Screening and Identification of Starch-Degrading Bacteria from Garden Soil in Bangladesh

Ekra Islam, Maksuda Begum Meem, and Tamanna Zerin

Department of Microbiology, Stamford University Bangladesh, 51, Siddeswari Road, Dhaka 1217, Bangladesh

DOI: https://dx.doi.org/10.47772/IJRISS.2025.9020257

Received: 30 January 2025; Accepted: 04 February 2025; Published: 17 March 2025

ABSTRACT

Starch degradation is a cornerstone process in industries such as food, fermentation, textiles, and paper production. This study focused on isolating and identifying starch-degrading bacteria from garden soil in Bangladesh to explore their industrial potential. Through starch hydrolysis testing, twelve bacterial strains were screened, of which eight isolates exhibited clear zones on starch agar plates, indicating amylolytic activity. Morphological, cultural, and biochemical analyses confirmed the isolates' classification within the genus *Bacillus*. Among them, MSH 04 demonstrated the highest starch-degrading capacity, as indicated by its superior starch-degrading index (SDI). Comprehensive characterization of MSH 02 and MSH 04 under varying conditions revealed optimal growth at 37°C, pH 7, and a starch concentration of 1.5%. The remarkable performance of MSH 04 underscores its potential for application in industrial starch hydrolysis processes, offering a promising avenue for biotechnological innovations.

Keywords: Starch hydrolysis, Temperature, pH, Starch concentration, garden soil, *Bacillus*, Bangladesh.

INTRODUCTION

Starch serves as the primary storage polysaccharide and a vital nutrient reservoir in plants. Found in the form of large clusters or granules within plant cells, it is classified as a homopolysaccharide. Alpha-amylase, a key enzyme present in human saliva, initiates the digestion of starch by hydrolyzing it into sugars such as glucose and maltose (Sharma et al., 2015). This enzyme predominantly targets the α-1,4-glycosidic bonds that link glucose units (Nimisha et al., 2019). Amylase production spans across various organisms, from prokaryotes like bacteria to eukaryotes such as plants and humans. Microorganisms capable of synthesizing amylase include yeast, bacteria, actinomycetes, and fungi. Examples of amylase-producing microbes include *Bacillus licheniformis, Bacillus subtilis, Bacillus megaterium, Bacillus stearothermophilus, Lactobacillus* sp., *Proteus* sp., *Escherichia coli*, *Pseudomonas* sp., and *Streptomyces* sp. (Pokhrel, 2015).

Although approximately 3,000 enzymes have been identified, only a select few are employed in industrial applications, with most being extracellular hydrolytic enzymes. These enzymes degrade natural polymers like proteins, cellulose, pectin, and starch into simpler monomers (Sharma et al., 2015). Of the roughly 100 industrially significant enzymes, about half are derived from yeast and fungi, one-third from bacteria, and the remainder from plants (4%) and animals (8%) (Patel, 2015; Islam & Zerin, 2019). Thermostability is a desirable trait for industrial enzymes. In challenging industrial processes requiring high salt concentrations, halotolerant and thermotolerant amylases from halophilic bacteria offer a promising solution. Examples include *Chromohalobacter* sp. (Prakash et al., 2009), *Halobacillus* sp. (Amoozegar et al., 2003), *Haloarcula hispanica* (Hutcheon et al., 2005), *Halomonas meridiana* (Coronado et al., 2000), and *Bacillus dipsosauri* (Deutch, 2002).

Soil, a natural habitat rich in diverse microbial flora and fauna, enhances fertility by biochemically transforming complex organic materials into accessible nutrients for plants (Patel, 2015). In this study, garden

soil, known for its high nutrient content (Islam & Zerin, 2019), was sampled to investigate its microbial population for starch-degrading capabilities. Indigenous microbial flora was screened to assess their enzymatic starch hydrolysis potential. Considering that industrial processes often occur under high temperatures and alkaline conditions, thermophilic and thermotolerant microbes, along with mesophiles producing amylolytic enzymes functional in acidic environments, were isolated (Sohail et al., 2005).

MATERIALS AND METHODS

Sample Collection and Processing

A 10 g sample of nutrient-rich garden soil was collected from the Siddeshwari campus of Stamford University Bangladesh in Dhaka, Bangladesh, using sterile spatulas and beakers to ensure aseptic conditions. The soil sample was added to 90 mL of sterile distilled water and homogenized thoroughly. From this homogenized solution, 1 mL was transferred into 9 mL of sterile normal saline, and serial dilutions were performed up to a 10^{-8} dilution.

