Pseudomonas litoralis sp. nov., isolated from Mediterranean seawater

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Strains 2SM5^T and 2SM6, two strictly aerobic chemo-organotrophic gammaproteobacteria, were isolated from Mediterranean seawater off the coast of Vinaroz, Castellón, Spain, in February, 1990. They were extensively characterized by a polyphasic study that placed them in the genus *Pseudomonas*. Phylogenetic analysis of 16S rRNA gene sequences showed that both strains shared 100 % sequence similarity and were closely related to members of the *Pseudomonas pertucinogena* clade, with less than 97.3 % similarity to strains of established species; *Pseudomonas xiamenensis* was the closest relative. Analysis of sequences of three housekeeping genes, *rpoB*, *rpoD* and *gyrB*, further confirmed the phylogenetic assignment of the Mediterranean isolates. Chemotaxonomic traits such as quinone and polar lipid composition also corroborated the placement of strains 2SM5^T and 2SM6 in the gammaproteobacteria. Other phenotypic traits, including fatty acid composition, enabled clear differentiation of both isolates from other species of *Pseudomonas*. We therefore conclude that strains 2SM5^T and 2SM6 represent a novel species of *Pseudomonas*, for which the name *Pseudomonas litoralis* is proposed; the type strain is 2SM5^T (=CECT 7670^T=KCTC 23093^T).

The genus *Pseudomonas* Migula 1894 was described originally to include Gram-negative, strictly aerobic rods that are motile by polar flagella. *Pseudomonas* includes a large number of species isolated from a variety of natural sources including soil, plants, water and clinical specimens, and has become a very heterogeneous taxon that is characterized by a high level of metabolic diversity (Rosselló *et al.*, 1991; Palleroni, 2005).

With the application of modern taxonomic techniques, including rRNA–DNA hybridization studies (Palleroni,

1984) and phylogenetic studies based on 16S rRNA gene sequence comparisons (Moore *et al.*, 1996; Anzai *et al.*, 2000), the genus *Pseudomonas* has been split into several genera, some even belonging to different subclasses in the phylum ‘Proteobacteria’, to accommodate bacteria originally described as species of *Pseudomonas*. In spite of this, the genus *Pseudomonas sensu stricto* comprises 120 species with names that have been validly published (<http://www.bacterio.cict.fr>; Yarza *et al.*, 2010). Based on partial sequences of four housekeeping genes (16S rRNA, *gyrB*, *rpoB* and *rpoD*), Mulet *et al.* (2010) have recently identified two main intrageneric groups (IG) among species of the genus *Pseudomonas*, called IG *Pseudomonas aeruginosa* and IG *Pseudomonas fluorescens*. In their study, some species were placed on phylogenetic branches apart from both lineages, namely *Pseudomonas oryzihabitans*, *Pseudomonas psychrotolerans*, *Pseudomonas luteola*, *Pseudomonas pertucinogena* and *Pseudomonas pachastrellae*; among them, the last two always formed the deepest branch. Although not considered in the study by Mulet *et al.* (2010), other recently described species closely related to *P. pertucinogena* (Kawai & Yabuuchi, 1975) and *P. pachastrellae* (Romanenko *et al.*, 2005) are *Pseudomonas pelagia* (Hwang *et al.*, 2009), *Pseudomonas sabulinigri* (Kim *et al.*, 2009), *Pseudomonas xiamenensis* (Lai & Shao, 2008) and *Pseudomonas xinjiangensis* (Liu *et al.*,

Abbreviations: ASW, artificial sea water; DPG, diphosphatidylglycerol; FAMES, fatty acid methyl esters; IG, intrageneric groups; ML, maximum-likelihood; MLSA, multilocus sequence analysis; MP, maximum-parsimony; NJ, neighbour-joining; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PHB, polyhydroxybutyrate.

The GenBank/EMBL/DDBJ accession numbers for strain 2SM5^T (16S rRNA gene, partial *rpoB* gene, partial *rpoD* gene, partial *gyrB* gene), strain 2SM6 (16S rRNA gene, partial *rpoB* gene, partial *rpoD* gene, partial *gyrB* gene), *P. sabulinigri* CECT 7679^T (partial *rpoB* gene, partial *rpoD* gene, partial *gyrB* gene) and *P. pelagia* CECT 7689^T (partial *rpoB* gene, partial *rpoD* gene, partial *gyrB* gene) are FN908483–FN908496, respectively.

