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Isolation And Characterization of Chitinase-Producing Microorganisms from Soil

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ABSTRACT
Chitinase-producing microorganisms are important players in the biodegradation process of chitin and are used in diverse fields such as agriculture, biotechnology, environmental management, and biological control. This study focuses on the isolation and characterization of chitinase-producing microorganisms using standard microbiological procedures. Fifty soil samples from agricultural, compost, garden, rhizosphere, and forest soils will be collected, from which 96 microbial isolates will be obtained through serial dilution and spread plate techniques. In primary screening using Colloidal Chitin Agar (CCA), 18.8% of the isolates were found to be strong chitinase producers, 32.3% moderate producers, 22.9% weak producers, and 26.0% are non-producers of chitinases. Eighteen isolates will be characterized using morphological and biochemical tests. Most of the isolates are Gram-positive (66.7%), rods (72.2%), positive for catalase test (94.4%), positive for starch hydrolysis (83.3%), oxidase (77.8%), and gelatin hydrolysis (72.2%). Genus's identification shows that Bacillus spp. (38.9%) is the prevalent chitinase-producing microorganism followed by Streptomyces spp. (22.2%), Pseudomonas spp. (16.7%), Serratia spp. (11.1%), Aspergillus spp. (5.6%), and Trichoderma spp. (5.6%). The results indicate that agricultural and compost soils are rich sources of chitinolytic microorganisms and that Bacillus spp. is the promising candidate for chitinase production. Such microorganisms are useful in various industries such as sustainable agriculture, biological control, biodegradation of chitin-containing wastes, and other industrial processes.

Keywords: Chitinase; Soil microorganisms; Bacillus spp.; Colloidal chitin agar; Microbial characterization; Biocontrol

INTRODUCTION

Growing demands for sustainable and environmentally friendly biological processes have resulted in the intensification of research in the production of enzymes from various microorganisms that have potential industrial and environmental significance (Nayak et al., 2020; Desai et al., 2026). Enzymes, such as chitinase, have drawn much interest because of their potential ability to break down chitin, which is a naturally occurring polymer found in the cell wall of fungi and the exoskeleton of arthropods like insects and crustaceans. Microorganisms with the ability to degrade chitin are vital in the process of the natural

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decomposition of organic material and are beneficial in agriculture, biotechnology, and pharmaceutics.

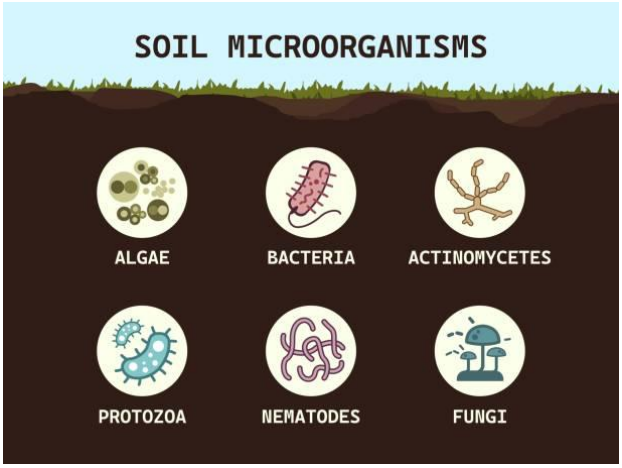


Figure 1: Soil Microorganisms

The soil is one of the most heterogeneous environments and an abundant source of microorganisms such as bacteria, fungi, and actinomycetes with the ability to produce different types of hydrolytic enzymes. Soil microorganism heterogeneity differs in relation to various characteristics like organic matter, moisture, vegetation, and environment (Banerjee & Mandal, 2019). Isolation of soil microorganisms for chitinase production is one of the best ways of obtaining efficient strains for biological control of fungi, biodegradation of agriculture and seafood wastes, and production of useful enzymes. The characterization of these microorganisms by morphological and biochemical tests helps in their identification and selection for industrial purposes.

Given the increasing significance of microbial chitinases, the current study was designed to isolate and characterize chitinase-producing microorganisms from various soil habitats. The current study mainly centered on investigating the diversity of microbial isolates, testing their chitinolytic activities, and determining efficient chitinase-producing microorganisms through standard microbiological methods (Shahbaz & Yu, 2020). The results obtained in this study could lead to the generation of environmentally friendly approaches for waste bioconversion, crop protection, and biotechnology based on enzymes.

