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Comparison of mineralization of solid-sorbed phenanthrene by polycyclic aromatic hydrocarbon (PAH)-degrading *Mycobacterium* spp. and *Sphingomonas* spp.

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Abstract The mineralization of ^{14}C-phenanthrene, sorbed to porous synthetic amberlite sorbents, i.e., IRC50, XAD7-HP, and XAD2, by three phenanthrene-degrading *Mycobacterium* soil isolates, i.e., strains VM552, VM531, and VM451 and three phenanthrene-degrading *Sphingomonas* soil isolates, i.e., strains LH162, EPA505 and LH227, was compared. In P-buffer and in the presence of IRC50, for all strains the maximum rate of mineralization of ^{14}C-phenanthrene was significantly higher (1.1–1.9 ng ml^{-1} h^{-1}) than the initial abiotic desorption rate (0.2 ng ml^{-1} h^{-1}), indicating that both *Mycobacterium* and *Sphingomonas* utilize sorbed phenanthrene with a higher rate than can be explained by abiotic desorption. Because all *Mycobacterium* and *Sphingomonas* strains belonged to different species, it can be suggested that this feature is intrinsic to those genera rather than a specific feature of a particular strain. The final mineralization extent in P-buffer in the presence of IRC50 was about a factor of two higher for the *Mycobacterium* strains compared to the *Sphingomonas* strains. Moreover, a significantly higher normalized phenanthrene mineralization ratio in the presence of IRC50 to the control (without IRC50) was found for the *Mycobacterium* strains compared to the normalized ratio found for the *Sphingomonas* strains. Addition of minimal nutrients had a more beneficial effect on phenanthrene mineralization by *Sphingomonas* compared to *Mycobacterium*, resulting into similar mineralization extents and rates for both types of strains in the presence of IRC50. Our results show that *Mycobacterium* is better adapted to utilization of sorbed phenanthrene compared to *Sphingomonas*, especially in nutrient-poor conditions.

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Introduction

Biodegradation of polycyclic aromatic hydrocarbons (PAHs) in PAH-contaminated soils is limited by the low bioavailability of these hydrophobic organic contaminants. The contaminants tend to sorb to organic matter or can be trapped into micropores leaving them poorly accessible to microbial attack. Nevertheless, bacteria that are able to use PAHs as the sole source of carbon and energy were frequently isolated from PAH-contaminated soils. Such PAH-degrading bacterial isolates belong to a very limited number of genera such as *Pseudomonas*, *Burkholderia*, *Sphingomonas*, and *Mycobacterium*. Especially soil bacteria from the genera *Mycobacterium* and *Sphingomonas* seem to be important PAH-degrading colonizers of PAH-contaminated soil indicating that those bacteria have adapted to the low bioavailability of PAHs and that they possess specific capacities to get access to sorbed PAHs or to survive in the apparent oligotrophic conditions encountered in such soils. At least *Mycobacterium* seems to display several physiological adaptations to low bioavailability such as attachment to the PAH substrate source and high specific affinity toward PAHs (Wattiau et al. 2002; Wick et al. 2001a, 2002).

To study the effect of sorption on the microbial degradation of hydrophobic organic contaminants, model systems using synthetic porous sorbents containing sorbed pollutants are used (Calvillo and Alexander 1996; Friedrich et al. 2000; Grosser et al. 2000; Guerin and Boyd 1997; Harms and Zehnder 1995; Nam and Alexander 1998; Scow and Alexander 1992; Tang et al. 1998). Although it was assumed that the desorption rate of an organic contaminant into the aqueous phase is the rate-limiting step for microbial degradation of sorbed organic contaminants (Bouchez et al. 1995; Harms and Bosma 1997; Harms and

Zehnder 1995; Ogram et al. 1985), these studies showed that the microbial utilization rate of a sorbed substrate can be higher than expected from its abiotic desorption rate, hence, it was suggested bacteria can display features to increase access to the sorbed substrate (Calvillo and Alexander 1996; Friedrich et al. 2000; Grosser et al. 2000; Guerin and Boyd 1992; Tang et al. 1998). Guerin and Boyd (1992) showed that soil-sorbed naphthalene had a different bioavailability to two different bacterial species. For *Pseudomonas putida* 17484, the rate of naphthalene mineralization exceeded the abiotic desorption rate and resulted in an enhanced rate of naphthalene desorption from soils. Tang et al. (1998) demonstrated that strain P5-2, a soil isolate enriched in the presence of phenanthrene sorbed to SM7 polyacrylic beads, degraded sorbed phenanthrene at a higher rate than can be expected from the abiotic desorption rate. However, despite the ubiquity of PAH-degrading *Mycobacterium* and *Sphingomonas* strains in PAH-contaminated soils, no or only poor data exist on degradation of sorbed phenanthrene by members of these genera (Cheung and Kinkle 2001; Leys et al. 2004, 2005). Grosser et al. (2000) demonstrated that a phenanthrene-mineralizing *Mycobacterium* strain, enriched in the presence of sorbed phenanthrene, mineralized sorbed phenanthrene at rates higher than the abiotic desorption rate. However, it is not known if this characteristic is a feature of the used *Mycobacterium* strain or a general feature of PAH-degrading *Mycobacterium* spp.

