Volume : 3 | Issue : 6 | June 2014 • ISSN No 2277 - 8179

Research Paper

Production of industrially important metabolite from raw glycerol

Biotechnology

KEYWORDS : Raw glycerol, Lactic acid, Lactobacillus delbrueckii, Biodiesel, Metabolite

*** Hiren Patel**	Department of Plant Molecular Biology and Biotechnology, Navsari Agricultural University, Navsari-396 450. *Corresponding Author
Khushbu Patel	Department of Plant Molecular Biology and Biotechnology, Navsari Agricultural University, Navsari-396 450.
Gaurav Shah	Department of Biotechnology, Veer Narmad South Gujarat University, Surat-395 007

ABSTRACT

Microbial conversion of glycerol into value added products was investigated aiming to facilitate the utilization of waste glycerol. The crude glycerol from biodiesel production represents an abundant and inexpensive source which can be used as raw material for lactic acid production. The first aim of this investigation was to select a suitable strain for producing lactic acid from glycerol with a high concentration and productivity. The second aim was to obtain the optimum fermentation conditions, as a basis for large-scale lactate production in the future. Lactobacillus delbrueckii strain gave higher production of lactic acid (4.3 gL-1) in batch flask fermentation having raw glycerol as carbon source under agitation at 200 rpm, pH 6.5 and temperature 420C. As compared with pure glycerol, Lactobacillus delbrueckii gave lower lactic acid production (1.6 gL-1) under same environmental condition. So, raw glycerol containing medium is more favorable for lactic acid production compare to pure glycerol containing medium.

INTRODUCTION

Renewable energy sources and biofuel, including biodiesel, have been gaining increasing attention recently as a replacement for fossil fuels. However, their implementation in the general market depends on making these fuels more competitive. A convenient way to lower the cost of biofuel is to use the by-products as a potential source of energy, rather than treating them as waste [1]. Biodiesel is a prominent candidate as alternative diesel fuel. It has few advantages compared to conventional diesel, including the status of renewable energy source and lower emissions. Advances against petroleum diesel fuel are represented by the terms of sulfur content, flash point, content of aromatic substances and biodegradability.

Glycerol is yielded at about 10% as a by-product during the process of biodiesel production [2]. The global biodiesel market is estimated to reach 37 billion gallons by 2016, growing at an average annual growth of 42%. Then, about 4 billion gallons of crude glycerol will be produced. According to the reported study, the crude glycerol represents an abundant and inexpensive source which can be converted to industrial products with high additional value, such as 1, 3-propanediol, polyhydroxyalkanoate, hydrogen, epichlorohydrin and also lactic acid [3] [4].

Lactic acid, its salts and esters have a wide range of potential uses and are extensively applied in diverse fields of the food, industrial, cosmetic and pharmaceutical industries, as well as in agriculture [5] [6]. Lactic acid was first produced worldwide from glucose or pure starch by fermentation. During the past 15 years, starchy hydrolyzates obtained from corn [7], cassava [8], barley [9], wheat [10] and potatoes [11] were also tested for their suitability as substrates for lactic acid fermentation. The aim of the current study is to develop a fermentation process based on the substitution of expensive nutrient supplements with cheaper materials from renewable resources [12]. It is reported that glycerol can be converted into lactic acid by a chemical route. Chemical conversion of glycerol into lactic acid requires high temperature, high pressure and expensive catalysts [13]. Therefore, much attention has recently been focused on the microbial conversion of glycerol to lactic acid since the process is relatively easy and has high yield.

MATERIALS AND METHODS

Materials

Crude glycerol was obtained from biodiesel plant of Central Salt & Marine Chemical Research Institute Bhavnagar, Gujarat India. All other chemicals were obtained commercially and of analytical grade.

Bacterial Strain

Four strains were used for lactic acid productions which were collected from NCLM Pune & two strains were collected from the MTCC Chandigardh (Table-1).

