International Research Journal of Biological Sciences ISSN 2278-3202
Vol. **15(3),** 28-31, August **(2026)** *Int. Res. J. Biological Sci.*

Short Communication

A study on chitinase of *Cytobacillus firmus* and its applied potential

Monal Ambekar and Sarina. P. Khabade*
Department of PG studies in Biotechnology, Nrupathunga University, NT road, Bangalore, India
sarinapk2008@gmail.com

Available online at: www.isca.in, www.isca.me
Received 22nd August 2024, revised 28th December 2025, accepted 20th May 2026

Abstract

Aim of this study deals with the isolation of chitinase enzyme from cockroach carcasses and to analyze its potential biocontrol activity. The sample of cockroach carcasses were collected aseptically and subjected to serial dilution to isolate the bacterial strain. Two microorganisms were isolated and were screened for lytic enzyme in colloidal chitin media. The biochemical tests were performed to prove their validity. Further sequencing was performed to determine the species. The isolates were screened based on the clear zone formed. These isolates were subjected to biochemical test and 16s ribosomal RNA sequencing. A bacterial strain, Cytobacillus firmus, gram-negative, non-motile organism was identified. The isolates analyzed for antifungal activity showed significant percentage of inhibition.

Keywords: Chitinase, Cytobacillus firmus, Antifungal activity and 16s rRNA sequencing.

Introduction

Chitin is a naturally occurring polysaccharide essential for the structure and function of many organisms, making it the second most abundant biopolymer on Earth after cellulose. It is found in various biological systems, including arthropod exoskeletons, fungal cell walls, and certain algae[1]. Chitin synthase is a conserved enzyme found in all chitin-synthesizing organisms, catalysing the formation of different chitin forms[2]. Chitin is a linear polymer made up of repeated units of N-acetylglucosamine, a glucose derivative, linked together by β-(1→4) glycosidic bonds, resulting in a long fibrous chain. The presence of acetyl groups in the N-acetylglucosamine units enhances the polymer's strength and rigidity, making it an ideal structural material[3-6]. Chitin is insoluble in water, alcohol, and dilute acids but dissolves in concentrated alkaline solutions and some solvents. Its deacetylated derivative, chitosan, is more soluble and versatile in various industrial processes[7]. Chitin is an effective chelating agent due to its high nitrogen content (6.89%)[8]. Chitinases are hydrolytic enzymes that degrade chitin, a long-chain polymer of N-acetylglucosamine found in arthropod exoskeletons and fungal cell walls[9,10]. They play important roles in nutrient cycling, defense mechanisms, and pathogenic interactions[11]. Chitinases are divided into two major families based on amino acid sequence similarity: glycoside hydrolase family 18 (GH18) and glycoside hydrolase families 19 (GH19). Important source of chitinases, particularly in the soil[12,13].

Insect chitinases, a member of the glycoside hydrolase family 18, break down β-(1,4)-glycosidic bonds in chitin[12]. They are controlled by insects, particularly those that eat chitin-containing materials. These enzymes help to prevent infections by breaking down pathogenic fungi and parasite cell walls[14].

Materials and Methods

Design of experiment: This research involved isolation of insect chitinase enzyme from cockroach carcasses to check its potential antifungal and biocontrol activity.

Materials: The chemical aga-agar peptone, carboxymethyl cellulose, dextrose, starch and sodium chloride was procured from HI-media.

Screening the isolates for the production of lytic enzymes: Preparation of 3% Starch-Nutrient Agar: The isolates were screened for amylase production on nutrient agar plates supplemented 3% starch produced colonies with clear zone. Amylase-producing colonies had a colorless zone. 3% starch nutrient agar was prepared by dissolving beef extract, peptone, sodium chloride, and agar agar in distilled water. The diluted sample was streaked onto the plates and incubated for 24 hours.

Preparation of 10% Milk-Nutrient Agar: The isolates were screened for caseinase-production on nutrient agar plates supplemented with 10% produced colonies with clear zone. Caseinase-producing colonies had a colorless zone. 10% Milk-Nutrient Agar was prepared by dissolved chemicals in distilled water, added to nutrient solution, and incubated for 24 hours in a bacterial incubator.

Morphological characterization: Isolates were characterized and Gram staining was performed to identify the organism. The process involved smearing culture onto a glass slide, flooded with various solutions, decolorized, and counterstained. The slide was then observed under a microscope under different magnifications.

Preparation of Colloidal Chitin Media: Colloidal Chitin was prepared using *Agaricus bisporus* paste as a nutrient source. A 0.1% colloidal chitin media was prepared by dissolving Chitin, Peptone, Sodium chloride, and Agar Agar in distilled water. The media was autoclaved, inoculated on Petri plates, and incubated for 24 hours in a bacterial incubator.

