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Lactic acid production from agricultural resources as cheap raw materials

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Abstract

Agricultural resources such as barley, wheat, and corn were hydrolyzed by commercial amylolytic enzymes and fermented into lactic acid by *Enterococcus faecalis* RKY1. Although no additional nutrients were supplemented to those resources, lactic acid productivities were obtained at >0.8 g/l h from barley and wheat. When 200 g/l of whole wheat flour was hydrolyzed by amylolytic enzymes after the pre-treatment with 0.3% (v/v) sulfuric acid and sterilized by filtration, *E. faecalis* RKY1 efficiently produced lactic acid with 2.6 g/l h of lactic acid productivity and 5.90 g/l of maximal dry cell weight without additional nutrients. Lactic acid productivity and cell growth could be enhanced to 31% and 12% higher values than those of non-adapted RKY1, by adaptation of *E. faecalis* RKY1 to CSL-based medium. When the medium contained 200 g/l of whole wheat flour hydrolyzate, 15 g/l of corn steep liquor, and 1.5 g/l of yeast extract, lactic acid productivity and maximal dry cell weight were obtained at 5.36 g/l h and 14.08 g/l, respectively. This result represented an improvement of up to 106% of lactic acid productivity and 138% of maximal dry cell weight in comparison to the fermentation from whole wheat flour hydrolyzate only.

Keywords: Lactic acid; Agricultural resources; Corn steep liquor; Submerged fermentation; *Enterococcus faecalis*

1. Introduction

Lactic acid has various applications in food, pharmaceutical, leather, and textile industries (VickRoy, 1985). Since lactic acid has high reactivity due to containing both hydroxyl (–OH) and carboxyl (–COOH) groups, it plays a major role as a chemical feedstock capable of being converted to various chemicals such as acrylic acid, propylene glycol, acetaldehyde, and 2,3-pentanedione (Varadarajan and Miller, 1999). The continuous increase in demand for lactic acid has been due to its increasing applications in preparation of biodegradable polymers, medical sutures, and green solvents (Datta et al., 1995; Litchfield, 1996). Lactic acid is industrially produced either by chemical synthesis or by microbial fermentation. A biological method has the advantage that an optically pure lactic acid can be obtained by choosing a strain of lactic acid bacteria, whereas chemical synthesis always results in a racemic mixture of lactic acid (Ryu et al., 2003). The presence of L(+)-lactic acid with high optical purity gives polylactic acids of high melting point and high crystallinity (Lunt, 1998; Yun and Ryu, 2001).

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Lactic acid bacteria are traditionally fastidious microorganisms and have complex nutrient requirements due to their limited ability to biosynthesize B-vitamins and amino acids (Fitzpatrick and O'Keeffe, 2001). Refined sugars such as glucose or sucrose have been more frequently used to produce lactic acid than raw starchy substrates such as barley, corn, or wheat (Hofvendahl and Hahn-Hägerdal, 1997). Furthermore, a considerable amount of expensive complex nitrogen source, such as yeast extract, must be added to the medium to produce lactic acid in a reasonable time. However, these are economically unfavorable because pure sugars and pure complex nitrogen sources are expensive but lactic acid is a relatively cheap product. Therefore, raw materials for industrial lactic acid production need to have several characteristics such as low cost, low levels of contaminants, rapid fermentation rate, high lactic acid yields, little or no by-product formation, and year-round availability (Ryu et al., 2003). According to Tejayadi and Cheryan (1995), the cost for raw material possessed 68% of the total cost for lactic acid production from whey permeate and yeast extract using *Lactobacillus* (*Lb.*) *bulgaricus*. Åkerberg and Zacchi (2000) also previously reported, through the simulation study of lactic acid fermentation process, that the operational cost including raw material, neutralizing agent, hydrolyzing enzyme, and membrane for electrodialysis possessed approximately 80% of the total cost for lactic acid production from wheat flour. Since raw material cost cannot be reduced by scaling-up the process, starchy material and/or corn steep liquor have been considered as attractive nutrient sources for industrial lactic acid production.

We report here the lactic acid production from agricultural and renewable resources (such as barley, corn, and wheat) as cheap raw substrates without additional nutrients. We also evaluated the effect of pre-treatment methods of agricultural resource, which can release fermentable glucose, on lactic acid production. The addition of corn steep liquor (CSL) was investigated to improve the lactic acid fermentations. Furthermore, we investigated the effect of cell adaptation to CSL-based medium on lactic acid fermentations.

2. Methods

2.1. Strain and growth medium

Enterococcus faecalis RKY1 (Yun and Ryu, 2001; Ryu et al., 1999, 2001; Oh et al., 2003), a homofermentative L(+)-lactic acid producer, was utilized in this work. Stock cultures were maintained at −20 °C in 5 ml vials containing 50% (v/v) glycerol until used. Unless otherwise mentioned, the medium for cell growth contained the followings (g/l): glucose 30, yeast extract 10, and K_2HPO_4 5. The growth medium for adaptation culture to CSL-based medium was composed of 30 g/l of glucose, 10 g/l of CSL (solid basis), 3 g/l of yeast extract, and 5 g/l of K_2HPO_4. Yeast extract was obtained from Difco Laboratories (Detroit, MI), glucose and K_2HPO_4 were purchased from Yakuri Chemicals Co. (Tokyo, Japan), and CSL was kindly offered from TS Corporation Research and Development Center (Incheon, Korea). The pH was adjusted to 7.0 prior to sterilization at 121 °C for 15 min.

2.2. Enzymatic hydrolysis

The agricultural resources such as barley, wheat, and corn were kindly provided by TS Corporation Research and Development Center, and they were minutely grinded by milling process. Up to 250 g of milled flour was suspended in 800 ml of tap water and the pH of this suspension was adjusted to 6.0. Three hundred microgram of α-amylase, Termamyl 120L, Type LS (Novo Nordisk A/S, Bagsvaerd, Denmark), was added to the suspension and then the mixture was heated to optimal temperature for enzymatic liquefaction at 95 °C for 30 min. The liquefied solution was cooled to room temperature, and then the pH was re-adjusted to 4.5. After the temperature of the liquefied solution reached below 60 °C, 300 μg of glucoamylase, AMG 300L (Novo Nordisk A/S) was added. This saccharification step was aseptically performed on a sterilized 2 l Erlenmeyer flask at 55 °C and 200 rpm for 24 h. The saccharified solution was filtrated through 2 μm filter paper, which was then used for main fermentation medium. If necessary, some other additives such as CSL and/or yeast extract were added to the saccharified solution.

2.3. Inoculum preparation and batch fermentation

E. faecalis RKY1 cells from stock cultures were transferred to 15 ml sterile growth medium in a 20 ml vial and incubated at 38 °C for 10 h. A 0.6 ml of this culture was then transferred to a new growth medium (15 ml) in a 20 ml vial every 10 h. After 4–5 consecutive propagation steps, 3 ml of this culture was then transferred to a 50 ml vial that contained 40 ml growth medium. The inoculum was incubated at 38 °C for 6 h on a shaking incubator (KMC-8480SF, Vision Scientific Co., Daejeon, Korea) at 200 rpm before inoculation at 4% (v/v) to the fermenter. Batch fermentations were performed on a KF-2.5L fermenter (Kobiotech Co., Incheon, Korea) containing 1 l working volume and controlled at 38 °C and 200 rpm. The culture pH during batch fermentations was maintained at 7.0 by automatic addition of 10 N NaOH. The samples were aseptically withdrawn at desired intervals and were frozen at −20 °C for further analysis.