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Degradation of Hexachlorocyclohexane Isomers by Two Strains of *Alcaligenes faecalis* Isolated from a Contaminated Site

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Cropping of high yield varieties has led to the proliferation of serious insect pests causing major losses in crop yield. The consequences are more severe in tropical countries like India because of the conducive climate for growth of insect pests. Therefore, there has been extensive and intense use of insecticides, particularly organochlorines, for an effective control of insects. Hexachlorocyclohexane (HCH) is a broad spectrum insecticide still used in some of the developing countries, though developed countries have banned or curtailed its use (Kaushik 1991). Commercial formulations of HCH contain four isomers α, β, γ, and δ. Even in those countries where the use of insecticide HCH has been discontinued for a number of years, the problem of residues of all isomers of HCH remains because of the high persistence and interconversion of these isomers in soil (Steinwandter and Schluter 1978). Insecticides in the soil have far- reaching consequences as they disturb the delicate equilibrium between micro-organisms and their environment. Microbial degradation of various pesticides in the soil environment is well documented (Matsumura and Benezet 1978). A perusal of literature on pesticide degradation also clearly indicates that soil bacteria exposed to a pesticide may be more effectively degrading in its subsequent use (Tu and Miles 1976). Although a few reports on the degradation of HCH in soil, including microbial, are present in literature (Kaushik 1989; Kaushik 1991), little information is available on the degradation efficiency of microbes after their acclimatization.

In the present study, an attempt has been made to isolate micro-organisms from the HCH contaminated soil, showing the capacity to degrade HCH and to acclimatize them under laboratory conditions to investigate their increased degradation efficiency under aerobic conditions on nutrient medium. Results obtained in the study with *Bacillus circulans* and *Bacillus brevis* are

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published elsewhere (Gupta *et al.* 2000) and those of *Alcaligenes faecalis* are presented here.

MATERIALS AND METHODS

All the isomers of HCH used were of 99% purity. The other chemicals were of analytical grade. All glassware were cleaned with acetone and oven dried. HCH degrading bacteria were isolated from the HCH contaminated soil from the factory premises of Hindustan Insecticides Limited, New Delhi, India. Soil samples were taken up to a depth of 5 cm from four different sites and pooled. The medium used for isolation and enrichment was nutrient agar (pH 6.9) that contained 5 g beef extract, 3 g peptone, and 8 g sodium chloride in 1 L of distilled water (Difco Manual 1953). HCH was added to the nutrient agar to yield a final concentration of 5 μg/ml of HCH. Five gram sample of soil was vigorously stirred with 100 ml of distilled water for half an hour. One ml of this suspension was subjected to serial dilution up to 10^{-6} to give the final inoculum. One ml of inoculum was transferred to each incubation flask containing 50 ml nutrient medium and 5μg/ml of HCH mixture. Flasks were incubated at 33±2°C for three days. One ml of the medium from this flask was subjected to serial dilution and the 10^{-3} dilution was used to inoculate the petri plates. Petri plates were again incubated for 3 days at 33 ± 2°C.

Round, well-defined colonies appearing on the surface of the medium were isolated and streaked on separate petri plates. After three to four streakings, only one type of colony persisted and the culture was considered pure. The resultant bacterial strains were tested for their efficiency to degrade HCH. The strains that were capable of slightly degrading HCH were acclimatized by regularly exposing them to HCH for >2 years and then used for further studies.

Subsequently, the isolated strains were inoculated in Erlenmeyer flasks containing 50 ml nutrient medium and 5 μg/ml of HCH isomer viz α, β, γ, and δ separately. The flasks were incubated at 33 ± 2°C. The medium was extracted at an interval of 2 days for the residues of α,γ, and δ isomers. For β isomer, however, this interval was 7 days. Uninoculated medium containing HCH acted as a control. Analysis was done for 8 days for α,γ, and δ and 28 days for β isomer. Similarly, the bacterial strains were also examined for their degradation efficiency at a concentration of 1 μg/ml.

Three extractions were done using 10 ml of hexane each time. A pinch of Na_2SO_4 was added to avoid emulsion formation. Pooled hexane portions were concentrated on Buchhi evaporator to dryness. Final volume was made to 5 ml with distilled hexane.