Enzymatic assay of chitinase: The enzymatic assay involved centrifuging the nutrient broth with 1% mushroom chitin, and the crude enzyme sample was analysed using 1% Carboxymethyl cellulose, 1% starch, and 1% Chitin. The substrates were dissolved in distilled water, and the enzyme reaction was carried out in a bacterial incubator. The absorbances were then read at 540 nm using a UV-Vis spectrophotometer. The process involved incubating the samples in different conditions and observing the colour change[15,16].

Biochemical test: The identification of microorganism was done by the methods suggested in the Bergey's Manual of Systematic Bacteriology[17].

Dual Plate Assay: The antifungal properties of isolates were determined using colloidal chitin media made of chitin, peptone, sodium chloride, and agar agar. The medium was autoclaved, poured into Petri plates, and inoculated with fungal cultures of *Aspergillus niger* and *Penicillin*. Colonies that inhibited fungal growth were deemed antifungal. The percentage of inhibition was calculated using the following formula:

% inhibition = 100 - (E/C x 100).

16s RNA sequencing: Pure culture plates were prepared by inoculating a single colony on the colloidal chitin media which was incubated overnight. DNA was isolated from the culture provided by the scientist. Its quality was evaluated on 1.0 % agarose gel, and a single band of high-molecular-weight DNA has been observed. Fragment of 16S rRNA gene was amplified by 27F and 1492R primers. A single discrete PCR amplicon band of ~1400 bp was observed when resolved on agarose gel.

The PCR amplicon was purified to remove contaminants. Forward and reverse DNA sequencing reaction of PCR amplicon was carried out with 785F and 907R primers using BDT v3.1 Cycle sequencing kit on ABI 3730xl Genetic Analyzer. The consensus sequence of the 16S rRNA gene was generated from forward and reverse sequence data using aligner software.

The 16S rRNA gene sequence was used to carry out BLAST with the 'nr' database of the NCBI GenBank database. Based on the maximum identity score, the first ten sequences were selected and aligned using the multiple alignment software program Clustal W. Distance matrix and the phylogenetic tree was constructed using MEGA 11[18-20].

Results and Discussion

Chitinase producing bacterialcolonies were isolates from cockroach carcasses of a garden cockroach. Totally, 4 different isolates (labeled as M1, M2, S1 and S2) were obtained out of which two isolates, M1 and S1, with a clear zone, were chosen for screening by using nutrient agar plates containing 3% starch and 10% milk and for morphological analysis. M1 was found to be gram-negative, rod-shaped, and bacillus species, whereas S1 was also gram-negative, rod-shaped, and bacillus-like. Both isolates were plated on colloidal chitin media and incubated for 24 hours to produce the chitinase enzyme.

Enzymatic Assay of Chitinase Enzyme: The partially purified crude enzyme was checked for its activity against different substrates.

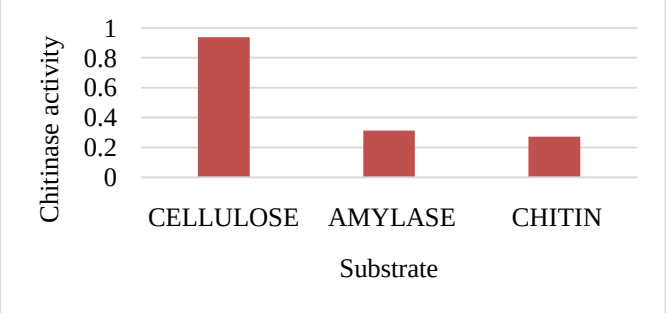

Figure-1: Enzymatic Assay of M1.

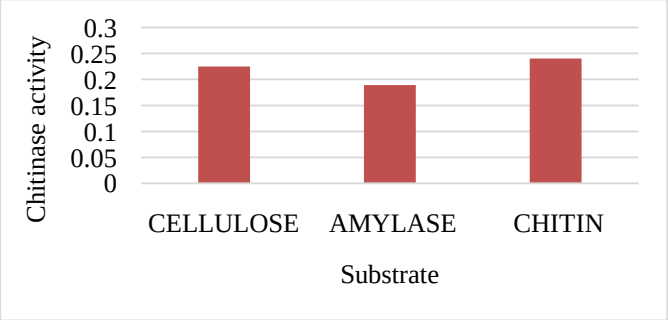

Figure-2: Enzymatic Assay of S1.

The two isolates were tested for enzymatic activity. Three distinct substrates (cellulose, amylase, and chitin) were used to assess the activity of the chitinase enzyme. The isolate M1's chitinase enzyme had the highest activity (495 U/ml/min) against cellulose, moderate activity (195 U/ml/min) against amylase, and the lowest activity (130 U/ml/min) against chitin. The isolate S1 chitinase enzyme had the highest activity (115 U/ml/min) against the substrate chitin, moderate activity (105 U/ml/min) against cellulose, and the lowest activity (90 U/ml/min) against the substrate amylase. Earlier studies have reports that the chitinase production by *Streptomyces chilikenis* showed Maximum activity in case of colloidal chitin, residual activity of 15% was observed with starch. However, no enzymatic activity was observed in case of CMC[21].

