INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY

TAXONOMIC DESCRIPTION

Gutierrez-Patricio *et al.*, *Int. J. Syst. Evol. Microbiol.* 2021;71:004942

DOI 10.1099/ijsem.0.004942

MICROBIOLOGY SOCIETY

OPEN ACCESS

Paracoccus onubensis sp. nov., a novel alphaproteobacterium isolated from the wall of a show cave

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Abstract

A novel facultatively anaerobic, non-motile, Gram-stain-negative, non-endospore-forming alphaproteobacterium, strain 1011MAR3C25^T, was isolated from a white biofilm colonizing the walls of the Andalusian show cave Gruta de las Maravillas (Huelva, Spain). Strain 1011MAR3C25^T grew at 8–42 °C (optimum, 20–30 °C), at pH 5.0–9.0 (optimum, pH 5.0–6.0) and in the presence of 0–12% (w/v) NaCl (optimum 3–5%). Cells were catalase- and oxidase-positive. The strain grew heterotrophically with various carbon sources and chemoautotrophically with thiosulfate under aerobic conditions. Results of phylogenetic analysis showed that strain 1011MAR3C25^T was related to *Paracoccus saliphilus* DSM 18447^T and *Paracoccus alkanivorans* LMG 30882^T (97.90% and 97.32% 16S rRNA sequence identity values, respectively). The major respiratory quinone was ubiquinone Q-10 and the predominant fatty acid was $C_{18:1}\omega7c$. The polar lipid profile consisted of diphosphatidylglycerol, phosphatidylcholine, phosphatidylglycerol, an unidentified aminolipid, an unidentified glycolipid and an unidentified polar lipid. The DNA G+C content was 60.3 mol%. Based on a polyphasic taxonomic study it is proposed that strain 1011MAR3C25^T (=CECT 9092^T=LMG 29414^T) represents a novel species of the genus *Paracoccus*, for which the name *Paracoccus onubensis* sp. nov. is proposed.

The genus *Paracoccus* belongs to the class *Alphaproteobacteria* and family *Rhodobacteraceae*. It was described by Davis *et al.* [1] with *Paracoccus denitrificans* as the type species. Members of this genus are Gram-stain-negative, non-spore-forming, usually non-motile, and catalase- and oxidase-positive. The major cellular fatty acid is $C_{18:1}\omega7c$. The G+C content of the DNA is 64–70% mol. Currently the genus *Paracoccus* includes 74 validly named species and 15 non-validly named species (https://lpsn.dsmz.de/genus/paracoccus). The general model of metabolism is chemoorganotrophic. Some species are able to grow as facultative chemolithoautotrophs using reduced sulphur compounds as substrates and others can live as facultative methylotrophs using methanol as a substrate [2]. Paracocci are ubiquitous and growth occurs in water [3] and soil [4–6], among other environments [7]. *Paracoccus cavernae* was isolated from a cave air [8]. In this study, a bacterial strain, 1011MAR3C25^T, was isolated from white biofilms coating the walls of a cave and characterized using a polyphasic approach to determine its taxonomic position. Strain 1011MAR3C25^T is proposed to represent a novel species within the genus *Paracoccus* named *Paracoccus onubensis* sp. nov.

ISOLATION AND ECOLOGY

Strain 1011MAR3C25^T was isolated from white biofilms coating the walls of Gruta de las Maravillas (37° 53′ 27.92″ N, 6° 33′ 57.25″ E). Gruta de las Maravillas is a cave located in Southwestern Spain. This was the first show cave open to tourism, in 1914. Its length is 2130 m from which around 1400 m are visited. Along the tourist trail, small, round, white-coloured colonies forming biofilms on the walls can be observed, which are particularly abundant close to the cave exit. White cave biofilms are composed of a large diversity of uncultured representatives of many different major evolutionary lineages [9, 10]; therefore, they represent an important source of novel bacteria. In October 2011, samples were obtained aseptically by scraping off the white-coloured colonies with a sterile scalpel, stored in sterile tubes at 5 °C, and analysed immediately upon arrival in the laboratory.

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Keywords: *Paracoccus*; *Proteobacteria*; show cave; whole genome average nucleotide identity.
Abbreviations: ANI, average nucleotide identity; DDH, DNA–DNA hybridization; EBS, E-basal salts.
The GenBank/EMBL/DDBJ accession numbers for the genome and 16S rRNA gene sequences of strain 1011MAR3C25^T are QZCG00000000 and LN867136, respectively.
One supplementary table and four supplementary figures are available with the online version of this article.

