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Isolation, identification and protein profiling of chitinase producing marine bacteria

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Keywords

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Chitinolytic activity,
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Abstract

The marine bacteria which were obtained from the sea showed high ability to produce an enzyme chitin. Chitin is one of the underutilized bioresources in the world as it is available on a large scale especially from the marine waste. It has got many biotechnological applications like it can be used as a product in fertilizer and is used in food additives. Due the wide range of applications of chitin researches started isolating chitin producing bacteria. Chitinase producing bacteria were isolated from marine sediments and the biochemical characterizations were done. Through this biochemical characterization they were able to identify 11 chitinase producing bacteria. By the SDS-PAGE technique it can be shown that the organism doesn't belongs to the same genera or species. The clear zone produced in the plate confirmed the chitinolytic activity of bacteria. The genomic DNA of the best isolates was obtained and the DNA is subjected to undergo restriction digestion analysis.

Introduction

Chitin is a long chain linear polymer of an N-acetyl glucosamine a derivative of glucose , and is found in many places throughout the natural world.it is the characteristic component of the cell walls of fungi, the exoskeleton of arthropods such as crustaceans and insects.it is a tough leathery insoluble substance to determine the chemical structure. Large quantities of chitin are produced in the oceans of the world each year. The structure of chitin was solved by Albert Hoffman in 1929. In the most arthropods it is modified, occurring largely as a component of composite materials, such as sclerotin a tanned proteinaceous matrix which form much of the exoskeleton of the insects. In vertebrates the chitin has a major role with allergic response. It is insoluble in water and in majority of organic solvents. Chitosan prepared from chitin through chemical N-acetylation is water soluble and possess biological properties such as high biocompatibility and anti-microbial activities (Maria *et al.,2014*).

Chitosan is widely used in medical applications including antitumor therapy and cholesterol control in medical membranes, wound dressings and controlled released medical materials. Chitosan has also been used as a natural substance for the enhancement of seed germination and plant growth and it also act as an ecofriendly bio pesticide to boost the innate plant defense mechanisms against fungal infection. From the data given by (John stone 1908) the abundance of co0pepods and the chitin contents estimated that just one subclasses of planktonic crustacean form 10-12 chitinous last in the developmental stages.

Chitin is attributed primarily to marine organisms especially fungi, bacteria, actinomyctes. Marine organisms that live under extreme conditions are responsible for bulk chitin recycling. By the action of light most of the bacterial chitinase are able to degrade much chitin in the sea, they are biological control agents for countering fungal infection in plant (Muzzarelli *et al* ., 1977). Hydrolysis of chitin by chitinase produce N-acetyl chitooligosaccharides (GlcNac) which have varied biological function and potential applications in wide range of field(Suzuki *et al* .,1985). GlcNac exhibit strong attracting responses to peritoneal exudates cells in mice, have tumor growth inhibitory effect against Meth-A solid tumor. Chitinase are also used in the preparation of protoplasts from yeast and fungi, as a protective isolated from numerous bacterial sources. These include aerobic bacteria of the genera *Streptomyces sp., Enterobacter sp., Serratia sp., and Pseudomonas sp.* From the given great diversity of possible chitin structures, it is not surprising that bacteria typically produce more than one type of chitnase. For example: *Bacillus sp* secrete six major chitinase, *Serratia* produce five chitinase, *Streptomyces lividans* produce three. In the present study chitinivorous bacteria were detected by inoculating chitin medium with samples of marine sediments, staining and biochemical characteristics and protein profiling of bacterial isolates collected from various places of Tamil Nadu and Kerala.

Materials and Methods

Collection of samples

The marine sediments were collected from various coastal regions of TamilNadu, Kerala and Mumbai. In sterile the sediment samples were taken to the laboratory in sterile screw cap bottles and used for isolation (M.Kuddas *et al.,* 2013).

Sterilization of water for media preparation

The collected water was sterilized, filtered and used for media preparation.

Isolation of bacterial colony

Dissolve the ingredient in sterilized marine water and adjust the pH 7.5 with sodium hydroxide and made it to one litre. The different bacterial colonies were isolated by spread plate method (Kuldeep kaur et *al.*,2012).

Preparation of colloidal chitin

Colloidal chitin was prepared by the method of *Berger et al.,* by partial hydrolysis of chitin with concentrated hydrochloric acid for an overnight incubation at room temperature. The chitin obtained was filtered through glass wool to 250ml of ice cold ethanol. The colloidal chitin was washed several times with larger volumes of distilled water to adjust the pH 7(Imanda *et al.,* 2015).

