Nocardioides salarius sp. nov., isolated from seawater enriched with zooplankton

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A rod-shaped marine bacterium, designated CL-Z59^T, was isolated from seawater enriched with zooplankton. Phylogenetic analyses based on 16S rRNA gene sequences showed that strain CL-Z59^T belongs to the genus *Nocardioides*, the highest level of sequence similarity (99.2 %) being found with respect to *Nocardioides marinisabuli* KCCM 42681^T. However, DNA–DNA hybridization experiments between strain CL-Z59^T and *N. marinisabuli* KCCM 42681^T revealed a mean relatedness value of 33 %. Cells were Gram-positive, strictly aerobic and non-motile. Strain CL-Z59^T grew optimally at pH 6–7 and 25–30 °C and at a NaCl concentration of 3 %. The isolate was characterized chemotaxonomically as having L-diaminopimelic acid in the cell wall. The major isoprenoid quinone was MK-8(H₄) and the predominant cellular fatty acid was iso-C_{16:0}. The DNA G + C content was 73.3 mol%. On the basis of phylogenetic, genotypic and phenotypic data, strain CL-Z59^T represents a novel species of the genus *Nocardioides*, for which the name *Nocardioides salarius* sp. nov. is proposed. The type strain is CL-Z59^T (=KCCM 42320^T=DSM 18239^T).

At the time of writing, since the description of the genus *Nocardioides* within the class *Actinobacteria* (Prauser, 1976), 27 species of the genus have been isolated from various environments. *Nocardioides simplex* (Jensen, 1934), *Nocardioides albus* (Prauser, 1976), *Nocardioides jensenii* (Suzuki & Komagata, 1983; Collins *et al.*, 1989), *Nocardioides luteus* (Prauser, 1984), *Nocardioides alkali-tolerans* (Yoon *et al.*, 2005a), *Nocardioides kribbensis* (Yoon *et al.*, 2005b), *Nocardioides dubius* (Yoon *et al.*, 2005c), *Nocardioides lentus* (Yoon *et al.*, 2006a), *Nocardioides kongjuensis* (Yoon *et al.*, 2006b), *Nocardioides insulae* (Yoon *et al.*, 2007a), *Nocardioides panacihumi* (An *et al.*, 2007), *Nocardioides hankookensis* (Yoon *et al.*, 2008) and *Nocardioides terrigena* (Yoon *et al.*, 2007b) were isolated from various soils. *Nocardioides pyridinolyticus* (Yoon *et al.*, 1997) and *Nocardioides oleivorans* (Schippers *et al.*, 2005) were isolated from the oxic zone of an oil shale column and crude oil, respectively. In addition, *Nocardioides plantarum* (Collins *et al.*, 1994) was isolated from herbage, and *Nocardioides nitrophenolicus* (Yoon *et al.*, 1999), *Nocardioides aquiterrae* (Yoon *et al.*, 2004) and *Nocardioides aromaticivorans* (Kubota *et al.*, 2005) were

isolated from non-saline aquatic environments (industrial wastewater, groundwater and a river, respectively). *Nocardioides daphniae* was isolated from a water flea originating from lake water (Tóth *et al.*, 2008). Furthermore, seven species were isolated from saline environments: *Nocardioides aquaticus* (Lawson *et al.*, 2000) was isolated from a saline lake in Antarctica, *Nocardioides aestuarii* (Yi & Chun, 2004a) and *Nocardioides ganghwensis* (Yi & Chun, 2004b) were isolated from tidal flats, *Nocardioides furvisabuli* (Lee, 2007), *Nocardioides marinisabuli* (Lee *et al.*, 2007) and *Nocardioides hwasunensis* (Lee *et al.*, 2008) were isolated from beach sand and *Nocardioides marinus* (Choi *et al.*, 2007) was isolated from seawater. In this study, strain CL-Z59^T was isolated from seawater enriched with zooplankton and was subjected to a polyphasic taxonomic analysis.

To isolate zooplankton-associated bacteria, seawater was sampled from the surface of the South Sea, Korea, filtered using a syringe filter (0.2 µm pore size) and dispensed into a 20 ml autoclaved glass vial. Then the 0.2 µm-filtered seawater was supplemented with zooplankton [collected using a zooplankton net (200 µm mesh size) and rinsed with 0.2 µm filter-sterilized seawater three times] and incubated at a temperature close to the *in situ* temperature

The GenBank/EMBL/DBJ accession number for the 16S rRNA gene sequence of strain CL-Z59^T is DQ401092.

(approx. 10–15 °C). After about 1 year, 50 µl aliquots were taken and spread on a plate of low-nutrient heterotrophic medium supplemented with 1 × mixed carbons (Cho & Giovannoni, 2004). After 1 month, each colony was streaked again on a plate of low-nutrient heterotrophic medium plus 1 × mixed carbons, and subsequently on marine agar 2216 (MA; Difco). Strain CL-Z59^T grew better on MA and was routinely cultivated on MA at 30 °C. The strain was maintained both on MA at 4 °C and in marine broth 2216 (Difco) supplemented with 30 % (v/v) glycerol at –80 °C.

Morphological, physiological and biochemical analyses were performed. Unless otherwise specified, all characteristics described hereafter were based on cultures grown on MA at 30 °C. Gram-staining was performed as described by Smibert & Krieg (1994). The cell morphology and motility of the isolate were observed by using phase-contrast microscopy (BX50; Olympus). Transmission electron microscopy (EX2; JEOL) was used to establish whether flagella were present. Anaerobic growth was checked on MA and ZOF medium (Lemos *et al.*, 1985) supplemented with additional agar (1.5 %), using the GasPak anaerobic system (BBL) at 30 °C for 15 days. Catalase and oxidase activities and decomposition of casein, hypoxanthine, xanthine and L-tyrosine were determined according to the protocols described by Smibert & Krieg (1994). Nitrate reductase activity and degradation of starch and Tween 80 were investigated as described by Hansen & Sørheim (1991).

The temperature range for growth was determined on the basis of the formation of single colonies visible on MA incubated at 5–45 °C (using increments of 5 °C). The pH range (between pH 5 and 10, using increments of 1 pH unit) for growth was determined by assessing changes in the OD₆₀₀ over time in marine broth 2216. The final pH was adjusted using 1 M NaOH and 1 M HCl solutions. The NaCl tolerance of strain CL-Z59^T was determined using synthetic ZoBell broth (containing, l⁻¹ distilled water: 5 g Bacto peptone, 1 g yeast extract, 0.1 g ferric citrate; Yi & Chun, 2004b) with various NaCl concentrations (0, 0.1, 0.3, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 and 20 %, w/v). Nitrate reduction, indole production, arginine dihydrolase, urease, gelatinase and β-galactosidase, acid production from glucose and hydrolysis of aesculin were tested using an API 20NE kit (bioMérieux) according to the manufacturer's instructions, except that the cell suspension was prepared using artificial seawater at half strength (containing, l⁻¹ distilled water: 12 g NaCl, 5.44 g MgCl₂·6H₂O, 2 g Na₂SO₄, 0.73 g CaCl₂·2H₂O, 0.35 g KCl, 0.1 g NaHCO₃, 0.05 g KBr, 0.0135 g H₃BO₃, 0.02 g SrCl₂·6H₂O and 0.015 g NaF; Lyman & Fleming, 1940) as the suspension medium. Other enzymic activities were determined using an API ZYM kit (bioMérieux) and half-strength artificial seawater as the suspension medium. Carbon utilization was tested using a modified basal broth medium (containing, l⁻¹ distilled water: 11.8 g NaCl, 0.32 g KCl, 2.26 g MgCl₂·6H₂O, 2.97 g MgSO₄·7H₂O,

0.65 g CaCl₂·2H₂O, 0.2 g NaNO₃, 0.2 g NH₄Cl and 0.05 g yeast extract; pH 6.7 ± 0.5) containing 0.2 % carbon source. Carbon utilization was scored as negative when growth was equal to, or less than, that in the negative control with no carbon source. Growth was measured by monitoring changes in the OD₆₀₀ after 8–12 days incubation at 30 °C.

Isoprenoid quinones were isolated according to the method of Minnikin *et al.* (1984) and were analysed by HPLC as described by Collins (1985) at the Korean Culture Center of Microorganisms, Seoul, Korea. The cell-wall diamino acids were determined as described by Stanek & Roberts (1974). The whole-cell fatty acid methyl esters in strain CL-Z59^T, grown on MA at 30 °C for 1 day, were analysed at the Korean Culture Center of Microorganisms, using gas chromatography according to the instructions of the Microbial Identification System (MIDI). Genomic DNA–DNA relatedness was determined by using dot-blot hybridization (Kim *et al.*, 2007). Prehybridization, hybridization and detection were performed using a DIG labelling and detection kit (Roche Molecular Biochemicals) according to the manufacturer's instructions. The experiment was repeated on different days.

For 16S rRNA gene amplification with PCR, DNA was extracted from a single colony by using a boiling method (Englen & Kelley, 2000). The crude extracts served as the DNA template for the PCR reactions, which involved *Taq* DNA polymerase (Bioneer) and primers 27F and 1492R (Lane, 1991). The PCR product was purified by using an AccuPrep PCR purification kit (Bioneer) and was cloned using the pGEM-T Easy vector (Promega). Sequencing of the 16S rRNA gene was performed with an Applied Biosystems automated sequencer (ABI3730XL) at Macrogen Corp. (Seoul, Korea). An almost-complete 16S rRNA gene sequence (1446 bp) of strain CL-Z59^T was obtained. The sequence was compared with those available in the GenBank database by using BLASTN searches (Altschul *et al.*, 1990). The 16S rRNA gene sequence of strain CL-Z59^T was manually aligned with those of the type strains of other species belonging to the genus *Nocardioides* and with phylogenetically related genera obtained from GenBank and the Ribosomal Database Project (Cole *et al.*, 2003) database by using known 16S rRNA gene secondary-structure information. Phylogenetic trees were obtained by using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods. An evolutionary distance matrix for the neighbour-joining method was generated according to the model of Jukes & Cantor (1969). The robustness of tree topologies was assessed by using bootstrap analyses based on 1000 replications for the neighbour-joining and maximum-parsimony methods. Alignment analysis was carried out using the jPHYDIT program (Jeon *et al.*, 2005) and phylogenetic analyses were carried out using MEGA3 (Kumar *et al.*, 2004). The DNA G+C content was determined at the Korean

Table 1. Characteristics that serve to differentiate strain CL-Z59^T from related *Nocardioide*s species

Strains: 1, strain CL-Z59^T; 2, *N. marinisabuli* SBS-12^T; 3, *N. kribbensis* KSL-2^T; 4, *N. plantarum* DSM 11054^T; 5, *N. pyridinolyticus* OS4^T; 6, *N. aquiterrae* GW-9^T; 7, *N. aestuarii* JC2056^T; 8, *N. lentus* KSL-17^T; 9, *N. aquaticus* EL-17K^T; 10, *N. ganghwensis* JC2055^T; 11, *N. marinus* CL-DD14^T; 12, *N. kongjuensis* A2-4^T; 13, *N. nitrophenolicus* NSP41^T. Data are from Yoon *et al.* (1997, 1999, 2004, 2005b, 2006a, b), Yi & Chun (2004a, b), Lawson *et al.* (2000), Choi *et al.* (2007) and Lee *et al.* (2007). All strains are positive for the Gram stain and for the decomposition of casein, catalase, esterase lipase (C8) and leucine arylamidase. All are negative for the decomposition of hypoxanthine and for β -glucuronidase, *N*-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase. +, Positive; –, negative; w, weakly positive; v, variable; ND, not determined.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13
Cell morphology	Rods	Rods	Short rods, cocci	Short rods, cocci	Rods, cocci	Rods, cocci	Rods	Rods, cocci	Cocci, short rods	Rods	Rods, cocci	Rods, cocci	Rods
Cell size (μ m)	0.3–0.6 $\times$ 0.6–1.6	0.6–0.8 $\times$ 1.4–2.1	0.8–1.0 $\times$ 1.5–2.0	ND	0.5–0.6 $\times$ 1.2–1.6	0.8–1.0 $\times$ 1.7–2.0	0.3–0.4 $\times$ 0.9–2.1	0.4–0.7 $\times$ 1.0–4.5	0.9–1.0 $\times$ 0.9–1.4	0.5–0.5 $\times$ 0.9–4.5	0.6–0.6 $\times$ 1.0–1.8	0.7–0.7 $\times$ 0.8–3.0	0.8–0.8 $\times$ 1.0–3.0
Colony colour	Cream	Pale yellow	Cream	ND	Cream	Cream	Ivory	Yellow	Dull orange	Ivory	Cream	Yellowish white	Yellowish white
pH range (optimum)	6–10 (6–7)	5.1–12.1 (7.1–8.1)	6–11 (9)	ND	5–9 (8)	(6–7)	6–10 (7)	6.5–9.5 (8)	5.5–9.5 (7–8)	6–10 (7)	6–9 (7–8)	5.5–10 (7–8)	6–10 (8)
Temperature range (optimum) ($^{\circ}$ C)	10–35 (25–30)	4–40	4–35 (30)	25	20–40 (35)	15–42 (30)	20–35 (30)	4–34 (28)	3–43.5 (16–26)	10–40 (30)	10–40 (25–30)	10–40 (30–37)	15–40 (30)
NaCl range (optimum) (%)	1–10 (3)	0–8	0–3	ND	ND	ND	0–8 (0–2)	0–5 (0.5)	0–15 (1–6)	0–8 (0–1)	0.5–8 (1–3)	0–5 (0)	ND
Motility	–	–	–	–	+	+	–	–	–	–	–	–	+
Oxidase	–	–	+	–*	–	+	–	w	–*	–	–	+	+
Nitrate reduction	–	–	+	–	+	+	–	+	+	+	–	–	–
Decomposition of:													
Starch	w	w	–	–	+	–	–	–	w†	+	–	–	+
Tween 80	+	–	+	+	–	+	+	+	+	+	+	+	+
L-Tyrosine	+	–	+	–	+	–	–	+	+	+	–	–	+
Xanthine	–	–	–	–	+	–	–	–	–	w	–	–	–
API ZYM													
Alkaline phosphatase	+	+	+	–	+	–	w	+	v	+	+	+	+
Esterase (C4)	+	–	+	+	–	–	+	+	w	v	w	–	–
Lipase (C14)	–	–	–	w	–	–	–	–	–	–	–	–	–
Valine arylamidase	+	–	–	w	w	–	+	–	w	+	w	–	+
Cystine arylamidase	+	–	–	+	–	w	–	–	v	w	–	–	w
Trypsin	+	–	–	–	+	+	+	–	w	–	+	–	+
α -Chymotrypsin	+	–	+	–	–	–	+	–	–	–	–	–	–
Acid phosphatase	+	–	+	w	+	+	w	+	+	w	w	+	+
Naphthol-AS-BI-phosphohydrolase	+	–	+	+	+	+	w	+	–	–	–	w	+
α -Galactosidase	–	–	–	–	–	–	–	–	–	+	–	–	–
β -Galactosidase	–	–	w	–	–	–	+	–	–	+	–	–	–

Table 1. cont.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13
α -Glucosidase	+	+	+	+	+	+	+	—	+	+	+	+	+
β -Glucosidase	—	—	—	+	—	W	—	+	—	—	—	—	W
Carbon sources utilized													
<i>N</i> -Acetylglucosamine	—	ND	ND	—*	—*	—*	—	ND	—*	+	—	ND	ND
L-Arabinose	—	—	—	—	—	—	—	W	—	+	—	—	—
L-Arginine	+	ND	ND	+	—*	—*	—	ND	—*	—	—	ND	ND
L-Asparagine	—	ND	ND	—*	+	+	—	ND	—*	+	+	ND	ND
Cellobiose	+	—	+	+	+	+	+	+	—	+	ND	—	—
Citrate	—	+	ND	+	—*	W*	+	ND	—*	+	+	—	—
D-Fructose	—	+	—	+	+	+	+	—	+	+	+	—	+
D-Galactose	—	+	+	—	+	+	+	+	—†	+	+	—	—
D-Glucose	+	—	+	+	+	+	+	+	+	+	+	+	+
Glycerol	—	—	ND	+	—*	—*	W	ND	+	+	—	ND	—
Inositol	—	—	—	—	+	—	—	—	W†	—	—	ND	—
Lactose	—	—	—	—	+	+	W	+	—	+	—	ND	—
L-Lysine	—	ND	ND	—*	—*	+	W	ND	—*	—	—	ND	ND
D-Mannitol	+	—	+	—	+	+	+	+	+	+	+	ND	—
D-Mannose	—	—	—	—	+	+	—	—	—	+	—	W	—
L-Ornithine	+	ND	ND	+	—*	—*	—	ND	—*	+	—	ND	ND
Raffinose	—	—	+	—	—	—	W	+	—	+	—	ND	—
L-Rhamnose	—	—	+	+	+	W†	—	+	+	—	+	ND	+
D-Ribose	+	—	+	—	+	W†	—	—	—	—	—	—	+
D-Salicin	—	—	ND	+	—*	—*	—	ND	—*	+	ND	—	ND
Succinate	—	ND	ND	+	+	+	+	ND	+	+	+	+	+
Sucrose	+	—	+	+	+	+	+	+	+	+	+	+	+
Trehalose	+	ND	+	+	+	+	ND	+	W	ND	+	+	+
D-Xylose	—	+	—	+	+	+	+	+	W‡	+	+	—	+
DNA G + C content (mol%)	73	73	74	69	73	73	70	75	69	72	73	72	71
Isolation source	Zooplankton-enriched seawater	Beach sand	Alkaline soil	Herbage	Oil-shale column	Ground-water	Tidal flat	Alkaline soil	Saline lake, Antarctica	Tidal flat	Seawater	Soil	Industrial waste-water

*Data from Yi & Chun (2004a).

†Negative results were reported by Yi & Chun (2004b).

‡Positive results were reported by Yi & Chun (2004b).

Culture Center of Microorganisms by using HPLC analysis (Tamaoka & Komagata, 1984).

Cells were Gram-positive, non-motile and strictly aerobic. During the exponential growth phase, cells of strain CL-Z59^T were rods (0.3–0.6 µm in width and 0.6–1.6 µm in length). Colonies on MA were circular, smooth, entire, convex and creamy white. Cells were positive for catalase activity and negative for oxidase and nitrate reductase. Cells were positive for the decomposition of casein, starch, Tween 80 and L-tyrosine but were negative for the decomposition of hypoxanthine and xanthine (Table 1).

Growth of strain CL-Z59^T was observed at temperatures in the range 10–35 °C, the optimum being 25–30 °C. Growth occurred at pH 6–10, the optimum pH being 6–7. The strain required NaCl for growth (1–10 %, w/v), the optimum concentration being 3 %. The other results of morphological, physiological and biochemical tests are given in the species description and in Table 1.

Strain CL-Z59^T contained a tetrahydrogenated menaquinone with eight isoprene units [MK-8(H₄)] as the major menaquinone, like most *Nocardioides* species (Holt *et al.*, 1994) and LL-diaminopimelic acid was the

Table 2. Cellular fatty acid composition (%) of strain CL-Z59^T and phylogenetically related *Nocardioides* species

Strains: 1, strain CL-Z59^T; 2, *N. marinisabuli* SBS-12^T; 3, *N. kribbensis* KSL-2^T; 4, *N. plantarum* DSM 11054^T; 5, *N. pyridinolyticus* OS4^T; 6, *N. aquiterrae* GW-9^T; 7, *N. aestuarii* JC2056^T; 8, *N. lentus* KSL-17^T; 9, *N. aquaticus* EL-17K^T; 10, *N. ganghwensis* JC2055^T; 11, *N. marinus* CL-DD14^T; 12, *N. kongjuensis* A2-4^T; 13, *N. nitrophenolicus* NSP41^T. Data are from Yoon *et al.* (1997, 1999, 2004, 2005b, 2006a, b), Lawson *et al.* (2000), Yi & Chun (2004a, b), Choi *et al.* (2007) and Lee *et al.* (2007). Fatty acids that represent <0.5 % in all strains have been omitted. –, Not detected or <0.1 %. Double bond position indicated by a capital letter is unknown (Yoon *et al.*, 2004).

Fatty acid	1	2	3	4	5	6	7	8	9	10	11	12	13
Saturated													
C _{10:0}	–	–	–	5.7	–	–	1.4	–	–	1.6	–	–	–
C _{15:0}	–	–	–	1.2	0.4	0.4	–	1.0	2.4	1.1	0.3	–	–
C _{16:0}	1.0	2.7	3.6	10.5	1.2	0.5	–	1.9	14.1	0.8	1.3	3.9	2.3
C _{17:0}	–	1.0	–	4.4	1.2	0.5	–	1.8	5.9	2.0	0.3	1.4	0.9
C _{18:0}	–	2.4	1.9	6.8	1.0	0.3	–	–	6.9	0.2	0.4	3.4	–
Unsaturated													
C _{17:1ω6c}	–	–	–	2.6	–	–	–	1.7	–	1.6	0.2	11.2	10.8
C _{17:1ω8c}	0.5	2.8	2.4	4.4	1.7	1.7	7.0	6.6	–	25.7	3.8	2.9	1.4
C _{18:1ω7c}	2.4	–	1.9	4.0	–	–	–	–	–	1.3	1.6	–	–
C _{18:1ω9c}	1.4	5.6	7.2	5.0	2.0	0.9	2.7	3.1	30.6*	5.7	4.4	5.0	4.5
Branched													
iso-C _{12:0}	0.3	–	–	2.0	–	–	–	–	–	0.6	–	–	–
iso-C _{14:0}	3.2	–	5.4	0.8	0.5	1.5	1.8	2.3	–	1.3	2.0	–	0.4
iso-C _{15:0}	3.4	3.8	9.7	2.5	5.1	4.9	1.2	0.7	–	2.7	0.3	2.2	3.1
anteiso-C _{15:0}	0.5	–	2.0	–	1.4	2.4	1.3	–	–	–	0.2	–	0.5
iso-C _{16:1} H	4.6	1.4	–	0.3	4.2	6.8*	14.5	–	–	4.4	0.3	–	1.3
iso-C _{16:0}	65.3	48.7	42.5	6.2	47.3	57.6	52.0	60.9	8.1	30.4	71.5	27.1	28.1
iso-C _{17:0}	2.5	10.8	5.7	2.1	1.7	0.7	–	1.0	–	2.4	0.6	6.3	12.3
anteiso-C _{17:0}	2.3	4.3	4.2	–	14.2	9.0	1.8	1.5	10.6	–	0.7	3.0	5.8
iso-C _{17:1ω9c}	–	2.7	2.3	0.5	–	–	–	–	–	1.6	–	–	–
anteiso-C _{17:1ω9c}	0.1	–	–	–	2.1	2.4	–	–	–	–	–	–	–
iso-C _{18:0}	1.4	2.2	1.4	0.2	0.5	0.2	–	1.5	1.3	1.2	2.1	1.4	1.6
10-Methyl													
C _{16:0}	5.2	–	–	–	5.0	3.4	1.3	0.9	–	–	1.4	3.2	3.6
C _{17:0}	1.4	1.7	1.5	6.0	5.5	4.1	3.1	5.8	–	3.9	5.1	6.0	5.1
C _{18:0} (TBSA)†	0.5	–	1.2	8.4	3.4	0.8	0.3	1.6	3.6	–	1.7	14.4	13.9
Hydroxy													
C _{17:0} 3-OH	0.7	–	–	–	–	–	–	2.7	–	–	–	–	–
Summed feature 3‡	3.2	2.9	3.1	0.6	0.8	0.7	1.3	1.8	–	1.9	1.3	2.3	–

*C_{18:1} (Lawson *et al.*, 2000).

†TBSA, Tuberculostearic acid.

‡Summed feature 3 contains iso-C_{15:0} 2-OH and/or C_{16:1ω7c}.

cell-wall diamino acid, as in *Nocardioides* species (Prauser, 1976). The fatty acid profile for strain CL-Z59^T was dominated by iso-C_{16:0} (65.3%; Table 2), which is typically found as a major component in members of the genus *Nocardioides* (Yoon *et al.*, 1999). The DNA G+C content of strain CL-Z59^T was 73.3 mol%, being within the range (67–75 mol%;

Prauser, 1976; Yoon *et al.*, 2006a) reported for recognized species of the genus *Nocardioides*.

Phylogenetic trees based on 16S rRNA gene sequences showed that strain CL-Z59^T falls within the radiation of the cluster comprising *Nocardioides* species (Fig. 1). Sequence similarity indicated that the closest relative of

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationships between strain CL-Z59^T and members of the genus *Nocardioides*. Bootstrap percentages (based on 1000 replications) greater than 60% are shown at nodes. Accession numbers are given in parentheses. Filled circles indicate that the corresponding nodes were also obtained in the maximum-parsimony tree. *Terrabacter tumescens* ATCC 6947^T (AF005023) was used as an outgroup. Bar, 0.01 nucleotide substitutions per site.

strain CL-Z59^T was *N. marinisabuli* (99.2 %); the other members of the genus *Nocardioide*s showed sequence similarities in the range 94.2–97.1 %. In all of the phylogenetic trees, strain CL-Z59^T formed a tight clade with *N. marinisabuli* SBS-12^T (Fig. 1). This clade was related to the nearest clade, containing *N. kribbensis*, *N. aquaticus* and *N. lentus* (Fig. 1). The levels of DNA–DNA relatedness between strain CL-Z59^T and *N. marinisabuli* KCCM 42681^T and between strain CL-Z59^T and *N. kribbensis* KSL-2^T were 33 ± 9 % and 18 ± 6 %, respectively, supporting the view that strain CL-Z59^T is a distinct species (Stackebrandt & Goebel, 1994). Furthermore, strain CL-Z59^T can be clearly distinguished from *N. marinisabuli* on the basis of phenotypic traits: pH, temperature and NaCl range for growth, the ability to decompose Tween 80 and L-tyrosine, the ability to produce acid phosphatase, α -chymotrypsin, cystine arylamidase, esterase (C4), naphthol-AS-BI-phosphohydrolase, trypsin and valine arylamidase and the utilization of cellobiose, citrate, D-fructose, D-galactose, D-glucose, D-mannitol, D-ribose, sucrose and D-xylose (Table 1). In addition, phenotypic, biochemical and chemotaxonomic features, including NaCl ranges for growth, optimal pH, the absence of oxidase and nitrate reductase, the carbon-utilization pattern and the fatty acid profiles, clearly served to differentiate strain CL-Z59^T from *N. kribbensis* (Tables 1 and 2). Notably, combinations of some phenotypic traits (e.g. alkaline phosphatase, α -chymotrypsin, α -galactosidase, α -glucosidase and utilization of L-arabinose, cellobiose, D-glucose, inositol, D-mannitol, succinate and D-xylose) can serve to differentiate species of the genus *Nocardioide*s (Table 1). In conclusion, based on the evidence from the polyphasic study, strain CL-Z59^T represents a novel species of the genus *Nocardioide*s, for which the name *Nocardioide salarius* sp. nov. is proposed.

Description of *Nocardioide salarius* sp. nov.

Nocardioide salarius (sa.la'ri.us. L. masc. adj. *saliarius* of, or belonging to, salt, referring to the isolation of this microorganism from seawater).

Cells are Gram-positive, non-motile, strictly aerobic rods approximately 0.6–1.6 μ m long and 0.3–0.6 μ m wide. Colonies on MA are creamy white and circular, smooth and convex with entire margins. Growth occurs at 10–35 °C (optimally at 25–30 °C) and at pH 6–10 (optimally at pH 6–7). Growth occurs at NaCl concentrations of 1–10 % (w/v) (optimally at 3 %). Positive for catalase but negative for oxidase and nitrate reductase. Casein, starch, Tween 80 and L-tyrosine are hydrolysed but hypoxanthine and xanthine are not. In the API 20NE system, aesculin hydrolysis and gelatinase are positive but nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease and β -galactosidase are negative. In the API ZYM system, acid phosphatase, alkaline phosphatase, α -chymotrypsin, cystine arylamidase, esterase

(C4), esterase lipase (C8), α -glucosidase, leucine arylamidase, trypsin, naphthol-AS-BI-phosphohydrolase and valine arylamidase are positive but *N*-acetyl- β -glucosaminidase, α -fucosidase, α -galactosidase, β -galactosidase, β -glucosidase, β -glucuronidase, lipase (C14) and α -mannosidase are negative. Utilizes L-arginine, cellobiose, D-glucose, D-mannitol, L-ornithine, D-ribose, sucrose and trehalose, but not *N*-acetylglucosamine, L-arabinose, L-asparagine, citrate, D-fructose, D-galactose, glycerol, inositol, lactose, L-lysine, D-mannose, raffinose, L-rhamnose, D-salicin, succinate and D-xylose as sole carbon sources. The major menaquinone is MK-8(H₄). The cell-wall diamino acid is LL-diaminopimelic acid. The major fatty acid is iso-C_{16:0} (65.3 %). The DNA G + C content of the type strain is 73.3 mol%.

The type strain, CL-Z59^T (=KCCM 42320^T=DSM 18239^T), was isolated from seawater enriched with zooplankton.

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