INDIAN JOURNAL OF SCIENCE AND TECHNOLOGY

RESEARCH ARTICLE

Harnessing the Proteolytic Power of *Aspergillus Terreus Sgtm2*

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OPEN ACCESS

Received: 22-10-2024

Accepted: 19-02-2025

Published: 10-04-2025

Citation: Amin T, Joshi D, Patni D, Mohil M (2025) Harnessing the Proteolytic Power of *Aspergillus Terreus Sgtm2*. Indian Journal of Science and Technology 18(12): 937-946. https://doi.org/10.17485/IJST/v18i12.3414

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Funding: None

Competing Interests: None

Published By Indian Society for Education and Environment (iSee)

ISSN
Print: 0974-6846
Electronic: 0974-5645

Abstract

Objectives: The research is focused on isolating and characterizing proteolytic microorganisms from soil samples collected from a cowshed. Additionally, it aimed to optimize the conditions for protease production by utilizing a selected microbial strain. **Methods:** Cowshed soil were used to isolate protease-producing fungi; they were cultured on PDA (Potato Dextrose Agar) plates and tested for their ability to produce proteolytic enzymes using milk agar plates. Morphological analysis and 18S rRNA sequencing identified the strains. Submerged fermentation was employed for protease production, with quantification conducted using the McDonald and Chen assay method. Process optimization was performed to maximize enzyme yield under varying conditions of inoculum size, temperature, pH, carbon, and nitrogen sources. **Findings:** Among five isolates (C1, C2, C3, C4, and C5), four exhibited proteolytic activity, with isolate C3 showing the highest protein hydrolysis. Morphological and molecular analysis identified the most efficient strain, SGTM2, as *Aspergillus terreus* with 99.10% similarity to *Aspergillus terreus* ATE1. Optimized production conditions (2% inoculum size, 45°C, pH 8.0, 1% maltose as carbon source, and 1.5% peptone as nitrogen source) resulted in a yield of 5.03 kg/m^3 protease, with peak production achieved on the 4th day of incubation. **Novelty:** This study demonstrates the industrial potential of *Aspergillus terreus* in producing high yields of protease under optimized conditions. The study shows that the species differ 0.9% from the existing species which leads to the better and high yield of protease (accession number PP474258). The study contributes to the development of cost-effective enzyme production processes, particularly for applications in detergents, food processing, pharmaceuticals, and waste management industries.

Keywords: Protease synthesis; Aspergillus terreus; Bio waste management enzymes; Protein hydrolysis; Cowshed soil fungi; Soil-derived fungi

1 Introduction

Proteases, or peptidases, are enzymes that degrade proteins by hydrolyzing their peptide bonds. They are essential for numerous types of biological activities, including protein turnover, metabolism, and cell signaling [1]. Despite their crucial importance, proteases have been widely applied across various industries, including food production and

detergent formulation, medicines, and biotechnology. This industrial application of proteases, particularly alkaline proteases, led to substantial studies into generating distinctive, more efficient, and cost-effective protease-producing microbes [2]. Fungi, particularly those from the *Aspergillus* species, have received attention due to their propensity to release large quantities of proteases externally.

The global demand for proteases is caused by the requirement for enzymes that can tolerate extreme industrial conditions like high pH and temperature while remaining stable over time. Despite their effectiveness, current proteases frequently fail to exceed these demanding standards, resulting in inefficiencies in a variety of industrial applications [3]. Although substantial advances in enzyme manufacturing and optimization, issues such as low yield, high costs of production and enzyme instability continue. Consequently, there is a continued need to discover novel protease sources that can be cultivated cost-effectively while maintaining high stability and efficiency under diverse conditions [4].

The soil surroundings, specifically manure-rich conditions including cowshed dirt, have a variety of microorganisms that are certain times disregarded in protease studies. These soils are filled with various organisms that have evolved to live in organic-rich conditions in which decaying feed and animal excreta provide a steady nutrition source. Such conditions have the potential to isolate new proteolytic fungi with unique enzyme characteristics that are useful for certain commercial applications [5].

