

ISOLATION OF SIX NOVEL SPECIES OF THERMOPHILIC BACILLI FROM SHALLOW MARINE VENTS

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Abstract

Six aerobic thermophilic bacilli, capable of growing at 65°C, were isolated from water and sediments samples collected at shallow hydrothermal vents of Vulcano, Lipari, Panarea and Stromboli islands of Eolian Archipelago (Italy). On the basis of physiological and genetic characteristics, these isolates were demonstrated to be different from other well described reference species of the genus *Bacillus* and were classified as novel species.

Key-words: bacteria, biodiversity, thermal vents

Around Eolian Islands many marine hydrothermal systems are from shallow to a depth of 800 m, with temperature values from 25 to 95 °C and rich in dissolved gases (CO₂, CH₄, H₂, and H₂S). From these sites, a wide variety of Eubacteria and Archea have been described reflecting the very varied physical and chemical conditions around the shallow thermal vents (1-7). In the framework of the European project (MAST III-95-0034) titled "Microorganisms in deep sea vents and marine hot springs as sources of potentially valuable chemicals", studies on bacterial diversity at hydrothermal system of Eolian Islands have been undertaken by direct and cultural techniques (8). Here we report the isolation of novel prokaryotes and their cultural, biochemical and genetic characteristics.

Materials and methods

The enrichment of aerobic, heterotrophic, thermophilic bacteria was obtained from water samples filtered through membrane filters. Filters were inoculated into Bacto Marine Broth 2216 (Difco) and liquid Medium D (9). The media were incubated at 65°C for three days in aerobic conditions. Isolation was made on the same media supplemented with agar (2%). Optimal growth was tested after incubation at different temperatures (range 37 - 75 °C), pH (5.5 - 9.0) and salt concentration (0 - 10%). Biochemical properties were screened (8). Genetic characterization was carried out according to Sunna *et al.* (10).

Results and discussion

Eighty-seven thermophilic bacilli were isolated from water and sediment samples collected close to the vents (11). Numerical analysis of their phenetic characteristics showed a high diversity in respect to thermophilic reference strains (8). Also the restriction patterns of their amplified 16S rDNA were different from those of reference strains. Eighteen representative strains from groups of isolates having identical patterns were selected for subsequent analyses. Genetic characterization of the selected isolates demonstrated the novelty of six of them. In fact, comparison of their 16S rDNA sequences with those of reference *Bacillus* spp. gave similarity values in a range of 93-99.1%. Phylogenetic analysis indicated the position of most of isolates within the radiation of the *Bacillus* rRNA group 5 including thermophilic strains. DNA-DNA reassociation studies yielded less than 70% homology between these six isolates and the phylogenetically closest species, finally allowing their classification as novel thermophilic *Bacillus* species. One of them, strain 3s-1, has been already described as *Bacillus vulcani* DSM 13174^T (12). Table 1 shows the chemo-taxonomic properties of the six novel species in comparison with thermophilic type strains of

Bacillus rRNA group 5. Strain 3s-1, strain 4-1, and strain 1bw were isolated from Vulcano, strain 7S from Panarea, strain 5-2 from Lipari, and strain 10-1 from Stromboli. The new species had optimal growth at 55-60 °C and neutral pH, with NaCl 2%, since of their marine origin. They were adhesive on substrate as polystyrene and were able to produce exopolysaccharides. All were resistant to heavy metals such as cadmium and zinc, and some of them also to mercury, silver and arsenic. They are indeed interesting for biotechnological applications as other thermophilic *Bacillus* spp. yet described (13).

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Table 1. Phenotypic characteristics of the new thermophilic isolates and taxonomically related reference strains

Characteristics	<i>B. thermo-</i> <i>denitrificans</i> DSM 465 ^T	<i>B. stearo-</i> <i>thermophilus</i> DSM 22 ^T	<i>B. thermo-</i> <i>leovorans</i> DSM 5366 ^T	strain 3s-1 <i>B. vulcani</i> DSM13174 ^T	<i>Bacillus</i> sp. strain 4-1	<i>Bacillus</i> sp. strain 1bw	<i>Bacillus</i> sp. strain 7s	<i>Bacillus</i> sp. strain 5-2	<i>Bacillus</i> sp. strain 10-1
G+C mol (%)	50.3	52.6	51-56	53	40.8	45	39	43.2	42.7
Growth at 37 °C	-	+	-	+	+	-	+	-	-
Growth at 70 °C	+	+	+	+	-	+	-	+	-
Growth at pH 5.5	+	-	-	+	-	-	-	-	-
Growth at pH 9	-	-	-	+	+	+	+	+	+
NaCl (0%)	+	+	+	+	-	-	-	+	+
NaCl (3%)	+	+	-	+	+	+	+	+	+
Nitrate reduction	+	+	+	+	-	-	-	-	+
Denitrification	+	-	v	nd	nd	nd	nd	nd	nd
Anaerobic growth	+	w	v	-	nd	nd	nd	nd	nd
Hydrolysis of:									
starch	+	+	+	+	+	+	-	-	+
casein	w	+	w	-	+	-	-	+	+
Utilization of:									
citrate	-	-	v	-	+	+	-	-	-
Production of:									
acetoin	-	+	-	-	+	+	-	+	-
Acid from:									
glucose	+	+	+	+	-	+	-	+	+
sucrose	+	+	+	+	-	+	+	+	+
mannose	+	+	+	+	-	+	-	+	+
fructose	+	+	+	+	+	+	-	-	+
cellobiose	+	-	+	+	-	+	-	-	-