Jundishapur J Microbiol. 2012;5(4):546-549. DOI: 10.5812/jjm.3524

Jundishapur Journal of Microbiology
www.jjmicrobiol.com

Study of Polyols Production by *Yarrowia lipolytica* in Batch Culture and Optimization of Growth Condition for Maximum Production

Gholam Reza Ghezelbash[1*], Iraj Nahvi[1], Mohammad Rabbani[1]

[1] Department of Biology, Faculty of Science, University of Isfahan, Isfahan, IR Iran

ARTICLE INFO

Article type:
Original Article

Article history:
Received: 13 Nov 2011
Revised: 6 Mar 2012
Accepted: 6 Mar 2012

Keywords:
Erythritol
Yarrowia lipolytica
Batch Culture
Optimization
Polyols

ABSTRACT

Background: Sugar alcohol erythritol is a non-caloric sweetener, non-cariogenic, and safe for diabetics because of no change to blood glucose and insulin levels after oral administration. Erythritol cannot be degraded by any enzymatic systems and must be eliminated from the blood through the kidney.

Objectives: The aim of this study was production and optimization of erythritol from glucose by *Yarrowia lipolytica*.

Materials and Methods: *Y. lipolytica* DSM70562 was cultivated at 30°C in a 250 mL Erlenmeyer containing 50 mL of production medium composed of 200 g/L glucose, 10 g/L yeast extract, 10 mg/L $MnSO_4.4H_2O$, and 2 mg/L CuSO4.5H2O. Erythritol was separated from the sugars and other polyols by thin layer chromatography. Total polyols was determined using colorimetric method of Bok and Demain, and erythritol was also eluted from the paper and determined by this colorimetric method.

Results: In a batch culture with 200 g/L glucose at pH 5.5, an erythritol producer of *Y. lipolytica* capable to produce 27.8 g/L erythritol after seven days was selected, corresponding to a 21.52% yield and a productivity of 0.165 g/L/h.

Conclusions: In this investigation we optimized the production medium and through altering medium components that resulted in a drastic change in polyol composition. Present study reports the production of erythritol for the first time by a *Y. lipolytica* strain DSM70562. Due to increasing demand for erythritol as a low caloric sweetener in food industry, its production via biological processes is becoming increasingly important.

Implication for health policy/practice/research/medical education:
Erythritol is one of the best low-calorie natural sweeteners. Unlike artificial sweeteners, erythritol does not have any significant side effects. In addition, erythritol does not raise plasma glucose or insulin levels.

Please cite this paper as:
Ghezelbash GR, Nahvi I, Rabbani M. Study of Polyols Production by *Yarrowia lipolytica* in Batch Culture and Optimization of Growth Condition for Maximum Production. *Jundishapur J Microbiol*. 2012;5(4):546-9. DOI: 10.5812/jjm.3524

1. Background

Polyols act as compatible solutes and can play a role in osmotic adjustment (1). Their role in membrane and protein protection is well established (2). The low molecular weight polyols, glycerol and erythritol, are more effective in osmotic adjustment than higher-molecular-weight compounds such as mannitol (3).

Erythritol is a four-carbon sugar alcohol with a molecular weight of 122. It is present in small quantities in fruits and fermented foods, 70–80 % as sweet as sucrose with a

* *Corresponding author:* Gholam Reza Ghezelbash, Department of Biology, Faculty of Science, University of Isfahan, Isfahan, P.O. Box: 81746-73441, IR Iran. Tel: +98-3117932456; Fax: +98-3117932456; *E-mail:* gh.r.ghezelbash@gmail.com

DOI: 10.5812/jjm.3524

very low caloric value of 0.2 calories per gram. This non-caloric sweetener is an important sugar alcohol being industrially produced only by fermentation (4, 5). Industrial production of erythritol began in Japan in 1990 and has been used as sugar substitutes for candies, chocolates, soft drinks, chewing gum, jellies, jams and yogurt. It has been approved in U.S.A. in 2001 and used as a flavor enhancer, formulation aid, humectant, nutritive sweetener, stabilizer, thickener, sequestrant and texturizer at maximum levels of 100% in sugar substitutes (6).

