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CATECHOL BIODEGRADATION BY *PSEUDOMONAS* STRAIN : A CRITICAL ANALYSIS

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ABSTRACT

Catechols are the most abundant organic pollutants of our environment. They are of major concern because of their persistence and toxicity and gave scientists a thinking to engineer/develop certain measures for its removal from the environment. Bacteria explore the environment for its nutritional requirement and degrade many of complex molecules available to them including the toxins and pollutants. Under biological process being the cheapest mode for removal of the catechols, the *Pseudomonas putida* has been found having the great potential to degrade them upto 500 mg/L. Such degradative bacteria, under normal circumstances, enzymatically convert xenobiotic, aromatic compounds to either catechol or protocatechuate. The most common catabolic pathway involves conversion of the parent molecules to chlorocatechols by the action of a monooxygenase or a dioxygenase. Many members of *Pseudomonas* carry plasmid that encodes enzymes capable of degrading aromatic and halogenated organic compounds. The degradation proceeds in two phases, first, an aromatic compound is prepared for ring cleavage by a variety of ring modification reactions. The second phase of degradation includes ring fission and subsequent reactions leading to the generation of tricarboxylic acid cycle intermediates. Two key enzymes involved in this β-ketoadipate pathway are catechol 1, 2- dioxygenase and 3, 4-PCD.

Key words : Catechols, *Pseudomonas putida*, β-Ketoadipate pathway, 1, 2-Dioxygenase, 3, 4-PCD

INTRODUCTION

Environmental pollutants are compounds that are toxic to living organisms; released into the ecosystem at high concentrations, usually as a consequence of human

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activities. Bacteria have developed nutritional strategies to obtain energy from virtually every compound available to them and play a crucial role in sustainable development of the biosphere and in biogeochemical cycles. The abundance of microorganisms, together with their great ability for horizontal gene transfer and their high growth rates, allow them to evolve quickly and to adapt to changing environmental conditions. The great genetic diversity of microorganisms accounts for their great metabolic versatility[1-3]. Among the most abundant environmental pollutants; catechol and related products are of major concern because of their long term persistence and the toxicity. Therefore, the removal of catechol is vital before letting it into the environment. Various treatment strategies are available for its removal; however, its removal by biological means is much cheaper, less energy consuming and above all, environment friendly. Members of the genus *Pseudomonas* are the most predominant group of soil microorganisms that degrade xenobiotic compounds. Biochemical assays have shown that various strains of *Pseudomonas* can degrade and, as a consequence, detoxify more than 100 different organic compounds. In many cases, one strain uses several different related compounds as a sole carbon source. *Pseudomonas putida* has the capability to degrade catechol upto 500 mg/L, sometimes the catechol may be at concentration as high as 1000 mg/L.

The biodegradation of complex organic molecules (Fig. 1) requires the combined effort of several different enzymes. The genes that code for the enzymes of these biodegradative pathways are sometimes located in the chromosomal DNA, although they are more often found on large (approximately 50 to 200 kb) plasmids. In some organisms including *Pseudomonas*, the genes that contribute to the degradative pathway are on both chromosomal as well as plasmid DNA. Degradative bacteria, in most cases, enzymatically convert xenobiotic, aromatic compounds to either catechol or protocatechuate. Then, through a series of ortho/meta cleavage reactions, catechol and protocatechuate are processed to yield either acetyl-CoA and succinate or pyruvate and acetaldehyde, that are readily metabolized by almost all organisms.

Significant efforts have been made during the recent past to study the biodegradation of organic pollutants and the mechanisms involved therein[4-7]. Although various metabolic interactions have been investigated, many aspects however, remain to be examined to obtain a detailed overview of this biodegradation and to optimize or predict *in situ* microbial degradation. Approaches to analyze and assess biodegradation have shifted towards the application of culture-independent methodologies to characterize natural and genetically engineered pollutant-degrading microbial associations. An outstanding characteristic of bioremediation is that it is carried out in non-sterile open environments that contain a variety of organisms. Although bacteria, such as those capable

of degrading pollutants (e. g. *Pseudomonas* sp., *E. coli*), play a key role in bioremediation, other organisms also affect the process.

