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PHOSPHATE DEGRADATION FROM DIMECRON AND BIDRIN BY SOME RICE RHIZOSPHERE BACTERIA

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Abstract

Sixteen bacterial species isolated from the rhizosphere of **Oryza sativa** *(L) were tested for their capabilites to bring into solution phosphorus from two organo phosphorus pesticides. Of the sixteen isolates only five were apparently able to bring into solution the organic phosphate from Dimecron, while ten isolates were able to do so for Bidrin. Bidrin was found to be more easily solubilized than Dimecron by the selected isolates.* **Azotobacter chroococcum** *and* **Alcaligenes recti** *were found to be the most potent phosphate dissolver for Dimecron and Bidrin respectively.*

INTRODUCTION

The practice of wide scale application of pesticieds, while improving crop yield, raises questions as to the short and long range effects of them on ecological balance. So, an ideal pesticide compound would be one that destroys the pest quickly and in turn, itself gets degraded to more elementary non-toxic substances within a considerably short time. One of the approaches to the study of the relationship between microorganisms and pesticides is that, as nearly all modern pesticides are organic, they could conceivably be metabolised with a resulting modification or destruction of their activity (Alexander 1978 . Organophosphorus pesticides are degraded in the soil environment by an initial co-metabolic attack (Hsu and Bartha, 1979). Several soil microorganisms degrade these toxic compounds from their complex organic structure to simple aliphatic structure, ultimately making those toxic compounds lose their toxicity.

Phosphate dissolving bacteria are present in soil and phosphorus availability is influenced by rhizosphere inhabitants. The number of individual group of microorganisms in the rhizosphere increases with plant age (Patel, 1969). Rhizosphere soil is known to contain greater number of phosphate dissolving microbes than non-rhizosphere soil (Molla *et al.*, 1983 ; Taha *et al.*, 1969 . Microorganisms isolated from rhizosphere of plants have been found to be more efficient in solubilizing phosphates than those from nonrhizosphere region (Sperber. 1958 ; Sobieszezanski, 1961). The soil is the "sink" which receives the pesticides and degrade it by soil microbiota (Dubey *et al.*, 1989). It is known that a number of soil microbes including fungi and bacteria degrade the complex pesticide molecules and uses it as a source of carbon (Dubey *et al.*, 1989). It is also possible that phosphate dissolving soil microbes would degrade the phosphate containing pesticides and play an important role in supplying the growing plants with their needs of P to certain extent while alleviating the problems of soil pollution.

The present investigation was taken up to isolate and identify phosphate dissolving microorganisms from the rhizosphere of *Oryza sativa* (L) and compare their efficiency in phosphate from organophosphorus pesticides in liquid medium.

MATERIALS AND METHODS

Nine soil samples were collected from the root zones of "Kataktara", "Panbira" and "BR3" varieties of rice grown at BRRI farm, Gazipur. pH of the collected soil samples was around neutrality (Table 1). The soils were air dried and sieved through a 0.5 mm sieve. The air dried samples were used to isolate bacterial colonies on dilution plates of NAM (nutrient agar medium) and modified azotobacter plate. From the nine soil samples, 85 individual colonies were isolated on nutrient agar plate and 10 on modified Azotobacter agar plate. A total of 95 bacterial colonies were studied and they were found to vary in their colony characters (Table 2). Out of these 95 bacterial colonies 16 were selected for further studies. Two of the isolates represented the *Azotobacter* group (A/2 & A/4) and the rest 14 were other soil bacteria. Final identification was made by comparing their morphology and nature of arrangement through microscopic study after simple and differential staining, physiological and biochemical characteristics following Bergy's manual of determinative bacteriology.

Table 1 : The collected soil samples with their corresponding rhizosphere and pH.

