



PRIFYSGOL
BANGOR
UNIVERSITY

Insights into the degradation capacities of *Amycolatopsis tucumanensis* DSM 45259 guided by microarray data

Bourguignon, Natalia; Bargiela, Rafael; Rojo, David; Chernikova, Tatyana; Lopez de Rodas, Sara A.; Garcia-Cantalejo, Jesus; Nather, Daniela J.; Golyshin, Peter; Barbas, Coral; Ferrero, Marcelo; Ferrer, Manuel

World Journal of Microbiology and Biotechnology

DOI:

[10.1007/s11274-016-2163-8](https://doi.org/10.1007/s11274-016-2163-8)

Published: 01/12/2016

Peer reviewed version

[Cyswllt i'r cyhoeddiad / Link to publication](#)

Dyfyniad o'r fersiwn a gyhoeddwyd / Citation for published version (APA):

Bourguignon, N., Bargiela, R., Rojo, D., Chernikova, T., Lopez de Rodas, S. A., Garcia-Cantalejo, J., Nather, D. J., Golyshin, P., Barbas, C., Ferrero, M., & Ferrer, M. (2016). Insights into the degradation capacities of *Amycolatopsis tucumanensis* DSM 45259 guided by microarray data. *World Journal of Microbiology and Biotechnology*, 32(201). <https://doi.org/10.1007/s11274-016-2163-8>

Hawliau Cyffredinol / General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal ?

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

1 Journal: *World Journal Microbiology and Biotechnology*

2 **Insights into the degradation capacities of *Amycolatopsis tucumanensis***
3 **DSM 45259 guided by microarray data**

4 **Natalia Bourguignon^{1,#}, Rafael Bargiela^{2,#}, David Rojo^{3#}, Tatyana N. Chernikova⁴,**
5 **Sara A López de Rodas⁵, Jesús García-Cantalejo⁵, Daniela J Näther⁶, Peter N**
6 **Golyshin⁴, Coral Barbas³, Marcela Ferrero^{1,§}, and Manuel Ferrer^{2,§*}**

7 ¹ Planta Piloto de Procesos Industriales Microbiológicos (PROIMI-CONICET),
8 Tucumán, Argentina.

9 ² Consejo Superior de Investigaciones Científicas (CSIC), Institute of Catalysis,
10 Madrid, Spain.

11 ³ Centro de Metabolómica y Bioanálisis (CEMBIO), Facultad de Farmacia,
12 Universidad CEU San Pablo, Campus Montepincipe, Boadilla del Monte, Madrid,
13 Spain.

14 ⁴ School of Biological Sciences, Bangor University, Gwynedd LL57 2UW, UK.

15 ⁵ Unidad de Genómica-Campus Moncloa, C.A.I. Genómica y Proteómica, Facultad
16 CC. Biológicas, Universidad Complutense de Madrid, Madrid, Spain.

17 ⁶ Institute for Microbiology, Goethe University, Biocentre, Frankfurt, Germany.

18

19 ***Correspondence**

20 Manuel Ferrer

21 mferrer@icp.csic.es

22

23 #These authors contributed equally to this work

24 §These authors contributed equally to this work

25 Running title: Catabolome of *A. tucumanensis*

26

27 **Abstract** The analysis of catabolic capacities of microorganisms is currently often
28 achieved by cultivation approaches and by the analysis of genomic or metagenomic
29 datasets. Recently, a microarray system designed from curated key aromatic catabolic
30 gene families and key alkane degradation genes was designed. The collection of genes
31 in the microarray can be exploited to indicate whether a given microbe or microbial
32 community is likely to be functionally connected with certain degradative phenotypes,
33 without previous knowledge of genome data. Herein, this microarray was applied to
34 capture new insights into the catabolic capacities of copper-resistant actinomycete
35 *Amycolatopsis tucumanensis* DSM 45259. The array data support the presumptive
36 ability of the DSM 45259 strain to utilize single alkanes (*n*-decane and *n*-tetradecane)
37 and aromatics such as benzoate, phthalate and phenol as sole carbon sources, which was
38 experimentally validated by cultivation and mass spectrometry. Interestingly, while in
39 strain DSM 45259 *alkB* gene encoding an alkane hydroxylase is most likely highly
40 similar to that found in other actinomycetes, the genes encoding benzoate 1,2-
41 dioxygenase, phthalate 4,5-dioxygenase and phenol hydroxylase were homologous to
42 proteobacterial genes. This suggests that strain DSM 45259 contains catabolic genes
43 distantly related to those found in other actinomycetes. Together, this study not only
44 provided new insight into the catabolic abilities of strain DSM 45259, but also suggests
45 that this strain contains genes uncommon within actinomycetes.

46 **Keywords** Alkanes · *Amycolatopsis tucumanensis* · aromatics · catabolome ·
47 degradation · microarray

48

49 **Introduction**

50 Imagine the microbial communities responding to pollutants-intake and how variable
51 this can be (Hazen et al. 2010; Kostka et al. 2011; Beazley et al. 2012; Guazzaroni et al.
52 2013; Gutierrez et al. 2013). The most obvious reaction will be their ability to react by
53 degrading such pollutants to intermediates feeding the central metabolism (Liang et al.
54 2011; Lu et al. 2012; Mason et al. 2012; Kimes et al. 2013; Mason et al. 2014). There is
55 great interest in identifying next-generation information that allows predicting the
56 diversity of pollutants that each community and the microorganisms conforming it, can
57 degrade and the catabolic genes implicated (Pérez-Pantoja et al. 2008; Pérez-Pantoja et
58 al. 2012; Guazzaroni et al. 2013; Bargiela et al. 2015a). The analysis of catabolic
59 capacities of microbial communities or single cultures begins by assessing gene
60 contents, which are currently often achieved using genomic or metagenomic data
61 (Guazzaroni et al. 2013; Bargiela et al. 2015a,b), followed by the analysis of the
62 annotated genome or metagenome and a reference catabolic database as input
63 information (Pérez-Pantoja et al. 2008; Pérez-Pantoja et al. 2012; Guazzaroni et al.
64 2013; Bargiela et al. 2015a,b). Further, catabolic network can be built, using as an input
65 potential protein-coding gene sequences obtained by direct sequencing of DNA material
66 and the web-based AromaDeg resource (Duarte et al. 2014; Bargiela et al. 2015a,b).

67 In case genome information is lacking, the identification of catabolic capacities
68 required extensive experimental efforts, i.e. by producing microcosms in which the
69 ability to degrade pollutants is investigated using labeled or not labeled compounds
70 (Watanabe and Hamamura 2003; Pandey et al. 2008). With the aim of easing this
71 process, a novel internally calibrated functional gene microarray system (the so-called
72 catabolome array) was recently developed (Vilchez-Vargas et al. 2013). It contains
73 optimally designed probes covering key aromatic catabolic gene families and key

74 alkane degradation genes. This enables identifying molecular functions of identified
75 genes in light of catabolic pathways by using DNA material, without the need of
76 genome sequencing. The microarray contains 3605 probes (50 mer) representing
77 catabolic gene subfamilies encoding key activities in hydrocarbon degradation
78 pathways, that included Rieske non-haem iron ring hydroxylating (di)oxygenases
79 (RHDO), extradiol dioxygenases of the vicinal chelate superfamily (EXDOI), intradiol
80 dioxygenases (INDO), soluble di-iron aromatic ring hydroxylating monooxygenases,
81 ferredoxins of multicomponent aromatic degradation enzymes (FERRE), muconate
82 cycloisomerases (MCIS), maleylacetate reductases (MACR), alkane hydroxylases of the
83 integral membrane-bound monooxygenases (ALKB), cytochrome P450, CYP153
84 alkane hydroxylases (CYP153), benzoyl coenzyme A reductases (BCOAR), and
85 benzylsuccinate synthases. Supplementary Table 1 provides information regarding
86 accession numbers for sequences and taxonomic origin of catabolic genes associated to
87 each of the probes. Briefly, most probes (circa 84%) derived from genomes from
88 cultivable bacteria of at least 182 different species, distributed among 70 genera that
89 included *Gordonia*, *Nocardioides*, *Rhodococcus*, *Prauserella*, *Mycobacterium*,
90 *Nocardia*, *Dietzia*, *Corynebacterium*, *Frankia* and *Janibacter* (Actinobacteria),
91 *Flavobacteria*, *Dokdonia*, *Polaribacter* and *Maribacter* (Bacteroidetes), *Geobacillus*
92 and *Desulfitobacterium* (Proteobacteria), *Acinetobacter*, *Sphingomonas*, *Alcanivorax*,
93 *Cycloclasticus*, *Pseudomonas*, *Legionella*, *Xanthomonas*, *Burkholderia*, *Oleiphilus*,
94 *Xanthobacter*, *Thalassolituus*, *Acidisphaera*, *Photorhabdus*, *Bdellovibrio*, *Ruegeria*,
95 *Rhodobacter*, *Ralstonia*, *Methylococcus*, *Bradyrhizobium*, *Hahella*, *Jannaschia*,
96 *Polaromonas*, *Paraburkholderia*, *Paracoccus*, *Marinobacter*, *Sulfitobacter*,
97 *Roseovarius*, *Oceanicola*, *Pseudoceanicola*, *Oceanicaulis*, *Loktanella*, *Maritimibacter*,
98 *Parvularcula*, *Roseobacter*, *Acidiphilium*, *Psychrobacter*, *Bermanella*,

99 *Stenotrophomonas*, *Blastochloris*, *Azoarcus*, *Magnetospirillum*, *Geobacter*, *Thauera*,
100 *Ensifer*, *Aromatoleum*, *Rhodopseudomonas*, *Syntrophobacter*, *Alkalilimnicola*,
101 *Desulfobacula*, *Parvibaculum*, *Sphingopyxis*, *Caulobacter*, *Erythrobacter* and
102 *Novosphingobium* (Proteobacteria). Probes from species of *Tetrahymena* (Eukaryotia),
103 *Neurospora* (Ascomycota) and *Methanopyrus* (Euryarchaeota) are also included. Note
104 that within bacterial species whose probes are included in the microarray, 30 belong to
105 10 genera (*Gordonia*, *Nocardioides*, *Rhodococcus*, *Prauserella*, *Mycobacterium*,
106 *Nocardia*, *Dietzia*, *Corynebacterium*, *Frankia* and *Janibacter*) of the order
107 Actinomycetales. Detailed information on all the probes on the array and the
108 evolutionary relationships are reported elsewhere (Vilchez-Vargas et al. 2013).

