Pseudomonas gallaeciensis sp. nov., isolated from crude-oil-contaminated intertidal sand samples after the Prestige oil spill

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ABSTRACT

Strains V113[T], V92 and V120 have been isolated from sand samples taken at the Atlantic intertidal shore in Galicia, Spain, after the Prestige oil spill. A preliminary analysis of the 16S rRNA and the partial rpoD gene sequences indicated that these strains belonged to the Pseudomonas genus, but they were distinct from any known Pseudomonas species. They were extensively characterized by a polyphasic taxonomic approach and phylogenetic data that confirmed that these strains belonged to the Pseudomonas pertucinogena group. Phylogenetic analysis of 16S rRNA, gyrB and rpoD gene sequences showed that the three strains were 99% similar and were closely related to members of the P. pertucinogena group, with less than 94% similarity to strains of established species; Pseudomonas pachastrellae was the closest relative. The Average Nucleotide Index based on blast values was 89.0% between V113[T] and the P. pachastrellae type strain, below the accepted species level (95%). The predominant cellular fatty acid contents and whole cell protein profiles determined by MALDI-TOF mass spectrometry also differentiated the studied strains from known Pseudomonas species. We therefore conclude that strains V113[T], V92 and V120 represent a novel species of Pseudomonas, for which the name Pseudomonas gallaeciensis is proposed; the type strain is V113[T] (= CCUG 67583[T] = LMG 29038[T]).

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Introduction

The genus *Pseudomonas* represents a group of Gram-negative bacteria within the *Gammaproteobacteria* that are non-spore forming rods that are motile by polar flagella [29]. The bacteria of the genus *Pseudomonas* are ubiquitous, metabolically versatile, and important for the recycling of elements in the environment. The genus was first described by Migula (1894) and currently comprises a large number of species, and new species are described continuously. At the time of this manuscript's composition, 167 species with valid names have been described (http://www.bacterio.cict.fr/p/pseudomonas.html) [8,30].

The characterization of *Pseudomonas* type strains by multilocus sequence analysis (MLSA), concatenating the sequences of the 16S rDNA, *gyrB*, *rpoD* and *rpoB* genes, permitted the establishment of two main lineages, *Pseudomonas fluorescens* and *Pseudomonas aeruginosa*, which were divided into several groups (G) and subgroups (SG) [25,27]. In recent years, the *Pseudomonas pertucinogena* G has experienced a significant increase in the number of newly described species compared with the other groups. In 2004, this group was represented only by *P. pertucinogena* [16]; today, it contains 16 species, the following of which have been recently described: *Pseudomonas aestusnigri* [34,11], *Pseudomonas salegens* [3], *Pseudomonas salina* [39], *Pseudomonas oceani* [36,10], *Pseudomonas populi* [4] and 'Pseudomonas saudimassiliensis' not yet validated [6].

Proteobacteria have played a key role in the bacterial community in the contaminated intertidal sand after the Prestige oil spill [1,2]. The characterization of the bacterial species present in this habitat is crucial to have a comprehensive knowledge of the microorganisms present in this environment. This paper is focused on the

genus *Pseudomonas* and a putative new species in the genus. During a study of the *Pseudomonas* diversity in crude-oil contaminated intertidal sand samples after the Prestige oil spill (Galicia, Spain) in September 2004, several isolates were considered representatives of possible new species. These results were confirmed by the analysis of partial sequences of sigma 70 subunit of RNA polymerase, encoded by the *rpoD* gene [26]. Three of these strains, V113[T], V92 and V120, have been characterized taxonomically in the present study. A new *Pseudomonas* species is proposed in the *P. pertucinogena* G, with the strain V113[T] as the type strain.

Materials and methods

Bacterial strains and growth conditions

Strains V113[T] and V120 have been isolated from "Boca do Rio" beach (42°50′11.52″N, 9°6′11.52″W) from buried weathered fuel. Strain V92 was isolated from an unpolluted sand sample from "Praia de Seda" beach (42°46′29.27″N, 9°7′27.08″W). The strains were isolated after growth in mineral basal medium (MMB) [5] with naphthalene (V92 and V113[T]) or hexadecane (V120) as energy and carbon sources [26]. A list of the bacterial strains studied as representatives of closely related species of the *P. pertucinogena* group is provided in Table S1 [13,18,20,21,31,37,38]. All bacteria were cultured at 30 °C on Luria-Bertani medium (LB) for 24–48 h.

DNA extraction, PCR amplification, DNA sequencing conditions

The DNA extraction, PCR amplification, primers used, purification of the amplified products and DNA sequencing conditions, as well as the sequence analysis procedures, were previously described [28].

