Food Sci. Technol. Res., **11** (4), 400–406, 2005

Production of L-Lactic Acid by Simultaneous Saccharification and Fermentation Using Unsterilized Defatted Rice Bran as a Carbon Source and Nutrient Components

Masayuki TANIGUCHI[1]*, Masahiro HOSHINA[1], Suguru TANABE[1], Yuki HIGUCHI[1], Kenji SAKAI[2], Sadami OHTSUBO[3], Kazuhiro HOSHINO[4] and Takaaki TANAKA[1]

[1] *Department of Materials Science and Technology, Niigata University, 2–8050 Ikarashi, Niigata, 950–2181, Japan*
[2] *Department of Applied Chemistry, Oita University, 700 Dannoharu, Oita, 870–1192, Japan*
[3] *Food Research Center, Niigata Agricultural Research Institute, 2–25 Shin-ei-cho, Kamo, Niigata 959–1381, Japan*
[4] *Department of Materials Systems Engineering and Life Science, Toyama University, 395 Gokufu, Toyama, 930–8555, Japan*

Received July 30, 2005; Accepted December 22, 2005

On the basis of growth rate at low pH, yield of lactic acid from glucose, and optical purity of lactic acid produced, we selected lactic acid bacteria favorable for production of optically pure L-lactic acid from defatted rice bran without sterilization. Of 21 strains tested, strains Nos. 13 and 16 produced 27–29 kg m^{-3} of lactic acid with high optical purity from 100 kg m^{-3} of unsterilized defatted rice bran in simultaneous saccharification and fermentation (SSF) with MRS medium at pH 4.5, a level at which the growth of indigenous lactic acid bacteria in defatted rice bran was suppressed. In a SSF process using strain No. 16 in which McIlvaine buffer (pH 4.5) was used instead of MRS medium, no growth of indigenous lactic acid bacteria was observed in defatted rice bran, and 28 kg m^{-3} of lactic acid with 92% L-type content was produced from 100 kg m^{-3} of unsterilized defatted rice bran. In SSF using McIlvaine buffer (pH 4.5), the protein fraction of defatted rice bran was found to play a significant role as a nitrogen source for the growth of lactic acid bacteria. By increasing the initial cell concentration to OD_{660}=1.0 for SSF using strain No. 16 and McIlvaine buffer (pH 4.5), the proportion of L-lactic acid produced was enhanced to 95%.

Keywords: L-lactic acid, rice bran, simultaneous saccharification and fermentation, nitrogen source

Introduction

Lactic acid, which contains both hydroxyl and carboxyl moieties and has one chiral carbon atom, is an important organic acid which is widely used in the food, pharmaceutical, and cosmetic industries (Ray and Sandine, 1992; Litchfield, 1996). Moreover, lactic acid can be polymerized to form the biodegradable and recyclable polyester poly (lactic acid), which may play an increasing role in solving a world-wide environmental problem (Datta *et al.,* 1995; Yin *et al.,* 2000; Stevens, 2002). Poly (lactic acid) is a potential substitute for plastics manufactured from petroleum derivatives. Highly optically pure lactic acid is necessary to obtain highly crystalline poly(lactic acid), on which the high strength and chemical and heat resistance of the polymer (Lunt, 1998) are based. Lactic acid can be produced commercially either by chemical synthesis from acetaldehyde and hydrogen cyanide or by microbial fermentation. Synthetic production results in a racemic mixture of the two isomers, while fermentative production can yield a racemate or either form alone, depending on the microorganism, substrate, and growth conditions used. Many researchers have conducted extensive investigations into methods of enhancing the productivity and economy of the processes for producing optically pure lactic acid, including use of cheap raw materials (Luo *et al.,* 1997; Moldes *et al.,* 1999; Sakai *et al.,* 2000), utilization of suitable microorganisms (Hujanen and Linko, 1996; Yin *et al.,* 1997, Dien *et al.,* 2002; Zhou *et al.,* 2003; Taniguchi *et al.,* 2004), and development of cultivation techniques and purification procedures (Hoshino *et al.,* 1991; Planas *et al.,* 1996; Börgardts *et al.,* 1998; Velázquez *et al.,* 2001).

One of the most serious problems associated with the biological production of lactic acid is the high cost of raw materials such as starch, which represent a large portion of the production price (Datta *et al.,* 1995; Åkerberg and Zacchi, 2000). Recently, in order to reduce the cost of raw materials for lactic acid and concurrently convert wastes to useful substances, many researchers have investigated the possibility of producing lactic acid from waste products, such as whey permeate (Tejayadi and Cheryan, 1995; Øyaas *et al.,* 1996; Senthuran *et al.,* 1997), beet molasses (Monteagudo and Aldavero, 1999), bakery waste (Oda *et al.,* 1997), waste water from paper (Schmidt and Padukone, 1997) and potatoes (Huang *et al.,* 2003), agricultural wastes (Garde *et al.,* 2002; Rivas *et al.,* 2004; Naveena *et al.,* 2005), and food waste (Sakai *et al.,* 2004). In particular, the utilization of agricultural by-products for lactic acid production is attractive because of their low price and high polysaccharide contents.