109 In this work, we exploit the catabolome array (Vilchez-Vargas et al. 2013) to get
110 new insights into the degrading capacities of *A. tucumanensis* strain DSM 45259, a
111 copper-resistant actinobacterium isolated from polluted sediments (Albarracín et al.
112 2010a). Note that the threshold for considering a signal as a true positive in the array
113 was set when hybridization occurred with a probe exhibiting > 80% sequence identity,
114 where it can be assumed that the target DNA is derived from a gene encoding a member
115 of the same subfamily as that for which the probe was designed (Vilchez-Vargas et al.
116 2013). This, together with the fact that the majority of the probes belong to bacteria,
117 including actinomycetes (see above), suggest that catabolic genes of the actinomycete
118 *Amycolatopsis tucumanensis* DSM 45259 will be detectable. Having said that, *A.*
119 *tucumanensis* DSM 45259 was widely studied for its remarkable copper-resistance
120 (Dávila Costa et al. 2011a,b; Dávila Costa et al. 2012). More recently, degradation of
121 naphthalene and phenanthrene was found to occur in minimal medium when growing
122 on glucose as co-substrate (Bourguignon et al. 2014). In the present study we provide
123 evidences that *A. tucumanensis* DSM 45259 has also the capacity to use aliphatic and

124 aromatic hydrocarbons such as *n*-decane, *n*-tetradecane, phthalate, benzoate and phenol
125 as sole carbon sources. These abilities, predicted by the microarray, were further
126 confirmed by cultivation tests and target mass spectrometry analysis. Although, such
127 degradation capacities are common within other actinomycetes, the results suggest that
128 strain DSM 45259 carries some catabolic genes distantly related to previous catabolic
129 genes of other actinomycetes. In addition to that, because good agreement with the
130 array-based predictions was observed after experimental validations, we suggest that the
131 strategy herein described represents a promising strategy for disentangling contexts-
132 specific catabolic phenotypes in any organism or microbial community, without the
133 need of genome or metagenome sequencing.

134 **Materials and Methods**

135 **Chemicals and basic culture conditions**

136 All chemicals used were of the purest grade available and were purchased from Fluka-
137 Aldrich-Sigma Chemical Co. (St Louis, MO, USA). *A. tucumanensis* strain DSM
138 45259, a copper resistant strain, was used in this study (Albarracín et al. 2010a; Dávila
139 Costa et al. 2012). Strain DSM 45259 was cultivated in Tryptic Soy Broth (TSB)
140 medium (tryptein: 17 g L⁻¹; soy peptone: 3 g L⁻¹; NaCl: 5 g L⁻¹; K₂HPO₄: 2.5 g L⁻¹;
141 glucose: 2.5 g L⁻¹; pH 7.3 ± 0.2) at 30 °C until late exponential growth phase. This
142 culture was used as pre-inoculum for a 30 ml Minimal Media (MM) broth ((NH₄)₂SO₄:
143 2 g L⁻¹; K₂HPO₄: 0.5 g L⁻¹; MgSO₄·7H₂O: 0.2 g L⁻¹; FeSO₄: 0.01 g L⁻¹; glucose: 1.25 g
144 L⁻¹; pH 7.0 ± 0.2) containing 0.2 mM naphthalene (from a 25 mM stock solution in
145 acetone) and glucose 1.25 g L⁻¹. Control culture without the addition of hydrocarbon
146 was performed. Cultures were incubated at 30 °C and 180 rpm for 96 h, after which

147 cultures were centrifuged (8000 rpm; 10 min; 4 °C) and cell pellets used for DNA
148 extraction.

149 **Catabolome microarray analysis**

150 The total DNA extraction was done by using cells harvested during late exponential
151 growth phase in cultures containing glucose and naphthalene as carbon sources, and the
152 hexadecyltrimethylammonium bromide (CTAB) method with some modifications
153 (Bailey et al. 1995). Briefly, harvested cells were re-suspended in 750 µL lysozyme-
154 CTAB extraction solution (8 mg mL⁻¹ lysozyme, 2 % CTAB, 1.4 M NaCl, 20 mM
155 ethylenediaminetetraacetic acid (EDTA), 100 mM Tris-HCl, pH 8, 50 mg L⁻¹ ARNase,
156 0.3 M sucrose). After incubation during 2 h at 37 °C to improve cell lysis, 250 µL
157 sodium dodecyl sulfate (SDS) 2% (w/v) were added, the solution was vortexed for 1
158 min, and then 2 µL β-mercaptoethanol added and incubated 30 min at 60 °C. To purify
159 DNA, 1 volume of chloroform:isoamyl alcohol (24:1) was added. The solution was
160 mixed and centrifuged (12000 rpm, 15 min). After separation of the aqueous phase, 1
161 volume of 2-propanol was added and the solution incubated at -20 °C during 1 h to
162 facilitate DNA precipitation. The precipitated DNA was washed with 1 volume of 70 %
163 (v/v) ethanol and dried. Finally, the DNA was re-suspended in 50 µL sterile distilled
164 water. Purity of extracted DNA was assessed by measuring the 260/280 and 260/230
165 ratios using a spectrophotometer. DNA concentration was measured using Quanti-iT
166 dsDNA Assay kit (Invitrogen, Paisley, UK).

167 For DNA fragmentation, the resulting genomic DNA was heat-fragmented at 95 °C
168 for up to 1.5 h. The aliquot of each digestion reactions was analyzed on 2% (w/v)
169 agarose gel and completed if the majority of DNA fragments had a size range of 200-
170 1000 base pairs (bp). This resulted in the production of fragments 200 to 1000 bases in

171 length. The resulted DNA was precipitated with isopropanol, suspended in 45 μ L of
172 MilliQ water and used for labelling. We labelled total DNA by direct incorporation of
173 Cy5-conjugated dUTP (GE Healthcare) using terminal deoxynucleotidyl transferase
174 (Thermo Scientific, Paisley, UK). Following a 4 h incubation at 37 °C, the reaction
175 terminated by addition of 0.5 M EDTA, pH 8.0. The labelled target was purified from
176 unincorporated dye molecules by adding 200 μ L of TE buffer and spinning through a
177 Microcone filter (Millipore, Hertfordshire, UK) for 15 minutes at 11000 rpm. The
178 purified, labelled target was precipitated with isopropanol, and resuspended to a final
179 volume of 20 μ L with MilliQ water. The dye incorporation was measured with a
180 NanoDrop spectrophotometer. Labelled DNA samples were vacuum-dried and stored at
181 -20° C until hybridization.

182 We used a chip previously designed and calibrated by Vilchez-Vargas et al. (2013).
183 For microarray hybridization, probes were printed on CodeLink Activated slides
184 (SurModics, Eden Prairie, USA) using MicroGrid TAS II spotter (BioRobotics,
185 Germany) at the University of Frankfurt (Frankfurt, Germany). Coupling of DNA
186 probes was performed by overnight incubation of slides in saturated NaCl chamber.
187 Post-coupling processing included the blocking of residual reactive groups and was
188 done as follows: slides were washed with 4x SSC (190 mM sodium chloride plus 20
189 mM tri-sodium citrate equivalent to sodium concentration of 250 mM), 0.1% (w/v)
190 SDS, for 30 min, then rinsed briefly with deionized water and dried by centrifugation
191 for 3 min at low-speed centrifuge. Prior to hybridization, labelled DNA was incubated
192 with herring sperm DNA (Invitrogen, Paisley, UK) for 5 min at 95 °C and then 80 μ L of
193 hybridization buffer was added. For hybridization, slides were inserted into
194 hybridization chamber and after that were covered by coverslips. The solution of Cy5 –

195 dUTP labelled DNA in hybridization buffer (100 μ L total volume) was carefully
196 infused through narrow gaps between slides and covers.

