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In Vitro Detoxification of Pendimethalin by Two *Actinomycetes* spp

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Bioremediation is one of the methods to remove toxic pollutant like pesticide (herbicide) from the environmental factors like air, water and soil by living species, e.g. microorganism. Herbicide pendimethalin has been found to persist in the top 10 cm of the soil and cause phytotoxicity to succeeding crop (Raj *et al.*, 2003; Dewan *et al.,* 1999). Phytotoxicity after application of pendimethalin to rice seedling is also documented (Pandit *et al.*, 2000). Kulshrestha *et al.* (2000) attempted degradation of this persistent herbicide and observed its enhanced degradation only in the surface soil (0-15 cm). Work was envisaged to study whether pendimethalin (Arora & Gopal, 2004) could be degraded by actinomycetes (Prescott *et al.*, 1990) cultured in mineral salt medium. Pendimethalin has earlier been degraded by soil microbes (Singh & Kulshrestha, 1991), chemical (Singh & Kulshrestha, 1992) and physical solarisation (Gopal & Mukherjee, 1999) methods but there is no report about its degradation by this filamentous Gram – positive bacteria (Prescott *et al.*, 1990). Degradation of pesticides by actinomycetes has been reviewed (Schrijver & Denot, 1999) but degradation of pendimethalin is not reported therein. The paper attempts to study rate of dissipation of pendimethalin in the presence of two actinomyctes sp.

MATERIALS AND METHODS

Pesticide degradation by standard *Streptomyces* procured from Microbiology Division, IARI, New Delhi and isolate A was tested in mineral salt medium (Sahu *et al.,* 1990) and their growth for use of inoculums was taken in Kenknight medium (Rao, 1986). The composition of mineral salt media used for degradation study as suggested by Sahu *et al.* (1990) was: $(NH_4)_2$ HPO_4 0.50 g, $MgSO_4 7H_2O$ 0.20 g, $FeSO_4.7H_2O$ 0.01 g, K_2HPO_4 0.10 g, Ca $(NO_3)_2$ 0.01g, distilled water – 1L and pH – 7. 25 mL mineral salt medium was added to each of the 77 culture tubes of 100 mL capacity. The actinomycetes strain *Streptomyces* sp and isolate A were allowed to grow in Kenknights medium. Its composition as per Rao, 1986 was: Glucose 1.0 g, K_2HPO_4 0.12g, $NaNO_3$ 0.1 g, KCl 0.1 g, $MgSO_4$ $7H_2O$ 0.1 g, distilled water 1L at pH 7. Since isolate A was powdery (not slimy like bacteria), and raised colonies on solid substrate, could be maintained/multiplied in the media recommended for actinomycetes (Shukla, 1993). The identification that the species present in isolate – A is *Streptomyces*

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Table 1. Biochemical tests carried out to characterize the microorganism.

Name of the Test	**Substrate Specific Activity**
Gram's stain	Positive
Pigments	-
H_2S Production	-
MacConkey Agar growth	-
Flourescence	-
Motility	-
Catalase	-
Oxidase	-
MR	-
VP	-
Indole	-
Citrate utilization	-
Starch	+
Casein	-
Geletin	+
Oxidation-Fermentation (OF)	-
Nitrate reduction	-
Anaerobic growth	-
Urease	-
Growth in 2% NaCl	+
5% NaCl	+
7%NaCl	+
9% NaCl	-
Growth at pH 5	-
pH 8	+
pH 9	-
Growth at 15^0C	-
25^0C	+
37^0C	+
42^0C	+
50^0C	-
Utilization of	
Glucose	+
Arabinose	+
Mannitol	+
Xylose	-
Meso-inositol	+
Raffinose	+w
Rhamnose	+
Salicin	-
Sucrose	+w
Galactose	+
Fructose	+
Spore Chain morphology	Rectiflexibles

was based on morphological observation about its filamentous nature under microscope and other biochemical tests (Table 1).

The tubes containing media were sterilized in an autoclave at 10555 kg/m^2 for 30 min. 250 µL of 1000 ppm (µg/mL) of pendimethalin in hexane were added to 63 tubes in order to have 63 tubes having media + pesticide. Out of these 4% inoculum of actinomycete (two species, one *Streptomyces* and other isolate A) was transferred aseptically in to 42 test tubes using sterilized tips with micropipette. Thus in one set of 21 tubes, inoculum of actinomycete *Streptomyces* sp. was added and in other sets of 21 tubes other strain present in actinomycetes isolate A of were added. 14 sets had only medium and pesticide and no inoculum was added to these.

The tubes were kept in an incubator shaker at 30^{0}C and separate tubes were drawn on 0, 1, 3, 5, 10, 15 and 20 d each time in triplicate. For extraction from mineral salt medium, the content of the test tubes from each replicate was poured separately into 150 mL separating funnel and 30 mL of 10% (W/V) sodium chloride solution was added to each followed by partitioning thrice with dichloromethane (3x15 mL).

