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研究課題名（和文）環境保全微生物に生じたDNAダメージと修復のアダクトミクスに関する研究

研究課題名（英文）Study on DNA adductomics related to DNA damage and repair involving environmental microorganisms

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研究成果の概要（和文）：環境汚染物質研究において、毒性の高い多環芳香族炭化水素(PAH)の環境循環を知ることはとても重要である。本研究では、汚染物質分解細菌として知られるスフィンゴビウム属KK22株のPAH分解能と、分解における代謝と毒性応答に注目した。KK22は、PAHをカテコール系代謝物へと変換することがわかった。一方で、このカテコール系代謝物は、KK22にDNA損傷を誘発させることがDNA付加体の網羅的解析によって示された。トランスクリプトームを用いた解析によって、カテコール系代謝物に対してのKK22の応答機構を解析した。その結果、KK22がDNAダメージ修復タンパクを発現させていることが示唆された。

研究成果の概要（英文）：Bacteria that biodegrade genotoxicants such as polycyclic aromatic hydrocarbons (PAHs) play important roles in carbon cycling of environmental pollutants. During PAH biodegradation, metabolite production may cause DNA damage and mutations which have implications for bacterial evolution. Biodegradation and exposure experiments were conducted with Sphingobium barthaii KK22. PAH-catechol metabolites were produced during biotransformation and gene expression related to PAH-catechol production was confirmed by RT-PCR. DNA adducts, including a PAH-catechol adduct were identified through DNA damage assessment by LC/ESI-MS/MS DNA adductomics and provided first direct evidence that bacteria are subjected to bulky DNA damage during PAH exposure. Increased levels of reactive oxygen species and oxidative DNA adducts were confirmed. RNA transcriptome analyses revealed that more than 600 genes were differentially expressed from PAH-catechol exposure including genes involved in DNA damage repair.

研究分野：　環境毒性学

キーワード：　DNAアダクトミクス　多環芳香族炭化水素　Biotransformation

１．研究開始当初の背景

Little is known about the manner by which aromatic hydrocarbon-degrading bacteria manage genotoxicity during growth on and biodegradation of environmental pollutants such as polycyclic aromatic hydrocarbons (PAHs) and their potentially reactive biotransformation products. During biodegradation of PAHs by bacteria, catechols may be produced that undergo redox cycling with aromatic quinones.

Quinones are a group of highly reactive compounds that may act as agents of genotoxicity and cause different types of damage to growing cells including DNA damage. DNA damage may occur through the production of reactive oxygen species (ROS) and through electrophilic attack of DNA bases to form DNA damage known as DNA adducts.

２．研究の目的

Towards the development of methods to investigate the conditions of bacterial cells during exposure and biodegradation of PAHs by bacteria, experiments were conducted with the PAH-degrading soil bacterium *Sphingobium barthaii* KK22 with aims, (1) to confirm catechol production during PAH biodegradation by this strain, (2) to develop methods for bacterial DNA extraction and preparation of 2'-deoxy-nucleosides for analyses by liquid chromatography electrospray ionization tandem mass spectrometry (LC/ESI-MS/MS), (3) to develop DNA adductomic methods to analyze 2'-deoxynucleosides for putative DNA adducts from this strain by multiple reaction monitoring mode (MRM), (4) where possible, to propose identities for DNA adduct candidates through LC/ESI-MS/MS product ion scan mode and comparison with authentic standards, (5) to develop methods to measure levels of ROS produced during exposure of this strain to reactive chemicals, and (6) to conduct comparative gene expression analyses by RT-PCR and RNA transcriptomics of bacterial cells under different exposure conditions.

３．研究の方法

(1) Biotransformation assays were conducted by exposing strain KK22 cells to the PAHs, naphthalene and phenanthrene, followed by liquid-liquid extraction of growth media, and analyses by LC/ESI-MS/MS full scan and product ion scan modes.

(2) DNA adductomics analyses were conducted by exposing strain KK22 cells to hydrogen peroxide and 1,2-naphthoquinone, followed by DNA extraction, digestion and purification of bacterial DNA 2'-deoxynucleosides. LC/ESI-MS/MS analyses were conducted by MRM and product ion scan modes. Proposals of DNA adduct identities were conducted by analyses of fragmentation patterns from product ion scan analyses and comparison to authentic DNA adduct standards. 1,2-naphthoquinone was reacted with 2'-deoxyguanosine to produce DNA adduct standard reaction products that were used for comparative purposes with putative DNA adduct targets.

(3) To measure levels of ROS in bacterial cells, cells were cultivated under different exposure conditions and an assay using 2',7'-dichlorodihydrofluorescin diacetate (DCFH-DA) for fluorescence detection of ROS was developed.

