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Original Article

Systematic Analysis of Microbial Degradation Pathway of 1-Naphthyl-N-Methyl Carbamate Generated by EAWAG Biocatalysis/Biodegradation Database - Pathway Prediction System

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ABSTRACT

Objective- This study tends to describe the use of In-silico computational methods as emerging important tools in designing and study of pesticide biodegradation experiments. **Method-** EAWAG-BBD PPS and eMolecules database have been used to predict the biodegradation pathways of 1-naphthyl-N-methyl carbamate, commonly called carbaryl. The relevance of results has been studied by comparing obtained pathway with the products from biodegradation experiments. **Result-** Generated pathway is aerobic in nature and confirms the breakdown of carbaryl in 1-naphthol as the major degradation product with the release of methylamine, which has been proven by several studies. Ring-cleavage of 1-naphthol is the major problem since it occurs much later in the pathway. Obtained pathway elucidates that some of the compounds viz. 1, 2-dihydroxynaphthalene, salicylate, catechol, gentisate, succinate and maleylacetate, have been reported by various studies. In addition, the plausible pathway also mentioned 2-oxopropanoate, (2Z, 4Z)-hexa-2, 4-dienedioate or 2-oxo-pent-4-enoate, 1, 2, 4-benzenetriol, (Z)-4, 6-dioxohept-2-enedioate, as intermediate products, which are yet to be proven by biodegradation experiments. **Conclusion-** Thus, EAWAG-BBD PPS can be used to design and study biodegradation/ bioremediation experiments.

INTRODUCTION

Environmental contamination with pesticides has increased in the recent past with the discovery and introduction of various new chemicals in fields and houses. These toxic substances have not only entered the soil and air, but have also reached the water table[1]. Current biodegradation practices need to pace up to control these contaminations. Identification and characterization of microorganisms for degradation of pesticides and their application for bioremediation is a lengthy process and has associated risks with it since sometimes, the chemical transforms into more toxic substance with tendency to accumulate in the environment[2,3]. Thus, before the application of microbial system, in silico study is desired for prediction of possible biodegradation pathways of any pollutant in the environment. Various

computational tools and databases like Kyoto Encyclopedia of Genes and Genomes (KEGG), METACYC and EAWAG-BBD (Swiss Federal Institute for Environmental Science and Technology-Biocatalysis/Biodegradation Database), etc. are available for this exploration[4]. It was formerly called University of Minnesota Biocatalysis/Biodegradation Pathway Prediction System (UMBBD-PPS) until 1st July 2014 when the rights had been sold to EAWAG. Such tools give possible products and biodegradation pathways, which make it easier to understand the nature of contaminant and aid in designing biodegradation experiments precisely. Further, PPS describes the chemical nature of the pesticides and possible step wise pattern of structural changes of degrading contaminants along with similar examples[5, 6].

In this study we have selected globally used carbamate insecticide 1-Naphthyl-N-methyl carbamate, commonly called carbaryl. It is formed by treating methyl isocyanate (MIC) with 1-naphthol, the process that led to leakage of MIC and resulted in Bhopal gas tragedy killed more than 11000 people and injured over 500000 people in India in 1984[7]. Its structure comprises of 1-naphthol ring to which hydroxyl ions are attached. Double ring hinders its degradation since ring cleavage is not easy. Studies disclose 1-naphthol as the major degradation product. Its structure comprises of a naphthalene ring to which a hydroxyl ion is attached at primary position. 1-naphthol has been a fluorescent organic compound recently listed as potentially carcinogenic[8]. The ester bond between N-methyl carbonic acid and 1-naphthol is responsible for carbaryl's toxicity, making 1-naphthol a potential groundwater contaminant[9]. Carbaryl has slight systemic activity and kills both beneficial and harmful insects[10]. It is categorized as class3 carcinogenic by European Union (EU) index and likely human carcinogen by United States Environmental Protection Agency (US EPA)[11,12].

