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Genomic, phylogenetic and catabolic re-assessment of the *Pseudomonas putida* clade supports the delineation of *Pseudomonas alloputida* sp. nov., *Pseudomonas inefficax* sp. nov., *Pseudomonas persica* sp. nov., and *Pseudomonas shirazica* sp. nov

Vahid Keshavarz-Tohid, Jordan Vacheron, Audrey Dubost, Claire Prigent-Combaret, Parissa Taheri, Saeed Tarighi, Seyed Mohsen Taghavi, Yvan Moenne-Loccoz, Daniel Muller

► **To cite this version:**

Vahid Keshavarz-Tohid, Jordan Vacheron, Audrey Dubost, Claire Prigent-Combaret, Parissa Taheri, et al.. Genomic, phylogenetic and catabolic re-assessment of the *Pseudomonas putida* clade supports the delineation of *Pseudomonas alloputida* sp. nov., *Pseudomonas inefficax* sp. nov., *Pseudomonas persica* sp. nov., and *Pseudomonas shirazica* sp. nov. *Systematic and Applied Microbiology*, 2019, 42 (4), pp.468-480. 10.1016/j.syapm.2019.04.004 . hal-02392254

HAL Id: hal-02392254

<https://hal.science/hal-02392254>

Submitted on 11 Dec 2020

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1 **Genomic, phylogenetic and catabolic re-assessment** of the *Pseudomonas putida* clade supports
2 the delineation of *Pseudomonas alloputida* sp. nov., *Pseudomonas inefficax* sp. nov.,
3 *Pseudomonas persica* sp. nov., and *Pseudomonas shirazica* sp. nov.

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20 **Journal:** Systematic and Applied Microbiology

21

22 ABSTRACT

23

24 Bacteria of the *Pseudomonas putida* group are studied for a large panel of properties ranging
25 from plant growth promotion and bioremediation to pathogenicity. To date, most of the
26 classification of individual pseudomonads from this group relies on 16S RNA gene analysis,
27 which is insufficient for accurate taxonomic characterization within bacterial species complexes
28 of the *Pseudomonas putida* group. Here, a collection of 20 of these bacteria, isolated from
29 various soils, was assessed via multi-locus sequence analysis of *rpoD*, *gyrB* and *rrs* genes. The
30 20 strains clustered in 7 different clades of the *P. putida* group. One strain per cluster was
31 sequenced and results were compared to complete genome sequences of type strains of the *P.*
32 *putida* group. Phylogenetic analyses, average nucleotide identity data and digital DNA
33 hybridizations, combined to phenotypic characteristics, resulted in the proposition and
34 description of four new species i.e. *Pseudomonas alloputida* Kh7^T (= LMG 29756^T = CFBP
35 8484^T) sp. nov., *Pseudomonas inefficax* JV551A3^T (= DSM108619^T = CFBP 8493^T) sp. nov.,
36 *Pseudomonas persica* RUB6^T (= LMG 29757^T = CFBP 8486^T) sp. nov. and *Pseudomonas*
37 *shirazica* VM14^T (= LMG 29953^T = CFBP 8487^T) sp. nov.

38

39 Introduction

40

41 *Pseudomonas* is one of the most complex and diverse bacterial genera [1, 2],
42 encompassing over 250 described species as of May 2018 [3, 4]
43 (<http://www.bacterio.net/pseudomonas.html>). Most species from this genus seem ubiquitous and
44 were isolated from a variety of distinctive habitats in water, soil, and eukaryotic hosts [5].
45 Although some species or strains were shown to be pathogenic for humans [6], animals [7-9] or
46 plants [10, 11], most *Pseudomonas* genotypes are inoffensive as commensal members of the
47 microbiota [2] or even beneficial to their eukaryotic hosts (e.g. plant growth-promoting
48 rhizobacteria [12-14]). Thus, this bacterial taxon presents a large variety of lifestyles, plays
49 diverse roles in biochemical cycles [15, 16], and produces various metabolites of
50 biotechnological interest [17], such as vitamin B12 [18], siderophores [19], antibiotics [14, 20-
51 22] or phytohormones [20, 21, 23].

52 Although species might emerge through ecological specialization [24, 25], current
53 bacterial species definition relies on molecular (genomic) homogeneity between strains of the
54 species. In brief, microbial species delineation relies on a polyphasic approach [26, 27] based
55 originally on (i) the change in melting temperature (or ΔT_m) of heteroduplex DNA formed upon
56 annealing of the DNAs from pairwise-tested strains, and (ii) DNA-DNA hybridization (DDH)
57 percentage [26, 27] or whole genome sequence identity computed as average nucleotide identity
58 (ANI) [28]. In addition, sequence comparison of DNA taxonomic markers (house-keeping genes
59 such as *rrs*, *gyrB* or *rpoD*) and characterization of phenotypic traits (morphological, biochemical
60 and/or enzymatic properties) are combined to assemble a set of strains in a species [26, 27].

61 Over 70 new *Pseudomonas* species have been described in the last ten years [2], and
62 recent analyses based on ANI calculations are suggesting the existence of several
63 uncharacterized species (or genomospecies [29]). Based on multilocus sequence analyses
64 (MLSA) with *rrs*, *gyrB*, *rpoD* and *rpoB* and ANI-based genome comparisons, the *Pseudomonas*
65 genus is divided in three main lineages, each subdivided in several phylogenetic groups : the *P.*
66 *fluorescens* lineage is constituted of 7 groups, the *P. aeruginosa* lineage 3 groups and the *P.*
67 *pertucinogena* lineage 1 group [2, 30, 31].

68 Within the *P. fluorescens* lineage, the *P. putida* group is the second largest one in the
69 number of described species [32]. These species are mainly studied for their biotechnological

70 potential [33], in relation to the production of particular chemicals [34-36] or phytobeneficial
71 properties [37, 38]. *P. putida* was isolated in 1889 [39, 40], and since then other species were
72 isolated from clinical samples (*P. mosselii* [41] and *P. monteilii* [42, 43]), infected animals (*P.*
73 *entomophila* L48 [44] and *P. plecoglossicida* [9]), soil (*P. soli* [45], *P. vranovensis* [46], *P.*
74 *taiwanensis* [47], *P. fulva*, *P. parafulva*, *P. cremoricolorata* [48], and *P. guariconensis* [49]) and
75 water (*P. donghuensis* [50, 51]). Many isolates of the *P. putida* group have been classified as *P.*
76 *putida* strains based on 16S rRNA gene homology. However, most of them do not belong to the
77 *P. putida* species *sensu stricto*, but to genospecies within the *P. putida* group [51-53].
78 Recently, Keshavarz-Tohid et al. [54] isolated bacteria from the bean rhizosphere, and 18 of the
79 isolates were affiliated to the *P. putida* group based on phylogenetic analysis of taxonomic
80 markers. These isolates were distributed over five phylogenetic clusters (termed Pp1 to Pp5) that
81 did not include type strains, raising the possibility that they might represent new species within
82 the *P. putida* group.

83 The objective of this work was to clarify whether the *Pseudomonas* isolates of
84 Keshavarz-Tohid et al. [54] could correspond to new species, and if so to establish these new
85 species in taxonomic terms. To this end, we inferred the phylogeny of the isolates and other
86 strains of the *P. putida* group, sequenced the genome of some of them, and compared *in silico*
87 these bacteria to the type strains from the *P. putida* group and a large panel of strains published
88 as *P. putida* (we will hereafter refer to them as *P. 'putida'* in cases where they were misnamed as
89 *P. putida*) and for which the genomic sequence was available. Thus, the phylogeny of 95 strains
90 of the *P. putida* group was investigated by MLSA, and they were assessed based on average
91 nucleotide identity (ANI), digital DNA hybridizations and phenotyping. This resulted in the
92 proposition of four new species within the *P. putida* group, i.e. *P. alloputida* sp. nov., *P.*
93 *inefficax* sp. nov., *P. persica* sp. nov. and *P. shirazica* sp. nov.

94

95 **Material and methods**

96

97 *Bacterial strains, culture conditions and DNA extraction*

98 Bacterial strains were previously isolated from different soils from Iran or France and
99 affiliated to the *Pseudomonas* genus [37, 54]. Briefly, 20 strains originated from rhizosphere of
100 bean plants from three Iranian provinces: strains RUB1 (previously VF16) and RUB2

101 (previously VF13) from Fars province; VKh2, VKh4, VKh9, VKh10, RUB6 (previously
102 VKh13), VKh7, VKh14, VKh17 from Khorasan province ; VM2, VM3, VM10, VM11, VM13,
103 VM14 and RUB5 (previously VM6) from Mazandaran province [54]. Three strains named
104 JV241A, JV551A1 and JV551A3 were isolated from the rhizosphere of maize grown in French
105 soil (Béligneux, 30 km East from Lyon) [37]. Besides this collection, *P. 'monteilii'* SB3078 [55]
106 (hereafter renamed *P. shirazica*), *P. 'putida'* W15Oct28 [56], *P. 'putida'* BW11M1 [57]
107 (hereafter renamed *P. mosselii*) and *P. cremoricolorata* DSM 17059^T [56] were obtained from
108 Aalborg University (Denmark), Université Libre de Bruxelles (Belgium), Catholic University
109 Leuven (Belgium) and University of Malaya (Malaysia), respectively. The 18 strains were
110 routinely cultivated overnight on King's B [58] or LB [59] media at 28°C with shaking (150
111 rpm). Bacterial genomic DNA was extracted from 500 µL of bacterial culture using the
112 NucleoSpin Tissue kit (Macherey-Nagel, Hoerd, France), following the manufacturer's
113 instructions. DNA was quantified spectrophotometrically and adjusted to 30 ng µL⁻¹.

114

115 *Phylogenetic analysis*

116 The phylogenetic analyses included 126 *Pseudomonas* strains with complete or draft
117 genome sequences (accession numbers available in Table S1), 13 *Pseudomonas* strains with
118 sequenced taxonomic markers (Table S2; see below), and *Cellvibrio japonicus* Ueda 107^T as
119 outgroup. Nucleotide sequences were retrieved from Genbank (Table S1) and aligned using
120 MUSCLE v3.8.31 [60]. Alignments were used to compute Maximum Likelihood trees using
121 PhyML [61], with 500 bootstraps, and SeaView v4 [62]. Phylogenetic trees from individual *rrs*
122 (16S rRNA gene), *gyrB* and *rpoB* sequences were generated (not shown), as well as a
123 phylogenetic tree based on concatenated sequences with a total length of 2963 nucleotides (1436
124 for *rrs*, 820 for *gyrB*, 707 for *rpoD*).

125

126 *Phenotypic profiling*

127 Strains RUB1, VKh7, RUB6, VKh14, VM14, JV551A1, JV551A3, *P. 'monteilii'*
128 SB3078, *P. 'putida'* W15Oct28, *P. 'putida'* BW11M1 and *P. cremoricolorata* DSM 17059^T
129 were tested for phenotypic characteristics, and results compared with published data on the other
130 strains. Cell morphology and number of flagella were investigated by using the method of
131 Heimbrook et al. [63]. Biochemical tests were done with Biolog GEN III MicroPlate (BIOLOG,

132 Hayward, CA), according to manufacturer's instructions, using 71 carbon sources and 23
133 chemical sensitivity assays over 48 h. All tests were done two times.

134

135 *Genome sequencing*

136 As described above, genomic DNA was extracted from strains JV241A, JV551A1,
137 JV551A3, RUB1, RUB6, VM14, VKh7 and VKh14 grown overnight in King's B [58]. The
138 resulting DNA samples were sent to Molecular Research LP (Shallowater, TX, USA), where
139 library preparation was performed using the Nextera DNA sample preparation kit (Illumina Inc.,
140 San Diego, CA, USA). Genomic DNA was then sequenced using Illumina MiSeq systems and
141 assembled using SeqMan NGen® version 12.0 (DNASTAR, Madison, WI, USA) with paired-
142 end sequencing parameters on the default settings. Genome annotation was done with the online
143 MicroScope platform [64]. The draft genome sequences can be found under bioprojects
144 PRJEB24813, PRJEB24814, PRJEB24815, PRJEB25064, PRJEB25066, PRJEB25068,
145 PRJEB25065, PRJEB25067 (Table S1).

