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CHARACTERIZATION OF CHITINASE PRODUCING BACILLUS SPECIES ISOLATED FROM CHILLI RHIZOSPHERES OF GUNTUR DISTRICT ANDHRA PRADESH

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ABSTRACT

Chitin is the second most abundant organic and renewable source in nature, after cellulose. Chitinases are chitin-degrading enzymes have great importance in enzyme market. A total of 10 bacterial isolates belonging to *Bacillus* species isolated from chilli rhizosphere, Guntur district of Andhra Pradesh, India. All the isolates were tested for chitinase production on colloidal chitin medium. Among them four isolates i.e. Bacillus sp. PB-3(2.22 IU/ mg), Bacillus sp. PB-5(2.75 IU/ mg), Bacillus sp. PB-6(2.22 IU/ mg), and Bacillus sp. PB-8(3.46 IU/ mg) showed chitinase production. Optimization studies like incubation period, pH, carbon and nitrogen sources were determined by these four isolates. Chitinase production was maximum on 6 days of incubation (3.46 IU/ mg) period and the optimum pH was found to be 7.0. Carbon and nitrogen sources greatly influenced the chitinase production. Among them 1% glucose and 0.5% glycine was the best for chitinase activity.

KEYWORDS: Bacillus, Chitinase, Carbon and nitrogen sources

1. INTRODUCTION

Screening of diversified microorganisms from different habitats and their optimum utilization in industrial sector is the need of the hour. The increasing energy demands has drawn worldwide attention on the utilization of renewable resources particularly agricultural and forest residues, the major components of which are cellulose, starch, lignin, xylan and pectin. These materials have attracted considerable attention as alternative feed stock and energy source. Since they are available abundantly several microbes are capable of using these

substances as carbon and energy sources by producing a vast array of enzymes in different environmental niches. (Antranikian *et.al*., 1992).

Industrial biotechnology, using microorganisms and biological catalysts "enzymes" to produce goods and services has come of age. Search for new enzymes for use in commercial applications with desirable biochemical and physico chemical characteristics and a low production costs have been the focus of much research (Neetha *et .al*, 2011).

Chitinases (EC 3.2.1.14) can catalyze the hydrolysis of chitin to its monomer *N*-acetyl-D-glucosamine. Chitinase is an inducible enzyme secreted by many microorganisms in cultures containing chitin or its oligomers as sole carbon source. Chitinolytic microorganisms are considered to be more effective antagonists of fungal pathogens because of the direct action of chitinase alone (Yu *et al*., 2008) or in combination with other antifungal compounds produced by the antagonist (De Boer *et al*., 1998). Chitinase is known to process diverse characteristics worthy of detailed enzymatic studies related to their biological role and structural elucidation (Thamthiankul, Suan-Ngay et al. 2001; Liu, Kao *et al*., 2003). In Andhra Pradesh Guntur is famous for chilli production. Majority of the soils contain chilli rhizosphere. However the present study reveals that the chitinase production by bacillus species isolated from chilli rhizosphere. Optimization studies also reveal the chitinase activity on these isolates.

2. MATERIALS AND METHODS

2.1 Isolation

One gram representative soil sample was suspended in 10 ml of sterile distilled water and shaken thoroughly for 10 minutes. Microorganisms were isolated from collected samples by the serial dilution plate technique using Nutrient Agar Medium (NAM). Serial dilutions up to 10^{-5} of each sample were prepared by using sterilized water. Sample dilutions were plated (in triplicates) on NAM and incubated at 35^0 C for 24 to 48 h. Pure Colonies were picked and maintained on NAM slants at 4^0 C and further assessed for enzyme production in liquid medium.

2.2 Screening of chitinolytic microorganisms

Chitinase activity can be qualitatively assayed by determining the clearance zone developed around the colonies growing on the colloidal chitin agar medium (Cody, 1989; Wirth and Wolf, 1990). The potency of the isolates for chitinase production is determined on the basis

of ratio of zone of clearance (CZ) to colony size (CS) (Cody, 1989). This procedure requires longer incubation time for about 2 to 7 days and is relatively less sensitive because of the poor visualization of the CZ. Screening the chitinolytic microorganisms by incorporating calcofluor white M2R (0.001% w/v) in chitin agar has been developed (Vadiya *et al*., 2003).

