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Isolation and Characterization of Multiple Enzyme Producer *Bacillus* Species from Saline Belt of Purna River

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Abstract: Isolation and screening of *Bacillus* species were carried out from soil samples of saline belt of Purna River for multiple extracellular enzymatic activity. A total of 60 soil samples were analysed from Purna river basin (30 each from Akola and Buldhana district) for protease, amylase and lipase production. Soil samples were cultured on skim milk agar (1%), Starch agar (1%) and Egg Yolk agar (1%) respectively at 37^0C for 24 and 48h. The highly potential isolates for protease, amylase and lipase were 11, 9, and 7 respectively. Out of these 27 isolates, 9 (33%) produced all 3 enzymes, as multiple extra cellular enzyme producers. While 7 (27%) isolates able to produce protease and amylase and 4 only protease and lipase. The above study revealed new and interesting perspectives of bacterial strains isolated from saline belt of Purna River as a source of proteases, amylase and lipase that can be exploited potentially for various industries for enzyme production.

Key words: Protease, Amylase, Lipase, *Bacillus* species, Purna river

INTRODUCTION

The enzymes are essential proteins for the metabolic system of all living organisms and have an important role in the degradation of organic matter, host infection and food spoilage. They may be isolated from animals, plants and microorganisms. The microorganisms are considered good sources of industrial enzymes with great diversity. The Proteases, amylases and lipases enzymes are used in large scale in the textile, detergents, food, paper and leather industries. These extracellular enzymes may be produced in liquid or solid media[10,3].

Among the various proteases, bacterial proteases are most efficient, compared with animal and fungal proteases, and *Bacillus* species are specific producers of extra cellular proteases, a most important industrial enzyme, accounting for nearly 60% of total worldwide enzyme market[2]. Proteases have wide applications in pharmaceutical, leather, detergent, food, waste management, dairy, baking, brewing, protein hydrolysis, diagnostic and Silver recovery[1,6]. Amylase hydrolyzes starch and produces dextrins and progressively smaller polymers composed of glucose units, constitutes approximately 25% of the enzyme market covering many industrial processes such as sugar, textile, paper, brewing and baking, distilling industries, preparation of digestive aids and production of cakes, fruit juices, starch syrups and pharmaceuticals[7]. Lipases find a number of potential applications in detergent, paper manufacturing, organic chemical processing, biosurfactant synthesis, nutrition, cosmetics, pharmaceuticals and agrochemical industry[11]. Hence attempt was made to isolate protease, amylase and lipase producing *Bacillus* species as multi-enzyme producer from salinity affected soil of Purna River basin of Vidarbha region of Maharashtra state.

MATERIALS AND METHODS

Isolation: A total of 60 soil samples (30 each from Akola and Buldhana district) were collected from 60 different villages situated in saline belt of Purna River. A 1g of each soil samples were dissolved in 10 mL sterile distilled water, and mixed thoroughly. The supernant of these suspensions was used for isolation of *Bacillus* species which can produce protease, amylase and lipase by plating on skim milk agar, starch agar and egg yolk agar respectively at 37°C for 24h for amylase and 48h for protease and lipase producing bacteria. Enzyme production was identified by clear zone around colonies of protease, amylase (after addition of iodine) and lipase producing bacteria. Isolated predominant, morphologically distinct colonies were selected by replica plate technique and all isolates were identified on the basis of cultural, morphological and biochemical characteristics.

Screening for alkaline Proteolytic Amylolytic and Lipolytic activity: The efficient bacterial isolates were

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re-subcultured onto alkaline skim milk agar, starch agar and egg yolk agar plates for detection of their enzyme production efficacies. Productions of these enzymes were studies at various pH (8, 9, and 10). A clear zone of hydrolysis on casein, starch (after addition of iodine) and lipid gave an indication of protease, amylase and lipase producing bacteria. The efficiencies of enzyme production were studies on the basis of diameter of zone of hydrolysis.

RESULTS AND DISCUSSION

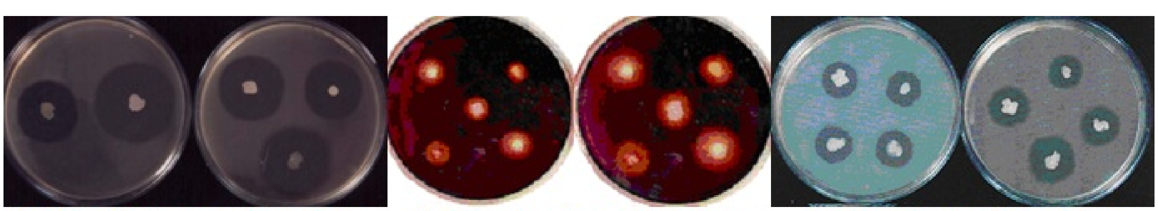
The sole protease producer was P_{32}, P_{35} and P_{43}; amylolytic producer A $_{40}$ and A_{55} and lipolytic producer was L_{46}. Enzymes from *Bacillus* are very efficient in breaking down a large variety of carbohydrate, lipids and proteins into smaller units. *Bacillus* species grow efficiently with very low cost carbon and nitrogen sources[9].

These 27 isolates of *Bacillus* species produced enzymes over the large range of pH investigated (7 to 10). However the maximum production observed at pH 7, 8 and 9. At pH 10, the isolates P_{24} and P_{45} showed the maximum zone of casein digestion. Isolates were inoculated at different pH that showed different activities. At pH 7 and 8, zone of starch hydrolysis was more than pH 9 and 10. At pH 10 only 4 isolates showed zone of starch hydrolysis. In lipolytic activity, at pH 7 and 8 isolates showed maximum activity while at pH 9 the zone of lipid hydrolysis decrease. At pH 10, out of 7 isolates only 4 isolates were able to show lipolytic activity (Table 2).

