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ISOLATION AND CHARACTERIZATION OF CHITINOLYTIC MICROORGANISMS FROM MARINE SOIL OF GUJARAT

Hiral Desai[1], Krusha Patel[1], Dr. Chetan G Prajapati[3], Sandip H. Bhatt[4], Dr. Anil Patani[1,5*], Dr. Megha Barot[6*], Dr. Sachidanand Singh[7], Dr. Dharmendra Prajapati[1,2*]

[1]Department of Biotechnology, Smt. S. S. Patel Nootan Science & Commerce College, Sankalchand Patel University, Visnagar- 384315, Gujarat, India
[2]Center for Research and Innovation, Sankalchand Patel University, Visnagar- 384315, Gujarat, India
[3]Humanities and Science Department, Government Engineering College, Palanpur-385001, Gujarat, India
[4]Chemical Engineering Department, L.D. College of Engineering, Ahmedabad, Gujarat, India
[5]Gujrat Biotechnology Research Center (GBRC), M.S. Building, Gandhinagar-382011, Gujarat, India
[6]Research and Development cell, Parul University, Vadodara- 391760, Gujarat, India
[7]Department of Biotechnology, School of Energy and Technology, Pandit Deendayal Energy University, Gandhinagar, Gujarat, India

ABSTRACT
The current study was focused on isolation and screening of chitinolytic bacteria from marine soil samples. Marine environments serve as an abundant source of chitin waste obtained from exoskeletons of crustaceans. In this regard, marine soil samples can be considered potential sources for chitin degrading bacteria. Total four samples of marine soils were collected and used for the isolation of microorganisms. Bacteria were isolated and tested for chitin-degrading ability by using the colloidal chitin agar medium. Isolation of chitinolytic microbes were done through serial dilution and spread plate method. Microbes producing chitinases were selected based on the presence of a clear zone of chitin degradation surrounding the colony. Total six bacterial colonies were isolated, out of six two bacterial isolates selected on the bases of high chitinolytic activity. These two potential strains were then characterized morphologically and biochemically. The molecular identification of the effective chitin degradation isolates was done using 16S rRNA gene sequencing, isolates J and Yz were identified as *Brevibacillus schisleri* and *Pseudomonas indica* respectively.

KEYWORDS: Chitinase, 16s rRNA sequencing, Chitin degradation, Marine soil

1. INTRODUCTION

Soil is a major habitat and consist of all kinds of additives, such as mineral along with organic substances for plants, and consist of a huge number of microorganisms, both beneficial and harmful for plants [1]. Since years ago, people have been isolating bacteria from various sources. Several types of bacteria have been found in a variety of habitats, including extreme environments, marine sediments, amorphous materials, waste water, etc. The traditional classification system divides them into prokaryotes and eukaryotes based on differences as well as similarities in their morphological characteristics. These groups are then further divided into different phyla, classes, orders, families, genera, and species. However, due of the differences in phenotypic traits, taxonomy classification using these techniques might be challenging [2]. Using DNA/RNA sequencing, Carl Wose and George E. Fox began analyzing and sequencing the 16S rRNA genes of different bacteria and utilized the results for phylogenesis [3].

Chitin is a nitrogenous polysaccharide comprising beta-1, 4 linked N-acetyl-d-glucose, which is structurally similar to cellulose but for the replacement of one hydroxyl group of each glucoside unit with either an acetylated or deacetylated amino group. Chitin is the second most common natural polymer and is found extensively as an essential element of the structure of crustaceans, insects, and other arthropods, in addition to being part of the cellular wall of almost all fungi and some algae. About 75% of the entire body weight of shellfish like krill, crab and shrimp is regarded as waste products, and chitin accounts for 20% to 58% of their dry weight. The chitinase enzyme has the ability to degrade the insoluble form of chitin into its constituent oligo and monomers, which exist in different organisms such as animals, insects, fungi, higher plants, bacteria and viruses. Endochitinase and exochitinase are two types of chitinases. The endochitinases act on chitin at internal sites to form oligomers of GlcNAc, while the exochitinases act by hydrolyzing chitin successively to yield GlcNAc, chitobiose. It has been shown that various strains belonging to the species of *Vibrio, Erwinia, Bacillus, Flavobacterium, Aeromonas, Arthrobacter, Chromobacterium, Enterobacter* are capable of chitin degradation under aquatic conditions [4].

However, most of the produced chitin is in the aquatic ecosystem. Even though the insoluble polymer is produced continuously, there are only minute traces of chitin in ocean sediments, suggesting that there must be an efficient chitin breakdown system in the ocean at the same time as its formation. The large-scale breakdown of chitin is brought about by marine chitinolytic bacteria, which have an essential role in nutrient recycling through the formation of carbon and nitrogen sources for the marine ecosystem [5].

