STUDIES ON SOIL WITH RESPECT TO CHITINOLYTIC *BACILLUS* FROM AURANGABAD AND AKOLA DISTRICT (M.S.) INDIA

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ABSTRACT

The cheapest source of isolation of bacillus is soil so it becomes necessary to know that which soil having more number of chitinolytic *bacillus* hence present comparative study was done on the soil from Aurangabad and Akola district. The method for isolation of chitinolytic *Bacillus* depends on basis of zone of clearance on chitin agar plate having calcofluor white M2R dye. One hundred and twenty samples of *Bacilli* were collected from Aurangabad and Akola districts. It showed specific staining, morphology and biochemical characters. All samples were isolated and tested for chitinase production. Percentage of chitinolytic Bacillus were estimated for both districts, finding of these studies reveal that occurrence of chitinolytic bacillus in Aurangabd soil is more as compared to Akola district with respect to observed groups of *Bacilli*. Aurangabad soil is more suitable for isolation of chitinolytic bacillus for research and other purpose as compare to Akola district for studied group of bacillus.

Key words: Bacillus, chitinase enzyme, Isolation of chitinolytic *Bacilli.*

INTRODUCTION

Chitin is a versatile and promising biopolymer with numerous industrial, medical and commercial uses (Haki and Rakshit 2003). The biodegradation of chitin requires the synergistic action of several hydrolytic enzymes for efficient and complete breakdown. The combined action of endochitinases (EC 3.2.1.14) and exochitinases (chitobiosidases) and β-N-acetyl hexosaminidase (EC 3.2.1.82) results in the degradation of chitin polymer into the soluble N-acetyl D-glucosamine (Gkargkas *et al*. 2004). These enzymes are found in a wide variety of organisms including bacteria, fungi, insects, plants, and animals (Gooday 1990). Chitnase enzymes have a wide range of biotechnological applications, especially in the production of chito-oligosaccharides and N-acetyl D-glucosamine (Pichyangkura *et al*. 2002), biocontrol of pathogenic fungi (Chernin *et al.* 1997 and Mathivanan *et al.* 1998), preparation of sphaeroplasts and protoplasts from yeast and fungal species (Mizuno *et al*. 1997 and Balasubramanium *et al*. 2003) and bioconversion of chitin waste to single cell protein (Vyas and Deshpande 1991).

Many species of bacteria are known to synthesize chitinase for the utilization of chitin as a source of carbon and nitrogen. Some chitinolytic bacteria have been the potential agents for biological control, both of the plant diseases caused by various phytopathogenic fungi and of insect pests, because fungal cell walls and insect exoskeletons both contain chitin as a major structural component (Chernin *et al*. 1997). Because of its thermostability chitinase enzymes are widespread in gram-positive bacteria, e.g., spore-forming genera *Bacillus* and *Clostridium*. *Bacillus* species are well known for their ability to produce many useful enzymes (eg.chitinase) and widely spread in nature especially in soil so the main objective of this study is to find out the soil having more occurance of chitinolytic *Bacillus* from Aurangabad and Akola district.

MATERIALS AND METHODS

Collection of soil sample

Different places like agricultural land, bank of river and play ground were selected for collection of soil samples. Upper part of soil approximately 10-15 CM was removed and about 500 gm of soil was collected from four different corners and middle region of above mentioned places of Aurangabad district in sterile polythene bags with the help of sterile sickle, like that different soil sample were collected from Akola district. The samples were preserved at 4°C till further use for microbial analysis.

Isolation of chitinolytic bacillus

Initial screening has been performed by serial dilutions techniques of different soil samples. Organisms screened by this technique were investigated on the basis of staining, morphology and biochemical characters. After confirmation of Bacillus further investigation have been performed for the production of chitinase enzyme by chitin agar plate having calcofluor white M2R dye (0.001% w/v) method(Vadiya *et.* al. 2003a). In this Bacillus were spread and inoculated on the chitin agar plate and incubated 24-48 hrs. for zone of clearance. Colonies that produce zone of clearance were selected and preserved for further investigation.

RESULT AND DISCUSSION

Based on initial investigation on cell morphology and biochemical characters total six group (Table 1, 2 and fig. 1.) of *Bacilli* (Rod, Gram positive, Motile) were prepared. *Bacilli* culture were isolated and placed into the respective group on the basis of the cell morphology and biochemical characters. Total 10 culture of bacillus for each individual group were isolated from Akola district and same type of isolation has been performing from Aurangabad district. At the end total 60 cultures of *Bacilli* from Akola district. And 60 cultures of bacillus from Aurangabad district were isolated.

