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A study of three bacteria isolated from marine sediment and description of *Micromonospora globispora* sp. nov.☆

Lorena Carro[a,b,*,1], Aysel Veyisoglu[c,1], Demet Cetin[d], José M. Igual[e], Hans-Peter Klenk[a], Martha E. Trujillo[b], Nevzat Sahin[f,**]

[a] School of Natural and Environmental Sciences, Newcastle University, Ridley Building, Newcastle upon Tyne, NE1 7RU, UK
[b] Departamento de Microbiología y Genética, Edificio Departamental, Campus Miguel de Unamuno, Universidad de Salamanca, 37007 Salamanca, Spain
[c] Department of Bioengineering, Faculty of Engineering and Architecture, Sinop University, 57000, Nasuhbasoglu-Sinop, Turkey
[d] Science Teaching Programme, Gazi Faculty of Education, Gazi University, Ankara, Turkey
[e] IRNASA-CSIC, Salamanca, Spain
[f] Department of Molecular Biology and Genetic, Faculty of Art and Science, Ondokuz Mayis University, 55139 Kurupelit-Samsun, Turkey

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ABSTRACT

During a study looking for the isolation of new actinobacteria strains with potential for antibiotic production from deep marine sediment, three strains were collected with a morphology similar to the one described for the *Micromonospora* genus. A polyphasic study was designed to determine the taxonomic affiliation of the strains S2901^T, S2903, and S2904. All the strains showed chemotaxonomic properties in line with their classification in the genus *Micromonospora*, *meso*-diaminopimelic acid in the wall peptidoglycan, a tetrahydrogenated menaquinone with nine isoprene units as major respiratory quinone, *iso*-$C_{15:0}$ and *iso*-$C_{16:0}$ as major fatty acids and diphosphatidylglycerol, phosphatidylethanolamine and phosphatidylinositol as major polar lipids. The 16S rRNA gene sequences of strain S2901^T, S2903, and S2904 showed the highest similarity (99.2%) with the type strain of *Micromonospora halophytica* DSM 43171^T, forming an independent branch in the phylogenetic gene tree. Their independent position was confirmed with *gyr*B gene and MLSA phylogenies. Whole genome sequences confirmed by digital DNA-DNA hybridization analysis that the isolates should be assigned to a new species within the genus *Micromonospora* for which the name *Micromonospora globispora* sp. nov. (S2901^T, S2903 and S2904) is proposed.

Micromonospora [1] is the type genus of the family *Micromonosporaceae* [2] within the phylum *Actinobacteria*. At the time of writing the genus is composed of 83 species according to the list of prokaryotic names with standing in nomenclature [3]. This genus of Gram-stain positive, aerobic, filamentous and spore-producing actinobacteria has characteristic carotenoid pigments giving orange colorations in most of their colonies [4]. Strains of *Micromonospora* have been isolated from a wide range of habitats, being soils [5–7] and plants [8–10] their most abundant sources. Several strains representing new species of the genus have been described from marine habitats, and more specifically marine sediments, exemplified by the type strains of *Micromonospora krabiensis* [11], *Micromonospora sediminicola* [12], *Micromonospora fluostatini* [13], *Micromonospora yasonensis* [14], *Micromonospora sediminis* [15] and *Micromonospora profundi* [16].

Polyphasic studies to determine the taxonomic affiliation of *Micromonospora* strains usually include 16 rRNA gene phylogeny; however, this gene marker is not sufficiently discriminative for the genus [17]. Alternatively, the use of the housekeeping gene *gyr*B [18,19], as well as multilocus sequence analysis including *atp*D, *rec*A, and *rpo*B genes provide better phylogenetic resolution [4,17]. Application of new genomic technologies has allowed the analysis of whole-genome sequences for a great number of bacteria, including a recent study of 42 different species of *Micromonospora* [20], greatly increasing the available information of the genus and giving the possibility of comparison with new described species that

☆ The GenBank/EMBL/DDBJ accession number for the 16S rRNA genes and the genomes of the strains S2901^T, S2903, and S2904 are KF818390, JN989305, and KF818391, and QGGF00000000, QGSU00000000, and QGSV00000000, respectively.

* Corresponding author at: Departamento de Microbiología y Genética, Edificio Departamental, Campus Miguel de Unamuno, Universidad de Salamanca, 37007 Salamanca, Spain.
** Corresponding author at:, Department of Molecular Biology and Genetic, Faculty of Art and Science, Ondokuz Mayis University, 55139 Kurupelit-Samsun, Turkey.
E-mail addresses: lcg@usal.es (L. Carro), nsahin@omu.edu.tr (N. Sahin).

