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Isolation and characterization of naphthalene biodegrading *Methylobacterium radiotolerans* bacterium from the eastern coastline of the Kingdom of Saudi Arabia

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Abstract: Bioremediation is based on microorganisms able to use pollutants either as a source of carbon or in co-metabolism, and is a promising strategy in cleaning the environment. Using soil contaminated with petroleum products from an industrial area in Saudi Arabia (Jubail), and after enrichment with the polycyclic aromatic hydrocarbon (PAH) naphthalene, a *Methylobacterium radiotolerans* strain (N7A0) was isolated that can grow in the presence of naphthalene as the sole source of carbon. *M. radiotolerans* is known to be resistant to gamma radiation, and this is the first documented report of a strain of this bacterium using a PAH as the sole source of carbon. The commonly reported *Pseudomonas aeruginosa* (strain N7B1) that biodegrades naphthalene was also identified, and gas chromatography analyses have shown that the biodegradation of naphthalene by *M. radiotolerans* and *P. aeruginosa* did follow both the salicylate and phthalate pathways.

Introduction

Petroleum-based products are major global pollutants, and it is estimated that more than a million liters of petroleum products per year contaminate the environment (Seo et al. 2009). Among the oil-derived pollutants are polycyclic aromatic hydrocarbons (PAHs). These are not only hazardous to the environment, but also carcinogenic and immunosuppressive in humans (Lah 2011). Thus, their elimination from the environment remains a priority, and the most promising approach is biodegradation, which exploits the ability of microorganisms to use hydrocarbons as sources of carbon. Several PHA biodegrading microorganisms have been reported, and some of them have been used in field conditions for remediation (Nzila 2013, Seo et al. 2009, Tyagi et al. 2011). In the Kingdom of Saudi Arabia (KSA), oil spills and the generation of oil-product waste in the environment are common. The KSA environment is characterized by harsh conditions with high temperatures and dryness (Al-Thukair 2002); it is conceivable that, over time, diverse groups or species of bacteria with unique biochemical features have been selected. The aims of the present work were to: 1) isolate and characterize novel microorganisms that can biodegrade the two-ring PAH naphthalene; and 2) study the possible metabolic pathways of naphthalene in these microorganisms by identifying key metabolites using gas chromatography-mass spectrometry (GC-MS) techniques.

Material and Methods

Chemicals

Sigma-Aldrich (St. Louis, MO, USA) supplied the naphthalene, salicylate, catechol, phenol, benzene, toluene, anthracene, phenanthrene, $(NH_4)_2SO_4$, KH_2PO_4, $CaCl_2 \cdot 7H_2O$, $MgSO_4 \cdot 7H_2O$, Na_2HPO_4 and $FeSO_4 \cdot 7H_2O$, and all of the chemicals used in the preparation of BH medium. Luria--Bertani Broth (LB) was purchased from Difco, USA.

Sample collection and in vitro culture

Soil samples were collected from an oil-contaminated area in the industrial city of Jubail, at the following location: 27°06′46.53″ N 49°22′24.53″ E. Isolation of naphthalene degrading bacteria was carried out after several enrichments with BH-naphthalene medium. One gram of soil sample was added to 100 mL of BH-naphthalene, and the suspension was vortexed and incubated in a shaker-incubator at 37°C and at 120 rpm for 7 days. Then, 0.5 mL of the enriched medium was transferred to 10 mL of fresh BH-naphthalene medium for a further 7 days. This enrichment process was repeated three times and thereafter, the cultures were streaked on BH-naphthalene agar solid plate medium, and incubated at 37°C for 7 days. Individual colonies were isolated and streaked again on the BH-naphthalene agar solid medium (and this was repeated two times) to ascertain the purity of the colonies, and these colonies were then used for further studies.

Monitoring of bacteria growth in the presence of naphthalene and other hydrocarbons

To assess naphthalene and hydrocarbon utilization bacteria were cultured in BH-naphthalene or BH supplemented with 0.1% of one of the following as a source of carbon (methanol, ethanol, salicylate, catechol, toluene, benzene, phenol, and phenanthrene). Bacteria growth was assessed at various temperatures (30, 35, 37.5, 40, 45 and 50°C), salinity (0, 0.1, 2, 4, and 6% of NaCl [wt/wt]) and pH (6, 7, and 8). The normal BH medium has a pH 7; solutions of NaOH (1.0 M) and HCl (1.0 M) were used to adjust the pH.

An aliquot of bacteria was cultured in LB liquid rich medium and the bacteria growth monitored by measuring the absorbance (optical density [OD]) of culture at 600 nm, the standard wavelength to measure bacterial growth. The OD measurements were carried out using an APEL PD-303 UV spectrophotometer (Japan). Bacteria were also measured by counting of CFU (colony forming unit) using serial dilution method following culture and counting of bacteria on a solid agar medium. The bacteria count of around 10^9 CFU/ml (an approximate OD of 0.8–1) was used as initial culture in all subsequent studies.

All experiments were carried out with 10^7 CFU (from the initial culture described above) in a total volume of 50 mL BH medium containing 1% of naphthalene or other substrates, and bacteria growth was monitored by measuring the OD. Thus, the initial bacteria concentration was around 2.10^5 CFU/ml. A culture without bacteria was used as the negative control, and absorbance values >0.75 were considered to represent excellent growth. High growth was associated with OD values between 0.75 and 0.5, OD values between 0.49 and 0.1 were considered to represent normal growth, moderate growth was associated with OD values of 0.09–0.01, while no growth was considered to be present when the OD was <0.01. In the experiment on naphthalene utilization, bacteria growth was measured both using OD, and colony counting (CFU).

