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TAXONOMIC DESCRIPTION
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Rossellomorea arthrocnemi sp. nov., a novel plant growth-promoting bacterium used in heavy metal polluted soils as a phytoremediation tool

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Abstract

Strain EAR8^T is a root endophyte isolated from *Arthrocnemum macrostachyum* plants collected from the Odiel marshes, Huelva (Spain). It presented *in vitro* plant growth-promoting properties and improved the plant growth and heavy metal accumulation in polluted soils playing an important role in phytoremediation strategies. Phenotypically, strain EAR8^T cells were Gram-positive, aerobic and non-motile rods with terminal oval endospores and non-swollen sporangia which form beige, opaque, butyrous, raised and irregular colonies with undulate margins. The strain was able to grow between 15–45 °C, at pH 6.0–9.0 and tolerated 0–25% NaCl (w/v) showing optimal growth conditions on trypticase soy agar plates supplemented with 2.5% NaCl (w/v) at pH 7.0 and 37 °C for 24 h. Chemotaxonomic analyses showed that the isolate has *meso*-diaminopimelic acid as the peptidoglycan in the cell wall and MK-7 as the major respiratory quinone. The predominant fatty acids were anteiso-$C_{15:0}$ and iso-$C_{15:0}$ and the polar lipid profile was composed of diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine. Phylogenetic analyses based on the whole proteomes of closest sequenced relatives confirmed that strain EAR8^T is affiliated to the genus *Rossellomorea* and forms a clade with *Rossellomorea vietnamensis* 15-1^T with maximum support. Genome analyses showed that EAR8^T has indole-3-acetic acid and siderophore biosynthesis and transporters genes and genes related to resistance against heavy metals. Phenotypic and phylogenomic comparative studies suggested that strain EAR8^T is a new representative of the genus *Rossellomorea* and the name *Rossellomorea arthrocnemi* sp. nov. is proposed. Type strain is EAR8^T (=CECT 9072^T=DSM 103900^T).

The genus *Rossellomorea*, belonging to the family *Bacillaceae* (phylum *Firmicutes*), is a very recenty genus proposed by Gupta *et al.* [1] to encompass previously described *Bacillus* species that can reliably be distinguished from all other *Bacillaceae* species by the presence of exclusively shared conserved signature indels in their protein sequences. At the time of writing, the genus comprises up to four validly named species [2], with *Rossellomorea aquimaris* as the type species. Species from genus *Rossellomorea* have mainly been isolated from different saline environments [3–5]. No representative of *Rossellomorea* have been isolated from plants so far.

Strain EAR8^T is an endophytic bacterium isolated from roots of *Arthrocnemum macrostachyum*, a halophyte plant growing in the Odiel marshes, Huelva, Spain [6]. Previous studies showed that EAR8^T is an efficient plant growth-promoting (PGP) bacterium able to produce siderophores and synthetise auxins [6]. This strain also tolerates up to 15 mM Ni, 9 mM Pb, 8 mM As and 2 mM Cd, among others [6]. Moreover, it was part of a bacterial consortium that improved seed germination, growth and heavy metal accumulation in roots of *A. macrostachyum* plants in heavy metal polluted soils, enhancing the potential of this halophyte to be used as a phytoremediation tool [6, 7].

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Abbreviations: ANI, average nucleotide identity; dDDH, digital DNA-DNA hybridization; IAA, indole-3-acetic acid; MA, marine agar; MK, menaquinone; PGP, plant growth-promoting; TLC, thin-layer chromatography; TSA, tryptic soy agar; TYGS, type genome server.
The GenBank/EMBL/DDBJ accession number for the complete 16S rRNA gene sequence is MZ416782. The GenBank/EMBL/DDBJ accession number for the draft genome is CAJGBF010000000.
Three supplementary tables and three supplementary figures are available with the online version of this article.

Based on the demonstrated efficiency of this PGP strain for bioaugmentation processes to recover heavy metal contaminated soils, it is worthwhile to determine its exact phylogenetic position. This study therefore aims to characterize plant endophyte strain EAR8^T as a potential novel species in the genus *Rossellomorea* by using a polyphasic approach.

