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The culturable mycobiota of a Mediterranean marine site after an oil spill: isolation, identification and potential application in bioremediation

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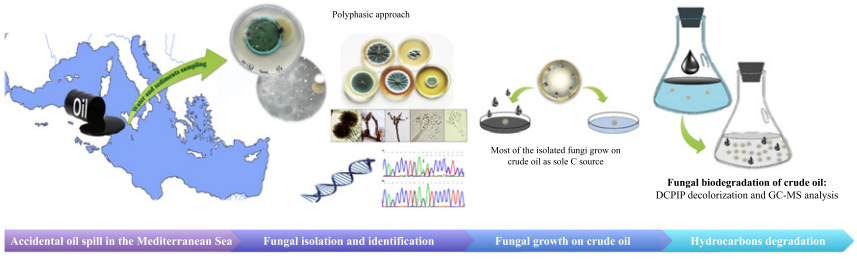
HIGHLIGHTS

- Fungi may restore marine environment after oil spills.
- First report about mycobiota of an oil contaminated Mediterranean Sea site
- The observed fungal biodiversity is higher in water than sediments.
- Most of isolated fungi grow on crude oil as sole carbon source.
- Being able to strongly degrade oil, two fungi may be used as bioremediation agents.

GRAPHICAL ABSTRACT



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ABSTRACT

Bioremediation of marine environment could be the response to oil spills threats. In the present study the fungal community from a Mediterranean marine site chronically interested by oil spills was investigated. Sixty-seven taxa were isolated from water sample and 17 from sediments; for many of the identified species is the first report in seawater and sediments, respectively. The growth of 25% of the fungal isolates was stimulated by crude oil as sole carbon source. Four strains were selected to screen hydrocarbons degradation using the 2,6-dichlorophenol indophenol (DCPIP) colorimetric assay. *A. terreus* MUT 271, *T. harzianum* MUT 290 and *P. citreonigrum* MUT 267 displayed a high decolorization percentage (DP ≥ 68%). *A. terreus* displayed also the highest decreases of hydrocarbons compounds (up to 40%) quantified by gas-chromatography analysis.
These results suggest that the selected fungi could represent potential bioremediation agents with strong crude oil degradative capabilities.

1. Introduction

Most of oil worldwide production is transported by sea, therefore costal and marine environments are strongly exposed to accidentally oil spills (McGenity et al., 2012; U.S. Energy Information Administration, EIA, 2014). The effects of such events are incalculable, influencing human life style, the cost environment and the whole wildlife. For instance, after the Prestige accident in 2002 the most affected beaches lost up to 66% of species richness (De la Huz et al., 2005); the Deepwater Horizon spill (2010) compromised biodiversity of vertebrates (Antonio et al., 2011) and metazoan meiofauna (Baguley et al., 2015).

Once released in the environment, oil prevents light diffusion and oxygen penetration. Its carcinogenicity and mutagenicity has been already demonstrated, driving also the U.S. EPA to list 16 Polycyclic Aromatic Hydrocarbons (PAHs) usually present in crude oil mixture as priority pollutants (Balachandran et al., 2012; Matsubara et al., 2006).

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Table 1
Water and sediments chemical-physical parameters.

	Redox potential (mV)	Dissolved oxygen (mg L^{-1})	Salinity (‰)	pH
Water	50	6.99	36	7.5
Sediments	−140	3.80	32	7.7

Nowadays crude oil removal is possible using different techniques, such as in situ burning or the use of chemical dispersants, but they have economic, ecologic and technic drawbacks: for instance the use of dispersant does not solve the problem but, just separate oil in another phase which cannot easily be removed from the environment (Schaum et al., 2010; Zheng et al., 2014). Bio-based systems offer interesting alternatives being an economic and environmental friendly method (Zhang et al., 2011). The use of bacteria and fungi is based on their capability to use crude oil and its derivatives as the sole carbon source (McGenity et al., 2012). Their adaptation skills and robustness to survive in extreme environments could lead them to be agents of bioremediation process. Bioremediation of crude oil spill is a well-known practice to treat polluted terrestrial environments (Marco-Urrea et al., 2015), but in marine ecosystems the knowledge is still rough, and its primarily focused on prokaryotic organisms. Harayama et al. (2004) demonstrated that bacteria such as *Alcanivorax* spp. and *Cycloclasticus* spp. are able to use aliphatic and aromatic hydrocarbons, respectively. Many bacteria can also produce biosurfactants, which are very important in order to reduce the surface tension and increase the uptake of crude oil (Das and Chandran, 2011; Yakimov et al., 1998). Nevertheless some factors could affect their efficiency as nutrient scarcity (i.e. C:N:P ratio) (Crisafi et al., 2016; Hassanshahian and Cappello, 2013; Jiménez et al., 2007) and crude oil composition, being saturated hydrocarbons most biodegraded than aromatic ones (Wang et al., 2013).