The aim of this study was to isolate and characterize potential chitinolytic microorganisms from marine soil samples, which can be used as biocontrol agents.

2. MATERIALS AND METHODS

2.1. Soil sample collection

A total of 4 samples of marine soil were collected from various coastal region of Gujarat (Porbandar, Veraval, Shivrajpur beach and fish market of Dwarka). The soil sample at a depth of about 10 cm was collected and kept in an icebox until taken to the laboratory for processing.

2.2. Isolation of chitinolytic bacteria from marine soil

Bacteria were isolated from marine soil by serial dilution and spread plate method. 5 gm of soil sample was mixed with 50 ml of distilled water and placed in rotary shaker incubator overnight at 120 rpm. Serial dilutions were done up to a dilution of 10^{-5}. A volume of 0.1 ml of the diluted sample was used for inoculation on the chitin agar media (Na_2HPO4 6g, NH_4Cl 1g, KH_2PO4 3g, NaCl 0.5g, Yeast extract 0.05g, colloidal chitin 1% per litter) and then spread on the media plates through the use of the spreader. Plates were incubated for 5-6 days at 37^0C. After incubation, plates were observed for the presence of clear zone around colonies indicated chitinolytic activity. Chitin degrading colonies were selected and purified by four flame method. Pure cultures of bacteria were preserved for subsequent research in glycerol stock [6].

2.3. Characterization of isolates

The isolated bacteria were characterized according to their molecular structure using 16S rRNA sequence analysis, Gram staining, and biochemical tests. The size, shape, color, type of colonies, and colony pigmentation were recorded. The bacteria were stained using Gram staining technique and observed under the microscope using high power lens. Biochemical characterization of bacteria was done using various tests such as the Citrate utilization test, Indole test, Voges-Proskauer's test and Methyl red test [7].

2.4. DNA extraction

Bacterial isolates were grown overnight in nutrient broth at 37^0 C. 5 ml of culture broth was centrifuged at 10,000 rpm for 5 min and pellet was collected. The bacterial cell pellets were suspended in 1 ml of extraction buffer (500 mM NaCl, 50 mM EDTA pH 8.0, 100 mM Tris HCl pH 8.0, 20 mg/ml lysozyme and 0.07% -mercaptoethanol). Later on, 50 µl of 10% SDS was added to the samples which were mixed and then heated at 65°C for 30 min. The samples were centrifuged at 10,000 rpm for 20 min and the supernatant was collected in a new tube. RNase was added and samples were incubated at 37^0 C for one hour. Samples were extracted thrice with an equal volume of chloroform / tris-saturated phenol (1:1). DNA isolation was carried out by adding 2.5 volumes of absolute ethanol to each sample along with $1/10^{th}$ sample volume of 5 M NaCl. The samples were incubated in –20°C for 2 h before centrifugation at 12,000 rpm for 20 min. The DNA pellet was washed with 70% ethanol, dried and then dissolved in TE (10 mM Tris HCl, 1 mM EDTA) buffer. Purity of genomic DNA was assessed using electrophoresis in 1.0% agarose gel [8].

2.5. 16S rRNA isolation and analysis

16S rRNA gene sequencing and analysis were carried out using PCR amplification. Electrophoresis on a 1.0% agarose gel was used to identify the replicated PCR products of the 16S rRNA bacterial gene fragments (Fig. 3). Column purification was used to eliminate impurities from the PCR amplicon. Purified fragments were sequenced (gene explore diagnostics and research center, Ahmedabad, Gujarat) by ABI 3500xl Genetic Analyzer. Using the BDT v3.1 Cycle Sequencing Kit and primers 27F and 1391R, the DNA sequencing reaction of the PCR amplicon was performed. The BLAST (Basic Local Alignment Search Tool) technique was then used to compare the acquired sequences with the NCBI (National Center for Biotechnology Information) nucleotide database. During this process, the sequences of the type strains showing similarity with the sequences of the isolates were used for further comparison [9].

2.6. NCBI sequence submission

The 16S rDNA sequences of isolates J and Yz, which are capable of producing chitinase, have been registered to the NCBI database with accession numbers PQ651467, PQ651468.

2.7. Bioinformatic analysis

The NCBI BLAST search program (http://blast.ncbi.nlm.nih.gov) was used to find non-chance sequence similarity in 2 sequences. Every single sequence was BLAST, and the BLAST hit with the lowest expect-value (a measure of the quantity of non-chance alignments) was chosen.