Primer sequences corresponding to enterobacterial repetitive intergenic consensus (ERIC) elements (ERIC 1R: 5′-ATGTAAGCTCCTGGGGATTCAC-3′ and ERIC2: 5′-AAGTAAGTGACTGGGGTGAGCG-3′) [35] and BOX elements (BoxA1R: 5′-CTACGGCAAGGCGACGCTGACG-3′) [17] were used for DNA fingerprinting.

Phylogenetic analysis

Individual trees based on the partial sequences of the 16S rRNA (1300 nucleotides) and *gyrB* (801 nucleotides) genes have been included in the analysis, together with the *rpoD* (737 nucleotides) gene sequence. An analysis of these three concatenated gene sequences was also performed with a total of 2838 nt [25]. An update (until 2018) of the type strains of all species in the *P. pertucinogena* phylogenetic group, as defined by Mulet and collaborators [27], was included and compared in the present study (Table S1). The Jukes–Cantor (JC) [14], maximum likelihood (ML) [9] and maximum parsimony (MP) [22] algorithms were used for the comparisons.

Genome sequencing and analysis

Genomic DNA was isolated from strain V113[T], using the Wizard Genomic DNA Purification kit (Promega) according to the manufacturer instructions. The Illumina HiSeq 2000 obtained paired-end library reads were *de novo* assembled using the Newbler Assembler v2.7 program (Roche). The draft genome was annotated using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP). The Whole Genome Shotgun project for strain V113[T] has been deposited in DDBJ/ENA/GenBank under the accession number LMAZ00000000. The version described in this paper is the first version, LMAZ01000000. Analysis and comparison of the functional annotation was done using the Kyoto Encyclopedia of genes and Genomes (KEGG Automatic Annotation Server [KAAS]) [24]. The presence of plasmids has been assessed manually and also with the Plasmid Finder *in silico* web tool [7].

The relatedness of the novel species *Pseudomonas gallaeciensis* V113[T] genome to whole genome shotgun sequences of all species type strains in the *P. pertucinogena* G available in public databases was determined based on the tetranucleotide frequency correlation coefficients (TETRA), average nucleotide identity (ANI) using the BLASTN algorithm (ANIb), and the MUMMER ultra-rapid aligning tool (ANIm) as well as genome-to-genome distance (GGDC) methods. ANIb and ANIm were calculated using the JSpecies software tool available at the webpage http://www.imedea.uib.es/jspecies. The recommended species cut-off was 95% for the ANIb and ANIm indices [32]. GGDC was calculated using the web service http://ggdc.dsmz.de [23] and using the recommended BLAST method. The GGDC results shown are based on the recommended formula 2 which is independent of the genome length and is thus robust against the use of incomplete draft genomes.

Cell morphology and physiological tests

Cell size, morphology and flagellar insertion were determined by transmission electron microscopy of cells from the exponential growth phase in LB. A Hitachi model H600 electron microscope was used at 75 kV. The samples were negatively stained with phospho-tungstic acid (1%, pH 7.0) as previously described [19].

The production of fluorescent pigments was tested on King B medium (*Pseudomonas* agar F, Difco), and pyocyanin production was tested on King A medium (*Pseudomonas* agar P, Difco). The strains V113[T], V92 and V120 were characterized phenotypically using API 20 NE strips (bioMérieux), Biolog GN2 and GENIII MicroPlates (Biolog, Hayward, CA). Growth temperatures (4, 6, 10, 15, 18, 25, 30, 37 and 42 °C) were determined in LB medium and growth in the presence of NaCl (0–15% w/v) and pH ranges (4–11) were observed in Nutrient Broth (Merck).

Chemotaxonomic analysis

The chemotaxonomic data obtained with matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) for strains V113[T], V92 and V120 and their closely related species type strains were obtained at the Scientific-Technical Services (University of Balearic Islands, Spain) and analysed as previously described [34].

Whole cell fatty acid methyl esters (FAME) analysis was performed at the Spanish Type Culture Collection, CECT, Valencia, Spain (http://cect.org/identificaciones) under high standardized conditions. Fatty acids were extracted, prepared and analysed as described in Ref. [34].

Results and discussion

Phylogenetic analysis

A preliminary analysis of the partial sequences of the *rpoD* gene compared among all of the *Pseudomonas* type strains revealed that strains V113[T], V92 and V120 could be representatives of a new species [26]. A complete phylogenetic analysis has now been accomplished.

