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THE ALGAL DIVERSITY IN THE PHLEGREAN FIELDS (CAMPANIA, ITALY) ARCHEOLOGICAL DISTRICTS: AN OVERVIEW

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HIGHLIGHTS

- Microbial communities in Phlegrean Fields.
- Different microhabitats in Phlegrean Fields.
- Algal diversity in the archaeological and geothermal areas.

ABSTRACT

The coexistence of several ecological conditions (hot springs, streams, mud, rock walls), characterized by different pH, temperature values, water potential and mineralogical makes the Phlegrean Fields as an ideal area for the analysis of the microalgal assemblages and their relationships with the different microhabitats occurring in the sites. Here we report an overview of the algal diversity within the archaeological and geothermal areas of Phlegrean Fields and the adaptability for most of them to acidic and thermal conditions in the volcanic soils.

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1. INTRODUCTION

Study on the biogeography of species provide clues on those mechanisms which are at the base of the genesis and the maintenance of biodiversity, like speciation, extinction, dispersion and interactions among species. The geographic distribution of plants and animals has been study object for the biologists since 1900 while research on biogeography of microorganisms is more recent. The application of more advanced biomolecular methodologies has allowed to analyze the real biodiversity of a microbial community. The small dimensions, the high abundance and the metabolic plasticity of the unicellular organisms would confer them a very wide dispersion capacity, not affected by ecological or geographic parameters. The endolithic and cryptoendolithic environments are ubiquitous habitat for microorganisms. These microorganisms form an important interface between biological and geological ecosystems. The biogeochemical characteristics, such as the physical and chemical properties of the rocks, climate, direction of exposure, influence the specific microbial composition of the endolithic biofilms (Walker and Pace, 2007). Algae are the most frequent organisms found on rock surfaces, after cyanobacteria and bacteria. However, microorganisms are able to obtain different elements for their metabolism by biosolubilization of the rock material. Such microbial biosolubilization involves the production of organic and inorganic acids by the metabolic activity of algae, lichens, fungi and bacteria. Phlegrean Fields represent an important archeological and volcanic area situated northwest of Naples, Italy, of undeniable fascination; one of the most famous archeological site of Cuma, and one of the forty volcanoes located in Phlegrean Fields, the worldwide known Solfatara and Pisciarelli; it is an ancient volcanic crater still active but quiescent that for nearly two millennia retains activity of sulphur dioxide fumaroles, boiling mud jets and high soil temperature (Chidini et al. 1996). What makes these two Phlegrean Fields even more fascinating, along with their history, is that both of them preserve the most ancient life forms which are at the base of the photosynthetic life. At the same time, considering that the algae living in these habitats cannot be considered solely as a threat, but also as an important source of biodiversity. In this review we reported the microalgal flora occurring in some Phlegrean sites, namely, Pisciarelli (Agnano, Italy), Sybil Cave (Cuma, Italy), Piscina Mirabilis (Bacoli, Italy), along with the type of mineral substratum, and exploited to examine the physico-chemical interactions between microorganisms and substrate and to provide an evaluation of the biodeteriogenic and/or bioprotective action carried out by the microorganisms based on experimental measurements, occurrence locations and references. The data gathered together here will be useful for the study of biological colonization in archaeological district and extreme environmental, and should provide a basis for ecological studies.

2. ENVIRONMENTS

2.1 Volcanic district

Solfatara and Pisciarelli (Figure 1) are a hydrothermal system located in the central part of the Campi Flegrei caldera (Cinigla et al 2009). At Solfatara, volcanites are basically represented by incoherent products often with trachytic occurences deposed on the trachytic lavas of Monte Olibano dated before 4000 years (Di Girolamo et al 1984). The geothermal system is vapour-dominant, with the hottest fumaroles reaching temperatures of 140-160°C, according to Cioni et al. (1984), Bolognesi et al. (1986) and Chiodini et al. (1996).