To understand the evolutionary trait, the phylogenetic tree of two isolated strains was developed using MEGA 11 [10]. To trace out the evolutionary pattern of the bacterial isolates and evaluate its relation to other sequences of choice at NCBI, Neighbor-Joining (NJ) method of mathematical averages with distance matrix was applied. The Jukes-Cantor technique was used to calculate the evolutionary distances, which are expressed in base substitutions per site. Eleven nucleotide sequences were used in this investigation. For every pair of sequences, all ambiguous locations were eliminated (pairwise deletion option). The final dataset contained 1275 locations in total. Phylogenetic trees are used to display the results (Fig. 5 & 6).

3. RESULTS

3.1. Isolation of chitinolytic microbes from marine soil

The marine soil samples were collected from 4 different sites. For the isolation of microbes, 5g of marine soil sample was added to 50 ml of sterile distilled water. Then, 1 ml of the soil suspension was serially diluted 5 times. 0.1 ml of the diluted soil suspension from each dilution was then spread on chitin agar plates. After incubation period, microbial colonies were seen on chitin agar plate. A total of 6 colonies were selected based on clear zone formation and morphological differences (Fig.1). Out of 6 isolates only 2 bacterial isolates (isolate J & Yz) (Fig. 2) represented high production of chitinase and those two isolates were re-purified by four flame method.

Figure 1: Isolation of chitinolytic microbes using the spread plate method (Dwarka fish market soil sample).

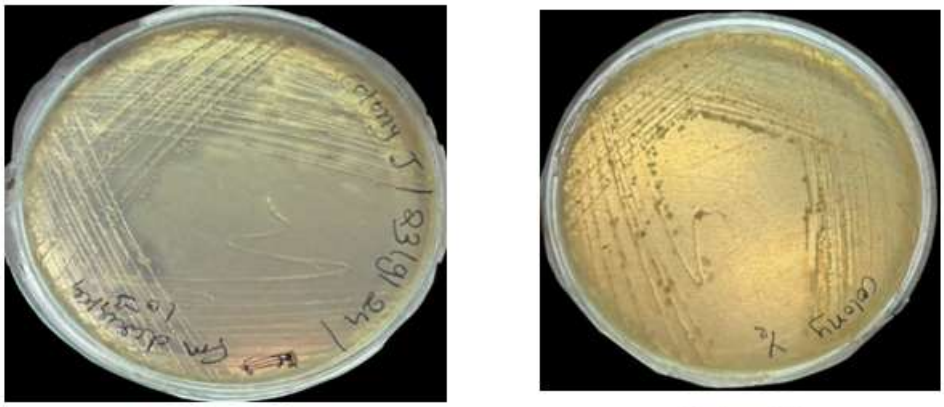

Figure 2: Purified colonies of isolates J and Yz.

3.2. Characterization of isolates

Characterization of two bacterial isolates were done by morphological (Table. 1) and biochemicals assays (Table. 2). One is Gram-positive, rod-shaped bacteria and one is Gram-negative, rod-shaped bacteria (Table. 3).

Table 1: Morphological characterization of isolates.

Colony characteristics	**Isolate J**	**Isolate Yz**
Size	Small	Small
Shape	Round	Round
Margine	Entire	Uneven
Elevation	Low convex	Embedded
Consistency	Moist	Dry
Texture	Smooth	Rough
Opacity	Translucent	Opaque

Table 2: Biochemical characterization of isolates.

Biochemical tests	**Isolate J**	**Isolate Yz**
Indole test	Negative	Negative
Citrate utilization test	Negative	Positive
Methyl-Red test	Negative	Negative
Vogas-Proskaur test	Negative	Negative

Table 3: Microscopic characterization of isolates.

Isolates	**Gram-staining**
Isolate J	Gram-positive
Isolate Yz	Gram-negative

3.3. Molecular identification of isolates

The bacterial isolates, J and Yz have maximum chitinolytic activity and were chosen for molecular identification using 16s rRNA sequencing and phylogenetic analysis. The size of PCR product from the bacterial test samples along with a 100 bp DNA ladder that served as a molecular size marker, was analysed on agarose gel electrophoresis. A single discrete band of ~1500 bp was observed in the test samples. This is a clear indication of a successful PCR process and the amplicon is suitable for sequencing.