ISOLATION AND ECOLOGY

Strain EAR8^T was isolated from roots of *A. macrostachyum* from the Odiel marshes, Huelva, Spain (37° 13′ N, 6° 57′ E) as described by Navarro-Torre *et al.* [6]. Briefly, after root surface disinfection, endophyte extraction was performed in 0.9% sterile saline (w/v) solution using a sterile mortar. The extract was plated on tryptic soy agar (TSA) plates supplemented with 0.3 M NaCl and incubated at 28 °C for 72 h. Isolates were identified and classified according to their colony characteristics and pure cultures were stored in 15% glycerol at −80 °C for long-term preservation.

16S RNA PHYLOGENY

Genomic DNA was extracted using an i-genomic BYF DNA Extraction kit (Intron Biotechnology) according to the manufacturer's instructions. The 16S rRNA gene was then amplified by PCR using the primers and conditions described by Navarro-Torre *et al.* [6] and the product was sequenced by StabVida (Portugal). 16S rRNA gene partial sequence was deposited in the GenBank/EMBL/DDBJ database under the accession number KU320872 (complete 16S rRNA gene sequence as extracted from the genome sequence can be found under accession number MZ416782). This sequence was aligned with the sequences of the closely related type strains using the Ez-Taxon-e service (www.ezbiocloud.net/eztaxon) [8] and pairwise similarities were determined as described by Meier-Kolthoff *et al.* [9]. A phylogenetic tree was created using the GGCD web server (http://ggdc.dsmz.de/) [10] according to Montero-Calasanz *et al.* [11].

The 16S rRNA gene sequence of strain EAR8^T confirmed that it belonged to the genus *Rossellomorea* showing the highest sequence similarities to *Rossellomorea aquimaris* TF-12^T (97.4%) and *Rossellomorea vietnamensis* 15-1^T (97.2%). These similarities were, nevertheless, lower than the threshold recommended (98.8–98.7%) to determine a new species within the phylum *Firmicutes* [9, 12], indicating the unambiguous novelty of strain EAR8^T within the genus *Rossellomorea*. Phylogenetic inferences also confirmed its affiliation by placing the isolate in a well-supported clade within the genus *Rossellomorea* (Fig. 1).

GENOME FEATURES

The whole genome of strain EAR8^T was sequenced by MicrobesNG (Birmingham, UK) using Illumina technology. Kraken was used to identify the closest available reference [13]. The quality of data was studied by mapping the reads with BWA mem [14], *de novo* assembly of the genome was performed using SPAdes [15] and, then, more quality parameters were checked with BWA mem. The whole genome was deposited in GenBank/EMBL/DDBJ under the accession number CAJGBF010000000. Genome annotation was performed with Prokka [16] and basic statistics about the genome were extracted using the RAST server version 2.0 [17], QUAST version 4.6.3 software [18], the SignalP 4.1 server [19], the TMHMM server v.2.0 [20], CRISPRFinder [21] and PlasFlow [22].

According to the genome sequence analyses, the draft genome sequence of strain EAR8^T had a total length of 4775586 bp and was formed of 107 contigs with a coverage of 80.6×. The N50 value was 199119 and the G+C content 42 mol% (Table S1).

A phylogenetic tree based on whole proteomes was inferred using the Type (Strain) Genome Server (TYGS; https://tygs.dsmz.de/) [23]. The phylogenetic inference was performed via FastME 2.1.4 including SPR postprocessing [24]. The tree was rooted at the midpoint [25] and visualized with PhyD3 [26].

The phylogenetic tree based on whole proteogenomes confirmed the affiliation within the genus *Rossellomorea* with maximum support for *R. vietnamensis* NBRC 101237^T as the closest species (Fig. 2).

Finally, taxogenomic analyses were carried out with the closely related species calculating the overall genome related indexes. Digital DNA–DNA hybridization (dDDH) and average nucleotide identity (ANI) tests were determined using the TYGS web server (https://tygs.dsmz.de/) [23] and JSpeciesWS server (http://jspecies.ribohost.com/jspeciesws) [27], respectively.

dDDH results showed 28.7 and 20.3% similarities with genomes of *R. vietnamensis* NBRC 101237^T (accession number BCVQ00000000) and *R. aquimaris* TF-12^T (accession number LQXM00000000), respectively. Furthermore, ANI tests reported 84.1% (ANIb) and 86.3% (ANIm) with *R. vietnamensis* NBRC 101237^T and 75.6% (ANIb) and 83.7% (ANIm) relatedness with *R. aquimaris* TF-12^T. Accordingly, all values were well below the recommended thresholds for species delineation [9, 28].