Rice bran, which is the cheap residue obtained from

* To whom correspondence should be addressed.
E-mail: mtanig@eng.niigata-u.ac.jp

brown rice during the polishing process, is one of the most abundant agricultural by-products in Japan. From the quantity of 8.73×10^6 t of rice produced in 2004 (Ministry of Agriculture, Forestry, and Fisheries of Japan, 2004) and the weight ratio of bran to whole brown rice (about 10%), the annual amount of rice bran produced in Japan may be estimated at about 8.7×10^5 t. After rice oil has been squeezed out, the residual defatted rice bran contains large amounts of starchy and cellulosic polysaccharides. Enzymatic or chemical hydrolysis is necessary to allow lactic acid bacteria to utilize the polysaccharides as carbon sources. Recently, the process of simultaneous saccharification and fermentation (SSF) has been shown to reduce the time and number of steps involved in bioprocesses for the production of lactic acid and ethanol from starchy and cellulosic biomass (Moldes *et al.,* 2000; Stenberg *et al.,* 2000; Nakamura *et al.,* 2002). It is known that minimal preprocessing and supplying of nutrients to a substrate consisting of inexpensive waste materials should be to great advantage in reducing the cost of production of lactic acid. It is very difficult to sterilize solid materials such as rice bran; much heat energy is required for complete sterilization prior to use as a substrate in SSF. In a previous report, we described a process of D-lactic acid production from polysaccharides in defatted rice bran by SSF in which the requirement for sterilization was bypassed by maintaining the pH of the MRS medium (De Man *et al.,* 1960) at a low level to suppress the growth of indigenous bacteria in the bran (Tanaka *et al.,* 2006).

In the present study, in order to produce L-lactic acid from polysaccharides in defatted rice bran by SSF without sterilization, we selected lactic acid bacteria which were optimal in terms of growth rate at a low pH, yield of lactic acid from glucose, and optical purity of lactic acid produced. Since rice bran not only contains a large amount of polysaccharides but is also 18% protein, defatted rice bran may play a role as a nitrogen source necessary for production of lactic acid in a reasonable time. We have also produced L-lactic acid by SSF from unsterilized defatted rice bran by maintenance of the pH of the medium at a low level and providing no additional nutrients except for buffer constituents.

Materials and Methods

Microorganisms and defatted rice bran Twenty-one strains of lactic acid bacteria isolated from different places in Japan were used for production of L-lactic acid. Defatted rice bran powder was received as a gift from Nagaoka Yuryo Co., Nagaoka, Japan. The components of rice bran are starch and dextrin (46.7%), cellulose and hemicellulose (11.3%), protein (18.4%), lipid (1.4%), ash (10.4%), and others (11.8%), as described previously (Tanaka, 2006).

Growth properties of lactic acid bacteria Lactic acid bacteria were cultivated at 37℃ in 10 cm^3 of modified MRS medium (pH 6.8) (de Man *et al.,* 1960) supplemented with 10 kg m^{-3} of glucose in a test tube with a butyl rubber stopper. The modified MRS medium contained 10 kg of Polypepton (Nihon Pharmaceuticals, Tokyo, Japan), 10 kg of fish meat extract (#01230, Kyokuto Pharmaceutical Industrial, Tokyo), 5 kg of yeast extract (Oriental Yeast, Tokyo), 2 kg of K_2HPO_4, 2 kg of diammonium hydrogen citrate, 0.2 kg of $MgSO_4\cdot7H_2O$, 0.05 kg of $MnSO_4\cdot4H_2O$, and 1 kg of Tween 80 per m^3. The test tubes were used for preparing seed culture and for evaluating the growth properties of lactic acid bacteria. The seed culture was allowed to grow for 12 hr to reach the logarithmic growth phase, and the cells were then added to the medium at an initial optical density (OD) of 0.1 at a wavelength of 660 nm. The growth properties examined were not only lactic acid yield from glucose and optical purity of lactic acid produced but also specific growth rate at pH 4.5. Lactic acid yield and optical purity were determined using culture broth obtained after cultivation for 24 hr. The specific growth rate was calculated on the basis of an initial increase in cell concentration.

Lactic acid production by SSF In this study, an enzyme mixture of 6.7 g m^{-3} amylase (Dabiase K-27, Nagase ChemteX, Osaka, Japan) and 3.3 g m^{-3} cellulase (Cellulase Y-NC, Yakult Pharmaceutical Industry, Tokyo, Japan) were used to saccharify the polysaccharides in the rice bran as described previously (Tanaka *et al.,* 2006). The optimal pH values of the amylase and cellulase were 4.5 and 4.0, respectively, according to the manufacturers' data. Lactic acid bacteria were cultivated in a 1-dm^3 bioreactor (TEJC-S1J, Chiyoda Seisakusho, Nagano, Japan) containing 0.7 dm^3 modified MRS medium without sterilization. The initial pH of the medium was adjusted to 4.5 using 4 N NaOH or 4 N HCl solution. The medium was supplemented with 100 kg m^{-3} unsterilized rice bran as a carbon source. The seed culture for the bioreactor was allowed to grow for 12 hr to reach the logarithmic growth phase, as described above. The cells were then added to the medium at an initial OD of 0.1 at a wavelength of 660 nm, and the mixed enzyme solution of amylase and cellulase described above was concurrently added without sterilization through a membrane filter. The temperature was kept at 37℃ and nitrogen gas was sparged at 0.3 vvm through the medium to maintain anaerobic conditions. An antifoaming agent (KM-72F, Shin-Etsu Chemical, Tokyo, Japan) was added when necessary. The pH of the medium was kept at 4.5 by addition of 4 N NaOH solution using a peristaltic pump coupled to a pH controller. The culture broth was agitated at 400 rpm for 8 hr and then agitated at 200 rpm to avoid sedimentation of the rice bran and the cells of lactic acid bacteria.

In other SSF experiments, McIlvaine buffer (a mixture of 0.1 M citric acid and 0.1 M sodium phosphate dibasic, pH 4.5 or 4.0) was used instead of MRS medium. The rice bran without sterilization was suspended at 100 kg m^{-3} in McIlvaine buffer. After addition of the seed culture and the amylase-cellulase mixture, the rice bran suspension in the buffer was incubated in a similar manner to the experiments using the modified MRS medium as described above.

