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Research Article

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Lactic acid production from rice straw using plant-originated *Lactobacillus rhamnosus* PN04

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ABSTRACT

The study focused on lactic acid (LA) production using rice straw as waste product using plant-originated Lactobacillus rhamnosus PN04 in different treatment of heat. Straw has been treated with heat before L. rhamnosus PN04 leading to the pH decrease gradually to 4.68 compared to the absence of L. rhamnosus PN04 (5.91) and heat untreated straw cases. By Thin layer chromatography (TLC) and Infrared spectroscopy (IR) analysis, there was strong evidence for LA production in this condition. The result has driven a cheap method to produce LA for pharmaceutical field, food and chemical industry.

Key words: lactic acid (LA), *Lactobacillus rhamnosus* PN04, pH, heat treatment, analysis

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INTRODUCTION

Recently, the demand for lactic acid (LA) has been increased gradually. In particularly, the demand was estimated to be 130,000–150,000 tons per year worldwide [1] LA was proved to be an important chemical having wide ranged applications in food, pharmaceutical as well as chemical industry [2]. Moreover, LA was also an important source in producing poly(lactic acid) polymer (PLA) which is an essential alternative material. LA could be produced by chemical synthesis or microbial fermentation. For LA bio-production, there are a great source of carbon is needed as substrate, normally, that source is sugar. However, using sugars as a substrate for LA production could increase the cost of the final product. As a result, finding an alternative source caused much attention [3]. The current dependence in sugars would be reduce by the use of lignocellulosic materials such as agriculture straw [3, 4]. However, there is one big limitation when using these alternative materials that most of the LA producer could not fermented cellulose directly but need the help of enzymatic hydrolysis of cellulose [3]. In addition, many byproducts in the materials could also inhibit the bacterial growth effecting the final product quality and quantity [5].

Therefore, study on a suitable strain that can use the cellulose as substrate for LA production is necessary. If these lignocellulosic materials could be converted into LA by biological fermentation, it not only reduce the cost of the LA products but also solve the environment pollution and resources wasting.

In this study, a potential candidate for LA production from straw was introduced which is *Lactobacillus rhamnosus* PN04 isolated from *Hottuynia cordata* [6].

EXPERIMENTAL SECTION

Materials

Bacterial Strain

L. rhamnosus PN04 isolated from *Hottuynia cordata* [6] was used in the study.

Straw source
Rice straw were obtained from Vietnamese farms – Nine Dragons river delta, Mekong Delta.

Methods
Straw soaking time optimization
5 grams of dried straw was soaked in 100mL distilled water with and without heat treatment. Before culturing, the soaking times were optimized in different days which were 3, 4, 5, 6 and 7 days by measuring the stabilities of the pH(s).

Straw treatment with L. rhamnosus PN04
To understand the best condition for LA production, pH was check before and after adding *L. rhamnosus* in straw with or without sterilization.

For straw without sterilization, before soaking straw in water, *L. rhamnosus* (10^9 CFU/mL) was cultured with the straw during the soaking time (optimal condition). After the soaking time, the bacteria were isolated from the medium by centrifugation (10000 rpm, 20 minutes, 4^{o}C). Next, pH values and cell pellet weights were measured for checking the living ability of the bacteria in straw medium. The changing in pH values was the preliminary step for investigating the LA production which was then proven by TLC. Moreover, the study also focused of *L. rhamnosus* (10^9 CFU/mL) added into straw fluid filtered after straw was soaked in optimal condition. After *L. rhamnosus* was cultured in the extracted fluid and the fluid was cultured in more 5 days. LA production was evaluated.

For straw without sterilization (120^{o}C, 15 minutes), *L. rhamnosus* was added similarly in straw as straw without sterilization.

Lactic acid purification
All the fluid in different treatment collected using centrifugation. The supernatant was added with $Ca(OH)_2$ to coagulate proteins, then, filtered with activated carbon to remove the colored substances. Noting that this addition would turn lactic acid into calcium lactate.

$$2CH_3CHOHCOOH + Ca(OH)_2 \rightarrow 2CH_3CHOHCOOCa + 2H_2O$$

Next, the filtered liquid was evaporated to reduce the excess amount of water. The following step was the addition of sulfuric acid (H_2SO_4) to precipitate calcium sulfate, which was then filtered out.

$$(CH_3CHOHCOO)_2Ca + H_2SO_4 \rightarrow 2CH_3CHOHCOOH + CaSO_4$$

RESULTS AND DISCUSSION

Determination of soaking time optimization
The pH of the medium after soaking straw in distilled water for different times were illustrated in figure 1. The pH values decreased during the soaking times. However, from the fifth day, the pH started to stabilize at around 6.30. For that reason, it was suggested that 5 days was the optimized condition for making culture medium from straw. As seeing in figure 1, pH was reduced when straw was soaked in water longer that was meant that there was some bacteria produce acid or straw produced some acid substances in water. To clarify, more detection should be done.