Four supplementary figures and three supplementary tables are available with the online version of this paper.

2009). It is interesting to note that although marine environments are not a primary source for the isolation of *Pseudomonas* spp. (Palleroni, 2005), three of these six species (*P. pachastrellae*, *P. pelagia* and *P. sabulinigri*), were isolated from marine habitats.

During a study of bacterial communities in Mediterranean seawater, two strains, 2SM5^T and 2SM6, were isolated from a seawater sample obtained at a depth of 10 m off the coast of Vinaroz, Castellón, Spain (40° 27' 24" N 0° 31' 36" E) in February, 1990. The seawater was decimally diluted in sterile seawater, plated on marine agar (MA) and incubated at 20–25 °C for up to 10 days. Pure cultures were obtained from isolated colonies on the plates. The strains were maintained at –80 °C as a suspension in marine broth (MB) supplemented with 20 % glycerol. Originally, both strains were included in a numerical taxonomic study with several aerobic marine Gram-negative bacteria obtained from similar samples, in which they remained as isolated operational taxonomic units (Ortigosa *et al.*, 1994).

Recently, the partial 16S rRNA gene sequences of strains 2SM5^T and 2SM6 were determined according to Lucena *et al.* (2010). A BLAST search in public databases (Zhang *et al.*, 2000) indicated that the almost complete sequences of both strains had a maximum of around 97.3 % similarity to the type strains of *P. xiamenensis*, *P. pertucinogena* and *P. sabulinigri*, thus providing a first indication that both Mediterranean isolates are representatives of a novel species belonging to the genus *Pseudomonas*. Therefore, we have studied these strains intensely using a polyphasic approach that included phylogenetic analyses based on the 16S rRNA gene and three housekeeping genes (*rpoB*, *rpoD* and *gyrB*), chemotaxonomic analysis [fatty acid methyl esters (FAMES), polar lipids, and isoprenoid quinones] and an extensive biochemical and physiological characterization to determine their taxonomic position.

Reference strains used in this study are as follows: *P. pelagia* CECT 7689^T, *P. pertucinogena* CECT 7776^T, *P. sabulinigri* CECT 7679^T, *P. xiamenensis* DSM 22326^T and *P. xinjiangensis* DSM 23391^T.

Strains 2SM5^T and 2SM6 and reference strains were routinely grown aerobically on MA or MB for 48–72 h at 20–25 °C, in the dark, for inocula or sample preparation. Unless otherwise indicated, all media were supplemented with half-strength artificial seawater (ASW; 400 mM NaCl, 100 mM MgSO₄·7H₂O, 20 mM KCl and 20 mM CaCl₂·H₂O). Methods for phenotypic characterization were performed as described previously by Macián *et al.* (2001, 2005) and Lucena *et al.* (2010). Both isolates were Gram-negative, strictly aerobic, and oxidase- and catalase-positive. Cell morphology, motility and flagellum arrangement were observed on wet mounts under phase-contrast microscopy (Leica DMRB fluorescence microscopy). Strains 2SM5^T and 2SM6 were short rods, 0.5–0.9 µm wide and 0.8–1.9 µm long, motile by a single polar flagellum, as seen after flagella staining according to the method of Heimbrook *et al.* (1989) (Supplementary Fig.

S1, available in IJSEM Online). On MA plates, they produced smooth, beige colonies with regular edges. They did not accumulate polyhydroxybutyrate (PHB) granules, as shown by staining with Nile Blue A (Smibert & Krieg, 1994) of cells grown on MA and basal medium agar [BMA; 50 mM Tris/HCl, pH 7.5; 19 mM NH₄Cl; 0.33 mM K₂HPO₄·3H₂O; 0.1 mM FeSO₄·7H₂O and 1.3 % (w/v) purified agar (Oxoid) on half-strength ASW; Baumann & Baumann, 1981] plus acetate for 5 days at 25 °C. They were not pigmented on King A and King B medium (King *et al.*, 1954). The Mediterranean isolates were able to grow at 15 and 37 °C, but not at 4 or 40 °C, and both grew without added NaCl and in up to 15 % (w/v) total salinity (Table 1). They were able to hydrolyse Tween 80 extracellularly but not alginate, starch, casein, gelatin or DNA after 1 week of incubation on the various media used.

