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Shallow hydrothermal vents in the southern Tyrrhenian Sea

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Shallow hydrothermal systems represent particularly suitable fields for studying the interactions between fluids and microorganisms inhabiting extreme environments. Coastal zones of the southern Tyrrhenian Sea are home to shallow hydrothermal systems, related to both active and extinct volcanism, located in the Flegrean area, at Cape Palinuro and around the Eolian Islands. A great diversity of Archaea and Bacteria has been reported at shallow hydrothermal vents, where they are key elements in ecosystem functioning. When compared with deep-sea locations, photosynthetic microorganisms appear to be the main characteristic of shallow hydrothermal vents, where both light and hydrothermal energy support a complex microbial community displaying primary productivity (phototrophic and chemotrophic) of mixed origin. Viriobenthos abundances are low compared with other shallow marine systems, suggesting that viruses are less tolerant than prokaryotes of high temperatures and consequently are believed to have a low impact on prokaryotic abundance and biodiversity. Microorganisms from shallow hydrothermal vents represent an almost unexploited resource for thermostable enzymes and biomolecules potentially valuable in biotechnology.

Keywords: Archaea; Bacteria; biodiversity; exopolysaccharides; shallow hydrothermal vents; viriobenthos

1. Introduction

Marine hydrothermal systems (shallow and deep-sea vents) are considered to be ‘extreme environments’ because they are characterised by high temperatures and unusual chemical conditions that are prohibitive for most organisms.

The southern Tyrrhenian Sea hosts a variety of shallow hydrothermal systems off the coastal zones at depths that allow investigations by diving. These shallow systems are mainly located in the Flegrean Fields area (Napoli Gulf), at Cape Palinuro (Salerno) and at the Eolian Islands (Figure 1).

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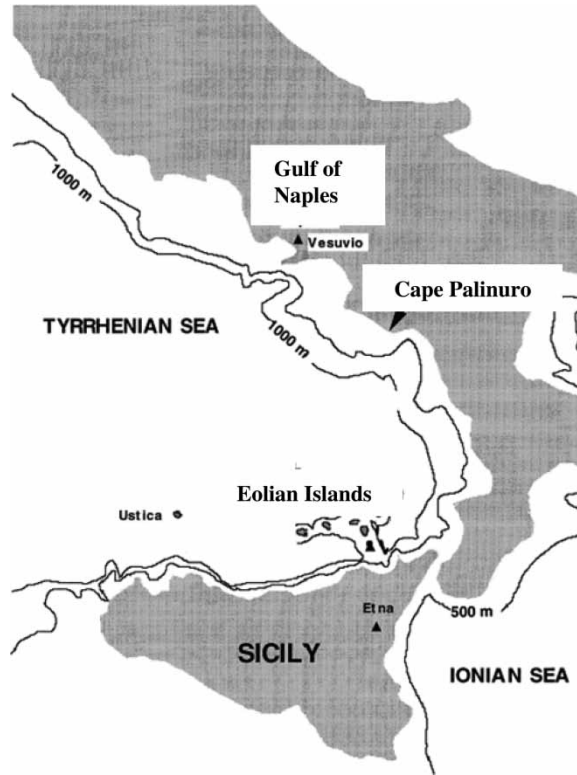


Figure 1. Hydrothermal areas in the Tyrrhenian Sea.

Submarine hydrothermal vents release both thermal waters and gases whose temperatures, detected at the emission points, are in the range 30–130 °C. In general, fluids come out from open fractures in the rocks or diffuse through the sandy sea-floor. The chemical composition of the thermal waters reveals that they are normally highly concentrated in salts as a consequence of high-temperature water–rock interactions [1–3]. The venting gases are CO₂ dominated by variable concentrations of reactive (H₂S, O₂, CH₄, CO, H₂) and inert (N₂, Ar, He) gases. The hydrothermal fluids might be carriers of significant amounts of heavy metals that can either precipitate around the vents or be transported as dissolved ions as a function of the thermodynamic conditions of the system. Based on the geochemistry of the released submarine fluids, Italiano [4] has recently proposed the existence of geothermal systems beneath all the Eolian Islands and has estimated the temperature at depth to be in the range 150–350 °C.

The most active shallow hydrothermal system is located off the island of Panarea, where several vents are spread over the sea-floor to a depth of 150 m. The area inside the islets (~4 km² wide) was also impacted by a volcanic crisis on 2 November 2002, when a low-energy submarine blast generated a crater from which a huge degassing (estimated to be in the order of 10⁹ L of CO₂ per day [4]) killed all the fish living in the area.

Apart from that extreme event, the venting of hydrothermal fluids at shallow depth influences the characteristics of the biological communities living in the immediate vicinity of the vents. Indeed, the direct effect of emissions on the biota is to exclude the majority of eukaryotic organisms which are less tolerant. Hydrothermal fluids have a low impact on prokaryotic microorganisms which, because of their often unique structural, physiological and genetic characteristics, are able to tolerate both strong physical and chemical gradients and so-called extreme conditions. Archaea

and Bacteria are the key elements in the functioning of hydrothermal ecosystems because they are involved in the transformation of inorganic compounds released through vent emissions and are at the base of the hydrothermal system food web [5,6].

Microorganisms thriving at shallow hydrothermal vents possess nutritional requirements and overall metabolic pathways ideally suited to hydrothermal ecosystem, however, their distribution and role in biogeochemistry have been poorly investigated.