Figure 1: Pisciarelli, Phlegrean Fields. *Source: photo by Claudia Ciniglia*

2.2 Archeological district

A wide research programme on the archaeological area of Southern Italy has revealed a valuable cultural heritage of monuments built in tuff rock. Tuff is a type of rock consisting of consolidated volcanic ash ejected from vents during a volcanic eruption (Sgobbo & Moccia, 2016). This type of rock has a high porosity and a high water capacity. These characteristics make it a perfect substrate for the colonization and the formation of biofilms. Two archeological sites, the Sybil Cave and the Piscina Mirabilis (Figure 2), are artificial caves dug in the yellow tuff and used during antiquity for various purposes (Mangoni & Sgobbo, 2013). Phlegrean Fields has been inhabited since prehistoric times and became an important centre of civilization during antiquity. Different underground habitats have been dug in the district, and two of them have remarkable dimensions. The most ancient is the so-called Sybil Cave (fourth century BCE), near Cuma, which is a long gallery cut into the soft tuff, lighted by multiple openings to the surface. The other large cave (dating back to the first century CE) is the Piscina Mirabilis, located in Bacoli, a cistern excavated in tuff, used as storage for drinking water. In addition, both caves were dug in the same geological material originated from the eruption of the Neapolitan Yellow Tuff (dated at 15 ka BP; Insinga et al., 2004), which produced the caldera collapse of the bay of Pozzuoli (Scarpati et al., 1993).

2.3 Mineralogical analysis

The products of the Phlegraean activity range in composition from K-basalt to phonolite: K-basalt (3%), trachybasalt (8%), latite (10%), trachyte (27%), alkali-trachyte (45%) and phonolite (7%) (Di Girolamo et al., 1984). The geothermal system is liquid-dominant with spring water temperatures up to 90°C. The geothermal waters are fed by steam which has a composition similar to that of the fumaroles emerging in the Solfatara crater (Chiodini et al. 1996). In both localities, gaseous sulphur in the fluids is totally represented by H_2S. Other gases in Solfatara fumaroles include major CO_2 and minor N_2, H_2, CH_4, Ar and He (Dall' Aglio et al., 1972; Tedesco et al., 1990; Martini et al., 1991; Allard et al., 1991; Chiodini et al., 1996).

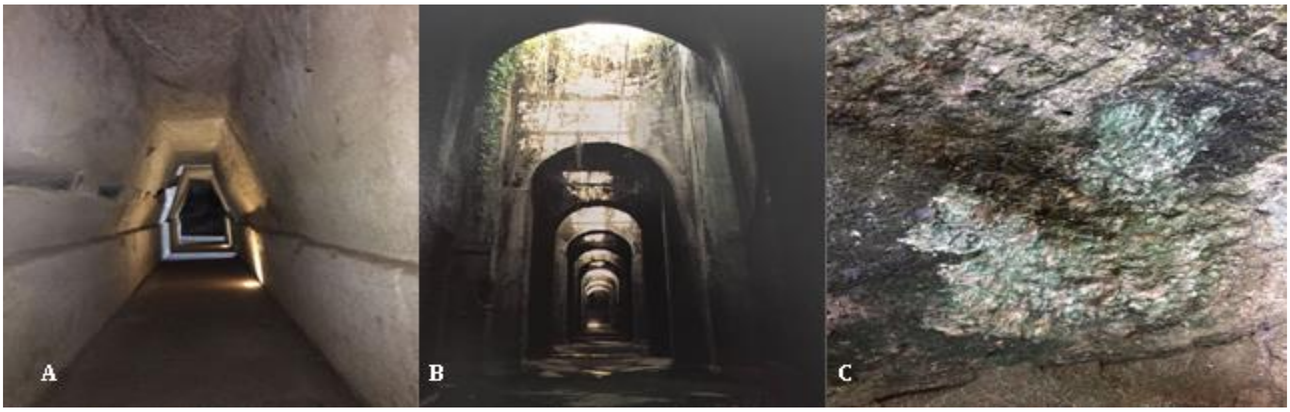

Figure 2: (A) Sybil Cave ; (B) Piscina Mirabilis; (C) exemple of algal communities. Paola *Cennamo & Claudia Ciniglia*

3. ALGAL COMMUNITIES

Table 1 and 2 lists the microorganisms present and isolated in the Plegrean area, together with the substratum. A total of 28 species were identified by molecular methods (18S rDNA); the largest group was represented by Cyanobacteria, followed by Chlorophyta, Rhodophyta and Bacillariophyta (Ciniglia et al. 2005; Cinglia et al. 2007, Pollio et al 2005; Cennamo et al 2012).

