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# Isolation, Screening and Identification of Bacteria Capable of Degrading Chlorpyrifos and Endosulfan

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## ABSTRACT

Soil samples rich in pesticides were collected from agricultural fields in and around Bangalore. Samples were inoculated into mineral salts medium with chlorpyrifos and endosulfan as the sole source of carbon and incubated for 90 days. Intermittent addition of pesticide was carried out for enrichment of cultures. Bacteria capable of degrading chlorpyrifos and Endosulfan were isolated on mineral salts agar medium. Out of 27 samples collected, 58 isolates capable of degrading chlorpyrifos and 68 isolates capable of degrading endosulfan were obtained, majority of them being gram negative in nature. Out of these 23 isolates for chlorpyrifos and 28 isolates for endosulfan were capable of showing good growth in mineral salts medium. Out of the 23 isolates for chlorpyrifos, 10 isolates and out of 28 isolates for endosulfan, 7 isolates were able to show evident colour change on medium with phenol red. Screening of the isolates for their efficiencies in degrading chlorpyrifos and endosulfan was carried out based on spectrophotometric analysis. Strains CHS23 and ENS10, showed the highest degradation of 38% and 42% respectively. CHS23 and ENS10 were identified as *Pseudomonas putida and Pseudomonas aeruginosa* respectively based on nucleotide sequence and phylogenetic tree analysis. Nucleotide sequences of *Pseudomonas putida and Pseudomonas aeruginosa* were deposited in NCBI gene bank with accession numbers JQ701740 and JX204836 respectively.

**Keywords:** Chlorpyrifos, Endosulfan, Biodegradation, *Pseudomonas putida, Pseudomonas aeruginosa*

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# INTRODUCTION

Chlorpyrifos [O, O-diethyl *O*-(3, 5, 6-trichloro-2-pyridyl) phosphorothioate] is one of the most commonly and widely used commercial organophosphate insecticide.[1] It is effective in controlling a variety of insects including cutworms, corn rootworms, cockroaches, grubs, flea beetles, flies, termites, fire ants and lice. It has been used as an insecticide on grain, cotton, fruit, nut and vegetable crops as well as on lawns and ornamental plants.[2] Chlorpyrifos is a neurotoxin and suspected endocrine disruptor and it has been associated with asthma.[3]

Endosulfan (6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,3,4-benzo-dioxathiepin-3-oxide) is a cyclodiene organo-chlorine. It is used extensively throughout the world as a contact and stomach insecticide and as an acaricide on field crops like cotton, paddy, sorghum, oilseeds, coffee, vegetables and fruit crops.[4] It is used to control chewing and sucking insects such as Colorado beetle, flea beetle, cabbage worm aphids and leaf hopper.[5] It has been implicated in mammalian gonadal toxicity[6], genotoxicity[7] and neurotoxicity.[8]

Considerable amount of work has been done on chlorpyrifos degradation by bacteria[9-12] and fungi[13-15] isolated from agricultural soil and other sources. There are also reports on endosulfan degradation by bacteria[16-19] and fungi.[20-22]

With the aim of isolating, screening and identifying efficient bacteria in degrading chlorpyrifos and endosulfan the present topic has been selected.

# EXPERIMENTAL SECTION

## PESTICIDE AND OTHER CHEMICALS

Commercial-grade insecticide chlorpyrifos and Endosulfan were procured from a pesticide selling shop in Bangalore (Fig. 1 and 2). Other chemicals were procured from Hi Media Pvt. Ltd. Mumbai.

