

DOI: [https://doi.org/10.71317/kjard.2.9\(s\).2026.620](https://doi.org/10.71317/kjard.2.9(s).2026.620)

Kashmir Journal of Academic Research and Development

Journal homepage: <https://rjsaonline.org/index.php/KJARD>

Valorization of Agro-Industrial By-Products for Enhanced Amylase Production by *Bacillus tropicus*: A Sustainable Biotechnological Approach

Sapna Shakir

Department of Microbiology, Faculty of Science, The University of Swabi, Anbar-Swabi, Khyber Pakhtunkhwa, Pakistan

Email: sapnashakir1@gmail.com**Johar Jamil**

Department of Microbiology, Faculty of Science, The University of Swabi, Anbar-Swabi, Khyber Pakhtunkhwa, Pakistan

Email: johar.jamil@uoswabi.edu.pk**Abid Sarwar**

Food and Biotechnology Research Center, PCSIR Laboratories Complex, Lahore, Punjab, Pakistan

Rubina Nelofer

Food and Biotechnology Research Center, PCSIR Laboratories Complex Lahore

Email: rubinanelofer@gmail.com**Awais Amin**

Department of Microbiology, University of Swabi, KPK, Pakistan

Email: awaismicrobiologist@gmail.com**Younas Khan**

Department of Microbiology, University of Swabi

Email: m.younaskhan950@gmail.com**Waqas Ahmed**

Department Of Microbiology, University Of Swabi, KPK, Pakistan

Email: waqas490.uos@gmail.com**Maawa Gul**

Department of Microbiology, University of Swabi

Email: maawagul4@gmail.com**Rizwan Ullah**

Department of Microbiology, University of Swabi

Email: std108413@uop.edu.pk

ARTICLE INFO

Received:

August 04, 2026

Revised:

August 21, 2026

Accepted:

September 03, 2026

Available Online:

September 15, 2026

Keywords:

Amylase, *Bacillus tropicus*, Agro-industrial waste, Submerged fermentation, Process optimization, 16S rRNA sequencing, Circular bioeconomy

Corresponding Author:

Sapna ShakirEmail: sapnashakir1@gmail.com

ABSTRACT

The increasing need for industrial enzymes, coupled with the growing need for sustainable waste management, has made it necessary to explore cost-effective bioprocessing strategies. In this study the possibility of using different agro-industrial wastes as alternative substrates for the production of amylase using a novel bacterial isolate from the sugarcane rhizosphere was explored. Bacterial strains were first tested on starch agar medium and the presence of amylolytic activity was verified by flooding with iodine. The isolate with the best morphology, biochemical and molecular characteristics was selected as the most promising. Gram staining showed that the strain was Gram-positive, rod-shaped, and 16S rRNA gene sequencing with universal primers (27F and 1492R) followed by Sanger sequencing and phylogenetic analysis confirmed the strain as *Bacillus tropicus* (Accession No. OQ674805). Wheat bran, rice polish, rice husk and soluble starch were used as substrates for amylase production by submerged fermentation. The optimization of the process was performed with the one factor at a time method. The amylase activity was the highest (299.068 IU/min/mL) when the substrate used was rice polish after incubation for 72 hours at 35°C and pH 7. Peptone (146.289 IU/min/mL) and urea (142.049 IU/min/mL) were the two nitrogen sources that greatly supported the production of enzymes. It is interesting to note that the addition of MnCl₂ caused a dramatic increase in amylase activity to 508.26 IU/min/mL, indicating a metal-dependent catalytic mechanism. The process is also industrially feasible as 345.982 IU/min/mL was obtained after 72 hours of fermentation in a 2 L stirred bioreactor. The enzyme was found to be most active at moderately thermophilic conditions and neutral pH, which are desirable for several industrial applications such as food processing, detergents, textiles, and biofuels. This study proposes *B. tropicus* as a potential microbial producer of amylase with renewable agro-industrial residues, which is also cost-effective and sustainable, in line with circular bioeconomy principles.