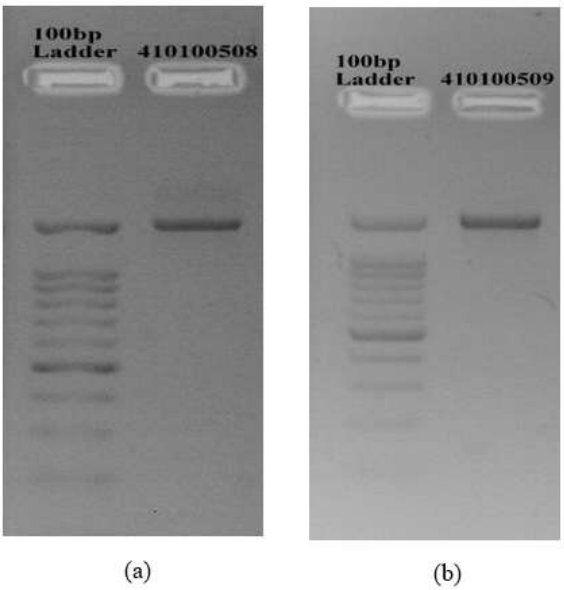

Figure 3: Agarose gel electrophoresis of 16s rRNA gene. (a) 16s rRNA gene of isolate Yz, (b) 16s rRNA gene of isolate J.

3.4. BLAST

The most comparable 16S rRNA gene sequences from the GenBank database (http://www.ncbi.nlm.nih.gov/blast) were found using a BLAST search of specific bacterial sequences. Results of BLAST are shown in (Table 4 and 5). Both strains queries had 0 E-values, indicating that all alignments were non-chance alignments. The percentage of queries covered varied between 97% and 100%. Most identical percent values (the percentage of the query sequence's similarity to the target sequence) fell between 96% and 100%.

According to sequence analysis, isolate Yz is identical to *Pseudomonas indica* with a maximum homology of 100% and isolate J is identical to *Brevibacillus schisleri* with maximum homology of 100%. Distribution of two bacterial isolates at genus level with number of blast hits is shown in (Fig. 5 & 6).

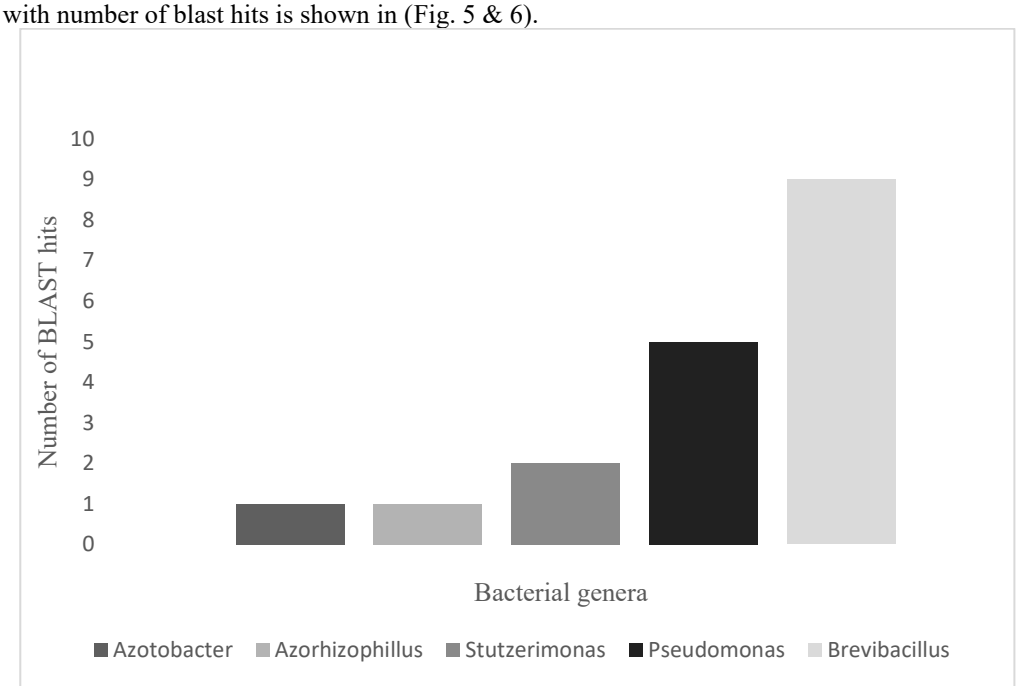

Figure 4: Taxonomic distribution of two bacterial isolates at genus level based on 16s rRNA gene BLAST analysis.

Table 4: Results of BLAST analysis of isolate Yz.

