Biotransformation of 3-Hydroxypropionaldehyde from Glycerol by Isolated Lactic Acid Bacteria

Preeyawan PHALIWONG[1], Mallika BOONMEE[2,3] and KorakochHAMSUPO[2,3,*]

[1]*Graduate School, KhonKaen University, KhonKaen 40002, Thailand*
[2]*Department of Biotechnology, Faculty of Technology, KhonKaen University, KhonKaen40002, Thailand*
[3]*Fermentation Research Center for Value Added Agricultural Products,Faculty of Technology, KhonKaen University, KhonKaen40002, Thailand*

([*]Corresponding author: korham@kku.ac.th)

Abstract

3-Hydroxypropionaldehyde (3-HPA) is a small chemical molecule which can be used as an antimicrobial substance and intermediate for the synthesis of acrylic acid. 3-HPA can be generated through the biotransformation of glycerol by glycerol dehydratase. Somestrains of lactic acid bacteria have glycerol dehydratase for 3-HPA production. Therefore, the biotransformation of glycerol to 3-HPA by isolated lactic acid bacteria from piglet intestine was investigated. This study used *Lactobacillu*s sp. LAB100 and *Lactobacillus* sp. LAB001 as cells for 3-HPA production by comparison with the reference strain, *Lactobacillus reuteri*DSM20016. A 30% glycerol solution (10 ml) was transformed to 3-HPA at 37°C for 24 h by cell producers (stationary phase). The 3-HPA concentration and antimicrobial activity were then determined. Results showed that *Lactobacillus* sp. LAB100, *Lactobacillus* sp. LAB001, and *L. reuteri* DSM20016 gave 3-HPA concentrations of 64.5, 58.2, and 73.7 g/l, respectively. The two isolates (LAB100 and LAB001) displayed antimicrobial activity against *Salmonella typhimurium* TISTR292, with similar activity toward the reference strain *L. reuteri* DSM 20016 (1600 AU/ml).

Keywords: Isolated lactic acid bacteria, biotransformation, 3-Hydroxypropionaldehyde (3-HPA)

Introduction

3-Hydroxypropionaldehyde (3-HPA)is a small chemical molecule, non-protein, water soluble substance, effective in a wide range of pH, resistant to proteolytic and lipolytic enzymes (Axelsson et al., 1998). It was reported that 3-HPA has antimicrobial activity toward a wide range of foodborne pathogens and spoilage organisms including both Gram-positive and Gram-negative bacteria, yeast, molds, and protozoa [1, 2]. Moreover, 3-HPA is a precursor to acrolein which can be used as an intermediate for making acrylic acid, 1,3-propanediol (1,3-PDO) and a variety of other useful industrial chemicals [3]. 3-HPA can be produced via a biological process. Glycerol can be transformed to 3-HPA by glycerol dehydratase and coenzyme B12 [4]. Currently, genera of bacteria that can transform glycerol into 3-HPA have been identified as*Bacillus welchii* [5], *Bacillus subtilis* CU12 [6], *Clostridium butyricum* [7], *Citrobactor fueundii* [8], *Enterobacter aerogenes* [8], *Enterobacter agglomerans* [9], *Klebsilla pneumonia* [8, 10, 11], and *Lactobacillus reuteri* [12-17]. *Lactobacillus reuteri* is mostly used for producing 3-HPA because this strain is a non-pathogenic bacteria, acknowledged to be safe for humans (Generally Recognized as Safe; GRAS) by the Food and Drug administration [18] and gives high concentration of 3-HPA [2]. In our laboratory, there were two *Lactobacillus* sp. (*Lactobacillus* sp. LAB001 and *Lactobacillus* sp. LAB100) which constituted promising candidates for 3-HPA production [19].

Therefore, the aim of this study was to investigate 3-HPA production by isolated lactic acid bacteria (LAB001 and LAB100) to promote production of 3-HPA on a large scale.