The samples were resuspended in a sterile 0.85% (w/v) NaCl solution and subsequently seeded in a 10-fold diluted marine agar medium (Difco). Strain 1011MAR3C25^T was isolated on marine agar after incubation at 30 °C for 7 days.

PHYLOGENY

Genomic DNA was extracted according to Marmur [11]. The 16S rRNA genes were amplified following the method described by Jurado *et al.* [12]. The identification of phylogenetic neighbours was determined using the global alignment algorithm on the EzTaxon-e database [13]. The phylogenetic analyses were carried out by aligning and comparing the nearly complete 16S rRNA gene sequence of strain 1011MAR3C25^T with corresponding sequences of members of the genus *Paracoccus*. In addition, to find more precise phylogenetic relationships of the species closest to strain 1011MAR3C25^T, phylogenomic treeing based on 139 single-copy genes was performed according to Campbell *et al.* [14]. Alignments were created using the multiple sequence alignment program MUSCLE [15]. Phylogenetic trees were reconstructed using the best substitution model selected by the software MEGA X [16]. The evolutionary history using the 16S rRNA gene sequences was inferred by using the maximum-likelihood method, Kimura's two-parameter model with a discrete Gamma distribution and invariant sites [17]. The evolutionary history of the concatenated genes was deduced using the maximum-likelihood method and the general time reversible model with a discrete gamma distribution and invariant sites [18]. The robustness of the tree was evaluated by bootstrap resampling (1680 replicates).

Sequencing of its 16S rRNA gene revealed that strain 1011MAR3C25^T belonged to the genus *Paracoccus*. The results of phylogenetic analysis based on 16S rRNA gene sequences showed that strain 1011MAR3C25^T was closely related to *Paracoccus saliphilus* DSM 18447^T, *Paracoccus alkanivorans* LMG 30882^T, '*Paracoccus siganidrum*' DSM 26381^T, *Paracoccus homiensis* DSM 17862^T and *Paracoccus aestuarii* DSM 19484^T, with identity values of 97.90, 97.32, 97.18, 96.96 and 96.96%, respectively. Besides, the phylogenetic trees showed that the closest relatives of strain 1011MAR3C25^T were *P. saliphilus* and *P. alkanivorans* (Figs 1, S1 and S2, available in the online version of this article) and were separated from the rest of the species within the genus *Paracoccus*. In the phylogenetic trees based on 16S rRNA gene sequences (Figs 1 and S1), the nodes were well-supported with bootstrap values greater than 60. The bootstrap value of 100 in the phylogenomic tree (Fig. S2) showed the high phylogenetic relatedness between strain 1011MAR3C25^T, *P. saliphilus* and *P. alkanivorans*.

GENOME FEATURES

The genomic DNA of strain 1011MAR3C25^T, *P. aestuarii* DSM 19484^T and '*P. siganidrum*' DSM 26381^T were sequenced using 250 bp paired-end reads on an Illumina HiSeq platform by means of a Nextera XT library prep kit. The read quality was checked with FastQC 0.11.5 (www.bioinformatics.babraham.ac.uk/projects/fastqc). Reads were adapter-trimmed using Trimmomatic 0.36 with a sliding window quality cutoff of Q15 [19]. *De novo* assembly of this draft genome was performed using SPAdes 3.7 [20]. The remaining genomes of the reference strains were taken from public databases. Calculations of whole genome average nucleotide identity (ANI) and TETRA values were carried out using the JSpecies software [21], whereas OrthoANI measurements were calculated as described by Lee *et al.* [22]. Additionally, the genomic relationship between strain 1011MAR3C25^T and its closest relatives was examined by classical DNA–DNA hybridization (DDH) as described by Urdiain *et al.* [23] and whole genome sequenced-based DDH predicted by the Genome-to-Genome Distance Calculator 2.1 (http://ggdc.dsmz.de) [24]. Gene prediction was carried out with Prodigal [25], whereas Sma3s [26] was implemented for functional annotations using the curated Uniprot-SwissProt database [27].

The relatedness between strain 1011MAR3C25^T and its phylogenetically closest type strains according to 16S rRNA gene sequences was assessed by ANI, TETRA and OrthoANI calculations. The genome sizes and accession numbers of strain 1011MAR3C25^T, *P. aestuarii* DSM 19484^T, '*P. siganidrum*' DSM 26381^T and *P. alkanivorans* LMG 30882^T were 4764139 bp (QZCG00000000), 3747355 bp (QZEV00000000), 4906790 bp (QZEW00000000) and 4662693 bp (QOKZ00000000), respectively. The genome of strain 1011MAR3C25^T contained a total of 40 contigs. The N50 value was 272503, and the largest contig was 518150 bp. The average G+C content was 60.3 mol%. A total of 4595 genes were detected, with three rRNA and 45 tRNA genes predicted and 4486 proteins (Table S1). Genome coverage was 30.0×.

