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STUDY OF THE APPLICATION OF BACTERIA CULTURE TO CONTROL ENVIRONMENTAL POLLUTION DUE TO FLY-ASH PRODUCED BY THERMAL POWER PLANTS

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Abstract : Experimental studies have been carried out to find the effects of microbes isolated from cow dung on fly –ash produced by coal-fired power plants. For these studies, selected microbes have been isolated from cow dung collected from local dairy of Midnapore district, West Bengal , India and fly-ash sample has been collected from Bandel Thermal power plant, Bandel, Hooghly, India. Calcium solubilization efficiency of these microbes and action capacity of plant nutrients, like – Zn, Cu, Ni, Cr, V, etc. from fly-ash, have been determined. It has been found that new Pseudomonas sp. Strain is highly effective for desired results. Studies have also been carried out to find the effect due to the bacterization of seeds for germination and hence plant growth on fly-ash. Thus, instead of dumping fly-ash on the bare lands or in the ash pond, it can be utilized in cultivated lands or unused lands for plant growth using method of bacteriology to control environmental pollution with promising results.
Keywords : Fly-ash, environmental pollution, bacteria culture, microbes, plant growth

1. INTRODUCTION

Bacteriology and its applications have been given due importance for the last several years [1,2]. One of the applications of this method is to control environmental pollution control [3,4]. Indian coal consists of high percentage of ash. So, when this coal is burned in the thermal power plants, a huge amount of fly-ash is produced as residue which causes environmental pollution when dumped in bare lands or in ash ponds. Several attempt have been made to utilize this fly-ash. For examples : Production of i) ceramic bricks using some binders [5] ii) in-organic polymers [6] iii) nano-structure materials [7] and iv) utilization in cement and concrete production.

It has been reported that some bacteria can solubilize water insoluble minerals – such as, Tri-calcium phosphate [Ca3(PO4)2], Manganese di-oxide (MnO2), Calcium silicate (CaSiO3), etc. [8] present in the fly-ash .

In this paper, an attempt has been made to present the results of the experiments conducted to i) isolate the pseudomonas bacteria from cow dung ii) application of isolated bacteria in fly-ash to extract plant nutrients and iii) to make a comparative study of the growth of plants in treated fly–ash, non-treated fly-ash and non-treated soil.

2. MATERIALS AND METHODS

2.1 Collection of cow dung and fly-ash samples

For experiments, , both the fresh cow dung(FCD) and stored cow dung(SCD) samples were collected from the local dairy of Midnapore (coordinates- 22.60 W to 87.560 N), West Bengal, India in a sterile auto-cleave polythene bags. The fly ash sample was collected from Bandel Thermal Power plant (coordinates - 22.40990 N to 87.85250 E),Bandel, Hooghly, India.

2.2 Isolation and identification of bacteria

Initially, 1 gm of both FCD and SCD samples were dumped separately in 100 ml. King's B broth (KB) and incubated at 280 C in Luria Bertani (LB) broth and incubated at 280 C. After three days, those were screened and media were serially diluted up to 10-10 times using sterile distilled water. Each serially diluted inoculums was pour-plated with KB agar (1.5%) medium and incubated at 280 C for 24 hours. Distinct single colonies were collected and sub-cultured to purify on the same medium. The bacteria were identified by following the tests given in Bergey's Manual of Determinative Bacteriology [7]. The purified each isolate was stored in 20% glycerol at 40 C.

2.3 Screening of different water insoluble salt solubilizating bacteria

For screening of salt- solubilizing bacteria, isolates were grown on different salt-agar medium [composition (in gm /100ml) : dextrose -1.0; WIS -0.5; (NH4)2 SO4 - 0.5, KCl -0.02; MgSO4 , 7H2O - 0.1; yeast extract – 0.5; agar-2.0 and Ph -7.0], containing salt separately as Aluminium trioxide (Al2O3); Chromium trioxide (Cr2 O3), Ferric Oxide (Fe2 O3),

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Ferrous Carbonate ($FeCO_3$), Calcium Oxide (CaO); Manganese dioxide (MnO_2); Calcium silicate ($CaSiO_3$) and Calcium di-hydrogen phosphate [$Ca(H_2PO_4)$ at room temperature (280 C) for 7 days.