Tabel 1: List of prokaryota microorganism in the sampling sites of the Phlegrean Fields

Species	Sampling Sites	Reference
PROKARYOTA		
CYANOPHYTA		
Aphanotecae sp.	Sybil cave	Ciniglia et al. 2005; Cennamo et al. 2012
Chroococcus lithophilus	Sybil cave	Ciniglia et al. 2005; Cennamo et al. 2012
Chroococcus varius	Sybil cave; Piscina Mirabilis	Ciniglia et al. 2005; Cennamo et al. 2012
Gloeocapsa sp.	Sybil cave	Ciniglia et al. 2005; Cennamo et al. 2012
Leptolyngbya foveolarum	Piscina Mirabilis	Ciniglia et al. 2005; Cennamo et al. 2012
Leptolyngbya fragilis	Piscina Mirabilis	Ciniglia et al. 2005; Cennamo et al. 2012
Lyngbya sp.	Piscina Mirabilis	Ciniglia et al. 2005; Cennamo et al. 2012
Nostoc commune	Piscina Mirabilis	Ciniglia et al. 2005; Cennamo et al. 2012
Oscillatoria sp.	Sybil cave; Piscina Mirabilis	Ciniglia et al. 2005; Cennamo et al. 2012

3.1 Cyanophyta

Aphanotecae sp. is a coccoid cyanobacterium growing in the litholitic substratum along with *Chroococcus* lithophilus, *Chroococcus varius* and *Gloeocapsa* sp. ; the genus *Leptolingbya* was the most widespread filamentous cyanobacterium, the species isolated were *Leptolungbya foveolarum*, *Leptolungbya fragilis*, *Lyngbya* sp. Other cyanobacteria were *Nostoc commune*, *Oscillatoria* sp., *Synechococcus bacillaris*, *Synechococcus* sp. All these cyanobacterial species were able to colonize different stone substrata and are typical of many archelogical sites (Cennamo et al. 2009; Cennamo et al. 2012; Cennamo et al. 2013; Cennamo et al. 2015). Since these genera are ubiquitous and therefore their presence is not strictly related to a specific lithic substratum or climate, all reference reported in the literature did not establish a clear relationship between organisms and the substratum. The tuff stone is considered as the best substratum for the growth of the cyanobacteria because of the porosity, roughness, highly hygroscopic, and capillary water absorption, which strongly influence water availability for micro-organisms. All these cyanobacteria are unable to grow on scarcely porous substrata and a low pH typical of acidic fumarole sites.

3.2 Bacillariophyceae

There are many studies on diatoms that live in acidic environments, Negoro reported the presence of *Nitzschia* and *Pinnularia* near fumaroles where the temperature reached 50 ° C. *Pinnularia obscura* Krasske is frequently present in acidic environments (De Nicola D., 2000). In Italy the presence of *Pinnularia* has been repeatedly reported in acidic areas of a different nature (Pinto et al., 1992; Ciniglia et al. 2007). In recent studies Cennamo et al. 2015 examined the biofilm of the vulcanic substrata (tuff and Piperno) and species belonging to *Pinnularia obsura* and *Navicula* sp. were abundant.

3.3 Chlorophyta

The Chlorophyta are the most abundant algal group present in the italian acidic environmental (Pinto et al. 1992, Huss et al. 2001; Pollio et al. 2005); *Chlorella saccharophila* var. *saccharophila* (Chlorococcales), *Auxenochlorella protothecoides* var. *acidicola*, (Huss et al. 2001); *Viridiella fridericiana* (Albertano et al., 1991, Huss et al. 2001), *Pseudococcomyxa simplex* (Albertano et al., 1990), *Stichococcus bacillaris* (Pinto et al. 1992; Cennamo et al. 2012), all these species are also present in the Phlegrean Fields. *Stichococcus sp., Gloeocystis* sp. *Scenedesmus arcuatu and Scotiellopsis terrestris* (Cennamo et al. 2012) are present only in the archeological area. *Chlorella* and *Pseudococcomyxa* are ubiquitous algae able to live both on the soil near fumaroles and in the archeological areas. Among the Chlorophyta, *Chlamydomonas acidophila* Negro and *Dunaliella acidophila*, were frequently isolated from thermoacidic sites (Pollio et al. 2005).