Erythritol can be produced by osmophilic yeasts belonging to the genus *Aureobasidium*, *Candida*, *Moniliella*, *Pichia*, *Pseudozyma*, *Trigonopsis*, *Trichosporon*, *Trichosporonoides* and *Yarrowia* (5). Industrially, erythritol has been produced by the use of a mutant of *Aureobasidium* sp. at a rate of 1.81 g/L/h with a high yield of 44 % in a medium containing 40% glucose (7-9). *Y. lipolytica* has been reported to produce several polyols and organic acids such as erythritol, glycerol and citric acid. The composition of polyols produced by *Y. lipolytica* depends on the nature, composition and concentration of media constituents. The objective of the present study was to improve polyols production from *Y. lipolytica* by optimization growth condition in batch culture to minimize formation of glycerol. In addition, the erythritol production of the strain was improved with the range of glucose in batch culture.

2. Objectives

The aim of this study was erythritol production by *Y. lipolytica*. This study is the first research for erythritol production in Iran.

3. Materials and Methods

3.1. Microorganisms and Media

Y. lipolytica DSM70562 was obtained from DSMZ collection culture. The growth medium for activation contained 100 g/L glucose and 10 g/L yeast extract. Yeast cultures maintained at 4°C and sub-cultured each 4 weeks. The production medium contained 200 g/L glucose, 10 g/L yeast extract, 10 mg/L $MnSO_4.4H_2O$, and 2 mg/L $CuSO_4.5H_2O$ in flask culture.

3.2. Culture Conditions

A single colony *Y. lipolytica* was inoculated into a 100 mL Erlenmeyer flask containing 10 mL of production medium and incubated at 30°C, 180 rpm for 48 h. Two and half milliliters of the broth was transferred into a 250 mL Erlenmeyer flask containing 50 mL production medium and incubated at 30°C for seven days on a reciprocating shaker with 180 rpm. Initial pH of the production medium was adjusted at 5.5 (4, 10). Fermentation samples were run and analyses usually were performed at 24 h intervals or when the fermentation was judged as complete. Samples were analyzed for residual sugar, total polyol concentration, erythritol concentration, and biomass density.

3.3. Optimization of Erythritol Production

Effects of environmental factors on erythritol production were studied. The process was examined in the presence of various carbon sources as well as nitrogen source, temperature, and initial pH.

3.4. Choice of Carbon Source

To study the effect of carbon sources on erythritol production by *Y. lipolytica*, glucose from production medium was substituted by sucrose at the concentration of 100 g/L. Respective media were inoculated with two and half milliliters of seed cultures from the activated culture and incubated at 30°C, 180 rpm on rotary shaker. After one week, samples were analyzed for biomass, residual glucose, and polyol concentration.

3.5. Effect of Yeast Extract

The effect of the yeast extract concentration on erythritol production in production medium was examined by 20 % glucose as a carbon source. Two and half milliliters of seed cultures were inoculated in 250 mL Erlenmeyer flasks with 50 mL medium of varying yeast extract concentrations ranging from 0.5 to 2.5 % and incubated at 30°C, 180 rpm for seven days. Samples were analyzed as described earlier.

3.6. Effect of Initial pH

Effect of initial pH on erythritol production was studied in shake flasks with 50 mL production medium. The pH of a production medium plays a vital role in the production of various products. In this experiment a range of pH from 4 to 7 was studied and used for final production. Incubation temperature of fermentation medium was maintained at 30°C.