API 20NE and API ZYM test strips (both bioMérieux) and Biolog GN2 microplates were applied to strains 2SM5^T and 2SM6, as well as to reference strains *P. pelagia* CECT 7689^T, *P. pertucinogena* CECT 7776^T, *P. sabulinigri* CECT 7679^T, *P. xiamenensis* DSM 22326^T and *P. xinjiangensis* DSM 23391^T. Cell suspensions for the API 20NE and API ZYM systems were made in half-strength ASW and results were recorded in triplicate. With the API 20NE system, strains 2SM5^T and 2SM6 were positive for assimilation of caprate and adipate. In the API ZYM system, both strains were positive for esterase lipase (C8), leucine arylamidase and naphthol-AS-BI-phosphohydrolase, and negative for all other tests. In the Biolog GN2 system after 48 h incubation, strains 2SM5^T and 2SM6 oxidized the 12 compounds, mainly organic acids, listed in the species description. Strain 2SM5^T weakly oxidized *i*-erythritol, monomethyl succinate, D-galacturonic acid, D-glucuronic acid, succinamic acid, L-leucine and L-proline; oxidation of dextrin, gentiobiose, alaninamide and thymidine was variable. Strain 2SM6 gave variable results for oxidation of L-arabinose, L-rhamnose, citric acid and D-glucuronic acid.

Sole carbon and energy sources, including carbohydrates, organic acids, amino acids and amines, used for growth were tested on BMA according to Baumann & Baumann (1981). This screening was performed in two independent trials. Substrates that supported growth of both isolates after 15 days incubation at 28 °C were acetate, pyruvate, propionate, 2-oxoglutarate, succinate, fumarate, malate, lactate, L-glutamate and L-alanine. Strain 2SM6 was also positive for 4-aminobutyrate and weakly positive for D-glycerate and butyrate. The rest of the carbon sources, from a total of 53, gave negative or variable results (Supplementary Table S1, available in IJSEM Online).

FAME analysis was performed at the CECT, Valencia, Spain. Fatty acids were extracted and prepared according to standard protocols as described for the MIDI Microbial Identification System (Sasser, 1990). Cellular fatty acid content was analysed by GC with an Agilent 6850 chromatographic unit, with the MIDI Microbial Identification System using the RTSBA6 method (MIDI, 2008) and the

Table 1. Differential characteristics of the novel isolates and strains of related species

Strains: 1, *P. litoralis* 2SM5^T and 2SM6; 2, *P. xiamenensis* DSM 22326^T; 3, *P. pertucinogena* CECT 7776^T; 4, *P. pelagia* CECT 7689^T; 5, *P. sabulinigri* CECT 7679^T; 6, *P. xinjiangensis* DSM 23391^T; and 7, *P. pachastrellae* KMM 330^T. Unless otherwise indicated, all data were obtained concurrently in this study. +, Positive; –, negative; NA, not available; v, variable.

Characteristic	1	2	3	4	5	6	7
Reduction of nitrate to nitrite (API 20NE)	–	+	–	+	–	–	– ^a
PHB accumulation	–	NA	– ^b	– ^c	NA	+ ^d	+ ^d
Growth at:							
4 °C	–	– ^e	– ^b	+ ^c	+ ^a	+ ^d	– ^f
40 °C	–	+ ^e	+ ^b	– ^c	– ^a	+ ^d	+ ^f
Maximum salinity tolerance (%)	15	8 ^e	5 ^e	8 ^c	10 ^a	6 ^d	10 ^f
Hydrolysis of:							
Tween 80	+	+ ^e	– ^b	– ^c	NA	+ ^d	+ ^f
Starch	–	– ^e	– ^b	+ ^c	NA	– ^d	– ^f
Enzymic activities (API ZYM):							
Alkaline phosphatase	–	–	+	+	+	+	– ^f
Lipase (C14)	–	v	+	–	+	–	– ^f
Trypsin	–	+	+	–	–	+	– ^f
Substrates oxidized in Biolog GN2:							
Methyl pyruvate	+	+	–	–	+	+	+ ^a
Acetic acid	+	+	+	–	+	+	– ^a
Citric acid	–	+	–	+	+	+	+ ^a
Lactic acid	+	+	–	+	+	–	+ ^a
Propionic acid	+	+	+	–	+	–	+ ^a
Sebacic acid	+	–	–	–	+	–	+ ^a
Succinic acid	+	+	+	+	–	+	+ ^a
L-Glutamic acid	+	–	+	–	+	–	– ^a
L-Proline	–	+	–	+	+	–	– ^a
Assimilation of (API 20NE):							
Caprate	+	–	–	–	+	–	+ ^a
Adipate	+	–	–	–	+	–	– ^a

