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Mini-review

Bacterial diversity losses: A potential extracellular driving mechanism involving the molecular ecological function of hydrophobic polycyclic aromatic hydrocarbons☆

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ABSTRACT

The DNA transformation is vital to the horizontal gene transfer (HGT). The low-efficiency transformation of bare plasmid exposed to hydrophobic polycyclic aromatic hydrocarbons (PAHs) decreases the gene transfer level, and is possibly related to the loss of bacterial diversity at present. PAHs have great affinity for bare DNA through dispersion force and π–π overlap between PAHs and bases. These noncovalent interactions between PAHs and bases reduced the transformational efficiency of plasmid into bacterial recipients. Meanwhile these low-efficiency transformations for plasmid are controlled by the ions like Ca^{2+} in environment, in turn, presence of 0.5 mmol L^{-1} Ca^{2+} recovered the efficiency from 3.2 (phenanthrene), 3.5 (pyrene) to about 4.45 and 4.75, respectively. The combination of Ca^{2+} with the —POO^-— groups in DNA forms strong electrovalent bonds, weakening the molecular effect of DNA on PAHs and in turn promoting the gene transfer exposed to PAHs.

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Until now, researchers, including ecologists and environmentalists, have generally attributed the losses in bacterial diversity caused by anthropological contaminants to merely the direct intracellular damages. Public document has proposed that the interaction of intracellular DNA with contaminants induces changes in genetic information via the effects of mutation, teratogenesis, and carcinogenesis [1–3], and hold that these effects result in the death of organisms. Such viewpoints are acted as the main theoretical basis for the bacterial diversity losses caused by hydrophobic organic contaminants. Although researchers recognize that these lateral transfers effectively change the ecological and pathogenic characteristics of bacterial species [4], few doubt that the diversity loss caused by anthropogenic contaminants is also dominated by the effects of contaminants on DNA transfer. The DNA transformation, which means transformation of competent cells through uptake of extracellular DNA, is vital to the horizontal gene transfer (HGT). The low-efficiency transformation of bare plasmid exposed to hydrophobic polycyclic aromatic hydrocarbons (PAHs) decreases the gene transfer level. Primary case study implies that the gene transfer of bare DNA affected by the interaction of DNA with polycyclic aromatic hydrocarbon (PAH) contaminants may be related to the loss of bacterial diversity [4,5].

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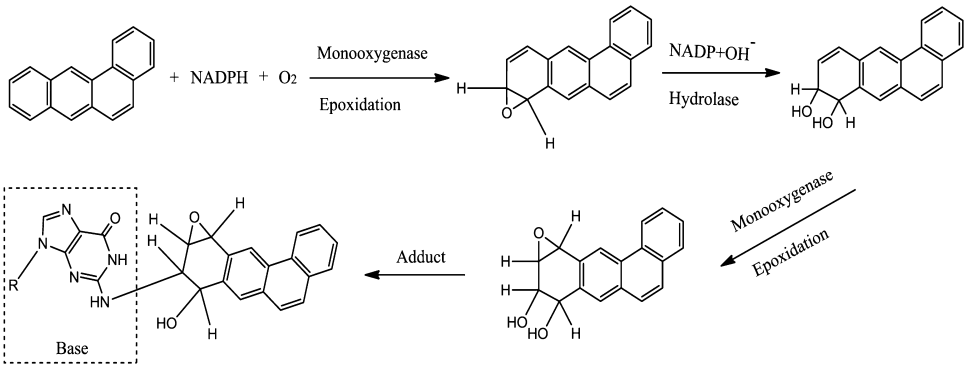

Fig. 1. The reported main pathway by which PAHs affect interacellular DNA [15].

1. Role of bare DNA in bacterial diversity and evolution

Horizontal gene transfer (HGT) is an important process by which a bacterium takes up exogenous free DNA and incorporates it into its own chromosome via homologous recombination or converts it into an autonomous extrachromosomal replicon [6,7]. This plays an important role in genetic variation and heredity, ecological and genetic diversity, and evolution [4,8]. On the death of an organism, the intracellular germplasm and extracellular materials are released into the soil and water, where they can be transferred to other living cells and expressed in the new host [9]. Many such gene transfers between different organisms have been reported [10]. For example, up to between 10% and 16% of *Escherichia coli* DNA has originated due to HGT [4,11]. In addition, *E. coli* isolated from the intestines of Japanese individuals was found to contain gene segments that originated from the ocean environment via edible seafood, which indicates that gene transfer between microorganisms and animals is ubiquitous in natural environments [12,13].

