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Research Article

ISOLATION AND IDENTIFICATION OF AMYLASE PRODUCING BACTERIA FROM SOIL RECEIVING KITCHEN AND AGRICULTURAL WASTE

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ABSTRACT

Amylases are one of the main enzymes used in industry. Such enzymes hydrolyze the starch molecules into polymers composed of glucose units. Amylases have potential application in wide number of industrial processes such as food, fermentation and pharmaceutical industries. Microbes are the most preferred sources of enzymes due to their broad biochemical diversity. The present study focused with the isolation of amylase producers from soil receiving kitchen and agricultural waste. From the 9 soil samples we had isolated 65 isolates out of which 29 isolates were selected based on amylase activity as clear zone on starch agar. Out of 29 isolates 10 isolates show excellent zone of hydrolysis in the secondary screening. The isolates showed prominent activity were further identified by standard conventional methods, which showed most of them belonging to genus *Bacillus* followed by *Pseudomonas spp., Serratia marcescens* and *Staphylococcus aureus.* This study can be further used for the study of amylase on different parameters.

INTRODUCTION

The use of enzyme in industrial processes is beginning to deliver its promise Enzyme have high catalytic rates and work in aqueous solution. Amylases is used in detergents (37%), textiles (12%), Starch (11%) baking (8%) and animal feed (6%) are the main industries, which use about 75% of industrially produced enzymes. Amylases constitute a class of industrial enzyme having approximately 25% of the enzyme market. An extra-cellular amylase, specifically raw starch digesting amylase has found important application in bioconversion, of starches and starch-based substrates. Amylolytic enzymes have great significance in biotechnological applications in food, fermentation, textile to paper industries (10).

The major advantage of using microorganisms for the production capacity and microbes are easy to manipulate to obtain enzymes of desired characteristic. Many microorganism produces amylase such as *Bacillus subtilis, B. cereus, B. polmyxa, B. amyloliquefaciens, B. coagulans, B. subtilis, Lactobacillus, Escherichia, Proteus, B. lincheniformis, Bacillus steriothermophilus Bacillus megaterium, Pseudomonas sp. etc.* Soil receiving the agricultural waste is one of the rich sources of microorganisms specially of starch degrading microorganisms (15). Biomass generate large amounts of organic wastes, agricultural wastes, such as wheat straw, corn carbohydrates, oat hulls, and sugarcane bagasse; residues from logging and timber milling; spoiled products and food-processing wastes; and urban solid waste such as paper, cardboard, kitchen and garden refuse (6) and it is of vital importance that waste is managed in such a way that it does not cause any harm to either human health or to the environment (14).

Cheap and readily available kitchen and agricultural waste which presently constitutes a menace to solid waste management, may be a rich source of amylolytic bacteria (18), therefore, the present study was put forth to isolate, identify and purify amylolytic bacteria from kitchen and agricultural waste sites to get some excellent amylolytic bacteria.

MATERIAL AND METHODS

Collection of soil samples

Different types of amylase producing bacteria were isolated from soil samples collected from different sites rich in carbohydrates and starch like kitchen wastes and agricultural wastes from Akola region.

Isolation of amylase producing bacteria

Amylase producing bacteria were isolated from collected soil samples by serial dilution of soil and spread on nutrient agar plates. Serial dilution was done by taking one gram of soil in 100 ml distilled water in a flask.1ml suspension from one to

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another, dilutions were made upto 0.0000010, 0.1ml of sample spread on Petri plates from last two dilutions and these plates were incubated at 37°C for 24 hours. After incubation mixed cultures were obtained which were purified by streaking on agar plates.

Screening for maximum amylase producing bacteria

Primary screening

Screening was done by the spot inoculation method (12). Isolated and culture were inoculated on the starch agar plates for the screening of amylase producing bacteria and plates were kept for incubation at 37°C for 24 hours, to screen the amylase activity of the obtained cultures. After incubation period plates were observed for formation of clear zone around the colonies by flooding Iodine solution on the plates.

Secondary screening

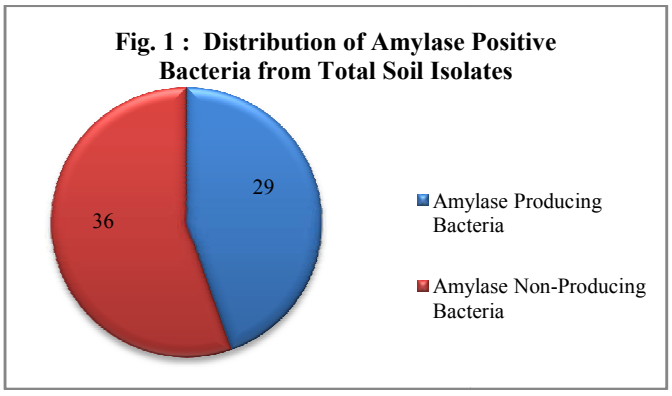
In this the nutrient broth was prepared and inoculated with each isolate in separate tubes and inoculated at 37° C for 48 hours. After inoculation the broth was centrifuged at 10,000 rpm for 15 minutes. The cell free supernatent was poured into the well of 5 mm size made by sterile cook borer into the starch agar medium. The plates then incubated at 37° C for 24 hours. After incubation the plates were flooded with iodine solution and the zone of clearance was observed. The isolates showed highest zone were selected as excellent amylase producer and selected for further study.