16S rRNA analysis and species identification

This analysis was carried out by the company Royal Life Science, Pvt. Ltd., Secunderabab, India. Briefly, colonies were picked up with a sterilised toothpick, suspended in 0.5 mL of sterile saline and centrifuged at 1,500 g for 3 min. The pellet was suspended in 0.5 mL of InstaGene Matrix to purify DNA for PCR analysis, according to the manufacturer (Bio-Rad, USA). The samples mixed with InstaGene Matrix were incubated at 56°C for 30 min and then heated at 100°C for 10 min, and after centrifugation (1,500 g for 10 min), the supernatant was collected ready to be used for PCR analysis. Template DNA (1.0 µL) was amplified with 27F primer (AGAGTTTGATCMTGGCTCAG) and 1492R primer (TACGGYTACCTTGTTACGACTT), in a total volume of 20 µL, under the following conditions: 35 amplification cycles at 94°C for 45 sec, 55°C for 60 sec, and 72°C for another 60 sec. The amplified genes of around 1,400 bp (base pairs) were sequenced by using Big Dye terminator cycle sequencing kit (Applied BioSystems, USA) with the following primers (518F: CCAGCAGCCGCGGTAATACG and 800R: TACCAGGGTATCTAATCC). Sequencing products were resolved on an automated DNA sequencing system (Applied BioSystems model 3730XL, USA). Species identification was carried out by Basic Local Alignment Search Tool (BLAST), a tool to establish the degree of homology of a DNA sequence with those available at the NCBI, National Center for Biotechnology Information Database (Anonymous, 2014a).

Detection of metabolites by GC-MS

GC-MS analyses were performed using an Agilent 5975B mass spectrometer attached to an Agilent 6890N gas chromatograph. GC was carried out on an HP-5 column (30 m, 0.25 mm [i.d.]) using helium as the carrier gas. The conditions for GC analyses were as follows: initial temperature of 50°C held for 2 min followed by an increase to 250°C at a rate of 5°C/min followed by holding at 250°C for 30 min. The mass analysis conditions: inlet temperature of 250°C and mass range of 15–550 m/z. The biodegradation of naphthalene was investigated by growing bacteria (ca. 10^7 CFU in 50 mL) in two separate flasks with 500 mL of BH-naphthalene in each. As a control, BH-naphthalene medium without bacteria was used. One of the BH-naphthalene flasks was analyzed after 7 days and the other after 14 days (the time corresponding to maximum growth of bacteria). To identify naphthalene metabolites in the media, each of the three media was filtered to remove cells, and the insoluble material and filter paper were washed with warm distilled water (200 mL). The filtrate was extracted with ethyl acetate (100 mL × 3). The organic fractions were combined, dried with $CaCl_2$, and concentrated under vacuum. The remaining residue was dissolved in chloroform (1.0 mL) before its analysis by GC-MS.

Results and discussion

After enrichment with naphthalene, the soil sample cultures led to the identification of two colonies, N7A0 and N7B1, that were able to grow in the presence of naphthalene as the sole source of carbon. Both colonies were Gram-negative and rod shaped. N7A0 colonies were pink, circular, and convex, with an entire margin, while N7B1 colonies were pale, circular, raised and with an undulating margin; N7B1 was fluorescent.

16S rRNA analysis and species identification

Individual colonies were isolated, and cultured in LB solid medium for the isolation of 16S rRNA genes. N7A0 and N7B1 amplified 16S rRNA genes were around 1400 nucleotides in length (GeneBank accession numbers KC776527 and KC776528). The "BLAST" query for the N7A0 sequence showed high similarity (≥98%) with bacteria of the genus *Methylobacterium*, and almost all strains of *Methylobacterium radiotolerans* characterized so far had a similarity of 99% with this strain. Based on this information, it was concluded that N7A0 strain belongs to the species *M. radiotolerans*. The genus *Methylobacterium* has been originally proposed by Patt *et al.* in 1978, and is essentially aerobic, Gram-negative and pink pigmented (Green 1992), as supported by the morphological data. One of the characteristics of *M. radiotolerans* species is that it is resistant to gamma radiation (Green and Bousfield 1983). BLAST analysis of the N7B1 16S rRNA gene was also carried out. The first 500 hits had 99% similarity with this strain and these hits were almost exclusively *Pseudomonas aeruginosa* strains. Thus, based on both morphological and genetic analyses, N7B1 would belong to the commonly found species *P. aeruginosa*. As this bacterium has already been studied extensively, subsequent studies were focused on *M. radiotolerans*, using *P. aeruginosa* for comparison.

Bacteria growth in the presence of naphthalene

The growth of these 2 strains was assessed in the presence of naphthalene by monitoring both the increase of the OD at 600 nm and the counting of CFU. *P. aeruginosa* N7B1 was a rapid growing bacterium in the presence of naphthalene, with a maximum growth being attained between days 7 and 12. Values up to 0.6 and 7.2 (log) could be reached for OD and CFU/mL, respectively. On the other hand, *M. radiotolerans* N7A0 was a slow growing bacterium. The maximum OD was 0.025 and CFU/ml of 5.2 (log) after 14 days. Thus, both microorganisms were able to thrive in the presence of naphthalene as the sole source of carbon.

The effect of temperature (25, 37, 40, 45 and 50°C), salinity (0, 0.1, 1, 2, 4 and 6% [wt/wt]) and pH (6, 7, and 8) was also tested on the growth of both strains in the presence of naphthalene by monitoring growth by measuring OD at 600 nm. The highest growth of *M. radiotolerans* N7A0 was observed at 45°C, 0.1% (wt/v) salinity and pH 7, while the best growth of *P. aeruginosa* N7B1 was associated with a temperature of 45°C, 1% (wt/v) salinity and pH 8. The biodegradation of naphthalene by *M. radiotolerans* has been mentioned but without provided supportive data (Anonymous, 2014b). Thus, to the best of our knowledge, this is the first documented report on a *M. radiotolerans* strain that can grow in the presence of naphthalene as the sole source of carbon.

