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CHARACTERIZATION OF CHITINOLYTIC MICROORGANISMS ISOLATED FROM DIFFERENT CHITIN RICH ENVIRONMENTS

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ABSTRACT

After cellulose chitin is abundant biopolymer which is widely dispersed in aquatic and terrestrial environment. Chitinase enzymes have more attention because of their extensive applications in the field of agriculture and environment. Chitinolytic microorganisms were isolated from different chitin-rich environments to study their different properties. Total 96 samples of soil and water were collected from various sampling sites in and around the Thane district of the Mumbai region. A total of 183 chitinolytic bacteria were isolated by using colloidal chitin agar (CCA), which was made by chitin flakes. All the isolates were qualitatively and quantitatively screened for their chitinolytic activity by Congo Red Assay and Schales Assay respectively. 30 potent isolates showing Relative Enzyme Activity (REA) ≥20 were selected and their enzyme units were also calculated. Biochemical and morphological methods were used to identify potent strains up to the genus level. Isolate SW26 showed excellent chitinase activity with 14.2 U/ml and it was further identified as Paenibacillus lautus by 16s rRNA sequencing. Chitinase production was done with optimum media by Paenibacillus lautus yields 30.21 U/ml of the enzyme. Fungal phytopathogens *Penicillium* sp., *Aspergillus niger*, *Aspergillus fumigatus*, *Curvuleria* sp., *Fusarium* sp. were isolated from infected fruits and leaves collected from the local market, gardens, and farms. Antifungal potential of partially purified chitinase was checked by disc diffusion method against isolated fungal phytopathogens. The partially purified enzyme showed excellent antifungal potential towards

Penicillium sp. and *Aspergillus niger*. Antifungal activity of chitinase is indicative of applications for the preservation of agricultural produce.

Keywords: Chitin, Chitinase, Relative enzyme activity, Antifungal, Phytopathogen

INTRODUCTION

Chitin (C8H13O5N)n is chemically equivalent to cellulose having β -1, 4-linkage of N-acetyl-D-glucosamine polysaccharide. It holds nitrogen and one hydroxyl groups of each glycosidic unit is substituted by an acetylated or deacetylated amino group **[1]**. Chitin is the second most common natural bio-polymer following to cellulose. It is primarily found in outer hard structure of the insects, and arthropods, and fungal and algal cell walls **[2]**. Even though the extensive occurrence of chitin in nature, the main commercial sources of chitin have been shells of crab, lobsters and shrimp **[3]**. Almost 50-70% mass of shellfish like crabs, krill, and shrimp, considered as waste, in which contribution of chitin is approximately 20–58%. Globally almost more than 1000 metric tons of chitin produced from aquatic ecosystems **[4]**. Chitinase enzyme is a family of evolutionarily glycoside hydrolases (GHs) characterized by their ability to cleave the β-1, 4-glycosidic linkage between the N-acetyl glucosamine (GlcNAc) units that form the chitin chains **[5, 6]**. Varieties of organisms are capable to produce chitinase comprising some bacteria, many of the fungi, insects, animals and plants which play important functional roles depending on their source **[7]**. Chitinases produced by numerous bacterial species; some of the prominent known genera are *Aeromonas* spp., *Vibrio* spp., *Streptomyces* spp., *Bacillus* spp., *Serratia* spp., *Saccharopolyspora* spp., *Nocardioides* spp., *Microbispora* spp., *Actinoplaes* spp., and *Pseudomonas* spp. **[8]**. On the basis of mode of action chitinases are categorized as endochitinases and exochitinases **[9]**. Chitinase has a numerous applications included production of pharmaceutically essential chitooligosachharides, single cell protein preparation, biocontrol of pathogenic fungi, and chitinous waste biodegradation **[10]**. Chito-oligomers have applications in medical, agricultural, and industrial sectors as antibacterial and antifungal mediator, antihypertensive agent as well as to enhance the food quality**[11]**. The present work deals with the isolation, identification, and characterization of chitinase producing microorganisms from different chitin rich environments.

MATERIALS AND METHODS

Chemicals, Reagents and Glasswares

Chitin flakes was procured from HiMedia. Growth media, reagents, dialysis bag and all the chemicals, used were purchased from HiMedia and some from the Thomas Baker. All glasswares were of borosilicate while plastic wares and were purchased from Tarsons.

Samples collection

Soil and water samples were collected from the different environments including Forest (Sanjay Gandhi National Park, Borivali, Matheran Forest, and Neral), Coastal area, and Bank of River (Gorai Beach, Borivali, Marine Lines Beach, Mumbai, Kali River, and Titwala), Dumping Ground, Ambernath and Mangroves area of Gorai Beach. Soil (~ 10grams from depth of 2-3 cm) collected in sterile zip lock bags and water (~ 30ml) collected in sterile polypropylene containers. Samples brought to the research laboratory and processed as early as possible or stored in the refrigerator until further processing **[12, 13]**.