2. Interaction between bare DNA and hydrophobic PAHs

Compared to DNA in the intracellular environment, “bare” DNA is quite sensitive and vulnerable to direct damage on exposure to

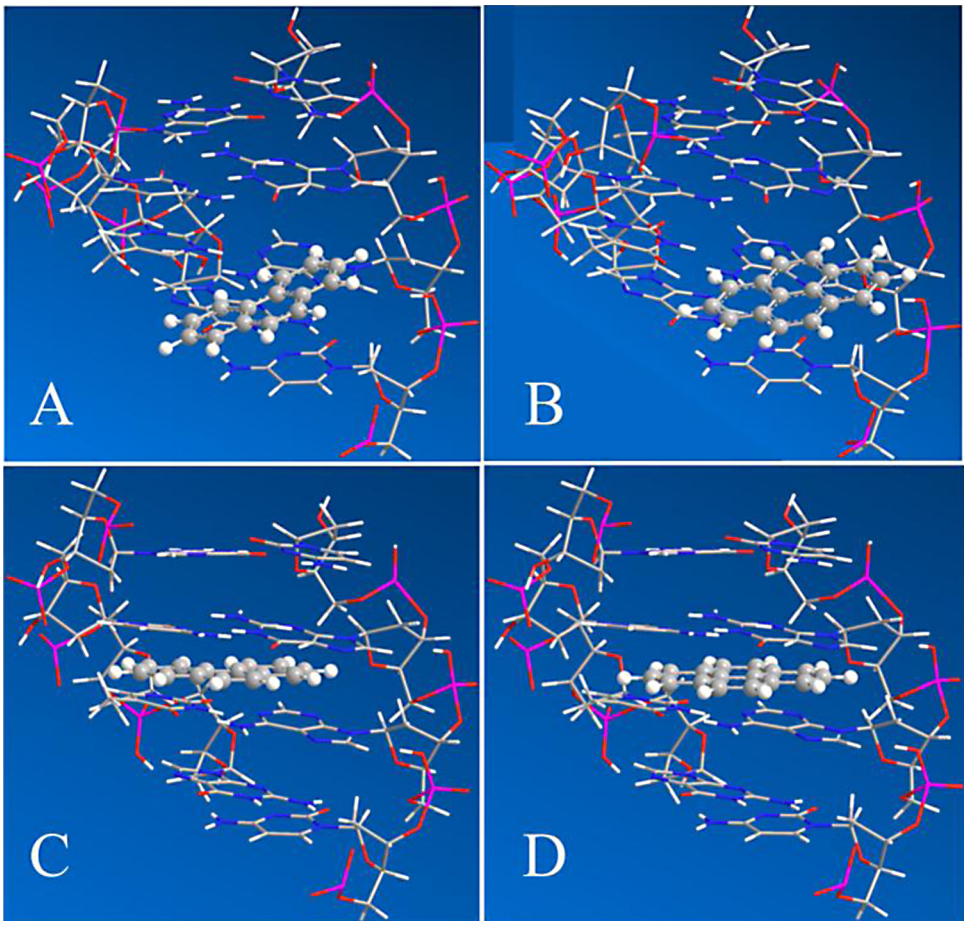

Fig. 2. Interaction sites between PAHs and DNA. Fig. 2A and B shows that phenanthrene and pyrene inserted into grooves in DNA; and Fig. 2C and D indicates that phenanthrene and pyrene inserted between bases through dispersion force and π–π overlap of PAHs-bases. The interaction between PAHs and DNA are calculated using the Autodock 4.2 [18], and are optimized using the Orca 1.8.1 (BLYP D3 GCP(DFT/SVP) def2-SVP def2-SVP/J) [19]. Solvent (water) effects were taken into consideration implicitly.