Isolated *Bacilli* were further investigated for chitinase production ability as zone of clearance by chitin agar plate method and percentage of chitinolytic Bacillus were estimated for each group from Akola and Aurangabad district. (Table 3 and 4)

Figure 1: Colony Morphology of six groups of *Bacillus* on chitin agar plates.

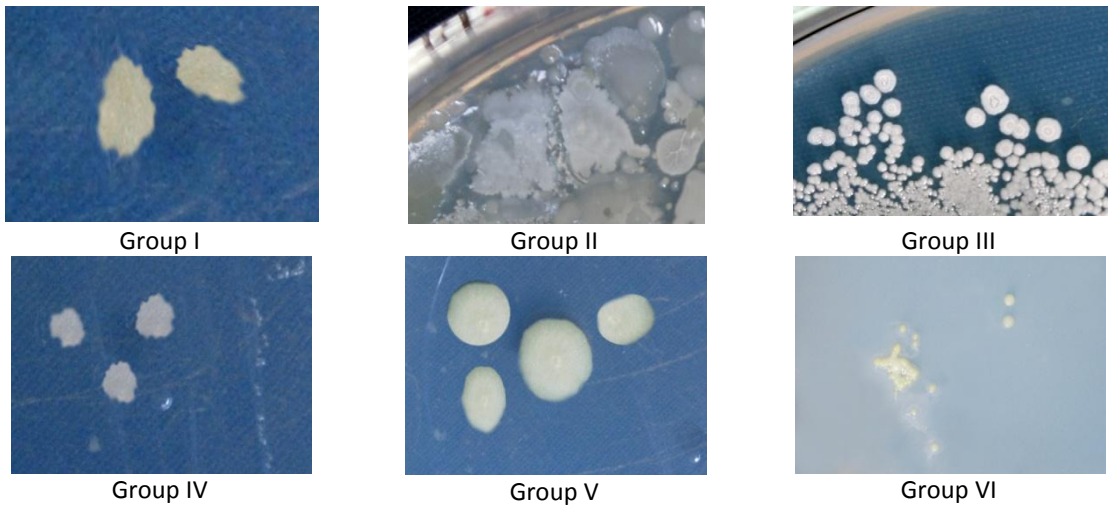

Table 1: Colony Morphological Characters of six groups of *Bacillus*

Isolates	Colony Morphology (From Agar Plates)				
	Shape	Elevation	Edge	Colour	Surface
Group I	Irregular	Flate	Lobate	Cream	Wrinkled
Group II	Irregular	Flate	Lobate	White	Wrinkled
Group III	Round	Flate	Entire	White	Smooth
Group IV	Round	Flate	Lobate	White	Wrinkled
Group V	Irregular	Flate	Entire	Cream	Smooth
Group VI	Round	Raised	Entire	Cream	Smooth

Fig. 2: Percentage analysis of Chitinolytic Bacillus for Akola & Aurangabad District

