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Research Article

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Enzyme Activities of Bacterial Isolates from Iron Mine Areas of Barbil, Keonjhar District, Odisha, India

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ABSTRACT

Microbial population and microbial activity are considered to be indicative measures of soil quality. In the present investigation it was found that bacterial load ranged between1.04X10^4 to 7.8 X 10^5 CFUgm^{-1} soil and 4.7X 10^3 to 6X10^4 CFUgm^{-1}soil of unexplored and explored iron mining areas respectively. Whereas, phosphate solubilizing bacterial load was found to be very low, 3X10^2 to 5.9X10^3 CFUgm^{-1} of soil. In toto 110 bacteria were isolated and identified as Bacillus sp. 64(58.2%), 4(3.6%) were Pseudomonas sp., 2(1.81%) Sulphobaccilus sp., and one (0.9%) each from Ancyclobacter sp., Enterobacter sp., Acetobacter sp. and Geomicrococcus sp. It was found that 33(30%), and 39(33.5%) of the isolates showed amylase and protease activity respectively. But, 24(21.8%) of the isolates showed both amylase and protease activities. Only 4(3.63%) isolates showed phosphatase activity. Phosphate solubilising activities of microbes in association with starch/complex polysaccharides and protein degrading capabilities make them a suitable component to maintain soil fertility.

Keywords: *Mining soil, Phosphatase, Amylase, Protease.*

INTRODUCTION

Microorganisms contribute to nutrient availability in soil, manage soil fertility by means of different biochemical processes and they contribute to the growth and success of the plants and overall ecosystem of a soil environment. Bacteria are the most abundant microorganism in the soil, and serve many important purposes, such as nitrogen fixation, phosphate solubulization, sulphur oxidation and other biochemical processes [1,2]. The microbial population and diversity are varied from ecosystem to ecosystem due to availability and types of nutrients. Due to high mining activity of mining soil leads to the loss of soil biota as the mining activities are invariably associated with the removal of fertile top soil organic layer enriched with vegetational cover. Therefore, any changes in microbial biomass ultimately affect nutrient cycling of soil organic matter[3]. Enzyme activity is a soil property that is chemical in nature, but has a direct biological origin. Since soil enzyme activities are very sensitive to pollution, enzymes have been suggested as potential indicator or monitoring tools to assess soil quality and health [4]. The soil enzyme like amylases, protease and phosphatase could be a good indicator to assess soil quality, i) they are strongly linked with important soil properties such as organic matter, microbial activity or biomass, ii) they have the tendency to change soil properties, iii) they involve relatively simple methods as compared to other soil parameter assessment of soil quality[5,6]. One of the important activities of soil microbes is the solubilization of phosphate and make it available to plants. Phosphate solubilizing activities of microbes in association with hydrolysis of Starch/complex polysaccharides, proteins make them a suitable candidate for maintenance of soil fertility. Hence, the aim of the present investigations is to isolate bacteria from mining soil samples and to screen for their enzymatic activities with wide range of tolerant capacity in adverse conditions in order to exploit their biotechnological potential both in industrial and agricultural sectors.

MATERIALS AND METHODS

Study area

The study was conducted in mining areas of Barbil, Keonjhar District of Odisha, India, situated at 22.12°N and 85.40°E. The area is located at an average elevation of 477 m from the sea level. Barbil is an industrialized area with mining activities throughout the year. The site is very rich in iron and manganese ores.

Collection of samples and physico-chemical analysis of soil

A total of 10 soil samples were collected from different sites of Barbil from explored and unexplored mining area (Forest area) following the method adopted by Baruah and Barthakur[7]. Physico-chemical analysis of the soil samples were studied for soil moisture, pH, temperature, salinity, phosphorous, potassium and organic carbon contents. Analysis was carried out in Soil Chemistry laboratory, Baripada, Odisha by standard soil analysis method[8].

Isolation and identification of isolates

Bacteria were isolated by serial dilution technique using spread plate method on both Nutrient Agar and Pikovskaya's Agar medium. Identification and characterization of the isolates were made on the basis of colony characteristics, individual morphology by gram staining and through an array of biochemical reactions[9,10].