2.3 Detection and quantification of chitinases activity

The activity of chitinases can be qualitatively assayed by using chitin agar plate either with or without fluorescent dye. Activity staining method can also be used for qualitative assay. Activity staining can be done by incorporating ethylene glycol chitin in the gel (Trudel and Asselin, 1989). However, this method has limitations since the gel can not be further used for protein staining and there is problem of mobility of chitinase in the gel because of the presence of polysaccharide in the gel. These problems have been over come by running protein sample in the gel without incorporating chitin followed by diffusion on chitin agar plate containing fluorescent dye (Gohel *et al*., 2005). Colloidal chitin with Remazol Brilliant Blue R was also used as a substrate for colorimetric assay of chitinase (Gómez Ramírez *et al*., 2004).

2.4 Optimization studies on chitinase production

In the present study a thematic attempt was made to investigate the effect of various parameters including incubation period, pH, temperature, carbon (Sucrose, Glucose, Maltose, Galactose and colloidal chitin) and nitrogen sources (sodium nitrate, ammonium sulphate, peptone, Yeast extract and glycine) on chitin broth medium. A classical approach using substrate fermentation medium under standard conditions mention earlier optimization studies like sub merged fermentation.

3. RESULTS AND DISCUSSION

3.1 Incubation period

A total of 10 isolates were obtained from chilli rhizosphere in the vicinity of Guntur, Andhra Pradesh. The preliminary characterization like cultural and biochemical characteristics of rhizobia was done by Bergey's manual of systemic bacteriology. All the isolates belong to Bacillus species according to their preliminary and biochemical studies. All the isolates were designated as Bacillus sp. PB-1 to Bacillus sp. PB-10 and tested for chitinase activity (Table-1). Among them four isolates i.e. Bacillus sp. PB-3(2.22 IU/ mg), Bacillus sp. PB-5(2.75 IU/ mg), Bacillus sp. PB-6(2.32 IU/ mg), and Bacillus sp. PB-8(3.46 IU/ mg) showed chitinase

production. Optimization studies like incubation period, pH, carbon and nitrogen sources were determined by these four isolates.

Table 1: Chitinase production (IU/mg) by Bacillus species.

Isolates	Chitinase production (IU/mg)
Bacillus sp. PB-1	-
Bacillus sp. PB-2	-
Bacillus sp. PB-3	**2.22**
Bacillus sp. PB-4	-
Bacillus sp. PB-5	**2.75**
Bacillus sp. PB-6	**2.32**
Bacillus sp. PB-7	-
Bacillus sp. PB-8	**3.46**
Bacillus sp. PB-9	-
Bacillus sp. PB-10	-

*Each data is an average of three replicates

3.2 Screening for chitinase production

Further the four strains were screened for chitinase production by different incubation periods like 2, 3, 4, 5, 6 and 7 days. After 2 days of incubation period the clear zone (CZ) was formed around the bacterial colony. Total colony size and clear zone was measured for 7 days of incubation period (Table-2). Maximum colony size was observed in 6 days of incubation period.

Table 2: Screening for chitinase production by Bacillus species.

S.No.	Bacillus Isolates	Clear zone around the colony (mm) at different incubation periods (days)					
		2	**3**	**4**	**5**	**6**	**7**
1	*Bacillus* sp. PB-3	-	2	4	6	8	8
2	*Bacillus* sp. PB-5	-	2	4	6	8	8
3	*Bacillus* sp. PB-6	2	4	6	8	10	10
4	*Bacillus* sp. PB-8	2	4	6	8	10	10

*Each data is an average of three replicates

3.3 Effect of incubation period on amylase production

Different incubation (2,3,4,5,6 and 7 days) periods were studied for the chitinase production on chitin broth medium. Maximum chitinase production was observed by 6 days of incubation period (Table-3). Maximum enzyme production could be obtained only after a certain incubation time which allows the culture to grow at a study state (Pandey *et al.*, 2000). Enzyme production of each strain is based on the specific growth rate of the strain.

