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Isolation, Screening Identification and optimization of Amylase Producing Yeast

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Abstract:

Amylases are glycoside hydrolases, which are enzymes that facilitate the hydrolysis of complex sugars, such as starch and glycogen, into smaller sugar molecules. A total of 25 diverse natural samples were used to isolate amylolytic yeast. Specifically, samples from apples, custard apples, wine, toddy, potatoes, and soil were collected from the Warangal District. Fifteen isolates, namely AVS1, AVS2, AVS3, AVS4, AVS5, AVS6, AVS7, AVS8, AVS9, AVS10, AVS11, AVS12, AVS13, AVS14, and AVS15, exhibited amylolytic activity according to the qualitative test results. Up to 10 yeast isolates do not exhibit obvious zones due to their inability to break down starch. The highest amylase index was found by AVS5 isolate with a value of 1,219 mm and the isolate with the lowest index was AVS15 with a value of 0.096 mm. The pH scale showed that 6 was the most desirable, followed by 5 and 7. 6.0 to 7.0. Enzyme synthesis by prospective yeast was influenced by temperature changes of 10, 20, and 30 C, with peak production occurring at 20 C. The incubation periods varied between 4, 8, and 12 days, with the highest observed amylase enzyme synthesis occurring after 8 days.

Key Words: Amylase, Yeast, Enzyme Production, Optimization

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Introduction:

Enzymes exhibit high efficiency as catalysts in biological reactions. Enzymes enhance the rate of chemical processes by facilitating an alternative reaction pathway that possesses a reduced activation energy. The initial molecules that serve as the target for enzymatic activity are referred to as substrates. Through enzymatic action, these substrates are transformed into distinct molecules known as products. These products play a crucial role in initiating and expediting numerous biochemical events within living cells. The majority of the volume is attributed to microbial enzymes. However, fewer than 50 species are utilized to generate the complete roster of microbial enzymes that hold commercial significance. There is clearly the possibility to seek for species that produce new enzymes or enzymes with improved characteristics and yield[1]. Amylases are glycoside hydrolases, which are enzymes that facilitate the hydrolysis of complex sugars, such as starch (a form of plant food storage) and glycogen (a form of animal food storage), into smaller sugar molecules[2]. They cleave the intermolecular bonds within subunits in polysaccharides, resulting in the formation of monosaccharides (glucose) and disaccharides (maltose). The starch polysaccharide consists of glucose subunits that are connected by glycosidic linkages formed by the amylose and amylopectin polymers[3,4]. Amylases are ubiquitous in living organisms, including plants, animals, fungi, and bacteria. There are other types of amylases, such as α (alpha) amylases, which can be found in animals and bacteria[5,6]. The β (beta) and γ (gamma)-amylase enzymes found in plants exhibit distinct methods of breaking down bonds inside complicated sugar molecules. Alpha-amylase catalyzes the hydrolysis of long-chain sugar molecules by breaking the bonds at random sites. It exhibits a higher rate of activity compared to other types of enzymes due to its ability to operate on the substrate at any location. In addition,

they possess conserved amino acids such as glutamate and aspartate in the catalytic site [7-8]. Amylases can be obtained from different sources, including plants, animals, and microorganisms. However, microbial sources are typically preferred in industrial settings due to the potential to enhance the production of microbial enzymes through classical genetic techniques, continuous culture selection, induction, or optimization of growth conditions for the desired enzyme. [9-14].Yeast amylases are favoured over other microbial sources due to their generally regarded as safe (GRAS) status, hyphal mode of growth, and ability to tolerate low water activity and high osmotic pressure. These characteristics make yeast highly efficient for converting solid substrates, making them increasingly popular as a source of amylolytic enzymes for industrial use [15-17]. This study was aimed at Screening, Isolation Identification and optimization of Amylase Producing Yeast for Amylase production

Material and Methods:

