Appl Biochem Biotechnol (2016) 178:285–293
DOI 10.1007/s12010-015-1871-0

Production of Optically Pure D-Lactic Acid by the Combined use of *Weissella* sp. S26 and *Bacillus* sp. ADS3

Qingxin Li[1] · **Mohammad Sufian Bin Hudari**[1] · **Jin Chuan Wu**[1]

Received: 4 August 2015 / Accepted: 23 September 2015 /
Published online: 30 September 2015
© Springer Science+Business Media New York 2015

Abstract Optically pure D-lactic acid was produced from glucose, xylose, or starch by the combined use of *Weissella* sp. S26 and *Bacillus* sp. ADS3, two native bacterial strains isolated from Singapore environment. *Weissella* sp. S26 was used to ferment various sugars to lactic acid rich in D-isomer followed by sterilization of the broth and inoculation of *Bacillus* sp. ADS3 cells to selectively degrade acetic acid (if any) and L-lactic acid. In a simultaneous saccharification and fermentation of starch by *Weissella* sp. S26 in 1 L of modified MRS medium containing 50 g/L starch at 30 °C, lactic acid reached 24.2 g/L (23.6 g/L of D-isomers and 0.6 g/L of L-isomers), and acetic acid was 11.8 g/L at 37 h. The fermentation broth was sterilized at 100 °C for 20 min and cooled down to 30 °C followed by inoculation of *Bacillus* sp. ADS3 (10 %, *v*/*v*), and the mixture was kept at 30 °C for 115 h. Acetic acid was completely removed, and L-lactic acid was largely removed giving an optical purity of D-lactic acid as high as 99.5 %.

Keywords Lactic acid · Fermentation · Bioprocessing · Acetic acid

Introduction

Lactic acid is widely used in food, feed, cosmetic, and pharmaceutical industries [2, 19] as well as in producing poly lactic acid (PLA), one of the most promising biodegradable polymers [15]. PLA can occur as poly (L-lactic acid) (PLLA), poly (D-lactic acid) (PDLA) or a stereocomplex of PLLA and PDLA which has significantly improved mechanical performances, thermal resistance, and hydrolysis resistance compared to PLLA or PDLA [12, 15].

✉ Jin Chuan Wu
wu_jinchuan@ices.a-star.edu.sg

[1] Institute of Chemical and Engineering Sciences, Agency for Science, Technology and Research (A*STAR), 1 Pesek Road, Jurong Island, Singapore 627833, Singapore

The demand for high optical purity of D-lactic acid is increasing due to its potential applications in making thermostable PLA. Therefore, it is of importance to produce optically pure D-lactic acid in a cost-effective way.

Optically pure lactic acids required for making PLA can be readily produced by microbial fermentation using different carbon sources [8, 14]. High optical purity of L-lactic acid has been commercially produced by microbial fermentation [8]. During microbial production of D-lactic acid, usually small amount of L-lactic acid is present. It has been reported that the optical purities of D-lactic acid were lower than 98.3 % using bacteria such as *E. coli* and *Sporolactobacillus* sp. [17, 18, 21]. It is thus important to obtain D-lactic acid with very high purity because the presence of a small amount of L-lactic acid would severely affect its commercial applications. For example, the optical purity of lactic acid is a crucial parameter for controlling the physical and thermochemical properties as well as the biodegradation rate of PLA [15].

Separation of a small amount of L-lactic acid from D-lactic acid in a cost-effective way is very difficult using the conventional methods such as chromatography and electrophoresis [6, 10]. It has been reported that mutagenesis of the strains can improve the optical purity of D-lactic acid. High yield of D-lactic acid with high optical purity can be obtained from a genetically modified strain under oxygen deprivation conditions [9]. If D-lactic acid or its polymer is to be used in food or personal care industries, microbes used for producing D-lactic acid might need to be carefully selected. Usually, non-GMO strains are preferred for the D-lactic acid production. Therefore, it is useful to explore an alternative way to provide D-lactic acid with high optical purity. Studies have shown that lactic acids can be produced by bacteria using diverse carbon resources [20]. It would be useful if bacterial stains can use economic carbon sources such as lignocelluloses and starch. If the carbon sources are from lignocellulose, acetic acid is unavoidably generated during the pre-treatment as a result of hydrolysis of the highly acetylated hemicellulose [5, 7]. In this case, the removal of acetic acid from lactic acid products in a cost-effective way is also very important.

