Short Communication

Isolation and characterization of phenol degrading yeast

Riddhi Patel and Shalini Rajkumar

Department of Biochemistry and Biotechnology, Institute of Science, Nirma University of Science and Technology, Sarkhej-Gandhinagar Highway, Ahmedabad Gujarat, India

A phenol degrading yeast isolate was identified and characterized from the soil sample collected from a landfill site, in Ahmedabad, India, by plating the soil dilutions on Sabouraud's Dextrose Agar. The microscopic studies and biochemical tests indicated the isolate to be *Saccharomyces cerevisiae*. The phenol degrading potential of the isolate was measured by inoculation of pure culture in the mineral medium containing various phenol concentrations ranging from 100 to 800 mg l^{-1} and monitoring phenol disappearance rate at regular intervals of time. Growth of the isolate in mineral medium with various phenol concentrations was monitored by measuring the turbidity (OD_{600} nm). The results showed that the isolated yeast was tolerant to phenol up to 800 mg^{-1}. The phenol degradation ranged from 8.57 to 100% for the concentration of phenol from 800 mg l^{-1} to 200 mg l^{-1}, respectively.

Keywords: Biodegradation / Phenol / *Saccharomyces cerevisiae*

Received: May 20, 2008; accepted: July 04, 2008

DOI 10.1002/jobm.200800167

Introduction

Increased developmental activities in various industrial and medical sectors have led to the release of pollutants in the form of waste-products and man-made xenobiotics [1]. Phenol is one such hazardous chemical discharged from the pharmaceutical, textile, paper and pulp, paint solvent, pesticide, dye, plastic, explosive, herbicide and steel industry [2–4]. Penetration of phenol from the top soil into subsoil results in contamination of groundwater [5]. So, complete removal of phenol from the industrial effluents is essential as the incomplete breakdown may result in products which are more harmful [1]. Bioremediation utilize microorganisms to convert the toxic compounds into harmless by products of the cellular metabolism like CO_2 and H_2O [6–10]. Adoption of micro flora to a soil specific condition is dependent upon the capacity of microorganism to survive in the new environment, utilize organic carbon present in the medium as carbon source and multiply and proliferate in the new surrounding. The normal population may perish in the toxic environment. But, newly developed species will grow as it acquires new genetic properties by mutation, substitution, and expression of new gene and develop where in they are enriched slowly and steadily in the process of "acclimation" [11].

The complex cell wall of yeast which is absent in bacteria, aids prevention of lysis and protection against harmful environmental conditions [12]. Therefore, this study aimed at isolating and identifying phenol degrading yeast from the soil sample collected from the landfill site and scrutinizing the potential of the isolated yeast to degrade phenol by measuring the phenol disappearance rate using colorimetric method.

Materials and methods

Soil samples from land-fill site, Vatva, G.I.D.C. (Gujarat Industrial Development Corporation) were the biological matrix for the yeast isolation. The soil samples were serially, diluted up to 10^{-7} fold and plated on the Sabauraud's dextrose agar. Investigations on morphological and physiological characteristics of the isolate were carried out. A series of conventional biochemical

Correspondence: Shalini Rajkumar, Department of Biochemistry and Biotechnology, Institute of Science, Nirma University of Science and Technology, Sarkhej-Gandhinagar Highway, Ahmedabad Gujarat 382 481 India
E-mail: shalini_rjk@yahoo.com
Phone: +91-2717-241901-04
Fax: +91-2727-241916

tests were done for the identification of the isolated yeast. The tests were based on the principle of pH change and substrate utilization. The substrates tested were glucose, melibiose, lactose, maltose, sucrose, galactose, cellobiose, inositol, xylose, glucitol, raffinose and trehalose [13].

The yeast isolate was tested for its tolerance and growth on Mineral medium (K_2HPO_4 0.4 g l^{-1}, KH_2PO_4 0.2 g l^{-1}, NaCl 0.1 g l^{-1}, $MgSO_4$ 0.1 g l^{-1}, $FeSO_4 \cdot 7\ H_2O$ 0.01 g l^{-1}, $MnSO_4 \cdot H_2O$ 0.01 g l^{-1}, $Na_2MoO_4 \cdot 2\ H_2O$ 0.01 g l^{-1}, $(NH_4)_2SO_4$ 0.4 g l^{-1}) containing varying concentrations of phenol ranging from 100 to 800 mg l^{-1}. Incubation was allowed for 5 days at room temperature on a rotary shake. Samples were periodically withdrawn from culture flasks for the measurements of growth and phenol concentrations. For estimating the phenol losses by stripping, flask containing mineral medium with 100 mg l^{-1} phenol without inoculation was monitored [14, 15].

