LGU Journal of
LIFE SCIENCES

LGU Society of Life Sciences

Research Article
Vol 2 issue 1 Jan-March 2018

LGU J.Life.Sci
ISSN 2519-9404
eISSN 2521-0130

Fermentation of Molasses by *Saccharomyces cerevisiae* for Bio-Ethanol Production

Shafeeq-ur-Rehman[1], Taimoor Khan[1], Farhan Rasheed[1], Shahid Raza[2*]

1 Department of Biotechnology, University of South Asia, Lahore, Pakistan
2 Lahore Garrison University, Lahore, Pakistan
*Corresponding Author's Email: dr.raza03@gmail.com

ABSTRACT: *Day by day demand of ethanol goes on increasing due to use of it in various industrial purposes such as alternative source of energy and also used as important chemicals with biofuels due to its eco-friendly nature, Molasses which is common by product of sugar industry is the least expensive source of ethanol because it does not need hydrolysis of starch. In this study Different yeast strains isolated from soil, environment and fruits were used for bio-ethanol production and were also tested for fermentation of molasses. Evolution of CO_2 confirmed the production of bio-ethanol. Green or greenish yellow color appeared in positive control (Baker's yeast) while no color appeared in negative control (sterilized molasses used as control) and orange color appeared in second negative control i.e. distilled water.*

Key words: *Saccharomyces cerevisiae, Fermentation substrate*

INTRODUCTION

Ethanol, which is an important chemical product has the high tendency to replace fossil fuels as biofuels,is an important chemical product.(Topcagic and Minteer 2006). An exchanged fuel that can easily be used in present day combustion engine by mixing with gasoline as eco-friendly biofuel. (Larsen and Johansen, 2009). Day by day demand of ethanol goes on increasing due to use of it in various industrial purposes such as alternative source of energy and also used as important chemicals with biofuels due to its eco-friendly nature. (Topcagic and Minteer 2006; Lee and Kessler, 2007). By using suitable microbial strains, fermentation substrate we can increase the yield of ethanol. (Lin and Tanaka, 2006). Microorganism that have improved flocculating potential, rapid fermented ability, osmo-tolerance, and thermo-tolerance can be used for ethanol production. Few yeast strains have been found to possess suitable characteristics for ethanol production (Panchal et al., 1982; Hacking et al., 1984) although no microbial strain has all these desirable qualities. (Anderson et al., 1986; Bai et al., 2008) and local fermented foods (Brooks 2008) and fermented pineapple juice.

(Eghafona et al., 1999). *Saccharomyces cerevisiae* is commonly used for the ethanol production, (Martın et al., 2002; Hahn-Hägerdal et al., 2007; Bai et al., 2008) Lot of strains which produces ethanol at high rate have been isolated and characterized. But during batch fermentation lot of parameter e.g, product or substrate concentration can inhibit the yield of yeast strains. That's why biologist have great interest to isolate and characterized those yeast strains which have ability to produce high yields of ethanol. Molasses which is common by product of sugar industry is the least expensive source of ethanol because it does not need hydrolysis of starch. Sugarcane molasses is a red brown liquid by-product of sugar extraction which contains about 40-45% sugar, some minerals, metals, vitamins, amino acids, organic acids and gel mass (Roukas 1996; Ren et al., 1997). For fermentation of glucose into bio-ethanol, Molasses is considered a cheap carbon source and it also contains many other suitable nutrient components required for microbial growth which makes it preferable choice for microbiologist (Roukas 1996; Eghafona et al., 1999). K_2HPO_4 is added in diluted molasses as a phosphorous source as molasses does not contain phosphorus in sufficient quantities required for yeast activities. In alcoholic fermentation, characteristic of many yeast species, the fermentation process starts with one molecule of the six carbon sugar - glucose, and terminates with two molecules of the two carbon alcohols - ethanol and two molecules of CO_2.Time of fermentation, ethanol yield, and fermentation efficiency can be improved with optimization of the fermentation process and implementation of new technologies.

MATERIALS AND METHOD

Isolation and purification of yeast strains:

1 gram of Soil was taken and serial dilution method was used to make dilution of sample. 100µl of dilution 10^{-6} and 10^{-7} were added in 2 tubes of YEPD broth separately. Tubes were incubated at 30ºC for 24 hours. 100µl of ach tube was spreaded over YEPD agar plates, incubated at 30ºC for 24 hours. Different colonies were selected and further purified to obtain pure colonies. Microscopic examination was performed for observation of yeast cell morphology. Baker's yeast was used as positive control for ethanol production.

Production of ethanol by using molasses as raw material:

Pre-treatment of Molasses: Molasses was diluted (1:10) with autoclaved distilled water and 2% (w/v) K_2HPO_4 was added in it and was dispensed in tubes (10ml in each)

Inoculum Preparation: Selected colonies were grown in YEPD broth under aerobic conditions for cell proliferation. O.D. was taken at 600nm and cell density was adjusted at 1. Baker's yeast was used as positive control for ethanol production.

Inoculation of yeast strains in molasses tubes: 100µl from each tube was inoculated in sterilized molasses tubes and were incubated at 30ºC under anaerobic conditions for 4 days. .

