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Biodegradation of n-hexadecane by *Aspergillus* sp. RFC-1 and its mechanism

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ABSTRACT

Fungi can use n-hexadecane (HXD) as a sole carbon source. But the mechanism of HXD degradation remains unclear. This work mainly aimed to study the degradation of HXD by *Aspergillus* sp. RFC-1 obtained from oil-contaminated soil. The HXD content, medium acidification and presence of hexadecanoic acid in the medium were determined by gas chromatography-mass spectrometry, and fungal growth was observed. Enzyme and gene expression assays suggested the involvement of an alkane hydroxylase, an alcohol dehydrogenase, and a P450 enzyme system in HXD degradation. A biosurfactant produced by the strain RFC-1 was also characterized. During 10 days of incubation, 86.3% of HXD was degraded by RFC-1. The highest activities of alkane hydroxylase (125.4 μmol mg^{-1} protein) and alcohol dehydrogenase (12.5 μmol mg^{-1} proteins) were recorded. The expression level of cytochrome P450 gene associated with oxidation was induced (from 0.94-fold to 5.45-fold) under the HXD condition by Real-time PCR analysis. In addition, HXD accumulated in inclusion bodies of RFC-1with the maximum of 5.1 g L^{-1}. Results of blood agar plate and thin-layer chromatography analysis showed RFC-1 released high lipid and emulsification activity in the fungal culture. Induced cell surface hydrophobicity and reduced surface tension also indicated the RFC-1-mediated biosurfactant production, which facilitated the HXD degradation and supported the degradation process.

1. Introduction

Petroleum hydrocarbons are considered the most common environmental pollutants. The increasing global demand for energy in recent years has resulted in water and soil deterioration by oil industry pollution (Hasan et al., 2010). Alkanes are highly abundant in the environment because of the broad usage of petroleum fuels and their derivatives (Meng et al., 2017). The low-molecular-weight alkanes are volatile in nature and easily degraded, whereas those with high molecular weight are highly persistent in the environment (Labinger and Bercaw, 2002).

n-Hexadecane (HXD) is a major alkane component, and is present in the aliphatic fragment of crude oil. The solubility of HXD in water is 5.21×10^{-5} mg L^{-1} at 15 °C and has high partitioning co-efficient 9.1 logKow (Stroud et al., 2007). HXD is present in highly contaminated oil sites, and it possesses well-characterized biodegradability, hence, HXD compound has been used as a model molecule for studying alkane hydrocarbon biodegradation (Schoefs et al., 2004).

There are three kinds of alkane hydroxylase monooxygenases involving in short-, medium-, and long-chain alkanes degradation. They are methane monooxygenase, membrane-bound nonheme alkane monooxygenase, and cytochrome P450 (CYP52) (Van Beilen and Funhoff, 2007). A major process of alkane degradation is the oxygenation of the terminal methyl group (Rehm and Reiff, 1981). Given that alkane-degrading microorganisms possess multiple genes for alkane hydroxylases, they can highly degrade a wide range of alkanes. Two major factors are responsible for the rapid n-alkane degradation from petroleum mixtures via microorganisms: metabolic enzyme activity for oxidizing n-alkanes and alkane transfer into cells. Little is known about the metabolic intermediates of alkane extracellularly before it is assimilated across the cell membranes, despite the understanding regarding the mechanisms by which long-chain fatty acids enter cells (Van Den Berg et al., 2004).

Many hydrocarbon-degrading microbial excrete biosurfactants, amphiphilic molecules of diverse chemical nature, which enhances the aptitude of microbial cells to use hydrophobic compounds as growth substrates (Kiran et al., 2009; Mahjoubi et al., 2013; Al-Hawash et al., 2018b). Extracellular biosurfactants and bioemulsifiers produced by

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