Saudi Journal of Biological Sciences (2016) xxx, xxx–xxx

King Saud University

Saudi Journal of Biological Sciences

www.ksu.edu.sa
www.sciencedirect.com

ORIGINAL ARTICLE

Potential of pure and mixed cultures of *Cladosporium cladosporioides* and *Geotrichum candidum* for application in bioremediation and detergent industry

Violeta D. Jakovljević [a,*], **Miroslav M. Vrvić** [b]

[a] *Institute for Biology and Ecology, Faculty of Science, University of Kragujevac, Radoja Domanovića 12, 34 000 Kragujevac, Serbia*
[b] *Department of Biochemistry, Faculty of Chemistry, University of Belgrade, Studentski trg 12-16, 11000 Belgrade, Serbia*

Received 26 May 2015; revised 13 November 2015; accepted 12 January 2016

KEYWORDS

Biomass;
Carbo-hydrates;
Organic acids;
pH;
Proteolytic activity

Abstract The effect of ethoxylated oleyl–cetyl alcohol (Henkel, "Merima", Serbia) on the growth and metabolic activity of *Cladosporium cladosporioides*, *Geotrichum candidum* and their mixed culture was in the focus of this paper. The cultures were grown in Czapek-Dox liquid nutrient medium with the addition of 0.5% pollutant and without it. The physico-chemical and biochemical changes of pH, the total biomass dry weight, the quantity of free and total organic acids, proteolytic activity and the quality of carbohydrates were evaluated from 4th to 19th day of fungal growth. The pollutant caused an inhibitory effect on biomass dry weight of *C. cladosporioides* and *G. candidum* for 10.36% and 4.65% respectively, and stimulatory effect on biomass of mixed culture for 3.80%. The pollutant had influence on the decrease in pH value of the media in the phase of culture growth, and pH changes were correlated with the amount of excreted total organic acids. The highest quantity of free and total organic acids was noted in media with pollutant of mixed culture and *C. cladosporioides*, respectively. The alkaline protease activities of *C. cladosporioides*, *G. candidum* and mixed culture were enhanced by addition of pollutant for 56.88%, 55.84% and 30.94% respectively. The obtained results indicate the potential of both pure and mixed cultures in mycoremediation environment contaminated by alcohol ethoxylated and detergent industry.

* Corresponding author. Tel.: +381 34336223; fax: +381 34335040.
E-mail addresses: jakovljevicvioleta@gmail.com (V.D. Jakovljević), mmvrvic@sezampro.rs (M.M. Vrvić).
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

1. Introduction

Fatty alcohol ethoxylates (FAEs) are the leading group of non-ionic ethoxylated surfactants (Szymanski et al., 2000) and make up the second highest volume group of surfactant after linear alkylbenzene sulfonate. By far, the greatest use of FAEs is in domestic detergents, household cleaners and

http://dx.doi.org/10.1016/j.sjbs.2016.01.020
1319-562X

Please cite this article in press as: Jakovljević, V.D., Vrvić, M.M. Potential of pure and mixed cultures of *Cladosporium cladosporioides* and *Geotrichum candidum* for application in bioremediation and detergent industry. Saudi Journal of Biological Sciences (2016), http://dx.doi.org/10.1016/j.sjbs.2016.01.020

personal care products such as shampoos, but they are also used as penetration promoters in insecticides. After use, surfactants as well as their products are mainly discharged into sewage treatment plant and then dispersed into environment by releasing effluents into surface waters and by sludge disposal on land (Olkowska et al., 2014). FAEs are easily biodegraded in aerobic and anaerobic conditions, although the massive dumping of these surfactants into sewage waters requires strict control to prevent the pollution of the aquatic environment. In order to perform the purification of wastewaters from different xenobiotics, combinations of physical, chemical and biological methods have been developed (Araujo et al., 2008; Mo et al., 2008). Due to the side effects that accompany the use of the physical and chemical methods, the application of new technologies such as bioremediation is necessary (Haritash and Kaushik, 2009).

Bioremediation is a process in which indigenous or inoculated microorganisms (e.g., fungi, bacteria, and other microbes) degrade (metabolize) organic contaminants found in soil and water, converting them to innocuous end products (Bavarva, 2015). Thus, the identification of microorganisms indigenous to high-contaminated environment ought to be the first step of bioremediation process. A large number of fungal enzymes (e.g., peroxidases, oxidoreductases, cellulolytic enzymes, proteases, amylases, etc.) have been reported to play an important role in an array of waste treatment application, because there is growing interest in application of fungi in bioremediation. Published papers (Fakhru'l-Razi and Molla, 2007) report the use of a wide variety of pure and mixed cultures of fungi, including micromycetes of genus *Aspergillus*, *Penicillium*, *Trichoderma*, *Fusarium*, *Cladosporium*, etc. for biopurification of soils and waters contaminated by different hydrocarbons.