146

147 *Computation of average nucleotide identities and digital DNA-DNA hybridizations*

148 Average Nucleotide Identity (ANI) was calculated for 126 complete or draft genomes
149 with fastANI [65] (Table S3 and Figure 1). In total, the dataset consisted of 15,750 pairwise
150 values (excluding the 126 pairwise comparisons of each genome with itself). The Genome-to-
151 Genome Distance Calculator GGDC 2.1 [66-68] was used to calculate the digital DNA-DNA
152 hybridization (dDDH) estimates between genomes of candidate strains, so as to define new
153 species in comparison with genomes of type strains from the *P. putida* group.

154

155 **Results**

156

157 *Phylogenetic classification of strains affiliated to the *P. putida* group*

158 A phylogenetic tree from concatenated *rrs*, *gyrB* and *rpoD* genes was generated for all
159 126 strains (Figure 2). In addition to 17 type strains and our 20 isolates of the *P. putida* group,
160 the tree contains strains from the *P. putida* group for which the sequenced genome is available in
161 a database (some of them shown here to be misnamed as *P. putida*), and a set of representative
162 type strains of the *P. fluorescens* group and the *P. aeruginosa* lineage. The different strains

163 affiliated so far to *P. putida* were retrieved in different *Pseudomonas* clades, with *P. 'putida'*
164 FDAARGOS121 branching within the *P. aeruginosa* lineage, and *P. 'putida'* CBB5 and MC4
165 5222 within the *P. fluorescens* group.

166 Only 3 of 65 non-type strains that were confirmed here to belong to the *P. putida* group
167 did correspond to the *P. putida* species (Figure 2). None of our 20 isolates [37, 54] belonged to
168 *P. putida* species; three of them were found close to type strains, i.e. *P. wadenswilersensis* CCOS
169 864^T [69] for strain JVA241A (96% sequence identity for the concatenated *rrs*, *gyrB* and *rpoD*
170 sequences) and *P. soli* LMG27941^T for cluster-Pp1 strains RUB1 and RUB2 (each with 98%
171 identity). The remaining 17 strains were distributed in four distinct clusters (Pp2, Pp3, Pp4, Pp5)
172 or close to cluster Pp3 (for strain JV551). Although clusters Pp2, Pp3, Pp4 and Pp5 include
173 various sequenced bacteria named *P. putida* or *P. monteili* so far, none of them contain the *P.*
174 *putida* type strain (or any other type strain). Based on previous use of multilocus sequence
175 analyses to identify putative new *Pseudomonas* species [53, 70], our findings point to the
176 occurrence of four if not five new species in the *P. putida* group, and hereafter we define four
177 proposed species i.e. *P. shirazica* (corresponding to cluster Pp4), *P. alloputida* (for cluster Pp5),
178 *P. persica* (for cluster Pp2) and *P. inefficax* (for French strains of the Lyon area).

179

180 *Genome sequence-based species delimitation*

181 ANIs calculated using whole genome data for the eight strains sequenced and the 117
182 sequenced *Pseudomonas* strains retrieved (including 19 type strains) pointed to 33 genomic
183 species (including the 19 species already described), based on ANI values $\geq 95\%$ (Figure 1 and
184 Table S3). The ANI values strengthened the proposal of the new species *P. shirazica*, *P.*
185 *alloputida*, *P. persica* and *P. inefficax*, in that strains within each of them displayed ANIs above
186 95% with one another but that were below the 95% threshold with all other related strains tested
187 (including type strains). This was the case for (i) *P. 'putida'* S16, *P. 'monteili'* SB3078 and
188 SB3101, and strains VM14, SF1, DLL-E4, S11, HB3267, MO2 and KG4 (now *P. shirazica*), (ii)
189 *P. 'putida'* BIRD-1, S12, PCL1760, YKD221, SJTE-1, DOT-TIE, PDI, TRO1, JCM 9802, B6-2,
190 INSali382, IDAHO, KT2440 and JLR11, and strains VKh7 (CFBP8484) and VKh14 (now *P.*
191 *alloputida*), (iii) strain RUB6 (now *P. persica*), and (iv) strains JV551A1 and JV551A3 (now *P.*
192 *inefficax*). Although the ANI value was below the 95% threshold (i.e. 94.9%) when comparing
193 the RUB1 genome (5.5 Mb) with the partially-sequenced genome (only 0.9 Mb released) of *P.*

194 *sol*i LMG 27941^T, the ANI was > 96% when comparing RUB1 to *P. soli* CCOS 191, suggesting
195 that RUB1 is a new strain of the *P. soli* species. The difference in genome sequence availability
196 had probably biased the ANI estimate with the type strain. As the genome sequence of *P.*
197 *wadenswilerensis* CCOS 864^T (the closest to JV241A) has not been released, it is not possible to
198 clarify whether JV241A belongs to *P. wadenswilerensis* or to another, yet-undescribed species.

199 In addition to ANI comparisons, the dDDH values determined using all type strains (with
200 GGDC 2.1 [66-68]) showed that for strains VKH7 (proposed type strain for *P. alloputida*),
201 VM14 (proposed type strain for *P. shirazica*), JV551A1 (proposed type strain for *P. inefficax*),
202 *P. 'putida'* W15Oct28 and RUB6 (proposed type strain for *P. persica*), the values were all
203 significantly lower than the cut-off of 70% (Table S4). This confirms, together with ANI, that the
204 five strains are representatives of novel species in the *P. putida* group. dDDH values calculated
205 with other sequenced strains from the *P. putida* group indicated that two of the proposed new
206 species contain several of these strains, which so far have been incorrectly affiliated to other
207 species (*P. putida* or *P. monteilii*; see Table S4). It is the case for (i) strains LS46, TRO1, B6-2,
208 YKD221, ND6, F1, JLR11, KT2440, INSali382, DOT-T1E, S12, S12_GCF, BIRD-1, VKh14,
209 IOFA19, PCL1760, PD1, SJTE-1, IOFA1, LF54, H, Idaho and JCM 9802, whose dDDH value
210 with *P. alloputida* Vkh7 (type strain) ranges from 70.6% (for TRO1) to ~100% (for VKh14 ;
211 Table S4), and (ii) strains S16, SB3078, SB3101, SF1, DLL-E4, S11, HB3267, MO2 and KG4,
212 whose dDDH value with *P. shirazica* VM14 (type strain) ranges from 78.8% (for S16) to 89.2%
213 (for HB3267).

214

215 *Morphological and biochemical features*

216 When grown on King's B agar, *P. soli* RUB1, *P. alloputida* Vkh7 (proposed type strain),
217 *P. persica* RUB6, VKh14, *P. shirazica* VM14 (proposed type strain), *P. inefficax* JV551A1
218 (proposed type strain), *P. inefficax* JV551A3, *P. shirazica* SB3078, *P. 'putida'* W15Oct28, *P.*
219 *mosselii* BW11M1 and *P. cremoricolorata* DSM 17059^T formed circular, convex colonies
220 producing fluorescent pigment(s). All were Gram-negative, aerobic and rod shaped.

221 Table 1 shows the phenotypic characteristics differentiating the 14 type strains of the *P.*
222 *putida* group (a more complete set of data is presented in Table S5). In the proposed species *P.*
223 *alloputida*, strains VKh7 (proposed type strain), KT2440 and VKh14 presented negative results
224 (GEN III MicroPlate, Biolog) for D-melibiose, *p*-hydroxy-phenylacetic acid, α -keto-butyric acid

225 and β -methyl-D-glucoside tests, whereas its closest relatives *P. putida* and *P. monteilli* were
226 positive for the same tests (Table 1). In the proposed species *P. persica*, strain RUB6 (proposed
227 type strain) gave negative results for bromo-succinic acid, D-galacturonic acid, D-glucuronic
228 acid, D-mannose and *p*-hydroxy-phenylacetic acid tests and positive results for acetic acid, D-
229 serine, glucuronamide and sucrose tests, in contrast to its closest relatives *P. guariconensis* and
230 *P. inefficax*. In the proposed species *P. inefficax*, strain JV551A3 (proposed type strain)
231 displayed positive results for bromo-succinic acid, D-galacturonic acid, D-glucuronic acid, D-
232 mannose and *p*-hydroxy-phenylacetic acid tests. In addition, *P. inefficax* JV551A1 and JV551A3
233 differed from *P. alloputida* VKh7 and VKh14 in that their formic acid tests were positive and
234 their sucrose tests negative. In the proposed species *P. shirazica*, strain VM14 (proposed type
235 strain) gave a positive result in the formic acid test and negative results for *N*-acetyl-D-
236 glucosamine, D-galacturonic acid, L-galactonic acid lactone and D-glucuronic acid tests.

237 For *P. putida* W15Oct28, particular morphological and phenotypic characteristics were
238 found. In contrast to members of related species *P. putida* and *P. fulva*, strain W15Oct28 could
239 not grow at 37°C and harbored 2 or 3 polar flagella. Contrarily to *P. alloputida* VKh7 and
240 VKh14, strain W15Oct28 gave a positive result for *p*-hydroxy-phenylacetic acid test and
241 negative results for sucrose, D-mannose and D-galacturonic acid tests. Taken together, genomic
242 and phenotypic data are suggesting that strain W15Oct28 is a member of a new genomic species.

243 244 Discussion

245
246 Members of the *P. putida* group play important ecological roles in various ecosystems,
247 especially in soils and sediments, and have received considerable research attention for plant
248 growth promotion, biodegradation of organic contaminants, and other biotechnological usages
249 [71, 72]. The prominent strain KT2440 (i.e. mt-2 / DSM 6125 / ATCC 47054) alone (cluster
250 Pp5; reclassified as *P. alloputida* in this work) has been the focus of several hundred
251 publications.

252 In 2011 [71], genomes of strains F1, KT2440, W619 and GB-1 were compared as
253 member of a same species, but in the present work we demonstrated that strains F1 and KT2440
254 are members of the *P. alloputida* species (cluster Pp5), whereas GB-1 (close to cluster Pp5) and
255 W619 (branching between *P. reidholzensis* CCOS 865^T and *P. taiwanensis* BCRC 17751^T) are

256 strains representing two new genomic species. Moreover, GB-1 represents the closest genomic
257 species to *P. alloputida* and strain W619 together with strain ATH43 form another
258 genomospecies more distantly related to *P. putida* since several other species are branching
259 between W619 and *P. putida sensu stricto* (see Figure 2 and Table S3). Interestingly, Wu et al.
260 [71] noticed that W619 presented the most different genome architecture (synteny around the
261 replication origin is not conserved with the other strains) and heavy metal gene organization
262 compared to *P. alloputida* strain KT2440 and strain F1 or to *P. 'putida'* GB-1. Similarly,
263 Lidbury et al. [73] analyzed the phosphorous scavenging capabilities of different *Pseudomonas*
264 species and grouped strains in different species, with strains KT2440 and BIRD-1 together (now
265 in *P. alloputida*), and GB-1 in a new genomic species and W619 in another new genomic
266 species.

267 The taxonomy of *P. putida* and related species has long been recognized in need of a
268 clarification [43, 51, 74], and it is in this context that the current taxonomic assessment was
269 carried out. Comparative genomic analysis using all the genome sequences available in public
270 databases enabled to define 33 genomic species encompassing the 19 already described species
271 (Figures 1, 2, Tables S3 and S4). The other 14 genomic species (currently unnamed)
272 encompassed strain with incorrect species affiliations (Table 2). Four genomic species among the
273 14 were assessed by differential phenotypic analysis, which enabled to propose four new species.

