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ACIDIC CHITINASE PRODUCTION BY *NEISSERIA SPECIES*

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ABSTRACT

The soil sample was collected from the Miraj (MS) fish market. The soil sample was enriched in minimal salt medium containing chitin as sole carbon source. 30 Bacterial species were isolated from enriched soil sample by serial dilution technique and spread plate technique on chitin agar medium. The isolated bacterial species were screened for chitinase production by primary and secondary screening. In the primary screening chitinase production were confirmed by hydrolysis zone and in secondary screening submerged fermentation were used for the chitinase production by using 1% colloidal chitin in nutrient broth. Among the 30 bacterial colonies R4 shown highest zone of hydrolysis and maximum chitinase activity at pH 6.0. Further R4 were characterized and identified as a *Neisseria* species according to Bergeys manual of Bacteriology.

KEYWORDS: Chitinase, Chitin, Screening, *Neisseria* species.

INTRODUCTION

Chitin is well- known as an insoluble structural polysaccharide that occurs in the exoskeleton and gut linings of many insects, invertebrates such as crustaceans, protozoa, fungi and diatoms which could be hydrolyzed by chitin degrading enzymes such as Chitinases (Kramer *et al*., 1986). Chitin is the linear β-1, 4-N- acetylglucosamine polysaccharide (Cabib *et al*., 1987) is the most abundant renewable resource after cellulose (Deshpande *et al.*, 2000).

Approximately 75% of the total weight of shellfish are considered as a waste and comprises 20- 58% of the dry weight of the said waste (Sugnita *et al.*, 2000). Chitinase (EC 3.2.11.14) enzyme is found in a variety of organisms including bacteria, viruses, fungi, insects, higher

plants and animals. Chitinases are hydrolytic and mycolytic in nature (Park *et al.*, 1992). Chitinases are also isolated from the stomach of human beings. Chitin catabolism occurs in two steps involving the initial cleavage of the chitin polymer by chitinase into chitin oligosaccharides, and then further cleavage to N- acetyl glucosamine monomers by chito-biases (Deshpande *et al.*, 2000). Chitinases are widely applied to control plant pathogenic fungi and insects, to control mosquito, for the production of chito-oligosccharides, Single cell protein production and in water purification (Gooday *et al.*, 1990; Deshpande *et al*., 1986; Cabib *et al.*, 1987).

MATERIALS AND METHODS

Chemicals

Chitin Powder, Shrimp well Chitin flakes, N- acetyl glucosamine powder, Nutrient agar medium, minimum salt medium was purchased from the Himedia laboratories Mumbai, India.

Sample collection

Soil sample were collected from the Miraj (MS) fish market from 4-5 cm depth of soil with the help of sterile spatula and put in sterile plastic bag which was brought to laboratory and were kept in refrigerator at 4^0C till further processing.

Preparation of colloidal chitin

Colloidal chitin was prepared from the shrimp well chitin flakes by Hsu and Lockhood method (1975). In this method the chitin flakes (40g) were slowly added to 600 ml of conc. HCL and kept at 30^0C for 60 min. with vigorous stirring. Chitin flakes was precipitated as a colloidal suspension by adding it slowly 20 ml of distilled water at 4^0C. The suspension was collected by filtration and washed by suspending it in about 51 ml of distilled water. Washing was repeated for 3 times until pH of the suspension was 3.5 and used as a substrate.

Enrichment of chitinase producers

1 g of soil sample was enriched in 100 ml of Minimal Salts medium (MSM). The enrichment was carried out at 25^0C with 180 rev/min. in incubator shaker and viable count was studied.

Composition of MSM medium; K_2HPO_4 - 0.03 g, KH_2PO_4 – 0.07 g, $MgSo_4$ – 0.05 g, $ZnSO_4.7H_2O$ – 0.001 g, $FeSo_4.7H_2O$ – 0.001 g and chitin powder – 1 g in 100 ml of distilled water , pH- 6.0.

Isolation of Chitinase producers

For the isolation of chitinase producers serial dilution and spread plate technique was used. Serially diluted soil sample were spread on nutrient agar medium plates. The plates were incubated at 37^0C for 24 – 48 hrs. Well grown isolated colonies were picked and sub-cultured on nutrient agar slants.

Screening of Chitinase producers

Primary screening

Well grown isolated bacterial colonies were spot inoculated on sterile colloidal chitin agar medium and were incubated at 37^0C for 24 – 48 hrs. After incubation the plates were flooded with 2% cango red and were examined for the zone of hydrolysis. On the base of zone of hydrolysis one chitinase positive producer were selected for secondary screening.

Secondary screening

Secondary screening were carried out by submerged fermentation in which chitinase positive R4 were inoculated in the sterile colloidal chitin broth medium (pH 6.0) and incubated at 37^0C for 5 – 8 days in incubator shaker at 180 rpm. The samples were removed from broth after every 24 hrs; centrifuged and were assayed for chitinase activity by DNS method using phosphate buffer pH 6.0 at 540 nm in colorimeter.

