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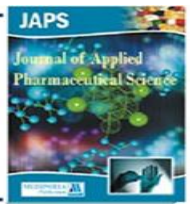

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Studies on Atrazine Mineralization by a Consortium of Bacteria Isolated from Sugarcane Field Soil

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ABSTRACT

Herbicides are anthropogenic chemicals that enter environment and can adversely affect non-target organisms and may be detrimental to human health. The herbicide atrazine is a halogenated herbicide that persist in soil and water causing environmental concern. In view of this problem the present work was carried out on the bioremediation aspects of atrazine. The soil and water samples were collected from atrazine applied sugarcane field and screened isolates were tested for their ability to utilize atrazine as sole carbon source. Their ability to degrade atrazine without others aid was determined.

Keywords: Atrazine, herbicide, *Pseudomonas sps*, bioremediation.

INTRODUCTION

Weeds act as a reservoir of disease organisms and as alternative hosts for pathogens and insects pests. They are major agricultural pests. The herbicide atrazine, 2-chloro-4-ethylamino-6-isopropyl amino-s- triazine is used throughout the world**.** Atrazine is a member of triazine group of herbicides used for control of grassy and broad leaf weeds in corn, sorghum, sugar cane crops but their use is restricted, which means applicators must have certification to purchase and use atrazine . Although atrazine is an effective herbicide, extensive toxicological investigations (Biradar and Rayburn, 1995; Allran and Karasov, 2000) have motivated and continue to motivate bioremediation directed research. Bioremediation is an environmental clean-up technique that is currently link investigated for use on a wide variety of chemicals. Bioremediation has been considered largely as the technology of choice for atrazine – compromised sites despite the availability of various alternative treatment and containment methods such as ozonation (Ma and Graham, 2000) photochemical degradation (Konstantinou *et al.,* 2001) diatomaceous earth remediation (Agdi, 2000) and powdered activated carbon absorption (Cames, 2000). Several researchers have reported the enrichement and isolation of microorganism which are able to dealkylate atrazine in a carbon limited medium (Behki and Kahn, 1986) mineralise and use atrazine as a sole carbon and energy source (Mandelbaum *et al.,* 1995 ; Yanzekontchou and Gschwind 1995 ; Topp, 2000).

Biostimulation involves the addition of nutrient that are deficient but required for biodegradation of the contaminate. The addition of nutrient cause an increase of microbial population, thereby increasing the number of indigenous microorganisms capable of degrading the contaminate. Alvey *et al.,* 1996, showed survival activity of atrazine bacterial consortium in rhizosphere soil. *Psudomonas sp.,* strain ADP metabolized atrazine to carbon dioxide and ammonia via the intermediate hydroxyatrazine. The genetic potential to produce hydroxyatrazine was attributed to 1.9 kb DNA fragment.

In the present study on atrazine bioremediation, microorganisms capable of atrazine biodegradation have been isolated, characterized and their role in degradation has been determined. Microbial consortia involving more than one microbe in atrazine degradation has been found.

MATERIALS AND METHODS

Sample collection

Soil samples were collected from four coners and in the center of atrazine applied sugarcane field and the samples were pooled together, brought to laboratory in sterile containers , processed within 24 hours. Commercially available atrazine (atrataf) (containing atrazine 50 % marketed by RALL'S INDIA) was incorporated into sterile nutrient agar.

Isolation of atrazine resistant bacteria

Serial dilutions of the sample was done and 0.1 ml of the sample was spread on nutrient agar plates incorporated with atrazine of 0.01% concentration. After incubation the colonies that appeared on the plates were selected as atrazine resistant colonies. These resistant colonies were then streaked on nutrient agar plate containing higher concentration of atrazine.

Phenotyping characterization of the isolates

Various morphological and biochemical test such as Gram staining, starch hydrolysis, Gelatin hydrolysis, Indole test, Voges-proskauer test, Citrate utilization test, Triple sugar Iron agar test, Catalase test, Oxidase test were done and the result were recorded, lactose utilization were identified using Mac Conkey agar medium (Buchnan and Gibbons, 1974).

Determination of atrazine degradation

To determine the atrazine degradation three different types of media were prepared. Minimal salt medium (focht, 1994), Minimal salt medium containing 1 mg/ml atrazine, Minimal salt medium containting 1mg/ml of atrazine and glucose (0.1 %). The saline suspended cells were used as inoculam for atrazine degradation studies. It was incubated at 37ºC for about 15 days, the optical density was measured at 620nm after the period of incubation.