In all individual and concatenated gene sequence trees studied, strains V113[T], V92 and V120 were located in the same branch, independent from the other type strains in the *P. pertucinogena* group, with *Pseudomonas pachastrellae* CCUG 46540[T] being the closest type strain to the group (Figs. 1 and 2). High bootstrap values supported the following JC tree branches: concatenated and *rpoD* gene Trees 100%, 16S rRNA gene Tree 90%, and *gyrB* gene Tree
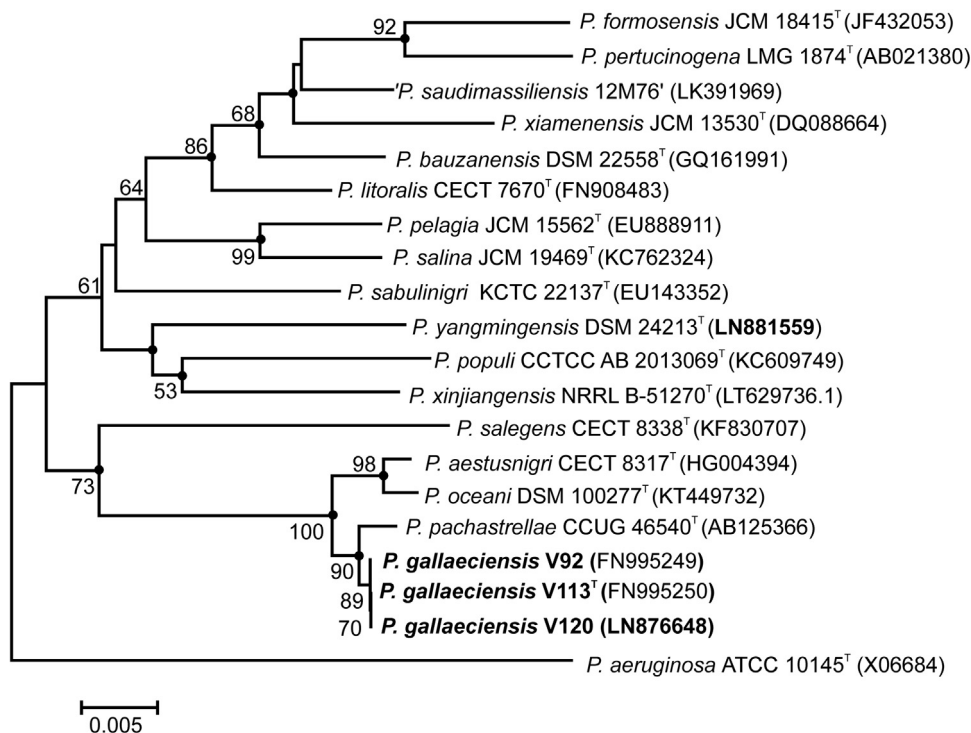
Fig. 1. Phylogenetic tree based on 16S rRNA gene of *Pseudomonas gallaeciensis* and phylogenetically close members of *Pseudomonas*. Distance matrices were calculated by the Jukes–Cantor method. Dendrograms were generated by the neighbour-joining method. *P. aeruginosa* ATCC 10145[T] was used as the outgroup. The bar indicates sequence divergence. Percentage bootstrap values higher than 50% (from 1000 replicates) are indicated at the nodes. Filled circles indicate that the corresponding nodes were also obtained in the trees generated with the maximum likelihood and maximum parsimony methods. GenBank accession numbers are given in parentheses. Accession numbers indicated in bold are for sequences determined in this study.

72% (*rpoD* and *gyrB* gene Tree data not shown). The three strains were 99% similar in the concatenated nucleotide sequences, and at least nine different nucleotides were detected in their concatenated sequences. The analysis of the concatenated sequences showed that the closest type strain to strains V113[T], V92 and V120 was *P. pachastrellae* CCUG 46540[T] (93.9, 94.0 and 93.8% similarity, respectively). Similar results with high bootstrap values were obtained when ML and MP algorithms were used. Common node branches of the three trees are indicated in Fig. 2. The intragroup average similarity value calculated for members of the *P. pertucinogena* group was 85.0 ± 3.6% (Table S2). The 94% similarity between strain V113[T] and *P. pachastrellae* affiliated this strain with the *P. pertucinogena* group; however, this similarity is lower than 97%, the threshold established to discriminate among species in the genus *Pseudomonas* by MLSA of three concatenated gene sequences [25]. Strains V113[T], V92 and V120 could not be affiliated with any *Pseudomonas* species previously described and should be considered representatives of a new species.

Phenotypic characteristics

Strain V113[T] was a Gram-negative, rod shaped bacterium (0.9–2.0 μm long and 0.6 μm wide) that was motile by means of a single polar flagellum (Fig. S1). Strains V113[T], V92 and V120 were positive for catalase and oxidase activities. After incubation for 48 h at 30 °C on LB plates, colonies were round, convex, beige in colour, bright and with entire margins (1–4 mm of diameter). Strains V113[T], V92 and V120 were able to grow in LB medium at 6–37 °C. No growth was detected at 4 °C or 42 °C. The optimum growth was between 25–30 °C. Growth was observed on Nutrient Broth in the presence of 2–13% NaCl (w/v), optimum 4-8%, and the bacteria tolerated a pH ranging from 5 to 10 (optimum 6) (Table 1). Strains V113[T], V92 and V120 and all the other strains