MATERIALS AND METHODS

The experiment was performed to isolate and characterize the microorganisms which produce chitinase enzyme from various soil samples using traditional microbiological methods (Kapur et al., 2018). This included soil sample collection, isolation of microbes, screening for chitinase producing ability, characterization of isolated microorganisms, identification, and statistical analysis.

Study Design and Soil Sample Collection

The current study utilized the experimental design in the laboratory to obtain and analyze the chitinase producing microorganisms from soil samples (Atheena & Rajesh, 2024). Fifty soil samples were obtained from five different habitats such as agricultural soil, compost soil,

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garden soil, rhizosphere soil, and forest soil. The soil samples were collected from a depth of 5 – 15 cm with the help of sterile tools, packed in sterile polyethylene bags, and kept in the laboratory at 4°C.

Isolation of Microorganisms

Isolation of microorganisms was done through the method of serial dilution and plating out. One gram of each of the soil samples was suspended in sterilized distilled water and serially diluted up to 10^{-6}. The aliquots from the serially diluted samples were plated out on Nutrient Agar for bacteria and Potato Dextrose Agar for fungi (Gonfa et al., 2023; Khaled, 2021). The plated cultures were incubated and purified through serial streaking. Ninety-six microbial isolates were obtained and stored for further analysis.

Primary Screening for Chitinase Production

All isolates that have been recovered were evaluated for their ability to produce chitinase using Colloidal Chitin Agar (CCA) medium. The isolates were spotted into the CCA plates and cultured in the laboratory. Chitinase production was observed based on the appearance of clear zones around the colony as a result of hydrolysis (Gasmi et al., 2019). The size of the clearance zones helped to classify the isolates as either strong (> 20 mm), moderate (10-20 mm), weak (< 10 mm), or non-chitinase producing isolates were selected for further characterization.

Morphological and Biochemical Characterization

Microbiological studies were done on the selected isolates producing chitinases using standard microbiological procedures. Characterization was based on both morphological and biochemical studies. Morphological studies involved Gram staining, determination of cell shapes, and colony colorations. On the other hand, biochemical studies comprised of catalase, oxidase, starch, gelatin, citrate and urease tests (Teshome, 2023). The results of these analyses were used to evaluate the physiological and enzymatic characteristics of the selected isolates.

Identification of Chitinase-Producing Microorganisms

The selected isolates were classified at the genus level by their morphology, microscopic appearance, Gram stain reactions, and biochemical tests. Microorganisms that were classified include Bacillus spp., Streptomyces spp., Pseudomonas spp., Serratia spp., Aspergillus spp., and Trichoderma spp (Ekundayo et al., 2022). Among these isolated organisms, Bacillus spp. was prevalent.

Statistical Analysis

The results of the experiment were collated and analyzed using Microsoft Excel 2021 and SPSS Version 26.0. Frequencies and percentages were generated to describe the distribution of microorganisms, the screening categories, morphological features, biochemical tests, and genera that have been identified. The results were illustrated using tables and graphs (Tran et al., 2020; Kotb et al., 2023).

RESULTS AND DISCUSSION

Table 1 and Figure 2 show the distribution of the isolates of microorganisms from different soil samples. A total of 96 isolates of microorganisms have been isolated from five different soils, namely, agricultural soil, compost soil, garden soil, rhizosphere soil, and forest soil. The

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agricultural soil gave the most isolates (34; 35.4%), followed by compost soil (24; 25.0%), garden soil (18; 18.8%), rhizosphere soil (14; 14.6%), and forest soil (6; 6.2%). The chart shown in Figure 1 shows clearly the variation in the distribution of the isolates in the different soils.