3.4 Chrysophyta

Ochromonas, along with *Chlamydomonas*, is one of the algal species commonly colonizing acidic lakes, where in many cases represent more than 50% of the entire algal population. The genus *Ochromonas* (Chrysophyta) includes at least 70 species widely distributed in heterotrophic waters (Ettl et al.,1978; Ettl et al., 1999). *Ochromonas* spp. cells can contain bacterial cells thus acting as mixotrophic and phagotrophic organisms; many genera sampled in acidic lakes in Lusatia (Germany) were described as phagotrophic (Sanders & Porter, 1988). *Ochromonas* species was detected on volcanic soils from Kurili Island, at pH 1.0 and 42°C, successively identified as *O. vulcania*. The presence of *Ochromonas* sp. in Italian acidic soils was reported for the first time in 1994 by Albertano et al. Morphological and ecophysiological analyses successively confirmed the identification of this strain as *Ochromonas vulcania* (Gromov et al. 1991).

3.5 Rhodophyta

One of the most intriguing groups of photosynthetic microorganisms is represented by Cyanidiophyceae (Rhodophyta), a group of red unicellular algae perfectly suited to the environmental extremes offered by the volcanic and post volcanic areas, where temperatures rise above 50°C, and high sulphuric acid concentrations, generated by the oxidation of sulphur gaseous emissions, greatly reduce the pH to values prohibitive for the majority of eukaryotic life forms (pH 0.5-3.0). Phylogenetic tools, and genome analyses of different algal taxa have shown that Cyanidiophyceae represent one of the ancient and largest algal groups which have diversified; their long evolutionary history began around 1.5 MYA, just before the formation of the supercontinent Rodinia (1.2 MYA), which resulted in an increase in volcanic activity that would have favored the diversification and dispersal of these thermoacidophilic algae.

Table 2: List of eukaryota microorganism in the sampling sites of the Phlegrean Fields

Species	Sampling Sites	Reference
EUKARYOTA		
BACILLARIOPHYTA		
Navicula sp.	Sybil cave; Piscina Mirabilis	Cennamo et al. 2012; Ciniglia et al. 2005
Pinnularia obscura	Sybil cave ; Pisciarelli	Cennamo et al. 2012; Cennamo et al. 2015; Ciniglia et al. 2005; Ciniglia et al. 2007; Pinto et al., 1992
CHLOROPHYTA		
Chlorella sp.	Sybil cave; Piscina Mirabilis	Cennamo 2005; Cennamo et al. 2012; Ciniglia et al. 2005
Chlorella saccharophila var. *saccharophila*	Sybil cave; Piscina Mirabilis; Pisciarelli	Cennamo et al. 2012; Cennamo et al. 2015; Ciniglia et al. 2005
Auxenochlorella protothecoides var. *acidicola*	Pisciarelli	Ciniglia et al. 2005; Huss et al. 2001
Chlamydomonas acidophila	Pisciarelli	Pollio et al. 2005
Dunaliella acidophila	Pisciarelli	Ciniglia et al. 2005
Gloeocystis sp.	Sybil cave; Piscina Mirabilis	Cennamo et al. 2012; Ciniglia et al. 2005
Pseudococcomyxa simplex	Sybil cave; Piscina Mirabilis; Pisciarelli	Albertano et al. 1990; Cennamo et al. 2012; Ciniglia et al. 2005
Stichococcus sp	Sybil cave; Piscina Mirabilis	Cennamo et al. 2012; Ciniglia et al. 2005
Stichococcus bacillaris	Pisciarelli	Pinto et al. 1992
Scenedesmus arcuatus	Sybil cave; Piscina Mirabilis	Cennamo et al. 2012; Ciniglia et al. 2005
Scotiellopsis terrestris	Sybil cave; Piscina Mirabilis	Cennamo et al. 2012; Ciniglia et al. 2005
Viridiella fridericiana	Pisciarelli	Albertano et al. 1991; Huss et al. 2001
CHRYSOPHYCEAE		
Ochromonas sp.	Pisciarelli	Albertano et al. 1994
RHODOPHYTA		
*Cyanidium chil*ense	Sybil cave; Piscina Mirabilis	Cennamo et al. 2012
Cyanidioschzon merole	Pisciarelli	Ciniglia et al. 2005; Pinto et al. 2003; Pinto et al. 2006
Galdieria sulphuraria	Pisciarelli	Ciniglia et al. 2005; Pinto et al. 2003; Pinto et al. 2006
Cianidium caldarium	Pisciarelli	Ciniglia et al. 2005; Pinto et al. 2003; Pinto et al. 2006
Phragnonema sordidum	Pisciarelli	Cennamo et al. 2012