As mentioned above, strain EAR8^T has some PGP properties such as siderophore and auxin production [6]. Using the RAST server version 2.0 [17], genes involved in these properties were searched for in the genome. Regarding the production of siderophores, genes related to the synthesis of different siderophores were annotated. In fact, all genes involved in pyochelin biosynthesis (*pchR*, *pchA*, *pchB*, *pchC*, *pchD*, *pchE*, *pchF* and *pchG*) described in *Pseudomonas aeruginosa* [29] were located into the EAR8^T genome (Table S2). In addition, genes encoding the enzymes isochorismatase and 2,3-dihydr o-2,3-dihydroxybenzoate dehydrogenase, key in the synthesis of bacillibactin, a siderophore produced by members of family *Bacillaceae* [30, 31], were identified. Moreover, other enzymes involved in the synthesis of enterobactin, vibriobactin, rhizobactin 1021, baumannoferrin and aerobactin were also observed. Finally, genes related to siderophore transport systems, such as permeases and ATPases components, and receptors were also present in the genome (Table S2).

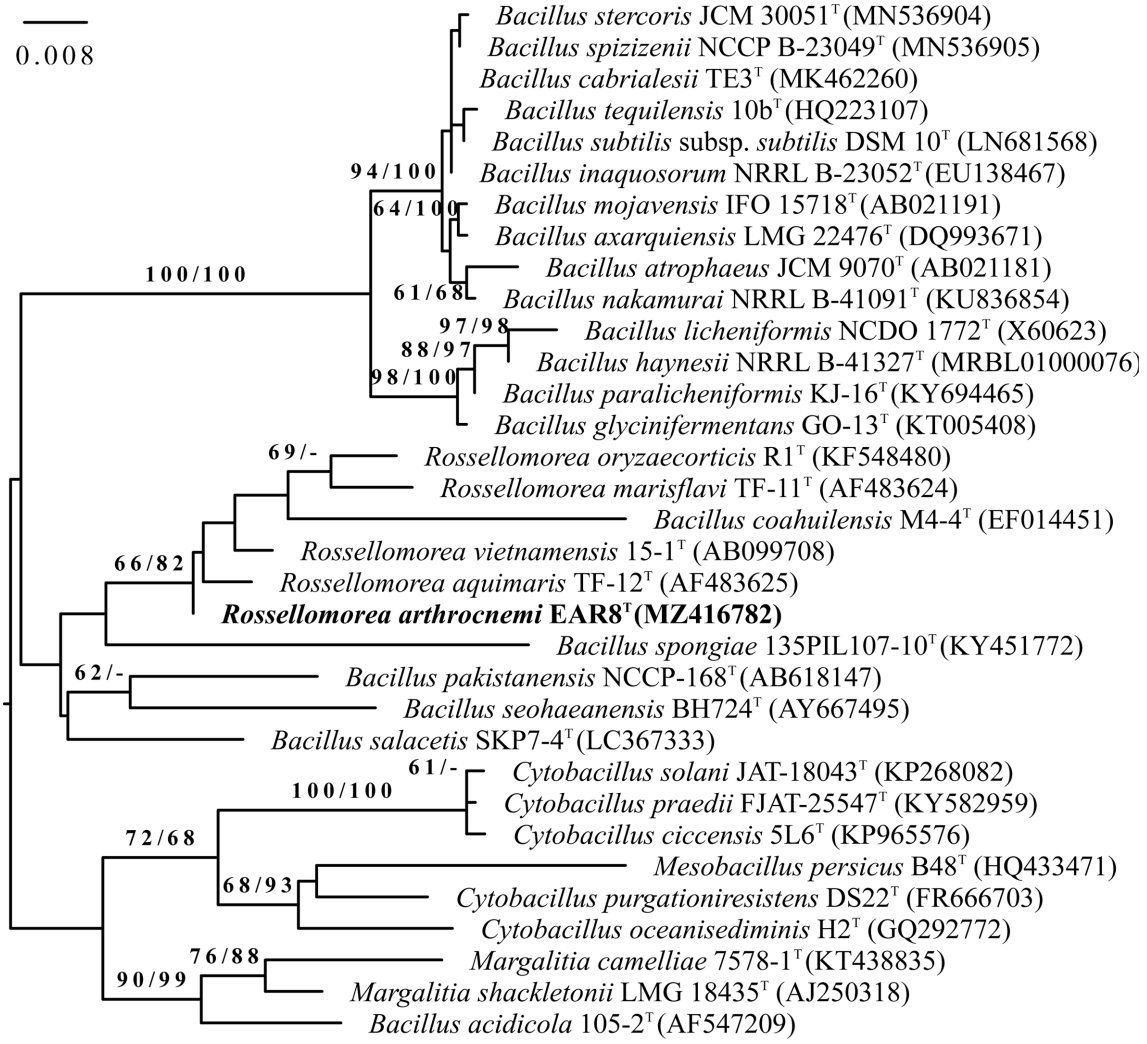

Fig. 1. Maximum-likelihood phylogenetic tree inferred from 16S rRNA gene sequences, showing the phylogenetic position of strain EAR8^T relative to type strains of species within the genus *Rossellomorea*. The branches are scaled in terms of the expected number of substitutions per site. Support values obtained from 1000 replicates from maximum-likelihood (left) and maximum-parsimony (right) bootstrapping are shown above the branches if ≥60%. Sequence accession numbers are given in parentheses.