Table 1: Distribution of Microbial Isolates Recovered from Different Soil Samples

Source of Soil Sample	Number of Isolates	Percentage (%)
Agricultural soil	34	35.4
Compost soil	24	25.0
Garden soil	18	18.8
Rhizosphere soil	14	14.6
Forest soil	6	6.2
Total	**96**	**100.0**

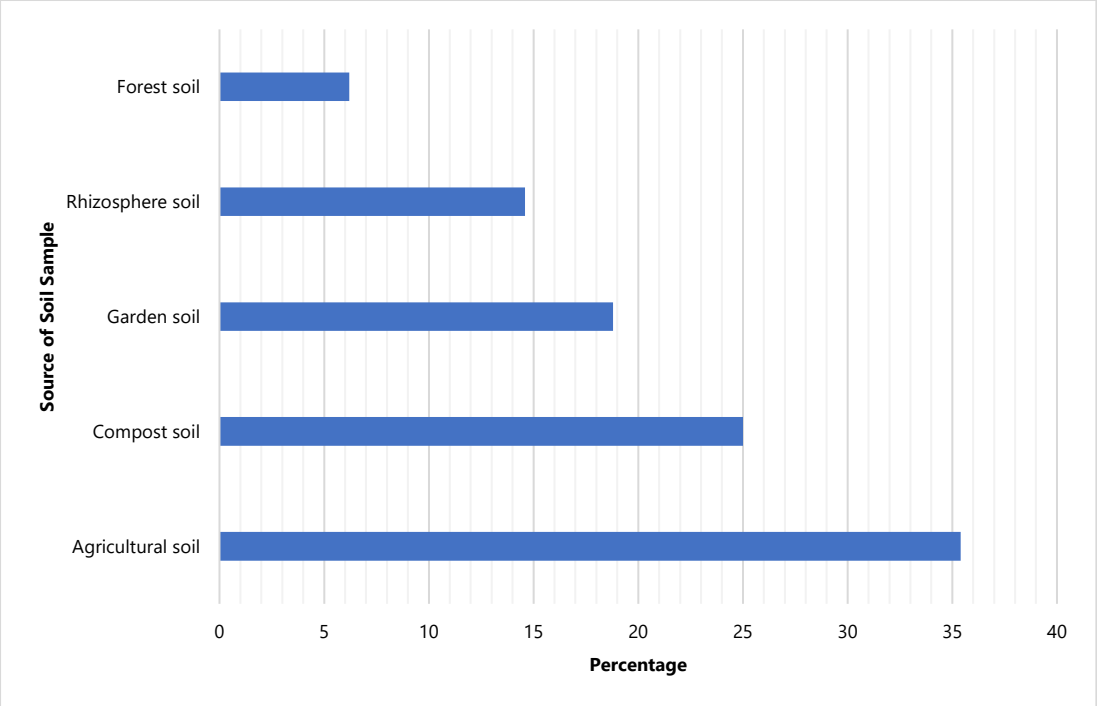


Figure 2: Graphical Representation of Distribution of Microbial Isolates Recovered from Different Soil Samples

From the findings, the highest microbial isolation was found in agricultural soils, which constituted over one-third (35.4%) of all the total microbial isolates. This could be due to the availability of abundant organic matter in such soils, continuous supply of nutrients, and suitable conditions for microbial growth. Secondly, compost soil also showed relatively high microbial isolation (25.0%). The reason being, it is rich in nutrients and involves active microbial activity. Lastly, forest soil was the one having the least number of microbial isolation

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(6.2%). Overall, it was seen that agricultural and compost soils have been good sources of isolating microbes capable of producing chitinase enzymes. Overall, the findings demonstrate that agricultural and compost soils are promising sources for isolating potential chitinase-producing microorganisms due to their greater microbial diversity and abundance.

Table 2 and Figure 3 show the results of the first step screening of 96 isolates for chitinase production on colloidal chitin agar (CCA). On the basis of the size of the clear zone around the colonies, the isolates were categorized into strong, moderate, weak, or non-chitinase producing isolates. Out of all the isolates, 31 (32.3%) were moderate chitinase producers with clear zones of 10-20 mm, while 25 (26.0%) were non-chitinase producing isolates. Strong chitinase producers, with clear zones greater than 20 mm, comprised 18 (18.8%) isolates, and 22 (22.9%) were weak chitinase producers with clear zones less than 10 mm. Figure 2 graphically illustrates the distribution of isolates across the four screening categories.