2.4 Seed Bacterization for Growth Promotion

The seeds of Cicer arietinum were selected for bacterization. Fly-ash sample collected from thermal power plant was dumped in sterilized culture tubes, 50 ml each. They were autoclaved at 15 ib/inch2 pressure for 15 min. and bacterized separately for 14 days. 200 ml of different bacterial inoculums, containing 3 x 108 cfu/ml, were separately centrifuged at 7000 rpm for 20 min. and supernatants were discarded. Bacterial pallets were washed thrice with sterile distilled water and finally suspended with carboxymethyl cellulose solution (1 mg CMC in 100 ml sterile distilled water). Then these bacterial solutions of different selected bacteria were added separately with the sterilized fly-ash samples kept in culture tubes. Surface- sterilized seeds of Cicer arietinum were kept separately on those treated fly-ash in culture tubes. Seeds also kept on non-treated fly-ash sample in a tube, taking it as control. A similar set was made where instead of fly-ash only soil was used and seeds were placed on it. After seven days incubation, plant growth promoting activity by selected microbes, were assed and seedling Vigour-Index (VI) for all the sets.

3. RESULTS

Data obtained, after doing some experiments as described in the previous section, were analyzed to get following results :

3.1 Preliminary Biological Characterization of the isolates

A total of 55 isolates were collected which were found to be Gram-negative, small-bacillus in shape as well as endospore negative. They gave circular colonies on KB –plates. Seven different bacterial species were selected on the basis of their phenotypic (Table 1) and biochemical characteristics. Two types of biochemical tests were conducted. First type has shown different types of acidic compounds production (Table 2). Second type has shown the different types of enzyme production (Table 3).

Table 1 : Morphological Characteristics of Bacteria

Tests/Assay name	E10-57	B10-11	P10-44	P10-76	P10-46	P10-7 1	P10-111
Gram-Staining	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Capsule-staining	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Endospore–straining	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Form of colony	Circular	Circular	Circular	Circular	Circular	Circular	Circular
Elevation of colony	Convex	Flat	Convex	Flat	Flat	Convex	Convex
Surface of Colony	Concentric	Smooth	Concentric	Smooth	Smooth	Smooth	Smooth
Cell shape bacillus	Small bacillus	Small bacillus	Small bacillus	Small bacillus	Small bacillus	Small bacillus	Small bacillus

Table 2: Results of Acidic compound production Tests

Tests	$B10^{-1}{}_1$	$P10^{-6}{}_4$	$P10^{-7}{}_6$	$P10^{-4}{}_6$	$P10^{-7}{}_1$	$P10^{-11}{}_1$	$E10^{-5}{}_7$
Methyl red test (Ph range: 4.2 -6.3)	-	+	+	+	+	+	-
Indope-3 acetic acid production	+	+	+	+	+	+	+
HCN production	+	-	+	+	+	+	+
H2S production	+	+	+	+	+	-	+

Table 3 : Results of Enzyme production Tests

Name of the enzyme	$B10^{-1}{}_1$	$P10^{-6}{}_4$	$P10^{-7}{}_6$	$P10^{-4}{}_6$	$P10^{-7}{}_1$	$P10^{-11}{}_1$	$E10^{-5}{}_7$
Alpha- Amylase	-	-	-	-	-	-	-
Catalase	+	+	+	+	+	+	+
Oxidase	-	+	+	+	+	+	+
Protease	-	+	-	+	+	+	+
Urease	+	+	+	+	+	+	+
Gilatinase	+	+	-	+	+	+	+

3.2 Evaluation of Salt Solubilizing activities

From the data of experimental results, it has been seen that isolates have shown two types of activities. All isolates have good growth on every salts containing plates but no one could produce halo-zones on aluminium trioxide (Al_2O_3), Chromium trioxide (Cr_2O_3), Ferric oxide (Fe_2O_3), Ferrous carbonate($FeCO_3$) calcium oxide (CaO). But other salt containing plates, different bacteria have shown growth characteristics and clear halo-zones with different solubilizing efficiency (SE) values. The experimental results are shown in Table 4.

