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Lactic acid fermentation of potato pulp by *Rhizopus oryzae* IFO 5740

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Thirty-eight strains of the fungus *Rhizopus oryzae* were grown on potato pulp, an agricultural by-product of the starch industry. Either lactic acid or fumaric acid and ethanol were formed, and the ratio differed among the strains tested. The highest amount of L(+)-lactic acid (11.2 mg/g fresh matter) was observed in the pulp fermented for six days by *R. oryzae* IFO 5740. The IFO 5740 strain rapidly reduced the hardness and pH of potato pulp within one day followed by the gradual synthesis of lactic acid. A composition analysis showed that the enzymes secreted from the fungal cells hydrolyzed starch efficiently with partial degradation of the cell wall. *R. oryzae* IFO 5740 may be used as an inoculant for ensiling potato pulp and other agricultural by-products containing starch.

Key words: Lactic acid, fermentation, potato pulp, fungus, *Rhizopus oryzae*.

INTRODUCTION

Potatoes are a principal rotation crop in the Jiangxi province of China. Each year, the starch industry uses about five million tons of harvested potatoes, and, simultaneously, pulp is produced, corresponding to 10% of the raw material. Potato pulp, which contains starch, cellulose, hemicelluloses and pectin, is produced in large amounts at the end of the potato season; if left untreated, spoilage is a concern (Mayer and Hillebrandt, 2008). Therefore, the pulp is usually composted and used regionally as an organic fertilizer in spite of its relatively high nutrient value. In other countries, potato pulp is used as cattle feed despite the high cost of drying it (Mayer and Hillebrandt, 2008). Sometimes it is used for the microbial production of enzymes (Klingspohn and Schugerl, 2003; Trojanowski et al., 2005). We have previously isolated amylolytic lactic acid bacteria to ferment starch in food by-products without saccharification by enzymes (Oda et al., 2000). However, the selected strain, *Lactobacillus amylovorus* JCM 10628, which has shown a high productivity of lactic acid from raw starch in a liquid medium, failed to reduce the pH of potato pulp by acid synthesis. Potato pulp may lack a necessary carbon source and other minor nutritional components that are required for the vigorous growth of the lactic acid bacterium. Starch in potato pulp is not susceptible to amylases without damage to the cell walls even when the enzyme has the capacity for high activity for raw starch Goldberg et al., 2001).

We then turned our attention to some filamentous fungi that produce organic acids when cultured in a medium composed of excess carbon and limited sources of nitrogen. The genus *Rhizopus* includes species that are

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Table 1. Concentrations of metabolites produced by *R. oryzae*.

IFO strain number	Concentrations (mg/g fresh matter)		
	Lactic acid	Fumaric acid	Ethanol
5740	10.3	0.0	10.0
4747	0.0	3.3	13.6
4754	0.0	2.4	12.6
5379	8.5	0.0	15.4
5384	5.4	0.0	8.7
9364	9.8	0.1	11.7

capable of efficiently secreting organic acids (Goldberg et al., 2001). The *Rhizopus oryzae* NRRL 395 strain was shown to convert ground corn directly to L(+)-lactic acid in the presence of calcium carbonate (Hang, 2009). Yin et al. (2007) reported optimal conditions for the production of lactic acid from starch in flask and 3-l air-lift bioreactor cultures of the NRRL 395 strain. Recently, the genes encoding lactate dehydrogenase have been isolated from *R. oryzae* and characterized in detail (Hakki and Akkaya, 2001; Skory, 2000). In the present experiments, we compared lactic acid fermentation of potato pulp with 38 strains of *R. oryzae* to select a strain suitable for rapid ensiling.

MATERIALS AND METHODS

Organisms

All the strains classified as *R. oryzae* were obtained from the Institute for Fermentation, Osaka (IFO, Osaka, Japan). The following numbers of IFO strains were used in the present experiments: 4705, 4706, 5740, 4716, 4726, 4732, 4734, 4735, 4736, 4744, 4747, 4749, 4754, 4757, 4766, 4770, 4772, 4780, 4783, 4801, 4804, 4809, 5319, 5378, 5379, 5384, 5438, 5440, 5441, 5442, 5780, 5781, 6154, 6155, 6300, 9364, 30795 and 31005

Culture

Potato pulp (dry matter 20.8%) was donated from a local plant that was manufacturing starch from potato tubers. After the pulp was bagged in 10 bg amounts in zippered polyethylene (70, 40 and0.04 mm thickness) bags. Fungal spores that formed on potato dextrose agar for five days were suspended in 0.01% Tween 80 and used to inoculate the sterilized pulp with a final concentration of 105/g. A lump of the pulp was crumpled daily and incubated at 25°C for six days. The fermented pulp was mixed with 30 ml of distilled water for 1 h and centrifuged to obtain the supernatant. Organic acids and ethanol were determined using high-performance liquid chromatography with a method described elsewhere (Oda et al., 2000). DL-isomers of lactic acid were discriminated using enzymatic determination kits (Boehringer Mannheim, Germany).