Determination of bacterial consortia involved in atrazine degradation

The isolates that showed maximum absorbance in minimal salts and atrazine were selected for the study. The selected isolates were designated as A, B,C. After the incubation period at room temperature for 15 days growth was assessed by measuring the optical density at 620 nm

Determination of dechlorination

Bomocresol purple medium was prepared in two batches , to one batch atrazine (1 mg/ml) was added and were inoculated with mixed inoculum of A,B and C to study dechlorination .

Modified minimal salt medium was used to study urea breakdown. Ammonia release during atrazine degradation was demonstrated by treating culture filtrate with Nesler's reagent. A biometer flask was used to demonstrate Co_2 liberation from atrazine contaminated soil

RESULTS AND DISCUSSION

The ten resistant isolates that developed following incubation were marked as "**potential atrazine resistant**" isolates. The results of the various biochemical test of the isolates are presented in Table - 1

Table. 1: D-Dextrose, L-Lactose, S- Sucrose, Nlf-Non- Lactose Fermentor.

Isolates	Shape	Grame Stain	Catalase	Oxidase	Citrate Test	Indole Test	Vp Test	Starch Hydrolysis	Growth On Macconkey Agar	Tsi Test
1	COCCI	Positive	+	+	+	_	_	_	NLF	_
2	ROD	Positive	_	+	_	_	_	_	NLF	_
3	ROD	Negative	_	+	+	_	_	_	NLF	D fermentation
4	ROD	Positive	+	+	+	_	_	+	NLF	_
5	ROD	Positive	+	+	+	_	_	_	NLF	_
6	ROD	Positive	+	+	_	_	_	+	_	_
7	ROD	Negative	+	+	_	_	_	_	_	_
8	ROD	Negative	+	+	_	_	_	_	NLF	D fermentation
9	COCCI	Positive	+	_	+	_	_	_	NLF	_
10	ROD	Positive	+	_	+	_	_	+	NLF	D,L,S fermentation

DETERMINATION OF ATRAZINE DEGRADATION

The ten atrazine resistant isolate were further analyzed for their ability to use Atrazine as a sole carbon source are shown in Table: 2

Table. 2:. Results of Atrazine Degradation.

Isolates	Msm	Absobbance At 620 Nm	
		Msm + Atrazine Concentrstion(0.1%)	Msm + Gulcose(0.1%) +Atrazine Concentration(0.1%)
1	_	0.08	0.13
2	_	0.10	0.12
3	_	0.09	0.15
4	_	0.07	0.14
5	_	0.09	0.15
6	_	0.17	0.20
7	_	0.19	0.19
8	_	0.21	0.25
9	_	0.10	0.18
10	_	0.07	0.15

From the results it was evident that the 10 isolate were capable of utilizing atrazine as sole carbon source. Only three isolate were found to use atrazine best without glucose. Based on the biochemical test performed the isolate no 6 – A identified as *Bacillus sp.,* the isolate no 7 – B identified as *Alkaligenes sp.,* the isolate no 8- C identified as *Pseduomonas sp.,* The bacterial consortia involved in atrazine degradation was studied. The growth of **AB (***Bacillus sps +Alkaligene sps*) and **CA** (*Pseudomonas +Bacillus*) were less pronounced in both media. The growth of **BC** (*Alkaligene sps.,* + *Pseudomonas sps.,*) well pronounced in both media. The bacterial consortium could do much better than what they do individually (Table : 3)

Table. 3: Determination of Bacterial Consortia Involved In Atrazine Degradation.

Inoculum	Absorbance At 620nm		
	Msm	Msm + Atrazine (1mg/ml)	Msm + 0.1 % Gulcose + Atrazine (1 mg/ml)
A	_	0.17	0.15
B	_	0.19	0.18
C	_	0.21	0.22
AB	_	0.19	0.18
BC	_	0.25	0.32
CA	_	0.19	0.25
ABC	_	0.26	0.38

Table. 4: Results On Urea Breakdown To Ammonia.

Tube	Medium	Nessler's Reagent		Ammonia Production
		Added	Not Added	
A	Modified Msm Only	_	√	**Nil**
B	Modified Msm Only	√	_	**Nil**
C	Modified Msm + Atrazine Only	_	√	**Nil**
D	Modified Msm + Atrazine Only	√	_	**Nil**
E	Modified Msm + Atrazine +ABC Inoculums	_	√	**No Brown ppt**
F	Modified Msm + Atrazine ABC Inoculums	√	_	**Yes (Brown Ppt)**

Formation of urea and its subsequent breakdown to ammonia and V Co_2 during atrazine degradation are shown in (table: 4). In experimental flask Co_2 liberation was seen due to atrazine mineralization by microbial consortium inoculated.

