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In-Silico Feasibility of Novel Biodegradation Pathways For1-Naphthyl Methylcarbamate

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Abstract: Bioremediation is a promising pollution treatment method in the elimination or minimization of anthropogenic compounds in the environment.*In-silico* approach using variety of computational tools to predict novel biodegradation pathways for pollutant degradation permits to explore the potential of microorganisms in cleaning up the particular compound from the environment.However, given the wealth of novel pathways obtained using prediction methods, it is necessary to evaluate their relative feasibility, particularly within the context of the cellular environment. In this study, we have utilized computational tools like UMBBD-PPS and the eMolecules database. We have identified several potent bacteria that can be used to accomplish bioremediation of 1-naphthyl methylcarbamate/Carbaryl.Through this work, computational tools are shown to be useful in the design and evaluation of novel xenobiotic biodegradation pathways and identifying cellular feasibility degradation routes.

Key words: Biodegradation · 1-Naphthyl Methylcarbamate · Carbaryl · *In-silico* · UMBBD-PPS · Insecticides

INTRODUCTION

Various chemicals are entering the environment due to anthropogenic activity like industrial production, agricultural activities etc. Industries generate useless by-products and waste materials with 1 to 10% of the quantity of parent chemicals during the production [1]. Several hundred pesticides of different chemical compositions are currently used for agricultural and control purposes all over the world. Because of their extensive use, they are detected in various environmental matrices such as soil, water and air [2]. The prevalence and widespread use of man-made chemicals/xenobiotics has led to a focused effort to establish new technologies to reduce or eliminate these contaminants from the environment. Commonly used pollution treatment methods such as incineration, landfilling and air stripping arehaving adverse effects on the environment [3,4]. Additionally, these methods are costly and sometimes inefficient. Therefore, it is important to develop alternative methods of biodegradation that are effective, minimally hazardous and economical. Biological methods for the bulk removal of these pollutants are therefore generally preferred [5]. One promising treatment method is to exploit the ability of microorganisms to use these pollutants as foreign substances for their maintenance and growth, a process known as bioremediation [6]. Bioremediation/biodegradation is the field of biology that incorporates the exploitation of biological catalysts/microorganisms for decontaminating the environmental pollutants. Some microorganisms have acquired the ability to catabolize chemical compounds that do not form part of their central metabolism as they face them in the environment [7]. Some bacteria, fungi, algae and yeast have special properties and functional groups in their cell walls that can decrease metal ion concentrations from ppt to ppb with high efficiency [8]. The objective of bioremediation study is to stimulate such microorganisms to destroy the contaminants. The technology can take advantage of a natural metabolic pathway or genetically modify an organism to have a particular toxic appetite [9]. Choice of specific

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microorganisms for the degradation remains the key factor which consumes moderate time and resources. *In-silico* approach can help to save this energy and resources as it will give a fair idea about chemical nature of contaminant, pathways involved in degradation and microorganisms responsible for such degradation.

Carbaryl/1-naphthyl methylcarbamate is a white solid crystalline chemical in the carbamate family used chiefly as an insecticide. Over 90% of synthetic insecticides are organophosphates, carbamates and pyrethroids with action on the nervous system [10]. Carbamate insecticides are reversible inhibitors of the enzyme acetylcholinesterase which interfere with nervous system and causes death. Carbaryl is widely used to control foliar insects but it also affects non-target soil organisms like earthworm. After 20 minutes of exposure to 0.125 ppm carbaryl, locomotion and geotaxis of earthworm get significantly affected; head abnormalities were also reported at this concentration. The hatching of cocoon alters at 0.5 ppm [11]. Solubility of Carbaryl in water is very low hence it remains in atmosphere for significant time.

Carbaryl is classified by the World Health Organization (WHO) as moderately hazardous (class-II) chemical [12]. An *ex-situ* study (bioremediation study away from the contamination site) carried out in laboratory shown *Pseudomonas sps*. strains C4, C5 and C6 utilize Carbaryl as the sole source of carbon and energy. Carbaryl is metabolized via 1-naphthol, 1,2 dihydroxynaphthalene and Gentisate [13]. This study was carried out in laboratory using culture media and not in the soil at contaminated site. Generally contaminants show positive results under laboratory conditions but *in-situ* degradation of such compounds become difficult because of non-availability of the desired microbial flora in the soil or some other field variables and hence the desired microbial flora is added from outside at the contaminated site. In such scenario, availability of information regarding maximum number of bacteria responsible for *in situ* biodegradation of the compound is necessary.