The growth of the yeast isolate was monitored at time intervals of 10 hours by measuring the turbidity (optical density) at 600 nm (OD_{600}). Phenol disappearance rate was estimated using 4-amino-antipyrine method [16, 17]. 150 µl of sample was transferred to Eppendorf tubes and centrifuged at 10,000 rpm for 5 min at 4 °C. 100 µl of the supernatant was transferred to the glass tubes and diluted 1000 folds for the colorimetric assay at 500 nm. The phenol concentrations were extrapolated using a standard curve taken as a reference [18]. Care was taken that samples did not stand more than 2 min before the readings were taken as the colour develops immediately.

Results and discussion

There are many reports indicating yeasts to be more efficient phenol degraders when compared to bacteria. *Candida tropicalis* was found to have better phenol degrading potential than *Cryptococcus terreus* and *Rhodotorula creatinivora* [19]. The isolate obtained in the present study grew at pH 6 (acidic) at 28 °C which is favourable for the growth of fungi. On Sabouraud's dextrose agar, the colony of isolated organism after 24 h of incubation was white to cream coloured, smooth, glaborous and yeast-like. The microscopic morphology of the isolate was large, globose to ellipsoidal, budding and 3.0–10.0 × 4.5–21.0 µm in size. In addition, the germ tube was not observed under the microscope and hence the germ tube test was negative, which further supported that the yeast might be *S. cerevisiae* as germ tube is a characteristic of *Candida* sp. [20]. Also, the organism grew on gentamicin suphate, an anti-bacterial but the growth was inhibited on cycloheximide, an anti-fungal. Cycloheximide has an inhibitory effect on the growth of almost all the species of yeast except *Candida*. Furthermore, the positive assimilation tests for raffinose, trehalose, maltose, melibiose, sucrose suggested that the isolated yeast was *Saccharomyces cerevisiae.* The yeast isolate did not assimilate lactose, galactose, cellobiose, inositol, and xylose, while melibiose was assimilated after prolonged incubation (Table 1).

The growth curve of isolate at 100 mg l^{-1} phenol recorded a prolonged lag phase followed by the exponential phase. At 200 and 300 mg l^{-1} the growth of isolate was significantly lower compared to the growth at 100 mg l^{-1} (Fig. 1a). No lag phase was observed during the growth of organism at phenol concentration above 100 mg l^{-1}. The prolonged lag phase probably

Table 1. Biochemical and growth characteristics of yeast isolate.

Test	Characteristic of *Sacccharomyces cerevisiae*	Characteristic of isolate
Growth on Sabuoraud's Dextrose agar	White to cream colored, smooth, glaborous	White to cream colored, smooth, glaborous
Microscopic studies	Large, globose to ellipsoidal budding yeast-like cells or blastoconidia	Large, globose to ellipsoidal budding yeast-like cells or blastoconidia
Capsule staining	Capsule absent	Capsule absent
Physiological test	Germ Tube test: negative Hydrolysis of urea: negative Growth on cycloheximide medium: negative	Germ Tube test: negative Hydrolysis of urea: negative Growth on cycloheximide medium: negative
Assimilation test		
Glucose	+	+
Maltose	+	+
Sucrose	+	+
Raffinose	+	+
Lactose	–	–
Cellobiose	–	–
Galactose	V	–
Trehalose	V	+
Xylose	–	–
Inositol	–	–
Glucitol	–	–
Melibiose	V	+
Potassium nitrite	–	–

+: positive; –: negative; V: variable.