Description	Max Score	Total Score	Query Cover	E - Value	% identical	Accession No.
Pseudomonas indica strain NBRC 103045 16S ribosomal RNA, partial sequence	2364	2364	100%	0	100	NR_114196.1
	2326	2326	100%	0	99.45	NR_028801.1

Pseudomonas indica strain IMT37 16S ribosomal RNA, partial sequence						
Stutzerimonas azotifigens strain 6H33b 16S ribosomal RNA, partial sequence	2187	2187	100%	0	97.5	NR_041247.1
Stutzerimonas urumqiensis strain T3 16S ribosomal RNA, partial sequence	2143	2143	100%	0	96.88	NR_171524.1
Azorhizophilus paspali strain NBRC 102228 16S ribosomal RNA, partial sequence	2137	2137	100%	0	96.8	NR_114054.1
Pseudomonas aeruginosa strain DSM 50071 16S ribosomal RNA, partial sequence	2135	2135	97%	0	97.59	NR_117678.1
Pseudomonas aeruginosa strain NBRC 12689 16S ribosomal RNA, partial sequence	2135	2135	97%	0	97.59	NR_113599.1
Pseudomonas aeruginosa strain ATCC 10145 16S ribosomal RNA, partial sequence	2135	2135	97%	0	97.59	NR_114471.1
Azotobacter vinelandii strain NBRC 102612 16S ribosomal RNA, partial sequence	2121	2121	100%	0	96.57	NR_114166.1

Table 5: Results of BLAST analysis of isolate J.

Description	**Max Score**	**Total Score**	**Query Cover**	**E – Value**	**% identical**	**Accession No.**
Brevibacillus schisleri strain ATCC 35690 16s ribosomal RNA, partial sequence	2353	2353	100%	0	100	NR_181635.1
Brevibacillus brevis strain DSM 30 16S ribosomal RNA, partial sequence	2338	2338	100%	0	99.76	NR_112204.1
Brevibacillus brevis strain NBRC 15304 16S ribosomal RNA, partial sequence	2337	2337	100%	0	99.76	NR_041524.1
Brevibacillus formosus strain NBRC 15716 16S ribosomal RNA, partial sequence	2333	2333	100%	0	99.69	NR_113801.1
Brevibacillus formosus strain DSM 9885 16S ribosomal RNA, partial sequence	2333	2333	100%	0	99.69	NR_040979.1
Brevibacillus antibioticus strain TGS2-1 16S ribosomal RNA, partial sequence	2314	2314	100%	0	99.45	NR_165725.1
Brevibacillus formosus strain NRRL NRS-863 16S ribosomal RNA, partial sequence	2311	2311	100%	0	99.29	NR_115591.1
Brevibacillus agri strain DSM 6348 16S ribosomal RNA, partial sequence	2311	2311	100%	0	99.37	NR_040983.1
Brevibacillus agri strain NBRC 15538 16S ribosomal RNA, partial sequence	2307	2307	100%	0	99.29	NR_113767.1

3.5. GC ratio and sequence length of isolated strains

The GC% influences gene regulation and DNA double helix stability. GC% is correlated with melting temperature, if GC% is high than melting temperature is also high. Here, GC% of both the strains (J and Yz) is same (Table 6). GC ratio of obtained sequences is calculated by oligo calculator (https://mcb.berkeley.edu/labs/krantz/tools/oligocalc.html).

Table 6: Percentage of GC ratio and sequence length of isolated strains.

Name of isolated strains	GC %	Sequence length	Molecular weight
Pseudomonas indica	55%	2976	921888
Brevibacillus schisleri	55%	3179	983959

3.6. Phylogenetic Tree

The phylogenetic tree has been separated into clusters and subclusters with bootstrap values between 42 and 100 for isolate J and 58 and 100 for isolate Yz using the Neighbour-Joining technique. Further sub clustering of these clusters confirmed that the bacterial isolate J was *Brevibacillus schisleri* and Yz was *Pseudomonas indica*.