Here, we report a method for producing optically pure D-lactic acid by the combined use of two native bacterial strains that we isolated from the natural environment in Singapore. An additional advantage is that acetic acid, which is usually produced in case of hetero-fermentation or unavoidably existed in lignocellulose hydrolysate, can also be efficiently removed by this method.

Materials and Methods

Microorganisms and Culture Media

Bacillus sp. ADS3 and *Weissella* sp. S26 were isolated from the soil samples collected in Singapore. Soil samples were mixed with autoclaved water. Water was then plated on LB plates. Colonies were inoculated into 2 mL of LB medium and cultured at 37 °C for 12 h. Any colony that can reduce the pH of the medium was further purified. Among these screened bacteria, two of them showed the ability to produce lactic acids, and they were identified as *Weissella* sp. and *Bacillus* sp., respectively, using the conventional bacterial identification methods. *Weissella* sp. S26 is capable of producing lactic acid rich in D-isomer (i.e., 95 %). *Bacillus* sp. ADS3 is able to preferentially digest acetic acid and L-lactic acid in the mixture of acetic acid, L- and D-lactic acids.

The agar plates for cultivating *Bacillus* sp. ADS3 contained (per liter) 20 g sodium acetate, 5.0 g $(NH_4)_2SO_4$, 5 g KNO_3, 2.0 g NaH_2PO_4, 0.1 g $MgSO_4 \cdot 7H_2O$, 0.1 g $MnSO_4 \cdot 4H_2O$, 0.1 g

$FeSO_4 \cdot 7H_2O$, 1.0 g yeast extract (BD), and 20.0 g agar (BD), with pH 7.0. The same medium containing no agar was used for cultivating *Bacillus* sp. ADS3 in submerged cultures. *Bacillus* sp. ADS3 was cultivated in 1-L flasks at 30 °C and 150 rpm.

The modified MRS medium was used for preparing seed culture of *Weissella* sp. S26. It contained (per liter) 10.0 g peptone, 8.0 g meat extract, 4.0 g yeast extract (BD), 20.0 g glucose (or xylose), 2.1 g sodium chloride, 1.0 g Tween 80, 2.0 g K_2HPO_4, 2.0 g triammonium citrate, 0.2 g $MgSO_4 \cdot 7H_2O$, and 0.05 g $MnSO_4 \cdot 4H_2O$, with pH 6.2.

Three media were used for producing D-lactic acid by *Weissella* sp. S26. The first one was glucose or xylose medium containing (1 L) 20.0 g or 50.0 g of glucose or xylose, 10.0 g peptone, 4.0 g yeast extract, and 2.0 g triammonium citrate, with pH 6.6. The second medium was starch-MRC medium containing (1 L) 10.0 g peptone, 8.0 g meat extract, 4.0 g yeast extract, 1.0 g Tween 80, 2.0 g K_2HPO_4, 2.0 g triammonium citrate, 0.2 g $MgSO_4 \cdot 7H_2O$, 0.05 g $MnSO_4 \cdot 4H_2O$, 2.1 g NaCl, and 50 g starch, with pH 6.0. The third medium was the same with the second one except that the tapioca starch (Ng Nam Bee Marketing Pte. Ltd, Thailand) was processed before adding to the mixture by hydrolyzing 50 g of starch in 500 mL of sterilized water using 0.5 mL of α-amylase (liquozyme, Novozymes) and 5 mL of glucoamylase (Spirizyme, Novozymes) at 85 °C for 2 h.