Analytical methods The cell concentration was de-

termined by measuring the OD at 660 nm. The numbers of living cells of inoculated lactic acid bacteria in the SSF broth and indigenous bacteria in defatted rice bran were concurrently measured as colony forming units (CFU) on a modified MRS agar plate at pH 6.8. The colonies of each type of lactic acid bacteria inoculated were distinguished from those of the indigenous bacteria based on the differences in color and form between the bacteria. The concentration of living cells for each type of bacteria was expressed as CFU cm^{-3}. The concentrations of organic acids were determined by high performance liquid chromatography (HPLC) using a Shim-pack SCR-102H column (Shimadzu, Kyoto, Japan) and an electrical conductivity detector. The optical purity of lactic acid was determined using a chiral column (SUMICHIRAL OA-5000 L, Sumika Chemical Analysis Service, Osaka, Japan). The concentrations of total soluble sugar and glucose were determined by the phenol-sulfuric acid method (Dubois *et al.,* 1956), and a glucose oxidase-peroxidase kit (Glucose CII-Test Wako, Wako Pure Chemical Industries, Osaka, Japan), respectively.

Results and Discussion

Selection of lactic acid bacteria Our previous results (Tanaka *et al.,* 2006) revealed that the indigenous lactic acid bacteria in defatted rice bran produced racemic lactic acid by SSF at pH 6.0–6.8 and that maintenance of the pH of the medium at a low level made it possible to suppress the growth of indigenous bacteria. On the basis of these findings, growth rate at a low pH, yield of lactic acid from glucose, and optical purity of lactic acid produced were employed as criteria for selecting lactic acid bacteria favorable for the production of optically pure L-lactic acid from defatted rice bran without sterilization. Figure 1 shows a comparison of growth properties and L-lactic acid production capabilities among twenty-one types of lactic acid bacteria. The bacteria, which were isolated from rivers, soils, rice, food wastes and so on before being stored in our laboratory, were tested as described in Materials and Methods. The bacteria were divided into three groups based on their lactic acid yields. The numbers of bacterial strains which produced yields of 0.6–0.75 kg/kg, 0.8–0.9 kg/kg, and more than 0.9 kg/kg were six, six, and nine, respectively. As shown in Fig. 1A, eleven strains had specific growth rates of more than 0.2 hr^{-1} at an initial pH of 4.5. Nine strains produced L-lactic acid of high optical purity, but of these, five strains did not produce a high yield, as shown in Fig. 1B. Of the nine bacterial strains which produced lactic acid with a yield of more than 0.9 g/g, none possessed both a high specific growth rate at pH 4.5 and the ability to produce pure L-lactic acid. However, there were four strains that could produce almost-pure L-lactic acid with a high yield, although their specific growth rates at pH 4.5 were around 0.1 hr^{-1}. These four strains (Nos. 13, 16, 18, and 19) were further investigated in detail.

Production of lactic acid by SSF using MRS medium Rice bran usually contains indigenous microorganisms. Since it is difficult to achieve sterilization of microorganisms attached to solid organic matter, much heat energy is required for complete sterilization. In our previous study, we showed that control of the pH of the MRS medium at 4.5–5.0 could suppress the growth of indigenous microorganisms in defatted rice bran without sterilization, and that 28 kg m^{-3} of optically pure D-lactic acid was successfully produced from 100 kg m^{-3} defatted rice bran by SSF at pH 5.0 by *Lactobacillus delbrueckii* NBRC (IFO) 3202 (Tanaka *et al.,* 2006). Based on the our previous results, we cultivated the four selected strains at pH 4.5 in MRS medium containing unsterilized defatted rice bran and an unsterilized mixture of amylase and cellulase. Figure 2 shows the results of L-lactic acid production from 100 kg m^{-3} defatted rice bran by each strain of lactic acid bacterium. Concentrations of total soluble sugar and glucose increased initially and then decreased after 4–8 hr. In all SSF processes, glucose formed by enzymatic hydrolysis was consumed completely in up to 12 or 24 hr by each bacterial strain, but about 5 kg m^{-3} of total soluble sugar remained after 36 hr. Arabinose and unknown sugars were detected among the remaining

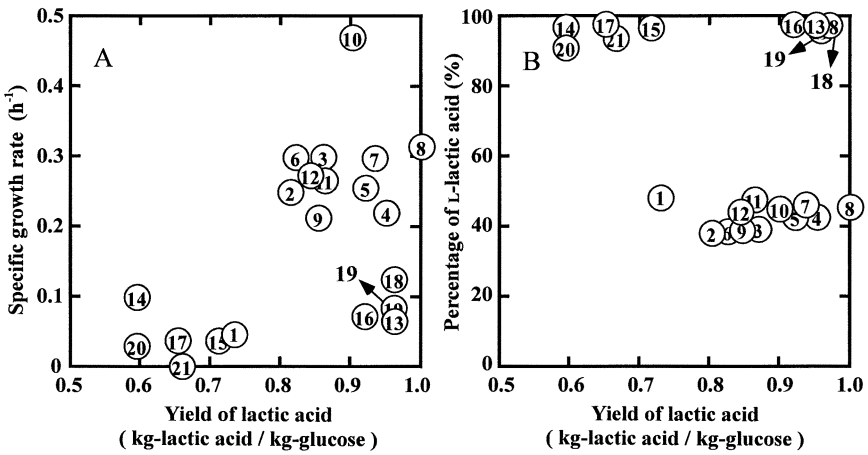

Fig. 1. Comparison of growth properties of lactic acid bacteria tested. (A) relationship between yield of lactic acid and specific growth rate at pH 4.5, (B) relationship between yield of lactic acid and percentage of L-lactic acid.