Strain 1011MAR3C25^T showed ANI and OrthoANI values below 85% with the closest species of the genus *Paracoccus* (Table 1). Values ranging from 84.52 to 79.57% and from 84.67 to 80.10 % were obtained for strain 1011MAR3C25^T and both *P. saliphilus* DSM 18447^T and *P. alkanivorans* LMG 30882^T, respectively. Values below 85% are lower than the generally accepted species boundary 94–96% proposed by Richter and Rosselló-Móra [21]. Moreover, the TETRA values were less than 0.99, supporting the ANI calculation and the species circumscription. The ANIb, ANIm, OrthoANI and TETRA results showed that strain 1011MAR3C25^T should be classified as a novel member of the genus *Paracoccus*.

DDH values among strain 1011MAR3C25^T and *P. saliphilus* DSM 18447^T, *P. aestuarii* DSM 19484^T, *P. homiensis* DSM 17862^T, *P. alkanivorans* LMG 30882^T and '*P. siganidrum*' DSM 26381^T were between 42.17–49.84% for classical DDH, and 20.40–23.70% for *in silico* DDH (Table 1). In both analyses, the percentages were below the recommended cutoff point of 70% DDH for species delineation and, therefore, indicated that strain 1011MAR3C25^T was not related at the species level with any of the five studied type strains.

Predicted and annotated genes with Prodigal and Sma3s were grouped according to the biological processes classified in the UniProt database (Fig. S3). Genes involved in the transport category were the most represented in strain 1011MAR3C25^T,