Production of D-Lactic Acid-Rich Product by *Weissella* sp. S26

Weissella sp. S26 seed was prepared by inoculating *Weissella* sp. S26 into 100 mL MRS medium in 500 mL flasks followed by shaking at 37 °C for 28 h. The mixture was centrifuged at 8000×*g* for 15 min, and the pellets were collected, re-suspended, and inoculated into a 2-L fermenter containing 1 L medium. When the second medium was used, 0.5 mL of liquozyme and 5 mL of Spirizyme were added into the fermenter together with the seed inoculum. The fermentation was carried out at 37 °C, 200 rpm with a N_2 flow rate of 0.2 vvm. The pH was maintained at 6.0 by automatic addition of 5.0 M NaOH or 10.0 M HCl during the fermentation. Samples were regularly collected for analysis during fermentation. After completion of the fermentation, the broth was heated at 100 °C for 20 min to kill the bacterial cells, and the pH was then adjusted to 8.0 with 5.0 M NaOH. The resulting product was used for further treatment by *Bacillus* sp. ADS3 to remove acetic acid and L-lactic acid.

Preparation of *Bacillus* sp. ADS3 Cells

Five to ten colonies of *Bacillus* sp. ADS3 were picked up from the agar plate and inoculated into 20 mL of liquid medium which was then incubated at 37 °C, 200 rpm overnight. The culture was transferred into a 200-mL medium and incubated at 37 °C, 200 rpm overnight. The culture was collected by centrifugation at 8000×*g* for 15 min. The cell pellets were ready for use in the subsequent steps for removing acetic acid and L-lactic acid.

Removal of L-Lactic Acid from the Fermentation Broth Rich in D-Lactic Acid by *Bacillus* sp. S3

Bacillus sp. ADS3 pellets from the 200-mL culture were added into 1 L of sterilized *Weissella* sp. S26 fermentation broth. The mixture was incubated at 30 °C, 150 rpm for several days. Samples were collected regularly for analysis by HPLC.

Degradation of Pure Acetic Acid or L-Lactic Acid by *Bacillus* sp. ADS3

To investigate the mechanism of acetic acid and L-lactic acid removal by *Bacillus* sp. ADS3, 100 mL of acetic acid (5 g/L) or L-lactic acid (5 g/L) whose pH was adjusted to 8.0 by 5 M NaOH was added into 200 g of wet *Bacillus* sp. ADS3 cells which were produced by cultivating in 200 mL of liquid medium for 16 h followed by centrifugation at 8000×*g* for 15 min. The mixture was stirred at 30 °C, 200 rpm continuously, and liquid samples were taken regularly for HPLC analysis. After completion of the conversion, the cells were harvested by centrifugation, and production of polyhydroxyalkanoates (PHAs) was analyzed. The supernatant was analyzed by LC/MS to detect the nominal masses of metabolites produced during the acetic acid or lactic acid digestion.

Analytical Methods

The optical purity of lactic acid was detected by HPLC (LC-10AT, refractive index detector SPD-10A, Shimadzu, Kyoto, Japan) with a Mitsubishi MCI® Gel CRS15W column (50× 4.6 mm, Mitsubishi Chemicals, Tokyo, Japan) at 30 °C using 2 mM of $CuSO_4$ as the mobile phase at 0.4 mL/min. The optical purity of D-lactic acid was calculated based on the following equation [20]:

$$\text{Optical purity} = \frac{\text{D-lactic acid} - \text{L-lactic acid}}{\text{D-lactic acid} + \text{L-lactic acid}} \times 100\%$$

The concentrations of xylose, glucose, and acetic acid were analyzed by a Shimadzu HPLC system equipped with a Bio-Rad Aminex HPX-87H column (300×7.8 mm, Bio-Rad, Herculse, CA, USA). A standard curve was first plotted using pure xylose, glucose, or acetic acid. Samples during fermentation were first cleared by centrifugation at 8000×*g*, 4 °C for 10 min before being injected to the column for analysis.

Results

Production of D-Lactic Acid from Glucose by Combined use of *Weissella* sp. S26 and *Bacillus* sp. ADS3

Weissella sp. S26 can produce lactic acid rich in D-isomer from various sugars. As the types and concentrations of sugars may affect the modes of lactic acid fermentation, we tested the lactic acid fermentation using five different media containing 2 % glucose, 5 % glucose, raw starch, starch hydrolysate, and 2 % of xylose, respectively.