This study investigates the protease-producing potential of microorganisms isolated from cowshed soil. The alkaline protease-producing organisms identified include *Aspergillus candidus, Aspergillus flavus, Aspergillus terreus, Aspergillus fumigatus, Aspergillus melleus, Fusarium sp., Paecilomyces lilacinus, Aspergillus Niger,* and *Aspergillus oryzae* [6]. Among these fungal isolates, *Aspergillus terreus* is identified as a particularly promising producer of proteases. A strain with strong proteolytic activity was chosen via a severe screening approach utilizing milk agar plates. The fermentation conditions were tuned to increase protease production. Submerged fermentation investigations demonstrated that *A. terreus* may manufacture proteases under alkaline conditions, with the highest activity at pH 8.0 and 45°C temperature [7]. This demonstrates its potential for commercial applications, notably in detergent manufacture, where enzymes must function in alkaline conditions.

A number of factors, including pH, temperature, and nutritional content, were carefully changed to determine the conditions that resulted in the maximum enzyme activity. This information shows that *A. terreus* might be a useful source of protease in a variety of industrial applications [8]. Furthermore, the molecular identification of the *Aspergillus* strain confirmed its taxonomic classification and provided insights into its genetic makeup, which could be important for future studies focused on enzyme enhancement and genetic optimization [9].

This study addresses key challenges in protease research, particularly the scalability and cost-effectiveness of enzyme production. While microbial sources like *Aspergillus terreus* show considerable potential, existing protease production methods face significant challenges in scalability and economic feasibility, especially in industries such as detergent manufacturing and leather processing. Furthermore, although Aspergillus species have been widely studied, much of microbial biodiversity, particularly from unconventional environments like cowshed soils, remains underexplored. The research investigates cowshed soil as a novel source of protease-producing fungi, focusing on *A. terreus*, and optimizes fermentation conditions to enhance protease yield. It also provides a foundation for scaling up enzyme production for industrial applications, with an emphasis on enzyme stability and cost-effectiveness. This study contributes to industrial enzyme production by identifying *A. terreus* as a potent protease producer, optimizing its production parameters, and demonstrating the potential of unconventional microbial habitats. The outcomes pave the way for future research aimed at improving enzyme yield, stability, and scalability to address the growing demand for high-performance proteases in various industrial sectors.

2 Material and Methods

2.1 Study site

The research was carried out between July 2023 and April 2024 at Sigma University, located in Vadodara, Gujarat. The study site, characterized by its advanced research facilities, provided an ideal environment for the objectives of the investigation.2.2. Collection of sample

A 0.01 kg sample was meticulously obtained from the cowshed in Halol, using a sterile container to prevent contamination. This container was then transported to the laboratory at Sigma University, where it was opened under aseptic conditions for subsequent analysis and further examination.

2.2 Preparation of media

Potato dextrose agar (PDA) has been used for this study because it is the medium used for the growth of fungal range. A total of 0.005 kg of PDA was weighed by an analytical balance, and 0.0002 kg of vancomycin was added in the PDA to inhibit bacterial

growth. The mixture was transferred into a 250 mL Erlenmeyer flask (0.00025 m^3), and 0.00015 m^3 of distilled water was added. The mixture was vigorously mixed to ensure dissolution. The flask was then sealed with cotton wool and covered in paper. The media was sterilized by autoclaving at 121°C for 15 minutes. After the sterilization process, the medium was removed from the autoclave and allowed to cool to room temperature. It was then poured into Petri plates and left to solidify. Once solidified, the fungal isolate was inoculated onto the medium for culture growth.

2.3 Screening for protease producing fungi

Fungal strains were isolated from the soil of a cowshed in Halol using the serial dilution plate method. Dilutions ranging from 10^{-2} to 10^{-} were plated onto Potato Dextrose Agar (PDA) medium, supplemented with 0.00005 kg of vancomycin, and incubated at room temperature. Post-incubation, fungal growth was monitored, and fungal spores exhibiting notable growth were subsequently transferred onto Skim Milk Agar plates for further screening to identify protease-producing fungi.

2.4 Identification of Effective Protease-Producing Isolates

2.4.1 Morphological characterization of protease producers

Fungal isolates exhibiting pronounced clearance zones, indicative of efficient enzyme production, were selected for further morphological characterization. The colony morphology was meticulously examined on Potato Dextrose Agar (PDA) plates. Additionally, a detailed microscopic examination of all protease-producing cultures was conducted through fungal staining, allowing for precise identification and characterization of the fungal species.

