

ISOLATION OF YEAST AND EXTRACTION OF SUPEROXIDE DISMUTASE & β GLUCANASE AND ITS APPLICATION IN AGRICULTURE

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1. Abstract

Enzymes are highly efficient biocatalysts researched for industrial-scale catalysis because of their several distinct advantages. The present research includes the extraction of enzymes that have agricultural applications from yeast. The yeast cells were used to produce enzymes that had agricultural applications. The yeast cells were first isolated from two different curd sources. Isolation was done by serial dilution followed by the spread plate technique was employed for the collected curd samples. The isolates were grown on specific media like Yeast Potato Dextrose (YPD) medium. Cultural, and morphological study was performed for the selected colonies. The isolated yeast cells were screened for the production of enzymes like superoxide dismutase and β Glucanase. These enzymes are Significant and have extensive applications in agriculture. The yeast cells were then grown on YPD broth and incubated at 30°C for 24 h in an orbital shaking incubator at 180 rpm. The cells were centrifuged and lysed with cell-specific buffer for extraction of enzymes. Qualitative and Quantitative analysis was performed on the crude enzyme suspension to understand the activity rate of superoxide dismutase and β glucanase. The main study involves the action of the enzymes, superoxide dismutase and β glucanase on seed germination and plant growth. These enzymes can be used in modern agriculture to gain tolerance to stress conditions and reduce reliance on chemical inputs, making agriculture more eco-friendly.

Keywords: Yeast, superoxide dismutase, β glucanase, seed germination, plant growth

2 . Introduction

Enzymes are proteins composed of hundreds and thousands of amino acids that act as catalysts. Catalysts accelerate any biochemical reaction. Enzymes that act on molecules are known as substrates, and they are converted into products. Enzymes can be produced in large quantities by microorganisms for industrial applications (Choi, J.-M., *et.al* 2015) The uses are extends to food production, preservatives, textual manufacture in the paper industries, pharmaceuticals, and agriculture. Various organisms produce enzymes: fungi account for 60%, bacteria 24%, higher animals 6%, and plants 4%. Currently, fungal enzymes account for more than 50% of the total enzyme market (Dhevagi. *et al.* 2021.) The commercial-scale enzyme production requirements for some species in Yeast, *Aspergillus*, *Trichoderma*, *Rhizomes*, and *Penicillium* genera can be partially attributed to their large market share(El-Gendi, H., Saleh,*el. al* 2021). Yeast cells has been used for biotechnological purposes, but very

few studies reported that yeasts have the ability to produce a group of plant growth-promoting activities and biocontrol activity. (Chaib, I 2024) Therefore, the main aim is to highlight the application of yeasts as biological agents in different sectors of sustainable farming practices. (Satyanarayana, T., & Kunze, G 2009.) Yeast cells are screened for the production of Superoxide Dismutase and β Glucanase for their applications in agriculture. Superoxide dismutase (SOD) is a class of enzymes biological oxidant enzyme which can respond to oxidative stress, lipid metabolism, inflammation, and oxidation. It prevents the toxic effects of free radicals and be effective in antitumor, anti-radiation, and anti-aging studies (Szöllősi, R. 2014), (Zheng, M., Liu, *et.al* 2023). Beta-glucanases are enzymes, believed to have appreciable ecological implications and have commercial usage in several industries such as food, feed, healthcare, environment and energy (Edison, L. K., & Pradeep, N. S. (2022)). Microorganisms are the most preferred sources for industrial beta-glucanases because of their easy obtainability and rapid growth rate. Plant-associated microbes have evolved in such a way that such associations now play an important role in a healthy ecosystem. (Jha, Y. 2022).

3 Material and Methods

3.1 Material

3.1.1 Media Requirements

Potato dextrose agar (PDA), Yeast extract peptone dextrose agar (YEPD), Yeast extract peptone dextrose broth (YPD Broth), Carboxymethylcellulose (CMC) Agar, Yeast Glucan Agar.