Microbial degradation of aromatic compounds

Microbial metabolism of halogenated aromatic compounds has been studied by many previous workers[8,9]. Biodegradation pathways of chlorobenzoic acids[9,10], chlorophenols[11, 12], chlorobenzene[13] and 1, 3-dichlorobenzene[14] have been thoroughly studied in pure cultures of bacteria. The most common catabolic pathways involve conversion of the parent molecules to chlorocatechols by the action of a monooxygenase[9] or a dioxygenase[9,13].

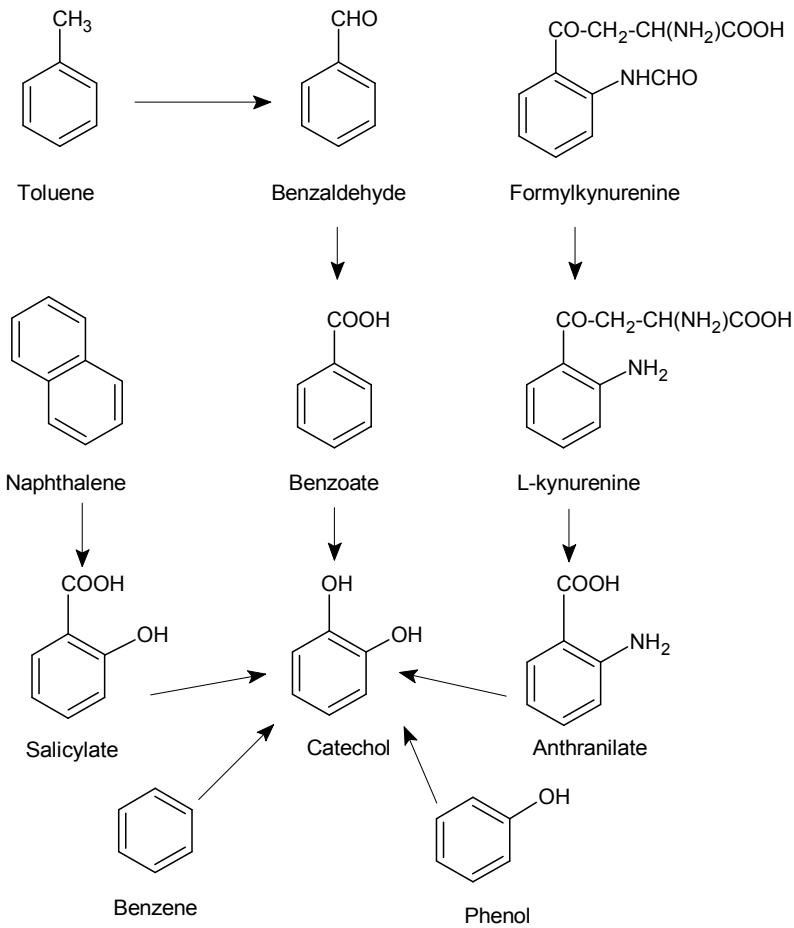

Fig. 1 : Pathways for the enzymatic conversion of aromatic compounds by degrading bacteria to catechol

Cleavage of the resultant chlorocatechols to form chloromuconic acids is catalyzed by nonspecific 1, 2-dioxygenases termed type II pyrocatechases by Dorn and Knackmuss[15].Cleavage by a 2, 3-oxygenase is unproductive and results in inactivation of the enzyme[16]. Chloride is eliminated by lactonization of chloromuconic acids and subsequent reactions to yield β-ketoadipic acid[9].

The benzene ring is the unit of chemical structure most widely spread in nature. Moreover, the thermodynamic stability of the benzene ring increases its persistence in the environment; therefore, many aromatic compounds are major environmental pollutants[17]. By expressing different catabolic pathways, microorganisms can use a wide array of aromatic compounds as sole carbon and energy sources[18].The general ability of bacteria to use such compounds is related to the fact that most of these compounds are commonly present in the environment as a result of the recycling of plant-derived material such as tannin[19].*Pseudomonas* species and closely related organisms have been the most extensively studied owing to their ability to degrade so many different contaminants[20].Many members of *Pseudomonas* carry plasmid that encodes enzymes capable of degrading aromatic and halogenated organic compound. In most cases, a single plasmid carries the gene encoding enzymes for a specific degradative pathway. It is possible to create an organism with multiple degradation capabilities. In addition, by genetic manipulation, it is possible to extent the range of substrate degradation by a particular enzymatic pathway.