Materials and methods

Microorganisms

Lactobacillus sp. LAB001, *Lactobacillus* sp. LAB100 and *Lb. reuteri* DSM20016 (German Collection of Microorganisms and Cell Cultures, Germany) were used as cell producers for 3-HPA production. The strains were preserved in MRS medium (HIMEDIA, India) containing 20%(v/v) glycerol (BHD, England) at -20ºC. The culture was propagated twice in MRS medium (24 h, 37 ºC) prior to use as an inoculum.

Salmonella typhimurium TISTR292 (Thailand Institute of Scientific and Technological Research) was used as the indicator strain for antimicrobial activity of 3-HPA. The strain was preserved in Nutrient Broth (HIMEDIA, India) containing 20%(v/v) glycerol (BHD, England) at -20ºC. The culture was propagated twice in MRS medium (24 h, 37 ºC) prior to use as an inoculum.

Production of 3-HPA by isolated lactic acid bacteria

3-HPA production was carried out using a two-step process consisting of the cultivation of cellproducers and the biotransformation of glycerol to 3-HPA.

In the first step, 10%(v/v) of isolated *Lactobacillus* sp. (LAB001 and LAB100) and *Lb. reuteri* DSM20016 were cultivated in MRS medium at 37ºC until the cell reached the stationary phase (16 h). The whole cells were then harvested by centrifugation at 5,000 rpm for 10 min,beforewashing twice with distilled water.The whole cells were used for the production of 3-HPA.

For the biotransformation process, the whole cellsfrom the first step were suspended in 30%(w/v) glycerol solution (10 ml) at 37ºC without shaking for 24h. After biotransformation, the samples were centrifuged (5,000 rpm, 10 min) to obtain the supernatant for analysis (glycerol concentration, 3-HPA concentration, and antimicrobial activity). All experiments were carried out in triplicate.

Analytical methods

Glycerol concentrations (initial glycerol and residue glycerol concentrations) were determined by the chromotropic acid method [20], and 99%(w/v) glycerol was used as standard for the assays. The value of residue glycerol concentration was removed from the value of the initial glycerol concentration to obtain avalue for consumed glycerol concentration. For calculation of 3-HPA concentration, 1 g of glycerol consumed converted to 0.8 g of 3-HPA. [3].

Antimicrobial activity of 3-HPA

The antimicrobial activity of 3-HPA was determined by paper disc method (modified from Loo et al. [21]). *Salmonella typhimurium* TISTR292 was used as the indicator strain. In this test, sterile paper discs (0.5 cm.) were placed on an agar plate where 10 µl of *S. typhimurium* TISTR292 (~10^6 CFU/ml) had been placed. Then, serial two-fold dilutions of 3-HPA-containing supernatants (10 µl) were dropped onto discs, and the plateswere left to incubate at 37^{o}C for 24 h. The inhibition area was revealed by the formation of a clear zone in the indicator bacterial cells. The diameter of the clear zone was measured with a vernier caliper in centimeters (cm). Antimicrobial activity was expressed in arbitrary units (AU) per ml of the original cultures calculated as follows: AU/ml is the highest dilution exhibiting inhibition zone per ml of dropping supernatant.

Statistical analysis

All experiments were carried out in triplicate and the data presented as an average value with standard deviation. Data were analyzed by one-way analysis of variance (one-way ANOVA) using SPSS software (version 19).

Results and discussion

30%(w/v) glycerol solution was used as the substrate for biotransformation by *Lactobacillu*s sp. LAB001 and *Lactobacillus* sp. LAB100 (stationary phase). After 24 h of incubation, the results showed that *Lactobacillus* sp. LAB001 gave 3-HPA concentration of 58.2 g/l (Table 1). Table 2 shows the results of biotransformation by *Lactobacillus* sp. LAB100. The average value of 3-HPA concentration was 64.5 g/l. The two isolatesof *Lactobacillu*s sp. (LAB001and LAB100) displayed antimicrobial activity against *Salmonella typhimurium* TISTR292of 1,400 and 1,600 AU/ml, respectively,and the diameters of the inhibition zones were slightly difference (Table 3).