in the *P. pertucinogena* group failed to produce either fluorescent pigments or pyocyanin when cultured for 24–48 h at 30 °C on King B or King A medium. The differential phenotypic characteristics in the API 20NE, Biolog GN2 and GENIII tests are indicated in Table 1. Strains V113[T], V92 and V120 shared the ability to assimilate capric acid, adipic acid, malic acid; positive for utilization of L-alanine, L-glutamic acid, glucuronamide, methyl pyruvate, L-lactic acid, Tween 40, β-hydroxy-D, L-butyric acid, acetoacetic acid, propionic acid, acetic acid, Tween 80, sebacic acid, L-asparagine and positive for the sensitivity of 1% sodium lactate, fusidic acid, D-serine, troleandomycin, rifamycin SV, lincomycin, guanidine HCl, niaproof 4, vancomycin, tetrazolium violet, tetrazolium blue, lithium chloride, potassium tellurite, aztreonam, sodium butyrate. Other 14 tests were variable, indicating a high intraspecies diversity. The rest of tests were negative or weak. In contrast, *P. pachastrellae* was unable to use L-alaninamide, D-alanine and L-proline. *P. aestusnigri* was unable to assimilate glucose, arabinose and malic acid, and negative for L-arabinose, cis-aconitic acid, L-alaninamide and D-alanine.

P. oceani could be differentiated from all type strains analyzed in this study by the ability to use L-fucose, gelatin, L-phehylalanine and α-keto valeric acid and was negative for the utilization of D-lactic acid. Regarding the oxidation tests performed with Biolog GN2 and GENIII, the type strains of the *P. pertucinogena* group and V113[T], V92 and V120 showed a limited ability to use carbon sources, with negative or weak results for a large number of substrates as previously published [15,33,34].

Chemotaxonomic analysis

The protein profiles obtained showed that the three strains, V113[T], V92 and V120, were closely related (100% similarity). The *P. pachastrellae* type strain was the closest strain (95% similarity), and the similarities to the other strains in the *P. pertucinogena* group
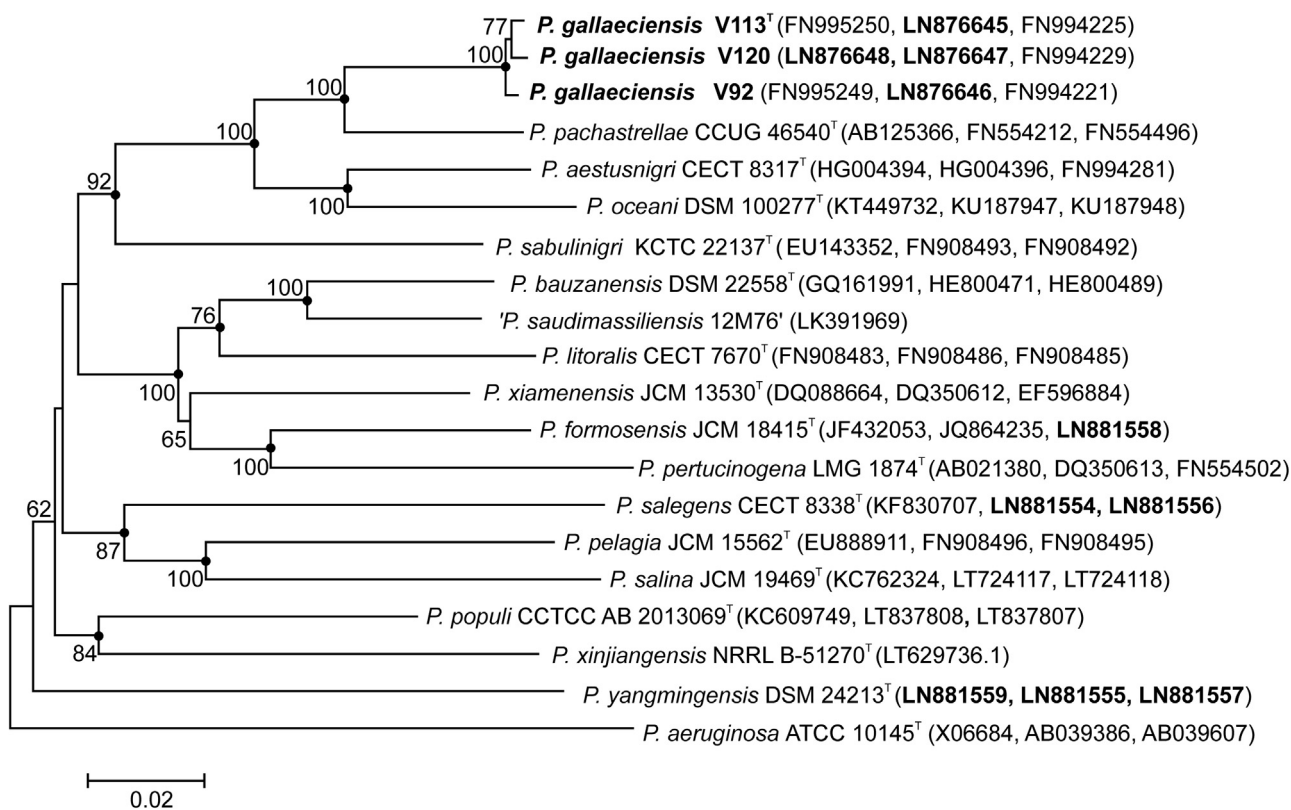
Fig. 2. Phylogenetic tree based on concatenated sequences of the 16S rRNA, *gyrB* and *rpoD* genes of *Pseudomonas gallaeciensis* and phylogenetically close members of *Pseudomonas*. Distance matrices were calculated by the Jukes–Cantor method. Dendrograms were generated by the neighbour-joining method. *Pseudomonas aeruginosa* ATCC 10145[T] was used as the outgroup. The bar indicates sequence divergence. Percentage bootstrap values higher than 50% (from 1000 replicates) are indicated at the nodes. Filled circles indicate that the corresponding nodes were also obtained in the trees generated with the maximum-likelihood and maximum parsimony methods. GenBank accession numbers are given in parentheses in the following order: 16S rRNA, *gyrB* and *rpoD* genes. Accession numbers indicated in bold are for sequences determined in this study.