197 The hybridization was performed at 55 °C for 18 h using hybridization buffer
198 consisting of 15% (v/v) dimethylsulfoxide, 25% (v/v) formamide, 1.25 x SSC, 0.15%
199 (w/v) SDS, 0.15% (w/v) Tween 20, 880 mM betaine, 5x TE buffer (50 mM Tris-HCl, 5
200 mM EDTA) and 0.1 mg L⁻¹ bovine serum albumin (BSA) in aqueous solution.
201 Following hybridization, slides were washed 5 min at 42 °C in 1x SSC containing 0.3%
202 (w/v) SDS, twice in 1x SSC (1 min, 42 °C), in 0.5x SSC (1 min, 20 °C), in 0.1x SSC
203 containing 0.3% (w/v) SDS (1 min, 42° C) and finally twice in 0.1x SSC (1 min, 20 °C).
204 Slides were dried at low speed in centrifuge for 30 seconds. Slides were scanned in a
205 GenePixR 4000B microarray scanner (Molecular Devices, Berkshire, UK) and images
206 analyzed by using the software of image analysis GenePixRPro 6.0 from Axon
207 Instruments / Molecular Devices Corp (Molecular Devices, Berkshire, UK).

208 Each query sequence from probes targeting catabolic genes (see accession numbers
209 in Supplementary Table S1) for which a positive signal was obtained in the microarray
210 was submitted to web-based AromaDeg resource (Duarte et al. 2014). Each sequence
211 was then associated with a catabolic enzyme performing an aromatic compound
212 degradation reaction.

213 **Quantification of the biodegradation of hydrocarbons by cultivation and liquid** 214 **chromatography-mass spectrometry**

215 Activation of the DSM 45259 strain was firstly done by transfers in minimal-mineral
216 medium with a low concentration of hydrocarbon (20 mg L⁻¹) for the adaptation of the
217 microorganism. The medium consisted of 2.6 g Na₂HPO₄, 1.33 g KH₂PO₄, 1 g
218 (NH₄)₂SO₄ and 0.20 g MgSO₄·7 H₂O dissolved in 1000 mL of demineralized water. The

219 medium was adjusted to pH 7.2 ± 0.3 . After sterilization, 5 mL of trace element solution
220 and 1 mL of vitamin solution were added. Both solutions were prepared as described in
221 DSMZ methanogenium medium 141 and autoclaved or sterile filtered separately
222 ([DSMZ 2012]). Substrates were sterilized separately and added aseptically at an
223 amount of 20 mg L^{-1} each. Cultivation was done at $30 \text{ }^\circ\text{C}$ with a 180 rpm constant
224 agitation during 72 h. The cell biomass was washed twice with 20 mM sodium
225 phosphate buffer pH 7.0 and used to produce cultures. Briefly, cell pellets were grown
226 in 30 mL of the same medium ($0.4 \text{ g wet cell pellet L}^{-1}$) with various aliphatic and
227 aromatic hydrocarbons such as *n*-decane, *n*-tetradecane, phenol, benzoate and phthalate
228 to serve as the sole source of carbon and energy. Substrates were sterilized separately
229 and added aseptically at an amount of 500 mg L^{-1} each. Cultivation was done at $30 \text{ }^\circ\text{C}$
230 with a 180 rpm constant agitation during 72 h. Two controls were done: a control test
231 without the addition of the cells (abiotic test) and a control test without the addition of
232 the hydrocarbon (biotic test).

233 The extraction of the hydrocarbons and their degradation intermediates was
234 performed by adding 1 volume of acetone to the cultures. After homogenization, flasks
235 were stand for 30 min, and then centrifuged at 13000 rpm during 10 min. The
236 supernatants were analyzed by target analysis by Liquid Chromatography (LC)-Mass
237 Spectrometry (MS) to confirm the degradation of the initial substrates as well as the
238 existence of degradation intermediates in test and control cultures. For that, the
239 following reagents and standards have been used: acetonitrile (LC-MS grade, Sigma-
240 Aldrich, Steinheim, Germany), formic acid (FA) (MS grade, Sigma-Aldrich, Steinheim,
241 Germany) and MilliQ® water (Millipore, Billerica, MA, USA). For reference masses
242 purine, hexakis(1H,1H,3H-tetrafluoropropoxy)phosphazine (HP) and ammonium
243 trifluoroacetate ($\text{TFA}(\text{NH}_4)$) from Agilent (API-TOF reference mass solution kit) were

244 used. The metabolic profile was achieved using a liquid chromatography system
245 consisting of a degasser, a binary pump, and an auto-sampler (1290 infinity II, Agilent).
246 Samples (0.5 μL) were applied to a reversed-phase column (Zorbax Extend C18 50 x
247 2.1 mm, 1.8 μm ; Agilent), which was maintained at 60 $^{\circ}\text{C}$ during the analysis. The
248 system was operated at a flow rate of 0.6 mL min^{-1} with solvent A (water containing
249 0.1% formic acid) and solvent B (acetonitrile containing 0.1% formic acid). The
250 gradient was 5% B (0–1 min), 5 to 80% B (1–7 min), 80 to 100% B (7–11.5 min), and
251 100 to 5% B (11.5–12 min). The system was finally held at 5% B for 3 min to re-
252 equilibrate the system (15 min of total analysis time). Data were collected in positive
253 and negative ESI modes in separate runs using QTOF (Agilent 6550 iFunnel). The
254 analyses were performed in both positive and negative ion modes in full-scan from m/z
255 50 to 1000. The capillary voltage was 3000 V and the nozzle voltage was 1000 V with a
256 scan rate of 1.0 spectrum per second. The gas temperature was 250 $^{\circ}\text{C}$, the drying gas
257 flow was 12 L min^{-1} , the nebulizer was 52 psi, the sheath gas temperature 370 $^{\circ}\text{C}$ and
258 the sheath gas flow 11 L min^{-1} . For positive mode, the MS-TOF parameters were as
259 follows: fragmentor 175 V and octopole radio frequency voltage 750 V. For negative
260 mode, the MS-TOF parameters included the following: fragmentor 250 V and octopole
261 radio frequency voltage 750 V. During the analyses, two reference masses were used:
262 121.0509 (purine, detected m/z $[\text{C}_5\text{H}_4\text{N}_4+\text{H}]^+$) and 922.0098 (HP, detected m/z
263 $[\text{C}_{18}\text{H}_{18}\text{O}_6\text{N}_3\text{P}_3\text{F}_{24}+\text{H}]^+$) in positive mode and 112.9856 (TFA(NH_4), detected m/z
264 $[\text{C}_2\text{O}_2\text{F}_3(\text{NH}_4)-\text{H}]^-$) and 966.0007 (HP+FA, detected m/z $[\text{C}_{18}\text{H}_{18}\text{O}_6\text{N}_3\text{P}_3\text{F}_{24}+\text{FA}-\text{H}]^-$) in
265 negative mode. The references were continuously infused into the system, enabling
266 constant mass correction. Samples were analyzed in randomized runs, during which
267 they were incubated in an auto-sampler at 4 $^{\circ}\text{C}$. The analytical runs for both polarities

268 were set up starting with the analysis of ten equilibrium injections followed by the
269 samples. A single injection per sample was done.

270 Based on a list of candidates, their accurate monoisotopic masses were searched for
271 in the MS chromatograms (± 10 ppm) using MassHunter Quantitative Analysis
272 (B.06.00, Agilent) and their identification confirmed by the analysis of the commercial
273 standards. Then, the corresponding peak areas were integrated using the same software.

274 **Determination of catechol dioxygenase activity**

275 To investigate the catechol 2,3-dioxygenase activity, a spectrophotometric method was
276 used, in which the formation of oxidation products is followed. Briefly, the strain was
277 pre-cultivated in minimal-mineral medium as described before at 30 °C until
278 exponential phase using benzoate and phenol as sole carbon sources (20 mg L⁻¹). This
279 culture was used to inoculate 30 mL of minimal-mineral medium containing 500 mg L⁻¹
280 of benzoate and phenol, respectively (for details see above). Control cultures without
281 the addition of hydrocarbons were done. Cultures were incubated at 30 °C at 180 rpm
282 during 72 h, after which cells were separated by centrifugation (8000 rpm; 10 min; 4
283 °C). The pellet was washed twice with 50 mM K/Na-phosphate (pH 7.5) buffer, and
284 then re-suspended in 5 mL of this buffer. For the preparation of protein cell extracts, the
285 cells were broken by three passages in a French Press® at 20000 psi, after which the
286 sample was centrifuged (10000 rpm; 10 min; 4 °C) to eliminate cell debris. Supernatant
287 was carefully aspirated and immediately used for activity assay. The assay was
288 performed in 96-well plates and 200 μ L of total volume, as described elsewhere
289 (Alcaide et al. 2013). Briefly, the catechol 2,3-dioxygenase activity was measured (in
290 triplicates) in a microplate reader (Synergy HT Multi-Mode Microplate Reader -
291 BioTek) by evaluating the increase of absorbance at 388 nm due to the formation of the

292 reaction product 2-hydroxy-6-oxohepta-2,4-dienoate (HOHD), in a reaction mixture that
293 contains 10 μL of protein extract containing catechol 2,3-dioxygenase to a substrate in
294 the presence of the following solution: 87 μL of K/Na-phosphate (pH 7.5) and 3 μL of
295 catechol solution in H_2O (10 mM) to achieve a final substrate concentration of 0.15
296 mM. Reactions were followed at 30 $^\circ\text{C}$ for 20 min (ϵ_{HOHD} at 388 nm = 13,800 $\text{M}^{-1} \text{cm}^{-1}$).
297 One unit (U) of enzyme activity was defined as the amount of enzyme required to
298 transform 1 μmol of substrate in 1 min under the assay conditions.

