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SHORT COMMUNICATION

Starchy Effluent from Rice Noodle Manufacturing Process as Feasible Substrate for Direct Lactic Acid Production by *Lactobacillus plantarum* S21

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Abstract In utilization of both starch containing wastewater and gelatinized starchy waste, *Lactobacillus plantarum* S21 demonstrated the high capability of lactic acid production directly from starchy effluent and maintained its potency even at high concentration of initial starchy substrate of 40, 60 and 80 g/L by maximum yielding 1.00±0.06, 0.89±0.03, 0.90±0.07 g/g substrate, productivity of 0.79±0.06, 0.98±0.00, 1.23±0.07 g/L·h and production efficiency of 94.6, 78.8, and 74.3%, respectively, at 48 h fermentation. This is the first report on direct conversion of starchy wastes to lactic acid by amylolytic lactic acid bacterium using high concentration of starchy substrate.

Keywords amylolytic lactic acid bacterium · lactic acid · *Lactobacillus plantarum* · rice noodles · starchy waste

Thailand was ranked as the sixth largest global rice producer in 2010–2011, and approximately 55% of the rice production has been distributed for domestic consumption. Apart from the rice being consumed as the main carbohydrate of the daily meal, the processing of rice into noodles is a significant factor involved in the high amount of rice that is consumed in the country, as rice noodles are the second most commonly consumed food item of Thai people, after rice. During the rice noodle manufacturing process, raw rice flour wastewater is generated during the washing, soaking, and milling of rice. Moreover, a high concentration of gelatinized flour is discarded daily after the steaming of rice flour slurry. Therefore, due to the generation of a large quantity of starchy waste, proper waste treatment steps are required. The bioconversion of bio-waste to higher value-added products has been intensively discussed and promoted, for the purposes of use as biofuel and organic acid (Pandey et al., 2000; Ohkouchi and Inoue, 2007). Owing to the increase in the global lactic acid demand, the conversion of starchy waste into lactic acid, which is of value in the food, textile, leather, and electronic industries, is of significant interest. Direct conversion of starch to lactic acid is an alternative strategy that has been used to produce lactic acid directly from starch by amylolytic lactic acid bacteria (ALAB). This fermentation strategy was studied and evaluated for its potential due to the high demand of amylase enzymes. Moreover, additional starch liquefaction and saccharification steps are discarded, because the ALAB are capable of producing lactic acid from starch. Therefore, it is beneficial to directly convert both a great deal of starchy wastewater and a high concentration of gelatinized starchy waste into lactic acid, which can be seen as a value-adding strategy for further applications of lactic acid. However, very few lactic acid productions were directly converted from starchy waste sources (Pintado et al., 1999; Kim et al., 2003; Ohkouchi and Inoue, 2006). These reports mentioned only the direct conversion of lactic acid from low concentrations of starch, which is impractical for large scale production. The present note observes feasibility in the production of lactic acid using both starchy effluent and gelatinized starchy waste from the rice noodle

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manufacturing process by highly efficient ALAB, *Lactobacillus plantarum* S21 (accession number KF 836428) isolated from Thai indigenous food products. The bacterium has been confirmed to have potentiality and suitability for lactic acid production from cassava starch (Rientrakoonchai, 2010).

Starchy effluent (SE) derived from a rice noodle factory in Chiang Mai, Thailand, was investigated. The factory processed rice at approximately 10–15 tons per day in order to distribute rice noodle products to Thai consumers in the upper northern region. The starchy wastewater was discharged at approximately 60 m^3 per day. A part of the starchy wastewater from the milling process was collected and used for lactic acid production. The basic composition of the starchy wastewater, including total solids, ash, total carbohydrates, starch, reducing sugar, total proteins, and moisture content, were analyzed prior to preparing the medium. *Lactobacillus plantarum* S21 was maintained in 15% glycerol and stored at −80°C. A single colony was placed on MRS agar containing 10 g/L of starch as a sole carbon source. The culture condition was performed at 37°C under static condition. The medium was provided based on modified MRS (mMRS) medium that consisted of (g/L) 5.0 g peptone, 5.0 g beef extract, 2.5 g yeast extract, 1.0 g K_2HPO_4, 2.5 $CH_3COONa \cdot 3H_2O$, 1.0 g di-sodium hydrogen citrate, 0.5 mL Tween80, 0.1 g $MgSO_4 \cdot 7H_2O$, and 0.1 g $MnSO_4 \cdot H_2O$. The composition described above was dissolved in SE to a final concentration of 10 g/L total carbohydrate. For a high starch concentration level, the super quality grade of rice starch (RS) was purchased from Bangkok Inter Food Co. ltd, Thailand, which was guaranteed by the company to have no protein, fat, minerals, and sugar contents, and was further adjusted to the desired concentration levels of total carbohydrates. Sterilization was performed by autoclaving at 121°C for 15 min. Seed inoculum was prepared by spiking a single colony of *L. plantarum* S21 in 50 mL of mMRS containing 10 g/L RS as a carbon source and was statically incubated at 37°C for 16–8 h.

