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## Tapping *In-Silico* For The Super Enzyme For Biodegradation Of Pesticides

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**ABSTRACT**

The objective of present study is to identify the diversity of pesticide degrading enzymes across various domains of life using *in-silico* methods. The amino acid sequence of nitrile hydratase (*Ensifer meliloti*, NCBI) and pyrethroid hydrolase enzyme (*Aspergillus amawori*) were retrieved from NCBI and these sequences were used to identify possible homologs in various phyla of microorganisms by *in-silico* method. The homologs were found to belong to several untapped phyla of bacteria and fungi. Appreciable identity for nitrile hydratase was found in 10 phyla of bacteria (Archaebacteria, Actinobacteria, Chloroflexi, Deinococcus-thermos, Firmicutes, alpha proteobacteria, beta proteobacteria, gamma proteobacteria, Synergistetes, Thermodesulfobacteria) and 2 phyla of fungi (Chytridiomycota, Microsporidia). For pyrethroid hydrolase, microorganisms from 1 phyla of eubacteria (Delta proteobacteria) and 3 phyla of fungi (Ascomycota, Basidiomycota, Zygomycota) showed identity more than the threshold value. These homologs when further analyzed by conserved sequence comparative analysis have been found to contain potential pesticide degrading domains. These undiscovered agents can be directly screened for the biodegradation of pesticides to check the residues formed after biodegradation and the approach can further be improved by using recombinant DNA techniques.

**Keywords:** BLAST homologs, bioremediation, enzymes, *in-silico*

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## INTRODUCTION

Indian economy has a major cornerstone in terms of agriculture. In order to ensure the high yield of crops for the increasing population, protection from pests is an important aspect [1]. Pesticides are the organic chemical substances that are premediated extensively to repel, kill any pest either acting broadly or specifically for a particular pest.[2] These are classified on the basis of mode of action, mode of entry, type of target organism, the chemistry, etc. On the basis of the chemical composition they are broadly classified as Organo halogen, Organophosphorous, Carbamates, Pyrethroids, Neonicotinoids, Spinosyns (spinosad), Pyrroles (chlorfenapyr) , Quinazolines (fenazaquin), Benzoylureas (diflubenzuron an IGR), Antibiotics (abamectin) etc., [3]. Pesticide use on global scale is increasing day by day. Almost 98% of these pesticides are contributing as a pollutant in various resources as they have the capability of high persistence in the soil. [4]The leaching out in the soil leads to ground water contamination and non-specific effect on the various organisms.[5] Soil is composed of several nutrients and harbors a diversity of microorganisms that contributes in the nutrient cycling, soil fertility and various beneficial interactions with plants.[6] Many of the pesticides have high persistence in the soil which do not degrade easily and effect the beneficial microflora of soil leading to deterioration of soil quality by their accumulation[7] The degradation of pesticides in the soil depends on many factors including the temperature, moisture content, pH of the soil and type of microorganisms present in the soil.[8] Various species of bacteria including *Bacillus sp., Micrococcus sp., Acidomonas sp., Streptomyces sp., Achromobacter sp., Pseudomas sp*. [9], are present in the soil and possess enzymes that can degrade the pesticides.

### Enzyme system for the biodegradation of pesticides

Zhou et al.,2014 successfully demonstrated that the nitrile hydratase enzyme of *Ensifer meliloti* CGMCC 7333 is responsible for the biotransformation of acetamiprid to the N-amidoamide metabolite which is unstable and degrades to form a chlorinated pyridyl methylmethanamine compounds [10]. The biochemical reactions for degradation are achieved through a number of different enzymes such as dehydrogenases, dioxigenases, ligninase [11]

| S.No | Pesticide class | Mode of Action | Target organisms | Persistence in soil | M/o Reported for Biodegradation | Enzymes Identified for Biodegradation |
|---|---|---|---|---|---|---|
| 1 | Organohalogens (Heptachlor) | Central nervous system stimulant GABA-gated chloride channel antagonist. weak androgen receptor antagonist. | Termites, Cutworms, Weevils, Wireworms, Japanese beetles | 4-30 years | *Phlebia tremellosa Phlebia acanthocystis, Nocardia spp. Fusarium spp.* | Hydrolases, Hydroxylases [17] [18] |
| 2 | Organophosphates (Monocrotophos) | Ability to inactivate the enzyme acetylcholinesterase Non-systemic, selective with stomach action. Mitochondrial complex III electron transport inhibitor (hydromethylnon) | Aphids, Common mires, Spiders, Ticks, Caterpillars | 1-35 days | *Aspergillus Oryzae* | Phosphatase [19] |
| 3 | Pyrethroids (Cypermethrin) | Prevent the closure of the voltage-gated sodium channels in the axonal membranes | Lepidoptera species | 2.4-58.3 days | *Micrococcus spp., Aspergillus sp.* | Esterase, 3-phenoxybenzaldehyde dehydrogenase, 3-phenoxybenzoate dioxygenase, phenol hydroxylase, |