Colloidal chitin preparation

Colloidal chitin prepared from flakes of chitin (Hi-Media). 4gm of chitin Flakes were treated with slow addition of 60ml of concentrated HCl along the sides of the bottle. The reaction mixture was then vigorously stirred for 1hr. The mixture of chitin and HCl then filtered through whatman filter paper No.1 kept in a plastic funnel seated in a tripod stand. The obtained filtrate was treated with chilled distilled water for the precipitation of colloidal chitin. After overnight incubation, the colloidal chitin thus obtained as filter cake was washed to neutralize it to pH 7. The obtained colloidal chitin was stored 4°Ce until further use **[14]**.

Enrichment and Isolation of chitinolytic microorganisms

Samples were enriched in Bushnell Hass broth medium ($MgSO_4$- 0.20g/L, $CaCl_3$-0.02g/L, KH_2PO_4 -1.0g/L, K_2HPO_4 -1.0g/L, NH_4NO_3 -1.0g/L, $FeCl_3$-0.05g/L) with colloidal chitin as the only source of carbon, for 24-48 hours on a rotary shaker. After enrichment of samples for 4-5 days, loop full suspension from enrichment flasks was streaked on sterile Colloidal chitin agar (CCA) plates and incubated at room temperature for 2-3days. Chitinase-producing bacteria shows clear zone around the colony, which were purified and maintained on CCA slants at 4°C **[15, 16]**.

Qualitative Screening

Qualitative screenings of all the isolates were done by the Congo Red plate assay method. Isolates were spot inoculated on sterile CCA and kept at room temperature for 48 hours. After incubation, congo red poured into plates (0.1 % w/v) for 15 minutes and then

drained off. After that, plates were further poured by 0.5 M NaCl for another 5 min to wash off any residuals of congo red. The plates observed for the zone of clearance around the colonies **[17, 18]**.

The diameter of colonies and the clear zone around it were measured **[19]**. Relative Enzyme Activity (REA) value was obtained with the formulae mentioned below:

Relative Enzyme activity (REA) = $\frac{D^2-d^2}{D^2}$

("D" is clear zone diameter and "d" is the colony diameter)

Quantitative screening

Chitinase assay: Activity of chitinase evaluated by the measuring production of reducing sugar after the action of chitinase on chitin by Schales' assay method **[20, 21]**. The Schales' reagent is yellow (0.5 M sodium carbonate and 0.5 g/L potassium ferricyanide solution in water) shows reduction of color intensity when interact with reducing sugars.1ml of each culture suspension was inoculated in Bushnell Hass Broth medium containing 1% colloidal chitin. Isolates were grown-up in erlenmeyer flasks with 50ml of the medium on rotary shaker (100 rpm) for 48 hrs at room temperature. Cells separated and supernatant collected by centrifugation (10000 rpm for 15 min at 4˚C). For detection of chitinase activity 1 ml of supernatant and 1ml of Schales' reagent incubated at 100°C for 15 minutes. Once it gets cool the absorbance was taken at 420 nm. Protein estimation of all the collected supernatants was done by Lowry's method **[22]**. Enzyme units were calculated from the above-collected data. Enzyme unit is defined as the total of enzyme that produced 1µmol of N-acetyl-D-glucosamine per minute.

Morphological & Biochemical identification of selected potent isolates

Isolates were identified on the basis of their colony characteristic, Gram's character, and their biochemical properties by performing different biochemical tests (Sugar Fermentation test, Indole test, Methyl red test, Vogesprausker test, Citratase, Lecithinase, amylase, starch hydrolysis, gelatinase). The biochemical analysis was performed based on Bergey's manual of determinative bacteriology **[23]**.

Molecular identification of the potent isolate

The potent isolate identified based on Gram's character and biochemical tests was reconfirmed by performing the 16S rRNA gene study at Genexplore Diagnostics and Research Centre Pvt. Ltd. Sequence of the identified isolate was submitted to NCBI and gene bank accession number was obtained **[24, 25]**.

Production and Purification Chitinase Enzyme

Lab Scale production of chitinase carried out in 5 L of the sterile glass fermenter using chitinase flakes are the sole source of carbon. Optimum conditions (Time, Temperature, pH, Substrate concentration standardized previously) for the production were maintained throughout the incubation period. After incubation cells were removed by centrifugation and cell-free supernatant used as a crude enzyme. Purification of chitinase was done by the salting-out

process. The crude chitinase was precipitated with different concentrations of ammonium sulfate. The obtained precipitate dissolved in 0.05M phosphate buffer of pH 7.0. The precipitate subjected to overnight dialysis in a 12000KD (Himedia) dialysis membrane against 0.05M phosphate buffer. This dialyzed chitinase enzyme was used for additional study **[26]**.