Importance of Media used

Potato dextrose agar: Potato Dextrose Agar (PDA) is used for fungi cultivation. Potato Dextrose Agar (PDA) is a general-purpose medium for yeasts and molds that can be supplemented with acid or antibiotics to inhibit bacterial growth. PDA can be used for growing clinically significant yeast and molds. The nutritionally rich base (potato infusion) encourages mold sporulation and pigment production in some dermatophyte Dextrose Agar (PDA)- Principle, Uses, Procedure & Characteristic (Stam, R. (2016))

Yeast Extract Peptone Dextrose Agar (YEPD)

YEPD consists of yeast extract, peptone, and glucose or dextrose. Yeasts grow well on a minimal medium containing only dextrose and salts. Carbon supplement in the form of dextrose, and nitrogen in the form of peptone are key ingredients in the media. This medium supports the enhanced growth of both natural and mutated types of all kinds of growing yeast (YEPD Agar Plates. (2011), YPD media. (2010), (Cold Spring Harbor Protocols, 2010))

Carboxymethylcellulose (CMC) Agar

CMC is used as a selected medium for isolation and used for enzyme activity study of microorganisms, especially observing its breakdown of the substrate present in the media. Used for Congo Red test Glucanase activity. (Triani, H. D., Marlida *et.al* (2024)).

3.1.2 Chemicals:

Gram staining Reagent (crystal violet, iodine, alcohol, safranin), Lactophenol cotton blue, Ethylenediaminetetraacetic acid (EDTA), Potassium dihydrogen phosphate (KH_2PO_4), Dipotassium phosphate (K_2HPO_4), Sodium acetate, Sodium hydroxide (NaOH), Acetic acid.

3.1.3 Instrumentation requirements:

Glassware, Autoclave(Shital scientific industries), incubator(Remi instruments), cold centrifuge(Remi C- 24BL), colorimeter(Digital colormeeter 112), orbital shaker, weighing balance(Infra digit), Microscope, Nanodrop UV VIS Spectrophotometer(Infra digi version 1.0), Laboratory stirrer(Remi 127/ A), Magnetic stirrer (Tarsons digital spinot)

3.2 Methods**3.2.1 Isolation of Yeast from Curd Samples****3.2.2 Serial Dilution of Curd Samples**

Take 1gm of curd and add it to a 100 ml sterile water flask. Take 10 sterile test tubes; from the stock solution 0.5ml is pipetted out into the 1st test tube, which contains 4.5ml of sterile water. The dilution is thoroughly mixed several times. .05 ml of the mixture is taken from the 10^{-1} dilution and transferred to the second tube. The same process is repeated for the remaining tube. Further, 0.1ml of the solution should be taken from the 1st test tube and plated in YPD agar at 37°C for 42 hours(Serial Dilution. (2010)

3.2.3. Morphological Identification**3.2.3.1 Cultural Characters**

The size, shape, color, elevation, opacity, nature, and margin of the isolated colonies were observed on PDA plates.

3.2.4 Gram Staining

Gram staining is one of the most basic and important techniques in microbiology. It was discovered by Hans Christian Gram in 1882. To perform the test. The well isolated colony is collected from an inoculated plate with the help of a sterile loop, making a smear with the help of distilled water on a clean glass slide. Heat fixes the slide and stain it with crystal violet, iodine for 60 sec, alcohol is used for 20 sec to de-stain the excess and finally, safranin is used for 60sec. the slide is left for air dry and observe it under a microscope(Dey, P. (2022).)

3.2.5. Lactophenol Cotton Blue (LPCB) Staining

Add a drop of water to a clean glass slide. Add the fungal specimen to the water drop using an inoculation loop (from a solid medium), depending on the sample used. Fungal mounted slide prepared in the above step was stained with Lactophenol Cotton Blue and further studied microscopically to identify its morphological characteristics (Kali, A. 2014).