Test for enzymatic activities

Phosphatase activity

Phosphatase activity of the isolates was tested on Pikovskaya's agar (PA) containing tricalcium phosphate as insoluble phosphate source. Pikovskaya's agar plates were prepared as per manufacturers (Hi-Media Pvt. Ltd. Mumbai, India) instructions. Freshly grown cultures of bacteria were spot inoculated on PA plates by the help of a sterile loop. Inoculated plates were incubated at 37^0C for 24h to 72h. Formation of a halo zone around the colonies is indicative of positive phosphatase activity. Solubilizing index (SI) of the isolates was determined by using the formula[11,12] as follows:

$$SI = \frac{\text{Halo zone + Colony diameter}}{\text{Colony diameter}}$$

Amylase activity (starch hydrolysis test)

Amylase activity of the isolates was initially tested in Starch Nutrient Agar plates for bacteria following the method Booth[13]. Freshly grown cultures of the isolates (on NA slants) were transferred onto Starch Nutrient Agar plates by the help of a inoculation loop. After 24 hrs at 37^0C of incubation for bacteria the plates were exposed to iodine vapour. Entire plate turned blue and a clear zone around the colony indicated positive amylase activity and/or starch hydrolysis by isolates. Enzymatic index was calculated using formula as follows:

$$\text{Enzymatic Index} = \frac{\text{Zone around the colony + Colony diameter}}{\text{Colony diameter}}$$

Protease activity (protein hydrolysis)

To test proteolytic activity the organisms were grown on Skimmed Milk Agar Plates. Skimmed milk (Skimmed milk 1%, Dextrose 2% and Agar 1.5%) plates were prepared and the isolates were spot inoculated by the help of a inoculation loop taking from freshly grown colonies of bacteria on NA plates. The plates were incubated at 24 hrs at 37^0C. The plates were observed after incubation period, a clear zone around the colony indicates positive protease activity. Enzymatic index was calculated by using formula as described earlier[13].

RESULTS AND DISCUSSION

Physicochemical properties of the Soil

Table 1: Physico chemical analysis of soil collected from different sites of Barbil mining area

Collection site	Moisture conent %	Temperature	Organic Carbon %	Acidity	Salinity(N)	P(Kg/hector)	K(Kg /hector)
Site -1	5.8	42	0.54	5.6	0.5	3	50
Site- 2	6.3	44	0.51	6	0.5	3.4	56
Site-3	8.1	39	0.16	6.4	0.5	2.6	145
Site – 4	8.6	40	0.24	6.2	0.5	2.4	56
Site – 5	5.6	41	0.14	6.2	0.5	3.2	59
Site -6*	9.3	38	0.97	5.5	0.5	4.4	210
Site-7*	13.4	37	0.81	6.1	0.5	3.8	203
Site -8*	8.7	38	0.73	5.8	0.5	4	280
Site- 9*	10.6	36	1.18	5	0.5	3.4	351
Site-10*	10.3	34	0.86	6.3	0.5	3.2	263

* Unexplored mining area

Altogether ten soil samples were collected from different sites of mining area of Barbil. Out of ten soil samples, five samples (1-5) were collected from explored mining areas and other five samples (6-10) were collected from unexplored mining area during April- May,2013 ((Table -1). The soil samples were studied for different physico-chemical properties. The results indicated that moisture content was found to be highest in the soil samples collected from unexplored area. Highest percentage of moisture (13.4%) was recorded from site-7. The moisture content of explored mining soil was comparatively lower and it varied from 5.8 to 8.6. Soil temperature of the studied area was found to be varied from 34 – 44^0C. The percentage of organic carbon was varied from 0.14 – 1.18 among the sites. Highest organic carbon percentage (1.18) was observed in site -9 of unexplored area, whereas, site-5 of explored area showed lowest organic C percentage. Salinity of the soil samples collected was found to be even. In general, all the soil samples were found to be acidic in nature. Phosphorus content of the soil was observed to be very less, varied from 2.4 to 4.4 Kg/h. In contrast potassium content was found to be highest in unexplored area (351 Kg/h), lowest (50 Kg/h) in site -1 of the explored area.