The lower organic phase was passed through anhydrous sodium sulfate, transferred into round-bottomed flask and evaporated under reduced pressure. 5 mL of hexane was added and evaporated to remove the traces of dichloromethane. Thereafter the flask was rinsed with 5 mL of hexane for gas chromatographic analysis of the herbicide. The residual amount of pendimethalin was analyzed using a Hewlett – Packard GLC fitted with a glass column (2 m long x 2 mm diameter) packed with 3% OV-17 coated on 80-100- mesh Chromosorb W.H.P., an electron capture detector (^{63}Ni) and computer operated integrator. The column, injector and detector temperatures were kept at 250^0, 275^0 and 280^{0}C, respectively. The retention time of pendimethalin was 1.75 min at the nitrogen flow rate of 30 ml/min. The standard solution of pendimethalin was injected after every four injection of the samples to get good comparison. The identity of the pendimethalin was confirmed by mass spectrometry by matching pendimethalin with the spectrum available in mass spectral library. The GC-MS was carried out using Fisons model Trio-1000. The ionization was done using electron impact and the analyses by quadrapole. The standardization for MS analyses was done using peaks of heptachlor at 69, 219, 264, and 502 for maximum signal intensity.

RESULTS AND DISCUSSION

Two actinomycetes one standard *Streptomyces* sp. and other designated as isolate -A were screened for their ability to degrade pendimethalin. *Streptomyces* and isolate-A were grown in salt medium that had and no organic compound except pendimethalin, which was to be degraded. After inoculation with isolate-A the amount of pendimethalin recovered was 174.22 µg/ml on day-5 whereas in control it was 226.24µg/ml (Table 2). The response to various biochemical tests carried out to characterize the actinomycete present in isolate-A is exhibited in

Table 1. Thus the actinomycete was identified at genus level and its species will be confirmed after 16S r DNA analysis. In comparison to these, pendimethalin degraded by only 1.49% in control during 5 day and 5.17% in 20 day period. Thus after a lag phase of 5 days, there was faster degradation. This was evident as on 20 days after inoculation, remaining concentration of the herbicide when estimated showed 47.91% degradation by *Streptomyces* while it degraded by 72.75% from the initial herbicide concentration in the presence of isolate-A. The degradation patterns of pendimethalin by these two actinomyctes (Shukla, 1993) are presented in Table 2.

Table 2. Degradation of pendimethalin by *Streptomyces* sp and isolate-A.

	Amount of herbicide (μg) ± Standard Deviation		
Days	*Streptomyces*	Isolate A	Control
0	227.64±2.5635 (-)	201.34±3.0643 (-)	229.67 ± 5.2593(-)
1	214.46±3.5354 (5.59)	190.69± 5.4996 (5.17)	229.40± 3.8088 (0.11)
3	209.14±2.9308 (6.71)	187.61± 5.5278 (5.4)	226.41± 4.5299 (1.41)
5	199.68±2.1040 (10.79)	174.22±4.9028 (11.97)	226.24±4.8893 (1.49)
10	158.37± 3.7612 (27.70)	67.41 ± 2.8248 (6379)	223.41±3.7858 (2.72)
15	128.52±5.5088 (38.82)	50.60 ± 4.2517 (70.14)	218.81± 3.7568 (4.72)
20	106.82± 4.6487 (47.91)	44.45± 4.4919 (72.75)	217.78± 3.6694 (5.17)

*Mean of three replicates, figurees in parenthesis show percent degradation

Table 3. Statistical data on half-life of the pendimethalin in presence of two actinonycetes.

Actinomycetes	Regression equation	r^2	Half life
Streptomyces sp	Y =(-)0.017X+2.361	0.9897	17.63
Isolate A	Y=(-)0.0383X+2.3308	0.9274	7.85

Y=log concentration of herbicide (mg); X= time in days

Assuming first order kinetics for degradation of pendimethalin in presence of two actinomycetes, the regression equation along with r^2 and half life (in days) were calculated and are presented in Table 2. The half-life of pendimethalin has been reported to be 28.1 day to 31.7 day when applied at different rates (0.75 – 2.0 kg ha^{-1}) and the rate of the dissipation had followed first order kinetics. The standard actinomycete (*Streptomyces* sp) was thus found less efficient for degrading this herbicide. Rai *et al.* (1999) have reported degradation of pendimethalin by enrichment cultures from different soils. They showed that rapid biodegradation of pendimethalin in mineral salt medium is possible through culture enrichment. The culture were reported to be able to degrade pendimethalin 97.3 to 99.8% in 45 days and half life was 11.2-19.8 days whereas in present study still

lower half life (7.86 days) could be attained by using a pure actinomycete.

The half-lives of the herbicides were 17.63 and 7.85 d respectively (Table 3) in presence of *Streptomyces* and isolate A. Since the half-life in control (without microorganism in media) was 273 d, the superiority of the isolate A for detoxifying this herbicide is proved. Thus the isolated actinomycete showed potential for degrading the herbicide pendimethalin.

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