Table 1. 3-HPA concentrations from glycerol biotransformation by *Lactobacillus* sp. LAB001

Experiment	3-HPA concentration (g/l)
1	57.45 ±0.79
2	61.39 ±3.15
3	55.88 ± 2.36
$\bar{x}$	58.24 ± 2.84

Table 2. 3-HPA concentrations from glycerol biotransformation by *Lactobacillus* sp. LAB100

Experiment	3-HPA concentration (g/l)
1	62.17 ±2.36
2	68.47 ±3.94
3	62.96 ± 1.57
$\bar{x}$	64.53 ± 3.43

Table 3. Diameter of inhibition zone from 3-HPA by isolates *Lactobacillus* sp. (LAB001andLAB100)

Isolate	Diameter of inhibition zone (cm)											Antimicrobial activity of 3-HPA (AU/ml)
	0x	2x	4x	6x	8x	10x	12x	14x	16x	18x	20x	
LAB001	1.1	1	1	0.9	0.9	0.9	0.9	0.9	0	0	0	1,400
LAB100	1.1	1	1	1	1	1	1	1	1	0	0	1,600

Note: x = concentration of analyzed sample (3-HPA).

The concentrations and antimicrobial activity of 3-HPA from glycerol biotransformation by *Lactobacillus* sp. LAB001, *Lactobacillus* sp. LAB100, and *Lb. reuteri* DSM20016 (the reference strain) are summarized in Table 4. The results showed that the 3-HPA concentrations of the 2 isolates of *Lactobacillus* sp. were lower than the 3-HPA concentration of *Lb. reuteri* DSM20016. However, the 2 isolates of *Lactobacillus* sp. displayed antimicrobial activity against *S. typhimurium* TISTR292 similar to the reference strain, *Lb. reuteri* DSM 20016.

Table 4. Concentration and antimicrobial activity of 3-HPA from glycerol biotransformation by *Lactobacillus* sp. LAB001, *Lactobacillus* sp. LAB100, and *Lb. reuteri* DSM20016

Producer strain	**3-HPA concentration (g/l)**	**Antimicrobial activity of 3-HPA (AU/ml)**
Lactobacillus sp. LAB001	58.24 ± 2.84	1,400
Lactobacillus sp. LAB100	64.53 ± 3.43	1,600
*Lb. reuteri*DSM20016	73.72 ± 1.64	1,600

Conclusions

This study showed that 3-HPA can be produced via biological process by isolated lactic acid bacteria (LAB001 and LAB100). However, the 3-HPA concentrations of the 2 isolates were lower than the 3-HPA concentration of *Lb. reuteri* DSM20016. Therefore, increasing 3-HPA concentration of the 2 isolates of *Lactobacillus* sp. underoptimal conditions is required for further study.

Acknowledgments

We would like to thank the Graduate School, Fermentation Research Center for Value Added Agriculture Products (FerVAAP) and the Department of Biotechnology, Faculty of Technology, Khon Kaen University for their support and provision of research facilities.

References

[1] L. Axelsson. Lactic acid bacteria: Classification and Physiology, pp. 1-72. In: S. Salminen and A.V.Wright, editors. Lactic Acid Bacteria: Microbiology and Functional Aspects. 2nd ed. B. Marcel Dekker, New York. (1998).

[2] S. Vollenweider, and C. Lacroix. 3-Hydroxypropionaldehyde: applications and perspectives of biotechnological production. *Appl Microbiol Biotechnol*. 64 (2004) 16-27.

[3] P.J. Slininger, R.J. Bothast, and K.L. Smiley. Production of 3-hydroxypropionaldehyde from glycerol. *Appl. Environ. Microbiol.* 46 (1983) 62-67.

[4] K.L. Smiley, and M. Sobolov. A cobamide-requiring glycerol dehydrase from an acrolein-forming Lactobacillus. *Arch. Biochem. Biophys.* 97 (1962) 538-543.

[5] F.B. Humphreys. Formation of acrolein from glycerol by *B. welchii*. *J. Infect. Dis.* (1924) 282-290.