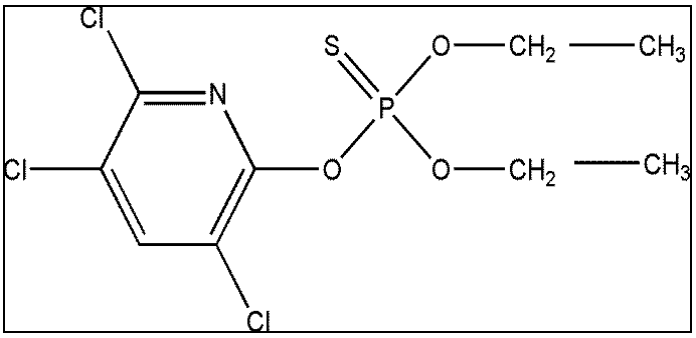


**Fig. 1: Structure of Chlorpyrifos**

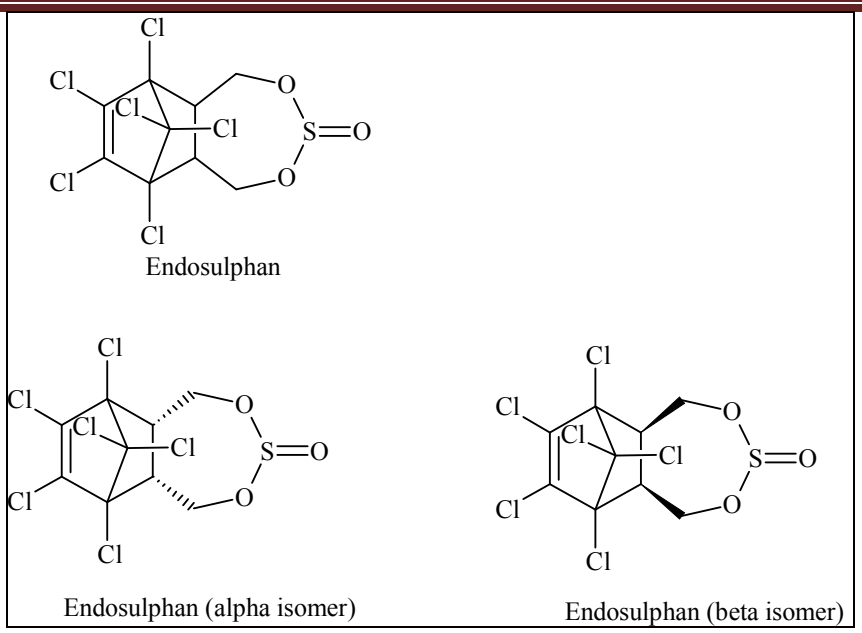


**Fig. 2: Structure of Endosulfan and its isomers**

## ISOLATION, ENRICHMENT AND SCREENING

Twenty seven soil samples were collected in sterile polythene bags from different agricultural sites in and around Bangalore having a history of repeated application of Chlorpyrifos and Endosulfan. One gram of each soil sample was inoculated into 100 ml of mineral salts media enriched with 1% Chlorpyrifos[23] and also into 100 ml of non-sulfur medium with 1% Endosulfan as the sole source of carbon.[24] Flasks were incubated for 90 days at 37°C under static condition with intermittent addition of pesticide for enrichment of cultures. Bacteria capable of degrading Chlorpyrifos and Endosulfan were isolated and pure cultures were maintained on mineral salts agar medium with 1% pesticide as sole source of carbon.

Loopfuls of each culture were inoculated into fresh mineral salts media with Chlorpyrifos and Endosulfan. These cultures were incubated till log phase. Five ml ($4x10^2$ CFU/ml) inoculum from each flask were inoculated into 100 ml of fresh mineral salts medium with pH 7 containing 2% pesticide and the flasks were incubated at 37°C under static condition. To monitor the growth of cultures O.D. was measured at 660 nm every 24 hrs. Loopfuls of cultures were also inoculated into mineral salts agar media containing 2% pesticide and 0.02% phenol red indicator.[25] Cultures showing higher O.D. by the end of 7 days and those showing maximum zone of colour change from red to yellow due to production of acidic products were selected for further studies.

Further screening of the isolates was carried out by estimating the amount of Chlorpyrifos according to Khan *et al.*[26] and Endosulfan according to Venugopal and Sumalatha.[27]

Percent degradation of the pesticide by the cultures was calculated using the formula: Percent degradation = [(Initial concentration-Final concentration)/Initial concentration] x 100

**IDENTIFICATION OF BACTERIA**

Preliminary identifications of all the cultures were done by Gram's staining. The isolate CHS23 and ENS10 which showed better degradation efficiency of chlorpyrifos and Endosulfan respectively were identified upto the genus level by biochemical tests[28] and upto species level based on nucleotide sequence and phylogenetic tree analysis carried out by Bioserve India Pvt. Ltd., Hyderabad and Bhat Bio-tech India Pvt. Ltd., Bangalore. Nucleotide sequences of both the cultures were deposited in NCBI Gene Bank.