299 **Results**

300 **Degradation capacities of DSM45259 guided by microarray data**

301 Catabolome microarray data from *A. tucumanensis* DSM 45259, grown in naphthalene
302 and glucose as co-substrate constituted the input information in our study. The complete
303 information about the microarray tests is described in the Materials and Methods
304 section. Following the restrictive criterion of fold-change above 6-fold higher than
305 background signal in the internally calibrated microarray system, we detect a total of 5
306 out of 3605 genes encoding proteins with proved catabolic functions (Table 1). As
307 mentioned, to detect signals with a high precision, only signals > 6 normalized intensity
308 (NI) were considered, as the use of internal positive controls for setting the correct
309 threshold according to the desired precision of the experiment revealed that, under
310 conditions described in Materials and Methods, any signal $> 6\text{-}8\text{NI}$ is highly unlikely to
311 be false positive (Vilchez-Vargas et al. 2013). Raw fluorescence signals for probes
312 targeting the 3605 genes are detailed in Supplementary Table 1. Within the 11 catabolic
313 genes families targeted by the microarray (Vilchez-Vargas et al. 2013), we detected the
314 presence of 4 covered by the 5 positive probes, which are summarized below.

315 Genes implicated in alkane degradation were found. Particularly, the probes
316 targeting *alkB* genes AJ833983 and AJ833926 for AlkB alkane hydroxylases (the so-
317 called ALKB catabolic gene family by Vilchez-Vargas et al. 2013) were strongly
318 detected (Table 1). AlkB participates in the initial attack of n-alkenes in the *n*-alkane
319 oxidation pathway (Fig. 1). Within ring hydroxylating dioxygenases (RHDO catabolic
320 gene family) we found AAD17377 by a high intensity of hybridization, followed by
321 AAD03558 (Table 1). According to AromaDeg (Duarte et al. 2014), AAD17377 gene
322 encodes a benzoate 1,2-dioxygenase (Bzt) that convert benzoate into *cis*-1,6-dihydroxy-
323 2,4-cyclohexadiene-1-carboxylic acid within the benzoate to catechol degradation
324 pathway, and AAD03558 a phthalate 4,5- dioxygenase (Pht) that converts phthalate into
325 protocatechuate. Finally, within ring hydroxylating monooxygenases (RHMO catabolic
326 gene family) we found Z36909, which was the probe with the highest level of
327 hybridization intensity (Table 1), and that encodes a phenol hydroxylase, an enzyme
328 that catalyzes the first step in the degradation of phenol into catechol.

329 Taken together, the microarray data support the ability of the DSM 45259 strain to
330 utilize single alkanes and aromatics such as benzoate and phenol (through conversion to
331 catechol) and phthalate (through conversion to protocatechuate) as carbon sources (Fig.
332 1). Interestingly *alkB* gene in the strain DSM 45259 matches with two probes
333 (AJ833983 and AJ833926) encoding the same protein, namely, an alkane hydroxylase
334 (AlkB) from actinomycete *Rhodococcus* species; this matches with the taxonomy of
335 strain DSM 45259. Indeed, several actinomycetes able to degrade C₅–C₁₀ alkanes
336 contain alkane hydroxylases as, for example, representatives from mycobacteria and
337 rhodococci (van Beilen et al. 2005; Sekine et al. 2006; Lincoln et al. 2015). By contrast,
338 genes encoding Bzt (AAD17377) and Pht (AAD03558) match with probes from
339 Proteobacteria (*Sphingobium* and *Burkholderia* spp.), and that of the phenol

340 hydroxylase (Z36909) to a probe from *Acinetobacter* sp. This suggests that *alkB* gene in
341 DSM 45259 strain is highly similar to that found in other actinomycetes, while the other
342 3 genes are quite divergent to those from actinomycetes.

343 Note that no any other gene implicated in the later stages of the degradation of
344 alkanes, apart from *alkB*, was detected in the microarray because it does not contains
345 such genes (Vilchez-Vargas et al. 2013). In the case of genes implicated in the later
346 stages of the degradation of protocatechuate, phenol and catechol, the microarray
347 contains probes encoding catechol-2,3-dioxygenases (Cat) within the so-called extradiol
348 dioxygenases (EXDO) catabolic gene family, and catechol 1,2-dioxygenases and
349 protocatechuate 3,4-dioxygenases (3,4-PCD) within the so-called intradiol dioxygenase
350 (INDO) catabolic gene family. None of those genes were detected in the microarray
351 according to 6 fold-change criterion, suggesting the absence or low expression level of
352 those genes in strain DSM 45259 under our assay conditions. Indeed, the DNA material
353 used for microarray hybridization was obtained from cells harvested during late
354 exponential growth phase in cultures containing glucose and naphtalene as carbon
355 sources, where those genes may be expressed at low level. This is in agreement with
356 cultivation, activity tests and mass spectrometry experiments (see below) that confirmed
357 that strain DSM 45259 contains 3,4-PCD and Cat activities when grown on phthalate
358 and benzoate (see below), and that in the absence of these substrates expression level of
359 those genes may be most likely low. This is not the case of the genes encoding catechol
360 1,2-dioxygenase whose presence in the genome of strain DSM 45259 could not be
361 confirmed both by array and cultivation tests (see below).

362 **Experimental validation by cultivation and mass spectrometry**

363 To prove the correctness of the predictions and to discard that the predictions are an
364 artifact derived from an inaccurate hybridization, experimental validation assays were
365 conducted. For that, cultures were set up with C₁₀ and C₁₄ alkanes (*n*-decane and *n*-
366 tetradecane), and the aromatics phthalate, phenol and benzoate as the only carbon
367 source, and after 0, 24, 48 and 72 h cultivation we examined the efficiency of strain
368 DSM 45259 to degrade them. A concentration of 500 mg L⁻¹ of each compound was
369 used. Target analysis by Liquid Chromatography-Mass Spectrometry (LC-MS) was
370 further used to confirm the consumption of the initial substrates and the formation of
371 key degradation intermediates in test cultures as compared to the abiotic (culture media
372 containing aromatics but no cells) and biotic (culture without the aromatics) control
373 cultures.

374 The level of degradation of *n*-decane and *n*-tetradecane could not be obtained as
375 both chemicals could not be detected under our analytical platform. However, *n*-
376 decanoic (in *n*-decane microcosm) and *n*-tetradecanoic acid (in the *n*-tetradecane
377 microcosm) were detected at high level (Table 2), demonstrating that the degradation of
378 both alkanes by strain DSM 45259 occurred. Degradation of phthalate, phenol and
379 benzoate was achieved at 49.7, 89.1 and 57.6%, respectively, at the end of the 72 h
380 assay. This was shown by measuring the remaining amount of these 3 compounds
381 (Table 2). Degradation was further demonstrated by identifying the increasing
382 abundance level of the phthalic-degradation products protocatechuic acid and 3-
383 oxoadipic acid (in phthalate microcosm) and the phenol- and benzoate-degradation
384 product catechol (in phenol and benzoate microcosms) during the follow-up assay
385 (Table 2). The identification of 3-oxoadipic acid in the cultures grown with phthalate in
386 combination to the identification of a gene encoding a phthalate 4,5-dioxygenase in the
387 microarray demonstrate that catabolism of phthalate proceeds via the proto-catechuic

388 *ortho* cleavage pathway in which a protocatechuate 3,4-dioxygenase may be implicated
389 (Fig. 1). In case of catechol degradation, no intermediates were detected above the
390 detection limit by LC-MS, possibly because they are rapidly converted and thus
391 accumulated at low level under cultivation conditions. However, the demonstration of
392 catechol 2,3-dioxygenase activity in protein extracts from cells obtained in cultures
393 grown on benzoate and phenol in MM broth (see Materials and Methods section)
394 revealed that the catechol *meta*-ring cleavage branch is fully operative in DSM 45259
395 (Fig. 1). Indeed, activity values of 0.86 ± 0.07 and 1.18 ± 0.05 unit mg^{-1} protein were
396 obtained under our experimental assay conditions.

397 Taken together, as shown in Table 2, signatures for the degradation of the 5
398 chemicals predicted as being used as carbon sources (Fig. 1) were experimentally found
399 (Table 2), thus confirming a total agreement with our predictions.

