Effect of Temperature on Production Intracellular and Extracellular Invertase by Potential Indigenous Strain Kluyveromyces Marxianus Using Sugarcane Molasses

A.Q Laghari
Chemical Engineering Department, Mehran University of Engineering & Technology, Jamshoro, Pakistan

S.Aziz
Chemical Engineering Department, Mehran University of Engineering & Technology, Jamshoro, Pakistan

Arshad Iqbal
Chemical Engineering Department, Mehran University of Engineering & Technology, Jamshoro, Pakistan

A.Sami
Chemical Engineering Department, Quaid-e-Awam University of Engineering Science & Technology, Nawabshah, Pakistan

Farman Ali Shah
Mehran University of Engineering & Technology, Jamshoro, Pakistan

Abstract

This study is focused on enzymology regarding enzyme purification technique and application of thermotolerant specie Kluyveromyces marxianus yeast in bio reactions for the research and optimization of fermentation temperature for intracellular and extracellular enzyme production. Fermentation studies were carried out in shake flask level to optimize intracellular and extracellular enzyme production by changing process conditions like temperature range from (30 to 55°C), the pH (5.5), speed (350). The substrate type sugar cane molasses 15% added as a carbon and ammonium sulphate (0.75%) as nitrogen source. The optimized fermentation temperature was found 45°C, at pH 5.5, and rpm was 350. The production of intracellular invertase was (890µmoles/min/g) while the extracellular (120µmoles/min/g) Kluyveromyces marxianus was greater efficiency as compared to other specie because of its metabolic activity, which express more heat stability and Invertase activity upto 65°C.

Keywords: Yeast, Fermentation, K.marxianus

DOI: 10.7176/JNSR/9-9-02

Publication date: May 31st, 2019

1. Introduction

Nowadays, most of the people are suffer from daies due insufficient nutrient supply in the diets of human and feed of animals food[10]. Because of to this inconvenience, it is essential to propagate the manufacturing of protein with the help of all available technique. The situation of malnutrition in people living in developed and developing countries have become a growing concern as a result of deficiencies in food protein[11]. With the rapid growth of the world's population, high-pressure effects on the industry of food and the feed for producing sufficient human food and animal feed to meet demands of nutrition. The continual population resource of developing countries calls for increased and improved supply of human food and animal feed. The world's growing demand for protein rich foods has affected the formulation of alternative protein sources to be counterpart to conventional protein sources.

This growing world demand for food and feed proteins has led to the search for unconventional protein sources for supplementing conventional protein source. The companies dealing with animals feed are also facing under priced and over affected by conventional components, [1]

2. MATERIALS AND METHODS

2.1 Purification of Strain

Purification of current strain Kluyveromyces marxianus was done at biochemical engineering laboratory of Chemical engineering Department Mehran University of Engineering and Technology Jamshoro. [2]
2.2 Maintenance of Culture
The practical work was carried out at biochemical laboratory of Department Chemical Engineering Mehran University of Engineering & Technology Jamshoro. All analytical grade chemicals, glassware were purchased, oxide, and Dae-Jung companies from AL-Bourne and Shabbir Scientific Store Hyderabad. The black strip liquid (molasses) for fermentation was purchased from Kharipur and Rani pur District hairpur Mir's. Kluyveromyces marxianus culture was maintained as per method [5, 6] on Saboraud’s Dextrose agar (SDA) slants and plates. Medium was prepared by the mixing of different analytical grade chemicals into distilled water one by one. The shaking volume was made up to 100 ml in an Erlenmeyer flask of 250 ml capacity. the pH of the medium was maintained up to 5.5 by using HCl and NaOH.

2.3 Agar Plates preparation
The following amounts of chemical composition were used for the preparation of agar plates as by. [2]

| Chemicals          | % (w/v) |
|--------------------|---------|
| Agar               | 3.0     |
| Glucose            | 2.0     |
| Peptone            | 0.5     |
| Sodium chloride    | 0.5     |
| Yeast extract      | 1.0     |

Above chemical composition were used for the preparation of nutrient agar plates method mentioned by. [2,3]. These chemicals used as medium for the growth of microorganisms. about 50 ml of distilled water was poured in 250 ml Erlenmeyer flask followed their shaking. After that further water is added maintained up to 100 ml and HCL and Sodium NaOH used for proper maintenance of pH upto5.5.