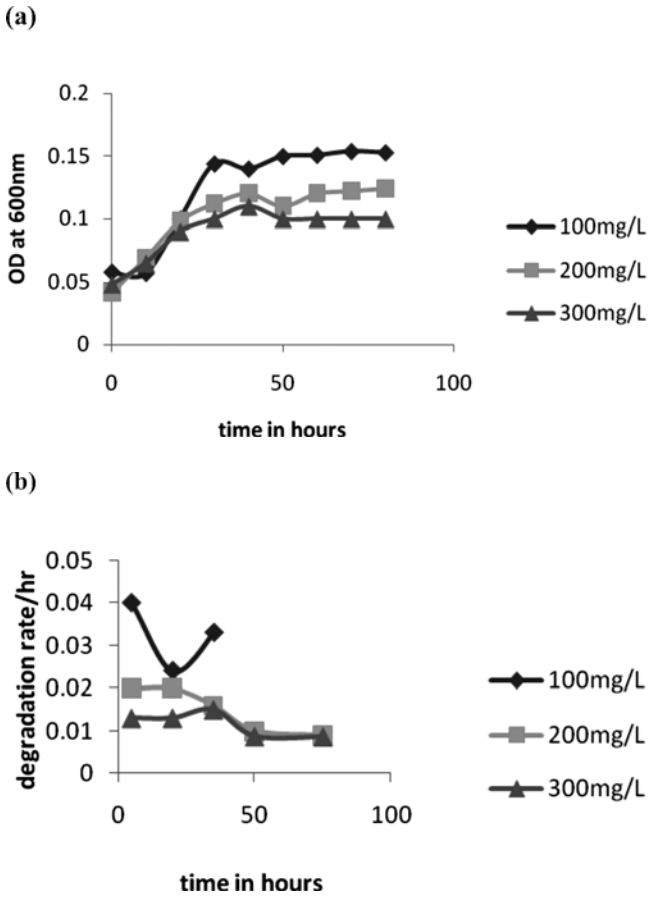

Figure 1. Growth (a) and phenol degradation (b) by yeast isolate.

represented adaptation of the organism to the phenol environment. It has been observed that during the lag period certain compounds are synthesized which reduces the toxic effect of the substrate and allows the cell to grow and reproduce in its presence [21]. Increase in phenol degradation rate after an initial decrease by the isolate was observed in mineral medium containing 100 mg l^{-1}. The isolate could degrade phenol completely up to 200 mg l^{-1} within 77 h (Table 2). At 200 and 300 mg l^{-1} of phenol, the isolate showed decrease in the phenol degradation rate after 40 hours that corresponded to onset of stationary phase (Fig. 1b). A significant decrease in the phenol degradation rate of isolate was observed at the concentration above 200 mg l^{-1}. This may be due to the inhibitory and the lytic effect of phenol [9]. It implied that the tolerance of organism to phenol toxicity decreased with increasing concentrations. The isolate was tolerant to phenol up to 800 mg l^{-1}. However, phenol degradation was only 8.75% in 77 h of incubation. The phenol degradation ranged from 17–53.3% for the concentrations 300–700 mg l^{-1} in 77 h (Table 2). Besides, none of the yeast

Table 2. Phenol degrading potential of the isolate.

Initial phenol concentration (mg l^{-1})	Phenol degraded (mg l^{-1})	Time taken (h)	Phenol degraded (%)
100	100	30	100
200	200	77	100
300	160	77	53.3
400	160	77	40
500	160	77	32
600	100	77	16
700	120	77	17
800	70	77	8.75

cultures became contaminated in the flask, which was also due to the antiseptic nature of phenol [6].

The yeast species, isolated from soil sample of a landfill site, on morphological and biochemical tests was identified as *S. cerevisiae*. The isolate completely degraded 200 mg l^{-1} of phenol in 77 h. The rate of phenol degradation and growth decreased with increase in phenol concentration. The phenol degrading potential of the isolate was 8.57% at 800 mg l^{-1} of phenol.

The phenol degrading potential of isolated *S. cerevisiae* when compared to degrading potential of other yeasts like *Candida tropicalis* was low. But the metabolic efficiency of the strain can be enhanced by genetically engineering the organism at two different levels: manipulating specific catabolic pathway or manipulating the host cells. In order to improve the rate of phenol removal, key enzyme of the involved catabolic pathway and regulatory mechanism controlling the expression of the catabolic genes can be manipulated.

References

[1] Kumaran, P., 1999. Phenolic waste treatment by specialised microbes. In: Goel, P.K. (ed.), Advances in Industrial Wastewater Treatment, pp. 380–395. Vedams eBooks (P) Ltd. New Delhi, India.

[2] Vijayagopal, V. and Viruthgiri, T., 2005. Kinetics of biodegradation of phenol using mixed culture isolated from mangrove soil. Pollution Research, **24**, 157–162.

[3] Edwin, A., 2008. Removal of phenol and *o*-cresol by adsorption onto activated carbon. E.-J. Chem., **5**, 224–232.