3.2.6. Extraction of Enzymes

Extraction of Superoxide Dismutase (SOD)

Yeast cells were produced on YPD broth. For the extraction, 250 ml of YPD Broth is inoculated with a loopful of isolated yeast cell culture and incubated at 32°C hrs, in the orbital shaker for 72hrs. Cell suspension was subjected through centrifugation of culture for 10 min at 4°C at 5000 rpm. The cell pellet was collected and washed with PPbuffer (Potassium phosphate buffer) twice. EDTA and PP buffer were taken in (1:1 ratio) was subjected to cell lysis. The cell were subjected to a magnetic stirrer with a magnetic bead for cell lysis (1800 rpm for 30 min). The cell debris is removed by centrifugation at 1300 rpm for 15 min. (Mesa-Herrera, F. *et.al* 2019, Dellomonaco, C., Amaretti, A *et.al.* 2006)

Extraction of β Glucanase

Yeast cells were produced on YPD broth. For the extraction, 250 ml of YPD Broth is inoculated with a loopful of isolated yeast cell culture and incubated at 32°C hrs in the orbital shaker for 72hrs. Cell suspension was subjected to centrifugation of culture for 10 min at 4°C at 5000 rpm. The cell pellet was collected and subjected to a magnetic stirrer with a magnetic bead for cell lysis Sodium acetate buffer. The cell debris is removed by centrifugation at 1300 rpm for 15 min (Wood, P. J., & Jørgensen, K. G. (1988).

3.2.7. Quantitative Analysis

3.2.7.1 Nano Drop Technique

NanoDrop spectrophotometer, quantifying a protein sample concentration is as easy as a click of the pipette, a push of a button, and a dab of tissue to clean up.

Blank the Nano Drop

Add 2 μ L of the buffer which is taken as blank. Lower the upper arm of the NanoDrop and click the “Blank” button on the software. Wait 20 seconds for the blank measurement to be made. lift the upper arm and dry the pedestal with a wipe.

Measure the Protein Sample

Add 2 μ L of the sample to the lower pedestal and lower the upper arm. Click the “Measure” button on the software and wait 20 seconds for measurement. (Nucleic Acid v1. (2017)

3.2.8 Qualitative Test

3.2.8.1 Pyrogallol Antioxidation Assay (SOD).

First, 1M Tris HCL was prepared then 10 ml, weight 0.0378 g of pyrogallol and dissolved in Tris HCL. Store at 4°C under dark conditions. Adjust pH to 8.2 with Tris and dilute to 50 ml with distilled water. The 2ml enzyme sample was incubated in 2 ml of the prepared reagent Measure the absorbance at 420nm every 30 sec for every 3 min (Mesa-Herrera, F., Quinto-Aleman, D., & Díaz, M. (2019).

3.2.8.2 Congo Red Test for Glucanase Detection

The method was useful for measuring enzyme activities using Congo Red Test. It is an rapid and qualitative assay used to detect glucanase activity by hydrolysis zones on an agar plate containing β -glucan substrates. The principle of the test relies on the ability of Congo Red dye to bind to intact glucans. When glucanase enzymes degrade the substrate, the dye no longer binds, creating clear zone around colonies or enzyme application sites. (Wood, P. J., & Jørgensen, K. G. (1988).

3.2.9 . Incubation of Seeds in Superoxide Dismutase and β Glucanase for Seed Germination

Seed samples such as Green Gram and Fenugreek were used. The seeds were soaked in enzymes for 16 hours and then soaked in sterile soil. The germination rate of the seeds is studied

4. Results and Discussion

4.1 Collection of Sample

Different curd sample was collected from different source

4.1.1 Isolation of Microorganisms

After incubation, different colony morphology was observed on incubated plates spread plate technique is used for the Inoculation of four different curd samples on PDA Plates by the spread plate technique



Fig -4.1.1 YEPD plate

4.1.2 Characteristics of Yeast Isolates

Table -4.1.2 : Characteristics of yeast isolates

Colony Morphology	
Colony colour	White, cream-yellow
Colony texture	Smooth, mucoid
Colony elevation	Flat

4.2. Morphological Identification

4.2.1. Gram Staining

Gram staining was performed for the fungal colonies. Four samples show gram-positive round oval budding cells under 100x were carried further for characterization

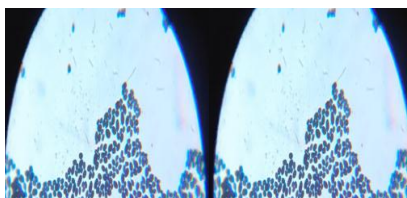


Fig- 4.2.1 Yeast cells under 100x microscope

4.2.2. Fungal staining

Staining was performed for the fungal colonies. samples show Purple color ,Oval Budding cells under 45x.

