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Isolation of Reactive Blue 13 Degrading Strain in Wastewater with High Salinity

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One strain of HXL-3, capable of degrading azo dyes reactive blue 13 (RB13), was isolated from a lab-scale sequence biological reactor wastewater with high salinity. The isolate was identified as *Candida* sp. based on its physiological characteristics, biochemical tests and 26S rDNA D1/D2 gene phylogenetic analysis.

Keywords: *Candida* sp., Reactive blue 13, Biodegradation, High salinity.

INTRODUCTION

Reactive azo dyes are widely used as textile colorants, typically for cotton dyeing, due to their variety of colour shades, high wet fastness profiles, ease of application, brilliant colours and minimal energy consumption. However, dye refining, bleaching step needed adding hydroxide needed sodium hypochlorite and other alkaline substances, dye wasterwater had a lot of salt. The dye containing effluents may cause serious problems since the cleavage of azo bonds produces aromatic amines, which are considered as mutagenic and carcinogenic[1-3]. Azo dyes are almost all xenobiotic compounds, which are characterized by containing one or more azo groups (-N=N-), representing a major group of dyes causing environmental concern because of their colour, biorecalcitrance and potential toxicity to animals and human[4].

In this study, the C.I. reactive blue 13, a representative of copper-containing reactive azo dyes, was chosen as target and a strain of Candida HXL-3 with high decolourization capability, was isolated from a sequence biological reactor (SBR), which used to treat azo dye wastewater with high salinity.

EXPERIMENTAL

Reactive blue 13 (RB13), a common azo dye, was obtained from Tian Yu Ltd., Zhejiang, China. The commercial product without further purification was used in this study. Stock solutions of the dyes were prepared at 1,000 mg L^{-1} and diluted before use. All other reagents used in this study were of analytical grade.

YPD medium and mineral salts medium (MSM) (1.0 g NH_4NO_3, 1.5 g K_2HPO_4, 0.5 g KH_2PO_4, 0.2 g $MgSO_4$ and 1.0 g NaCl per liter, pH 7.0) were used in this study. The screening medium (SM) was MSM supplemented with 50 mg L^{-1} RB13. GMM medium was SM medium supplemented with 200 mg L^{-1} glucose. Solid medium plates were prepared by adding 1.5 % (wt/vol) agar.

General procedure: Sludge sample (5.0 g) collected from the long-term RB13 water sequence biological reactor was added to 100 mL GMM medium and incubated at 28 ºC for 7 days in a rotary shaker at 150 rpm. Five milliliters of enriched cultures, showing degradation of RB13 were transferred to 100 mL fresh GMM medium. Three subcultures were performed before the isolation of effective strains. The final enriched culture was spread on GMM plates. Different colonies formed were picked up and tested for their RB13-degrading capabilities. One strain, designated as HXL-3, which possessed the highest degradation capacity, was selected for further investigation.

The isolated strain HXL-3 was identified by biochemical and physiological investigation. The morphological and physiological properties of the isolate were determined according to the standard methods of yeast identification[5,6]. Utilization of carbon and nitrogen sources was conducted at 25 ºC with occasional shaking. Total genomic DNA was prepared from strain HXL-3 by high-salt precipitation[7]. A pair of universal primers: 5'-gcatatcaataagcggaggaaaag-3' (NL-1) and 5'-ggtccgtgtttcaagacgg-3' (NL-4) were used to amplify

the 26S rDNA D1/D2. The PCR product was purified by UNIQ-10 column DNA gel extraction kit (Shanghai Sangon Biological Engineering Technology & Services Co., Ltd.) Sequencing was conducted by Invitrogen Biological Technology Service, Shanghai, China. The obtained sequences were compared with those deposited at the GenBank database (National Center for Biotechnology Information, NCBI) and identified by using the Basic Local Alignment Search Tool (BLAST) also available in NCBI. Phylogenesis was analyzed with MEGA version 3.0 software and distance was calculated using the Kimura 2-parameter distance model. Unrooted trees were built using the neighbor-joining method. Each dataset was bootstrapped 1,000 times.