Surprisingly, around 600 million years ago, a mesophilic lineage, named as *Cyanidium chil*ense, evolved from the extremophilic ones, colonizing caves, which can be considered as extreme environments, as well, where nutrient input, light intensity, temperature and humidity are limiting factors for microorganisms. Members of the genus *Cyanidium* have been frequently found in caves worldwide occurring, and enthusiastically it has been also detected inside the archeological park of Cuma (Naples, Italy), specifically in the Sybil cave (Cennamo et al. 2012). *Cyanidium chilense* lives intermingled with a microbial community composed by bacteria, cyanobacteria, green and brown unicellular algae and fungi colonizing both the tuff cave walls of Sybil Cave and the tracheite cave walls and the natural fumarole caves in Solfatara (Ciniglia et al. 2005; Cennamo et al. 2012).
The hydrothermal system of Pisciarelli is located on the eastern edge of the Solfatara crater in the central part of the Phlegrean Fields Caldera (Napoli, Italy), characterized by thermal pools fed by meteoric waters intersected by fumarolic gases rising from a deep boiling aquifer. The gaseous sulphur in the fumarolic fluids is totally represented by H_2S. The microbial community is composed of different groups of prokaryotes, such as Archaea (Kvist et al., 2005), chemotrophic sulphur bacteria, anoxygenic phototrophic bacteria and eukaryotic algae (Brock, 1978); these latter are mainly represented by Cyanidiophytina assemblages, growing diffusely over the entire site. The sulphur fumes favored the development of a large, thick algal mat characterized by algal communities in which only members of the Cyanidiophytina were observed; green patches, formed exclusively by Cyanidiophytina, were distributed along the station at temperatures ranging from 33° C to 45°C and at very low pH values (0.5–1.5). Among them, the relative abundance of *Galdieria sulphuraria* varied from about 20% to 50%, whereas *Cyanidium caldarium* was the dominant species, occurring at a relative percentage of 48–67%. Finally *Cyanidioschzon merolae* was the least represented species in the Cyanidiophytina assemblages (Pinto et al. 2003; Pinto et al. 2006).

4. CONCLUSION

Microbial communities are selected by their habitat and by the interactions within the community. They are typically characterized by few dominating organisms and are often formed in extreme environments, acidic hydrothermal habitats being a classical example. A rich nutrient or energy source is a prerequisite for communities formation, and the Phlegrean area these are present. Lithological formation of Pisciarelli/Solfatara area are the result of the water-rock interaction and the microorganisms-rock interaction. The water-rock interaction is enhanced by high temperatures and low pHs of the fluids and strongly influenced by the direct or indirect mediation of microorganisms. The process causes a colonization only of thermophilic and acidophilic organisms can be considered the most important agents of rocks alteration. Cryptoendolithic algae, exclusively made of Galdieria, collected in the altered side of local volcanic rocks. As previously reported (Hoffmann, 1989; Bell, 1993; Gross et al.., 1998), the cryptoendolithic growth is probably a strategy adopted by the algae to avoid desiccation, thanks to the alunite-opale layers of the altered trachytic volcanic rock in which only few fissures are present. Dunaliella has been found in this site since 1970 and in no other sites; probably, the absence of pools and water stream with variable pH values have given such stable environmental conditions that allowed *Dunaliella* to persist in this site, while *Ochromonas* has never been found.
The interaction between microorganisms (algae and cyanobacteria) and vulcanites or volcanic rocks (tuff) is also detectable in the archelogical sites. The major microbial biodiversity on the tuff rock depends on the climatic parameter, are suscetible of variations as a consequence of weather seasonal fluctuations. Here, "opportunistic" green unicellular algae grow. The development of extremely different biological communities has been observed in different microenvironments: from the

extremophilic algae "sensu stricto", -Cyanidiophytina, Diatoms and flagellate green unicellular algae (*Chlamydomonas*, *Dunaliella*)- to more tolerant ones (Chlorophyceae).

Certain organisms found only in geothermally heated environments are quite possibly relicts of the earliest biosphere that have remained essentially unchanged by the vagaries of time and evolution for more than three billion years. Several extreme thermophiles have optimal growth rates near the boiling point of water. Some are found in geothermal aquifers hundreds of meters below the surface, or in deep oceanic volcanic vents. algal communities. Subsequently, sampling point sites will be identified where the algal communities will be collected.

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