In this study, we used porous synthetic amberlite sorbents containing sorbed ^{14}C-labeled phenanthrene to compare mineralization of phenanthrene sorbed to solids by three *Mycobacterium* and three *Sphingomonas* strains, previously isolated from PAH-contaminated soils for their phenanthrene-degrading capacity. The *Sphingomonas* strains were isolated by classical enrichment in liquid minimal medium (Bastiaens et al. 2000; Mueller et al. 1990), while the *Mycobacterium* strains were isolated by enrichment in a liquid aqueous/organic biphasic system or by direct plating of a soil extracted with aqueous phase or an organic phase (D. Springael, unpublished observation). The facts that the used *Mycobacterium* and *Sphingomonas* strains all belonged to different species and that the three *Mycobacterium* strains were obtained using different isolation techniques will allow us to make conclusions whether the capacity to degrade solid-sorbed phenanthrene at a rate higher than the abiotic desorption rate is associated with a particular strain or is intrinsic to a genus. In addition, as previous mineralization experiments examining degradation of sorbed PAHs are either performed in P-buffer (as resting cell assays) (Crocker et al. 1995; Guerin and Boyd 1992, 1997) or in a nutrient-rich minimal medium (Calvillo and Alexander 1996; Grosser et al. 2000; Nam and Alexander 1998; Tang et al. 1998), we examined the influence of minimal nutrients on the mineralization of sorbed substrates by one *Mycobacterium* and one *Sphingomonas* inoculum. The availability of nonlimiting amounts of minimal nutrients in the medium might indeed influence the outcome of the test by sustaining the strains activity, but is a condition far from the starvation conditions often encountered by bacteria in natural environments and in industrial PAH-contaminated soils, in particular.

Materials and methods

Bacterial strains, media, and culture conditions

The PAH-degrading *Mycobacterium* and *Sphingomonas* strains used in this study were isolated from PAH-contaminated soils and phenanthrene was utilized as the sole source of carbon and energy. *Mycobacterium gilvum* VM552 was isolated by enrichment in a minimal medium/heptamethylnonane biphasic system with pyrene dissolved in the organic phase (D. Springael, unpublished observation). *Mycobacterium frederiksbergense* VM531 and *Mycobacterium austroafricanum* VM451 were isolated by direct plating of an aqueous soil extract or a soil extracted with silicone oil, respectively, on plates with phenanthrene crystals (D. Springael, unpublished observation). *Sphingomonas* strains LH162 and LH227 (Bastiaens et al. 2000) and *Sphingomonas paucimobilis* EPA505 (Mueller et al. 1990) were isolated by enrichment in minimal medium with phenanthrene crystals. Based on 16S rRNA sequence analysis, these strains belong to different species (Bastiaens 1998). All strains were grown at 25°C on a rotary shaker (125 rpm) in 250 ml Erlenmeyer flasks containing 100 ml of phosphate-buffered minimal medium (MM) (pH 7.5), as described by Harms and Zehnder (1994) and Pfennig and Lippert (1966), with about 2 g l^{-1} phenanthrene crystals as sole carbon source. The MM contained (per liter) 0.875 g $Na_2HPO_4.2H_2O$, 0.1 g KH_2PO_4, 1 g $(NH_4)_2SO_4$, 0.2 g $MgCl_2.6H_2O$, 0.1 g $Ca(NO_3)_2.4H_2O$, and 5 ml of a trace elements solution [(per liter) 800 mg titriplex III, 300 mg $FeCl_2$, 10 mg $MnCl_2.4H_2O$, 4 mg $CoCl_2.6H_2O$, 1 mg $CuSO_4$, 3 mg $Na_2MoO_4.2H_2O$, 2 mg $ZnCl_2$, 0.5 mg LiCl, 0.5 mg $SnCl_2.2H_2O$, 1 mg H_3BO_3, 2 mg KBr, 2 mg KI and 0.5 mg $BaCl_2$].