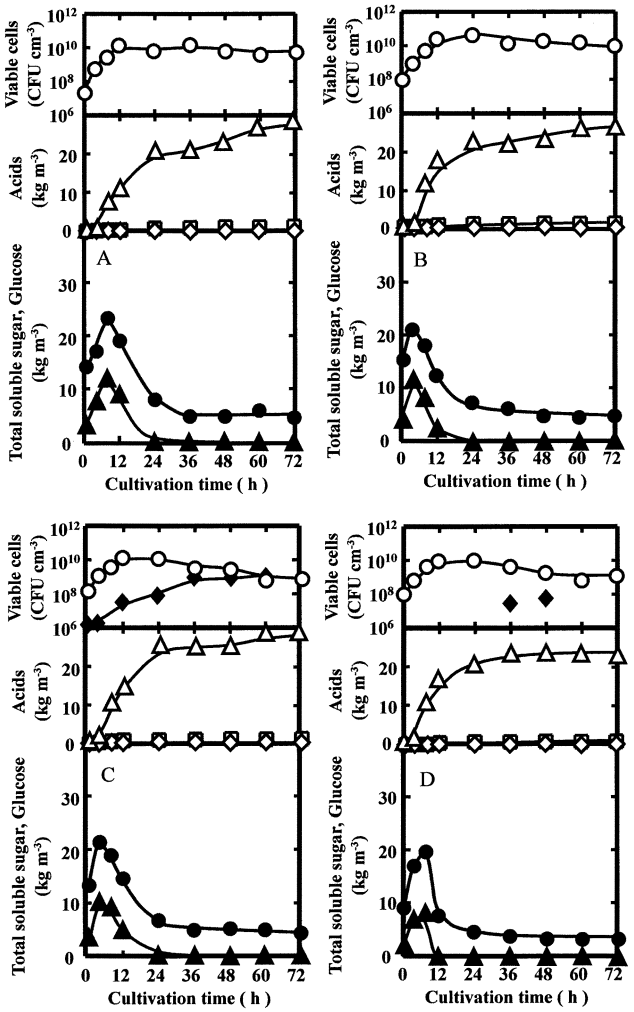

Fig. 2. Production of lactic acid from unsterilized defatted rice bran by SSF using MRS medium (pH 4.5) with (A) strain No. 13, (B) strain No. 16, (C) strain No. 18 and (D) strain No. 19. (○) Viable cells of each lactic acid bacterium inoculated, (◆) viable cells of indigenous lactic acid bacterium, (△) lactic acid, (□) acetic acid , (◇) formic acid, (●) total soluble sugar, (▲) glucose.

sugars by HPLC (data not shown). Lactic acid was produced from 4 hr and the final concentrations were more than 25 kg m^{-3}. Little or no formic or acetic acid was detected in the medium. However, as shown in Figs. 2C and 2D, growth of indigenous lactic acid bacteria was observed when strains Nos. 18 and 19 were used for SSF. Consequently, the optical purity of lactic acid produced was low, as described below (see Table 1). When strain No. 13 or No. 16 was inoculated into MRS medium without sterilization, the concentrations of living cells increased to more than 10^{10} CFU cm^{-3} at 10 hr and then remained at high levels. Although unsterilized defatted rice bran was added to the MRS medium, no other type of lactic acid bacterium was detected. The results were identical to those obtained for D-lactic acid production by *L. delbrueckii* NBRC (IFO) 3202 (Tanaka *et al.*, 2006). When strain No. 13 or No. 16 was used, the SSF process resulted in production of lactic acid with high optical purity from unsterilized defatted rice bran, as described below (see Table 1).

Production of lactic acid by SSF using McIlvaine buffer

Lactic acid bacteria are known to be fastidious microorganisms and have complex nutrient requirements due to their low capability of biosynthesizing B-vitamins and amino acids. In previous works on lactic acid production (Hofvendahl and Hahn-Hägerdal, 2000), a considerable amount of an expensive complex nitrogen source such as yeast extract was added as a supplement to the medium when raw starchy materials such as barley, corn, and wheat, as well as refined sugars such as glucose and sucrose, were used as carbon sources. However, economic analysis of lactic acid production has shown that yeast extract is the largest cost contributor, accounting for about 38% of the total production cost (Tejayadi and Cheryan, 1995). To enhance the economics of lactic acid production by lactic acid bacteria, there have been many studies on the possibility of replacing the costly yeast extract with less expensive sources of nitrogen and amino acids (Hujanen and Linko, 1996; Yoo *et al.*, 1997; Kurbanoglu and Kurbanoglu, 2003; Bustos *et al.*, 2004). In these studies, corn steep liquor (CSL), whey protein

Table 1. Production of L-lactic acid from defatted rice bran by simultaneous saccharification and fermentation.