When 2 % of glucose was used for the lactic acid production by *Weissella* sp. S26, the final lactic acid titer reached 15 g/L at 20 h. Acetic acid was also detected at 1.3 g/L (Fig. 1a). After adding *Bacillus* sp. ADS3 cells into the sterilized fermentation broth, both acetic acid and L-lactic acid started to be degraded (Fig. 1b). Acetic acid was completely degraded within 45 h when the D-lactic acid optical purity reached 96.6 % (Fig. 1b). When glucose (5 %) was used for the fermentation (Fig. 2), the lactic acid titer reached 31.1 g/L at 26 h. Acetic acid was completely removed at 60 h when the D-lactic acid optical purity reached 98.2 % (Fig. 2b).

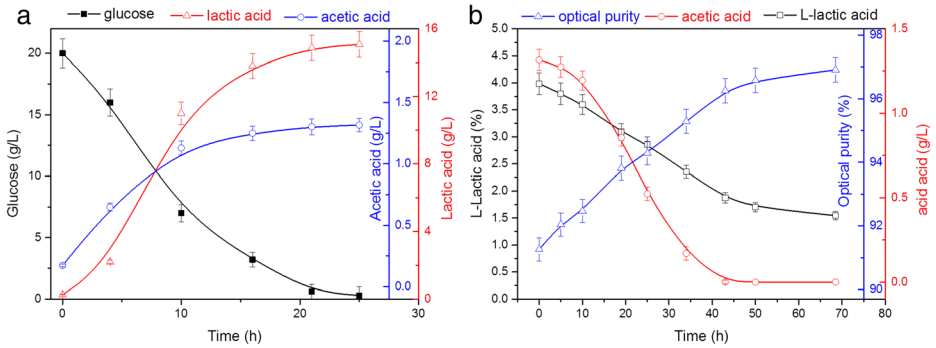

Fig. 1 Time courses of lactic acid production by *Weissella* sp. S26 (**a**) and removal of L-lactic acid and acetic acid from the fermentation broth by *Bacillus* sp. ADS3 (**b**). After fermentation, the broth was sterilized at 100 °C for 20 min and cooled down to 30 °C before adding *Bacillus* sp. ADS3 cells

Production of D-Lactic Acid from Starch by Combined use of *Weissella* sp. S26 and *Bacillus* sp. ADS3

Starch was used as a cheaper carbon source to produce lactic acid in two cases, simultaneous saccharification and fermentation and saccharification followed by fermentation.

For the simultaneous saccharification and fermentation, the starch was added into the medium together with liquozyme and Spirizyme to start the process. The lactic acid titer reached 24.2 g/L at 37 h (Fig. 3a). Longer fermentation time did not improve the lactic acid production, which might be attributed to the fact that the cell density reached the maximum at which point bacterial cells do not produce lactic acid. The acetic acid concentration reached 11.8 g/L at 67 h (Fig. 3a). After sterilization, the addition of *Bacillus* sp. ADS3 cells completely removed the acetic acid within 115 h (Fig. 3b) when the D-lactic acid optical purity reached up to 99.5 % (Fig. 3b).

In the case of starch saccharification followed by fermentation, the lactic acid titer reached 24 g/L at 23 h, while the acetic acid concentration continuously increased and reached 5.8 g/L at 45 h (Fig. 4a). After sterilization, the addition of *Bacillus* sp. ADS3 cells completely removed the acetic acid at 90 h (Fig. 4b) when the optical purity of D-lactic acid reached up to 99.5 %.