Mineralization experiments were performed in P-buffer or minimal mineralization medium. The 0.1 M P-buffer (pH 5.8) contained 1.48 g l^{-1} K_2HPO_4 and 12.45 g l^{-1} KH_2PO_4. The minimal mineralization medium (pH 5.7) was prepared as described by Ortega-Calvo et al. (1995).

Model sorbents used and sorption–desorption of ^{14}C-phenanthrene

Properties of the model synthetic amberlite sorbents IRC50, XAD7-HP, and XAD2 (Supelco) used are listed in Table 1. The phenanthrene distribution coefficient K_d for the sorbents ranged from 3.0 to 4.2 l kg^{-1} (expressed as log K_d). The sorbents are porous resins with an average pore diameter of 30–300 nm for IRC50 and 9 nm for XAD7-HP and XAD2. The sorbents were washed before use as described by Cornelissen et al. (1998) and dried overnight at 70°C. Unlabeled phenanthrene (Fluka, purity >97%) dissolved at 200 μg ml^{-1} methanol and [9-^{14}C]-phenanthrene (Sigma, 13.1 mCi $mmol^{-1}$, dissolved in methanol;

radiochemical purity 98.9%) were mixed in a 500-ml Erlenmeyer flask. After evaporation of methanol (0.5–1.4 ml), the ^{14}C-labeled phenanthrene mixture in the flask was dissolved in P-buffer or mineralization medium at a final radioactivity of 0.008 mCi l^{-1} and a final concentration of 0.50 mg l^{-1} total phenanthrene, which was below its water solubility (1.18 mg l^{-1} at 25°C; Miller et al. 1985). In 15 ml Pyrex tubes, 100 mg of the model sorbents were equilibrated for 7 days at 20°C on a rotary shaker at 150 rpm with 4.5 ml of the phenanthrene containing P-buffer or mineralization medium. Phenanthrene desorption from the model sorbents was examined by sequential extraction with P-buffer during 27 days. The supernatant was periodically removed and replaced with 5 ml of fresh P-buffer. One ml of the removed P-buffer was mixed with 5 ml of liquid scintillation cocktail (Ultima Gold, Perkin-Elmer, Boston, MA, USA) and radioactivity was measured in a liquid scintillation counter (Packard Tri-Carb 1600CA, Perkin-Elmer). Triplicate desorption curves were established for all model sorbents. The maximum desorption rate was calculated as the slope of the linear regression line between five successive points of the desorption curve for the time period 6–93 h (Calvillo and Alexander 1996).

^{14}C-phenanthrene mineralization

Bacterial cultures in the stationary phase (after 8 days for VM552, 22 days for VM531 and VM451, 6 days for LH162, 3 days for EPA505, and 7 days for LH227) were passed through a 40- to 100-μm pore glass frit to remove the remaining phenanthrene crystals and were reincubated for 1 day at 25°C on a rotary shaker at 125 rpm to remove residual phenanthrene in the solution. The cells were collected by centrifugation (3,256 g, 15 min), washed twice with P-buffer or mineralization medium, and resuspended in P-buffer or mineralization medium to an optical density (660 nm) of 0.2. Viable cells in the suspensions were counted as colony-forming units (cfu) after plating serial dilutions (10^{-2} M $MgSO_4$) on solid agar MM plates containing 2 g l^{-1} glucose and 15 g l^{-1} agar (Select Agar, Invitrogen). The plates were incubated for 8–10 days at 29°C in the dark. Half a milliliter of the bacterial culture was added to Pyrex tubes containing 4.5 ml P-buffer or minimal mineralization medium containing the sorbents with sorbed ^{14}C-phenanthrene in equilibration with the solution. The same inoculum was added to Pyrex tubes with the same solution containing the same concentration of ^{14}C-phenanthrene but no sorbent, as a control system. The tubes were closed with Teflon-lined stoppers equipped with alkali traps (1 ml of 0.5 M NaOH) to measure $^{14}CO_2$ produced from ^{14}C-phenanthrene and were incubated at 20°C on a rotary shaker at 150 rpm. The NaOH solution was periodically removed from the trap and replaced with fresh alkali. The removed NaOH solution was mixed with 5 ml of liquid scintillation cocktail and the mixture was kept in the dark for at least 8 h for dissipation of chemiluminescence. Radioactivity was measured as described above. Cumulative mineralization curves were established in triplicate for the control and the model sorbents. The final mineralization extent was the cumulative extent of ^{14}C-phenanthrene mineralized after 27 days. The maximum mineralization rate for the control was calculated as the slope of the linear regression line between two of the first three successive points of the mineralization curve where the amount of $^{14}CO_2$ showed the largest increase. The maximum mineralization rate for the model sorbents was calculated as the slope of the linear regression line between the first five successive points of the mineralization curve.