Table 1
Differential phenotypic characteristics of *Pseudomonas gallaeciensis* from related *Pseudomonas* type strains. Strains: *P. gallaeciensis* (1. V113[T], 2. V92, 3. V120), 4. *P. pachastrellae* CCUG 46540[T], 5. *P. aestusnigri* CECT 8317[T], 6. *P. oceani* DSM 100277[T].

Characteristics	1	2	3	4	5	6
Temperature (°C)	6–37	10–37	6–37	7–41	6–37	4–41
NaCl (%) (w/v)	2–13	2–12	2–12	0–10[a]	2–10[b]	0–10[c]
pH	5–10	6–10	5–9	ND	6–10[b]	6–10[c]
Reduction of nitrate (API 20NE test)	−	+	−	−	−	−
Growth on (API 20NE test)						
Glucose	w	+	+	+	−	+
Arabinose	w	w	w	+	−	W
Malic acid	+	+	+	+	−	W
Trisodium citrate	+	−	+	+	−	+
Biolog GENIII test						
L-Fucose	−	−	−	−	−	+
Minocycline	−	w	−	+	−	−
Gelatin	−	−	−	−	−	+
Biolog GN2 test						
α-Cyclodextrin	−	w	−	+	−	− or w[c]
L-Arabinose	+	w	w	+	−	− or w[c]
cis-Aconitic acid	+	w	+	+	−	− or w[c]
α-Keto valeric acid	−	−	−	−	−	+[c]
D-Lactic acid	+	+	+	+	+	−[c]
L-Alaninamide	+	w	+	−	−	+[c]
D-Alanine	+	w	+	−	−	+[c]
L-Phenylalanine	−	−	−	−	−	+[c]
L-Proline	+	w	+	−	+	+[c]
Putrescine	w	w	−	−	−	+[c]

Positive (+), negative (−), weak (w) and not determined (ND). Unless otherwise indicated data were obtained in this study. [a,b,c] data taken from [a]Romanenko et al. [33], [b]Sanchez et al. [34] and [c]Wang and Sun [36]. Oxidase and catalase tests were positive for all strains.

Table 2

Cellular fatty acid composition (%) of *Pseudomonas gallaeciensis*, and its closest related species type strains of the genus *Pseudomonas*.

Fatty acid (%)	1	2	3	4	5	6
C12:0	9.3	8.7	10.1	10.1	8.9	8.5
C14:0	1.1	0.9	1.2	1.6	0.9	1.3
C16:0	14.8	12.8	14.6	16.9	16.2	20.5
C18:0	1.0	0.8	0.8	0.6	0.7	1.1
C10:0 3-OH	5.0	4.4	5.7	3.9	5.3	4.9
C12:0 3-OH	4.2	4.1	4.8	5.1	4.7	3.8
C17:0 cyclo	0.0	0.0	0.0	0.0	1.3	0.8
Summed features 3	29.3	26.7	32.3	34.5	25.3	24.4
Summed features 8	33.4	35.1	30.0	26.9	34.5	30.9

All strains were cultured on tryptone soy agar (TSA) and incubated at 28 °C for 48 h. Strains: *P. gallaeciensis* (1. V113[T]; 2. V92; 3. V120), 4, *P. pachastrellae* CCUG 46540[T], 5. *P. aestusnigri* CECT 8317[T], 6. *P. oceani* DSM 100277[T]. Data for *P. aestusnigri* CECT 8317[T] were from Sanchez et al. [34]. Summed feature represents groups of two or more fatty acids that could not be separated by the Microbial Identification System. Summed Feature 3, $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$; Summed feature 8, $C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$.

were lower than 80%. In the MALDI-TOF MS analysis, V113[T], V92 and V120 showed three common m/z peaks (supermass values) that were not present in *P. pachastrellae*, the closest type strain: 4808 m/z, 6901 m/z and 9617 m/z (Fig. S2). These data further supported the conclusion that these strains represented a distinct species that was separated from all other species of the *Pseudomonas* genus, even at the level of expression of the most abundant cellular proteins.