Table 1. Growth of bacterial strains in the presence of various substrates. Growth was monitored by measuring the optical density (OD) of the culture at 600 nm and at 14 days of incubation

	Bacterial growth	
Substrate	**M. radiotolerans N7A0**	**P. aeruginosa N7B1**
Methanol	+	+/–
Ethanol	+++	+
2-Propanol	+	+
Salicylate	+/–	++
Catechol	+/–	++
Toluene	+/–	+/–
Benzene	+/–	+
Phenol	+/–	+
Naphthalene	+	+
Anthracene	+/–	+
Phenanthrene	–	+

Optical density (OD) >0.75 = +++ (excellent growth); 0.75–0.5=++ (high growth); 0.49–0.1=+ (normal growth); 0.09–0.01=+/– (moderate growth); <0.01= – (no growth).

Substrate utilization

The ability of *M. radiotolerans* N7A0 to grow in the presence of various carbon substrates was also investigated under conditions of optimum growth in the presence of naphthalene. The analysis first focused on the monocyclic aromatic compounds benzene, toluene, phenol, salicylate and catechol; as well as PAHs such as naphthalene, anthracene and phenanthrene. One of the common traits of bacteria of the genus *Methylobacterium* is that they can grow in the presence of methanol, a volatile organic compound emitted by vegetation. This explains why *Methylobacterium* is often found in association with plants (Abanda-Nkpwatt et al. 2006, Lidstrom and Chistoserdova 2002). Thus, in addition to the aforementioned aromatic compounds, methanol, ethanol, and 2-propanol were also included in this analysis. Among the compounds tested, ethanol was the source of carbon that was associated with excellent growth in *M. radiotolerans* N7A0 as shown in Table 1. Normal growth was observed with methanol and 2-propanol. The assimilation of ethanol has been proposed in *Methylobacterium extorquens*, and this occurs through the C2 pathways involving the ethylmalonyl-CoA pathway (Anthony 2011). Thus, it is likely that the N7A0 strain expresses enzymes involved in this C2 pathway. Interestingly, the growth of this strain was only moderately promoted by methanol, the most common source of carbon for *Methylobacterium* (Anthony 2011). This is not surprising since many *Methylobacterium* strains are known to be non-methanol utilizing bacteria (Anthony 2011, Green 1992, Urakami et al. 1993).

Table 1 also shows that *P. aeruginosa* N7B1 can grow in the presence of most of the tested substrates. The members of the genus *Pseudomonas* are known to have diverse metabolic pathways and to grow using different substrates as a source of carbon. For instance, *P. aeruginosa* can grow in the presence of a variety of aliphatic and aromatic compounds such as lactate (Gao et al. 2012), n-docosane, n-triacontane, n-tetracontane (Zhang et al. 2011), polyurethane (K Mukherjee et al. 2011), aromatic fluoranthene, phenanthrene (Zhang et al. 2011), hexadecane, benzene, toluene (S Mukherjee et al. 2010), paracetamol (Hu et al. 2013), and 4-chlorobenzoate (Hoskeri et al. 2011). Similar wide range of assimilation of substrates has been reported with other species of the same genus, such as *Pseudomanas putida* with biodegradation of aromatic compounds (Diaz et al. 2008, Ebrahimi and Plettner 2013, El-Naas et al. 2009, Fernandez et al. 2012, Hwang et al. 2009, Li et al. 2011, Q Lin and Jianlong 2010, Phale et al. 2013, Takeo et al. 2006, You et al. 2013) and alkane derivatives (Dunn et al. 2005, Johnson and Hyman 2006, Smith and Hyman 2004). In line with previous reports, *Pseudomonas aeruginosa* strain N7B1 had a wide range of substrate specificity, thus is an important bacterium that could be used in bioremediation strategies.

Detection of metabolites by GC-MS

Degradation of naphthalene starts through the multi-component enzyme naphthalene dioxygenase, which converts naphthalene to *cis*-naphthalene dihydrodiol. The latter is transformed to 1,2-dihydroxynaphthalene by the action of *cis*-dihydrodiol dehydrogenase. At this point, two pathways are possible (Figure 1). The ring fission of 1,2-dihydroxynaphthalene leads to the formation of *o*-phthalic acid ("phthalic pathway"), which is subsequently converted to intermediates in the Krebs cycle(Haritash and Kaushik 2009, Seo et al. 2009). In the second pathway, 1,2-dihydroxynaphthalene is converted to salicylate, which is either transformed to catechol or gentisate ("salicylate pathway"). The ring fission of these two aromatic molecules results in the formation of intermediates in the Krebs cycle (Figure 1). The second pathway has been studied the most, and genes encoding for these catabolic enzymes are generally found in bacterial extra-chromosome plasmids, and plasmids from several bacterial species have been

characterized, including the plasmid NAH7 of *Pseudomonas putida* strain G (Fernandez et al. 2012), pAK5 of *Pseudomonas putida strain* AK5 (Izmalkova et al. 2013), and plasmid of *Gordonia* sp. strain CC-NAPH129-6 (Izmalkova et al. 2013).

The analysis showed a GC peak at a retention time of 14.1 min (Figure S1, S3), with a molecular mass of 132, and fragmentation pattern (Figure S2a) consistent with 4-hydroxy-2-oxovaleric acid, a metabolite in the catechol pathway (Figure 1; Table 2). This compound was detected in *P. aeruginosa* after 7 and 14 days of growth. However, the same metabolite was only detected after 14 days (not after 7 days) in *M. radiotolerans*. This result could be explained by the slow growth of the latter bacterium in the presence of naphthalene, thus requiring more time for 4-hydroxy-2-oxovaleric acid (which is downstream the pathway) to accumulate in the medium (Figure 1).