Because microorganisms grow in extremely steep temperature gradients and even in chemically fluctuating conditions, it may be expected that they have an unusually broad range of activity and thermostability. Thermophiles, like other extremophiles, represent a unique group of microorganisms whose genomes and biomolecules have evolved to exist and even to thrive in extreme environments, which often mimic industrial conditions.

This article collects information on the microbial diversity and geochemistry of the fluids vented at the shallow hydrothermal systems of the southern Tyrrhenian Sea, and highlights the existence of new microorganisms exhibiting interesting features which are potentially valuable in biotechnology.

2. Flegrean area

The Flegrean area, located close to Naples (Italy), consists of continental sites in Pozzuoli and two main islands of volcanic origin, Ischia and Procida. High thermal activity occurs both along the coast and in terrestrial places with emissions of thermal fluid of a sulphurous nature.

Polysaccharides in microorganisms play a major role in protecting cells from stress in extreme habitats. The production of exopolysaccharides by thermophilic organisms may serve as a boundary between the bacterial cell and its immediate environment. The chemical structure and physical properties of exopolysaccharides from extremophiles are of interest for their possible applications in many sectors of industry [7–9].

Thermophilic microorganisms have been isolated from hot marine springs around Ischia and from hydrothermal vents in the Gulf of Naples. Mucous colonies have been selected for the recovery of new strains producing exopolysaccharides. Fifteen strains (4001–4014), described as aerobes or facultative anaerobes, endospore forming with optimal growth temperatures in the range 60–80 °C, were studied for their ability to show a mucoid phenotype on solid medium and to produce viscous broth.

Strain 4001 (DSM 14431) was isolated from 'Secca-Fumosa', a locality named Lucrino (Pozzuoli, Italy). Sampling was carried out at 15 m depth, 1 km from the coast, at temperature of 80 °C and pH 7.6. Strains 4002, 4003 and 4004 (DSM 14422) were isolated from the seashore of 'Maronti' (Ischia, Italy), where ground surface temperatures reached 90 °C at pH 7.6, close to the fumarole vents. Strain 4005 was isolated from 'Cartaromana' (Ischia, Italy), at 0.7 m depth near the rock coast, the water temperature and pH were 70 °C and 6.9, respectively. Strains 4006–4011 were isolated from 'Sorceto' (Ischia, Italy) where samples of muddy soil were collected near the edge of a fresh water pond, which had a consistently higher temperature (65 °C) than the rest of the environment. Strains 4012–4014 were isolated from hydrothermal vents at 'Castiglione' (Ischia, Italy) at 85 °C and pH 7.6. The isolates grew aerobically between 50 and 85 °C with an optimum temperature between 60 and 85 °C. The optimum pH was 7.6 for all strains [7]. Although the isolates grew on a wide range of carbohydrates, only trehalose and saccharose sustained the growth of isolates 4002, 4004 and 4011; cellobiose and fructose strongly supported the growth of isolate 4012; mannose and galactose were well utilised by isolates 4003, 4005, 4006, 4008, 4009, 4010 and 4012. To induce the biosynthesis of new exopolysaccharides, different sugars were tested as carbon sources in the media.

Table 1. Main features of exopolysaccharides from extremophilic microorganisms isolated from Ischia Island in comparison with those from *Bacillus thermantarcticus* and *Haloarcula japonica* strain T5.

Properties	4001	4004	<i>Bacillus thermantarcticus</i>	<i>Haloarcula japonica</i> strain T5
Carbohydrate (%)	81.0	65.0	95.0	70.0
Protein content (%)	7.0	2.6	2.0	4.0
Molecular mass	380.000 Da	>1.000.000 Da	300.000 Da	/
Optical rotation	(+40.9)	(−199.7)	(−90)	/
Sugar analysis	Man/Glu/Gal 1/0.1/Tr	Gal/Man/GluN/Ara 1/0.8/0.4/0.2	Man/Glu 1/0.7	Man/Gal/Gluc. ac. 2/1/3
Repeating unit	Eptasaccharide	Pentasaccharide	Eptasaccharide	Pentasaccharide
Configuration	α and β	<i>Gluc</i> - <i>galacto</i> and <i>manno</i>	α - <i>manno</i> and β - <i>glucogalacto</i>	α and β

None of isolates showed the presence of ether lipids with isoprenoid chains; after methanolysis, all isolates possessed only fatty acid methyl esters as ‘core lipids’ [9]. Thus, the lipid analyses allowed us to assign the isolates to the domain Bacteria.

Attempts to identify strain 4001 (DSM 14431), using morphological and biochemical tests, showed an affiliation to the genus *Bacillus* [8]. Strains 4001 and 4004, grown on standard medium Marine Broth 2216 (Difco), are producers of exopolysaccharides. The biosynthesis of new exopolysaccharides from new thermophilic marine isolates is strongly induced by adding the appropriate sugar to the growth media; for example, the production of exopolysaccharides in strain 4009 reached $60 \text{ mg} \cdot \text{L}^{-1}$ using trehalose as the carbon source, increasing the yield by ~ 1000 -fold. These results are therefore encouraging; in fact, by manipulating the growth conditions we are able to both increase the yield of the biopolymer and induce the biosynthesis of polysaccharides. The increase in yield is essential to define the primary structure of the polymer on which the biotechnological features depend. The 4001 exopolysaccharide was chosen from among other exopolysaccharides for a better characterisation. It was a mannan with a molecular mass of $\sim 380\,000$ Da and positive optical rotation. A nuclear magnetic resonance spectrum of the exopolysaccharide was indicative of a complex primary structure, and confirmed the presence of a repetitive unity formed by seven monosaccharides, six with an α -gluco/galacto configuration and one with a β conformation (Table 1). More detailed studies on the mannan structure are necessary to obtain more insights into the physical properties of this new polysaccharide.