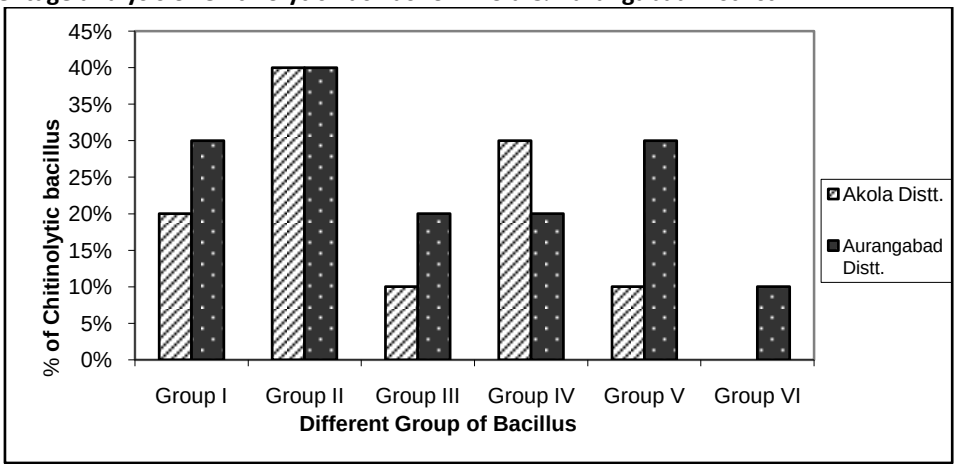

Table 2: Biochemical Characters of six groups of *Bacillus*

Characters	Group I	Group II	Group III	Group IV	Group V	Group VI
Catalase test	+	+	+	+	+	+
Anaerobic growth	+	+	+	+	+	+
Voges-Proskauer test	+	+	+	+	+	+
Methyl red test	-	+	-	+	-	+
Hydrolysis of Casein	+	+	+	+	+	-
Hydrolysis of Gelatin	+	+	+	-	-	+
Hydrolysis of Starch	+	+	+	+	+	+
Hydrolysis of Chitin	+	+	+	+	+	+
Hydrolysis of Urea	-	-	-	+	-	-
Utilization of citrate	+	+	+	+	+	+
Lipase	+	+	+	+	+	+
β-galactosidase	+	+	+	-	+	-
Arginine dihydrolase	+	-	+	+	+	-
Gas from nitrate	+	+	+	-	+	+
Acid from D-glucose	+	+	+	+	+	+
Acid from Mannitol	-	-	+	+	+	-
Acid from Inositol	+	-	+	+	+	+
Acid from L-arabinose	+	-	+	-	+	+
H_2S production	-	-	-	-	-	-

Table 3: Percentage analysis of chitinolytic Bacillus for different groups in Akola district

	Group I		Group II		Group III		Group IV		Group V		Group VI	
	No.of culture	Chit.	No.of culture	Chit.	No.of. culture	Chit.	No.of culture	Chit	No.of culture	Chit	No.of culture	Chit
	1	+	1	-	1	-	1	-	1	-	1	-
	2	-	2	-	2	-	2	-	2	-	2	-
	3	+	3	-	3	-	3	-	3	+	3	-
	4	-	4	+	4	-	4	+	4	-	4	-
	5	-	5	+	5	-	5	-	5	-	5	-
	6	-	6	+	6	-	6	+	6	-	6	-
	7	-	7	-	7	-	7	-	7	-	7	-
	8	-	8	-	8	+	8	+	8	-	8	-
	9	-	9	+	9	-	9	-	9	-	9	-
	10	-	10	-	10	-	10	-	10	-	10	-
No.of chitinolytic bacillus culture	2		4		1		3		1		0	
% of chitinolytic bacillus culture	20%		40%		10%		30%		10%		0%	

The above observation table 3 for Akola district shows that Group I having 20% chitinolytic bacillus, Group II having 40% chitinolytic bacillus, Group III having 10% chitinolytic bacillus, Group IV having 30% chitinolytic bacillus, Group V having 10% chitinolytic bacillus, Group VI having 0% chitinolytic bacillus. Observation table 4 for Aurangabad district showed that Group I having 30% chitinolytic bacillus then II having 40%, III 20%, IV 20%, V 30%, VI 10% respectively.

The above fig. 2 showed the percentage wise variation of number of chitinolytic bacillus for Aurangabad and Akola district with respect to the groups, in that group I, III and V of Aurangabad district having more percentage of chitinolytic Bacillus than group I, III and V of Akola district. Group II of Aurangabad district having equal percentage of chitinolytic *Bacillus* than group II of Akola district. Group IV of Aurangabad district having less percentage of chitinolytic *Bacillus* as compsre to IV of Akola district. Group VI of Aurangabad district having 10 percent. of chitinolytic Bacillus while group VI of Akola district is zero percent.

CONCLUSION

The finding of present study shows that out of six groups, four groups of Aurangabad district having more percentage of chitinolytic *bacillus* as compared to Akola district. One group having equal

and one group having less percentage that means overall percentage of occurrence of chitinolytic bacillus in Aurangabd soil is more as compared to Akola district with respect to observed six group.

Aurangabad soil is more suitable for isolation of chitinolytic bacillus for research and other purpose as compared to Akola district for studied group of bacillus.