Table 2: Primary Screening of Isolates for Chitinase Production on Colloidal Chitin Agar

Screening Category	**Number of Isolates**	**Percentage (%)**
Strong producer (Clear zone >20 mm)	18	18.8
Moderate producer (10–20 mm)	31	32.3
Weak producer (<10 mm)	22	22.9
No detectable activity	25	26.0
Total	**96**	**100.0**

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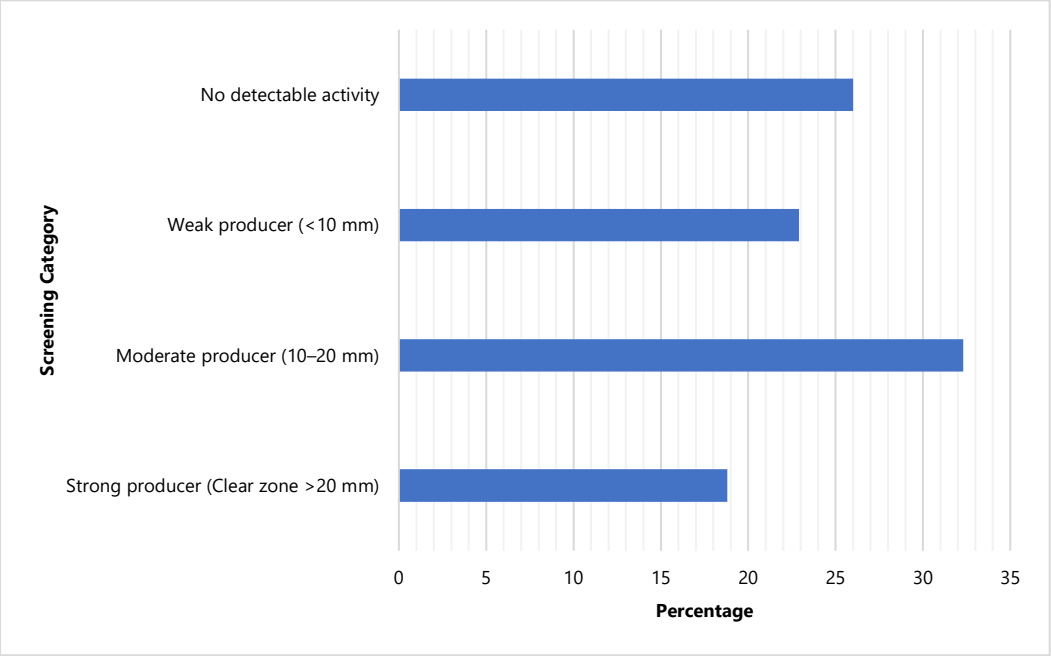


Figure 3: Graphical Representation of Primary Screening of Isolates for Chitinase Production on Colloidal Chitin Agar

From the results of the screening, it is evident that slightly over half of the isolated bacteria (51.1%) were capable of moderate to good chitinase-producing abilities, thereby emphasizing the presence of a significant number of chitinolytic microorganisms in the soil samples. Since there was a higher prevalence of moderately active isolates (32.3%), it is clear that many soil microorganisms have the ability to breakdown chitin, but only a few (18.8%) had good enzyme production potential for further studies. On the other hand, 26.0% of the bacteria isolated did not possess any chitinase producing ability. It means that not all soil microorganisms can degrade chitin. Generally, the results prove that soil is a rich source of chitinase producing microorganisms. Overall, these findings demonstrate that soil is a valuable source of chitinase-producing microorganisms and that primary screening on colloidal chitin agar is an effective method for identifying promising isolates for subsequent biochemical and molecular characterization.

Table 3 and Figure 4 show the morphological features of the 18 chitinase producing selected microorganisms. The results of Gram staining showed that 12 organisms (66.7%) were Gram positive, and 6 microorganisms (33.3%) were Gram negative. In terms of cell shape, 13 organisms (72.2%) showed a rod shape cell structure, and 5 organisms (27.8%) were cocci. When it comes to colony morphology, 11 organisms (61.1%) showed creamy or white colony structures, whereas 7 organisms (38.9%) showed pigment colonies. Figure 3 provides a graphical representation of the distribution of these morphological characteristics among the selected isolates.