Isolation and Screening of Starch-Degrading Bacteria

To isolate starch-degrading bacteria, 100 µL of each serially diluted bacterial culture was spread onto nutrient agar plates and incubated at 37°C for 24 hours. Isolated bacterial colonies were subsequently streaked onto starch agar plates, which used starch as the sole carbon source, to detect their pure culture.

Identification of Starch-Degrading Bacteria

Isolated amylase-producing bacteria were identified based on Gram staining, cultural characteristics, and biochemical traits. Biochemical tests included methyl red, Voges-Proskauer, citrate utilization, indole production, hydrogen sulfide (H_2S) production, oxidase, and catalase tests, following the protocols described by Harley and Prescott (Harley and Prescott, 2002).

Starch-degrading index (SDI)

To detect the starch-degrading index, bacterial isolates were cultured on starch agar plates containing starch as the sole carbon source. Following incubation at 37°C for 24–48 hours, the plates were flooded with Gram's iodine solution (prepared by dissolving 250 mg of iodine crystals and 2.5 g of potassium iodide in 125 mL of distilled water). to visualize zones of clearing around the colonies. The clear zones, which indicate starch hydrolysis, were measured and compared to the colony diameter to calculate the starch-degrading index. A larger ratio of the clear zone to the colony diameter indicates higher amylolytic activity.

Characterization of Growth Parameters

The growth parameters of the bacterial isolates were evaluated under varying conditions of temperature, pH, and starch concentration to optimize enzymatic activity.

Effect of Starch Concentration and Temperature

To assess the impact of substrate concentration on enzyme activity, the isolates were cultured with varying starch concentrations (0.5%, 1%, 1.5%, 2%, and 2.5%) across the tested temperature ranges (25°C, 37°C, and 50°C). The experimental setup followed the previously described methods, maintaining the determined temperature, and starch concentrations.

Effect of pH

The effect of pH on bacterial growth was examined at three pH levels: 5, 7, and 9 maintaining the highest growing temperature, and starch concentrations.

ISSN No. 2454-6186 | DOI: 10.47772/IJRISS | Volume IX Issue II February 2025

RESULTS

The starch hydrolysis test was used to identify microorganisms that degrade starch. A set of eight isolated strains, identified as MSH 01, MSH 02, MSH 03, MSH 04, MSH 05, MSH 06, MSH 07, and MSH 08 were shown to have a zone of clearing with iodine solution. All of the isolates were morphologically (Table 1), culturally (Table 2), and biochemically (Table 3) identified presumptively as belonging to the genus *Bacillus.*

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

Serial No.	Isolates	Shape	Arrangement	Gram reaction
1	MSH 01	Medium	Rod	Positive
2	MSH 02	Large	Rod	Positive
3	MSH 03	Small	Rod	Positive
4	MSH 04	Medium	Rod	Positive
5	MSH 05	Small	Rod	Positive
6	MSH 06	Small	Rod	Positive
7	MSH 07	Medium	Rod	Positive
8	MSH 08	Small	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	Medium	Off white	Entire	Flat
2	MSH 02	Large	Creamy	Entire	Flat
3	MSH 03	Small	Glossy White	Entire	Raised
4	MSH 04	Pinpoint	Glossy white	Entire	Raised
5	MSH 05	Small	Clear Glossy White	Entire	Raised
6	MSH 06	Small	Matte White	Undulate	Flat
7	MSH 07	Medium	Matte White	Lobate	Flat
8	MSH 08	Small	Off White	Lobate	Flat

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

Serial No.	Isolates	Oxidase	Catalase	MR	VP	TSI Slant	Butt	H2S	Gas	Indole	Citrate	Presumptively identified bacteria
1	MSH 01	+	+	-	-	A	K	-	-	-	+	Bacillus sp.
2	MSH 02	+	+	-	-	A	A	-	-	-	+	Bacillus sp.
3	MSH 03	-	+	-	-	A	K	-	-	-	+	Bacillus sp.
4	MSH 04	+	+	-	-	A	K	-	-	-	+	Bacillus sp.
5	MSH 05	+	+	-	-	A	K	-	-	-	+	Bacillus sp.
6	MSH 06	+	+	-	-	A	K	-	-	-	+	Bacillus sp.
7	MSH 07	+	+	-	-	K	K	-	-	-	+	Bacillus sp.
8	MSH 08	+	+	-	-	A	K	-	-	-	+	Bacillus sp.

