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Degradation pathway of cephalosporin antibiotics by *in vitro* cultures of *Lentinula edodes* and *Imleria badia*

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ABSTRACT

Bioremediation is a promising method to degrade active pharmaceutical ingredients with additional safety compared to conventional methods. This study provides the basis for a new, practical procedure to remove antibiotics contamination, particularly cephalosporins, spread on environmental matrix. For the experiment the fruiting bodies and their mycelia from liquid *in vitro* cultures of two edible species of mushrooms (*Imleria badia* and *Lentinula edodes*) were choosing because of their unique nutritional and medicinal properties and enzymatic content. The study was conducted at different time intervals by testing the possibility and speed of antibiotic mycoremediation using cefuroxime axetil in different doses. The identification of the degradation products in biomass and medium was performed on the basis of UPLC/MS analysis and supported with fragmentation patterns obtained from MS/MS experiments. Both mushroom mycelia exhibit a certain metabolic activity which causes declining cefuroxime axetil levels in the wide range of concentrations. The high removal rate (about 100% within 7 days of the experiment) is particularly effective, and could be one of the most important tools in removing soil and water pollutants.

1. Introduction

Environmental pollution is a global problem that concerns either developing and developed countries (Suresh and Ravishankar, 2004). The most common organic contaminants are pesticides, oil spills, antibiotics (classified as active pharmaceutical ingredients (API)) and personal care products. The wide scale of production and use of antibiotics in veterinary, clinical medicine and agriculture causes a significant increase in the quantity of antibiotics in the environment (Kümmerer, 2009; Aust et al., 2008; Martinez, 2009; Grumezescu et al., 2014). Xenobiotic contamination has been recognized as a global occurrence (Zhang and Li, 2011). Antibiotic resistance brings the environmental risk in view of residual antibiotics released into the ecosystems. Water and soil pollution influence the structure and function of ecosystem vitally. Most of harmful pollutants can get to human body through food chain and expose human's health in connection with their potential carcinogenic, teratogenic and mutagenic properties (Fent et al., 2006). Taking into account the antimicrobial properties, biodegradation of antibiotics is complicated (Crocker et al., 2016). Antibiotics can be removed through traditional ways (flocculation, sedimentation, filtering, biological processes) but these are not efficient enough, that's way new methods were expanded (ozonation, fenton and photofenton reactions, chlorination, photolysis, photo-catalysis) (Prakash et al., 2015). Plant growth-promoting rhizobacteria is often used to remove organic pollutants from contaminated soil (Zhuang et al., 2007; Ma and Zhai, 2014). Corn (*Zea mays* L.), green onion (*Allium cepa* L.) and cabbage (*Brassica oleracea* L.) grown in dunk contained antibiotics absorbed chlortetracycline with the accumulation of antibiotic in plant tissue (Kumar et al., 2005). There are few information available about organic pollutants toxicity on health of alive organisms and environmental (Daughton and Ternes, 1999; Boxall et al., 2004; Rodriguez-Mozaz et al., 2007; Ferrer and Thurman, 2003). Pollution of soil with herbicides, antibiotics and other chemicals affects a negative influence on human health and microbial activities. Oxytetracycline is translocated by plants and have a toxic impact on plant growth (Kong et al., 2007; Liu et al., 2009). Tested probes of isolated cultures of *Azotobacter* sp. from polluted soils showed high resistance to cotrimoxazole, chloramphenicol and nitrofurantoin compared to those isolated from uncontaminated soil (Abo-Amer et al., 2014). Used so far conventional methods for the APIs remediation proved to be costly. Therefore, several low-cost effective methods are being developed, such as the application of $FeCl_3$ as a coagulant with electrode systems for

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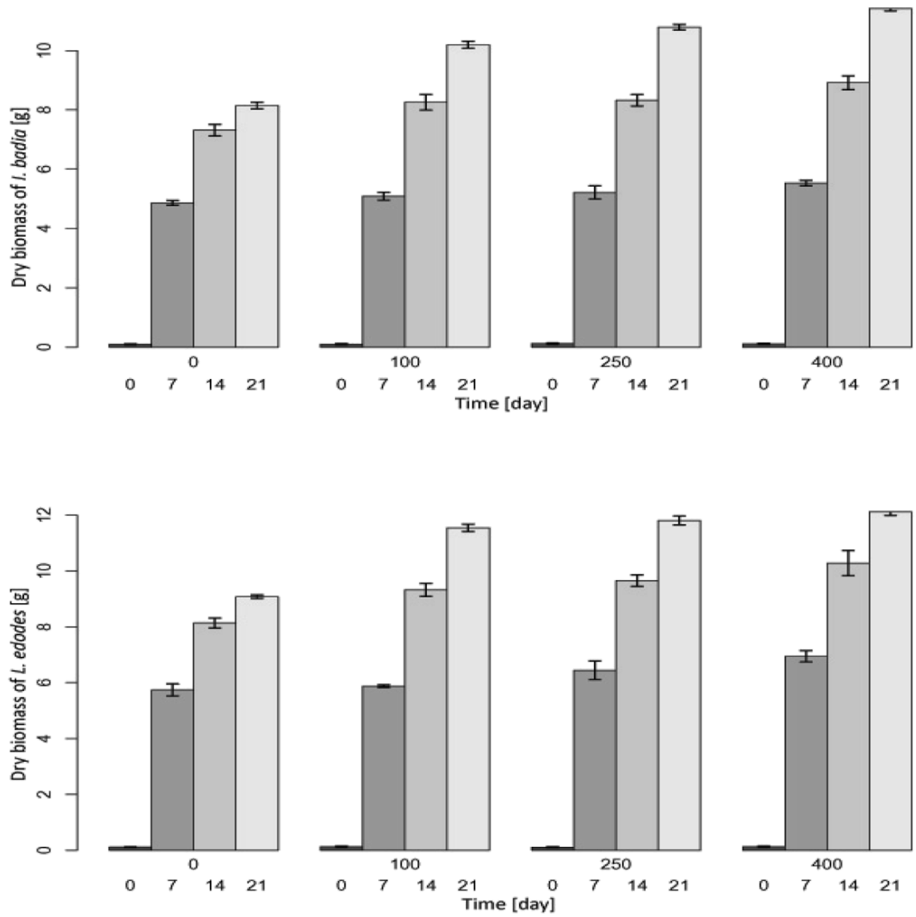