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Table 3: Morphological Characterization of Selected Chitinase-Producing Isolates

Characteristic	Number of Isolates	Percentage (%)
Gram-positive bacteria	12	66.7
Gram-negative bacteria	6	33.3
Rod-shaped	13	72.2
Cocci	5	27.8
Pigmented colonies	7	38.9
Cream/white colonies	11	61.1

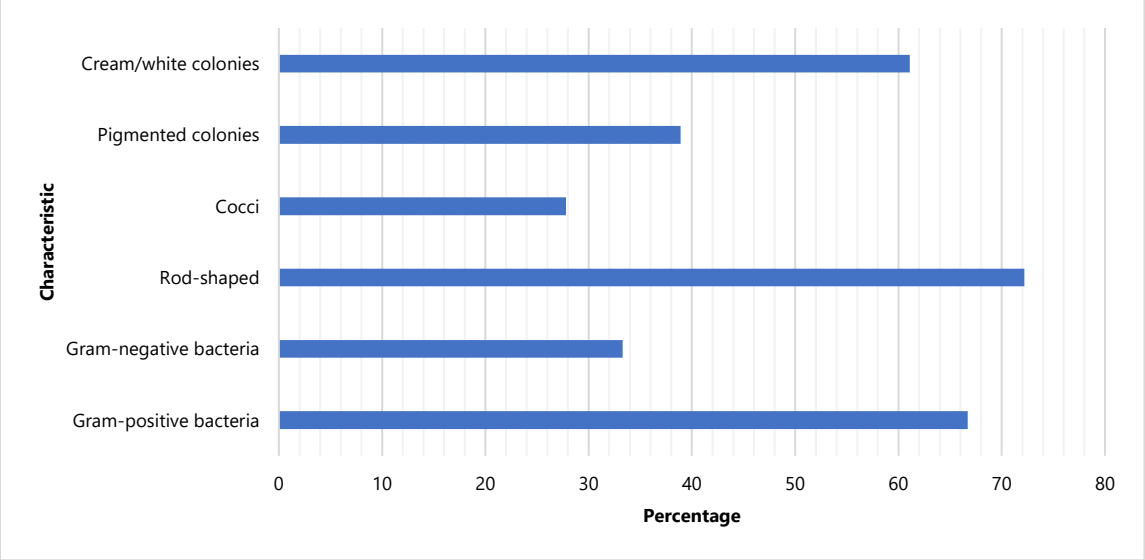


Figure 4: Graphical Representation of Morphological Characterization of Selected Chitinase-Producing Isolates

According to the morphological features, it can be concluded that the majority of chitinase producing isolates are Gram-positive and rod-shaped with prevalence of 66.7% and 72.2%, respectively. This means that groups of bacteria like Bacillus and other similar species can be considered as main sources of chitinase production in the tested soil samples. The presence of more cream or white colonies (61.1%) than pigmented ones (38.9%) show diversity of isolated microorganisms with predominance of non-pigmented ones. In general, these morphological features show that the isolated microorganisms have various phenotypes with predominance of Gram-positive, rod-shaped, cream- or white colony forming bacteria representing the most abundant group of potential chitinase producers identified during the study.

Table 4 and Figure 5 illustrate the biochemical traits of the 18 selected chitinase-producing isolates. In terms of catalase test, the highest positivity occurred with 17 isolates (94.4%) producing positive results. It was followed by starch hydrolysis with 15 isolates (83.3%), oxidase test with 14 isolates (77.8%), and gelatin hydrolysis with 13 isolates (72.2%). Also, 11 isolates (61.1%) had positive results for citrate utilization, and 8 isolates (44.4%) showed

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urease test. Figure 4 depicts the results of positive biochemical reactions of the selected chitinase-producing isolates.

Table 4: Biochemical Characteristics of Selected Chitinase-Producing Isolates

Biochemical Test	Positive Isolates	Percentage (%)
Catalase	17	94.4
Oxidase	14	77.8
Starch hydrolysis	15	83.3
Gelatin hydrolysis	13	72.2
Citrate utilization	11	61.1
Urease production	8	44.4