In order to fully explore the capabilities of microorganisms in cleaning up the environment, the use of computational tools to predict novel biodegradation pathways for pollutants and to gain a better understanding of the fate of these compounds in the environment would be valuable [14]. Prediction methods such as the Pathway Prediction System (PPS) [15], META [16] and others [17-21] rely on databases of rules describing bio-transformations that occur in cellular and environmental processes. In this work, we describe the evaluation of novel pathways and capable bacteria to degrade the Carbaryl using University of Minnesota Biocatalysis/Biodegradation Pathway Prediction System (UMBBD-PPS) which can help us to design *in-situ* as well as *ex-situ* experiment to verify the role of these bacteria in Carbaryl degradation.

MATERIALS AND METHODS

Compound details of Carbaryl like molecular weight, molecular formula, SMILES, source and compound ID were collected from eMolecules database.eMolecules is a database for chemical molecules which contains more than 7.0 Million unique molecules from commercial suppliers, like Acros, ASINEX, ChemBridge, ChemDiv, Comgenex, Enamine Ltd, Fluka, InterBioScreen, Key Organics, Life Chemicals, Maybridge, Otava, Sigma-Aldrich, Specs and many more. The database also contains all entries provided by NIST, PubChem and DrugBank. Duplicated/non-unique index numbers are resolved automatically. Two public available search modules are provided where the standard search allows querying for names, substructures and suppliers. The expert search feature on the other hand allows interactive searching using a molecular weight range, CASnumbers, suppliers, etc. The search can be used to widen or restrict already found results. The eMolecules can be accessed by using the URL: http://www.emolecules.com/

Information regarding Carbaryl compound like molecular weight (201.221), molecular formula ($C_{12}H_{11}NO_2$), structural formula, SMILES [CNC(=O)OC1CCCC2CCCCC12] and CAS number (63-25-2) was obtained from database eMolecules. SMILES retrieved for Carbaryl is used in UMBBD-PPS to predict the biodegradation pathway.

The UMBBD-PPS predicts microbial catabolic reactions using substructure searching, a rule-base and atom-to-atom mapping. The PPS recognizes organic functional groups found in a compound and predicts transformations based on biotransformation rules. These rules are based on reactions found in the UMBBD database or the scientific literature. The pathway prediction system can be accessed at the UM-BBD Pathway Prediction page which can be reached from the 'Pathway Prediction' link on the UMBBD home page, or by using the URL: http://umbbd.msi.umn.edu/predict/

The PPS predicts biodegradation of user compounds. Users can choose if they will view all or only the more likely aerobic transformations. The PPS uses Chemaxon's Marvin Sketch and MarvinView Java applets as plugins. A user enters compound into the system by one of two methods, 'Drawing the Structure and Generating SMILES' or 'Entering SMILES directly'.

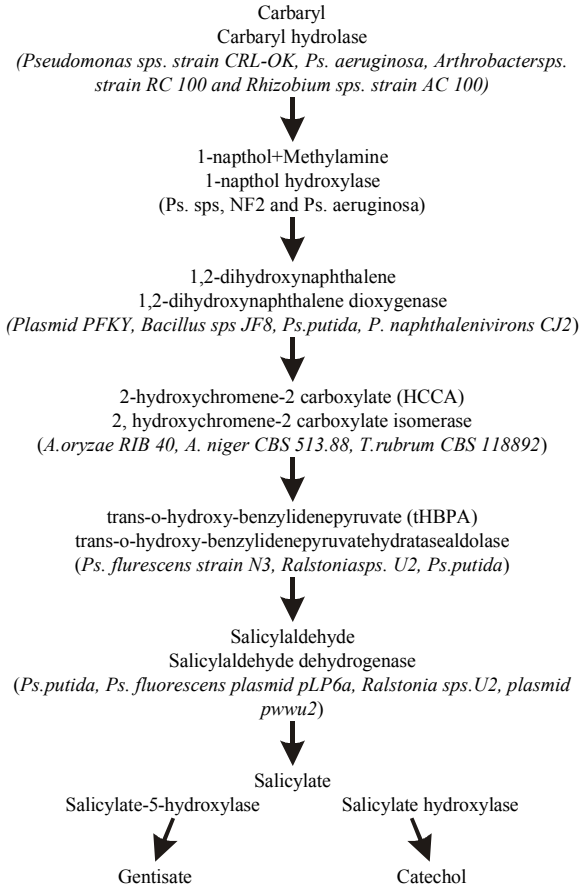

Fig. 1: Biodegradation pathway of Carbaryl according to UMBBD-PPS.
Figure shows the sequential biodegradtion of Carbaryl compound involving specific bacterial species/strains and their associated enzymes.