[6] C. Wise, L. Novitsky, A. Tsopmo, and T.J. Avis. Production and antimicrobial Activity of 3-hydroxypropionaldehyde from *Bacillus subtilis* strain CU12. *J. Chem. Ecol.* 38 (2012) 1521-1527.

[7] H. Malaoui, and R. Marczak. Purfication and characterization of the 1-3- propanediol dehydrogenase of *Clostridium bytyricum* E5. *Enzyme Microb. Technol.* 27 (2000) 399-405.

[8] J.E. Vancauwenberge, P.J. Slininger, and R.J. Bothast. Bacterial conversion of glycerol to beta-hydroxypropionaldehyde. *Appl. Environ. Microbiol.* 56 (1990) 329-332.

[9] F. Barbirato, J.P. Grivet, P. Soucaille, and A. Bories. 3-Hydroxypropionaldehyde, an inhibitory metabolite of glycerol fermentation to 1, 3-propanediol by enterobacterial species. *Appl. Environ. Microbiol.* 62 (1996) 1448-1451.

[10] P.J. Slininger, and R.J. Bothast. Optimizing aerobic conversion of glycerol to 3-hydroxypropionaldehyde. *Appl. Environ. Microbiol.* 50 (1985) 1444-1450.

[11] C. Ulmer, and A.P. Zeng. Microbial production of 3-hydroxypropionaldehyde from glycerol bioconversion. *Chem Biochem Eng Q.* 21(2007) 321-326.

[12] T.L. Talarico, and W.J. Dobrogosz. Chemical characterization of an antimicrobial substance produced by *Lactobacillus reuteri*. *Antimicrob. Agents Chemother.* 33 (1989) 674-679.

[13] Q. Lüthi-Peng, F. Dileme, and Z. Puhan. Effect of glucose on glycerol bioconversion by *Lactobacillus reuteri*. *Appl Microbiol Biotechnol*. 59 (2002) 289-296.

[14] Y. Doleyres, P. Beck, S. Vollenweider, and C. Lacroix. Production of 3-hydroxypropionaldehyde using a two-step process with *Lactobacillus reuteri*. *Appl Microbiol Biotechnol*. 68 (2005) 467-474.

[15] M. Tobajas, A.F. Mohedano, J.A. Casas, and J.J. Rodríguez. A kinetic study of reuterin production by *Lactobacillus reuteri* PRO 137 in resting cells. *Biochem. Eng. J.* 35 (2007) 218-225.

[16] C. Guo, Y. Daomao, X. Yaqin, and C. Hongwen. Influence of conditions on reuterin accumulation by the resting cell biotransformation process. *Chinese J Chem Eng.* 19 (2011) 1023-1027.

[17] S.K. Mishra, R.K. Malik, G. Manju, N. Pandey, G. Singroha, P. Behare, and J.K. Kaushik. Characterization of a Reuterin-producing *Lactobacillus reuteri* BPL-36 strain isolated from human infant fecal sample. *Probiotics Antimicrob Proteins.* 4 (2012) 154-161.

[18] The Food and Drug Administration [FDA]. Pasteurized Milk Ordinance, U.S. Department Health and Human Services. Public Health Service, Food and Drug Administration, Publication No. 229 (1989); Available at: http:// www.fda.gov/downloads/Food/Guidance Regulation/UCM209789.pdf.html., accessed January 2014.

[19] K. Hamsupo, M. Boonmee, P. Udomsri, and P. Putongpan. Screening of Lactic acid bacteria production 3-Hydroxypropionaldehyde from various sources. International Conference on Science and: Applications in Industry & Education (ICSTIE 2008), 12-13 December 2008, University Teknologi MARA, PulauPiang, MALAYSIA.

[20] E. Van Handel. Suggested modifications of the micro determination of triglycerides. *Clin. Chem.* 7 (1961) 249-251.

[21] Y.H. Loo, P.S. Skell, H.H. Thornberry, J.Ehrlich, J.M. McGuire, G.M. Savage, and J.C. Sylvester. Assay of streptomycin by the paper-disc plate method. *J Bacteriol.* 50 (1945) 701.