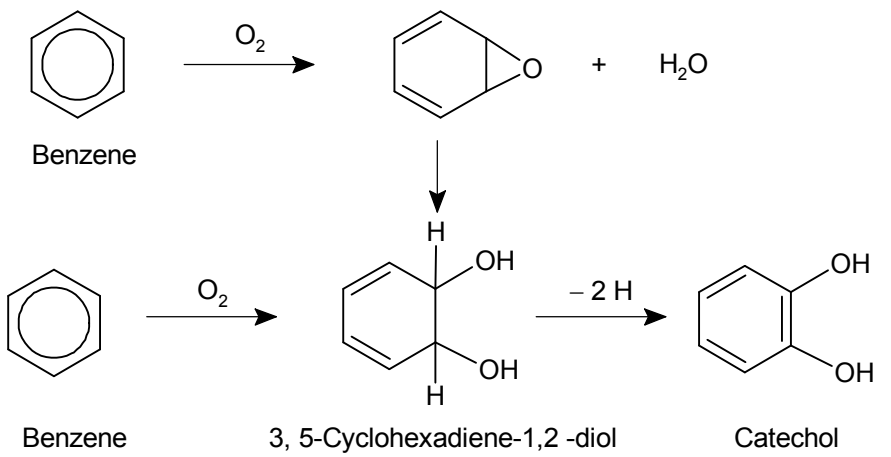

Fig 2 : Microbial degradation of benzene to catechol

Catechol is a reaction intermediate in the bacterial metabolism of catechin [the juice of *Mimosa catechu* (*Acacia catechu*)], naphthalene, benzene (Fig. 2), phenol and

other compounds[21]. In some species of *Pseudomonas* the benzene nucleus of catechol is cleaved by a pyrocatechase to give cis, cis muconic acid[22,23], whereas in other species catechol is oxidized to 2-hydroxymuconic semialdehyde by an enzyme that was designated catechol 2, 3-oxygenase[5].

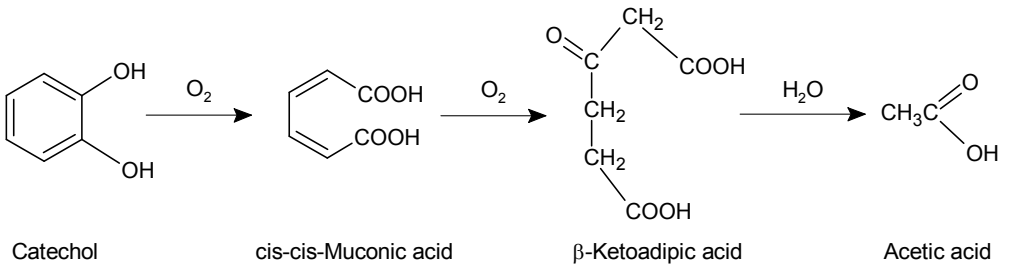

Fig 3 : Degradation of catechol

Evolutionary genetics of biodegradation pathways

The genes responsible for biodegradation pathways are usually arranged in clusters that comprise : (i) catabolic genes encoding the enzymatic steps of the catabolic pathway, (ii) transport genes responsible for active uptake of the compound and (iii) regulatory genes that adjust expression of the catabolic and transport genes to the presence of the compound to be degraded[24].Bacteria have developed a physiological response that controls and adjusts the specific regulation of catabolic operons to the physiological and metabolic state of the cells[25]. The catabolic clusters are usually present in mobile genetic elements, such as transposons and plasmids, which facilitate their horizontal transfer of the respective genes and, therefore, rapid adaptation of microorganisms to the new pollutants in a particular ecosystem[26, 27].