Table 4: Efficiency (SE) values of different salts from seven bacteria

Isolates	$Ca(H_2PO_4)_2$	$CaSiO_3$	MnO_2
$B10^{-1}_{1}$	241.68	575.20	81.00
$E10^{-5}_{7}$	200.00	380.00	100.00
$P10^{-6}_{4}$	200.00	271.50	112.50
$P10^{-7}_{6}$	153.80	312.80	128.60
$P510^{-4}_{6}$	260.00	600.00	125.50
$P510^{-7}_{1}$	200.00	320.00	100.00
$P510^{-11}_{1}$	250.00	380.00	120.05

3.3 Response to Seed germination and Plant growth

In all cases, two seeds per each have been germinated (germination energy 100%) and sprouts have given highest average growth, in each case. Among seven isolates, P10-76 has been seen to be best among the seven isolates. Mean vigor index (VI) values and percent disease incidence (PDI) values of sprouts after a week influenced by different bacteria have been noted (as shown in Table 5). The Pictures 1 (A) and (B) show the comparative growth of different species.

Table 5 : Seed germination and Plant growth

Isolates	*Mean VI values of non-treated seed's sprouts on treated TPP*	*Mean VI values of treated seed's sprouts on non treated*	*Mean VI values of treated seed's sprouts on non treated*	*Percent Disease incidence (PDI) of total sprouts treatedy by bacteria*	*Germination index (GI) of total sprouts treated by bacteria*	*Germination energy (GE) of total seeds treated by bacteria*
Control	885	0	90	50.00	0.16	33.33
B10-11	4210	1860	1220	25.00	0.33	66.67
B10-57	4700	2250	5180	00.00	0.42	83.33
P10-64	3170	5370	1600	00.00	0.42	83.33
P10-76	4940	4560	4850	00.00	0.51	100.00
P510-46	2830	2000	300	25.00	0.33	66.67
P510-71	1680	1485	1800	00.00	0.24	50.00
P510-111	3680	0	2850	00.00	0.25	50.00

(A) (B)

Picture 1: (A) – Sprout growth on treated fly-ash (B) Sprout growth on treated, non-treated fly-ash and on soil

4. DISCUSSION:

The data shown in Table 1, gives the morphological characteristics of different types of bacteria isolated from the cow dung. The Table 2 refers to the results of acidic components production tests for seven isolates. Table 3 shows the results of 6 types of enzyme production tests for seven isolates. Table 4 depicts the solubilizing efficiency values of seven bacteria for tree types of salts. Table 5 shows the results of the response to seed germination and plant growth. Among the seven bacteria , P10-76 is found to be best for plant growth. The identification of best isolate is done by16s rRNA Gene sequencing. Pictures 1(A) and (B) shows the real fact of the plant growth activity on fly-ah by bacteria. The results may vary if we use buffalo dung, pig manures, poultry dropping, etc. instead of cow dung. The results may also vary if we use the fly-ash of other power plants.

5. CONCLUSION:

The fly-ash of coal-fired thermal power plants is an important source of silica, aluminium, iron and calcium salts which play as micronutrients for plant growth. So, microbes not only play vital role in physical weathering of parent rocks for generation of fertile lands or soil but also help in in plant nutrition by microbes –plant symbiosis phenomena. The solubilization of insoluble materials into soluble materials by bacteria is done due to secretion of acidic substances by them. In case of treated ash, roots of plants enter into the ash more than the un-treated seed or ash.

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