2.4.2 Assessments for protease-producing fungi

Skim Milk Agar (SMA) media were used for this study because it contains milk from which the protein has to be degraded by Protease-producing fungi. The plates were inoculated with fungal isolates and incubate at room temperature for 5-7 days to grow into different colonies. The presence of a clear zone or zone of protein hydrolysis around the colonies is let to determine protease activity. The fungal isolates that produced protease were subsequently examined using the Lacto phenol cotton blue staining technique for identification. These protease-producing fungal isolates were then subculture onto PDA (Potato Dextrose Agar) plates and kept at 4°C until the protease enzyme was produced and optimized.

Table 1. The skim milk agar medium has the following composition

Ingredients	Amount
Milk	10 ml.
Yeast extract	0.25 grams
Dextrose	0.01 g; NaCl: 0.005 g.
Agar	1.5 grams
Distilled water	100 ml

2.5 Identification of fungi by 18S rRNA Sequencing

A total of four fungal strains were isolated from the soil of a cowshed, with one strain, demonstrating significant proteolytic enzyme activity, being selected for further analysis. The primary focus of this investigation was to explore the enzymatic capabilities of this particular fungal isolate through 18S rRNA sequencing, facilitating its accurate identification and characterization.

2.6 Production of Protease by Submerge Fermentation

The secondary screening of protease was done by submerge fermentation process. The selected Protease producing fungal culture was inoculate in the production media and incubate at rotatory shaker at 110rpm for 7 days. The composition of the production broth medium is as follows: 0.4 grams of K2HPO4, 2 grams of glucose, 1 gram of peptone, and 30 ml of milk are added to 200 ml of distilled water.

2.7 Enzyme Extraction

The production medium is taken then the selected fungal culture was added in the medium and incubates it on rotatory shaker for 7 days at 110 rpm. After the incubation period, the medium was harvested and centrifuged at 10,000 rpm for 10 minutes. The resulting mixture was filtered using filter paper, and the supernatant was collected.

2.8 Protease quantification

For the Qualitative assay biuret Method was performed and for Quantative assay folin lowry Method was performed [10].

2.8.1 Biuret Method

When protein solutions are rendered strongly alkaline and a dilute copper sulfate solution is introduced, a purplish to pink-violet coloration is observed, with the specific hue varying according to the complexity of the proteins present. Proteins typically yield a purplish-violet color, while proteases and peptones impart a pink color. Peptides, in contrast, produce a very light pink tint, and gelatin results in a nearly blue color. Certain polyalcohols, such as glycerol and ethylene glycol, can also form a similar complex. To perform the assay, 2×10^{-3} L of the test protein solution is added to a test tube, followed by 2×10^{-3} L of 10% NaOH solution and 5–7 drops of a 10 g/L $CuSO_4 \cdot 5H_2O$ solution. The contents are mixed thoroughly, and a shift from blue to violet indicates a positive result, while a transition from light blue to dark blue suggests a negative reaction. This color change is attributed to the alteration of $CuSO_4$ when subjected to an alkaline environment [11].

2.8.2 Folin-Lowry Technique

In the Lowry protein assay, copper ions interact with the peptide bonds in proteins under alkaline conditions, forming a complex that facilitates the quantification of protein concentration [12].

2.9 Optimization of Protease producing fungi

2.9.1 Impact of temperature on protease producing fungi

The skim milk agar was made and poured in the Petri plate and allowed it to solidified then add lop full of selected protease producing fungal culture and placed in plate. Then incubate the plates at 5 different temperatures. The impact of temperature on fungal colony growth was assessed under varying conditions. At 4°C, no growth was detected. Temperatures between 25-30°C resulted in slight growth, whereas 30°C and 37°C produced medium growth. Notably, the temperature of 50°C showed the highest growth.

2.9.2 Impact of pH on protease producing fungi

The pH of the medium was meticulously adjusted using K_2HPO_4, NaOH, citric acid, and boric acid in Skim Milk Agar, which was then poured into Petri plates and allowed to solidify. A loopful of the selected protease-producing fungal culture was introduced onto the surface of the solidified agar. The plates were subsequently incubated under five distinct pH conditions. The growth of fungal colonies was carefully monitored at each pH level. Notably, maximal growth was observed at pH 5, while moderate growth occurred at pH 6. No growth was detected at pH levels 7 and 8.