274

275 **Taxonomic characterization of the new isolates**

276 Phylogenetic and genomic analyses showed that certain isolates could be classified as members
277 of a new *Pseudomonas* species, and strain Kh7 (= LMG 29756 / CFBP 8484; cluster Pp5) was
278 designated as the type strain for *Pseudomonas alloputida* sp. nov. (Table 3). The new species
279 includes strain KT2440, which had been recognized as not belonging to the *P. putida* species
280 [75]. Indeed, DNA–DNA hybridization between strains *P. putida* DSM 291^T and KT2440 was
281 estimated to be 50.5% [75], i.e. below the 70% threshold that delimits bacterial species. The
282 phenotypic characterization shows profiles (Tables 1 and S5) of all representative type strains of
283 the *Pseudomonas putida* group. From the main characteristics, the *P. alloputida* species is
284 characterized by Gram-negative cells forming white colonies on Kings' B medium,
285 approximately 3 mm in diameter after 24 h growth. The cells were rods, motile and 1.6–5 µm in
286 length, with a growth temperature optimum of 28°C and an optimum pH of 7.0. Their genome

287 size ranged from 5.7 to 6.48 Mb with a GC% between 61.39% and 61.99% (Tables 4, S6 and
288 S7).

289 Phenotypic and phylogenetic analyses enabled to propose strain RUB6 (= LMG 29757 /
290 CFBP 8486; cluster Pp2) as the type strain of the new species *Pseudomonas persica* (detailed
291 characteristics are given in Table 5). From the main characteristics, *P. persica* corresponds to
292 Gram-negative cells forming white colonies on Kings' B medium, approximately 3 mm in
293 diameter after 24 h of growth. Compared with neighboring (sister) species found in the MLSA
294 tree (Figure 2), *P. persica* cannot use bromo-succinic acid, D-galacturonic acid, D-gluronic acid
295 but was able to use sucrose (unique among the tested species of the *P. putida* group). The cells
296 were rods, motile, and 1.6–5 µm in length, with an optimum growth temperature of 28°C and
297 optimum pH of 7.0. Genome size was 5.42 Mb, with a GC% of 62.92% (Tables 4, S6 and S7).

298 Comparative genomic analysis enabled to propose *Pseudomonas shirazica* sp. nov, with
299 VM14 (= LMG 29953 / CFBP 8487; within cluster Pp4) proposed as the type strain (Table 6). *P.*
300 *shirazica* encompasses strains that were isolated from various environments and that exhibit a
301 variety of phenotypes. Indeed, strain VM14 was isolated from bean rhizosphere [54], SB3078
302 and SB3101 degrade benzene, toluene, and ethylbenzene [55, 76], S16 degrades nicotine [77],
303 and HB3267 is a clinical isolate with high pathogenic potential [78]. *P. shirazica* is characterized
304 by Gram-negative cells forming white colonies on Kings' B medium, approximately 3 mm in
305 diameter after 24 h of growth. The cells are rods, motile, 1.6–5 µm in length, with a growth
306 temperature optimum of 28°C and an optimum pH of 7.0. Genome size ranges from 5.51 to
307 6.48 Mb with a GC% between 61.96% and 63.02% (Tables 4 and S7).

308 The last newly described species encompasses two strains (JV551A3 and JV551A1;
309 between clusters Pp2 and Pp3 ; see Figure 2) isolated from maize rhizosphere [37, 79], with
310 strain JV551A3 (= DSM 108619 / CFBP 8493) proposed as type strain for *P. inefficax* (Table 7).
311 None of the two strains presented any plant growth promotion effects when tested on maize or
312 *Arabidopsis* plants [37, 79]. Detailed species characteristics are summarized in Table 8. The cells
313 are rods, motile, 1.6–5 µm in length, with a growth temperature optimum of 28°C and an
314 optimum pH of 7.0. Genome size ranges from 6.42 to 5.88 Mb with a GC% between 62.85% and
315 62.75% (Tables 4, S6 and S7).

316

317 **Acknowledgments**

318 This research was supported by Academic Research cluster 3 of *Région Rhône-Alpes*, and CNRS
319 (France). We thank the Vice-Chancellor's office for Research and Technology in Agricultural
320 Science and Environmental Research of Khuzestan, Iran, for their support. We thank Danis
321 Abrouk (iBio), Florence Gerin and Laurence Loiseau (PARMIC) from UMR Ecologie
322 Microbienne for technical help and discussion. We are very grateful to Jian Woon and Chan Kok
323 Gan (University of Malaya) for strain DSM 17059^T, Maarten Ghequire (Catholic University
324 Leuven) for strain BW11M1, Sandra Matthijs and Pierre Cornelis (Université Libre de
325 Bruxelles) for strain W15Oct28, Morten Simonsen Dueholm (Aalborg University) for strain
326 SB3078. We also acknowledge the LABGeM and the National Infrastructure *France Génomique*
327 for support within the MicroScope annotation platform.

328

329 **Conflicts of interest**

330 The authors declare that there are no conflicts of interest.

331 **Figure legends**

332

333 **Figure 1** Genomic relationship between strains in the *P. putida* group based on ANI values
334 (%), which were determined with fastANI [65]. Type strains are in bold and the four new
335 species in red. A more exhaustive comparison is provided in Table S2.

336

337 **Figure 2** Maximum Likelihood phylogeny based on concatenated *rrs-gyrB-rpoB* genes of 138
338 *Pseudomonas* strains, i.e. 126 *Pseudomonas* strains with complete or draft genome sequences
339 (indicated with red dots for genomes obtained during the current project) and 13
340 *Pseudomonas* strains with sequenced taxonomic markers (blue dots). *Cellvibrio japonicus*
341 Udeea 107^T was used as outgroup. In the figure, the groups and phylogenetic lineages
342 proposed previously [2, 30, 74] are indicated; type strains and proposed type strains are in
343 bold. Within the *Pseudomonas putida* group, the *Pseudomonas putida* clusters proposed
344 previously [54] are highlighted in blue rectangles; the dotted lines are delimiting clusters of
345 strains belonging to the same species according to ANI data. Concatenated sequences were
346 used to compute the tree using PhyML [61], with 500 bootstraps, and SeaView v4 [62]. The
347 tree was visualized using iTOL software [80].

348

Table 1

Selected differential phenotypic characteristics of species of the *P. putida* group. Data are shown for the type strain or (when available) the mean data of type strain and related strains of the same species. Literature data are shown for *P. plecoglossicida* ATCC 700383^T [9], *P. guariconenesis* PCAVU^T [49], *P. parafulva* DSM 17004^T [44], *P. fulva* IAM 1529^T [44], *P. entomphila* L48^T [44], *P. putida* ATCC 12633^T [44] and *P. monteilii* ATCC 700476^T [44]. A full list of tested phenotypes is given in Table S5. Presented data were determined via GEN III MicroPlate (Biolog) metabolic tests. Type strains are in bold.

	<i>P. plecoglossicida</i> ATCC 700383 ^T	<i>P. entomphila</i> L48 ^T	<i>P. mosseli</i> ^a	<i>P. soli</i> ^b	<i>P. guariconenesis</i> PCAVU ^T	<i>P. persica</i> ^c	<i>P. inefficax</i> ^d	<i>P. shirazica</i> ^e	<i>P. parafulva</i> DSM 17004 ^T	<i>P. fulva</i> IAM 1529 ^T	<i>Pseudomonas</i> sp. W15Oct28	<i>P. putida</i> ATCC 12633 ^T	<i>P. monteilii</i> ATCC 700476 ^T	<i>P. alloputida</i> ^f
Flagellation	multiple polar	single polar	single polar	single polar	two polar	single polar	single polar	single polar	single polar	single polar	2 to 3 polar	single polar	ND	single polar
GEN III MicroPlate (28°C)														
6% NaCl	-	+	+	+	ND	-	-	-	+	+	-	-	+	-
Acetic acid	+	+	+	+	-	+	+	+	+	+	-	+	+	+
Bromo-succinic acid	+	+	+	+	W	-	+	-	+	+	-	+	+	D
D-Arabitol	-	+	+	-	-	-	-	-	-	-	-	-	-	-
D-Fructose	D	+	+	+	W	+	+	+	-	-	+	+	+	+
D-Galacturonic acid	-	-	-	-	-	-	+	-	-	-	-	+	+	+
D-Glucuronic acid	D	-	-	-	-	-	+	-	-	-	+	+	+	+
D-Mannitol	-	+	+	+	-	-	-	-	-	-	-	-	-	-
D-Mannose	+	+	+	+	-	-	+	+	+	+	-	+	+	+
D-Melibiose	+	-	-	-	-	-	-	-	-	W	-	+	+	-
D-Serine	+	+	-	-	-	+	+	D	+	+	+	+	+	D
D-Sorbitol	+	-	-	-	-	-	-	-	-	-	-	-	-	-
Dextrin	+	-	-	-	-	-	-	-	-	-	-	+	+	-
Glucuronamide	-	-	D	-	-	+	+	+	ND	ND	+	+	ND	+
L-Fucose	-	+	W	-	-	-	-	+	-	-	-	-	-	-
L-Pyroglutamic acid	ND	+	+	+	+	+	+	+	ND	ND	-	-	ND	+
<i>p</i> -Hydroxyphenylacetic acid	D	+	+	+	-	-	+	-	+	+	+	+	+	-
Propionic acid	+	+	+	+	+	+	+	+	+	+	-	+	+	+
Quinic acid	+	-	+	+	+	+	+	+	-	-	+	-	+	+
Sucrose	-	-	-	-	-	+	-	-	-	-	-	-	-	D
Tween 40	-	+	+	+	+	W	D	D	+	+	-	+	+	D
α -Hydroxybutyric acid	+	D	W	D	-	-	-	-	+	+	-	D	+	-
α -Keto-butyric acid	D	D	+	-	-	-	D	-	+	+	-	+	-	-
β -Methyl-D-glucoside	D	D	D	D	-	-	-	-	-	W	-	w	+	-
Formic acid	+	+	+	D	-	+	+	+	+	-	-	+	+	D

-, negative; + positive; ND, not done; W, weak; D, depends on the tested strain or experiment.

^a For *Pseudomonas mosseli*, data for type strain ATCC 700476^T [44] and strain BW11M1 were combined.

^b For *P. soli*, data for type strain F-279,208^T [45] and strain F16 were combined.

^c For *P. persica*, data for type strain RUB6^T and strain VM16 were combined.

^d For *P. inefficax*, data for type strain JV551A3^T and strain JV551A1 were combined.

^e For *P. shirazica*, data for type strain VM13^T, strain VM14 and strain SB3078 were combined.

^f For *P. alloputida*, data for type strain VKh7^T, strain VKh14 and strain KT2440 were combined.

Table 2. Proposed species affiliation of *P. putida* group strains for which the previous name proved incorrect or that were named in this work.