Identification of amylase producers

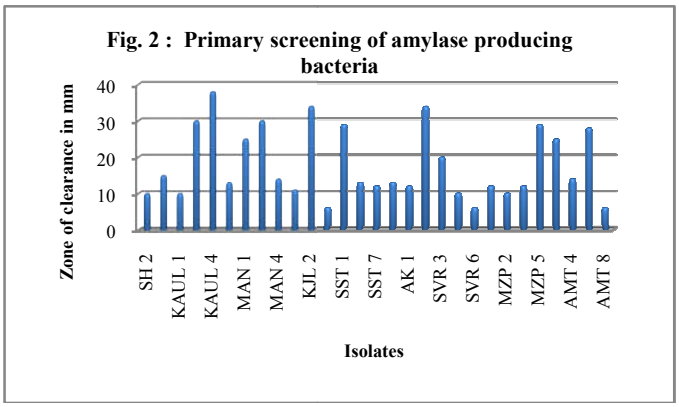
The isolated bacteria were identified on the basis of Bergey's manual of determinative bacteriology (3). Gram character, motility, growth characteristics on different media and biochemical properties like sugar fermentation, indole production, MR - VP reaction, citrate utilization, catalase, urease, oxidase, gelatinase using standard protocol were performed to detect the name of isolates.

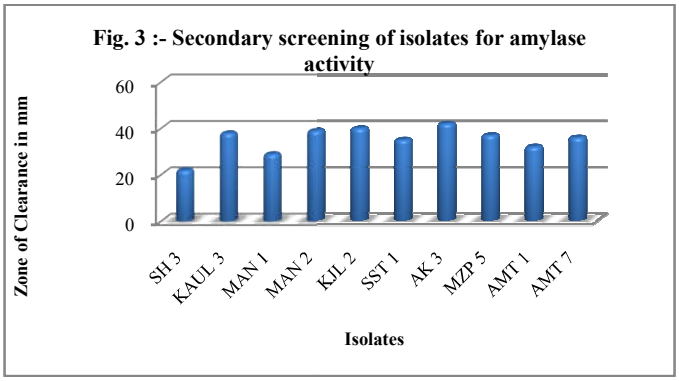
RESULTS AND DISCUSSION

Isolation of amylase producing bacteria from soil sample was done by serial dilution method. The obtained mixed cultures were further purified by streaking on nutrient agar plates. Total 65 soil isolates were obtained which were later screened for amylolytic activity by primary and secondary screening(Fig 1).



Primary screening was done by the spot inoculation method (12). In this the 65 isolates were inoculated as spot on starch agar medium followed by observing zone of clearance around the colonies. 29 bacterial colonies showed zone of clearance which further were selected for secondary screening. These isolates were classified as good and fair producers for amylase The secondary screening of amylase producing bacteria was done by the agar well diffusion method to get the excellent amylase producing bacteria. Excellent producers were selected on the basis of diameter of zone (Fig. 3).





All the 29 isolates from primary screening which showed the amylolytic activity were identified by performing and comparing various morphological, cultural and biochemical test according to the Bergy's Manual of Systemic bacteriology (Table 1). It was found that total 21 isolates were belongs to the genus Bacillus which includes *Bacillus firmus* (Four), *Bacillus licheniformis* (Two), *Bacillus psycharasaccharolyticus* (Three), *Bacillus sterothermophylus* (Two), *Bacillus cereus* (Two), *Bacillus mycoides* (One), *Bacillus coagalons* (One), *Bacillus subtilis* (Two), *Bacillus thuringinsis, Bacillus lentus, Bacillus alrei, Bacillus megaterium*.

In the screening most of the Bacillus showed good to excellent amylase production. The Bacillus genus was followed by *Pseudomonas spp*., *Serratia* and *Staphylococcus*. Amongst *Pseudomonas*, two isolates were found to be of *Pseudomonas fluroscence* and one of *Pseudomonas aeruginosa*. Amongst 3 isolates of *Serratia marcescence. Bacillus spp.* AK3 was found to be the excellent amylase producer. Both isolates of *Staphylococcus aureus* were found fair producers of amylase. The Identification of 10 isolates from secondary screening is shown in Table No. 1

DISCUSSION

Amylase is an enzyme that catalyses the hydrolysis of starch into sugar, three types of amylase is found that alpha-amylase, Beta-amylase, Gama-amylase. Amylase can be obtained from several sources such as plants, animals and microbes (7).