[1] These authors have participated equally to this work.

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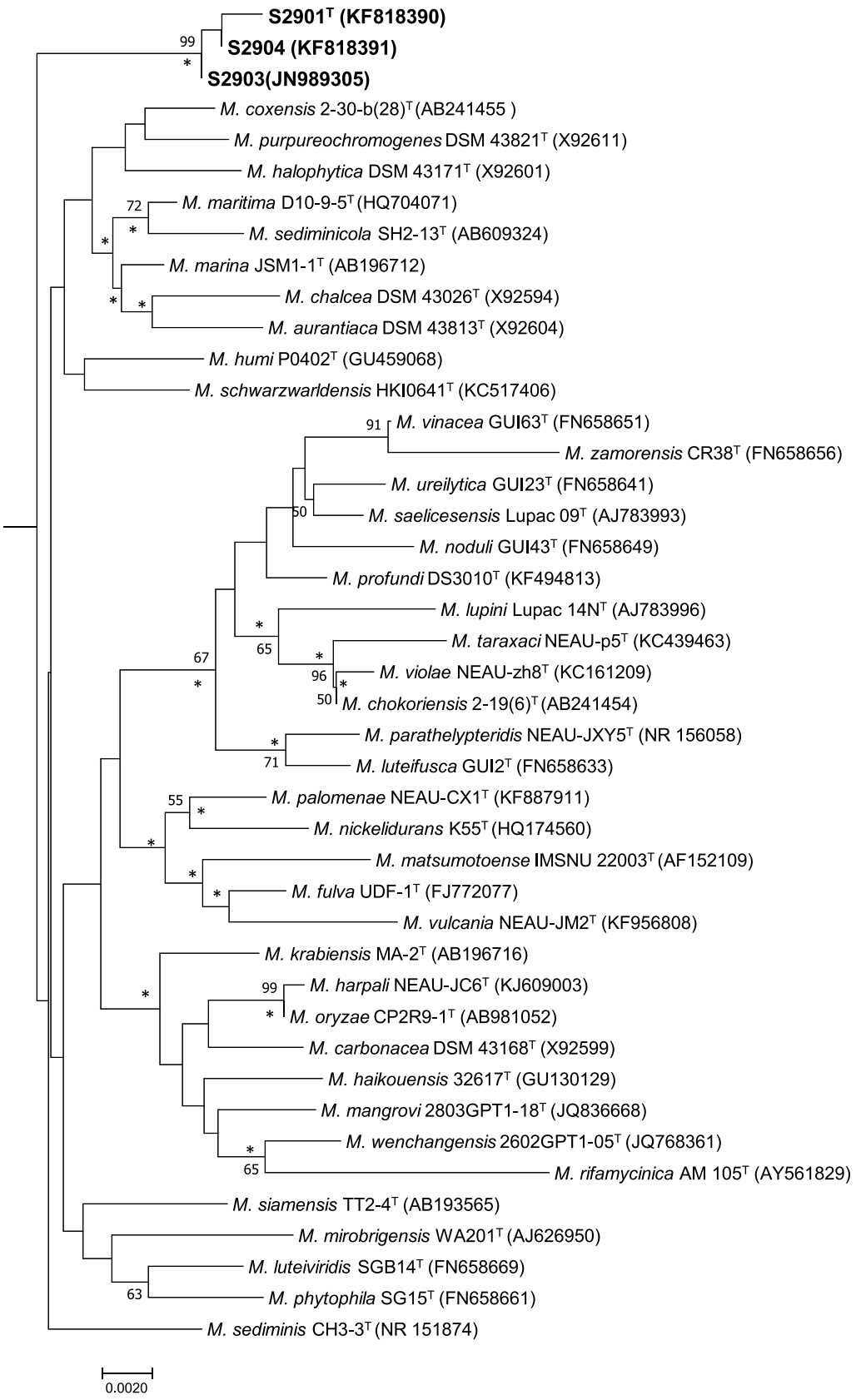

Fig. 1. Neighbour-joining phylogenetic tree section based on 16S rRNA gene sequences of strains S2901T, S2903 and S2904 and closely related species in the genus *Micromonospora*. A total of 1547 nt were analysed. Asterisks indicate that the corresponding nodes were also recovered in the maximum-likelihood tree. Numbers at the nodes indicate levels of bootstrap support (%); only values over 50% are shown. Bar, 0.002 substitutions per nucleotide position.

