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Extracellular enzyme production by environmental strains of *Serratia* spp. isolated from river Narmada

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Serratia a gram-negative enteric bacterium is generally recovered from clinical samples as an opportunistic human pathogen and rarely from water and soil. The extracellular enzymes produced by pathogen add to its virulence. In the present study, the extracellular enzymes secretion by 26 environmental strains of *Serratia* spp., isolated from different stations of river Narmada was investigated. Majority of isolates were capable of producing extracellular enzymes i.e., amylase, protease, lipase and chitinase, suggesting that they can be exploited as biocontrol and biodegrading agents. All the isolates, except *S. fonticola* were found to be potent protease producers, while only five isolates of *S. marcescens* produced chitinase.

Keywords: *Serratia*, extracellular enzymes, protease, chitinase, lipase, amylase.

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Serratia is an opportunistic gram-negative bacterium, belonging to the tribe *Klebsiellae* and the large family Enterobacteriaceae. It occurs naturally in soil, water, as well as in the intestine[1]. It is a potent producer of industrially important enzymes, and secretes several extracellular proteins, including chitinase, a lecithinase, a hemolysin, siderophores, lipase, proteases and a nuclease[2,3]. Except hemolysin, all are hydrolytic in nature and may contribute directly to cellular toxicity[4]. *Serratia* is also a potential insect pathogen and chitinase secreted by it plays an important role in its virulence, together with protease and lecithinase[5].

The characterization of extracellular microbial enzymes is important for understanding their role in the pathogenesis of infectious diseases, as they play a major role in causing cytotoxicity in mammalian cells, as well as to improve their application in biotechnology[4]. Although, the production of extracellular enzymes has been reported by *Serratia* spp. from clinical isolates[2,3,6], little or no work has been done on the enzymatic profile of the environmental isolates. In the present study, the twenty-six environmental isolates of *Serratia* spp., obtained from the water samples of eleven stations of river Narmada from its origin (Amarkantak, M.P.) to end (Bharuch, Gujarat) were screened for the production of extracellular enzymes amylase, protease, lipase and chitinase in culture media.

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Materials and Methods

Bacterial isolates

Bacterial isolates of *Serratia* spp. were collected from eleven different stations of river Narmada (viz., Amarkantak, Dindori, Mandla, Narsinghpur, Jabalpur, Hoshangabad, Omkareshwar, Koral, Bharuch, Ankaleshwar and Dahej). Water samples were brought to the laboratory under ice-cold conditions and bacteria were isolated on nutrient agar medium using standard pour plate dilution agar technique[7]. Morphologically distinct colonies were pure cultured on nutrient agar slants and were presumptively identified up to species level, on the basis of biochemical characteristics as described[8], and probabilistic identification of bacteria computer kit (PIB)[9]. They were further confirmed by molecular characterization using randomly amplified polymorphic DNA (RAPD), amplified ribosomal DNA restriction analysis (ARDRA), ribosomal intergenic spacer region analysis (RISA), enterobacterial repetitive intergenic consensus sequence (ERIC-PCR), flagellin genotyping and polymorphic GC rich repetitive sequence analysis (PGRS) typing methods[10]. Isolates were given BGCC (Bacterial germplasm collection centre) numbers and maintained in Bacteriology Lab, Dept. of Biosciences, R.D. University, Jabalpur.

Extracellular enzymes production and estimation

Amylase

Starch-enriched agar medium (1% starch, *p*H 7.2) plates were inoculated and incubated at 35±1°C for 24 hr. Lugol's iodine solution [Lugol's iodine:distilled water (1:5)] was flooded over the plates. A zone of clearance around the colony indicated production of amylase[11]. α- and β-Amylase activities were

assayed[11,12] as described. One unit of α-amylase is defined as the amount of enzyme, which caused a decrease of optical density by 0.05 in starch iodine color under assay condition. β-Amylase unit was defined as the amount of enzyme producing 1 micromole of maltose per min.

Protease

Isolates were streaked on gelatin-enriched nutrient agar medium (gelatin 4%, *p*H 7.2). After 24 hr of incubation at 37±1°C, the plates were flooded with 10% mercuric chloride reagent. A zone of clearance around the colony indicated production of proteases[12]. Protease activity was measured by casein digestion method[16], modified by Marrink and Gruber[14]. The absorbance of TCA soluble peptides was measured at 280 nm in UV-vis spectrophotometer (Systronics 118) against a control prepared by adding TCA prior to casein solution used as substrate. One enzyme unit was defined as 0.02 increase in absorbance.