Maria *et al.,*[5]. recorded several enzymatic activities with predominance of polygalacturonase (96%), followed by amylase (84%), protease (82%) and lipase (66%) in their studies.

Table 1: Multienzyme producer *acillus* species isolated from Purna River Basin

District	Village	Taluka	Enzyme Isolates	Protease	Amylase	Lipase	Probable Bacillus species
Akola district							
	Dudhala	Akola	L_{18}	+++	+++	+++	B.subtilis
	Ghusarwadi	Akola	P_{24}	+++	+++	+++	*B.subtilis*
	Ghusarwadi	Akola	L_{24}	+++	++	+++	*B.subtilis*
	Kasali	Akola	P_{27}	+++	++	+++	*B.subtilis*
	Mandala	Akola	L_{17}	+++	++	+++	*B.subtilis*
	H.tamaswadi	Akola	P_{21}	+++		+++	B.subtilis
	Khobarkhed	Akola	P_{29}	+++		+++	*B.subtilis*
	Khambora	Akola	A_{20}	++		+++	*B.megaterium*
	Nimbhora	Akola	A_{9}	++	+++		*B.polymyxa*
	Sangwi	Akola	A_{5}	++	+++		*B.polymyxa*
	Aagar	Akola	A_{11}		++	++	*B.mycoides*
Buldhana district							
	Dudhalgaon	Malkapur	L_{31}	+++	+++	+++	*B.subtilis*
	Gigaon	Nandura	P_{45}	+++	+++	+++	*B.subtilis*
	Koderkhed	Nandura	L_{47}	+++	+++	+++	B.subtilis
	Taklivatfal	Nandura	P_{44}	+++	++	++	*B.subtilis*
	Hingna balapur	J.Jamod	A_{54}	+++	+++		*B.polymyxa*
	Mandwa	J.Jamod	A_{59}	+++	+++		*B.polymyxa*
	Dolarkhed	Nandura	A_{52}	+++	+++		*B.polymyxa*
	Koderkhed	Nandura	P_{47}	+++	+++		*B.cereus*
	Hingna nagapur	Malkapur	L_{35}	+++		+++	B.subtilis
	Dudhalgaon	Malkapur	P_{31}	+++		++	*B.subtilis*
	Hingna gavan	Nandura	P_{43}	++			*B.brevis*
	Hingna nagapur	Malkapur	P_{35}	++			*B.brevis*
	Waghoda	Malkapur	P_{32}	++			*B.brevis*
	Pimpri Koli	Nandura	A_{40}		+++		*B.brevis*
	Dudhalgaon	J.Jamod	A_{55}		++		*B.cereus*
	Mamulwadi	Nandura	L_{46}		+++		*B.subtilis*



Activities of Proteases on Skim milk Agar　Activities of amylase on Starch Agar　Activities of lipase on Egg yolk Agar

Photo plate 1: Enzymatic activities of isolated Bacilli spp.

Table 2: Effect of pH on enzyme activities (Zone of hydrolysis in mm).

	Sources	Code	pH 7	pH 8	pH9	pH 10
Effect of proteases on Milk agar	Dudhalgaon	P_{31}	38	27	20	16
	Gigaon	P_{45}	42	31	26	21
	Gusarwadi	P_{24}	43	32	26	21
	Hingna Gavhan	P_{43}	39	27	20	18
	Hingna Nagapur	P_{35}	41	27	21	19
	H. Tamaswadi	P_{21}	38	26	21	16
	Kasali	P_{27}	42	31	25	20
	Khoberkhed	P_{29}	41	30	25	20
	Koberkhed	P_{47}	39	25	23	20
	Takali Watfal	P_{44}	40	30	25	20
	Waghoda	P_{32}	39	28	25	20
Effect of amylase on Starch agar	Aagar	A_{11}	27	16	15	-
	Dadulgaon	A_{55}	29	16	20	-
	Dolarkhed	A_{52}	30	20	16	15
	Hingna balapur	A_{54}	28	16	16	18
	Khambhora	A_{20}	27	17	17	-
	Nimbhora	A_{9}	28	19	16	-
	Pimpri koli	A_{40}	28	18	15	15
	Sangawi	A_{5}	28	17	15	-
Effect of lipase on egg yolk agar	Dudhala	L_{18}	24	20	16	10
	Dudhalgaon	L_{31}	21	19	16	11
	Gusarwadi	L_{24}	21	18	15	-
	Hingna Nagapur	L_{35}	22	20	17	12
	Koderkhed	L_{47}	23	20	17	11
	Mamulwadi	L_{46}	22	18	15	-
	Mandala	L_{17}	21	19	15	-

In present study, 27 *Bacillus* strains, isolated from soil of Saline belt of Purna River showed multiple enzyme activity by producing protease (41%), amylase (33%) and lipase (26%). All isolates were identified as *Bacillus* species (Photo plate 1). All 3 enzymes were produced by 9 isolates and showed multienzyme production capacity. Shah *et al.,*[8]. worked on amylase, protease and lipase enzyme and showed out of 25 isolates, 8 were able to produce all 3 enzymes. Limpon and Kalila[4]. isolated 24 strains of *Bacillus* and reported, 21, 19, 15 and 9 isolates exhibited protease, amylase, lipase and cellulase activities respectively. Similar findings were recorded in present study where total 27 isolates, assayed on skim milk agar, starch agar and egg yolk agar respectively and 11, 9 and 7 isolates exhibited protease, amylase and lipase activity. The above study clearly revealed new and interesting perspectives showing that bacterial strains isolated from Saline belt of Purna river represents a source of proteases, amylase and lipase that can be exploited potentially for various industries for enzyme production, mainly in detergent industry.

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