To examine the possible toxicity of the model sorbents to the bacteria, 10 μl of additional ^{14}C-phenanthrene (2.4 μCi ml^{-1} methanol) was added to three Pyrex mineralization tubes at the same time as the bacterial inoculum and accumulated $^{14}CO_2$ was measured after 3–6 h. To examine the residual activity of the bacteria at the stationary phase of the mineralization curve, 10 μl of additional ^{14}C-phenanthrene (2.4 μCi ml^{-1} methanol) was added to three Pyrex mineralization tubes after 20 days of incubation and accumulated $^{14}CO_2$ was measured 7 days later.

Statistical analysis

The Tukey test (SAS 9.1; Cary, NC, USA) ($P<0.05$, $n=3$) was used for all statistical comparisons of mineralization extent, maximum mineralization rate, and desorption rate.

Results

Desorption of ^{14}C-phenanthrene from model sorbents

The initial abiotic desorption rates were calculated for the time period 6–93 h and were 0.19, 0.13, and 0.02 ng ml^{-1} h^{-1} for IRC50, XAD7-HP, and XAD2, respectively. The

Table 1 Properties of the model synthetic amberlite sorbents

Amberlite	Chemical nature[a]	Particle size (μm)[a]	Pore diameter (nm)[a]	Surface area (m^2 g^{-1})[a]	Phenanthrene log K_d (l kg^{-1})[c]
IRC50	Carboxylic acid	297–1190	30–300	ND[b]	3.0
XAD7-HP	Acrylic ester	250–840	9	450	3.5
XAD2	Divinyl benzene	250–840	9	300	4.2

[a]Data provided by Supelco
[b]No data available
[c]Distribution coefficient K_d is from Grosser et al. (2000)

decreasing desorption rate for IRC50 to XAD7-HP to XAD2 correlates with their increasing log K_d from 3.0 to 4.2 l kg^{-1} (Table 1). The phenanthrene log K_d values for soils vary depending on the organic carbon content but often range from 2.5 to 3.5 l kg^{-1} (Friedrich et al. 2000; Grosser et al. 2000), which is in the same range as the phenanthrene log K_d values for the sorbents.

Mineralization of ^{14}C-phenanthrene by *Mycobacterium* spp. and *Sphingomonas* spp. in P-buffer

The cumulative mineralization curves of ^{14}C-phenanthrene to $^{14}CO_2$ by *Mycobacterium* sp. VM552 or *Sphingomonas* sp. LH162 in the absence of sorbents (control) and in the presence of the model sorbents IRC50 and XAD7-HP in P-buffer are shown in Fig. 1a. Mineralization curves obtained with *Mycobacterium* strains VM531 and VM451 were similar to the curves obtained with strain VM552, while mineralization curves obtained with *Sphingomonas* strains LH227 and EPA505 were similar to the curves obtained with strain LH162. The final mineralization extent after 27 days and the maximum mineralization rate for the six strains in the absence (control) and presence of sorbents in P-buffer are shown in Table 2. Although for all strains the maximum mineralization rates in the absence of sorbents were significantly higher than in the presence of IRC50 (Tukey test, $P<0.05$), for all *Mycobacterium* strains the final mineralization extent in the presence of IRC50 (53−62%) was significantly higher than the extent of mineralization recorded in the absence of sorbents (18−52%) ($P<0.05$). In contrast, all *Sphingomonas* strains showed final mineralization extents that were significantly higher in the absence of sorbents (49–65%) than in the presence of IRC50 (25–29%) ($P<0.05$).

In the presence of IRC50, maximum mineralization rates for all six strains were recorded, which were significantly higher (1.1–1.9 ng ml^{-1} h^{-1}) than the initial abiotic desorption rate for IRC50 during the time period of 6–93 h (0.2 ng ml^{-1} h^{-1}). This indicates that *Mycobacterium* and *Sphingomonas* can utilize sorbed phenanthrene with a higher rate than can be explained by abiotic desorption. The maximum mineralization rate in the absence of sorbents was significantly higher for the three *Sphingomonas* strains than for the three *Mycobacterium* strains. On the contrary, the mineralization rate in the presence of IRC50 was significantly higher for the three *Mycobacterium* strains than for the *Sphingomonas* strains except for strain EPA505. As such, the reduction in the mineralization rate caused by reduced phenanthrene bioavailability was significantly different between the *Mycobacterium* strains