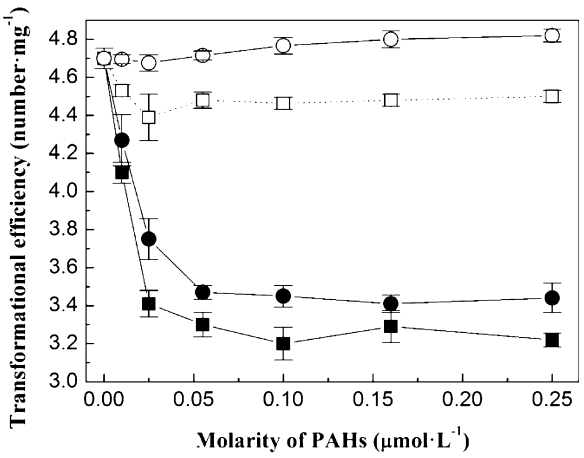

Fig. 3. The Ca^{2+}-influenced transformational efficiency of plasmids exposed to phenanthrene (■), pyrene (●), 0.5 mmol L^{-1} Ca^{2+} plus phenanthrene (□), and 0.5 mmol L^{-1} Ca^{2+} plus pyrene (○). The transformational index was calculated as follows: transformational efficiency = $\log_{10}$[the ratio of the number of transformants (unit number) versus the mass of the added plasmid DNA (mg)]. Error bars represent one standard deviation ($n = 3$). The method for DNA transformation was referenced according to the reference [14] and [19]. In brief, two kinds of pUC19 plasmid DNA solutions were prepared: one only contains PAHs in designed concentration gradients and another one contains 0.5 mmol L^{-1} Ca^{2+} besides PAHs. After incubation for 2 h, 5 μL of the plasmid DNA solution without PAHs (using ultrafiltration centrifuge tube) was transferred into the competent cells of *E. coli DH5a*. The mixtures were heat-shocked at 42 °C for 90 s, then placed into a ice-water bath for 3 min. With an addition of SOC liquid culture media, the solutions were incubated for 45 min at 37 °C, and then spread on Luria-Bertani solid culture media containing 100 mg L^{-1} of ampicillin sodium. After about 36 h, CFU was counted and transformation efficiency was calculated.

hydrophobic PAHs. These persistent lipophilic organic contaminants with high biological affinity are ubiquitous in the environment [14]. Owing to their strong hydrophobic properties, PAHs have greater affinity for such organic substances as compared to other organic contaminants or heavy metals. Therefore, the PAHs in the same environmental background may be capable of partitioning organic substances. Any "bare" germplasm released into the soil or water is directly exposed to these hazardous materials.

The extracellular interaction of DNA with PAHs is completely different from that in an intracellular environment. Fig. 1 shows the main pathway by which PAHs affect intracellular DNA. In it, the PAH molecules are first catalyzed into "OH–PAH" by a series of enzymes, and the active "—OH" functional groups in the PAH molecules combine with the bases of DNA by forming chemical "DNA adducts" based on chemical bonds [15].

In contrast, the interaction of PAHs with free DNA in the extracellular environment is based on weak molecular forces. Although changes in the structure, backbone composition, and guanine constituents of DNA induced by PAHs which can be inserted into double strands have been observed, and imidazole-like derivatives are produced from the combination of imidazole rings with pyrene [5,17], PAHs lack active functional groups related to the functional sites of DNA, and no enzyme catalysis occurs in the extracellular environment. Therefore, the changes in DNA seen in the extracellular environment cannot be attributed to the formation of chemical bonds between DNA and PAHs, but are linked to the weak molecular forces between DNA molecules and PAHs. In other words, polar DNA molecules can induce relative displacement between the electron cloud and atomic nucleus of non-polar PAHs, causing the appearance of dipoles with excellent induction forces in PAH molecules. These induction forces of the PAH molecules then attract polar DNA molecules with their innate dipoles [15]. PAHs are inserted into grooves in DNA (Fig. 2A and B) or between bases (Fig. 2C and D) through dispersion force and π–π overlap between PAHs and bases.