2.10 Optimization of Protease production *Aspergillus terreus* SGTM2 and its Activity

2.10.1 Impact of Incubation Time on production of protease

To find effect of time period fermentation broth was inoculated by 0.001 L inoculum. Incubate for the fungal growth. Time is required for the maximum growth of microorganism, produced higher amount enzyme production. After every 24 hours, Checked protease production by enzyme assay of McDonald and Chen method.

2.10.2 Impact of Temperature on Protease Activity

To determine the optimum temperature for protease production, a broth containing raw milk was incubated at varying temperatures: 25°C, 30°C, 37°C, 45°C, 50°C, and 55°C. To each sample, 0.002 L of protease enzyme obtained from *Aspergillus terreus* SGTM2 was added. After 24 hours of incubation, the broth was analyzed for protease activity, and enzyme activity was subsequently assayed.

2.10.3 Impact of pH on Protease Activity

The broth was prepared at pH values of 5, 6, 7, 8, 9, and 10. Each broth was inoculated with 0.002 L of protease enzyme obtained from *Aspergillus terreus* SGTM2 and incubated at 50°C to assess enzyme activity. After 24 hours of incubation, protease activity was evaluated, and enzyme activity was subsequently assayed.

2.10.4 Impact of Carbon Source on Protease Activity

Various carbon sources were evaluated for their impact on protease activity, aiming to enhance enzyme yield. The carbon sources tested included glucose, starch, sucrose, glycerol, lactose, mannitol, maltose, dextrose, and fructose. In the experimental setup, the broth was supplemented with the selected carbon sources, and 2 mL of protease enzyme obtained from *Aspergillus terreus* SGTM2 was added. The medium was adjusted to a pH of 8 and incubated at 50°C. After 24 hours of incubation, protease production was assessed, and enzyme activity was determined using the McDonald and Chen method.2.11.5. Impact of Nitrogen Source on Protease Activity:

To optimize the nitrogen source for enzyme production, Raw Milk in the broth were replaced by various inorganic and organic nitrogen sources like Casein, Beef extract, Peptone, Yeast extract, and Meat extract, Urea, Tryptone, Ammonium Nitrate, Asparagine, Sodium Nitrate and Ammonium Sulphate respectively in medium. Protease activity was carried out using 0.002 L of protease enzyme obtain from *Aspergillus terreus* SGTM2, pH of 8 and temperature of 50 °C and 1% as carbon source. Enzyme activity was checked after 24 hrs and the enzyme activity was assayed

3 Results and Discussion

3.1 Purification and Identification of protease producing microorganisms

Morphologically different 5 strains were isolated from different collected samples using PDA media plates. Isolated cultures were separately screened for their proteolytic activity. Out of 5 different cultures, 4 isolates shown proteolytic activity on milk agar plates. So that Protease production was confirmed with zone of protein hydrolysis on milk agar plate. The cultures were named as C1, C2, C3, C4, and C5. Protease activity was measured by zone of hydrolysis after 48 hrs of incubation. Zone of protein hydrolysis on Milk agar plate by various cultures were shown in Figure 1.