## RESULTS AND DISCUSSION

Fifty eight isolates capable of degrading chlorpyrifos and 68 isolates capable of degrading endosulfan were obtained from twenty seven samples. Out of these 23 isolates for chlorpyrifos (Table 1) and 28 isolates for endosulfan (Table 2) were capable of showing good growth in mineral salts medium. Out of the 23 isolates for chlorpyrifos, 10 isolates and out of 28 isolates for endosulfan, 7 isolates were able to show evident colour change on medium with phenol red (Fig. 3). Majority of the isolates were gram negative in nature. Among all isolates CHS23 and ENS10 were able show maximum of 38% and 42% degradation of chlorpyrifos and endosulfan respectively.

Based on biochemical tests the isolate CHS23 and ENS10 were tentatively identified as *Pseudomonas* spp. Based on nucleotide sequence and phylogenetic tree analysis, CHS23 was identified as *Pseudomonas putida* (Fig. 4) and ENS10 as *Pseudomonas aeruginosa* (Fig. 5). The nucleotide sequence of *Pseudomonas putida* and *Pseudomonas aeruginosa* was deposited in NCBI gene bank with accession numbers JQ701740 and JX204836 respectively.

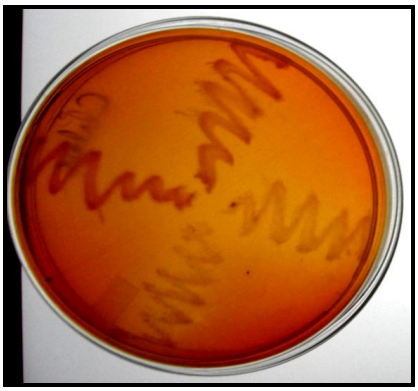

**Fig. 3: Growth of isolates on medium with phenol red**

**Table 1: Isolates for Chlorpyrifos degradation**

| Isolate | O.D. at 660nm | % Degradation | Gram Character |
|---|---|---|---|
| CHS01 | 0.05 | 25 | Gram negative coccobacilli |
| CHS05 | 0.07 | 28 | Gram Negative short Rods |
| CHS09 | 0.05 | 26 | Gram Negative long Rods |
| CHS11 | 0.03 | 21 | Gram Negative short Rods |
| CHS14 | 0.04 | 24 | Gram Negative short Rods |
| CHS15 | 0.03 | 20 | Gram Negative short Rods |
| CHS18 | 0.03 | 21 | Gram negative coccobacilli |
| CHS20 | 0.03 | 24 | Gram Negative short Rods |
| CHS22 | 0.04 | 25 | Gram Negative short Rods |
| **CHS23** | **0.10** | **38** | **Gram negative short rods** |
| CHS30 | 0.06 | 25 | Gram Negative short Rods |
| CHS32 | 0.04 | 20 | Gram Negative short Rods |
| CHS37 | 0.01 | 19 | Gram Negative short rods |
| CHS38 | 0.01 | 20 | Gram negative coccobacilli |
| CHS40 | 0.03 | 22 | Gram Negative short Rods |
| CHS43 | 0.01 | 20 | Gram Negative long Rods |
| CHS46 | 0.01 | 19 | Gram Negative short Rods |
| CHS48 | 0.04 | 26 | Gram Negative short Rods |
| CHS49 | 0.01 | 18 | Gram Positive Rods |
| CHS50 | 0.03 | 23 | Gram negative coccobacilli |
| CHS52 | 0.04 | 21 | Gram Negative short Rods |
| CHS56 | 0.08 | 29 | Gram Positive Rods |
| CHS57 | 0.02 | 19 | Gram negative coccobacilli |

