Biological Forum – An International Journal **15(5): 450-454(2023)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Isolation and Screening of Amylolytic Yeast from various Natural Sources

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(Received: 27 February 2023; Revised: 17 April 2023; Accepted: 19 April 2023; Published: 20 May 2023)
(Published by Research Trend)

ABSTRACT: The objective of this research is to get the isolates of yeast which have the potential to produce amylase. Amylase enzymes are the examples of the types of enzymes that play an important role in biotechnology and industry. Amylase (E.C.3.2.1.1) are the enzyme that works as catalyst in the hydrolysis of starch into simple monomers. Amylase enzymes are widely used in various industrial fields such as textile, food, paper and other industries. Compared to other organisms, yeasts can produce enzymes more effectively and safer for the environment. Amylolytic yeast can be isolated from flower substrates as it contains sugar for the very limited condition of yeast growth.

Amylase is one of most important group of enzymes. Amylolytic yeast strains were isolated from different natural sources. These are of ubiquitous occurrence and hold the maximum marked share of enzyme sales. Which hydrolyze the starch it is observed by the zone of inhibition by the adding of iodine. Starch converts to product as maltose and glucose molecules. Amylase having yeasts were isolated on the YEPD agar media. These isolated colonies were centrifuged at 8000 rpm for 15min by adding methanol as a substrate. The crud was extracted and all isolates were screened on YEPS (Yeast extract Peptone Starch) media, for the amylase activity crude extract possess zone of inhibition by the lysis of starch. The maximum amylase activity having isolate was subjected to 18s rRNA gene sequencing.

Keywords: Amylase enzyme, amylolytic Yeast, Starch, YEPD media and YEPS media.

INTRODUCTION

Enzymes are biological catalysts, which initiate and speed up thousands of biochemical reactions in living cells. Enzymes are specific molecules due to the selective binding site each type of enzyme possesses for its respective substrate (Aiyer 2005; Sivaramakrishnan *et al.*, 2006). Amylase (E.C.3.2.1.1) is an enzyme that has widely used in bioindustry application since it can hydrolyze starch polymers into glucose monomer units (Gupta *et al*., 2003; Hong *et al.*, 2002). About 30% of the world's enzyme production industry is amylase enzymes. Amylase enzyme works to degrade starch polymers by breaking down α-1,4-glycosidic bonds. The α-amylase enzyme is used for various industrial needs such as bakery, paper industry, detergent, food and pharmaceutical industries. Amylase enzymes can be obtained from various organisms such as yeast. Yeasts are single-celled eukaryotic organisms. There are more than 1,500 species of yeast, but some species were important in industrial applications (Mobini-Dehkordi and Javan 2012; Chandimala *et al.*, 2022). Yeast derived from Latinized Greek meaning "sugar-fungus" (Kuno, 2022). This group of yeasts includes strains of brewer's yeast and baker's yeast (Cubillos *et al.*, 2019), used for producing our favorite carb-heavy treats: bread and alcohol. They convert sugars, CO and alcohol, given enough time (Maicas, 2020). Most commercial yeasts are manufactured by different companies but amylolytic yeasts gain edits fame over others. saccharomyces cerevisiae yeast, most belongs to phylum Ascomycota, only a few being Basidiomycota (Giavasis *et al.*, 2019). Yeasts are found worldwide in soils and on plant surfaces and are especially abundant in sugary mediums such as flower nectar and fruits (Boekhout *et al.*, 2022). Yeast's well-known applications in wine, beer and alcohol production, it is also a work horse discovery and manufacturing tool across many industries. Yeast has been a powerful model organism for understanding human biology and diseases. Amylases are one among the main enzymes used in industry. Amylase enzyme hydrolyze the starch molecules. Amylases have potential application in industrial processes such as food, fermentation and pharmaceutical industries. Amylases are often obtained from plants, animals and microorganisms. The amylases of microorganisms have a broad spectrum of industrial applications as they are more stable than when prepared with plant and animal amylases. The major advantage of using microorganisms for the production of amylases is to economical bulk production capacity and the fact that microbes are easy to manipulate to obtain enzymes of desired characteristics. Amylase has been derived from several fungi, yeasts and bacteria (Patil *et al.*, 2021). However, enzymes from fungal and bacterial sources have dominated applications in industrial