Fatty acid profiles were similar in strains V113[T], V92 and V120 (Table 2). Strains V113[T], V92 and V120 exhibited very similar profiles, and summed feature 8 ($C_{18:1}$ $\omega6c$ and/or $C_{18:1}$ $\omega7c$, 35.1–30.0%), summed feature 3 ($C_{16:1}$ $\omega7c$ and/or $C_{16:1}$ $\omega6c$, 32.3–26.7%), $C_{16:0}$ (14.8–12.8%), $C_{12:0}$ (10.4–8.7%), $C_{10:0}$ 3-OH (5.7–4.4%) and $C_{12:0}$ 3-OH (4.8–4.1%) were the most abundant fatty acids. The fatty acid profiles of strains V113[T], V92 and V120 were similar to those of species of the *P. pertucinogena* group, and they had the three fatty acids typically present in the genus *Pseudomonas*, which are $C_{10:0}$ 3-OH, $C_{12:0}$ 2-OH and $C_{12:0}$ 3-OH, according to Palleroni [29].

Total DNA fingerprinting

Total DNA fingerprinting by ERIC and BOX PCRs showed that isolates V113[T], V92 and V120 were representatives of three different strains (Fig. S3). The three *P. gallaeciensis* strains had six identical bands in the ERIC-PCR (425 bp, 525 bp, 1150 bp, 1200 bp, 1350 bp and approximately 2500 bp); strain V113[T] had three additional bands (750 bp, 925 bp and approximately 1900 bp) and V120 had five additional bands (475 bp, 600 bp, 850 bp, 1300 bp and 1900 bp). In the BOX-PCR, strain V113[T] had two additional bands (425 bp and 830 bp), V92 had three (400 bp, 600 bp and 800 bp) and V120 had four (200 bp, 475 bp, 775 bp and 1100 bp). The DNA profiles were also different from those of the type strains of *P. pachastrellae* and *P. aestusnigri*.

General taxonomic genome features of strain V113[T]

The draft genome was assembled in 18 scaffolds (71 contigs) with an average of 75× coverage. No plasmids were detected. The main characteristics of the whole-genome sequence of strain V113[T] are depicted in Table 3. All genome relatedness values of strain V113[T] calculated by the algorithms ANIb, ANIm and GGDC against the species type strains of the *P. pertucinogena* group were clearly below the established cut-off for each (ANIb: 95%, ANIm: 95%, GGDC: 70%), confirming that strain V113[T] represents a novel genomic species in the group. The results are shown in Table S3.

Table 3

Genomic characteristics of *Pseudomonas gallaeciensis* V113[T] sp. nov.

Characteristics	V113[T]
GeneBank ID	LMAZ00000000
Genome size (bp)	4,246,542
No. scaffolds/contigs	18/71
N50 scaffold/contig size	509,657/154,985
Largest contig size	1,749,682
Q40 Plus Bases (%)	4,232,689
Q39 Minus Bases (%)	502
GC-content (%)	61,2%
Total genes	3905
Protein-coding genes (CDS)	3806
No. hypothetical proteins	1321
RNA genes (clusters)	1
tRNAs	46
Pseudogenes[a]	49
Mobilome:	
Integrases	2
Transposases	11

[a] The number of total Pseudogenes indicated includes genes with ambiguous residues, frameshifted genes, incomplete genes, genes with internal stops or other multiple problems.

Insights from the genome sequence

Three thousand eight-hundred and six of the 3,905 total genes were predicted as protein-coding genes (CDS) in the genome of strain V113[T]. Genome analysis allowed the prediction of several relevant metabolic traits. Nitrogen could be assimilated by reducing nitrate to nitrite and ammonia, and sulfate and sulfur reduced to sulfide for biosynthesis. Ferric enterobactin was detected as siderophore for iron adquisition. Sugars might be metabolized by the glycolytic or the pentose phosphate pathways.

