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Screening of Amylase Producing *Bacillus* Sp. Isolated from Banana Rhizosphere

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ABSTRACT

Amylases are one of the most important industrial enzymes that account for about 20% of the world's enzyme production and a wide range of applications such as conversion of starch to sugar syrups, food, feed fermentation, textile, detergent, and paper industries. The present study deals with the characterization of 23 bacterial cultures were isolated from Banana rhizosphere, Guntur region, Andhra Pradesh, India. Among them, 10 isolates showed the Amylolytic activity on Starch Agar Medium. All the bacterial isolates differed in cultural, morphological and biochemical characteristics were observed. These 10 isolates were identified according to Bergeys Manual of systemic bacteriology. The probability of all these isolates related to Bacillus sp. All the isolates showed round and irregular shaped, cream, light yellow and orange color colonies on SAM. The isolates DS1, DS2, DS6 and DS 8 showed the gram-negative rods and any of the isolates does not produce spores, The remaining strains DS3, DS4, DS5, DS7, DS9 and DS 10 showed gram-positive rods and producing spores. The diameter of inhibition zones was ranged between 10 to 22 (mm) in size. The two isolates DS3 and DS7 showed the maximum solubilization in the screening.

INTRODUCTION

Amylases are the most important enzymes in industries, they catalyze the breakdown of starch into sugar. Amylases were derived from several sources such as plants, animals, and microbes, the microbial amylases meet industrial demands; literature available in a large number of such available commercially and they have almost completely replaced chemical hydrolysis of starch in starch processing industries. The main advantage of using microorganisms for production capacity is their amino ability, microbial manipulation to obtain the enzyme production enzymes of desired characters [1]. Bacteria and fungi secrete amylases to the outside and inside of their cell to carry out extracellular and intracellular enzyme. And they have broken down the insoluble starch, the soluble end products produce such as glucose or maltose [2].

Many of the soil microorganisms used in α-Amylases and β-amylases production including bacterial strains such as *Bacillus subtilis, B. cereus, B. polymyxa, B. amyloliquefaciens, B. coagulans, B. subtilis, Lactobacillus, Escherichia, Proteus, B. lincheniformis, Bacillus stearothermophilus, Bacillus megaterium, Streptomyces* sp., and *Pseudomonas* sp. *etc.*. Amylases from plant and microbial sources have been employed as food additives. Barely amylases have been used in the brewing industry. Amylases also found in spoiled food waste. The soil bacteria, *Bacillus* sp. is widely used for thermostable α-amylase production to meet industry. The filamentous filling has been used for the production of amylases for centuries [3-4].

Enzymes are thought of as natural biocatalysts that promote specific chemical reactions. Most enzymes are produced by the fermentation of bio-based materials [5]. Microbial enzymes are most popular to those from each plant and animal sources because they are inexpensive to produce and their enzyme contents are more expected, convenient to handle and reliable [6]. Enzymes are mainly performing in the conversion of macromolecules to body energy and new materials, also for growth, repair, and cell maintenance. The source of enzymes is animal, plant, and microorganisms; however, the industrial applications of commercial enzymes, microorganisms are the foremost vital source of assorted enzymes [7].

Furthermore, Amylases are also used in the textile industry; amylases are used for removal of starch sizing and as additives in detergents [8]. However, the cost of producing this enzyme is high and the cost of procurement by developing countries can be even higher as a result of

importation. Cheap and readily available agricultural waste such as Potato peels, Banana peels which presently constitutes a menace to solid waste management, may be a rich source of Amylolytic bacteria [9]. Banana peel contained 14.6% glucose and 56% sucrose [10]. It has also been reported that banana fruit stalk contained 56.8% total sugar, 27.0% starch, 4.65% reducing sugar and 4.3% protein on a dry weight basis, and *Bacillus subtilis* isolated from banana wastes could produce α-amylase at significant levels compared to other strains recovered from the same source [9].

Hence, in the present investigation, an attempt was made to develop a suitable process for the production of α-amylase, using banana rhizospheres. However, there is no information available on the characterization of Amylolytic bacteria from Banana rhizospheres in Guntur region, Andhra Pradesh, India. Therefore the main objective of the present study describes the isolation, cultural, morphological and biochemical characteristics of the Amylolytic isolates and these used for further studies.

MATERIALS AND METHODS

Soil sample collection

Soil samples were collected from various Banana rhizospheres in Guntur region, Andhra Pradesh, India.

Isolation of Bacteria

The bacterial isolates capable of producing raw starch digestive enzymes were isolated from the Banana rhizosphere by serial dilution method (Microbiology by Prescott, Ed. VII). The soil suspension of 10^{-4} dilution was added to the raw starch solution and incubated for 48 hrs for the specific growth of amylase producing bacteria. This sample was serial diluted and inoculated into Starch agar plates by pour and spread plating methods. Starch Agar Medium (1000 ml), contains starch-20gm, beef extract-3gm, peptone-5gm, agar-15 gm which facilitates the growth of amylase producing bacteria. The plates were then incubated at 37°C for 48 hours in an incubator. The individual colonies of the culture were streaked separately onto different starch agar plates. Thus formed cultures were subjected to screening. [11].