*Data from: a, Kim *et al.* (2009); b, Kawai & Yabuuchi (1975); c, Hwang *et al.* (2009); d, Liu *et al.* (2009); e, Lai & Shao (2008); f, Romanenko *et al.* (2005).

Microbial Identification Sherlock software package version 6.1. *P. pelagia* CECT 7689^T, *P. pertucinogena* CECT 7776^T, *P. sabulinigri* CECT 7679^T, *P. xiamenensis* DSM 22326^T and *P. xinjiangensis* DSM 23391^T were included for comparative purposes. All strains were cultured on tryptone soy agar (TSA) and incubated at 28 °C for 48 h. Strains 2SM5^T and 2SM6 exhibited very similar profiles in which C_{18:1}ω7c and/or C_{18:1}ω6c, C_{16:0}, C_{17:0} cyclo and C_{16:1}ω7c and/or C_{16:1}ω6c were the most abundant fatty acids. The fatty acid profiles of strains 2SM5^T and 2SM6 were similar to those of related species of the *P. pertucinogena* clade (Table 2). It has to be mentioned that fatty acid compositions of the type strains of *P. xiamenensis* and *P. xinjiangensis* obtained in our laboratory were in sharp contrast to the original data

Table 2. Cellular fatty acid contents (%) of *P. litoralis* strains 2SM5^T and 2SM6 and related species of the intragenetic group '*P. pertucinogena*'

Strains: 1, *P. litoralis* 2SM5^T; 2, *P. litoralis* 2SM6; 3, *P. xiamenensis* DSM 22326^T; 4, *P. pertucinogena* CECT 7776^T; 5, *P. pelagia* CECT 7689^T; 6, *P. sabulinigri* CECT 7679^T; 7, *P. xinjiangensis* DSM 23391^T; 8, *P. pachastrellae* DSM 17577^T (Kim *et al.*, 2009). All data are from this study except where marked. All strains were grown on TSA for 48 h at 28 °C, except for *P. pachastrellae* DSM 17577^T, which was grown for 72 h at 30 °C. –, Not detectable; TR, trace amount (<1 %).

Fatty acid	1	2	3	4	5	6	7	8
Saturated								
C _{12:0}	7.9	7.5	9.3	6.1	8.1	7.6	7.0	10.2
C _{13:0}	–	TR	–	1.3	TR	TR	TR	–
C _{14:0}	TR	1.0	TR	1.5	TR	1.0	TR	–
C _{15:0}	–	–	–	–	–	–	–	1.8
C _{16:0}	18.5	17.4	16.1	7.9	11.8	18.5	3.3	16.9
C _{17:0}	TR	1.8	TR	3.3	TR	TR	–	1.3
C _{18:0}	1.2	1.2	1.1	TR	1.0	1.5	TR	1.1
Unsaturated								
C _{15:1} ω6c	TR	TR	–	1.5	TR	TR	TR	–
C _{17:1} ω8c	TR	1.6	TR	5.3	1.1	TR	TR	1.1
Branched								
iso-C _{16:0}	–	–	–	–	–	–	7.0	–
iso-C _{17:0}	TR	TR	–	1.6	TR	1.2	–	–
iso-C _{18:0}	–	–	–	–	–	–	1.5	–
anteiso-C _{17:0}	–	–	–	TR	–	1.4	–	–
Hydroxy								
C _{10:0} 3-OH	3.0	2.9	3.5	3.7	3.1	2.5	1.6	2.7
C _{11:0} 3-OH	TR	TR	–	1.7	TR	TR	–	–
C _{12:0} 3-OH	4.2	3.9	4.5	3.4	4.4	4.0	4.2	4.2
C _{18:0} 3-OH	–	–	–	2.0	–	–	–	–
Cyclo								
C _{17:0} cyclo	8.9	8.5	5.1	9.0	4.4	1.2	2.0	1.4
C _{19:0} cyclo ω8c	1.8	1.4	3.0	3.8	1.3	TR	2.2	–
Summed features								
C _{16:1} ω7c/C _{16:1} ω6c	18.1	18.3	12.8	14.3	24.6	20.4	33.3	21.6
C _{18:1} ω7c/C _{18:1} ω6c	32.6	31.8	42.5	25.7	35.4	36.8	34.5	35.2