**Table 2: Isolates for Endosulfan Degradation**

| Isolate | O.D. at 660nm | % Degradation | Gram Character |
|---|---|---|---|
| ENS01 | 0.09 | 37 | Gram Positive cocci |
| ENS02 | 0.01 | 25 | Gram Negative Long rods |
| ENS05 | 0.01 | 27 | Gram Negative Long rods |
| ENS07 | 0.02 | 24 | Gram Negative Short rods |
| ENS08 | 0.06 | 32 | Gram NegativeCoccobacilli |
| **ENS10** | **0.29** | **42** | **Gram Negative Short Rods** |
| ENS11 | 0.04 | 26 | Gram Negative long rods |
| ENS15 | 0.01 | 24 | Gram Negative long rods |
| ENS17 | 0.03 | 24 | Gram Positive Rods |
| ENS19 | 0.03 | 25 | Gram Positive Rods |
| ENS21 | 0.03 | 25 | Gram Negative long rods |
| ENS24 | 0.04 | 26 | Gram Negative long rods |
| ENS26 | 0.06 | 27 | Gram Negative Short rods |
| ENS29 | 0.04 | 25 | Gram Negative Long rods |
| ENS32 | 0.10 | 35 | Gram Negative long rods |
| ENS35 | 0.06 | 28 | Gram Negative Long rods |
| ENS38 | 0.01 | 25 | Gram Negative Short rods |
| ENS41 | 0.05 | 30 | Gram Positive rods |
| ENS45 | 0.13 | 32 | Gram Negative Short rods |
| ENS46 | 0.22 | 34 | Gram Negative Long rods |
| ENS49 | 0.05 | 29 | Gram Negative Long rods |
| ENS52 | 0.02 | 23 | Gram Negative Short rods |
| ENS58 | 0.05 | 31 | Gram Positive rods |
| ENS60 | 0.15 | 32 | Gram Negative Long Rods |
| ENS62 | 0.01 | 25 | Gram Negative Long Rods |
| ENS64 | 0.22 | 36 | Gram Negative Short Rods |
| ENS66 | 0.01 | 24 | Gram Negative Short Rods |
| ENS67 | 0.07 | 39 | Gram Negative Short Rods |