sectors. Amylolysis process is conversion of the starch in to sugar by the action of amylase enzyme. Yeast converts the sugars to CO_2, alcohol, energy. Starch or Amylum is a polymeric carbohydrate found chiefly in seeds, fruits, tubers, roots and stem pith of plants, notably in corn, potatoes, wheat, and rice amyluman important food stuff and used (Cripwell *et al.,* 2021). Amylases hydrolyze starch molecule into glucose, maltose and dextrin. They have many applications in some industry like baking industry and production of bread, liquefaction of starch and saccharification, textile desizing, paper and detergent industry, analysis in medical and clinical chemistry, food and pharmaceutical industries.

MATERIALS AND METHODS

Among 29 different natural samples amylolytic yeast isolation was carried by the collection of6samples like apples, custard apples, wine, toddy, potato and soil samples were processed for amylolytic yeast isolation from in and around Warangal District. T.S. and placed on YEPD. All yeasts were transferred to cultivate in a YEPD (Yeast extract, Potato and Dextrose) medium that containing 1% (w/v) dextrose, 0.5% (w/v) yeast extract and 1%(w/v) peptone. Plates were incubated at 35ºC for 24hrs for the observation of yeast colonies picked and transferred on to new YEPD plates for making pure cultures.

Screening of amylolytic yeast. Screening of amylolytic yeast carried on YEPS (Yeast Extract Pepton Starch) agar (1% starch) medium. All the 6 isolated yeasts trains were examined for their amylolytic activity as follows: All the isolated different yeast strains were grown in separate flasks (250 ml) containing 50 ml sterile amylase(s) production medium and were inoculated with 1% of yeast cell suspension (Fig. 1). Then, the flasks were incubated for 24h at 35˚C. At the end of incubation period, the content of each flask was centrifuged by adding methanol as a solvent at 8000 rpm for 15 min. and filtered through Whatman No. 1 filter paper. The supernatant collected as crude enzyme extract which used to determine the enzyme productivity of the strain through measurement enzyme activity (Plate 1 and 2). Then, twenty ml of sterilized medium were poured aseptically in each sterilized Petri-dish, the YEPS agar medium were allowed to cool and solidify. One well was made per each plate by a sterilized cork borer (10mm diameter), 0.1 ml of the crude enzyme extract was introduced in to the well, and then, the plates were incubated for 24hrs. At 35˚C. At the end of incubation period, plates were flooded with iodine($1.5gl^{-1}$). Production of amylase enzyme is indicated by the clearing zone around the well. The prospective colonies were maintained on YEPD media at 4˚C in refrigerator for future investigations.

RESULT

Total 6 yeast strains were isolated from the different sources like toddy, wine, apple, soil, custard apple and potato. The results are mention in Table 1.

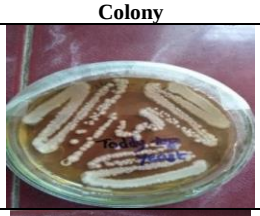
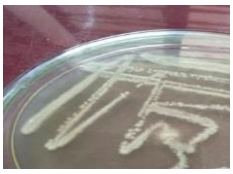
Isolated yeast strains were screened based on clear zone of inhibition by the starch hydrolysis.

Based on amylolytic activity among 6 yeasts one was selected for further study. Isolated and subjected to 18s rRNA gene sequencing at NCL Pune The selected strain showed maximum identity with *Pichia manshurica*. It is isolated from toddy sample. Phylogenic tree was prepared (Fig. 2) amylase is producing after 24 hrs. incubation period 35ºC as the culture appeared yellow in color.

