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Microbial degradation mechanism of pyridine by *Paracoccus* sp. NJUST30 newly isolated from aerobic granules

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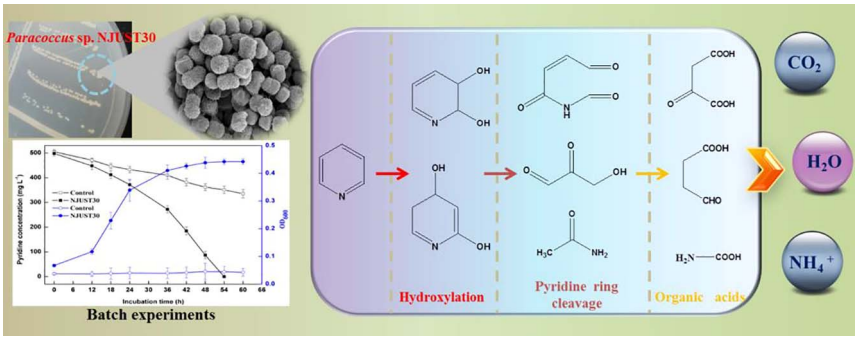
HIGHLIGHTS

- NJUST30 could utilize pyridine as the sole carbon and nitrogen source for growth.
- Pyridine could be completely mineralized during biodegradation by NJUST30.
- Major metabolites during pyridine biodegradation by NJUST30 were identified.
- A new pathway for pyridine biodegradation by NJUST30 was firstly proposed.

GRAPHICAL ABSTRACT



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ABSTRACT

In this study, a pyridine-degrading strain namely NJUST30 was isolated from aerobic granules capable of degrading pyridine and was identified as *Paracoccus* sp. At incubation temperature of 30 °C, initial pH of 7.0 and initial pyridine concentration of 500 mg L^{-1}, complete pyridine removal could be achieved within incubation period as short as 54 h. Pyridine biodegradation profile by NJUST30 could be well fitted by the first-order decay kinetic model, suggesting the recalcitrant nature of pyridine and the inhibitory effect of pyridine towards NJUST30. Major intermediates during pyridine biodegradation by NJUST30, including 2,4-dihydroxy-2H-pyridine-3-one, 2-carbonyl-succinic acid, 1,2-dihydro-pyridin-2-ol, piperidin-2-ol and 4-formylamino-butyric acid were identified through HPLC/MS. Based on these intermediates, a distinct pyridine biodegradation pathway via hydroxylation, pyridine ring cleavage, carbonylation and carboxylation was revealed for the first time.

1. Introduction

Nitrogenous heterocyclic compounds (NHCs) have been being discharged into the ecosystem, causing serious environmental pollution [1]. Among various NHCs, pyridine has even more potential application in agrochemical and pharmaceutical industries because it is an excellent organic solvent or intermediate for the manufacture of dyes, explosives, pesticides, herbicides and drugs [2–4]. Thus, pyridine inevitably finds its way into the environment through the discharge of wastewater from the related industries if these wastewaters contaminated by pyridine were not treated properly and effectively [3,5]. Pyridine is classified as a hazardous substance and listed as priority pollutants by The United States Environmental Protection Agency due to its toxic, teratogenic and carcinogenic properties [6,7].

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Consequently, there is an urgent need to exploit an efficient and economical treatment method to remove pyridine from wastewater.

Various technologies have been employed for pyridine removal from contaminated wastewater, such as advanced oxidation processes [3,8,9], physical adsorption [10,11] and membrane separation [12], etc. The application of various physicochemical methods is limited due to high energy consumption, high cost and serious secondary pollution [9,13]. However, biological treatment has turned out to be a promising and economical approach to deal with various recalcitrant pollutants such as pyridine, because of its merits such as low cost and environmental friendliness [14–16]. Nevertheless, due to the high toxicity and resistance of pyridine, the application of biological method for pyridine removal is very confined [17].

For the bioremediation of sites contaminated by pyridine, previous investigation was mainly focused on the isolation of pyridine-degrading microorganisms, including *Bacillus* [18], *Pseudomonas* [19], *Pseudonocardia* [20], *Streptomyces* [21], *Arthrobacter* [22], *Alcaligenes* [23], *Nocardiodes* [24], *Shinella* [17], *Shewanella* [1], *Rhodococcus* [6] and *Paracoccus* [16], et al. For the remediation of wastewater contaminated by pyridine, bioaugmentation strategy through the inoculation of these functional species into various biological systems such as activated sludge tank has been developed [16,25–27]. However, more attention should be paid onto pyridine biodegradation mechanism, in order to guide the engineer to achieve an effective bioaugmentation strategy.

This study addressed a detailed investigation on pyridine biodegradation by a newly isolated pyridine-degrading strain, namely *Paracoccus* sp. NJUST30. The effect of various abiotic factors on pyridine biodegradation performance was investigated. Major pyridine biodegradation intermediates were identified. A new degradation pathway for pyridine biodegradation by *Paracoccus* sp. NJUST30 was proposed for the first time.