Isolation of Fungal Phytopathogens

Infected fruits and leaves samples were collected from farms, markets, and gardens. Infected parts were surface sterilized, cut down, and inoculated in sterile potato dextrose broth (PDB) medium for enrichment for 96-120 hours. After enrichment samples were spot inoculated on Potato Dextrose Agar (PDA) and observed growth after 72-96 hours. Fungal growth was checked and purified fungal isolates were identified and stored at 4°C on the PDA slant **[27, 28]**.

Antifungal potential of partially purified chitinase Antifungal potential of the partially purified chitinase was evaluated against the isolated fungal phytopathogens by the disc diffusion method. Spore suspension of test fungal cultures was uniformly spread on PDA plates by sterile cotton swabs. Discs that were used, soaked with the partially purified enzyme (dialyzed and unanalyzed) for 10-15 min, the control disc soaked in buffer solution. The soaked filter paper discs were laid on the center of agar plates and incubated at 25°C for 72 hours and observed for the activity. Mycelia growth from the central disc containing the control (buffer) and discs containing partially purified chitinase was compared for antifungal activity. The zone of inhibition around the discs were measured **[29, 30]**.

RESULTS AND DISCUSSIONS

Samples collection

Soil and water are rich sources of different types of microorganisms **[31, 32].** Total 96 samples were collected from different sampling sites which comprise included 55 soil samples and 41 water samples. Details of sampling sites and the number of samples collected from each location are mentioned in

(Table I).

Colloidal chitin preparation

For isolation of chitinolytic microorganism colloidal chitin was prepared by the procedure described above. From 4gram of chitin flakes approximately 30grams (wet weight) of colloidal chitin was obtained which is displayed in **Figure 1**. Obtained colloidal chitin was white, soft, and soluble, unlike chitin flakes. This form can be used for the isolation of chitinolytic microorganisms. Prepared colloidal chitin kept at 4°C in a glass bottle for further study.

Enrichment and Isolation of Chitinolytic Microorganisms

All the soil and water samples were subjected to an enrichment process which ensured the proliferation of chitinase-producing organisms. Total 183 chitinolytic microorganisms were purified after enrichment, in which 85 from soil samples and 98 from water samples were obtained. All purified bacterial isolates were transferred on CCA slants and put in storage at 4°C for study. Details of isolates obtained from different sampling sites are depicted in **Table 1**. Chitinolytic microorganisms on CCA plates showing zone of clearance can be seen in **Figure 2**. Gupta *et al*, isolated 28 chitinolytic microorganism from the soil samples from different environments **[33]**.

The micro-organisms isolated from the enrichment process were further subjected to qualitative and quantitative screening.

Qualitative Screening

All 183 isolates were subjected to qualitative screening in which zone of clearance around each isolate was examined on CCA plates by Congo red plate assay. About 81.96% of isolates obtained from the forest were found to be positive for chitinolytic activity followed by dumping ground where the percentage of positive isolates was 80%. The coastal area and riverbank also showed a significant number of positive isolates. Total 143 (78.14%) isolates showed positive activity and the remaining 40 (21.85%) isolates showed no activity i.e. no zone of clearance around the colony. Relative enzyme activity (REA) of 143 isolates were calculated. Total 30 isolates showing REA value ≥20 were selected as potent microorganisms for quantitative screening. Krithika *et al* (2016) presented a reported that only 14 isolates from 35 showed zone of clearance when incubated colloidal chitin media and observed with congo red assay **[34].** The qualitative screening revealed that although some colonies from the sample were consistently observed throughout the enrichment process, not all the isolates were capable of chitinase production. REA values

of selected 30 isolates are depicted in **Table 2** and the bacterial growth showing zone of clearance by Congo red assay is displayed in **Figure 3**.

Quantitative screening

Thirty potent isolates were studied for maximum production of chitinase during their growth in broth media supplemented with colloidal chitin. In Quantitative screening reducing sugar released by enzymatic action on substrate colloidal chitin was measured and protein content of the supernatant was also checked. The isolates were checked for their activity in enzyme units. Isolate **SW26** gave a maximum yield of **14.2 U/ml** of chitinase enzyme amongst all selected 30 isolates followed by the isolate **SW6 (12.6 U/ml)** and **SS5 (11.2 U/ml)**. After the qualitative, quantitative screening, and statistical evaluation **isolate SW26** was found to be the most effective producer of chitinase enzyme. The isolate was subjected to biochemical and molecular identification in the further experiment. Enzyme units for a total of 30 isolates are presented in **(Table 3)**.