Regarding auxin production, the EAR8^T genome showed genes involved in tryptophan biosynthesis and indole-3-acetic acid (IAA) biosynthesis. Tryptophan is the main precursor to IAA synthesis. It is involved in five different well-studied pathways. That is, the indol-3-acetamide pathway, the indole-3-pyruvate pathway, the tryptamine pathway, the tryptophan side-chain oxidase pathway and the indole-3-acetonitrile pathway [32]. The tryptamine and indole-3-acetonitrile pathways have been described as the main IAA routes in the closer genus *Bacillus* [33, 34]. Both pathways were also identified in the EAR8T´s genome sequence in addition to complete sets of genes involved in the indol-3-acetamide and indole-3-pyruvate pathways (Table S2). It supported the *in vitro* IAA production observed for this strain in previous works [6].

Finally, the presence of genes related to heavy metal tolerance (Table S3) confirmed the resistance to As, Cu, Pb, Zn, Cd, Hg and Ni previously observed [6].

PHYSIOLOGY AND CHEMOTAXONOMY

Growth conditions were studied on TSA plates supplemented with 0.3 M NaCl at different temperatures (4, 15, 20, 25, 28, 30, 32, 37 and 45 °C) and at different pH values (pH 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0; at 28 °C) for 6 days. pH values were adjusted using a citrate–phosphate buffer (0.1 M citric acid and 0.2 M dibasic sodium phosphate) and a Tris–HCl buffer [0.1 M Tris (hydroxymethyl) aminomethane and 0.1 M HCl]. NaCl tolerance was examined by incubating the isolate on membrane tryptone–glucose extract agar plates with varying NaCl concentrations (0, 0.5, 2.5, 5, 7.5, 10, 12.5, 17.5, 20, 25