Table 4- Percentage analysis of chitinolytic Bacillus for different groups in Aurangabad district

	Group I		**Group II**		**Group III**		**Group IV**		**Group V**		**Group VI**	
	No.of culture	Chit.	No.of culture	Chit.	No.of. culture	Chit.	No.of culture	Chit	No.of culture	Chit	No.of culture	Chit
	1	-	1	-	1	-	1	+	1	-	1	-
	2	-	2	+	2	+	2	-	2	-	2	-
	3	-	3	+	3	-	3	-	3	-	3	-
	4	-	4	-	4	-	4	-	4	-	4	-
	5	-	5	-	5	-	5	+	5	-	5	+
	6	+	6	-	6	-	6	-	6	+	6	-
	7	-	7	+	7	-	7	-	7	-	7	-
	8	+	8	+	8	-	8	-	8	+	8	-
	9	-	9	-	9	-	9	-	9	-	9	-
	10	+	10	-	10	+	10	-	10	+	10	-
No.of chitinolytic bacillus culture		3		4		2		2		3		1
% of chitinolytic bacillus culture		30%		40%		20%		20%		30%		10%

Chit. = Chitanase enzyme

LITERATURE CITED

Balsubramanium N, Juliet G.A, Srikalaivani P and Lalithakumari D. 2003. Release and regeneration of protoplasts from the fungus *Trichothecium roseum*. *Canadian Journal of Microbiology*. **49**(4):263-268.

Chernin LS, De La Fuente L, Sobolev V, Haran S, Vorgias CE, Oppenheim AB and Chet I. 1997. Molecular cloning, structural analysis, and expression in Escherichia coli of a chitinase gene from Enterobacter agglomerans**.** *Applied Environmental Microbiology***. 63**(3): 834-839

Gooday GW. 1990. The ecology of chitin degradation. *Advances in Microbial Ecology*.**11**: 387**–** 430.

Gkargkas Konstantinos, Mamma Diomi, Nedev George, Topakas Evangelos, Christakopoulos Paul, Kekos Dimitris and Macris Basil J. 2004. Studies on N acetyl-ß- D- glucosaminidase produced by *Fusarium oxysporum* F3 grown in solid-state fermentation. *Process Biochemistry*. **39**(11): 1599-1605.

Haki GD, Rakshit SK. 2003. Developments in industrially important thermostable enzymes: a review. *Bioresour. Technol*. **89**:17-34.

Mathivanan N, Kabilan V and Murugesan K. 1998. Purification, characterization and anti- fungal activity from *Fusarium chlamydosporum*, a mycoparasite to groundnut rust, *Puccinia arachidis*. *Canadian Journal of Microbiology*.**44** (7): 646-651

Mizuno Katsushige, Kimura Osamu and Tachiki Takashi. 1997. Protoplast formation from *Schizophyllum commune* by a culture filtrate of *Bacillus circulans* KA-304 grown on a cell-wall preparation of *S. commune* as a carbon source. *Bioscience Biotechnology and Biochemistry*. **61**(5): 852-857.

Lindsay GJH, Gooday GW. 1985. Chitinolytic enzymes and the bacterial microflora in the digestive tract of cod, Gadus morhua. *J.Fish Biol*. **26**:255**–** 265

Pichyangkura Rath, Kudan Sanya, Kultiyawong Kamontip, Sukwattanasinitt Mongkol and Aiba Sei Ichi. 2002. Quantitative production of 2-acetamido-2-deoxy-D-glucose from crystalline chitin by bacterial chitinase. *Carbohydrate Research*. **337**(6): 557-559.

Vaidya RJ, Macmil SLA, Vyas PR, chhatpar HS. 2003. The novel method for isolating chitinolytic bacteria and its application in screening for hyperchitinase producing mutant of *Alcaligenes xylosoxydans*. *Lett. Appl. Microbiol*. **36**:129-13

Vaidya RJ, Shah IM, Vyas PR, Chhatpar HS.2001. Production of chitinase and its optimization from a novel isolate *Alcaligenes xylosoxydans*:potential in antifungal biocontrol world. *J.Microbiol.Biotechnol*.**17**:691-696

Vaidya RJ, Macmil SLA, Vyas P, Ghetiya LV, Thakor KJ, Chhatpar HS. 2003. Biological control of Fusarium wilt of pigeonpea Cajanus cajan(L) Millsp with chitinolytic *Alcaligenes Xylosoxydans*. *Indian J Exp.Biol*. **41**:1469-1472

Vaidya RJ, Vyas P, Chhatpar HS. 2003.Statistical optimization of medium components for the production of chitinase by *Alcaligenes Xylosoxydans*. *Enzyme Microb. Technol*. **33**:92-96

Vyas PR and Deshpande MV. 1991. Enzymatic hydrolysis of chitin by *Myrothecium verrucaria*chitinase complexand its utilization to produce SCP. *Journal of Generaland AppliedMicrobiology*.**37**(3):267-275.