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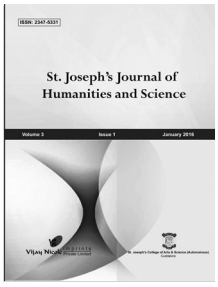

Studies on Chitinase Producing Bacillus Species from Agricultural Soil Sample and Shrimp Waste

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ABSTRACT

Chitinase are wide spread among organisms being produced by bacteria, algae, fungi, protozoa, gastropods, insects, crustaceans and vertebrates etc, among these bacterial strains has the high activity of chitinase production. Bacteria produce chtinolytic enzymes to meet nutritional needs. In fungi, insects and crustaceans, these enzymes are invoved in morphogenesis. In plants and probably vertebrates they play a role in defense mechanism against pathogens, chitinases have found many industrial and pharmaceutuical applications including biocontrol agents and management of chitinous wastes. In this study we characterized the enzyme activity and its potential ability at various pH , carbon sources and enzyme immobilization techniques. Out of 17 different samples 50 Bacillus species were isolated and identified in Hicrome Bacillus Agar Four different species of Bacillus were isolated such as *Bacillus pumilus*, *Bacillus subtilis, Bacillus megaterium, Bacillus cereus* were almost predominant in chitinous waste. chitinase producing bacteria isolated using Chitinase Agar medium. screening of chitin utilizers were done by using chitinase plate assay method. The selection of working strain was made on the basis of their activity determined qualitatively. The observed colonies have undergone several phenotypic characteristics.

Keywords: Bacillus; Chitinase; Shrimp Waste; Hicrome Bacillus Agar

1.0 INTRODUCTION

Chitin can be regarded as the second most common polysaccharide on earth, after cellulose. Formally, chitin can be derived from cellulose by substitution of the hydroxyl group on carbon atom 2 of glucose with a acetylated ammonia group. Among 50 chitin decomposing bacteria isolated from agricultural soil,the following genera are represented *Flavobacteri um,Bacillus,Pseudomonas,Streptomyces, Nocardia* and *Micromonospora.* Among the fungi that can decompose chitin, *Aspergillus, Mucor* and *Mortiella* species are included up to 10 -6 organism / g of agricultural soil are able to utilize chitin. *Paenibacillus chitinolyticus* is another important bacteria which degrades chitin. Chitin occurs as a support medium in the animal as well as in the plant kingdom and forms the exoskeleton of many non-vertebrates. Chitin is also continuously produced in soil as the main cell wall component of many Basidiomycetes and Ascomycetes..chitinases have found many industrial and pharmaceutical applications including biocontrol of plant pathogenic fungi and insects, production of chitooligosaacharides and management of chitinous wastes.

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2.0 MATERIALS AND METHODS

Isolation and identification: Soil samples from different areas were collected randomly from different agricultural field at different places such as fish market soil, poultry wastes, shrimp wastes and coastal soil samples in and around cuddalore. The soil samples and other wastes were collected in a sterile container and transported to laboratory for further processing, hence a total of 17 different soil samples and wastes were collected from 17 different sites. The chitinase producing bacteria was isolated by using Minimal salt Agar and then further it was screened and its phenotypic character was studied by using Hicrome Bacillus agar for Identifying Bacillus species. The isolates was identified based on cellular morphology, Gram staining, endospore staining and biochemical tests.

3.0 OPTIMIZATION PARAMETERS FOR CHITINASE ACTIVITY

3.1 Effect of pH on Chitinase Production

The isolated culture such as *Bacillus cereus, Bacillus pumilus*, *Bacillus megaterium* and *Bacillus subtilis* inoculated in Minimal salt broth at different pH ranges from pH 4 to 10 and incubated at 37°C for 48 hours, the lipase activity was observed in UV spectrophotometer at 560nm

3.2 Effect of Carbon Source on Chitinase Production

The isolated cultures such as *, Bacillus cereus, Bacillus pumilus*, *Bacillus subtilis* and *Bacillus megaterium* inoculated in minimal salt broth supplemented with various carbon sources such as glucose, sucrose, maltose and mannitol were incubated at 37°C for 48 hours, the chitinase activity was observed in UV spectrophotometer at 560nm.

4.0 Stability of Chitinase Enzyme by Immobilization

The stability of chitinase enzyme was studied by immobilization

5.0 Hydrolysis of Starch & Casein

The isolated cultures such as *Bacillus cereus, Bacillus pumilus*, *Bacillus subtilis* and *Bacillus megaterium* inoculated in starch agar plates and skim milk agar plates .

6.0 Lecthinase activity: The isolated cultures such as*, Bacillus cereus, Bacillus pumilus*, *Bacillus subtilis* and *Bacillus megaterium* inoculated in egg yolk media.