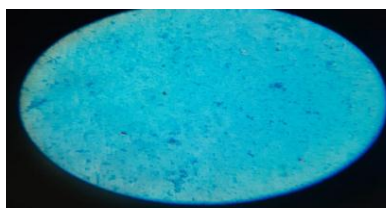


Fig -4.2.2 Yeast cell under 45x microscope

4.3. Extraction of Enzymes

4.3.1 Superoxide Dismutase

The enzyme was obtained after centrifugation after cell lysis

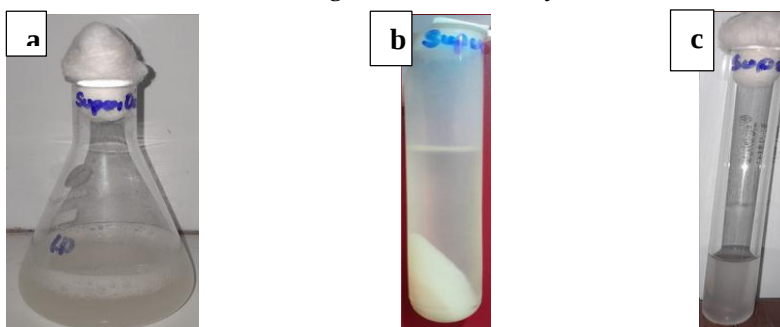


Fig 4.3.1 a- Cells treated with lysis buffer, b- Cell debris and enzyme, c- Extracted enzyme of Superoxide Dismutase

4.3.2 β Glucanase

The enzyme is obtained after centrifugation

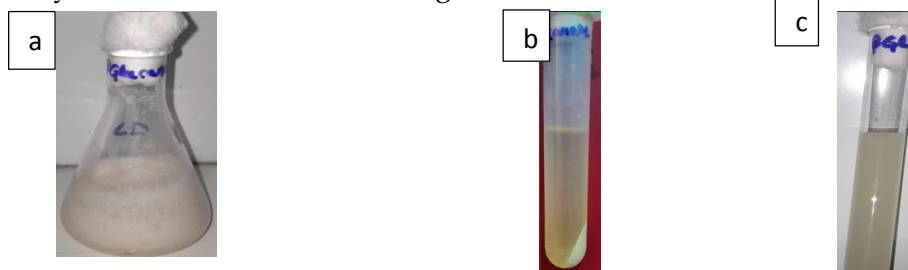


Fig -4.3 a- Cell treated with lysis buffer, ,b- Cell debris and enzyme, c-Extracted enzyme. of β Glucanase

4.4. Quantitative analysis:

4.4.1. Nanodrop analysis

4.4.1.1 Superoxide Dismutase

The protein concentration for Superoxide Dismutase was found to be 8.854 mg/ml under 340nm. This result confirms that the enzyme prepared is suitable for further enzymatic study.

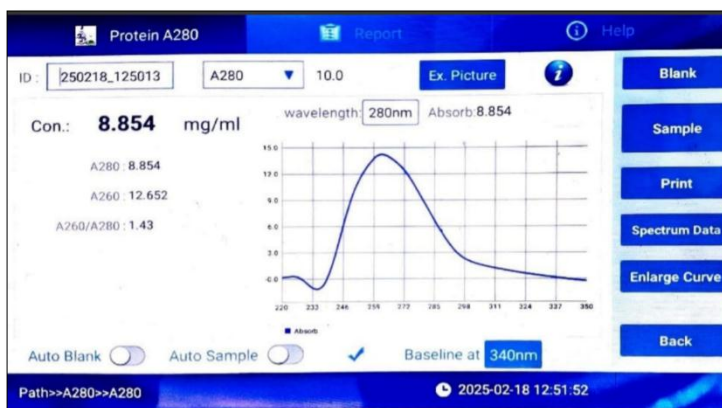


Fig- 4.4.1.1 Nano drop analysis of Superoxide

4.4.1.2. Beta Glucanase: The protein concentration for Beta glucanase was found to be 9.220 mg/ml under 340nm. This result confirms that the Glucanase prepared is suitable for further enzymatic study.