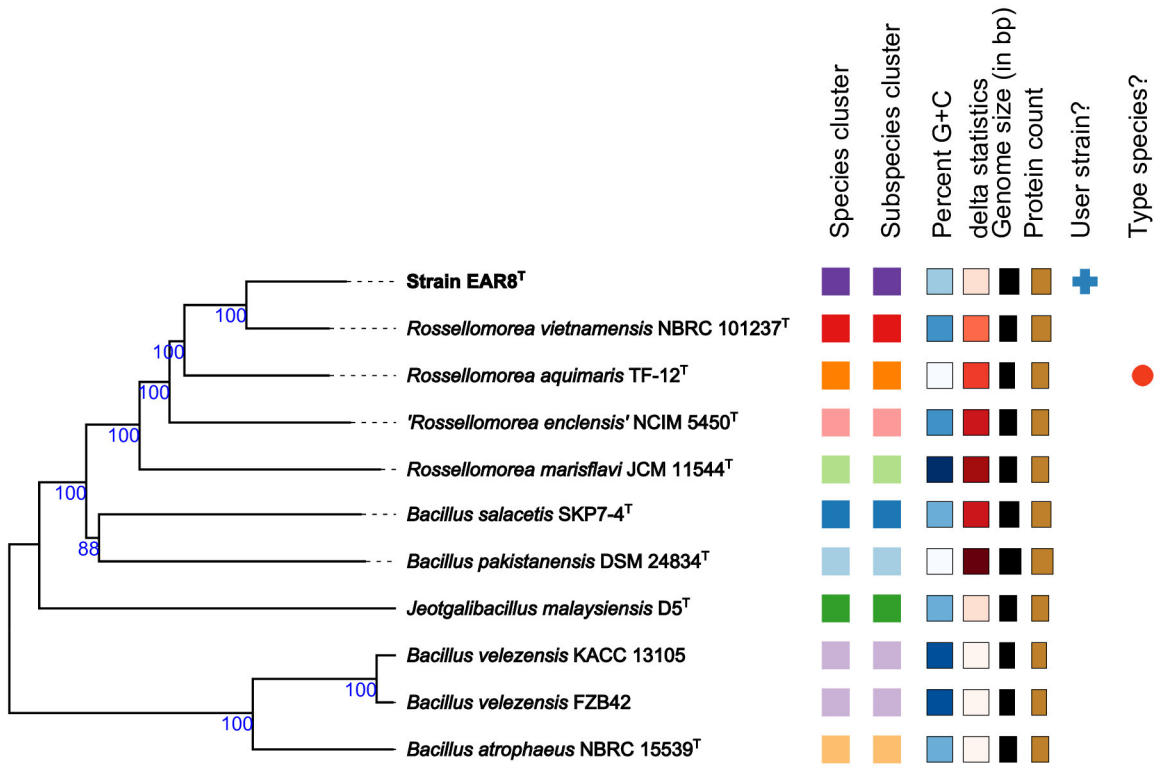

Fig. 2. Phylogenomic tree inferred with GBDP from whole proteomes showing the phylogenetic position of strain EAR8^T relative to closest species. The numbers above branches are GBDP pseudo-bootstrap support values from 100 replications.

and 30%; w/v) at 28 °C for 6 days [35]. Anaerobic respiration was tested in tubes with semisolid TSA supplemented with 2.5% NaCl (w/v) covered with 2 ml agar (2% w/v) and, then, with 2 ml paraffin. Tubes were incubated at 28 °C for 10 days [36]. Additional cultural features were tested on marine agar (MA) and two selective media, cetrimide agar and MacConkey agar, both supplemented with 2.5% NaCl (w/v). Colony characteristics were observed on TSA plates supplemented with 2.5% NaCl (w/v) grown at 37 °C after 24 h using a stereoscopic microscope (Olympus SZ61). Colony colour was determined by the colour chart RAL D2 Design. Cell morphology was examined in the growth phase using an optical microscope with a ×100 objective (Olympus CX41) after Gram staining [37]. Finally, cell motility was studied by observing a drop of liquid culture under optical microscopy with a ×40 objective [6].

Cells of strain EAR8^T were Gram-stain-positive, aerobic and non-motile rods of 0.3–0.4×1.4–2 µm (in growth phase) occurring singly. Terminal oval endospores and non-swollen sporangia were also identified (Fig. S1) in concordance with related species [3, 4, 38] and in line with the genus description [1]. However, the absence of motility is a trait only shared with a few species of the closer genus *Bacillus* i.e. *Bacillus anthracis*, *Bacillus megaterium* and *Bacillus mycoides* [39], being the first non-motile representative in *Rossellomorea*. After 24 h at 37 °C on TSA 2.5% NaCl (w/v) plates, cells formed beige (RAL 090 90 10), opaque, butyrous, raised and irregular colonies with an undulate margin whose diameter was 3.8 mm. After 7 days, a curled margin appeared. Growth was observed at pH from pH 6.0 to 9.0 (optimum pH at 7.0–8.0) and at temperature from 15 to 45 °C (optimum temperature at 37 °C). The temperature range for growth was similar to those shown by the reference strains (Table 1). Regarding NaCl tolerance, strain EAR8^T tolerated up to 25% NaCl (w/v) and it was able to grow in absence of NaCl, so it could be considered an extremely halotolerant bacterium according to the classification suggested by Ventosa *et al.* [40]. Closely related species were also described as halotolerant [3–5, 38], but strain EAR8^T was shown to be the most salt tolerant strain in this study (Table 1).