The 4-hydroxy-2-oxovaleric acid, which was found in both bacterial species, is an intermediate that is produced after catechol, a clear indication that naphthalene was biodegraded through a "salicylate pathway" in these two strains. In support of this, these bacteria were able to grow in the presence of salicylic acid or catechol (two important intermediates in the "salicylate pathway"), as the sole source of carbon (Table 1, Figure 1). This growth was limited with the *M. radiotolerans* strain but was very high with *P. aeruginosa* (Table 1), thus the latter strain can efficiently express catabolic enzymes of the "salicylate pathway". The existence of this pathway has already been proven in *P. aeruginosa* (Civilini et al. 1999, Takizawa et al. 1999), and in other bacteria of the genus *Pseudomonas* (Izmalkova et al. 2013, Phale et al. 2013). The growth of *M. radiotolerans* in the presence of salicylic acid or catechol is lower than in the presence of naphthalene, yet one would have expected these single ring molecules to be easily catabolised, thus supporting a higher bacteria growth. Assuming that salicylic acid or catechol are efficiently transported in the cell, these results may indicate that the salicylate pathway is not predominant in *M. radiotolerans*. However, we did not test the effect of "o-phthalic pathway metabolites" on bacteria growth for comparison, thus it will be speculative to conclude which of the 2 pathways is predominant in *M. radiotolerans*.

GC-MS analysis also showed a peak at a retention time of 32.2 min (Figure S1, Figure S2b) with a fragmentation pattern (Figure S2b) that was consistent with *o*-phthalic acid, dibutyl phthalate (Table 2) in both strains. In the biodegradation of naphthalene by *P. aeruginosa*, another peak was noted in the GC-MS analysis with a retention time 21.9 min (Figure S3), and its MS fragmentation pattern (Figure S4a) was consistent with hydroxy-phthalic acid. Another peak (retention time 30.5 min, Figure S3) was identified in *P. aeruginosa*, and its MS fragmentation (Figure S4b) was consistent with acetoxy--phthalic acid, the acetate derivative of hydroxy-phthalic acid. However, it is interesting to note that this hydroxy-phthalic acid metabolite was not identified in *M. radiotolerans*. As discussed previously in relation to 4-hydroxy-2-oxovaleric acid, the absence of hydroxy-phthalic acid in *M. radiotolerans*

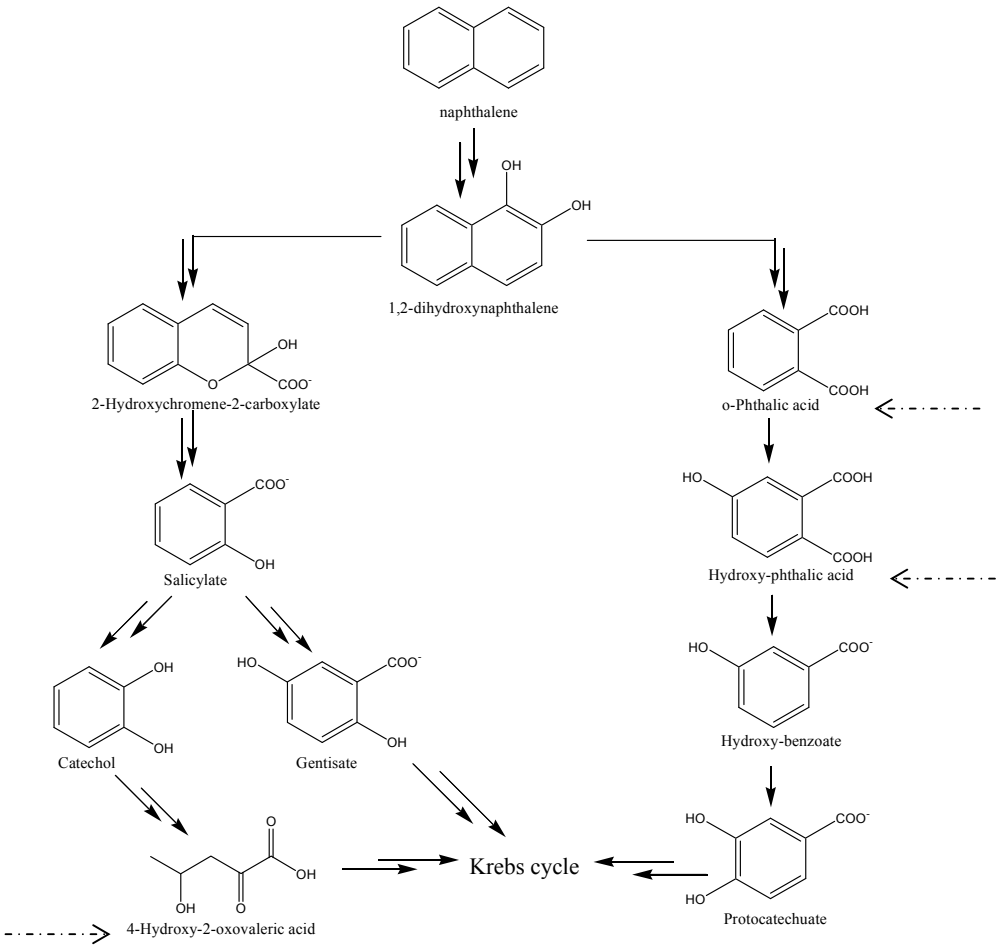


Fig. 1. Naphthalene biochemical biodegradation pathways. Discontinuous arrows show molecules identified by gas chromatography (GC) analysis

Table 2. GC-MS analysis of naphthalene metabolites of *M. radiotolerans* N7A0 and *P. aeruginosa* N7B1

	Metabolite	Retention time (min)	m/z (% relative intensity) [molecular ion]	Compound
***M. radiotolerans* N7A0**	I	14.1	132 (3) [M$^+$], 131 (3), 87 (13), 75 (22), 57 (93), 45 (100)	4-Hydroxy-2-oxovaleric acid
	II	32.2	223 (5) [M+1], 205 (4), 167 (1), 149 (100), 121 (3), 104 (6)	dibutyl phthalate
***P. aeruginosa* N7B1**	I	14.1	132 (3) [M$^+$], 131 (3), 87 (13), 75 (22), 57 (93), 45 (100)	4-Hydroxy-2-oxovaleric acid
	II	32.2	223 (5) [M+1], 205 (4), 167 (1), 149 (100), 121 (3), 104 (6)	dibutyl phthalate
	III	21.9	182 (100) [M$^+$], 181 (27), , 121 (28), 91 (14)	Hydroxy-phthalic acid
	IV	30.5	224 (12) [M$^+$], 181 (100), 136 (8), 121 (9), 91 (3)	Acetoxy-phthalic acid