Figure 5: Phylogenetic tree of genus *Brevibacillus.*

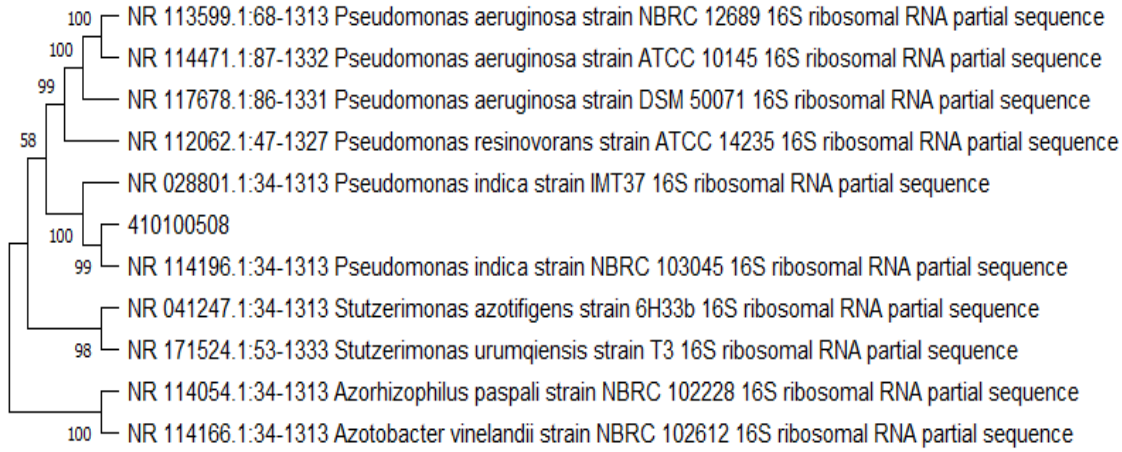

Figure 6: Phylogenetic tree of genus *Pseudomonas.*

4. DISCUSSION

The current study, presents successful isolation and identification of chitinolytic bacteria from marine soil samples obtained from the coastal region of Gujarat, thereby providing evidence that marine soil is a suitable environment for various microorganisms that can utilize chitin as their source of carbon and energy. Marine environments, especially coastal regions, contain abundant chitin, which serves as an excellent niche for the growth of chitinolytic bacteria.

Chitin degrading bacterial strains were isolated from fish market soil of Dwarka, Gujarat. Isolation of chitin degrading bacteria was done by serial dilution and spread plate method on chitin agar plate. Total six bacterial strains were isolated, of which two bacterial strains (isolate J and Yz) were further narrow down on the basis of formation of clear zone of chitin degradation on chitin agar plate, which indicates their ability to produce extra cellular chitinase enzyme. Characterization of these two isolates were done by biochemical tests and 16s rRNA sequencing. Total four biochemical tests (methyl-red test, indole test, citrate utilization test, vogas-Proskaur test) were done, based on biochemical tests, it could be assumed that isolates J and Yz could be *Bacillus sp.* and *Pseudomonas sp.* Molecular characterization was done by 16s rRNA sequencing. Based on molecular characterization, isolates J and Yz were identified as *Brevibacillus schisleri* and *Pseudomonas indica* respectively.

The Previous research studies shown isolation of chitinolytic bacterial strains of *Pseudomonas* and *Brevibacillus*. Gonfa et al., 2023, isolated total 29 chitinolytic bacterial strains including *Pseudomonas putida* from riverbank soil, Ambo, Western Ethiopia [11]. Isolation was done by spread plate method on chitin agar plate. All 8 chitinolytic bacterial strains were selected based on zone of chitin degradation. Out of 29 only 8 bacterial strains produced clear zone of chitin

degradation. Characterization of that 8 bacterial strains were done by biochemical tests and molecular identification by MALDI - TOF MS. Total four biochemical tests were performed for all 8 chitinolytic bacterial strains including methyl red test, oxidase test, motility test, indole test Catalase test, citrate utilization test, vogus-proskaur test, starch hydrolysis, chitin hydrolysis, gelatine hydrolysis. *Pseudomonas putida*, among 8 chitinolytic strains, give negative results of methyl-red test, indole test, citrate utilization test, vogus-proskaur test, which supports our results of biochemical test. *Pseudomonas lini,* a chitinolytic bacterial strain was isolated by [12] from nematode infected pepper field in Qalyubia Governorate in North-Eastern of Egypt. *Brevibacillus laterosporus* was also proven as chitinolytic bacterial strain as it gives clear zone of chitin degradation on colloidal chitin agar plate, which is isolated from mangrove marsh soil in Andhra Pradesh, India [13]. In another study, *Brevibacillus reuszeri* and *Lysinibacillus fusiformis* a chitinolytic bacterial strain was isolated from shrimp shell waste by spread plate method. Total 10 bacterial strains were isolated, out of 10 only 2 bacterial strains were selected on basis of high chitinolytic index [14]. Khatun et al., 2025 isolated chitinolytic bacterial strain S3C1 from soil of fish market, Bangladesh[15]. Isolation was done on colloidal chitin agar plate at 30^0C. Biochemical characterization was done by various biochemical tests, The KOH test, urea hydrolysis, citrate utilization, catalase test, and lactose fermentation test gives positive results, whereas the oxidase test, indole, starch hydrolysis, and mannitol salt agar tests give negative results. Molecular characterization was done by 16s rRNA sequencing where bacterial isolate identified as *Klebsiella variicola.* A total 35 chitinolytic bacterial strains were isolated from fish market soil of Tambaram and Vanagaram areas in Chennai, Tamil Nadu, India. Among 35, five bacterial strains were best chitinase producer and identified as *Brevibacillus borstelensis, Acinetobacter venetianus*, *Bacillus badius*, *Acinetobacter sp*., *Chitiniphilus shianonensis* by 16s rRNA sequencing [16].