Table -1. Different strains used in lactic acid production

Sr.No.	Name of the cultures	NCIM NO.	MTCC NO.
1	Lactobacillus casei	2125	-
2	Lactobacillus delbrueckii	2025	-
3	Lactobacillus pentosus	2912	-
4	Bacillus laevolacticus	2464	-
5	Lactobacillus plantarum	-	2912
6	Lactococcus lactis subsp. lactis	-	3041

Screening & Enrichment of the strains

All strains under study were screened for the efficient utilization of raw glycerol & production of lactic acid. For screening, the suspension of all the strain was prepared. Take 1 mL of 10^{6-} fold diluted culture broth and spread the suspension on agar plates containing yeast extract 2 gL^{-1}, beef extract 5 gL^{-1}, peptone 3 gL^{-1}, glycerol 20 g L^{-1}, NaCl 3 gL^{-1}, K_2HPO_4 2 gL^{-1}, Agar 20 $gL^{-1,}$ $CaCO_3$ 10 gL^{-1}(L-1 Medium). The initial pH was 7.0 regulated by 1 N NaOH. Glycerol in the medium was used as substrate to favor the growth of glycerol-utilizing microorganisms. All the plates were incubated at 37 ^{0}C for 12 h. The production of lactic acid was indicated by visualization of clear zone around the growth of organism dew to solubalization $CaCO_{3.}$ Clear zone was observed around the colony [12] [13].

Batch flask fermentation

Selected strains were used for lactic acid fermentation. The composition of preculture medium (L-2) was $K_2^{HPO}{}_4$ 3 $gL^{-1,}$ $(NH_4)2SO_4$ 5 gL^{-1}, yeast extract 4 gL^{-1}, peptone 5 gL^{-1}, glycerol 20 gL^{-1}. The colonies were transferred into a 250 mLflask with 50 mL preculture medium and incubated in a rotary shaker at 37 ^{0}C and 150 rpm for 12 h.

The medium (L-3) was used for batch fermentation. The medium used for batch flask fermentation contained the following components: yeast extract 4 gL^{-1}, peptone 7 gL^{-1}, glycerol 20 gL^{-1}, K_2HPO_4 10 gL^{-1}, $(NH_4)2SO_4$ 10 gL^{-1} [14] [15]. 1mL of preculture medium (L-2) was transferred into a 250 mL flask containing 100 mL medium (L-3) and then incubated. The optimization of lactic acid production was done at different environmental parameters. The data are the averages of triplicate experiments.

Analytical methods

Lactic acid production was confirmed by TLC method. For quantitative analysis of lactic acid, 4-Hydroxy diphenyl method was carried out [16].

Further qualitative and quantitative analysis was carried out by HPLC method. Glycerol, acetic acid, and ethanol were analyzed by Water 2695 alliance separation module high performance liquid chromatography (HPLC) system with a waters 2414 Refractive detector, and lactic acid and succinic acid were analyzed by a UV detector at 210 nm. The stationary and mobile phases were a supelcogel make of supelcogel (sigma Aldrich) (300 × 7.8 mm) and a 0.1% H_2SO_4 solution in water at 0.5 mL min^{-1}, respectively. The column temperature was controlled at 65 ^{0C} and pressure 550-600 psi.

RESULTS

Table :- 2. Lactic Acid Production (gL-1) by different strain in raw glycerol at various parameters *

	Lactobacillus casei	Lactobacillus delbrueckii	Lactobacillus pentosus	Bacillus laevolacticus	Lactobacillus plantarum	Lactococcus lactis subsp. lactis
pH						
4.0	-	0.5	0.3	-	-	0.2
4.5	-	0.6	0.4	-	-	0.2
5.0	-	1.1	0.6	0.2	0.2	0.3
5.5	-	1.5	0.7	0.2	0.3	0.4
6.0	-	1.9	1.0	0.3	0.3	0.5
6.5	-	3.7	1.1	0.6	0.4	1.0
7.0	-	3.1	1	0.5	0.3	0.9
Temperature (^{0}C)						
30	-	-	-	-	-	-
32	-	0.2	-	-	-	-
35	-	0.3	0.2	0.1	-	0.1
37	-	0.8	0.6	0.2	0.1	0.3
40	-	1.8	0.9	0.4	0.2	0.7
42	-	3.9	1.2	0.7	0.5	1.1
45	-	2.4	1.1	0.6	0.4	1.0
rpm						
100	-	0.2	-	-	-	0.1
120	-	0.8	0.2	0.1	-	0.1
140	-	1.1	0.3	0.2	0.1	0.2
160	-	1.9	0.6	0.3	0.2	0.3
180	-	2.9	0.9	0.4	0.4	0.8
200	-	4.3	1.4	0.7	0.6	1.4