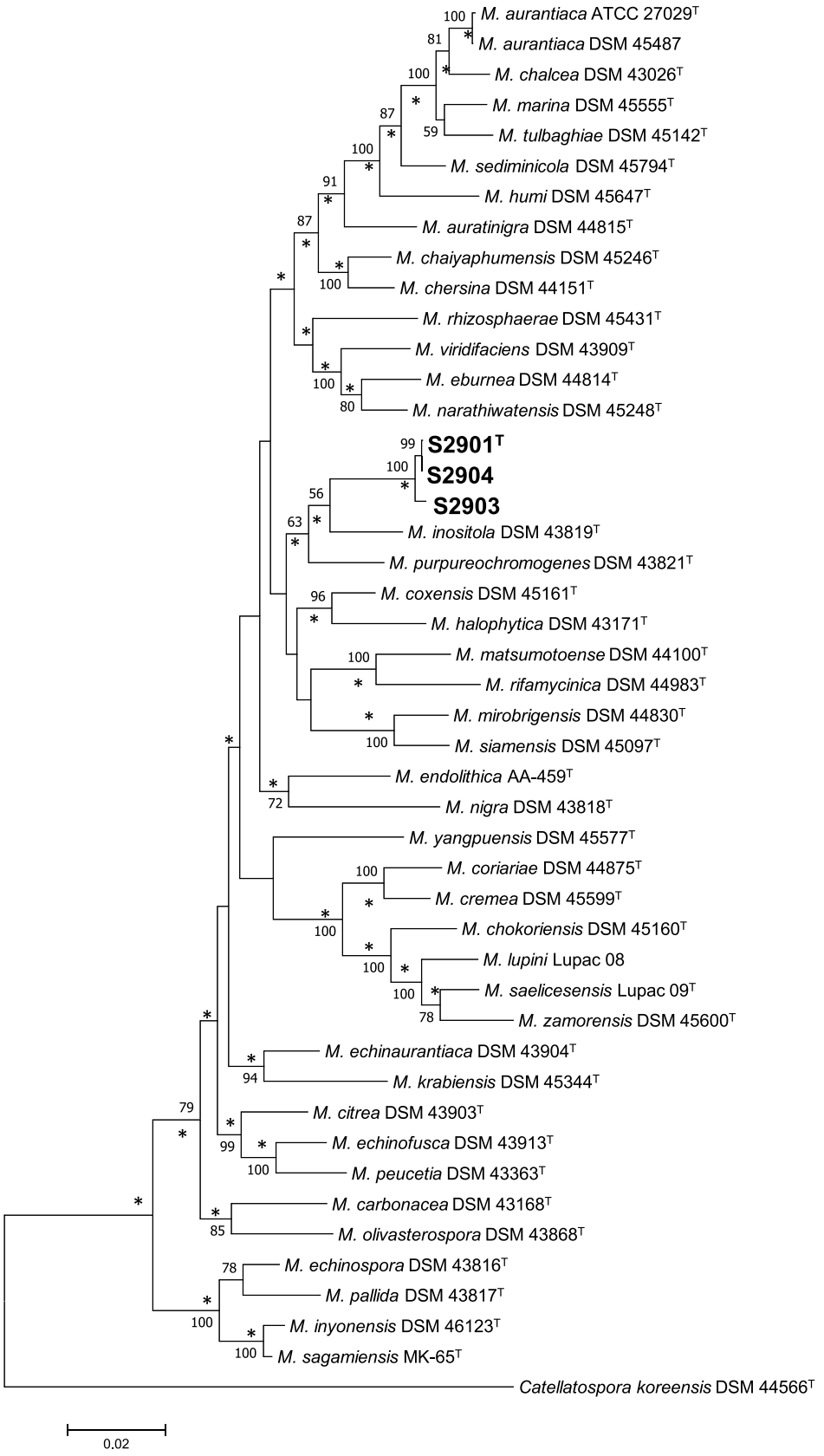

Fig. 2. Maximum-likelihood phylogenetic tree based on multilocus sequence analysis of 16S rRNA, *gyr*B, *rpo*B, *atp*D, and *rec*A sequences of strains S2901^T, S2903 and S2904 and closely related species in the genus *Micromonospora*. A total of 9049 nt were analysed. Asterisks indicate that the corresponding nodes were also recovered in the neighbour-joining tree. Numbers at the nodes indicate levels of bootstrap support (%); only values over 50% are shown. Bar, 0.02 substitutions per nucleotide position.

