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MARINE FUNGI FROM A CRUDE-OIL POLLUTED SITE IN MEDITERRANEAN SEA: DIVERSITY AND SELECTION OF POTENTIAL BIOREMEDIATION AGENTS

FUNGHI MARINI DI UN SITO CONTAMINATO DA PETROLIO NEL MAR MEDITERRANEO: BIODIVERSITÀ E SELEZIONE DI POTENZIALI AGENTI DI BIORISANAMENTO

Abstract - *Oil spill represents one of the main threats for marine ecosystems and human health. Nowadays different techniques contribute to crude oil removal although with several environmental inconvenients. Bioremediation the use of microorganisms to clean polluted environments, is an economic and environmental friendly method to counteract crude oil persistence in marine water. Since the mycoflora adapted to these extreme ecosystems is barely known, in the present study the fungal community of a Mediterranean marine site, chronically and recently interested by oil spills, was investigated and preliminary bioremediation tests were performed.*

Key-words: *fungi, oil spills, marine environment, biodiversity, bioremediation.*

Introduction - Most of oil worldwide production is shipped by sea. As a consequence costal and marine environments are constantly exposed to accidental oil spills. Oil is carcinogenic and mutagenic and, once released in the environment, prevents light diffusion and oxygen penetration, causing serious problems to marine life (Balachandran *et al*., 2012). Nowadays crude oil can be removed by means of techniques, such as in situ burning or the use of chemical dispersants, but they present economical, ecological and technical drawbacks (Schaum *et al*., 2010; Zheng *et al*., 2014). Bioremediation can be a viable alternative both from an environmental and economic perspective. Bioremediation of crude oil spills is a well-known practice to treat polluted terrestrial environment, but knowledge and applications in marine ecosystems are still rough and primarily focused on prokaryotic organisms. The aim of this work was to isolate and to identify the fungal community from a crude-oil polluted site in the Mediterranean Sea, and to test the isolates for bioremediation purposes.

Materials and methods - Samples of seawater and sediments were collected from a marine site chronically and recently contaminated by a crude-oil spill in Gela (Sicily). Aliquots of seawater and sediments were plated on Petri dishes containing a salty medium; the developing fungi were isolated in pure culture for taxonomic identification according to a poliphasic approach (morpho-physiological, molecular and phylogenetic studies) and deposited at the *Mycotheca Universitatis Taurinensis* (MUT). The number of colony forming units (CFU) per 100 ml of water and per gram of dry sediments was calculated. Moreover, fluorescent Brightener (FB) staining was used to detect actively growing hyphae and fungal spores in raw samples. All the isolated fungi were tested for their abilities to grow on crude oil as sole carbon source. The mycelium growth was evaluated after 4, 7 and 14 days. The four most promising strains were selected to evaluate their capability to degrade oil in liquid cultures, using DCPIP as colorimetric assay (Varjani and Upasani, 2013): aliquots of solution were periodically sampled for the spectroscopy measurements. At the end of the experiment, the biomasses were collected and dried to assess the fungal development.

Results - Fungal hyphae and spores were directly detected by staining with FB, both in water and sediments. The water sample showed a total fungal load of about 30 CFU 100 ml^{-1} and a high fungal biodiversity: the cultivable mycoflora consisted of 67 taxa belonging to 29 genera. Almost all the recorded taxa belonged to Ascomycota (94%). As for the sediments, the total fungal load was about 4,000 CFU g^{-1} dw, and the cultivable mycoflora consisted of 17 taxa belonging to 12 genera. Ascomycota predominated (94%) over Basidiomycota (6%). The 142 isolated fungi, were tested for their capability to grow in presence of crude oil: about 25% of the organisms were significantly stimulated by its presence. The highest stimulation was observed for *Lulworthiales* sp. Kohlm, 2000 (MUT 263), *Penicillium citreonigrum* Dierckx, 1901 (MUT 267), *Aspergillus terreus* Thom, 1918 (MUT 271) and *Trichoderma harzianum* Rifai, 1969 (MUT 290). The ability of these strains to degrade crude oil in liquid culture was evaluated in presence of the redox dye DCPIP. *A. terreus* showed the highest rate of discoloration (88%). Also *P. citreonigrum* and *T. harzianum* activated an oxido-reductase cascade and were able to oxidize about 70% of DCPIP, while *Lulworthiales* sp. was less effective.

Conclusions - Crude oil spill is a huge threat for marine ecosystems and wildlife biodiversity, and the use of adapted fungi capable of cleaning these contaminated environments is an actual challenge. The high biodiversity and the presence of hyphae in the samples highlighted by FB demonstrated the existence of an important living fungal community immediately after the oil spill, as already suggested by other authors (Bik *et al.*, 2012). Noteworthy, the selection of three strong crude-oil degraders for bioremediation purposes: *A. terreus*, *P. citreonigrum* and *T. harzianum*. In conclusion, the understanding of the fungal biodiversity and of its ability to use crude oil as sole carbon source, is a key step that would lead to the development of a microbial consortium useful for bioremediation purposes.

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