The growth study of *L. plantarum* S21 was investigated by transferring 10% (v/v) inoculum to mMRS medium containing either SE or RS as the sole carbon source. Lactic acid and viable cells were monitored every 6 for 48 h. Total carbohydrates was also investigated in order to calculate the lactic acid yield. To observe the possibility of direct conversion of high starch concentrations from starchy wastewater, 10% of the inoculum size was transferred to the mMRS medium containing a starchy carbon source at total carbohydrate levels of 10, 20, 40, 60, and 80 g/L. During the fermentation stage, the pH of the culture was maintained at 7.0 by the addition of 10 N NaOH. Lactic acid, total carbohydrates, and amylase activity were determined. Lactic acid yield was defined as the formation of lactic acid to total carbohydrate consumption ($\Delta P/\Delta S$). The productivity is defined as the rate of lactic acid formation ($\Delta P/\Delta T$).

Quantification of starch was determined by measuring the light absorption of the iodine-starch complex color at a wavelength of 620 nm (Xiao et al., 2006). Reducing sugar was determined by DNS method (Miller et al., 1959). Total protein was determined according to Kjeldahl method (1883). Lactic acid was determined by HPLC using Rezex ROA-Organic acid H+(8%) column (300 ×7.8 mm) (Phenomenex, USA) running in 0.005 N H_2SO_4 as a mobile phase at flow rate of 0.5 mL/min. The column temperature was maintained at 40°C, and the separated sample was then assessed by UV detector at 210 nm. Viable cells were determined by pour plate on MRS agar. Total carbohydrate was determined by phenol-sulfuric method (Dubois et al., 1956). Amylase activity was assayed using a reaction mixture containing 0.25 mL of 0.5% (w/v) of soluble starch substrate in 0.1 M Na-phosphate buffer pH 6.5 and 0.25 mL of proper dilution of enzyme. After 10 min of incubation at 37°C, 0.5 mL of DNS solution was added to stop the reaction to measure the amount of reducing sugar liberated from the starch. The solution was placed in boiling bath for 10 min and left to cool, followed by addition of 5 mL of distilled water prior to measuring the absorbance at 540 nm. One unit of enzyme was defined as the enzyme that liberated 1 μmole of reducing sugar under assay conditions. The significant difference among lactic acid, lactic acid yield, and productivity were determined by complete randomized design of analysis of variance. The mean values were analyzed by the least significant difference using Statistix software, version 8.0 (Analytical software, USA).

The starchy effluent as a by-product of the rice noodle manufacturing process used in this study was derived from the broken rice caused by washing and milling processes, from which 60 m^3 of effluent was discarded per day. It basically contained 18.0±0.9 total carbohydrates, 14.4±0.2 starch content, 1.8±0.1 reducing sugar, and 0.6±0.02 g/L total protein with a pH value of 3.39±0.10 (Table 1). The starch content of this SE is close to that of the food waste collected from cafeterias (Ohkouchi and Inoue, 2006) and mussel processing waste (Pintado et al., 1999), and was used for the investigation of direct lactic acid production. Probst et al. (2013) mentioned that bio-waste with high acidic and high organic content is the preferred habitat of lactic acid bacteria, thus, it could be an alternative substrate for lactic acid production. Few attempts have been made to utilize starchy waste for lactic acid production (Pintado et al., 1999; Kim et al., 2003; Ohkouchi and Inoue, 2006). Not only have the soaking and milling processes generated starchy effluent, but the steaming process has also been found to discard a great deal of high concentrations of gelatinized flour. Therefore, the growth study of *L. plantarum* S21 in SE was determined based on mMRS medium in comparison with mMRS medium alone with 10 g/L of total carbohydrates. Results showed that it was possible to grow *L. plantarum* S21 in mMRS containing SE, which remarkably produced more lactic acid than RS (Fig. 1), indicating that SE did not affect the bacterial growth and was consumed equally to that of RS. The lactic acid amounts obtained from both media were insignificantly different at 24 and 48 h of fermentation (9.0±0.3, 9.4±0.4 g/L from RS and 8.9±0.2, 9.2±0.1 g/L from SE), in agreement with the lactic acid yields. This demonstrated insignificant differences between the two media at the above-mentioned stages. The results indicated that *L. plantarum* S21 displays clear feasibility for direct conversion of SE