3. Submarine caves of Cape Palinuro

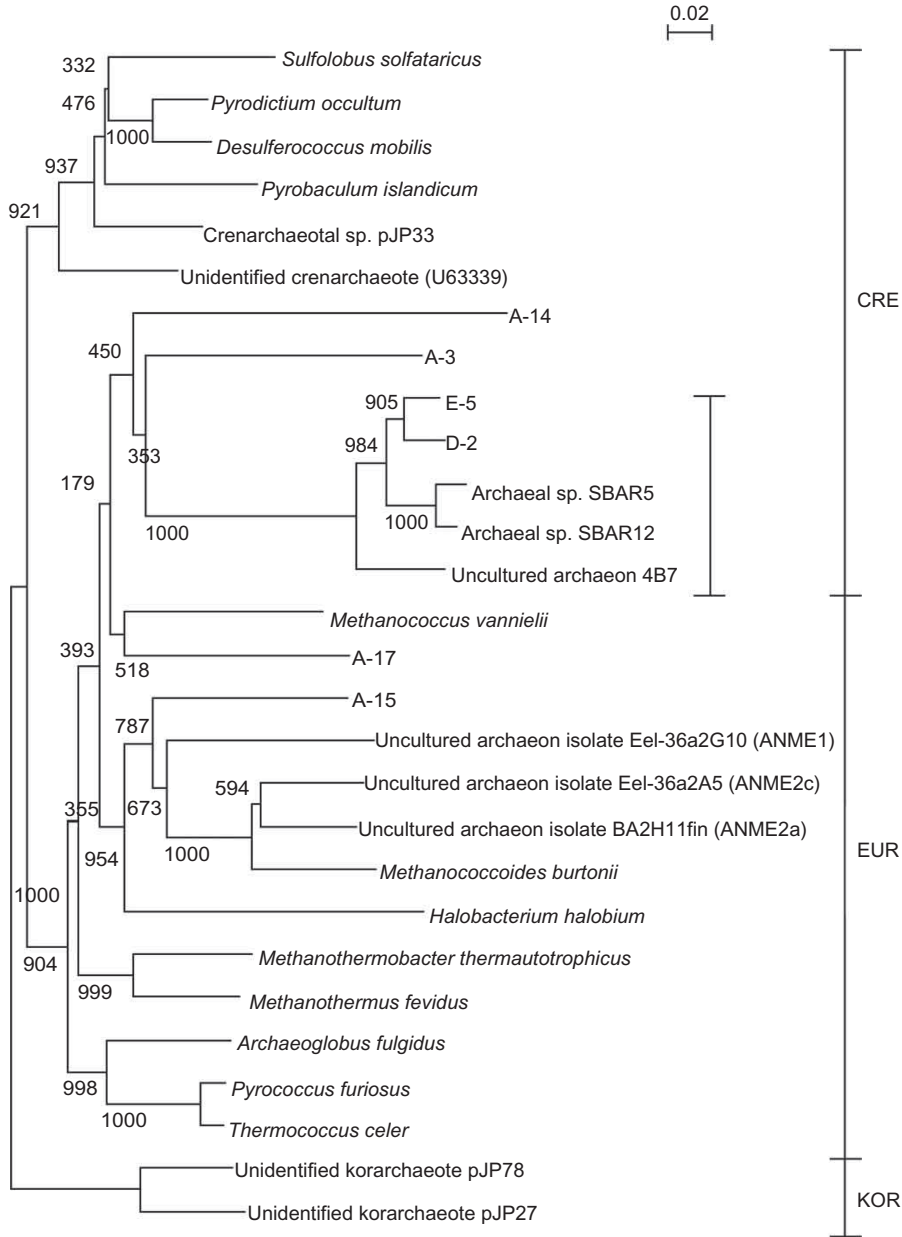
Submarine caves are usually described as non-conventional shallow marine ecosystems because of the absence of sun irradiation which leads to the poor development of biota compared with common coastal habitats. The microbiology of such ecosystems, including blue and black holes, is remarkable and is often key to understanding the ecology and biochemistry of the investigated site [10,11].

Along the coast between Campania and Basilicata (southern Italy), the Cape of Palinuro is characterised by numerous submarine caves, a few of which show inner H_2S -enriched vents. Grotta Azzurra and Grotta Sulfurea are the two caves exhibiting the most significant venting activity and the development of anoxic niches is remarkable.

Anoxic freshwaters are clearly separated from aerobic seawater by a definite chemocline; eukaryotic life exists only in the oxygenated water layers, whereas below the chemocline only prokaryotic microorganisms are observed.

