnosus* PN 04.

EXPERIMENTAL SECTION

Lactic acid bacteria cultivation

Lactobacillus rhamnosus PN04 was cultured in MRS broth [13]. The cultivation was optimized in different periods from 24h to 48h.

Sample preparation

After incubation, the culture medium was centrifuged at 13000 rpm, 4^{o}C for 10 min. All supernatants were taken and freeze-dried. The formed powders were suspended with the one mL of methanol. The soluble methanol fractions were collected and let at room temperature in 24h to evaporate methanol. The newly formed powders were dissolved in distilled water and ready for high performance liquid chromatography (HPLC) and thin layer chromatography (TLC). Standard vitamins (B6, B9, B12) were prepared at a concentration of 2µg/mL in distilled water.

Thin layer chromatography

The optimization of mobile phase was performed and UV light (245 nm) was used for detection. Therefore, the mobile phase systems were optimized as the system including chroloform -ethanol- H2O in a ratio (2:2:1) [14] or system (Toluene – methanol – acetone – acetic acid in ratio of 14:4:1:1) or system (Ethyl acetate – acetic acid - H2O – ethanol in a ratio 16:2:2:1). The samples applied on TLC were 20-40 µL and standard vitamins (2µL) were used. TLC analysis for vitamin detection was based on Rf and spot properties.

High performance chromatography (HPLC)

Mobile phase was a mixture of acetonitrile: methanol (9:1) according to United state pharmacopoeia (USP 23). Detection vitamin based on retention time. Concentration was determined based on the peak area and dilution factor.

RESULTS AND DISCUSSION

Thin layer chromatography

Different mobile phases were screened for detection. Vitamins could not be detected in the mobile phase system (chloroform - ethanol - H2O) and (toluene - methanol - acetone - acetic acid) (Data not shown). However, vitamin B6 produced in *L. rhamnosus* could be detected in the system including in ethyl acetate - acetic acid - H2O - ethanol (16:2:2:1) under UV light (254 nm) (Figure 1).

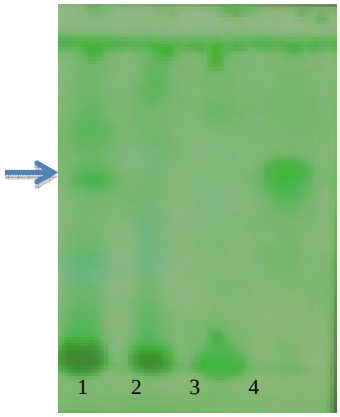

Figure 1: Thin layer chromatography to detect vitamin B6 in *L. rhamnosus*. (1): Extract of *L. rhamnosus* cultured MRS within 48 h; (2): *L. rhamnosus* cultured MRS within 24 h; (3): MRS used as negative control; (4): Standard B6. The arrow showed the spots of B6

The spot detected in *L. rhamnosus* gave the dark blue, similarly to standard B6. Also, the Rf of this spot equaled to standard B6 (Rf=0.6). Obviously, *L. rhamnosus* could produce B6 in MRS nutrient with enough amount to be detectable.

Otherwise, the other vitamins couldn't be detected that might be their low concentration. In order to ensure the production of the other vitamins, HPLC should be done.

High performance liquid chromatography

The *L. rhamnosus* culture after dried and solubilised in distilled water were qualified and quantitative using HPLC. The HPLC results were summarized in table 1 and 2.

The results showed there was the existence of the peak of vitamin B6 at 4.870 min, similarly to standard B6 (4.896 min). The results were aceptable because the retention time in different samples was evaluated using standard deviations. Based on the United States Pharmacopoeia (USP) method, the tailing factors of these chromatograms did not exceed 2 and the standard deviation of the retention time of the tested sample was less than 2% in the comparison with the retention time of the standard vitamins. Therefore, the recorded peaks of the vitamins in samples were concluded similarly. By calculating the peak area and based on dilution factor, concentration of vitamin B6 was 0.062 mg/mL. Table 2 showed the existence of the peak of vitamin B9 at 17.833 min, similarly to standard B9 (17.833). From that, concentration of vitamin B9 was 0.002 mg/mL.