Isolation of Amylolytic Yeast

A total of 25 diverse natural samples were used to isolate amylolytic yeast. Specifically, samples from apples, custard apples, wine, toddy, potatoes, and soil were collected from the Warangal District. These samples were processed for amylolytic yeast isolation and subjected to T.S. and placed on YEPD. The yeasts were transferred to a YEPD (Yeast extract, Potato and Dextrose) medium containing 1% (w/v) dextrose, 0.5% (w/v) yeast extract, and 1% (w/v) peptone for cultivation. The plates were placed in an incubator at a temperature of 35ºC for a duration of 24 hours. This was done in order to observe the yeast colonies that were selected and then transferred onto new YEPD plates, with the purpose of creating pure cultures.

Screening of amylase-producing yeast

The selection process involved inoculating yeast onto a YPSA medium including starch agar and peptone yeast. By using Agar diffusion method based on [18]. The composition of YPSA includes 10 gm of soluble starch, 5 gm of peptone, 2 gm of yeast extract, 0.1 gm of $MgSO_4.7H_2O$, 0.1 gm of $CaCl_2.7H_2O$, and 0.5 gm of KH_2PO_4, with a total volume of 1000 ml. The process of creating wells using aseptic straws was performed on YPSA agar, which had been divided into four quadrants. The measurement of amylase activity was conducted using the iodine-starch method [19]. Approximately 20 µL of yeast solution was introduced into the medium. The media composition involved creating a yeast suspension by adding 5 mL of sterile distilled water to the yeast isolates in the working culture. The resulting suspension was then homogenised using a vortex. The incubation process was conducted at a temperature of 30 °C for a duration of 72 hours. The presence of amylase activity is shown by the formation of a distinct transparent area surrounding the colony upon iodine application [20]. The relationship between the diameter of the clear zone and the diameter of the colonies is quantified as relative enzyme activity [21].

The amylolytic index was measured to evaluate the capacity of yeast isolates to generate amylase. The amylase enzyme measurements were replicated three times for each sample [22]. The amylolytic index value is determined using Goldbeck's formula, which is IA = 2.6. Yeast morphology observation [23]. Yeast isolates with the highest capacity to generate amylase enzymes are observed. The macroscopic inspection of yeast morphology encompasses the examination of the colony's texture, colour, surface, profile, and edge [24].

Biochemical and morphological Characterization

The morphology of yeast and vegetative cells was determined by culturing them in yeast extract mannitol agar (YEMA) media, using the techniques described by Kreger-Van Riij [25] and Kurtzman and Fell [26]. Every isolate was subjected to a series of biochemical assays, including assessments for amylase reduction, catalase activity, urease activity,

cellulase generation, hydrogen sulphide production, casein hydrolysis, citrate utilisation, lipase activity, and carbohydrate fermentation (specifically glucose, lactose, sucrose, dextrose, and mannitol).

Selection of Organism

Among 15 yeast strains, one strain showed highest levels of amylase activity according to the results of amylolytic index, i.e., AVS5 which was selected for further studies.

Optimization studies

Effect of pH

Incubation was carried out at pH 5, 6, and 7 using yeast inoculation culture media that contained enzymes. A solution of 0.1N HCl and 0.1N NaOH was used to alter the pH of the growth medium.

Effect of Temperature

The evaluation of enzyme production was conducted by varying the temperature at three different levels: 10, 20, and 30°C. Following incubation, the culture medium was filtered and examined for the degree of variance expressed as a percentage.

Effect of different nutrient sources

The nutrient source was optimised by selecting specific carbon sources (glucose), nitrogen sources (peptone extracts), phosphorous source (Dipotassium phosphate), and trace element (zinc sulphate) for probable fungal species. Following incubation, the culture medium was filtered and examined for the degree of variance expressed as a percentage.

Results and Discussion:

Despite the significant influence of genetics and microbial physiology on enzyme production, it is emphasised by multiple reviewers (Steele and Stowers 1991; Bull et al., 1992) that

screening programmes to identify microorganisms capable of producing bioactive molecules remain a crucial aspect of biotechnology.