Fig. 1. Dry biomass of *I. badia* and *L. edodes* grown with various doses (0, 100, 250, 400 mg) of antibiotic. Other details of the experiment are described in Materials and Methods section. The *error bars* indicate the standard error of three independent experiments.

treatment of liquid swine manure (Laridi et al., 2005), advanced oxidation processes (UV radiation, hydrogen peroxide and TiO_2, ozone) (Li et al., 2008; Zwiener and Frimmel, 2000), or a micelle-clay system (pre-adsorbed on montmorillonite) for removing of the antibiotics from water (Polubesova et al., 2006).

Nowadays, the investigation on conversion of industrial or agroindustrial wastewater into other non-toxic forms is still in progress. Bioremediation include methodologies that employ living organisms to remove damaging substances from the environment. The remediation does not require the transfer of the contaminated material to the site of treatment, so it can give some savings on transportation and storage costs of polluted materials (Pletsch et al., 1999). Compared to the conventional remediation, bioremediation is simpler, less harmful to the environment and does not induce secondary pollution. Remediation through fungi, ‘mycoremediation’, exerts mycelia and fruiting bodies of mushrooms with their ability to degrade a wide variety of environmentally pollutants, transforms wastes into harmless products by multiplicity methods (biodegradation, biosorption, bioconversion) (Purnomo et al., 2013; Zhu et al., 2013). The success of mycoremediation depends on: accessibility of fruiting bodies of mushrooms, availability of contaminants, and remediation capability of the strains used. Removal of waste from the environment by mushrooms possess many benefits, and it can be done both in *in situ* and *ex situ* conditions. Simultaneously it presents a challenge for the researchers (Durali et al., 2005; Kryczyk et al., 2017). Mushroom mycelia were used as sorbents and biological filters; their surface structures consist of large biomasses with strong construction and affinity to pollutants (Volesky and Holan, 1995). Damodaran et al. reported the bioaccumulation potential of *Galerina vittiformis*, isolated from municipal waste dump yards for *in vitro* removal of heavy metals (Damodaran et al., 2014). The hyphae of mushrooms produce enzymes, typically induced by their substrates, such as extracellular peroxidases, pectinases, ligninase (laccase, lignin peroxidase, manganese dependent peroxidase), xylanases, cellulases and oxidases (Nyanhongo et al., 2007; Novotný et al., 2004; Akinyele et al., 2011; Zhang et al., 2013), which are able to oxidize stubborn pollutants *in vitro*. In the fruiting bodies of mushrooms, a large quantity of phenolic compounds was found, like protocatechuic, p-hydroxybenzoic, p-coumaric and cinnamic acids applying strong antioxidant potential (Reis, 2012).

The aim of our work was to conduct the pilot tests for the evaluation the ability of mushroom-based remediation to degrade cephalosporin antibiotics. For presented experiment two edible species of mushrooms and their mycelia from liquid *in vitro* cultures were choosing because of their enzymatic content and unique nutritional and medicinal properties. The fruiting bodies of *Imleria badia* are commonly found in Europe and North America, mostly in coniferous and mixed forests. *Lentinula edodes* fruiting bodies are used from ancient times in medicine because of their immunostimulating activity. Fruiting bodies of this species are rich in bio-active polysaccharides such as *β*-D-glucans for example: lentinan, xylomannan, free sugars (arabinose, arabitol, mannose, mannitol, trehalose, glycerol), vitamins and eritadenine (S-adenosyl-L-homocysteine hydrolase). The extracts from *L. edodes* also presented anti-oxidative and anti-microbial properties (Bisen et al., 2010). Furthermore, both of these species are rich source of enzymes with lignolithic activity for example cellulases, laminarinases, and xylanases (Mata et al., 2016).

Our research article was prepared to underline the importance of monitoring residual antibiotics as one of the essential points for environmental safety, and to evolve new ability for reducing their environmental risks, to discuss future directions for remediation. Here, we proposed structures of degradation products and likely path of decomposition of cefuroxime axetil using mycelia from liquid *in vitro*