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ISOLATION OF STARCH DEGRADING BACTERIA FROM RHIZOSPHERIC SOIL OF MYMENSINGH, BANGLADESH

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Starch degrading bacteria are vital for various industries like food, fermentation, textile, and paper. The aim of this study is to isolate and characterize bacteria able to degrade starch from the rhizosphere of soil located in Mymensingh, Bangladesh. Collected soil sample was serially diluted in sterilized peptone water, poured on sterilized starch agar plates and incubated at 37ºC for 24 hours. The representative colonies showing different morphology was randomly picked up using the streaking method on nutrient agar media. A total of 8 bacterial colonies were isolated and labelled as MSH 01, MSH 02, MSH 03, MSH 04, MSH 05, MSH 06, MSH 07, and MSH 08. Biochemical characteristics showed that all the isolates belonged to the genera *Bacillus*. Among those, MSH 06 showed the highest starch degrading index (SDI) followed by MSH 02 and MSH 05. Surveillance to identify microbial species with enhanced starch hydrolyzing potential might be helpful in biotechnology industries.

Keywords: *Bacillus*, Rhizosphere, Starch degrading index, Amylase, Mymensingh

INTRODUCTION

There has been considerable revived interest in soil microorganisms and their key role in numerous terrestrial ecosystems. Indeed, soil microbial communities are shown to contribute chiefly in complicated processes like biogeochemical cycles, plant nutrition and health or soil structure and fertility (1, 2, 3, 4). Starch degrading amylolytic enzymes are most significant in the biotechnology industries with vast applications in food, fermentation, textile and paper industries (5). Amylases can be obtained from several sources such as plants, animals and microbes (6). The microbial amylases are most well-liked to completely different sources of amylases due to its plasticity and big accessibility. It has nearly surpassed the substitute sources in many industries (5). Among the bacterial species, *Bacillus* spp., *Bacillus amyloliquefaciens*, *Bacillus licheniformis* and Pseudomonas spp. are widely used for enzyme production. Other species like *Bacillus cereus* and *Bacillus subtilis* are also used for production of amylase enzyme. Amylases produced from *Bacillus licheniformis*, *Bacillus stearothermophilus* and *Bacillus amyloliquefaciens* showed potential in an exceeding range of commercial applications in processes like food, fermentation, textile and paper industries (7, 8).

There are several reports on starch degrading microorganisms from various sources and their respective amylase activity (9-11). The present investigation dealt with the isolation of starch degrading Rhizobacteria from soil of Mymemsingh, Bangladesh.

MATERIALS AND METHODS

Description of Study Area: The soil sample was collected from Mymensingh, a major city and the capital of Mymensingh Division, Bangladesh settled on the bank of Brahmaputra River, about 120 km (75 miles) north from Dhaka.

Samples Collection: Around 10 grams of soil sample was collected from the rhizospheres. The soil sample was placed within the sterilized bag and transferred at once to the laboratory of Stamford University Bangladesh.

Sample Preparation and Isolation of Starch Degrading Bacteria: Ten gram of soil sample was mixed with 90 ml of sterile peptone water in different 250 ml conical flax and homogenized for ten minutes using orbital shaker at 110 rpm. Then, one ml of each sample was transferred aseptically into 9 ml of sterile peptone water and mixed thoroughly using vortex. The homogenates were serially diluted to 10^{-8}. Thenceforth, 0.1 ml aliquots of dilutions were spread properly on starch agar plates. The plates were incubated at 37ºC for 24 hours. The microbial colonies showing completely different morphology were selected and subcultured by streak plate technique (12).

Identification of Bacterial Isolates: The microbial isolates were subjected to identification by Gram reaction, colony morphologyand biochemical tests including oxidase, catalase, TSI, MRVP, citrate test, and indole test using Bergey's Manual of Determinative Bacteriology, 8th edition.

Screening for Amylase Activity (Starch Iodine Test): Isolated strains were picked up from every plate containing pure culture and patterned in straight lines in starch agar plates where starch was used as carbon source. The plates were incubated at 37ºC for 24 hours. After incubation, the plates were flooded with Gram's iodine solution, (250 mg iodine crystals added to 2.5 g potassium iodide solution and 125 ml of distilled water), to develop deep blue color.

Determination of Starch Degrading Index (SDI): Ability of those isolates to degrade starch was delineated by the starch degrading index (SDI) by the ratio of the total diameter of clear zone and colony diameter. According to the degrading index, potential colonies with the best efficiency were selected as best starch degrading colonies (13).

RESULTS & DISCUSSION

The morphological features, colony morphology, biochemical characteristics of the isolated colonies were summarized in Tables 1, 2, and 3. In the present study, based on their gram reaction, all of the 8 isolates were gram-positive and rod shape in chains except isolate

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MSH 01 and 08 showed cocci shape in cluster and discrete rod arrangement, respectfully (Table 1). In bacterial colony morphology, all of the isolates showed off-white pigmentation where isolate MSH 01 was small in size and the rest of them were medium in size. Isolates MSH 01, 02 and 05 showed raised elevation and others showed flat elevation. Biochemical characteristics of the bacterial species showed that isolate MSH 01 was only VP negative but MR positive while rest of the isolates showed the opposite results. Isolates MSH 02 to 08 showed alkaline or acid slant but acid butt with no gas or H_2S production. Those isolates showed indole negative but citrate, oxidase and catalase positive. On the contrary, isolate MSH 01 showed indole and oxidase positive but citrate and catalase negative (Table 3). Taken all the biochemical test results, colony and bacterial cell morphology into account, it was presumptively identified as MSH 01 is *Streptococcus* spp. and MSH 02 to 08 was *Bacillus* spp. One study conducted in Ethiopia showed that soil samples from rhizosphere of various plant species showed 72% were *Bacillus* spp. while the remaining 28% were *Pseudomonas* spp. (14).

