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Isolation and identification of an iopromide-degrading strain and its application in an A^2/O system

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HIGHLIGHTS

- Isolate and identify an iopromide-degrading strain I-24.
- Evaluate the effects of various factors on IOPr degradation by strain I-24.
- Examine the enzymatic activity of I-24 supplied with different carbon sources.
- Apply strain I-24 to a lab-scale waste water treatment process.
- Analyse the 16S rDNA fragments obtained from the reactor by DGGE and UPGMA cluster.

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ABSTRACT

An iopromide (IOPr)-degrading bacterium was isolated from activated sludge of a wastewater treatment plant in Shanghai. Based on its morphology, physiological-biochemical characteristics and a phylogenetic analysis of its 16S rRNA sequence, the bacterium was identified and named as *Pseudomonas* sp. *I-24*. The optimum condition for degrading IOPr was at 30 °C and pH 7.0. After 5 days, strain I-24 could degrade 30 mg/L IOPr by 99% in a basal salts medium with a 5% (V/V) inoculum and 200 mg/L starch as the primary substrate. When applied to an Anaerobic-Anoxic/Aerobic (A^2/O) process, with the coexistence of other bacteria, the strain I-24 got lower (61.3%) IOPr removal, but in two A^2/O systems (with and without I-24 inoculation), the $CODc_r$ removal were both approximately 95%. The trial dosed with strain I-24 showed better IOPr removal than the un-dosed one. I-24 sustained its abundance in the A^2/O system during the experiment.

1. Introduction

Iodinated X-ray contrast media (ICM) are body imaging video diagnostic drugs that are used for imaging soft tissues, internal organs, and blood vessels. Containing a free carboxyl group and non-ion reagents, they can be roughly divided into ionic and non-ionic reagent groups. Iopromide (IOPr) is a typical non-ionic ICM (Gartiser et al., 1996; Sacher et al., 2001). Other common ICMs are non-ionic iomeprol (IOM), iohexol and iopamidol and the ionic compound meglumine diatrizoate. IOPr is difficult to biodegrade (Kormos et al., 2010; Rahman et al., 2010). In clinical diagnosis, ICM are administered at high daily doses up to 200 g/day and excreted mainly in non-metabolised forms. IOPr and some other ICM are detected at trace levels (ng/L to μg/L) in wastewater treatment plant effluent, surface runoff and even drinking water worldwide (Gartiser et al., 1996; Shane et al., 2006; Ratola et al., 2012; Ryu et al., 2011; Yoon et al., 2010), but compared with other trace contaminants, the concentrations of ICM are much higher (POSEIDON, 2006). Although there have been few studies reporting that trace ICM in aqueous environments pose a risk to human health, their high polarity and persistence in water environments have caused concern, and ICM have been regarded as potential carcinogens (Krause et al., 2009; Seitz et al., 2008). Because IOPr could not be effectively removed by ozonation, photo-catalytic or other advanced oxidation processes (Krause et al., 2009; Seitz et al., 2008; Li et al., 2012), biodegradation is an important transformation pathway (Kormos et al., 2010). Some research found that the nitrifying bacteria (Batt et al., 2006) or anaerobes (Lecouturier et al., 2008) could degrade ICM. The objective of this study was to identify an IOPr-degrading bacterial strain isolated from return activated sludge of a secondary sedimentation tank in an urban sewage treatment plant and to investigate its IOPr degradation characteristics in an A^2/O system.

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2. Methods

2.1. Materials

IOPr solution (99.5%, Dr. Ehrenstorfer Corporation, Germany), IOPr injection (Ultravist 370, 370 mg I/mL, Luoheshi Bayer Pharmaceutical Co., Ltd.), and acetonitrile (Chromatographic purity, Honeywell Burdick & Jackson Corporation) were obtained from the indicated manufacturers, and other chemicals were purchased from SCR (Sinopharm Chemical Reagent Co., Ltd.). All chemicals used were of analytical grade. Solutions used in this experiment were prepared using ultra-pure water that was produced using a Milli-Q device (Millipore, USA).