Identification of chitinase producer

The morphological and biochemical characterization of selected R4 chitinase producer was carried out to identify the culture. Biochemical tests were performed as described in Bergey's manual of systematic Bacteriology.

RESULTS

Isolation of chitinase producers

10 isolates were obtained. They were labeled as R1, R2, R3 up to R10 and were sub-cultured on sterile nutrient agar slants in duplicates and stored at 4^0C. The results were shown in table I.

Screening of chitinase producers

Primary screening

All R10 isolates were screened for chitinase production on sterile colloidal chitin agar medium. Among the R10 isolates R4 were showing maximum chitin hydrolysis zone. The results were shown in figure; I and table; I

Secondary screening

The chitinase positive R4 were selected for secondary screening and they were screened for chitinase production. The maximum chitinase activity shown by R4, 6.03µg/ml/mol at pH 6.0. The results were shown in Figure II and table: II

Identification of chitinase Producer

The selected R4 chitinase producer were identified as *Neisseria spp* and it's morphological and biochemical characteristics were shown in table : III & IV

Table I; Colony size and zone of chitin hydrolysis shown by isolates in primary screening.

Isolate No.	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10
Colony size(cm)	0.2	0.1	0.1	0.2	0.2	0.1	0.2	0.2	0.2	0.1
Zone of hydrolysis	0.3	0.3	0.2	0.6	0.4	0.3	0.3	0.3	0.4	0.4

Figure I: Isolation and Primary screening of *Neisseria spp.*

Table II; Selection of R4 on the basis of their colony size, zone of hydrolysis and chitinase activity.

Isolate number	Colony size (cm)	Zone of hydrolysis(cm)	Chitinase activity µg/ml/mol
R4	0.2	0.6	6.03

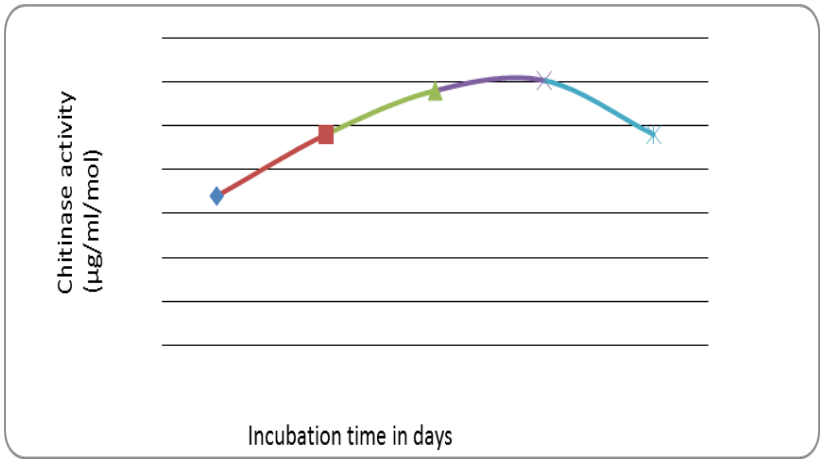

Figure II: Production of chitinase by submerged fermentation.

Table III; Morphological characteristics of R4 isolates.

Size (cm)	Shape	Color	Margin	Texture	Gram's nature	Motility
0.2	Circular	Pink	Entire	Mucoid	Gram negative cocci	Non-motile

Table IV; Biochemical characteristics of R4 isolates.

Sr.No.	Biochemical test	Isolate R4
1	Sugar utilization	
	Lactose	-
	Fructose	+
	Mannose	+
	sucrose	+
	Glucose	+
	Xylose	+

2	Catalase test	+
3	Indole test	+
4	Methyl red	+
5	V-P test	-
6	Citrate utilization	-
7	Starch hydrolysis	+
8	Casein hydrolysis	+
9	Oxidase test	+
10	Chitin hydrolysis	+

DISCUSSION

Numerous studies have investigated several aspects of chitin degradation on nematicidal activity. In the present study soil samples were collected from the fish market from which 10 chitinase producers were isolated by using colloidal chitin agar medium. These isolation methods were similar with Gooday *et al.*, 1990 and Deshpande *et al.*, 1986. In the primary screening plates containing isolated bacterial organisms were flooded with 2% Congo red to check chitin hydrolysis. The similar method for confirmation of chitin hydrolysis was reported by Bansode V. B and Bajekal S.S 2006. After primary screening R4 got as a highest chitinase producer that is why R4 were selected for secondary screening by submerged fermentation at pH 6.0. On 7^{th} days of incubation period R4 shows 6.03µg/ml/mol of chitinase activity by DNS method. These results are accordance with those shown by Sudhakar *et al.*, 2011; and Deshpande *et al.*, 1986. For the study of purification of R4

bacterial colony Bergey's manual were used and confirmed that R4 were belongs to genera *Neisseria* but I got very less literature about *Neisseria spp*.

CONCLUSION

This study concludes that Chitinases would be effectively produced by *Neisseria spp* at acidic pH and at 37^0 C in colloidal chitin broth medium. It shows highest chitinase activity against chitin but the production takes much more time. The production of Chitinases widely applied in the field of chemistry, biochemical, biotechnological, agricultural an environmental rotection.

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