CONCLUSION

The present study of atrazine bioremediation involes the screening the bacteria that can degrade atrazine in sugarcane field soil. Many atrazine resistant bacteria utilize atrazine as the sole carbon source. It was also found that the presence or absence of glucose in the medium didn't affect atrazine degradation. Three main degradation pathways of atrazine have been studied. They include biological hydrolysis,biological dealkylation and chemical hydrolysis. Biological dealkylation is the most well studied pathway. Several metabolites are dechlorinated and further degraded to biuret and urea. Urea is finally broken down into Co_2 and ammonia. The hydrochlorination of atrazine in the inoculated medium indicates the role of the isolates in atrazine dealkylation and de hydrochlorination. Ammonia release from urea breakdown was also demonstrated using nessler's reagent. But release of ammonia from atrazine took more time of incubation. A biometer flask was used to demonstrate Co_2 liberation from atrazine contaminated soil. So the present study concludes that the bacteria probably with other bacterial population in soil could completely mineralize atrazine and thus help in atrazine bioremediation. The combined effect of the three bacterial consortium was more than the individual isolate on atrazine degradation.

By modifying microorganisms either through conventional mutations or by r-DNA techniques, novel bugs can be created and released in to contaminated sites for rapid mineralizations of the Xenobiotic compounds.

REFERENCES

Assaf, N.A and Turco ,R.F. Accelerated biodegradation of atrazine by a microbial consortium is possible in culture and soil. Biodegradation 1994; 5:29-35.

Alvey, S.A and Crowley ,D.E. Influence of organic amendments on biodegradation of atrazine as a nitrogen source . *J.Environ. Qual* . 1995; 24: 1156-1162.

Alvey, S.A and Crowley, C.E. Survival and activity of atrazine mineralizing bacterial consortium in rhizosphere soil. *Environ. Sci. tecnol* . 1996 ; 30: *1596-1603.*

Agdi , K. Removal of atrazine and four organophosphorus pesticides from environmental waters by diatomauous earth- remediation method. *J.Environ. monitor* . 2000; 2: 420-423.

Allran, J.W and karasov, W.H. Effects of atrazine and nitrate on *Rana pipens* larvae exposed in the laboratory . *Environ. Toxicol. Chem*. 2000 ; 19: 2850-2855.

Bekhi, R.M and khan ,S.U . Degradation by *Pseudomonas* n-dealkylation and dehalogenation of atrazine and its metabolites .*J.Agric. food chem*.1986 ; 34: 746-749.

Biradar,D.P and Rayburn A.L . Chromosomal damage induced by herbicide contamination at concentrations observed in public water supplies.*J.Eniron.Qual* 1995 ; 24: 1222-1225.

Buchnan, R.E and Gibbons, M.E. Bergy's manual of determinative bacteriology.1974 The willams and wilkins Co., Baltimore.

Campos ,C . Atrazine remorval by powdered activated carbon in floc blanket reactors. *Water Res*. 2000 ; 34: 4070-4080

Focht,D.D. Microbiological procedures for bio-degradation research 1994; 407-426

Gebendinger, N and Radosevich, M. Inhibition of atrazine degradation by cyanazine and exogenous nitrogen in bacterial isolate M9 13-3 *Appl. Microbial. Biotechnol.* 1999 ; 51: 375-381

Konstantinou, I.K. and Albanis, T.A. Photodegradation of selected conditions. *J.Environ. Qual*. 2001 ; 30 : 121-130

Mandelbaum. R.T., Allan D.L. and L.P.Wackett. Isolation and characterization of a *Pseudomonas sp* that mineralizes the S-triazine herbicide atrazine *Appl. Environ. Microbiol* 1993 ; 61: 1451-1457

Ma, J. and Graham, N.J.D. Degradation of atrazine by manganese catalysed ozonation – Influence of radical scavengers. *Water Res*. 2000; 34 : 3822-3828.

Topp, E. A comparison of three atrazine-degrading bacteria for soil bioremediation. *Biol. Test. Soils*. 2001 ; 33: 529-534.

Yanze-kantchou, C. and Gschwine, N. Mineralisation of the herbicide atrazine in soil inoculate with a *Pseudomonas strain. J. Agricul. Food Chem*. 1995; 43 : 2291- 2294