7.0 Immobilization of Chitinase enzyme:

Take 48 hours broth culture and centrifuge at 4000 rpm for a duration of 20minutes. Collect the supernatant with the clear sterile Pasteur pipette without disturbing the other layer. The bacterial cell mass is found in the form of pellet in the bottom of the centrifuge tubes. The supernatant is used as a crude chitosanase in the preparation of immobilized bead. Add 5ml of sodium alginate solution from the stock solution to the test tube using 5ml micropipette. Immediately add 5ml of crude chitosanase enzyme solution from the stock to the alginate solution in the test tube and mix it thoroughly using a cyclomixer. Take 40ml of calcium chloride stock solution in a 50ml clean and dry test tube and put it in the stand. Pipette 5ml of alginate and enzyme solution from the test tube using 5ml micropipette. From a height of 10cm release the mixture from the pipette, one drop at a time into the calcium chloride solution in the test tube. The beads containing the enzyme will form in the calcium chloride solution. Leave the beads to harden for at least 10minutes in the calcium chloride solution itself, Stir the beads in the calcium chloride solution by shaking the solution and pour the beads into a beaker and measure the beads.

8.0 RESULTS AND DISCUSSION

8.1 Morphological and physiological characteristics

Morphological and physiological characteristics of bacterial isolates of chitinase producers were investigated according to the methods described in Bergey's Manual of Determinative Bacteriology. (Table:1) It was identified as a member of *Bacillus* species. The colonial appearance of this *Bacillus* species in Hicrome Bacillus Agar (Plate:1) possess different colonies such as *Bacillus pumilus* had produced light green to green color colonies, *Bacillus subtilis* shows yellowish green to green colonies; *Bacillus megaterium* shows yellow mucoid colonies, *Bacillus cereus* produces light blue, large flat with blue centred colonies.

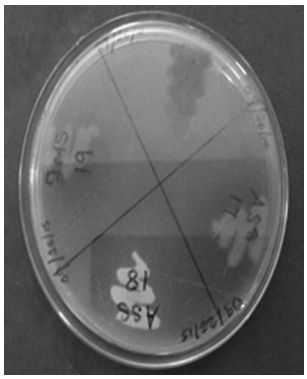
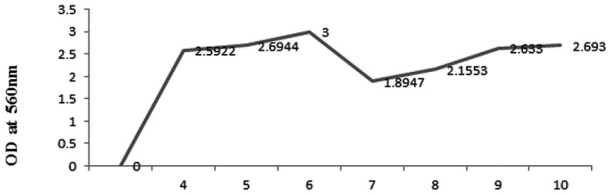


Plate 1: Growth of Different Bacillus sps in Hicrome Bacillus Agar

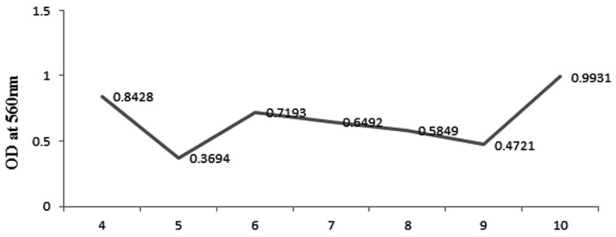
Table:1 Biochemical Characterization

Biochemical Characteristics	*B.pumilus*	*B.subtilis*	*B.cereus*	*B.megaterium*
Cellular morphology& Gram staining	Gram positive rods	Gram positive rods	Gram positive rods	Gram positive rods
Spore staining	Spore former	Spore former	Spore former	Spore former
Activity of Catalase	Positive	positive	positive	Positive
Activity of oxidase	Positive	positive	positive	Positive
Indole reaction	Negative	negative	negative	Negative
Methyl red reaction	Negative	negative	negative	Negative
Voges proskauer reaction	Positive	positive	positive	Negative
Citrate utilization	Positive	positive	positive	Positive
Starch hydrolysis	Positive	positive	positive	Positive
Casein hydrolysis	Positive	positive	positive	Positive
Lecthinase activity	Positive	positive	positive	Positive
Urease activity	Negative	negative	negative	Negative
TSI	k/A	k/A	k/k	k/k
LIA	k/k	K/K	K/K	k/k
Mannitol motility test	Nonmotile	nonmotile	nonmotile	nonmotile

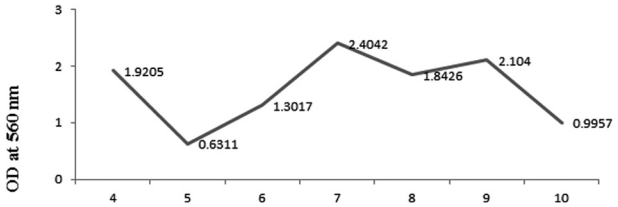
8.2 Optimization of chitinase at various pH



