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Isolation and characterisation of amylase producing microbes from different soil samples of agricultural wastes

Ramakrishna D*
Department of Microbiology, Government College (Autonomous), Kalaburagi, Karanataka, India

Abstract
Enzymes from bacterial and fungal sources have been increasingly applied in industrial sectors [1]. Amylases are one of the enzymes which can be derived from several sources such as plants, animals, bacteria and fungi. Microbial production of amylase is more fruitful than that of other sources like plant or animals, because of the short growth period, biochemical diversity and the ease with which enzyme concentrations might be increased by environmental and genetic manipulation. Hence, the enzymes from microbial sources generally meet industrial demands [2].
As per our studies I totally isolated from agricultural waste soil samples from different area in Kalaburagi region and serially dilution method & I screened 30 strains amylase producing microbes. All the strains are morphological, biochemical studies are carriedout.05 isolates are giving good enzyme acivity.

Keywords: Degradation, Morphological, medicinal, indole

Introduction
Amylases are one of the enzymes which can be derived from several sources such as plants, animals, bacteria and fungi. Microbial production of amylase is more fruitful than that of other sources like plant or animals, because of the short growth period, biochemical diversity and the ease with which enzyme concentrations might be increased by environmental and genetic manipulation. Hence, the enzymes from microbial sources generally meet industrial demands [2]. Several *Bacillus* species and thermostable *Actionmycetes* including *Thermomonospora* and *Thermoactinomyces* are versatile producers of theenzymes. The bacterial amylases were derived from *Bacillus subtilis*, *B.licheniformis*, *B.amyloliquifaciens*, *B.stearothermophilus*, *B.gavealeus*, *B.mesentericus*, *B.myocodes*, *B.polymyxa*, *B.vulgates*, *B.aterrimus*,*B.coagulance*, *B.cereus*, *Lactobacillus* sp., *Escherichia coli*, *Proteus* sp. And *Pseudomonas* sp. etc. The genus *Bacillus* produces large variety of extracellular enzymes, of which amylases and proteases are of significant 24 industrial importance. *B.subtilis*, *B.stearothermophilus*, *B.lecheniformis* and *B.amyloliquefaciens* are known to be the good producers of thermostable α – amylase [5]. The thermophilic bacterium *B.stearothermophilus* offers an alternative for commercial production of thermostable α-amylases. Alkaline and thermotolerant amylases were produced by *B.licheniformis* and *B.halodurans* [4].
The isolation may begin with pretreatment of samples which favour the survival of the preferred organism. This is followed by growth on selective or non selective media and often associated with batch or continuous enrichment. Classical methods of screening to obtain suitable organisms are very time consuming expensive and often without any guarantee [3].
Amylase have a wide range of application in various industries such as in the food, bread making, paper industries, textiles, sweetneners, glucose and fructose srups, fruit juices detergents fuel ethanol fron starc, alcoholic bevarages, digestive aids and spot tem overs in dry cleaning. Bacterial alpha amylase are also being in clinical, medicinal and analytical chemistry. These are widely used thermostable enzymes in starch industry are the amylases.

Materials & Methods
Collection of different types of soil samples
Soil samples were collected from different sources and areas aseptically in the polythene bags and bring to the laboratory for further studies isolation of amylase producing microbes by using different collected soil samples.

Isolation and screening of amylase producing microbes from different collrcted soil samples
By using different collected soil samples are used for isolation and screening of amylase producing microbes by different methods in that serially dilution method. take 1gm of soil sample and serially diluted the sample upto seven dilutions and then serially diluted samples inoculated to the media starch agar media and spread the sample then kept for incubation at 370c for about 24 hrs. After incubation colonies will appear then further tested for screening of amylase producing microbes.

Screening for Amylase Activity (Starch Iodine Test)
Isolated colonies were picked up from each plate containing pure culture and streaked in straight lines in starch agar plates with starch as the only carbon source. After incubation at 37ºC for 24-48 hrs., individual plates were flooded with Gram's iodine (Gram's iodine- 250 mg iodine crystals added to 2.5gm potassium iodide solution, and 125ml of water, stored at room temperature) to produce a deep blue colored starch-iodine complex. In the zone of degradation no blue colour forms, which is the basis of the detection and screening of an amylolytic strain. The colonies which were showing zone of clearance in starch agar plates were maintained on to nutrient agar slants.