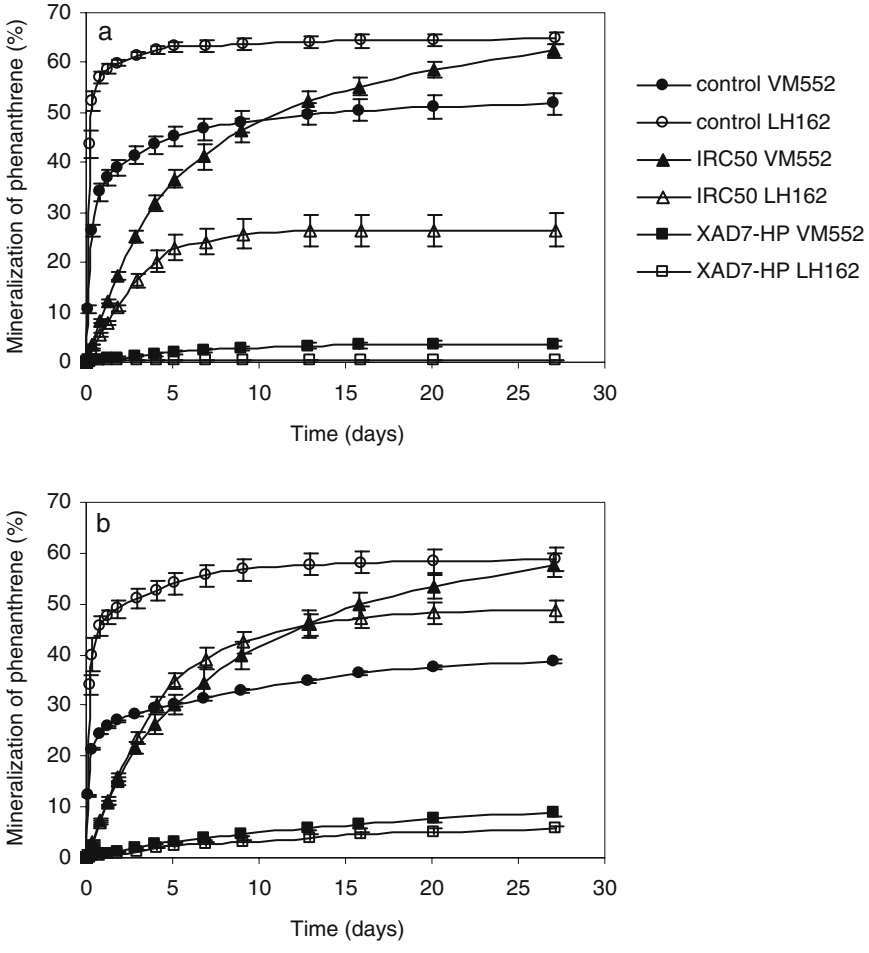

Fig. 1 **a** Mineralization of ^{14}C-phenanthrene (as $^{14}CO_2$) in P-buffer or **b** in minimal mineralization medium by *Mycobacterium* sp. VM552 or *Sphingomonas* sp. LH162 in the absence of sorbents (control) and in the presence of the model sorbents IRC50 and XAD7-HP. Error bars represent one standard error from three replicates

Table 2 Mineralization extent and maximum rate of ^{14}C-phenanthrene mineralization in P-buffer or minimal mineralization medium by *Mycobacterium* strains VM552, VM531, and VM451 or *Sphingomonas* strains LH162, EPA505, and LH227 in the absence of sorbents (control) and in the presence of the model sorbents IRC50, XAD7-HP, and XAD2

Medium	P-buffer						Minimal medium	
Bacterial strains	VM552	VM531	VM451	LH162	EPA505	LH227	VM552	LH162
Cfu ml^{-1}	3.2×10^7	2.5×10^6	2.7×10^7	3.0×10^7	5.0×10^7	1.3×10^6	3.2×10^7	3.0×10^7
Final mineralization extent* (%)								
Control	51.7 B	34.8 C	18.2 D	64.9 A	52.7 B	48.9 B	38.6 b	58.7 a
IRC50	62.4 A	52.9 B	54.7 AB	26.4 C	25.3 C	29.3 C	57.7 a	48.6 a
XAD7-HP	3.7 A	1.3 BC	2.0 B	0.5 C	1.0 BC	0.3 C	9.0 a	5.7 b
XAD2	0.4	0.2	0.2	0.3	0.2	0.1	0.4	0.3
Maximum mineralization rate (ng ml^{-1} h^{-1})[a]								
Control	17.08 C	9.82 D	3.93 D	41.38 A	31.62 B	45.58 A	15.88 b	32.52 a
IRC50	1.90 A	1.66AB	1.52 B	1.21 C	1.90 A	1.12 C	1.70 a	1.70 a
XAD7-HP	0.10 A	0.03 B	0.08AB	0.04 B	0.12 A	0.03 B	0.14 a	0.09 b
Ratio IRC50/control[b]	0.11 B	0.17AB	0.39 A	0.03 D	0.06 C	0.02 D	0.11 a	0.05 b
Mineralization extent additional ^{14}C-phenanthrene[a] (%), added at day 20								
Control	9.2 B	22.4 A	17.1 A	0.6 C	0.7 C	0.6 C	ND[c]	ND
IRC50	21.9 B	4.9 C	33.6 A	0.5 C	0.2 C	0.2 C	ND	ND
XAD7-HP	5.6 A	1.7 B	0.4 C	0.3 C	0.3 C	0.1 C	ND	ND