[4] Gurujeyalakshmi, G. and Oriel, P., 1989. Isolation of phenol-degrading *Bacillus stearothermophilus* and partial characterization of the phenol hydroxylase. Appl. Environ. Microbiol., **55**, 500–502.

[5] Lenke, H., Pieper, D., Bruhn, C. and Knackmuss, H., 1992. Degradation of 2,4-dinitrophenol by two *Rhodococcus erythropolis* strains, HL 24-1 and HL 24-2. Appl. Environ. Microbiol., **58**, 2928–2931.

[6] Thakur, I., 1995. Degradation of Xenobiotic Compounds. Environmental Biotechnology Basic Concepts and Application, pp. 261–288.

[7] Dean, B., 1978. Genetic toxicology of benzene, toluene, xylenes and phenols. Mutat. Res., **47**, 75–97.

[8] Fogel, M., Taddeo, A. and Fogel, S., 1986. Biodegradation of chlorinated ethenes by a methane-utilizing mixed culture. Appl. Environ. Microbiol., **51**, 720–724.

[9] Karlson, U. and Frankenberger, W., 1989. Microbial degradation of benzene and toluene in groundwater. Bull. Environ. Contam. Toxicol., **43**, 505–510.

[10] Yan, J., Jianping, W., Hongmei, L., Suliang, Y. and Zongding, H., 2005. The biodegradation of phenol at high initial concentration by yeast *Candida tropicalis*. Biochem. Eng., **24**, 243–247.

[11] Colvin, R. and Rozich, A., 1986. Phenol growth kinetics of heterogeneous populations in a two-stage continuous culture system. Wat. Pollut. Cont. Fed., **58**, 326–332.

[12] Lagorce, A., Hauser, N., Labourdette, D., Rodriguez, C., Martin-Yken, H. *et al*., 2003. Genome-wide analysis of the response to cell wall mutations in the yeast. J. Biol. Chem., **278**, 20345–20357.

[13] Yarrow, D., 1998. Methods for the isolation, maintenance, and identification of yeasts. In: Kurtzman, C.P. and Fell, J.W. (eds.), The Yeasts – A Taxonomic Study, pp. 77–100. Elsevier Science Amsterdam.

[14] Ruiz-Ordaz, N., Ruiz-Lagunez, J., Castanon-Gonzalez, J., Hernandez-Manzano, E. *et al.,* 2001. Phenol biodegradation using a repeated batch culture of *Candida tropicalis* in a multistage bubble column. Rev. Latinoam. Microbiol., **43**, 19–25.

[15] Yan, J., Jianpng, W., Xiaoquiang, J., Qinggele, C. and Zongding, H., 2007. Mutation of *Candida tropicalis* by irradiation with a He–Ne laser to increase its ability to degrade phenol. Appl. Environ. Microbiol., **73**, 226–231.

[16] Martin, R., 1949. Rapid colorimetric method for phenol estimation. Anal. Chem., **21**, 1419.

[17] Neumann, G., Teras, R., Monson, L., Kivisaar, M., Schauer, F. and Heipieper, H., 2004. Simultaneous degradation of atrazine and phenol by *Pseudomonas* sp. strain ADP: Effects of toxicity and adaptation. Appl. Environ. Microbiol., **70**, 1907–1912.

[18] Folsom, B., Chapman, P. and Pritchard, P., 1990. Phenol and trichloroethylene degradation by *Pseudomonas cepacia* G4: Kinetics and interactions between substrates. Appl. Environ. Microbiol., **56**, 1279–1285.

[19] Krallish, I., Gonta, S., Savenkova, L., Bergauer P. and Margesin, R., 2006. Phenol degradation by immobilized cold-adapted yeast strains of *Cryptococcus terreus* and *Rhodotorula creatinivora*. Extremophiles, **10**, 441–449.

[20] Arthur, C., Theresa, D. and Kristin, K., 1996. Comparison of the murex *C. albicans*, Albicans-sure, and Bacticard Candida test kits with the germ tube test for presumptive identification of *Candida albicans.* J. Clin. Microbiol., **34**, 2616–2618.

[21] Martinez-Garcia, G., Williams, C., Burgoyne, A. and Edyvean, R., 2003. Aerobic treatment of alpechin by *Candida tropicalis.* XI Symposium, Científico-Técnico Expoliva, Jaén, Spain.