Bacterial population

Isolation and enumeration of bacteria were carried out by spread plate and pour plate methods on both NA and PA medium. The phosphate solubulization bacterial loads were studied on Pikovaskay's medium. The bacterial loads were found to be more in the soil sample collected from unexplored area in comparison to mining activity area. But interestingly, the phosphate solubilizing bacterial population was less in comparison to total bacterial loads on NA plate, in both explored and unexplored area and it was found to be $3X10^2$ to $5.9X10^3$. The highest bacterial load was found to be 7.8 X 10^5 $CFUgm^{-1}$ of soil in site-10 of unexplored area and $9.3X10^4$ CFU/gm in explored area (Table 2). It was observed in our study that, the bacterial load drastically fall down in case of explored area in comparison to unexplored area.

The microbial population in the soil are regulated by different biotic and abiotic factors. The bacterial flora is always more in the soil containing highest organic and moisture content was reported by different researcher[14],[15]. In corroboration to this, during this investigation we observed more microbial load in soil samples collected from unexplored area (forest area) in comparison to explored areas with human interferences, it could be attributable to presence of less organic carbon and moisture content in explored sites. Observations of lowest bacterial population was found in the iron mining soil during the investigation may be due to the contamination of soil with heavy metals, that can have a detrimental effect on microbial activity and function, decreases soil respiration and microbial biomass [16,17]. Since the presence or abundance of an organism may be limited by a variety of environmental factors, biotic as well as abiotic, and since the limiting effect may be due to two or more interacting factors rather than a single isolated one. Therefore, in the mining areas all limiting factors like high percentage of heavy metals, low organic carbon percentage, high temperature, low moisture content, that drives the physical and physiological change of adopted microbes to survive in that area and became dominants. Among the

isolates *Bacillus* sp were observed to be the dominant species, in these mining soils. This is mainly due to their wide distribution, sporulating nature, tolerance capacity of the genus to adverse conditions

Table 2: Bacterial load of explored and unexplored area of Barbil, Keonjhar

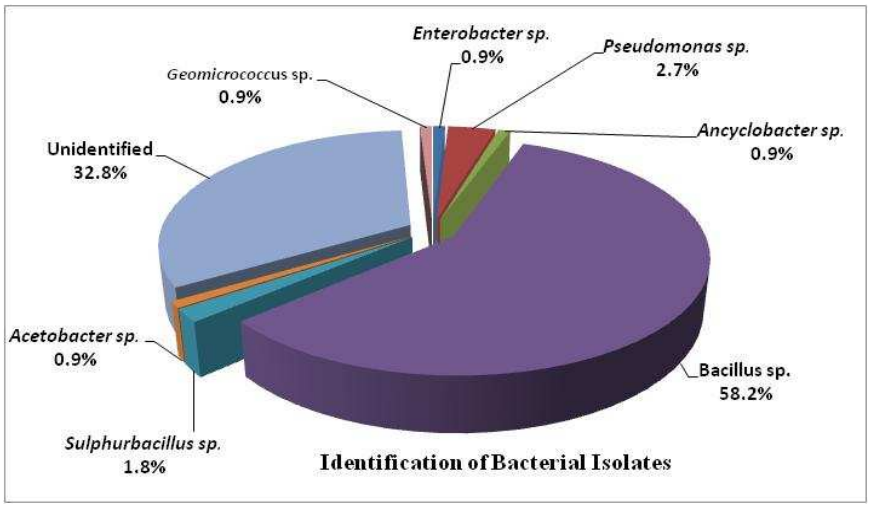
Sample No.	NA		PA	
	Pour Plate	Spread Plate	Pour Plate	Spread Plate
Site-1	4.3 $X10^3$	1.8 $X10^4$	3.2$X10^2$	3.4$X10^3$
Site-2	3.1 $X10^4$	8.9 $X10^3$	3.3 $X10^2$	7.2 $X10^3$
Site-3	4.7 $X10^3$	4.6 $X10^4$	3 $X10^2$	3.7 $X10^2$
Site-4	6.0 $X10^4$	9.3 $X10^4$	3.5 X 10^3	4 X 10^3
Site-5	1.02 $X10^4$	3.8 $X10^4$	4.1 X 10^3	5.3 $X10^3$
Site-6*	1.04 $X10^4$	4.6 $X10^4$	3.8$X10^3$	3.1 $X10^3$
Site-7*	1 X 10^5	7.3 X 10^4	4 $X10^3$	3.8 $X10^3$
Site-8*	6 X 10^4	8.5 $X10^4$	3.5 $X10^3$	4 $X10^3$
Site-9*	1.28 X 10^5	1.18 $X10^5$	3.1 $X10^2$	5.9 $X10^3$
Site-10*	6.4 X 10^5	7.8 X 10^5	4.4 $X10^3$	3.4 $X10^3$