During the last decade, several investigations have been carried out in these caves with regard to the autochthonous microflora, and preliminary data on the population structure have been

obtained using scanning electron microscopy analyses. Subsequent experiments were focused on the metabolic patterns exhibited by microorganisms in different niches and on isolation of the most representative heterotrophic bacteria. Further electron microscopy studies were performed and molecular DNA analyses were carried out to obtain new insights into the microbial composition of the overall prokaryotic population, particularly the Archaea. Extraction of total DNA,



CRE: Crenarchaeota, EUR: Euryarchaeota, KOR: Korarchaeota

Figure 2. Phylogenetic tree of Archaea from samples collected inside the Grotta Azzurra of Cape Palinuro (Salerno).

purification and polymerase chain reaction amplification were performed as described by DeLong [12]. Screening of 16S rRNA gene clones was carried out as reported by Kato et al. [13]. The sequences of 16S rDNAs were checked and aligned as reported by Saitou and Nei [14].

As regards the archaeal tree (Figure 2), strains A-17 and A-15 are somewhat related to the methanogenic archaeal group, but the phylogenetic distance between them is great. Strains E-5 and D-2 are related to the members of marine crenarchaeota group I (MG-1), representing the most abundant and widely distributed *Archaea* in the global ocean biosphere from surface to bottom waters and from polar to tropical regions.

In cold-seep environments, we have identified a microbial community common in the sulphur circulation model, which involved SO_x, a SRB–ANME2 consortium and methanogens [15]. Compared with the cold-seep environment, the cave microbial community might be incomplete, and not related to archaeal communities, ANME and/or methanogens. This suggested that the sulphur circulation system in the cave might not be concerned with methane production and carbon circulation, and this is a big difference from the cold-seep habitat.

The opportunity to discover novel taxonomic species in the Palinuro Cave microflora is certainly remarkable, nevertheless it should be noted that the potential application of some cultivable strains of bioactive molecules (hydrolytic enzymes, bacteriocins, etc.) will be detected either at intra- and extracellular level.

4. Shallow hydrothermal vents of the Eolian Islands

The Eolian Archipelago, located ~25 km NORTH of Sicily (Italy), consists of seven main islands, all of which are volcanic in origin. Shallow submarine hydrothermal systems off the Eolian Islands (Vulcano and Panarea) have been known to be active since historical times, although only recently have scientific studies reported the presence of deep-located emissions (up to 800 m depth) off the eastern coast of Vulcano Island [2]. The submarine hydrothermal fluids are characterised by

Table 2. Analytical data of hydrothermal fluids from selected sites of the Eolian Islands. (Upper) Data from Vulcano Island. Gas concentration in mL gas · L⁻¹ seawater. (Lower) Data from Panarea Island. Gas concentration in vol%.

Site	Depth (m)	T°C	pH	CO ₂	H ₂ S	CH ₄	N ₂	O ₂	Reference		
Vulcano Is.											
Levante Harbour	6	32–38	4.4–6.3	nd	bdl-8.4	nd	nd	bdl-5.7	[19]		
Levante Harbour	2	35	6.11	1010	1.8	nd	nd	0.60	[32]		
Levante Harbour	0.8	60	5.75	992	5.1	nd	nd	0.20	[32]		
La Roja	3	49	6.03	582	0.01	0.004	7.24	0.75	[17]		
P. Conigliara	15	45	6.09	1492	bdl	0.001	6.32	0.24	[17]		
Reference site	Sea level	15	8.10	0.24	bdl	bdl	9.60	4.80	[17]		
Site	Depth (m)	T°C	pH	CO ₂	H ₂ S	He	H ₂	CH ₄	N ₂	O ₂	Reference
Panarea Is.											
Bottaro	8	52	5.28	98.1	1.04	6 × 10 ⁻⁴	4 × 10 ⁻⁴	5 × 10 ⁻⁴	0.29	bdl	[33]
Bottaro	8	55	5.42	95.9	bdl	8 × 10 ⁻⁴	1 × 10 ⁻³	6 × 10 ⁻⁴	0.28	bdl	[33]
Bottaro	16	28	7.60	nd	nd	nd	nd	nd	nd	3.90*	[38]
Campo 7	21	65	4.91	98.5	1.8	7 × 10 ⁻⁴	bdl	bdl	bdl	bdl	[33]
Campo 7	21	60	4.92	96.4	bdl	8 × 10 ⁻⁴	5 × 10 ⁻⁴	2 × 10 ⁻²	0.30	0.03	[33]
Black Point	23	130	3.30	99.1	0.5	1 × 10 ⁻³	nd	6 × 10 ⁻²	0.37	0.02	[36]
Black Point	23	110	7.46	nd	nd	nd	nd	nd	nd	4.62*	[38]
La Calcara	20	95	5.10	97.5	0.3	9 × 10 ⁻⁴	5 × 10 ⁻⁴	2 × 10 ⁻¹	2.20	0.04	[20]
Reference site	12	15	8.10	1.64	bdl	2.8 × 10 ⁻⁴	bdl	bdl	65.6	32.8	[33]

Notes: nd, Not determined; bdl, below analytical limits. *Gas concentration in mL · L⁻¹.

Table 3. Thermophilic and hyperthermophilic strains isolated from Vulcano Island.