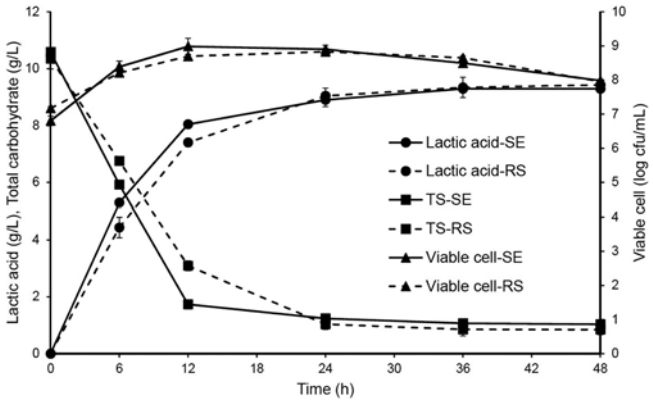

Fig. 1 Profile of lactic acid production, total carbohydrate consumption and viable cells during lactic acid fermentation by *L. plantarum* S21 at 37°C in mMRS broth containing 10 g/L rice starch (RS) and starchy effluent (SE).

Table 1 Content of starchy effluent and gelatinized starchy waste based on wet weight

Components	Starchy wastewater % (g/100 g)*	Gelatinized starchy waste % (g/100 g)*
Total solid	2.55±0.12	10.37±0.01
-Ash	0.72±0.21	0.21±0.01
-Total protein	0.06±0.00	0.07±0.00
-Total carbohydrate	1.78±0.40	10.10±0.26
-Starch	1.43±0.02	9.58±0.30
-Reducing sugar	0.17±0.07	0.48±0.01
Moisture	97.45±0.12	89.63±0.01
pH	3.39±0.10	ND**

*Mean values are presented with standard deviation (SD).
**ND = Not determined.

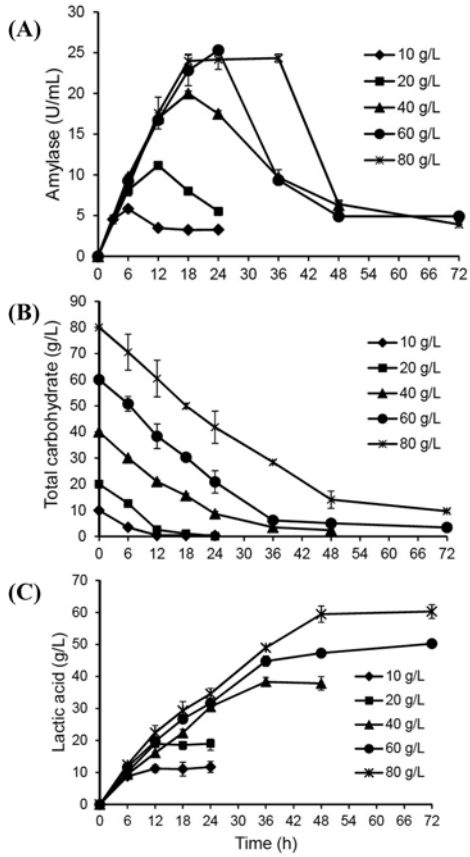

Fig. 2 Profile of amylase activity (A), total carbohydrate (B) and lactic acid (C) during direct conversion of rice noodle factory effluent containing various concentrations of the starchy carbon source to lactic acid by *L. plantarum* S21 at 37°C.

to lactic acid.

In conventional rice noodle manufacturing, high concentration of gelatinized flour is typically discarded from the steaming process, and the remainder accumulated on filtered cloth sheets after steaming. Commonly, flour slurry for noodle manufacturing was adjusted to concentrations ranging from 30–40% (w/v) (Hormdok and Noomhorm, 2007), but gelatinized starchy waste achieved from this steaming process had only 10.10±0.26 g/100 g gelatinized starchy waste with 9.58±0.30 g starch/100 g calculated to approximately 100 g/L of total carbohydrates, as well as other components presented in Table 1. This was considered an interesting form of waste, because the ingredients did not possess too much complicated components as described by Chiemchaisri et al. (2007). No reports have yet described the utilization of this starchy material for lactic acid production. Therefore, it is considered considerably challenging and attractive to utilize this waste for the direct production of lactic acid. The possibility of producing lactic acid from high concentration of starch in order to utilize both gelatinized starchy waste and the starchy effluent from the rice noodle factory by *L. plantarum* S21was evaluated. Before creating the pilot scale of lactic acid production, the SE was used as a part of the carbon source in mMRS medium by supplementation of RS to initiate the final total carbohydrate content of 10–80 g/L.