Table No 1 Morphological and cultural characteristic of isolates from secondary screening

Test performed		SH3	KOUL 3	MAN 1	MAN 2	KJL 2	SST 1	AK 3	MZP 5	AMT 1	AMT 7
Size in mm		4 mm	2 mm	3 mm	3 mm	2 mm	3 mm	4 mm	2 mm	3 mm	3 mm
Shape		Oval	Circular	Circular	Eleptical	Circular	Eleptical	Circular	Eleptical	Eleptical	Circular
Margin		Entire	Entire	Entire	Entire	Wavy	Entire	Wavy	Flat	Wavy	Entire
Colour of colony		Cream	White	Off white	Creamy	Off white	Off white	Off white	Off white	Off white	White
Elevation		Raised	Raised	Flat	Flat	Flat	Flat	Flat	Entire	Flat	Raised
Opacity		Opaque	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque
Gram Character		Gram +ve short rod	Gram +ve short rod	Gram +ve short rod	Gram +ve short rod	Gram +ve long rod	Gram +ve short rod	Gram +ve short rod	Gram +ve short rod	Gram +ve short rod	Gram +ve short rod
						Suger fermentation					
Glucose	Acid	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
	*G*as	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve
Sucrose	Acid	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
	*G*as	-ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Mannitol	Acid	+ve	+ve	-ve	-ve	+ve	-ve	+ve	+ve	-ve	+ve
	Gas	-ve	-ve	-ve	-ve	+ve	-ve	+ve	+ve	-ve	+ve
						IMViC Test					
Indole		-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve
MR		+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
VP		-ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve
Citrate		-ve	-ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve	+ve
						Enzyme Test					
Catalase		+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve	+ve
Oxidase		+ve	+ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve
Urease		-ve	+ve	-ve	+ve	-ve	-ve	-ve	-ve	+ve	-ve
Gelatinase		+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Desulphurase		+ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Nitrace Reductase		+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve	+ve
Name of probable isolate		*B. firmus*	*B. psycharasaccharolyticus*	*B. cereus*	*B. mycoides*	*B. substilis*	*B. thuringiensis*	*B. subtilis*	*B. lentus*	*B. alrei*	*B. megaterium*

Large number strains of microorganisms such as bacteria, fungi, actinomycetes have ability to degrade amylase.

Microbial enzymes are widely used in industrial processes such as brewing, baking, textiles, pharmaceuticals, starch processing and detergents, alpha-amylase are some of the most versatile enzymes in the industrial enzyme sector and account for approximately 25% of the enzyme market (19).

Biomass generate large amounts of organic wastes: agricultural wastes, such as wheat straw, corn carbohydrates, oat hulls, and sugarcane bagasse; residues from logging and timber milling; spoiled products and food-processing wastes; and urban solid waste such as paper, cardboard, kitchen and garden refuse (6) and it is of vital importance that waste is managed in such a way that it does not cause any harm to either human health or to the environment (14).

In the present study, isolation and screening of amylase producing bacteria was carried out from the agricultural and kitchen waste soil. For this soil samples were collected from different places. Similar sources for isolation were also preferred by other researchers (11,17,8). The isolation was carried out first by serial dilution of soil samples on starch agar medium. Similar method has been used by Clark *et al*.(1958); Abe *et al*.,(1979).

The serially diluted soil samples were screened for amylase producing bacteria on starch agar plate. Out of 65 bacteria, 29 bacteria showed zone of clearance after pouring iodine solution in primary screening. In secondary screening 10 isolates showed high zones of clearance out of 29 isolates. All the bacterial isolates were characterized based on gram staining and several biochemical and enzyme reactions, The probable isolates were found to be as, *Pseudomonas fluroscence, Bacillus firmus, Bacillus licheniformis, Bacillus psycharasaccharolyticus, Bacillus stearothermophylus, Serratia marcescens, Bacillus cereus, Bacillus mycoides, Bacillus coagalons, Bacillus subtillus, Bacillus thuringiensis, Bacillus lentus, Bacillus megaturium, Staphylococcus aureus, Pseudomonas fluroscence, Pseudomonas aeruginosa,* and *Serratia marcescens.*

In the study most of the amylase positive bacteria that obtained from different soil samples were belong to *Bacillus* genus. This is in agreement with other studies (20,10,5,9,16,11). Who also reported many species of bacillus are good amylase producer. Amongst the other isolates which showed amylolytic activities *Pseudomonas fluroscence, Pseudomonas aeruginosa, Serratia marcescens* and *Staphylococcus aureus* were also found as good outcome of the study. The present results showed similarity with other studies as Raju and Divakar (2013) reported production of amylase by *Pseudomonas aeruginosa* isolated from garden soil, Further Alariya *et al.,* (2013) reported *Pseudomonas fluroscence,* and *Serratia marcescens* as one of the potent amylase producers. Our findings also correlated with these studies as *Serratia marcescens* and *Pseudomonas fluroscence* was found to show good amylolytic activities.

CONCLUSION

The present study showed that isolate Ak-3 and KJL-2 showed highest amylolytic activity. The isolates belongs to the genus *Bacillus spp.* as well as *Pseudomonas spp.* and *Serratia marcescens,* and they showed their ability to produce amylase. Our research is useful for further enzyme optimization studies which are required for the enhanced enzyme activity which helpful for exploitation of these isolates for commercial production.

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