RESULTS AND DISCUSSION

Isolation and characterization of the RB13-degrading strain HXL-3: A RB13-degrading strain designated as HXL-3 was enriched and isolated from the long-term RB13 waste water. The strain HXL-3 degraded over 90 % of total RB13 at a concentration of 50 mg L^{-1} in GMM medium in 48 h. The optimal temperature and pH value for strain wax to grow was at 25-30 ºC and 2.0-7.0 on GMM medium, respectively. The colony morphology of the isolated strain was observed on agar plate after 30 days of culturing at 25 ºC. Further information on the cell morphology of the isolated strain was observed after cultivation in liquid medium for 3 days by SEM (Fig. 1). After 3 days of growth at 25 ºC, the cells are ovoid, elongate, 3.0-5.0 µm × 5.6-10.2 µm. After one month of growth on wort agar at 25 ºC, the streak culture is crisp, light brow, delicately wrinkled and has an erose margin. Well-developed tree-like pseudomycelia were abundantly formed on corn meal agar Dalmau plate. It can fermente D-glucose, but negative for maltose, alactose, lactose, sucrose, rsffinose. It can use DL-lactic acid, succinic acid, while not for galactose, L-arabinose, sucrose, D-ribose, maltose, L-rhamnose, cellobiose, erythritol, trehalose, ribitol, lactose, D-mannitol, melibiose, rsffinose, soluble starch, citric acid, D-xylose, inositlo. Utilizations of the following substrate as nitrogen: Vitamin-free medium growth,while not for nitrat, cadaverine. 2HCl, L-lysine. Negative in tests for activities of urease and the hydrolysis of gelatin.

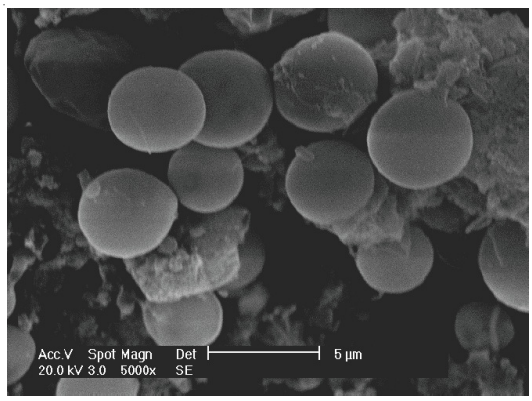

Fig. 1. SEM image of the isolated *Candida* sp. HXL-3

The phylogenetic tree of the 26S rDNA D1/D2 gene sequence is shown in Fig. 2. Strain HXL-3 was related to the *Candida* sp. lineage and closely clustered with two type strains

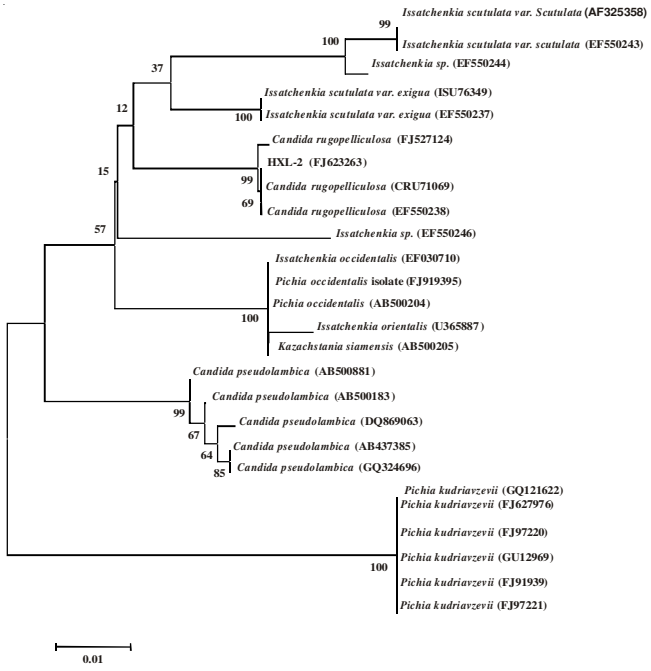

Fig. 2. Phylogenetic analysis of strain HXL-3 and related species by the neighbor-joining approach. Bootstrap values obtained with 1,000 resamplings are indicated as percentages at all branches. The scale bars represent 0.005 substitutions per nucleotide position. The GenBank accession number for each microorganism is shown in parentheses after the species name

Candida rugopelliculosa CRU71069T and *Candida rugopelliculosa* strain NRRL Y-17079T, with sequence similarity scores of 99 % and, respectively. The result of this phylogenetic analysis was consistent with that of the morphological characteristics and biochemical tests. Therefore, strain HXL-3 was identified as *Candida* sp. FJ623263.

Conclusion

At present, the domestic real high salt wastewater processing is still in laboratory test phase,Practical application there is a large distance. China has rich salt resources, you can make full use of this resource and the mechanism of screening Halophiles work order for the halophilic.The actual high-salt wastewater treatment bacteria provide a theoretical basis.

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