Morphological and Biochemical identification

All the selected 30 bacterial isolates were differentiated by the Gram staining in which 57% isolates were found to be Gram-Positive and 43% are Gram-Negative. Based on biochemical test results, isolates were identified up to the genus level by Bergey's Manual of Bacteriology, 9th edition. The biochemical test results were tabulated as below in **Table 4** and **Figure 5**. About 10 different genera of chitinolytic bacteria were identified by the biochemical Testing. Potent isolate SW26 was reconfirmed by the molecular identification of 16s rRNA identification technique.

Molecular identification of potent isolate SW26

Potent isolate SW26 was identified as *Bacillus* spp. based on biochemical properties and Gram's characteristics were reconfirmed by performing the 16S rRNA gene analysis. Identification studies were carried out at Genexplore Diagnostics and Research center Pvt. Ltd. 16s rRNA gene sequences of NCBI GeneBank database compared with 16s rRNA gene sequence of isolate SW26 by BLAST search and phylogenetic analysis revealed the highest identity and evolutionary relatedness (93%) of isolate SW26 (**Res 1377 in Figure 6**) with *Paenibacillus lautus*.

Kim *et al* (2017) isolated chitinase-producing bacteria from soil which collected from tomato cultivation fields in Korea later identified as *Paenibacillus elgii* HOA73 **[35]**.

Another study by Tariq and others reported that bacterial isolate from chitinous waste shows excellent antifungal activity against fungal pathogens such as *Aspergillus fumigates Fusarium solani* and*, Aspergillus parasiticus later identified as Paenibacillus elgii* **[36]**.

Production and Purification Chitinase Enzyme

Designing a medium is of critical importance because composition of the medium can considerably affect the yield of enzymes. On the basis of optimization study results, the production of chitinase was carried out on a laboratory scale. Enzyme content was checked after each Ammonium sulfate concentration used for purification which shows **30.21 U/ml** at 60% saturation after dialysis.

Antifungal potential of partially purified chitinase

Fungal phytopathogens cultures namely *Aspergillus niger, Aspergillus fumigatus, Fusarium* sp., *Curvuleria* sp., and *Penicillium* sp. isolated from the infected fruits leaves. Isolated fungal phytopathogens were tested against partially purified chitinase as well as crude supernatant (Undialyzed).

In the present study Undialyzed (crude) as well as purified chitinase by *Paenibacillus lautus*. shows inhibition against fungal phytopathogens *Penicillium* sp., *Aspergillus niger, Aspergillus fumigatus*, *Curvuleria* sp. The inhibition revealed varying levels of susceptibility. It was noted that the dialyzed enzyme shows the inhibitory activity against almost all the phytopathogens while Un-dialyzed enzyme also shows inhibitory activity but not against the *Fusarium* sp. A zone of inhibition was observed and measured **(Figure 7)**. Maximum inhibition was observed against the *Aspergillus nige*r and *Aspergillus fumigates* by the dialyzed enzyme and also there is not much difference in the activity against *Curvuleria* sp. The enzyme shows similar activity against the *Penicillium* sp. and *Fusarium* sp. Hoster et al. reported similar results with crude and purified enzymes from *Streptomyces aureofaciens*, resulting in cell wall lysis in different phytopathogenic fungi **[37]**. Similarly, Mukerjee and Sen reported that the chitinase purified from the *Streptomyces* P10 shows mycelial growth inhibition of *A. niger, A. alternata* and *H. sativum,* fungal phytopathogens **[38]**.

Table 1: Sampling site geographical location details and number of isolates obtained from each sampling site (soil and water samples)

Source of Sampling	Geographical location	Soil samples	Water samples	Total samples	Number of isolates Obtained
Forest	19°13'58.0"N,72°52'45.0"E Sanjay Gandhi National park, Borivali.	10	11	21	122
	18°58'48.0"N,73°16'12.0"E Matheran, Neral	10	08	18	
Bank of river	19°19'43.1"N,72°52'45.0"E Kalu river, Titwala	10	10	20	26
Dumping ground	19°11'32.72"N, 73°12'24.7"E Dumping ground Ambernath	10	-	10	5
Coastal area	19°14'31.04"N ,72°46'50.98"E Gorai beach Borivali	05	05	10	23
	18°57'17.1"N ,72°48'49.57"E Girgaon Chowpatty, Mumbai	05	07	12	
Mangroves	19°14'12.67"N ,72°49'1.74"E Magroves area , Borivali	05	-	05	7
Total		55	41	96	183