Growth rate and enzyme synthesis of the culture are the two main characteristics which are mainly influenced by incubation time (Ellaiah *et al*., 2002).

Table 3: Effect of incubation period on Chitinase production by Bacillus species.

Incubation periods	*Bacillus* sp. PB-3	*Bacillus* sp. PB-5	*Bacillus* sp. PB-6	*Bacillus* sp. PB-8
2	0.22	0.75	0.88	0.66
3	0.25	1.05	1.11	0.98
4	0.45	1.45	1.67	1.45
5	0.78	2.33	2.11	1.98
6	2.22	2.75	2.32	3.46

3.4 Effect of pH

Different pH levels were maintained for the chitinase production by all the four species. All the isolates showed maximum chitinase production on neutral pH. Acidic phase to a neutral phase enzyme activity increased up to a pH of 4.0 and upon further increase in pH, enzymatic activity decreased (Table-4). Different organisms have different pH optima and any modification in their pH optima could result in a decrease in their enzyme activity (Adinarayana et al, 2005).

Table 4: Effect of pH on Chitinase production by Bacillus species.

Ph	*Bacillus* sp. PB-3	*Bacillus* sp. PB-5	*Bacillus* sp. PB-6	*Bacillus* sp. PB-8
pH-4	0.11	0.23	0.34	0.23
pH-5	0.88	0.97	0.88	1.23
pH-6	1.26	1.55	1.65	2.45
pH-7	2.22	2.75	2.32	3.46
pH-8	1.76	1.23	1.67	1.89

3.5 Influence of carbon sources

Several carbon substrates like Sucrose, Glucose, Maltose, Galactose and colloidal chitin were tested to evaluate the enzyme production. Among them glucose contain the medium produced maximum chitinase production (Table-5). Various reports supported to this type of results like, Glucose and starch when supplemented as additional carbon substrate to the medium has resulted in enhanced enzyme production. Among the tested substrate sucrose and glucose resulted in enhanced enzyme production (Prakasham 2007).

Table 5: Effect of different Carbon sources on Chitinase production by Bacillus species.

Additional Carbon sources	*Bacillus* sp. PB-3	*Bacillus* sp. PB-5	*Bacillus* sp. PB-6	*Bacillus* sp. PB-8
Control	0.23	0.34	0.45	0.67
Sucrose	1.78	1.11	1.31	1.79
Maltose	2.01	1.23	1.26	1.98
Galactose	1.05	1.76	1.89	2.44
Glucose	2.22	2.75	2.32	3.46
Colloidal chitin	1.11	1.99	1.76	1.77

3.6 Influence of nitrogen sources

Various nitrogen sources (sodium nitrate, ammonium sulphate, peptone, Yeast extract and glycine were induced the chitinase production among them glycine influenced the maximum (3.46 IU/mg) chitinase production (Table-6).

Table 6: Effect of nitrogen sources on Chitinase production by Bacillus species.

Nitrogen sources	*Bacillus* sp. PB-3	*Bacillus* sp. PB-5	*Bacillus* sp. PB-6	*Bacillus* sp. PB-8
Control	0.11	0.19	0.23	0.88
NaNo3	1.09	1.67	1.88	1.29
(NH4)SO4	1.16	1.90	1.75	2.89
Yeast extract	1.89	2.22	1.99	2.33
Glycine	2.22	2.75	2.32	3.46
Peptone	1.88	2.42	1.72	2.56

5. CONCLUSION

In the present study four bacillus species produced chitinase production on colloidal chitin medium. Among them *Bacillus* sp. PB-8 showed maximum chitinase production on the basis of optimization studies. Six days of incubation period and neutral pH was suitable for maximum chitinase for this strain. Maximum chitinase production was observed by using Glucose and glycine as carbon and nitrogen sources.

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