Considering the amylase activity during lactic acid fermentation, we found that the higher concentrations of the starchy carbon source induced *L. plantarum* S21 to produce higher amount of amylase (Fig. 2A). The highest amylase activity of 24 U/mL came from the fermentation of 60 and 80 g/L of total carbohydrates. Amylase activity was increased to the maximum at 6, 12, 18, 24, and 36 h of lactic acid fermentation from 10, 20, 40, 60, and 80 g/L total carbohydrate, respectively, and then decreased thereafter. This finding was in accordance with the reasoning that amylase is the inducible enzyme, which is generally induced in the presence of starch or its hydrolytic product maltose, Gupta et al. (2003) and Gangadharan et al. (2006). It was associated with cell growth and was produced at the first stage of fermentation, as reported by Ohkouchi and Inoue (2006). After the reduction of total carbohydrates in each fermentation medium, *L. plantarum* S21 amylase decreased noticeably (Fig. 2A and B). Amylase catalyzed the hydrolysis of starch and released sugars to serve *L. plantarum* S21 for the growth and production of lactic acid. The maximum lactic acid of

Table 2 Comparison of lactic acid, lactic acid yield and lactic acid productivity of *L. plantarum* S21 from starchy effluent with various concentrations of rice starch. The presented values were obtained from the steady state of lactic acid formation in each substrate concentration

Total carbohydrate (g/L)	Time (h)*	Lactic acid (g/L)*	Lactic acid yield (g/g)*	Productivity (g/L·h)*
10	12	11.2±1.1^{e}	1.17±0.14^{a}	0.94±0.13^{cd}
20	12	19.0±0.3^{d}	1.09±0.02^{ab}	1.58±0.03^{a}
40	48	37.8±2.1^{c}	1.00±0.06^{bc}	0.79±0.06^{d}
60	48	47.3±0.2^{b}	0.86±0.03^{d}	0.98±0.00^{c}
80	48	59.4±2.5^{a}	0.90±0.07^{cd}	1.23±0.07^{b}

*Mean values are presented with standard deviation (SD).
$^{a-e}$represent significant difference at p <0.05.

11.2±1.1 and 19.0±0.3 g/L with lactic acid productivity of 0.94±0.13 and 1.58±0.03 g/ L·h, which were obtained at 12 h of fermentation of medium containing 10 and 20 g/L of starch, respectively. The result revealed a rapid consumption of the starchy carbon source of *L. plantarum* S21 and apparently produced lactic acid, as shown in Fig. 2B and C. In addition, lactic acid of 37.8±2.1, 47.3±0.2, and 59.4±2.5 g/L were obtained from the use of 40, 60, and 80 g/L of total carbohydrates at 48 h of fermentation. At higher concentration levels, total carbohydrate readings of 60 and 80 g/L seemed to limit lactic acid production since lactic acid yield was decreased significantly to the range of 0.90 g/g (Table 2), however, the total lactic acid content (59.4±2.5 g/L) and productivity (1.23±0.07 g/L·h) obtained from 80 g/L initial total carbohydrates were the highest when compared to those of 40 and 60 g/L. This data was of great value and meaningful in the research of direct lactic acid production using food waste by amylolytic lactic acid bacterium, since high concentrations of lactic acid, as well as greater yields and higher productivity were certainly obtained from the experiment. So far, Pintado et al. (1999) used amylolytic lactic acid bacteria, including *L. plantarum* A6, *L. manihotivorans* LMG18010, *L. plantarum* R10101/2 and *Pediococcus* sp. VA403, for direct conversion of mussel processing wastes for lactic acid production, but the study reported that the conversion rate of food waste contained low concentrations of either starch or total carbohydrates. High concentration levels of food waste were perfectly converted into lactic acid by *L. manihotivorans* LMG18011 (Ohkouchi and Inoue, 2006), with the highest yield of 1.11 g/g and similar production efficiency (81.2%) to that of our report, but the concentration levels of the carbon source and the achieved lactic acid were obviously lower. Few papers have assessed the direct conversion of a high concentration of starch into lactic acid since substrate inhibition has typically been found (Son and Kwon, 2013). Hence, this study is of particular interest, because the capabilities of *L. plantarum* S21 are distinct when compared other previously mentioned studies. The results revealed that the starchy effluent from the rice noodle manufacturing process is feasible for using as a substrate in the direct conversion of starch to lactic acid by *Lactobacillus plantarum* S21. We therefore expect to combine the starchy effluent and gelatinize starchy waste for the direct conversion of starchy substrate to lactic acid in the next study in order to utilize starch effluent most efficiently.

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