Majority of microbes form 1-naphthol as the major degradation product and further degradation has been shown by very few[9,13-16]. Major problem associated with pesticide degradation has been difficulty in breakdown of the ring structure, which is not easy. Also the resultant degradation products themselves are more toxic than the parent compound itself. Same is the case with carbaryl. This study has been designed to analyze possible pathways of carbaryl biodegradation using EAWAG-BBD-Pathway Prediction System. Its relevance has been studied by comparing compounds obtained in the resultant pathway with similar reactions listed and proven by various biodegradation studies. Intermediate compounds have been named using ChemDraw Ultra 8.0.6.

MATERIALS AND METHODS

Compound details of carbaryl like molecular weight (201.221gmol^{-1}), molecular formula ($C_{12}H_{11}NO_2$), SMILES (Simplified Molecular Input Line Entry System) [O=C (Oc2cccc1ccccc12) NC], CAS number (63-25-2), source and compound ID were collected from eMolecules database, which contains finer details of commercially supplied chemicals from various companies along with all the entries listed in NIST, PubChem and DrugBank. It automatically resolves all the duplicated or non-indexed numbers. There are two search modules; standard search that allows searching suppliers, names and substructures and expert search feature that allows specific search including molecular weight, CAS numbers, etc. The eMolecules can be accessed by using the URL: http://www.emolecules.com/.

The EAWAG-BBD PPS uses substructure searching, a rule-base, and atom-to-atom mapping to predict catabolic reactions of microorganisms. PPS recognizes the organic functional groups and predicts the pathway using the biotransformation rules listed in the database. Biotransformation rules covered in the database are based on reactions found in scientific literature (on the basis of similar reactions). PPS (Pathway Prediction System) can be accessed from http://eawag-bbd.ethz.ch/. It predicts biodegradation using two plugins Chemaxon's MarvinSketch and MarvinView Java applets. User can select whether to view all or only most likely reactions (aerobic). The compound can be entered either by "Drawing Structure and Generating SMILES" or by "Entering Smiles Directly". By default, PPS shows "Aerobic" biotransformation, show up to

6 levels, display compounds containing 3 or more carbon atoms and stop where there are more than 10 compounds. These options are changeable throughout the process. "Aerobic Likelihood" refers to reactions occurring in aerobic conditions, exposed to soil with moderate moisture or water at neutral pH and 25°C with no competing or other toxic compounds in soil. This transformation is indicated by green arrow[17]. List of rules can be viewed by the link: http://eawag-bbd.ethz.ch/servlets/pageservlet?ptype=allrules.

Retrieved SMILES of Carbaryl compound were entered directly in EAWAG-BBD; the prediction was done for aerobic biotransformation and the resultant pathway was studied along with the literature that listed the degradation compounds found in the obtained pathway. Carbaryl biodegradation pathway by *Pseudomonas* species (spp.) proven by Swetha and Phale, mentioned in the list of pathways of EAWAG website, has been represented in Figure 1[17]. List of pathways can be accessed using the link http://eawag-bbd.ethz.ch/servlets/pageservlet?ptype=allpathways. Obtained most probable aerobic biodegradation pathway of carbaryl has been shown in Figure 2.

RESULT AND DISCUSSION

EAWAG-BBD PPS generated a cascade of reactions that were aerobic in nature. Hydrolysis of carbaryl lead to the formation of 1-naphthol, with the release of methylamine as a result of conversion of phenyl carbamate derivative into phenol derivative and amine (Rule Bt0389). This reaction differs with carbaryl formation reaction since methylamine was released instead of MIC. 1-naphthol formation has been proven by *Pseudomonas aeruginosa*, *Arthrobacter* spp. strain RC 100 and *Rhizobium* spp. strain AC 100 and is known to be catalyzed by carbaryl hydrolase[9, 13-17]. Methylamine, being a toxic gas, although less toxic than MIC, limits further degradation since it can be noxious to some microorganisms. It enters glycophosphate pathway and form pyruvate.