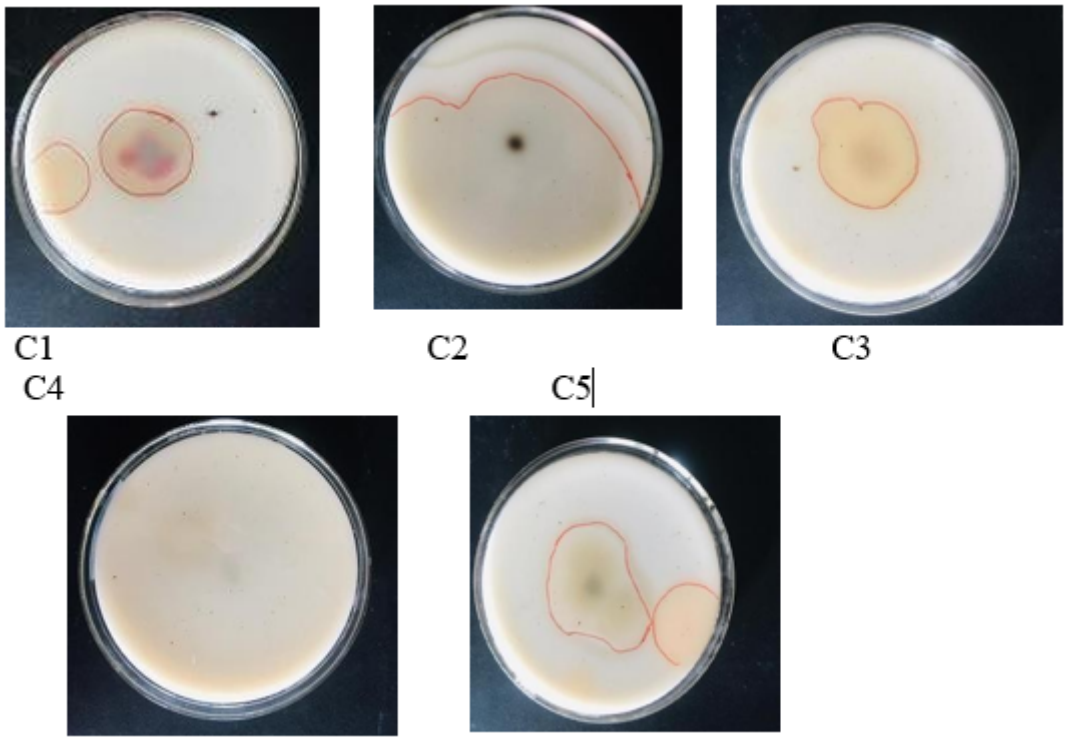

Fig 1. Milk agar plates, Zone of Protein Hydrolysis by protease producers

Morphological identification of protease producers

3.2.1 Cultural characteristics

5 isolates which shown proteolytic activity on milk agar plates were checked for their gram's reaction and zone of protein hydrolysis were measured in mm diameter .

3.2.2 Microscopic observation

Microscopic observation was observed by fungal staining. 5 cultures were shown in Lacto phenol cotton Blue Stain. Out of 4 isolates, 1 culture was selected based on higher zone of protein hydrolysis, which are more efficient producers of protease enzyme.

3.3 Identification of SGTM2 Strain by 18sr RNA Sequencing

The 18S rRNA sequencing analysis of the strain SGTM2 revealed a striking similarity to three known strains of *Aspergillus terreus*. Specifically, SGTM2 exhibited a 99.10% similarity to *Aspergillus terreus* strains ATE1 (Accession: KM462826.1), MP1 (Accession: MN744345.1), and AN4 (Accession: KF660536.1). Utilizing the Weighbor tree neighbor joining method, a phylogenetic tree was generated to visualize the relationships between these strains. Upon submission to the gene Bank database, the sequencing data for SGTM2 was assigned the accession number PP474258. Analysis of the phylogenetic tree conclusively identified SGTM2 as belonging to the species *Aspergillus terreus*. Remarkably, this analysis also unveiled SGTM2 as a novel strain within this species.

Aligned Sequence Data of Sample – SGTM2 (1666 bp)