Domain	Reference
	Crenarchaeota
	<i>Acidianus brierleyi</i> Simmons and Norris [48]
	<i>Acidianus infernus</i> Segerer et al. [49]
	<i>Pyrodictium occultum</i> Stetter et al. [51]
	<i>Staphylothermus marinus</i> Fiala et al. [52]
Archaea	<i>Thermoplasma maritimum</i> Stetter [53]
	Euryarchaeota
	<i>Archeoglobus fulgidus</i> Stetter [56]
	<i>Ferroplasma placidus</i> Hafenbradl et al. [57]
	<i>Paleococcus helgesonii</i> Amend et al. [58]
	<i>Pyrococcus furiosus</i> Fiala and Stetter [50]
	<i>Pyrococcus woesei</i> Zillig et al. [59]
	<i>Thermococcus acidaminovorans</i> Dirmeier et al. [60]
	<i>Thermococcus alcaliphilus</i> Keller et al. [54]
	<i>Thermococcus celer</i> Zillig et al. [55]
	<i>Thermoplasma volcanium</i> Segerer et al. [61]
	<i>Acidithiobacillus caldus</i> Simmons and Norris [48]
	<i>Acidithiobacillus thiooxidans</i> Simmons and Norris [48]
	<i>Aquifex aeolicus</i> Deckert et al. [62]
Bacteria	<i>Bacillus aeolius</i> Gugliandolo et al. [63]
	<i>Geobacillus</i> sp. strain 1bw Maugeri et al. [28]
	<i>Geobacillus vulcani</i> Caccamo et al. [64]
	<i>Thermotoga maritima</i> Huber et al. [65]
	<i>Thiobacillus prosperus</i> Huber and Stetter [66]

the contemporaneous presence of gases and thermal waters with temperatures ranging from 28 to 130 °C (Table 2). The chemical characteristics of the Eolian shallow vents were similar to those reported for hydrothermal solutions from deep-sea oceanic vents, showing that similar processes occur at hydrothermal vents independent of their location [1,2]. Elemental sulphur, varying in colour from white to yellow to orange, is a common mineral found in sediments around the vents.

From the thermal springs of Vulcano Island, new thermophilic and hyperthermophilic microorganisms belonging to the Archaea and Bacteria, and of interest in both pure and applied research have been isolated (Table 3).

Despite the chemical characteristics being different from the surrounding seawater and the seemingly unfavourable habitat, these sites are inhabited by bacteria able to adapt to the stress of the environmental conditions and displaying specific nutritional pathways [16–18].

The microbial community of the studied shallow vents includes different autotrophic (phototrophic and chemolithotrophic) bacterial populations which show a wide range of temperature tolerance. The chemosynthetic bacteria retrieved from these sites are mainly represented by sulphur-oxidising species involved in the turnover of reduced sulphur compounds (H_2S , $\text{S}_2\text{O}_3^{2-}$, S, etc) and partially oxidised sulphur compounds (polysulphides and polythionates) released as vent emissions. The chemosynthetic activity of sulphur-oxidising bacteria adds to the primary productivity of photosynthetic microorganisms living in the thermal water and at the sediment surface [19,20]. The resident heterotrophs of shallow sites can be supported by both autochthonous and allochthonous organic matter, because the presence of amino acids and sugars has been demonstrated in the immediate vicinity of hydrothermal sites [21,22].

Samples of water and sediment were collected from a submarine solfataric area at 1–5 m depth along the southern coast of the Vulcano Island. Many heterotrophic bacteria were isolated and characterised using physiological and biochemical analyses, such as substrate utilisation, growth temperature range, growth pH range, NaCl tolerance, SDS/PAGE, volatile fatty

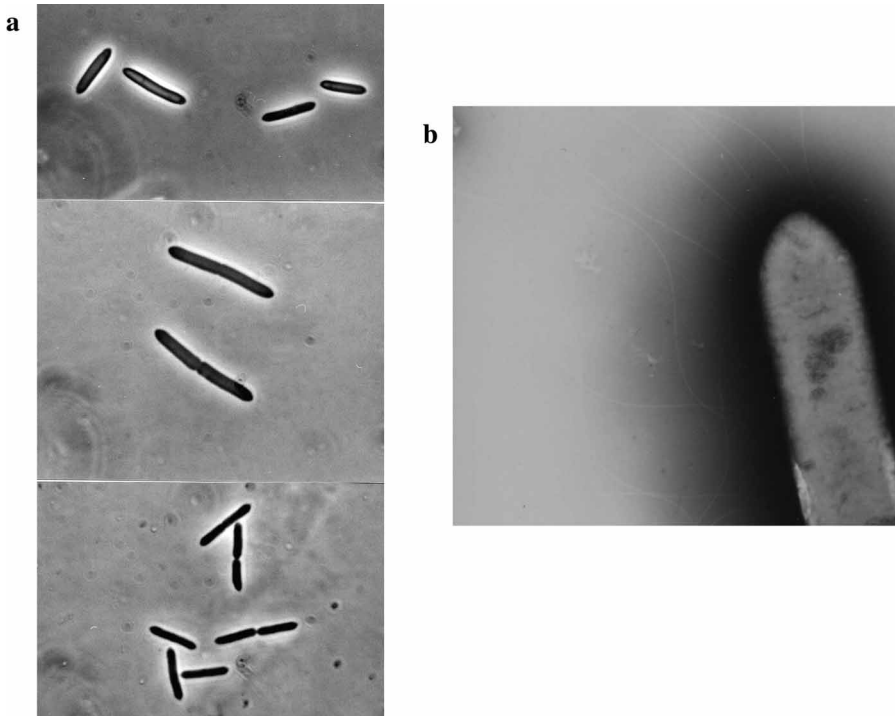


Figure 3. Morphological characteristics of *Clostridium* sp. strain EO isolated from Vulcano Island.