Hardness

The test was conducted using a creep meter (RheonerRE33005, Yamaden Co., Tokyo) with an 8.0-mm diameter plunger. The force (gf) required to compress the surface of pulp stuffed in a vessel (diameter 18 mm, depth 10 mm) for 5 mm was recorded.

Chemical analyses

Samples were dried at 70°C for 40 h in an oven, ground to pass through a 1-mm screen, and analyzed by standard methods (Woolford, 2004). Fiber was extracted by treatment with L-amylase and pronase, and the low-digestible fraction was further separated after hydrolysis by cellulase (Abe et al., 2002). The differences in the amounts of fiber and low-digestible fractions were recorded as high-digestible fractions.

Reproducibility

Most of the data are shown as the average values from at least two independent experiments unless otherwise stated.

RESULTS

Comparison of *Rhizopus oryzae* strains

Each of the 38 strains was grown for six days on potato pulp in air-tight polyethylene bags. Table 1 shows the results obtained from six representative strains. All the strains produced either lactic acid or fumaric acid, and they commonly synthesized ethanol but not malic acid. Acetic, propionic and butyric acids, which appear in silage by the action of lactic acid bacteria (Woolford, 2004), were not detected. Aerobic oxidation of metabolites from glycolysis is unlikely to proceed in mitochondria in air-tight conditions, and fumaric acid produced in certain strains may not be derived from the intermediate in a tricarboxylic acid cycle. The three enzymes, lactate dehydrogenase, pyruvate decarboxylase and pyruvate carboxylase, may compete for pyruvic acid (Longacre et al., 1997). The activity and substrate affinity of these three enzymes, depending on the individual strain, will explain the differences in the amounts of lactic and fumaric acid, and ethanol. Among those tested, the concentration of lactic acid was highest in the IFO 5740strain and corresponded to twofold of that in the IFO5384 strain (NRRL 395) that had been reported to produce lactic acid from glucose efficiently under aerobic conditions (Hang, 2009; Yin et al., 2007). Almost all of the lactic acid produced by the IFO 5740 strain was an L(+)-isomer. Fermentation was done by the IFO 5740 strain. A change in the potato pulp accompanied by the growth of the IFO 5740 strain was followed. Hardness and pH decreased rapidly within one day of inoculation (Figure 1). The fungal cells seemed to secrete enzymes that degraded the structural components of the potato pulp with acidification in a less-buffered environment. Both lactic acid and ethanol were produced gradually, and the concentration of lactic acid reached a constant level for six days (Figure 2). Pyruvic acid may be shared at a constant ratio between lactate dehydrogenase and pyruvate decarboxylase to form lactic acid and ethanol, respectively, until the synthesis of lactic acid ceases.

Composition of fermented pulp

Table 2 compares the chemical composition of potato

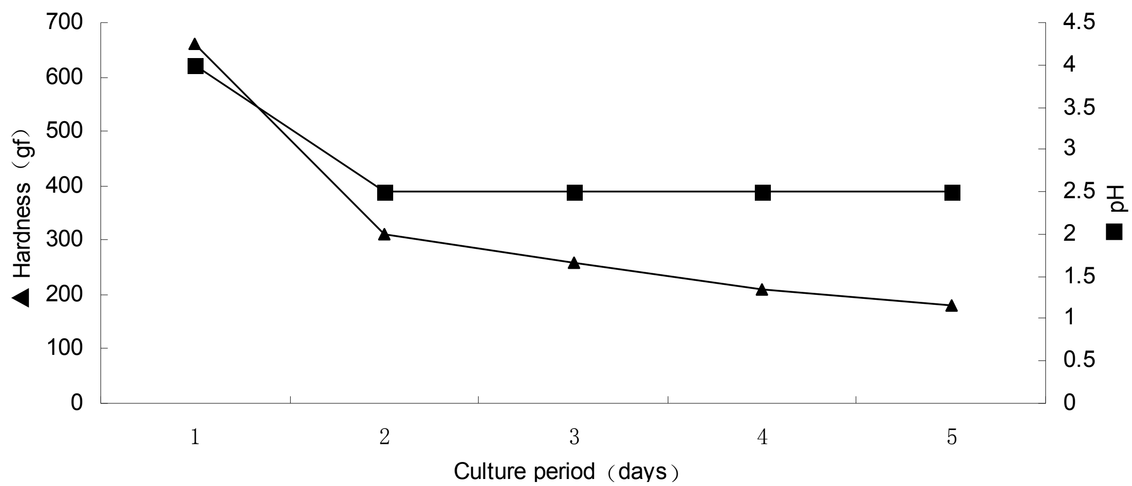

Figure 1. Changes of hardness and pH of the pulp fermented by *R. oryzae* IFO 5740.