Strain	Medium	pH (−)	Initial O.D. (−)	Lactic acid (kg m^{-3})	Acetic acid (kg m^{-3})	Formic acid (kg m^{-3})	Content of L-lactic acid (%)	Remarks
No. 13	MRS	4.5	0.1	28.7	0.32	ND[a)]	93	Fig. 2A
	Buffer	4.5	0.1	24.9	1.39	1.67	48	Fig. 3A
No. 16	MRS	4.5	0.1	26.9	1.03	0.19	89	Fig. 2B
	Buffer	4.5	0.1	27.9	1.12	0.28	92	Fig. 3B
	Buffer	4.0	0.1	27.8	1.09	0.23	92	Fig. 4A
	Buffer	4.5	1.0	26.7	1.97	0.35	95	Fig. 4B
No. 18	MRS	4.5	0.1	28.4	0.99	ND	71	Fig. 2C
	Buffer	4.5	0.1	30.3	2.83	ND	68	Fig. 3C
No. 19	MRS	4.5	0.1	26.7	1.12	ND	47	Fig. 2D
	Buffer	4.5	0.1	31.1	2.74	ND	68	Fig. 3D

[a)]ND: Not detected.

hydrolysate, barley malt sprouts, and grass extract were found to be the best economical alternatives. Recently, Oh *et al.*, (2005) reported that *Enterococcus faecalis* produced lactic acid with 2.6 kg m^{-3} hr^{-1} of lactic acid productivity and 5.9 kg m^{-3} of maximal dry cell weight from whole wheat flour hydrolysed by amylolytic enzymes after pretreatment with 0.3% (v/v) sulfuric acid, without additional nutrients. The authors also showed that the addition of 15 kg m^{-3} of CSL and 1.5 kg m^{-3} of yeast extract to the hydrolysate resulted in 5.4 kg m^{-3} hr^{-1} of high lactic acid productivity and 14.1 kg m^{-3} of maximal dry cell weight.

We attempted to produce lactic acid from unsterilized defatted rice bran by SSF at pH 4.5 without addition of MRS medium constituents. McIlvaine buffer (pH 4.5) was used for the SSF process instead of MRS medium, as described in Materials and Methods. Figure 3 shows the results of L-lactic acid production from 100 kg m^{-3} unsterilized defatted rice bran by each lactic acid bacterial strain. Concentrations of total soluble sugar and glucose initially increased and then decreased after 8 hr. Concentrations of total soluble sugar and glucose formed by enzymatic hydrolysis were higher than those obtained in SSF processes using MRS medium, suggesting decreased consumption by lactic acid bacteria. Except for strain No. 13 (Fig. 3A), the lactic acid bacteria consumed glucose completely up to 24 hr, but 5 kg m^{-3} of total soluble sugar remained after 36 h, similar to the pattern shown in Fig. 2. Strain No. 13 was unable to grow in the SSF process when the buffer was used, and growth of indigenous lactic acid bacteria in the rice bran was observed, as shown in Fig. 3 A. For strains Nos. 18 and 19, lactic acid was produced from 4 or 8 hr, and the final concentrations were more than 26 kg m^{-3}, which is higher than those of the SSF processes using MRS medium. These results suggested that the protein fraction in defatted rice bran supported the growth of lactic acid bacteria, including some indigenous lactic acid bacteria. In fact, growth of indigenous lactic acid bacteria was observed as shown in Fig. 3, except for strain No. 16, and concentrations of acetic acid detected were significantly higher than those of SSF processes using MRS medium as described below (see Table 1). In addition, the growth of indigenous lactic acid bacteria resulted in the production of lactic acid with low optical purity, as described below (see Table 1). When strain No. 16 was inoculated into defatted rice bran suspension, the initial growth rate was slower than that observed for SSF using MRS medium. However, no growth of any indigenous lactic acid bacteria was observed, and the concentrations of living cells increased to more than 10^{10} CFU cm^{-3} at 24 hr and then remained at high levels throughout the SSF process. The use of strain No. 16 allowed production of lactic acid with relatively high optical purity from unsterilized defatted rice bran as described below (see Table 1). The results were similar to that of L-lactic acid production by SSF using MRS medium.

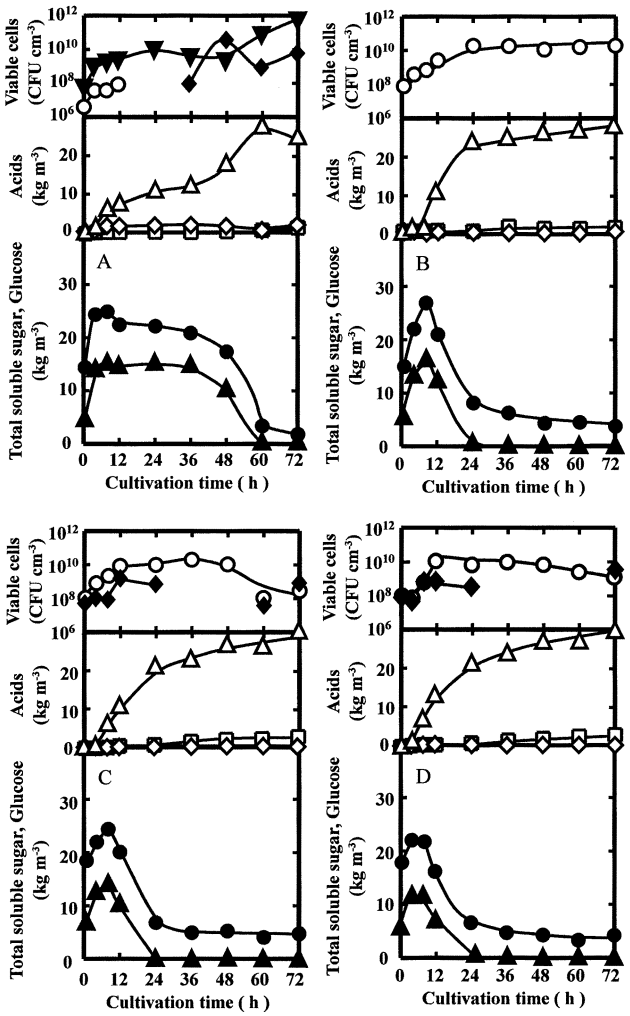

Fig. 3. Production of lactic acid from unsterilized defatted rice bran by SSF using McIlvaine buffer (pH 4.5) with (A) strain No. 13, (B) strain No. 16, (C) strain No. 18 and (D) strain No. 19. (▼) Viable cells of other indigenous lactic acid bacterium. Other symbols are the same as shown in Fig. 2.