Strain name	Former species affiliation	New species
<i>P. mosselii</i> species		
1A00316	<i>Pseudomonas putida</i>	<i>Pseudomonas mosselii</i>
250J	<i>Pseudomonas</i> sp.	<i>Pseudomonas mosselii</i>
BW11M1	<i>Pseudomonas putida</i>	<i>Pseudomonas mosselii</i>
<i>P. soli</i> species		
RUB1=VF6	<i>Pseudomonas soli</i>	<i>Pseudomonas soli</i>
CCOS 191	<i>Pseudomonas soli</i>	<i>Pseudomonas soli</i>
<i>P. guariconensis</i> species		
MTCC5279	<i>Pseudomonas putida</i>	<i>Pseudomonas guariconensis</i>
<i>P. persica</i> species		
RUB5 =VM6	<i>Pseudomonas</i> sp.	<i>Pseudomonas persica</i>
RUB6 ^T = VKh13 ^T =LMG 29757 ^T =CFBP 8486 ^T	<i>Pseudomonas</i> sp.	<i>Pseudomonas persica</i>
<i>P. inefficax</i> species		
JV551A1=CFBP 8492	<i>Pseudomonas</i> sp.	<i>Pseudomonas inefficax</i>
JV551A3 ^T =DSM 108619 ^T =CFBP 8493 ^T	<i>Pseudomonas</i> sp.	<i>Pseudomonas inefficax</i>
<i>P. shirazica</i> species		
SB3101	<i>Pseudomonas monteilii</i>	<i>Pseudomonas shirazica</i>
SB3078	<i>Pseudomonas monteilii</i>	<i>Pseudomonas shirazica</i>
S16	<i>Pseudomonas putida</i>	<i>Pseudomonas shirazica</i>
DLL-E4	<i>Pseudomonas putida</i>	<i>Pseudomonas shirazica</i>
SF1	<i>Pseudomonas putida</i>	<i>Pseudomonas shirazica</i>
S11	<i>Pseudomonas putida</i>	<i>Pseudomonas shirazica</i>
MO2	<i>Pseudomonas monteilii</i>	<i>Pseudomonas shirazica</i>
HB3267	<i>Pseudomonas putida</i>	<i>Pseudomonas shirazica</i>
VM14 ^T =LMG 29953 ^T =CFBP 8487 ^T	<i>Pseudomonas</i> sp.	<i>Pseudomonas shirazica</i>
HB13667	<i>Pseudomonas putida</i>	<i>Pseudomonas shirazica</i>
<i>P. fulva</i> species		
S610	<i>Pseudomonas putida</i>	<i>Pseudomonas fulva</i>
<i>P. monteilii</i> species		
B001	<i>Pseudomonas putida</i>	<i>Pseudomonas monteilii</i>
HB4184	<i>Pseudomonas putida</i>	<i>Pseudomonas monteilii</i>
<i>P. alloputida</i> species		
LS46	<i>Pseudomonas putida</i>	<i>Pseudomonas alloputida</i>
TRO1	<i>Pseudomonas putida</i>	<i>Pseudomonas alloputida</i>
B6-2	<i>Pseudomonas putida</i>	<i>Pseudomonas alloputida</i>
YKD221	<i>Pseudomonas putida</i>	<i>Pseudomonas alloputida</i>
ND6	<i>Pseudomonas putida</i>	<i>Pseudomonas alloputida</i>
ATCC =DSM 6899 =BCRC 17059 =F1	<i>Pseudomonas putida</i>	<i>Pseudomonas alloputida</i>
JLR11	<i>Pseudomonas putida</i>	<i>Pseudomonas alloputida</i>
ATCC 47054 =DSM 6125 =NCIMB 11950 = KT2440	<i>Pseudomonas putida</i>	<i>Pseudomonas alloputida</i>
INSali382	<i>Pseudomonas putida</i>	<i>Pseudomonas alloputida</i>
DOT-T1E	<i>Pseudomonas putida</i>	<i>Pseudomonas alloputida</i>
S12	<i>Pseudomonas putida</i>	<i>Pseudomonas alloputida</i>
S12_GCF	<i>Pseudomonas putida</i>	<i>Pseudomonas alloputida</i>
BIRD-1	<i>Pseudomonas putida</i>	<i>Pseudomonas alloputida</i>
VKh14 =LMG 29758 =CFBP 8485	<i>Pseudomonas</i> sp.	<i>Pseudomonas alloputida</i>

VKh7 ^T =LMG 29756 ^T =CFBP 8484 ^T	<i>Pseudomonas</i> sp.	<i>Pseudomonas alloputida</i>
IOFA19	<i>Pseudomonas monteilii</i>	<i>Pseudomonas alloputida</i>
PCL1760	<i>Pseudomonas putida</i>	<i>Pseudomonas alloputida</i>
PD1	<i>Pseudomonas putida</i>	<i>Pseudomonas alloputida</i>
SJTE-1	<i>Pseudomonas putida</i>	<i>Pseudomonas alloputida</i>
IOFA1	<i>Pseudomonas putida</i>	<i>Pseudomonas alloputida</i>
LF54	<i>Pseudomonas putida</i>	<i>Pseudomonas alloputida</i>
H	<i>Pseudomonas putida</i>	<i>Pseudomonas alloputida</i>
Idaho	<i>Pseudomonas putida</i>	<i>Pseudomonas alloputida</i>
JCM 9802	<i>Pseudomonas putida</i>	<i>Pseudomonas alloputida</i>
<i>P. putida</i> genomic species 1		
GB-1	<i>Pseudomonas putida</i>	<i>Pseudomonas</i> sp.
<i>P. putida</i> genomic species 2		
W15Oct2018	<i>Pseudomonas putida</i>	<i>Pseudomonas</i> sp.
<i>P. putida</i> genomic species 3		
S13 1 2	<i>Pseudomonas putida</i>	<i>Pseudomonas</i> sp.
SJ13	<i>Pseudomonas putida</i>	<i>Pseudomonas</i> sp.
OUS82	<i>Pseudomonas putida</i>	<i>Pseudomonas</i> sp.
H8234	<i>Pseudomonas putida</i>	<i>Pseudomonas</i> sp.
JCM 18798	<i>Pseudomonas putida</i>	<i>Pseudomonas</i> sp.
<i>P. putida</i> genomic species 4		
KG4	<i>Pseudomonas putida</i>	<i>Pseudomonas</i> sp.
<i>P. putida</i> genomic species 5		
T2 2	<i>Pseudomonas putida</i>	<i>Pseudomonas</i> sp.
KB9	<i>Pseudomonas putida</i>	<i>Pseudomonas</i> sp.
<i>P. putida</i> genomic species 6		
GTC 10897	<i>Pseudomonas monteilii</i>	<i>Pseudomonas</i> sp.
<i>P. putida</i> genomic species 7		
PC2	<i>Pseudomonas putida</i>	<i>Pseudomonas</i> sp.
<i>P. putida</i> genomic species 8		
GM84	<i>Pseudomonas</i> sp.	<i>Pseudomonas</i> sp.
<i>P. putida</i> genomic species 9		
W619	<i>Pseudomonas putida</i>	<i>Pseudomonas</i> sp.
SQ1	<i>Pseudomonas putida</i>	<i>Pseudomonas</i> sp.
ATH43	<i>Pseudomonas putida</i>	<i>Pseudomonas</i> sp.
<i>P. putida</i> genomic species 10		
USDA-ARS-USMARC-56711	<i>Pseudomonas monteilii</i>	<i>Pseudomonas</i> sp.
<i>P. putida</i> genomic species 11		
PA14H7	<i>Pseudomonas putida</i>	<i>Pseudomonas</i> sp.
<i>P. putida</i> genomic species 12		
CBF10-2	<i>Pseudomonas putida</i>	<i>Pseudomonas</i> sp.
CSV86	<i>Pseudomonas putida</i>	<i>Pseudomonas</i> sp.
<i>P. putida</i> genomic species 13		
ABAC8	<i>Pseudomonas putida</i>	<i>Pseudomonas</i> sp.
UASWS0946	<i>Pseudomonas putida</i>	<i>Pseudomonas</i> sp.
<i>P. putida</i> genomic species 14		
ABAC63	<i>Pseudomonas putida</i>	<i>Pseudomonas</i> sp.
MR3	<i>Pseudomonas putida</i>	<i>Pseudomonas</i> sp.

Table 3

Description of *Pseudomonas alloputida* sp. nov. according to Digital Protologue TA00611 assigned by the www.imedeia.uib.es/dprotologue website

Taxonumber	TA00611
Species name	<i>Pseudomonas alloputida</i>
Genus name	<i>Pseudomonas</i>
Specific epithet	<i>alloputida</i>
Species status	sp. nov.
Species etymology	al.lo.pu'ti.da. Gr. masc. adj. <i>allos</i> , other; L. fem. adj. <i>putida</i> , rotten, stinking; specific epithet of a <i>Pseudomonas</i> ; N.L. fem. adj. <i>alloputida</i> , another <i>putida</i> .
Authors	Vahid Keshavarz-Tohid, Jordan Vacheron, Audrey Dubost, Claire Prigent-Combaret, Parissa Taheri, Saeed Tarighi, Seyed Mohsen Taghavi, Yvan Moënne-Loccoz, Daniel Muller
Title	Genomic, phylogenetic and catabolic re-assessment of the <i>Pseudomonas putida</i> clade supports the delineation of <i>Pseudomonas alloputida</i> sp. nov., <i>Pseudomonas inefficax</i> sp. nov., <i>Pseudomonas persica</i> sp. nov., and <i>Pseudomonas shirazica</i> sp. nov.
Submitter	Daniel MULLER
E-mail of the submitter	daniel.muller@univ-lyon1.fr
Designation of the type strain	VKh7
Strain collection numbers	CFBP 8484 = LMG 29756
16S rRNA gene accession number	LT718459
Genome accession number [EMBL]	PRJEB25065
Genome status	Draft
Genome size	5707279
GC mol %	61.99
Country of origin	Iran
Region of origin	Khorasan Razavi province
Date of isolation	14/07/2014
Source of isolation	Rhizosphere of bean root
Sampling date	12/07/2014
Geographic location	Neyshaboor city
Latitude	36° 11' 42.5" N
Longitude	58° 49' 44.8" E
Altitude	1250
Number of strains in study	3
Source of isolation of non-type strains	Bean rhizosphere
Growth medium, incubation conditions	King's B agar (KBA) at 28°C
Gram stain	Negative

Cell shape	Rod
Motility	Motile
If motile	Flagellar
If flagellated	Single polar
Sporulation (resting cells)	None
Colony morphology	Colonies were fluorescent, approximately 3 mm in diameter, circular and convex with an entire margin
Lowest temperature for growth	15
Highest temperature for growth	30
Temperature optimum	28
pH optimum	7.5
pH category	Neutrophile
Lowest NaCl concentration for growth	1%
Highest NaCl concentration for growth	8%
Relationship to O₂	Halotolerant (optimum < 1 % NaCl and growth observed at > 6 % NaCl)
O₂ conditions for strain testing	Aerobe
Positive tests with BIOLOG	Aerobiosis
Negative tests with BIOLOG	α -keto-glutaric acid, <i>a</i> -D-glucose, acetic acid, citric acid, D-fructose, D-galacturonic acid, D-gluconic acid, D-glucuronic acid, D-mannose, D-saccharic acid, glucuronamide, glycerol, inosine, L-alanine, L-aspartic acid, L-glutamic acid, L-histidine, L-lactic acid, L-pyroglutamic acid, L-serine, propionic acid, quinic acid, γ -amino butyric acid, β -hydroxybutyric, aztreonam, vancomycin, guanidine HCl, lincomycin, minocycline, niaproof 4, rifamycin SV, tetrazolium blue, tetrazolium violet, troleandomycin, 1% NaCl, 1% sodium lactate, 4% NaCl, 8% NaCl, D-serine, D-fucose, fusidic acid, L-arginine, L-galactonic acid lactone, L-malic acid, lithium chloride, mucic acid, nalidixic acid, pH 5, pH 6, potassium tellurite, sodium bromate, sodium butyrate
Energy metabolism	α -D-lactose, D-arabitol, D-cellobiose, D-galactose, D-glucose-6-PO ₄ , D-mannitol, D-melibiose, D-raffinose, D-sorbitol, D-trehalose, dextrin, gentiobiose, L-fucose, L-rhamnose, myo-inositol, <i>N</i> -acetyl-D-galactosamine, <i>N</i> -acetyl-D-glucosamine, <i>p</i> -hydroxy-phenylacetic acid, turanose, α -hydroxybutyric acid, α -keto butyric acid, β -methyl-D-glucoside, gelatin, acetoacetic acid, pectin, 3-methyl glucose, D-aspartic acid, D-lactic acid methyl ester, D-malic acid, D-maltose, D-salicin, glycyl-L-proline, <i>N</i> -acetyl neuraminic acid, <i>N</i> -acetyl- β -D-mannosamine,
Variable tests with BIOLOG	bromo-succinic acid, D-serine, methyl pyruvate, sucrose tween 40, formic acid
Positive tests with API	ADH, GLU—assim, MNE, GNT, CAP, MLT, CIT, PAC, OX
Negative tests with API	NO ₃ , TRP, GLU_ Ferm, URE, ESC, GEL, PNPG, ARA, MAN, NAG, MAL, ADI
Energy metabolism	Chemoorganotroph

Table 4

Genome size and GC% content of species in the *P. putida* group. For species that contain more than one sequenced strain, data are shown as means \pm standard deviations. Raw data are shown in Table S6.