TCTAAGTATAAAGCACTTTATACTGTGAAACTGCGAATGGCTCATTAAATCAGTTATCGTTTATTT GATAG-
TACCTTACTACATGGA TACCTGTGGTAATTCTAGAGCTAATACATGCTAAAAACCCCGACTTCGGAAGGGGT-
GTATTTATT AGATAAAAAACCAATGCCCTTC GGGGCTCCTTGGTGATTCATAATAACTTAACGAATCG-
CATGGCCTTGCGCCGGCGATGGTTCATTC AAATTTCTGCCCTATCAACTT TCGATGGTAGGATAGTG-
GCCTACCATGGTGGCAACGGGTAACGGGGAATTAGGGTTCGATTCCG GAGAGGGAGCCTGAGAAACG-
GCT ACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCCGACACGGGGAGGTAGTGACA
ATAAATACTGATACGGGGCTCTT TCGGGTCTCGTAATTGGAATGAGTACAATCTAAATCCCTTAACGAG-
GAACAATTGAGGGCAATCT GGTCCAGCAGCGCGGAATTCCA GCTCCATAGCGTATTTAAAGTTTTGCAGT-
TAAAAGCTTGTAGTGAACCTTGGTCTGGCTGGCCGGT CCGCTCACCGCGAGTACTGGT CCGGCTGACCTTTC-
CTTCTGGGGAATCCCATGGCCTTCACTGGCTGTGGGGGAACCAGGACTTTTA CTGTGAAAAAATTAGAGTGTT
CAAAGCAGGCCTTTGCTCGAATACATTAGCATGGAATAATAGAATAGGACGTGCGGTTCTATTTT GTTGGTTTC-
TAGGACCGCCGTA ATGATTAATAGGGATAGTCGGGGGCGTCAGTATTCAGCTGTCAGAGGTGAAATTCTTG-
GATTTGC TGAAGACTAACTACTGCGAAAG CATTCGCCAAGGATGTTTTCATTAATCAGGGAACGAAAGT-
TAGGGGATCGAAGACGATCAGATA CCGTCGTAGTCTTAACCATAAAC TATGCCGACTAGGGATCGGGCGGT-
GTTTCTATGATGACCCGCTCGGCACCTTACGAGAAATCAAA GTTTTTGGGTTCTGGGGGGAGT ATGGTCG-
CAAGGCTGAAACTTAAAGAAATTGACGGAAGGGCACCACAAGGCGTGGAGCCTGCGG CTTAATTTGACTCAA-
CACGGGGA AACTCACCAGGTCCAGACAAAATAAGGATTGACAGATTGAGAGCTCTTTCTTGATCTTTTGGATG
GTGGTGCATGGCCGTTCTTAGT TGGTGGAGTGATTTGTCTGCTTAATTGCGATAACGAACGAGACCTCGGCC-
CTTAAATAGCCCGGT CCGCATTTGCGGGCCGCTGGCT TCTTAGGGGGACTATCGGCTCAAGCCGATGGAAGT-
GCGCGGCAATAACAGGTCTGTGATGCCCTT AGATGTTCTGGGCCGCACGCGC GCTACACTGACAGGGCCAGC-
GAGTACATCACCTTGGCCGAGAGGTCTGGGTAATCTTGTTAAACC CTGTCGTGCTGGGGATAGAGCA TTGCAAT-
TATTGCTCTTCAACGAGGAATGCCTAGTAGGCACGAGTCATCAGCTCGTGCCGATTAC GTCCCTGCCCTTTG-
TACACACC GCCCGTCGCTACTACCGATTGAATGGCTCGGTGAGGCCTTCGGACTGGCTCCAGGGGGTTGGCAA
CGACCCCCCAGAGCCGGAAAGT
TGGTCAAAACCTG

Sample: SGTM2

- The Microbe was found to be *Aspergillus terreus* isolate ATE1 small subunit ribosomal RNA gene

1. Sequence ID: KM462826.1
2. The closest homologue was identified as the *Aspergillus terreus* isolate MP1 small subunit ribosomal RNA gene.
3. Sequence ID: MN744345.1

The organisms and their corresponding accession numbers with percentage match are as follows:

1. *Aspergillus terreus* isolate ATE1, small subunit ribosomal RNA gene (Accession No. KM462826.1) - 99.10% match
2. *Aspergillus terreus* isolate MP1, small subunit ribosomal RNA gene (Accession No. MN744345.1) - 99.10% match
3. *Aspergillus terreus* strain AN4, 18S ribosomal RNA gene (Accession No KF660536 1 - 99 10% match
4. *Aspergillus sp*. isolate 68, small subunit ribosomal RNA gene (Accession No. MT649560.1) - 99.09% match