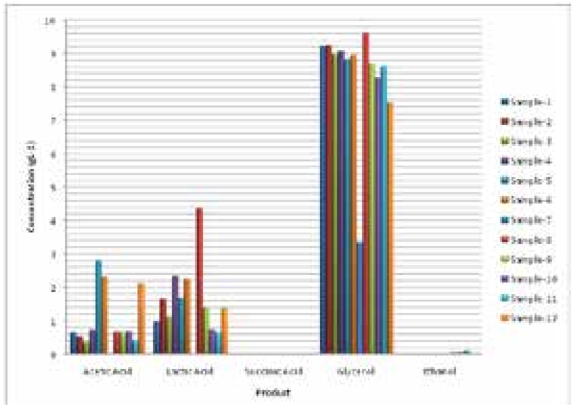
***All the results are the average of the triplicate experiments**

Table-3 Broth compositions in aerobic flask culture containing initial glycerol concentration 20 (gL-1), fermentation time 48 h, pH 6.5, Temperature 42 0C at 200 rpm.

Strain	Tested Sample No. for HPLC	Acetic Acid (gL^{-1})	Lactic Acid (gL^{-1})	Succinic Acid (gL^{-1})	Glycerol (gL^{-1})	Ethanol (gL^{-1})
Lactobacillus casei	1	0.66	0.99	-	9.226	-
Lactobacillus delbrueckii	2	0.53	1.68	-	9.25	-
Lactobacillus pentosus	3	0.38	1.12	-	9.00	-
Bacillus laevolacticus	4	0.74	2.35	-	9.09	-
Lactobacillus plantarum	5	2.82	1.70	-	8.83	-
Lactococcus lactis subsp.lactis	6	2.33	2.26	-	8.97	-
Lactobacillus casei	7	-	-	-	3.35	-
Lactobacillus delbrueckii	8	0.68	4.37	-	9.63	-
Lactobacillus pentosus	9	0.67	1.43	-	8.71	0.067
Bacillus laevolacticus	10	0.70	0.75	-	8.3	0.056
Lactobacillus plantarum	11	0.42	0.66	-	8.64	0.124
Lactococcus lactis subsp.lactis	12	2.13	1.40	-	7.55	-

Note :- Sample no 1-6 contain pure glycerol and sample no 7-12 contain raw glycerol.

Fig- 1 Comparison of different metabolite products in different samples



DISCUSSION

Screening and enrichment

All the strains were spread on medium (L-1) plate and incubated at 37 ^{0}C. Next day, all strains displayed the clear zone around their colonies, dew to solubalization of $CaCO_3$. It indicated that most of the strains were capable of producing organic acid. Then selected colonies were transferred into the preculture medium (L-2) for further enrichment at 37 ^{0}C, 150 rpm. Here culture medium had been screened for the presence of lactic acid production by TLC method for conformation.

Batch fermentation

In batch fermentation, all strains were inoculated in medium (L-3) containing raw glycerol at various parameters. Different strains showed different parameters for optimization of lactic acid. Among all of them Lactobacillus delbrueckii showed higher production in medium containing glycerol 20 gL^{-1}, 200 rpm, pH-6.5 at 42 ^{0}C as shown in table-2. Lactobacillus casei was not able to produced lactic acid in medium containing raw glycerol. Also to confer the more suitable source of glycerol for lactic acid production, all strains were inoculated in medium containing pure glycerol for batch fermentation providing same optimum condition. Comparing the results of both the raw glycerol and pure glycerol batch fermentation, it was concluded that raw glycerol medium showed a higher lactic acid production (4.37 gL^{-1}) compared to pure glycerol (1.68 gL^{-1}) by Lactobacillus delbrueckii. While other strains like Lactobacillus casei, Lactobacillus pentosus, Bacillus laevolacticus, Bacillus laevolacticus, Lactococcus lactis subsp.lactis showed higher lactic acid production in pure glycerol containing medium.