In the present investigation, we have successfully isolated a yeast strain from Custard apple.

A total of 25 diverse natural samples were used to isolate amylolytic yeast. Specifically, samples from apples, custard apples, wine, toddy, potatoes, and soil were collected from the Warangal District. The yeast is inoculated from isolates that have been examined and found to grow at a temperature of 35°C and a pH range of 4-5. The qualitative testing involved the administration of iodine, which resulted in the creation of distinct clear zones. Starch substrate utilized for amylase activity assay. Starch, a kind of carbohydrate, necessitates the presence of the amylase enzyme for digestion. The presence of amylase-producing yeast isolates can be detected by observing the emergence of transparent areas surrounding the yeast colonies. In order to ascertain the existence of a distinct area devoid of bacteria, a concentrated starch medium that had been excessively colonized by bacteria was treated with a solution of lugol's iodine. Upon application of the solution, the region beyond the clear zone will exhibit a purplish-blue coloration due to the reaction between lugol's iodine solution and unhydrolyzed starch. The absence of staining in clear zones is attributed to the hydrolysis of starch present in the zone, resulting in the formation of simpler molecules like disaccharides or monosaccharides.

Fifteen isolates, namely AVS1, AVS2, AVS3, AVS4, AVS5, AVS6, AVS7, AVS8, AVS9, AVS10, AVS11, AVS12, AVS13, AVS14, and AVS15, exhibited amylolytic activity according to the qualitative test results (Table 1). The presence of a distinct area devoid of any growth surrounding the yeast colonies indicates that the isolate possesses the ability to break down starch by hydrolysis and has the capacity to generate amylase enzymes. Up to 10 yeast isolates do not exhibit obvious zones due to their inability to break down starch. This is

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because each isolate requires various carbon sources. The occurrence of distinct regions in yeast has the capacity to generate amylase enzymes due to the conversion of starch into simpler compounds such as disaccharides and monosaccharides through hydrolysis [27]. The lack of distinct regions surrounding the colonies suggests that there has been a chemical interaction between iodine reagents and starches that have not undergone hydrolysis in the YPSA medium. [28].

The Amylolytic Index values generated by 15 isolates exhibit variations. This suggests that each isolate exhibits variation in their ability to break down starch in YPSA medium. The AVS5 isolates had the greatest amylolytic index value of 1,219 mm, followed by the AVS09 isolates with a value of 0.958 mm (Figure 1). The AVS15 isolates had the lowest amylolytic index value of 0.096 mm.

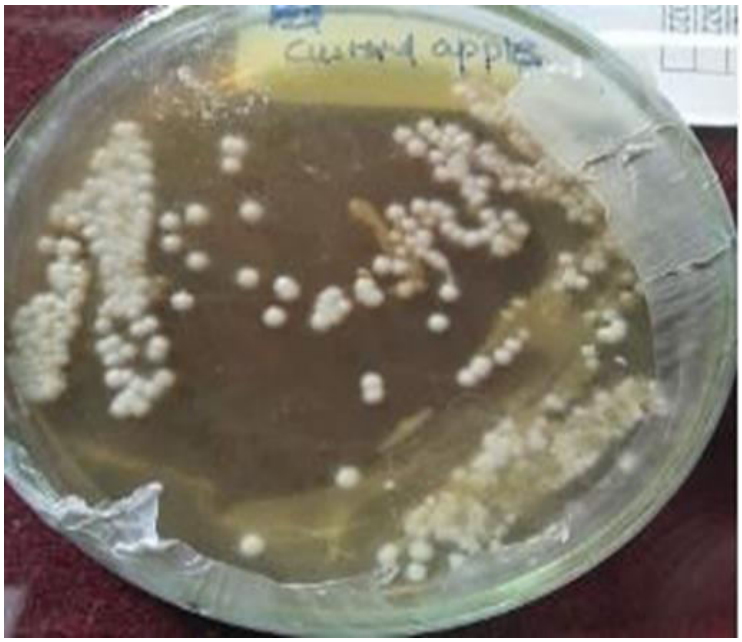

Figure: 1 Represents the pure culture of *Candida parapsilosis* (OR793848.1)