Improvement of lactic acid production by SSF using McIlvaine buffer As described above, strain No. 16 successfully produced about 28 kg m^{-3} of optically pure L-lactic acid from 100 kg m^{-3} defatted rice bran by SSF in McIlvaine buffer without additional nutrients. However, similarly to SSF processes using the other strains, increases in the concentrations of acetic and formic acids were observed, as shown in Fig. 3. Since the MRS agar plate assay at pH 4.5 made it impossible to detect all living cells in the SSF process, satisfactory repression of the growth of indigenous microorganisms in unsterilized defatted rice bran may not be possible only by maintaining a pH of 4.5. To improve the optical purity of the lactic acid produced, we attempted to produce lactic acid from unsterilized rice bran by SSF using a buffer in which the pH was controlled at 4.0 or the initial cell concentration was elevated to OD_{660}=1.0. Figures 4A and 4B show the results of lactic acid production by SSF with a controlled pH of 4.0 (OD_{660}=0.1) and with an initial cell concentration of OD_{660}=1.0 (pH 4.5), respectively. By maintaining the pH at 4.0, the growth rate of strain No. 16 was

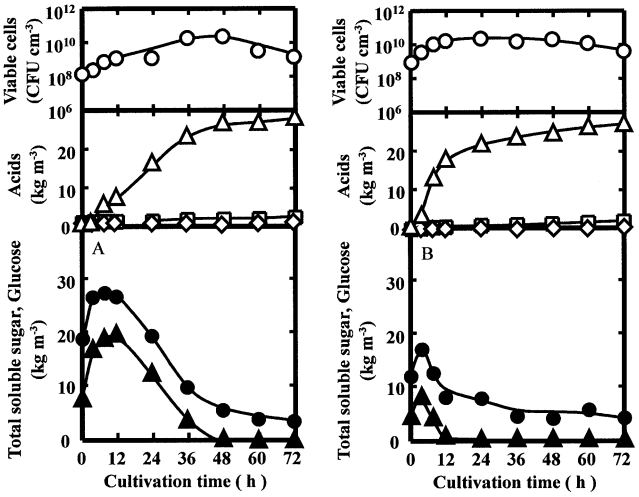

Fig. 4. Production of lactic acid from unsterilized defatted rice bran by SSF using McIlvaine buffer with strain No. 16. Conditions: (A) pH controlled at 4.0 and initial cell concentration of $OD_{660}=0.1$, (B) pH controlled at 4.5 and initial cell concentration of $OD_{660}=1.0$. Symbols are the same as shown in Fig. 2.

lowered, and sugar consumption and lactic acid production were both retarded, as shown in Fig. 4A. However, a high lactic acid production rate was observed for cells at a higher initial concentration, as shown in Fig. 4B. In SSF with pH 4.0, the concentration of lactic acid produced and its optical purity were similar to those obtained in SSF with pH 4.5 (Fig. 3B). When the initial cell concentration was $OD_{660}=1.0$, the concentration of lactic acid produced was almost the same as that obtained in SSF with pH 4.5 (Fig. 3B), but the optical purity of the lactic acid increased slightly.

Comparison of lactic acid production from defatted rice bran Table 1 shows a comparison of lactic acid production from unsterilized rice bran among different SSF processes. When strains Nos. 18 and 19 were used, the optical purity of lactic acid produced was low due to production of racemic lactic acid by indigenous lactic acid bacteria in the defatted rice bran. Strain No. 13 produced about 29 kg m^{-3} of relatively optically pure L-lactic acid from 100 kg m^{-3} defatted rice bran by SSF using MRS medium. However, for SSF using strain No. 13 and McIlvaine buffer, the optical purity of lactic acid produced was low due to production of racemic lactic acid by indigenous lactic acid bacteria in rice bran. In SSF using strain No. 16, 27–28 kg m^{-3} of relatively optically pure L-lactic acid was produced from 100 kg m^{-3} defatted rice bran when both MRS medium and McIlvaine buffer were used as media. Moreover, control of the pH at 4.0 had little or no effect on the optical purity of lactic acid produced, but enhancement of the initial cell concentration of strain No. 16 resulted in a slight increase in optical purity. The ratio of L- and D-lactic acids was 95: 5. The increase in optical purity seems to be attributable to suppression of indigenous lactic acid bacteria in defatted rice bran in the early phase of SSF.

We conducted identification tests on strain No. 16 using an API 50 CHL bacterial identification kit, which tests acid formation from various sugars and sugar alcohols (data not shown). By this analysis, the strain was identified as *Lactobacillus rhamnosus*. The results obtained will be reported elsewhere, together with the biochemical properties of the strain.

Conclusion

On the basis of our previous findings, we selected lactic acid bacteria favorable for production of optically pure L-lactic acid from defatted rice bran without sterilization. SSF processes using strains Nos. 13 and 16 in MRS medium at pH 4.5 resulted not only in lack of growth of indigenous lactic acid bacteria in defatted rice bran but also in the production of 27–29 kg m^{-3} of lactic acid with high optical purity from 100 kg m^{-3} of unsterilized defatted rice bran. In an SSF process using McIlvaine buffer (pH 4.5) instead of MRS medium, no growth of indigenous lactic acid bacteria was observed, and strain No. 16 produced 28 kg m^{-3} of relatively optically pure lactic acid from 100 kg m^{-3} of unsterilized defatted rice bran. The protein fraction in defatted rice bran was found to support the growth of lactic acid bacteria. When the initial cell concentration of strain No. 16 was elevated to $OD_{660}=1.0$ in a SSF process using McIlvaine buffer (pH 4.5), the amount of L-type lactic acid produced increased to 95%.

