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Substrate specificity of angular dioxygenase from carbazole-degrading bacterium *Neptuniibacter* sp. strain CAR-SF

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ABSTRACT

Carbazole-degrading bacteria have been shown to have broad substrate specificity towards various contaminants. Carbazole 1,9a-dioxygenase (CARDO) from *Neptuniibacter* sp. strain CAR-SF composed of terminal oxygenase component CarAa, ferredoxin component CarAc, and ferredoxinreductase component CarAd.Expression vector encoding carbazole 1,9a-dioxygenase (CARDO) from *Neptuniibacter* sp. strain CAR-SF CARDO, pETCARA1 was constructed and dioxygenase activity was assessed by monitoring the blue-indigo production in Luria broth media and SDS-PAGE. Gas chromatography-mass spectrometry analysis revealed the angular dioxygenation of dibenzofuran at angular position adjacent to oxygen atom to yield 2,2',3-Trihydroxybiphenyl. CARDO also demonstrated activity towards dibenzothiophene and fluorene by converting the substrates into monooxygenation products, dibenzothiophene-5-oxide and 9-Fluorenone respectively. Cis-dihydrodiols and monohydroxylated products were also seen in the biotransformation of naphthalene, biphenyl and fluoranthene. These diverse oxygenations illustrated by CARDO revealed the broad versatility in its action on polyaromatic compounds and thus will make it as an excellent tool for bioremediation application.

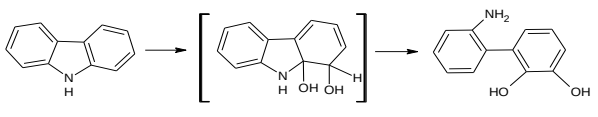
Keywords: Carbazole 1,9a-dioxygenase, angular dioxygenase, substrate specificity, carbazole

INTRODUCTION

Polycyclic and heterocyclic aromatic compounds are well known environmental pollutants that result largely from the incomplete combustion of coal, oil, petrol and wood. The fate of polyaromatic compound and its derivatives in nature is of great environmental concern due to their carcinogenic properties and also to be recalcitrant molecules. Based on the data deposited by Agency for Toxic Substances and Disease Registry (ATSDR), 17 polycyclic aromatic hydrocarbons (PAHs) have been identified as priority substances that pose a significant danger to public health. Their carcinogenic properties therefore call for a great attention in studying such chemicals and targeting their degradation pathways in order to detoxify and mineralize these ubiquitous environmental contaminants.

PAH dioxygenases are well known to have broad substrate specificities and perform various oxidation reactions, including *cis* dihydroxylation, mono- and di-oxygenation. In the field of bioremediation, it is imperative to have pollutant-degrading bacteria or enzymes with versatile oxidation activities since it is more often that bacteriawould be exposed to more than one organic compound in natural environment. Thus, angular dioxygenase is the most favourable dioxygenase as it can participate in various oxidation reactions. Besides, the initial oxidation to break the chemically stable aromatic ring is the most critical point in the degradation pathway of aromatic compounds in which the intermediate would become more accessible for degradation by other enzymes.

Previously, carbazole-degrading genes governing the angular attack on carbazole have been reported by Nagashima*et al.* from marine bacterium *Neptuniibacter* sp. strain CAR-SF. The terminal dioxygenase enzyme, known as carbazole 1,9a-dioxygenase (CARDO) composed of terminal oxygenase (CarAa), ferredoxin (CarAc) and ferredoxinreductase (CarAd). CARDO catalyzes the angular dioxygenation of carbazole to give the unstable dihydroxylated intermediate that will be converted to 2'-aminobiphenyl-2,3-diol (Fig. 1). Concerning that there is still a lack of knowledge on the CARDO from marine bacteria, it willbe quite interesting to investigate the similarities and at the same time to compare the substrate recognition by CARDO from terrestrial bacterium CA10 and marine bacterium CAR-SF. In this paper, we obtain the information on the substrate recognition of CARDO from marine bacterium *Neptuniibacter* sp. CAR-SF and identified theproducts generated by $CARDO_{CARSF}$from various aromatic compounds. This is the first report on the CARDO enzyme governing the angular attack from a marine bacterium.



Carbazole (CAR) 2'-Aminobiphenyl-2,3-diol

Fig.1.Conversion of CAR catalyzed by CARDO from *Neptuniibacter* sp. strain CAR-SF. The structure shown in bracket is an unstable intermediate that has not been characterized