Introduction

Enzymes are biological catalysts that accelerate biochemical reactions essential for numerous industrial processes. Amylases are one of the vast range of industrial enzymes which account for about 25–33% of the global enzyme market and are used in various industries (Al-Bedak et al., 2025). According to Market Research (2025), the global amylase market was worth around USD 1.9 billion in 2025 and will grow at a compound annual rate of 6.18% to reach USD 3.1 billion by 2033. These enzymes break down starch into simpler sugars like glucose, maltose, and dextrin, by removing the α -1,4-glycosidic linkages (Al-Bedak et al., 2025). Amylases are used in various fields, including food and beverage processing, detergents, textiles, paper, pharmaceuticals, animal feed, and biofuel production (Saeed et al., 2025). Alpha-amylase is the most dominant enzyme with around 48–55% market share as it is widely used in starch liquefaction and the food and beverage industry (Mordor Intelligence, 2025). The microbial sources are preferred for the production of industrial amylase as they grow fast, produce more amount of amylase, and are easily manipulated genetically (Al Sharif et al., 2025). *Bacillus* spp. are known to produce extracellular amylases with desirable properties like thermostability and wide pH tolerance, making them stand out among bacterial genera for this production (Saeed et al., 2025; Al Sharif et al., 2025). *Bacillus* spp. are the most prominent producers of amylase, accounting for about 50% of the global enzyme market, among which *Bacillus subtilis*, *B. licheniformis*, *B. amyloliquefaciens* and *B. cereus* are the most studied species (Isabel & Premalatha, 2025). In recent years, novel high-performance amylase-producing strains have been identified, such as *Bacillus spizizenii*, which can produce 34,121 U/g and is stable over a wide range of pH (4–9) and temperature (30–80°C) (Al Sharif et al., 2025). Cost of raw materials is a critical factor for economic viability of industrial enzyme production. Agro-industrial wastes are abundant, renewable and low-cost substrates that can be used as carbon and nitrogen sources for microbial fermentation (Saeed et al., 2025; Nguyen et al., 2025). Lignocellulosic residues are the by-products of agricultural processing industries that are produced in large amounts, such as mango peels, wheat bran, rice bran, potato peel, and sugarcane bagasse (Saeed et al., 2025; Mojumdar & Deka, 2024). The use of these waste streams in enzyme production will not only lower the production cost but also help mitigate environmental issues related to waste disposal that are consistent with the principles of a circular bioeconomy (Nguyen et al., 2025; Gillet et al., 2024). For example, starch industry wastewater has proven to be a suitable source for producing amylase with *Bacillus amyloliquefaciens*, with an optimized production rate of 5.73 U/mL (Nguyen et al., 2025). The submerged fermentation (SmF) process is generally used for the production of enzymes in industrial processes because it provides good process control, homogenous nutrient distribution and is easily scaled up (Nguyen et al., 2025). Optimal fermentation conditions such as substrate concentration, nitrogen sources, temperature, pH, and metal ions are crucial for enzyme production (Saeed et al., 2025; Al-Bedak et al., 2025). The statistical methods like response surface methodology (RSM) and Plackett-Burman design (PBD) have been useful for determining the critical factors and optimizing amylase production by 1.2-fold or more (Saeed et al., 2025). The present study aimed to isolate and characterize amylase producing bacteria from sugarcane rhizosphere, screen different agro industrial wastes as substrate for amylase production and optimize fermentation condition for the production of amylase. This research is part of the efforts to develop sustainable bioprocesses for the production of enzymes in industries, utilizing renewable resources. The results also hold significance for other biotechnological applications such as the development of enzyme-based antimicrobial strategies and sustainable veterinary therapeutics (Muneeb et al., 2025; Khan et al., 2025).

Materials and Methods

Study Location

This investigation was conducted at the Food and Biotechnology Research Center, PCSIR Laboratories Complex, Lahore, Punjab, Pakistan.

Soil Sample Collection

Soil samples were collected from the sugarcane rhizosphere in Lahore, Punjab, Pakistan. Samples were obtained from a depth of 10 cm below the soil surface using sterile plastic containers and transported to the laboratory for immediate processing.

Media Preparation

Bacterial cultures were maintained on nutrient agar medium. Starch agar medium was used for screening amylase-producing microorganisms. The fermentation medium for amylase production contained (g/L): soluble starch 1.0, peptone 1.0, yeast extract 1.0, KH_2PO_4 0.2, MgSO_4 0.1, NaCl 0.1, CaCl_2 0.1, and Tween 80 (1 mL), with the pH adjusted to 7.0 (Dash et al., 2015).

Isolation of Bacterial Strains

Bacterial strains were isolated using serial dilution and pour plate techniques. One gram of soil sample was suspended in 10 mL of normal saline and vortexed thoroughly. Serial dilutions (10^{-1} to 10^{-6}) were prepared, and 100 μL aliquots from each

dilution were spread onto nutrient agar plates. Plates were incubated at 37°C for 24 hours, and colonies were morphologically examined and sub-cultured for purification (Khan et al., 2025a).

Screening of Amylase-Producing Bacteria

Isolated bacterial colonies were screened for amylolytic activity on starch agar medium containing (g/L): peptone 5.0, beef extract 3.0, soluble starch 2.0, and agar 15.0. Inoculated plates were incubated at 37°C for 3 days. Following incubation, iodine solution (1 g iodine crystals and 2 g potassium iodide in 100 mL distilled water) was flooded onto the plates. The appearance of clear hydrolysis zones around bacterial colonies indicated amylolytic activity (Figure 1).