1-naphthol further transformed into 1, 2-dihydroxynaphthalene since 1-hydroxy-2-unsubstituted aromatic compounds transform into 1, 2-dihydroxyderivatives (Rule Bt0014). The reaction catalyzed by 1, 2-dihydroxynaphthalene dioxygenase. Multiple species and strains of *Pseudomonas* degrade 1-naphthol and form 1, 2-dihydroxy-naphthalene[9,13,15,17,19,20]. However, it must be noted that when 2 ring positions are available for hydroxylation, both the products are produced, but the enzyme produces only one of them; it is not possible to predict specifically which one will it produce. 1, 2-dihydroxynaphthalene enters naphthalene pathway and can further transform either into salicylate or 2-oxopropanoate. This reaction deals with the conversion of 1, 2-dihydroxypolyaromatics to 1-hydroxy-2-carboxyaromatics (Rule Bt0340*). Salicylate-5-hydroxylase and salicylate hydroxylase catalyze the conversion of salicylate into gentisate and catechol respectively.

This conversion has been supported by few studies, especially by *Pseudomonas species* and *Achromobacter*[9,14,20,21]. 2-oxopropanoate then underwent pyruvate metabolism. Further 1, 2-dihydroxynaphthalene can transform either into catechol (Rule Bt0060) or gentisate (Rule Bt0064). Formation of catechol was the result of the conversion of hydroxyl-carboxy-aromatic into catechol derivative while gentisate formation results from the conversion of 1-hydroxy-4-unsubstituted benzenoid into 1, 2-dihydroxybenezenoid derivative.

Swetha and Phale proposed the pathway of carbaryl degradation by *Pseudomonas* species, strains C4, C5 and C6 and it has also been listed by EAWAG-BBD PPS[9]. The pathway has been mentioned below: Carbaryl → 1-naphthol → 1, 2-dihydroxynaphthalene → Salicylaldehyde → Salicylate → Gentisate → Maleylpyruvate[9].

Catechol formation leads to initiation of the breakdown of the ring structure. Formation of catechol has been proven by a strain of *Achromobacter* and some other microbes as well[14,21-25]. It can undergo two degradation paths depending on the organism. It can either form (2Z, 4Z)-hexa-2, 4-dienedioate or 2-oxo-pent-4-enoate and formate with the application of different biodegradation rules. (2Z, 4Z)-Hexa-2, 4-dienedioate is formed as a result of intra-diol ring cleavage of vic-substituted dihydroxy-aromatic (Rule Bt0254). It is interesting to note that compounds cleaved by intra-diol or extra-

diol ring cleavage can also be broken down by certain other microbes in certain environmental conditions. (2Z, 4Z)-Hexa-2, 4-dienedioate further breaks down into succinate and acetate ions (Rule Bt0348). Catechol can also transform into 2-oxo-pent-4-enoate and formate. This transformation is the result of the conversion of vic-substituted dihydroxybenzenoid into 2-oxopent-4-enoate derivative and carboxylate (Rule Bt0351). This rule handles extra-diol (meta) ring cleavage. Formate is used in methane metabolism while 2-oxo-pent-4-enoate is used in benzoate pathway.

Gentisate formation has been listed by *Arthrobacter* sp. strain RC100, *Pseudomonas* sp. (NCIB 12042 & 12043) and *Rhodococcus* sp. (NCIB 12038)[9,21-25]. Gentisate can transform either into 1, 2, 4-benzenetriol or into (Z) -4, 6-dioxohept-2-enedioate on the application of different biodegradation rules. Formation of 1, 2, 4-benzenetriol is the result of transformation of vic-substituted hydroxycarboxyaromatic compound into catechol derivative (Rule Bt0060). Conversion of 1, 4-dihydroxy-benzenoid into maleylacetate governs the formation of (Z)-4, 6-dioxohept-2-enedioate from gentisate (Rule Bt0357). It further transforms into fumarate since maleylacetoacetate is converted to fumarate and acetoacetate (Rule Bt0358). It can further transform into 2-oxopropanoate, which is utilized in pyruvate metabolism.