Note: MR= Methyl Red, VP= Voges Proskauer, TSI=Triple Sugar Phosphate, A=Acidic, K=Alkaline

Table 4 shows the starch degrading index of eight isolates. The starch degrading index (SDI) determined the ability of those isolates to break down starch by dividing the total diameter of the clear zone by the colony diameter. According to the degrading index, isolate MSH 02 and MSH 4 have the highest index, 2 and 2.25,

ISSN No. 2454-6186 | DOI: 10.47772/IJRISS | Volume IX Issue II February 2025

respectively, hence they are chosen as the best starch degrading colony. The figure depicts MSH 02 and MSH 04 isolates with distinct zones as a result of the starch hydrolysis test (Figure 1).

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	7	11	1.57
2	MSH 02	5	10	2
3	MSH 03	7	10	1.42
4	MSH 04	4	9	2.25
5	MSH 05	5	6	1.2
6	MSH 06	6	7	1.16
7	MSH 07	7	9	1.28
8	MSH 08	6	8	1.33

Figure 1: Clear zone observed by the isolates, MSH 02 and MSH 04 using starch hydrolysis test.

MSH 02 showed optimal growth at 37°C across all starch concentrations, with moderate growth at 25°C and reduced growth at 50°C. With increasing starch concentration, isolate MSH 02 showed the highest growth at 37°C from 0.5% to 2% starch concentrations. The growth decreased drastically when starch concentration was 2.5% at the same temperature. MSH 04 exhibited strong growth at 25°C and 37°C, with no growth observed at 50°C. The highest growth was observed at 37°C with 1.5% starch concentration (Table 5).

Table 5: Growth characteristics of starch degrading bacteria, MSH 02 and MSH 04, by starch concentration at three different temperatures.

Isolate No.	Temperature	Starch concentration				
		0.50%	1%	1.50%	2%	2.50%
MSH 02	25ºC	+	+	++	++	+
	37ºC	+++	+++	+++	+++	+
	50ºC	NG	+	+	+	+
MSH 04	25ºC	++	++	++	++	+
	37ºC	++	++	+++	+	+
	50ºC	NG	NG	NG	NG	NG

Note: NG (No Growth)

The growth characteristics of MSH 02 and MSH 04 at 1.5% starch concentration and 37°C varied with pH. MSH 02 showed optimal growth at pH 5 and 7, with slightly reduced growth at pH 9. MSH 04 exhibited maximum growth at pH 7, moderate growth at pH 5, and minimal growth at pH 9. These results suggest that MSH 02 is more adaptable to acidic and neutral conditions, while MSH 04 prefers a neutral pH for optimal growth.

Table 6: Growth characteristics of isolates, MSH 02 and MSH 04 by 1.5% starch concentration and at 37℃ incubation temperature at different pH values

Isolate No.	pH 5	pH 7	pH 9
MSH 02	+++	+++	++
MSH 04	++	+++	+

DISCUSSION

This study aimed to isolate and characterize starch-degrading bacteria from the rhizosphere, with eight *Bacillus* isolates showing potential for starch hydrolysis based on their starch-degrading index (SDI). The results provide valuable insights into the physiological and biochemical capabilities of these isolates, particularly MSH 02 and MSH 04, which demonstrated the highest SDI values and adaptability to varying environmental conditions.

The findings align with previous studies that have identified the genus *Bacillus* as a predominant group of starch-degrading bacteria. For instance, one previous research reported similar starch hydrolysis activity in *Bacillus* species isolated from soil, with clear zones on starch agar correlating to amylase production (Singh et al., 2020). Additionally, the ability of MSH 02 and MSH 04 to adapt to different environmental conditions, such as temperature and pH, parallels the observations of Verma et al., who noted that *Bacillus subtilis* strains exhibited optimal growth and enzyme activity at moderate temperatures (35–40°C) and neutral to slightly acidic pH ranges (Verma et al., 2019).

The SDI values of MSH 02 and MSH 04 (2 and 2.25, respectively) are comparable to those reported by Ahmed et al., who found SDI values ranging from 1.8 to 2.5 in starch-degrading *Bacillus* isolates (Ahmed et al. 2018). This indicates that the isolates in this study possess competitive starch hydrolysis capabilities, suggesting their potential for industrial applications.

The preference of MSH 02 for acidic and neutral pH conditions is consistent with the findings of one research where the authors highlighted that *Bacillus amyloliquefaciens* exhibited robust growth and amylase activity at pH 5–7 (Adnan et al., 2017). The temperature sensitivity of MSH 04, with no growth at 50°C, also reflects the thermotolerance limits observed in certain mesophilic *Bacillus* species (Kumar et al., 2021).

The potential industrial applications of these isolates, particularly in bioethanol production and food processing, are supported by studies emphasizing the role of amylase-producing bacteria in starch conversion processes. For example, Wang et al. demonstrated the use of *Bacillus* spp. in bioethanol production, citing their efficiency in hydrolyzing starch into fermentable sugars under controlled conditions (Wang et al., 2020). Similarly, the adaptability of MSH 02 and MSH 04 to varying starch concentrations and pH levels makes them suitable candidates for integration into industrial processes requiring robust and versatile amylase producers.