Fig- 4.4.1.2 Nano drop analysis on Glucanase

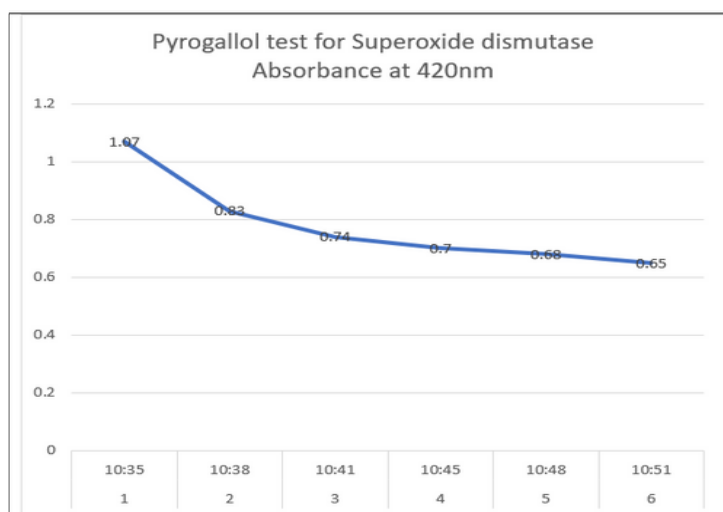
4.5. Qualitative Test

4.5.1. Pyrogallol Antioxidation Assay (SOD)

Superoxide is an antioxidant that prevents the oxidation of pyrogallol as time increases there in a reduced in the absorbance rate

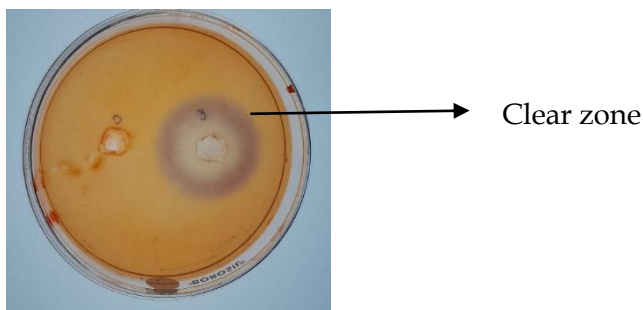
Table -4.5.1 : Pyrogallo test results

Pyrogallol Test for Superoxide Dismutase		
s.no	time	Absorbance at 420nm
1	10: 35	1.07
2	10:38	0.83
3	10:41	0.74
4	10: 45	0.70
5	10:48	0.68
6	10:51	0.65

**Fig- 4.5.1 Absorbance graph**

4.5.2. Congo Red Test for Glucanase Detection

When glucanase enzymes hydrolyze β -glucans, the dye-bound polymer is degraded, leading to a change in color or Clear zones around colonies or enzyme application sites indicating glucanase activity (β -glucan degradation).

**Fig-4.5.2 Congo test plate with clear zone**

4.6. Application of Enzymes

4.6.1 Superoxide Dismutase

Superoxide dismutase (SOD) isozymes are compartmentalized in higher plants and play a major role in combating oxygen radical-mediated toxicity. In plants provides oxidative stress resistance, correlating age, species, and specificity of plants during development.

It's an important component in plant defense machinery. SODs constitute the first defense line against abiotic stress-acquired enhanced ROS and its reaction products, where SODs catalyze the dismutation of O_2 to H_2O_2 and O_2 . Here under, recent reports available on the modulation of SODs in abiotic-stressed plant. (Szöllősi, R., Pinmanee *et.al* 2022)

4.6.2. β Glucanase

β Glucanase helps plant gametes during sexual reproduction and also protects the cells against fungal infection by degrading its cell wall. (Jha, Y. (2022)

4.7 Incubation of Seeds in Enzyme

Table 4.7: Seed Observations

s.no	Picture	Seed Germination	Radical Formation	Shoot Formation	Leaf Formation	Flower
Day 0		-	-	-	-	-
Day 1		-	-	-	-	-
Day 4	 Green gram Glucanase	✓	✓	Sample1 2.8	Sample 1- 1.4	-
		✓	✓	Sample 2 - 6	Sample2 -1	-
		-	-	Sample3 -7	Sample3 -2	-
		-	-	Sample 1- 1	Sample 1-0.5	-
		-	-	Sample2 -0.8	Sample3 -2	-