The occurrence of *Geotrichum candidum* and *Cladosporium cladosporioides* in sewage and industrial wastewater and sewage sludge is reported in studies of Cooke and Pipes (1970), Diener et al. (1976) and Fluery (2007). The ability of *G. candidum* to degrade various organic wastes such as phenolics (Garcia Garcia et al., 1997, Landeka Dragičević et al., 2010) and glycerol trinitrate (Singh and Ward, 2004) or its ability to decolorize different azo and anthraquinone dyes (Kim et al., 1995) is well documented. On the other hand, *C. cladosporioides* is effective in degradation of [*o*,*o*-diethyl-*o*-(3,5,6-trichloro-2-pyridyl)phosphorothioate] and its hydrolysis product (Chen et al., 2012), of concrete (Wei et al., 2013) and different azo and triphenylmethane dyes (Vijaykumar et al., 2006). However, no literature data exist about the role of these fungi in biodegradation ethoxylated alcohol or other non-ionic surfactants (NSs). Our previous studies (Jakovljević et al., 2014; Stojanović et al., 2011a,b) confirmed that several species of fungi (*Fusarium oxysporum*, *Aspergillus niger*, *Trichothecium roseum*, etc.) originated from municipal wastewaters, able to grow and metabolize EOCA at a wide concentration range 0.01–1%. Taking into consideration the fact that literature provides the evidence of the powerful biodegradation potential of *G. candidum* and *C. cladosporioides* and due to the fact that they are abundant in man-made contaminated wastewater, these particular, abovementioned fungal species are chosen as test organisms in this study.

The current study was conceived in order to investigate the effect of EOCA on the growth and changes of metabolic activity of pure cultures *G. candidum* and *C. cladosporioides* and their mixed culture isolated from wastewater, since these parameters are crucial for the application of fungi in mycoremediation. Additionally, stability and high activity of fungal alkaline proteases in the presence of tested pollutant can have a practical application in the detergent industry.

2. Experimental

2.1. Isolation and identification of fungi from wastewater

Pure cultures of micromycetes *C. cladosporioides* (Fresen) G. A. de Vries (1952) and *G. candidum* link (1809) were isolated from samples of wastewater river basin of Lepenica (Kragujevac, Serbia) (the place of wastewater flood, sewage) according to the standard procedure. The isolation and identification of pure cultures from a sample of wastewater were carried out based on the macroscopic and microscopic morphology at the Faculty of Biology, University of Belgrade, Serbia. The fungi were raised on PDA plates at (28 ± 2 °C) from 3- to 5-days until sporulation. After having sufficient population of spores, the plates were stored at (4 ± 0.5 °C) and subcultured monthly in sterile conditions.

2.2. Spore inocula preparation

Inoculum suspensions were prepared from fresh, mature cultures as described above. The colonies were covered with 5 mL of distilled sterile water. The inoculums were achieved by carefully rubbing the colonies with a sterile loop; the isolates were shaken vigorously for 15 s with a Vortex mixer and then transferred to a sterile tube. The inoculum sizes were adjusted to 1.0×10^6 spores/mL by microscopic enumeration with a cell-counting hematocytometer (Neubauer chamber).

2.3. Experimental procedure and culture condition

Three sets of triplicate 250 mL Erlenmeyer flasks were prepared for screening of *C. cladosporioides* and *G. candidum* and their mixed culture. Each flask contained 100 mL of Czapek Dox liquid nutrient media, which was prepared according to the procedure presented in Table 1. The pH value of nutrient media was adjusted at 4.8 with 0.1 mol L^{-1} HCl before sterilization. All flasks were sterilized at 121 °C in an autoclave for 15 min. After cooling the liquid media to room temperature, 1 mL spore suspension of individual and mixed cultures of fungi was inoculated in liquid media in aseptic condition. Inoculated flasks were incubated on an electric shaker (Kinetor-*m*, Ljubljana) at 150 rpm and ambient temperature for 19 days. The flasks were harvested at 4th, 7th, 10th, 14th and 19th day after inoculation. To observe the fungal growth, mycelium was removed by filtration of fermentation broth, according to the procedure described below. Fermentation broth was used for determination of pH, organic acids, carbohydrates and protease activity.

2.4. Determination of dry weight biomass

After the separation of the mycelium from the fermentation broth by filtration through a filter paper of a known weight, the mycelium was washed with deionized water several times.

Please cite this article in press as: Jakovljević, V.D., Vrvić, M.M. Potential of pure and mixed cultures of *Cladosporium cladosporioides* and *Geotrichum candidum* for application in bioremediation and detergent industry. Saudi Journal of Biological Sciences (2016), http://dx.doi.org/10.1016/j.sjbs.2016.01.020