The strains were isolated from a highly polluted area after a crude-oil spill. Therefore, the presence of genes for the biodegradation of aromatic and lineal hydrocarbons was studied. A complete set of 16 genes with a regulatory protein of the Fis family was detected for the degradation pathway of monoaromatics, related to the catabolism of toluene, benzene and phenol. A multicomponent phenol hydroxylase (P0, P1, P2, P3, P4 and P5) predicted to produce catechol or 4-methylcatechol, which might be meta cleaved by catechol 2,3-dioxigenase to produce pyruvate and acetyl–CoA. This set of genes was 97-100% identical to the corresponding genes of the close-related species *P. aestusnigri* and *P. pachastrellae* type strains [11,12]. A complete set of genes related to alkane degradation pathway were detected. The alkane 1-monooxygenase and the rubredoxin conducting to the corresponding fatty acid were 98% identical to the homologous proteins in *P. pachastrellae.*

Genes related with the interaction with the environment, like those related to secretion systems, biofilm formation or motility were studied in detail. Types II and VI secretion systems were detected, but no evidence of Types I, III, IV or V was found. Additionally, systems related to quorum sensing pathways (*BarA*/*Uvry*/*CsrA* system), to biofilm formation (*GacA*/*Rsm pathway*) and involved in alginate biosynthesis have been found. Genes related to efflux pumps as *mexH*, *mexI* and *oprM* involved in betalactam resistance have been found and also the multidrug resistance efflux pump *mtd* genes. Together with flagellation, genes for twitching motility and swarming were present.

The mobilome represents one of the main contributors to bacterial intraspecies variability. The prophage contribution to the bacterial genome is highly variable. A cluster of 28 genes, sixteen bacteriophage structural proteins, and 12 hypotetical proteins related to phages were found in scaffold 3 (ASB58_11015 to 11150). Additionally, 11 transposases and 2 integrases were found scattered in the chromosome.

Table 4
Protologue for *Pseudomonas gallaeciensis* sp. nov.

Taxonumber	TA00074
Species name	*Pseudomonas gallaeciensis*
Genus name	*Pseudomonas*
Specific epithet	*gallaeciensis*
Species status	sp. nov.
Species etymology	N.L. fem. adj. gallaeciensis, pertaining to Galicia, Spain, where the type strain was isolated
Designation of the type strain	V113
Strain collection numbers	CCUG 67583, LMG 29038
16S rRNA gene accession number	FN995250
Alternative housekeeping genes	*rpoD* [FN994225], *gyrB* [LN876645]
Genome accession number	LMAZ00000000
Genome status	draft
Genome size	4246542
GC mol %	61.2
Country of origin	Spain
Region of origin	Galicia
Other	Lariño municipality
Date of isolation	01 September 2004
Source of isolation	Sand contaminated by Prestige crude oil
Sampling date	01 September 2004
Geographic location	Praia da Seda beach
Latitude	42°46′29.2″N
Longitude	9°7′27.1″W
Depth	0
Altitude (alti)	0
Number of strains in study	3
Source of isolation of non-type strains	Praia da Seda beach, Lariño municipality, Galicia, Spain
Growth medium, incubation conditions used for standard cultivation	Luria-Bertani medium (LB) at 30 °C
Gram stain	NEGATIVE
Cell shape	rod
Cell size (length or diameter)	0.9–2.0 µm long and 0.6 µm wide
Motility	Motile
If motile	Flagellar
If flagellated	One polar flagellum
Sporulation (resting cells)	None
Colony morphology	Round, convex, colour beige, bright and with entire margins (1–4 mm diameter) on LB plates after incubation for 48 h at 30 °C
Temperature range	6–37
Lowest temperature for growth	6
Highest temperature for growth	37
Temperature optimum	25–30
Lowest pH for growth	5
Highest pH for growth	10
Lowest NaCl concentration for growth	2
Highest NaCl concentration for growth	13
Salinity category	Moderate halophile (optimum 7–15% NaCl)
Positive tests with BIOLOG	L-Alanine, L-glutamic acid, glucuronamide, methyl pyruvate, L-lactic acid, Tween 40, β-hydroxy-D,L-butyric acid, acetoacetic acid, propionic acid, acetic acid, Tween 80, sebacic acid, L-asparagine, 1% sodium lactate, fusidic acid, D-serine, troleandomycin, rifamycin SV, lincomycin, guanidine HCl, niaproof 4, vancomycin, tetrazolium violet, tetrazolium blue, nalidixic acid, lithium chloride, potassium tellurite, aztreonam, sodium butyrate
Negative tests with BIOLOG	Negative all strains: D-maltose, D-trehalose, D-cellobiose, gentiobiose, sucrose, turanose, stachyose, D-raffinose, α-D-lactose, D-melibiose, b-methyl-D-glucoside, D-salicin, N-acetyl-D-glucosamine, N-acetyl-β-D-mannosamine, N-acetyl-D-galactosamine, N-acetyl-neuraminic acid, α-D-glucose, D-mannose, D-fructose, D-galactose, 3-methyl glucose, D-fucose, L-fucose, L-rhamnose, inosine, D-sorbitol, D-mannitol, D-arabitol, myo-Inositol, D-glucose-6-PO4, D-fructose-6-PO4, D-aspartic acid, D-serine, gelatin, glycyl-L-proline, L-arginine, L-aspartic acid, L-histidine, L-pyroglutamic acid, L-serine, pectin, D-galacturonic acid, D-galactonic acid lactone, D-gluconic acid, D-glucuronic acid, mucic acid, quinic acid, D-saccharic acid, p-hydroxy-phenylacetic acid, D-lactic acid methyl ester, D-malic acid, gamma-amino-butyric acid, α-keto-butyric acid, formic acid, sodium bromate, α-cyclodextrin, i-erythritol, lactulose, D-psicose, xylitol, D-glucosaminic acid, itaconic acid, α-keto valeric acid, malonic acid, L-alanyl-glycine, glycyl-L-aspartic acid, L-histamine, hydroxy-L-proline, L-leucine, L-ornithine, L-phenylalanine, L-threonine, D,L-carnitine, urocanic acid, uridine, thymidine, phenylethylamine, 2,3-butanediol, glycerol, D,L-α-glycerol phosphate, glucose-1-phosphate Weak or negative: dextrin, glycogen, α-hydroxy butyric acid, adonitol, glycyl-L-glutamic acid, putrescine, 2-aminoethanol
Variable tests with BIOLOG	Minocycline, citric acid, α-keto glutaric acid, L-malic acid, bromo-succinic acid, nalidixic acid, acetoacetic acid, L-arabinose, mono-methyl-succinate, cis-aconitic acid, succinic acid, succinamic acid, L-alaninamide, D-alanine, L-proline
Positive tests with API	CAP, ADI, MLT
Negative tests with API (APIN)	NEGATIVE: TRP, GLU, ADH, URE, ESC, GEL, PNPG, MNE, MAN, NAG, MAL, GNT, PAC, WEAK: ARA
Variable tests with API (APIV)	NO3, GLU (fermentation), CIT
Commercial kits used	BIOLOG GENIII, BIOLOG GN2, API 20NE
Energy metabolism	Chemoorganotroph