2. Materials and methods

2.1. Growth medium

Enrichment and isolation of the strains capable of degrading pyridine was performed in liquid mineral salts medium (MSM) containing phosphate buffer (7 mmol L^{-1}, pH = 7.0), $MgSO_4$ (0.1 g/L), $CaCl_2$ (0.05 g/L), and trace element solution SL-4 (1 mL L^{-1}) according to our previous study [28]. In order to keep pH constant, KH_2PO_4 and Na_2HPO_4 $12H_2O$ were used as the phosphate buffer in MSM. Pyridine was added as the sole carbon and nitrogen sources at desired concentration. Before use, all the media were autoclaved at 121 °C for 30 min. Store culture was maintained on LB plates containing 500 mg L^{-1} pyridine and was stored at 4 °C for further study.

2.2. Enrichment, isolation and identification of pyridine-degrading strain

Pyridine-degrading strains were isolated from aerobic granules collected from a sequencing batch reactor (SBR), which was used to treat wastewater containing pyridine. Several aerobic granules were ground and then 5 g grinding sample was added into 100 mL liquid MSM supplemented with 200 mg L^{-1} pyridine in 250 mL Erlenmeyer flasks. Subsequently, these flasks were incubated on a rotary shaker at 30 °C and 180 rpm for the enrichment of pyridine-degrading strains. Three days later, 5 mL of the inoculum was transferred into fresh MSM with pyridine concentration increased gradually to 300 mg L^{-1} for another round enrichment. After then, pyridine concentration in fresh MSM was increased step by step and finally increased to 500 mg L^{-1}. After successive transfer for three times, the diluted suspensions (10^{-1}–10^{-10}) were spread onto the pre-sterilized LB agar plates containing 500 mg L^{-1} pyridine. The morphologically distinct colonies were selected, purified and preserved on the MSM agar plates and MSM agar slant, respectively. All of the isolates obtained were inoculated into 100 mL pre-sterilized liquid MSM containing 500 mg L^{-1} pyridine to explore their ability to metabolize pyridine. Finally, a bacterial strain named after NJUST30, which could utilize pyridine as the sole carbon and nitrogen sources for growth, was chosen for further study.

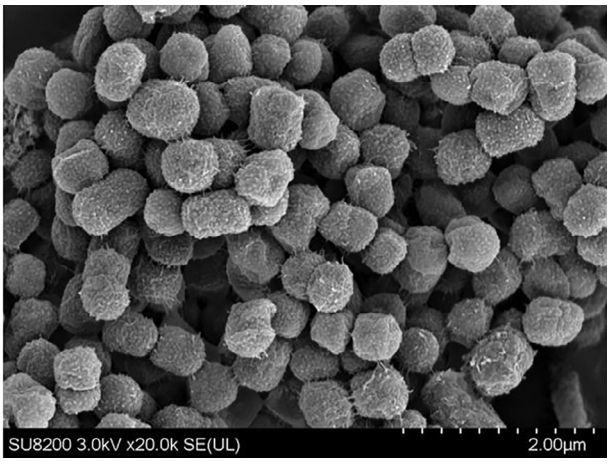

Fig. 1. Scanning electron microscopy of strain NJUST30.

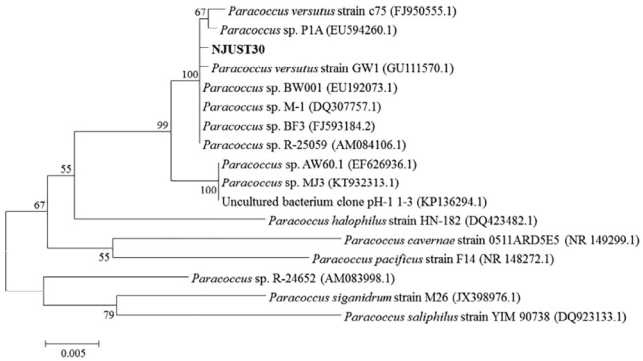

Fig. 2. Phylogenetic tree of strain NJUST30 based on the analysis of 16S rRNA gene sequence.

For the identification of the obtained pyridine-degrading strain, a comparative 16S rRNA gene sequence analysis was performed in accordance with our previous study [29]. The amplification of genes was carried out using primer 1492R (5′-GGTTACCTTGTTACGACTT-3′) and 27F (5′-AGTTTGATCMTGGCTCAG-3′). The nucleotide sequences were deposited in the GenBank database and the BLAST analysis was performed by the National Center for Biotechnology Information (NCBI) sequence database. The sequences were further analyzed using Molecular Evolutionary Genetic Analysis (MEGA, version 5.1) [30].

2.3. Biodegradation assays

Pyridine biodegradation assays by NJUST30 were carried out in MSM using a series of 250 mL Erlenmeyer flasks as batch reactors. After being cultured in liquid LB medium on a rotary shaker at 180 rpm and 30 °C for 48 h, cells of NJUST30 were harvested by centrifugation (6000*g*, 4 °C, 10 min) and washed for three times with 100 mL sterilized MSM. Finally, the inocula used for further study was prepared through the dilution of the bacterial deposit using sterilized liquid MSM, until an optical density at wavelength 600 nm (OD_{600}) reached around 2.0. In the following study, the inocula prepared were transferred into 100 mL liquid MSM at inoculum size of 5% (v/v).

To study the effect of incubation temperature on pyridine degradation, pyridine biodegradation was carried out in 100 mL MSM containing 500 mg L^{-1} pyridine at initial pH of 7.0 (7 mM phosphate buffer), with the incubation temperature varied from 25 °C to 40 °C. The effect of the initial pH value was investigated at incubation temperature