BRAZILIAN JOURNAL OF MICROBIOLOGY 47 (2016) 18–24

BRAZILIAN JOURNAL OF MICROBIOLOGY

http://www.bjmicrobiol.com.br/

Environmental Microbiology

Isolation and characterization of phenol degrading yeasts from wastewater in the coking plant of Zarand, Kerman

Maryam Karimi[a,b], *Mehdi Hassanshahian*[c,*]

[a] *Department of Microbiology, Kerman Branch, Islamic Azad University, Kerman, Iran*
[b] *Department of Microbiology, Kerman Sience and Reaserch Branch, Islamic Azad University, Kerman, Iran*
[c] *Department of Biology, Faculty of Sciences, Shahid Bahonar University of Kerman, Kerman, Iran*

ARTICLE INFO

Article history:
Received 12 January 2014
Accepted 15 August 2014

Associate Editor: Lara Durães Sette

Keywords:
Biodegradation
Coal tar
Coking plant
Yeast
Zarand

ABSTRACT

Phenol and phenolic compounds are environmental pollutants present in industrial wastewaters such as coal tar, oil refineries and petrochemical plants. Phenol removal from industrial effluents is extremely important for the protection of environment. Usually, phenol degradation is carried out by physicochemical methods that are costly and produce hazardous metabolites. Recently, phenol biodegradation has been considered. Yeasts are the most important phenol biodegraders. In this study, the phenol-degrading yeast from environmental samples (soil and wastewater) was isolated from the coking plant of Zarand, Kerman. Then total heterotrophic yeasts were counted. The soil samples had higher rates of yeast degrader, in comparison to wastewater samples. After three passages, four yeasts (K1, K2, K7 and K11) that had the highest growth rate were selected for further study. Also, these yeasts were able to remove phenol measured by Gibbs reagent. The effect of four different concentrations of phenol (50, 125, 200 and 275) $mg L^{-1}$ was measured and three degradation patterns in these yeasts were observed. The hydrophobicity and emulsification activity were measured in all eleven yeasts. Finally, strong yeasts in phenol degrading yeasts were identified by molecular method using amplification of 18S rRNA gene region. The sequencing results showed that these isolated yeasts belonged to *Candida tropicalis* strain K1, *Pichia guilliermondii* strain K2, *Meyerozyma guilliermondii* strain K7 and *C. tropicalis* strain K11.

© 2015 Sociedade Brasileira de Microbiologia. Published by Elsevier Editora Ltda. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Introduction

Due to the toxic properties of both phenol and chlorophenol, the efficient removal of these compounds from industrial aqueous effluents is of great practical significance for environmental protection. Because of the improper treatment of these materials, they have widely contaminated soil and groundwater, and their toxicity seriously affects living organisms. Once wastewater containing phenolic compounds is discharged into the receiving body of water, it endangers fish life, even at a relatively low concentration,

* *Corresponding author.*
E-mails: mshahi@uk.ac.ir, hasanshahi@gmail.com (M. Hassanshahian).
http://dx.doi.org/10.1016/j.bjm.2015.11.032
1517-8382/© 2015 Sociedade Brasileira de Microbiologia. Published by Elsevier Editora Ltda. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

e.g., 5–25 mg L^{-1}.[1–3] After physical and chemical purification procedures, the phenol concentration is decreased to 0.3–0.4 g L^{-1}.[4] For many years, activated sludge was used to change phenolic contamination.[4–6] Beside physical and chemical methods, biological methods for removal play an important role in wastewater management. To treat phenolic compounds, biodegradation techniques have recently been developed for their economical advantages and the low possibility of the production of byproducts.[6] Phenol is not readily degradable and can be very toxic to most types of microorganisms at sufficiently high concentration. Phenol can inhibit the growth rate, even among those species that have the metabolic capability of using it as a substrate for growth. Various phenol-degrading microorganisms have been extensively studied to develop and improve the technological processes of biodegradation. A number of studies with prokaryotic microorganisms have been carried out.[7–11] However, the use of such technology is confined due to phenolic toxic properties, especially 4-cholorophenol, for microorganisms. Some yeast strains are reported to have the capability of utilizing phenol.[4] Only some members of the yeast genera, including *Rhodotorula, Trichosporon*, and *Candida*, can metabolize phenolic compounds as the only source of carbon and energy.[9] Some *Rhodotorula* species show a significant capacity to degrade phenol, catechol, cresol, resorcinol, 3-methoxybenzoic acid and hydroquinones.[12] Basak et al.,[13] worked on the biodegradation of 4-chlorophenol using *Candida tropicalis* PHB5 by optimizing physicochemical parameters. It was found that the yeast was able to grow on 4-chlorophenol and metabolize this substrate.[13] Phalgune et al.[14] explained the process of phenol biodegradation by the yeast *C. tropicalis* NCIM 3556 in aqueous medium using DOSY NMR techniques. This test indicated that the phenol was completely degraded to carbon dioxide and water within approximately 20 h after incubation.[14] Hassanshahian et al.,[15] described the ability of two different yeast strains, *Yarrowia lipolytica* PG-20 and PG-32, in decomposing aromatic hydrocarbons.[16] Chandran and Das[17] isolated *C. tropicalis* from some oil contaminated soil that had high ability to produce biosurfactants capable of degrading diesel oil during ten days.[17] The coking plant of Zarand is located seven kilometers road from Zarand, in the province of Kerman, Iran. This factory has an area of 100 ha. There are many toxic pollutants in the wastewater of this coking plant, such as phenol, Ammonium (NH3), cresol, etc., but the main pollutant of this factory is phenol. The aim of this study was characterization of phenol degrading yeasts from the wastewater of this coking plant in Zarand, Kerman.