Species name	Genome size (bp)	GC (%)
<i>Pseudomonas japonica</i>	6.66×10^6	64.16
<i>Pseudomonas alkylphenolia</i>	5.76×10^6	60.63
<i>Pseudomonas vranovensis</i>	5.70×10^6	61.53
<i>Pseudomonas donghuensis</i>	5.64×10^6	62.42
<i>Pseudomonas cremoricolorata</i>	4.66×10^6	63.50
<i>Pseudomonas taiwanensis</i>	5.42×10^6	61.87
<i>Pseudomonas plecoglossicida</i>	5.34×10^6	62.99
<i>Pseudomonas entomophila</i>	5.89×10^6	64.16
<i>Pseudomonas mosselii</i>	$5.81 (\pm 0.33) \times 10^6$	64.35 ± 0.25
<i>Pseudomonas soli</i> ^a	$5.79 (\pm 0.22) \times 10^6$	64.18 ± 0.01
<i>Pseudomonas guariconensis</i> ^a	5.20×10^6	62.48
<i>Pseudomonas persica</i>	5.42×10^6	62.92
<i>Pseudomonas inefficax</i>	$6.06 (\pm 0.18) \times 10^6$	62.80 ± 0.05
<i>Pseudomonas shirazica</i>	$5.99 (\pm 0.25) \times 10^6$	62.45 ± 0.22
<i>Pseudomonas parafulva</i>	5.09×10^6	63.46
<i>Pseudomonas fulva</i>	$4.68 (\pm 0.08) \times 10^6$	61.89 ± 0.12
<i>Pseudomonas putida</i>	$6.27 (\pm 0.10) \times 10^6$	62.12 ± 0.14
<i>Pseudomonas monteilii</i>	$6.19 (\pm 0.25) \times 10^6$	61.57 ± 0.15
<i>Pseudomonas alloputida</i>	$6.06 (\pm 0.27) \times 10^6$	61.70 ± 0.19

^a Strains for which the entire genome was not sequenced were not included (including the type strains of *P. soli* and *P. guariconensis*).

Table 5

Description of *Pseudomonas persica* sp. nov. according to Digital Protologue TA00711 assigned by the www.imedea.uib.es/dprotologue website

Taxonnumber	TA00711
Species name	<i>Pseudomonas persica</i>
Genus name	<i>Pseudomonas</i>
Specific epithet	<i>Persica</i>
Species status	sp. nov.
Species etymology	per'si.ca. L. fem. adj. <i>persica</i> , Persian.
Authors	Vahid Keshavarz-Tohid, Jordan Vacheron, Audrey Dubost, Claire Prigent-Combaret, Parissa Taheri, Saeed Tarighi, Seyed Mohsen Taghavi, Yvan Moënne-Loccoz, Daniel Muller
Title	Genomic, phylogenetic and catabolic re-assessment of the <i>Pseudomonas putida</i> clade supports the delineation of <i>Pseudomonas alloputida</i> sp. nov., <i>Pseudomonas inefficax</i> sp. nov., <i>Pseudomonas persica</i> sp. nov., and <i>Pseudomonas shirazica</i> sp. nov.
Submitter	Daniel MULLER
E-mail of the submitter	daniel.muller@univ-lyon1.fr
Designation of the type strain	RUB6
Strain collection numbers	CFBP 8486=LMG 29757
16S rRNA gene accession number	LT718462
Genome accession number [EMBL]	PRJEB25066
Genome status	Draft
Genome size	5425242
GC mol %	62.92
Country of origin	Iran
Region of origin	Khorasan Razavi province
Date of isolation	14/07/2014
Source of isolation	Rhizosphere of bean
Sampling date	12/07/2014
Geographic location	Neyshaboor city
Latitude	36° 11' 42.5" N
Longitude	58° 49' 44.8" E
Altitude	1250
Number of strains in study	2
Source of isolation of non-type strains	Bean rhizosphere
Growth medium, incubation conditions	King's B agar (KBA) at 28°C
Gram stain	Negative
Cell shape	Rod
Motility	Motile

If motile	Flagellar
If flagellated	Single polar
Sporulation (resting cells)	None
Colony morphology	Colonies were fluorescent, approximately 3 mm in diameter, circular and convex with an entire margin
Lowest temperature for growth	15
Highest temperature for growth	37
Temperature optimum	28
pH optimum	7.5
pH category	Neutrophile
Lowest NaCl concentration for growth	1%
Highest NaCl concentration for growth	4%
Relationship to O₂	Aerobe
O₂ conditions for strain testing	Aerobiosis
Positive tests with BIOLOG	D-fructose, D-gluconic acid, D-saccharic acid, D-serine, glucuronamide, glycerol, inosine, L-alanine, L-aspartic acid, L-glutamic acid, L-histidine, L-pyroglutamic acid, L-serine, methyl pyruvate, propionic acid, quinic acid, sucrose, γ -amino butyric acid, β -hydroxybutyric, formic acid, vancomycin, guanidine HCl, lincomycin, minocycline, niaproof 4, rifamycin SV, tween 40, aztreonam, acetoacetic acid, tetrazolium blue, tetrazolium violet, troleandomycin, 1% NaCl, 4% NaCl, 1% sodium lactate, D-serine, D-fucose, fusidic acid, L-arginine, L-malic acid, lithium chloride, mucic acid, nalidixic acid, pH 5, pH 6
Negative tests with BIOLOG	Bromo-succinic acid, D-arabitol, D-cellobiose, D-galacturonic acid, D-glucose-6-PO ₄ , D-glucuronic acid, D-mannitol, D-mannose, D-melibiose, D-raffinose, D-sorbitol, D-trehalose, dextrin, gentiobiose, L-fucose, L-rhamnose, <i>N</i> -acetyl-D-galactosamine, <i>N</i> -acetyl-D-glucosamine, <i>p</i> -hydroxy-phenylacetic acid, turanose, α -hydroxybutyric acid, α -keto butyric acid, β -methyl-D-glucoside, gelatin, pectin, 3-methyl glucose, 8% NaCl, D-aspartic acid, D-lactic acid methyl ester, D-malic acid, D-maltose, D-salicin, glycyl-L-proline, L-galactonic acid lactone, <i>N</i> -acetyl neuraminic acid, <i>N</i> -acetyl- β -D-mannosamine, sodium bromate, sodium butyrate, stachyose, D-fructose-6-PO ₄
Energy metabolism	Chemoorganotroph

Table 6

Description of *Pseudomonas shirazica* sp. nov. according to Digital Protologue TA00712 assigned by the www.imedea.uib.es/dprotologue website

Taxonnumber	TA00712
Species name	<i>Pseudomonas shirazica</i>
Genus name	<i>Pseudomonas</i>
Specific epithet	<i>Shirazica</i>
Species status	sp. nov.
Species etymology	shi.ra'zi.ca. N.L. fem. adj. <i>shirazica</i> pertaining to Shiraz (a city in Iran)
Authors	Vahid Keshavarz-Tohid, Jordan Vacheron, Audrey Dubost, Claire Prigent-Combaret, Parissa Taheri, Saeed Tarighi, Seyed Mohsen Taghavi, Yvan Moënne-Looccoz, Daniel Muller
Title	Genomic, phylogenetic and catabolic re-assessment of the <i>Pseudomonas putida</i> clade supports the delineation of <i>Pseudomonas alloputida</i> sp. nov., <i>Pseudomonas inefficax</i> sp. nov., <i>Pseudomonas persica</i> sp. nov., and <i>Pseudomonas shirazica</i> sp. nov.
Submitter	Daniel MULLER
E-mail of the submitter	daniel.muller@univ-lyon1.fr
Designation of the type strain	VM14
Strain collection numbers	CFBP 8487 = LMG 29953
16S rRNA gene accession number	LT718474
Genome accession number [EMBL]	PRJEB25068
Genome status	Draft
Genome size	5,514,185
GC mol %	62.84
Country of origin	Iran
Region of origin	Mazandaran province
Date of isolation	15/07/2014
Source of isolation	Rhizosphere of bean
Sampling date	12/07/2014
Geographic location	Behshahr city
Latitude	36° 44' 54.7" N
Longitude	53° 32' 42.9" E
Altitude	-15
Number of strains in study	5
Source of isolation of non-type strains	Bean rhizosphere
Growth medium, incubation conditions	King's B agar (KBA) at 28°C
Gram stain	Negative
Cell shape	Rod
Motility	Motile

If motile	Flagellar
If flagellated	Single polar
Sporulation (resting cells)	None
Colony morphology	Colonies were fluorescent, approximately 3 mm in diameter, circular and convex with an entire margin
Lowest temperature for growth	4
Highest temperature for growth	37
Temperature optimum	28
pH optimum	7
pH category	Neutrophile
Lowest NaCl concentration for growth	1%
Highest NaCl concentration for growth	4%
relationship to O₂	Aerobe
O₂ conditions for strain testing	Aerobiosis
Positive tests with BIOLOG	acetic acid, D-fructose, D-gluconic acid, D-mannose, D-saccharic acid, glucuronamide, glycerol, L-alanine, L-aspartic acid, L-fucose, L-glutamic acid, L-histidine, L-pyroglutamic acid, L-serine, methyl pyruvate, propionic acid, quinic acid, γ -amino butyric acid, β -hydroxybutyric, formic acid, vancomycin, guanidine HCl, lincomycin, minocycline, niaproof 4, potassium tellurite, rifamycin SV, tetrazolium blue, tetrazolium violet, troleandomycin, 1% NaCl, 1% sodium lactate, 8% NaCl, D-fucose, L-arginine, L-malic acid, mucic acid, pH 5, pH 6
Negative tests with BIOLOG	bromo-succinic acid, D-arabitol, D-cellobiose, D-galacturonic acid, D-glucose-6-PO ₄ , D-glucuronic acid, D-mannitol, D-melibiose, D-raffinose, D-sorbitol, D-trehalose, dextrin, gentiobiose, L-rhamnose, <i>N</i> -acetyl-D-galactosamine, <i>N</i> -acetyl-D-glucosamine, <i>p</i> -hydroxy-phenylacetic acid, sucrose, turanose, α -hydroxybutyric acid, α -keto butyric acid, β -methyl-D-glucoside, gelatin, aztreonam, acetoacetic acid, pectin, 3-methyl glucose, D-serine, D-aspartic acid, D-lactic acid methyl ester, D-malic acid, D-maltose, D-salicin, glycyl-L-proline, L-galactonic acid lactone, <i>N</i> -acetyl neuraminic acid, <i>N</i> -acetyl- β -D-mannosamine, sodium bromate, stachyose, D-fructose-6-PO ₄
Variable tests with BIOLOG	D-serine, inosine, tween 40, 4% NaCl, fusidic acid, nalidixic acid, sodium butyrate
Energy metabolism	Chemoorganotroph

Table 7

Description of *Pseudomonas inefficax* sp. nov. according to Digital Protologue TA00715 assigned by the www.imedea.uib.es/dprotologue website

Taxonnumber	TA00715
Species name	<i>Pseudomonas inefficax</i>
Genus name	<i>Pseudomonas</i>
Specific epithet	<i>inefficax</i>
Species status	sp. nov.
Species etymology	From <i>in.ef.fi.cax</i> . L. fem. adj. <i>inefficax</i> , inefficient. The strains have no effect on plant growth
Authors	Vahid Keshavarz-Tohid, Jordan Vacheron, Audrey Dubost, Claire Prigent-Combaret, Parissa Taheri, Saeed Tarighi, Seyed Mohsen Taghavi, Yvan Moënne-Loccoz, Daniel Muller
Title	Genomic, phylogenetic and catabolic re-assessment of the <i>Pseudomonas putida</i> clade supports the delineation of <i>Pseudomonas alloputida</i> sp. nov., <i>Pseudomonas inefficax</i> sp. nov., <i>Pseudomonas persica</i> sp. nov., and <i>Pseudomonas shirazica</i> sp. nov.
Submitter	Daniel MULLER
E-mail of the submitter	daniel.muller@univ-lyon1.fr
Designation of the type strain	JV551A3
Strain collection numbers	CFBP8493 = DSM 108619
16S rRNA gene accession number	PRJEB24815
Genome accession number [embl]	PRJEB24815
Genome status	Draft
Genome size	6240036
GC mol %	62.85
Country of origin	France
Region of origin	Béligneux "AIN 01"
Date of isolation	14/01/2014
Date of isolation unknown (< yyyy)	2014
Source of isolation	Soil
Sampling date	12/07/2014
Geographic location	Béligneux "AIN 01"
Latitude	45°52'18.9"N
Longitude	5°07'18.2"E
Altitude	~270
Number of strains in study	2
Source of isolation of non-type strains	Maize rhizosphere
Growth medium, incubation conditions	King's B agar (KBA) at 28°C
Gram stain	Negative
Cell shape	Rod