presented in their species descriptions, most probably due to methodological differences (Supplementary Table S2, available in IJSEM Online).

Analyses of respiratory quinones and polar lipids of the isolates were performed independently at the Istituto di Chimica Biomolecolare, CNR, Pozzuoli, Italy. Quinone analysis of strains 2SM5^T and 2SM6 was done according to Romano *et al.* (2001) and both isolates had a ubiquinone (Q-9) system, which is in agreement with the genus description of *Pseudomonas* (Palleroni, 2005). Polar lipids of strains 2SM5^T and 2SM6, together with *P. xiamenensis* DSM 22326^T and *P. pertucinogena* CECT 7776^T as references, were extracted from 3.0 g freeze-dried cells grown in MA for 48 h at 25 °C. Polar lipids were separated by two-dimensional silica-gel TLC and specific functional groups were detected as described previously (Tindall, 1990a, b), whereas total lipid

Fig. 1. Neighbour-joining trees showing the phylogenetic positions of strains 2SM5^T and 2SM6, and some related *Pseudomonas* species based on partial 16S rRNA (a), *rpoB* (b), *rpoD* (c), *gyrB* (d) and concatenated gene sequences (e). Bars, 0.005 (a) or 0.05 (b–e) expected nucleotide substitutions per site. Only bootstrap values $\geq 75\%$ are shown (1000 resamplings) at branch points. According to NCBI, sequence AJ717459 in Fig. 1(b) originates from strain *P. stutzeri* CIP 11199^T; however, the correct designation should be *P. stutzeri* LMG 11199^T.

material was stained with 0.1% $\text{Ce}(\text{SO}_4)_2$ followed by heating at 100 °C for 5 min (Romano *et al.*, 2001). All four strains had phosphatidylethanolamine (PE), diphosphatidylglycerol (DPG) and phosphatidylglycerol (PG) as major components (Supplementary Fig. S2, available in IJSEM Online), which is in agreement with data published previously for *Pseudomonas* species (Cámara *et al.*, 2007; Romanenko *et al.*, 2005, 2008; Stolz *et al.*, 2007; Xin *et al.*, 2009). In addition, a moderate amount of an unknown phospholipid (PL1) was detected in strains 2SM5^T and 2SM6 and in *P. xiamenensis* DSM 22326^T but not in *P. pertucinogena* CECT 7776^T. Since some *Pseudomonas* species exhibit phosphatidylcholine in a similar position, Dragendorff's reagent was applied (analysis done at CECT) but no spot was stained, an indication that quaternary amines are not present.

The presence of 3-hydroxy fatty acids ($\text{C}_{10:0}$ 3-OH and $\text{C}_{12:0}$ 3-OH), together with ubiquinone Q-9, and their polar lipid profile confirm the placement of the Mediterranean isolates in the genus *Pseudomonas sensu stricto* (Oyaizu & Komagata, 1983).

Isolation of genomic DNA was done using a microbial DNA isolation kit (Ultra Clean; Mo Bio Laboratories). Amplification and partial sequencing of coding genes were performed as described previously by Lucena *et al.* (2010) (16S rRNA gene), Ait Tayeb *et al.* (2005) (*rpoB* gene) and Pascual *et al.* (2010) (*rpoD* and *gyrB* genes). The almost complete 16S rRNA gene sequences of strains 2SM5^T and 2SM6 as well as partial *rpoB*, *rpoD* and *gyrB* gene sequences of 2SM5^T, 2SM6, *P. sabulinigri* CECT 7679^T and *P. pelagia* CECT 7689^T were obtained in this study. The rest of the sequences analysed in this paper were obtained from public databases and their accession numbers are displayed in the phylogenetic trees.