3. Ca^{2+}-controlled transfer of DNA exposed to PAHs

Free calcium ions enhance the efficiency of DNA transformation into bacterial recipients by forming hydroxyl–calcium phosphate complexes in DNA [6]. The interaction between "bare" DNA and PAH molecules is based on a weak molecular force, which implies that such weak molecular forces are more strongly affected by the chemical bonds of Ca-DNA. Fig. 3 supports this viewpoint. The transformational efficiency of DNA plasmids (pUC19) with no PAHs and Ca^{2+} is 4.7 (PAHs are exposed to plasmid DNA and did not directly contact with host cell (*E. coli DH5a*)). Isolated phenanthrene and pyrene clearly resulted in low-efficiency transformation; the efficiency decreased to about 3.2 and 3.5 with increasing PAH concentrations up to 0.25 μmol L^{-1}, respectively. The presence of Ca^{2+} significantly promoted the low-efficiency transformation of plasmid exposure to PAHs, and the presence of 0.5 mmol L^{-1} Ca^{2+} recovered the efficiency from 3.2, 3.5 to about 4.45 and 4.75, respectively [15].

Compared to the enhanced transformational efficiency caused by higher concentrations of Ca^{2+} (>80 mmol L^{-1}) (results found in Refs. [6,16]), these results explain how a very tiny amount of Ca^{2+} can enhance gene transfer involving isolated DNA via PAHs. Although previous reports postulated that a Ca^{2+} concentration >80 mmol L^{-1} significantly enhanced the DNA transformation via the formation of hydroxyl–calcium phosphate complexes in DNA [6,16],Fig. 3 indicates that the

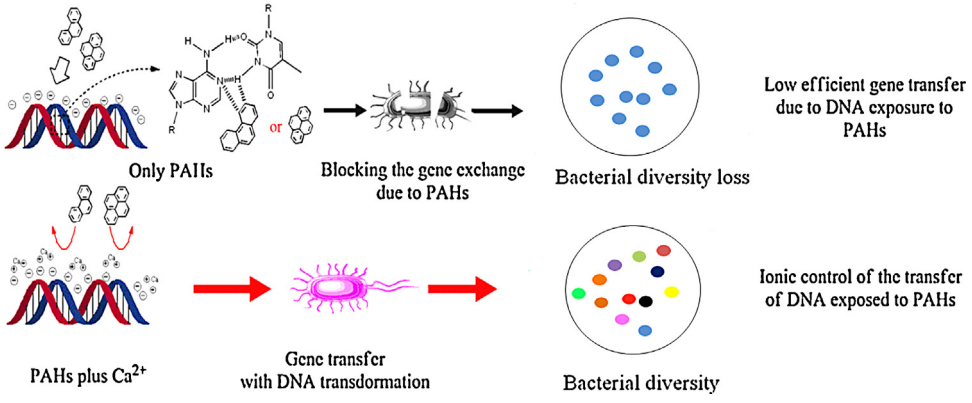

Fig. 4. The relationship among the low efficient transformation of DNA, gene transfer, ionic control, and bacterial diversity loss. In plate, the different colors represent the various bacteria. The blue dots in plate point to the oneness of bacteria. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

necessary Ca^{2+}concentration of 0.5 mmol L^{-1} obviously promoted the transfer efficiency of plasmid DNA exposed to PAHs. In other words, the enhancement of DNA transformation on exposure to PAHs cannot be attributed to the formation of hydroxyl–calcium phosphate by anti-DNase in DNA, but is related to the isolation of the DNA from PAHs by Ca^{2+}.

Based on this experimental evidence, such a Ca^{2+}-controlled mechanism for the transfer of genetic material exposed to PAHs may involve the combination of Ca^{2+} with the —POO^-— groups in DNA to form strong electrovalent bonds. Because —POO^-— groups and Ca^{2+} are different in electric charges, each Ca^{2+} will theoretically bond two —POO^-—, resulting in a chain of —POO^-— groups that may lock up neighboring nucleotides [15]. This will weaken the molecular effect of DNA on PAH and promote the low-efficiency transfer of DNA plasmids exposed to PAH contaminants (Fig. 4).

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