Pak. J. Biotechnol. 9 (2) 49- 56 (2012) ISSN 1812- 1837

BIODEGRADATION OF SENCOR HERBICIDE BY SOME TREPTOMYCETES IN LIQUID CULTURE

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ABSTRACT

This work was designed to study the role of streptomycetes in the biodegradation of Sencor herbicide and its persistence in liquid culture. Testing the biodegradation abilities of nine isolates of *Streptomyces in vitro* revealed that they differed greatly in the number of degradation-products produced from Sencor. Gas liquid chromatographic analysis revealed that all tested isolates began to degrade Sencor after 15 days from incubation. Number of compounds varied from one to five depending upon the isolate used, as the streptomycete isolates varied greatly in their abilities to degrade Sencor as indexed by the number of compounds produced from Sencor degradation. The products of Sencor degradation were more demonstrated after 30 and 45 days from incubation. Half life period of the herbicide was reached after 15 days in *S. aureomonopodiales* and mixture treatments, while was reached in the other treatments after 30 days. The present results could be considered as an additional prove for the *in-vitro* abilities of these streptomycete isolates to degrade and utilize Sencor as a sole nitrogen source in culture medium.

Key words: Biodegradation, Sencor, *Streptomyces*, herbicide, Liquid culture.

INTRODUCTION

All the pesticide groups could be metabolized by micro-organisms (MacRae,1989). Several investigators reported that micro-organisms are considered as the most active biological agent for biodegradation of pesticides (her-bicides) in the environment (Cho *et al*., 2008; Getenga *et al*., 2009; Govantes *et al*., 2009 and Tappin *et al*., 2012).

Goring *et al.* (1967) found that 1000 ppm of picloram (as tordon) in media had no effect on *S. scabies,* while trifluralin at rates up to 445 ppm favoured growth of actinomycetes, altering greyish aerial mycelium into colorless. Hemphill and Fields (1967) reported that no adverse effects were found with DMPA incorporated into media and the degradation products 2,4-dichloro-phenol and O-methyl-isopropyl- phosphoramidothiate, respectively, suppressed and stimulated the growth of many actinomycete species. Gusterov *et al*., (1972) reported that triazines such as desmctryne inhibited 50% of actionmycetes in culture at concentrations of 8-100 ppm. Govantes *et al*., (2009) showed that atrazine is an herbicide of the s-triazine family that is used primarily as a nitrogen source by degrading micro-organisms. They also reported that atrazine and

terbuthylazine was mineralized by an *Arthrobacter* sp. isolated from a sugarcane-cultivated soil in Kenya in laboratory experiments.

Tappin *et al.*, (2012) reported that atrazine is a globally applied herbicide, contamination by which may lead to direct and indirect ecotoxicological impacts. Although a common contaminant of surface waters, microbial biodegradation of atrazine in this environment has been little studied, with most work focused on soils by means of selected, atrazine-degrading bacteria-enriched cultures.

The aim of this work was determination of the ability of the three effective *Streptomyces* isolates (Clu-40, Pt-71 and Wu-86) to degrade the Sencor in liquid culture and its half life during 45 days were also determined.

MATERIALS AND METHODS

Streptomycete isolates: A number of nine streptomycete isolates (B1, B3, BB24, Clo40, Cot44, Cor47, G55, P71 and W86) which were able to utilize Sencor herbicide as a sole carbon and/or nitrogen source were tested for their abilities to degrade this herbicide *in vitro*.

Standard inoculums of spores: Based on the method of Waksman and lechevalier (1961) starch nitrate slants inoculated with strepto-mycete isolates were incubated at 28°C±2 for 10 days. The heavy spores from the surface of the growth were scraped in the presence of five ml sterilized distilled water. Each ml of this suspension contains about 10^5 spores.

Biodegradation of Sencor herbicide by streptomycete isolates in liquid culture: Standard inoculum of spores for each isolate was inoculated into starch nitrate broth supplemented with Sencor at the rate of 0.75 g/L and incubated on a rotary shaker (160 rpm min^{-1}) for 45 days at 28^oC±2. Three controls were used, medium alone, medium supplemented with Sencor and medium inoculated with streptomycete isolate. Samples were taken from treatments and controls at intervals of 0, 15, 30 and 45 days for gas liquid chromatographic determination. Each culture was filtered through Whatman No.1 filter paper.