acid determination, membrane lipids composition and 16S rDNA sequencing [10,23]. After isolation of heterotrophic bacteria and clustering, 35 representative strains were selected by SDS-electrophoresis and taxonomically characterised. Among these isolates, *Clostridium* sp. strain EO was fully described. Its morphological characteristics under the optical microscope during the early and late exponential phase and electron microscopy observations reveal the presence of flagella (Figure 3). Strain EO was able to grow on acetate, formate, fructose, dextrose, mannose, starch, peptone and glycerol; cellulose, xylitol, arabinose and pullulan were not utilised. The optimal growth temperature was 36–38 °C, and the tolerated temperature range was between 24 and 44 °C. The optimal pH for growth was around 7 and no development was observed at pH 4 and 10.5. The G + C percentage was 68.6. Following results obtained with 16S rDNA sequencing, further investigations were performed showing the lowest homology of EO with its closest relatives. Clustering and discrimination among representative isolates allowed the identification of strain EO as a new subspecies of the previously described *Clostridium ganghwense* [24].

Several thermoresistant and thermotolerant strains of *Bacillus* and *Geobacillus* were isolated from the Eolian Islands and are of taxonomic and biotechnological interest as producers of new biomolecules for biotechnology and pharmacology.

The new exopolysaccharides possess unique physico-chemical properties, antiviral and immunomodulatory effects [7,25–28]. The thermophilic strains, isolated from different Eolian thermal sites, showed enzymatic activity (hydrolase, lipase, protease, etc.) that may be useful in various industrial applications [29].

Molecular methods, independent of cultivation, are considered the best tool to study the microbial community composition [30], and techniques based on 16S rRNA have been successfully used to reveal the intrinsic genetic diversity in the hydrothermal system of Vulcano. Studies on thermophilic and hyperthermophilic microbial communities from hydrothermal seeps on Vulcano

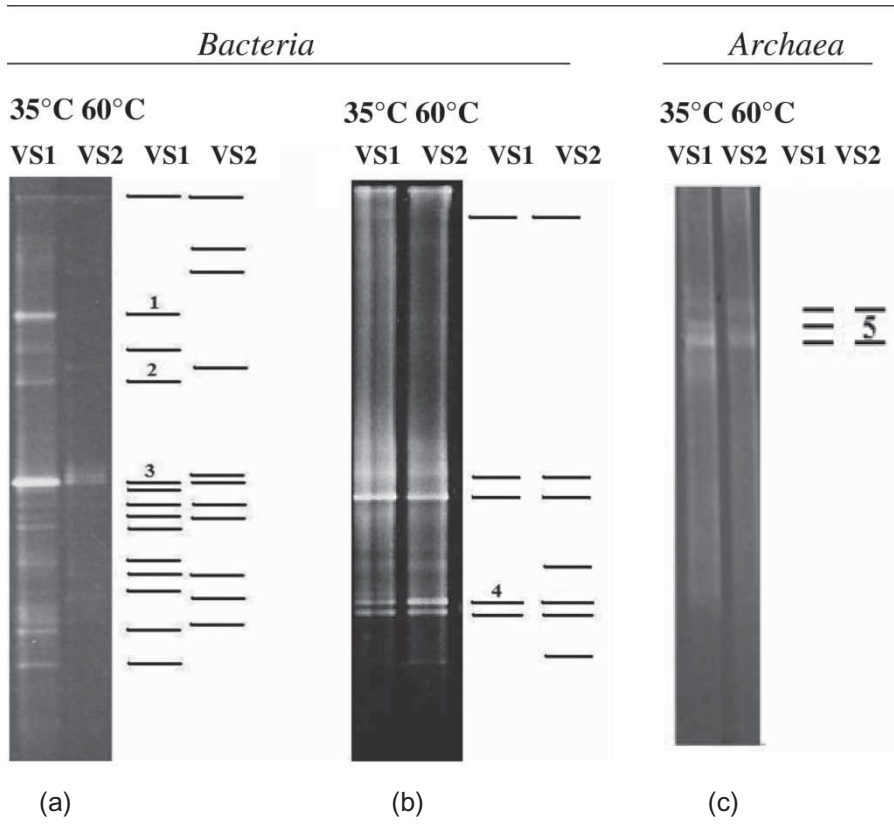


Figure 4. DGGE profiles of bacterial PCR-amplified 16S rDNA (a) V3 region and (b) V9 region, and (c) of archaeal 16S rDNA obtained from sediment samples (VS1 and VS2) with different temperature of Vulcano vents.

Island, using fluorescent *in situ* hybridisation, have shown that groups of Bacteria and Archaea are equally represented [31].

Fragments resolved by PCR/DGGE of 16S rRNA from Bacteria and Archaea from sediment samples collected at two Vulcano vents, characterised by different temperatures (35 and 60°C, respectively), indicated that the richness of Archaea was lower than that of Bacteria in the hydrothermal vent system of Vulcano [32] (Figure 4). Dominant phylotypes at the shallow marine vents of Vulcano belonged to Proteobacteria (alpha, epsilon and gamma subclasses) (Figure 5), and *Natronorubrum thiooxidans* (Halobacteriaceae), a hyperhalophilic archeon currently detected only in other extreme habitats.