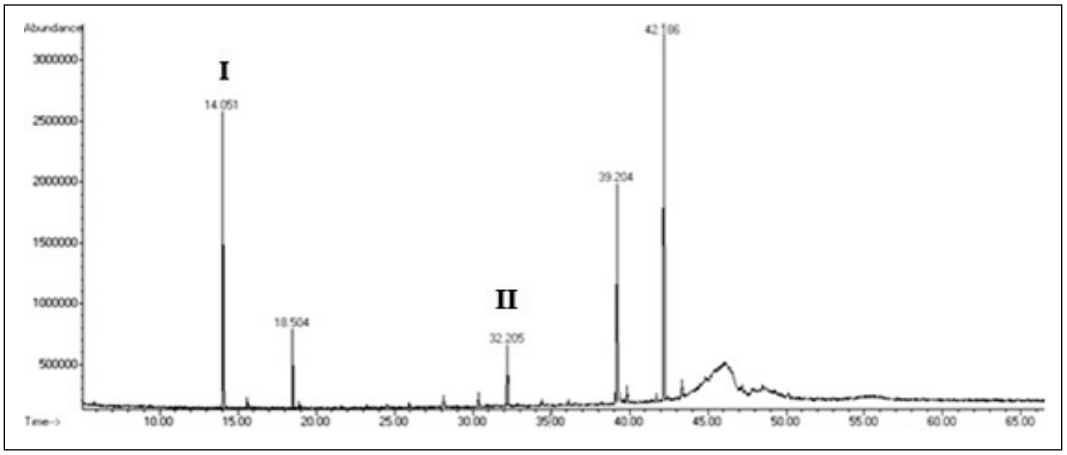
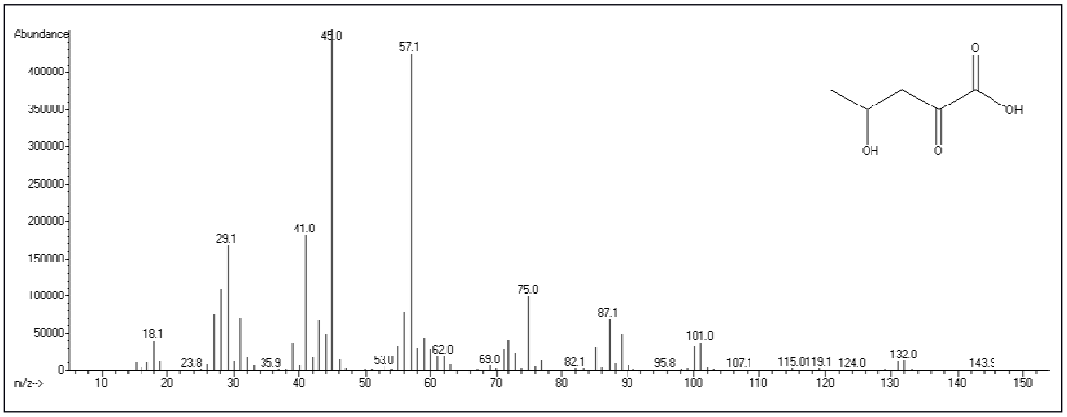


Fig. S1. GC chromatogram of naphthalene metabolites by *Methylobacterium radiotolerans* N7A0. I : 4-hydroxy-2-oxovalic acid, II: dibutyl phthalate

(a)



(b)

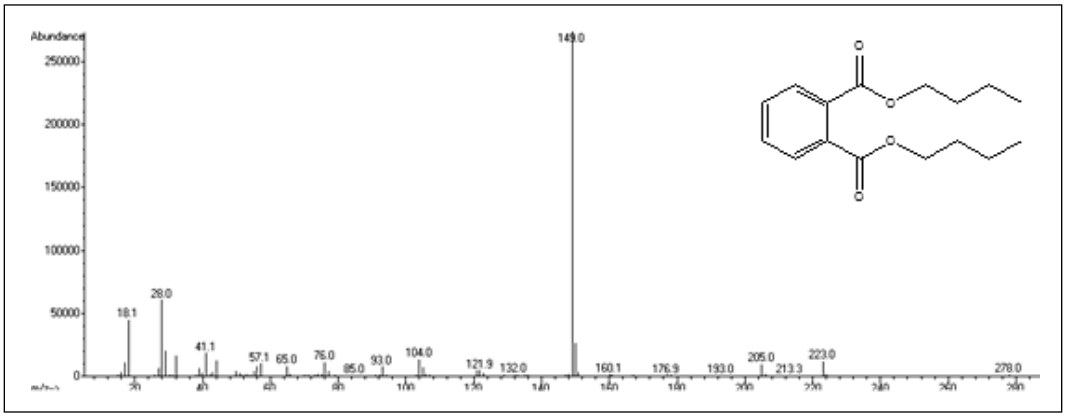


Fig. S2. Mass spectra of naphthalene metabolites by *Methylobacterium radiotolerans* N7A0. (a) 4-hydroxy-2-oxovalic acid, (b) dibutyl phthalate