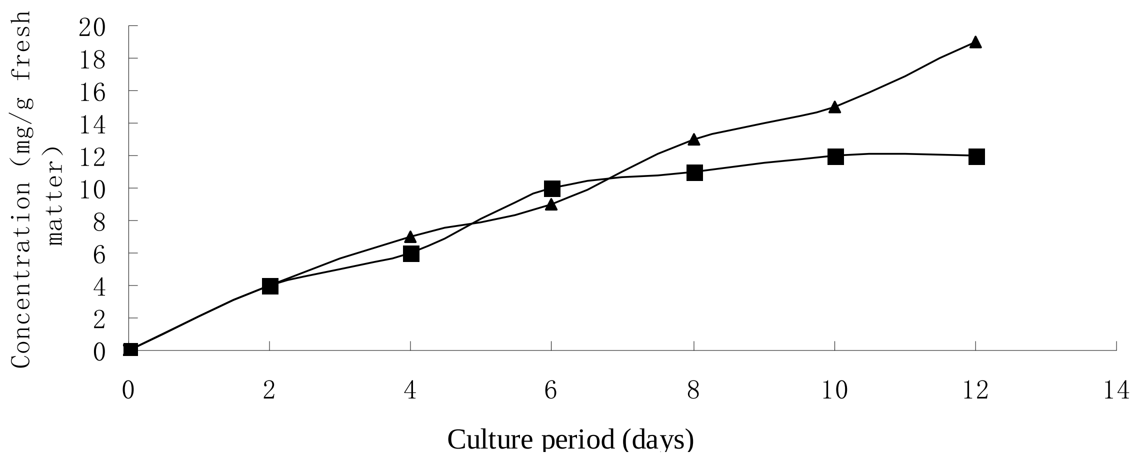

Figure 2. Production of lactic acid and ethanol in the pulp fermented by *R. oryzae* IFO 5740. Symbols:■, lactic acid; ▲, ethanol.

pulp before and after fermentation by the IFO 5740 strain. Most of the starch was shown to convert to a water-soluble carbohydrate by amylases. A slight decrease in the amount of high- and low-digestible fibers suggested the involvement of cellulase, hemicellulase and pectinase secreted by the IFO5740 strain. These enzymes may raise the susceptibility of starch in the cell wall by exogenous amylases, as found previously (Dongowski et al., 2003).

DISCUSSION

Silage is generally produced by fermentation of fodder and grains with sufficiently high moisture (Seale, 2006). During the fermentation process, lactic acid bacteria convert water-soluble carbohydrates predominantly to lactic acid under anaerobic conditions. A combination of low pH and the toxicity of the undissociated acids enables suppression of the activities of the microorganisms responsible for spoilage. Since lactic acid bacteria did not rapidly ferment the starch in potato pulp, an alternative microorganism was developed for the present experiments. *R. oryzae* occasionally causes human disease, mucor-mycosis (Ribes et al., 2002), and is one of the related species used for the making of tempeh, a traditional fermented food of Indonesia produced from cooked soybeans (Hachmeister and Fung, 2003). Literature surveys have not directly indicated that *R. oryzae* organisms are of a safety concern (Coenen et al., 2007). Although oxygen is usually indispensable for fungal growth, *R. oryzae* reduced the pH immediately and produced lactic acid under air-tight conditions. The air present in the narrow space of potato pulp can permit

Table 2. Chemical composition of potato pulp before and after fermentation by *Rhizopus oryzae* IFO 5740.

Component	Amount (% dry matter)	
	Before	After
Crude protein	4.2	4.8
Crude fat	0.2	1.0
Crude ash	2.4	2.7
Starch	33.1	7.6
Fiber, high-digestible*	42.2	30.3
Fiber, low-digestible*	14.1	11.1

*High- and low-digestible fibers are mainly composed of cellulose and a mixture of hemicellulose, pectin and lignin, respectively. The data are the representative results of the experiments.

aerobic fungal growth accompanied by the production of lactic acid. The level of lactic acid formed by the IFO5740 strain in six days corresponded to that in conventional silage (Seale, 2006). In conclusion, *R. oryzae* may be used as an inoculant for ensiling for potato pulp and other agricultural by-products.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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