The large physical and chemical variations seen at Vulcano and Panarea, characterised by different depths and temperatures, have little influence on the total prokaryotic abundance and viability [33,34]. Recent investigations have been carried out in different vents off Panarea Island, where the most active submarine hydrothermal system of the Eolian islands is located [35], to study the structure and biodiversity of the entire microbial community [33]. This study revealed that primary production at the shallow hydrothermal system of Panarea was supported by a complex microbial community composed by both phototrophs and chemolithotrophs. DGGE profiles obtained by amplification of 16S rRNA regions demonstrated that the resident microbial community at different Panarea vents is composed of few dominant populations of Bacteria and Archaea, more phylogenetically diverse than those found at non-hydrothermal sites. Specialised

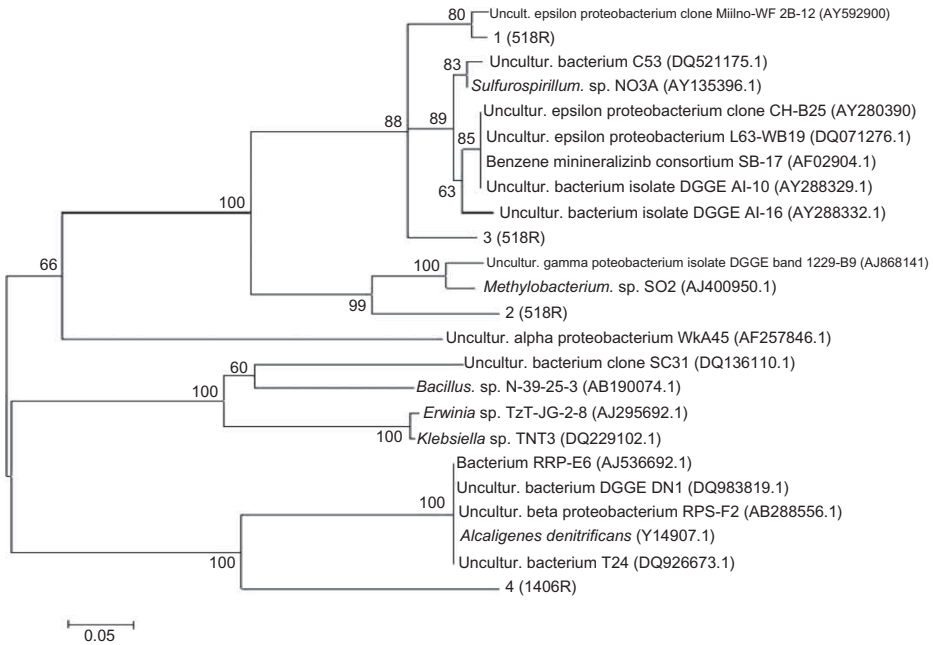


Figure 5. Neighbour-joining phylogenetic tree showing the position of bacterial 16S rRNA gene sequences from sediment samples of Vulcano vents. In bold are represented the sequences retrieved from excised DGGE bands (1, 2, 3 and 4; see Figure 4) using 357–518R (labelled 518R) and 1070–1406R (labelled 1406R) primer sets. Numbers at nodes depict the percentage bootstrap values obtained in neighbor-joining/maximum parsimony analyses (500 replicates).

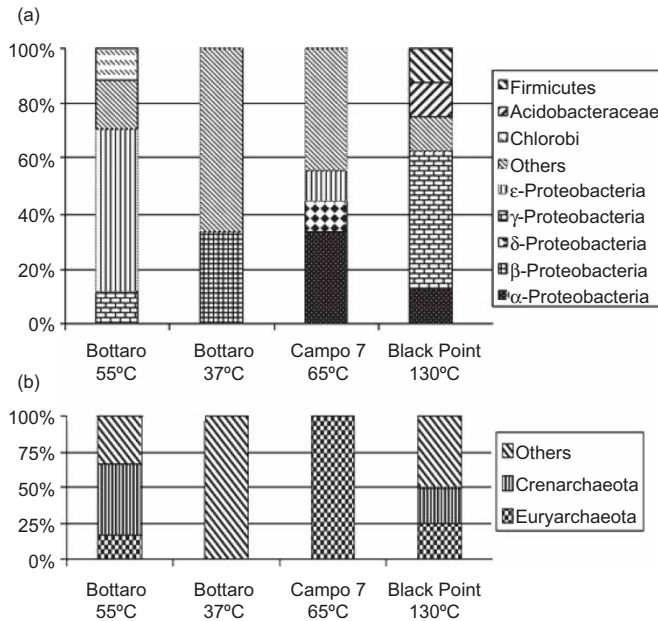


Figure 6. Microbial community structure from Panarea vents based on 16S rDNA gene sequences of excised bands from bacterial (a) and archaeal (b) DGGE profiles.