The ring structure of 1, 2, 4-benzenetriol broke down to form (2E, 4Z)-3-hydroxy hexa-2, 4-dienediaote or 4-hydroxy-2-oxopent-4-enoate. Intermediate (2E,4Z)-3-hydroxy hexa-2, 4-dienediaote was formed as a result of intra-diol ring cleavage (Rule Bt0254); further it transformed into maleylacetate as enol form was converted into keto (Rule Bt0044). Further, transformation shows conversion of maleylacetate into 3-oxoadipate. When other intermediate 4-hydroxy-2-oxopent-4-enoate is formed, the resultant is acetylpyruvate (Rule Bt0044). Acetylpyruvate further broke down to form pyruvate and acetate. Pyruvate formation has been reported by *Achromobater* spp.[14].

CONCLUSION

EAWAG-BBD PPS predicted the biodegradation pathway of carbaryl including all the major transformations. The obtained cascade elucidated the breakdown of carbaryl in 1-napthtol with the release of methylamine. Formation of 1, 2-dihydroxynaphthalene, salicylate, catechol, gentisate, succinate, acetate, formate, acetylpyruvate and maleylacetate, have been proven by various studies either in the same form or in ionic or acidic form. The pathway also indicates the formation of 2-oxopropanoate, (2Z, 4Z)-hexa-2, 4-dienedioate or 2-oxo-pent-4-enoate and (Z)-4, 6-dioxohept-2-enedioate, which still need to be proven by microbiological studies. Further, 1-naphthol, reported as the major degradation product by various studies, can be broken down into succinate and acetate or maleylacetate and acteylpyruvate. To a certain extent, plausible pathway showed similarity with catechol and naphthalene degradation pathway. Moreover, few compounds of catechol and naphthalene degradation pathways are yet to be reported in carbaryl by biological studies. Also, bacterial degradation extensively supports the results while fungi need to be exploited to study this biodegradation pathway. Hence, EAWAG-BBD PPS is a handy tool that performs computational analysis for assisting in designing, understanding and studying the biodegradation pathways and also in identification of end products.

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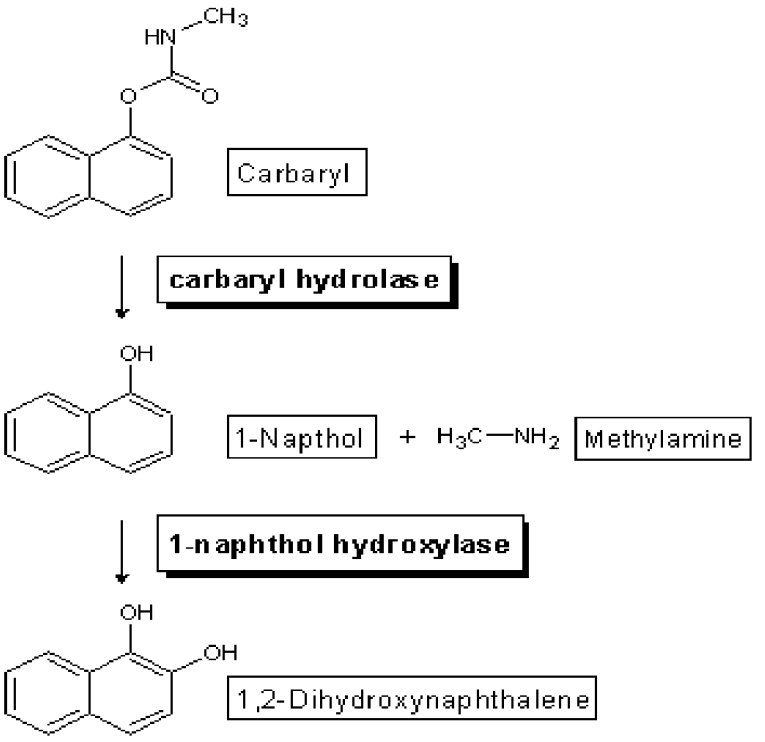

Figure 1. Carbaryl biodegradation pathway by *Pseudomonas* spp. by Swetha and Phale (2005)[9]

Source: http://eawag-bbd.ethz.ch/cbl/cbl_image_map.html.