Table 1
Differential characteristics of study strains of study and their closest phylogenetic relatives. Strains: 1, S2901^T; 2, S2903; 3, S2904; 4, *M. coxensis* DSM 45161^T; 5, *M. halophytica* DSM 43171^T; 6, *M. inositola* DSM 43819^T; 7, *M. purpureochromogenes* DSM 43821^T;. DPG: diphosphatidylglycerol, PE: phosphatidylethanolamine, PI: phosphatidylinositol, GL: glycolipid, PL: unidentified polar lipids, Ara: arabinose, Gal: galactose, Glu: glucose, Man: mannose, Rha:rhamnose, Rib: ribose, Xyl: xylose. Numbers in parentheses for chemotaxonomic data represent the percentages detected for each compound.

Characteristics	1	2	3	4	5	6	7
Major menaquinones (%)	MK-9(H_4) (63)	MK-9(H_4) (70)	MK-9(H_4) (73)	MK-9(H_4)[a]	MK-9(H_4)[a]	MK-9(H_4)[a]	MK-9(H_4)[a]
	MK-9(H_6) (10)	MK-9(H_6) (9)	MK-9(H_6) (9)	MK-9(H_6)[a]			
	MK-10(H_4) (5)	MK-10(H_4) (5)	MK-9(H_2) (9)	MK-9(H_8)[a]			
	MK-9(H_2) (4)	MK-9(H_2) (5)					
Polar lipid profile	DPG, PE, PI, GLs, 2PLs	DPG, PE, PI, GLs, 3PLs	DPG, PE, PI, GLs, 2PLs	DPG, PE, PIM, PG[a]	ND	ND	ND
Major fatty acids (%)	*iso*-$C_{16:0}$ (23)	*iso*-$C_{15:0}$ (26)	Sum fea. 9 (21)	10 methyl-$C_{17:0}$ (22)[a]	*iso*-$C_{16:0}$ (26)[a]	*iso*-$C_{16:0}$ (19)[a]	*iso*-$C_{16:0}$ (26)[a]
	iso-$C_{15:0}$ (20)	*iso*-$C_{16:0}$ (11)	*iso*-$C_{15:0}$ (19)	*iso*-$C_{17:1}$ ω9c (13)[a]	*iso*-$C_{17:0}$ (16)[a]	*iso*-$C_{15:0}$ (14)[a]	*iso*-$C_{15:0}$ (19)[a]
	Sum fea. 9 (16)	Sum fea. 9 (19)	*iso*-$C_{16:0}$ (16)	*iso*-$C_{15:0}$ (12)[a]	*anteiso*-$C_{17:0}$ (13)[a]	*iso*-$C_{17:0}$ (8)[a]	9 methyl-$C_{16:1}$ (9)[a]
	iso-$C_{17:0}$ (12)	*iso*-$C_{17:0}$ (17)	*anteiso*-$C_{17:0}$ (10)	*anteiso*-$C_{17:0}$ (9)[a]	$C_{16:0}$ (12)[a]	10 methyl-$C_{17:0}$ (8)[a]	
	anteiso-$C_{18:0}$ (8)	*anteiso*-$C_{18:0}$ (10)	*iso*-$C_{17:0}$ (9)	*iso*-$C_{16:1}$ (8)[a]	$C_{17:0}$ (10)[a]		
Whole-cell sugars	Man, Xyl	Gal, Glu, Man, Rib, Xyl	Gal, Man, Xyl	Ara, Gal, Glu, Man, Rha, Rib, Xyl[a]	ND	ND	ND
Tolerence tests							
pH 5	+	+	+	+	–	–	–
pH 6	+	+	+	+	–	–	–
pH 10	+	+	+	–	–	+	–
20 °C	–	–	–	+	–	+	–
37 °C	+	+	+	+	–	+	+
40 °C	+	+	+	–	–	+	–
1% (w/v) NaCl	–	–	–	+	+	+	+
2% (w/v) NaCl	–	–	–	+	+	+	–
3% (w/v) NaCl	–	–	–	+	–	+	–
Degradation (%, w/v) of							
Tween 40 (% 1)	–	–	–	+	–	–	+
Tween 80 (% 1)	–	–	–	+	–	–	–
Carbon source utilization (1.0%, w/v)							
D-Arabinose	–	+	–	+	+	–	–
D-Arabinose	+	+	+	–	+	+	+
D-Cellobiose	+	+	+	+	–	+	+
D-Fructose	+	+	–	+	+	+	+
D-Galactose	+	+	+	+	–	+	+
meso-Inositol	+	+	+	–	–	+	–
Maltose	+	+	+	+	–	+	+
D-Mannose	+	+	+	+	–	+	+
D-Mannitol	+	+	+	–	–	–	–
D-ribose	+	+	+	+	–	–	–
Xylose	–	+	+	+	–	–	–

[a] Data from Carro et al. [37].

include whole genome sequences. The combination of genomic and phenotypic information will allow a faster and more reliable classification of new isolates of the genus *Micromonospora*.