Analysis was done on Gas Chromatograph (GC) having methyl silicone (1μ film thickness) capillary column (25 m, 0.25 mm i.d.) and ^{63}Ni electron capture detector. The temperatures of oven, column and detector were 175, 175 and 240°C, respectively. Nitrogen (carrier gas) flow was 10 ml/min.

RESULTS AND DISCUSSION

Two strains of *Alcaligenes faecalis* were selected as HCH degrading micro- organisms (Table 1.)

Both the strains are found to degrade HCH isomers viz α,γ, and δ very efficiently but the strain S-2 could not degrade β -HCH at any of the two concentrations (Fig.1). *Alcaligenes faecalis* (S-1) was observed to degrade β HCH up to 25% and 32% at 5 μg/ml and 1 μg/ml, respectively, after 28 days of incubation (Fig.1b,d). β-HCH is the most stable isomer of HCH due to strainless bonds and absence of internal repulsion. The degradation is higher at low concentration, which may be due to the fact that a higher concentration is inhibitory for bacteria. These two strains of *Alcaligenes faecalis*, isolated from the same source differed in their ability to degrade β HCH. The increased versatility towards β - HCH degradation of *Alcaligenes faecalis* S-1 may have been due to the broadening of the dehalogenase specificity by either mutational events or by plasmid exchange and recombination (Miguez et al. 1990).

Rest of the three isomers are degraded very effectively by both strains. HCH was degraded up to 90 % and 97 % at 5 μg/ml and 1 μg/ml, respectively on eighth day by *Alcaligenes faecalis* S-1 (Fig.1a,c) whereas degradation was 93 and 100% for 5 μg/ml and 1 μg/ml, respectively by *Alcaligenes faecalis* S-2 (Fig. 1 e,f). All γ -HCH was degraded by the fourth day and eighth day at 1μg/ml and 5 μg/ml, respectively by both strains. The percent degradation of δ-HCH on eighth day of incubation was 91 and 80 % at 5 μg/ml and 1 μg/ml, respectively, by strain S-1. Strain S-2 was more efficient in degrading δ-isomer. The observed

Table 1. Characteristics of two strains of *Alcaligenes faecalis* (S_1,S_2) showing differential degradation of HCH

Characteristic	Strain S_1	Strain S_2
Shape	Rod	Rod
Growth on nutrient agar	Round, smooth & convex colonies	Round, smooth & convex colonies
Motility	Motile	Motile
Gram's reaction	-	-
Growth temperature		
37°C	+	+
42°C	+	-
55°C	-	-
Growth on NaCl (%)		
2.5	+	+
5.0	weak	-
7.0	-	-
Growth on Mac Conkey agar Lacfermenter	-	-
Starch hydrolysis	-	-
Nitrate reduction	+	+
Nitrite reduction	-	+
Indole test	-	-
H_2S production	-	-
Oxidase and catalase test	+	+
Casein hydrolysis	-	-
Methyl red test	-	-
Voges Proskauer test	-	-
Citrate utilization	+	+
Pigment formation	-	-
Acid production from carbohydrates:		
Mannose	-	±
Maltose, Fructose, Sucrose & Lactose	-	-
Xylose	-	+