3.7. Analytical Methods

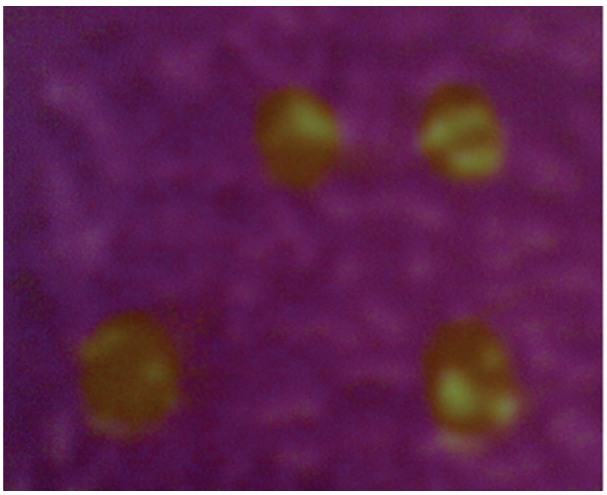
Yeast growth was measured by centrifuging a sample of the fermented liquor, washing the yeast twice with distilled water, and drying at 80 °C in 12 h. Glucose was measured using dinitrosalicylic acid method (9) after cell removal by centrifugation. The supernatant liquid from the yeast centrifugation was used for further analyses. Erythritol was separated from the sugars and other polyols by thin layer chromatography (TLC). TLC was conducted using a solvent system composed of ethyl acetate: 2-butanol: water in the ratio of 6:3:1 by volume. Polyols were stained and detected by spraying 1 % $NaIO_4$ on the samples followed by 1 % $KMnO_4$ (11). Polyols create yellow spots on purple background. Total polyols were determined by colorimetric method of Bok and Demain (12); the same method was applied to elute and determine erythritol from the paper. Yield of erythritol production was calculated by this formula: yield (%) = (erythritol / carbon source consumption) ×100 %.

4. Results

4.1. Optimization of Polyols Production (Choice of Carbon Source)

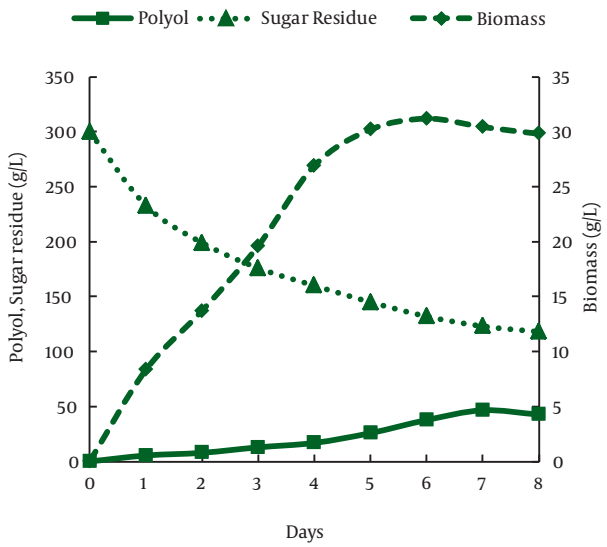
Figure 1 shows a sample of TLC which stained by 1 % $NaIO_4$ and 1 % $KMnO_4$. This strain produces maximum amount of erythritol after seven days *(Figure 2)*. Despite of sucrose and lactose, glucose showed to keep organism growing and producing erythritol well. Glucose at concentration of 20% revealed maximum production and yields *(Figure. 3)*.

Figure 1. Separation of Polyols by TLC



Lane 1: Erythritol; Lane 2: Glycerol; Lane 3: Products of *Y. lipolytica* DSM70562 in Fermentation Medium

Figure 2. Glucose Consumption and Cell Growth During Polyol Production by *Y. lipolytica*



(■) Polyol, (▲) sugar residue, (♦) Biomass (dry weight)