Figure 1: Colloidal chitin preparation from chitin flakes

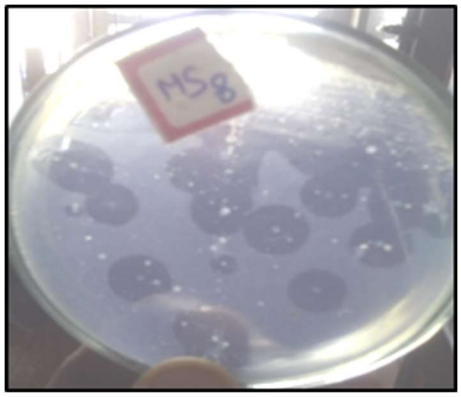

Figure 2: Isolation of Chitinolytic microorganisms on CCA plates

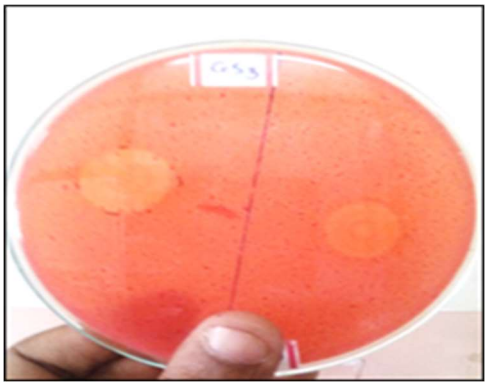

Figure 3: Qualitative plate assay for chitinolytic activity with Colloidal chitin as substrate and Congo red as staining solution

Table 2: Relative Enzyme Activity (REA) of selected 30 potent Isolates					
Isolate	**REA**	**Isolate**	**REA**	**Isolate**	**REA**
SS4	**24.00**	**SW30**	**53.39**	**SW15**	**41.25**
SS5	**53.39**	**SW31**	**22.04**	**SW19**	**83.02**
SS6	**23.17**	**SW32**	**41.25**	**SW24**	**26.58**
SS9	**39.96**	**SW33**	**65.69**	**SW26**	**29.5**
SS11	**39.96**	**SW34**	**52.77**	**SW28**	**22.04**
SS20	**42.89**	**SW35**	**24.00**	**TW15**	**25.44**
SS22	**32.64**	**SW36**	**25.26**	**TW18**	**24.00**
SW3	**20.00**	**SW47**	**20.00**	**TW20**	**31.49**
SW9	**33.51**	**SW48**	**26.04**	**TS1**	**36.51**
SW11	**20.00**	**SW49**	**36.51**	**TS3**	**27.77**

Table 3: Enzyme Units/ml obtained from selected 30 Isolates obtained from Schales assay and protein estimation

Isolate	U/ml	Isolate	U/ml	Isolate	U/ml
SS4	**8.3**	**SW30**	**7.0**	**SW15**	**10.6**
SS5	**11.2**	**SW31**	**7.1**	**SW19**	**7.8**
SS6	**12.6**	**SW32**	**6.0**	**SW24**	**7.3**
SS9	**8.9**	**SW33**	**10.3**	**SW26**	**14.2**
SS11	**6.9**	**SW34**	**6.3**	**SW28**	**5.6**
SS20	**7.7**	**SW35**	**3.2**	**TW15**	**7.1**
SS22	**5.6**	**SW36**	**9.4**	**TW18**	**9.4**
SW3	**9.7**	**SW47**	**5.0**	**TW20**	**9.5**
SW9	**7.6**	**SW48**	**6.9**	**TS1**	**8.3**
SW11	**6.9**	**SW49**	**7.4**	**TS3**	**6.6**

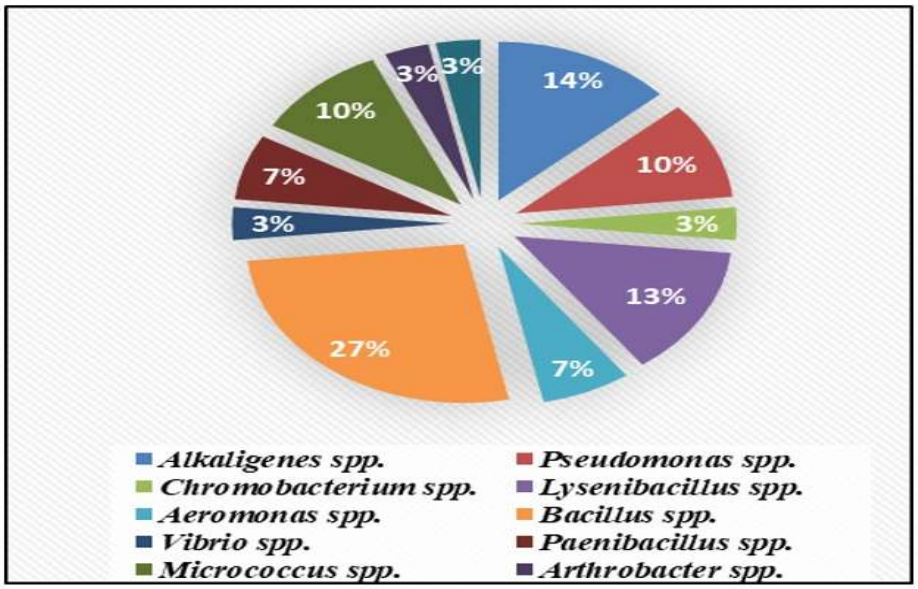

Figure 4: Identification of 30 isolates up to genus level by microscopic observation and biochemical tests results by Bergey's Manual of Bacteriology 9th edition