In this study the taxonomic status of three *Micromonospora* strains isolated from marine sediments in Turkey was determined. The strains, S2901^T, S2903 and S2904, were compared between them and with the closest validly published species of *Micromonospora* genus using a range of phenotypic and genotypic characteristics. The comparison showed that the strains represent a new species within the genus *Micromonospora* for which the name *Micromonospora globispora* sp. nov. is proposed.

Strains S2901^T, S2903 and S2904 were isolated from marine sediment collected using a dredge at a depth of 45 m off the Yason Peninsula on the southern Black Sea coast near Ordu, Turkey (41°08.184′ N and 37°41.126′ E). The sediment samples were stored at −20 °C until processed using the standard dilution plate method. The strains were isolated in SM1 medium (Stevenson's medium) [21] supplemented with filter sterilised cycloheximide (50 μg ml^{-1}), nalidixic acid (10 μg ml^{-1}), novobiocin (10 μg ml^{-1}) and nystatin (50 μg ml^{-1}) to avoid the development of fungi and Gram-negative bacteria, following incubation at 30° C for 30 days. The isolates were purified on yeast extract-malt extract agar (International *Streptomyces* Project medium 2 (ISP 2), [22]); maintained on agar slopes at 4 °C and preserved as suspensions of mycelial fragments and spores in glycerol (20%, v/v) at −20 °C and −80 °C.

The study strains together with closely related type strains were examined for a range of morphological, cultural and physiological properties. Temperature, pH and NaCl tolerances were determined using ISP 2 (pH 7.2) as the basal medium. Growth at different temperatures (4, 10, 20, 28, 30, 37, 40, 45, 50 and 55 °C) was determined after incubation for 14 days at pH 7.2. NaCl tolerance against 1-10% NaCl (w/v) (at intervals of 1.0 NaCl unit) and pH tolerance (4.0, 5.0, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 10.0, 11.0 and 12.0) were evaluated at 30 °C for 14 days; KH_2PO_4/HCl, KH_2PO_4/K_2HPO_4 and K_2HPO_4/NaOH buffer systems were used to maintain the pH values of the media. Established methods were followed to determine whether the strains degraded Tweens 40 and 80 [23]; the remaining degradation tests were examined using previously described methods [24]. Carbon-source utilization was examined using ISP 9 (pH 7) medium [22] supplemented with a final concentration of 1% (w/v) of the carbon sources. Utilization of amino acids as sole nitrogen sources was determined according to Williams et al. [24] using a final concentration of 0.1% (w/v) of each nitrogen source. In addition, cultural characteristics of the strains were determined on Czapek's agar [25], ISP media 2–7 [22], modified Bennett's agar (MBA; Jones 1949), nutrient agar [26] and tryptic soy agar (TSA;

Table 2
Fatty acids profiles of the strains S2901^T, S2903, and S2904.

Fatty acids	S2901^T	S2903	S2904
Saturated			
$C_{14:0}$	0.2	0.3	0.2
$C_{16:0}$	0.8	1.0	0.8
$C_{18:0}$	1.8	1.4	1.5
$C_{19:0}$	0.3	0.2	0.2
Saturated branched			
iso-$C_{13:0}$	0.2	0.2	0.2
iso-$C_{14:0}$	2.0	0.8	0.6
iso-$C_{15:0}$	19.6	19.2	26.8
iso-$C_{16:0}$	23.1	16.6	10.8
iso-$C_{17:0}$	11.6	8.7	17.3
iso-$C_{18:0}$	2.5	1.2	1.5
iso-$C_{19:0}$	0.2	0.2	0.4
anteiso-$C_{15:0}$	4.6	4.7	4.5
anteiso-$C_{16:0}$	0.3	0.4	0.3
anteiso-$C_{17:0}$	7.9	10.0	10.5
Unsaturated straight			
$C_{17:1}\omega8c$	0.9	2.0	0.5
$C_{17:1}\omega5c$	–	–	0.2
$C_{18:1}\omega9c$	1.1	2.3	0.8
Unsaturated branched			
iso-$C_{15:1}$ F	0.5	0.5	0.6
iso-$C_{16:1}$ H	2.9	3.7	1.6
anteiso-$C_{17:1}\omega9c$	1.4	3.6	1.6
Methyl branched			
10-methyl $C_{18:0}$, TBSA	1.1	0.9	–
*Summed features			
3	0.3	0.5	0.2
6	0.4	–	–
9	16.1	21.4	18.8

*Summed features: 3 ($C_{16:1}$ ω7c/$C_{16:1}$ ω6c; $C_{16:1}$ ω6c/$C_{16:1}$ ω7c), 6 ($C_{19:1}$ ω11c/$C_{19:1}$ ω9c), 9 (10-methyl $C_{16:0}$; *iso*-$C_{17:1}$ ω9c).