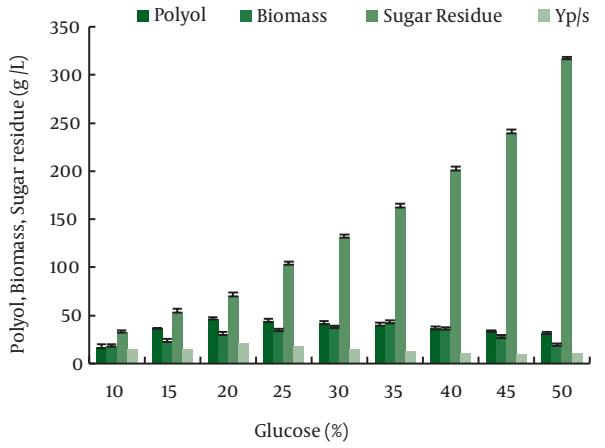


Figure 3. Effects of Concentration of Glucose on Polyol Production

4.2. Effect on Yeast Extract

Yeast extract concentrations varied from 0.5 to 2.5 %. At the highest level of yeast extract concentration, yeast growth was excessive and polyols yields were very low. *Figure 4* shows that 1.0% of yeast extract is the best amount of this source for polyols production. TLC also showed that 1.0% of yeast extract is the best concentration for erythritol production. Based on sugar consumed, at the lowest level of yeast extract concentration, erythritol yields, appeared well, however sugar utilization was very slow as a result of low cell concentrations.

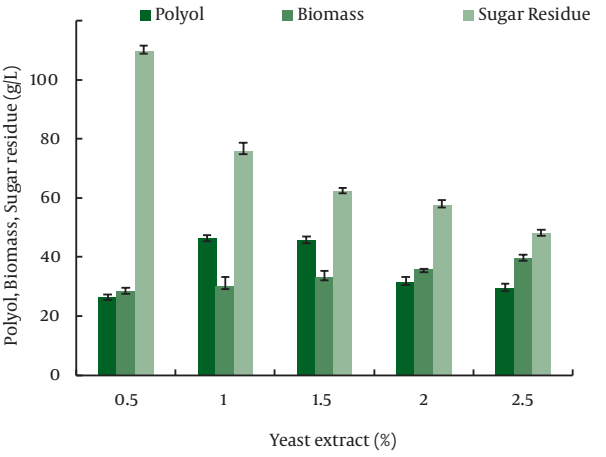


Figure 4. Effects of Concentration of Yeast Extract on Polyol Production

4.3. Effect of Initial pH

Yarrowia grows in a broad spectrum of pH but the highest amount of polyols production occurred at pH 5.5 *(Figure. 5)* and detected by TLC; an alkaline pH led to an increase in glycerol production.

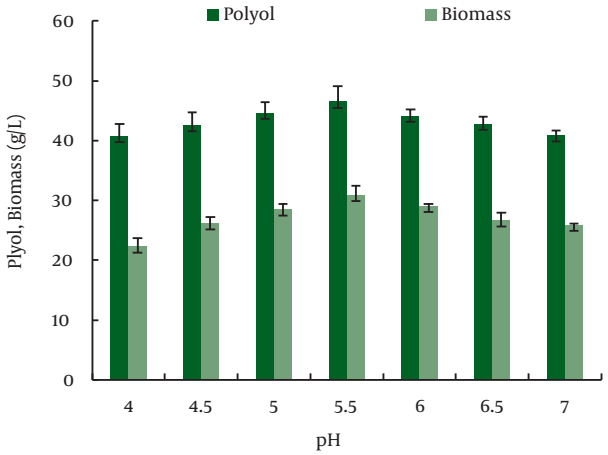


Figure 5. Optimal pH for Production of Polyol

5. Discussion

The concentration and pattern of polyols produced by osmotolerant yeasts are strongly influenced by medium composition and environmental conditions (10). The pathways for polyols biosynthesis and catabolism can be different among organisms (11). It was observed that *Y. lipolytica* produced 60% erythritol out of total produced polyols. The preliminary screening experiments (one-factor at a time) suggested that glucose - as carbon source - and yeast extract - as nitrogen source - were critical medium components for erythritol formation. Pattern of polyol production can be drastically altered by changing the medium components (10, 13).