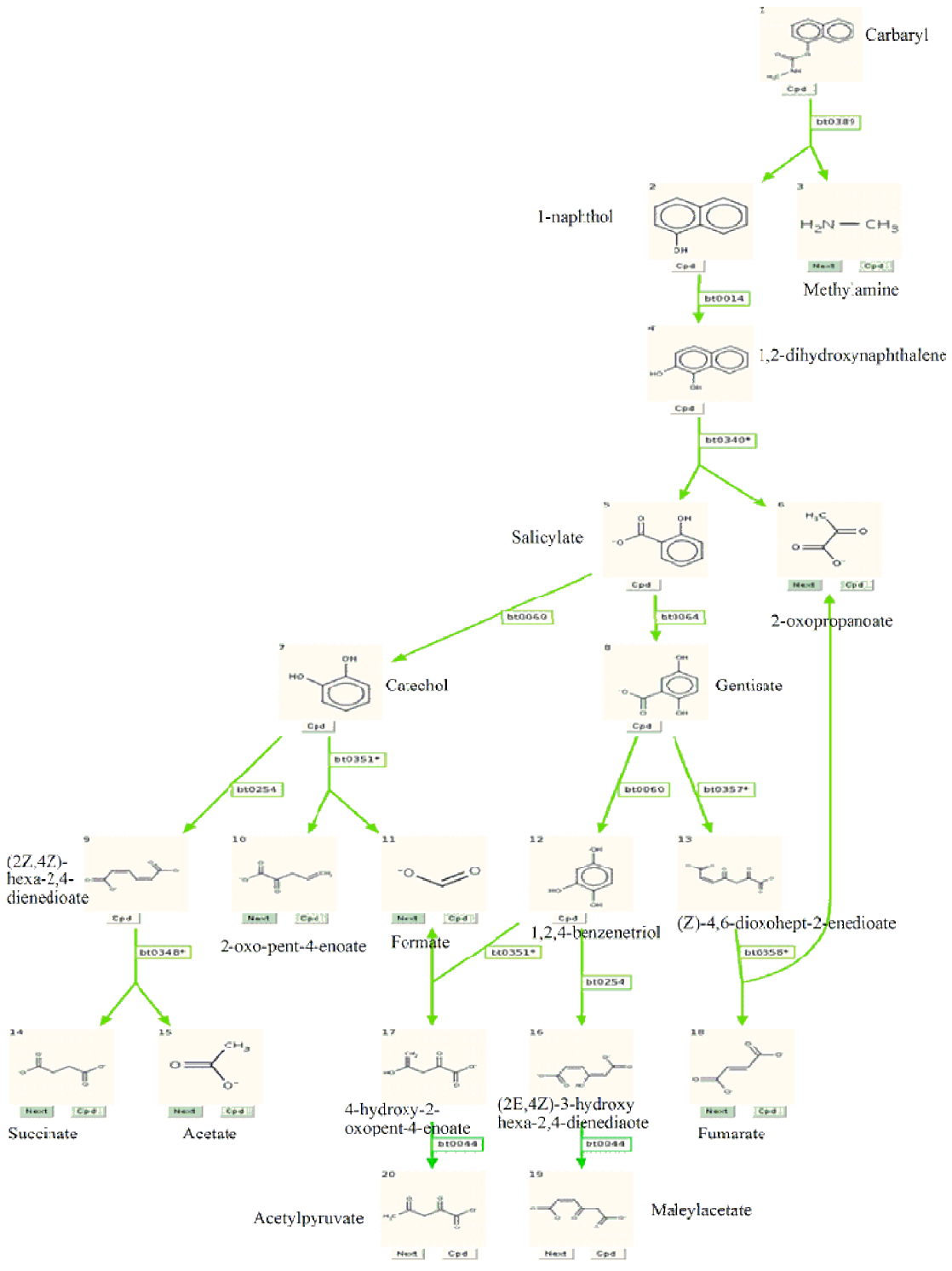

Figure 2. Carbaryl biodegradation pathway predicted by EAWAG-BBD PPS