Table 3
DNA-DNA distances of strains S2901^T, S2903 and S2904 and closest type strains of *Micromonospora* evaluated through GGDC and OrthoANI methods.

	S2901^T	S2903	S2904
M. coxensis DSM 45161^T	35.5/86.3	36.6/86.8	35.5/86.1
M. halophytica DSM 43171^T	35.6/86.2	36.5/86.7	35.6/86.2
M. mirobrigensis DSM 44830^T	34.9/86.1	36.1/86.4	34.9/86.1
M. purpureochromogenes DSM 43821^T	36.4/86.8	37.6/87.0	36.4/86.8
M. inositola DSM 43819^T	42.3/89.6	43.4/89.9	42.3/89.6
S2903	96.8/99.6	–	–
S2904	100/100	96.8/99.6	–

Difco) following incubation at 30 °C for 14 days. The ISCC-NBS colour charts were used to determine colony colours [27]. Furthermore, the micromorphological properties of the strains were determined by examining gold coated dehydrated specimens of 43-day cultures grown on N-Z-Amine agar (DSMZ-medium 554) using a JEOL JSM 6060 instrument.

Biomass for the chemotaxonomic analyses was harvested from shake flasks (200 revolutions per minute) of GYM (DSMZ medium 65) after 14 days at 28 C°, washed three time in sodium chloride solution (0.9%, w/v) and freeze dried. Biomass for fatty acid analyses was recovered from five days-old cultures in GYM media incubated at 28 °C. Standard thin-layer chromatographic procedures were used to establish the chemotaxonomic profiles of the strains, including the determination of the isomers of diaminopimelic acid (A2pm) [28], predominant isoprenologues [29,30], diagnostic sugars and polar lipids using the procedure of Minnikin et al.[31]. Samples for cellular fatty acids were analysed by GC using the standard MIDI system (Sherlock version 4.5) and peaks were named using the database RTSBA6 [32].

Genetic profiles were obtained after amplification with the primer M13 (5′- GAGGGTGGCGGTTCT-3′). DNA was extracted using a REDExtract-N.Amp kit (Sigma) and amplified following the manufacturer's instructions. The thermal cycling parameters were: 7 min at 95 °C, 35 cycles of 1 min at 94 °C, 1 min at 45 °C and 2 min at 72 °C, followed by a 6 min final extension at 72 °C. A 1.5% agarose gel containing ethidium bromide was loaded with 10 μl of each of the PCR products and electrophoresis run at 85 V for 90 min in freshly prepared 1x TBE-EDTA buffer at pH 8.0 using a Bio-Rad PowerPac 300 power supply. Photographs of the electrophoresis results were recovered as TIFF files. Genomic DNA extraction, PCR-mediated amplification and 16S rRNA gene sequencing were performed as described by Chun and Goodfellow [33] using an ABI PRISM 3730 XL automatic sequencer. Clustal W was used to align nearly full-length 16S rRNA gene sequences obtained in this study with the type strains of *Micromonospora* species retrieved from GenBank database. The housekeeping gene *gyr*B was amplified and sequenced following the protocol described by Garcia et al. [18]. Phylogenetic analyses were performed with MEGA 7.0 using the neighbor-joining and maximum likelihood algorithms as previously described [4]. Whole-genome sequencing was carried out at MicrobesNG using an Illumina HiSeq 2500 platform with 2 × 250 bp paired-end reads. All strains were analysed through a standard pipeline, identifying the closest available reference genome using Kraken and mapping the reads using BWA mem. A *de novo* assembly of the reads was carried out using SPAdes. Variant calling was performed using VarScan and reordered and reoriented relative to a reference genome based on a MUMmer whole-genome alignment. An automated annotation was performed using Prokka. The digital DNA–DNA hybridization (dDDH) value between the draft genome of the strains and closely related type strains from the genus *Micromonospora* were calculated using formula 2 of the GGDC web server available at http://ggds.dsmz.de/phylo_form.php and OrthoANI value using OAT version 0.93.1 [34]. Housekeeping genes were retrieved and verified from the genome sequences to construct the multilocus sequence analysis proposed by Carro et al. [17] for the genus *Micromonospora*.