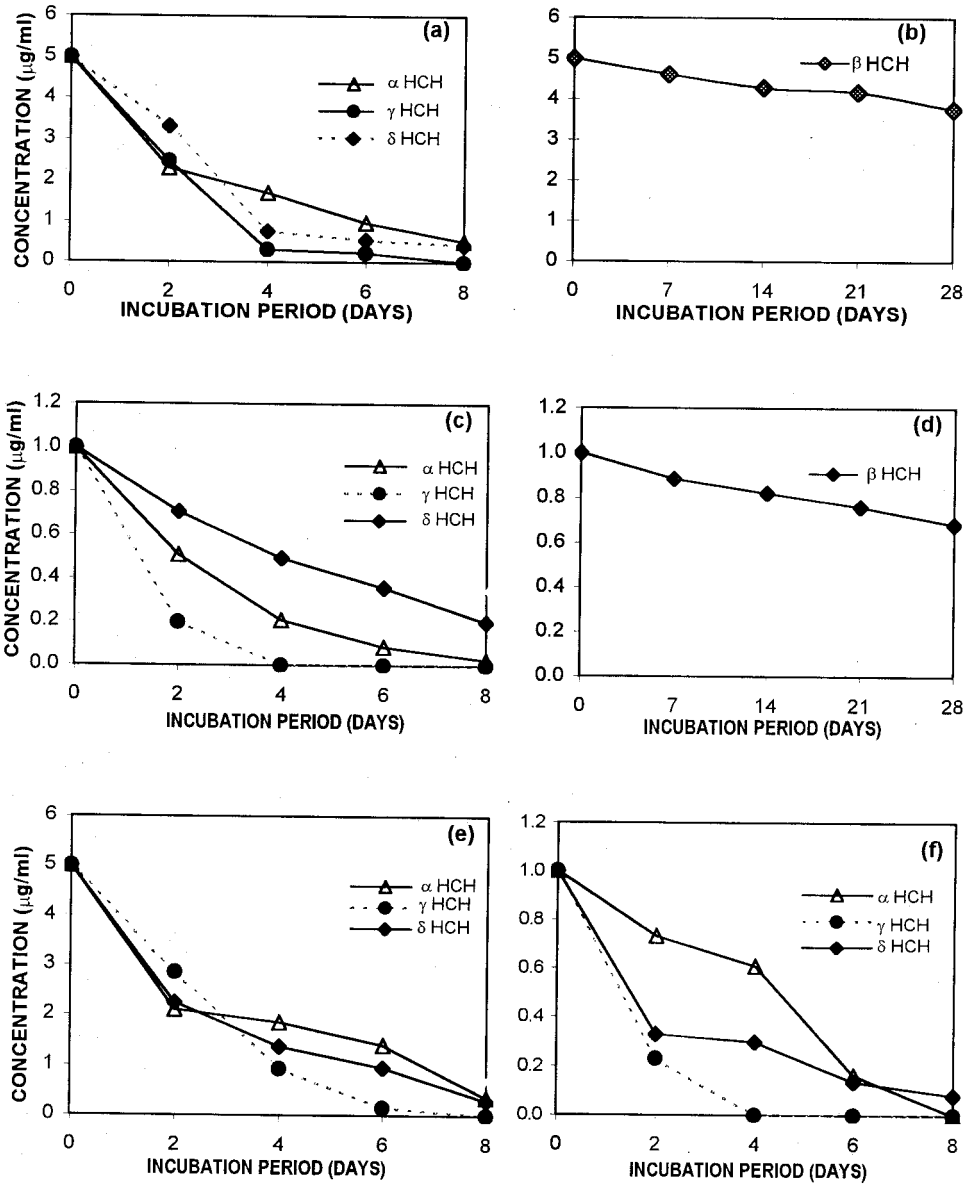

Figure 1. Degradation of HCH - isomers by acclimiatized *Alcaligenes faecalis* (S-1) at initial concentration of 5μg/ml (a,b); 1μg/ml (c,d); and by *A. faecalis* (S-2) at 5 μg/ml (e); and 1 μg/ml(f)

degradation was 94 and 92% at 5µg/ml and 1µg/ml, respectively on eighth day of incubation.

In the present study initial lag period for the degradation of HCH isomers was not observed. This can be attributed to the fact that these strains have been highly adapted to the HCH environment for more than two years and initial lag in degradation becomes shorter with successive application of pesticides due to enrichment of pesticide degrading micro–organisms (Sethunathan *et al.* 1978). In a long term experiment, Senoo *et al.* (1996) reported that the strains got adapted to HCH concentration if exposed during multiplication and afterwards degraded HCH without a lag period.

Earlier workers reported anaerobic conditions to be more suitable for microbial degradation of chlorinated hydrocarbon pesticides (Mac Rae *et al.* 1967; Castro and Yoshida 1974). However, Tu (1976) isolated several aerobic species of bacteria and fungi, which were able to degrade γ HCH. Some reports are now available which indicate that aerobic conditions are more feasible for HCH degradation (Bachmann 1988; Sahu *et al.* 1992; Gupta *et al.* 2000).

Bioremediation is a potential field and requires considerable attention directed towards exploitation of diverse bacterial isolates from the contaminated areas showing differential capacity of pesticide degradation. Very little information is available on this aspect. Further work, therefore, needs to be directed to identify the induced changes at the molecular level, which can be exploited for developing technologies for cleaner environment.

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