DISCUSSION

Micro-organisms in particular have been regarded as treasure of useful enzymes. There is a great variation between various genera as to their ability to produce a specific enzyme, the production of particular enzymes varies with the particular medium, temperature, pH and incubation time. In recent years, the uses of microorganisms have become a huge importance to food, textile, baking and detergent industries and sparked a large interest into the exploration of enzyme activity in microorganisms (Baghban *et al.,* 2019). This study was focused on assessing the ability of yeast which have the potential to produce amylase, at different temperature, time and pH. Different natural sources are promising for novel amylolytic yeast. Among all yeast have immense potential to produce enzymes and one such important enzyme is amylase. To isolate amylolytic yeast from the different natural sources YEPD media was employed, on this media morphologically distinct yeast were isolated. Amylolytic yeasts were selected based on intensity of morphology and color (Chakraborty, 2020). Amylase activity of isolated species was screened on YEPS medium for several studies. Earlier demonstrated that amylase producing yeast, among 6 amylolytic yeast isolates one is white color shiny round shaped, one is white color rounded one is white color oval shaped, one is light yellow color round shaped and one is light cream color round shape. The amylolytic activity of yeast had been reported previously by Kwon *et al.* (2020). The active isolates were identified morphologically and by the starch hydrolysis. Uma Maheswar and Satyanarayana (2003) reported that the different carbon sources have varied influence on the extracellular enzymes especially amylase strains. Also Bajpai and Bajpai (1989) found that the different carbon sources can greatly influence the production of amylase. Starch is generally accepted as nutritional component for induction of amylolytic enzymes. The cell pellets were used from culture broth methanol organic solvent have been employed to extract the amylase enzyme presents in pellets (Moshfegh *et al.,* 2013). The cell pellets were screened by the starch hydrolysis. This result was in agreement with that of Alli *et al.* (1998); Oboh (2006). Thus, the selected white color shiny round shaped yeast strain showed a very good amylase activity then 18s rRNA gene sequencing-based approaches have become a standard for studying the phylogeny (Lukša *et al.,* 2020).

Table 1: Isolation of yeast.

Sr. No.	Sample	Location	Code of Isolate	Colony morphology	Colony
1.	Toddy	Siddipet	TOD-1	White colour, round shape and shiny colony	
2.	Apple	Hanumakonda	APP-1	White color and oval shape	
3.	Wine	Microbiology Lab in CDU	WIN-1	Light yellow colour and round shape	
4.	Custardapple	Hanumakonda	CUA-1	White colour and round shape	
5.	Soil	Flour mill at Jammikunta	SOL-1	Light cream colour and round shape	
6.	Potato	Vegetable market at Hasanparti	POT-1	White colour and round shape	