 Superoxide    Fenugreek Glucanase 			Sample3 -5 - -	-	-

	 Superoxide Dismutase   					
Day 5	 Green Gram Glucanase  	✓ ✓ ✓ -	✓ ✓ - -	Sample1 -9.7 Sample2 -13.3 Sample3 -11.7 - - -	Sample1 -3 Sample2 -2.5 Sample3 -2.7 - - -	- - - -



Superoxide



**Fenugreek
Glucanase**



	Superoxide 					
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4.7. Discussion

The current study is done on Isolation of yeast and the screening of enzymes Superoxide and Glucanase, were treated on seeds and then planted in the soil. Endosperm cell wall started to break down on 3 day and leaf appeared on the 4 day. This study is compared to Wang *et al.*,(2024) studies who carried out the experiment for 3-5 months has observed break down of the endosperm cell wall of seed after 2- 5 days conducted on tomato plant.(Wang *et al.*,(2024)

In Green gram breakdown of endosperm was observed on the 3rd day, which was coated with Glucanase and Superoxide, similar observations on cotton seeds was recorded earlier by Zhu *et al.*, in the year 2022. (Zhu *et al*(2022)

Gobatto, D. R., Carneiro *et. al* in the year 2025, incubated wheat in cool conditions using Superoxide Dismutase and observed effective seed proliferation, In present study the seed were incubated with the enzyme in cooled condition then transferred to the soil in normal room temperature and has shown fast seed germination and no pathogenic activity on 5th day and enhance root formation was observed which has shown similar results as Hwang, D. H., Kim, S. T *et.al.*, in 2007.(Hwang, D. H., Kim, S. T.*et.al.*, (2007).

No pathogenic growth was observed when green gram was treated with Glucanase and superoxide, which was compared to González García's study performed in 2024, which was experimented on grape vine(*Vitis vinifera*) and Meshram, S., Gogoi, R. *et.al* (2022), experimented on Maize plant.(Meshram, S., Gogoi, R *et.al* (2022),(Lee angel, I. P. (2017))

El-Kinany, R. G., Ahmed, M *et.al.*in (2025) performed a study on *Dianthus caryophyllus* for 60 day and monitored physiological responses, flowering time and oxidation stress and shown shoot length to be 35-40 cm on day 45 when compared to the present study Green gram (*Vigna radiata*) has shown 11.3 cm of shoot length on 5th day. Further study on plant growth and pathogen defence mechanism is still going on. (El-Kinany, R. G., Ahmed, M *et.al.*(2025).

5. Appendix

Yeast Extract Peptone Dextrose Agar (YEPA)

Composition

Peptone - 20gm

Dextrose - 20gm

Yeast extract -10gm

Agar -15gm

Yeast Extract Peptone Dextrose Broth

Composition

Peptone - 20gm

Dextrose - 20gm

Yeast extract -10gm

Potato Dextrose Agar

Composition

Potato infusion - 200gm

Dextrose - 20gm

Agar - 20gm

Distilled water - 1000 ml

Carboxymethylcellulose (CMC) Agar

Composition

Carboxymethylcellulose -10gm

Peptone -5gm

Yeast extract -2gm

NaCl -5gm

Dextrose -5gm

Agar -15gm

Distilled water - 1000gm

Ethylenediaminetetraacetic acid (EDTA)

Composition

EDTA -37.22gm

NaOH- ph 8

Distilled water – 800ml

Potassium Phosphate Buffer

Composition

KH₂PO₄ -13.6 gm

K₂HPO₄ - 17.4 gm

Distilled water – 1000ml

Sodium Acetate Buffer

Composition

Sodium acetate – 8.2 gm

Acetate – ph 5

Distilled water 800 ml

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32. Potato Dextrose Agar (PDA)- Principle, Uses, Procedure & Characteristics
YEPD Agar- Composition, Principle, Preparation, Results, Uses
Lactophenol Cotton Blue (LPCB) Staining - Microbe Notes
Figure S6: Comparison of DNA concentration yields obtained from QUBIT and Nanodrop. (n.d.). <https://doi.org/10.7717/peerj.4827/supp-6> Microsoft Word - Measuring DNA Concentration by NanoDrop.docx
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