Table 1: Retention time and concentration of vitamin B6

Sample/standard	RT (min)	Concentration (mg/mL)
Vitamin B6	4.896	0.132
L. rhamnosus	4.870	0.062

Table 2: Retention time and concentration of vitamin B9

Sample/standard	RT (min)	Concentration (mg/mL)
VitaminB9 (folic acid)	17.838	0.125
L. rhamnosus	17.833	0.002

As a result, *L. rhamnosus* PN04 could produce vitamin B6 and B9 in MRS medium. The optimal results suggested the vitamin production occurs when the nutrients or cofactors are required properly. Because this strain was isolated from plant, vitamin was produced because there might be the interaction of this strain with plant, leading to their biosynthesis gene clusters to be able to promote easily. In the study, MRS medium might not be the suitable for producing vitamin B2, B3, B12, PP *L. rhamnosus* PN04. It is important to point out that the results were also interesting as they provided enough data to consider the condition for vitamin production in *Lactobacillus* originated in plants.

CONCLUSION

The present study has primarily detected the vitamin production in *Lactobacillus rhamnosus* PN04. Although these bacteria contains the biosynthesis genes for vitamin production, vitamin production was not ready to be produced when the conditions were not suitable.

This study investigate the potency for the production of vitamins by LAB and look for the conditions for vitamin production. Now a day, most of vitamin are been producing from plant or organic synthesis but vitamin source from LAB did not investigated so much. More researches about this subject will be carried out so far. In addition, vitamin producing LAB could be a worthwhile products, LAB served as inimitable source for developing novel products and applications, especially those that can satisfy the increasing consumer's demands for natural products and health benefits.

REFERENCES

[1] AR Patel; C Lindström; A Patel; JB Prajapati; O Holst, *Int. J. Fermented foods*, **2012**, 1, 87-101.
[2] N Shah; JB Prajapati, *J. Food Sci. Technol.*, **2014**, 51(12), 3886-3893.
[3] V Capozzi; P Russo; MT Dueñas; P López; G Spano, *Appl. Microbiol. Biotechnol.*, **2012**, 96,1383-1394.
[4] AY Tamine; M Saarela; AS Korslund; VV Mistry; NP Shah, Production and maintenance of viability of probiotic micro-organisms in dairy products, In: Tamine AY, editor. Probiotic DairyProducts. Blackwell Publishing Ltd, Oxford, UK., **2005**, 39-72.
[5] TM Marques; R Wall; P Ross; GF Fitzgerald; A Ryan; C Stanton, C., *Curr. Opinion Biotechnol.*, **2010**, 21, 149-156.
[6] S Keuth; B Bisping, *J. Appl. Bio.*, **1993**, 75, 427-434.
[7] JG LeBlanc; JE Laino; M Juarez del Valle; V Vannini; D van Sinderen; MP Taranto; G Font de Valdez; G Savoy de Giori; F Sesma, *J. Appl. Microbiol.*, **2011**, 111, 1297-1309.
[8] C Burgess; M O'Connell-Motherway; W Sybesma; J Hugenholtz, D van Sinderen, *Appl. Environ. Microbiol.*, **2004**, 70, 5769-5777.
[9] MR D'Aimmo; P Mattarelli; B Biavati; NG Carlsson; T Andlid, *J. Appl. Microbiol.*, **2012**, 112, 975-984.
[10] S Jayashree; K Jayaraman; G Kalaichelvan, *Recent Res. Sci. Technol.*, **2010**, 2 (1), 83-88.
[11] W Sybesma; M Starrenburg; L Tijsseling; MHN Hoefnagel; J Hugenholtz, *Appl. Environ. Microbiol.*, **2003**, 69, 4542-4548.
[12] T Nguyen; V Doan; L Ha; H Nguyen, *Indian J. Microbiol.*, **2013**, 53(4), 385-390.
[13] JC De Man; M Rogosa; ME Sharpe, *J. Appl. Bacteriol.*, **1960**, 23(1), 130-135.
[14] EL Ponder; B Fried; J Sherma, *Acta chromatogrphica*, **2004**, 14, 70.