Identification of Amylase-Producing Bacteria

Gram Staining

Gram staining was performed using a modified Cheesebrough method. Bacterial smears were prepared on clean glass slides, air-dried, and heat-fixed. Slides were stained with crystal violet for 1 minute, rinsed with distilled water, treated with Gram's iodine for 1 minute, decolorized with 50% acetone-ethanol solution, and counterstained with safranin for 45 seconds. Slides were examined under an optical microscope at 40X to 100X magnification (Khan et al., 2025b) (Figure 2).

DNA Extraction

Bacterial DNA was extracted from a single colony suspended in phosphate-buffered saline and centrifuged. The pellet was resuspended in 600 μ L of cell lysis buffer, mixed gently, and incubated for 10 minutes. Isopropanol (600 μ L) was added, and the solution was mixed until white DNA strands became visible. DNA was spooled using a pipette tip, transferred to a fresh vial, and washed with 500 μ L of 70% ethanol. The DNA was precipitated by centrifugation at 10,000 rpm for 10 minutes, air-dried, and resuspended in 50 μ L of 1X TE buffer by incubation at 55-66°C for 5 minutes (Khan et al., 2025b).

PCR Amplification and 16S rRNA Gene Sequencing

The 16S rRNA gene was amplified using universal primers 27F and 1492R in a PCR thermocycler. Amplified products were verified by gel electrophoresis and sequenced using the Sanger sequencing method. Sequences were analyzed using Chromas Light software, and similarity searches were performed using NCBI BLAST. Phylogenetic trees were constructed using ClustalW and the neighbor-joining method (Dash et al., 2015) (Figure 3).

Substrate Screening

Four agro-industrial wastes (rice husk, rice polish, wheat bran, and soluble starch) were evaluated as substrates for amylase production under submerged fermentation conditions.

Screening of Nitrogen Sources

Various organic and inorganic nitrogen sources including ammonium nitrate, yeast extract, tryptone, ammonium sulfate, peptone, sodium nitrate, and urea were examined at 1% (w/v) concentration.

Primary Production Media

Amylase production under submerged fermentation was carried out in 250 mL Erlenmeyer flasks containing fermentation medium. The medium was autoclaved at 121°C for 20 minutes, and various substrates were added as specified in each experiment (Dash et al., 2015).

Optimization of Culture Conditions in Shake Flask

The one-factor-at-a-time (OFAT) method was employed to optimize fermentation parameters. The effects of the following factors were systematically evaluated:

- Substrate concentration: 1-4% (w/v)
- Nitrogen sources: 1% (w/v)
- Incubation temperature: 30, 35, 37, and 45°C
- Incubation period: 24, 48, 72, and 96 hours
- pH: 4.5, 5.5, 6.5, and 7.0

- Metal ions: CuCl_2 , MnCl_2 , NaCl , CaCl_2 , sorbitol, and Triton X-100

Lab-Scale Bioreactor Fermentation

Scale-up experiments were conducted in a 2 L laboratory-scale stirred bioreactor (B. Braun, Germany) with a working volume of 1.5 L. The bioreactor was equipped with two six-blade turbine impellers, pH electrode, temperature probe, and dissolved oxygen electrode. Fermentation was carried out at 37°C, pH 7.0, and 200 rpm for 72 hours using rice polish (150 g) as substrate.

Enzyme Assay

Amylase activity was determined using the dinitrosalicylic acid (DNS) method (Miller, 1959). The reaction mixture contained 1 mL of diluted enzyme, 1 mL of 1% starch solution in citrate buffer (pH 4.8), and 1 mL of citrate buffer. After incubation at 50°C for 30 minutes, the reaction was stopped by adding 1.5 mL of DNS reagent, and the mixture was heated in a boiling water bath for 15 minutes. After cooling, absorbance was measured at 540 nm. One unit of amylase activity was defined as the amount of enzyme required to liberate 1 μmole of reducing sugar per minute under assay conditions.

Statistical Analysis

All experiments were conducted in triplicate. Data were analyzed using SPSS version 11.0 (SPSS Inc., Chicago, IL). Mean values and standard deviations were calculated.

Results

Isolation and Primary Screening of Amylase-Producing Bacteria

Bacterial strains were successfully isolated from sugarcane rhizosphere soil samples. Primary screening on starch agar medium followed by iodine flooding revealed clear hydrolysis zones around several bacterial colonies, indicating amylolytic activity (Figure 1). The most promising isolate, designated as the highest amylase producer, was selected for further characterization and fermentation studies.



Figure 1. Zone of clearance produced by isolated bacterial strains on starch agar medium after iodine flooding. The clear zones around bacterial colonies indicate amylolytic activity, confirming the presence of amylase-producing bacteria.

Identification of the Selected Isolate

Morphological and Biochemical Characterization

The selected isolate exhibited white, rod-shaped colonies on nutrient agar. Gram staining confirmed the bacterium as Gram-positive (Figure 2). Biochemical tests showed positive results for catalase and oxidase (Table 1).