Motility	Motile
If motile	Flagellar
If flagellated	Single polar
Sporulation (resting cells)	None
Colony morphology	Colonies were fluorescent, approximately 3 mm in diameter, circular and convex with an entire margin
Lowest temperature for growth	4
Highest temperature for growth	37
Temperature optimum	28
pH optimum	7
pH category	Neutrophile
Lowest NaCl concentration for growth	1%
Highest NaCl concentration for growth	4%
relationship to O₂	Aerobe
O₂ conditions for strain testing	Aerobiosis
Positive tests with BIOLOG	D-arabitol, D-cellobiose, D-glucose-6-PO ₄ , D-mannitol, D-melibiose, D-affinose, D-sorbitol, D-trehalose, dextrin, gentiobiose, L-fucose, L-rhamnose, <i>N</i> -acetyl-D-galactosamine, <i>N</i> -acetyl-D-glucosamine, sucrose, turanose, α -hydroxybutyric acid, β -methyl-D-glucoside, gelatin, acetoacetic acid, pectin, 3-methyl glucose, D-aspartic acid, D-lactic acid methyl ester, D-malic acid, D-maltose, D-salicin, glycy-L-proline, <i>N</i> -acetyl neuraminic acid, <i>N</i> -acetyl- β -D-mannosamine, stachyose, D-fructose-6-PO ₄
Negative tests with BIOLOG	acetic acid, bromo-succinic acid, D-fructose, D-galacturonic acid, D-gluconic acid, D-glucuronic acid, D-mannose, D-saccharic acid, D-serine, glucuronamide, glycerol, inosine, L-alanine, L-aspartic acid, L-glutamic acid, L-histidine, L-pyroglutamic acid, L-serine, methyl pyruvate, <i>p</i> -hydroxy-phenylacetic acid, propionic acid, quinic acid, γ -amino butyric acid, β -hydroxybutyric, formic acid, aztreonam, vancomycin, guanidine HCl, lincomycin, minocycline, niaproof 4, rifamycin SV, tetrazolium blue, tetrazolium violet, troleandomycin, 1% NaCl, 1% sodium lactate, 4% NaCl, D-serine, D-fucose, fusidic acid, L-arginine, L-galactonic acid lactone, L-malic acid, lithium chloride, mucic acid, nalidixic acid, pH 5, pH 6, sodium bromate, sodium butyrate
Variable tests with BIOLOG	Tween 40, α -keto butyric acid
Energy metabolism	Chemoorganotroph

	<i>Pseudomonas</i> sp. JV241A	<i>Pseudomonas donghuensis</i> HYS ^T	<i>P. vranovensis</i> DSM 16006 ^T	<i>Pseudomonas alkylphenolia</i> KL28 ^T	<i>Pseudomonas</i> sp. USDA-ARS-USMARC-56711	<i>P. cremoricolorata</i> DSM 17059 ^T	<i>P. plecoglossicida</i> NBRC 103162 ^T	<i>P. taiwanensis</i> DSM 21245 ^T	<i>Pseudomonas mosselii</i> BW11M1	<i>Pseudomonas mosselii</i> ATCCBAA 99 ^T	<i>Pseudomonas soli</i> LMG27941 ^T	<i>Pseudomonas soli</i> RUB1	<i>Pseudomonas entomophila</i> L48 ^T	<i>P. guariconensis</i> LMG27394 ^T	<i>Pseudomonas fulva</i> CIP106765 ^T	<i>Pseudomonas parafulva</i> DSM117004 ^T	<i>Pseudomonas putida</i> NBRC 14164 ^T	<i>Pseudomonas</i> sp. W15Oct28	<i>Pseudomonas alloputida</i> VKh14	<i>Pseudomonas alloputida</i> VKh7 ^T	<i>Pseudomonas alloputida</i> KT2440	<i>Pseudomonas monteilii</i> DSM14164 ^T	<i>Pseudomonas monteilii</i> SB3078	<i>Pseudomonas shirazica</i> VM14 ^T	<i>Pseudomonas inefficax</i> JV551A3 ^T	<i>Pseudomonas inefficax</i> JV551A1	<i>Pseudomonas persica</i> RUB6 ^T
<i>Pseudomonas</i> sp. JV241A	100	93.3	86.9	87.4	82.8	83.2	84.3	83.3	84.7	84.7	83.5	84.5	84.6	81.2	81.9	83.1	84.0	84.1	83.4	83.4	83.5	83.9	84.3	84.2	84.2	84.1	84.5
<i>Pseudomonas donghuensis</i> HYS^T	93.4	100	87.0	87.2	82.8	83.1	84.4	83.4	84.7	84.7	83.6	84.4	84.5	81.0	81.9	83.2	84.0	84.0	83.4	83.4	83.4	83.8	84.3	84.3	84.0	84.1	84.4
<i>Pseudomonas vranovensis</i> DSM 16006^T	87.0	86.8	100	87.3	82.1	82.4	83.5	82.7	83.7	83.7	82.9	83.5	83.6	79.8	81.5	82.5	83.1	83.4	82.7	82.7	82.8	83.2	83.3	83.4	83.2	83.3	83.5
<i>Pseudomonas alkylphenolia</i> KL28^T	87.5	87.3	87.3	100	82.2	82.5	83.6	82.8	83.8	83.7	82.6	83.7	83.7	80.2	81.6	82.7	83.2	83.5	82.7	82.8	82.9	83.1	83.4	83.3	83.4	83.3	83.6
<i>Pseudomonas</i> sp. USDA-ARS-USMARC-56711	83.0	82.7	82.1	82.2	100	83.3	83.7	82.8	84.2	84.1	83.2	83.8	84.0	79.6	82.3	83.4	83.3	83.3	82.8	82.8	82.9	83.3	83.6	83.7	83.5	83.5	83.9
<i>Pseudomonas cremoricolorata</i> DSM 17059^T	83.4	83.2	82.4	82.5	83.3	100	84.4	83.4	84.5	84.5	83.4	84.4	84.3	80.3	82.5	83.8	83.8	83.9	83.5	83.6	83.4	83.7	84.1	84.3	84.0	84.0	84.4
<i>Pseudomonas plecoglossicida</i> NBRC 103162^T	84.3	84.3	83.5	83.5	83.6	84.1	100	86.5	87.1	87.0	86.2	86.8	86.9	82.1	83.8	84.9	87.3	87.6	86.6	86.6	86.7	87.2	87.7	87.9	87.8	87.8	88.0
<i>Pseudomonas taiwanensis</i> DSM 21245^T	83.3	83.3	82.8	82.9	83.0	83.2	86.4	100	85.7	85.7	84.9	85.5	85.6	80.8	83.3	84.1	85.8	86.1	85.5	85.5	85.5	86.0	86.5	86.4	86.4	86.4	86.6
<i>Pseudomonas mosselii</i> BW11M1	84.8	84.7	83.7	83.8	84.1	84.5	87.1	85.8	100	99.2	90.7	91.3	89.6	82.6	83.6	85.3	86.2	86.4	85.7	85.7	85.8	86.2	86.8	86.9	86.7	86.7	86.9
<i>Pseudomonas mosselii</i> ATCCBAA 99^T	84.6	84.7	83.7	83.7	83.9	84.5	87.0	85.7	99.2	100	90.7	91.2	89.6	82.7	83.6	85.3	86.3	86.3	85.8	85.9	86.0	86.8	86.9	86.9	86.9	86.6	87.0
<i>Pseudomonas soli</i> LMG27941^T	83.7	83.6	82.9	82.9	83.2	83.4	86.2	84.9	90.9	90.8	100	94.7	88.3	70.0	83.0	84.6	85.3	85.5	84.7	84.7	84.8	85.2	85.9	86.2	86.1	86.1	86.3
<i>Pseudomonas soli</i> RUB1	84.4	84.5	83.6	83.7	83.9	84.3	86.9	85.5	91.3	91.3	94.9	100	89.1	82.4	83.4	85.0	86.3	86.3	85.5	85.5	85.7	86.1	86.5	86.7	86.6	86.5	86.8
<i>Pseudomonas entomophila</i> L48^T	84.4	84.4	83.6	83.6	83.8	84.3	86.8	85.6	89.6	89.5	88.4	89.1	100	82.7	83.4	85.2	86.2	86.3	85.7	85.7	85.8	86.2	86.8	86.8	86.8	86.7	87.0
<i>Pseudomonas guariconensis</i> LMG27394^T	81.3	80.9	79.9	80.1	79.7	80.0	81.9	81.1	82.9	82.6	70.0	82.4	82.7	100	78.4	80.5	81.5	81.6	81.4	81.4	82.7	81.6	82.3	82.4	82.5	82.4	83.2
<i>Pseudomonas fulva</i> CIP106765^T	82.0	81.9	81.5	81.5	82.3	82.5	84.0	83.4	83.6	83.5	83.1	83.4	83.4	79.0	100	82.8	84.3	84.4	83.9	84.0	84.0	84.3	84.4	84.5	84.5	84.5	84.6
<i>Pseudomonas parafulva</i> DSM117004^T	83.3	83.2	82.7	82.9	83.5	83.8	84.9	84.1	85.5	85.4	84.4	85.2	85.4	80.7	82.8	100	84.3	84.4	84.0	84.1	84.0	84.2	84.9	84.8	84.5	84.5	84.8
<i>Pseudomonas putida</i> NBRC 14164^T	83.9	83.9	83.0	83.2	83.2	83.8	87.2	85.8	86.1	86.3	85.2	86.2	86.1	81.6	84.2	84.3	100	94.7	90.2	90.1	90.2	90.5	90.1	90.2	90.0	89.9	90.2
<i>Pseudomonas</i> sp. W15Oct28	83.9	83.9	83.4	83.5	83.2	83.8	87.4	86.0	86.3	86.2	85.4	86.3	86.3	81.8	84.3	84.3	94.7	100	90.1	90.1	90.1	90.7	90.2	90.4	90.2	90.1	90.6
<i>Pseudomonas alloputida</i> VKh14	83.5	83.4	70.0	82.8	82.6	83.3	86.6	85.5	85.7	85.9	84.4	85.5	85.7	81.5	83.8	83.8	90.2	90.2	100	100	97.1	89.5	89.5	89.6	89.5	89.4	89.7
<i>Pseudomonas alloputida</i> VKh7^T	83.4	83.4	70.0	82.8	82.6	83.3	86.7	85.5	85.7	85.8	84.6	85.5	85.6	81.2	83.9	83.8	90.3	90.2	100	100	97.1	89.5	89.5	89.7	89.5	89.4	89.8
<i>Pseudomonas alloputida</i> KT2440	83.5	83.5	82.9	83.0	82.9	83.4	86.7	85.5	85.8	86.1	85.0	85.7	85.9	82.7	83.9	84.0	90.3	90.2	97.1	97.1	100	89.7	89.8	89.7	89.6	89.6	89.8
<i>Pseudomonas monteilii</i> DSM14164^T	83.9	83.9	83.2	83.1	83.2	83.6	87.2	85.9	86.3	86.8	85.2	86.1	86.3	81.3	84.4	84.2	90.6	90.9	89.6	89.6	89.7	100	90.0	90.2	90.0	89.9	90.5
<i>Pseudomonas monteilii</i> SB3078	84.3	84.2	83.3	83.4	83.4	83.9	87.7	86.5	86.9	86.9	86.1	86.6	86.8	82.5	84.3	84.7	90.2	90.3	89.5	89.5	89.8	90.0	100	97.5	94.4	94.4	93.7
<i>Pseudomonas shirazica</i> VM14^T	84.3	84.2	83.4	83.4	83.5	84.1	87.8	86.4	86.9	86.9	86.1	86.8	86.8	82.1	84.4	84.6	90.2	90.4	89.6	89.6	89.7	90.1	97.5	100	94.7	94.6	93.8
<i>Pseudomonas inefficax</i> JV551A3^T	84.2	84.0	83.3	83.4	83.5	84.0	87.8	86.3	86.8	87.0	86.0	86.5	86.8	82.1	84.4	84.5	90.0	90.2	89.6	89.5	89.6	90.0	94.3	94.5	100	99.9	93.6
<i>Pseudomonas inefficax</i> JV551A1	84.3	84.1	83.2	83.4	83.4	84.0	87.7	86.3	86.7	86.6	85.9	86.5	86.7	81.9	84.3	84.4	89.9	90.1	89.4	89.4	89.5	89.9	94.3	94.6	99.9	100	93.5
<i>Pseudomonas persica</i> RUB6^T	84.5	84.5	83.6	83.5	83.7	84.2	87.9	86.5	87.0	87.0	86.1	86.9	87.0	83.0	84.6	84.8	90.4	90.7	89.8	89.7	89.9	90.3	93.8	93.8	93.7	93.7	100