[a]The mineralization extents and rates are the mean of three replicates. Means within one row with a different uppercase letter are significantly different among the bacterial strains in P-buffer. Means within one row with a different lowercase letter are significantly different among VM552 and LH162 in minimal medium. Statistical significance was determined by a Tukey test ($P<0.05$)
[b]Ratio of mean maximum mineralization rate ($n=3$) in the presence of IRC50 to control. Ratios within one row with a different letter are significantly different among the bacterial strains based on 95% confidence limits
[c]ND: not determined

on the one hand, and the *Sphingomonas* strains on the other hand, which is shown by the normalization of the maximum mineralization rate for each strain in the presence of IRC50 to that in the control environment (Table 2). This comparison factored out small differences in the set-up of the systems such as differences in bacterial cell numbers used and effect of the growth stadium of the cultures used. The ratio of the maximum mineralization rate of IRC50 to control was significantly higher for the three *Mycobacterium* strains compared to the ratio calculated for the three *Sphingomonas* strains. Similarly, the final mineralization extent in the presence of IRC50 was about a factor of two higher for the *Mycobacterium* spp. compared to the *Sphingomonas* spp. The final extent in the presence of XAD7-HP was low for all strains, i.e., 1.3–3.7% for the *Mycobacterium* spp. and 0.3–1.0% for the *Sphingomonas* spp. The final mineralization extent in the presence of XAD2 was less than 0.5% for all strains and therefore the maximum mineralization rate in the case of XAD2 was not calculated (Table 2).

None of the sorbents were toxic to the used bacterial strains. The mineralization extent of the additional ^{14}C-phenanthrene, simultaneously added with the inoculum, was in the range of 15–55% in the presence of IRC50, 12–48% in the presence of XAD7-HP, and 1–7% in the presence of XAD2 for the six strains. In contrast with the *Mycobacterium* strains, the *Sphingomonas* strains lost all PAH-mineralizing activity at the stationary phase of the mineralization. While the *Mycobacterium* strains still mineralized 9–22 or 5–34% of the additional ^{14}C-phenanthrene added at day 20 in the absence of sorbents or in the presence of IRC50, respectively, the *Sphingomonas* strains mineralized less than 1% of the additional ^{14}C-phenanthrene (Table 2).

Mineralization of ^{14}C-phenanthrene by *Mycobacterium* sp. VM552 and *Sphingomonas* sp. LH162 in minimal mineralization medium

The cumulative mineralization curves of ^{14}C-phenanthrene to $^{14}CO_2$ by *Mycobacterium* sp. VM552 or *Sphingomonas* sp. LH162 in the absence of sorbents (control) and in the presence of the model sorbents IRC50 and XAD7-HP in minimal mineralization medium are shown in Fig. 1b. The final mineralization extent after 27 days and the maximum mineralization rate in minimal mineralization medium are shown in Table 2. As observed in P-buffer, the final mineralization extent for *Mycobacterium* sp. VM552 in the presence of IRC50 (58%) was significantly higher than in the absence of sorbents (39%) ($P<0.05$), while for *Sphingomonas* sp. LH162, the final mineralization extent in the absence of sorbents (59%) was significantly higher than in the presence of IRC50 (49%) ($P<0.05$). For LH162, the availability of nutrients had a significantly positive effect on phenanthrene mineralization in the presence of IRC50 while this was not the case for VM552. The final extent and the mineralization rate recorded for LH162

increased significantly from 26 to 49% and from 1.2 to 1.7 ng ml^{-1} h^{-1}. In the presence of XAD7-HP, the final extent increased significantly from 4 to 9% for VM552 and from 0.5 to 6% for LH162. In minimal medium, the final extent and the mineralization rate in the presence of XAD7-HP were still significantly higher for VM552 compared to LH162. Also, the ratio of maximum mineralization rate in the presence of IRC50 to control was still significantly higher for VM552, compared to LH162.