Morphological and Biochemical Characteristics
Amylase producing strains are further studied by using different morphological and biochemical methods like Gram staining, motility, indole production, methyl red, Vogues

Proskauer's, citrate utilization, triple sugar iron, nitrate reduction, catalase, oxidase, gelatin liquefaction, urease, hydrolysis of casein, hydrolysis of starch were carried out.

Enzyme production medium

Take 5gram of pepton, 3gram of beef extract, 20gram of starch 12.5gram of agar agar in 1000ml of distilled water.10 ml of medium was taken in a 100 ml conical flask. The flasks were sterilized in autoclave at 121 oC for 15 min and after cooling the flask was inoculated with overnight grown bacterial culture. The inoculated medium was incubated at 37 oC in shaker incubator for 24 hr. At the end of the fermentation period, the culture medium was centrifuged at 5000 rpm for 15 min to obtain the crude extract, which served as enzyme source

Composition of preparation of medium starch agar

Pepton=5gms
Beef extract=3gms
Starch=20gms
Agar agar=12.5gms
Distilled water=1000ml

Results & Discussions

Totally we isolated from different soil samples from different area soils by serially dilution method and I screened for 25 strains amylase producing microorganisms from different soil samples.

Totally I isolated 20 strains for further screening by using all strains by morphological and cultural characteristics methods like colony characters and some morphological tests as follows

Table 1: Isolated strains are tested by Morphological cultural characters

Strain No	Form	Consistancy	Colour	Gram's stain	Motility	Spore form
1	Regular	opaque	creamy	Positive	motile	Spore former
2	circular	transparent	White	Positive	motile	Spore former
3	irregular	opaque	white	positive	motile	Spore former
5	irregular	transparent	creamy	positive	motile	Spore former
6	Regular	opaque	creamy	positive	motile	Spore former
7	circular	transparent	creamy	positive	motile	Spore former
8	circular	opaque	White	positive	motile	Spore former
9	irregular	opaque	white	positive	motile	Spore former
10	Regular	transparent	white	positive	motile	Spore former
11	irregular	opaque	white	positive	motile	Spore former
12	circular	opaque	creamy	positive	motile	Spore former
13	Regular	opaque	creamy	positive	motile	Spore former
14	irregular	opaque	creamy	positive	motile	Spore former
15	circular	transparent	creamy	positive	motile	Spore former
16	irregular	transparent	White	positive	motile	Spore former
17	Regular	opaque	White	positive	motile	Spore former
18	circular	opaque	White	positive	motile	Spore former
19	circular	transparent	Creamy	positive	motile	Spore former
20	Regular	transparent	creamy	positive	motile	Spore former

Biochemical tests

Indole test

This test is used to determine the ability of an organism to split tryptophan to form the compound indole. The test strains were inoculated individually in to typtophan broth and incubated for 24 hrs then add 10 drops of Kovac's reagent was added them deep red colour developed due to indole production.

MR Test

All the isolates are tested using this test is used to determine tha ability of an organism to produce acid production and develops red colour by using MR indicator then test is positive if no red colour test is negative.

VP Test

All the isolates are tested by using the VP Test Pyruvic acid, the compound formed is to fermentative degradation of glucose is further metabolized products.cheif end products of glucose metabolism and form quantity of acid in the presence of atmospheric oxygen and KOH 40%,acetone is converted in to diacetyl and alpha napthol serve asa catalyst to ring out crimson to ruby red (pink) coloured complex.the organisms are individually inoculated into the MRVP broth and then incubated at 37^0C. if colour change observation is positive.

Citrate test

This test is used to ability of an organism to use sodium citrate as a carbon source and inorganic ammonium salts as its nitrogen source. All the isolates are inoculates citrate agar media and incubated, bromo thymol blue as indicator form green to blue. After incubation we observe test is positive or negative.

Catalase test

This test is used to production of catalase enzyme here loopfull of cultures were placed on glass slide individually the cells were mixed with 3% hydrogen peroxide and an immediate air bubbles appears indicates test is positive then there is no air bubble indicates test is negative.

Remaining tests starch hydrolysis, gelatin and urease test are also conducted as per the results given in the below table and results are shown in the table-2.