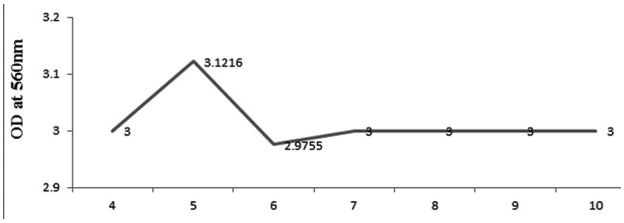
Graph: 1 Effect of pH on Chitinase Production by Bacillus pumilus



Graph: 2 Effect of pH on Chitinase Production by Bacillus cereus

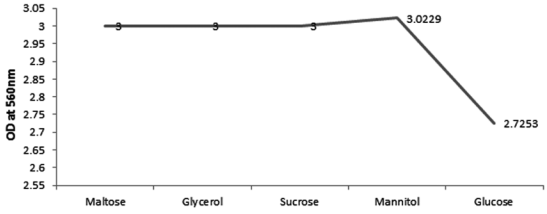


Graph: 3 Effect of pH on Chitinase Production by Bacillus subtilis

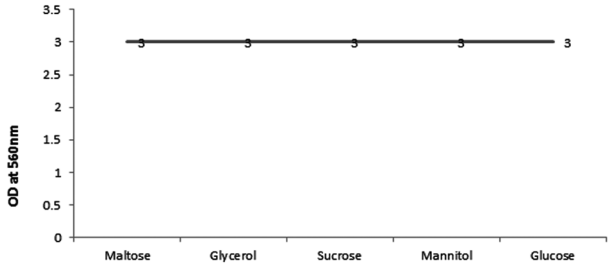


Graph: 4 Effect of pH on Chitinase Production by Bacillus megaterium

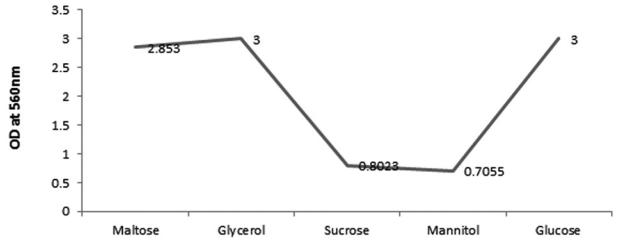
8.3 Effect of Carbon Source on Chitinase Production



Graph: 5 Effect of Carbon Source on Chitinase Production by Bacillus pumilus



Graph: 6 Effect of Carbon Source on Chitinase Production by Bacillus cereus



Graph: 7 Effect of carbon source on Chitinase Production by Bacillus megaterium

Graph: 8 Effect of Carbon Source on Chitinase Production by Bacillus subtilis

9.0 Hydrolysis of Starch

The isolated cultures such as *Bacillus pumilus*, *Bacillus cereus*, *Bacillus subtilis* and *Bacillus megaterium* inoculated in starch agar plates and incubated at 37°C for 24 hrs after incubation zone of clearance was observed by using Grams iodine solution , the hydrolyzed area forms clear zone and the un hydrolyzed area appears dark brown color.

10.0 Hydrolysis of Casein

The isolated cultures such as *Bacillus pumilus*, *Bacillus cereus*, *Bacillus subtilis* and *Bacillus megaterium* inoculated in skim milk agar plates and incubated at 37°C for 24 hrs. After incubation zone of clearance was observed which indicates the organism has hydrolysed casein.

11.0 Lecthinase activity

The isolated cultures such as *Bacillus pumilus*, *Bacillus cereus*, *Bacillus subtilis* and *Bacillus megaterium* inoculated in egg yolk media after incubation the production of lecthinase is observed by cream white color colonies.

12.0 Enzyme immobilization technique

Production of extracellular crude chitinases has been remarkable in ordinary growth media; for commercial production extracellular chitinases can be stabilized in immobilization method; in this preparation we used Sodium alginate as the carrier molecule; the enzyme beads formed can be preserved and it can be used for further application.

13.0 Significance and impact of the study

The present study was focused to isolate and characterize chitinase producing bacteria from various sources. Different species of *Bacillus* such as *Bacillus pumilus, Bacillus subtilis, Bacillus megaterium,* and *Bacillus cereus* was isolated and screened. All these chitinase producers appeared promising good source of chitinase but it requires a detail characterization of growth and nutrient conditions. The optimization process of chitinase enzyme at various pH with different isolates was standard, and with different carbon sources was also reasonable in producing chitinase. This enzyme is needed for degradation of marine wastes which contains higher amount of chitin and isolated strains which can be exploited for an assortment of applications for industrial source.

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