Discussion

In this study, we compared the mineralization of phenanthrene sorbed to porous synthetic amberlite sorbents as model for soil by phenanthrene-degrading *Mycobacterium* spp. and *Sphingomonas* spp. In the presence of IRC50, the maximum mineralization rate of ^{14}C-phenanthrene as a measure of biodegradation rate was significantly higher for all *Mycobacterium* and *Sphingomonas* strains compared to the abiotic rate of desorption from IRC50. This indicates that the rate of mass transfer from the IRC50 sorbent to the aqueous phase is not necessarily the rate-limiting step in the biodegradation of sorbed PAHs in our systems. Similarly, Guerin and Boyd (1992) reported on a *Pseudomonas* strain that mineralized soil-sorbed naphthalene faster than its abiotic desorption rate while various PAH-degrading bacteria enriched in the presence of sorbed PAHs displayed a higher degradation rate of sorbed PAHs than the abiotic desorption rate compared to strains isolated with nonsorbed PAHs (Grosser et al. 2000; Tang et al. 1998). The desorption rate for phenanthrene from preloaded solid phases was measured by repeated extraction with aqueous phase (Grosser et al. 2000; Nam and Alexander 1998). Sorbed PAHs will dissolve into the aqueous phase until the equilibrium aqueous concentration is reached while bacterial uptake will continuously reduce the aqueous concentration (Wick et al. 2001b) and therefore, in this study and in other studies, the abiotic desorption rate was probably underestimated. On the other hand, if desorption to the aqueous phase was the rate-limiting step for the mineralization of phenanthrene, it would be expected that the reduction in degradation rates caused by reduced phenanthrene bioavailability, i.e., the normalized mineralization ratios, would not vary considerably among the strains or that all strains would be similarly affected regarding mineralization rate by the presence of IRC50. This was not the case. Therefore, it can be stated that at least all *Mycobacterium* strains and *Sphingomonas* strain EPA505 degraded phenanthrene sorbed to IRC50 more rapidly than abiotic desorption rates and that these strains, one way or another, increased the phenanthrene flux from the IRC50 sorbent. To improve bioavailability, PAH-degrading bacteria seem to have developed strategies such as substrate source attachment and high specific substrate affinity toward PAHs to increase the diffusive flux to the cells and production of biosurfactants, to increase the apparent dissolved concentration of PAHs (Johnsen and Karlson 2004; Johnsen et al. 2005; Wick et al. 2001b). Because the tested *Mycobacterium* strains belonged to different species and all strains showed this increased mineralization, this characteristic seems to be a general feature of the PAH-degrading members of this genus, rather than a feature of a specific species or strain. However, the *Mycobacterium* isolate SM7.6.1 showed, in contrast with the strains tested in our study, efficient degradation of phenanthrene sorbed to SM7, a model sorbent similar to XAD7-HP (Grosser et al. 2000). This might be due to physicochemical differences and hence differences in PAH sorption capacity between SM7 and XAD7-HP, as the sorbents were purchased from different suppliers, or to different experimental procedures used. Alternatively, as the *Mycobacterium* strain used in the study of Grosser et al. (2000) was an isolate enriched with phenanthrene sorbed to SM7, this strain might be specially adapted to the degradation of sorbed phenanthrene. Such strains were not included in our study. It might indicate that differences in the capacity to access sorbed phenanthrene exist between different *Mycobacterium* isolates.

Both in P-buffer and minimal mineralization medium, interesting differences in effects of sorption could be observed between the *Mycobacterium* and most of the *Sphingomonas* strains. The addition of IRC50 increased the mineralization extent for all *Mycobacterium* strains while it decreased this extent for the *Sphingomonas* strains. For *Mycobacterium* strains VM531 and VM451, values increased from 18–35% in the absence of IRC50 to a value above 50% in the presence of IRC50 although mineralization rates significantly decreased in the latter case. For *Mycobacterium* strain VM552, the mineralization extent even increased from 52 to 62%. Mineralization extents from 50 to 60% might suggest the complete consumption of ^{14}C-phenanthrene with the rest being converted into biomass (Garcia-Junco et al. 2003). This difference in mineralization extent with sorbed and nonsorbed phenanthrene is difficult to explain but might suggest that mycobacteria switch their metabolism, converting more PAH mass to CO_2 and hence energy instead of cell mass in the presence of IRC50. The decrease of the mineralization extent in the presence of IRC50 for the *Sphingomonas* strains can be explained by a combination of the observed decrease in mineralization rate due to a different phenanthrene bioavailability (see below) and the recorded lower viability of *Sphingomonas* compared to *Mycobacterium* in nutrient-poor conditions. Indeed, the decrease in mineralization extent in the presence of IRC50 was clearly smaller when minimal medium was used instead of P-buffer (see below). Following up the complete mass balance of ^{14}C-phenanthrene should give more clarity about the effect of sorbents on mineralization extent for both *Mycobacterium* and *Sphingomonas*.