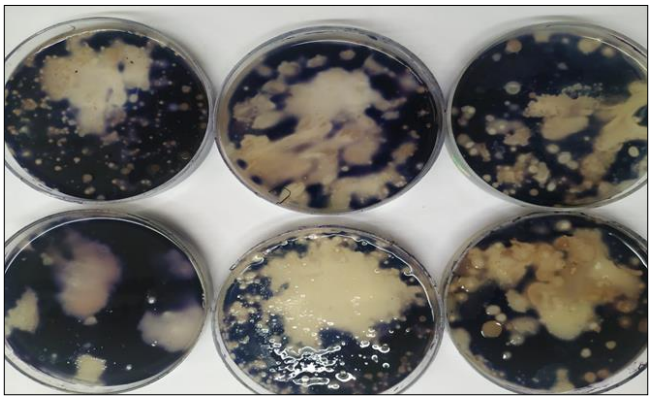

Fig 1: Shows the amylase producing microbes are isolated & screened by using the starch agar media clear zone shows colonies they utilizes starch as a carbon source

Table 2: Isolated strains are further tested by using different methods biochemical Characters as follows the above methods

Strain No	Indole	MR Test	VP Test	Citrate	Catalase	Oxidase	Starch test	Getatin	Urease
1	-ve	-ve	-ve	+ ve	+ ve	+ ve	+ ve	-ve	+ ve
2	-ve	-ve	-ve	+ ve	+ ve	+ ve	+ ve	-ve	+ ve
3	-ve	-ve	-ve	+ ve	+ ve	+ ve	+ ve	-ve	+ ve
4	-ve	-ve	-ve	+ ve	+ ve	+ ve	+ ve	-ve	+ ve
5	-ve	-ve	-ve	+ ve	+ ve	+ ve	+ ve	-ve	+ ve
6	-ve	-ve	-ve	+ ve	+ ve	+ ve	+ ve	-ve	+ ve
7	-ve	-ve	-ve	+ ve	+ ve	+ ve	+ ve	-ve	+ ve
8	-ve	-ve	-ve	+ ve	+ ve	+ ve	+ ve	-ve	+ ve
9	-ve	-ve	-ve	+ ve	+ ve	+ ve	+ ve	-ve	+ ve
10	-ve	-ve	-ve	+ ve	+ ve	+ ve	+ ve	-ve	+ ve
11	-ve	-ve	-ve	+ ve	+ ve	+ ve	+ ve	-ve	+ ve
12	-ve	-ve	-ve	+ ve	+ ve	+ ve	+ ve	-ve	-ve
13	-ve	-ve	-ve	+ ve	+ ve	+ ve	+ ve	-ve	-ve
14	-ve	-ve	-ve	+ ve	+ ve	+ ve	+ ve	-ve	-ve
15	-ve	-ve	-ve	+ ve	+ ve	+ ve	+ ve	-ve	-ve
16	-ve	-ve	-ve	+ ve	+ ve	+ ve	+ ve	-ve	-ve
17	-ve	-ve	-ve	+ ve	+ ve	+ ve	+ ve	-ve	-ve
18	-ve	-ve	-ve	+ ve	+ ve	+ ve	+ ve	-ve	-ve
19	-ve	-ve	-ve	+ ve	+ ve	+ ve	+ ve	-ve	-ve
20	-ve	-ve	-ve	+ ve	+ ve	+ ve	+ ve	-ve	-ve

Summary & conclusion

All the isolates are from different soil samples collected from different areas and screened for isolation of amylase producing microbes by using different methods here figure -1 shows the zone inhibition clear zone indicates amylase producing microbes.

All 20 isolated strains from different soil samples and those strains are used for testing primarily morphological tests like colony characters, gram.'s staining, motility and spore staining results are given as per the table no -1. Then secondly we screening all the isolates by using different methods od biochemical and cultural methods like indole test, MR test, VP test, citrate test, catalase test, Oxidase test, starch hydrolysis, gelatin and Urease test, all the isolates are tested and given the results as per in the table no-2. Amylase have a wide range of application in various industries such as in the food, bread making, paper industries, textiles, sweetneners, glucose and fructose srups, fruit juices detergents fuel ethanol fron starc, alcoholic bevarages, digestive aids and spot temovers in dry cleaning.

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