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Outlines of Pathways of Degradation of High-molecular-weight Polycyclic Aromatic Hydrocarbons by Mycobacteria

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Ability to degrade high-molecular-weight polycyclic aromatic hydrocarbons (HMW-PAHs) is present in diverse species of fast-growing mycobacteria which are widely distributed in heavily contaminated sites and an uncontaminated humus soil (Kim et al., in press). Mycobacterial isolates from humus soil and heavily contaminated sites were numerically differentiated into the two strain groups showing significant differences in the utilization of carbohydrates, including inositol, adonitol, D-arabitol, L-arabitol, erythritol, rhamnose, sorbitol, and xylitol, but both of them exhibited similar substrate utilization patterns for primary alcohols from ethanol to pentanol, 1,4-butanediol, benzyl alcohol, hexadecane, ethyl acetate, fluoranthene, phenanthrene and pyrene as the sole C/E sources. The comparative sequence analysis of 16S ribosomal RNA gene proved that patterns of the variable V2 and V3 regions were useful for discriminating fast-growing and thermosensitive HMW-PAH-degrading mycobacteria into ten subgroups, being consistent with the phylogenetic tree.

The degradation of HMW-PAHs by mycobacteria is beneficial for the yield of carbon and energy, but it is disadvantageous to more or less extents in certain circumstances leading to the accumulation of toxic PAHs and intermediates, e.g., PAH catechol. The redox cycle between semiquinone and quinone is greatly related to the generation of reactive oxygen species and DNA adducts. Against the toxicity problems, PAH *o*-quinone reductase (PQR) plays a role in the detoxification of PAH *o*-quinones and the prevention of the depletion of PAH catechol substrates from PAH degradation (Kim et al., 2003). A pyrene-degrading *Mycobacterium* sp. strain PYR100 possessed two PQR1 and PQR2 for the reduction of 9,10-phenanthrenequinone (9,10-PQ) and/or 4,5-pyrenequinone (4,5-PyQ) constitutively. PQR1 containing a flavin-cofactor was a monomer (20.1 kDa), and PQR2 containing no flavin cofactor was a homodimer (26.5 kDa subunit). There was no match between the two *N*-termini of those proteins. PQR2 was inhibited by dicumarol and quercetin to greater extents than PQR1. PQR2 showed much higher specificity constants (k_{cat}/K_m, $\times 10^8$ M^{-1} s^{-1}) for menadione (3.5 ± 2.2) and 9,10-PQ (120 ± 100) than PQR1 for menadione (0.71 ± 0.20) and 9,10-PQ (3.8 ± 1.6). Additionally, PQR2 exhibited broad substrate specificities and high specificity constants for 1,4-

naphthalenequinone (9.5 ± 3.8), 1,2- naphthalenequinone (39 ± 18) and 4,5-PyQ (320 ± 260). The contents of PQR1 and PQR2 in strain PYR100 varied with the pH of culture media.

Similar PQR activities exist in *Mycobacterium vanbaalenii* strain PYR-1 (DSM 7251^T), by which the HMW-PAH degradation has been most intensively studied. Exogenous catechol inhibitors, including alizarin, anthrarobin, 2,3-dihydroxynaphthalene and esculetin, had effects in lowering the PQR activities towards 9,10-PQ and 4,5-PyQ (Kim et al., 2004). Of them, anthrarobin and esculetin (final concn., 100 μM for each) inhibited the PQR activity for 4,5-PyQ reduction up to 64 % - 92%, and anthrarobin resulted in the highest O-methylation (1.06 ± 0.04 nmole (mg-protein)$^{-1}$ for 30 min) catalyzed by a constitutive catechol-O-methyltransferase (COMT) activity in this strain. The COMT activity was involved in the O-methylation of 1,2-dihydroxyphenanthrene to form 1-methoxy-2-hydroxyphenanthrene and 1,2-dimethoxyphenanthrene from phenanthrene degradation, and in the O-methylation of 1,2-dihydroxypyrene to give 1-methoxy-2-hydroxypyrene, 1-hydroxy-2-methoxy-pyrene and 1,2-dimethoxypyrene from pyrene degradation. The COMT activity of strain PYR-1 plays an eminent role in the detoxicification of non-metabolizable PAH catechols in combination with the PQR activities.

Various pathways of the degradation of phenanthrene and pyrene by strain PYR-1 have been postulated based on the identification of metabolites from mass and NMR spectral analyses (Kim et al., 2005). To elicit why the contents of COMT and PQRs varied with the pH of culture media, the growth of strain PYR-1 and the whole cell biotransformation of phenanthrene and pyrene were investigated at pH 6.5 and 7.5. In tryptic soy broth strain PYR-1 grew more rapidly at pH 7.5 (μ'=0.058 ± 0.0076 h^{-1}) than at pH 6.5 (μ'=0.028 ± 0.049 h^{-1}). However, the resting cells suspended in phosphate buffers with the same pH values displayed a shorter lag time for the degradation of phenanthrene and pyrene at pH 6.5 (6 h) than at pH 7.5 (48 h). The one unit pH drop enhanced the degradation rate for phenanthrene from 7.3 to 31.1 nmole h^{-1} (mg-protein)$^{-1}$, and similarly for pyrene from 7.2 to 26.7 nmole h^{-1} (mg-protein)$^{-1}$. Higher levels of both compounds were detected in the cytosol fractions obtained at pH 6.5. This implicates that an acidic pH renders the mycobacterial cells more permeable to hydrophobic substrates, and that higher substrate concentrations in the cytosol accelerate the induction of PAH degradation and enhanced the degradation rates. The metabolic pathways were likely initiated by the PAH oxidation at the K-region. The resultant phenanthrene-9,10-dihydrodiol and pyrene-4,5-dihydrodiol were metabolized via transient catechols to the ring fission products, 2,2'-diphenic acid and 4,5-dicarboxyphenanthrene, respectively. These metabolic pathways converged to form phthalic acid. In contrast, strain PYR-1 produced higher levels of the *O*-methylated derivatives of non-K-region phenanthrene-diol and pyrene-diol at pH 6.5. Other non-K-region products, including *cis*-4-(1-hydroxynaphth-2-yl)-2-oxobut-3-enoic acid, 1,2-dicarboxynaphthalene and benzocoumarin-like compounds, were also detected in the culture fluids. The non-K-region oxidation reactions must be a significant burden to the cell due to accumulation of toxic metabolites.

References

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