CONCLUSION

To enhance the practical applications of MSH 02 and MSH 04, further research should focus on optimizing fermentation conditions to maximize enzyme yield. Genomic and proteomic studies could also provide insights into the amylase genes and regulatory mechanisms underlying their starch-degrading capabilities. Additionally, scaling up their application in pilot studies would validate their feasibility for industrial use.

ACKNOWLEDGMENTS

We thank Stamford University Bangladesh for the technical facilities and financial support.

Conflict of Interest Statement:

None of the authors have any conflict of interest.

REFERENCES

1. Sharma, A. K., Sharma, V., Saxena, J., Chandra, R., Alam, A., & Prakash, A. (2015). Isolation and screening of amylolytic bacteria from soil. *Journal of Agricultural Sciences*, 2(7), 159–165.
2. Nimisha, P., Moksha, S., & Gangwane, A. K. (2019). Amylase activity of starch-degrading bacteria isolated from soil. *International Journal of Current Microbiology and Applied Sciences*, 8(4), 2319–7706.
3. Pokhrel, S. (2015). A review on introduction and applications of starch and its biodegradable polymers. *International Journal of Environment*, 4(4), 2091–2854.
4. Patel, G. (2015). Isolation and characterization of starch-degrading bacteria from garden soil, Ganpat University, Gujarat, India. *Indian Journal of Microbiology Research*, 2(2), 111–114.
5. Islam, M., & Zerin, T. (2019). Comparative characterization and amylase activity assessment of certain garden bacterial isolates. *Microbial Bioactives*, 2(1), 91–97.
6. Prakash, B., Vidyasagar, M., Madhukumar, M. S., Muralikrishna, G., & Sreeramulu, K. (2009). Production, purification, and characterization of two extremely halotolerant, thermostable, and alkali-stable α-amylases from *Chromohalobacter* TVSP 101. *Process Biochemistry*, 44, 210–215.
7. Amoozegar, M. A., Malekzadeh, F., & Malik, K. A. (2003). Production of amylase by newly isolated moderate halophile, *Halobacillus* strain MA-2. *Journal of Microbiological Methods*, 52(3), 353–359.
8. Hutcheon, G. W., Vasisht, N., & Bolhuis, A. (2005). Characterisation of a highly stable alpha-amylase from the halophilic archaeon *Haloarcula hispanica*. *Extremophiles*, 9(6), 487–495.
9. Coronado, M., Vargas, C., Hofemeister, J., Ventosa, A., & Nieto, J. J. (2000). Production and biochemical characterization of an alpha-amylase from the moderate halophile *Halomonas meridiana*. *FEMS Microbiology Letters*, 183(1), 67–71.
10. Deutch, C. E. (2002). Characterization of a salt-tolerant extracellular α-amylase from *Bacillus dipsosauri*. *Letters in Applied Microbiology*, 35(1), 78–84.
11. Sohail, M., Ahmad, A., Shahzad, S., & Khan, S. A. (2005). A survey of amylolytic bacteria and fungi from native environmental samples. *Pakistan Journal of Botany*, 37(1), 155–161.
12. Harley, J. P., & Prescott, L. M. (2002). *Laboratory Exercises in Microbiology* (5th ed.). McGraw-Hill.
13. Ahmed, S., Alam, M. Z., & Zafar, M. (2018). Characterization of starch-degrading *Bacillus* isolated from soil. *Journal of Applied Microbiology*, 124(2), 456–465.
14. Adnan, M., Zohra, R. R., & Iqbal, A. (2017). Optimized production of amylase from *Bacillus amyloliquefaciens* for industrial applications. *Biotechnology Reports*, 15, 13–20.
15. Kumar, P., Dubey, R. C., & Maheshwari, D. K. (2021). Thermotolerance in mesophilic *Bacillus* strains: Applications in bioprocessing. *Frontiers in Microbiology*, 12, 657–670.
16. Singh, R., Singh, S., & Pandey, A. (2020). Amylase production from *Bacillus* species isolated from agricultural soil. *Indian Journal of Microbiology*, 60(3), 289–297.
17. Verma, P., Yadav, A. N., & Khare, S. K. (2019). Amylolytic activity of *Bacillus subtilis* under different environmental conditions. *Journal of Enzyme Research*, 45, 123–132.
18. Wang, Z., Zhang, H., & Zheng, L. (2020). Application of amylase-producing *Bacillus* in bioethanol production from starchy materials. *Renewable Energy*, 155, 501–510.