Oxidase activity was determined adding 1% *N*,*N*,*N*′,*N*′-tetramethyl-*p*-phenylenediamine powder (Becton, Dickinson and Company) to the bacterial biomass. Oxidase test was considered positive if the colour of the biomass turned blue. For the catalase activity, a drop of 3% H_2O_2 was added to the bacterial biomass. The presence of bubbles indicated if the test was positive.

Strain EAR8^T was catalase-positive but oxidase-negative (Table 1) in correlation with previous descriptions for most species of the genus.

Biochemical characterizations of strain EAR8^T were performed using the API 20NE, API 20Strep and API ZYM galleries (bioMérieux) according to the manufacturer's instructions. In addition, GEN III MicroPlates (Biolog) were

Table 1. Differential characteristics among strain EAR8^T and the closely related type strains of the genus *Rossellomorea*.

Strains: 1, Strain EAR8^T; 2, *R. aquimaris* TF-12^T; 3, *R. vietnamensis* 15-1^T; 4, *R. marisflavi* TF-11^T; 5, *R. oryzaecorticis* R1^T. +, positive; –, negative; W, weak; ND, no data available.

Characteristics	1	2	3	4	5
NaCl range for growth (%, w/v)	0–25	0–18[a]	0–15[c]	0–16[a]	0–9[b]
Temperature range for growth (°C)	15–45	10–44[a]	10–40[c]	10–47[a]	15–45[b]
Optimum temperature for growth (°C)	37	30–37[a]	31–40[c]	30–37[a]	37[b]
Oxidase activity	–	–[a]	+[c]	–[a]	+[b]
Hydrolysis of:					
Aesculin	+	–[a]	+[c]	+[a]	ND
Gelatin	+	–[b]	+[c]	+[b]	–[b]
Urease activity	+	–[a]	–[c]	–[a]	–[b]
Assimilation of:					
L-Arabinose	+	–[b]	–[c]	–[b]	+[b]
D-mannose	+	–[b]	–[c]	+[b]	+[b]
D-mannitol	+	–[b]	+[c]	+[b]	+[b]
N-Acetyl-glucosamine	+	–[b]	+[c]	W[b]	–[b]
Potassium gluconate	–	ND	+[c]	ND	+[b]
Acid production from:					
D-Ribose	–	+[a]	+[c]	+[a]	W[d]
D-Mannitol	–	–[a]	+[c]	+[a]	+[d]
Lactose	+	–[a]	–[c]	–[a]	–[d]
D-Raffinose	–	–[a]	+[c]	W[a]	–[d]
Glycogen	–	+[a]	+[c]	–[a]	+[d]
Oxidation of:					
β-Gentiobiose	–	–	+	–[b]	–[b]
Turanose	–	+	+	–[b]	–[b]
β-Methyl-D-glucoside	+	+	–	ND	–[b]
D-Salicin	+	–	–	+[b]	–[b]
D-Mannose	+	–	–	ND	ND
3-*O*-Methyl-D-glucose	–	+	+	ND	ND
D-Fucose	+	+	–	–[b]	–[b]
D-Arabitol	–	+	+	ND	–[b]
myo-Inositol	+	–	–	–[b]	–[b]
D-Glucuronic acid	+	–	–	ND	ND
D-Saccharic acid	+	–	–	ND	ND
Citric acid	+	–	–	ND	ND
L-Malic acid	+	–	–	ND	ND
β-Hydroxy-butyric acid	+	–	–	ND	ND
Propionic acid	–	+	+	ND	ND

Continued

Table 1. Continued

Characteristics	1	2	3	4	5
Resistance to:					
Nalidixic acid	–	+	+	ND	ND
1% Sodium lactate	–	+	+	ND	ND
Sodium formate	–	+	+	ND	ND

a, Data from Yoon *et al.* [3].
b, Data from Hong *et al.* [38].
c, Data from Noguchi *et al.* [4].
d, Data from Daroonpunt *et al.* [49].

used to test carbon source utilization and chemical sensitivity. the GEN III MicroPlates were inoculated using liquid cultures resuspended in a viscous inoculating fluid C supplemented with 2.5% NaCl (w/v) at 90–95% transmittance. Then, those were incubated at 30 °C for 3 days in an Omnilog device (Biolog). Obtained results were analysed with the opm package for R version 1.3.72 [41, 42]. Reference strains *R. aquimaris* DSM 16205^T and *R. vietnamensis* DSM 18898^T were tested in parallel experiments.