Sample No.	Date of collection	Corrosponding rhizosphere	pH of the soil sample
1	26.7.84	Kataktara	6.8
2	26.7.84	Kataktara	6.8
3	26.7.84	Kataktara	6.9
4	7.8.84	Panbira	6.7
5	7.8.84	Panbira	6.9
6	7.8.84	Panbira	6.7
7	20.8 84	BR_3	7.0
8	20.8.84	BR_3	7.0
9	20.8.84	BR_3	6.9

Table 2 : Plate count of the microbial colonies on nutrient agar (pH 7.0) and modified Azotobactar media (pH 8.0) and the shapes of the colonies.

No. of samples used for isolation	Total no. of isolated colonies		Shape of the colonies					
	Nutrient Agar Media	Modified Azotobacter Media	Circular	More or less circ.	Filamentous	Punctiform	Rhizoidal	Irregular
9	85	10	49	19	7	3	4	13

Two organophosphorus pesticides, namely Dimecron ane Bidrin, commonly used in Bangladesh, were obtained from Ciba-Geigy and Burma-Eastern respectively. The chemical composition of Dimecron 100 SCW is $C_{10}H_{19}O_5$ NCIP and of Bidrin 850 g/l WSC is $C_8H_{16}O_5$PN.

The concentration of the individual pesticides, at which no growth inhibition occurred was chosen for the study of the phosphate degradation capacity of the isolates (Khan and Shaikh, 1988). The 16 isolates were incubated for 2 weeks in broth or in broth containing individually 0 05% Dimecron and 0.8% Bidrin. After two weeks of incubation the media were filtered through a millipore filter paper of 0.45 μm pore size. The orthophosphate present in the filtrates were then determined colorimetrically by vanado-molybdate yellow color method (IRRI, 1976). The experiment was repeated twice each time having four replications. The results presented are averages of eight individual replicates.

RESULTS AND DISCUSSION

Results indicating the values for soluble phosphates after incubation are presented in table 3. Of the 16 isolates, 1/9, 1/10. 1/18, 3/10 and 3/16 were from the rhizosphere of Kataktara, 2 & 23 were from the rhizosphere of Panbira and A/1, A/2, A/4, A/5, A/6, A/7, A/8, A/11 & R/3 were from the rhizosphere of BR3. These bacteria have been identified to be different strains of *Bacillus circulans*, *B. pulvifaciens*, *Alcaligenes recti*, *Flavobacterium rigense*, *Brevibacterium lipolyticum* (rhizosphere of Kataktara), *B. circulans*, *B. pulvifaciens* (rhizosphere of Panbira), *Achromobacter superficialis*, *Azotobacter beijerinckii*, *A. chroococcum*, *Achromobacter liquefaciens* *Flavobacterium rigense*, *Achromobactar guttatus*, *Micrococcus luteus*, *Escherichia freundii* and *Pseudomonas pseudomallei* (rhizosphere of BR_3) respectively.

It appears that the pesticide Bidrin degrades more easily and rapidly than Dimecron. Of the 16 strains of bacteria, 10 could degrade phosphate from Bidrin in two weeks time while only 5 strains were able to do so for Dimecron. That Bidrin is easily and quickly degraded was also evident from the fact that the strains could tolerate this pesticide at a higher concentration (0.8%) than Dimecron (0.05%) without having any growth inhibition (Khan and Shaikh, 1988). When the capability of the individual strains is considered, it appeared that *Azotobacter*

Phosqhate degradation by Bacteria

Table 3: Soluble phosphate concentration in nutrient broth medium containing Dimecron (0.05%) and Bidrin (0,8%) incubated with selected bacterial strains for two weeks.