Screening

The bacterial isolates capable of producing raw starch digestive enzymes were isolated from the local soil by serial dilution method. The soil suspension of 10^{-4} dilution was added to the raw starch solution and incubated for 48 hrs for the specific growth of amylase producing bacteria. This sample was serially diluted and inoculated into Starch Agar plates by pour and spread plating methods. Starch agar medium was used to facilitate the growth of amylase producing bacteria. Cultural, Morphological and Biochemical tests were used to identify the microorganisms.

The screening of bacterial isolates was carried out on the basis of their enzyme producing capability on Starch agar medium. The following steps were involved in the screening procedure: i) The plates were incubated until clear colonies were formed. ii) Then the plates were flooded with Iodine solution (Iodine- 3 gm, KI- 2gm, distilled water - 300ml). iii) The culture that is capable of producing amylase enzyme produces a clear zone around the colony, leaving rest of the plate turning blue. iv) For cross checking a blank was prepared with the same medium but was not inoculated with any bacteria.

Cultural characteristics

Colonies are described as to such properties like Colour, Shape, Margin, Consistency, and Zone of observation on growth medium [12].

Morphological characteristics

Morphological characteristics include gram staining, shape, spore staining, texture, and pigmentation were done by all the 10 Amylolytic isolates.

Gram Staining

Gram staining method is used to distinguish the unknown bacteria bacterial species in gram positive and gram negative. Bacterial cell wall composed of the multilayered or thick layer of peptidoglycan, but some bacterial species contained monolayer on the thin layer of Peptidoglycan [13-14]. The bacterial smear was prepared on the glass slide. And added few drops of crystal violet and incubated for 1 minute, then carefully washed slide carefully with water. Now added few drops of iodine and incubated for 1 minute. Carefully washed slide carefully with water. Added few drops of acetone and washed again. Used few drops of

safranin and incubated for 1 minute. Carefully washed slide carefully with water. A microscope was used for observation.

Biochemical characterization of *Bacillus* isolates

The isolates were identified by conducting various biochemical tests includes, Methyl red test, Indole test, Citrate test, Urease test, Gelatin test, Catalase test, Maltose test, Sucrose test and the results were analyzed through Bergey's Manual of Microbiology [15].

RESULTS AND DISCUSSION

The present study deals with the isolation of 10 amylase producing bacteria from Banana soil, Guntur, India. This was performed by the serial dilution spread plate technique. The similar method has been used by [16-17]. The probability of Identification of selected *Bacillus* isolates was identified on the basis of standard morphological and biochemical tests according to Bergey's Manual of Determinative Bacteriology [18]**.** Isolated *Bacillus* strains were primarily screened for the production of amylase was done by starch agar plate method [19]. Then 10 bacterial isolates from soil were tested for production of amylase by the starch hydrolysis test [20]. Cultural characteristics of the 10 isolates, few showed rod-shaped and few of them showed irregular shape. All the 10 isolates showed the zone of inhibition on SAM. All the isolates showed variations in color of the colony i.e. light yellow, creamy and orange in color (Table-1). The isolates DS1, DS2, DS7 and DS 8 showed negative rods and does not produce spores. DS3, DS4, DS5, DS6, DS9, and DS10 showed the positive rods and these are produced spores (Table-2).

Table 1. Cultural characteristics of *Bacillus* sp. isolated from Banana soil

Isolate no.	Shape	Margin	Consistency	Colour	Observation
DS1	Round	Uneven	Moist	Light yellow	Zone of inhibition observed
DS2	Irregular	Uneven	Moist	Orange	Zone of inhibition observed
DS3	Round	Uneven	Moist	Creamy	Zone of inhibition observed
DS4	Irregular	Uneven	Moist	Orange	Zone of inhibition observed
DS5	Round	Uneven	Moist	Orange	Zone of inhibition observed
DS6	Round	Uneven	Moist	Orange	Zone of inhibition observed
DS7	Round	Uneven	Moist	Creamy	Zone of inhibition observed
DS8	Irregular	Uneven	Moist	Creamy	Zone of inhibition observed
DS9	Irregular	Uneven	Moist	Creamy	Zone of inhibition observed
DS10	Round	Uneven	Moist	Creamy	Zone of inhibition observed

Table 2. Morphological characteristics of *Bacillus* sp. isolated from Banana soil