400 Discussion

401 In this report, we described new insights into the degradation capacities of the copper-
402 resistant actinomycete *A. tucumanensis* DSM 45259 using microarray data. Our
403 approach was based on the utilization of the catabolome microarray presented by
404 Vilchez-Vargas et al. (2013). However, we adapt the output of the microarray data to
405 incorporate a prediction tool based on the utilization of the web-based AromaDeg
406 resource (Duarte et al. 2014), and identify unambiguously genes encoding catabolic
407 proteins of this microorganism. Further, with cultivation and metabolomics approaches
408 being developed, we provided experimental validation. Based on the microarray and
409 experimental data presented we unambiguously identified that *A. tucumanensis* DSM
410 45259 has the ability to use alkanes (i.e., *n*-decane and *n*-tetradecane), phthalate, phenol
411 and benzoate as sole carbon sources. Degradation occurred in the absence of glucose as

412 co-substrate that was previously reported to be required for the degradation of
413 naphthalene and phenanthrene (Bourguignon et al. 2014).

414 Actinomycetes possess potent capacities to metabolize aliphatic and aromatic toxic
415 hydrocarbons. Species of the genera *Mycobacterium*, *Streptomyces* and *Nocardia* (that
416 contain genera such as *Gordonia* and *Rhodococcus*), commonly found in contaminated
417 soils, are the best characterized members. Thus, by using cultivation, phylogenetic,
418 phenotypic, and/or genomic information approaches, members of these genera have
419 been shown to use as sole carbon and energy sources, to different extend, a wide range
420 of compounds. They include, crude oil, diesel oil, rapeseed oil, linear and branched
421 medium-to-long chain alkanes (up to C₃₆), alkenes, haloalkanes, monocyclic aromatic
422 compounds (benzoate, catechol, gentisate, salicylate, phenol, phenylethanol, thymol,
423 alkylbenzenes, xylene, toluene, phthalate) and poly-aromatic compounds (biphenyl,
424 naphthalene, anthracene, fluoranthrene, phenanthrene, coronene, pyrene, chrysene,
425 naphthacene, acenaphthene, benzo[a]pyrene, 7,12-dimethylbenz[a]anthracene), as well as
426 organic sulfur compounds (i.e. benzothiophene and dibenzothiophene) and nitro-
427 aromatics (Kelley and Cerniglia, 1995; Lloyd-Jones and Hunter, 1997; Bastiaens et al.
428 2000; Monticello, 2000; Stingley et al. 2004; Kumar et al. 2006; Larkin et al. 2006;
429 Zeinali et al. 2007; Yang et al. 2011; Balachandran et al. 2012; Fathepure, 2014; Luo et
430 al. 2014; Sudhir et al. 2014). In some case biodegradation was only observed when
431 growing on glucose as co-substrate (Pizzul et al. 2006). Members of the genus
432 *Arthrobacter* and *Streptomyces* can also degrade halogenated pesticides (Bourguignon
433 et al. 2014). *Terrabacter* isolates have been also shown to degrade dibenzofuran and the
434 heterocyclic nitrogen compound carbazole (Iida et al. 2002). Therefore, we can
435 conclude that the degrading capacities herein reported for *A. tucumanensis* DSM 45259
436 are within common abilities for other actinomycetes.

437 A solid basis of genomic understanding on degradation capacities of actinomycetes
438 has been mainly established, particularly for *Mycobacterium* and *Nocardia* isolates.
439 Thus, genome analysis of *Mycobacteria* isolates has contributed to the characterization
440 of key enzymes such as the initial ring-hydroxylating dioxygenases participating in the
441 degradation of substrates such as biphenyl, naphthalene, anthracene, fluoranthene,
442 pyrene, phenanthrene, phthalate and benzoate (Brezna et al. 2003; Stingley et al. 2004;
443 Kim et al. 2006; Kim et al. 2007; Kim et al. 2008; Kallimanis et al. 2011; Zhang et al.
444 2012; Kwak et al. 2014). Genome sequence of *Rhodococcus* strains revealed they
445 contain not only multiple alkane hydroxylase genes (*alkB*) and from 27 to 73
446 cytochrome P450 monooxygenases and other catabolic genes predicted to be involved
447 in the metabolism of alkanes and nitroalkanes, as well as an array of cyclic ketones,
448 halogenated aromatics and aromatic hydrocarbons (e.g., benzoate, catechol, gentisate,
449 salicylate, homogentisate, naphthalene, phenanthrene, anthracene, and benzo[a]pyrene)
450 (McLeod et al. 2006; Chen et al. 2013; Pathak et al. 2013; Zhang et al. 2014; Lincoln et
451 al. 2015; Qu et al. 2015). In addition to that, phylogenetic, phenotypic, and genomic
452 information for 27 completely genome-sequenced mycobacteria revealed a total of 9532
453 genes conforming the so-called “PAH-degrading” node, of which 3533 genes belong to
454 the core-genome that is present in each strain and 5999 genes belong to the dispensable
455 genome that is absent in one or more strains (Kweon et al., 2015). Among the 3533 core
456 genes, only 136 common genes were tentatively identified to be involved in the
457 degradation of aromatic hydrocarbons, which indicate the high variability of gene
458 sequences and degradation abilities within isolates. Some of these common genes, such
459 as the ones needed for pyrene degradation, have been demonstrated to be acquired by
460 horizontal transfer (DeBruyn et al. 2012). This high genomic variability was also
461 supported by the present study which suggests that at least 3 genes from strain DSM

462 45259 (those encoding Bzt, Pht and phenol hydroxylase) are quite divergent (<80%
463 sequence identity) to those of other actinomycetes. This was suggested as no
464 hybridization signals of such genes were found with any actinomycete-probes of the
465 same subfamily present in the microarray, while hybridizing with those from
466 Proteobacteria.

467 In conclusion, we report here new insights into the catabolic abilities of the first
468 member of the *Amycolatopsis* genus and identify a variety of genomic signatures which
469 seems to be uncommon within actinomycetes. This work also contributed to deepening
470 into the degradation capacities of actinomycetes, whose knowledge is mostly limited for
471 *Mycobacterium* and *Nocardia* isolates. Note that the genus *Amycolatopsis* has been
472 classified in the family Pseudonocardiaceae and it currently contains 39 species with
473 validly published names (<http://www.bacterio.cict.fr/a/amycolatopsis.html>). Recent
474 studies indicate that the chemotaxonomic characteristics of this genus, which relate to,
475 but differentiate from *Streptomyces* and *Nocardia*, are intrinsically determined by the
476 molecular phylogeny of their encoding genes (Xu et al. 2014). Its closest relatives are
477 *Amycolatopsis* sp. ATCC 39116, *A. methanolica* 239 and *A. thermoflava* N1165.
478 *Amycolatopsis* sp. ATCC 39116 (previously known as *S. setonii*) harbors genes
479 encoding canonical pathways for catabolism of catechol, benzoate, protocatechuate,
480 phenylacetate, and methylated aromatic compound (Davis et al. 2012). *A. methanolica*
481 239 (previously known as *Streptomyces* sp. strain 239 or as *Nocardia* sp. strain 239) can
482 grow in mineral medium broth containing methanol, ethanol, 1-propanol, 1-butanol,
483 2,3-butanediol, acetone, benzoic acid methylester, benzylamine, 3- and 4-
484 hydroxybenzoates, 3,4-dihydroxybenzoate, phenylacetate, phenylacetaldehyde,
485 phenyllactate, phenylpyruvate, 4-hydroxyphenylacetate, 4-hydroxyphenylpyruvate, D-
486 phenylalanine, gentisate, and homogentisate as sole sources of carbon. Also, it contains

487 degradation pathways for benzoate, fluorobenzoate, toluene, xylene, styrene,
488 naphthalene and other related polycyclic aromatic hydrocarbons (Wattam et al. 2014).
489 *A. thermoflava* N1165 has been shown to degrade atrazine, naphthalene, anthracene,
490 tetrachloroethene, 1- and 2-methylnaphthalene, 2,4-dichlorobenzoate, toluene, xylene,
491 biphenyl, hexachlorocyclohexane, trinitrotoluene, ethylbenzene, and styrene (Chun et
492 al. 1999).

493 Finally, cultivation and mass spectrometry evidences are provided that
494 demonstrated that the catabolome array can aid in the understanding of degrading
495 capacities without previous genome, and possibly metagenome, sequence knowledge.

496 **Acknowledgement** This work was supported by the CSIC (Programa CSIC de Cooperación
497 Científica para el Desarrollo i-COOP; ref. COOPB20077), the European Community project
498 KILLSPILL (FP7-KBBE-2012-312139), and the Spanish Ministry of Economy and
499 Competitiveness (CTQ2014-55279-R). N.B. is a recipient of a fellowship from the Consejo
500 Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina. We thank Drs.
501 Dietmar H. Pieper and Ramiro Vilchez-Vargas for their support in providing resources for this
502 study.

503 **Compliance with ethical standards**

504 **Conflict of interest** All authors declare that they have no conflict of interest.

505 **Human and animal rights** This article does not contain any studies with human participants or
506 animals performed by any of the authors.

507 **References**

508 Albarracín VH, Alonso-Vega P, Trujillo ME, Amoroso MJ, Abate CM (2010a) *Amycolatopsis*
509 *tucumanensis* sp. nov., a copper-resistant actinobacterium isolated from polluted sediments.
510 Int J Syst Evol Microbiol 60(2):397-401.

511 Albarracín VH, Amoroso MJ, Abate CM (2010b) Bioaugmentation of copper polluted soil
512 microcosms with *Amycolatopsis tucumanensis* to a copper for *Zea mays* plants.
513 *Chemosphere* 79(2):131-137.