Table 4 (*Continued*)

Oxidase	Positive
Catalase	Positive
Negative tests	Fluorescence on King A and King B agar
Major fatty acids	Summed feature 8 ($C_{18:1}$ $\omega6c$ and/or $C_{18:1}$ $\omega7c$, 35.1–30.0%), summed feature 3 ($C_{16:1}$ $\omega7c$ and/or $C_{16:1}$ $\omega6c$, 32.3–26.7%), $C_{16:0}$ (14.8–12.8%), $C_{12:0}$ (10.4–8.7%), $C_{10:0}$ 3-OH (5.7–4.4%) and $C_{12:0}$ 3-OH (4.8–4.1%)
Biosafety level	1
Habitat	Beach sand (http://purl.obolibrary.org/obo/ENVO_00002138)
Biotic relationship	Free-living
Known pathogenicity	None

Conclusion

The chemotaxonomic data supported by MALDI-TOF and cell fatty acid methyl esters of the strains V113^T, V92 and V120 clearly locate these strains in the *Pseudomonas* genus. The genomic sequences of the housekeeping genes studied (16S rRNA, *rpoD*, *gyrB*) indicate that the three strains are representative of a new species. The ANIb genome analysis confirms this presumption. Strains V113^T, V92 and V120 were isolated from different sites of the intertidal coast and are members of a new bacterial species able to persist in a contaminated environment.

Considering the phylogenetic, chemotaxonomic and phenotypic characteristics presented, we propose a new species, *P. gallaeciensis* sp. nov., with *P. gallaeciensis* V113^T as the type strain. The full description of the new taxon is shown in Table 4 together with the description of the closest species in Table S4 as obtained from the Digital Protologue website (http://imedea.uib-csic.es/dprotologue/) in which the new species was registered under reference TA00074.

Note

The GenBank/EMBL/DDBJ accession numbers for the nucleotide sequences reported in this study are as follows: LN876648 and LN881559 (strain *P. gallaeciensis* V120 and *Pseudomonas yangmingensis* DSM 24213^T for the 16S rRNA), LN876645, LN876646, LN876647, LN881554, LN881555, LT724117 and LT837808 (strain *P. gallaeciensis* V113^T, V92, V120, *P. salegens* CECT 24213^T, *P. yangmingensis* DSM 24213^T, *P. salina* JCM 19469^T and *P. populi* CCTCC AB 2013069^T for the *gyrB* gene), LN881556, LN881557, LN881558, LT724118 and LT837807 (strain *P. salegens* CECT 8338^T, P. *yangmingensis* DSM 24213^T, *Pseudomonas formosensis* JCM 18415^T, *P. salina* JCM 19469^T and *P. populi* CCTCC AB 2013069^T for the *rpoD* gene).

Acknowledgements

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.syapm.2018.03.008.

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