Isolate no.	Gram staining		Spore staining	Texture	Pigmentation
	Grams reaction	Shape			
DS1	Gram-negative	Rod	Spores not observed	Smooth	Nil
DS2	Gram-negative	Rod	Spores not observed	Smooth	Nil
DS3	Gram-positive	Rod	Spores observed	Smooth	Nil
DS4	Gram-positive	Rod	Spores observed	Smooth	Nil
DS5	Gram-positive	Rod	Spores observed	Smooth	Nil
DS6	Gram-positive	Rod	Spores observed	Smooth	Nil
DS7	Gram-positive	Rod	Spores not observed	Smooth	Nil
DS8	Gram-negative	Rod	Spores not observed	Smooth	Nil
DS9	Gram-positive	Rod	Spores observed	Smooth	Nil
DS10	Gram-positive	Rod	Spores observed	Smooth	Nil

Diameter of Inhibition zones

Out of twenty-three isolates, ten bacteria showed the zone of clearance on starch agar media and among ten, DS3 and DS7 showed the maximum zone of clearance on the starch agar medium i.e. 20 mm and 22 mm in diameter (Table-3). Similarly, 6.0 to 28 mm in diameter of the inhibition zones resulted in *Bacillus licheniformis* [22]. Whereas the DS 9 showed the lowest diameter (10 mm) of inhibition zones on starch agar media. Least zone observed the area of minimum clearance of 1.0 mm on starch agar medium [21].

Table 3. Amylase activity of *Bacillus* sp. isolated from Banana soil

Isolate no.	Probable identity	Diameter of inhibition zones (mm)
DS1	Bacillus sp.	16
DS2	Bacillus sp.	14
DS3	Bacillus sp.	20
DS4	Bacillus sp.	16
DS5	Bacillus sp.	13
DS6	Bacillus sp.	13
DS7	Bacillus sp.	22
DS8	Bacillus sp.	14
DS9	Bacillus sp.	10
DS10	Bacillus sp.	14

Biochemical characteristics of *Bacillus* isolates

Different Biochemical tests such as Catalase Test, Citrate Utilization Test, Oxidase Test, Methyl red test, Voges Proskauer test, Indole Production test and Starch Hydrolysis test were used which confirmed the bacterial isolates were chosen for enzyme (amylase) potential because it has shown maximum zone of clearance on starch agar plates (Table-4). All the 10 bacterial strains differed in biochemical characteristics. Similar results also proved in the bacterial strain by *Bacillus licheniformis* [22].

Table 4. Biochemical characteristics of *Bacillus* sp. isolated from Banana soil

Isolate no.	Indole test	Methyl red test	Vogues Proskeur test	Citrate test	Oxidase test	Starch production test	Catalase test
DS1	Positive	Positive	Negative	Positive	Negative	Positive	Positive
DS2	Positive	Positive	Negative	Positive	Negative	Positive	Negative
DS3	Positive	Positive	Negative	Negative	Positive	Positive	Negative
DS4	Positive	Positive	Negative	Negative	Positive	Positive	Positive
DS5	Positive	Positive	Negative	Positive	Positive	Positive	Positive
DS6	Positive	Positive	Negative	Positive	Negative	Positive	Negative
DS7	Negative	Positive	Positive	Positive	Negative	Positive	Negative
DS8	Positive	Positive	Positive	Negative	Positive	Positive	Positive
DS9	Positive	Positive	Negative	Negative	Positive	Positive	Negative
DS10	Positive	Positive	Negative	Positive	Negative	Positive	Positive

Sugar fermentation tests

All the strains (DS 1 to DS 10) showed the positive results of sugar fermentation tests like dextrose, glucose, mannitol, lactose, and sucrose (Table-5). Among the ten isolates, three isolates i.e. DS3, DS5, and DS7 showed positive results and the remaining isolates showed negative results on maltose test.

Table 5. Characterization of Sugar fermentation on *Bacillus* sp. from Banana soil

Isolate no.	Dextrose	Glucose	Mannitol	Maltose	Lactose	Sucrose
DS1	Positive	positive	Positive	Negative	Positive	Positive
DS2	Positive	positive	Positive	Negative	Positive	Positive
DS3	Positive	positive	Positive	Positive	Positive	Positive
DS4	Positive	positive	Positive	Negative	Positive	Positive
DS5	Positive	positive	Positive	Positive	Positive	Positive
DS6	Positive	positive	Positive	Negative	Positive	Positive
DS7	Positive	positive	Positive	Positive	Positive	Positive
DS8	Positive	positive	Positive	Negative	Positive	Positive
DS9	Positive	positive	Positive	Negative	Positive	Positive
DS10	Positive	positive	Positive	Negative	Positive	Positive

CONCLUSION

The present study reveals the isolated Amylolytic bacterial strain- DS3 and DS7 showed the maximum zone on Starch Agar medium. Based on cultural, morphological and biochemical studies, also proved to these two strains can be used for the industrial application like the starch modification.

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