Previous data from Navarro-Torre *et al.* [6] also indicated that strain EAR8^T hydrolyses casein and DNA but not starch, Tween 80, cellulose, chitin and pectin. Additional biochemical results from the API 20NE and API ZYM galleries and the GEN III MicroPlates can be found in Table 1 and Fig. S2. A detailed list of them are also provided in the protologue.

Table 2. Cellular fatty acid patterns (%) of strain EAR8^T and closely related species

Strains: 1, EAR8^T; 2, *R. aquimaris* DSM 16205^T; 3, *R. vietnamensis* DSM 18898^T. –, Not detected; TR, values below 1%. Values below 1% in all columns are not displayed. All data were obtained in this study.

Fatty acid	1	2	3
iso-$C_{14:0}$	4.0	1.1	2.2
iso-$C_{15:0}$	21.6	19.9	15.0
anteiso-$C_{15:0}$	52.2	34.8	36.3
$C_{16:1}$ ω7*c* alcohol	4.0	–	TR
iso-$C_{16:0}$	2.2	1.9	6.8
$C_{16:0}$	TR	2.4	10.4
Summed feature 4*	3.7	TR	TR
iso-$C_{17:0}$	TR	4.3	11.4
anteiso-$C_{17:0}$	5.7	16.5	12.7
$C_{18:0}$	TR	2.9	TR
anteiso-$C_{19:0}$	–	6.5	–
$C_{20:0}$	–	3.4	–

*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. Summed feature 4 was listed as iso-$C_{17:1}$ and/or anteiso-$C_{17:1}$.

For chemotaxonomic analysis, respiratory quinones and polar lipids were extracted from freeze-dried biomass using an aqueous methanol–petroleum ether (1:1, v/v) solution following the combined protocol established by Minnikin *et al.* [43]. Respiratory quinone residue was then dissolved in isopropanol and identified by high-performance liquid chromatography [44]. Recovered polar lipids extracts were analysed by 2D thin-layer chromatography (TLC) [43]. Polar lipid identification was carried out by spraying different reagents over the TLC plates (0.2% ninhydrin in acetone, α-naphtol-sulphuric acid, 1.3% molybdenum blue, Dragendorff reagent and 5% molibdatophosphoric acid in ethanol to detect amino-groups, glyco-groups, phospho-groups, choline-groups and all total lipids, respectively) [45, 46]. In addition, 40 mg fresh biomass grown on TSA plates supplemented with 0.3 M NaCl for 24 h at 28 °C wase harvested to extract fatty acids following the protocol outlined by Sasser [47]. Reference strains indicated previously were analysed in parallel experiments using the same growth conditions. The identification of fatty acids was performed using the Microbial Identification System (MIDI) Sherlock version 6.1 (TSBA40 database). Lastly, Staneck and Roberts's protocol [48] was used to identify the stereoisomer of diaminopimelic acid in the peptidoglycan of strain EAR8^T.

Strain EAR8^T presented *meso*-diaminopimelic acid in the cell-wall peptidoglycan and MK-7 as the major respiratory quinone (88%), similarly to what was already described for other *Rossellomorea* representatives. Minor MK components (<10%) of MK-6 (4%), MK-8 (3%) and MK-9 (0.3%) were also identified. The fatty acid pattern was mainly composed of anteiso-$C_{15:0}$ and iso-$C_{15:0}$ (Table 2) as outlined for other related species used in this study and in correlation with what was already observed in other species [3, 38]. Finally, the polar lipid profile comprised diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine (Fig. S3). A similar pattern was observed for other species in the genus such as *Rosseallomorea oryzaecorticis* [38].

Considering the results of phylogenetic analysis and dDDH and ANI relatedness studies, and on the basis of phenotypic evidence, we propose that strain EAR8^T represents a novel species in the genus *Rossellomorea*, for which the name *Rossellomorea arthrocnemi* sp. nov. is proposed.

DESCRIPTION OF *ROSSELLOMOREA ARTHROCNEMI* SP. NOV.

Rossellomorea arthrocnemi (ar.thro.cne'mi. N.L. gen. neut. n. *arthrocnemi*, of *Arthrocnemum macrostachyum*, where the type strain was isolated from).