* Unexplored mining area

Identification of the isolates

In toto 110 bacteria were isolated from 10 soil samples studied and were identified on the basis of colony morphology and by a series of biochemical reaction and Gram's reaction. All the 110 bacterial isolates were primarily identified through Gram reaction and was observed that 100(90%) were Gram positive rods, 8(7.2%) and 2 (1.8%) were Gram negative Rod and Gram positive cocci respectively. By the help of series of biochemical and Gram's reaction the bacterial isolates were identified upto genus level. Out of 110 bacterial isolates, only 74 isolates could be identified and assigned to genera *Bacillus* sp. 64(58.2%), *Pseudomonas* sp. 4(3.6%), *Sulphobaccilus* sp. 2(1.81%), and one (0.9%) each from *Ancyclobacter* sp., *Enterobacter* sp., *Acetobacter* sp. and *Geomicrococcus* sp. However, 36 isolates remained unidentified.

Fig.1: Identification of bacterial isolates



Enzyme activities

Amylase and protease are two important industrial enzymes which solubilize starch and protein respectively. Therefore, it was investigated to find out amylase and protease activity of all 110 bacterial isolates. Among the isolates 33(30%) showed amylase activity, the highest efficiency was showed by the isolate DS-142 followed by DS-139, DS-141 and DS-114. 39(33.5%) of the isolate showed protease activity, the highest efficiency was observed with the isolate DS-127, followed by DS-105, DS-142 and DS-108 (Fig-2). 24(21.8%) of the isolates showed both amylase and protease activity. Whereas, only 4 (3.6%) isolates showed Phosphate solubilizing activity while studied on PA plates. The isolate DS -122 showed the highest phosphatase efficiency.

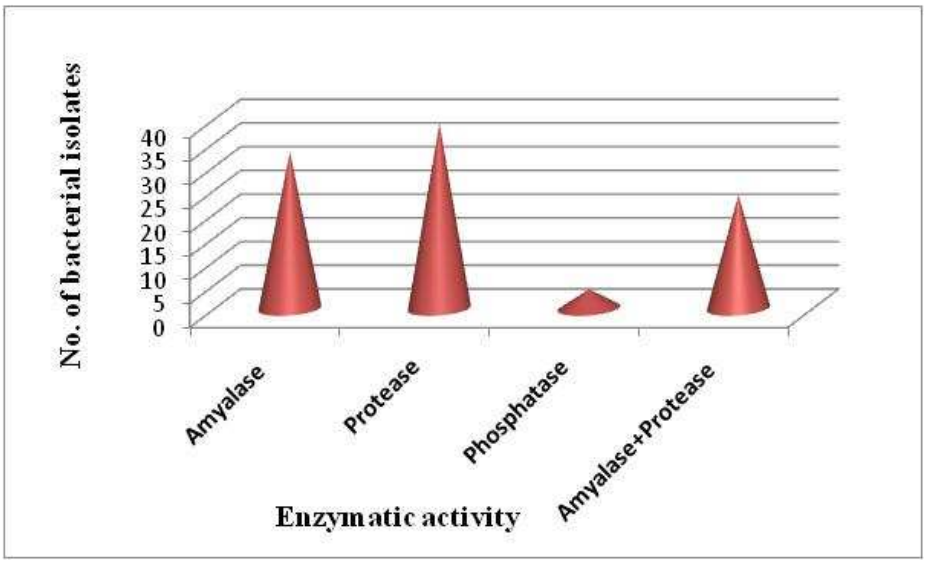
Microbial enzymes like amylase, protease and phosphatase are extracellular enzymes that play vital role in nutrient cycling of the soil[18]. However, soil microbial enzymes are considered to be indicative