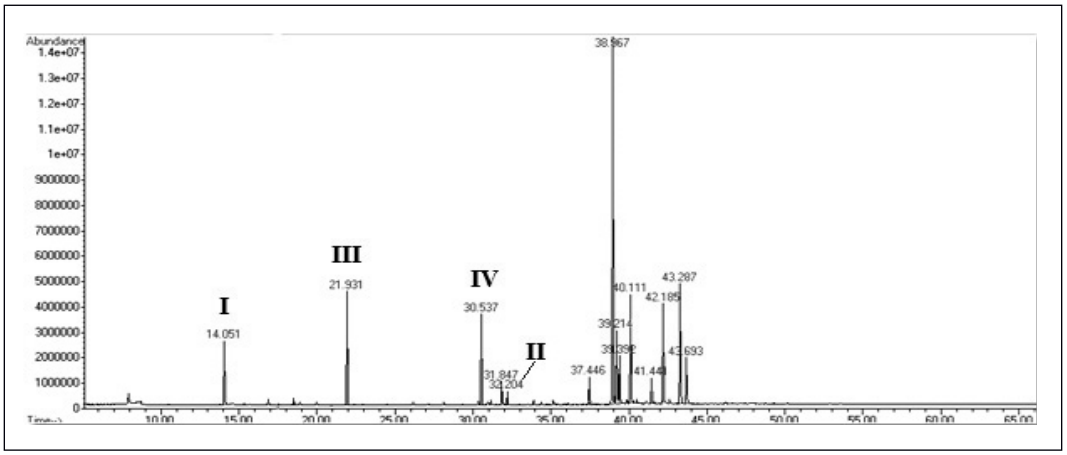


Fig. S3. GC chromatogram of naphthalene metabolites by *Pseudomonas aeruginosa* N7B1. I : 4-hydroxy-2-oxovalic acid, II: dibutyl phthalate, III: hydroxyl-phthalic acid, IV: Acetoxy-phthalic acid

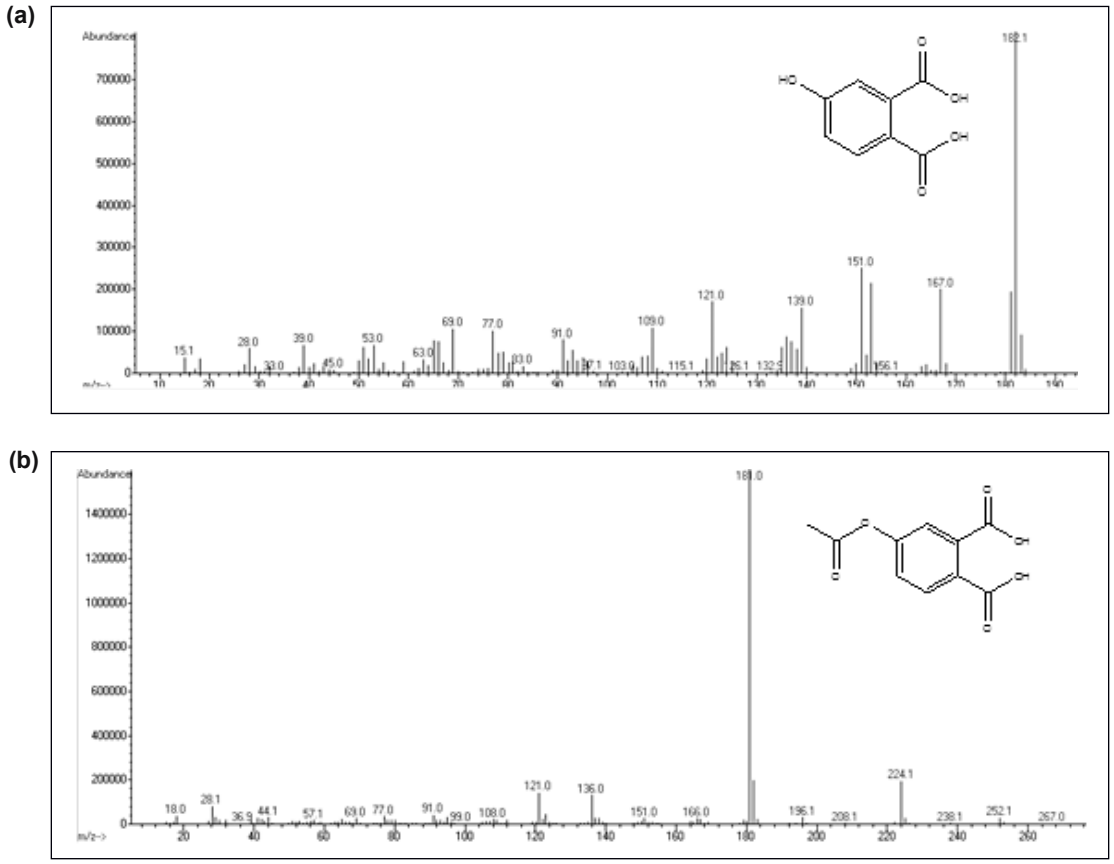


Fig. S4. Mass spectra of naphthalene metabolites by *Pseudomonas aeruginosa* N7B1. (a) hydroxyl-phthalic acid, (b) Acetoxy-phthalic acid

could be explained by the slow growth of this bacterium, thus, not allowing a sufficient identifiable quantity of this metabolite to accumulate in the medium.

The identification of *o*-phthalic acid in both strains was a clear indication that these two bacteria were able to utilize the "phthalic pathway" to biodegrade naphthalene. Hydroxy--phthalic acid, which is an intermediate metabolite only arising after *o*-phthalic acid, was identified in *P. aeruginosa* but not in *M. radiotolerans*. The existence of this pathway has been proven in many different bacteria including in *Pseudomona*s sp. (Jia et al. 2008), *Bacillus fusiformis* (C Lin et al. 2010), *Bacillus thermoleovorans* (Annweiler et al. 2000), and *Geobacillus* sp. (Bubinas et al. 2008).

From these analyses, both salicylate and phthalate pathways were shown to exist in the biodegradation of naphthalene by these bacteria, especially in *P. aeruginosa*. The existence of naphthalene biodegrading bacteria through these two pathways has already been reported. Indeed, Jia *et al.* (2008) have reported a strain of *Pseudomonas* sp. that is able to degrade naphthalene through both the salicylate and phthalate pathways. Similar results have been reported using *Arthrobacter* sp. although, in this strain, the phthalic pathway

is more expressed than the salicylate pathway (Seo et al. 2006). The existence of both pathways has also been proposed in the biodegradation of naphthalene by the thermophilic *Bacillus thermoleovorans* (Annweiler et al. 2000). Thus the existence of such dual pathways is not uncommon.