Figure 1 Genomic relationship between strains in the *P. putida* group based on ANI values (%), which were determined with fastANI [65]. Type strains are in bold and the four new species in red. A more exhaustive comparison is provided in Table S3

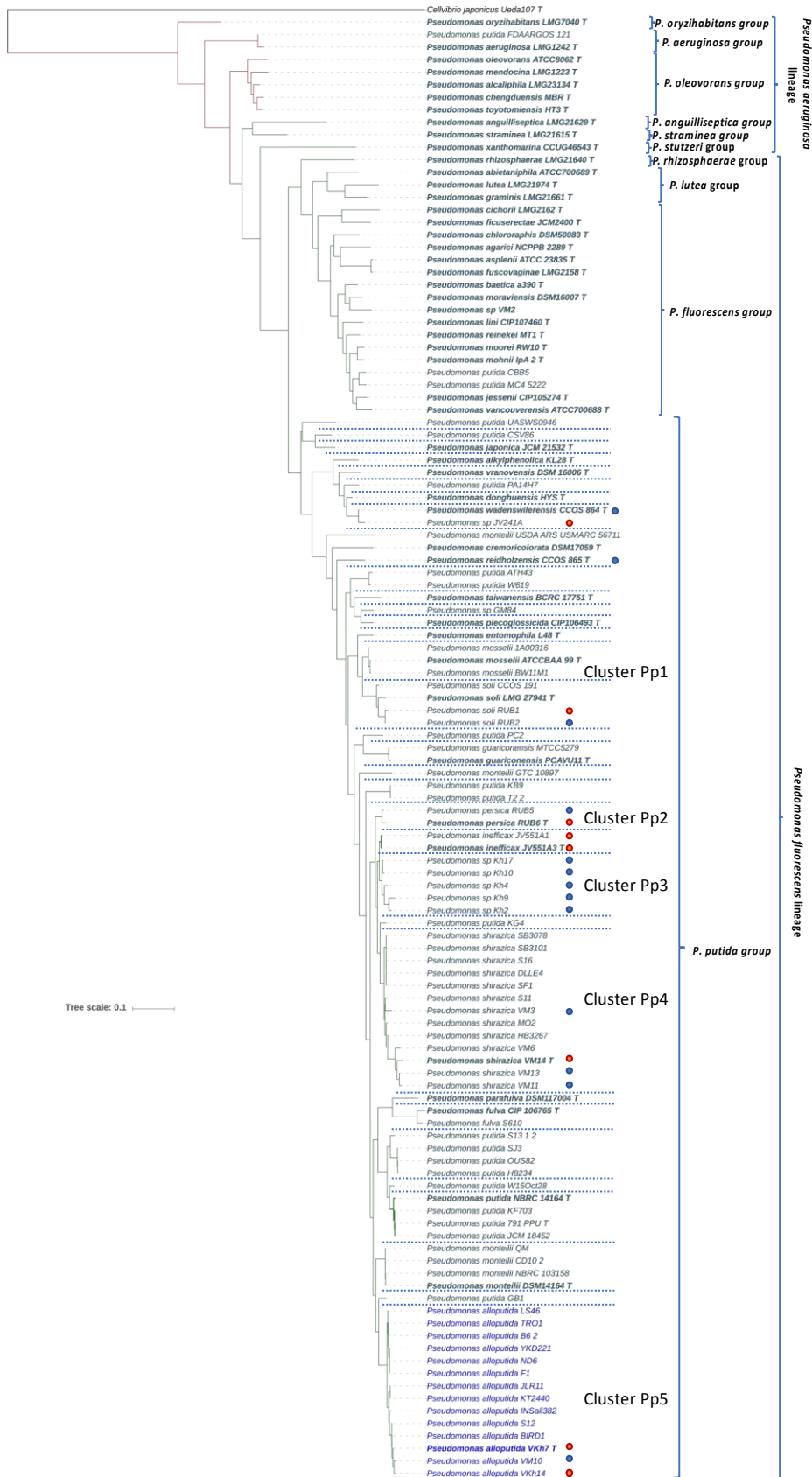


Figure 2 Maximum Likelihood phylogeny based on concatenated *rrs-gyrB-rpoB* genes of 138 *Pseudomonas* strains, i.e. 126 *Pseudomonas* strains with complete or draft genome sequences (indicated with red dots for genomes obtained during the current project) and 12 *Pseudomonas* strains with sequenced taxonomic markers (blue dots). *Cellvibrio japonicus* Ueda 107^T was used as outgroup. In the figure, the groups and phylogenetic lineages proposed previously [2, 30, 74] are indicated; type strains and proposed type strains are in bold. Within the *Pseudomonas putida* group, the *Pseudomonas putida* clusters proposed previously [54] are highlighted in blue rectangles; the dotted lines are delimiting clusters of strains belonging to the same species according to ANI data. Concatenated sequences were used to compute the tree using PhyML [61], with 500 bootstraps, and SeaView v4 [62]. The tree was visualized using iTOL software [80]

Supplementary tables

Added in an Excel file:

Table S1. Genome accession numbers.

Table S2. *rrs*, *rpoD* and *gyrB* accession numbers for strains without available genome sequence.

Table S3. Genomic relationship between strains in the *Pseudomonas* genus based on ANI values (%), which were determined with fastANI [65]. Type strains are in bold and the four new species in red. Blue strains are not presented in the main figures or tables.

Table S4. Digital DNA-DNA hybridization (dDDH) estimates computed using Genome-to-Genome Distance Calculator GGDC 2.1 [66-68]. Strains are listed according to the new species proposed.

Table S5. Differential phenotypic characteristics of strains of the *P. putida* group. Data are shown for the type strain as well as other related strains of the same species. Reference is cited for tests found in the literature. When no references are indicated data were obtained in present work. In yellow the mean phenotype of the species.

Table S6. Genome size and GC% content of strains in the *P. putida* group.

Table S7. Features of genomes used in the study.

References

1. Palleroni, N.J., The *Pseudomonas* story. *Environmental Microbiology*, 2010. 12(6): p. 1377-1383.
2. Peix, A., M.-H. Ramírez-Bahena, and E. Velázquez, The current status on the taxonomy of *Pseudomonas* revisited: An update. *Infection, Genetics and Evolution*, 2018. 57: p. 106-116.
3. Euzéby, J.P., List of bacterial names with standing in nomenclature: a folder available on the internet. *International Journal of Systematic and Evolutionary Microbiology*, 1997. 47(2): p. 590-592.
4. Parte, A.C., LPSN – List of prokaryotic names with standing in nomenclature (bacterio.net), 20 years on. *International Journal of Systematic and Evolutionary Microbiology*, 2018. 68(6): p. 1825-1829.
5. Silby, M.W., et al., *Pseudomonas* genomes: diverse and adaptable. *FEMS Microbiology Reviews*, 2011. 35(4): p. 652-680.
6. de Bentzmann, S. and P. Plésiat, The *Pseudomonas aeruginosa* opportunistic pathogen and human infections. *Environmental Microbiology*, 2011. 13(7): p. 1655-1665.
7. Bergan, T., Human- and animal-pathogenic members of the genus *Pseudomonas*, in the prokaryotes: a handbook on habitats, isolation, and identification of bacteria, M.P. Starr, et al., Editors. 1981, Springer Berlin Heidelberg: Berlin, Heidelberg. p. 666-700.
8. López, J.R., et al., *Pseudomonas baetica* sp. nov., a fish pathogen isolated from wedge sole, *Dicologlossa cuneata* (Moreau). *International Journal of Systematic and Evolutionary Microbiology*, 2012. 62(4): p. 874-882.
9. Nishimori, E., K. Kita-Tsukamoto, and H. Wakabayashi, *Pseudomonas plecoglossicida* sp. nov., the causative agent of bacterial haemorrhagic ascites of ayu, *Plecoglossus altivelis*. *International Journal of Systematic and Evolutionary Microbiology*, 2000. 50(1): p. 83-89.
10. Xin, X.-F. and S.Y. He, *Pseudomonas syringae* pv. tomato DC3000: a model pathogen for probing disease susceptibility and hormone signaling in plants. *Annual Review of Phytopathology*, 2013. 51(1): p. 473-498.
11. Patel, H.K., et al., Identification of virulence associated loci in the emerging broad host range plant pathogen *Pseudomonas fuscovaginae*. *BMC Microbiology*, 2014. 14(1): p. 274.
12. Haas, D. and G. Défago, Biological control of soil-borne pathogens by fluorescent pseudomonads. *Nature Reviews Microbiology*, 2005. 3(4): p. 307-319.
13. Ramette, A., et al., *Pseudomonas protegens* sp nov., widespread plant-protecting bacteria producing the biocontrol compounds 2,4-diacetylphloroglucinol and pyoluteorin. *Systematic and Applied Microbiology*, 2011. 34(3): p. 180-188.
14. Almario, J., et al., Rhizosphere ecology and phytoprotection in soils naturally suppressive to *Thielaviopsis black* root rot of tobacco. *Environmental Microbiology*, 2014. 16(7): p. 1949-1960.
15. Richardson, A.E., et al., Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. *Plant and Soil*, 2009. 321(1): p. 305-339.
16. Peix, A., et al., *Pseudomonas rhizosphaerae* sp. nov., a novel species that actively solubilizes phosphate in vitro. *International Journal of Systematic and Evolutionary Microbiology*, 2003. 53(6): p. 2067-2072.