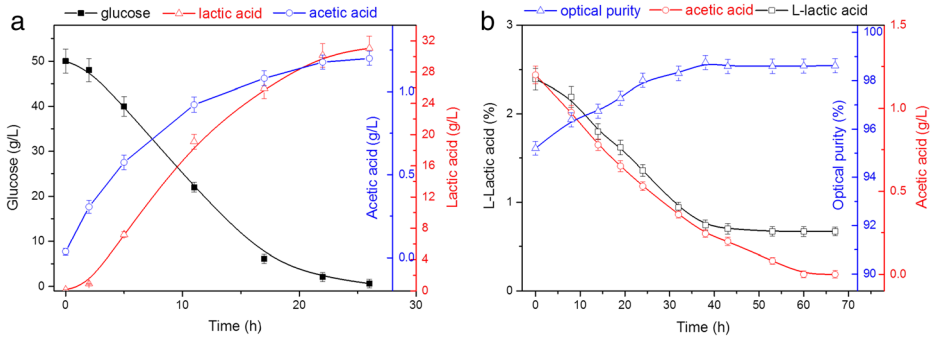

Fig. 2 Time courses of D-lactic acid production by *Weissella* sp. S26 using 5 % glucose (**a**) and removal of L-lactic acid from the fermentation broth by *Bacillus* sp. ADS3 (**b**). After fermentation, the broth was sterilized at 100 °C for 20 min and cooled down to 30 °C before adding *Bacillus* sp. ADS3 cells

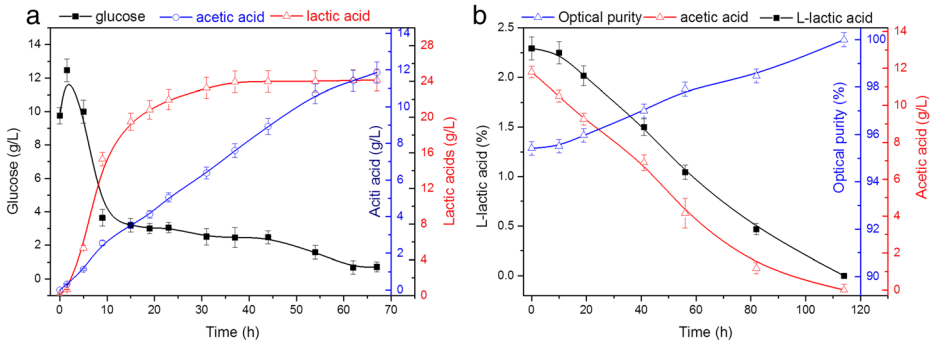

Fig. 3 Time courses of D-lactic acid production by *Weissella* sp. S26 in simultaneous saccharification and fermentation of starch (**a**) and removal of L-lactic acid and acetic acid from the fermentation broth by *Bacillus* sp. ADS3 (**b**). After fermentation, the broth was sterilized at 100 °C for 20 min and cooled down to 30 °C before adding *Bacillus* sp. ADS3 cells

Production of D-Lactic Acid from Xylose by Combined use of *Weissella* sp. S26 and *Bacillus* sp. ADS3

Xylose is a potential carbon source for industrial production of lactic acid as it is rich in lignocellulose. When 2 % of xylose was used, the lactic acid concentration reached 13.7 g/L at 48 h with 13.2 g/L of D-lactic acid and 8.5 g/L of acetic acid (Fig. 5a). The broth was sterilized and treated with *Bacillus* sp. ADS3 cells. The acetic acid was completely removed at 120 h when the optical purity of D-lactic acid reached 98.5 % (Fig. 5b).

Discussion

D-lactic acid has not yet been commercially produced, although its market demand is increasing due to the production of thermostable PLA. A lot of researches have been reported for D-lactic acid production, but almost all of them used genetically modified strains. In many cases, a small amount of L-lactic acid is still contained in the D-lactic acid product after the fermentation. The removal of this small amount of L-lactic acid from D-lactic acid in a cost-