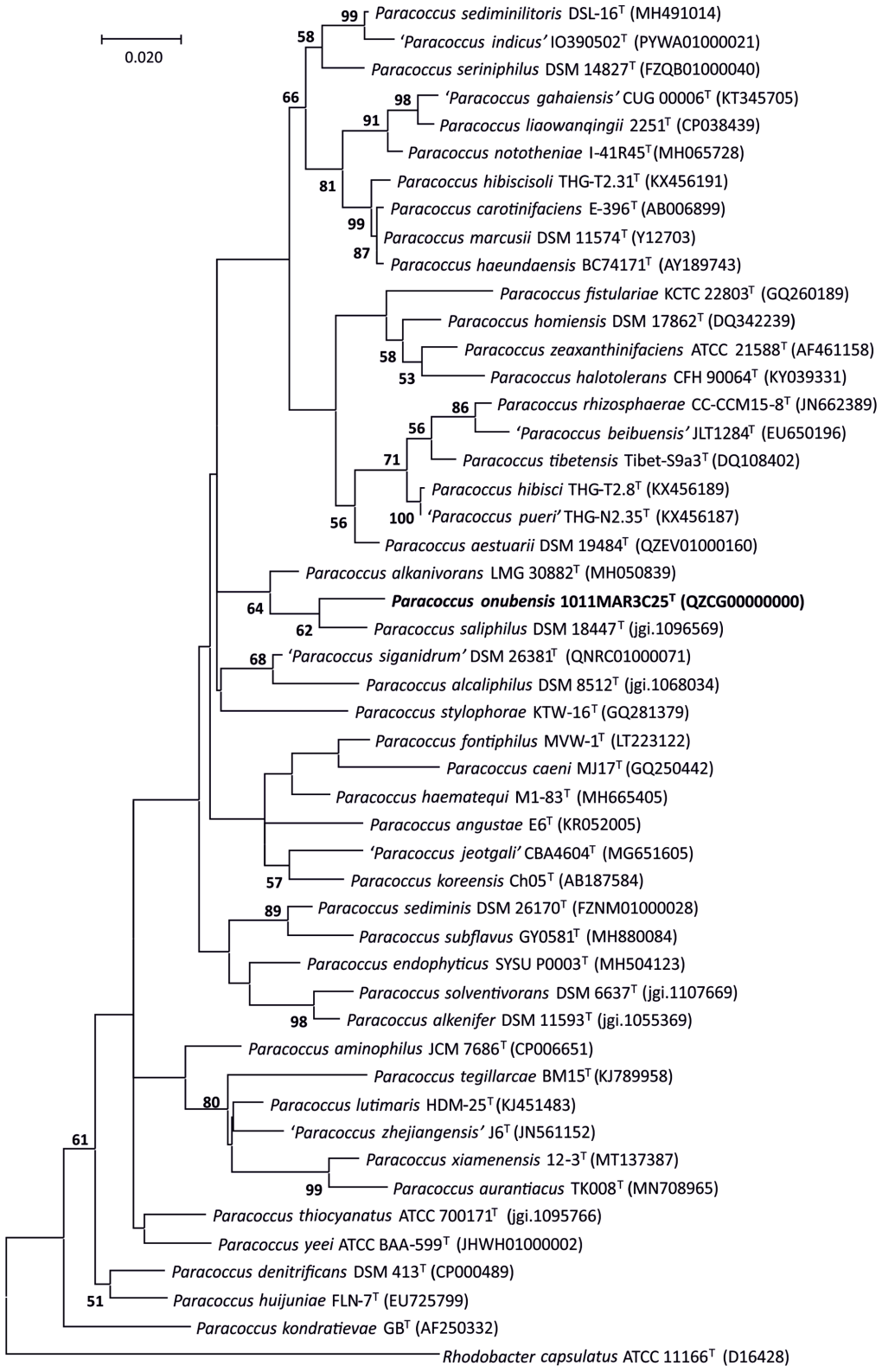

Fig. 1. Maximum-likelihood phylogenetic tree based on 16S rRNA gene sequences showing the relationship of strain 1011MAR3C25^T with other species of the genus *Paracococcus*. Bootstrap values (>50%) are expressed as percentages of 1680 replicates. There were a total of 1418 positions in the final dataset. The 16S rRNA gene sequence of *Rhodobacter capsulatus* ATCC 11166^T (D16428) was used as an outgroup. Bar, 0.02 substitutions per nucleotide position.

Table 1. Results of ANI calculations (%), TETRA correlations and DDH values (%) of strain 1011MAR3C25^T and other species of the genus *Paracoccus*

Taxa: 1, 1011MAR3C25^T (QZCG00000000); 2, *P. saliphilus* (FTOU00000000); 3, '*P. siganidrum*' (QNRC00000000); 4, *P. homiensis* (FOHO00000000); 5, *P. aestuarii* (QZEV00000000); 6, *P. alkanivorans* (QOKZ00000000). ND, Not determined, – duplicate data

	1	2	3	4	5	6
ANIb						
1	100	78.21	75.54	74.75	73.86	78.76
2	78.03	100	76.63	75.86	74.71	84.70
3	76.25	77.28	100	77.23	78.14	77.88
4	75.21	76.47	77.44	100	76.40	76.77
5	74.63	75.57	78.68	76.76	100	76.02
6	78.81	84.86	77.24	76.22	75.41	100
ANIm						
1	100	84.53	83.79	83.39	83.50	84.66
2	84.52	100	84.10	83.77	83.43	87.87
3	83.79	84.09	100	84.38	84.69	84.32
4	83.39	83.77	84.39	100	84.20	83.87
5	83.52	83.43	84.70	84.21	100	83.76
6	84.67	87.87	84.31	83.87	83.76	100
OrthoANI						
1	100	–	–	–	–	–
2	79.57	100	–	–	–	–
3	77.33	78.78	100	–	–	–
4	76.51	77.94	78.77	100	–	–
5	75.77	77.00	79.64	77.87	100	–
6	80.10	86.45	79.33	78.25	77.24	100
TETRA						
1						
2	0.95674	–	–	–	–	–
3	0.75838	0.86604	–	–	–	–
4	0.85423	0.89725	0.88234	–	–	–
5	0.69568	0.81374	0.93282	0.90284	–	–
6	0.961	0.98794	0.85662	0.89001	0.80492	–
WGS-based DDH	100	23.20	21.40	20.40	20.40	23.70
Classical DDH value	100	49.84	42.17	44.14	46.56	ND

with 22.22% of the total. This category includes the movement of ions, molecules, complexes and organelles into, out of or within the bacterium through agents such as transporters, pores or motor protein.

In silico analysis for the genome annotation of strain 1011MAR3C25^T confirmed the *in vivo* physiological studies detailed below for nitrogen and carbon metabolism. Thus, the assimilation of nitrate and urea was confirmed after the prediction of *nasAD* and *ureABCDEFG* genes in the genome, respectively, as well as the presence of mechanisms for the assimilation of inorganic carbon, such as the CO_2 fixation process, through the presence of ribulose bisphosphate carboxylase large chain (*cbbL*) and the ribulose bisphosphate carboxylase small subunit (*cbbS*), among others.