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| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | | | protocatechuate-3,4-dioxygenase and catechol-1,2-dioxygenase, Pyrethroid Hydrolase [20] |
| 4 | Neonicotinoids (Imidachloprid) | Selectively bind and interact with the insect nicotinic acetylcholine receptor site. | Plant hoppers, Aphids, Termites, Colorado bettle, Fleas, White grubs | 77-341 days | *Bacillus aerophilus, Klebsiella pneumoniae BCH1.* | Nitrile Hydratase [21] |
| 5 | Spinosyns (Spinosad) | Targeting binding sites on nicotinic acetylcholine receptors (nAChRs) of the insect nervous system that are distinct from those at which other insecticides have their activity. | Thrips, Leaf miners, Spider mites, Fruit flies | 9-17 days | | |
| 6 | Acaricides (Bifenthrin) | Non-systemic with contact action, inhibits oxidative phosphorylation | Fleas, Armyworms, Earwigs, Sowbugs | 54.2-173 days | *Sphingobium sp. Strain JZ-2* | Pyrethroid hydrolase [22] |
| 7 | Miticides (Dimethonate) | Inhibits electron transfer binding the center at complex I in mitochondria | Thrips, Leaf miners, Spider mites, Fruit flies | 2.1-4.3 days | *Aspergillus Niger* | Esterases, Phosphatases [23] |

**Table 1. Classification of pesticides their respective mode of action, target organisms and micro-organisms involved in biodegradation.**

## MATERIAL AND METHODS

### Data collection

An extensive literature survey was done to identify various enzymes reported for biodegradation of Pyrethroids and Neonicotinoids. There are a number of enzymes from various micro-organisms reported to cause biodegradation of these pesticides, but the amino acid sequences for only a few was found to be available. The sequences used as query were nitrile hydratase enzyme of *Ensifer meliloti* CGMCC 7333, responsible for the biotransformation of acetamiprid and pyrethroid hydrolase of *Aspergillus awamori* which causes biodegradation of pyrethroid. The amino acid sequences of these enzymes were retrieved in FASTA format from NCBI database.

### BLAST (Basic Local Alignment Search Tool)

The sequences selected were then used to conduct a PSI-BLAST search in various domains of life to identify homologs of these proteins. The search was conducted in bacteria, algae and fungi using blast-psi with default BLOSUM-62. The BLASTp hits obtained were surveyed such that the matching sequences with an expect value equal to or lower than $10^{-5}$ to at least one species of each of the phyla of Eubacteria were selected. The protein hits obtained were verified by CDART [12] for the domain present and hence classified as involved in pesticide degradation or other functions.

### Conserved domain search

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Conserved domains of the species showing identity more than 40% were reviewed from NCBI and the domains which were similar to the nitrile hydratase and Pyrethroid Hydrolase were recorded.

## RESULTS

Out of 31 bacteria phyla only 9 phyla showed species having more than 40% identity for the enzyme nitrile hydratase, and only 2 phyla in fungi showed more than 40% similarity for the same enzyme. After doing the conserved domain search, only 6 phyla showed the nitrile hydratase beta subunit domain. Zhou et al., 2014, found nitrile hydaratase in *Ensifer meliloti* of alpha proteobacteria, whereas the presence of same enzyme is likely to present in other phyla as well that are not identified yet.

In case of pyrethroid hydrolase 10 phyla of kingdom eubacteria and 2 phyla of fungi showed identity more than the threshold, as shown in table. Only ascomycetes showed the pyrethroid hydrolase conserved domain, representing that since the query sequence belongs to Ascomycota thus probability of finding more species consisting of same enzyme is Ascomycota only[13] found pyrethroid hydrolase in *Aspergillus sp*. of Ascomycota phyla, the conserved domain search showed the presence of the same enzyme in 30 more species that are not found yet.