Confirmation of ethanol by potassium dichromate test:

For detection of ethanol potassium dichromate test was performed.

Potassium dichromate test: Take 2ml of each sample in separate test tubes and add 2.5ml of conc. H_2SO_4 in these tubes and mix them gently. Add 20% K_2Cr2O_7 in each tube drop-wise until green or greenish gray color appears in tubes containing ethanol and yellow or orange color appears in negative control (distilled water).

Confirmation of ethanol by potassium dichromate test: Ethanol production was confirmed by potassium dichromate test as discussed above.

RESULTS

Isolation and purification of yeast strains:

Different colonies appeared on YEPD plates and 4 colonies were selected on basis of their colony morphology and microscopic examination.

Production of ethanol by using molasses as raw material:

Bubble production proves evolution of CO_2 under anaerobic conditions and is indication of glucose fermentation and bio-ethanol production. Increase in volume of medium also indicated CO_2 is dissolved in medium.

Ethanol detection in supernatant: Green or greenish yellow color appeared in positive control (Baker's yeast) and sample tubes (supernatant obtained after centrifugation) while no color appeared in negative control (sterilized molasses used as control) and orange color appeared in second negative control i.e. distilled water.

Confirmation of ethanol by potassium dichromate test:

Green color was observed in sample and positive control and orange color was observed in negative control.

DISCUSSION

Ethanol, due to its wide range use, is very important and its large scale production is usually required. Yeast strains and different cheap sources used as raw materials (like molasses) for bioethanol production are more commonly under investigation to fulfill the ethanol demand which is increasing day by day and , with a rising potential as a biofuel to replace fossil fuels, is an important chemical product. (Topcagic and Minteer 2006). An exchanged fuel that can easily be used in present day combustion engine by mixing with gasoline as eco-friendly biofuel. (Larsen et al., 2009). Ethanol has an increasing demand in various industrial purposes such as alternative source of energy, industrial solvents, cleansing agents and preservatives, has necessitated increased production of this alcohol. (Topcagic and Minteer 2006; Lee et al., 2007) Chemical synthesis of petrochemical substrates and microbial conversion of carbohydrates present in agricultural products usually accomplish ethanol production. Our selected strains have ability to produce bio-ethanol by using molasses as carbon source under laboratory conditions. In future, quantification and purification of the ethanol in extract can be done. Strain identification and modification is also necessary before their use at commercial scale.

REFERENCES

1. Anderson P, McNeil K and Watson E (1986). High-efficiency carbohydrate fermentation to ethanol at temperatures above 40 C by *Kluyveromyces marxianus* var. *marxianus* isolated from sugar mills. Appl. Environ. Microbiol. 51(6): 1314-1320.

2. Bai F, Anderson WA and Moo-Young M (2008). Ethanol fermentation technologies from sugar and starch feedstocks. Biotech. Adv. 26(1): 89-105.

3. Brooks A (2008). Ethanol production potential of local yeast strains isolated from ripe banana peels. Afr. J. Biotech. 7(20).

4. Eghafona NO, Aluyi HAS and Uduchi IS (1999). Alcohol yield from pineapple juice: Comparative study of Zymomonas mobilis and *Saccharomyces uvarum*. Niger. J. Microbiol. 13: 117-122.

5. Hacking A, Taylor I and Hanaz CM (1984). Selection of yeast able to produce ethanol from glucose at 40 C. Appl. Microbiol. Biotech. 19(5): 361-363.

6. Hahn-Hägerdal B, Karhumaa K, Fanseca C, Spencer-Martin I and Gorwa-grauslund MF (2007). Towards industrial pentose-fermenting yeast strains. Appl. Microbiol. and Biotech. 74(5): 937-953.

7. Larsen U, Johansen T and Schrammy J (2009). Ethanol as a fuel for road transportation. Main Report. IEA-AMF report 100.

8. Lee S, Kessler S, Forberich O, Buchwar C and Greemspan DC (2007). Cosmetic, personal care, cleaning agent, and nutritional supplement compositions and methods of making and using same, Google Patents.

9. Lin Y, Tanaka S (2006). Ethanol fermentation from biomass resources: current state and prospects. Appl. Microbiol. Biotech. 69(6): 627-642.

10. Martın C, Galbe M, Wahlbom CF and Jonsson LJ (2002). Ethanol production from enzymatic hydrolysates of sugarcane bagasse using recombinant xylose-utilising Saccharomyces cerevisiae. Enz. Microbial Technol. 31(3): 274-282.

11. Panchal C, Peacock L and Stewart GG (1982). Increased osmotolerance of genetically modified ethanol producing strains of Saccharomyces Sp. Biotechnology Letters 4(10): 639-644.

12. Ren, NQ, Wang B and Huarg JC (1997). Ethanol-type fermentation from carbohydrate in high rate acidogenic reactor. Biotech. Bioeng. 54(5): 428-433.

13. Roukas T. (1996). Ethanol production from non-sterilized beet molasses by free and immobilized *Saccharomyces cerevisiae* cells using fed-batch culture. Journal of Food Engineering 27(1): 87-96.

14. Topcagic S, Minteer SD (2006). Development of a membraneless ethanol/oxygen biofuel cell. Electrochimica Acta 51(11): 2168-2172.