514 Alcaide M, Tornés J, Stogios PJ, Xu X, Gertler C, Di Leo R, Bargiela R, Lafraya A, Guazzaroni
515 ME, López-Cortés N, Chernikova TN, Golyshina OV, Nechitaylo TY, Plumeier I, Pieper
516 DH, Yakimov MM, Savchenko A, Golyshin PN, Ferrer M (2013) Single residues dictate
517 the co-evolution of dual esterases: MCP hydrolases from the α/β hydrolase family.
518 *Biochem J* 454(1):157-166.

519 Bailey MJ, Lilley AK, Thompson IP, Rainey PB, Ellis RJ (1995) Site directed chromosomal
520 marking of a fluorescent pseudomonad isolated from the phytosphere of sugar beet;
521 stability and potential for marker gene transfer. *Mol Ecol* 4(6):755-763.

522 Balachandran C, Duraipandiyar V, Balakrishna K, Ignacimuthu S (2012) Petroleum and
523 polycyclic aromatic hydrocarbons (PAHs) degradation and naphthalene metabolism in
524 *Streptomyces* sp. (ERI-CPDA-1) isolated from oil contaminated soil. *Bioresour Technol*
525 112:83-90.

526 Bargiela R, Gertler C, Magagnini M, Mapelli F, Chen J, Daffonchio D, Golyshin PN, Ferrer M
527 (2015) Degradation network reconstruction in uric acid and ammonium amendments in oil-
528 degrading marine microcosms guided by metagenomic data. *Front Microbiol* 6:1270.

529 Bargiela R, Mapelli F, Rojo D, Chouaia B, Tornés J, Borin S, Richter M, Del Pozo MV,
530 Cappello S, Gertler C, Genovese M, Denaro R, Martínez-Martínez M, Fodelianakis S,
531 Amer RA, Bigazzi D, Han X, Chen J, Chernikova TN, Golyshina OV, Mahjoubi M,
532 Jaouanil A, Benzha F, Magagnini M, Hussein E, Al-Horani F, Cherif A, Blaghen M,
533 Abdel-Fattah YR, Kalogerakis N, Barbas C, Malkawi HI, Golyshin PN, Yakimov MM,
534 Daffonchio D, Ferrer M (2015) Bacterial population and biodegradation potential in
535 chronically crude oil-contaminated marine sediments are strongly linked to temperature.
536 *Sci Rep* 5:11651.

537 Bastiaens L, Springael D, Wattiau P, Harms H, deWachter R, Verachtert H, Diels L (2000)
538 Isolation of adherent polycyclic aromatic hydrocarbon (PAH)-degrading bacteria using
539 PAH-sorbing carriers. *Appl Environ Microbiol* 66(5):1834-1843.

540 Beazley MJ, Martinez RJ, Rajan S, Powell J, Piceno YM, Tom LM, Andersen GL, Hazen TC,
541 Van Nostrand JD, Zhou J, Mortazavi B, Sobecky PA (2012) Microbial community analysis
542 of a coastal salt marsh affected by the Deepwater Horizon oil spill. *PLoS One* 7:e41305.

543 Bourguignon N, Fuentes MS, Benimeli CS, Cuozzo SA, Amoroso MJ (2014) Aerobic removal
544 of methoxychlor by a native *Streptomyces* strain: Identification of intermediate metabolites.
545 *Int Biodeter Biodegr* 96:80-86.

546 Bourguignon N, Isaac P, Alvarez H, Amoroso MJ, Ferrero MA (2014) Enhanced polyaromatic
547 hydrocarbon degradation by adapted cultures of actinomycete strains. *J Basic Microbiol*
548 54(12):1288-1294.

549 Brezna B, Khan AA, Cerniglia CE (2003) Molecular characterization of dioxygenases from
550 polycyclic aromatic hydrocarbon-degrading *Mycobacterium* spp. *FEMS Microbiol Lett*
551 223(2):177-183.

552 Chen BS, Otten LG1, Resch V1, Muyzer G2, Hanefeld U1 (2013) Draft genome sequence of
553 *Rhodococcus rhodochrous* strain ATCC 17895. *Stand Genomic Sci* 9(1):175-184.

554 Chun J, Kim SB, Oh YK, Seong CN, Lee DH, Bae KS, Lee KJ, Kang SO, Hah YC, Goodfellow
555 M (1999) *Amycolatopsis thermoflava* sp. nov., a novel soil actinomycete from Hainan
556 Island, China. *Int J Syst Bacteriol* 49:1369-1373.

557 Colin VL, Castro MF, Amoroso MJ, Villegas LB (2013) Production of bioemulsifiers by
558 *Amycolatopsis tucumanensis* DSM 45259 and their potential application in remediation
559 technologies for soils contaminated with hexavalent chromium. *J Hazard Mater* 261:577-
560 583.

561 Dávila Costa JS, Albarracín VH, Abate CM (2011a) Cupric reductase activity in copper-
562 resistant *Amycolatopsis tucumanensis*. *Water Air Soil Pollut* 216:527-535.

563 Dávila Costa JS, Albarracín VH, Abate CM (2011b) Responses of environmental *Amycolatopsis*
564 strains to copper stress. *Ecotoxicol Environ Saf* 74(7):2020-2028.

565 Dávila Costa JS, Kothe E, Abate CM, Amoroso MJ (2012) Unraveling the *Amycolatopsis*
566 *tucumanensis* copper-resistome. *Biometals* 25(5):905-917.

567 Davis JR, Goodwin LA, Woyke T, Teshima H, Bruce D, Detter C, Tapia R, Han S, Han J,
568 Pitluck S, Nolan M, Mikhailova N, Land ML, Sello JK (2012) Genome sequence of
569 *Amycolatopsis* sp. strain ATCC 39116, a plant biomass-degrading actinomycete. *J*
570 *Bacteriol* 194(9):2396-2397.

571 DeBruyn JM, Mead TJ, Sayler GS. Horizontal transfer of PAH catabolism genes in
572 *Mycobacterium*: evidence from comparative genomics and isolated pyrene-degrading
573 bacteria. *Environ Sci Technol* 46(1):99-106.

574 Duarte M, Jauregui R, Vilchez-Vargas R, Junca H, Pieper DH (2014) AromaDeg, a novel
575 database for phylogenomics of aerobic bacterial degradation of aromatics. *Database*
576 (Oxford) 2014:bau118.

577 Fathepure BZ (2014) Recent studies in microbial degradation of petroleum hydrocarbons in
578 hypersaline environments. *Front Microbiol* 5:173.

579 Guazzaroni M-E, Herbst FA, Lores I, Tamames J, Peláez AI, López-Cortés N, Alcaide M, Del
580 Pozo MV, Vieites JM, von Bergen M, Gallego JL, Bargiela R, López-López A, Pieper DH,
581 Rosselló-Móra R, Sánchez J, Seifert J, Ferrer M (2013) Metaproteogenomic insights
582 beyond bacterial response to naphthalene exposure and bio-stimulation. *ISME J* 7(1):122-
583 136.

584 Gutierrez T, Singleton DR, Berry D, Yang T, Aitken MD, Teske A (2013) Hydrocarbon-
585 degrading bacteria enriched by the Deepwater Horizon oil spill identified by cultivation
586 and DNA-SIP. ISME J 7(11):2091-2104.

587 Hamzah RY, Al-Baharna BS (1994) Catechol ring-cleavage in *Pseudomonas cepacia*: the
588 simultaneous induction of ortho and meta pathways. Appl Microbiol Biotechnol 41(2):250-
589 256.

590 Hazen TC, Dubinsky EA, DeSantis TZ, Andersen GL, Piceno YM, Singh N, Jansson JK, Probst
591 A, Borglin SE, Fortney JL, Stringfellow WT, Bill M, Conrad ME, Tom LM, Chavarria KL,
592 Alusi TR, Lamendella R, Joyner DC, Spier C, Baelum J, Auer M, Zemla ML, Chakraborty
593 R, Sonnenthal EL, D'haeseleer P, Holman HY, Osman S, Lu Z, Van Nostrand JD, Deng Y,
594 Zhou J, Mason OU (2010) Deep-sea oil plume enriches indigenous oil-degrading bacteria.
595 Science 330(6001):204-208.

596 Iida T, Mukouzaka Y, Nakamura K, Kudo T (2002) Plasmid-borne genes code for an angular
597 dioxygenase involved in dibenzofuran degradation by *Terrabacter* sp. strain YK3. Appl
598 Environ Microbiol 68(8):3716-3723.

599 Kallimanis A, Karabika E, Mavromatis K, Lapidus A, Labutti KM, Liolios K, Ivanova N,
600 Goodwin L, Woyke T, Velentzas AD, Perisynakis A, Ouzounis CC, Kyripides NC,
601 Koukkou AI, Drainas C (2011) Complete genome sequence of *Mycobacterium* sp. strain
602 (Spyr1) and reclassification to *Mycobacterium gilvum* Spyr1. Stand Genomic Sci 5(1):144-
603 153.

604 Kelley I, Cerniglia CE (1995) Degradation of a mixture of high-molecular-weight polycyclic
605 aromatic hydrocarbons by a *Mycobacterium* strain PYR-1. J Soil Contam 4(1):77-91.