Acknowledgements We wish to thank Nagaoka Yuryou Co., Nagaoka, Japan, for the kind gift of defatted rice bran. We are grateful to Messrs. Y. Ozaki and S. Kanemoto of Satake Co., Higashihirosima, Japan, for helpful discussion. The study was partially supported by a Grant-in-Aid for the Research Project for Production of Lactic Acid from Biomass Resources from the Ministry of Agriculture, Forestry, and Fisheries of Japan.

References

Åkerberg, C. and Zacchi, G. (2000). An economic evaluation of the fermentative production of lactic acid from wheat flour. *Bioresource Technol.,* **75**, 119–126.

Börgardts, P., Krischke, W., Trösch, W. and Brunner, H. (1998). Integrated bioprocess for the simultaneous production of lactic acid and dairy sewage treatment. *Bioprocess Eng.,* **19**, 321–329.

Bustos, G., Moldes, A.B., Alonso, J.L. and Väzquez, M. (2004). Optimization of D-lactic acid production by *Lactobacillus coryniformis* using response surface methodology. *Food Microbiol.,* **21**, 143–148.

Datta, R., Tsai, S.P., Bonsignore, P., Moon, S.H. and Frank, J.R. (1995). Technological and economic potential of poly (lactic acid) and lactic acid derivatives. *FEMS Microbiol. Rev.,* **16**, 221–231.

De Man, J.C., Rogosa, M. and Sharpe, M.E. (1960). A medium for the cultivation of lactobacilli. *J. Appl. Bacteriol.,* **23**, 130–135.

Dien, B.S., Nichols, N.N. and Bothast, R.J. (2002). Fermentation of sugar mixture using *Escherichia coli* catabolite repression mutants engineered for production of L-lactic acid. *J. Indust. Microbiol. Biotechnol.,* **29**, 221–227.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A. and Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Anal. Chem.,* **28**, 350–356.

Garde, A., Jonsson, G., Schmidt, A.S. and Ahring, B.K. (2002).

Lactic acid production from wheat straw hemicellulose hydrolysate by *Lactobacillus pentosus* and *Lactobacillus brevis*. *Bioresource Technol.,* **81**, 217–223.

Hofvendahl, K. and Hahn-Hägerdal, B. (2000). Factors affecting the fermentative lactic acid production from renewable resources. *Enzyme Microbial Technol.,* **26**, 87–107.

Hoshino, K., Taniguchi, M., Marumoto, H., Shimizu, K. and Fujii, M. (1991) Continuous lactic acid production from raw starch in a fermentation system using a reversibly soluble-autoprecipitating amylase and immobilized cells of *Lactobacillus casei*. *Agric. Biol. Chem.,* **55**, 479–485.

Huang, L.P., Jin, B., Lant, P. and Zhou, J. (2003). Biotechnological production of lactic acid integrated with potato wastewater treatment by *Rhizopus arrhizus*. *J. Chem. Technol. Biotechnol.,* **78**, 899–906.

Hujanen, M. and Linko, Y.Y. (1996). Effect of temperature and various nitrogen sources on L(+)-lactic acid production by *Lactococcus casei*. *Appl. Microbiol. Biotechnol.,* **45**, 307–313.

Kurbanoglu, E.B. and Kurbanoglu, N.I. (2003). Utilization for lactic acid production with a new acid hydrolysis of ram horn waste. *FEMS Microbiol. Lett.* **225**, 29–34.

Litchfield, J.H. (1996). Microbiological production of lactic acid. *Adv. Appl. Microbiol.,* **42**, 45–95.

Lunt, J. (1998). Large-scale production, properties and commercial applications of polylactic acid polymers. *Polym. Degrad. Stab.,* **59**, 145–152.

Luo, J., Xia, L., Lin, J. and Cen, P. (1997). Kinetics of simultaneous saccharification and lactic acid fermentation processes. *Biotechnol. Prog.,* **13**, 762–767.

Ministry of Agriculture, Forestry, and Fisheries of Japan (2004). Production of Paddy Field Rice and Upland Rice, 2004, Ministry of Agriculture, Forestry, and Fisheries of Japan, Tokyo.

Moldes, A.B., Alonso, J.L. and Parajó, J.C. (1999). Cogeneration of cellobiose and glucose from pretreated wood and bioconversion to lactic acid: a kinetic study. *J. Biosci. Bioeng.,* **87**, 787–792.

Moldes, A.B., Alonso, J.L. and Parajo, J.C. (2000). Multi-step feeding systems for lactic acid production by simultaneous saccharification and fermentation of processed wood. *Bioprocess Eng.,* **22**, 175–180.

Monteagudo, J.M. and Aldavero, M. (1999). Production of L-lactic acid by *Lactobacillus delbrueckii* in chemostat culture using an ion exchange resins system. *J. Chem. Technol. Biotechnol.,* **74**, 627–634.

Nakamura, Y., Sawada, T. and Komatsu, A. (2002). Ethanol production from raw starch by a recombinant yeast having saccharification and fermentation activities. *J. Chem. Technol. Biotechnol.,* **77**, 1101–1106.

