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Direct production of L(+)-lactic acid from starch and food wastes using *Lactobacillus manihotivorans* LMG18011

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Abstract

This study describes several essential factors for direct and effective lactic acid production from food wastes by *Lactobacillus manihotivorans* LMG18011, and optimum conditions for simultaneous saccharification and fermentation using soluble starch and food wastes as substrates. The productivity was found to be affected by three factors: (1) initial pH, which influenced amylase production for saccharification of starch, (2) culture pH control which influenced selective production of L(+)-lactic acid, and (3) manganese concentration in medium which improved in production rate and yield of lactic acid. The optimum initial pH was 5.0–5.5, and the fermentation pH for the direct and effective fermentation from starchy substrate was 5.0 based on the yield of L(+)-lactic acid. Under these conditions, 19.5 g L(+)-lactic acid was produced from 200 g food wastes by *L. manihotivorans* LMG18011. Furthermore, the addition of manganese stimulated the direct fermentation significantly, and enabled complete bioconversion within 100 h.

Keywords: *Lactobacillus manihotivorans*; Starch fermentation; Culture conditions; Amylase; L(+)-lactic acid production from food wastes

1. Introduction

Organic solid wastes, such as food wastes, are high in moisture and rich in carbon. Annual generation rate of organic wastes in Japan is around 19.4 million tons (Ministry of Environment, 2000). Most of it has been directly incinerated with other combustible wastes, and residual ash has been disposed in landfills. However, this intermediate treatment system has several problems; for example, combustion energy loss is caused by high moisture content of food wastes, and undesirable byproducts such as dioxin-related compounds are formed (Sakai et al., 2001). Since the remaining capacity of landfills is very limited, methods for recycling organic wastes are urgently required in Japan. Recently, several organic wastes such as sludge, food wastes and livestock excreta have been recycled by conversion to compost or biogas. Although these bioconversion processes have been effective for organic fraction of these various wastes, the balance of supply and demand for recycled products should be carefully evaluated. In Japan, it was pointed out that the recycling system depending on compost production only was unsuitable from the standpoint of material balances of nitrogen and phosphate in agricultural field (Mishima, 2002). Therefore new processes for converting organic wastes to more valuable recycled products are required.

Lactic acid is one of the useful compounds utilized in food, pharmaceutical and chemical industries. Stereoselective two isomers exist for having a chiral carbon. It can be polymerized to biodegradable plastics, i.e. poly

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lactic acid, which has great potential for replacing petrochemical plastics (Datta et al., 1995). In the polymerization process, the stereospecificity of lactic acid is very important, and selective production of stereospecific lactic acid has been carried out by lactic acid bacteria (LAB) or fungi, *Rhizopus oryzae* (Park et al., 1998; Tay and Yang, 2002). Agricultural products such as corn, potato and wheat containing large amounts of starchy substrate have been preferred as raw materials (Hofvendahl and Hahn-Hagerdal, 2000). However, supplies of these materials are import dependent in Japan, so it is expected that food wastes, which are supplied constantly at lower costs and are rich in carbohydrate, could be suitable renewable resources in substitution of agricultural products. The food wastes contain polysaccharides, such as starch, as well as various oligosaccharides. Sakai et al. (2004) reported L(+)-lactic acid production from food wastes pretreated by adding commercial glucoamylase. Considering that addition of commercial enzyme is required for every fermentation, simultaneous saccharification and fermentation using LAB strain, which produces amylase, has several advantages in operational cost and space than two-step fermentation using commercial enzyme treatment. Several amylolytic LAB, such as *Lactobacillus amylophilus* (Nakamura and Crowell, 1979; Yumoto and Ikeda, 1995), *Lactobacillus amylovorus* (Zhang and Cheryan, 1991) and *Lactobacillus plantarum* A6 (Giraud et al., 1994) can convert starch directly to lactic acid. Among these strains, only *L. amylophilus* was reported as L(+)-isomer producer, but its ability to produce lactic acid seemed to be lower than the other two strains. Yumoto and Ikeda (1995) reported that the production rate of *L. amylophilus* decreased with higher substrate concentration, and that it required more than 150 h to produce 30 g lactic acid from 50 g soluble starch. In the case of L(+)-lactic acid production from food wastes, which are generated daily and are difficult to pool owing to their putrefactive, it is suggested that new L(+)-isomer producer with a higher bioconversion rate is necessary to shorten processing time. *L. manihotivorans* was isolated during cassava sour starch fermentation and was reported as homofermentor that produces only L(+)-lactic acid (Morlon-Guyot et al., 1998). The strain LMG18010^T exhibits extracellular amylase activity, but the strain LMG18011 exhibits cell-linked amylase activity. However, there is little information on the fermentative kinetics of strain LMG18011.

In this study, we have characterized the chemical composition of several food waste samples to evaluate their suitability as raw materials for lactic acid production. To achieve more rapid and cost-effective L(+)-lactic acid production from food wastes, we have attempted direct fermentation using one of the amylolytic LAB, *L. manihotivorans* LMG18011. We have examined the influence of factors, the initial pH, culture pH, and minerals of medium, on productivity by this strain using starch as a model substrate. We have also applied these factors to direct L(+)-lactic acid production from food wastes by this strain, and discussed its yield and feasibility.

2. Methods

2.1. Chemical composition of food wastes used for experiment

The food waste samples from the cafeteria in the National Institute for Environmental Studies (NIES) were collected once a month, in January, May, June, July and September of year 2002, and analyzed according to methods described by Sugahara and Maekawa (2000). The moisture was determined by loss of weight after drying at 105 °C for 15 h, and the ash was determined after combustion at 600 °C for 2 h. Total carbon, nitrogen and sulfur contents were determined using a CHNS Elemental Analyzer vario EL III (Elementar Analyzensysteme GmbH, Hanau, Germany) after the samples were lyophilized in a LFD-600 (Laytant Life Science, Tokyo, Japan). The concentration of manganese and other metals were determined with an Agilent 7500 ICP-MS (Agilent Technology Inc., Palo Alto, CA, USA) after decomposition of organic matter by treatment with HCl and HNO_3.

The fraction extracted with hot 80% ethanol was prepared for quantification of oligosaccharides. The fraction extracted with 52% perchloric acid from non-extractive residue was used for quantification of starch. The concentration of total sugars in both fractions was determined using the phenol–sulfuric acid method (Dubois et al., 1956).

2.2. Bacterial strains, culture conditions and medium preparation

L. manihotivorans strain LMG18011 was purchased from BCCM™/LMG Bacteria Collection (Gent, Belgium) and preserved in 20% glycerol at −80 °C. This strain was cultivated at 30 °C in modified MRS medium (DeMan et al., 1960); 10 g tryptone, 5 g yeast extract, 10 g beef extract, 2 g KH_2PO_4, 2 g triammonium citrate, 0.02 g $MgSO_4 \cdot 7H_2O$, 0.05 g $MnSO_4 \cdot 4H_2O$, 1 mL Tween 80, and 10 g carbon source per litre. All fermentations were performed with shaking at 20 rpm under micro-aerobic conditions (Guyot and Morlon-Guyot, 2001). All chemicals were purchased from Wako Chemicals (Osaka, Japan) except starch from rice, which was purchased from Merck KgaA (Damstadt, Germany).

Fresh food wastes disposed from the cafeteria in NIES, which corresponded to September samples described in Section 2.1, were prepared by homogenizing