Table 1. Morphological and biochemical characteristics of the amylase-producing strain.

Characteristic	Result
Morphological Features	
Color	White

Characteristic	Result
Shape	Rods
Gram Staining	Positive
Biochemical Features	
Catalase	Positive
Oxidase	Positive

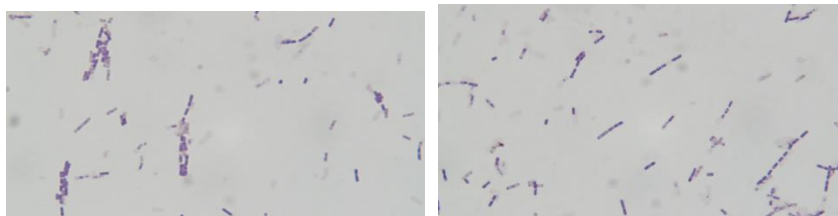


Figure 2. Gram staining micrograph of *Bacillus tropicus* showing Gram-positive rod-shaped cells. The image was captured at 100X magnification under oil immersion.

Molecular Identification

16S rRNA gene sequencing and phylogenetic analysis confirmed the isolate as *Bacillus tropicus* (Accession No. OQ674805). The sequence showed 93% query coverage and 87.56% identity with *B. tropicus* OQ674805.1 (Table 2). The phylogenetic tree demonstrated close evolutionary relationship with *B. tropicus* strains (Figure 3).

Table 2. Top 10 BLAST sequence matches for the 16S rRNA gene sequence of the isolated strain.

Scientific Name	Max Score	Total Score	Query Coverage	E-value	Identity (%)	Accession
<i>Bacillus tropicus</i>	1175	1175	93%	0.0	87.56	OQ674805.1
<i>Bacillus cereus</i>	1170	1170	94%	0.0	87.31	LT548959.1
<i>Bacillus albus</i>	1170	1170	93%	0.0	87.49	OM232457.1
<i>Bacillus cereus</i>	1168	1168	92%	0.0	87.61	MG516207.1
<i>Bacillus tropicus</i>	1166	1166	93%	0.0	87.07	MT835152.1

Scientific Name	Max Score	Total Score	Query Coverage	E-value	Identity (%)	Accession
Bacillus sp.	1157	1157	94%	0.0	87.12	MZ311875.1
Bacillus anthracis	1146	1146	94%	0.0	86.94	ON242114.1
Bacillus sp. YXA3-25	1144	1144	93%	0.0	86.97	JF701942.1
Bacillus anthracis	1140	1140	94%	0.0	86.85	MF092956.1
Bacillus sp.	1133	1133	94%	0.0	86.84	MT491145.1

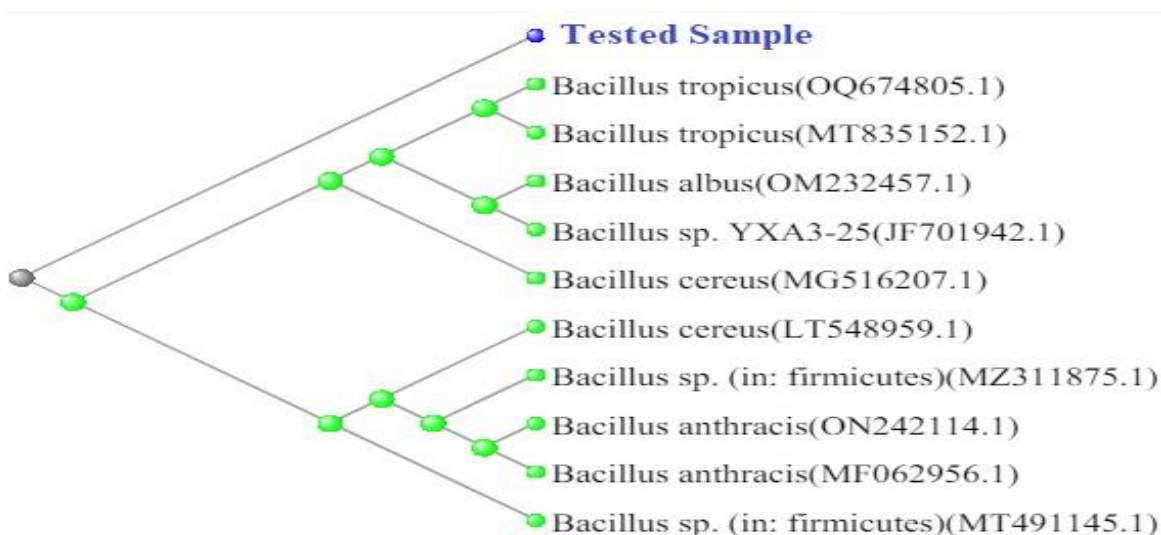


Figure 3. Phylogenetic tree of *Bacillus tropicus* and related species constructed using the neighbor-joining method based on 16S rRNA gene sequences. The tested sample is highlighted in blue, showing close evolutionary relationship with *B. tropicus* strains.