Furthermore, in the presence of IRC50 and both in P-buffer and minimal mineralization medium, normalized maximum mineralization ratios were significantly higher for the *Mycobacterium* strains than for the *Sphingomonas* strains, indicating that *Mycobacterium* is better adapted to

biodegradation of sorbed PAHs, compared to *Sphingomonas*. This might explain why in the study of Bastiaens et al. (2000), *Mycobacterium* was preferentially enriched and selected over *Sphingomonas* in case phenanthrene was sorbed to hydrophobic carriers. Moreover, *Sphingomonas* clearly demonstrated higher mineralization rates in the absence of sorbents, explaining why they might be enriched over *Mycobacterium* in enrichments with crystalline PAHs or water-dissolved PAHs as observed in the study of Bastiaens et al. (2000). It indicates that *Sphingomonas* displays higher affinities for dissolved phenanthrene compared to *Mycobacterium*, while for *Mycobacterium* the phenanthrene uptake is, one way or another, less influenced by sorption. Because *Mycobacterium* does not produce biosurfactants (Johnsen and Karlson 2004), a possible alternative explanation for the latter observation can be that *Mycobacterium* is more competitive in substrate source attachment, which results into efficient pollutant depletion at low concentrations at the cell surface, steeper concentration gradients, and higher substrate transfer rates (Wick et al. 2001b). Indeed, although bacterial attachment on the model sorbents was not examined in this study, *Mycobacterium* is well known for its attachment and biofilm formation on the PAH substrate source (Wattiau et al. 2002; Wells et al. 2005; Wick et al. 2002). However, although until now substrate attachment was not reported for phenanthrene-degrading *Sphingomonas*, formation of biofilms on fluorene crystals was described for the fluorene-degrading *Sphingomonas* sp. LB126 (S. Wuertz, unpublished observation) and attachment to phenanthrene-containing model sorbents was reported for a phenanthrene-degrading *P. putida* (Wells et al. 2005). Therefore, it can be suggested that *Mycobacterium*, in addition to substrate source attachment, might display other features to increase bioavailability (Wells et al. 2005).

Phosphate-buffered saline and P-buffer are often used in mineralization experiments as resting cell assays (Crocker et al. 1995; Guerin and Boyd 1992, 1997; Ortega-Calvo et al. 1999). Our results with the minimal mineralization medium showed that the addition of nutrients had a significantly positive effect on the mineralization rate and the final extent of mineralization for LH162 in the presence of IRC50. The final mineralization extent for VM552 and LH162 in the presence of XAD7-HP also increased significantly in the MM. As such, the significantly higher maximum mineralization rate and final mineralization extent with IRC50 for VM552 compared to LH162 observed in the presence of P-buffer, disappeared when minimal mineralization medium was used. Apparently, *Sphingomonas* sp. LH162 benefited more from the additional mineral nutrients compared to *Mycobacterium* sp. VM552. It is possible that *Sphingomonas* needs specific nutrients to utilize a sorbed PAH compound in terms of a strategy to improve the PAH bioavailability. Moreover, after 20 days in P-buffer, the three *Mycobacterium* strains could still mineralize a significant amount of freshly added ^{14}C-phenanthrene (5–34%) in the control and in the presence of IRC50, while the *Sphingomonas* strains showed no significant mineralization activity. Both observations indicate a better activity of *Mycobacterium* in nutrient starvation conditions compared to *Sphingomonas*. Such nutrient-poor conditions might often be encountered in environmental habitats such as PAH-contaminated soil. It is interesting to note that previously, *Mycobacterium* was reported to show adaptation, such as low maintenance requirement (Wick et al. 2002) and multiple-substrate utilization (Wick et al. 2003), to low carbon concentrations.

In conclusion, our results show that *Mycobacterium* spp. are better adapted to biodegradation of sorbed phenanthrene compared to *Sphingomonas* spp., especially in nutrient-poor conditions. Moreover, utilization of sorbed phenanthrene significantly increased, especially for *Sphingomonas*, when nutrients were not limited. Because soils, depending on the organic carbon content, show PAH sorption capacities similar to those of the sorbents used, the different utilization of sorbed PAHs between *Mycobacterium* and *Sphingomonas* might contribute to the definition of specific niches for both types of bacteria and competition between them in PAH-contaminated soil (Leys et al. 2004, 2005).

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