Biochemical Test: These isolates were subjected to identification through biochemical tests which are mention in Table-1.

Table-1: Biochemical Tests.

Test	M1	S1
Gram staining	Gram negative	Gram negative
Motility test	Non motile	Non motile
Starch hydrolysis	+	+
Casein hydrolysis	+	+
Gelatin hydrolysis	+	+
Oxidase test	+	+
Catalase test	+	+
Indole test	-	-

Dual-Plate Assay: The cockroach carcasses were used to isolate bacterial isolates M1 and S1 using a dual plate assay method. The isolates showed good inhibition against fungal pathogens *Aspergillus niger* and *Penicillin*, with a calculated percentage of 86.7% and 80.2% respectively.

Earlier studies conducted on Antifungal chitinase activity on phytopathogenic fungi was investigated in growing cultures and on soybean seeds infested with *Sclerotium rolfsii have reports* Fungal inhibition of 55% to 82% for *Aspergillusterreus*, *Aspergillus flavus*, *Nigrospora* sp, *Rhizopus* sp, *Aspergillus. niger*, *Fusarium* sp, *Aspergillus candidus*, *Absidia* sp, and *Helminthosporium* sp; 45% for *Curvularia* sp; and 10% for *Aspergillus fumigatus* ($P < 0.05$)[20].

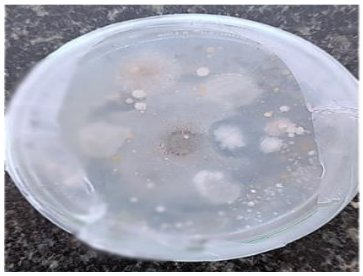

Figure-3: Dual-Plate Assay.

16s RNA Sequencing: The isolates were subjected to 16s rRNA sequencing process. DNA was isolated from a culture and evaluated on a 1.0% agarose gel. A high-molecular-weight DNA band was observed. The 16S rRNA gene fragment was amplified using 27F and 1492R primers. A PCR amplicon band of approximately 1400 bp was observed. DNA sequencing was performed using BDT v3.1 Cycle kit on ABI 3730xl Genetic Analyzer. The consensus sequence of the 16S rRNA gene was generated using aligner software. The first ten sequences were selected and aligned using Clustal W.

The results showed M1 and S1 to be Cytobacillus firmus as the primary organism. Subsequently, phylogenetic analysis and nucleotide homology revealed a high degree of similarity between them.

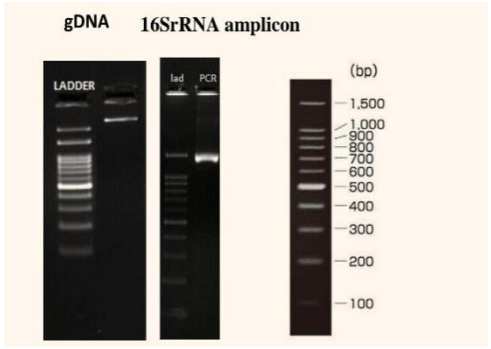

Figure-4: 16S rRNA Amplicon.

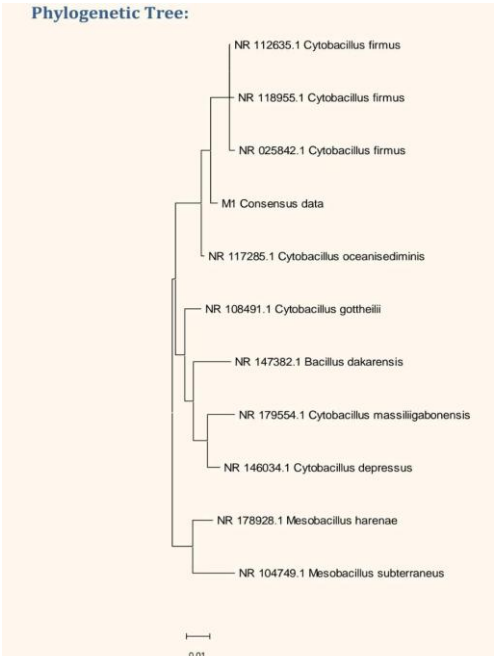

Figure-5: Phylogenetic Tree.

Conclusion

Chitinase enzyme is a natural enzyme found in insects, arthropod exoskeletons and crustaceans. The chitinase enzyme isolated from the cockroach biological role as an antifungal agent. The present results showed promising antifungal activities against *Aspergillus niger* and *Penicillin*. On the other hand, chitinase did not show any antifungal activity against Rhizopus.