Materials and methods

Sampling

For the isolation of phenol degrading yeasts, soil and wastewater samples were collected from the coking plant of Zarand (Kerman, Iran). The soil and wastewater samples were collected from three regions of this coking plant (37°30, N; 49°15, E). The soil samples were taken from 2 cm below the surface and wastewater samples were obtained from 1 cm below the surface. The samples were collected into sterile jars, placed on ice, and immediately transported to the laboratory for further analysis.

Total count of heterotrophic and degradative yeasts in the collected samples

For the enumeration of heterotrophic and degradative yeasts in the collected samples, serial dilutions were performed in PDA (Potato Dextrose Agar) and BHMS (Bushnell Hass Mineral Salt) media, respectively. The plates were incubated at 30 °C. After two days, the numbers of grown colonies were counted.

Isolation of phenol-degrading yeasts

A synthetic Bushnell Hass Mineral Salts medium (BHMS) was used for the isolation of degrading yeasts. BHMS medium contained (g L^{-1}): KH_2PO_4, 1; K_2HPO_4, 0.2; $MgSO_4 \cdot 7H_2O$, 0.2; $CaCl_2$, 0.02; NH_4NO_3, 1; $NaCl_2$; and 2 droplets of 60% $FeCl_3$. The pH was adjusted to 5/5–6. The BHMS medium was supplemented with 1% (v/v) phenol as the sole source of carbon and energy. To inhibit the growth of bacteria, 400 μL chloramphenicol (0.05% v/v) was added to the BHMS medium. A portion of the soil (5 g) or wastewater (5 mL) sample was added to 250 mL Erlenmeyer flasks containing 100 mL of the BHMS medium; the flasks were incubated for 10 days at 30 °C on a rotary shaker (INFORS AG, Germany) operating at 200 × *g*. Then, 5 mL aliquots were transferred to fresh BHMS medium. After a series of three further subcultures, inoculums from the flask were streaked out, and phenotypically different colonies were purified on Sabro dextrose agar medium.[15]

Growth rate and phenol biodegradation by the isolated strains

Growth rate of the isolates were routinely assessed indirectly by turbidity measurement (O.D. at 600 nm) in a UV–visible spectrophotometer (Shimadzu UV-160, Japan). The phenol removal assay was carried out using 2,4 dichloro-quinon-4-chloroimide dyes (Gibb's reagent). By this method, 150 mL medium was centrifuged (6000 × *g* for 10 min), 30 mL Na_2 HCO_3 and 20 mL Gibb's reagent were then added to the supernatants, and the colors developed were read at 630 nm.[18]

Growth of the selected strains on different concentrations of phenol

The effect of different concentrations of phenol (50, 125, 200, 275 mg L^{-1}) on the growth of selected yeast strains was measured. For this purpose, BHMS medium was supplemented with various concentrations of phenol. The flasks were incubated for 10 days at 30 °C on a rotary shaker operating at 180 rpm. Growth was routinely assessed indirectly by turbidity measurement (O.D. at 600 nm) in a UV–visible spectrophotometer (Shimadzu UV-160, Japan).

Measurement of emulsification activity and bacterial adherence to hydrocarbons (BATH)

The emulsification activity (E_{24}) was determined by combining equal volumes of hexadecane and cell-free culture

Download English Version:

https://daneshyari.com/en/article/4356609

Download Persian Version:

https://daneshyari.com/article/4356609

Daneshyari.com