Cells are Gram-stain-positive, aerobic and non-motile rods. Endospores are terminal and oval in non-swollen sporangia. Colonies are beige, opaque, butyrous, raised and irregular with undulate margins on TSA 2.5% NaCl (w/v) plates at 37 °C for 24 h (optimal growth conditions). Growth ranges are pH 6.0–9.0 (optimum at 7.0–8.0) and 15–45 °C (optimum at 37 °C). It tolerates 0–25% NaCl (w/v) and grows on MA plates but not on cetrimide or MacConkey agar media.

Catalase-positive and oxidase-negative. Aesculin, casein, DNA and gelatin are hydrolysed, butnot cellulose, chitin, pectin, starch and Tween 80 are not. Reduces nitrates to nitrites; assimilates D-glucose, maltose, D-mannitol, D-mannose, L-arabinose, malic acid and *N*-acetyl-D-glucosamine; ferments D-glucose; but cannot assimilate adipic acid, capric acid, phenylacetic acid, potassium gluconate and trisodium citrate. Negative for arginine dihidrolase and indole production and positive for urease activity. Acid is produced from lactose, trehalose and starch, but not from D-mannitol, D-raffinose, D-ribose, D-sorbitol, glycogen, inulin and L-arabinose. Positive for the Voges–Proskauer test and negative for leucine aminopeptidase and pyrrolidonyl arilamidase activities. Strong enzymatic activity is observed for alkaline phosphatase and β-glucosidase; weak activity for acid phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, naphthol-AS-BI-phosphohydrolase and α-glucosidase; and no activity for acid phosphatase, cystine arylamidase, lipase (C14), *N*-acetyl-β-glucosaminidase, trypsin, valine arylamidase, α-chymotrypsin, α-fucosidase, α-galactosidase, α-mannosidase, β-galactosidase and β-glucuronidase. Acetic acid, acetoacetic acid, citric acid, dextrin, D-fructose, D-fructose-6-phosphate, D-fucose, D-gluconic acid, D-glucuronic acid, D-glucose, D-lactic acid methyl ester, maltose, D-mannitol, D-mannose, D-saccharic acid, D-salicin, trehalose, gelatin, glycerol, glycyl-L-proline, L-arginine, L-aspartic acid, L-galactonic acid-γ-lactone, L-glutamic acid, L-histidine, L-lactic acid, L-malic acid, L-pyroglutamic acid, L-serine, methyl pyruvate, *myo*-inositol, *N*-acetyl-D-glucosamine, *N*-acetyl-β-D-mannosamine, pectin, sucrose, Tween 40, α-keto-glutaric acid, β-hydroxy-butyric acid and β-methyl-D-glucoside are oxidized but not bromo-succinic acid, butyric acid, D-arabitol, D-aspartic acid, cellobiose, D-galactose, D-galacturonic acid, D-glucose-6-phosphate, D-malic acid, melibiose, D-raffinose, D-sorbitol, turanose, serine, glucuronamide, inosine, L-alanine, L-fucose, L-rhamnose, 3-methyl-glucose, mucid acid, *N*-acetyl-D-galactosamine, *N*-acetyl-D-neuraminic acid, *p*-hydroxy-phenylacetic acid, propionic acid, quinic acid, stachytose, lactose, α-hydroxy-butyric acid, α-keto-butyric acid, β-gentiobiose and γ-amino-butyric acid. It tolerates aztreonam, lithium chloride and potassium tellurite, but not fusidic acid, guanidine HCl, lincomycin, minocycline, nalidixic acid, niaproof 4, rifampicin SV, sodium bromate, sodium formate, 1% sodium lactate, tetrazolium blue, tetrazolium violet, troleandomycin and vancomycin.

Meso-diaminopimelic acid is present in the the cell-wall peptidoglycan and MK-7 is the major respiratory quinone. The predominant fatty acids are anteiso-$C_{15:0}$ and iso-$C_{15:0}$. The polar lipid profile is composed of diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine. The genome of strain EAR8^T has a total length of 4775586 bp, formed of 107 contigs and has a coverage of 80.6×. The N50 value is 199119 and the G+C content is 42 mol%.

The type strain, EAR8^T (=CECT 9072^T=DSM 103900^T), was isolated as a root endophyte of the halophyte *Arthrocnemum macrostachyum*.

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

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