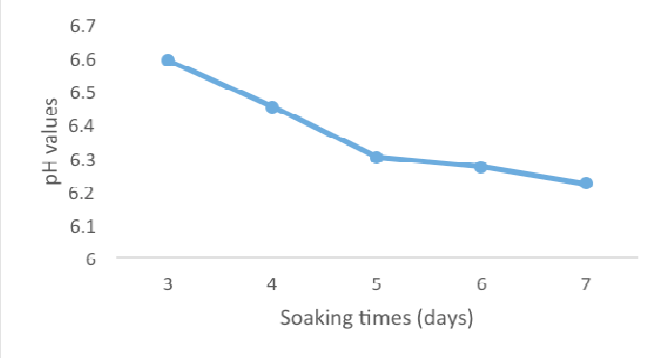

Figure 1. pH measurement of straw medium in different soaking times

pH(s) and cell pellet weights measurement

The Table 1 and 2 showed the results in pH(s) changing and cell weight determination of adding the bacteria before and after soaking time of heat untreated straw, respectively. All the experiments were performed 3 times.

Table 1. pH values and cell pellet weights when adding *L. rhamnosus* PN04 before soaking time

	pH values		cell pellet weights (gram) after 5 days
	Day 0	After 5 days	
With *L. rhamnosus*	7.00±0.00*	6.81±0.02*	0.78±0.03*
Without *L. rhamnosus*		6.30±0.02*	0.49±0.02*

**Mean±SEM(standard error of the mean)*

As can be seen in table 1, there was a small different between the pH of with and without *L. rhamnosus* PN04 addition after 5 days of culturing. It was meant that there was other acid producing in straw but killed by *L. rhamnosus* PN04. This was more obvious when *L. rhamnosus* PN04 was added in fluid obtained after soaking. The pH of fluid of straw was acid (Table 2) that pointed that there was other acid producing bacteria in straw. However, pH increased after without *L. rhamnosus* PN04, pointing that there was some bacteria in fluid producing alkaline. In case of adding *L. rhamnosus* PN04, *L. rhamnosus* PN04 and other bacteria had some effects like killing together or pH neutralization and so on, leading to pH increased. More effects will be studied soon.

Table 2. pH values and cell pellet weights when adding *L. rhamnosus* PN04 after soaking time

	pH values			cell pellet weights (gram) after 5 days
	Day 0	After 5 days of soaking	After 5 days of culturing	
With *L. rhamnosus*	7.00±0.00*	6.30±0.02*	7.06±0.03*	0.71±0.02*
Without *L. rhamnosus*			7.08±0.02*	0.60±0.02*

**Mean±SEM(standard error of the mean)*

The competition between our interest bacteria and the other microorganisms could be a strong barrier for LA producing from *L. rhamnosus*, therefore, before culturing, there should be a treatment step that sterilize the straw.
In table 3, straw has been treated with heat before adding the bacteria. With the presence of *L. rhamnosus* PN04, the pH was gradually decrease to 4.68 while the pH was 5.91 in case of the absence of *L. rhamnosus* PN04. As a result, there was strong evidence for LA production in sterilized straw but incubated with *L. rhamnosus* PN04. The samples were taken for TLC analysis for confirming the production of LA (Figure 2).

Table 3. pH values and cell pellet weights when culturing *L. rhamnosus* PN04 with heat treated straw medium

	pH values		cell pellet weights (gram) after 5 days
	Day 0	After 5 days	
With *L. rhamnosus*	7.00±0.00*	4.68±0.01*	1.33±0.01*
Without *L. rhamnosus*		5.91±0.01*	0.03±0.02*

**Mean±SEM(standard error of the mean)*

Lactic acid determination by Thin layer chromatography (TLC)

For further evidence of LA production, TLC analysis was performed. In heat treated straw with *L. rhamnosus* PN04, lactic acid production was higher than the other sample. The spot in heat treated straw with *L. rhamnosus* PN04 had the same Rf with the spot of standard LA (Figure 2). It strongly confirmed the success of the LA production from *L. rhamnosus* PN04 in with heated straw. Moreover, when using sterilized straw, the undesired products produced by other microorganisms were eliminated, leading the LA purification process more easily.