Lipase

Tributyrin (1%) containing agar medium (*p*H 7.5) was point inoculated and incubated at 37±1°C for 18-24 hr. A zone of clearing around the growth area indicated the production of lipase[13]. Lipase activity was assayed as described[15]. Consumption of 0.1 ml of 0.05 N NaOH to neutralize acid liberated by the enzyme was taken as one enzyme unit.

Chitinase

Chitinase activity was determined by preparing 0.2% colloidal chitin by acid hydrolysis. Colloidal chitin was prepared by dissolving 0.1% chitin in 10 ml of conc. H_2SO_4, which was then diluted 15 times with distilled water. Thereafter, it was centrifuged at 10,000 rpm for 15 min, supernatant was discarded and the pellet was washed twice with distilled water. Pellet was collected and 0.2% of this colloidal chitin was added to chitin hydrolysis agar base medium (*p*H 7.0). Media was autoclaved at 121°C at 15 lbs for 20 min and poured in sterilized petriplates. Heavy growth from an 18-24 hr old pure culture was point inoculated and incubated at 37±1°C for 36-72 hr. Halo zone around the colony indicated a positive test[13].

Chitinase produced by test strains was assayed as described[16] and modified[17]. The method is based on the estimation of substrate degradation by enzyme and involves the measurement of the turbidity variation of a colloidal chitin suspension during chitinolysis. One chitinase unit is equal to the amount of enzyme mediating 1% decrease in absorbance of reaction mixture.

Statistical analysis

Standard deviation (SD), Standard error (SE) and coefficient of variation (CV) were calculated as described[18].

Results and Discussion

Twenty-six strains of *Serratia* spp. were screened qualitatively and quantitatively for the production of extracellular enzymes (Table 1). Ten strains of *S. marcescens* (BGCC 1-3, 11-13, 20-22 and 25), 4 strains of *S. marinorubra* (BGCC 15, 17, 23 and 24), 5 strains of *S. plymuthica* (BGCC 5, 6, 10, 18 and 19), 4 strains of *S. rubidaea* (BGCC 9, 14, 16 and 26), 2 strains of *S. odorifera* (BGCC 7 and 8) and 1 of *S. fonticola* (BGCC 4) were used in the study. Eighteen isolates were found positive for amylase, 25 for protease, 21 for lipase and 5 for chitinase production. The data for the enzymatic production has shown considerable standard deviation (mean ±S.D) and coefficient of variance (CV) values. However, for *S. marinorubra* and *S. rubidaea*, the values of enzymes produced appear to be consistent, due to smaller values of CV (Table 1).

Amylases are industrially and ecologically valuable[19,20] enzymes. Seven strains of *S. marcescens*, all the strains of *S. marinorubra*, four strains of *S. plymuthica*, and three strains of *S. rubidaea* were found to be amylase producers. *S. marcescens* (BGCC 25) produced maximum α-amylase (205 EU/ml) and *S. plymuthica* (BGCC 5) the lowest (65 EU/ml). *S. marcescens* (BGCC 21) produced maximum β-amylase (62 EU/ml) and *S. plymuthica* (BGCC 5) the lowest (15 EU/ml).

Protease production was exhibited by all the strains, except *S. fonticola*. The maximum protease (110 EU/ml) was produced by *S. marcescens* (BGCC 12 and 21) and *S. rubidaea* (BGCC 26), while *S. marcescens* (BGCC 2) produced the lowest (16 EU/ml). Isolates were recovered from different stations of river Narmada, which are either surrounded by dense human population or by many textile industries and gelatin factory that might be responsible for the existence of the proteolytic bacteria in river water. Similar observations were made earlier[21-23], where the presence of *Bacillus* spp.,

Table 1—Enzymatic spectrum of isolates of *Serratia* spp. obtained from different stations of river Narmada

[Enzyme activities are given in EU/ml]

