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

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Abstract
This study is focused on enzymolgy 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 55^0C), 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 45ºC, 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 activty upto 65^0C.
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1.Introduction

Nowadays, most of the people are suffer from daises 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 greade 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 Khairpur and Rani pur District hairpur Mir's. Kluyveromyces marxianus culture was maintained as per methood [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]

TABLE I. CHEMICALS OF NUTRIENT AGAR PLATE

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.

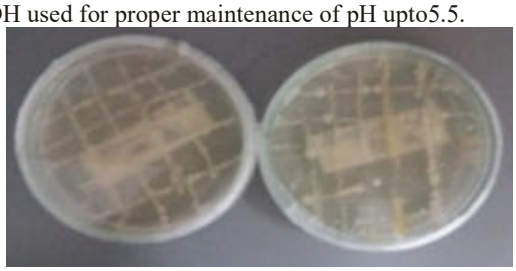

Fig. 1. *Kluyveromyces marxians* strain before purification

Fig. 2. *Kluyveromyces marxians* strain after purification

Purity of culture was checked in compound microscope before preparing inoculum the Fig. 1 shows the growth of *Kluyveromyces marxians* 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 use 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

Fermantation 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°t 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 ºC 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= ºC	**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 (30ºC to 55ºC) in fermentation process in order to investigate the optimized temperature for enzyme production. At 45ºC temperature the maximum production of extracellular enzyme was obtained. The extracellular enzyme production was (890 µmoles/min/g).

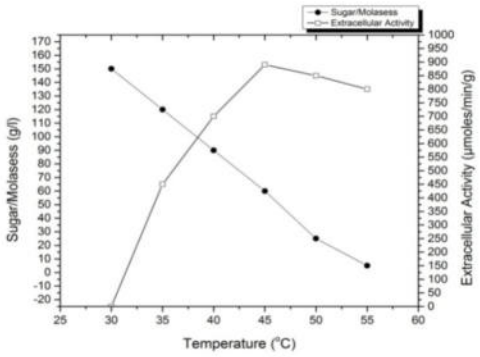

Fig. 3. Temperature Effect On The Production Of Intracellular Activity at various temperatures.

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

Temp= ^{o}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^{o}C to 55^{o}C) was applied from (30^{o}C to 55^{o}C) in fermentation process in order to determine optimized temperature for production of enzyme. The best temperature was 45^{o}C for production of intercellular enzyme. The intercellular enzyme production was (150 µmoles/min/g).

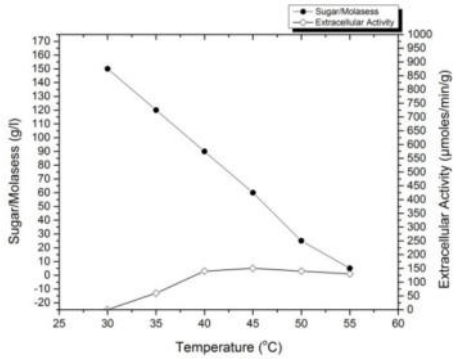

Fig. 4. Temperature Effect On The Production Of Intracellular Activity at various temperatures.

4. CONCLUSION

It is concluded that the indigenous strain Kluyveromyces marxianus can work at high temperature up to 65 ^{0}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.

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