Screening of chitinase producing bacteria

Screening was performed with bacterial isolates on the colloidal chitin agar medium incubated at 37^{O}c. Bacterial isolates were selected on the basis of a large hydrolysis zone after 96hrs of incubation and further screened for maximum enzyme production in nutrient broth media. The culture were centrifuged at 10,000rpm for 15minutes for 4^{O}c and crude was used for chitinase assay (Vincy *et al.,* 2014).

Assay of chitinase activity

The chitinase activity was assayed by measuring the reducing sugar released by the colloidal chitin. The crude enzyme (150µl) was added to the mixture consisting of 0.1% colloidal chitin (300µl) and (150µl) of 0.1%phosphate buffer Ph7. After incubation at 55^{O}c for 10 minutes, the mixture is centrifuged at 10,000rpm for 5 minutes. After centrifugation, the supernatant obtained was added with Schales reagent and boiled for 10minutes. Then the mixture is cooled and the absorbance was obtained at 420nm (Saima *et al., 2013).*

Biochemical identification of bacteria

The various biochemical test namely carbohydrate fermentation test, catalase test, litmus milk reaction, hydrogen sulphide production, indole production test, IMVICtest, citrate utilization, urease test, starch hydrolysis and the simple and gram staining procedures were carried out for the characterization of isolates.after obtaining the bacteria, the protein profiling of bacteria was done in SDS-PAGE(Kuldeep kaur *et al.*,2012).Finally protein profiling of bacteria were carried out using SDS-PAGE.

Results

Isolation and identification of bacteria

Sediment sample collected in sterile containers from different place were analyzed. The sample were serially diluted up to 10 and plated in nutrient agar plates. The growth of the bacteria was observed from the second day of incubation and about 11isolates were obtained. The colonies obtained were sub cultured on nutrient agar plates which appeared white and slightly yellow in colour.

Table 1-Best Chitinase Producing Bacteria

Organism	Zone of clearance (mm)
Bacillus licheniformis (std)	28
Isolate no.1	24
Isolate no.2	24
Isolate no.3	24
Isolate no.4	12
Isolate no.5	12
Isolate no.6	12
Isolate no.7	18
Isolate no.8	14
Isolate no.9	18
Isolate no.10	14
Isolate no.11	18

Table 2- Staining the bacteria

Organism	Gram staining
Isolate no.1	Gram positive rods
Isolate no.2	Gram positive rods
Isolate no.3	Gram positive rods
Isolate no.4	Gram negative rods
Isolate no.5	Gram negative rods
Isolate no.6	Gram negative rods
Isolate no.7	Gram negative rods
Isolate no.8	Gram negative rods
Isolate no.9	Gram negative rods
Isolate no.10	Gram negative rods
Isolate no.11	Gram negative rods