| S.No. | Bacteria | Nitrile hydratase | Conserved domain |
|---|---|---|---|
| (1) | Archaebacteria | 8 | Nitrile hydratase beta subunit (8) |
| (2) | Actinobacteria | 125 | Nitrile Hydratase Beta subunit (>40) |
| (3) | Firmicutes | 8 | Nitrile hydratase beta subunit (8) |
| (4) | Alpha proteobacteria | 148 | Nitrile Hydratase Beta Subunit (>40) |
| (5) | Beta proteobacteria | 97 | Nitrile Hydratase Beta Subunit (>40) |
| (6) | Gamma proteobacteria | 84 | Nitrile Hydratase Beta Subunit (>40) |
| (7) | Synergistetes | 1 | Oxidoreductase |
| (8) | Thermodesulphobacteria | 3 | Oxidoreductase |
| (9) | Chloroflexi | 7 | Beta subunit (7) |
| (10) | Deinococcus- thermos | 1 | Domain of unknown function |

**Table 1.1 homologous sequences and conserved domains of nitrile hydaratase in different phyla of kingdom Eubacteria.**

| S.No. | Fungi | Nitrile Hydratase (Homologous sequence) | Conserved Domain |
|---|---|---|---|
| (1) | Chytridiomycota | 1 | Malate Synthase |
| (2) | Microsporidia | 1 | Thioesterase domain |

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**Table 1.2 homologous sequences and conserved domain of nitrile hydratase in different phyla of kingdom Fungi.**

| S.No. | Kingdom | Phylum | Pyrethroid Hydrolase | Conserved Domain |
|---|---|---|---|---|
| (1) | Bacteria | Actinobacteria | 1 | Transketolases |
| (2) | Fungi | Ascomycota | 39 | Alpha/Beta hydrolase |
| | | Basidiomycota | 11 | Transketolases and primase domain |
| | | Zygomycota | 1 | Lipase domain |

**Table 1.3 Homologous sequences and conserved domains of pyrethroid hydrolase in different phyla of kingdom Eubacteria and Fungi.**

## DISCUSSION

Most of the microbes reported till date to be involved in biodegradation of pesticides belong to proteobacteria. The current study reports possibility of pesticide degrading microbes from various untapped phyla. After performing the conserved domain search based on BLAST homology only Ascomycota division of kingdom Fungi represents alpha/beta domain of pyrethroid hydrolase, as the enzyme origin is Ascomycota so probability of finding similar domain in Ascomycota is more. Other enzyme domains including transketolase and lipase are also observed in the BLAST homologs. The lipases carry out the breakdown of triglycerides into free-fatty acids and glycerols, thus can be used in bioremediation of oil-spills [14] but reports on involvement of lipases in pesticide degradation is unreported till date. The homology observed in the current study represents some degree of evolutionary relationship between the lipases and pyrethroid hydrolases. The transketolases have not been reported to be carrying out pesticide degradation, but as reported by [15], the presence of chorpyrifos lead to an upregulation of transketolase transcript in *Anabaena*.

For Nitrile Hydratase most of the results were tapped in Actinobacteria and Proteobacteria, few results were found for the same domain in Firmicutes. Other than the targeted domain, other domains were also found in BLAST homologs including thioesterase, oxidoreductase and malate synthase. Thioesterases catalyses the conversion of esters into alcohols, thus involved in pesticide degradation. Malate synthase is involved in glyoxylate pathway, oxidoreducatses catalyses the detoxification of various toxins and consist of oxygenases that carry out the oxygenation of aromatic organic compounds and increase their water solubility. The appreciable homology between the query sequence and the above-mentioned domains shows an evolutionary relationship between them and could have some role in pesticide degradation. Thioesterases have been reported to be involved in Phenylacetyl-CoA catabolic pathway (phenylacetyl-CoA is a major environmental contaminant). Oxidoreductases have also been reported to be involved in degradation pathways for heterocyclic aromatic compounds [16]. Malate synthase is present in all domains except in archaea, in different forms but a report for involvement in pesticide degradation is not available till date.

The study shows the dominant phyla with enzymes linked with degradation of pesticides. The information on presence of such enzymes in nature through non-cultivable approach shows the possibility of presence of an array of unexplored organisms and enzymes which can be used directly or made more efficient by metabolic engineering for biodegradation of contaminants.

## CONCLUSION

Pesticides have potential harmful effect on natural environment and human health. These pesticides can be degraded by microorganisms in lesser toxic forms and is one of the most important strategy for the removal of pollutants. Several enzymes have been reported that are involves in the biodegradation of pesticides but still there is a need to find the more efficient enzymes. These enzymes cab be tapped in-silico in different phyla of micro-organisms that have the potential to degrade pesticides efficiently. In the study, amino acid sequences of enzymes involved in biodegradation of neonicotinoids and pyrethroids were retrieved and their homologs in different phyla were tapped. The homologs with similar conserved domain can be directly screened

for biodegradation of pesticides and furthermore efficient recombinant strains can be formed to increase the efficacy of biodegradation.

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