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Degradation of *m*-cresol via the *ortho* cleavage pathway by *Citrobacter farmeri* SC01

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ABSTRACT

Traditionally two pathways are used to biodegrade *m*-cresol: (1) a *meta*; and (2) gentisate. However, my new study shows a promising third pathway. The study first isolates *Citrobacter farmeri* SC01 from an aerobic basin of a coking wastewater treatment. Then the isolated *C. farmeri* SC01 was used to study the metabolism of *m*-cresol. The findings show that the isolated *C. farmeri* SC01, if grown on *m*-cresol as the sole carbon source, can metabolically biodegrade *m*-cresol. By the methods of HPLC, LC–MS, FT-IR and GC–MS, the study identifies the metabolic intermediates of *m*-cresol biodegraded by *C. farmeri* SC01 under aerobic process. These intermediates are 3-hydroxybenzoic acid, *cis*,*cis*-munconate, and muconolactone, the typical *ortho*-cleavage metabolites formed and consumed during the degradation of *m*-cresol. Moreover, when growing on *m*-cresol as a sole carbon and energy source, *C. farmeri* SC01 can induce the production of catechol 1,2-dioxygenase. The findings contribute to the study of the characteristics of biodegradation, the metabolic mechanism, the properties of enzymes and the expression, and the regulation of the genes involved in the degradation of *m*-cresol.

1. Introduction

Phenol is primarily used in the production of the phenolic resins which is widely used in such industries as plywood, construction, automotive, and appliance. Phenol is also applied in the production of caprolactam and bisphenol A, the two compounds that serve as intermediates in the manufacture of nylon and epoxy resins, respectively. However, if ingested, contacted, or inhaled, even at low concentrations, these compounds are toxic to human beings and animals. For instance, phenol is toxic to many aquatic organisms, such as *Daphnia magna* (48 h EC_{50} 24.2–32.7 mg/L) and *Oncorhynchus mykiss* (96 h LC_{50} 11.9–14.1 mg/L) [1]. Therefore it is necessary to minimize the quantity of or even entirely remove, if possible, the phenolic compounds in water. If the concentration is below 1000 mg/L, biological methods are more economically feasible than physical or chemical approaches. In the biological approach, various microorganisms have been isolated and screened so that they employ phenol as their sole carbon and energy source [2–4]. Bacteria that degrade the phenolic compounds have been used in studies that aim to reach the kinetics models and to understand the involved complex biochemistry. The kinetics models contribute to the bioremediation of the toxic compounds [5,6].

It is indispensable to understand the metabolic pathway in the biological treatment. The pathway can usually be defined through the analysis of intermediates and biodegrading enzymes. Such analytical techniques as GC–MS, LC–MS, and NMR have been developed to detect biological metabolites and used in analytical approaches to accurately identify a number of pre-defined target metabolites, or produce fingerprints of metabolic changes without individually determining metabolite identities [7]. Three key dihydroxyaromatic intermediates produced during the biodegradation of phenol and other aromatic compounds have been identified: catechol, protocatechuic acid, and gentisate acid [6], from which the three metabolism pathways were named. These intermediates further undergo ring fission to yield such metabolites as pyruvic acid, acetic acid, succinic acid, and acetyl-CoA, for the Krebs cycle [8,9]. Catechol is the most common metabolite before ring cleavage when the three dihydroxyaromatic intermediates are degraded. Catechol and the primary substrates are formed when the aromatic precursor incorporate molecular oxygen [10]. Both single ring benzene and

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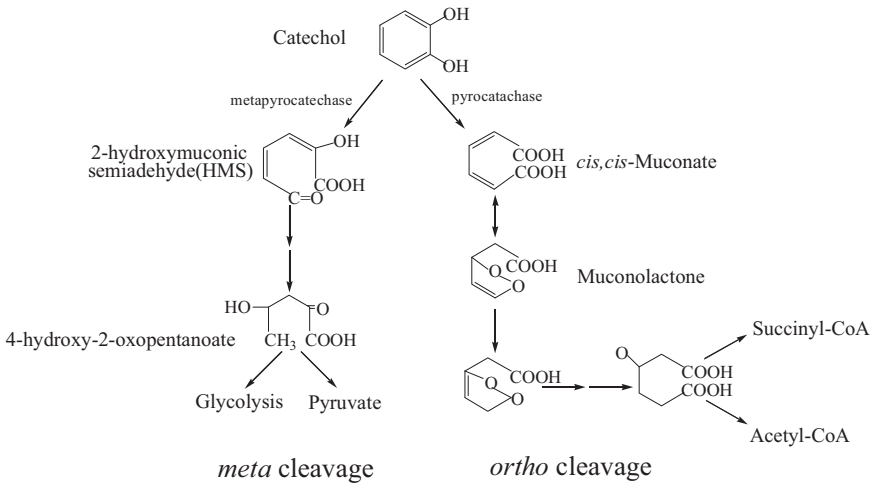

Fig. 1. Metabolism of catechol through the *meta*- and *ortho*-cleavage pathways.

three-ring phenanthrene can be funneled into catechol [11]. Once catechol is formed, it can be degraded through either the *meta* or the *ortho* (also commonly know as β-ketoadipate) ring cleavage pathway by the enzymes, catechol 2,3-dioxygenase and catechol 1,2-dioxygenase, yielding 2-hydroxymuconicsemialdehyde (HMSA) and *cis*,*cis*-muconate, respectively. Fig. 1 shows the *meta*- and *ortho*-cleaving pathway of catechol. From this diagram, it is clearly that the formation of *cis*,*cis*-muconate or 2-hydroxymuconic semiadehyde is a key indicator in distinguishing the pathways.