Extraction of Sencor and its degradation products: Based on the method of Jerczyk (1983) Sencor and its degradation products were extracted. Sample (50 ml) of each treatment was transferred into a separatory funnel and then 100 ml of the dichloromethane: ethyl acetate mixture (9:1) was added followed by shaking for 3-5 minutes. The lower dichloromethane /ethyl acetate phase was filtered over 10g of sodium sulphate through a fluted filter paper. The upper phase was re-extracted with 2 volumes of dichloromethane: ethyl acetate mixture (9:1v:v) were added and the lower phase was filtered as mentioned in before. This was repeated two times. All the lower phases were poured together in a rotary-evaporate to dryness.

Gas liquied chromatography (GLC) analysis: The gas liquid chromate-graph was carried out as recommended by Thornton and Stanley (1977). The residues were dissolved in 2ml of methanol. 1 μl of methanol extract were injected into the gas liquid chromatography model Hewlett-Packard 5890 series II plus., fitted with dual flame ionization and electron capture detector, electron pressure control

system (EPC), and Hp-1 capillary column (cross linked methyl silicone gun) 25 mx 0.32 mm x 0.52 um flime thickness and attached with computer unit and printer. The applied temperatures were 230°C, 180°C and 230°C for injection, column and detector respectively. The nitrogen gas was applied as carrier gas at flow rate 5 ml/min hydrogen and air for the flame at rates of 300 and 30 ml/min respectively. Under this condition the retention time of Sencor was 4.45 min. 2 µl of standard solution of metribuzin (2µg/µl) was injected into the GLC as standard. The peaks were compared with the standard and the amount of metribuzin in each sample was determined automatically by the computer unit. Each sample was injected two times as replicates.

RESULTS AND DISCUSSION

Researchers have demonstrated that atrazine has toxic effects in algae, aquatic plants, aquatic insects, fishes and mammals. Due to the toxicity and persistence of atrazine in the environment, the search of microbial strains capable of degrading it is fundamental to the development of bioremediation processes, as corrective tools to solve the current problems of the irrational use of agrochemicals (Sene *et al*., 2010).

Biodegradation of Sencor herbicide by some streptomycete isolates in liquid culture: The aforementioned results revealed that only nine isolates were able to utilize Sencor as a sole nitrogen source. Therefore, the present experiment was conducted to follow Sencor degradation up to 45 days in liquid shake culture. Gas liquid chromatographic analysis revealed that all tested isolates began to degrade Sencor after 15 days from incubation (Table 1). However, the products of Sencor degradation were more demonstrated after 30 and 45 days from incubation. This is probably due to high potential of the isolates to degrade Sencor as time proceeded. Ngigi *et al*., (2012) reported the degradation of atrazine was followed by measuring residual atrazine in liquid cultures over a given time period by high performance liquid chromatography.

Table-1: Degradation of Sencor by some streptomycete isolates* grown in starch nitrate broth.

Isolates	Sencor Degradation (0.75 g/L) after			
	0	15	30	45 days
B1	–	+**	+**	+**
B3	–	+	+	+
BB24	–	+	+	+
Clo40	–	+	+	+
Cot44	–	+	+	+
Cor47	–	+	+	+
G55	–	+	+	+
P71	–	+	+	+
W86	–	+	+	+

*: Isolates able to grow on starch nitrate medium deficient in nitrogen source. **: New compound appeared. B: Barley. BB: Broad bean. Clo: Clover. Cot: Cotton. Cor: Corn. G: Grapes. P: Pepper. W: Wheat.

Data summarized in Table-2 and illustrated by Figures 1-4 show that the tested streptomycete isolates varied greatly in their abilities to degrade Sencor as indexed by the number of compounds produced from Sencor degradation. This is clear sense isolates # B1, B3, Cot44, Cor47 and G55 (Figure 2) produced only one compound F (3.93 retention time) from Sencor. Isolate No. BB24 (Figure 3), produced two compounds namely A (1.26 RT) and F (3.95 RT). Isolate No. Clo40 (Figure 4) produced 3 compounds namely A, D and F. Isolate P71 (Figure 4) produced 4 compounds namely A, C, E and F. Finally, isolate W86 (Figure 4) was characterized by producing all the aforementioned compounds except for E compound. It is clear that all tested isolates were capable of producing the compound F. On the other direction the two isolates P71 and W86 were characterized by producing B and E compounds respectively. For each isolate, the same compounds produced after 30 days were also detected after 45 days of incubation. As far as we know, no studies were conducted concerning the biodegra-dation of Sencor by streptomycetes *in vitro*. However, we will refer latter to the studies of Sencor degradation in soil. The present results could be considered as an additional prove for the *in-vitro* abilities of these streptomycete isolates to degrade and utilize Sencor as a sole nitrogen source in culture medium. The results of this study was in harmony with that reported by Shelton *et al*., (1996), who conducted experiments to assess the ability of *Streptomyces* (strain PS1/5) to metabolize twelve herbicides representing several different classes including: acetanilides, triazines, ureas, uracils, and imidazoles. Incubations in aqueous culture with dextrin as carbon source and either ammonium or Casamino acids as nitrogen source resulted in transformations (> 50%) of eight of the herbicides tested: alachlor, metolachlor, atrazine, prometryne, ametryne, linuron, tebuthiuron, and bromacil; the remaining four herbicides (cyanazine, diuron, metribuzin, and imazapyr). Siripattanakul *et al*., (2009) enriched stable mixed cultures from atrazine-contaminated soil. The cultures were examined for their atrazine biodegradation efficiencies in comparison with J14a, a known atrazine-degrading strain of *Agrobacterium radiobacter*. For all cultures, atrazine was removed 33-51% within 7 days. Wang *et al.,* (2011) isolated a bacterial strain (HB-5) capable of utilizing atrazine as sole carbon and nitrogen sources for growth from an industrial wastewater sample by enrichment culture.