Of interest, the annotated genome of strain 1011MAR3C25^T suggested the presence of genes involved in the synthesis

of the flagellum, with the presence of five genes, *flhGHI* and *fliIP*, which contrasted with the *in vivo* analyses where no motility was observed. These annotations could be also related to the stress response and virulence factors in this bacterium being part of these biological processes.

PHYSIOLOGY AND CHEMOTAXONOMY

The investigation of morphological, chemotaxonomic and physiological characteristics of strain 1011MAR3C25^T and its phylogenetically closest strains, *P. saliphilus* DSM 18447^T, '*P. siganidrum*' DSM 26381^T, *P. homiensis* DSM 17862^T and *P. aestuarii* DSM 19484^T was performed in triplicate on marine agar medium at 30 °C, unless otherwise indicated.

Cell morphology and dimensions were examined in a Zeiss Axioscope two phase-contrast microscope equipped with image-analysing Axio Vision 2.05 software, and by field emission scanning electron microscopy (FESEM). For FESEM observations, the biomass was collected from the agar surface and fixed with 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) at 4 °C for 2 h. The cells were separated from the mixture by centrifugation and washed twice in cacodylate buffer for 5 min each and post-fixed in 1% osmium tetroxide for 1 h at 4 °C. After dehydration by subsequent dilution series in ethanol and acetone, the bacteria were dried in a critical point drying device (CPD 030, Balzers) at 34.5 °C. Finally, the bacterial cells were sputter-coated with a thin gold–palladium film and examined in a JEOL JSM-7001F microscope. FESEM examinations were operated in secondary electron detection mode with an acceleration potential of 15 kV. Colony morphology was analysed using 3–5 days old cultures grown on marine agar medium utilizing a stereo microscope. Motility was examined on nutrient broth or marine broth media containing 0.3% agar [28] and confirmed by staining with flagella stain droppers (BD; according to the manufacturer's instructions) and observation in a phase contrast microscope. Oxidase activity was tested by monitoring the oxidation on BBL DrySlide Oxidase (BD). Catalase activity was determined by the production of bubbles after addition of a drop of 3% (v/v) hydrogen peroxide on cellular biomass smeared on a glass slide. Acid production from carbohydrates, assimilation tests and enzymatic activities were identified with the API 50CH, API 20NE and API ZYM galleries (bioMérieux), according to the manufacturer's instructions. The Gram reaction was performed following the method of Hucker [29] and was confirmed by KOH-lysis test [30]. The temperature range for growth was tested at 3, 4, 8, 10, 15, 20, 25, 30, 37, 40, 42 and 45 °C. Tolerance to NaCl was assessed on nutrient agar medium supplemented with 0, 3, 5, 7, 9, 10, 13, 14, 15 and 16% (w/v) NaCl. Growth at different pH values were assessed with marine broth adjusted to pH 3.0–13.0 (at intervals of 1.0 pH unit) by using the following buffer system: pH 3.0–5.0 with 0.1 M citric acid/0.1 M sodium citrate; pH 6.0–8.0 with 0.2 M sodium phosphate dibasic/0.2 M sodium phosphate monobasic; pH 9.0–11.0 with 0.1 M sodium bicarbonate/0.1 M sodium carbonate; pH 12.0–13.0 with 0.2 M potassium chloride/0.2 M sodium hydroxide. Negative controls were used to verify each buffer. Utilization of ammonium sulphate (7.8 g l^{-1}), sodium glutamate (20 g l^{-1}), sodium nitrate (7.8 g l^{-1}), casamino acids (10 g l^{-1}) and peptone (10 g l^{-1}) as sole nitrogen sources were tested in medium N (artificial seawater containing 1.0 g l^{-1} glucose and 0.2 g l^{-1} NaHCO3, pH 7.0) [31]. The oxygen requirement for bacterial growth was tested in anaerobic chambers (GENbag anaer, bioMérieux). Endospore production was tested on oatmeal agar (Difco), nutrient agar (Difco) and Bennett's agar media [32]. DNA and casein hydrolysis were tested by using DNAse test agar (Difco) and milk agar (Merck), respectively, according to Barrow and Feltham [33]. Lypolytic activity was examined in Tweens 20, 40 and 80 [34]. In addition to chemoorganotrophic metabolism, strain 1011MAR3C25^T was tested for chemolithoautotrophy and methylotrophy using reduced sulphur compounds: as thiosulphate and methanol, respectively. Strain 1011MAR3C25^T was grown aerobically and anaerobically at 28 °C in E-basal salts (EBS) [35], supplemented with agar (15.00 g l^{-1}) and 20 mm sodium thiosulfate [36]. Culture of strain 1011MAR3C25^T on methanol (50 mm) was performed in a liquid medium [37]. As a control, EBS supplemented with glucose (1.0 g l^{-1}) was used. Cellular fatty acid profiles were analysed in triplicate after collecting biomass from a culture grown on trypticase soy broth agar for 72 h at 30 °C following the methodology described by Jurado *et al.* [38]. Analyses of quinones and polar lipids were carried out by the Identification Service, Leibniz-Institut DSMZ (Deutsche Sammlug von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany).

