Isolation and Identification of a Novel Strain of Klebsiella Oxytoca with Phenol-Degrading Activity

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Abstract: A new phenol-degrading strain was isolated from activated sludge, it was identified as Klebsiella oxytoca using sequencing the entire 16 s rRNA gene. The kinetics of phenol biodegradation by Klebsiella oxytoca were studied. The whole-cell kinetic properties of phenol biodegradation for strain Klebsiella oxytoca thus revealed a significant biodegradation rate.

Key words: Klebsiella oxytoca; phenol biodegradation; kinetic properties
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Phenolic compounds are widely distributed in the environment from various industrial as well as natural sources, for example chlorinated phenolic compounds are specifically utilized as insecticides, herbicides, detergents, solvents, wood preservatives and antimicrobial agents. Biolpgical methods are preferable methods to treat aromatic compounds because it is economical and there is a low possibility of the production of by-products[1]. Several microorganisms used are usually aerobes, including Pseudomonas putida[2-3], Ochrobactrum sp.[4], Rhodococcus sp.[5], Burkholderia sp.[6]. These aerobes are more efficient at degrading toxic compounds because they grow faster than anaerobes and usually transform organic compounds to inorganic compounds. Except for these reports above, there is almost no published information available in the literature regarding the existence of aromatic ring cleavage activities within different species of genus Klebsiella.

In this paper, a new phenol-degrading strain was isolated from return activated sludge used in the biological treatment of waste-water of Jizhuangzi wastewater plant in Tianjin. The sludge enriched on phenol was used as a source of phenol— degrading bacteria. The enrichment was maintained for 2 years with bimonthly transfers of 20% inocula to fresh revised aerobic mineral medium. Cells were grown in a stirred chemostat at room temperature under stationary conditions. Approximately 10 mmol/L phenol 0.2 mmol/L dichlorophenol as the carbon source was added every week. pH 6.5[7-8].

We confirmed the identification by sequencing the entire 16 s rRNA gene. The 16 s rDNA was amplified using a pair of forward (5'-AGAGTTTGATCCTGGCTCAG—3') and reverse (5'-TACGGCTACCTTGTTACGACT-3') primers following genomic DNA extraction and the corresponding PCR. 16 s rDNA containing plasmid vectors were constructed using the pGEM-T Easy vector and used to transform E. coli cells for the nucleotide sequence analysis of the selected clones. The 16 s rDNA sequences presents the strain that can be identified as Klebsiella oxytoca. This is first time to report about the Klebsiella oxytoca could be applied to degradating phenol.

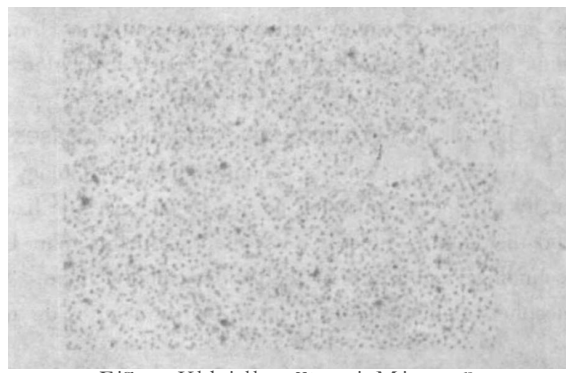

Fig. 1 Klebsiella oxytoca in Microscope

To clarify the level of phenol biodegradation in strain Klebsiella oxytoca, this activity was investigated. Kinetic parameters for phenol degradation by cells were

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determined by measuring phenol disappearance rates. Phenol were determined by monitoring changes in phenol concentration, using a modified extended Kalman filter assay[7-8]. The most important factor of influence reaction rate is temperature, for example, 1400 mg/L phenol can be reduced to 1.0 mg/L by Klebsiella oxytoca that 30 hours is needed at 32℃, but 70 h is needed at 25℃.

The determined rates of phenol degradation were fit to Haldane's equation and treated with the Lineweaver-Burk double reciprocal plot the half-saturation constant and the theoretical maximum degradation rate were estimated to be 19.7 mg/L and 0.65 mg/L min mg protein, at 32℃ respectively. Compared to known phenol degraders[2, 3] with K_S values, strain Klebsiella oxytoca showed a K_S 12 times higher in magnitude and a V_S times higher. The whole-cell kinetic properties of phenol biodegradation for strain Klebsiella oxytoca thus revealed a significant biodegradation rate.

In conclusion, a new phenol-degrading strain, Klebsiella oxytoca, was isolated from phenol-activated sludge in Tianjin. Phenotypic characteristics and phylogenetic analysis indicated the strain belong Klebsiella oxytoca; it is first time report that the Klebsiella oxytoca is being applied to taken the phenol biodegradation.

References:

[1] ALASDAIR H N. An Environmental Perspective on the Biodegradation of Organochlorine Xenobiotics [J]. International Bioakteriporation & Biodegradation, 1996, 3: 21-24.

[2] TARIK A, Emine B. Kinetics model for growth of Pseudomonas putida F1 during benzene, toluene and phenol biodegradation [J]. Process Biochem, 2004, 39: 983-988.

[3] GURUSAMY A, JUANG Ruey-shin. Microbiological degradation of phenol using mixed liquors of Pseudomonas putida and activated sludge [J]. Waste Manage, 2002, 22: 703-710.

[4] VELASCO J, ROMERO C, LOPEZ-GONI I, et al. Evaluation of the relatedness of Brucella spp. and Ochrobactrum anthropi and description of Ochrobactrum intermedium sp. nov., a new species with a closer relationship to Brucella spp. [J]. Int J Syst Bacteriol, 1998, 48: 759-768.

[5] MARGESIN R, FONTEYNE P A. Low-temperature biodegradation of high amounts of phenol by Rhodococcus sp. and basidiomycetous yeasts [J]. Res Microbiol, 2005, 156: 68-75.

[6] MOUSTAFA E S. Biological degradation of substrate mixtures composed of phenol, benzoate and acetate by Burkholderia cepacia G4 [D]. Biochemical Engineering Division of GBF, Mascheruder Weg1, Germany, 2003.

[7] WANG Q, MA P-S. Kinetics of phenol degradation by streptococcus [C]. Proceedings of 9th CEST, Greece, 2005: 1622-1626.

[8] WANG Q, MA P-S. Nonlinear Kalman filter simultaneous determination of phenol and o-chlorophenol [J]. Spectroscopy and Spectrum Analysis, 2006, 26: 899-904.

一株苯酚降解产酸克雷伯菌的分离与鉴定

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摘　要：从活性污泥中分离出一降解苯酚菌株，经 16 s rRNA基因测序确定属于产酸克雷伯菌。研究了产酸克雷伯菌降解苯酚动力学行为，显示出该菌株具有显著的降解苯酚能力。

关键词：产酸克雷伯菌；苯酚生物降解；动力学特征

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