Cresols are one of the phenolic compounds in the US EPA Substance Priority List [12]. Yeast (*Candida tropicali*) [2], *Pseudomonas* sp. [13], fungi (such as *Aspergillus*, *Cladosporium*, *Fusarium*, *Monicillium*, *Trichoderma*, *Penicillium*, *Pleurotus*, *Phanerochaete*) [14], and two sulfate-reducing bacteria (OX39 and *Desulfobacterium cetonicum*) [15] have been utilized to degrade *m*-cresol. Hopper et al. [16] and Jain et al. [17] studied different *m*-cresol metabolic pathways with *Pseudomonas putida* NCIMB9869 grown on 3,5-xylenol or *m*-cresol. Growing on 3,5-xylenol, it metabolizes *m*-cresol via *m*-hydroxybenzoate following the gentisate pathway [18]. However, if growing on *m*-cresol, the bacterium metabolizes *m*-cresol via 3-methylcatechol, which is further metabolized through the catechol pathway via meta-fission (Fig. 2, re-drawn based on Hopper and Taylor, 1975). Gentisate- and catechol-pathways are the most common biodegradation pathways of *m*-cresol.

An efficient *m*-cresol-degrading microorganism, named *Citrobacter farmeri* SC01 has been isolated in an aerobic basin of a coking wastewater treatment plant. In the previous studies, the authors investigated the identification, biodegradation characteristic and kinetic model, finding that catechol 1,2-dioxygenase (at 260 nm) augments obviously during *m*-cresol biodegradation [19,20]. The study finds no change in the wavelength at 330 nm, 340 nm, and 375 nm that corresponds to 3-maleylpyruvate, 3-fumarylpyruvate, and 2-hydroxymuconate semialdehyde, respectively, the metabolites of catechol or gentisate. Therefore, it is assumed that *C. farmeri* SC01 has degraded *m*-cresol in a new pathway. Fig. 3 shows the hypothetical pathway of *m*-cresol biodegradation by *C. farmeri* SC01.

In this paper, the intermediates of *m*-cresol metabolized by the strain *C. farmeri* SC01 were analyzed and identified by methods of HPLC, LC–MS, FT-IR, and GC–MS. This contributes to the study of the biodegradation, metabolic mechanism, properties of the enzymes, and the expression and regulation of the genes involved in the degradation of *m*-cresol.

2. Materials and methodologies

2.1. Strains

The organism *C. farmeri* SC01 used in this experiment was isolated from an aerobic basin of a coking wastewater treatment plant. The physiological and biochemical characteristics, phylogenetic relationships, degradation efficiency of *m*-cresol, and degradation kinetics have been described previously [20].

2.2. Media

Cells grew in a synthetic mineral medium containing ($g\,L^{-1}$) KH_2PO_4 2.74, K_2HPO_4 2.24, $(NH_4)_2SO_4$ 1.0, $MgCl_2$ 0.2, $CaCl_2$ 0.01, $FeCl_3{\cdot}6H_2O$ 0.02, NaCl 0.1, and pH 6.8–7.0. Then, *m*-cresol

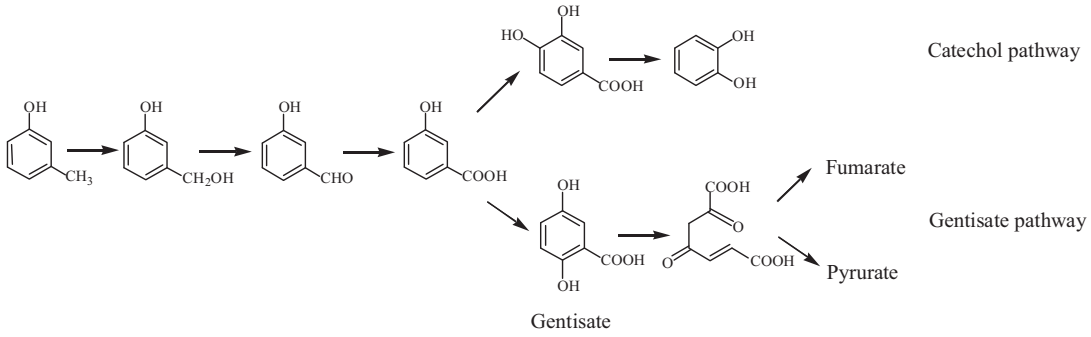

Fig. 2. Different pathways for *m*-cresol degradation, after growing with 3,5-xylenol or *m*-cresol. *P. putida* NCIMB 9869 uses the gentisate or catechol pathways for oxidizing *m*-cresol.