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Isolates	Diameter (mm) Clear Zone
AVS1	0,351
AVS2	0,361
AVS3	0,722
AVS4	0,9
AVS5	**1,219**
AVS6	0,206
AVS7	0,220
AVS8	0,655
AVS9	0,958
AVS10	0,255
AVS11	0,693
AVS12	0,225
AVS13	0,148
AVS14	0,544
AVS15	0,096

Table 1. Amylolytic index value (IA) of the yeast isolates from the YPSA medium.

Using the internal transcribed spacer (ITS) sections of the rRNA gene as a barcode, the specific strain and isolate that produces amylase were identified. The BLAST n identity score match > 99.99 percent against sequences in the GenBank was used to identify the species level. The isolate of Candida parapsilosis (OR793848.1) (Figure 2)(Table 2) was shown to be an ethanologenic yeast. We next got accession numbers and deposited each isolate's sequence in GenBank, a database maintained by the National Centre for Biotechnology Information. The present isolate and related strains' sequences were obtained from GenBank, aligned with ClustalW, and a phylogenetic tree was built using MEGA software version 11.0 to assess their phylogenetic position and infer their evolutionary history. The neighbour joining

method was used to generate the phylogenetic tree from the evolutionary distance data obtained from the Jukes-Cantor model.

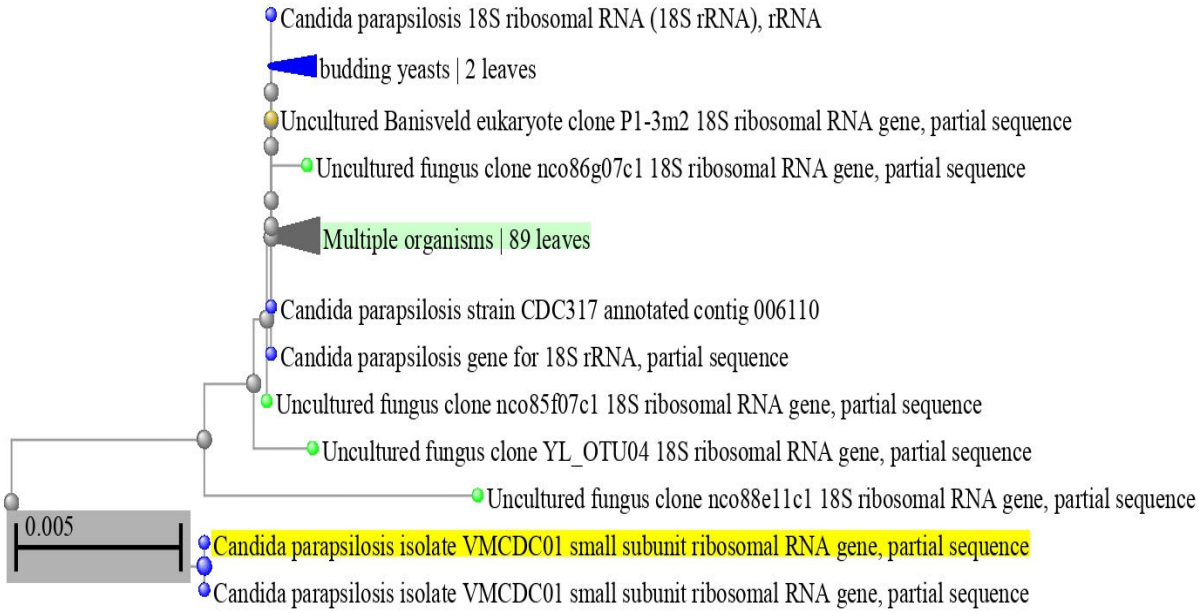

Figure: 2 Phylogenetic Tree of *Candida parapsilosis* (OR793848.1)