Table-2: Number of compounds produced from Sencor degradation by some streptomycete isolates. = Retention time (minutes)

Isolates	No. compounds	Compounds produced from Sencor degradation					
		A (1.26)*	B (1.74)	C (1.95)	D (2.67)	E (2.95)	F (3.95)
B1	1	-	-	-	-	-	+
B3	1	-	-	-	-	-	+
BB24	2	+	-	-	-	-	+
Clo40	3	+	-	-	+	-	+
Cot44	1	-	-	-	-	-	+
Cor47	1	-	-	-	-	-	+
G55	1	-	-	-	-	-	+
P71	4	+	-	+	-	+	+
W86	5	+	+	+	+	–	+

Figure-1: Gas liquid chromatographic analyses of starch nitrate liquid medium (A); metribuzin (active ingredient of Sencor herbicide) (B); commercial Sencor (C) and starch nitrate liquid medium supplemented with Sencor (D).

Figure-2: Gas liquid chromatographic analyses of Sencor degradation by streptomycete isolates B1, B3, Cot44, Cor47 and G55 in starch nitrate liquid medium (after 30 days incubation at 28±2°C on a rotary shaker at 160 rpm min^{-1}).

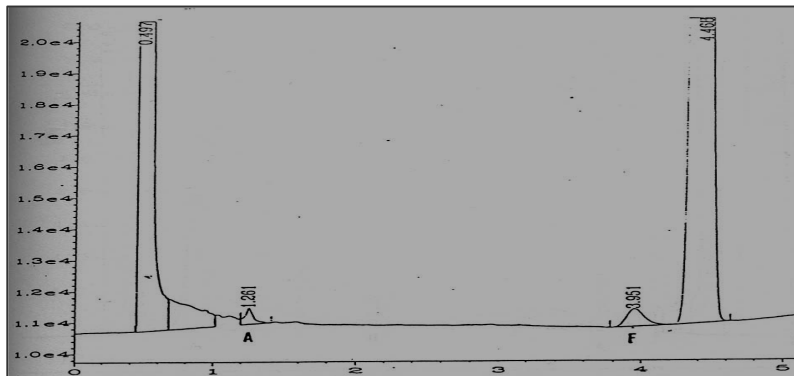

Figure-3: Gas liquid chromatographic analysis of Sencor degradation by streptomycete isolate BB24 in starch nitrate liquid medium (after 30 days incubation at 28±2°C on a rotary shaker at 160 rpm min^{-1}).