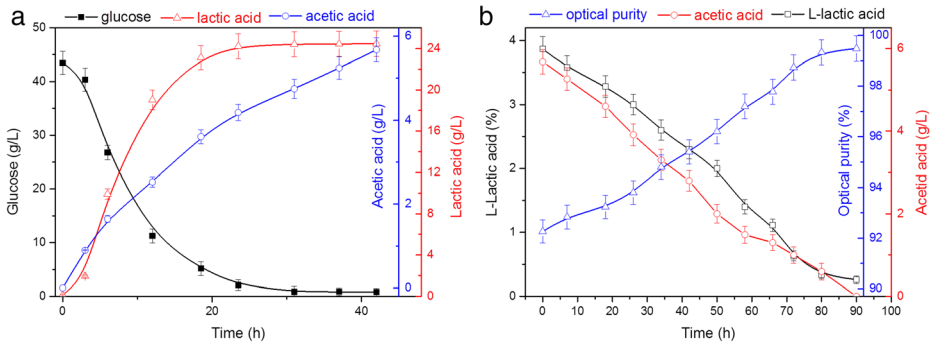

Fig. 4 Time courses of D-lactic acid production by *Weissella* sp. S26 in saccharification followed by fermentation of starch (**a**) and removal of L-lactic acid and acetic acid from the fermentation broth by *Bacillus* sp. ADS3 (**b**). After fermentation, the broth was sterilized at 100 °C for 20 min and cooled down to 30 °C before adding *Bacillus* sp. ADS3 cells

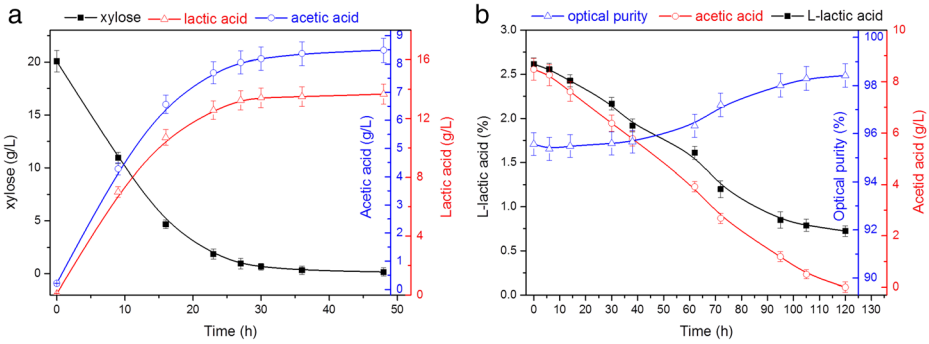

Fig. 5 Time courses of D-lactic acid production from xylose by *Weissella* sp. S26 (**a**) and removal of L-lactic acid and acetic acid from the fermentation broth by *Bacillus* sp. ADS3 (**b**). After fermentation, the broth was sterilized at 100 °C for 20 min and cooled down to 30 °C before adding *Bacillus* sp. ADS3 cells

effective way is very difficult [6, 10]. Genetically modified *Corynebacterium glutamicum* was shown to be able to produce D-lactic acid in a minimal medium in 30 h, which showed potential application of such strains [9]. In this study, we show a method that can produce D-lactic acid using economic carbon sources and non-genetically modified bacteria. This method will be useful if the D-lactic acid or its product is used in food industry or using economic carbon resources as a carbon source for fermentation. Our method can not only remove L-lactic acid from D-lactic acid, but also simultaneously remove acetic acid from the lactic acid product. The contamination of lactic acid product by acetic acid is usually unavoidable especially when the sugars are from lignocellulose hydrolysate in which acetic acid is inevitably existed as a result of the hydrolysis of the acetylated hemicellulose. It has been noted that the processing time is long in the current study especially since economic carbon sources are used (Fig. 2). Recycling of bacterial cells may be a way to reduce the operation cost. This method is advantageous in terms of its simple operation and simultaneous removal of acetic acid and L-lactic acid. Although it needs some time in the step of L-lactic acid removal, no special equipment is required. *Bacillus* sp ADS3 can also be used to process the fermentation product of other D-lactic acid-producing bacteria. It is expected to be a cost-effective and commercially competitive method for producing D-lactic acid from various sugars especially when the sugars are from lignocellulose where the removal of acetic acid is essential. The D-lactic acid produced by the combined use of two native strains is also very suitable for applications where non-GMO products are needed, such as in food and personal care industries.