All strains studied presented morphology and phenotypic properties that fits with the characteristics described for the genus *Micromonospora* [35]. Isolates S2901^T, S2903, and S2904 presented bright orange colonies that turned darker upon the production of spores showing differential abilities to grow on several media (Table S1) and single spores on the tip of the hyphae (Supplementary Fig. S1 in the online version at DOI: 10.1016/j.syapm.2018.11.003). None of the strains produced soluble pigments in the media tested. Growth occurred between 28 and 40 °C in the absence of NaCl with an optimum at 28 °C; S2901^T, S2903, and S2904 grow at a pH between 5 and 10. The use of D-mannitol and their inability to grow in the presence of 1% (w/v) NaCl are characteristics that differ the three isolates from all the closely related type strains. Other phenotypic features evaluated for the strains and closely related species are shown in Table 1. Differences were observed between the strains isolated, mainly in carbon source utilization, and with the reference type strains, in carbon and nitrogen sources utilization, as well as in tolerance test. All the strains analysed presented *meso*-diaminopimelic acid in their cell walls, while whole-cell sugar profiles were different from each other although all contained the characteristic sugars xylose and mannose. Moreover, galactose was detected in strains S2903 and S2904, and glucose and ribose in S2903. The three isolates presented tetrahydrogenated menaquinone with nine isoprene units as the predominant isoprenologue (63, 70, and 73%). All the strains presented similar profiles for polar lipids, including phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylinositol, glycolipids and some unidentified polar lipids. The fatty acid profile of the isolated strains is shown in Table 2. Major fatty acids detected, *iso*-$C_{15:0}$ and *iso*-$C_{16:0}$, are typically found in the genus *Micromonospora* [35], although some variations were observed between the strains

Table 4
Description of *Micromonospora globispora* sp. nov. according to Digital Protologue TA00710 assigned by the www.imedea.uib.es/dprotologue website.

Taxonumber	TA00710
Species name	*Micromonospora globispora*
Genus name	*Micromonospora*
Specific epithet	*globispora*
Species status	sp. nov.
Species etymology	glo.bi.spo'ra. L. masc. n. *globus*, globe; N.L. fem. n. *spora* (from Gr. fem. n. spora), a spore; N.L. fem. adj. *globispora*, with air-balloon shaped spores
Authors	Lorena Carro, Aysel Veyisoglu, Demet Cetin, Jose Mariano Igual, Hans-Peter Klenk, Martha E. Trujillo, Nevzat Sahin
Title	A study of bacteria isolated from marine sediment and description of *Micromonospora globispora* sp. nov.
Corresponding author	Lorena Carro García
E-mail of the corresponding author	lcg@usal.es
Submitter	Lorena Carro
E-mail of the submitter	lcg@usal.es
Designation of the type strain	S2901
Strain collection numbers	KCTC 29212T = DSM 45936
16S rRNA gene accession number	KF818390
Genome accession number [EMBL]	QGGF00000000
Genome status	Draft
Genome size	6600000
GC mol %	71.7
Country of origin	Turkey
Region of origin	Ordu
Source of isolation	Sediment of Black Sea
Sampling date	01 Jan 2012
Number of strains in study	3
Source of isolation of non-type strains	Marine sediment
Growth medium, incubation conditions [temperature, pH, and further information] used for standard cultivation	M65, 28 °C, pH 7, without NaCl
Gram stain	POSITIVE
Cell shape	Filamentous
Motility	Nonmotile
Sporulation	Exospores
Mycelium	Substrate
Colony morphology	Orange
Temperature range	28-40
Temperature optimum	28
Highest pH for growth	5
Lowest pH for growth	10
pH optimum	7
pH category	Neutrophile
Lowest NaCl concentration for growth	0
Highest NaCl concentration for growth	0
Salinity optimum	0
Salinity category	Nonhalophile
Relationship to O2	Aerobe
Carbon source used [specific compounds]	D-arabinose, D-cellobiose, D-galactose, D-mannose, D-mannitol, D-ribose, dextrin, inulin, lactose, L-rhamnose, maltose, meso-inositol, succinic acid, sucrose
Carbon source not used [specific compounds]	Adonitol, D-sorbitol, dextran, L-glutamate, L-sorbose, xylitol
Carbon source variable [specific compounds]	D-fructose, L-arabinose, xylose
Nitrogen source	α-isoleucine, D-phenylalanine, glycine, L-alanine, L-arginine, L-asparagine, L-cysteine, L-hydroxyproline, L-methionine, L-phenylalanine
Energy metabolism	Chemoorganotroph
Biochemical tested properties	Starch is degraded but not adenine, casein, hypoxanthine, gelatine, guanine, Tween-40, Tween-80, xanthine or xylan compounds. Aesculin and arbutin are hydrolysed but not allantoin and urea. Nitrate reduction is positive.
Quinone type	MK-9(H4) and MK-9(H6)
Major fatty acids	*iso*-$C_{15:0}$, *iso*-$C_{16:0}$, *iso*-C17:0, *anteiso*-$C_{17:0}$, Sum in feature 9 (*iso*-$C_{17:1}$ω9c/10 methyl-$C_{16:0}$)
Peptidoglycan type	*meso*-diaminopimelic acid
Phospholipid pattern	Diphosphatidylglycerol, phosphatidylethanolamine, and phosphatidylinositol
Biosafety level	1
Habitat	Marine sediment ENVO:03000033
Biotic relationship	Free-living
Known pathogenicity	None