3.4 Protease production of culture using submerged fermentation

Bovin serum albumin curve for protein estimation[13] is shown in Figure 2

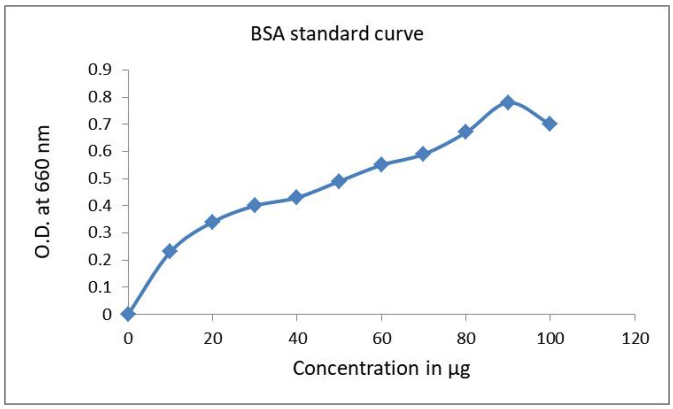

Fig 2. BSA (Bovine Serum Albumin) standard curve for protease Estimation

3.5 Enhancing Protease Production Efficiency using *Aspergillus terreus* SGTM2 and its Activity

3.5.1 Impact of Incubation Time on Protease Production

The synthesis of enzymes is closely related to cellular growth, resulting in a correlation between incubation time and subsequent enzyme production.The graph of protease production proceeded gradually reaching a maximum value at 4th day. Optimum protease production was observed on day 4. *Aspergillus terreus* spp. has been reported to achieve maximum protease production at 72 -90 hrs. It was shown In Figure 3. In another study, the production of alkaline protease by *Aspergillus terreus* was evaluated in which the highest enzyme activity, 22.5±0.11 U/mg protein, was observed at 45°C[14].

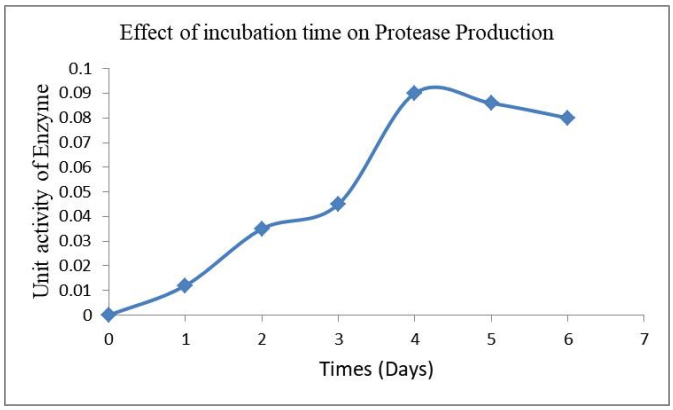

Fig 3. Effect of Incubation time on production of protease from *Aspergillus terreus* spp SGTM2

3.5.2 Impact of Temperature on protease Activity

The optimal temperature for maximal protease production was determined by incubating the inoculated media at temperature range from 25°C to 55°C. The highest enzyme production was observed at 50°C (Figure 4). However, protease activity was completely inhibited at both extremely low and high temperatures. Similar research shows, the optimal temperature for protease production by *A. terreus* was 37°C, resulting in an enzymatic activity of 250 units /ml and a specific activity of 320 units /mg[15].

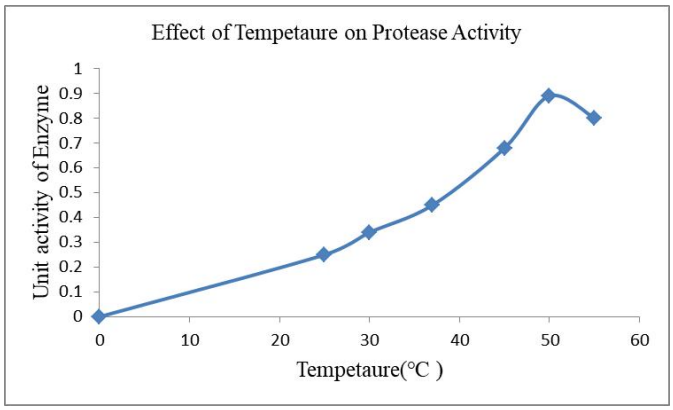

Fig 4. Effect of Temperature on protease Activity

3.5.3 Impact of pH on Protease Activity

In pH optimization study, demonstrated in Figure 5, conclusively indicated the protease was most effective at pH 8.0. Protease activity gradually increased as the pH ranged from 7.0 to 9.0. In a separate study, the optimal protease activity for *Aspergillus terreus* was observed at pH 7, with an enzyme activity of 120 IU/ml[16].