The results from the phylogenetic analysis and whole genome comparisons indicated that strain 1011MAR3C25^T was different from the previously published type strains. Thus, morphological, physiological and biochemical analyses of strain 1011MAR3C25^T were only compared with the closest type strains: *P. saliphilus* DSM 18447^T, *P. alkanivorans* LMG 30882^T and other *Paracoccus* species (Table 2).

Strain 1011MAR3C25^T and the reference strains had many differences (Table 2). These differences were mainly related to NaCl tolerance, temperature and pH conditions for growth, hydrolysis of Tweens 20 and 40, reduction of nitrate, growth on different substrates, enzymatic activity, and acid produced from different sugars.

Strain 1011MAR3C25^T tolerated concentrations of 0–12% (w/v) NaCl, like *P. homiensis* DSM 17862^T, *P. alkanivorans* LMG 30882^T and *P. saliphilus* DSM 18447^T. *P. aestuarii* DSM 19484^T and '*P. siganidrum*' DSM 26381^T tolerated 5 and 10% (w/v) NaCl, respectively. Optimal growth conditions also differed from one species to another. All the studied strains grew up to a temperature of 42 °C, except *P. saliphilus* DSM 18447^T, which grew up at 37 °C. Only *P. aestuarii* DSM 19484^T grew at 4 °C. All the studies strains grew within pH 5–8; however, the pH optimum differed between them. Strain 1011MAR3C25^T, '*P. siganidrum*' DSM 26381^T and *P. aestuarii* DSM 19484^T produced acid from

Table 2. Differential characteristics of strain 1011MAR3C25^T and its closest relatives

Strains: 1, 1011MAR3C25^T; 2, *Paracoccus saliphilus* DSM 18447^T; 3, *'Paracoccus siganidrum'* DSM 26381^T; 4, *Paracoccus homiensis* DSM 17862^T; 5, *Paracoccus aestuarii* DSM 19484^T; 6, *Paracoccus alkanivorans* LMG30882^T. Results from this study were obtained from cells grown under the same conditions. All data from the present study unless otherwise indicated. +, Positive; –, negative; (+), weakly positive.

Characteristics	1	2	3	4	5	6
Growth conditions:						
10% NaCl	(+)	+	(+)	+	–	+
12% NaCl	(+)	+	–	+	–	(+)
16% NaCl	–	(+)	–	–	–	–
Optimum NaCl	3–5	5–7	0–5	3–5	3	0–3
4 °C	–	–	–	–	(+)	–
6 °C	–	+	(+)	–	+	–
42 °C	(+)	–	+	(+)	+	+
Optimum pH	5	6	5	5	6	5
Hydrolysis of:						
Tween 20	+	+	+	–	–	+
Tween 40	–	–	+	+	–	+
Reduction of nitrate to nitrite	+	–	+	–	–	+
Urease	–	–	–	+	–	–
Growth on:						
Adipate	+	–	+	–	(+)	–
Citrate	+	+	+	–	+	+
Phenylacetate	+	–	+	–	(+)	–
Casamino acids	+	+	+	+	–	+
Enzymatic activity:						
α-Galactosidase	–	–	–	+	+	–
β-Galactosidase	–	–	–	+	+	–
Acid produced from:						
D-Adonitol	(+)	–	+	–	(+)	–
Amygdalin	–	+	–	–	+	+
D-Arabinose	+	–	+	–	(+)	+
L-Arabinose	+	–	+	–	(+)	+
D-Arabitol	–	–	–	–	+	+
L-Arabitol	(+)	–	+	–	(+)	(+)
Arbutin	–	–	–	–	+	–
Cellobiose	–	–	–	–	+	+
Erythritol	v	–	+	–	–	–
D-Fructose	+	–	+	–	(+)	+
D-Fucose	+	–	+	–	(+)	+
L-Fucose	(+)	–	–	–	–	+

