NOTE

***Bacillus vulcani* sp. nov., a novel thermophilic species isolated from a shallow marine hydrothermal vent**Daniela Caccamo,¹ Concetta Gugliandolo,¹ Erko Stackebrandt² and Teresa L. Maugeri¹Author for correspondence: Teresa L. Maugeri. Tel: +39 090 6765523. Fax: +39 090 393409.
e-mail: tmaugeri@unime.it¹ Dipartimento di Biologia Animale ed Ecologia Marina, Università Messina, Salita Sperone, 31, 98166 Messina, Italy² DSMZ – Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Mascheroder Weg 1b, D38124 Braunschweig, Germany**A thermophilic spore-forming bacterium was isolated from sediment of a shallow hydrothermal vent at Vulcano Island (Italy). After phenotypic and molecular analyses, it was identified as a novel *Bacillus* species, for which the name *Bacillus vulcani* is proposed. The type strain is strain 3s-1^T (= DSM 13174^T = CIP 106305^T).****Keywords:** *Bacillus vulcani* sp. nov., thermophilic *Bacillus*, phenotypic characterization, 16S rDNA sequence, DNA–DNA hybridization

The taxonomy of the genus *Bacillus* shows that thermophilic species are members of *Bacillus* rRNA group 5 (Ash *et al.*, 1991; Rainey *et al.*, 1994), including *Bacillus stearothermophilus*, *Bacillus thermoglucosidasius*, *Bacillus thermodenitrificans*, *Saccharococcus thermophilus*, '*Bacillus thermoantarcticus*' (Nicolaus *et al.*, 1996) and the emended species *Bacillus thermoleovorans*, combining *Bacillus thermoleovorans*, *Bacillus kaustophilus*, *Bacillus thermocatenulatus*, '*Bacillus caldolyticus*', '*Bacillus caldotenax*' and '*Bacillus caldovelox*', which are genomically homogeneous (Sunna *et al.*, 1997).

In this paper, we report the characteristics of a novel thermophilic *Bacillus* species isolated from a shallow marine thermal vent of the Eolian Islands, Italy.

A sediment sample was collected at a depth of 3 m during an oceanographic cruise (26/08/1996–06/09/1996) at the site Vulcano – La Roya (14° 58' N 38° 25' W). The sample was inoculated into Bacto Marine Broth 2216 (Difco) and Medium D (Degryse *et al.*, 1978) and incubated aerobically at 65 °C for 3 d. Positive cultures were plated on Bacto Marine Agar 2216 (Difco). All isolates were spore-formers and their phenotypic characters were studied in comparison with eight thermophilic *Bacillus* reference strains. Several isolates were selected for further molecular investigations. The taxonomic properties of strain 3s-1^T will be presented in this note.

The growth optimum was determined after incubation in Marine Broth at 37, 55, 60, 65, 70 and 75 °C and pH 5.5, 6.0, 7.0, 8.0 and 9.0. Halotolerance was tested in Nutrient Broth (Oxoid) with 0, 2, 3, 5, 7 and 10 % (w/v) NaCl. Biochemical characteristics were screened by API 20 E, API 20 NE, API 50 CHB and API ZYM (bioMérieux) according to Sharp *et al.* (1980) and Logan & Berkeley (1984). Sensitivity to nalidixic acid (30 µg), polymyxin B (300 U), chloramphenicol (30 µg), bacitracin (10 U), kanamycin (30 µg), tetracycline (30 µg), novobiocin (30 µg), penicillin G (1.5 U), streptomycin (10 µg) and erythromycin (10 µg) was tested. Tolerance to lysozyme and NaN₃, and xylan and dextran hydrolysis were tested according to White *et al.* (1993). Gelatin, starch and casein hydrolysis were tested according to Marteinsson *et al.* (1996). Lipolytic activity was tested according to Degryse *et al.* (1978).

Fatty acid and polar lipid content were determined as described by Nicolaus *et al.* (1995).