It is worth mentioning that although *Bacillus* sp. ADS3 is capable of digesting both acetic acid and lactic acid, it preferentially digests acetic acid if both are available. For the mixture of L- and D-lactic acids, it preferentially digests L-lactic acid than D-lactic acid, possibly due to the less toxicity of L-lactic acid than of D-lactic acid [11]. Therefore, stopping the digestion reactions at a right time is crucial for getting high purity of lactic acid free of acetic acid or high optical purity of D-lactic acid free of L-lactic acid.

Regarding the mechanism of acetic acid and L-lactic acid removal by *Bacillus* sp. ADS3, we preliminarily presumed that polyhydroxyalkanoates (PHAs) might be enriched in the cell biomass, which are usually accumulated by some bacteria as an intracellular energy reserve under nutrient-starvation conditions or even under nutrient non-limiting conditions by some bacteria such as *Ralstonia eutropha* and *Alcaligenes latus* [13, 17]. It has also been reported that some organic acids such as acetic acid, propionic acid, and lactic acid can be used by

PHA-producing strains for PHA synthesis [16]. Some *Bacillus* species such as *Bacillus thuringiensis* R1 [3], *Bacillus megaterium* [4], *Bacillus subtilis*, *Bacillus firmus*, *Bacillus sphaericus*, and *Bacillus pumilus* [1] have been reported to accumulate PHAs in various amounts. However, after analyzing the compositions of the cell biomass, we could not detect any production of PHAs in both cases of pure acetic acid and pure L-lactic acid degradation by *Bacillus* sp. ADS3. However, we did detect the production of larger amounts of HCO_3^- in the liquid phases by LC/MS analysis (data not shown). Therefore, it is highly possible that both acetic acid and lactic acid are converted to CO_2 by *Bacillus* sp. ADS3. Further investigation of the mechanism is still needed to have a better understanding of the biological process.

In summary, we show that *Weissella* sp. S26 can produce lactic acid using various carbon sources. D-lactic acid with high optical purity can be obtained by combined use of *Weissella* sp. S26 and *Bacillus* sp. ADS3. Our study provides an economic way to produce high quality D-lactic acid.

Acknowledgments This work was supported by the Science and Engineering Research Council (SERC) of the Agency for Science, Technology and Research (A*STAR), Singapore, under the Biomass to Chemicals Program (SERC Grants No. 1124004027 and No. 1326004117). Thanks are given to Ms. Ooi Kim Yng for her help in PHA analyses.

References

1. Belma, A., Zehra, N.Y., & B.Y., (2002) Determination of PHB growth quantities of certain Bacillus species isolated from soil. *Turkish Electric J Biotechnol* Special issue.
2. Connolly, E. (2013). D(−)-lactic acid-producing bacteria safe to use in infant formulas. *Natrafoods, 3*, 37–49.
3. Desetty, R., Mahajan, V., Khan, B. M., & Rawal, S. (2008). Isolation and heterologous expression of PHA synthesising genes from bacillus thuringiensis R1. *World Journal of Microbiology and Biotechnology, 24*, 1769–1774.
4. Gouda, M. K., Swellam, A. E., & Omar, S. H. (2001). Production of PHB by a bacillus megaterium strain using sugarcane molasses and corn steep liquor as sole carbon and nitrogen sources. *Microbiological Research, 156*, 201–207.
5. Ismail, K. S., Sakamoto, T., Hasunuma, T., Zhao, X. Q., & Kondo, A. (2014). Zinc, magnesium, and calcium ion supplementation confers tolerance to acetic acid stress in industrial saccharomyces cerevisiae utilizing xylose. *Biotechnology Journal, 9*, 1519–1525.
6. Kodama, S., Yamamoto, A., Matsunaga, A., Soga, T., & Minoura, K. (2000). Direct chiral resolution of lactic acid in food products by capillary electrophoresis. *Journal of Chromatography A, 875*, 371–377.
7. Li, Q., Ng, W. T., Puah, S. M., Bhaskar, R. V., Soh, L. S., Macbeath, C., Parakattil, P., Green, P., & Wu, J. C. (2013). Efficient production of fermentable sugars from oil palm empty fruit bunch by combined use of acid and whole cell culture-catalyzed hydrolyses. *Biotechnology and Applied Biochemistry*. doi:10.1002/bab.1188.
8. Ma, K., Maeda, T., You, H., & Shirai, Y. (2014). Open fermentative production of l-lactic acid with high optical purity by thermophilic Bacillus coagulans using excess sludge as nutrient. *Bioresource Technology, 151*, 28–35.
9. Okino, S., Suda, M., Fujikura, K., Inui, M., & Yukawa, H. (2008). Production of D-lactic acid by Corynebacterium glutamicum under oxygen deprivation. *Applied Microbiology and Biotechnology, 78*, 449–454.
10. Paik, M. J., Nguyen, D. T., Yoon, J., Chae, H. S., Kim, K. R., Lee, G., & Lee, P. C. (2011). Chiral separation of lactic acid in culture media and cells of Lactobacillus delbrueckii subsp. lactis as O-pentafluoropropionylated. *Nihon Geka Gakkai Zasshi, 32*, 2418–2422.
11. Papagaroufalis, K., Fotiou, A., Egli, D., Tran, L. A., & Steenhout, P. (2014). A randomized double blind controlled safety trial evaluating d-lactic acid production in healthy infants Fed a lactobacillus reuteri-containing formula. *Nutrition and Metabolism Insights, 7*, 19–27.
12. Sun, J., Wang, Y., Wu, B., Bai, Z., & He, B. (2015). Enhanced production of d-lactic acid by Sporolactobacillus sp.Y2-8 mutant generated by atmospheric and room temperature plasma. *Biotechnology and Applied Biochemistry, 62*, 287–292.

