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Assessing the effectiveness of *Byssochlamys nivea* and *Scopulariopsis brumptii* in pentachlorophenol removal and biological control of two *Phytophthora* species

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ABSTRACT

Bioremediation and biological-control by fungi have made tremendous strides in numerous biotechnology applications. The aim of this study was to test *Byssochlamys nivea* and *Scopulariopsis brumptii* in sensitivity and degradation to pentachlorophenol (PCP) and in biological-control of *Phytophthora cinnamomi* and *Phytophthora cambivora*. *B. nivea* and *S. brumptii* were tested in PCP sensitivity and degradation in microbiological media while the experiments of biological-control were carried out in microbiological media and soil. The fungal strains showed low PCP sensitivity at 12.5 and 25 mg PCP L^{-1} although the hyphal size, fungal mat, patulin, and spore production decreased with increasing PCP concentrations. *B. nivea* and *S. brumptii* depleted completely 12.5 and 25 mg PCP L^{-1} in liquid culture after 28 d of incubation at 28 °C. Electrolyte leakage assays showed that both fungi have low sensitivity to 25 mg PCP L^{-1} and produced no toxic compounds for the plant. *B. nivea* and *S. brumptii* were able to inhibit the growth of the two plant pathogens in laboratory studies and reduce the mortality of chestnut plants caused by two *Phytophthorae* in greenhouse experiments. The two fungal strains did not produce volatile organic compounds able to reduce the growth of two plant pathogens tested.

Introduction

Bioremediation is defined as the process whereby living organisms are able to transform environmental contaminants into less toxic forms. It uses naturally occurring bacteria, fungi or plants to degrade or detoxify substances hazardous to human health and/or the environment (Crawford & Crawford 1997; Vidali 2001; Olguin 2003; Petroselli *et al.* 2014).

Biological control (biocontrol) refers to the purposeful utilization of introduced or resident living organisms to suppress the activities and populations of one or more plant

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pathogens to improve plant health. Disease suppression by biocontrol agents is the sustained manifestation of interactions among the plant, the pathogen, the antagonist, the microbial community on and around the plant, and the physical environment (Handelsman & Stab 1996; Howell 2003; Lorito et al. 2010).

The use of beneficial fungi and bacteria for bioremediation and biocontrol of plant pathogens has made tremendous strides in numerous biotechnology applications. In recent years the need to find a global approach to environmental and agricultural issues has set the challenge to discover microorganisms which are useful for both bioremediation and biocontrol research (Griffin 2014). This approach is primarily focused on how to use microorganism strains to inhibit the dangerous advance of plant pathogens and to deplete environmental contaminants (Sylvia et al. 2005; Singh et al. 2011).

The fungi that play important roles in biocontrol and bioremediation strategies are numerous; some of these are grouped as mycorrhizal and endophytic fungi (Griffin 2014). The endophytic fungus *Phomopsis* sp. has been found to use the 4-hydroxybenzoic acid as the only carbon source (Chen et al. 2011) and to inhibit the growth of *Physocnemum brevilineum* which is a vector of *Ceratocystis ulmi*, an important elm pathogen (Webber 1981). The fungi belonging to genus *Glomus* are arbuscular mycorrhizas used in the biocontrol of soil-borne plant pathogens (Azcón-Aguilar & Barea 1996) and as heavy metal biosorbents in soil (Leyval et al. 1997). Filamentous fungal species belonging to the genus *Trichoderma* are able to counteract some plant pathogens by means of mycoparasitism and antibiosis (Howell 2003: Lorito et al. 2010) and simultaneously to deplete pollutants including heavy metals, pentachlorophenols (Bosso & Cristinzio 2014) and polycyclic aromatic hydrocarbons (PAHs) (Tripathi et al. 2013). *Aspergillus flavus* was used as a biosorbent of heavy metals (Deepa et al. 2006), phenol degrader (Ghanem et al. 2009; Bosso & Cristinzio 2014) and promoter of *Phytophthorae*'s growth inhibition (Evidente et al. 2009). *Penicillium* spp. has demonstrated an excellent ability to degrade different xenobiotic compounds such as phenolic compounds, PAHs and heavy metals (Leitão 2009). Nevertheless some strains of genus *Penicillium* were also used in biocontrol. In fact, *Penicillium funiculosum* and *Penicillium janthinellum* have been able to limit the *Phytophthorae* root rots of azalea (Ownley & Benson 1992; Fang & Tsao 1995). Some species of genus *Verticillium* have been found to remove petroleum products and PAHs in soil (Gadd 2001; Singh et al. 2011) and to control numerous plant pathogens such as fungi, bacteria and nematodes (Mérillon & Ramawat 2012). Other interesting ecological groups are for example the white-rot fungi. *Trametes versicolor* has shown promising biocontrol activities against *Fusarium oxysporum* (Ruiz-Dueñas & Martínez 1996) and represents one of the most important organisms used in bioremediation actions (Gadd 2001; Singh 2006; Bosso & Cristinzio 2014).