References

1. Bakkers, J., Kijne, J. W. and Spaink, H. P. (1999). Function of chitin oligosaccharides in plant and animal development. EXS 87, 71-83.

2. Glaser, L. and Brown, D. H. (1957). The synthesis of chitin in cell-free extracts of Neurospora crassa. *J. Biol. Chem*., 228(2), 729-742.

3. Jaworski, E., Wang, L. and Margo, G. (1963). Synthesis of chitin in cellfree extracts of Prodeniaeridania. *Nature*, 198, 790.

4. Shahidi, F., & Abuzaytoun, R. (2005). Chitin, chitosan, and co-products: chemistry, production, applications, and health effects. *Advances in food and nutrition research*, 49(4526), 49003-49008.

5. Kumar, M. N. V. R., Muzzarelli, R. A. A., & Sashiwa, H. (2011). Chitin and chitosan: Chemistry and pharmaceutical perspectives. *Pharmaceutical Research*, 28(1), 45-50.

6. Rinaudo, M. (2006). Chitin and chitosan: Properties and applications. *Progress in Polymer Science*, 31(7), 603-632.

7. Muzzarelli, R. A. (1999). Clinical and biochemical evaluation of chitosan for hypercholesterolemia and overweight control. *Exs*, 87, 293-304.

8. Suginta, W., Robertson, P. A. W., Austin, B., Fry, S. C., & Fothergill-Gilmore, L. A. (2000). Chitinases from Vibrio: activity screening and purification of chiA from Vibrio carchariae. *Journal of applied microbiology*, 89(1), 76-84.

9. Bhattacharya, D., Nagpure, A., & Gupta, R. K. (2007). Bacterial chitinases: properties and potential. *Critical reviews in biotechnology*, 27(1), 21-28.

10. Mendonsa, E. S., Vartak, P. H., Rao, J. U., & Deshpande, M. V. (1996). An enzyme from Myrothecium verrucaria that degrades insect cuticles for biocontrol of Aedes aegypti mosquito. *Biotechnology letters*, 18, 373-376.

11. Funkhouser, J. D., & Aronson, N. N. (2007). Chitinase family GH18: evolutionary insights from the genomic history of a diverse protein family. *BMC evolutionary biology*, 7, 1-16.

12. Henrissat, B., & Davies, G. J. (2000). Glycoside hydrolases and glycosyltransferases. *Family classification in CAZy*. *Acta Crystallographica Section D: Biological Crystallography*, 56(6), 841-844.

13. Kramer, K. J., & Koga, D. (1986). Insect chitin: physical state, synthesis, degradation and metabolic regulation. *Insect biochemistry*, 16(6), 851-877.

14. Sahai, A. S., & Manocha, M. S. (1993). Chitinases of fungi and plants: their involvement in morphogenesis and host—parasite interaction. *FEMS Microbiology Reviews*, 11(4), 317-338.

15. Harman, G. E., Hayes, C. K., Lorito, M., Broadway, R. M., Di Pietro, A., Peterbauer, C., & Tronsmo, A. (1993). Chitinolytic enzymes of Trichoderma harzianum: purification of chitobiosidase and endochitinase. *Phytopathology*, 83(3), 313-318.

16. Williams, M. J.; Hammond, A. C.; Butts, W. T. and Kunkle, W. E. (1989). Dynamics of a tropical grass legume sward in the subtropics and its effects on animal performance. *Proceedings of the XVI International Grassland Congress*, 4-11 October 1989, Nice, France. 1989, 1021-1022

17. Mahboub, M. T., Hassan, M. I., Bream, A. S., Mohamed, A. F., & Abdel-Samad, M. R. (2021). Evaluation of the antibacterial and antifungal activities of chitosan prepared from the American cockroach, (Periplaneta americana). *Egyptian Academic Journal of Biological Sciences, E. Medical Entomology & Parasitology*, 13(1), 39-46.

18. Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of molecular evolution*, 16, 111-120.

19. Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA11: molecular evolutionary genetics analysis version 11. *Molecular biology and evolution*, 38(7), 3022-3027.

20. Ray, L., Panda, A. N., Mishra, S. R., Pattanaik, A. K., Adhya, T. K., Suar, M., & Raina, V. (2019). Purification and characterization of an extracellular thermo-alkali stable, metal tolerant chitinase from Streptomyces chilikensis RC1830 isolated from a brackish water lake sediment. *Biotechnology Reports*, 21, e00311.

21. Reyes-ramírez, A., Escudero-Abarca, B., Aguilar-Uscanga, G., Hayward-Jones, P., & Barboza-Corona, J. E. (2004). Antifungal Activity of Bacillus thuringiensis Chitinase and Its Potential for the Biocontrol of Phytopathogenic Fungi in Soybean Seeds. *Journal of Food Science*, 69(5).