Bioresource Technology 146 (2013) 672–681

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

A novel production process for optically pure L-lactic acid from kitchen refuse using a bacterial consortium at high temperatures

Yukihiro Tashiro [a,b], Hiroko Matsumoto [a], Hirokuni Miyamoto [c], Yuki Okugawa [a], Poudel Pramod [a], Hisashi Miyamoto [d], Kenji Sakai [a,*]

[a] Laboratory of Soil Microbiology, Division of Applied Molecular Microbiology and Biomass Chemistry, Department of Bioscience and Biotechnology, Faculty of Agriculture, Graduate School, Kyushu University, 6-10-1 Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan
[b] Institute of Advanced Study, Kyushu University, 6-10-1 Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan
[c] Japan Eco-science Co. Ltd., 11-1 Shiomigaokacho, Chuo-ku, Chiba, Chiba 260-0034, Japan
[d] Miroku Co. Ltd., Iwaya 706-27, Kitsuki City, Oita 873-0021, Japan

HIGHLIGHTS

- We achieved selective L-lactic acid fermentation using MAR composts.
- The results of this study suggest the new academic concept of meta-fermentation.
- *Bacillus coagulans* was the main producer of L-lactic acid in meta-fermentation.

ARTICLE INFO

Article history:
Received 11 June 2013
Received in revised form 21 July 2013
Accepted 23 July 2013
Available online 29 July 2013

Keywords:
L-Lactic acid fermentation
Kitchen refuse
Bacterial consortium

ABSTRACT

We investigated L-lactic acid production in static batch fermentation of kitchen refuse using a bacterial consortium from marine-animal-resource (MAR) composts at temperatures ranging from 30 to 65 °C. At relatively low temperatures butyric acid accumulated, whereas at higher temperatures L-lactic acid was produced. In particular, fermentation at 50 °C produced 34.5 g L^{-1} L-lactic acid with 90% lactic acid selectivity and 100% optical purity. Denaturing gradient gel electrophoresis indicated that dominant bacteria present in the original MAR composts diminished rapidly and *Bacillus coagulans* strains became the dominant contributors to L-lactic acid production at 45, 50 and 55 °C. This is the first report of the achievement of 100% optical purity of L-lactic acid using a bacterial consortium.

1. Introduction

Food waste is generated by human activity worldwide, with the total amount estimated as 1.3 billion tons per year, according to the Annual report of FAO, 2011. One classification of food waste is by origin, i.e., household and food industries including food and drink manufacturing, distribution, retail, or eating facility (Parfitt et al., 2010). In Japan, ca. 70% of food waste (more than 2 million tons per year) is generated by food industries and has been reutilized in several ways including as compost, feed, and substrate for methane fermentation, as reported by the Ministry of Agriculture, Forestry and Fisheries of Japan. As for kitchen refuse (the food waste discarded by households), little is reutilized and most is incinerated or landfilled. Yet, kitchen refuse is an abundant and sustainable biomass resource and can be fermented by various microorganisms, with its high nutrient content promoting cell growth (Sakai et al., 2000). To date much effort has gone into the study of the fermentative production of various valuable products including ethanol (Ma et al., 2009), methane (Sasaki et al., 2009), and lactic acid (Sakai et al., 2006) from kitchen refuse.

Lactic acid is employed for various applications in several industries including the food, cosmetic, pharmaceutical, and chemical industries (Tashiro et al., 2011). In particular, attention has been focused on lactic acid as a raw material for polylactic acid (PLA) plastic over the past two decades, given that PLA is a recyclable biodegradable plastic material (Sakai et al., 2012). Highly optically pure lactic acid (OPLA) is required for the synthesis of high quality plastic, and poly-L-lactic acid (PLLA) and poly-D-lactic acid (PDLA) are produced from L-lactic acid and D-lactic acid, respectively (Abdel-Rahman et al., 2011). Recently, there has been great interest in a stereocomplex of PLLA and PDLA, owing to its higher melting point and faster biodegradability compared with that of their respective single polymers (Tashiro et al., 2011). These situations have encouraged OPLA production by microbial fermentation, given that some microorganisms produce OPLA, whereas

* Corresponding author. Tel./fax: +81 92 642 2861.
E-mail address: kensak@agr.kyushu-u.ac.jp (K. Sakai).