Optimization of Amylase Production

Effect of Various Substrates

Among the tested agro-industrial wastes, rice polish (2% w/v) supported the highest amylase production (167.97 IU/min/mL), followed by rice husk (2%), starch (1%), and wheat bran (4%) (Table 3).

Table 3. Effect of various agro-industrial substrates on amylase production by *Bacillus tropicus*.

Substrate	Concentration	Enzyme Activity (IU/min/mL)
Starch	1%	215.41

Substrate	Concentration	Enzyme Activity (IU/min/mL)
Rice polish	2%	167.97
Rice husk	2%	131.12
Wheat bran	4%	101.76

Effect of Nitrogen Sources

Among the various nitrogen sources tested, peptone (146.289 IU/min/mL) and urea (142.049 IU/min/mL) were most effective in enhancing amylase production. Inorganic nitrogen sources such as sodium nitrate (120.141 IU/min/mL) also supported enzyme production, while ammonium sulfate (54.975 IU/min/mL) and yeast extract (55.370 IU/min/mL) resulted in lower yields (Table 4).

Table 4. Effect of various organic and inorganic nitrogen sources on amylase production by *Bacillus tropicus*.

Nitrogen Source	Enzyme Activity (IU/min/mL)
Ammonium nitrate	80.303
Yeast extract	55.370
Tryptone	77.660
Ammonium sulfate	54.975
Peptone	146.289
Sodium nitrate	120.141
Without nitrogen source	46.964
Urea	142.049

Effect of Incubation Temperature

Maximum amylase production (276.968 IU/min/mL) was achieved at 35°C. Enzyme activity decreased at higher temperatures, with 64.698 IU/min/mL at 45°C (Table 5).

Table 5. Effect of incubation temperature on amylase production by *Bacillus tropicus*.

Temperature	Enzyme Activity (IU/min/mL)
30°C	126.855
35°C	276.968
37°C	146.692
45°C	64.698

Effect of Incubation Period

Optimal amylase production (299.068 IU/min/mL) was observed after 72 hours of incubation. Prolonged incubation (96 hours) resulted in decreased enzyme activity (110.762 IU/min/mL) (Table 6).

Table 6. Effect of incubation period on amylase production by *Bacillus tropicus*.

Time Interval	Enzyme Activity (IU/min/mL)
24 hours	151.459
48 hours	172.932
72 hours	299.068
96 hours	110.762

Effect of pH

The optimal pH for amylase production was 7.0, yielding 249.5 IU/min/mL. Acidic pH values (4.5 and 5.5) resulted in significantly lower enzyme activities (89.46 and 210.59 IU/min/mL, respectively) (Table 7).

Table 7. Effect of initial pH of fermentation medium on amylase production by *Bacillus tropicus*.

pH	Enzyme Activity (IU/min/mL)
4.5	89.46
5.5	210.59
6.5	107.7
7.0	249.5

Effect of Metal Ions

The addition of MnCl_2 dramatically enhanced amylase activity to 508.26 IU/min/mL, representing the highest activity observed among all metal ions tested. Other metal ions including NaCl (82.94 IU/min/mL), sorbitol (99.06 IU/min/mL), CaCl_2 (50.22 IU/min/mL), and Triton X-100 (57.12 IU/min/mL) showed comparatively lower effects (Table 8).

Table 8. Effect of various metal ions and additives on amylase activity of *Bacillus tropicus*.

Metal Ion/Additive	Enzyme Activity (IU/min/mL)
NaCl	82.94
MnCl_2	508.26
Sorbitol	99.06
CaCl_2	50.22
Triton X-100	57.12

Amylase Production in Bioreactor

Scale-up fermentation in a 2 L stirred bioreactor yielded maximum enzyme activity of 345.982 IU/min/mL after 72 hours at 37°C, pH 7.0, and 200 rpm. Extended fermentation (144 hours) resulted in decreased activity (109.143 IU/min/mL) (Table 9).

Table 9. Amylase production by *Bacillus tropicus* in a 2 L stirred tank bioreactor under optimized conditions.

Time Interval	Enzyme Activity (IU/min/mL)
72 hours	345.982
144 hours	109.143

Discussion

In the present study it was successfully established that a potent amylase producing bacterium, *Bacillus tropicus* was isolated from sugarcane rhizosphere soil and identified. The microbiological diversity and organic matter of the rhizosphere environment were found to be a good source for isolating industrially useful microorganisms (Anbessa et al., 2026). The isolate was identified as *B. cereus* group by 16S rRNA gene sequencing and phylogenetic analysis, including the newly described species *B. tropicus*, *B. albus* and *B. paranthracis* (Liu et al., 2017).