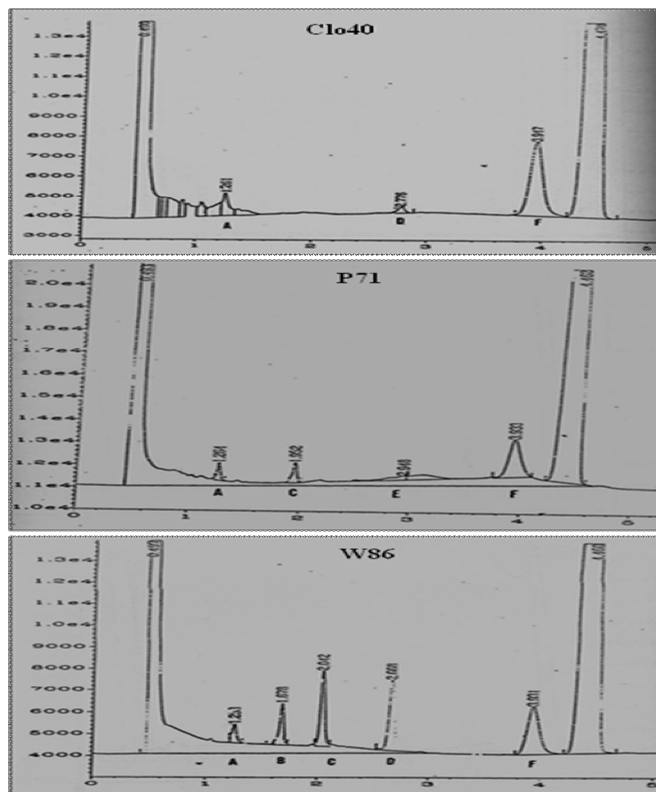

Figure-4: Gas liquid chromatographic analysis of Sencor degradation by streptomycete isolates: Clo40, P71 and W86 in starch nitrate liquid medium (after 30 days incubation at 28±2°C on a rotary shaker at 160 rpm min^{-1}).

REFERENCES

Cho, Y.S., B.U. Lee and K.H. Oh, Simultaneous degradation of nitro-aromatic compounds TNT, RDX, atrazine, and simazine by *Pseudomonas putida* HK-6 in bench-scale bioreactors. Journal of Chemical Technology and Biotechnology 83 (9): 1211-1217(2008).

Getenga, Z., U.Dorfler, A.Iwobi, M. Schmid and R. Schroll, Atrazine and terbuthylazine mineralization by an Arthrobacter sp. isolated from a sugarcane-cultivated soil in Kenya. Chemosphere 77(4): 534-539(2009).

Govantes, F., O.Porrua, V.Garcia-Gonzalez and E.Santero, Atrazine biodegra-dation in the lab and in the field: enzymatic activities and gene regulation. (Special Issue: Bioremediation.). Microbial Biotechnology 2(2): 178-185(2009).

Groing, C.A.I., J.D. Jriffith, F.C.O' Melia, H.H. Scott and C.R. Youngson, The effect of tordon on microorgnisms and soil biological processes. Down to Earth 22: 14-17(1967).

Gusterov, G., R.Brankova and S.S. Vilagov, The influence of some herbicides on the development and antibiotic activity of actinomycete-antagonistic with anti-fungal activity spectra. Symp. Biol. Hung. 11: 359-363 (1972).

Hemphill,D.D. and M.L.Fields, Effects of prolonged use of certain herbicides on soil microorganisms. Abstr. Meet. Weed Soc. Am. 36 (1967).

Jarczyk, H.J., Method for gas chromatographic determination of sencor residues in plant material, soil and water with an N-specific detector. Pflanzens-chutzNach-richten- Bayer 36(1):63-72 (1983).

MacRae, I.C., Microbial metabolism of pesticides and structurally related compounds. Reviews of Environmental Contamination and Toxicology 109: 1-23 (1989).

Ngigi, A.N., Z.M. Getenga, H.L.Boga and P.K.Ndalut, Biodegradation of s-triazine herbicide atrazine by *Enterobacter cloacae* and *Burkholderia cepacia* sp. from long-term treated sugarcane-cultivated soils in Kenya. Journal of Environmental Science and Health Part-B, Pesticides, Food Contaminants, and Agricultural Wastes 47(8): 769-778 (2012).

Sene, L., A.Converti, G.A.R.Secchi and R.deC.G.Simao, New aspects on atrazine biodegradation. Brazilian Archives of Biology and Technology 53(2): 487-496 (2010).

Shelton, D.R., S.Khader, J.S.Karns and B.M.Pogell, Metabolism of twelve herbicides by streptomyces. Biodegradation 7 (2): 129-136 (1996).

Siripattanakul, S., W.Wirojanagud, J.Mc Evoy, T.Limpiyakorn and E.Khan, Atrazine degradation by stable mixed cultures enriched from agricultural soil and their characterization. Journal of Applied Microbiology 106(3): 986-992 (2009).

Tappin,A.D., J.PLoughnane, A.J.Mc Carthy and M.F.Fitzsimons, Removal of atrazine from river waters by indigenous microorganisms. Environmental Chemistry Letters 10(1):89-96 (2012).

Thornton, J.S. and C.W.Stanley, Gas chromatographic determination of Sencor and metabolites in crops and soil. J. Agric. Food Chem. 25(3): 380-386 (1977).

Waksman,S.A. and H.A.Lechevalier, The actinomycetes Vol. II. Classification, identification and descriptions of genera and species. The Williams and Wilkins, Co. Baltimore, USA (1961).

Wang, J.H., L.S.Zhu, A.J.Liu, T.T.Ma, Q.Wang, H.Xie, J.Wang. T.Jiang and R.S.Zhao, Isolation and characterization of an Arthrobacter sp. strain HB-5 that transforms atrazine. Environmental Geo-chemistry and Health 33(3): 259-266 (2011).