Enzymes in the degradation of catechol

Microbial enzymes are useful catalysts for the degradation of organic pollutants in bioremediation but also for the synthesis of added value products in biocatalyst applications. Numerous bacterial strains, such as those of *Pseudomonas* species[28,29] provide enzymes that are able to transform functional groups of organic compounds under aerobic conditions. Enzymes involved in the degradation of catechol (Fig. 3) are mainly oxygenases. Oxygenases that utilize both atoms of dioxygen in their substrates are known as dioxygenases[30]. In general, degradation proceeds in two phases first; an aromatic compound is prepared for ring cleavage by a variety of ring modification reactions. The second phase of degradation includes ring fission and subsequent reactions leading to the generation of tricarboxylic acid cycle intermediates. Ring fission is catalyzed by

dioxygenases and is termed orthocleavage, when it occurs between the hydroxyl groups (intradiol cleavage) and meta cleavage, when it occurs adjacent to one of the hydroxyls (extradiol cleavage). Ortho-cleavage is commonly known as β-ketoadipate pathway. The latter name derives from the fact that β-ketoadipate is a key intermediate of the ortho-pathway. Two key enzymes involved in this β-ketoadipate pathway are catechol 1, 2-dioxygenase and 3, 4-PCD. All nine enzymes catalyzing the conversion of PCA and catechol to tricarboxylic acid cycle intermediates have been purified and characterized and in several cases, crystal structures are available[19]. The best studied enzyme is 3, 4-PCD from *P. putida*[31]. Protocatechuate 3, 4-dioxygenase and protocatechuate 4, 5-dioxygenase activities in their cell free extracts of *A Bacillus sp.*[32]. It followed both; ortho and meta cleavage pathways for protocatechuate degradation.

Protocatechuate 3, 4-dioxygenase catalyzes the intradiol cleavage of protocatechuate by incorporating two atoms of molecular oxygen to form β-carboxy cis, cis-muconate. Enzyme activity requires the participation of a ferric ion[16]. Catechol 1, 2-dioxygenase catalyzes the incorporation of oxygen to form cis, cis-muconate and resembles 3, 4-PCD because it is a dimeric, ferric iron-containing enzyme[33]. Most catechol 1, 2-dioxygenases studied are homodimers of identical subunits[34]. Conversion of 3-chlorocatechol by catechol 2, 3-dioxygenase in *Pseudomonas putida* GJ31 was identified[35].

Recombinant bacteria for bioremediation

To enhance the metabolic efficiency of a microorganism for a particular environmental application, genetic engineering is carried out at two different levels : (i) manipulation of the specific catabolic pathway and (ii) manipulation of the host cell. In order to improve the rate of pollutant removal and broaden the range of substrates of a catabolic pathway, manipulation of the key enzymes of both the pathway and the regulatory mechanisms that control the expression of the catabolic genes is required[1,3]. Protein stability and protein activity can be altered and/or improved by protein engineering and rationally directed molecular evolution techniques (DNA shuffling and other *in vitro* recombination methods)[3, 36]. Metabolic engineering also allows the generation of novel hybrid pathways by assembling catabolic modules from different origins in the same host cell, thus leading to pathway expansion to new substrates, completion of incomplete pathways, the creation of new routes and construction of bacteria with multiple pathways[3, 37-39].The rational combination of catabolic pathways may allow the complete metabolism of xenobiotics, as has been shown with the development of bacteria capable of mineralizing PCBs and can prevent the formation of dead-end products and toxic metabolites by misrouting of the pollutants[3, 37].

Bacteria used to remediate pollutants may undergo environmental stress due to high concentrations of toxic contaminants, toxic solvents, extreme pH, temperature, ionic strength, etc.[3].The combination in a single bacterial strain of different degradative abilities with genetic traits that provide selective advantages in the target site is a successful strategy for *in situ* bioremediation33.For instance, solvent-resistant strains may be ideal hosts to construct genetically engineered microorganisms for the removal of wastes with high solvent contents[37]. Heavy metals, including Hg, Cd and As, are currently major sources of pollution and cannot be destroyed or biodegraded. Recombinant microorganisms have been developed to accumulate and/or immobilize heavy metals present in soil and water[40,41]. As some metals can serve as terminal electron acceptors in microbial respiration, anaerobes that use them have been applied to reduce the soluble oxidized form of the metal to the insoluble form; thereby preventing its further spread in the environment[42]. Some polluted environments contain a mixture of organic wastes, heavy metals and high-energy radionuclides. In this context, radiation-resistant bacteria are being genetically engineered with biodegradation genes to render them suitable for the treatment of such mixed wastes[37,43,44].

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