Agro-industrial wastes were screened as substrates for amylase production and it was found that rice polish is the most effective carbon source (Table 3). Rice polish is a by-product of rice milling process, which contains approximately 34–62% carbohydrate, 11–15% protein and 7–11% crude fiber (Alauddina et al., 2017) and thus can provide essential nutrients for the growth of bacteria and enzyme production. A recent study by Tariq et al. (2026) found that rice polish is an excellent low cost substrate for enzyme production with a starch content of 79.5%, total sugars of 35.2%, and N content of 8.5%. The superiority of rice polish over other substrates could be explained by its balanced nutrient composition and the soluble sugars present

which are easily metabolized by *B. tropicus*. The same was observed by Saeed et al. (2025) who showed efficient amylase production by using mango peels as an agro-industrial waste substrate with the help of *Bacillus amyloliquefaciens*.

Optimization of nitrogen source showed that organic sources especially peptone and urea had a significant effect on amylase production (Table 4). Peptone is a complex source of peptides and amino acids that help bacteria grow and produce enzymes, making it an easily digestible source of nitrogen and growth factors (Saeed et al., 2025). Urea is an inorganic source, but is capable of being hydrolyzed to ammonia and carbon dioxide, which can be used for amino acid synthesis. The results corroborate with earlier reports of superiority of organic nitrogen sources to produce amylase (Chowdhury et al., 2026). By contrast, a thermophilic *Bacillus* spp. When grown on urea as a nitrogen source, WN11 did not grow, indicating that there may be strain-specific nitrogen utilization patterns (Eman et al., 1999).

The temperature range for amylase production (35°C) suggests that *B. tropicus* is a mesophilic organism with moderate thermostability of the enzymes produced (Table 5). This temperature optimum is in agreement with the previous studies conducted with the *Bacillus* species which generally showed the optimum enzyme production in the range of 30-40°C (Chowdhury et al., 2026). A study on *Bacillus subtilis* reported the optimal temperature of 35 °C and wheat bran as a submerged fermentation substrate for amylase production (Irfan et al., 2016). The decrease in enzyme activity with increasing temperature could be due to the thermal denaturation of the enzyme proteins or inhibition of bacterial growth. Irreversible thermoinactivation of *Bacillus* α -amylases is caused by monomolecular conformational scrambling, which leads to incorrect protein structures at elevated temperatures (Tomazic and Klibanov, 1988).

The incubation period of 72 hours is optimal as it takes the maximum amount of time for bacteria to grow and secrete enzymes (Table 6). A comparative study of *B. licheniformis* MTCC 2618 revealed that the maximum amylase production was on the 3rd day (72 hours) of fermentation (Divakaran et al., 2011). The following reduction in enzyme activity after 96 hours could be explained by the depletion of nutrients, formation of inhibitory metabolites, or proteolytic degradation of the enzyme (Saeed et al., 2025). Amylase production by *B. cereus* AS2 exhibited maximum production at 72 hours, similar temporal profile as reported for amylase production by *B. cereus* AS2 (Rehman et al., 2019).

The optimum pH for amylase production is 7.0, which is a neutral pH (Table 7). This is in line with the typical pH range for *Bacillus* amylases which are generally active at pH 6.0–8.0 (Divakaran et al., 2011). The optimum pH for amylase production by *B. cereus* AS2 was reported to be 7.0 (Rehman et al., 2019), and for *B. paralicheniformis* AOLT3, it was 7.5 (Chowdhury et al., 2026). The sharp decrease in the enzyme activity at acidic pH values indicates that the activity of the enzyme becomes impaired when the pH is acidic, either due to change in the enzyme's active site or its integrity.

The remarkable increase in amylase activity with MnCl_2 (508.26 IU/min/mL) is noteworthy from the findings of this study (Table 8). Manganese ions are known cofactors for a number of enzymes, including some amylases, in which they stabilize the enzyme structure or are involved in the catalysis. A study on the effect of metal ions reported that Ca^{2+} and Mg^{2+} showed positive effect in the production of α -amylase by *B. amyloliquefaciens* with the highest concentration of 7 g/L for Ca^{2+} which gave 0.439 IU/ml/min (Sudha, 2012). The observed activation of enzyme activity with the addition of MnCl_2 indicates a metal dependent mechanism of activation, which could be utilized in an industrial process.

Scale-up fermentation in stirred bioreactor resulted in higher enzyme activity of 345.982 IU/min/mL than shake flask culture (299.068 IU/min/mL) which shows that the production of enzyme at industrial scale is possible (Table 9). Increased enzyme production rate was observed when the *B. amyloliquefaciens* was scaled up from shake flask to stirred tank bioreactor, which was found to be 28–54% due to the enhanced oxygenation and mixing (El-Enshasy, 2007). The better performance in the bioreactor could be due to the better control of the process parameters like pH, temperature, dissolved oxygen, and better mixing and mass transfer (El-Enshasy, 2007).