Figure 1- Zone of clearance of best chitinase producing bacteria

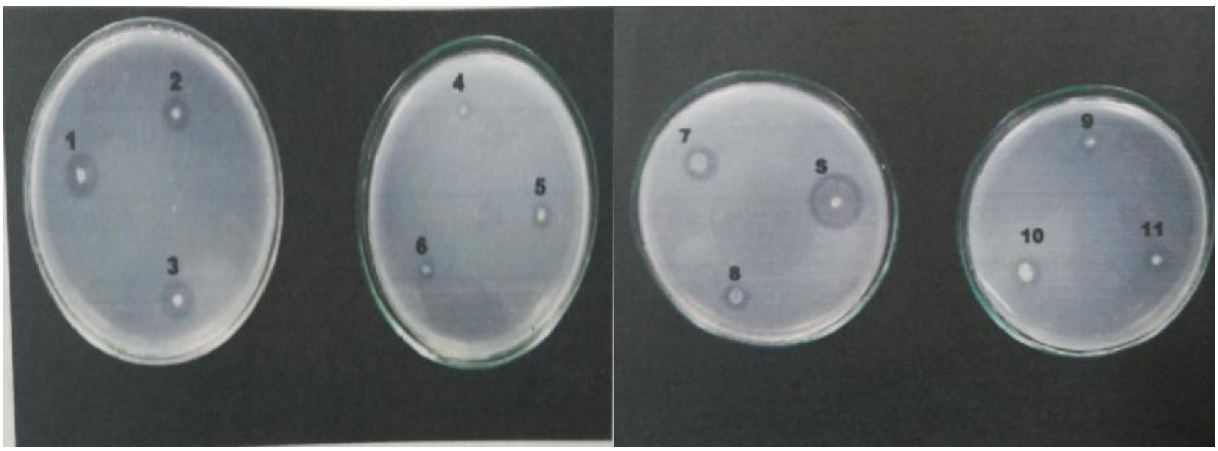

Table 3- Biochemical test results

Organism/ Number of isolates	Test									
	Sucrose	Glucose	Starch	Gelatin	Catalase	Urease	Lactose	Mr	Vp	Indole
Isolate1	A	A	+VE	+VE	-VE	-VE	-VE	-VE	+VE	-VE
Isolate2	A	A	+VE	+VE	-VE	-VE	-VE	-VE	+VE	-VE
Isolate3	A	A	+VE	+VE	-VE	-VE	-VE	-VE	+VE	-VE
Isolate4	-VE	-VE	-VE	-VE	+VE	-VE	-VE	-VE	-VE	-VE
Isolate5	-VE	-VE	-VE	-VE	+VE	-VE	-VE	-VE	-VE	-VE
Isolate6	-VE	-VE	-VE	-VE	+VE	-VE	-VE	-VE	-VE	-VE
Isolate7	AG	AG	-VE	+VE	+VE	-VE	-VE	-VE	+VE	-VE
Isolate8	-VE	-VE	-VE	-VE	+VE	-VE	-VE	+VE	-VE	-VE
Isolate9	A	AG	-VE	+VE	+VE	-VE	-VE	-VE	+VE	-VE
Isolate10	-VE	-VE	-VE	-VE	+VE	-VE	-VE	+VE	-VE	-VE
Isolate11	A	AG	-VE	+VE	+VE	-VE	-VE	-VE	+VE	-VE

A=Acid G=Gas +VE=Positive -VE=Negative

Screening for chitinase producing bacteria

The isolates were screened using chitin as a sole carbon source. About 11 isolates were choosen, which grow in chitinase detection agar plates. The strains grew well at room temperature (37◦C). The bacterial strains were capable of growing well on the agar plate which contains 1% of colloidal chitin .The clear zone of inhibition were observed at 2.6mm (Table 1). The result confirm that the isolates were obtained from marine sediments were chitin degrading organism. High level of chitinase were produced in a medium containing 1% colloidal chitin, as lactose is a carbon source where as low level of chitinase activity was observed in a medium containing other carbohydrates. Some of the organism was able to produce an enzyme chitin deacetylase which release chitosan. Therefore isolates were screened for chitin deacetylase activity using the diagnostic strip test.

Staining and biochemical test

In gram staining 11 isolates obtained which may be either gram positive or gram negative were identified as rod shaped bacteria. From the biochemical test the result were obtained that the standard organism which were used was *Bacillus licheniformis.* The 11 isolates were subjected to biochemical and morphological characterization to determine the species (Table 2).

All the strains showed negative result for urease activity, lactose utilization, methyl red reaction, indole test and hydrogen sulphide production .the isolates 1,2,3 showed similar characteristics *Bacillus sp* isolates, 7,8,9 showed *Serratia* sp, isolate 8 showed *Pseudomonas sp,* isolate 5,6,4 showed *Alcaligenes sp* (Table 3). Through SDS-PAGE analysis characteristics banding pattern were observed in the gel. The different pattern showed the isolate belong to different genera species.

Discussion

The result investigated that the marine bacteria which were obtained from the sea showed high ability to produce an enzyme chitin. Chitinase are essential enzymes, catalyzing the conversion of insoluble chitin to its monomeric components. It belong to family 18-glycosyl hydrolases have been isolated from a wide variety of sources including bacteria, yeast, fungi vertebrates. The fungi species such as *Metarhizium anisopliae,* Bacteria species such as *Bacillus cereus, Bacillus pumilus, Serratia marcescens,* and actinomytes such as *Streptomyces sp* were identified. About 11 isolates of the marine bacteria were obtained which are the best chitin producers compared to *Bacillus licheniforms.* The chitin degrading bacteria were obtained more in the Chennai and Mumbai samples.

The biochemical, morphological and characteristics of *Bacillus* sp, *Alteromonas* sp, *Serratia* sp, *Pseudomonas* sp. The isolates which were obtained are subjected to SDS-PAGE and characteristics banding pattern show that the isolate belong to different genera or species was determined. The genomic DNA was isolated from the best isolated and the restricted band was viewed under uv-transilluminator.

Conclusion

Chitin is one of the underutilized bioresources in the world as it is available on a large scale especially from the marine waste. It has got many biotechnological applications like it can be used as a product in fertilizer and is used in food additives. Due the wide range of applications of chitin researches started isolating chitin producing bacteria. Chitinase producing bacteria were isolated from marine sediments and the biochemical characterizations were done. Through this biochemical characterization they were able to identify 11 chitinase producing bacteria. By the SDS-PAGE technique it can be shown that the organism doesn't belongs to the same genera or species. The clear zone produced in the plate confirmed the chitinolytic activity of bacteria. The genomic DNA of the best isolates was obtained and the DNA is subjected to undergo restriction digestion analysis.

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