In conclusion, for the first time with supportive data, a *M. radiotolerans* strain was shown to be able to utilize naphthalene as the sole source of carbon, and this strain of bacterium grows efficiently in the presence of ethanol.

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References

Abanda-Nkpwatt, D., Musch, M., Tschiersch J., Boettner, M. & Schwab, W. (2006). Molecular interaction between Methylobacterium extorquens and seedlings: growth promotion, methanol consumption, and localization of the methanol emission site, *Journal of Experimental Botany*, 57, 15, pp. 4025–4032.

Al-Thukair, A.A. (2002). Effect of oil pollution on euendolithic cyanobacteria of the Arabian Gulf, *Environmental Microbiology*, 4, 2, pp. 125–129.

Annweiler, E., Richnow, H.H., Antranikian, G., Hebenbrock, S., Garms, C., Franke, S., Francke, W. & Michaelis, W. (2000). Naphthalene degradation and incorporation of naphthalene-derived carbon into biomass by the thermophile Bacillus thermoleovorans, *Applied and Environmental Microbiology*, 66, 2, pp. 518–523.

Anonymous. (2014)a. (http://blast.ncbi.nlm.nih.gov/Blast.cgi (30.01.2014)).

Anonymous. (2014)b. Naphthalene degradation – Methylobacterium radiotolerans (http://www.kegg.jp/entry/mrd00626 (20.07.2014)).

Anthony, C. (2011). How half a century of research was required to understand bacterial growth on C1 and C2 compounds; the story of the serine cycle and the ethylmalonyl-CoA pathway, *Science Progress*, 94, 2, pp. 109–137.

Bubinas, A., Giedraitytė, G., Kalėdienė, L., Nivinskiene, O. & Butkiene, R. (2008). Degradation of naphthalene by thermophilic bacteria via a pathway, through protocatechuic acid, *Central European Journal of Biology*, 3, 1, pp. 61–68.

Civilini, M., de Bertoldi, M. & Tell, G. (1999). Molecular characterization of Pseudomonas aeruginosa 2NR degrading naphthalene, *Letters in Applied Microbiology*, 29, 3, pp. 181–186.

Diaz, L.F., Munoz, R., Bordel, S. & Villaverde, S. (2008). Toluene biodegradation by Pseudomonas putida F1: targeting culture stability in long-term operation, *Biodegradation*, 19, 2, pp. 197–208.

Dunn, H.D., Curtin, T., O'Riordan, M.A. Coen, P., Kieran, P.M., Malone, D.M. & O'Connor, K.E. (2005). Aromatic and aliphatic hydrocarbon consumption and transformation by the styrene degrading strain Pseudomonas putida CA-3, *FEMS Microbiology Letters*, 249, 2, pp. 267–273.

Ebrahimi, P. & Plettner, E. (2013). Biodegradation of 1-allyloxy-4--propoxybenzene by selected strains of Pseudomonas putida, *Biodegradation*, 25, 1, pp. 31–39.

El-Naas, M.H., Al-Muhtaseb, S.A. & Makhlouf, S. (2009). Biodegradation of phenol by Pseudomonas putida immobilized in polyvinyl alcohol (PVA) gel, *Journal of Hazardous Materials*, 164, 2–3, pp. 720–725.

Fernandez, M., Niqui-Arroyo, J.L., Conde, S., Ramos, J.L. & Duque, E. (2012). Enhanced tolerance to naphthalene and enhanced rhizoremediation performance for Pseudomonas putida KT2440 via the NAH7 catabolic plasmid, *Applied and Environmental Microbiology*, 78, 15, pp. 5104–5110.

Gao, C., Hu, C., Ma, C., Su, F., Yu, H., Jiang, T., Dou, P., Wang, Y., Qin, T., Lv, M. & Xu, P. (2012). Genome sequence of the lactate-utilizing Pseudomonas aeruginosa strain XMG, *Journal of Bacteriology*, 194, 17, pp. 4751–4752.

Green, P.N. (1992). *The genus Methylobacterium*. In: The Prokaryotes (2nd edn, pp. 2342–2349), Balows, A., Trüper, H.G., Dworkin, M., Harder, W. & Schleifer, K.H. (Eds.), New York, Springer 1992.

Green, P.N. & Bousfield, I.J. (1983). Emendation of Methylobacterium Patt, Cole, and Hanson 1976, Methylobacterium rhodinum (Heumann 1962) comb. nov. corrig., Methylobacterium radiotolerans (It0 and Iizuka 1971) comb. nov. corrig., and *Methylobacterium mesophilicum* (Austin and Goodfellow 1979) comb. nov., *International Journal of Systematic Bacteriology*, 33, 4, pp. 875–877.

Haritash, A.K. & Kaushik, C.P. (2009). Biodegradation aspects of polycyclic aromatic hydrocarbons (PAHs): a review, *Journal of Hazardous Materials*, 169, 1–3, pp. 1–15.

Hoskeri, R.S., Mulla, S.I., Shouche, Y.S. & Ninnekar, H.Z. (2011). Biodegradation of 4-chlorobenzoic acid by Pseudomonas aeruginosa PA01 NC, *Biodegradation*, 22, 3, pp. 509–516.

Hu, J., Zhang, L.L., Chen, J.M. & Liu Y. (2013). Degradation of paracetamol by Pseudomonas aeruginosa strain HJ1012, *Journal of Environmental Science and Health, Part A, Toxic/Hazardous Substances & Environmental Engineering*, 48, 7, pp. 791–799.

Hwang, G., Park, S.R., Lee, C.H. Ahn, I.S., Yoon, Y.J. & Mhin, B.J. (2009). Influence of naphthalene biodegradation on the adhesion of Pseudomonas putida NCIB 9816-4 to a naphthalene--contaminated soil, *Journal of Hazardous Materials*, 172, 1, pp. 491–493.