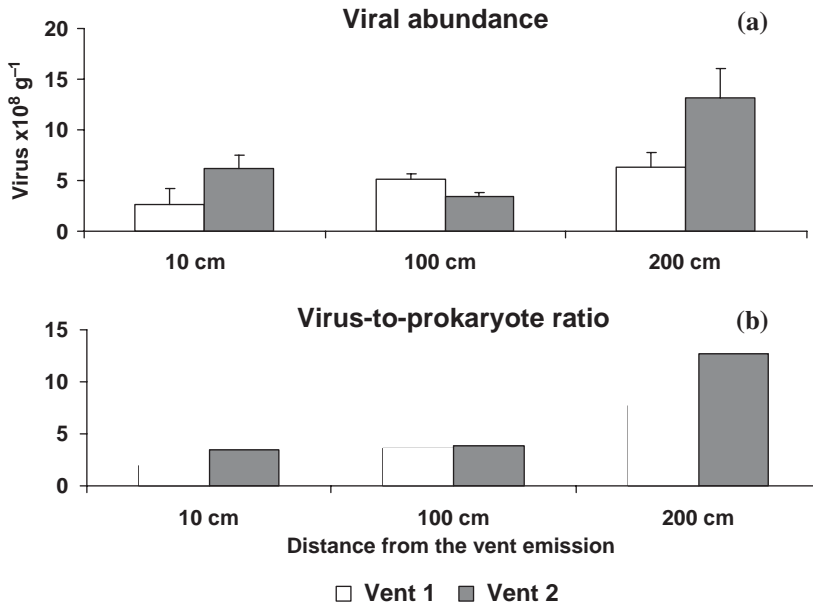


Figure 7. (a) Viral abundance in two vents off Panarea Island; (b) virus-to-prokaryote abundance ratio (modified from Manini et al. [38]).

bacterial and archaeal populations mainly involved in the oxidative phase of the sulphur cycle were the dominant component of the microbial community at Panarea sites. Bacterial richness, evaluated as the number of DGGE bands, and biodiversity, estimated by phylogenetical analysis, were always greater than in Archaea, and this was also the case at Black Point, the warmest (130 °C) and deepest (23 m) site (Figure 6) [36]. These findings agree with previously reported data on the diversity of Bacteria and Archaea in other shallow sites at Milos Island (Aegean Sea) and near Panarea [37,38].

To date, viral ecology in hydrothermal vents has been neglected almost completely. To our knowledge, the only available information deals with sparse investigations carried out on plume waters of deep-sea hydrothermal vents located in the Pacific Ocean [39,40]. It is known that marine viruses have the potential to control prokaryote abundance, and recent ecological models suggest that viruses might also increase prokaryote diversity by ‘killing the winner’ [41,42]. Manini et al. [38], in shallow hydrothermal vents at Panarea Island, showed that viral abundance in the sediments, evaluated as previously reported [43], increased significantly with increasing distance from the vent emission (Figure 7a). Also, the virus-to-prokaryote abundance ratio, utilised as a proxy for potential viral infection [44–46], was lowest in vent samples and increased significantly in control samples (Figure 7b). These results allow us to hypothesise that viruses may be less tolerant than prokaryotes to the high temperatures and fluid emissions, and consequently have a low impact on prokaryote abundance and biodiversity. At the same time, we cannot exclude that low viral abundance around the vent is related to lysogenic rather than lytic infections, because lysogeny could be an important survival mechanism in harsh environments [47]. Further studies are therefore needed to investigate the potential impact of lysogeny on prokaryote abundance and diversity in shallow hydrothermal vents. Moreover, although the dynamics of vent associated organisms are related with geothermal activity we cannot exclude that viral abundance can change with time.

5. Conclusions

Shallow hydrothermal vents of the Tyrrhenian Sea represent a unique, easily accessible natural system where studies to improve the understanding of the relationships between geosphere and biosphere, and the interactions between hydrothermal fluids and microbial communities living in extreme conditions have been developed over the last decade.

Because of the location at shallow depths, both light and hydrothermal energy support a complex microbial community living at these submarine hydrothermal vents, displaying a mixed origin of the primary productivity (phototrophic and chemotrophic). The presence of photosynthetic microorganisms appears to be the main characteristic of shallow hydrothermal vents, when compared with deep-sea locations, where primary productivity is exclusively chemotrophic.

Apart from the occurrence of high-energy events (e.g. submarine blasts) that have not yet been investigated, geochemical variations, linked to temporal and spatial variations, have a negligible influence on total prokaryotic counts and their viability, but at the same time exert a strong pressure on the microbial structure and composition of the resident microbial community.

Results obtained using molecular fingerprinting methods suggest that a few dominant, highly specialised populations of Archaea and Bacteria are significant components of the microbial community at shallow hydrothermal vents. Richness and biodiversity always appeared greater in Bacteria than Archaea, even at the hottest and deepest vents of the Eolian Islands. Although further investigations are needed to elucidate the relationship between Archaea and Bacteria and their relative abundances at shallow vents, the predominance of Bacteria over Archaea currently represents the major difference between shallow and deep-sea hydrothermal vents.

Viruses appeared to be less tolerant than prokaryotes to the high temperatures and fluid emissions, and are consequently believed to have a low impact on prokaryotic abundance and biodiversity.

As shown by the use of cultivation methods and molecular approaches, the hydrothermal vents of Tyrrhenian Sea are rich sources of unique bacterial and archaeal species. Microorganisms from shallow hydrothermal vents represent an almost unexploited resource for thermostable enzymes and biomolecules potentially valuable in biotechnology.

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