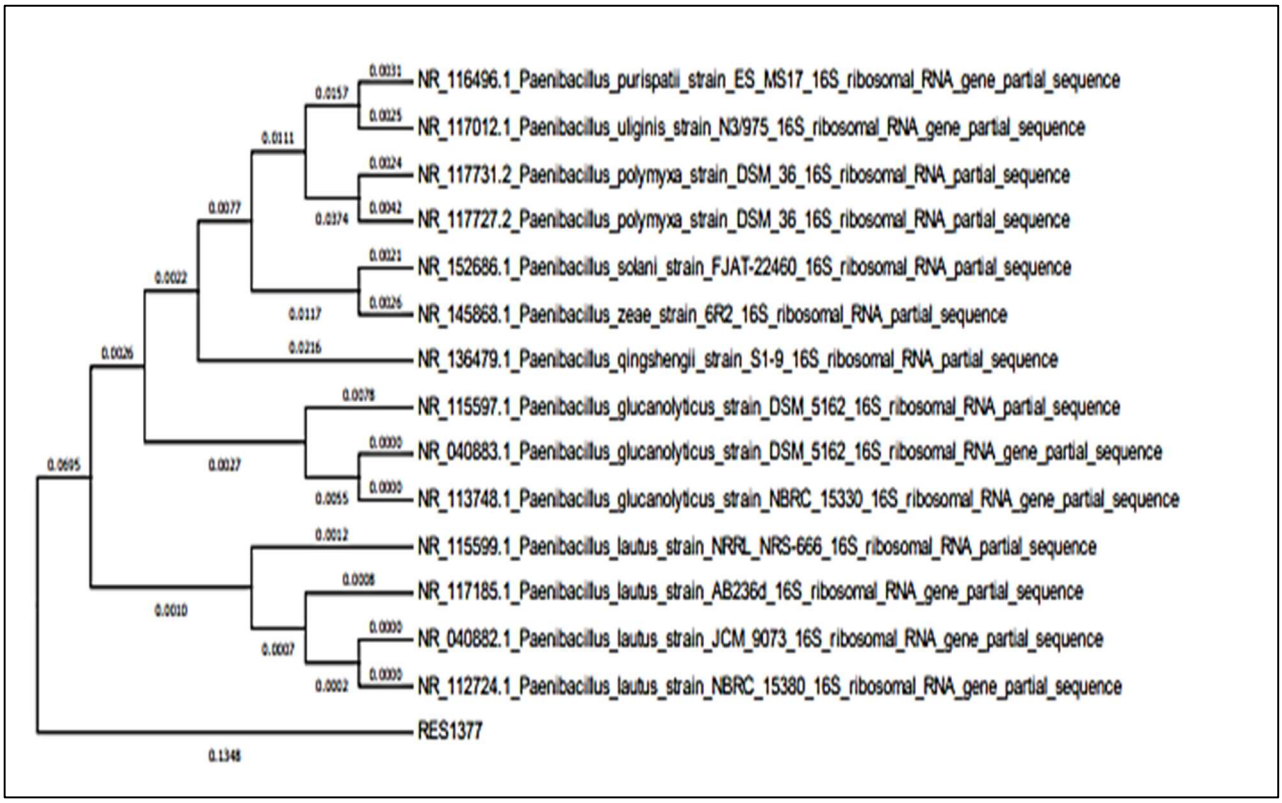

Figure 5: Evolutionary relationship of taxa

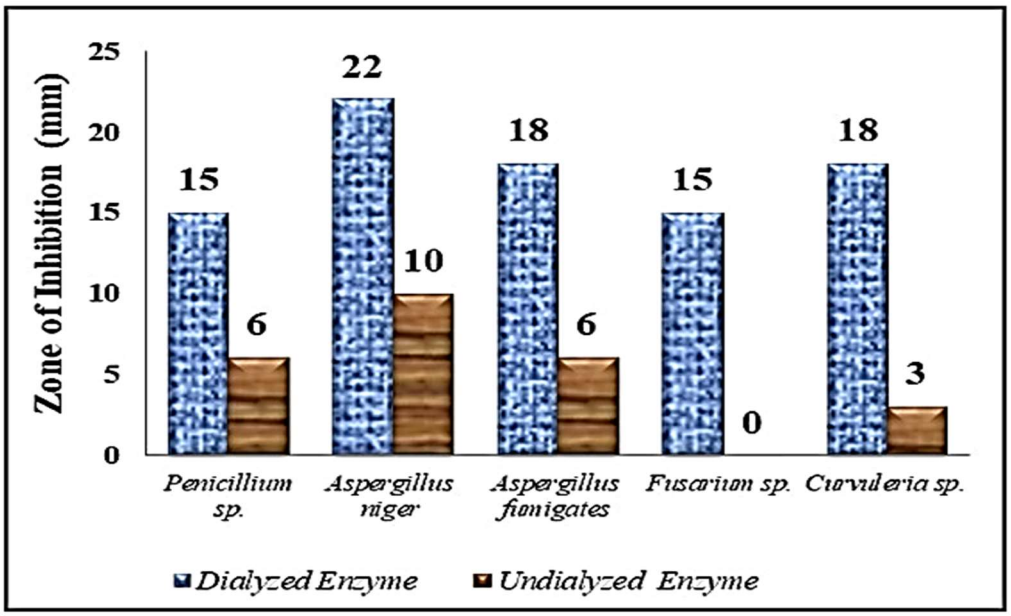

Figure 6: Comparative study of the zone of inhibition by Undialyzed and Dialyzed chitinase extracted from *Paenibacillus lautus* by disc diffusion method. The diameter of the zone of inhibitions is the o mm scale

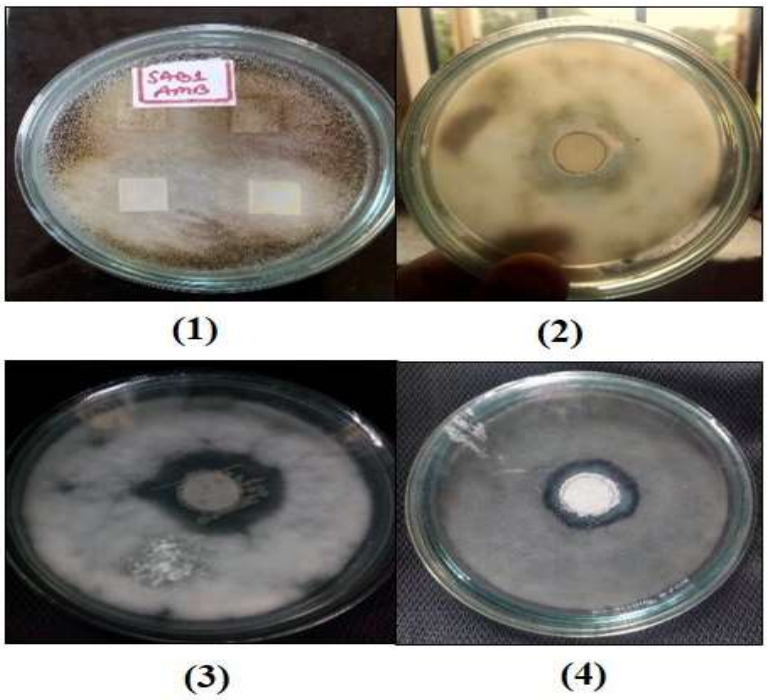

Figure 7: Antifungal potential of partially purified chitinase enzyme. 1.*Aspergillus niger* 2. *Aspergillus fumigatus 3. Fusarium* sp. 4. *Curvuleria* sp

Table 4: Identification of selected 30 potent chitinolytic isolates by performing biochemical test and Gram staining upto genus level *KEY: A – Acid Production, AG- Acid and Gas Production, (+) - Positive, (-) - Negative