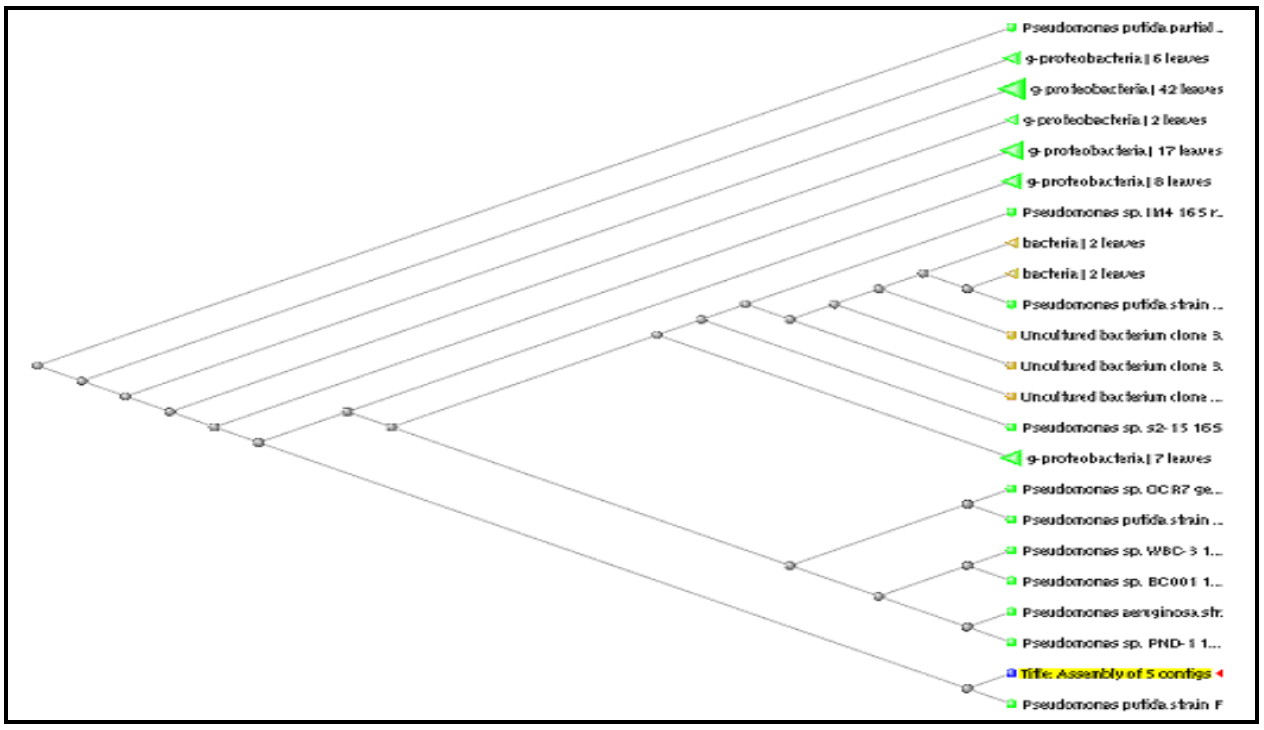


**Fig. 4: Phylogenetic tree of CHS23**

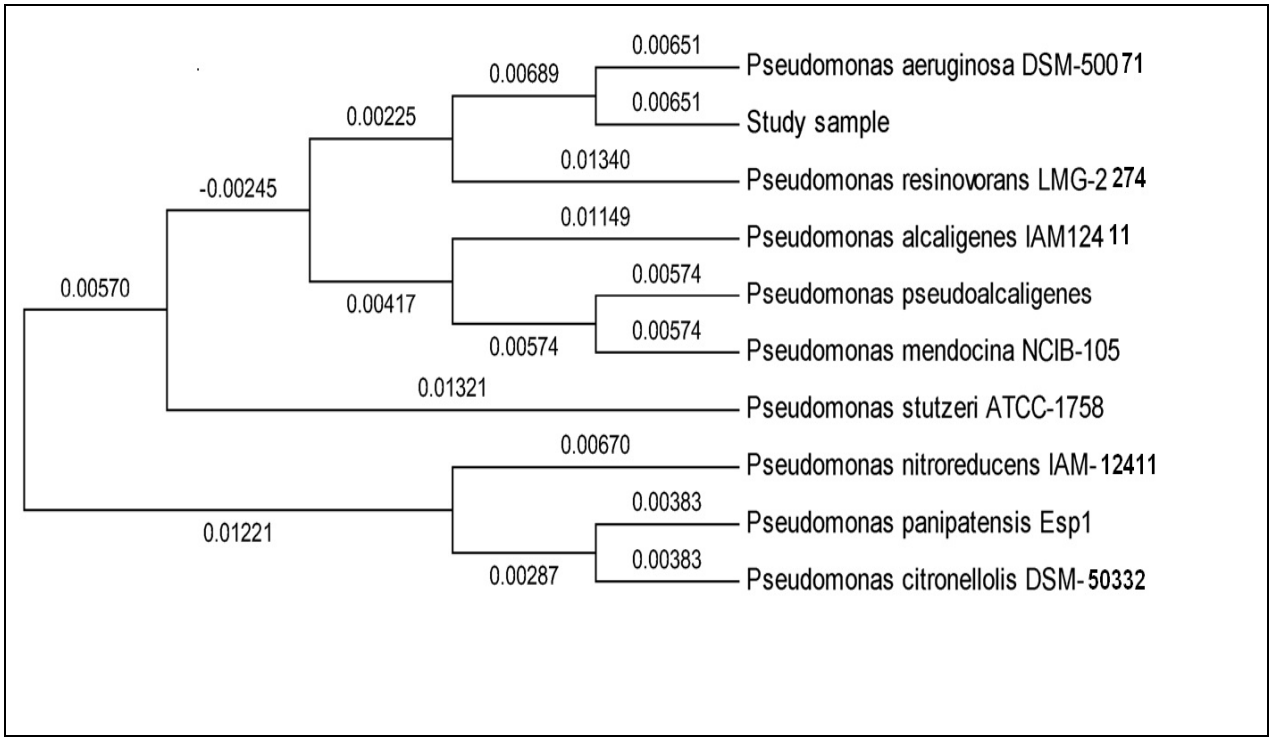


**Fig. 5: Phylogenetic tree of ENS10**

## CONCLUSION

Among the isolates obtained for degradation of both chlorpyrifos and endosulfan, majority were gram negative in nature. There are literatures available on efficiencies of gram negative bacteria in degradation of pesticides.[29-31]Further few species of *Pseudomonas* are known to be more potent in biodegradation of pesticides.[32-33] *Pseudomonas putida* and *Pseudomonas aeruginosa* isolated in the current study were found to be efficient in degrading chlorpyrifos and endosulfan respectively compared to others and can be used for bioremediation of soils contaminated with these pesticides.

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