Table 1: Morphological features of the bacterial species isolated from rhizosphere.

Serial No.	Isolates	Shape	Arrangement	Gram reaction
1	MSH 01	Small	Cluster/Cocci	Positive
2	MSH 02	Medium	Chain/ rod	Positive
3	MSH 03	Medium	Chain/rod	Positive
4	MSH 04	Medium	Chain/rod	Positive
5	MSH 05	Medium	chain/rod	Positive
6	MSH 06	Medium	Chain/ rod	Positive
7	MSH 07	Medium	Chain/ rod	Positive
8	MSH 08	Medium	Discrete/rod	Positive

Table 2: Colony morphology of the bacterial species isolated from rhizosphere on starch agar media.

Serial No.	Isolates	Size	Pigmentation	Margin	Elevation
1	MSH 01	Small	Off white	Entire	Raised
2	MSH 02	Medium	Off white	Entire	Raised
3	MSH 03	Medium	Off white	Undulate	Flat
4	MSH 04	Medium	Off white	Entire	Flat
5	MSH 05	Medium	Off white	Entire	Raised
6	MSH 06	Medium	Off white	Entire	Flat
7	MSH 07	Medium	Off white	Entire	Flat
8	MSH 08	Medium	Off white	Entire	Flat

Table 3: Biochemical characteristics of the bacterial species isolated from rhizosphere.

Serial No.	Isolates	Oxidase	Catalase	MR	VP	TSI				Indole	Citrate	Presumptively identified bacteria
						Slant	Butt	H_2S	Gas			
1	MSH 01	+	–	+	–	ND	ND	ND	ND	+	–	*Streptococcus* sp.
2	MSH 02	+	+	–	+	K	A	–	–	–	+	*Bacillus* sp.
3	MSH 03	+	+	–	+	K	A	–	–	–	+	*Bacillus* sp.
4	MSH 04	+	+	–	+	A	A	–	–	–	+	*Bacillus* sp.
5	MSH 05	+	+	–	+	A	A	–	–	–	+	*Bacillus* sp.
6	MSH 06	+	+	–	+	A	A	–	–	–	+	*Bacillus* sp.
7	MSH 07	+	+	–	+	A	A	–	–	–	+	*Bacillus* sp.
8	MSH 08	+	+	–	+	K	A	–	–	–	+	*Bacillus* sp.

Note: MR= Methyl Red, VP= VogesProskauer, TSI= Triple Sugar Iron, K=Alkaline, A=Acid, ND=Not Done.

Table 4: Starch degrading index of isolated colonies (SDI).

Serial No.	Isolates	Diameter of colony (mm)	Diameter of Clear zone (mm)	SDI
1	MSH 01	1	No zone	-
2	MSH 02	4	15	3.75
3	MSH 03	2	3	1.5
4	MSH 04	4	12	3
5	MSH 05	4	15	3.75
6	MSH 06	3	13	4.33
7	MSH 07	4	12	3
8	MSH 08	5	13	2.6

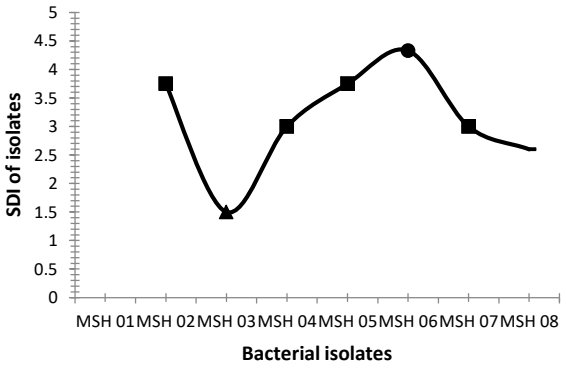

Figure 1: SDI value of the isolates isolated from rhizosphere.

Previous studies reported that the most dominant isolates of Gram-positive rod shaped bacteria was *Bacillus* spp., the main starch degrading rhizobacteria isolated from starch agar plates (15, 16). *Bacillus* sp. was also predominantly isolated with amylase activity from soil of kitchen waste (17). However, gram positive rhizobacteria showing the highest value of starch degrading index and enzyme production is directly correlated to the time period of incubation (18). Rhizobacteria can secrete amylases to the outside of their cells to carry out extra-cellular digestion and facilitate other different organic matters for plants to easily absorb and manufacture their food (19). Gram-positive bacteria almost showed characteristics of *Bacillus* species, capable of tolerating different factors, produced amylase enzymes to degrade starch into a soluble form and are applicable to food, industrial and leather industries.

CONCLUSION

The present study revealed that the soil sample was taken from Mymensingh was inhabited with microorganisms mostly of *Bacillus* genera with high potential to degrade starch. Though enzyme is often not inheritable from several plants and animals due to its limitations, microbial enzyme typically meets industrial demand. Surveillance to identify microbial species with enhanced starch hydrolyzing potential might be helpful in biotechnology industries.

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