Naveena, B.J., Altaf, M., Bhadrayya, K., Madhavendra, S.S. and Reddy, G. (2005). Direct fermentation of starch to L(+)lactic acid in SSF by *Lactobacillus amylophilus* GV6 using wheat bran as support and substrate: medium optimization using RSM. *Process Biochem.,* **40**, 681–690.

Oda, Y., Park, B., Moon, K. and Tonomura, K. (1997). Recycling of bakery wastes using amylolytic lactic acid bacterium. *Bioresource Technol.,* **60**, 101–106.

Oh, H., Wee, Y-J., Yun, J-S., Han, S.H., Jung, S. and Ryu, H-W. (2005). Lactic acid production from agricultural resources as cheap raw materials. *Bioresouce Technol.,* **96**, 1492–1498.

Øyaas, J., Storrø, I. and Levine, D.W. (1996). Uptake of lactose and continuous lactic acid fermentation by entrapped non-growing *Lactobacillus helveticus* in whey permeate. *Appl. Microbiol. Biotechnol.,* **46**, 240–249.

Planas, J., Rådström, P., Tjeneld, F. and Hahn-Hägerdal, B. (1996). Enhanced production of lactic acid through the use of a novel aqueous two-phase system as an extractive fermentation system. *Appl. Microbiol. Biotechnol.,* **45**, 737–743.

Ray, B. and Sandine, W. (1992). Acetic, propionic, and lactic acids of starter culture bacteria as biopreservatives. In "Food biopreservatives of microbial origin," ed. by Ray, B. and Daeschel, M. CRC Press, Boca Raton, pp. 103–136.

Rivas, B., Moldes, A.B., Dominguez, J.M. and Parajó, J.C. (2004). Lactic acid production from corn cobs by simultaneous saccharification and fermentation: a mathematical interpretation. *Enzyme Microbial Technol.,* **34**, 627–634.

Sakai, K., Murata, Y., Yamazumi, H., Tau, Y., Mori, M., Moriguchi, M. and Shirai, Y. (2000). Selective proliferation of lactic acid bacteria and accumulation of lactic acid during an open fermentation of kitchen refuse with intermittent pH adjustment. *Food Sci. Technol. Res.,* **6**, 140–145.

Sakai, K., Taniguchi, M., Miura, S., Ohara, H., Matsumoto, T. and Shirai, Y. (2004). Making plastics from garbage — a novel process for poly-L-lactate production from municipal food waste. *J. Indust. Ecol.,* **7**, 63–73.

Schmidt, S. and Padukone, N. (1997). Production of lactic acid from wastepaper as a cellulosic feedstock. *J. Ind. Microbiol. Biotechnol.,* **18**, 10–14.

Senthuran, A., Senthuran V., Mattiasson, B. and Kaul, R. (1997). Lactic acid fermentation in a recycle batch reactor using immobilized *Lactobacillus casei*. *Biotechnol. Bioeng.,* **55**, 841–853.

Stenberg, K., Bollok, M., Reczey, K., Galbe, M. and Zacchi, G. (2000). Effect of substrate and cellulase concentration on simultaneous saccharification and fermentation of steam-pretreated softwood for ethanol production. *Biotechnol. Bioeng.,* **68**, 204–210.

Stevens, E.S. (2002). Green plastics: an introduction to the new science of biodegradable plastics. Princeton University Press, Princeton and Oxford.

Tanaka, T., Hoshina, M., Tanabe, S., Sakai, K., Ohtsubo, S. and Taniguchi, M. (2006). Production of D-lactic acid from defatted rice bran by simultaneous saccharification and fermentation. *Bioresource Technol.,* **97**, 211–217.

Taniguchi, M., Tokunaga, T., Horiuchi, K., Hoshino, K., Sakai, K. and Tanaka, T. (2004). Production of L-lactic acid from a mixture of xylose and glucose by co-cultivation of lactic acid bacteria. *Appl. Microbiol. Biotechnol.,* **14**, 74–80.

Tejayadi, S. and Cheryan, M. (1995). Lactic acid from cheese whey permeate. Productivity and economics of a continuous membrane bioreactor. *Appl. Microbiol. Biotechnol.,* **43**, 242–248.

Velázquez, A.C., Pometto, A.L., Ho, K.L.G. and Demirei, A. (2001). Evaluation of plastic-composite supports in repeated fed-batch biofilm lactic acid fermentation by *Lactobacillus casei*. *Appl. Microbiol. Biotechnol.,* **55**, 434–441.

Yin, M., Simmons, T.L. and Baker, G.L. (2000). Synthesis and properties of polymers derived from substituted lactic acid. In "Chemicals and materials from renewable resources," ed. by Bozell, J. J., American Chemical Society, Washington, D.C., pp. 147–159.

Yin, P., Nishina, N., Kosakai, Y., Yahiro, K., Park, Y. and Okabe, M. (1997). Enhanced production of L(+)-lactic acid from corn starch in a culture of *Rhizopus oryzae* using an air-lift bioreactor. *J. Ferment. Bioeng.,* **84**, 249–253.

Yoo, I-K., Chang, H.N., Lee, E.G., Chang, Y.K. and Moon, S-H. (1997). Effect of B vitamin supplementation on lactic acid production by *Lactobacillus casei*. *J. Ferment. Bioeng.,* **84**, 172–175.

Zhou, S., Shanmugam, K.T. and Ingram, L.O. (2003). Functional replacement of the *Escherichia coli* D-(−)-lactate dehydrogenase gene (*ldhA*) with the L-(+)-lactate dehydrogenase (*ldhL*) from *Pediococcus acidiactici*. *Appl. Environ. Microbiol.,* **69**, 2237–2244.