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## Mycobacteria in environmental clean-up

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### Introduction

Mycobacteria are a diverse group of rod-shaped acid-fast bacteria that include more than 70 different species. The obligate *Mycobacterium tuberculosis* and *Mycobacterium leprae* cause tuberculosis and leprosy respectively. Most other members of the soil and water in both rural and urban settings throughout the world. There is no standard naming convention for the group of microorganisms. They may be referred to as nontuberculous mycobacteria (NTM), mycobacteria other than tuberculosis (MOTT), atypical mycobacteria and/or environmental mycobacteria. They can be found in aerosols, rivers and swamps, in treated city water, public swimming pools, hot spas, humidifiers, aquariums, garden soils, food, and many other places. Because they are protected by their waxy lipid-rich cell wall, mycobacteria are resistant to disinfectants and water treatment measures. Almost half of the NTM species identified are associated with opportunistic infections in animals and humans, and several have caused sporadic outbreaks. But, certain species of mycobacteria play an important role in environmental clean-up processes such as biodegradation of polycyclic aromatic hydrocarbons (PAHs), crude oils and chemical dyes and other recalcitrant molecules. This article will focus on the role of certain species of NTM in bioremediation of major environmental pollutant, PAHs.

### PAHs

PAHs are a group of organic compounds of two or more fused benzene rings in linear, circular or cluster arrangements. Contamination by PAHs is widespread and has been detected in air, water, soil and sediment (Johnson *et al.*, 2005)(Fig.1). Although PAHs are present in the terrestrial environment in low concentrations, pollution is mainly due to human activities. PAHs are produced from incomplete combustion of organic materials, fossil fuels, petroleum product spillage and various domestic and industrial activities.



Fig. 1. Oil (PAH) Contaminated site

Processes and sources that can produce or contain PAHs are given in table 1.

Table 1. Sources and processes of PAHs	
Natural oil seeps	Combustion of fossil fuels
Refinery and oil storage waste	Tobacco and cigarette smoke
Accidental spills from oil tanks and other ships	Forest and prairie fires
Municipal and urban wastewater discharge runoff	Rural and urban sewage sludge
River-borne pollution	Refuse and waste incineration
Atmospheric fallout of fly ash particulates	Coal gasification and liquefaction processes
Petrochemical industrial effluents	Creosote and other wood preservative wastes
Coal tar and other coal wastes	Chronic input associated with boating activities
Automobile engine exhausts	

**Source:** Johnson *et al.* (2005)

PAHs, when discharged into the environment, pose serious health hazards owing to their carcinogenic, mutagenic and genotoxic properties. Based on their ecotoxicity, United States Environmental Protection Agency has prioritized 16 PAHs as environmental pollutants (Mohanty *et al.*, 2010). Some important PAHs include naphthalene, fluorene, phenanthrene, anthracene, Benzo(a)fluorene, pyrene, chrysene and benzo(a)pyrene. Based on their molecular weight, PAHs are divided into high molecular weight PAHs (compounds with four or more benzene rings) and low molecular weight PAHs (compounds with two or three benzene rings) (Law *et al.*, 2002). High molecular weight PAHs are resistant to degradation and pose a concern to human health because of their carcinogenic potential. Low molecular weight PAHs do not pose a risk to human health as carcinogens but they are toxic to fish and other marine organisms. The persistence of these toxic pollutants in the environment can be attributed to their various physical and chemical properties (Tab.2). The solubility and volatility of these chemicals are decreased due to its hydrophobic nature and the presence of more number of benzene rings.

Table 2. Some physico-chemical properties of certain PAHs

PAH	Rings	Melting point (°C)	Boiling point (°C)	Solubility (mg/l)
Phenanthrene	3	101	340	1.29
Anthracene	3	216	340	0.07
Fluoranthene	4	111	250	0.26
Benz(a)anthracene	4	158	400	0.014
Pyrene	4	149	360	0.14
Chrysene	4	255	488	0.002
Benzo(a)pyrene	5	179	496	0.0038
Dibenz(a,h)anthracene	5	262	524	0.0005

Considering their chemical and physical properties, PAHs are not easily degradable and may persist in the environment. For example, the half life of tricyclic phenanthrene ranges from 16-126 days in soil, whereas for the five ringed high molecular weight PAH benzo(a)pyrene, the half life may range from 229 -1400 days (Mrozik *et al.*, 2003).

#### Remediation of PAHs from the environment

The removal of PAHs has been demonstrated by various means including chemical oxidation, photo-oxidation, volatilization and bioremediation. Bioremediation seems to be the most promising method for the removal of these toxic pollutants using microbial populations. The microbial mediated approach is cost effective, exploits naturally existing diverse bacterial communities with high specificity for degradation and produces innocuous end products. But the success of bioremediation with microbes is limited to low molecular weight compounds. The high molecular weight compounds are resistance to bioremediation is due to various reasons such as their structural stability, low solubility and high tendency to interact with non-aqueous phases. In fact, the bioavailability of PAHs has been shown to decrease logarithmically in relation to the increasing molecular weight (Johnson *et al.*, 2005). However, microbial degradation has been identified as a potential means of successfully removing PAHs from contaminated environments (Fig.2). Several researchers have reported the biodegradation kinetics of individual and mixture of PAHs by various microbial species.