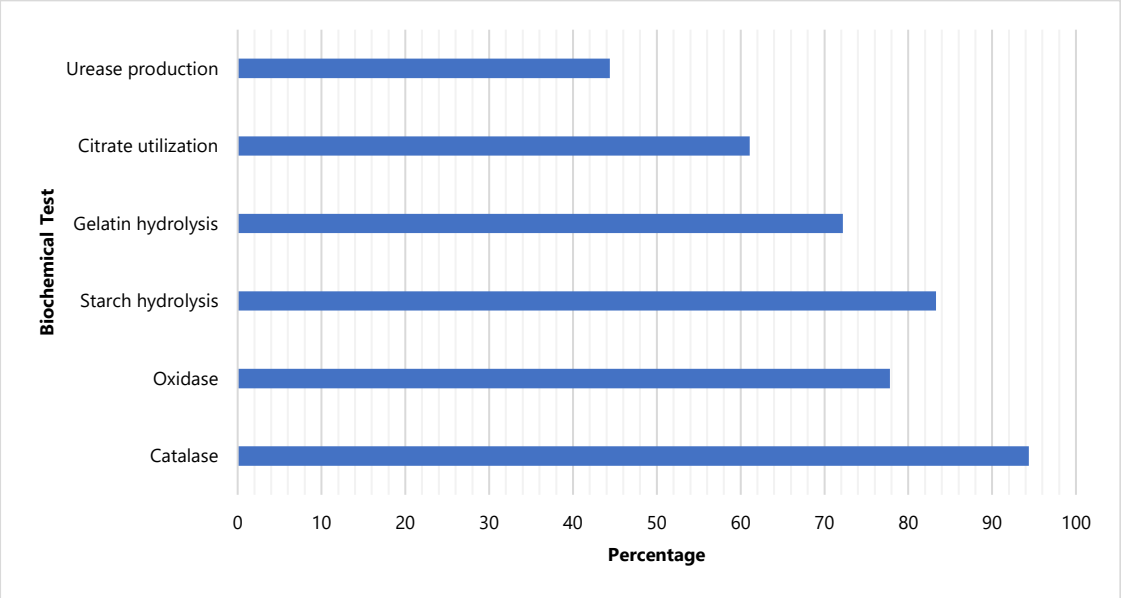


Figure 5: Graphical Representation of Biochemical Characteristics of Selected Chitinase-Producing Isolates

Biochemical analysis showed that most of the chosen isolates had several enzymatic and metabolic functions. High percentages of catalase-positive isolates (94.4%) indicate that most microorganisms were able to protect themselves from oxidative stress through hydrogen peroxide decomposition. At the same time, a high percentage of isolates positive for starch hydrolysis (83.3%) and gelatin hydrolysis (72.2%) implies the ability to synthesize extracellular hydrolytic enzymes, which usually means good chitin decomposition ability. Oxidase positivity of 77.8% of the isolates implies active aerobic metabolism, while citrate utilization (61.1%) implies metabolic flexibility of the isolates. Fewer isolates produced urease (44.4%), thus showing that the presence of this property was not very common among the chosen microbial population. In conclusion, the biochemical features show that the chosen isolates have a number of different physiological and enzymatic characteristics, making them

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promising candidates for effective chitinase producers.

The identification of the selected 18 microorganisms that produce chitinases is shown in Table 5 and Figure 6 below at the genus level. Of the identified isolates, the most predominant genus was the Bacillus spp., which included 7 isolates (38.9%), followed by the Streptomyces spp. with 4 isolates (22.2%). The Pseudomonas spp. comprised 3 isolates (16.7%) and the Serratia spp. had 2 isolates (11.1%). Furthermore, Aspergillus spp. and Trichoderma spp. each had 1 isolate (5.6%).

Table 5: Identification of Potential Chitinase-Producing Microorganisms

Identified Genus	Number of Isolates	Percentage (%)
Bacillus spp.	7	38.9
Streptomyces spp.	4	22.2
Pseudomonas spp.	3	16.7
Serratia spp.	2	11.1
Aspergillus spp.	1	5.6
Trichoderma spp.	1	5.6
Total	**18**	**100.0**