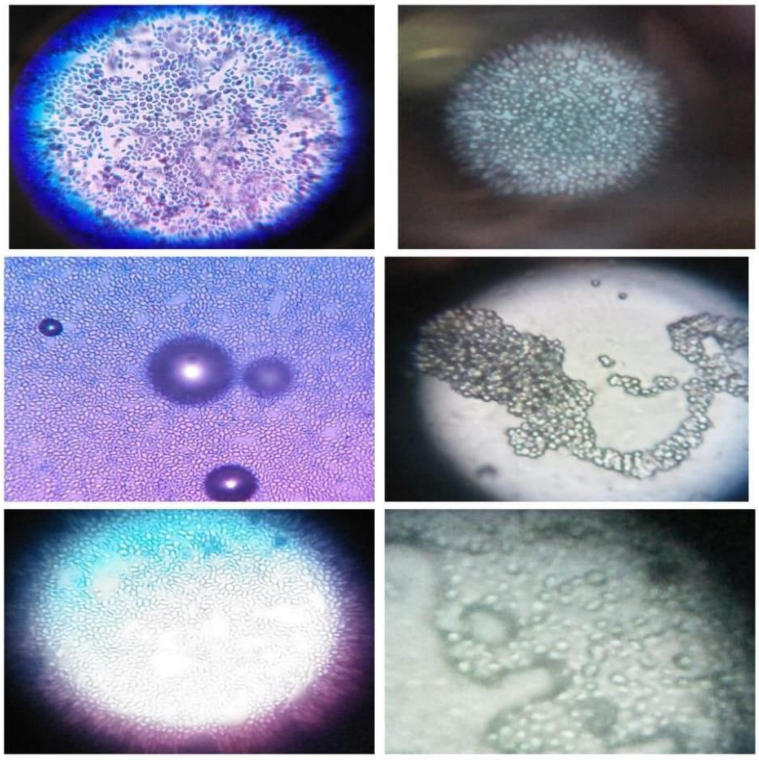


Plate 1. Microscopic observation.

Plate 2. Screening of yeast strains for amylase production for using amylase clearing zone (A.C.Z) assay.

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Toddy Custard apple Potato wine

Fig. 1. Represents the various substrate sources for the growth of yeast strain.

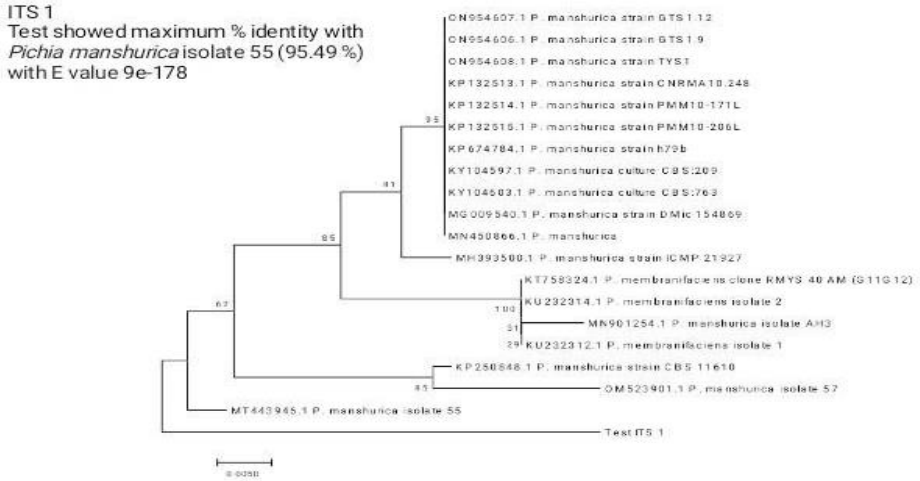

Fig. 2. Phylogenetic tree.

CONCLUSIONS

In conclusion total 6 yeast strains were isolated from the different sources like toddy, wine, apple, soil, custard apple and potato. Isolated yeast strains were screened based on clear zone of inhibition by the starch hydrolysis. Based on amylolytic activity among 6 yeasts one was selected and subjected to 18s rRNA gene sequencing the selected isolate can be characterized further for various useful industrial purposes.

FUTURE SCOPE

Amylases are among the most important enzymes used in various industries. Research on amylase has progressed very rapidly over the last five decades and potential industrial applications of the enzyme

especially in solid waste management have been identified. Major impediments to exploit the commercial potential of amylase are the yield, stability and cost of amylase production. Although, fungal isolates have been extensively studied by many researchers. Further, there arises a need for more efficient amylases in various sectors, which can be achieved either by chemical modification of the existing enzymes or through protein engineering. In the light of modern biotechnology, amylases are now gaining importance in biopharmaceutical applications. Still, their application in food and starch-based industries is the major market and thus the demand of amylases would always be high in these sectors.

Acknowledgement. The authors are thankful to Dr. Ch. V. Purushotham Reddy, Chancellor, Chaitanya (Deemed to be university), Warangal urban, Telangana for his encouragement and support in carrying out this work.
Conflict of Interest. None.

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How to cite this article: A. Veena and B.S. Anuradha (2023). Isolation and Screening of Amylolytic Yeast from various Natural Sources. *Biological Forum – An International Journal*, *15*(5): 450-454.