Name of Isolate	Strain No.	Amylase		Protease	Lipase	Chitinase
		α	β			
S. marcescens	BGCC1	-	-	106	359	210
	BGCC2	-	-	16	-	180
	BGCC3	-	-	100	490	160
	BGCC11	135	45	96	-	-
	BGCC12	180	50	110	285	-
	BGCC13	165	48	100	-	118
	BGCC20	195	60	77	380	-
	BGCC21	178	62	110	465	-
	BGCC22	182	45	65	364	-
	BGCC25	205	44	36	372	215
	SD	20.71	7.435	23.66	69.14	39.72
	CV	11.19	14.7	26.4	17.83	22.49
	SE	7.83	2.81	7.49	26.13	17.76
S. marinorubra	BGCC15	80	22	90	350	-
	BGCC17	95	36	90	175	-
	BGCC23	80	36	23	180	-
	BGCC24	85	40	35	160	-
	SD	7.07	7.895	35.56	89.57	-
	CV	8.317	23.56	59.76	41.42	-
	SE	3.535	3.947	17.78	44.78	-
S. plymuthica	BGCC 5	65	15	32	268	-
	BGCC6	68	17	36	360	-
	BGCC10	-	-	28	254	-
	BGCC18	78	25	27	175	-
	BGCC19	86	35	30	179	-
	SD	9.60	9.09	3.578	75.94	-
	CV	12.93	39.52	11.69	30.72	-
	SE	4.8	4.54	1.597	33.90	-
S. rubidaea	BGCC9	-	-	41	469	-
	BGCC14	105	40	26	354	-
	BGCC16	110	30	18	164	-
	BGCC26	110	35	110	282	-
	SD	2.886	5.0	41.93	127.9	-
	CV	7.529	14.28	86.01	40.32	-
	SE	1.67	2.89	20.96	63.95	-
S. odorifera	BGCC7	-		66	-	-
	BGCC8	-	-	27	255	-
	SD	-	-	27.5	-	-
	CV	-	-	59.2	-	-
	SE	-	-	19.5	-	-
S. fonticola	BGCC4	-	-	-	-	-

SD, Standard deviation; CV, coefficient of variation; SE, standard error; -, represents absence of enzyme.

Micrococcus spp., *Serratia* spp. and *Vibrio* spp. was reported in the effluents of gelatin factory. The detection of protease activity in the water and wastewater could be useful in determining the level of proteinaceous matter[20]. Earlier, extracellular protease production by *Serratia* spp. was found to be responsible for the exportation of foreign proteins across the outer membrane[24].

Lipase activity was shown by seven strains of *S. marcescens* and all other strains, except one strain of *S. odorifera* (BGCC 7) and *S. fonticola* (BGCC 4). *S. marcescens* (BGCC 3) produced the maximum lipase (490 EU/ml), while *S. marinorubra* (BGCC 24) the minimum (160 EU/ml). The occurrence of lipolytic bacteria at different stations indicates the mixing of sewage or wastewater in the river. The oil-degrading property of these bacteria could be useful in sewage treatment for decomposing lipids and biodegradation of wastes and hydrocarbons.

Only five strains of *S. marcescens* (BGCC 1, 2, 3, 13 and 25), out of twenty-six isolates showed chitinase production. BGCC 25 produced the maximum chitinase (215 EU/ml), while BGCC 13 the minimum (118 EU/ml). Earlier reports showed that the bacterial species like *Pseudomonas*, *Serratia*, *Bacillus* and *Arthrobacter* play an important role in biological control of fungal pathogens due to chitinase production[25,26,27], but only the chitinase machinery of *Serratia* is best-characterized[6]. Although chitinase production has also been reported in *S. plymuthica* and *S. ficaria*, but *S. marcescens* is a well-known chitinase producer and one of the most effective bacteria for the degradation of chitin, the second most abundant polymer on earth[28]. This property of *Serratia* spp. may be exploited for the control of fungal and insect pathogens[28,29]. Chitinase also plays a significant role in the virulence of this bacterium together with protease[29].

The present study is an effort to physiologically characterize different *Serratia* spp. on the basis of extracellular enzyme production. *Serratia* spp. isolated from the river Narmada are potential producers of hydrolases, which could serve as major virulence factors for aquatic insects and other fauna.

Amylase activity by environmental isolates of *Serratia* is not reported earlier, therefore its detection in the water (also waste water) could be useful in determining the level of carbohydrates, which could be useful in sewage and industrial effluent treatment[20]. Also, the five isolates of *S. marcescens* which were found to be potential chitinase producer could be exploited to inhibit chitinase-dependent pathogens, such as *Plasmodium falciparum*[30] and *Leishmania*[31]. Interestingly, the enzymes obtained from *S. marcescens* and *S. rubidaea* are promising, compared to earlier studies[2,3], indicating that *Serratia* spp. isolated from river water may be useful as an efficient biocontrol as well as biodegrading agent of wastes and hydrocarbons.

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