It has been found that the species of *Pseudomonas* and *Brevibacillus* are used as a biological control of certain phytopathogenic fungi. Abo-Zaid et al., 2023, reported that production of siderophores by *Pseudomonas fluorescens* JY3 and *P. aeruginosa* inhibit the growth of *Fusarium oxysporum* and *Rhizoctonia solani,* which cause damping off disease in wheat plant [17]. The formulations of siderophore producing *P. aeruginosa* and *Pseudomonas fluorescens* inhibited the fungal growth F. oxysporum for 40% and 80% respectively, whereas the inhibition percentage against R. solani was found to be 87.5% and 62.5% respectively. Total 53 chitinolytic bacterial strains were isolated from rhizospheric soil which inhibit the growth of *Fusarium udum,* causing wilt disease in pigeon pea. Only 2 bacterial strains *Pseudomonas spp*. and *Bacillus spp*. gives high chitinolytic activity. The disease severity levels in planta with the treatments of *Bacillus spp.* NS-22 and *Pseudomonas spp.* NS-1 were found to be only 14.62% and 23.08%, respectively, compared to that of the pathogen control 67.34% [18]. Wekesa et al., 2022, isolated total 13 bacterial strains and one of them is *Brevibacillus brevis*, which used as a biocontrol agent against *Fusarium solani* fungi [19]. 37 isolates of chitinolytic bacteria isolated from soil samples and tested for their antifungal activity against the *Fusarium, Rhizoctonia, Aspergillus* and *Colletotrichum* infecting tomato plant. Among which *Bacillus subtilis* and *Bacillus cereus* supressed the growth of *Rhizoctonia solani* about 40% and *Fusarium oxysporum* about 80%. In pot assay, Bacillus subtilis isolate potentially reduced the disease incidence of Fusarium wilt and damping off by 50%, which indicates its biocontrol potential [20].

Overall, the results from this study are similar to those reported in the literature suggesting that marine soil is a great reservoir of chitin-degrading bacteria. The bacteria isolated in this study, *Brevibacillus schisleri* and *Pseudomonas indica* have great potential for further studies to be used in the biodegradation of chitinous waste products and the biocontrol of fungal diseases.

5. CONCLUSION

The current study effectively isolated and identified chitinolytic bacteria from marine soil, proving that these environments act as a useful source for chitinolytic microbes. Both species (*Brevibacillus* and *Pseudomonas*) have been known for their capacity to produce extracellular chitinase enzymes responsible for the breakdown of chitin found in marine organic materials. The isolation of these chitinolytic bacteria suggests their role in the processes of nutrient cycling and biodegradation in marine environments. Furthermore, the isolated bacterial strains can be use as biocontrol agents since both the genera *Bacillus* and *Pseudomonas* are well-known for their antimicrobial properties and antifungal activities.

REFERENCES

1. E. Wołejko, A. Jabłońska-Trypuć, U. Wydro, A. Butarewicz, and B. Łozowicka, “Soil biological activity as an indicator of soil pollution with pesticides – A review,” Applied Soil Ecology, vol. 147, p. 103356, Mar. 2020, doi: 10.1016/j.apsoil.2019.09.006.
2. Anshul Sharma Manjul and Poonam Shirkot, “16S rRNA gene sequencing for bacterial identification of pullulanase synthesizing thermophilic bacteria contributing to big data”.
3. C. R. Woese and G. E. Fox, “Phylogenetic structure of the prokaryotic domain: The primary kingdoms,” Proceedings of the National Academy of Sciences, vol. 74, no. 11, pp. 5088–5090, Nov. 1977, doi: 10.1073/pnas.74.11.5088.
4. T. G. Gonfa, A. K. Negessa, and A. O. Bulto, “Isolation, screening, and identification of chitinase-producing bacterial strains from riverbank soils at Ambo, Western Ethiopia,” Heliyon, vol. 9, no. 11, p. e21643, Nov. 2023, doi: 10.1016/j.heliyon.2023.e21643.
5. T. V. A. Tran et al., “Isolation and characterization of chitinolytic fast-growing bacteria from marine environment in Vietnam,” Journal of Genetic Engineering and Biotechnology, vol. 23, no. 4, p. 100587, Dec. 2025, doi: 10.1016/j.jgeb.2025.100587.
6. P. Pawar, J. A. Doshi, P. Dandekar, and K. Poornima, “The characterization of chitinolytic soil bacterial isolates for their antagonistic activity against root-knot nematode Meloidogyne incognita: An effort towards developing ‘green’ nematicidal agents.,” Nov. 15, 2022. doi: 10.21203/rs.3.rs-2105494/v1.