Continued

Table 2. Continued

Characteristics	1	2	3	4	5	6
D-Galactose	+	+	+	–	–	+
Gentibiose	–	–	(+)	–	(+)	+
D-Glucose	+	–	+	–	(+)	+
Glycerol	(+)	–	+	–	–	+
Inositol	–	–	–	–	(+)	+
Lactose	–	–	–	–	+	+
D-Lyxose	(+)	–	+	–	–	–
Methyl α-D-glucopyranoside	–	–	–	–	+	–
Maltose	(+)	–	+	–	+	+
D-Mannitol	–	–	–	–	(+)	+
D-Mannose	+	–	+	+	(+)	+
Melibiose	(+)	–	+	+	(+)	+
N-Acetylglucosamine	–	–	–	–	+	+
Raffinose	–	–	–	–	(+)	+
L-Rhamnose	–	–	(+)	–	–	+
D-Ribose	+	–	+	–	+	–
Sucrose	(+)	–	(+)	–	+	+
Salicin	–	–	–	–	+	–
D-Sorbitol	–	–	–	–	(+)	+
D-Tagatose	–	–	(+)	–	–	–
Trehalose	(+)	–	(+)	–	+	–
Turanose	–	+	+	–	+	(+)
Xylitol	–	–	–	–	(+)	–
D-Xylose	+	(+)	+	–	(+)	+
L-Xylose	+	–	+	–	–	–
G+C content (mol%)*	60.3	60.3[a]	64.3[b]	63.0[c]	62.0[d]	61.4[e]

**a*, Wang *et al.* [6]; *b*, Liu *et al*. [39]; *c*, Kim *et al*. [40]; *d*, Roh *et al*. [3]; *e*, Zhang *et al*. [41].

a greater number of sugars than *P. saliphilus* DSM 18447^T, *P. alkanivorans* LMG 30882^T and *P. homiensis* DSM 17862^T.

All strains used sodium nitrate, ammonium sulphate, sodium glutamate and peptone as sole nitrogen sources. All strains, except *P. aestuarii* DSM 19484^T, used casamino acids. Strain 1011MAR3C25^T was able to oxidize thiosulfate but it did not grow anaerobically with this sulphur compound. Neither did it grow on methanol as an energy source. Strain 1011MAR3C25^T had a fatty acid pattern similar to that of the genus *Paracoccus* [2] (Table 3). The most abundant fatty acid was $C_{18:1}$ ω7*c* (72.55%), followed by others in minor proportions ($C_{18:0}$, $C_{19:0}$ cyclo *ω8c*, $C_{16:0}$, $C_{14:0}$ 3-OH and/or $C_{16:1}$ iso I, $C_{10:0}$ 3-OH, $C_{17:1}$ ω8*c*). The main difference from its closest reference strain was the presence of the fatty acid $C_{17:1}$ ω8*c* and the proportion of some others such as $C_{16:0}$. Strain 1011MAR3C25^T contained ubiquinone-10 as the major respiratory quinone like other species of the genus *Paracoccus*. The composition of its polar lipids included diphosphatidylglycerol, phosphatidylcholine, phosphatidylglycerol, an unidentified aminolipid, an unidentified glycolipid and an unidentified polar lipid.

Based on the results of the phenotypic and genotypic analyses presented in this study, it is proposed that the alphaproteobacterium strain 1011MAR3C25^T represents a novel species within the genus *Paracoccus*. We propose the name *Paracoccus onubensis* for strain 1011MAR3C25^T.

Table 3. Major fatty acid compositions of strain 1011MAR3C25^T and its related species

Strains: 1, 1011MAR3C25^T; 2, *Paracoccus saliphilus* DSM 18447^T; 3, '*Paracoccus siganidrum*' DSM 26381^T; 4, *Paracoccus homiensis* DSM 17862^T; 5, *Paracoccus aestuarii* DSM 19484^T; 6, *Paracoccus alkanivorans* LMG30882^T. Data in columns 1–5 are from this study and were obtained from cells grown under the same conditions. –, Not detected.