Accession	Description
KY118177.1	*Candida parapsilosis* isolate XS2
KY118176.1	*Candida parapsilosis* isolate XS1
KY263515.1	*Candida parapsilosis* strain SZ5
KC674155.1	Uncultured fungus clone nco85c10c1gene
XR_005013742.1	*Candida parapsilosis*
HE605209.1	*Candida parapsilosis* strain CDC317
EU091828.1	Uncultured Banisveld eukaryote
AY520248.2	*Candida parapsilosis* strain BG02-7-18-013C-C-1
KC674614.1	Uncultured fungus clone nco86g07c1sequence
KY118181.1	*Candida orthopsilosis* isolate XS6

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Table 2: Table representing the identified species with their respective accession number while sequence alignment with culture *Candida parapsilosis* (OR793848.1)

One of the most important steps in employing microorganisms in fermentation technology is optimizing growing conditions [29].

A variety of yeasts were tested for their potential to optimize enzyme output. High production and an excellent zone of measurement were observed in *Candida parapsilosis* (OR793848.1). The pH scale showed that 6 was the most desirable, followed by 5 and 7. High temperatures may render the gene encoding the starch-degrading enzyme inactive, according to a previous study (Aiba et al., 1983) [30]. According to Gupta et al. (2003) [31], the majority of the starch-degrading bacterial strains can be grown and enzymes can be produced within a pH range of 6.0 to 7.0. Enzyme synthesis by prospective yeast was influenced by temperature changes of 10, 20, and 30 $_{\beta}$C, with peak production occurring at 20 ^{k}C. The incubation periods varied between 4, 8, and 12 days, with the highest observed amylase enzyme synthesis occurring after 8 days. According to research by Sujeeta et al. (2017), when incubated at 30°C for longer periods of time, yeast isolates' amylase activity increases from 2.08 to 9.36 U/ml[32].

The present study involved the manipulation of nutritional content to investigate the effects on the amylase enzyme produced by *Candida parapsilosis* (OR793848.1). The concentration of glucose, a nutrient and carbon source, was varied. The production at a concentration of 1.0 mg/l of glucose was higher compared to other concentrations. The addition of peptone as a nitrogen source at a concentration of 1.0mg/l resulted in a significant increase in enzyme production. Peptone is the optimal nitrogen source for amylase production, whereas bacto-tryptone promoted the highest level of development. While Adenosine mono phosphate facilitated the highest level of growth, K_2HPO resulted in the highest level of amylase

production. The presence of calcium ion resulted in the highest levels of growth and amylase production for both C1 and C2, as observed in a study conducted by Chandrima in 2010[33]. The α-Amylase is a calcium metalloenzyme that contains at least one calcium ion bound to its molecule. The augmented proliferation of bacteria and synthesis of enzymes could be attributed to the heightened accessibility of calcium ions [34].

The concentration of dipotassium phosphate was modified to 0.5, 1.0, and 1.5 mg/l as phosphorus content. A concentration of 1.2 mg/l was found to be optimal for the synthesis of amylase enzyme. In contrast, the iron content was varied at 0.2, 0.4, and 0.6 mg/l in the treatment. A concentration of 0.18 mg/l was shown to be optimal for the generation of amylase enzymes.

Conclusions:

There were 15 yeast isolates which showed amylolytic activity from various natural substrates with the codes AVS1to AVS15. The highest amylase index was found by AVS5 isolate with a value of 1,219 mm and the isolate with the lowest index was AVS15 with a value of 0.096 mm. The pH scale showed that 6 was the most desirable, followed by 5 and 7. 6.0 to 7.0. Enzyme synthesis by prospective yeast was influenced by temperature changes of 10, 20, and 30 C, with peak production occurring at 20 C. The incubation periods varied between 4, 8, and 12 days, with the highest observed amylase enzyme synthesis occurring after 8 days. This isolate can be used industrially for amylase production.

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