The enzyme obtained in the present study from *B. tropicus* has some characteristics that are desirable for industrial use such as moderate thermostability, neutral pH optimum and enhancement by metal ions. These properties make it ideal for different industrial applications such as starch processing, food and beverage, detergents, textiles, and biofuels (Anbessa et al., 2026). In addition, recent *B. tropicus* strains with potential probiotic properties have been isolated, further emphasizing the industrial versatility of this species (Athulya et al., 2024).

Conclusion

This study successfully isolated and characterized a potent amylase-producing bacterium, *Bacillus tropicus*, from sugarcane rhizosphere soil. The bacterium demonstrated efficient amylase production using low-cost agro-industrial wastes, particularly rice polish, as substrate under submerged fermentation conditions. Process optimization revealed optimal production conditions of 2% rice polish, 72 hours incubation, 35°C, and pH 7.0, yielding 299.068 IU/min/mL in shake flask cultures. The addition of MnCl_2 significantly enhanced enzyme activity to 508.26 IU/min/mL, suggesting a metal-dependent catalytic

mechanism. Scale-up fermentation in a stirred bioreactor yielded 345.982 IU/min/mL, demonstrating industrial feasibility. The enzyme's moderate thermostability and neutral pH optimum make it suitable for various industrial applications. This study contributes to the development of sustainable, cost-effective bioprocesses for industrial enzyme production using renewable agro-industrial residues, aligning with circular bioeconomy principles.

References

- Al Sharif, B., Golchini, M.M., Soorni, A. and Mehrabi, R., 2025. Genomic and enzymatic insights into α -amylase-producing *Bacillus spizizenii* strains isolated from Isfahan province, Iran. *Plos one*, 20(12), p.e0333668.
- Alauddina, M., Islama, J. and Shirakawaa, H., 2017. Conversion of Rice Bran into a Superfood/Functional. *Superfood and Functional Food: An Overview of Their Processing and Utilization*, p.291.
- Al-Bedak, O.A., Moharram, A.M., Ameen, F., Stephenson, S.L., Idres, M.M. and Yasser, M.M., 2025. Optimizing conditions for augmented production of amylase by *Talaromyces islandicus* AUMC 11391. *Electronic Journal of Biotechnology*, 75, pp.39-48.
- Anbessa, M., Desalegn, A., Bai, F.Y. and Tigu, F., 2026. The α -Amylase Producing Bacteria From Bishoftu Lakes: Extraction, Purification and Application. *Natural Product Communications*, 21(4), p.1934578X261444501.
- Athulya, P.A., Chandrasekaran, N. and Thomas, J., 2024. *Bacillus* spp. isolated from intestine of *Oreochromis mossambicus*: Identifying a potential probiotic for tilapia culture. *Aquaculture Reports*, 36, p.102067.
- Chowdhury, D., Ojha, A. and Das, D., 2026. Sustainable bioprocessing of kitchen waste for amylase production by an autochthonous *Bacillus paralicheniformis* AOLT3: process optimization and enzyme characterization. *Systems Microbiology and Biomanufacturing*, 6(4), p.101.
- Dash, B.K., Rahman, M.M. and Sarker, P.K., 2015. Molecular identification of a newly isolated *Bacillus subtilis* BI19 and optimization of production conditions for enhanced production of extracellular amylase. *BioMed Research International*, 2015(1), p.859805.
- Divakaran, D., Chandran, A. and Pratap Chandran, R., 2011. Comparative study on production of α -amylase from *Bacillus licheniformis* strains. *Brazilian Journal of Microbiology*, 42(4), pp.1397-1404.
- El Enshasy, H., 2007. Bioprocess development for the production of alpha amylase by *Bacillus amyloliquefaciens* in batch and fed-batch cultures. *Research Journal of Microbiology*, 2(7), pp.560-568.
- Gillet, S.A., Silva, M.N., Carvalho, E.A., da Silva, E.G.P., dos Santos Moreau, P., Uetanabaro, A.P.T. and da Costa, A.M., 2024. Utilization of Peach-palm waste for cost-effective amylase production by *Trichoderma stromaticum*: Stability and industrial potential. *Mycology*, 15(4), pp.690-701.
- Irfan, M., Nadeem, M., Syed, Q., Abdullah Shakir, H. and Iqbal Qazi, J., 2016. Study on some properties of calcium-dependent α -amylase from *Bacillus subtilis* through submerged fermentation of wheat bran. *Chemical and Biochemical Engineering Quarterly*, 30(4), pp.429-437.
- Isabel, J.B. and Premalatha, M., 2025. Harnessing amylase producing novel bacterial strains from industrial waste: A bioprocessing and optimization approach. *International journal of biological macromolecules*, p.146769.
- Khan, A.Y., Effendi, M.H., Shaukat, A. and Muneeb, M., 2026. Prevalence, risk factors, and innovative therapies for methicillin-resistant *Staphylococcus aureus* in livestock and companion animals in Pakistan. *Open Veterinary Journal*, 16(3), p.1438. <https://doi.org/10.5455/ovj.2026.v16.i3.3>
- Khan, A.Y., Effendi, M.H., Tyasningsih, W., Ullah, S., Khan, I.U., Shah, M.K., Zaman, A., Muneeb, M. and Shabbir, G., 2025a. Antimicrobial potential of indigenous medicinal plants against Multi Drugs Resistant Enterohemorrhagic *Escherichia coli* O157: H7 Isolated From Canine in Pattoki Tehsil, Punjab Province, Pakistan. *Egyptian Journal of Veterinary Sciences*, 56(11), pp.2997-3008. <https://doi.org/10.21608/ejvs.2025.342425.2543>
- Khan, A.Y., Shaukat, A., Muneeb, M., Shah, M.K., Shaukat, I., Tahir Aleem, M., Afzal, F., Ur Rehman, T., Khan, S., Ud Din, Z. and Ahmadova, K., 2025b. Prevalence of Blood Protozoa and Impact of Chemotherapy in Exotic Cattle, With Special Reference to Haematological Parameters. *Egyptian Journal of Veterinary Sciences*, pp.1-8. <https://doi.org/10.21608/ejvs.2025.416243.3067>