During HPLC analysis of different samples, other useful by-products like ethanol and acetic acid were also found (table-3 and Fig-1). It means that some microbes are able to produce other useful byproduct also from raw glycerol. Figure-1 clearly indicated that higher production of acetic acid, lactic acid and ethanol were produced by Lactococcus lactis subsp.lactis, Lactobacillus delbruecki and Lactobacillus plantarum respectively in raw glycerol. All these byproducts also have great economic value in the market.

Conclusion:-

The use of renewable waste substrates is an ecofriendly choice that also contributes to the reduction of waste treatment costs and increases the economic value of by-products. This bioconversion would directly benefit the environment by obtaining biodegradable polymers, promoting the use of biodiesel and reducing petroleum dependency. The development of processes for converting inexpensive glycerol into higher value added metabolite is expected to make biodiesel production more economical and will thus help establish more biorefineries. Future research will focus on the isolation and characterization of more microorganisms that can use glycerol as a carbon source and generate valuable metabolites with unusual properties.

REFERENCE

1. Hartlep, M., Hussmann, W., Prayitno, N., Meynial-Salles, I., and Zeng, A. P. Study of two-stage processes for the microbial production of 1, 3-propanediol from glucose. Appl. Microbiol. Biotechnol., 2002; 60:60–66. | 2. Wang, L., Du, W. and Liu, D.H. Lipase-catalyzed biodiesel production from soybean oil deodorizer distillate with absorbent present intertbutanol system. J.Mol.Catal B: Enz., 2006; 43:29–32. | 3. Zeng, A.P. and Biebl, H. Bulk Chemicals from Biotechnology. The Case of 1, 3-propanediol production and the new trends. Adv Biochem Eng Biotechnol., 2002; 74:239–259. | 4. Freddy, G. Solvay will make epichlorohydrin from glycerol. Ind Bioprocess, 2006; 28:8–9. | 5. Datta, R, Tsai, S.P., Bonsignore, P., Moon, S.H. and Frank, J.R. Technological and economic potential of poly-lactic acid and lactic acid derivatives. FEMS Microbiol Rev,1995; 16:221–231. | 6. Rathin, D. and Michael, H. Lactic acid: recent advances in products, processes and technologies-a review. J. Chem. Technol. Biotechnol., 2006; 81:1119–1129. | 7. Vishnu, C., Seenayya, G. and Reddy, G. Direct conversion of starch to L(+)-lactic acid by amylase producing Lactobacillus amylophilus GV6. Bioprocess Eng., 2000; 23:155–158. | 8. Xiaodong,W., Xuan, G. and Rakshit, S.K. Direct fermentative production of lactic acid on cassava and other starch substrates. Biotechnol. Lett., 1997; 19:841–843. | 9. Hujanen M. and Linko Y.Y. Effect of temperature and various nitrogen sources on L(+)-lactic acid production by Lactobacillus casei. Appl Microbiol Biotechnol, 1996; 45:307–313. | 10. Akerberg, C. and Zacchi, G. An economic evaluation of the fermentative production of lactic acid from wheat flour. Bioresource Technol,2000; 75:119–126. | 11. Zielinska, K.J., Stecka, K.M., Miecznikowski, A.H. and Suterska, A.M. Degradation of raw potato starch by the amylases of lactic acid bacteria. Pr Inst Lab Badaw Przem Spozyw., 2000; 55:22–29. | 12. Venus, J. and Richter, K. Production of lactic acid from barley: strain selection, phenotypic and medium optimization. Eng. Life Sci., 2006; 6:492–500. | 13. Liu, H.C., Shen, Y.H. and Li, H.J. Method for synthesizing lactic acid by using glycerol. Chinese Patent CN101225041 (A), 2008. | 14. El-Ziney, M.G., Arneborg, N., Uyttendaele, M., Debevere, J. and Jakobsen, M. Characterization of growth and metabolite production of Lactobacillus reuteri during glucose/glycerol cofermentation in batch and continuous cultures. Biotechnol Lett, 1998; 20:913–916. | 15. Kazuhisa K. Method for production lactic acid. Japan Patent WO2008126667 (A2), 2008. | 16. Barker ,S. B. and Summerson, W. H. Determination of lactic acid in biological material. J. Biol. Chem., 1941; 138:535-554. |