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INSECTICIDE SPECIFIC EMULSIFIER PRODUCTION BY HEXACHLOROCYCLOHEXANE UTILIZING PSEUDOMONAS TRALUCIDA Ptm$^+$ STRAIN.

Anu Appaiah, K.A.[1] and Karanth, N.G.K.*

1 Dept. of Applied Botany, Manasa Gangothri, Mysore-6.
* Infestation Control and Protectants, Central Food Technological Research Institute, Mysore-13, INDIA.

Abstract: The culture supernatant of Pseudomonas tralucida (Ptm$^+$ strain) produces an agent which can emulsify the insecticide hexachlorocyclohexane (HCH), which was secreted by resting cells. Emulsifying agent is most active with α-HCH, and has some activity towards other chlorinated and phosphatic insecticides. The emulsifier is a heat stable macromolecule and asssociated with growth of the organism on α-HCH.

Introduction: Emulsifiers or surfactants are surface active compounds which reduce the surface tension of a medium and allow insoluble substances to remain in a single phase (Cooper and Zazic, 1980). Bacterial utilization of lipophilic organochlorine insecticides as a sole source of carbon and energy is known to elicit formation of emulsifying substances for rapid uptake of the compounds (Banerjee et al., 1983). Previously we had reported growth of Pseudomonas tralucida Ptm$^+$ strain on α-HCH (Karanth et al., 1985). It was of interest to find out if this culture might produce specific emulsifying agent for growth with HCH.

Material and Methods: Culture conditins for Ptm$^+$ strain: Ptm$^+$ strain was routinely maintained on Seubert's mineral medium (Seubert, 1960) with 20ppm α-HCH, 500 mg% mannitol and 250 mg% yeast extract. 10% of 24h old culture was inoculated to Luria broth and was grown for 10h on a magnetic stirrer. This seed culture was further inoculated @ 2.5% to a basal salt medium (BSM) containing 7 g K_2HPO_4, 3 g KH_2PO_4, 0.1 g $MgSO_4$, 0.455 g urea with 20 g of mannitol in 1l of distilled water and pH adjusted to 7.2. 100ml of this medium was dispersed into 500 ml Erlenmeyer flasks, autoclaved and cultures were grown with 100ppm of α-HCH added as acetone solution (0.1ml), on a rotary shaker at 32°C and 200 rpm for 30h. The culture broth was centrifuged at 12000g for 15 m. The supernatant was collected and used as crude emulsifier.

Emulsifier Assay: 20mg of α-HCH taken as acetone solution (0.2ml) was added to 5ml of the above supernatant in a 150 x 20mm test tube, vortexed for 1m. The colloidal supernatant was carefully decanted after allowing the excess pesticide to settle for 2h at ambient conditions. Activity was measured at 660nm, using distilled water as blank. Basal salt medium treated in a similar way served as control.

Results and Discussions: In initial experiments it was observed that the culture broth centrifuged at 5000g for 15m. left an opaque supernatant. Serial dilution plating of this opaque liquid did not show growth of bacterial colonies. However, the supernatant after centrifuging at 12,000g for 15m. became clear. Careful examination showed the presence of slimy substance at the bottom of the tube, which could be sucked out using a Pasteur pipette.

The slimy pellet was extracted with n-hexane and the organic layer contained α-HCH as revealed by tlc analysis. At the sametime the clear supernatant turned turbid after vortexing with HCH. Therefore it appeared that growth of Ptm^+ strain with HCH led to the production of surface active compounds that could emulsify HCH.

In a separate experiment the surface tension of the medium was measured before and during the cell growth, and reduction in the surface tension from 70 dynes/cm^2 to 24.5 dynes/cm^2 was recorded.

Data suggested that growth peak and emulsifier activity peak occur at different times (table1). Turbidimetric studies indicated that in Luria broth, maximum growth (late log phase) occurred at 12h and emulsifier activity peak appeared at 48h. However, in BSM the culture reached the late log phase after 28h and the emulsifier peak at 32h. The wide time interval between growth peak and activity peak in Luria broth (36h) and short time lag (4h) in BSM suggests HCH is involved in the production and release of the surfactant. Further, the 2 fold increase in the emulsifier activity observed in BSM containing HCH supports the above view.

TABLE 1. EFFECT OF MEDIUM COMPOSITION ON THE GROWTH AND EMULSIFIER ACTIVITY IN PSEUDOMONAS TRALUCIDA Ptm^+ STRAIN.

Medium	Growth peak A_{600}^{nm} (h)[a]	Appearance of Emulsifier* activity at late log phase (A_{660}^{nm})	Emulsifier peak (h)	Maximum* emulsifier activity (A_{660}^{nm})
Luria Broth	12	0.13	48	0.52
Basal salt medium with α-HCH	28	0.35	32	0.96

(a) Cell growth was measured turbidmetrically at 600 nm against distilled water blank.
* Emulsifier activity was checked as described in the text.

Different lipophylic pesticides were added to the 12,000g supernatant and the emulsification activity was measured. Results in table 2 show that this bioemulsifier produced by Ptm^+ strain is specific to α-HCH, with negligible activity for various Organophosphates and organochlorines, in agreement to the results of Banerjee et al., (1983) and Patel and Gopinathan (1986). Working with 2, 4, 5-trichlorophenoxy acetic acid

(2,4,5-T), Banerjee et al., (1983) have reported that compounds which do not form the growth substrates for the organism are not emulsified. We have not tested the growth of our organism in the insecticides included in table 2 except α-HCH.

TABLE 2. SUBSTRATE SPECIFICITY OF BIOEMULSIFIER PRODUCED BY Ptm$^+$ STRAIN

Pesticides[a]	Nature of the chemical	Activity (%)
a. Organochlorine		
1. α-HCH	Solid	100.00
2. DDT*	Solid	36.86
3. Heptachlor	Solid	5.10
4. Chlorodane	Liquid	-
b. Organophosphatic		
1. Ethion	Liquid	6.99
2. Endosulphan	Solid	1.51
3. Bromophos	Solid	-

a All pesticides were added to 5 ml culture supernatant at 20mg level as acetone solution (0.2 ml). The mixture was vortexed for 1m., kept at room temperature for 2h. before taking the reading.
* DDT = Dichloro diphenyl trichloroethane.

Preliminary studies of the properties of this surfactant (table 3) shows that the emulsifier specific to α-HCH is heat stable and non-dialysable. This observations is consistent with the results of Patel and Gopinathan (1986). Surfactant produced by Pseudomonas cepacia degrading 2,4,5-T has been known to be non-dialysable but heat labile molecule (Banerjee et al., 1983).

TABLE 3. EFFECT OF PHYSICAL TREATMENTS ON THE EMULSIFICATION ACTIVITY OF PSEUDOMONAS TRALUCIDA Ptm$^+$ STRAIN CULTURE SUPERNATANT.

Treatment	Activity (%)
1. Supernatant without any treatment	100.00
2. Boiled at 100°C for 30 min	89.34
3. Evoporated to dryness by direct heating*	76.37
4. Autoclaved (121°C for 20 min)	60.55
5. Dialysis[a]	91.21

* After complete evoporation the volume was adjusted to orginal volume with distilled water.
a Culture supernatant was dialysed 3 times against 100 volumes of distilled water in 12,000 cutout dialysis sac.

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