Isolate	Carbohydrate Fermentation																					
	Glucose	Lactose	Sucrose	Maltose	Mannitol	Mnnitol	Inulin	Galactose	Indole	MR	VP	Citrate	Nitratase	Urease	Chitin	Amylase	Gelatinase	Lecithinase	Casein	Grams character	Identified as
SS4	A G	A G	+	+	+	AG	A G	A G	+	+	-	+	-	+	+	-	-	-	-	Gram Negative Rods	*Alkaligene paradoxus*
SS5	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+	-	+	-	Gram Negative Rods	*Pseudomonas fluoroscence*
SS6	+	+	+	+	+	+	+	+	-	-	-	+	-	+	+	-	-	-	+	Gram Negative Rods	*Alkaligenes faecalis*
SS9	+	+	+	+	+	+	+	+	-	-	-	+	-	+	+	+	-	-	+	Gram Negative Coccobacilli	*Chromobacterium* sp.
SS11	+	+	+	+	+	+	+	+	-	-	-	-	-	+	+	-	-	+	-	Gram Negative Coccobacilli	*Pseudomonas fluoroscence*
SS20	+	+	+	+	+	+	+	+	-	-	-	+	-	+	+	-	-	+	+	Gram Negative Rods	*Alkaligenes* sp.
SS22	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+	-	+	+	Gram-Positive Rods	*Lysinibacillus sphaericus*
SW3	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	-	-	+	+	Gram-Positive Rods	*Lysinibacillus sphaericus*
SW9	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+	-	+	-	Gram Negative Rods	*Aeromonas* sp.
SW11	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	-	-	+	-	Gram-Positive Rods	*Lysinibacillus sphaericus*
SW15	+	A G	+	A G	A G	AG	A G	A G	-	-	+	+	-	+	+	-	-	-	+	Gram Positive Rods	*Bacillus* sp.
SW19	+	+	A G	+	+	+	+	+	+	+	-	+	-	+	+	-	-	-	+	Gram Negative Rods	*Vibrio* spp.
SW24	A G	+	+	+	+	+	A G	+	+	+	-	+	-	+	+	-	-	-	+	Gram Negative Rods	*Aeromonas* sp.
SW26	+	+	+	+	+	+	+	+	-	-	-	+	-	+	+	+	-	+	+	Gram Positive Rods	*Bacillus* sp.
SW28	+	+	+	-	+	-	A	A G	-	+	-	+	-	+	+	-	-	+	+	Gram Positive Rods	*Paenibacillus* sp.
SW30	+	A G	+	+	+	+	+	+	+	+	-	+	-	+	+	+	-	+	+	Gram Positive Rods	*Bacillus cereus*
SW31	+	-	+	+	-	-	+	-	-	-	-	-	-	+	+	-	-	-	+	Gram Positive Rods	*Bacillus subtilis*
SW32	+	-	+	+	-	-	+	+	-	-	-	-	-	+	+	-	-	-	+	Gram Positive Rods	*Bacillus* sp.
SW33	A G	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	+	Gram Positive Rods	*Lysinibacillus* sp.
SW34	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	-	-	-	+	Gram Positive Rods	*Rummelibacillus* sp.
SW35	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+	-	+	+	Gram Positive Rods	*Geobacillus* sp.
SW36	A G	+	+	+	+1	+	A G	+	+	+	-	+	-	+	+	-	-	-	+	Gram Positive Cocci	*Micrococcus* sp.
SW47	+	+	+	+	+	+	+	+	-	-	-	+	-	+	+	-	-	+	+	Gram Negative Short Rods	*Pseudomonas* sp.
SW48	+	A G	+	+	A G	+	+	+	+	+	-	+	-	+	+	+	-	+	+	Gram Negative Coccobacilli	*Alkaligenes* sp.
SW49	+	+	A G	+	+	+	+	+	+	+	-	+	-	+	+	+	-	+	+	Gram Positive Cocci	*Micrococcus* sp.
TW15	+	+	+	+	+	+	+	+	-	+	-	+	-	+	+	-	-	-	+	Gram Positive Short Rods	*Bacillus* sp.
TW18	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+	-	+	+	Gram Positive Cocci	*Micrococcus* sp.
TW20	-	-	+	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	+	Gram Positive Rods	*Paenibacillus* sp.
TS1	+	+							-	-	+	-	-	+	+	-	-	-	+	Gram Negative Rods	*Arthrobacter* sp.
TS3	+	+	+	A G	+	+	+	+	+	+	-	+	-	+	+	+	-	+	+	Gram Negative Coccobacilli	*Citrobacter* sp.

CONCLUSION

Though chitinase production and characterization done from different sources, it is quiet essential to discover some new sources for the chitinase production with more efficient and enriched properties to magnify their effectiveness. In the present study chitinolytic bacteria of various genera were isolated from different sources of soil and water samples. It can be concluded that **forest soil is the rich source** for the isolation of chitinolytic microorganisms.

The continuous process of decomposition of insects and other arthropods (exoskeleton rich in chitin) in the soil of the forest could be the reason for the adaptation of such chitinolytic flora in its soil.

It was noted that chitinase production increased under the appropriate optimized conditions. It suggests that the chitinase production by the selected isolate was inducible and the same could be increased for any test isolate through optimizations. Further extorter carried out industrial production of chitinase enzyme and possibly genetic engineering tools.The results of antifungal activity of partially purified chitinase prove it **effective in the management of fungal phytopathogens**.These isolates can be used potentially used for a variety of environmental, agricultural, and industrial applications wherein chitinase plays an important role. Further, isolates can also be used for remediation of soil and water dumped with huge heaps of chitinous waste.

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