13. Suwannasing, W., Imai, T., & Kaewkannetra, P. (2015). Cost-effective defined medium for the production of polyhydroxyalkanoates using agricultural raw materials. *Bioresource Technology, 194*, 67–74.
14. Tongpim, S., Meidong, R., Poudel, P., Yoshino, S., Okugawa, Y., Tashiro, Y., Taniguchi, M., & Sakai, K. (2014). Isolation of thermophilic l-lactic acid producing bacteria showing homo-fermentative manner under high aeration condition. *Journal of Bioscience and Bioengineering, 117*, 318–324.
15. Tsuji, H. (2005). Poly(lactide) stereocomplexes: formation, structure, properties, degradation, and applications. *Macromolecular Bioscience, 5*, 569–597.
16. Wang, J., & Yu, J. (2001). Kinetic analysis on formation of poly(3-hydroxybutyrate) from acetic acid by Ralstonia eutropha under chemically defined conditions. *Journal of Industrial Microbiology & Biotechnology, 26*, 121–126.
17. Wang, L., Zhao, B., Li, F., Xu, K., Ma, C., Tao, F., Li, Q., & Xu, P. (2011). Highly efficient production of D-lactate by Sporolactobacillus sp. CASD with simultaneous enzymatic hydrolysis of peanut meal. *Applied Microbiology and Biotechnology, 89*, 1009–1017.
18. Wang, Y., Tian, T., Zhao, J., Wang, J., Yan, T., Xu, L., Liu, Z., Garza, E., Iverson, A., Manow, R., Finan, C., & Zhou, S. (2012). Homofermentative production of D-lactic acid from sucrose by a metabolically engineered Escherichia coli. *Biotechnology Letters, 34*, 2069–2075.
19. Wee, Y. J., Kim, J. K., & Ryul, H. (2006). Biotechnological production of lactic acid and its recent applications. *Food Technology and Biotechnology, 44*, 163–172.
20. Ye, L., Hudari, M. S., Zhou, X., Zhang, D., Li, Z., & Wu, J. C. (2013). Conversion of acid hydrolysate of oil palm empty fruit bunch to L-lactic acid by newly isolated Bacillus coagulans JI12. *Applied Microbiology and Biotechnology, 97*, 4831–4838.
21. Yoshida, S., Okano, K., Tanaka, T., Ogino, C., & Kondo, A. (2011). Homo-D-lactic acid production from mixed sugars using xylose-assimilating operon-integrated lactobacillus plantarum. *Applied Microbiology and Biotechnology, 92*, 67–76.