Izmalkova, T.Y., Sazonova, O.I., Nagornih, M.O., Sokolov, S.L., Kosheleva, I.A. & Boronin, A.M. (2013). The organization of naphthalene degradation genes in Pseudomonas putida strain AK5, *Research in Microbiology*, 164, 3, pp. 244–253.

Jia, Y., Yin, H., Ye, J.S. Peng, H., He, B.Y., Qin, H.M., Zhang, N. & Qiang, J. (2008). Characteristics and pathway of naphthalene degradation by Pseudomonas sp. N7, *Huan Jing Ke Xue*, 29, 3, pp. 756–762. (in Chinese)

Johnson, E.L. & Hyman, M.R. (2006). Propane and n-butane oxidation by Pseudomonas putida GPo1, *Applied and Environmental Microbiology*, 72, 1, pp. 950–952.

Lah, K. (2011). Polycyclic Aromatic Hydrocarbons. (http://toxipedia.org/display/toxipedia/Polycyclic+Aromatic+Hydrocarbons (30.03.2014)).

Li, S., Li, X., Zhao, H. & Cai, B. (2011). Physiological role of the novel salicylaldehyde dehydrogenase NahV in mineralization of naphthalene by Pseudomonas putida ND6, *Microbiological Research*, 166, 8, pp. 643–653.

Lidstrom, M.E. & Chistoserdova, L. (2002). Plants in the pink: cytokinin production by methylobacterium, *Journan of Bacteriology*, 184, 7, pp. 1818.

Lin, C., Gan, L. & Chen, Z.L. (2010). Biodegradation of naphthalene by strain Bacillus fusiformis (BFN), *Journal of Hazardous Materials*, 182, 1–3, pp. 771–777.

Lin, Q. & Jianlong, W. (2010). Biodegradation characteristics of quinoline by Pseudomonas putida, *Bioresource Technology*, 101, 19, pp. 7683–7686.

Mukherjee, K., Tribedi, P., Chowdhury, A., Ray, T., Joardar, A., Giri, S. & Sil, A.K. (2011). Isolation of a Pseudomonas aeruginosa strain from soil that can degrade polyurethane diol, *Biodegradation*, 22, 2, pp. 377–388.

Mukherjee, S., Bardolui, N.K., Karim, S. Patnaik V.V., Nandy, R.K. & Bag, P.K. (2010). Isolation and characterization of a monoaromatic hydrocarbon-degrading bacterium, Pseudomonas

aeruginosa from crude oil, *Journal of Environmental Science and Health, Part A, Toxic/Hazardous Substances & Environmental Engineering*, 45, 9, pp. 1048–1053.

Nzila, A. (2013). Update on the cometabolism of organic pollutants by bacteria, *Environmental Pollution*, 178, pp. 474–482.

Phale, P.S., Paliwal, V., Raju, S.C. Modak, A. & Purohit, H.J. (2013). Genome sequence of naphthalene-degrading soil bacterium Pseudomonas putida CSV86, *Genome Announcements*, 1, 1, pp. 234–212.

Seo, J.S., Keum, Y.S., Hu, Y., Lee, S.E. & Li, Q.X. (2006). Phenanthrene degradation in Arthrobacter sp. P1-1: initial 1,2-, 3,4- and 9,10-dioxygenation, and meta- and ortho-cleavages of naphthalene-1,2-diol after its formation from naphthalene-1,2--dicarboxylic acid and hydroxyl naphthoic acids, *Chemosphere*, 65, 11, pp. 2388–2394.

Seo, J.S., Keum, Y.S., & Li, Q.X. (2009). Bacterial degradation of aromatic compounds, *International Journal of Environmental Research and Public Health*, 6, 1, pp. 278–309.

Smith, C.A. & Hyman, M.R. (2004). Oxidation of methyl tert-butyl ether by alkane hydroxylase in dicyclopropylketone-induced and n-octane-grown Pseudomonas putida GPo1, *Applied and Environmental Microbiology*, 70, 8, pp. 4544–4550.

Takeo, M., Prabu, S.K., Kitamura, C., Hirai, M., Takahashi, H., Kato, D. & Negoro, S. (2006). Characterization of alkylphenol degradation gene cluster in Pseudomonas putida MT4 and evidence of oxidation of alkylphenols and alkylcatechols with medium-length alkyl chain, *Journal of Bioscience and Bioengineering*, 102, 4, pp. 352–361.

Takizawa, N., Iida, T., Sawada, T., Yamauchi, K, Wang, Y.W., Fukuda, M. & Kiyohara, H. (1999). Nucleotide sequences and characterization of genes encoding naphthalene upper pathway of Pseudomonas aeruginosa PaK1 and Pseudomonas putida OUS82, *Journal of Bioscience and Bioengineering*, 87, 6, pp. 721–731.

Tyagi, M., da Fonseca, M.M. & de Carvalho, C.C. (2011). Bioaugmentation and biostimulation strategies to improve the effectiveness of bioremediation processes, *Biodegradation*, 22, 2, pp. 231–241.

Urakami, T., Araki, H., Suzuki, K. & Komagata, K. (1993). Further studies of the genus Methylobacterium and description of Methylobacterium aminovorans sp. nov., *International Journal of Systematic Bacteriology*, 43, 3, pp. 504–513.

You, Y., Shim, J., Cho, C.H. Ryu, M.H., Shea, P.J., Kamala-Kannan, S., Chae, J.C. & Oh, B.T. (2013). Biodegradation of BTEX mixture by Pseudomonas putida YNS1 isolated from oil-contaminated soil, *Journal of Basic Microbiology*, 53, 5, pp. 469–475.

Zhang, Z., Hou, Z., Yang, C., Ma, C., Tao, F. & Xu, P. (2011). Degradation of n-alkanes and polycyclic aromatic hydrocarbons in petroleum by a newly isolated Pseudomonas aeruginosa DQ8, *Bioresources Technology*, 102, 5, pp. 4111–4116.