Purity of culture was checked in compound microscope before preparing inoculum the Fig. 1 shows the growth of Kluyveromyces marxianus strain before purification when it was at raw state, after treatment the strain was purified shown in Fig. 2 for the proper application in the fermentation process for the production intracellular and extracellular enzyme production.[7,8,9]

2.4 Sterilization
The media was sterilized, at 121°C, 15 psi pressure for 15 min. then purity of medium was confirmed after different time interval ,24,48,72 hours. (Madihah et al., 2008; Madigan and Martinko, 2005).

2.5 Preparation of Inoculum
For yeast medium the inocula was prepared according to the following composition (w/v):[3]
TABLE II. CHEMICAL COMPOSITION OF INOCULUM COMPOUND

| Chemical     | % (w/v) |
|--------------|---------|
| Yeast Extract| 1.0     |
| Sodium Chloride| 0.5   |
| Glucose      | 2.0     |
| Peptone      | 0.5     |
| PH           | 5.5     |

2.6 Preparation of Fermentation Medium

Following composition of chemicals are used for the media preparation for fermentation.[2]

TABLE III: COMPOSITION OF CHEMICAL FOR FERMENTATION MEDIA

| Chemical                                      | (%) |age |
|-----------------------------------------------|-----|----|
| Carbon source (Sugar cane molasses)          | 15  |    |
| Nitrogen source (Di ammonium sulphate)       | 0.75|    |
| PH                                            | 5.5 |    |
| Inoculums                                     | 0.5 |    |

3. RESULT AND DISCUSSION

Fermentation studies were undertaken to optimize the temperature by changing process temperature. Fermentation for growth of K. marxianus in the presence of various temperatures, (30°C to 65°C) speed (350 rpm) pH (5.5) for the substrate consumption and invertase production. Molasses were employed to study their effect on growth and production. The intracellular and the extracellular enzyme production at 45 °C by indigenous strain K. marxianus with 15 % sugar concentration of substrate gave the maximum amount of intracellular (890 µmoles/min/g) and the extracellular (150 µmoles/min/g) enzyme. The optimum intracellular and the extracellular enzyme was observed after 48h of fermentation with media containing blackstrap molasses (15% total reducing sugars), the optimized temperature was 45°C, pH 5.5 and speed 300 rpm.

Table IV: Effect of temperature on the extracellular enzyme production at pH 5.5, 350rpm and 48 hours.

| Temp= °C | Sugar/Molasses g/l | Extracellular Activity (µmoles/min/g) |
|----------|---------------------|-------------------------------------|
| 30       | 150                 | 0                                   |
| 35       | 120                 | 450                                 |
| 40       | 90                  | 700                                 |
| 45       | 60                  | 890                                 |
| 50       | 25                  | 850                                 |
| 55       | 5                   | 800                                 |

The above mentioned table IV, the various temperatures was applied from (30°C to 55°C) in fermentation process in order to investigate the optimized temperature for enzyme production. At 45°C temperature the maximum production of extracellular enzyme was obtained. The extracellular enzyme production was (890 µmoles/min/g).

![Fig. 3. Temperature Effect On The Production Of Intracellular Activity at various temperatures.](image-url)
Table V. Effect of temperature on the extracellular enzyme production at pH 5.5, 350rpm and 48 hours.

| Temp°C | Sugar/Molasses g/l | Intracellular activity (µmoles/min/g) |
|--------|--------------------|--------------------------------------|
| 30     | 150                | 0                                    |
| 35     | 120                | 60                                   |
| 40     | 90                 | 140                                  |
| 45     | 60                 | 150                                  |
| 50     | 25                 | 140                                  |
| 55     | 5                  | 130                                  |

In table 5, the various temperatures (30°C to 55°C) was applied from (30°C to 55°C) in fermentation process in order to determine optimized temperature for production of enzyme. The best temperature was 45°C for production of intercellular enzyme. The intercellular enzyme production was (150 µmoles/min/g).