in the concentrations detected for these fatty acids. Other differences found included the absence of 10-methyl $C_{18:0}$ in the strain S2409. Genetic profiles of the samples shown that the three strains were not identical and could be clearly differentiated (Supplementary Fig. S2 in the online version at DOI: 10.1016/j.syapm.2018.11.003). EzBioCloud results of 16S rRNA gene sequences showed a closest relationship of the study strains with the type strain *Micromonospora halophytica* DSM 43171^T with percentages

of 99.2% in S2901^T, S2903, and S2904, followed by *Micromonospora purpureochromogenes* DSM 43821^T, *Micromonospora mirobrigensis* WA201^T and *Micromonospora coxensis* 2-30-b(28)^T, with 99.0, 99.0, and 98.9% similarity with S2901^T, S2903, and S2904, respectively. Phylogenetic analysis of the 16S rRNA gene sequences showed an independent branch within the genus *Micromonospora* that include the three closely related strains isolated during this study, which was supported by a bootstrap value of 99% (Fig. 1 and Supplementary Fig. S3 in the online version at DOI: 10.1016/j.syapm.2018.11.003). This result was confirmed by the *gyr*B gene and multilocus phylogenies, in which a well-separated branch including the three strains was also generated and supported by high bootstrap values of 99% and 100%, respectively (Supplementary Fig. S4 in the online version at DOI: 10.1016/j.syapm.2018.11.003 and Fig. 2), but showing a close relation with the type strain of *Micromonospora inositola* DSM 43819^T and well as *M. purpureochromogenes* DSM 43821^T in the MLSA tree.

Whole genome sequences of the strains S2901^T, S2903, and S2904 were generated with a genome size of 6673817, 6454517, and 6579413 bp, respectively. The G + C content of the genomic DNA based on the genome sequence is 71.7, 71.6, and 71.9 for S2901^T, S2903, and S2904, respectively. Digital DNA-DNA hybridization (dDDH) similarities were determined between the strains and its close phylogenetic neighbours using the GGDC server [36]. *In silico* DNA:DNA pairing between the strains and the type strains of *M. coxensis, M. halophytica, M. mirobrigensis*, *M. inositola*, and *M. purpureochromogenes,* genomes previously published in Carro et al. [37], were found to be below the recommended 70% cut-off point for the delineation of species [38] (Table 3). dDDH values carried out between the isolated strains showed that all of them had hybridization values over the cut-off point (Table 3). Similar results were observed when OrthoANI values were calculated for each pair, obtaining values under 90% with the type strains of *Micromonospora* and over 99% between the three new strains. These results confirm that our isolates represent a new taxon within the genus *Micromonospora*.

The three strains from this study were isolated from sediment sample at the Black Sea coast; however, none of the strains were able to grow even at 1% of sodium chloride (w/v), which is a main difference with their closest type strains, tolerant to concentrations up to 3%. Although they seem to be closely related, enough phenotypic and genotypic differences have been shown to separate them into a different clade. All the results presented in this study clearly indicate that the strains S2901^T, S2903, S2904 are closely related and represent a new species within the genus *Micromonospora* and we propose the name *Micromonospora globispora* sp. nov.

The formal description of the new species *Micromonospora globispora* sp. nov. is given in Table 4 with the Taxonumber TA00710.

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