Fatty acids	1	2	3	4	5	6†
$C_{16:0}$	3.27	1.23	1.40	1.27	1.14	<1
$C_{17:0}$	<1%	–	–	–	–	1.2
$C_{18:0}$	7.90	8.74	4.51	7.86	6.30	5.7
$C_{17:1}$ ω8c	1.41	–	<1	<1	–	–
$C_{18:1}$ ω7*c*	72.55	83.17	85.95	76.70	85.01	57.4
$C_{18:1}$ *ω7c*-methyl	–	–	–	6.81	–	–
$C_{19:0}$ cyclo *ω8c*	6.66	2.11	1.14	–	–	22.4
$C_{10:0}$ 3-OH	2.89	3.14	2.92	3.34	3.90	6.0
$C_{18:0}$ 3-OH	–	–	–	1.25	–	–
Summed feature 2*	3.10	1.17	3.34	2.06	3.17	3.2

*Summed feature 2 contains one or more of the following fatty acids: $C_{12:0}$ aldehyde/$C_{14:0}$ 3-OH and/or $C_{16:1}$ iso I. Summed features are fatty acids that cannot be resolved reliably from another fatty acid using the chromatographic conditions chosen. The MIDI system groups these fatty acids together as one feature with a single percentage of the total.
†Data from Zhang *et al*. [41].

DESCRIPTION OF *PARACOCCUS ONUBENSIS* SP. NOV.

Paracoccus onubensis (o.nu′ben.sis, N.L. masc. adj. *onubensis*, pertaining to Onuba, the Roman name of Huelva, the province where the organism was first isolated).

Cells are facultatively anaerobic, non-motile, Gram-stain-negative and non-endospore-forming. They are 0.5–0.7 µm×1.2–1.8 µm in diameter, grow in pairs or singly (Fig. S4). Colonies on marine agar are 0.5–1.3 mm in diameter, circular, convex, smooth and have entire margins. Good growth at 10–37 °C, optimal at 20–30 °C and weak growth at 8 and 42 °C. Tolerates up to 12% NaCl, with optimum growth within 3–5% (v/w) NaCl. Growth occurs at pH 5.0–9.0, with an optimum at pH 5.0–6.0. Catalase- and oxidase-positive. It grows heterotrophically with various carbon sources and chemoautotrophically with thiosulfate under aerobic conditions. D-Glucose, L-galactose, D-mannose, D-mannitol, *N*-acetylglucosamine, maltose, potassium gluconate, adipate, malate, citrate and phenylacetate can be utilized as carbon sources. Utilizes sodium nitrate, ammonium sulphate, sodium glutamate, casamino acids and peptone as sole nitrogen sources. Produces acid from D,L-arabinose, aesculin, D-fructose, D-fucose, D-galactose, D-glucose, D-mannose, D-ribose and D,L-xylose; weakly from D-adonitol, L-arabitol, L-fucose, glycerol, D-lyxose, maltose, melibiose, sucrose and trehalose. Negative for amygdalin, D-arabitol, arbutin, dulcitol, gentibiose, glycogen, inositol, inulin, lactose, melezitose, methyl α-D-glucopyranoside, methyl α-D-mannopyranoside, methyl β-D-xylopyranoside, *N*-acetylglucosamine, potassium gluconate, potassium 2-ketogluconate, potassium 5-ketogluconate, raffinose, L-ramnose, salicin, D-sorbitol, L-sorbose, starch, D-tagatose, turanose and xylitol. Reduces nitrate to nitrite. Hydrolyses aesculin and Tween 20, and does not hydrolyse DNA, casein or Tweens 40 and 80. Produces alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and α-glucosidase. Assimilates glucose, arabinose, mannose, mannitol, *N*-acetyl-glucosamine, maltose, potassium gluconate, adipic acid, malate, trisodium citrate and phenylacetic acid, but does not assimilate capric acid. The most abundant fatty acid is $C_{18:1}$ ω7*c*. Ubiquinone-10 is the major respiratory quinone. The polar lipids are diphosphatidylglycerol, phosphatidylcholine, phosphatidylglycerol and an unidentified aminolipid, an unidentified glycolipid and unidentified polar lipid.

The G+C content of the type strain is 60.3 mol%. The type strain, 1011MAR3C25^T (=CECT 9092^T=LMG 29414^T), was isolated from white biofilms on the walls of the cave Gruta de las Maravillas, Aracena (Huelva, Spain).

Funding information
This study was supported by project 0483_PROBIOMA_5_E, co-financed by the European Regional Development Fund within the framework of the Interreg V A Spain–Portugal program (POCTEP) 2014–2020.

Acknowledgements
We thank Isabel Galocha (IRNAS-CSIC) for technical assistance.

Author contributions
S.G.P., J.L.G.P., A.Z.M. and B.H.: data acquisition. V.L.: writing–original draft. C.S.J.: writing–review and editing.

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

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