16S rDNA sequencing was performed according to Sunna *et al.* (1997). The 16S rDNA sequence obtained was manually aligned with reference sequences of *Bacillus* spp. using the ae2 editor (Maidak *et al.*, 1997). Evolutionary distances were calculated by the method of Jukes & Cantor (1969). Phylogenetic dendrograms were constructed using the method of DeSoete (1983) and the neighbour-joining method (Felsenstein, 1993). A bootstrap analysis was used for the neighbour-joining tree by performing 500 resamplings (Felsenstein, 1988). Determination of mol% G+C content and DNA–DNA hybridization were performed according to Sunna *et al.* (1997).

The GenBank accession number for the 16S rDNA sequence of *Bacillus vulcani* is AJ293805.

Table 1. Differential phenotypic and chemotaxonomic properties of *Bacillus* strain 3s-1^T and related thermophilic bacilli

w, Weak reaction; v, variable.

	<i>Bacillus</i> strain 3s-1 ^T	<i>B. thermoleovorans</i> *	<i>B. stearothermophilus</i> DSM 22	<i>B. thermodenitrificans</i> DSM 465
G + C mol % DNA	53.0	51–56	52.6	51.5
Catalase	—	v	—	+
Oxidase	—	v	—	+
Growth at 75 °C	—	+	+	—
Optimal temperature	60 °C	55–70 °C	60 °C	65 °C
Growth with:				
3 % NaCl	+	—	—	+
5 % NaCl	—	—	—	+
Anaerobic growth	—	v	+	—
Formation of acetoin	+	—	+	—
Citrate utilization	+	v	—	—
Nitrate reduction	—	+	+	—
Denitrification	—	v	—	+
Hydrolysis of:				
Casein	—	w	+	—
Tween 20	+	—	—	—
Tween 80	—	+	—	—
Polymyxin B resistance	+	—	—	—
Acid from:				
Mannose	+	+	+	—
Mannitol	—	+	—	—
Cellobiose	+	+	—	+
Sucrose	+	v	+	+
Glycogen	+	+	+	—
Galactose	+	v	—	—
Glycerol	+	+	+	—
Xylose	+	+	—	+
Melibiose	+	v	+	—
Raffinose	+	v	+	—
Sorbitol	—	—	—	+
Amygdalin	+	v	—	—
Arbutin	+	v	—	+
Salicin	+	+	+	—
Gentiobiose	+	v	—	—
Turanose	+	v	+	+
Gluconate	+	+	—	—
α-Glucosidase	—	v	+	—
Alkaline phosphatase	—	v	—	+
Esterase (C4)	—	v	+	+
Esterase lipase (C8)	—	v	+	+

* Showed typical phenotypic character results of the group *B. thermoleovorans*, '*B. caldolyticus*' DSM 405, '*B. caldotenax*' DSM 406, '*B. caldovelox*' DSM 411, *B. kaustophilus* DSM 7263 and *B. thermocatenulatus* DSM 730, which were combined in one species, *B. thermoleovorans* DSM 5366^T (Sunna *et al.*, 1997).

Cells of strain 3s-1^T were Gram-positive, spore-forming, motile rods. Optimal growth occurred aerobically at 60 °C (range 37–72 °C) and pH 6.0 (range 5.5–9.0) with 2 % NaCl (range 0–3 %). Sensitivity to all tested antibiotics apart from polymyxin B and to lysozyme and sodium azide was observed. Strain 3s-1^T and reference strains were all able to hydrolyse starch,

gelatin and aesculin, ferment glucose, fructose, maltose, trehalose, ribose, 2-keto-gluconate and 5-keto-gluconate, and were unable to produce indole and hydrogen sulfide, hydrolyse urea, and ferment arabinose and sorbitol. Differential phenotypic characters of strain 3s-1^T and *Bacillus* reference strains are shown in Table 1.