606 Kim SJ, Kweon O, Freeman JP, Jones RC, Adjei MD, Jhoo JW, Edmondson RD, Cerniglia CE
607 (2006) Molecular cloning and expression of genes encoding a novel dioxygenase involved

608 in low- and high-molecular-weight polycyclic aromatic hydrocarbon degradation in
609 *Mycobacterium vanbaalenii* PYR-1. Appl Environ Microbiol 72(2):1045-1054.

610 Kim SJ, Kweon O, Jones RC, Edmondson RD, Cerniglia CE (2008) Genomic analysis of
611 polycyclic aromatic hydrocarbon degradation in *Mycobacterium vanbaalenii* PYR-1.
612 Biodegradation 19(6):859-881.

613 Kim SJ, Kweon O, Jones RC, Freeman JP, Edmondson RD, Cerniglia CE (2007) Complete and
614 integrated pyrene degradation pathway in *Mycobacterium vanbaalenii* PYR-1 based on
615 systems biology. J Bacteriol 189(2):464-472.

616 Kimes NE, Callaghan AV, Aktas DF, Smith WL, Sunner J, Golding B, Drozdowska M, Hazen
617 TC, Suflita JM, Morris PJ (2013) Metagenomic analysis and metabolite profiling of deep-
618 sea sediments from the Gulf of Mexico following the Deepwater Horizon oil spill. Front
619 Microbiol 4:50.

620 Kostka JE, Prakash O, Overholt WA, Green SJ, Freyer G, Canion A, Delgardio J, Norton N,
621 Hazen TC, Huettel M (2011) Hydrocarbon-degrading bacteria and the bacterial community
622 response in gulf of Mexico beach sands impacted by the deepwater horizon oil spill. Appl
623 Environ Microbiol 77(22):7962-7974.

624 Kubota M, Nodate M, Yasumoto-Hirose M, Uchiyama T, Kagami O, Shizuri Y, Misawa N
625 (2005) Isolation and functional analysis of cytochrome P450 CYP153A genes from various
626 environments. Biosci Biotechnol Biochem 69(12):2421-2430.

627 Kwak Y, Park GS1, Lee SE1, Li QX2, Shin JH3 (2014) Genome sequence of *Mycobacterium*
628 *aromaticivorans* JS19b1(T), a novel isolate from Hawaiian soil. J Biotechnol 186:137-138.

629 Kweon O, Kim SJ, Blom J, Kim SK, Kim BS, Baek DH, Park SI, Sutherland JB, Cerniglia CE
630 (2015) Comparative functional pan-genome analyses to build connections between
631 genomic dynamics and phenotypic evolution in polycyclic aromatic hydrocarbon
632 metabolism in the genus *Mycobacterium*. BMC Evol Biol 15:21.

633 Larkin MJ, Kulakov LA, Allen CC (2006) Biodegradation by members of the genus
634 *Rhodococcus*: biochemistry, physiology, and genetic adaptation. *Adv Appl Microbiol* 59:1-
635 29.

636 Liang Y, Van Nostrand JD, Deng Y, He Z, Wu L, Zhang X, Li G, Zhou J (2011) Functional
637 gene diversity of soil microbial communities from five oil-contaminated fields in China.
638 *ISME J* 5(3):403-413.

639 Lincoln SA, Hamilton TL, Valladares Juárez AG, Schedler M, Macalady JL, Müller R, Freeman
640 KH (2015) Draft genome sequence of the piezotolerant and crude oil-degrading bacterium
641 *Rhodococcus qingshengii* strain TUHH-12. *Genome Announc* 3(2) pii: e00268-15.

642 Lloyd-Jones G, Hunter DW (1997) Characterization of fluoranthene- and pyrene-degrading
643 *Mycobacterium*-like strains by RAPD and SSU sequencing. *FEMS Microbiol Lett*
644 153(1):51-56.

645 Lu Z, Deng Y, Van Nostrand JD, He Z, Voordeckers J, Zhou A, Lee YJ, Mason OU, Dubinsky
646 EA, Chavarria KL, Tom LM, Fortney JL, Lamendella R, Jansson JK, D'haeseleer P, Hazen
647 TC, Zhou J (2012) Microbial gene functions enriched in the Deepwater Horizon deep-sea
648 oil plume. *ISME J* 6(2):451-460.

649 Luo Q, Hiessl S, Steinbüchel A (2014) Functional diversity of *Nocardia* in metabolism. *Environ*
650 *Microbiol* 16(1):29-48.

651 Mason OU, Hazen TC, Borglin S, Chain PS, Dubinsky EA, Fortney JL, Han J, Holman HY,
652 Hultman J, Lamendella R, Mackelprang R, Malfatti S, Tom LM, Tringe SG, Woyke T,
653 Zhou J, Rubin EM, Jansson JK (2012) Metagenome, metatranscriptome and single-cell
654 sequencing reveal microbial response to Deepwater Horizon oil spill. *ISME J* 6(9):1715-
655 1727.

656 Mason OU, Scott NM, Gonzalez A, Robbins-Pianka A, Bælum J, Kimbrel J, Bouskill NJ,
657 Prestat E, Borglin S, Joyner DC, Fortney JL, Jurelevicius D, Stringfellow WT, Alvarez-

658 Cohen L, Hazen TC, Knight R, Gilbert JA, Jansson JK (2014) Metagenomics reveals
659 sediment microbial community response to Deepwater Horizon oil spill. ISME J 8(7):1464-
660 75.

661 McLeod MP1, Warren RL, Hsiao WW, Araki N, Myhre M, Fernandes C, Miyazawa D, Wong
662 W, Lillquist AL, Wang D, Dosanjh M, Hara H, Petrescu A, Morin RD, Yang G, Stott JM,
663 Schein JE, Shin H, Smailus D, Siddiqui AS, Marra MA, Jones SJ, Holt R, Brinkman FS,
664 Miyauchi K, Fukuda M, Davies JE, Mohn WW, Eltis LD (2006) The complete genome of
665 *Rhodococcus* sp. RHA1 provides insights into a catabolic powerhouse. Proc Natl Acad Sci
666 USA 103(42):15582-7.

667 Monticello DJ (2000) Biodesulfurization and the upgrading of petroleum distillates. Curr Opin
668 Biotechnol 11(6):540-6.

669 Nozaki M, Kagamiyama H, Hayaishi O (1963) Metapyrocatechase. I. Purification,
670 crystallization and some properties. Biochemische Z 338:582-590.

671 Pandey J, Chauhan A, Jain RK (2009) Integrative approaches for assessing the ecological
672 sustainability of in situ bioremediation. FEMS Microbiol Rev 33(2):324-375.

673 Pathak A, Green SJ, Ogram A, Chauhan A (2013) Draft genome sequence of *Rhodococcus*
674 *opacus* strain M213 shows a diverse catabolic potential. Genome Announc 1(1) pii:
675 e00144-12.

676 Pérez-Pantoja D, De la Iglesia R, Pieper DH, González B (2008) Metabolic reconstruction of
677 aromatic compounds degradation from the genome of the amazing pollutant-degrading
678 bacterium *Cupriavidus necator* JMP134. FEMS Microbiol Rev 32(5):736-794.

679 Pérez-Pantoja D, Donoso R, Agulló L, Córdova M, Seeger M, Pieper DH, González B (2012)
680 Genomic analysis of the potential for aromatic compounds biodegradation in
681 Burkholderiales. Environ Microbiol 14(5):1091-1117.

682 Pizzul L, Castillo MP, Strenström J (2006) Characterization of selected actinomycetes
683 degrading polyaromatics hydrocarbons in liquid culture and spiked soil. *World J Microbiol*
684 *Biotechnol* 22(7):745-752.

685 Qu J, Miao LL, Liu Y, Liu ZP (2015) Complete genome sequence of *Rhodococcus* sp. Strain
686 IcdP1 shows diverse catabolic potential. *Genome Announc* 3(4) pii: e00711-15.

687 Sekine M., S. Tanikawa, S. Omata, M. Saito, T. Fujisawa, N. Tsukatani, T. Tajima, T.
688 Sekigawa, Kosugi H, Matsuo Y, Nishiko R, Imamura K, Ito M, Narita H, Tago S, Fujita N,
689 Harayama S (2006) Sequence analysis of three plasmids harboured in *Rhodococcus*
690 *erythropolis* strain PR4. *Environ Microbiol* 8(2):334–346.

691 Seo JS, Keum YS, Li QX (2009) Bacterial degradation of aromatic compounds. *Int J Environ*
692 *Res Public Health* 6(1):278-309.

693 Seo JS, Keum YS, Li QX (2012) *Mycobacterium aromativorans* JS19b1(T) degrades
694 phenanthrene through C-1,2, C-3,4 and C-9,10 dioxygenation pathways. *Int Biodeterior*
695 *Biodegradation* 70:96-103.

696 Shen FT, Lin JL, Huang CC, Ho YN, Arun AB, Young LS, Young CC. Shen FT, Lin JL, Huang
697 CC, Ho YN, Arun AB, Young LS, Young CC (2009) Molecular detection and phylogenetic
698 analysis of the catechol 1,2-dioxygenase gene from *Gordonia* spp. *Syst Appl Microbiol*
699 32(5):291-300.

