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Genotoxic activation of 3,6-Dinitrobenzo[e]pyrene in SOS/umu assay

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Keywords: 3,6-Dinitrobenzo[e]pyrene; Metabolic activation; Genotoxicity; umu test system.

3,6-Dinitrobenzo[e]pyrene (DNBeP) is a potent mutagen identified in surface soil in two metropolitan areas of Japan. To clarify whether human cytochrome P450 (CYP) enzymes and human N-acetyltransferases (NATs) contribute to the bioactivation of DNBeP, an umu assay was used on Salmonella typhimurium strains expressing bacterial O-acetyltransferase (O-AT), and eight human CYP enzymes, NADPH-cytochrome P450 reductase and O-AT, and human NAT1 and NAT2. Genotoxicity was measured by induction of umuC gene expression. The dose-dependent induction of umuC by DNBeP was observed at concentrations between 0.01 and 1 nM in O-AT-expression strain, but not in O-AT-deficient strain. Although the induction of DNBeP was exhibited in NAT1-expressing strain, the induction was 10-fold lower concentrations than in the NAT2-expressing strain. In the CYP3A4+, CYP1A1-, and CYP1A2-expressing strains, DNBeP was found to be activated to reactive metabolites that cause induction of umuC gene expression compared with other CYP-expressing strains. These results suggest that human CYP3A4, 1A1 and 1A2 as well as human NAT2 contributed to the high genotoxicity of DNBeP and its metabolites.

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Development of bacterial tester strains to detect the mutagenicity of polycyclic aromatic hydrocarbons sensitively and specifically

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Keywords: polycyclic aromatic hydrocarbons, DNA polymerase IV, Ames test.

Benzo[a]pyrene (B[a]P), one of polycyclic aromatic hydrocarbons (PAH), is a ubiquitous environmental pollutant and a potent mutagen and carcinogen. We have constructed Salmonella typhimurium strain YG5161 by introduction of plasmid pYG768 carrying E. coli dinB encoding DNA polymerase IV to strain TA1538. This strain displays seven and four times higher sensitivity to the mutagenicity of B[a]P than do strain TA1538 and strain TA98, respectively. However, strain YG5161 also detects the mutagenicity of aromatic amines and nitroarenes with high sensitivity, which veils the mutagenicity of PAHs in complex mixtures. To increase the specificity to PAHs, we disrupted a nitroreductase gene, i.e., nfsB, of strain TA1538 1,8-DNP, which lacks O-acetyltransferase and another nitroreductases, i.e., NfsA. These enzymes collectively increase the sensitivity of Ames tester strains to aromatic amines and nitroarenes. The triple knockout strain YG7156 exhibited reduced sensitivity to 1-nitropyrene. After introducing plasmid pYG768 to the knockout strain, the resulting strain would detect the mutagenicity of PAHs more specifically and sensitively compared with YG5161 and YG7156.

多環芳香族炭化水素の変異原性を高感度、特異的に検出するバクテリアテスト株の開発
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