There are few evidences indicating that under some circumstances, fungi could be better degraders of petroleum than traditional bioremediation techniques recruiting bacteria (Saravanan and Sivakumar, 2013). Actually, fungi have been recently reported for their capability to use PAHs as benzo[*a*]pyrene (Passarini et al., 2011) and to produce biosurfactants Kiran et al. (2009). Unfortunately the knowledge of the fungal community associated to marine contaminated sites is scarce, being the central topic of just few researches (Al-Nasrawi, 2012; Elshafie et al., 2007; Sadaba and Sarinas, 2010; Salmanov et al., 2008; Salvo et al., 2005; Simister et al., 2015).

The aim of this work was to isolate and to identify the fungal community of an Italian marine site contaminated by an oil spill, in order to better understand the role of fungi in marine crude oil contaminated environments. The capability of the isolated fungi to use crude oil as sole carbon source was also assessed.

2. Materials and methods

2.1. Study area and sampling survey

Samples were collected from a marine site chronically and recently contaminated by oil spill in Gela, Sicily (37°03′31.58″ N; 14°15′27.32″ E). The last episode took place on June 4, 2013, when more than 13.500 L of heavy crude oil entered into the sea. Three days after the oil spill, 1.5 L of seawater at one-meter depth and about 200 g of sediments near the shorelines were collected, stored at 4 °C and transported to the laboratory. Main physical-chemical parameters (pH, temperature, salinity, redox potential) were measured using a multiparameter probe Waterproof CyberScan PCD 650 (Eutech Instruments, The Netherlands). Determination of oxygen concentration at chosen depths was carried out using the Winkler method (Carpenter, 1965) with an automatic endpoint detection burette 716 DNS Titrino (Metrohm AG, Herisau, Switzerland). Sediments and water parameters are listed in Table 1.

2.2. Fungal isolation and identification

One hundred aliquots of 10 mL of seawater sample were filtered through sterile nitrocellulose membranes (50 mm diameter, 0.45 μm pore, VWR); the membranes were transferred onto 9 cm diameter Petri dishes containing 20 mL of Corn Meal Agar Sea Salt medium (CMASS: 2 g corn meal, 20 g Sigma sea salt mix, 15 g agar, 1 L H_2O) added with antibiotics (streptomycin 0.015 g L^{-1}, chloramphenicol 0.05 g L^{-1}). Plates were incubated at 24 °C and monitored daily for one month to allow the isolation of slow-growing colonies. Each strain was isolated in pure culture for the taxonomic identification. The number of colony forming units (CFU) per 100 mL of seawater (CFU 100 mL^{-1}) was calculated both for the total mycoflora and for each species or morphotype. The relative abundance (RA) was estimated as CFU for each species out of the total CFU. Moreover, 10 sterile cottons swabs were scraped on the inner wall of the bottle containing the sample (where a visual analysis highlighted the presence of mycelium onto

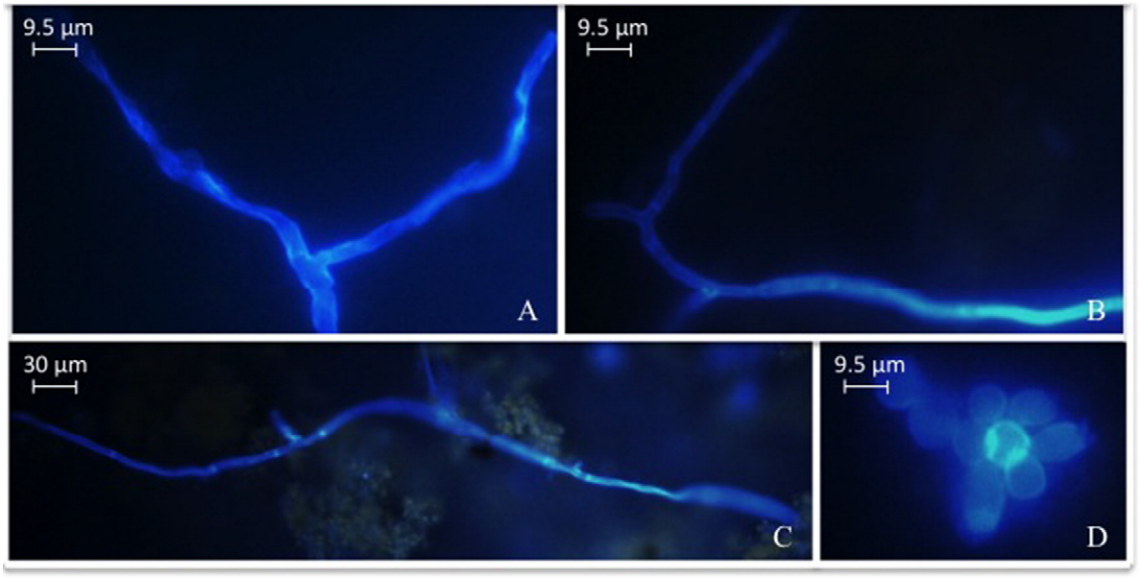

Fig. 1. Fungal structure detected by FB at epifluorescence microscope: A) hyphae in water sample; B) and C) hyphae in sediments; D) conidia in sediment.