7. “ISOLATION, SCREENING, AND CHARACTERIZATION OF CHITINASE PRODUCING BACTERIA FROM MARINE WASTES Original Article S. KRITHIKA1, C. CHELLARAM2,3* ”.
8. “Syed-Ab-Rahman, S. F., Carvalhais, L. C., Chua, E., Xiao, Y., Wass, T. J., & Schenk, P. M. (2018). Identification of soil bacterial isolates suppressing different Phytophthora spp. and promoting plant growth. Frontiers in Plant Science, 9, 1502.”.
9. P. Gao, G. Ye, and Y. Ma, “The Bacterial Community Characteristics of Hippophae rhamnoides Subsp. sinensis Rousi in Different Distribution Areas of the Qinghai–Tibet Plateau and Their Responses to Habitat Factors,” Biology (Basel)., vol. 14, no. 9, p. 1304, Sep. 2025, doi: 10.3390/biology14091304.
10. K. Tamura, G. Stecher, and S. Kumar, “MEGA11: Molecular Evolutionary Genetics Analysis Version 11,” Mol. Biol. Evol., vol. 38, no. 7, pp. 3022–3027, Jun. 2021, doi: 10.1093/molbev/msab120.
11. T. G. Gonfa, A. K. Negessa, and A. O. Bulto, “Isolation, screening, and identification of chitinase-producing bacterial strains from riverbank soils at Ambo, Western Ethiopia,” Heliyon, vol. 9, no. 11, p. e21643, Nov. 2023, doi: 10.1016/j.heliyon.2023.e21643.
12. 2*, Tahany Mohamed Ali Abd-Elrahman 1, Mohsen Abou Elela Sayed 1, Atef Abd El-Aziz Hassan Ragab2, Dina Salah-Eldin SeragEldin Ibrahim3 Aya Abdalrahman Mohamed Abdellatif1, “Isolation, Identification and Biocontrol Activity of Novel Chitinolytic Bacteria against Meloidogyne incognita Infecting Capsicum annuum L.”.
13. L. Prasanna, V. G. H. Eijsink, R. Meadow, and S. Gåseidnes, “A novel strain of Brevibacillus laterosporus produces chitinases that contribute to its biocontrol potential,” Appl. Microbiol. Biotechnol., vol. 97, no. 4, pp. 1601–1611, Feb. 2013, doi: 10.1007/s00253-012-4019-y.
14. M. Masri, E. SUKMAWATY, and L. ADITIA, “Novel chitinolytic bacteria from the shrimp shell processing waste,” Biodiversitas, vol. 22, no. 5, Apr. 2021, doi: 10.13057/biodiv/d220527.
15. M. Khatun et al., “Isolation, Purification, and Characterization of Endochitinase Producing Bacteria as Potential Bio-control Agents of Fungal Phytopathogens,” Nat. Prod. Commun., vol. 20, no. 6, Jun. 2025, doi: 10.1177/1934578X251342013.
16. C. C. S. KRITHIKA1, “ISOLATION, SCREENING, AND CHARACTERIZATION OF CHITINASE PRODUCING BACTERIA FROM MARINE WASTES”.
17. G. A. Abo-Zaid et al., “Evaluation of Bio-Friendly Formulations from Siderophore-Producing Fluorescent Pseudomonas as Biocontrol Agents for the Management of Soil-Borne Fungi, Fusarium oxysporum and Rhizoctonia solani,” Agriculture, vol. 13, no. 7, p. 1418, Jul. 2023, doi: 10.3390/agriculture13071418.
18. A. Dukare, S. Paul, and A. Arambam, “Isolation and efficacy of native chitinolytic rhizobacteria for biocontrol activities against Fusarium wilt and plant growth promotion in pigeon pea (Cajanus cajan L.),” Egypt. J. Biol. Pest Control, vol. 30, no. 1, p. 56, Dec. 2020, doi: 10.1186/s41938-020-00256-7.
19. T. B. Wekesa, V. W. Wekesa, J. M. Onguso, E. N. Wafula, and N. Kavesu, “Isolation and Characterization of Bacillus velezensis from Lake Bogoria as a Potential Biocontrol of Fusarium solani in Phaseolus vulgaris L.,” Bacteria, vol. 1, no. 4, pp. 279–293, Nov. 2022, doi: 10.3390/bacteria1040021.
20. M. S. Malik et al., “Biological control of fungal pathogens of tomato (Lycopersicon esculentum) by chitinolytic bacterial strains,” J. Basic Microbiol., vol. 62, no. 1, pp. 48–62, Jan. 2022, doi: 10.1002/jobm.202100512.