Fig. 2. Remediation of oil contaminated site

#### Mycobacteria in PAHs remediation

A large number of PAH-degrading microorganisms have been isolated from PAH contaminated environmental communities and characterized. Almost all PAH-degrading isolates are aerobic and able to use PAHs as sole carbon and energy source. Members of the genera *Pseudomonas*, *Sphingomonas* and *Mycobacterium* are well known for their degradation potential towards PAHs and have acquired diverse capabilities to inhabit a wide range of environments.

Mycobacteria seem to be more specialized in the degradation of high molecular weight PAHs such as pyrene. They are also unique in using hydrophobic sorbed/organic dissolved PAHs while *Pseudomonas* and *Sphingomonas* strains prefer aqueous liquid systems. In *Sphingomonas* and *Pseudomonas* strains, PAH-catabolic genes are often located in conjugative plasmids while in *Mycobacterium* species they seem to be chromosomal. So far, all PAHs degrading mycobacterial isolates could be placed in the phylogenetic branch of fast growing *Mycobacterium* species. Most PAH-degrading mycobacteria are scotochromogenic and produces smooth,

round yellow colonies on solid media. The PAH-degrading mycobacterial isolates were, based on 16S rRNA gene sequence, often assigned to the species *M. frederiksborgense*, *M. gilvum*, *M. austroafricanum*, *M. vanbaalenii*, *M. holderi*, *M. flavescens*, *M. anthracenicum* and *M. chelonae*.

*Mycobacterium vanbaalenii*, a fast growing species of *Mycobacterium* present in soil, is an exceptional microbe in its ability to oxidatively degrade a great variety of low and high molecular weight PAHs in soil. Phenanthrene, a tricyclic, low molecular weight PAH, is considered as a prototype PAH and is often used to detect PAH contamination. The *M. vanbaalenii* can mineralise 90% of added phenanthrene in about 14 days. PAH degradation by *M. vanbaalenii* is catalyzed by dioxygenases and monooxygenases, based on metabolites identified. Another study indicated the role of *M. vanbaalenii* in degradation of one of the most potent carcinogenic PAH, benzo(a)pyrene. Degradation of benzo(a)pyrene is confirmed to be a metabolic detoxification process based on the noncarcinogenic properties of the metabolites produced (Moody *et al.*, 2004). Mohanty *et al.* (2010) analysed the biodegradation of PAHs such as pyrene, anthracene and naphthalene by fast growing mycobacterium, *M. frederiksborgense*. The results showed that the PAH removals varied 54-81% when each PAH was at low concentrations in the mixture and 67-89% at higher concentrations. Herwijnen *et al.* (2003) had reported the degradation of anthracene by *Mycobacterium* sp. (strain LB501T) and concluded that the degradation proceeded via a novel pathway through O-phthalic acid. Cultures of *Mycobacterium* sp. (Strain PYR-1) were dosed with anthracene/phenanthrene and after 14 hours of incubation had degraded 92 and 90% of the added anthracene and phenanthrene, respectively (Moody *et al.*, 2001). Boldrin *et al.* (1993) reported the degradation of phenanthrene, fluorine, fluoranthene and pyrene by a *Mycobacterium* sp. Strain BB1 isolated from a former coal gasification site. Lopez *et al.* (2008) reported the simultaneous biodegradation of creosote by a pyrene-degrading *Mycobacterium* sp. AP1. Miller *et al.* (2004) isolated three *Mycobacterium* strains from creosote wood preservative-contaminated soil and they were able to rapidly degrade phenanthrene. The phylogeny of the 16S rDNA analysis showed that they were distinct from other mycobacterial isolates with PAH degrading activities. Catalase and superoxide dismutase (SOD) isozyme profiles confirmed that each isolate was distinct from each other and from the PAH degrading *Mycobacterium*, *M. vanbaalenii*. Doss-Ross and Cerniglia (1996) reported that *Mycobacterium flavescens* could degrade pyrene. Interestingly, mycobacteria have been repeatedly isolated as bacteria that are able to degrade high molecular weight PAHs, pyrene and benzo(a)pyrene. These bacteria are known for their comparatively slow growth. However, their growth on PAHs is faster than other bacteria: for example, the growth rate of *Mycobacterium* sp. BB1 was twice faster than *Rhodococcus* sp. UW1 (Heinkamp *et al.*, 1988).

Certain ongoing experiments to improve biodegradation have proposed the use of surfactants or organic solvents to improve the bioavailability of PAHs. The use of chemical surfactants increases the concentration of hydrophobic compounds in the aqueous phase by the process of emulsification and hence, promotes bioavailability of substrates. It has also been hypothesized that mycolic acids, the major cell wall components of mycobacteria may act as a type of biosurfactant and play an important role in increasing the efficiency of degradation of PAHs by reducing the surface tension of the molecules (Johnson and Korlson, 2004). So it is conceivable that the versatility of mycobacteria in degradation of PAHs makes it a potential target for use in PAH remediation. However, more research is needed for its efficient application.

### Conclusion

The diversity of fast growing *Mycobacterium* species in the environment is still greatly unknown but could be of major interest for bioremediation of PAH contaminated soils. Therefore, methods for community analysis and monitoring of indigenous and/or inoculated fast growing PAH-degrading *Mycobacterium* species in soil are needed.

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