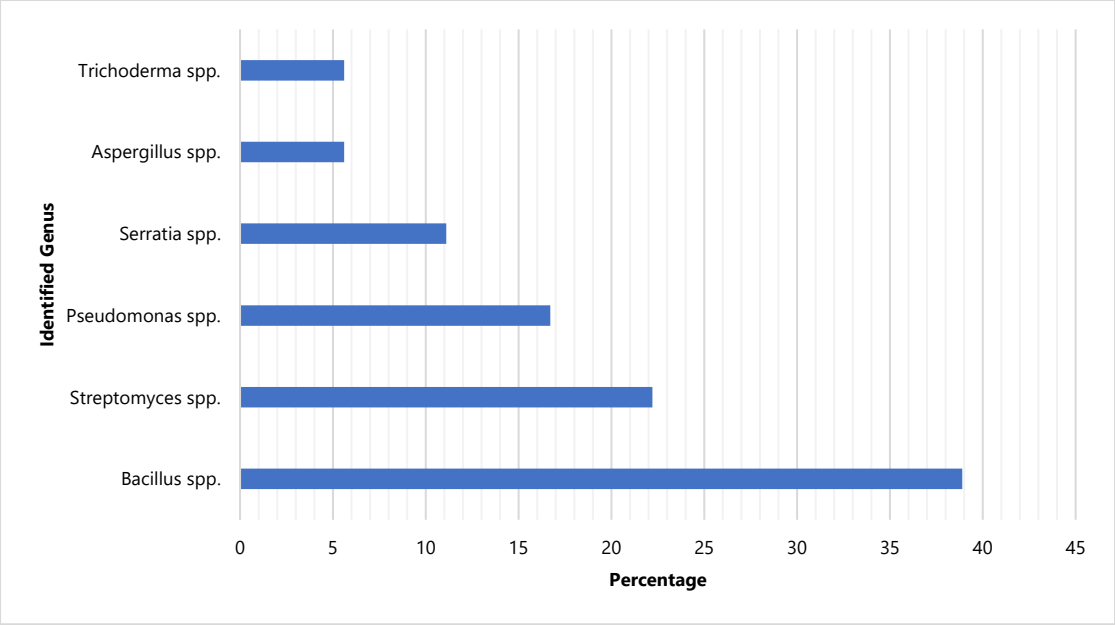


Figure 6: Graphical Representation of Identification of Potential Chitinase-Producing Microorganisms

From the analysis of the results obtained, it was clear that Bacillus spp. was the predominant producer of chitinase in the soil samples with a total of 38.9% of all the selected isolates. This shows that Bacillus spp. is well adapted to living in the soil environment and has good ability to degrade chitin by virtue of producing chitinase enzymes. Other important microorganisms

in the biodegradation of chitin were Streptomyces spp. (22.2%) and Pseudomonas spp. (16.7%). It should also be noted that there were some other chitin-degrading microorganisms, like Serratia spp., Aspergillus spp., and Trichoderma spp. It is obvious that both bacterial and fungi produce chitinase, but Bacillus spp. seems to be the best one in the production of the enzymes.

DISCUSSION

The results obtained from this study showed that soil is a rich source of microorganisms capable of producing chitinase (Singh et al., 2022; Sasi et al., 2020). The maximum number of isolates was obtained from agricultural and compost soils, and more than half of the isolated microorganisms were moderately to highly active in chitinase production. The dominance of Gram-positive, rod-shaped bacteria with positive biochemical traits suggested their ability to produce enzymes (Khatun et al., 2025; Dukariya & Kumar, 2020).

The most prominent of the microbes that were found included Bacillus spp. which was followed by Streptomyces spp Shaikh et al., 2018; Akeed et al., 2020). and Pseudomonas spp., thereby emphasizing the importance of these microbes in the degradation process of chitin. It is noteworthy that there are both bacterial and fungal sources of chitinase and they could be put to use in agriculture (Thakur et al., 2022).

CONCLUSION

The current research shows that soil acts as a rich source of microorganisms having chitinase-producing capabilities and good industrial potential. An adequate number of microorganisms are isolated in the present research from various types of soils, with the highest numbers of isolates obtained from agricultural soils and composts. A number of microorganisms are found to produce chitinase through primary screening, and further morphological and biochemical tests show that Gram-positive rod-shaped bacteria with highly active enzymes are the most abundant in the present study. Genus identification shows that among other microorganisms, Bacillus spp. are the most common chitinase-producers, followed by Streptomyces spp., Pseudomonas spp., Serratia spp., Aspergillus spp., and Trichoderma spp., which indicates the diversity of chitinolytic microorganisms present in soil. Overall, it has been concluded in the present study that soil acts as an excellent source of isolating microorganisms with chitinase-producing capabilities and emphasizes the wide application of these microorganisms in sustainable agriculture, biological control of plant pathogens, biodegradation of chitin-containing wastes of agriculture and sea food industry, enzyme production, and various eco-friendly biotechnological processes.

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