The lengths of the fragments used for further analysis ranged from 1403 to 1537 nt (16S rRNA gene), 909 to 1140 nt (*rpoB*), 723 to 857 nt (*rpoD*), 798 to 1164 nt (*gyrB*), and 3882 to 4569 nt (four concatenated genes). Multiple sequence alignments were obtained using CLUSTAL_X (Thompson *et al.*, 1997) taking into account the corresponding amino acid alignments for protein-coding genes. Nucleotide sequence alignments were inspected visually to identify positions of uncertain alignment, usually at the ends of the sequences, to be corrected or omitted for further analysis.

Phylogenetic analysis was performed using the program PAUP* version 4.0b10 (Swofford, 2002). Neighbour-joining (NJ; with Kimura's two-parameter correction), maximum-parsimony (MP; heuristic search option) and maximum-likelihood (ML) analyses were done for each of the genes and the concatenated sequence. Since the lengths of the sequences used were uneven, analyses were performed using a pairwise deletion method for gaps and missing sites, using all available comparative data from each sequence pair. For ML, the optimal models of nucleotide substitution were estimated through the program jModelTest (Posada, 2008) using the Akaike Information Criterion. Bootstrap analyses

were performed using 1000 replications for NJ and MP, and 500 replications for ML. The different trees obtained with the NJ, MP and ML methods are shown in Fig. 1, and Supplementary Figs S3 and S4 (available in IJSEM Online), respectively. Supplementary Table S3 shows the pairwise sequence similarities (%) of each dataset. All phylogenetic analyses of the 16S rRNA, *rpoB*, *rpoD* and *gyrB* genes individually and concatenated, display strains 2SM5^T and 2SM6 together. Irrespective of the tree reconstruction method applied, all trees analysed except for the *gyrB* trees showed the type strains of *P. xiamenensis* and *P. pertucinogena* as the nearest node to strains 2SM5^T and 2SM6, with all four strains forming a robust clade supported by high bootstrap values. The relative positions of the rest of the species belonging to the *P. pertucinogena* clade, *P. pachastrellae*, *P. pelagia*, *P. sabulinigri* and *P. xinjiangensis*, varied depending on the method applied (NJ, MP and ML) and the set of sequences used. Amino acid sequences were also analysed through MP and NJ methods applying the Poisson model (not shown). In all eight trees obtained, strains 2SM5^T and 2SM6 joined the same immediate neighbours as in the nucleotide-based trees, although other aspects of the tree topologies could be different.

Since multilocus sequence analysis (MLSA) uses a higher number of nucleotides than a single gene analysis, and avoids possible effects that the horizontal gene transfer could generate on phylogenetic relationships, it could be considered that MLSA shows the correct evolutionary pattern among the species included in this study. Even so, screening of recombination events in the four genes studied by using the RDP3 Beta 27 program (Martin *et al.*, 2005) and according to Pascual *et al.* (2010), did not reveal any signal of horizontal gene transfer in the sequences of each gene analysed.

The similarity values obtained between isolates 2SM5^T and 2SM6 in all four gene sequences analysed individually was always 100%, thus confirming their phylogenetic placement in the same species (Supplementary Table S3). The highest interspecific sequence similarities found between both isolates and their phylogenetic neighbours in the *P. pertucinogena* clade were 97.3% (16S rRNA), 88.7% (*rpoB*), 82.3% (*rpoD*), 84.3% (*gyrB*) and 89.4% (MLSA of four genes concatenated) to *P. xiamenensis* in all cases (Supplementary Table S3). On the one hand, the maximum interspecific similarity found with the 16S rRNA gene sequences is slightly above the generally accepted threshold value (97%) that differentiates prokaryotic species (Tindall *et al.*, 2010). On the other hand, the maximum interspecific similarities found with the house-keeping genes individually are clearly lower than the maximum interspecific similarity found among the rest of the *Pseudomonas* species analysed, 93.6% (*rpoB*), 87.1% (*rpoD*), 88.8% (*gyrB*) and 92.6% (MLSA) (Supplementary Table S3). Furthermore, the maximum interspecific values obtained in this work are in agreement with those previously observed (Lai & Shao, 2008; Mulet *et al.*, 2010), thus confirming the separate species status of strains