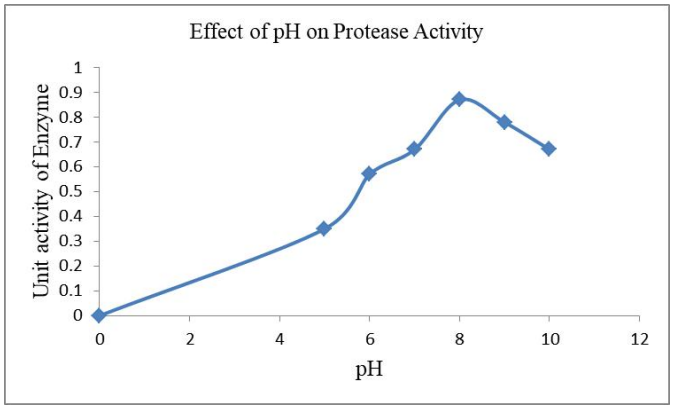

Fig 5. Effect of pH on protease Activity

3.5.4 Impact of Carbon Source on protease Activity

Figure 6 indicate there were seven carbon sources (0.01 L/kg) Glucose, Starch, Sucrose, Glycerol, Lactose, Manitol, Maltose, Dextrose and Fructose were used for the determination of protease production. The highest production of protease occurred in maltose. Other researcher reported that glucose was the pre dominant carbon source for protease production in *Aspergillus terreus*, resulting in a maximum enzyme activity of 1300 U/ml[17].

3.5.5 Impact of Nitrogen Source on protease production Aspergillus terreus spp

The nitrogen source is essential for organism growth, leading to an increase in enzyme or metabolite production and activity (Figure 7). The optimum nitrogen source for enzyme activity, eleven different nitrogen sources (1%) Beef extract, Ammonium

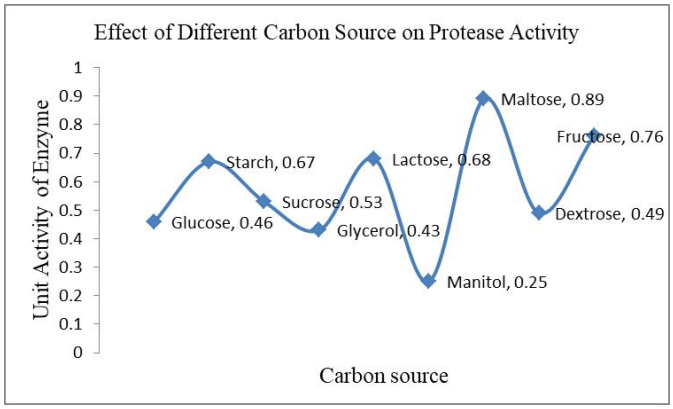

Fig 6. Effect of Carbon sources onprotease Activity

sulphate, Tryptone, Ammonium nitrate, Casein, Yeast extract, Meat extract, asparagine and Urea were used for determination of protease production. The maximum activity of protease was observed in Peptone. In other study, the highest protease activity of 322 U/ml was observed when ammonium nitrate was used as the nitrogen source, with a biomass of 0.654 mg [18].

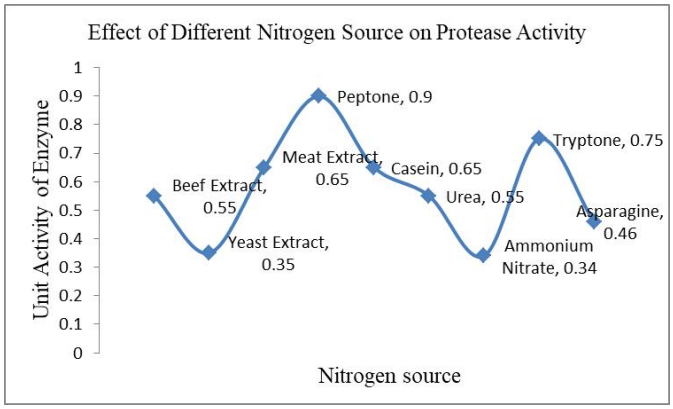

Fig 7. Effect of Nitrogen sources on protease Activity

4 Conclusion

This study indicates the potential of *Aspergillus terreus* SGTM2, a fungal strain isolated from cowshed soil, as a promising source of protease production for industrial applications. The research emphasizes the value of exploring unconventional microbial ecosystems, offering new insights into microbial biodiversity and enzyme production. The protease produced by *A. terreus* SGTM2 demonstrates considerable stability under alkaline and moderately thermophilic conditions, making it effective for various industrial applications such as detergents and waste management. While the study provides valuable optimization strategies for growth conditions and substrate utilization, further research is required to address the full characterization of enzyme including its substrate specificity and industrial scalability. This study underscores the value of unconventional microbial sources such as cowshed soil in discovering industrially relevant enzymes. Our research focused on refining enzyme properties, investigating the underlying genetic mechanisms, and scaling up production. *A. terreus* SGTM2 shows great promise as an industrial enzyme source.

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