Pentachlorophenol (PCP) is a toxic compound which is widely used as a wood treatment agent and general biocide. It is persistent in the environment and has been classified as a priority contaminant to be reclaimed in many countries. In fact, uncontrolled PCP uses and releases have caused contamination of soil, water and ground water. Although PCP is recalcitrant to biodegradation, numerous bacterial and fungal isolates have been reported to be able to degrade it (Gadd 2001; Singh 2006; Bosso & Cristinzio 2014).

In the present study, two fungal strains which were isolated from artificially PCP-contaminated soil during a long-term bioremediation study and identified as *Byssochlamys nivea* (Westling 1909) and *Scopulariopsis brumptii* (Salvanet-Duval 1935) (Scelza et al. 2008; Bosso et al. 2011; Bosso et al. 2015b), were tested in laboratory and greenhouse experiments to reach the following goals:

1 to evaluate their sensitivity to PCP and their capacity for contaminant degradation;
2 to assess their antagonistic effect *vs Phytophthora cinnamomi* (Rands 1922) and *Phytophthora cambivora* (Buisman 1927), soil-borne pathogens causing disease on many woody hosts (root and collar rot of adult trees and of seedlings in nurseries, plantations and forests) especially on plants belonging the genus *Castanea* (Vannini & Vettraino 2001).

Materials and methods

Fungal strains and cultivation conditions

Byssochlamys nivea and *Scopulariopsis brumptii* were stored into slant tubes containing potato dextrose agar (PDA; 5 g L^{-1} potato; 20 g L^{-1} dextrose; 15 g L^{-1} agar) at 20 °C at the laboratories of Forest Pathology of the Department of Agriculture (University of Naples Federico II, Italy).

Chemicals

PCP (>99 % purity) was purchased from Sigma–Aldrich (USA). All solvents and chemicals reagents were purchased from Carlo Erba Reagents (Italy).

Sensitivity test to PCP in plate culture

Fungal sensitivity to PCP was evaluated in Petri dishes. The fungal strains were grown onto PDA at different PCP concentrations (12.5 and 25 mg L^{-1}) prepared previously by dissolving the suitable amounts of PCP in 5 ml of methanol and then in PDA (final volume 1 L). This step was necessary because the PCP is weakly insoluble in water. The controls were cultured on PDA having 5 ml L^{-1} of methanol but without PCP. All samples were incubated at 25 °C for 7 d. Sensitivity to PCP was determined by measuring the diameter (Ø) of the colony, daily mycelium growth rate (Tomasini et al. 2001), hyphal size (this was achieved measuring with an ocular micrometer at total magnification of 100× the hyphal thickness in μm) and spore production (St-Arnaud et al. 1996). A number of 100 measurements were carried out for each replicate to determine hyphal thickness and spore production.

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