Fig. 1. Phylogenetic tree showing the position of strain 3s-1^T within the radiation of the *Bacillus* 16S rRNA group 5. Bar, 1 inferred nucleotide substitution per 500 nucleotides.

The fatty acid composition of strain 3s-1^T was dominated by iso- (52%) and anteiso-branched acids (13.6%), accounting for approximately 66% of the total. The major acids were iC17:0 (21%) and iC15:0 (16.6%); iC16:0 (14.6%), aiC17:0 (11.4%) and aiC15:0 (2.2%) were also found. Unsaturated acids were absent while *n*C18:0 were present (13%). Although the fatty acid profile was consistent with that reported for *Bacillus* species (O'Leary & Wilkinson, 1988), it should be noted that the quite high amount of *n*C18 was unusual for *Bacillus* species analysed so far (Kämpfer, 1994).

The lipid content was 4–5% of cellular dry weight. The major polar lipids were phospholipids (dominated by amino- and glycerophospholipids), as has been found for other *Bacillus* species (O'Leary & Wilkinson, 1988), while the absence of glycolipids was unusual. Phosphoethanolamine was present, but not cardiolipin or phosphatidylglycerol. NMR data confirm the presence of unusual polar lipids.

The DNA of strain 3s-1^T had a G+C content of 53.0 mol%. The 16S rDNA sequence analysis revealed that this strain was closely related to the members of the *Bacillus* rRNA group 5 (Fig. 1), sharing 99.1–99.7% sequence similarity with the six strains of *B. thermoleovorans* DSM 5366^T. The DNA–DNA reassociation values found between strain 3s-1^T and the closest strains, *B. kaustophilus* DSM 7263, '*B. caldolyticus*' DSM 405 and '*B. caldotenax*' DSM 406, were 60.7, 59.7 and 51.4%, respectively. A lower value (41.2%) was found for strain 3s-1^T and *B. thermodenitrificans* DSM 465. The hybridization of *B. kaustophilus* against *B. stearothermophilus* DSM 22 and *B. thermodenitrificans*, and of this latter against *B. stearothermophilus*, produced reassociation values of 61.4, 40.4 and 48%, respectively. All these values were lower than the recommended threshold value of about 70% accepted as the definition of a genospecies (Wayne *et al.*, 1987).

On the basis of all obtained data, we conclude that strain 3s-1^T is a novel thermophilic *Bacillus* species, for which the name *Bacillus vulcani* is proposed.

Description of *Bacillus vulcani* sp. nov.

Bacillus vulcani (vul.ca'.ni. L. gen. m. n. *vulcani* of the volcano, pertaining to the Eolian Island Vulcano where the organism was isolated from a shallow marine hydrothermal vent).

Cells are Gram-positive, motile rods, 4–7 µm long and 0.6–0.8 µm wide, with terminal and oval endospores. Catalase- and oxidase-negative. Optimal growth occurs aerobically at 60 °C (range 37–72 °C, pH 6.0 (range 5.5–9) with 2% NaCl (range 0–3%). Carbon sources supporting growth: glucose, mannose, trehalose, fructose, cellobiose, saccharose, maltose, lactose, galactose, mannitol, gluconate, adipate, malate, citrate and phenylacetate. Acid production from: glucose, glycerol, ribose, D-xylose, galactose, fructose, mannose, amygdalin, arbutin, aesculin, salicin, cellobiose, maltose, lactose, melibiose, saccharose, trehalose, raffinose, starch, glycogen, gentiobiose, D-turanose. Citrate is utilized. Production of acetoin, but not indole or H₂S. Nitrates are not reduced. Starch, aesculin, gelatin and Tween 20 are hydrolysed, but not casein or urea. The DNA G+C content is 53.0 mol%.

Type strain: *Bacillus vulcani*, 3s-1^T, deposited in Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany, as strain DSM 13174^T, and in Collection Institut Pasteur, Paris, France, as strain CIP 106305^T.

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