measures of soil fertility and bioremediation activities[19]. In this investigation, it was found that mining soils harbour fewer microorganisms with less diversity. Therefore, microbial function in the mining soil could be attributable to low microbial diversity and confined to a specific group of microorganisms[20]. Soil of Barbil area of Keonjhar district contains high percentage of Iron. Presence of toxic metals like iron in soil affects the microbial load as well as their activity[21,22,23]. In agreement to this we also reported lower population of bacteria in iron containing soil of Barbil with less enzymatic activities

Table 3: Enzymatic activity of the isolates

S. No.	Lab no./ Isolate	Enzymatic Efficiency (SI)		
		Amylase	**Protease**	**Phosphatase**
1	DS -100	1.15	1.05	-
2	DS -105	1.42	2.3	-
3	DS -106	1.16	1.3	-
4	DS -107	0.20	1.05	-
5	DS -108	-	1.35	-
6	DS -109	-	1.5	-
7	DS -110	-	0.30	-
8	DS - 112	1.30	1.01	-
9	DS - 113	1.33	-	-
10	DS- 114	1.6	-	-
11	DS -115	-	1.31	-
12	DS 116	1.15	1.05	2.2
13	DS 117	0.20	1.15	-
14	DS -119	0.20	1.43	-
15	DS -120	0.7	-	-
16	DS -121	-	1.50	-
17	DS -122	1.06	1.15	2.4
18	DS-123	-	0.6	-
19	DS -124	1.4	0.5	-
20	DS - 125	-	1.3	-
21	DS -127	-	2.5	-
22	DS 128	-	1.4	-
23	DS -129	-	1.2	1.8
24	DS - 130	0.4	0.3	-
25	DS - 131	1.7	-	2.0
26	Ds1 - 132	1.01	1.08	-
27	DS-138	1.09	1.1	-
28	DS -139	1.80	-	-
29	DS -140	-	1.3	-
30	DS -141	1.75	0.2	-
31	DS-142	1.83	2.1	-
32	DS - 143	1.50	1.7	-
33	DS -146	1.50	-	-
34	DS -147	1.37	1.2	-
35	DS -148	-	0.6	-
36	DS-149	0.5	-	-
37	DS - 150	-	0.9	-
38	DS -151	1.31	1.22	-
40	DS -156	0.20	-	-
41	DS - 157	1.55	0.8	-
42	DS - 158	-	0.3	-
43	DS- 159	1.3	-	-
44	DS-160	0.7	0.9	-
45	DS - 180	-	0.6	-
46	DS -184	-	1.42	-
47	DS -187	0.2	1.8	-
48	DS -201	1.3	1.15	-
49	DS- 208	1.4	1.06	-
50	DS- 210	1.4	1.7	-

Fig.2: Enzymatic activities of bacterial isolates



CONCLUSION

In conclusion it can be told that though it is a preliminary endeavour, through this investigation we reported low bacterial population with enzymatic activities (amylase, protease and phosphatase) of soil samples collected from mine areas (both explored and unexplored sites). The exploration of microbial wealth of these ecological niches (in mine soils of Barbil & Keonjhar districts in Odisha) is of its kind. Therefore, studies such as this, is a prerequisite to explore their biotechnological implication in industries and agriculture.

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