17. Puchałka, J., et al., Genome-scale reconstruction and analysis of the *Pseudomonas putida* KT2440 metabolic network facilitates applications in biotechnology. *PLOS Computational Biology*, 2008. 4(10): p. e1000210.
18. Kang, Z., et al., Recent advances in microbial production of δ -aminolevulinic acid and vitamin B12. *Biotechnology Advances*, 2012. 30(6): p. 1533-1542.
19. Cornelis, P., Iron uptake and metabolism in pseudomonads. *Applied Microbiology and Biotechnology*, 2010. 86(6): p. 1637-1645.
20. Loper, J.E., et al., Comparative genomics of plant-associated *Pseudomonas* spp.: insights into diversity and inheritance of traits involved in multitrophic interactions. *PLoS Genetics*, 2012. 8(7): p. e1002784.
21. Hesse, C., et al., Genome-based evolutionary history of *Pseudomonas* spp. *Environmental Microbiology*. 20(6): p. 2142-2159.
22. Weller, D.M., *Pseudomonas* biocontrol agents of soilborne pathogens: looking back over 30 years. *Phytopathology*, 2007. 97(2): p. 250-256.
23. Flury, P., et al., Insect pathogenicity in plant-beneficial pseudomonads: phylogenetic distribution and comparative genomics. *The ISME Journal*, 2016. 10(10): p. 2527-42.
24. Lassalle, F., D. Muller, and X. Nesme, Ecological speciation in bacteria: reverse ecology approaches reveal the adaptive part of bacterial cladogenesis. *Research in Microbiology*, 2015. 166(10): p. 729-741.
25. Lassalle, F., et al., Ancestral genome estimation reveals the history of ecological diversification in *Agrobacterium*. *Genome Biology and Evolution*, 2017. 9: p. 3413-3431.
26. Wayne, L.G., et al., Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *International Journal of Systematic and Evolutionary Microbiology*, 1987. 37(4): p. 463-464.
27. Stackebrandt, E., et al., Report of the ad hoc committee for the re-evaluation of the species definition in bacteriology. *International Journal of Systematic and Evolutionary Microbiology*, 2002. 52(3): p. 1043-1047.
28. Li, X., Y. Huang, and W.B. Whitman, The relationship of the whole genome sequence identity to DNA hybridization varies between genera of prokaryotes. *Antonie van Leeuwenhoek*, 2015. 107(1): p. 241-249.
29. Tran, P.N., M.A. Savka, and H.M. Gan, In-silico taxonomic classification of 373 genomes reveals species misidentification and new genospecies within the genus *Pseudomonas*. *Frontiers in Microbiology*, 2017. 8: p. 1296.
30. Mulet, M., J. Lalucat, and E. García-Valdés, DNA sequence-based analysis of the *Pseudomonas* species. *Environmental Microbiology*, 2010. 12(6): p. 1513-1530.
31. García-Valdés, E. and J. Lalucat, *Pseudomonas*: Molecular phylogeny and current taxonomy. In *Pseudomonas: Molecular and Applied Biology*. 2016. p. 1-23. Springer, Cham.
32. Garrido-Sanz, D., et al., Classification of isolates from the *Pseudomonas fluorescens* complex into phylogenomic groups based in group-specific markers. *Frontiers in Microbiology*, 2017. 8: p. 413.
33. Poblete-Castro, I., et al., Industrial biotechnology of *Pseudomonas putida* and related species. *Applied Microbiology and Biotechnology*, 2012. 93(6): p. 2279-2290.
34. Schmid, A., et al., Industrial biocatalysis today and tomorrow. *Nature*, 2001. 409: p. 258.
35. Wackett, L.P., *Pseudomonas putida*—a versatile biocatalyst. *Nature Biotechnology*, 2003. 21: p. 136.

36. Ward, P.G., et al., A Two Step Chemo-biotechnological conversion of polystyrene to a biodegradable thermoplastic. *Environmental Science & Technology*, 2006. 40(7): p. 2433-2437.
37. Vacheron, J., et al., Fluorescent *Pseudomonas* strains with only few plant-beneficial properties are favored in the maize rhizosphere. *Frontiers in Plant Science*, 2016. 7: p. 1212.
38. Agaras, B.C., A. Iriarte, and C.F. Valverde, Genomic insights into the broad antifungal activity, plant-probiotic properties, and their regulation, in *Pseudomonas donghuensis* strain SVBP6. *PLoS ONE*, 2018. 13(3): p. e0194088.
39. Migula, W., *Über ein neues System der Bakterien, in Arbeiten aus dem Bakteriologischen Institute der Technischen Hochschule Zu Karlsruhe*. 1894, Otto Nemnich: Karlsruhe.
40. Trevisan, V., I generi e le specie delle Batteriacee. Zanaboni Gabuzzi Milan, 1889: p. 1–35.
41. Dabboussi, F., et al., *Pseudomonas mosselii* sp. nov., a novel species isolated from clinical specimens. *International Journal of Systematic and Evolutionary Microbiology*, 2002. 52(2): p. 363-376.
42. Elomari, M., et al., *Pseudomonas monteilii* sp. nov., isolated from clinical specimens. *International Journal of Systematic and Evolutionary Microbiology*, 1997. 47(3): p. 846-852.
43. Molina, L., et al., Specific gene loci of clinical *Pseudomonas putida* isolates. *PLoS ONE*, 2016. 11(1): p. e0147478.
44. Mulet, M., et al., Taxonomic characterisation of *Pseudomonas* strain L48 and formal proposal of *Pseudomonas entomophila* sp. nov. *Systematic and Applied Microbiology*, 2012. 35(3): p. 145-149.
45. Pascual, J., et al., *Pseudomonas soli* sp. nov., a novel producer of xantholysin congeners. *Systematic and Applied Microbiology*, 2014. 37(6): p. 412-416.
46. Tvrzová, L., et al., *Pseudomonas moraviensis* sp. nov. and *Pseudomonas vranovensis* sp. nov., soil bacteria isolated on nitroaromatic compounds, and emended description of *Pseudomonas asplenii*. *International Journal of Systematic and Evolutionary Microbiology*, 2006. 56(11): p. 2657-2663.
47. Wang, L.-T., et al., *Pseudomonas taiwanensis* sp. nov., isolated from soil. *International Journal of Systematic and Evolutionary Microbiology*, 2010. 60(9): p. 2094-2098.
48. Uchino, M., et al., Recharacterization of *Pseudomonas fulva* Iizuka and Komagata 1963, and proposals of *Pseudomonas parafulva* sp. nov. and *Pseudomonas cremoricolorata* sp. nov. *The Journal of General and Applied Microbiology*, 2001. 47(5): p. 247-261.
49. Toro, M., et al., *Pseudomonas guariconensis* sp. nov., isolated from rhizospheric soil. *International Journal of Systematic and Evolutionary Microbiology*, 2013. 63(12): p. 4413-4420.
50. Gao, J., et al., *Pseudomonas donghuensis* sp. nov., exhibiting high-yields of siderophore. *Antonie van Leeuwenhoek*, 2015. 107(1): p. 83-94.
51. Yonezuka, K., et al., Phylogenetic analysis reveals the taxonomically diverse distribution of the *Pseudomonas putida* group. *The Journal of General and Applied Microbiology*, 2017. 63(1): p. 1-10.
52. Mulet, M., E. García-Valdés, and J. Lalucat, Phylogenetic affiliation of *Pseudomonas putida* biovar A and B strains. *Research in Microbiology*, 2013. 164(4): p. 351-359.
53. Gomila, M., et al., Phylogenomics and systematics in *Pseudomonas*. *Frontiers in Microbiology*, 2015. 6: p. 214.

54. Keshavarz-Tohid, V., et al., Phylogenetic diversity and antagonistic traits of root and rhizosphere pseudomonads of bean from Iran for controlling *Rhizoctonia solani*. *Research in Microbiology*, 2017. 168(8): p. 760-772.
55. Dueholm, M.S., et al., Complete genome sequences of *Pseudomonas monteilii* SB3078 and SB3101, two benzene-, toluene-, and ethylbenzene-degrading bacteria used for bioaugmentation. *Genome Announcements*, 2014. 2(3): p. e00524-14.
56. Ye, L., et al., Draft genome sequence analysis of a *Pseudomonas putida* W15Oct28 strain with antagonistic activity to Gram-positive and *Pseudomonas* sp. pathogens. *PLoS ONE*, 2014. 9(11): p. e110038.
57. Ghequire, M.G.K., et al., Draft genome sequence of *Pseudomonas putida* BW11M1, a banana rhizosphere isolate with a diversified antimicrobial armamentarium. *Genome Announcements*, 2016. 4(2): p. e00251-16.
58. King, E.O., M.K. Ward, and D.E. Raney, Two simple media for the demonstration of pyocyanin and fluorescin. *Translational Research*, 1954. 44(2): p. 301-307.
59. Bertani, G., STUDIES ON LYSOGENESIS I.: The mode of phage liberation by lysogenic *Escherichia coli*. *Journal of Bacteriology*, 1951. 62(3): p. 293-300.
60. Edgar, R.C., MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, 2004. 32(5): p. 1792-1797.
61. Guindon, S., et al., New algorithms and methods to estimate Maximum-Likelihood phylogenies: assessing the performance of PhyML 3.0. *Systematic Biology*, 2010. 59(3): p. 307-321.
62. Gouy, M., S. Guindon, and O. Gascuel, SeaView version 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. *Molecular Biology and Evolution*, 2010. 27(2): p. 221-224.
63. Heimbrook, M.E., W.L. Wang, and G. Campbell, Staining bacterial flagella easily. *Journal of Clinical Microbiology*, 1989. 27(11): p. 2612-2615.
64. Vallenet, D., et al., MicroScope—an integrated microbial resource for the curation and comparative analysis of genomic and metabolic data. *Nucleic Acids Research*, 2013. 41(D1): p. D636-D647.
65. Jain, C., et al., High throughput ANI analysis of 90K prokaryotic genomes reveals clear species boundaries. *Nature Communications*, 2018. 9(1): p. 5114.
66. Meier-Kolthoff, J.P., et al., Genome sequence-based species delimitation with confidence intervals and improved distance functions. *BMC Bioinformatics*, 2013. 14(1): p. 60.
67. Meier-Kolthoff, J.P., et al., Complete genome sequence of DSM 30083T, the type strain (U5/41T) of *Escherichia coli*, and a proposal for delineating subspecies in microbial taxonomy. *Standards in Genomic Sciences*, 2014. 9(1): p. 2.
68. Meier-Kolthoff, J.P., H.-P. Klenk, and M. Göker, Taxonomic use of DNA G+C content and DNA–DNA hybridization in the genomic age. *International Journal of Systematic and Evolutionary Microbiology*, 2014. 64(2): p. 352-356.
69. Frasson, D., et al., *Pseudomonas wadenswilerensis* sp. nov. and *Pseudomonas reidholzensis* sp. nov., two novel species within the *Pseudomonas putida* group isolated from forest soil. *International Journal of Systematic and Evolutionary Microbiology*, 2017. 67(8): p. 2853-2861.
70. Mulet, M., et al., Concordance between whole-cell matrix-assisted laser-desorption/ionization time-of-flight mass spectrometry and multilocus sequence analysis approaches in species discrimination within the genus *Pseudomonas*. *Systematic and Applied Microbiology*, 2012. 35(7): p. 455-464.

71. Wu, X., et al., Comparative genomics and functional analysis of niche-specific adaptation in *Pseudomonas putida*. *FEMS Microbiology Reviews*, 2011. 35(2): p. 299-323.
72. Loeschcke, A. and S. Thies, *Pseudomonas putida*—a versatile host for the production of natural products. *Applied Microbiology and Biotechnology*, 2015. 99(15): p. 6197-6214.
73. Lidbury, I.D.E.A., et al., Comparative genomic, proteomic and exoproteomic analyses of three *Pseudomonas* strains reveals novel insights into the phosphorus scavenging capabilities of soil bacteria. *Environmental Microbiology*, 2016. 18(10): p. 3535-3549.
74. Hesse, C., et al., Genome-based evolutionary history of *Pseudomonas* spp. *Environmental Microbiology*, 2018. 20(6): p. 2142-2159.
75. Regenhardt, D., et al., Pedigree and taxonomic credentials of *Pseudomonas putida* strain KT2440. *Environmental Microbiology*, 2002. 4(12): p. 912-915.
76. Dueholm, M.S., et al., Survival and activity of individual bioaugmentation strains. *Bioresource Technology*, 2015. 186: p. 192-199.
77. Wang, S.N., et al., Characterization of environmentally friendly nicotine degradation by *Pseudomonas putida* biotype A strain S16. *Microbiology*, 2007. 153(5): p. 1556-1565.
78. Fernández, M., et al., Analysis of the pathogenic potential of nosocomial *Pseudomonas putida* strains. *Frontiers in Microbiology*, 2015. 6: p. 871.
79. Vacheron, J., et al., Differential contribution of plant-beneficial functions from *Pseudomonas kilonensis* F113 to root system architecture alterations in *Arabidopsis thaliana* and *Zea mays*. *Molecular Plant-Microbe Interactions*, 2017. 31: p. 212-223.
80. Letunic, I. and P. Bork, Interactive tree of life (iTOL) v3: an online tool for the display and annotation of phylogenetic and other trees. *Nucleic Acids Research*, 2016. 44(W1): p. W242-W245.