(4) Gene expression was measured by RT-PCR analyses whereby strain KK22 cells were grown and exposed to different chemicals. Cellular RNA was extracted, cDNAs synthesized and RT-PCR analyses were conducted on genes related to cellular metabolism.

(5) RNA transcriptomics was conducted by RNA-seq method whereby strain KK22 cells were incubated under different exposure conditions and comparative analyses were made to determine relative levels of gene expression using data annotated from the whole genome sequence of strain KK22.

４．研究成果

(1) The model organism, *Sphingobium barthaii* strain KK22 was decided based upon its abilities to grown on and biodegrade PAHs.

(2) Whole genome sequencing and gene annotation of strain KK22 showed that at least seven oxygenase genes were present, of which at least three genes were potential dioxygenase genes. Primers for RT-PCR analyses were constructed based upon gene sequences from genome annotation. RT-PCR analyses of A1f dioxygenase gene expression confirmed its relationship to phenanthrene biodegradation.

(3) Metabolic pathways for the

biodegradation of the PAHs, naphthalene and phenanthrene were evaluated by biotransformation assays and dihydroxy-metabolites of phenanthrene and naphthalene (PAH-catechols) were identified by LC/ESI-MS/MS.

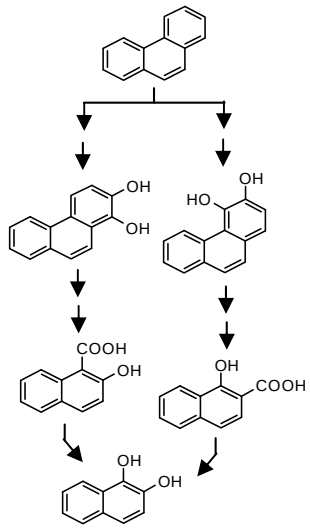

Fig. 1 Biodegradation of phenanthrene led to the production of PAH-catechols including 1,2-dihydroxynaphthalene (LC/ESI-MS/MS).

(4) Methods for extraction and preparation of prokaryotic DNA for adductome analyses were developed and confirmed.

(5) DNA adductome mapping of bacterial cells exposed to hydroperoxide revealed numerous putative DNA adducts and the identities of some, such as 8-oxo-2'-deoxyguanosine and 1,N6-etheno-2'-deoxy-adenosine were confirmed.

(6) DNA adductome mapping of bacterial cells exposed to 1,2-naphthoquinone revealed numerous putative DNA adducts and some appeared to be exposure-specific.

(7) Synthesis and LC/ESI-MS/MS analyses of DNA adduct standards of 2'-deoxyguanosine supported that bulky dihydroxynaphthalene DNA adducts appeared to have occurred in strain KK22 from exposure to 1,2-naphthoquinone.

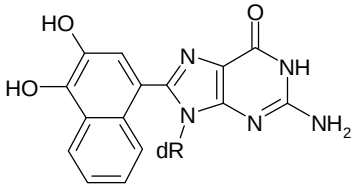

Fig. 2 Proposed structure of DNA adduct detected from strain KK22 cells after exposure to 1,2-naphthoquinone; dR: 2'-deoxyribose.

(8) An assay for the evaluation of ROS in bacterial cells was established. Results of ROS studies showed that ROS levels increased in cells that were exposed to hydroperoxide and 1,2-napththoquinone relative to unexposed controls. These data corroborated data from 1,2-napththo-quinone adductome analyses which showed that the oxidative adduct, 8-oxo-2'-deoxyguanosine was detected at higher levels in cells exposed to 1,2-napththo-quinone.

(9) Results of comparative RNA transcriptome analyses of cells exposed to 1,2-napthoquinone and cells not exposed to 1,2-napththoquinone revealed approx-imately 600 genes that were up-regulated in exposed cells. Data analyses are ongoing.

５．主な発表論文等

〔雑誌論文〕（計 5 件）

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(6) Fukuoka, K., and **R.A. Kanaly**. 2015. Investigation of DNA adducts in PAH-degrading bacterial cells after exposure to hydrogen peroxide by DNA adductomics. *The 44th Annual Meeting of the Japanese Environmental Mutagen Society*, Nov. 27-28, Fukuoka, Japan. Abstr. P-027.

(7) **Kanaly, R A.,** K. Fukuoka, and R. Micheletto. 2015. Analysis of DNA damage in bacterial cells by DNA adductomics, *7th International Congress of the Asian Society of Toxicology (ASIATOX 2015)*, June 23-26, Jeju, South Korea, Abstr. Abstr. N315.

６．研究組織

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