SMILES of Carbaryl compound was entered directly in UMBBD-PPS; prediction was done for aerobic as well as anaerobic biotransformation and the result of pathway prediction was studied as represented in the flowchart format (Fig. 1).

RESULTS AND DISCUSSION

UMBBD-PPS generated a reaction network involving a wealth of novel pathways describing the biodegradation of Carbaryl to form compounds with known metabolism. The enzyme actions involved in the known pathway were encoded in the UMBBD framework and applied to Carbaryl. The Carbaryl pathway elucidated by UMBBD-PPS is Carbaryl -> Methylamine + 1-napthol -> 1,2 dihydroxynaphthalene -> 2-hydroxychromene-2 carboxylate -> trans-o-hydroxy-benzylidenepyruvate ->salicylaldehyde -> Salicylate ->Gentisate and Catechol. The metabolic pathway of carbarylstarts from 1-napthol to 1,2-dihydroxynaphthalene and then into the napthalene pathway for degradation into intermediary metabolism. The organisms which can initiate a pathway are *Pseudomonas sps*.strain CRL-OK, *Ps. aeruginosa*, *Arthrobactersps*. strain RC 100 and *Rhizobiumsps*.strain AC 100. The enzyme Carbaryl hydrolase initiate this catabolic reaction.Carbaryl compound breaks into Methylamine and Napthol.

Napthol breaks down in presence of enzyme 1-napthol hydroxylase into 1,2 Dihydroxynaphthalene. Methylamine which is formed as byproduct enters Glycophosphate pathway to form Pyruvate. The possible bacteria for this reaction according to UMBBD-PPS are *Pseudomonas sps*. NF2 and *Ps. aeruginosa*.

1, 2 dihydroxynaphthalene enters naphthalene pathway. 1,2-dihydroxynaphthalene in presence of 1,2dihydroxy-naphthalene-dioxygenase enzyme breaks into 2-hydroxychromene-2 Carboxylate (HCCA). *Bacillus sp.JF8, Ps. putida, Polaromonas naphthalenivorans* CJ2 and Plasmid PFKY can degrade the 1, 2-dihydroxynaphthalene. 2-hydroxychromene-2 Carboxylate breaks down to trans-o- Hydroxy-benzylidenepyruvate (tHBPA) by enzyme 2, hydroxychromene-2-carboxylate isomerase and possible bacteria for this reaction are *Aspergillusoryzae, A.niger and Trichophytonrubrum.* Enzyme trans-o-hydroxy-benzylidenepyruvate-hydratasealdolase converts trans-o- Hydroxy-benzylidenepyruvate to Salicylaldehyde. Bacteria *Ps. fluorescens strain N3, Ralstoniasps. U2* and *Ps. putida* secretes this enzyme.Salicylaldehyde breaks down into Salicylate by enzyme salicyladehyde dehydrogenase and *Ps. putida* plasmid, *Ps. fluorescenes* plasmid pLP6a, *Ralstonia* sps.U2 plasmid can bring this break down. Salicylate finally breaks down into Gentisate and Catechol by the enzymes salicylate-5-hydroxylase and salicylate hydroxylase.

This study shows that the bacteria like *Pseudomonas sp. strains, Arthrobacter sp. strains, Rhizobium sp. strains, Bacillus sp. strains, Ps. putida, Polaromonas naphthalenivorans, Aspergillusoryzae, As. niger, Trichophytonrubrum, Ps. fluorescens, Ralstonia sp*. and certain plasmids have potential of carrying out degradation of the Carbaryl.Consortia of these microorganisms can be used to metabolize Carbaryl sequentially or in concert and at the same time it may help in biodegradation of multiple compounds present at contaminated site via variety of these bacteria.

This study has generated a biodegradation pathway describing the degradation metabolism of chemically harmful Carbarylto non-toxic compounds with known metabolism. It gives approximate estimation of the ability

of specific bacteriato utilize this novel pathway for growth with carbaryl as the sole carbon source. Using pathway prediction system, we identified physiologically feasible biodegradation pathway that is attractive alternative to the known *in- vitro* method of biodegradation. To our knowledge, this is the first study of the use of bioinformatics tools for prediction of degradation pathway of Carbaryl compound. This work will provide a direction to the *ex-situ* as well as *in-situ* degradation and will help to foresee the probable results of Carbaryl degradation before starting the actual experiment. Based on this study one can select specific bacteria to allow the degradation of Carbaryl which will save resources, time and efforts of the scientists.

Future *in-situ* work is aimed for addressing the issue of agricultural soil contamination by Carbaryl in order to fully understand the fate of this compound in the environment and to verify the above results.

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