Fig. 4. Temperature Effect On The Production Of Intracellular Activity at various temperatures.

4. CONCLUSION
It is concluded that the indigenous strain Kluyveromyces marxianus can work at high temperature up to 65 °C and at optimum temperature 40°C, pH 5.5, and speed 300rpm it gives maximum intracellular and the extracellular enzyme production. It is economically feasible for large scale production because it reduces the cooling cost. In Pakistan it can be use to produce the enzyme which can be utilize as food supplement to overcome on the malnutrition problem.

5. ACKNOWLEDGEMENT
My deepest feeling of thanks and appreciations are to my Department of Chemical Engineering, Mehran University of Engineering and Technology Jamshoro, Pakistan for providing me best research facilities and a great deal of knowledge that helped me to achieve my goals and Objective of my research.

I feel a great pleasure and honor to express my heart full gratitude to my supervisor Prof. Dr. Shaheen Aziz, and Co-supervisor, Prof. Dr. Syed Farman Ali Shah, for their proper guide line, technical support and sympathetic attitude throughout my experimental work at MUET.

References
[1] G. Suman, M. Nupur, S. Anuradha, B. Pradeep"International Journal of current micro biology and applied science" Single Cell Protein Production, Vol. 4, No. 9, pp. 251-262, 2015
[2] S. Aziz, H. Rehman Memon, F. Ali Shah, M.Ibrahim Rajoka, S. Ahmed Soomro” Production of Ethanol by Indigenous Wild and Mutant Strain of Thermotolerant Kluvyeromyces Marxianus Under Optimized Fermentation Conditions” Pak. J. Anal. Environ. Chem. Vol. 10, No. 1 & 2 2533, 2009
[3] F. Ali Shah, S. Aziz, H. Rahman Memon, Z. M Ali, M.Ibrahim Rajoka "Enhanced Production of Invertase from Thermotolerant Yeast Through Black Strap Molasses a Waste Product of Sugar Industry” Australian Journal of Basic and Applied Sciences, Vol.5 No.1, 48-54, 2011
[4] M. Shuler "Investigation of the Performance and Kinetics of Anaerobic Digestion at 45°C "Journal of Water Resource and Protection. Vol.7 No.14, 2015
[5] M. Ibrahim Rajoka, F. Latif, S. Khan and R. Shahid, "Kinetics of improved productivity of β galactosidase by a cycloheximide resistant mutant of Kluvyeromyces marxianus. Biotechnol" Lett. 26 (2004) 714.
[6] M. Ibrahim Rajoka, S. Khan, R. Shahid" Regulation of Galactosidase Production from K. marxianus" Food Technol. Biotechnol. Vol.41, No.4, pp.315–320 2003
[7] I. Banat, R.Merchant, “Isolation of thermotolerant, fermentative yeasts growing at 52 °C and producing ethanol
at 45 °C and 50 °C”. World J. Microbial Biotech. V.8, pp. 259-263, 1992.

[8] S. Limtong, W. Yongmanitchai, “Production of fuel ethanol at high temperature from sugar cane juice by a newly isolated Kluyveromyces marxianus,” Letters in App. Microbial. V. 98, pp.3367-3374. 2007

[9] Brady, D. Logan, S.R, McHale, “The effect of soluble alginate and calcium on b-galactosidase activity produced by the thermotolerant, ethanol-producing yeast strain Kluyveromyces marxianus” Bioproc Eng, V.18, pp. 101–104, 2007

[10] U. Bacha, M. Nasir, A. Khalique, A. A. Anjum, and M. A. Jabbar “comparative assessment of various agro-industrial wastes for saccharomyces cerevisiae biomass and its quality evaluation as single cell protein” The Journal of Animal & Plant Sciences, Vol. 21, No.4, pp. 844-849, 2011, Page:

[11] K. Hanim, A. Rahman, S. J Hanim, M. Yusof Z. Zakaria “Bioproteins Production from Palm Oil Agro-Industrial Waste” Pertanika J. Trop. Agric. Sci. Vol.39, No. 1, pp. 29 - 39, 2016