In this study characterized that at concentration higher than 1.0% of yeast extract, glycerol production rate was much higher than that of erythritol. These results indicate that the addition of yeast extract facilitated and enhanced glycerol production. Alkaline pH led to an increase in glycerol production. The optimum pH for erythritol production was determined to be 5.5which was almost the same as optimum pH (pH 5.6) for *Aureobasidium* sp. reported by Wako *et al.* (14). Erythritol is commercially produced by fermentation of *Aureobasidium* sp., *Torula* sp., and *Moniliella pollinis* (5). Erythritol production by *Y. lipolytica* DSM70562 needs gene engineering such as overexpression of erythrose reductase, and deletion or decrease of glycerol production by ultraviolet irradiation.

Acknowledgements

The current study was supported by the grant of Postgraduate Administration Office of the University of Isfahan to Gh. R. Ghezelbash for obtaining Ph.D. degree

Financial Disclosure

None declared.

Funding Support

None declared.

References

1. Hallsworth JE, Magan N. Culture Age, Temperature, and pH Affect the Polyol and Trehalose Contents of Fungal Propagules. *Appl Environ Microbiol*. 1996;**62** (7):2435-42.
2. Carpenter JF, Crowe JH. Modes of stabilization of a protein by organic solutes during desiccation. *Cryobiology*. 1988;**25** (5):459-70.
3. Chirife J, Favetto G, Ferro Fontan C. Microbial growth at reduced water activities: some physicochemical properties of compatible solutes. *J Appl Bacteriol*. 1984;**56** (2):259-68.
4. Kim KA, Noh BS, Lee JK, Kim SY, Park YC, Oh DK. Optimization of culture conditions for erythritol production by Torula sp. *J Microbiol Biotechnol*. 2000;**10** (1):69-74.
5. Moon HJ, Jeya M, Kim IW, Lee JK. Biotechnological production of erythritol and its applications. *Appl Microbiol Biotechnol*. 2010;**86** (4):1017-25.
6. Park YC, Lee DY, Lee DH, Kim HJ, Ryu YW, Seo JH. Proteomics and physiology of erythritol-producing strains. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2005;**815** (1-2):251-60.
7. Ishizuka H, Wako K, Kasumi T, Sasaki T. Breeding of a mutant of Aureobasidium sp. with high erythritol production. *J Ferment Bioeng*. 1989;**68** (5):310-4.
8. Lee JK, Ha SJ, Kim SY, Oh DK. Increased erythritol production in Torula sp. by Mn^{2+} and Cu^{2+}. *Biotechnol Lett*. 2000;**22** (12):983-6.
9. Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal Chem*. 1959;**31** (3):426-8.
10. Yang SW, Park JB, Soo Han N, Ryu YW, Seo JH. Production of erythritol from glucose by an osmophilic mutant of Candida magnoliae. *Biotechnol Lett*. 1999;**21** (10):887-90.
11. Lee KJ, Lim JY. Optimized conditions for high erythritol production by Penicillium sp. KJ-UV29, mutant of Penicillium sp. KJ81. *Biotechnol Bioprocess Eng*. 2003;**8** (3):173-8.
12. Bok SH, Demain AL. An improved colorimetric assay for polyols. *Anal Biochem*. 1977;**81** (1):18-20.
13. Savergave LS, Gadre RV, Narayanan BK. Strain improvement and statistical media optimization for enhanced erythritol production with minimal by-products from Candida magnoliae mutant R23. *Biochem Eng J*. 2011;**55** (2):92-100.
14. Wako K, Ishizuka H, Kawaguchi G, Kubo N, Kasumi T, Hayashi K. Erythritol production by Aureobasidium sp. SN-115. *J Ferment Technol*. 1988;**66** (4):479-83.