Bacterial biotransformation of the PAH nitrogen heterocycle, 9H-carbazole and metabolite analyses by liquid chromatography tandem mass spectrometry

OSATOKO Mizumura, SYUNSUKE UNO, ROBERT KANALY

Yokohama City University

Key Word: PAH, LC/ESI-MS/MS, carbazole, biodegradation

Azarenes, nitrogen-containing heterocyclic polycyclic aromatic hydrocarbons (NPAHs) are environmental contaminants that are more water soluble than their PAH counterparts and as such their ultimate environmental fates are of deep interest. A newly isolated soil bacterial strain was found to biotransform approximately 30% of 50 mg/L of the NPAH, 9H-carbazole in 6 days. Extraction and HPLC analyses of biodegradation microcosms by UV detection indicated the presence of multiple metabolites in pH 7 and pH 2 extracts of ethyl acetate and these extracts were subsequently transferred into methanol and analyzed by liquid chromatography electrospray ionization mass spectrometry (LC/ESI-MS) operating in negative ionization mode. Mass spectral scans of sample extracts obtained after 3 and 6 days, revealed the presence of various biotransformation products of 9H-carbazole that were not detected in the abiotic and biotic controls, including peaks that corresponded to the pseudo-molecular ions: [230-H]-, [204-H]+ and [136-H]-. LC/ESI(−)-MS/MS spectra were then acquired in product ion and precursor ion scanning modes under varying collision cell energies to obtain more detailed fragmentation pattern information for these metabolites. Analyses of diagnostic ions revealed the identities of three biotransformation products of 9H-carbazole and they are proposed to be: 1) 2-(2-carboxy-vinyl)-1H-indole-3-carboxylic acid, 2) indole-2,3-dicarboxylic acid, and 3) anthranilic acid (2-aminobenzoic acid). The detection of these three ortho-ring fission products indicates that the bacterial strain biotransforms 9H-carbazole by lateral dioxygenation. Further LC/ESI-MS/MS investigation is underway to determine the structures of other suspected metabolites.

e-mail: no95261f@yokohama-cu.ac.jp

Liquid chromatography tandem mass spectrometry analyses of the bacterial biotransformation products of the sulfur heterocycle benzothiophene

OYUUKI NEMOTO, ROBERT KANALY

Yokohama City University

Key Word: PAH, heterocycle, LC/ESI-MS/MS, biodegradation

Sulfur-containing heterocyclic polycyclic aromatic hydrocarbons (PAHS) are environmental contaminants and common constituents of fossil fuels. There is much interest to know their ultimate environmental fates through microbial processes and to investigate the potential use of microorganisms for the fuel desulfurization. A soil bacterium, recently isolated from a hydrocarbon-degrading consortium was found to biotransform the sulfur heterocycle, benzothiophene following induction by growth on the 3-ring PAH phenanthrene. Bacterial cells were grown for 6 days, harvested, washed and starved and subsequently inoculated into 100-ml size microcosms that consisted of 100 mg/L benzothiophene in 23 mL of Staniers basal medium each. Microcosms were incubated at 28°C for 7 days and samples were extracted at different intervals with ethyl acetate after lowering the pH to 2. Extracts were analyzed by liquid chromatography electrospray ionization mass spectrometry (LC/ESI-MS) operating in negative ionization mode and mass spectral scans of sample extracts revealed the presence of various biotransformation products of benzothiophene that were not detected in the abiotic and biotic controls. LC/ESI(−)-MS/MS spectra were then acquired in product ion and precursor ion scanning modes under varying collision cell energies to obtain more detailed fragmentation pattern information for these metabolites and analyses of diagnostic ions revealed the identities of at least three biotransformation products of benzothiophene proposed to be: 1) 2-hydroxy-3-oxo-2,3-dihydrobenzothiophene [165-H]-, 2) 2-mercaptophenylglyoxaldehyde, [165-H]-, and 3) what appears to be an oxidation product of 2′-mercaptomandeldehyde and 2′-mercaptoenylglyoxalate [347-H]-. The detection of these metabolites indicates that this bacterial strain biotransforms benzothiophene through the five-membered ring.

e-mail: no95261f@yokohama-cu.ac.jp