0960-8524/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biortech.2013.07.102

simple chemical production routes from petroleum generally yield racemic lactic acid. To date, many OPLA producing-bacteria have been reported, including lactic acid bacteria (Tashiro et al., 2011), *Bacillus* strain (Sakai and Yamanami, 2006), and genetically engineered *Escherichia coli* (Wang et al., 2012). In addition to these bacteria, fungi such as *Rhizopus oryzae* (Wu et al., 2011), and genetically engineered *Saccharomyces cerevisiae* (Ishida et al., 2005) have been reported to be OPLA producers.

Bacterial consortia have been utilized for production of fuels such as methane (Patra and Yu, 2012), butanol (Cheng et al., 2012), and ethanol plus hydrogen (Chu et al., 2012) using mixed microorganisms of sewage sludge as seeds. Marine-animal-resource (MAR) composts are produced from coffee-extracted residues of MAR such as small fishes, shrimps, and crabs by repeated fed-batch aerobic fermentation, where the fermentation temperature reaches ca. 75 °C without heating. A bacterial community structure analysis of MAR composts showed relatively stable bacterial community structure with several *Bacillus* strains (Niisawa et al., 2008). Because *Bacillus* strains are known to be OPLA producers, we expected that OPLA could be produced with this bacterial consortium of MAR compost as the seed. To date, there are no reports on OPLA production from kitchen refuse as the substrate by composts of bacterial consortia.

This study aimed to investigate the effects of temperatures on OPLA production from kitchen refuse using MAR compost as the seed, and to analyze the bacterial community structures of their fermentations. We were able to produce high L-lactic acid at an extremely high optical purity under thermal conditions. Denaturing gradient gel electrophoresis (DGGE) analysis revealed that *Bacillus coagulans* was the dominant strain contributing to OPLA production.

2. Methods

2.1. Seed of bacterial consortia

MAR compost was used as the source of bacterial consortia in this experiment. MAR compost was produced by Japan Eco-science Co. Ltd. (Chiba, Japan) from several materials including coffee-extracted residues and MAR such as small fishes, shrimps, and crabs. MAR compost production was performed in repeated fed-batch fermentations with three successive aerobic bioreactors at high temperature (max. 75 °C) without heating (Niisawa et al., 2008). A previous study indicated that bacteria belonging to the Firmicute phylum were dominant in MAR compost (Niisawa et al., 2008). One gram of MAR compost was suspended in 9 mL of sterilized water and used as a seed.

2.2. Media

Model kitchen refuse (MKR) medium was used for the fermentation experiments as follows, and contained the following compounds per liter of tap water: vegetables (66.7 g of carrot peel, 66.7 g of cabbage, and 66.7 g of potato peel), fruit (50 g of banana peel, 50 g of apple peel, and 50 g of orange peel), 70 g of baked fish, 50 g of rice, and 30 g of used tea leaves. After homogenization of the above materials, the medium was sterilized at 121 °C for 15 min. Industrial glucozyme (#200000, Nagase ChemteX, Osaka, Japan) was added to the sterilized MKR medium at 300 ppm and saccharification was performed at 50 °C for 2 h at pH 5.8. After saccharification, the pH of the MKR medium was adjusted to 7.0 with 10% ammonia solution, and thereafter, we directly used it as the fermentation medium without separation of the solid fraction. In this study, although the moisture content in each of the food compounds was not adjusted, the prepared MKR medium exhibited an ca. 80% moisture content in our preliminary experiments, and this value was quite similar to the ca. 79% moisture content in prepared food wastes generated by the food industry (Sakai et al., 2004).

