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Research Article

Phenotypic and Molecular Characterization of Two Enterobacter Strains, Isolated from a Phytodepuration-Based Wastewater Treatment Plant in Prato (Tuscany, Italy)

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Abstract

Environmental pollution and presence of pollutants in water environments are main concerns for society worldwide. Environmental-friendly methods for the clearance of these compounds are continuously developed and improved to provide efficient and economical solutions. Phytodepuration has been exploited over the years, since it is based on the remedying abilities of plants and their rhizospheric microorganisms. In this preliminary work, we describe the phenotypic and molecular characterization of two *Enterobacter* strains isolated from a wastewater treatment plant, which uses the common reed *Phragmites australis*. Both strains were able to resist against multiple antibiotics, growing in the presence of a wide range of pH and several pollutants (such as those containing metals and metalloids). They were also able to produce indole-3-acetic acid and to use diesel fuel as the sole carbon and energy source. In conclusion, this work represents the beginning for a deep characterization of the *P. australis* microbiota used for phytodepuration, to discover new metabolic abilities. These results might be used for the engineering of super-degrader strains with advantageous features such as a broad degradation activity against recalcitrant compounds and ability to grow in polluted environments.

Keywords: *Enterobacter*; *Phragmites australis*; Phytodepuration; Wastewater

Abbreviations

WW: Wastewater; CW: Constructed Wetlands; LFL: Landfill Leachate; WWTP: Wastewater Treatment Plant; MBR: Membrane Bio-Reactor; RAPD: Random Amplified Polymorphic DNA; RDP: Ribosomal Database Project; MIC: Minimal Inhibitory Concentration; TSB: Tryptic Soy Broth; TSA: Tryptic Soy Agar; IAA: Indole-3-Acetic Acid; CMC: Carboxymethyl Cellulose; EcC: *Enterobacter cloacae* Complex; MDR: Multi-drug Resistant; SWW: Synthetic Wastewater

Introduction

Environmental pollution, and above all water pollution, represents an issue of considerable relevance worldwide. In this context, phytodepuration, the group of technologies that utilize plants and their rhizospheric microorganisms for the removal and/or transformation of contaminants present in water and Wastewater (WW) [1], is considered as both an environmental-friendly and a valuable solution for environmental cleanup, especially in the case of wastewater treatment. Moreover, phytodepuration is appreciated because it is cost effective, presents aesthetic advantages, and allows long-term applicability.

Constructed Wetlands (CW) are engineered systems that mimic the self-purification properties of natural wetlands. CW have been successfully exploited in WW treatment and are considered as an environment-friendly management option for treatment of WW [2]. Indeed, they have been used successfully for removal of urban, rural, and industrial WW pollutants [3]. This ability relies on the interactions involving plants, microorganisms, soil, and pollutants [4], with the rhizosphere being the principal effector involved [5]. To this purpose, *Phragmites australis* (globally known as "common reed") is among the most employed plant species. It provides several advantages, such as its ability to grow well in marshy areas and its resistance to both heavy metals (i.e. As, Ni, and Fe) [6] and high salt concentrations [7]. It has recently been demonstrated that the endophytic bacteria associated with the roots of *P. australis* are beneficial to plants growing in contaminated sites, promoting the degradation of xenobiotic compounds [8].

To date, however, only a few studies have been carried out to understand and to better evaluate the role of the endophytic microorganisms associated to *P. australis* in the CW, especially to prove whether this association really represents an advantage. In literature, the effectiveness of the use of CW in the treatment of sewage containing heavy metals [9] and high salinity is reported. In this regard, the pilot plant in Calice (Prato, Italy), managed by G.I.D.A. SpA, has the goal to characterize and improve the remediation properties in tertiary treatment of Landfill Leachate (LFL) [10].

This paper describes the phenotypic and molecular characterization of two bacterial strains belonging to the genus *Enterobacter*, isolated from the root of *P. australis*. The two strains

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