Strain No.	Bacterial species	Soluble Phosphate concentration in medium containing Dimecron (0.05%)		Soluble Phosphate concentration in medium containing Bidrin (0.8%)	
		Initial (ppm)	Final (ppm)	Initial (ppm)	Final (ppm)
1/9	*Bacillus circulans*	18.51	15.62	15.20	17.71
1/10	*Bacillus pulvifaciens*	18.55	18.13	14.70	15.70
1/18	*Alcaligenes recti*	19.40	14.78	13.19	17.71
3/10	*Flavobacterium rigense*	17.29	17,88	14.30	16.04
3/16	*Brevibcterium lypolyticum*	14.78	16 04	15.20	16-04
2	*Bacillus circulans*	17.29	17.13	15.23	16.04
23	*Bacillus pulvifaciens*	19.39	16.96	15.70	15,20
A/1	*Achromobacter superficialis*	12.69	11.01	10.01	11.85
A/2	*Azotobacter beijerinckii*	12.69	13.52	13.52	13.52
A/4	*Azotobacter chroococcum*	0.42	6.07	11.01	15.20
A/5	*Achromobacter liquefaeiens*	7.49	11.18	14.36	16.04
A/6	*Flavabacterium rigense*	16.62	14.53	9.67	13.52
A/7	*Achromobacter guttatus*	11.01	11.01	14.36	13.52
A/8	*Midroccccus luteus*	15.20	14.19	16.04	15.20
A/11	*Escherichia freundii*	10.68	10.34	15.20	13.52
R/3	*Peseudomonas pseudomallei*	13.52	11.18	15.30	10.68

chroococeum is the most potent one followed by *Achromobacter liquefaciens*. The strains of these two bacteria could degrade phosphate from both pesticides. These two bacteria were followed by *Brevibacterium lypoliticum* and strain 3/10 of *Flavobacterium rigense*. While considering the capabilities of the strains to degrade the two pesticides the following order could be obtained respectively :

Azotobacter chroococcum > *Achromobacter liquefaciens* > *Brevibacterium lypoliticum* > *Azotobacter beijerinckii* > *Flavobacterium rigense* strain no. 3/10 and *Alcaligense recti* > *Azotobacter chroococcum* > *Flavobacterium rigense* strain no. A/6 > *Bacillus cirulans* strain no.1/9 > *Achromobacter superficialis* > *Flavobacterium rigense* strain no.2/10, *Achromobacter liquefaciens* > *Bacillus pulvifcciens* > *Brevibacterium lypoliticum*, *Bacillus circulans* strain no. 2.

The negative values obtained could possibly be explained as the temporary immobilization through assimilation. Precipitation of the soluble phosphates to an insoluble form could be another reason for such negative values. It is known that bacteria are able to convert soluble inorganic compound to the insoluble form and also absorb in their cell composition. Dugan (1970) reported inorganic compounds precipitated within extracellular fibrillar polymer net work which also trapped bacteria. Khan and Williams (1978) also worked on the effect of $CuSO_4$ on the growth of actinomycetes and observed that at high concentration of Cu, some mycellial growth appeared after a period of about 8 to 10 weeks and a conspicuous blue colored precipitate was found at the bottom of the flask. On closer examination, the precipitates were found to be composed of regular globose bodies made up of numerous tiny needle like crystals pointed at both ends.

Variation observed in the degradation capacity of the isolates could be related to the differences in their physiological and nutritional requirements as well as to the diffirence in the degradability of the two chemicals. It is known that the organophosphorus pesticides constitute a more labile and water soluble series of compounds than the chlorinated hydrocarbon insecticides (Nash, 1981). In the present case, Dimecron has chlorine in its composotion while Bidrin does not have it. Dimecron, thus falls in the second category of pesticides mentioned above.

Organo-phosphates thus solubilized are readily absorbed by plants growing in soil containing these compounds (Nash, 1981). It is therefore, not unlikely that bacteria can also absorb phosphorus released from these pesticides. From the light of the present observation it could be assumed that organophosphorus pesticides introduced into soil could be utilized by soil microbes as their substrate and it is quite likely that the metabolic activity of these microorganisms could either assimilate the phosphate in their cell composition or render it soluble and make availbe to growing plants. Moreover, the residual toxic effect of the two pesticides under study could, at least, in the rice rhizosphere be eliminated to a certain extent in relatively shorter period of time thus eliminating the possiblity of soil pollution.

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