2.3. Batch fermentation in MKR medium

One milliliter of seed was inoculated into 30 mL of MKR medium in a 50-mL plastic tube, and batch fermentation was performed statically at temperatures of 30, 37, 40, 45, 50, 55, 60, and 65 °C. The pH was adjusted every 24 h to 7.0 using 10% ammonia solution. Samples were periodically withdrawn for analysis of products, substrate, and bacterial community structure.

2.4. Analytical procedures

Organic acids (lactic acid, acetic acid, butyric acid, propionic acid, and formic acid) in the broth supernatant were determined using a specific HPLC system (Organic Acid Analyzer; Shimadzu, Kyoto) equipped with an ion-exclusion column (Shim-pack SCR-102H; Shimadzu, Japan) at 40 °C and with an electric conductivity detector (CDD-10AVP; Shimadzu). The mobile phases A (5 mM ρ-toluenesulfonic acid) and B (20 mM bis(2-hydroxyethyl)iminotris(hydroxymethyl)methane, 5 mM ρ-toluenesulfonic acid, 100 μM EDTA·2Na) were used, each at a flow rate of 0.8 mL min^{-1}. D-Lactic acid and L-lactic acids were measured with HPLC (Organic Acid Analyzer, Shimadzu) equipped with a MCI GEL CRS 10 W (Shimadzu) at 25 °C, with 2 mM $CuSO_4$ as the mobile phase (0.5 mL/min flow rate). Total hexose concentration was quantified by the phenol–sulfuric acid method at 490 nm (Dubois et al., 1956).

2.5. Calculations

The optical purity of L-lactic acid ($OP_{L\text{-}LA}$) was calculated as follows:

$$OP_{L\text{-}LA}(\%) = (C_{L\text{-}LA} - C_{D\text{-}LA}) \times 100/(C_{L\text{-}LA} + C_{D\text{-}LA})$$

where $C_{L\text{-}LA}$ and $C_{D\text{-}LA}$ are the respective concentrations (g L^{-1}) of L- and D-lactic acid produced.

Selectivity for lactic acid (S_{LA}) and selectivity for butyric acid (S_{BA}) were calculated as follows:

$$S_{LA}(\%) = (C_{L\text{-}LA} + C_{D\text{-}LA}) \times 100/(C_{L\text{-}LA} + C_{D\text{-}LA} + C_{AA} + C_{BA} + C_{PA} + C_{FA})$$

$$S_{BA}(\%) = C_{BA} \times 100/(C_{L\text{-}LA} + C_{D\text{-}LA} + C_{AA} + C_{BA} + C_{PA} + C_{FA})$$

where C_{AA}, C_{BA}, C_{PA}, and C_{FA} are the respective concentrations (g L^{-1}) of acetic acid, butyric acid, propionic acid, and formic acid produced.

2.6. DNA extraction and PCR amplification of partial 16S rRNA gene

Total DNA was isolated with a PowerSoil™ DNA Isolation Kit (MO BIO Laboratories, CA, USA) from centrifugation pellets of 1-mL fermentation broth according to the manufacturer's instructions. The partial 16S rRNA gene (V3 region) was amplified from the extracted DNA with universal primer pairs for eubacteria 357f-GC (5′-CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGGCCTACGGGAGGCAGCAG-3′) and 518r (5′-ATTACCGCGGCTGCTGGGCAC-3′) in a total volume of 50 μL containing 1.25 U of Ex Taq DNA polymerase (Takara Bio, Shiga, Japan), 0.2 mM dNTPs, 0.8 μM both primers, and ca. 0.2 ng/μL DNA template. The PCR cycling conditions were as follows: 94 °C for 3 min, 52 °C for 1 min, 72 °C for 1 min, followed by 30 cycles, and final extension at 72 °C for 10 min. PCR products were purified using the QIAquick PCR purification kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions.