

## *Paenibacillus selenitireducens* sp. nov., a selenite-reducing bacterium isolated from a selenium mineral soil

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A Gram-stain-positive, rod-shaped, facultatively anaerobic bacterium, designated strain ES3-24<sup>T</sup>, was isolated from a selenium mineral soil. The isolate was endospore-forming, nitrate-reducing and motile by means of peritrichous flagella. The major menaquinone was menaquinone 7 (MK-7) and the predominant fatty acids (>5 %) were anteiso-C<sub>15:0</sub>, iso-C<sub>16:0</sub>, C<sub>16:0</sub> and anteiso-C<sub>17:0</sub>. The major polar lipids were diphosphatidylglycerol, phosphatidylethanolamine and two unknown aminophospholipids. Strain ES3-24<sup>T</sup> contained meso-diaminopimelic acid in the cell-wall peptidoglycan and the DNA G+C content was 49.6 mol%. According to phylogenetic analysis based on the 16S rRNA gene sequence, strain ES3-24<sup>T</sup> was most closely related to *Paenibacillus terrigena* A35<sup>T</sup>, with 16S rRNA gene sequence identity of 98.3 %, while the other members of the genus *Paenibacillus* had 16S rRNA gene sequence identities of less than 95.0 %. DNA–DNA relatedness between strain ES3-24<sup>T</sup> and *P. terrigena* CCTCC AB206026<sup>T</sup> was 39.3 %. In addition, strain ES3-24<sup>T</sup> showed obvious differences from closely related species in major polar lipids, nitrate reduction and other physiological and biochemical characteristics. The data from our polyphasic taxonomic study reveal that strain ES3-24<sup>T</sup> represents a novel species of the genus *Paenibacillus*, for which the name *Paenibacillus selenitireducens* sp. nov. is proposed. The type strain is ES3-24<sup>T</sup> (=KCTC 33157<sup>T</sup>=CCTCC AB2013097<sup>T</sup>).

In 1991, the genus *Bacillus sensu lato* was divided into five highly divergent 16S rRNA groups (Ash *et al.*, 1991). The genus *Paenibacillus* was then established to accommodate the members of 16S rRNA group 3 (Ash *et al.*, 1993). Slot-blot hybridization using a specific 16S rRNA gene probe (Ash *et al.*, 1993) and PCR amplification using group 3-specific 16S rRNA gene primers (Shida *et al.*, 1996, 1997) were useful tools to distinguish members of the genus *Paenibacillus* from the other members of *Bacillus sensu lato*. At the time of writing, there are 145 species and four subspecies with validly published names in the genus *Paenibacillus* (<http://www.bacterio.net/p/paenibacillus.html>)

and the type species is *Paenibacillus polymyxa* (Stansly & Schlosser, 1947; Ash *et al.*, 1993). Most of these strains are Gram-reaction-positive, but some young cells react variably or negatively (Uetanabaro *et al.*, 2003; Valverde *et al.*, 2008). Most members of the genus *Paenibacillus* are rod-shaped, facultatively anaerobic or strictly aerobic (Lim *et al.*, 2006), non-pigmented, endospore-forming and motile by means of peritrichous flagella (Ludwig *et al.*, 2009). The DNA G+C content of members of the genus *Paenibacillus* ranges from 39 to 59 mol% (Ash *et al.*, 1993; Takeda *et al.*, 2005; Ludwig *et al.*, 2009; Park *et al.*, 2011; Tang *et al.*, 2011) and the cell-wall peptidoglycan diamino acid is meso-diaminopimelic acid. Menaquinone-7 (MK-7) is the major menaquinone and the predominant cellular fatty acid is anteiso-C<sub>15:0</sub> (34–80 %; Ludwig *et al.*, 2009), except for *Paenibacillus thermophilus* WP-1<sup>T</sup>, *Paenibacillus macerans* DSM 24<sup>T</sup> (Zhou *et al.*, 2012) and *Paenibacillus fonticola* BCRC 17579<sup>T</sup> (Chou *et al.*, 2007), which contain C<sub>16:0</sub> as the major fatty acid. To date, all members of the genus *Paenibacillus* for which polar lipid data are available show diphosphatidylglycerol (DPG) as the major polar lipid. In addition, some species contain phosphatidylethanolamine (PE) (Kim *et al.*, 2010; Tang *et al.*, 2011), phosphatidylglycerol (PG) (Chou *et al.*, 2009; Zhou *et al.*,

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Abbreviations: APL, aminophospholipid; DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PGL, phosphoglycolipid.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain ES3-24<sup>T</sup> is KC815539.

Six supplementary figures and a supplementary table are available with the online version of this paper.

2012), unknown aminophospholipids (APL) (Valverde *et al.*, 2010; Kämpfer *et al.*, 2012) and unknown phosphoglycolipids (PGL) (Chou *et al.*, 2007, 2009; Tang *et al.*, 2011). In this study, strain ES3-24<sup>T</sup> was the subject of a polyphasic taxonomic characterization following general instructions for prokaryotic strains (Tindall *et al.*, 2010) and the minimal standards for description of aerobic, endospore-forming bacteria (Logan *et al.*, 2009).

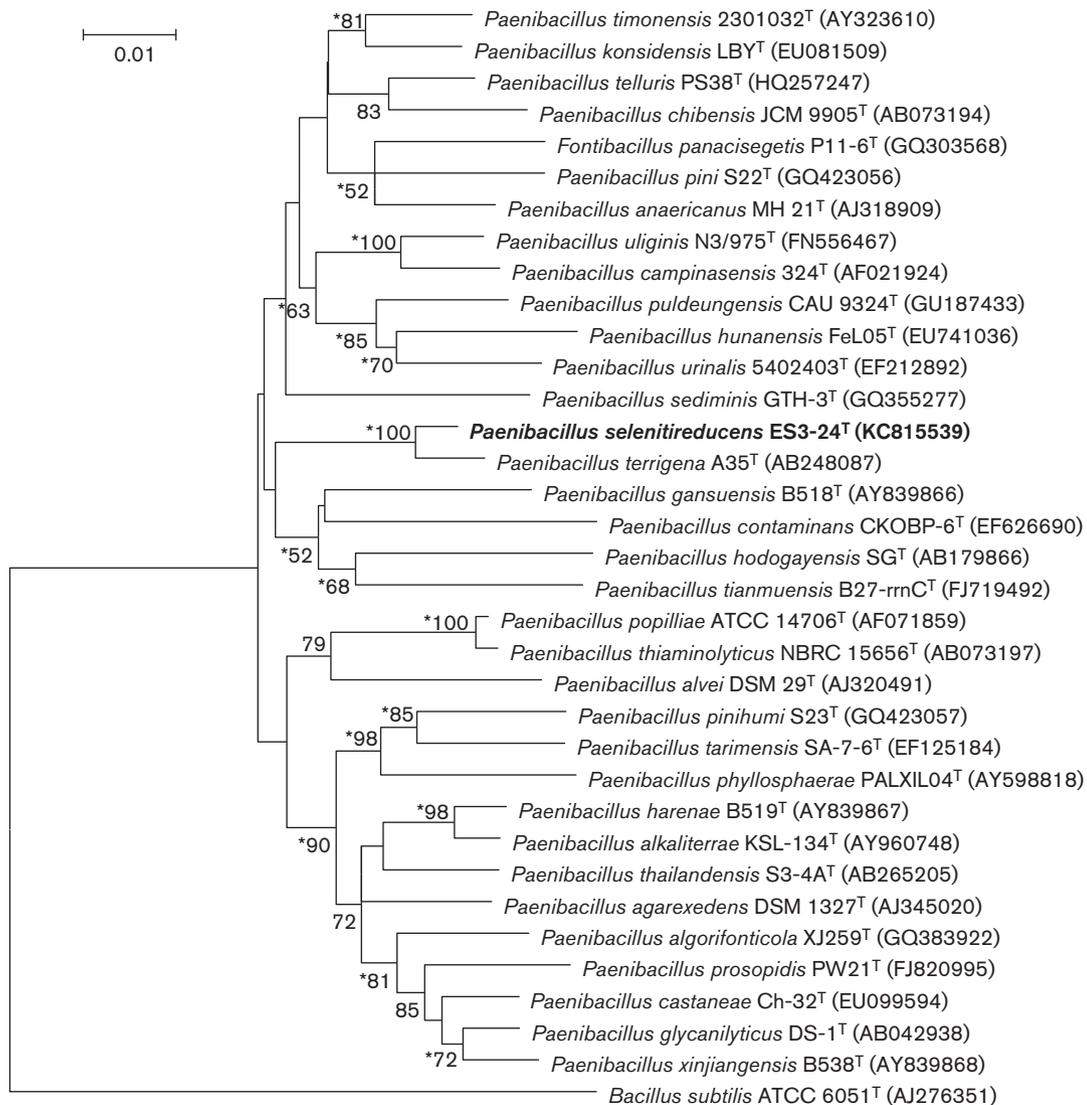
Strain ES3-24<sup>T</sup> was isolated from a selenium mineral soil collected in Enshi selenium mine (30° 17' 54" N 109° 28' 16" E) in Hubei province, PR China. Bacterial isolation was performed using 1/10 TSA (tryptic soy agar, pH 7.3; Difco) containing 1 mM Na<sub>2</sub>SeO<sub>3</sub> and incubated at 28 °C for 2 days. The total soil C, N, P and K concentrations and pH were determined as described by Okkenhaug *et al.* (2011). Cr and Se concentrations were determined by atomic absorption spectrometry and an atomic fluorescence spectroscopy, respectively. The pH of the soil was 4.5 and the total soil C, N, P, K, Cr and Se concentrations were 65.65, 5.03, 0.50, 16.37, 0.12 and 0.02 g·(kg dry weight soil)<sup>-1</sup>, respectively.

The nearly complete 16S rRNA gene sequence (1470 bp) of strain ES3-24<sup>T</sup> was amplified using 16S rRNA gene primers 27F and 1492R as described by Fan *et al.* (2008). The 16S rRNA gene sequence was compared with sequences available in the EzTaxon-e server (Kim *et al.*, 2012) and aligned with its close relatives using the CLUSTAL\_X program (Thompson *et al.*, 1997). Neighbour-joining (NJ; Saitou & Nei, 1987) and maximum-parsimony (MP; Fitch, 1971) trees were reconstructed using MEGA version 4.0 software (Tamura *et al.*, 2007), and the maximum-likelihood (ML) tree was reconstructed with the PHYML online web server (Guindon *et al.*, 2010). Distances were calculated based on Kimura's two-parameter method (Kimura, 1980) and bootstrap analysis was performed according to 1000 resamplings (Felsenstein, 1985). *Bacillus subtilis* ATCC 6051<sup>T</sup> was used in the phylogenetic trees as an outgroup. In the NJ tree, strain ES3-24<sup>T</sup> formed a unit branch with *Paenibacillus terrigena* A35<sup>T</sup> (98.3% 16S rRNA gene sequence identity; Xie & Yokota, 2007) and near a cluster containing *Paenibacillus gansuensis* B518<sup>T</sup> (Lim *et al.*, 2006), *Paenibacillus contaminans* CKOBP-6<sup>T</sup> (Chou *et al.*, 2009), *Paenibacillus hodogayensis* SG<sup>T</sup> (Takeda *et al.*, 2005) and *Paenibacillus tianmuensis* B27-rrnC<sup>T</sup> (Wu *et al.*, 2011) with 94.8, 93.7, 94.0 and 93.7% 16S rRNA gene sequence identity, respectively (Fig. 1). The ML and MP trees showed similar topologies (Figs S1 and S2, available in IJSEM Online). Hence, the closest related strain *P. terrigena* CCTCC AB206026<sup>T</sup> (=A35<sup>T</sup>) and the type strain of the type species, *P. polymyxa* CCUG 7426<sup>T</sup>, were analysed together as reference strains in this study.

For analysis of morphological, physiological and biochemical characteristics, strain ES3-24<sup>T</sup>, *P. terrigena* CCTCC AB206026<sup>T</sup> and *P. polymyxa* CCUG 7426<sup>T</sup> were grown on nutrient agar (NA; Difco) at 28 °C for 2 days unless otherwise mentioned. Sporulating cells and the flagellation

type were examined using a transmission electron microscope (H-7650; Hitachi) and a phase-contrast microscope (BX51M; Olympus) after cultivation in 1/10 TSB (tryptic soy broth, pH 7.3; Difco) for 3 days and 0.3% semi-solid NA for 2 days, respectively. Motility tests were performed using NA with 0.3% agar. The Gram staining reaction was observed using a Gram staining kit (Jiancheng Biotech) according to Dussault (1955) and combined with the KOH (3%) lysis method (Ryu, 1938). Growth at 4, 15, 20, 28, 32, 37 and 42 °C on NA and with 0–7% (w/v) NaCl (1% intervals) and at pH 3–10 (1 pH unit intervals) in nutrient broth (NB; Difco) was tested after 7 days of incubation at 28 °C. NB at pH 3–10 (at 1 pH unit intervals) (Sorokin, 2005) was prepared with the following buffer systems (filtered to remove bacteria): pH 3.0–7.0, 0.1 M citric acid/0.2 M Na<sub>2</sub>HPO<sub>4</sub>; pH 8.0–9.0, 0.2 M Tris/0.2 M HCl; pH 10.0, 0.05 M NaHCO<sub>3</sub>/0.1 M NaOH. Anaerobic growth was determined in an anaerobic chamber (produced by Mitsubishi Gas Chemical; <1% O<sub>2</sub>, 16% or more CO<sub>2</sub> and N<sub>2</sub>) after incubation for 7 days on NA. Growth was also tested on TSA, R2A agar and Luria–Bertani (LB) agar (all from Difco). Nitrate and nitrite reduction, production of H<sub>2</sub>S and indole, hydrolysis of DNA, starch and cellulose, D-glucose fermentation and methyl red and Voges–Proskauer tests were tested according to Dong & Cai (2001). Hydrolysis of casein and Tweens 20 and 80 was detected according to Cowan & Steel (1965). Production of precipitates on egg yolk agar was tested on NA with 10% egg yolk emulsion (cat. no. 17148; Sigma). Catalase activity was determined by assessing bubble production from 3% (v/v) hydrogen peroxide solution. Oxidase activity, hydrolysis of aesculin, urea, gelatin, p-nitrophenyl β-D-galactoside (PNPG) and o-nitrophenyl β-D-galactoside (ONPG), utilization of substrates as sole carbon and energy sources, acid production from carbohydrates and enzyme activities were tested using traditional methods and with the API 20NE, ID 32GN, API 50CHB and API ZYM systems (bioMérieux) according to the manufacturer's instructions. The results of API ZYM test strips were observed after incubation at 28 °C for 6 h. The API 50CHB, API 20 NE and ID 32 GN tests were examined after incubation at 28 °C for 48 h. Sole nitrogen and energy source utilization was determined with 0.1% (w/v) each of source (urea, potassium nitrate, glutamate, ammonium nitrate, ammonium dihydrogen phosphate, sodium nitrite, methionine, histidine, L-proline, L-serine and cysteine) as described by Dong & Cai (2001). The ability to reduce selenite (Na<sub>2</sub>SeO<sub>3</sub>) was observed on NA with the formation of elemental Se (red colour) or by testing the content of Se<sup>4+</sup> using HPLC hydride-generation atomic fluorescence spectroscopy (Beijing Titan Instruments Co.) (Liao *et al.*, 2013) after incubation for 2 days in NB. The results of the different characters are given in Table 1. The cell morphology is shown in Fig. S3, and other characters are listed in the species description.

The DNA G+C content of the isolate was determined by HPLC according to the method of Tamaoka & Komagata



**Fig. 1.** NJ phylogenetic tree showing the position of strain ES3-24<sup>T</sup>. Bootstrap values (>50%) based on 1000 replications are shown at branch nodes. Nodes that were also recovered in trees reconstructed by both the ML and MP methods are indicated by asterisks. Bar, 0.01 substitutions per nucleotide position.

(1984). For analysis of cellular fatty acids, bacterial biomass of strain ES3-24<sup>T</sup>, *P. terrigena* CCTCC AB206026<sup>T</sup> and *P. polymyxa* CCUG 7426<sup>T</sup> was collected when the bacteria reached the mid-exponential phase with similar OD<sub>600</sub> (incubated in TSB at 28 °C for about 20 h) and harvested by centrifugation (10 min, 7000 g) and then analysed using the Sherlock Microbial Identification System (MIDI Sherlock version 4.5; MIDI database TSBA40 4.10) (Kroppenstedt, 1985; Sasser, 1990). The menaquinone of strain ES3-24<sup>T</sup> was extracted using lyophilized cells grown in NB at 28 °C for 2 days and then analysed by the HPLC method as described by Xie & Yokota (2003). Polar lipids of strain ES3-24<sup>T</sup> and *P. terrigena* CCTCC AB206026<sup>T</sup> were extracted and examined as described by Minnikin *et al.* (1979) and

identified by two-dimensional TLC (Collins & Jones, 1980). Peptidoglycan in the cell wall was analysed using the method described by Schumann (2011). The level of genomic DNA–DNA relatedness between strain ES3-24<sup>T</sup> and *P. terrigena* CCTCC AB206026<sup>T</sup> was evaluated by DNA–DNA hybridization according to the thermal denaturation and re-naturation method (Huss *et al.*, 1983). Primers PAEN515F (5'-GCTCGGAGAGTGACGGTACCTGAGA-3') and 1377R (5'-GGCATGCTGATCCGCGATTACTAGC-3') were used in a PCR to produce a 0.9 kb *Paenibacillus*-specific 16S rRNA gene amplicon according to the method of Shida *et al.* (1996, 1997) and Chung *et al.* (2000) except that the annealing temperature used was 62 °C. DNA of two members of 16S rRNA group 2 (Ash *et al.*, 1991), *Lysinibacillus boronitolerans*

**Table 1.** Differential physiological characteristics between strain ES3-24<sup>T</sup> and type strains of phylogenetically related species of the genus *Paenibacillus*

Strain: 1, ES3-24<sup>T</sup>; 2, *P. terrigena* CCTCC AB206026<sup>T</sup>; 3, *P. polymyxa* CCUG 7426<sup>T</sup>; 4, *P. gansuensis* B518<sup>T</sup> (data from Lim *et al.*, 2006); 5, *P. contaminans* CKOBP-6<sup>T</sup> (Chou *et al.*, 2009); 6, *P. hodogayensis* SG<sup>T</sup> (Takeda *et al.*, 2005); 7, *P. tianmuensis* B27-rrnC<sup>T</sup> (Wu *et al.*, 2011). Data are from this study unless indicated. +, Positive; -, negative; w, weak reaction; v, variable; ND, no data available. Acids were produced from maltose, but not from D-adonitol, by all strains.

Characteristic	1	2	3	4	5	6	7
Anaerobic growth	+	+	+	-	+	-	ND
Oxidase	-	-	-	-	+	+	v
Catalase	+	+	+	-	+	+	+
Ranges for growth							
pH	5.0–10.0	3.0–10.0	6.0–9.0	7.0–7.5	6.5–8.0	ND	6.0–8.0
Temperature (°C)	15–37	4–37	4–42	10–45	10–37	ND	22–37
NaCl concentration (% w/v)	0–2	0–4	0–3	ND	0–2	ND	0–2
Nitrate reduction	+	-	-	-	-	-	+
Acid formation from:							
Aesculin	+	+	+	+	-	+	+
Gentiobiose	+	+	+	ND	+	-	w
D-Mannose	+	w	+	+	-	-	-
Mannitol	-	-	+	-	-	+	-
Methyl α-D-mannoside	-	w	-	ND	-	w	-
Lactose	+	+	+	+	-	w	-
Melibiose	+	+	+	+	+	+	-
Glycogen	-	-	+	ND	-	-	-
D-Ribose	+	w	+	+	-	w	+
D-Xylose	+	-	+	+	-	-	-
N-Acetylglucosamine	+	+	-	ND	-	w	+
Sucrose	-	+	+	+	+	+	+
Trehalose	+	-	+	+	+	+	+
Raffinose	+	+	+	+	-	-	-
Enzyme activity							
Alkaline phosphatase	+	-	-	ND	-	ND	ND
Esterase lipase (C8)	+	-	+	ND	-	ND	ND
N-Acetylglucosaminidase	-	+	-	ND	+	ND	ND
Leucine arylamidase	+	+	-	ND	+	ND	ND
α-Galactosidase	+	-	+	ND	-	ND	-
Utilization of:							
Citrate	-	-	-	ND	+	-	+
Potassium nitrate	-	-	+	ND	ND	ND	ND
Ammonium nitrate	+	+	-	ND	ND	ND	ND
Hydrolysis of:							
Starch	-	-	+	-	-	ND	w
Casein	-	-	-	+	-	ND	+
Gelatin	-	+	+	ND	-	-	+
Tween 20	-	-	+	ND	-	ND	ND
Tween 80	-	+	-	+	-	ND	ND
DNA G + C content (mol%)	49.6	48.1 <sup>a*</sup>	43.0–46.0 <sup>b</sup>	50.0	52.1	55.0	55.4–55.5
Major polar lipids†	DPG, PE, 2APL	DPG, PG, PE, 2APL	DPG, PG, PE, 3PL, GL <sup>c</sup>	ND	DPG, PE, PG	ND	ND

\*Data taken from: a, Xie & Yokota (2007); b, Shida *et al.* (1997); c, Kämpfer *et al.* (2006).

†DPG, Diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; APL, unknown aminophospholipid; GL, unknown glycolipid; PL, unknown phospholipid.

NBRC 103108<sup>T</sup> (Ahmed *et al.*, 2007) and *Lysinibacillus manganicus* CCTCC AB2012916<sup>T</sup> (Liu *et al.*, 2013), was used in the PCR amplification as a negative control.

The DNA G + C content of strain ES3-24<sup>T</sup> was 49.6 mol%, which is in the range reported for members of the genus *Paenibacillus* (39–59 mol%; Ash *et al.*, 1993; Takeda *et al.*,

2005; Ludwig *et al.*, 2009; Park *et al.*, 2011; Tang *et al.*, 2011). The fatty acids included anteiso-C<sub>15:0</sub> (55.5%), C<sub>16:0</sub> (13.4%), iso-C<sub>16:0</sub> (9.2%) and anteiso-C<sub>17:0</sub> (8.5%) as major components (>5%). Consistent with other species of the genus *Paenibacillus*, the predominant cellular fatty acid of the isolate was anteiso-C<sub>15:0</sub> (Table S1). The major menaquinone of strain ES3-24<sup>T</sup> was found to be MK-7, and the cell-wall peptidoglycan contained meso-diaminopimelic acid, which is the same as other tested members of the genus *Paenibacillus*. Strain ES3-24<sup>T</sup> and *P. terrigena* CCTCC AB206026<sup>T</sup> both contained DPG. The major polar lipids of strain ES3-24<sup>T</sup> were DPG, PE and two APL; *P. terrigena* CCTCC AB206026<sup>T</sup> contained DPG, PE, PG and two APL (Fig. S4). Similar to the two reference strains of *Paenibacillus*, a 0.9 kb specific PCR product was generated from strain ES3-24<sup>T</sup>, but not from the two strains of the genus *Lysinibacillus* (Fig. S5). The above results indicated that strain ES3-24<sup>T</sup> belonged to the genus *Paenibacillus*. However, DNA–DNA relatedness between strain ES3-24<sup>T</sup> and *P. terrigena* CCTCC AB206026<sup>T</sup> was 39.3 ± 0.8% ( $\Delta T_m = 71.5^\circ\text{C}$ ), which was significantly lower than the threshold value (70%) recommended for species delineation. This value provides evidence to classify strain ES3-24<sup>T</sup> within a novel species of the genus *Paenibacillus* (Stackebrandt & Goebel, 1994; Tindall *et al.*, 2010). In addition, strain ES3-24<sup>T</sup> showed large differences from the closely related type strain *P. terrigena* CCTCC AB206026<sup>T</sup> in major polar lipids (DPG, PE and two APL vs DPG, PE, PG and two APL; Table 1), amounts of iso-C<sub>16:1</sub>ω11c (1.1 vs 11.1%; Table S1), the morphology of colonies on NA (Fig. S6), nitrate reduction, acid production from D-xylose, trehalose and sucrose, activities of *N*-acetylglucosaminidase, α-galactosidase, alkaline phosphatase and esterase lipase (C8) and hydrolysis of gelatin and Tween 80 (Table 1). Compared with the type strain of the type species, *P. polymyxa* CCUG 7426<sup>T</sup>, strain ES3-24<sup>T</sup> also differed in major polar lipids (DPG, PE, PG, an unknown glycolipid and three unknown phospholipids vs DPG, PE and two APL; Table 1), amounts of cellular fatty acids (C<sub>16:0</sub>, 13.4 vs 1.5%; anteiso-C<sub>17:0</sub>, 8.5 vs 40.1%; Table S1), nitrate reduction, acid production from *N*-acetylglucosamine and sucrose, activities of leucine arylamidase and alkaline phosphatase, hydrolysis of gelatin and Tween 20 and utilization of ammonium nitrate and potassium nitrate as sole nitrogen and energy sources (Table 1).

Based on the results of 16S rRNA gene phylogenetic analysis, production of a specific PCR product, major menaquinone, cell-wall peptidoglycan, fatty acid profile and DNA G+C content, strain ES3-24<sup>T</sup> represents a member of the genus *Paenibacillus*. However, strain ES3-24<sup>T</sup> could be differentiated from the closely related species *P. terrigena* in terms of DNA–DNA relatedness, colony morphology, major polar lipids and some biochemical characteristics (Table 1). The data described above provide sufficient evidence to recognize strain ES3-24<sup>T</sup> as a representative of a novel species of the genus *Paenibacillus* according to the recommendations of Tindall *et al.* (2010)

and Logan *et al.* (2009). This strain was isolated by its capacity for selenite reduction and was associated with bioremediation. The name *Paenibacillus selenitireducens* sp. nov. is proposed for this novel species.

### Description of *Paenibacillus selenitireducens* sp. nov.

*Paenibacillus selenitireducens* [se.le.ni'ti.re.du'cens. N.L. n. *selenis* -itis selenite; L. part. adj. *reducens* (from L. v. *reducere*) leading back, bringing back and, in chemistry, converting to a different oxidation state; N.L. part. adj. *selenitireducens* selenite reducing].

Cells are rod-shaped, 0.7–1.2 μm wide and 5.4–6.2 μm long after incubation at 28 °C on NA for 2 days, Gram-stain-positive, catalase-positive, oxidase-negative, facultatively anaerobic and motile. Subterminal ellipsoidal spores are formed in swellings of the sporangium. Colonies are white-cream, flat, smooth, semi-transparent and circular with irregular edges, usually 0.8–1.3 mm in diameter after 2 days on NA at 28 °C. Growth occurs at 15–37 °C (optimum at 28 °C), pH 5.0–10.0 (optimum at pH 7.0–8.0) and 0–2% (w/v) NaCl. Can grow on NA, LB, TSA and R2A medium. Positive for reduction of nitrate to nitrite, PNPG test, ONPG test and hydrolysis of aesculin, but negative for production of indole and H<sub>2</sub>S, nitrite reduction, methyl red and Voges–Proskauer tests, egg yolk reaction and hydrolysis of DNA, arginine, Tweens 20 and 80, casein, gelatin, cellulose, urea and starch. Acid, but not gas, is produced from D-glucose. From the API 50 CHB test results, acid is produced from glycerol, D-ribose, D-xylose, methyl β-D-xyloside, D-galactose, D-glucose, D-mannose, *N*-acetylglucosamine, amygdalin, aesculin, cellobiose, maltose, lactose, melibiose, trehalose, raffinose and gentiobiose and produced weakly from L-arabinose, D-fructose, methyl α-D-glucoside, salicin and turanose; acid is not produced from erythritol, D-arabinose, L-xylose, methyl α-D-mannoside, D-arbutin, sorbose, L-rhamnose, dulcitol, inositol, mannitol, sorbitol, sucrose, inulin, melezitose, glycogen, xylitol, D-lyxose, D-tagatose, D- or L-fucose, D- or L-arabitol, gluconate, 2-ketogluconate or 5-ketogluconate. In API ZYM tests, shows activity of alkaline phosphatase, esterase lipase (C8), α-galactosidase, β-fucosidase, leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-galactosidase, α-glucosidase and β-glucosidase, but no activity of esterase (C4), lipase (C14), valine arylamidase, cystine arylamidase, trypsin, chymotrypsin, α-mannosidase, *N*-acetylglucosaminidase or β-glucuronidase. Maltose, D-mannose, *N*-acetylglucosamine, D-ribose, D-glucose, melibiose, urea, glutamate, ammonium nitrate, ammonium dihydrogen phosphate and methionine are assimilated as sole sources of carbon and/or nitrogen and energy, but rhamnose, inositol, itaconate, suberate, malonate, acetate, DL-lactate, L-alanine, 5-ketogluconate, glycogen, 3-hydroxybenzoate, L-fucose, sorbitol, propionate, caprate, valerate, citrate, 2-ketogluconate, 3-hydroxybutyrate, 4-hydroxybenzoate, adipate, malate, phenylacetate, L-proline, L-serine, histidine, sodium nitrite, potassium nitrate

and cysteine are not assimilated (API 20NE, ID 32GN and traditional methods). The polar lipids are DPG, PE, an unknown lipid and two unknown APL. The predominant menaquinone is MK-7. The cell-wall peptidoglycan diamino acid is *meso*-diaminopimelic acid. The major cellular fatty acids (>5%) are anteiso-C<sub>15:0</sub>, iso-C<sub>16:0</sub>, C<sub>16:0</sub> and anteiso-C<sub>17:0</sub>. The type strain is able to reduce selenite (Na<sub>2</sub>SeO<sub>3</sub>) to elemental selenium.

The type strain is ES3-24<sup>T</sup> (=KCTC 33157<sup>T</sup>=CCTCC AB2013097<sup>T</sup>), isolated from a selenium mineral soil, Enshi, Hubei province, PR China. The DNA G+C content of the type strain is 49.6 mol%.

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## References

- Ahmed, I., Yokota, A., Yamazoe, A. & Fujiwara, T. (2007). Proposal of *Lysinibacillus boronitolerans* gen. nov. sp. nov., and transfer of *Bacillus fusiformis* to *Lysinibacillus fusiformis* comb. nov. and *Bacillus sphaericus* to *Lysinibacillus sphaericus* comb. nov. *Int J Syst Evol Microbiol* **57**, 1117–1125.
- Ash, C., Farrow, J. A. E., Wallbanks, S. & Collins, M. D. (1991). Phylogenetic heterogeneity of the genus *Bacillus* revealed by comparative analysis of small-subunit-ribosomal RNA sequences. *Lett Appl Microbiol* **13**, 202–206.
- Ash, C., Priest, F. G. & Collins, M. D. (1993). Molecular identification of rRNA group 3 bacilli (Ash, Farrow, Wallbanks and Collins) using a PCR probe test. Proposal for the creation of a new genus *Paenibacillus*. *Antonie van Leeuwenhoek* **64**, 253–260.
- Chou, J. H., Chou, Y. J., Lin, K. Y., Sheu, S. Y., Sheu, D. S., Arun, A. B., Young, C. C. & Chen, W. M. (2007). *Paenibacillus fonticola* sp. nov., isolated from a warm spring. *Int J Syst Evol Microbiol* **57**, 1346–1350.
- Chou, J. H., Lee, J. H., Lin, M. C., Chang, P. S., Arun, A. B., Young, C. C. & Chen, W. M. (2009). *Paenibacillus contaminans* sp. nov., isolated from a contaminated laboratory plate. *Int J Syst Evol Microbiol* **59**, 125–129.
- Chung, Y. R., Kim, C. H., Hwang, I. & Chun, J. (2000). *Paenibacillus korensis* sp. nov., a new species that produces an iturin-like antifungal compound. *Int J Syst Evol Microbiol* **50**, 1495–1500.
- Collins, M. D. & Jones, D. (1980). Lipids in the classification and identification of coryneform bacteria containing peptidoglycans based on 2,4-diaminobutyric acid. *J Appl Bacteriol* **48**, 459–470.
- Cowan, S. T. & Steel, K. J. (1965). *Manual for the Identification of Medical Bacteria*. London: Cambridge University Press.
- Dong, X. Z. & Cai, M. Y. (2001). *Determinative Manual for Routine Bacteriology*. Beijing: Scientific Press.
- Dussault, H. P. (1955). An improved technique for staining red halophilic bacteria. *J Bacteriol* **70**, 484–485.
- Fan, H., Su, C., Wang, Y., Yao, J., Zhao, K., Wang, Y. & Wang, G. (2008). Sedimentary arsenite-oxidizing and arsenate-reducing bacteria associated with high arsenic groundwater from Shanyin, Northwestern China. *J Appl Microbiol* **105**, 529–539.
- Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.
- Fitch, W. M. (1971). Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* **20**, 406–416.
- Guindon, S., Dufayard, J. F., Lefort, V., Anisimova, M., Hordijk, W. & Gascuel, O. (2010). New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Syst Biol* **59**, 307–321.
- Huss, V. A. R., Festl, H. & Schleifer, K. H. (1983). Studies on the spectrophotometric determination of DNA hybridization from renaturation rates. *Syst Appl Microbiol* **4**, 184–192.
- Kämpfer, P., Rosselló-Mora, R., Falsen, E., Busse, H.-J. & Tindall, B. J. (2006). *Cohnella thermotolerans* gen. nov., sp. nov., and classification of '*Paenibacillus hongkongensis*' as *Cohnella hongkongensis* sp. nov. *Int J Syst Evol Microbiol* **56**, 781–786.
- Kämpfer, P., Falsen, E., Lodders, N., Martin, K., Kassmannhuber, J. & Busse, H.-J. (2012). *Paenibacillus chartarius* sp. nov., isolated from a paper mill. *Int J Syst Evol Microbiol* **62**, 1342–1347.
- Kim, K. K., Lee, K. C., Yu, H., Ryoo, S., Park, Y. & Lee, J. S. (2010). *Paenibacillus sputi* sp. nov., isolated from the sputum of a patient with pulmonary disease. *Int J Syst Evol Microbiol* **60**, 2371–2376.
- Kim, O. S., Cho, Y. J., Lee, K., Yoon, S. H., Kim, M., Na, H., Park, S. C., Jeon, Y. S., Lee, J. H. & other authors (2012). Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. *Int J Syst Evol Microbiol* **62**, 716–721.
- Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* **16**, 111–120.
- Kroppenstedt, R. M. (1985). Fatty acid and menaquinone analysis of actinomycetes and related organisms. In *Chemical Methods in Bacterial Systematics* (Society for Applied Bacteriology Technical Series no. 20), pp. 173–199. Edited by M. Goodfellow & D. E. Minnikin. London: Academic Press.
- Liao, S. J., Zhou, J. X., Wang, H., Chen, X., Wang, H. F. & Wang, G. J. (2013). Arsenite oxidation using biogenic manganese oxides produced by a deep-sea manganese-oxidizing bacterium, *Marinobacter* sp. MnI7-9. *Geomicrobiol J* **30**, 150–159.
- Lim, J. M., Jeon, C. O., Lee, J. C., Xu, L. H., Jiang, C. L. & Kim, C. J. (2006). *Paenibacillus gansuensis* sp. nov., isolated from desert soil of Gansu Province in China. *Int J Syst Evol Microbiol* **56**, 2131–2134.
- Liu, H. L., Song, Y. M., Chen, F., Zheng, S. X. & Wang, G. (2013). *Lysinibacillus manganicus* sp. nov., isolated from manganese mining soil. *Int J Syst Evol Microbiol* **63**, 3568–3573.
- Logan, N. A., Berge, O., Bishop, A. H., Busse, H.-J., De Vos, P., Fritze, D., Heyndrickx, M., Kämpfer, P., Rabinovitch, L. & other authors (2009). Proposed minimal standards for describing new taxa of aerobic, endospore-forming bacteria. *Int J Syst Evol Microbiol* **59**, 2114–2121.
- Ludwig, W., Schleifer, K. H. & Whitman, W. B. (2009). Family IV. *Paenibacillaceae* fam. nov. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 3, p. 269. Edited by P. De Vos, G. M. Garrity, D. Jones, N. R. Krieg, W. Ludwig, F. Rainey, K. H. Schleifer & W. B. Whitman. New York: Springer.
- Minnikin, D. E., Collins, M. D. & Goodfellow, M. (1979). Fatty acid and polar lipid composition in the classification of *Cellulomonas*, *Oerskovia* and related taxa. *J Appl Bacteriol* **47**, 87–95.
- Okkenhaug, G., Zhu, Y. G., Luo, L., Lei, M., Li, X. & Mulder, J. (2011). Distribution, speciation and availability of antimony (Sb) in soils and terrestrial plants from an active Sb mining area. *Environ Pollut* **159**, 2427–2434.
- Park, M. H., Traiwan, J., Jung, M. Y., Nam, Y. S., Jeong, J. H. & Kim, W. (2011). *Paenibacillus chungangensis* sp. nov., isolated from a tidal-flat sediment. *Int J Syst Evol Microbiol* **61**, 281–285.

- Ryu, E. (1938). On the Gram-differentiation of bacteria by the simplest method. *J Jpn Soc Vet Sci* 17, 58–63.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 4, 406–425.
- Sasser, M. (1990). *Identification of bacteria by gas chromatography of cellular fatty acids*, MIDI Technical Note 101. Newark, DE: MIDI Inc.
- Schumann, P. (2011). Peptidoglycan structure. *Methods Microbiol* 38, 101–129.
- Shida, O., Takagi, H., Kadowaki, K. & Komagata, K. (1996). Proposal for two new genera, *Brevibacillus* gen. nov. and *Aneurinibacillus* gen. nov. *Int J Syst Bacteriol* 46, 939–946.
- Shida, O., Takagi, H., Kadowaki, K., Nakamura, L. K. & Komagata, K. (1997). Transfer of *Bacillus alginolyticus*, *Bacillus chondroitinus*, *Bacillus curdlanolyticus*, *Bacillus glucanolyticus*, *Bacillus kobensis*, and *Bacillus thiaminolyticus* to the genus *Paenibacillus* and emended description of the genus *Paenibacillus*. *Int J Syst Bacteriol* 47, 289–298.
- Sorokin, D. Y. (2005). Is there a limit for high-pH life? *Int J Syst Evol Microbiol* 55, 1405–1406.
- Stackebrandt, E. & Goebel, B. M. (1994). Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* 44, 846–849.
- Stansly, P. G. & Schlosser, M. E. (1947). Studies on polymyxin: isolation and identification of *Bacillus polymyxa* and differentiation of polymyxin from certain known antibiotics. *J Bacteriol* 54, 549–556.
- Takeda, M., Suzuki, I. & Koizumi, J. I. (2005). *Paenibacillus hodogayensis* sp. nov., capable of degrading the polysaccharide produced by *Sphaerotilus natans*. *Int J Syst Evol Microbiol* 55, 737–741.
- Tamaoka, J. & Komagata, K. (1984). Determination of DNA base composition by reversed-phase high-performance liquid chromatography. *FEMS Microbiol Lett* 25, 125–128.
- Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007). MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol Biol Evol* 24, 1596–1599.
- Tang, Q. Y., Yang, N., Wang, J., Xie, Y. Q., Ren, B., Zhou, Y. G., Gu, M. Y., Mao, J., Li, W. J. & other authors (2011). *Paenibacillus algorifonticola* sp. nov., isolated from a cold spring. *Int J Syst Evol Microbiol* 61, 2167–2172.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL\_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* 25, 4876–4882.
- Tindall, B. J., Rosselló-Móra, R., Busse, H. J., Ludwig, W. & Kämpfer, P. (2010). Notes on the characterization of prokaryote strains for taxonomic purposes. *Int J Syst Evol Microbiol* 60, 249–266.
- Uetanabaro, A. P., Wahrenburg, C., Hunger, W., Pukall, R., Spröer, C., Stackebrandt, E., de Canhos, V. P., Claus, D. & Fritze, D. (2003). *Paenibacillus agarexedens* sp. nov., nom. rev., and *Paenibacillus agaridevorans* sp. nov. *Int J Syst Evol Microbiol* 53, 1051–1057.
- Valverde, A., Peix, A., Rivas, R., Velázquez, E., Salazar, S., Santa-Regina, I., Rodríguez-Barrueco, C. & Igual, J. M. (2008). *Paenibacillus castaneae* sp. nov., isolated from the phyllosphere of *Castanea sativa* Miller. *Int J Syst Evol Microbiol* 58, 2560–2564.
- Valverde, A., Fterich, A., Mahdhi, M., Ramírez-Bahena, M. H., Caviedes, M. A., Mars, M., Velázquez, E. & Rodríguez-Llorente, I. D. (2010). *Paenibacillus prospidis* sp. nov., isolated from the nodules of *Prosopis farcta*. *Int J Syst Evol Microbiol* 60, 2182–2186.
- Wu, X. H., Fang, H. H., Qian, C. D., Wen, Y. P., Shen, X. B., Li, O. & Gao, H. C. (2011). *Paenibacillus tianmuensis* sp. nov., isolated from soil. *Int J Syst Evol Microbiol* 61, 1133–1137.
- Xie, C. H. & Yokota, A. (2003). Phylogenetic analyses of *Lampropedia hyalina* based on the 16S rRNA gene sequence. *J Gen Appl Microbiol* 49, 345–349.
- Xie, C. H. & Yokota, A. (2007). *Paenibacillus terrigena* sp. nov., isolated from soil. *Int J Syst Evol Microbiol* 57, 70–72.
- Zhou, Y., Gao, S., Wei, D. Q., Yang, L. L., Huang, X., He, J., Zhang, Y. J., Tang, S. K. & Li, W. J. (2012). *Paenibacillus thermophilus* sp. nov., a novel bacterium isolated from a sediment of hot spring in Fujian province, China. *Antonie van Leeuwenhoek* 102, 601–609.

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