700 Stingley RL, Brezna B, Khan AA, Cerniglia CE (2004) Novel organization of genes in a
701 phthalate degradation operon of *Mycobacterium vanbaalenii* PYR-1. *Microbiology*
702 150(11):3749-3761.

703 Sudhir KS, Hai G, Modi DR, Joyotsana KP (2014) Growth potential assessment of
704 actinomycetes isolated from petroleum contaminated soil. *J Bioremed Biodeg* 5:7

705 van Beilen J.B., R. Holtackers, D. Luscher, U. Bauer, B. Witholt, W.A. Duetz (2005)
706 Biocatalytic production of perillyl alcohol from limonene by using a novel *Mycobacterium*

707 sp. cytochrome P450 alkane hydroxylase expressed in *Pseudomonas putida*. Appl Environ
708 Microbiol 71(4):1737–1744.

709 van Beilen JB, Funhoff EG, van Loon A, Just A, Kaysser L, Bouza M, Holtackers R,
710 Röthlisberger M, Li Z, Witholt B (2006) Cytochrome P450 alkane hydroxylases of the
711 CYP153 family are common in alkane-degrading eubacteria lacking integral membrane
712 alkane hydroxylases. Appl Environ Microbiol 72(1):59-65.

713 Vila J, López Z, Sabaté J, Minguillón C, Solanas AM, Grifoll M (2001) Identification of a novel
714 metabolite in the degradation of pyrene by *Mycobacterium* sp. strain AP1: actions of the
715 isolate on two- and three-ring polycyclic aromatic hydrocarbons. Appl Environ Microbiol
716 67(12):5497-5505.

717 Vilchez-Vargas R, Geffers R, Suárez-Diez M, Conte I, Waliczek A, Kaser VS, Kralova M,
718 Junca H, Pieper DH (2013) Analysis of the microbial gene landscape and transcriptome for
719 aromatic pollutants and alkane degradation using a novel internally calibrated microarray
720 system. Environ Microbiol 15(4):1016-1039.

721 Watanabe K, Hamamura N (2003) Molecular and physiological approaches to understanding the
722 ecology of pollutant degradation. Curr Opin Biotechnol 14(3):289-295.

723 Wattam AR, Abraham D, Dalay O, Disz TL, Driscoll T, Gabbard JL, Gillespie JJ, Gough R,
724 Hix D, Kenyon R, Machi D, Mao C, Nordberg EK, Olson R, Overbeek R, Pusch GD,
725 Shukla M, Schulman J, Stevens RL, Sullivan DE, Vonstein V, Warren A, Will R, Wilson
726 MJC, Seung Yoo H, Zhang C, Zhang Y, Sobral BW (2014). PATRIC, the bacterial
727 bioinformatics database and analysis resource. Nucl Acids Res 42 (D1): D581-D59.

728 Whyte LG, Smits TH, Labbé D, Witholt B, Greer CW, van Beilen JB (2002) Gene cloning and
729 characterization of multiple alkane hydroxylase systems in *Rhodococcus* strains Q15 and
730 NRRL B-16531. Appl Environ Microbiol 68(12):5933-5942.

731 Xu L, Huang H, Wei W, Zhong Y, Tang B, Yuan H, Zhu L, Huang W, Ge M, Yang S, Zheng
732 H, Jiang W, Chen D, Zhao GP, Zhao W (2014) Complete genome sequence and
733 comparative genomic analyses of the vancomycin-producing *Amycolatopsis orientalis*.
734 BMC Genomics 15:363.

735 Yang X, Rui Xue, Chong Shen, Shuren Li, Chong Gao, Qi Wang, Xiaoxia Zhao (2011) Genome
736 sequence of *Rhodococcus* sp. strain R04, a polychlorinated-biphenyl biodegrader. J
737 Bacteriol 193(18):5032-5033.

738 Zeinali M, Vossoughi M, Ardestani SK (2007) Characterization of a moderate thermophilic
739 *Nocardia* species able to grow on polycyclic aromatic hydrocarbons. Lett Appl Microbiol
740 45(6):622-628.

741 Zhang C, Anderson AJ (2012) Polycyclic aromatic hydrocarbon degrading gene islands in five
742 pyrene-degrading *Mycobacterium* isolates from different geographic locations Can J
743 Microbiol 58(1):102-111.

744 Zhang Y, Qin F, Qiao J, Li G, Shen C, Huang T, Hu Z (2012) Draft genome sequence of
745 *Rhodococcus* sp. strain P14, a biodegrader of high-molecular-weight polycyclic aromatic
746 hydrocarbons. J Bacteriol 194(13):3546.

747 Zhao K, Guo X, Gong J (2013) A novel benzoate-degrading *Rhodococcus* strain contains three
748 *catA* genes with one being transcriptionally active during the growth on benzoate. J
749 Environ Biol 34(2):401-407.

Table 1 List of probes associated to the 4 catabolic genes families that were found to be targeted by the microarray in *A. tucumanensis* DSM 45259.

Probe ID	Fluorescence (signal-background) ¹			Fold Change ¹	AromaDeg annotation ²
ALKB_AJ833983	5360	6022	5757	43,634	Alkane hydroxylase (AlkB)
ALKB_AJ833926	2655	2781	2220	21,416	
RHDO_AAD17377	1968	3694	3733	20,655	Benzoate dioxygenase (Bzt)
RHDO_AAD03558	361	772	1433	6,302	Phthalate 4,5-dioxygenase (Pht)
RHMO_Z36909	6173	6054	5585	44,98	Phenol hydroxylase (PH)

¹Signal corresponding to triplicates with standard deviation shown in Supplementary Table S1. Average (for triplicates) fold change of probe signal compared to background signal in the microarray is shown.

²AromaDeg-based annotations obtained when each query sequence from probes targeting catabolic genes for which a positive signal was obtained in the microarray was submitted to web-based AromaDeg resource (Duarte et al. 2014).

Table 2 List of chemical signatures of key metabolites known to participate in the degradation of alkanes and aromatics in culture and control microcosms.

	Abundance (a.u.) ^{1,2}			
	0 h	24 h	48 h	72 h
<i>n</i> -Decanoic acid ³	0	2071359	2736496	4518090
<i>n</i> -Tetradecanoic acid ⁴	0	2458412	3202831	4382836
Phthalic acid ⁵	79095538	64001448	52624286	39758652
Protocatechuic acid ⁵	0	0	770651	1112138
3-Oxadipic acid ⁵	4110	26955989	40657623	25657381
Phenol ⁶	862601	498098	196556	93768
Catechol ⁶	165585	60114743	71964381	98848577
Benzoate ⁷	1874035	1376927	1106361	794551
Catechol ⁷	532343	34588533	49513068	58615247

¹Abundance (in arbitrary units) was calculated (in triplicates) as the area of the peak (calculated on the basis of *m/z* and/or standards) of chemicals determined by LC-MS (positive [+] and negative [-] polarities) in cultures containing the selected pollutants. Strain DSM 45259 was cultivated on minimal mineral medium with *n*-decane, phenol, benzoate and phthalate (500 mg L⁻¹) as the sole source of carbon and energy, at 30 °C and 180 rpm during 72 h. Quantification of the biodegradation was further performed by extraction and target analysis of the substrates by LC-MS. Detailed conditions for cultivation and analytics are given in Materials and Methods section. ²Abundance levels for biotic and abiotic controls were considered for background corrections. ³Abundance levels of the initial pollutant and degradation intermediates in cultures with *n*-decane. ⁴Abundance levels of the initial pollutant and degradation intermediates in cultures with *n*-tetradecane. ⁵Abundance levels of the initial pollutant and degradation intermediates in cultures with phthalic acid; the presence of small amount of oxoadipic acid may be due to the presence of small amount of cells added at the beginning of the assay (see Materials and Methods section for details). ⁶Abundance levels of the initial pollutant and degradation intermediates in cultures with phenol. ⁷Abundance levels of the initial pollutant and degradation intermediates in cultures with benzoate.

Figure legends

Figure 1 Potential alkane and aromatic catabolic abilities of *A. tucumanensis* DSM 45259 guided by microarray data. Solid lines represent single step reactions while dotted lines represent degradation steps where multiple reactions are involved. Enzyme codes as follows: Alkane hydroxylase (AlkB); Benzoate dioxygenase (Bzt); Phthalate 4,5-dioxygenase (Pht); Phenol hydroxylase (PH). Red color names indicated enzymes encoded by genes targeting probes in the catabolome microarray, whereas those with blue color indicates those whose presence was unambiguously demonstrated by the presence of degradation intermediates (see also molecules in blue color) formed by the action of such enzymes. As shown in Table 2, the presence of *n*-decanoic acid, catechol, protocatechuate and 3-oxoadipate was confirmed by target LC-MS analysis. The degradation of catechol was confirmed by measurement of catechol-2,3-dioxygenase activity. Note: *n*-decane has been used as example of the ability of strain DSM 45259 to degrade alkanes, although cultivation tests and target metabolomics analysis also demonstrated its ability to degrade *n*-tetradecane.