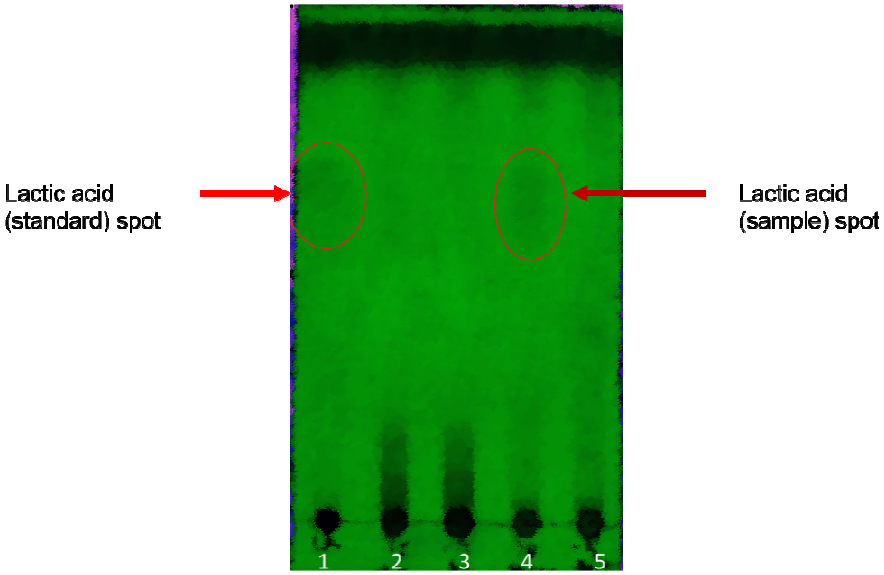

Figure 2. Lactic acid detection on thin layer chromatography. 1: acid lactic (standard), 2: heat untreated straw with *L. rhamnosus*, 3: heat untreated straw without *L. rhamnosus*, 4: heat treated straw with *L. rhamnosus*, 5: heat treated straw without *L. rhamnosus*

Lactic acid determination by Infrared spectroscopy (IR)

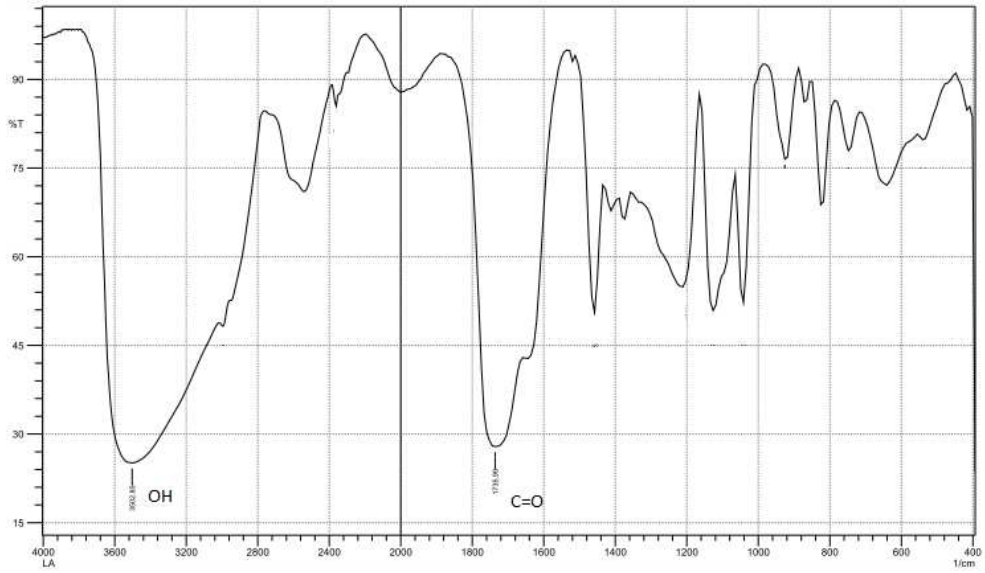

Figure 3. IR spectrum of collected sample for lactic acid detection

The IR was conducted to strengthen the success of lactic acid production. The band shifts related to the C=O group stretch can be observed at 1643 cm^{-1} in the spectrum (Figure 3) and the band around 3500 cm^{-1} is related to the stretching of OH group. Figure 3 reflected lactic acid production in straw using *L. rhamnosus* PN04.

CONCLUSION

Many strains of *Lactobacillus rhamnosus* are being used as probiotics which for improving human health. In this study, plant-originated *Lactobacillus rhamnosus* PN04 was used to produce lactic acid from straw, an agriculture waste product.

This study gave out the stable process for lactic acid production from rice straw using plant-originated *L. rhamnosus* PN04. By this, we could utilize the waste from agriculture for production not only to reducing the cost, but also solve the problem of environment impacts and resources wasting.

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