2SM5^T and 2SM6. Additionally, analysis of amino acid sequences, which are typically more conserved than their nucleotide counterparts, confirmed the results already presented. The intraspecific amino acid sequence distance between strains 2SM5^T and 2SM6 was 100 % in all three protein-coding genes. On the other hand, the highest interspecific amino acid sequence similarity found between both isolates and their closest phylogenetic neighbours was 96.3 % (*rpoB*), 82.6 % (*rpoD*) and 93.7 % (*gyrB*). These values are lower than those found among other species belonging to the *P. pertucinogena* clade (data not shown).

As shown in Table 1, our Mediterranean isolates can be distinguished from their closest phylogenetic neighbour *P. xiamenensis* based on several traits: nitrate reduction ability; growth at 40 °C; salt tolerance range; presence of trypsin activity; oxidation of citric acid, sebacic acid, L-glutamic acid and L-proline in Biolog GN2; and assimilation of caprate and adipate in API 20NE strips. Moreover, strains 2SM5^T and 2SM6 could be also distinguished from the rest of the species in the *P. pertucinogena* clade by no less than seven differential characteristics including, among others, the inability of our isolates to accumulate PHB, to grow at 4 and 40 °C, and their moderate salt tolerance (up to 15 % NaCl) (Table 1).

Phenotypic, including chemotaxonomic, and phylogenetic results presented here support the proposal of strains 2SM5^T and 2SM6 as members of a novel species of *Pseudomonas*, *Pseudomonas litoralis* sp. nov., with strain 2SM5^T as the type strain.

Description of *Pseudomonas litoralis* sp. nov.

Pseudomonas litoralis (li.to.ra'lis. L. fem. adj. *litoralis* of or belonging to the seashore).

Cells are Gram-negative, short rods, 0.5–0.9 µm wide and 0.8–1.9 µm long, motile by a single polar flagellum. Chemo-organotrophic, strictly aerobic, and oxidase- and catalase-positive. Grows on MA producing smooth, beige colonies with regular edges. Does not accumulate PHB granules and is not pigmented on King A or King B media. Both isolates grow at 15 and 37 °C, but not at 4 or 40 °C. Growth is observed without added NaCl and in up to 15 % total salinity. In API 20NE, positive for assimilation of caprate and adipate and negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, hydrolysis of aesculin and gelatin, β-galactosidase, and assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, N-acetylglucosamine, maltose, potassium gluconate, malic acid, trisodium citrate and phenylacetic acid. Hydrolyses Tween 80 extracellularly but not alginate, starch, casein, gelatin or DNA. In the API ZYM system, positive for esterase lipase (C8), leucine arylamidase and naphthol-AS-BI-phosphohydrolase. In the Biolog GN2 system, oxidizes Tween 40, Tween 80, methyl pyruvate, acetic acid, DL-lactic acid, propionic acid, sebacic acid, succinic acid, bromosuccinic acid, D-alanine, L-alanine and L-glutamic acid. On BMA, utilizes acetate, pyruvate, propionate, 2-oxoglutarate,

succinate, fumarate, malate, lactate, L-glutamate and L-alanine, out of 53 carbon sources tested, as sole carbon and energy sources. The most abundant FAMES are C_{18:1}ω7c and/or C_{18:1}ω6c, C_{16:0}, C_{17:0} cyclo and C_{16:1}ω7c and/or C_{16:1}ω6c. Ubiquinone Q-9 is the isoprenoid quinone, and diphosphatidylglycerol, phosphatidylethanolamine and phosphatidylglycerol are major polar lipids plus a moderate amount of an unknown phospholipid and traces of another unknown phospholipid and an unknown polar lipid.

The type strain is 2SM5^T (=CECT 7670^T=KCTC 23093^T), isolated from a seawater sample obtained at a depth of 10 m off the coast of Vinaroz, Castellón, Spain. Strain 2SM6 (=CECT 7669=KCTC 23094), isolated from the same source, is a reference strain.

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