- Liu, Y., Du, J., Lai, Q., Zeng, R., Ye, D., Xu, J. and Shao, Z., 2017. Proposal of nine novel species of the *Bacillus cereus* group. *International journal of systematic and evolutionary microbiology*, 67(8), pp.2499-2508.
- Mamo, G. and Gessesse, A., 1999. Effect of cultivation conditions on growth and α -amylase production by a thermophilic *Bacillus* sp. *Letters in applied microbiology*, 29(1), pp.61-65.
- Market Research (2025) Global Amylase Industry Trends Analysis Report 2025, Forecast to 2033
- Mojumdar, A. and Deka, J., 2019. Recycling agro-industrial waste to produce amylase and characterizing amylase-gold nanoparticle composite. *International Journal of Recycling of Organic Waste in Agriculture*, 8(Suppl 1), pp.263-269.
- Mordor Intelligence (2025) Carbohydrase Market Size, Share & 2031 Growth Trends Report.
- Muneeb, M., Khan, A.Y., Shaukat, A. and Qu, Y., 2026. Bacteriophage cocktails for combating multidrug-resistant *Staphylococcus aureus* infections in animals: A comprehensive review across pets, livestock, and wildlife. *Microbial Pathogenesis*, p.108774. <https://doi.org/10.1016/j.micpath.2026.108774>
- Nguyen, V., Ndao, A., Blais, J.F. and Adjallé, K., 2025. Evaluation of industrial wastewaters as low-cost resources for sustainable enzyme production by *Bacillus* species. *Clean Technologies*, 7(2), p.45.
- Rehman, A., Saeed, A., Asad, W., Kiran, T., Baloch, M.N. and Eijaz, S., 2019. Optimization of physicochemical parameters for maximum amylase production by indigenously isolated *Bacillus cereus* AS2 strain. *Pakistan Journal of Pharmaceutical Sciences*, 32, p.889.
- Saeed, S., Shakir, H.A. and Qazi, J.I., 2025. Statistical optimization of amylase production from *Bacillus amyloliquefaciens* using agro-industrial waste (mango peels) under submerged fermentation by response surface methodology. *Journal of Microbiological Methods*, p.107208.
- Saeed, S., Shakir, H.A. and Qazi, J.I., 2025. Statistical optimization of amylase production from *Bacillus amyloliquefaciens* using agro-industrial waste (mango peels) under submerged fermentation by response surface methodology. *Journal of Microbiological Methods*, p.107208.
- Sudha (2012) 'Effect of different concentrations of metal ions on alpha amylase production by *Bacillus amyloliquefaciens*', TathQeef Scientific Publishing.
- Tariq, H., Yunus, F.U.N., Ullah, N., Sarwar, A., Bashir, F., Awan, A., Khan, A.A., Alwaili, M.A. and Al-Hoshani, N., 2026. Valorization of rice polish biomass through acid and enzymatic hydrolysis for fermentable sugar production. *BioResources*, 21(3), p.651.
- Tomazic, S.J. and Klivanov, A.M., 1988. Why is one *Bacillus* alpha-amylase more resistant against irreversible thermoinactivation than another?. *Journal of Biological Chemistry*, 263(7), pp.3092-3096.



2026 by the authors; Journal of The Kashmir Journal of Academic Research and Development. This is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC-BY) license (<http://creativecommons.org/licenses/by/4.0/>).