Isolated strains were observed using a JSM-5600LV scanning electronic microscope (Japan). IOPr was analysed using an Agilent 1100-HPLC (HITACHI Corporation) and a Chromatogram data workstation (Eclipse XDB C18-Analysis column (4.6 × 250 mm, 5 μm)). A Constant-Temperature Incubator (Shanghai DuKe automation equipment Co., Ltd.) was used for strain incubation.

norganic salts culture medium (ISC) (K_2HPO_4 4.35 g/L, KH_2PO_4 1.7 g/L, NH_4C1 2.1 g/L, $CaC1_2{\cdot}2H_2O$ 0.03 g/L, $FeS0_4{\cdot}7H_2O$ 0.01 g/L, $MgSO_4$ 0.2 g/L, $MnSO_4$ 0.05 g/L, pH 6.8–7.0), enrichment culture medium (EC) (beef extract 3.0 g/L, Peptone l0.0 g/L, NaCl 5.0 g/L, IOPr 30 mg/L, pH 7.0–7.2) and solid culture medium (18 g/L agar powder added to the enrichment culture medium) were used in this experiment.

2.2. Isolation and identification of IOPr-degrading bacteria

Sludge samples were collected from the return sludge of the secondary sedimentation tank in the urban sewage treatment plant in Shanghai Songjiang. Two grams of each sludge sample were added to 100 mL ISC medium.

The samples were cultured using the gradient method for two months at 30 °C. The isolates were cultured away from light on a rotary shaker at 160 r/min, and at different times, 5% of the bacteria were removed for incubation in IOPr concentration gradients of 10, 50, 80, 100, 130 and 150 mg/L. The culture medium was diluted by the gradient from 10^{-1} to $\sim10^{-10}$ and streaked onto an EC medium plate. According to the colour, shape and size of the colonies, single colonies were inoculated into EC and incubated at 30 °C for 48 h. Subsequently, the bacteria were transferred to 100 mL ISC medium with 30 mg/L IOPr. The residual IOPr in the inorganic medium was measured after culture at 30 °C for 6 days. The isolated strains were tested for IOPr removal efficiency as described above; the strain whose IOPr removal was the highest was inoculated onto an EC medium slant and preserved in a refrigerator at 4 °C.

Bacteria from the EC slant were inoculated into an Erlenmeyer flask with EC medium and incubated at 30 °C for 24 h. The culture was then used for DNA extraction (Hiraishi, 1992). To reduce the possible bias caused by PCR amplification, rDNA was amplified in triplicate tubes, and these were combined for the subsequent cloning step. The rDNA fragments were cloned into plasmids using the TA cloning kit. Clonal rDNAs were prepared from randomly selected recombinants and used as templates for sequencing, which was conducted by the Shanghai Invitrogen Biotechnology Co., Ltd., China. The sequences obtained were aligned using MEGA 5.1 software, and an evolutionary distance tree was created based on the neighbour-joining method according to reference sequences from GenBank. The nucleotide sequences obtained were filed in GenBank under accession number JN226398.

2.3. Electron microscopic observation

The isolated IOPr degradation bacteria were cultured in EC medium to the logarithmic growth period. After centrifugal collection, the bacteria were treated by gradient dehydration and freeze-drying processing. Afterwards, the bacteria were observed using a JSM-5600 LV scanning electron microscope at the Donghua University Testing Centre.

2.4. General identification of the isolated strain

The morphological and biochemical characteristics of the strain were tested according to methods reported in the literature (Shen and Chen, 2007; Dong and Cai, 2001). The cell morphology of the strains was evaluated by an optical microscope combined with a digital camera (OLYMPUS CX31). Briefly, the following biochemical tests were performed: oxidase, starch hydrolysis, hydrogen sulphide production, aerobic tests, urease, glucose production, methyl red, V-P gram staining, glucose fermentation, indole, gelatin and simon salt of citric acid tests.

2.5. Degradation analysis of IOPr degradation bacterium I-24

I-24 cells were transferred from the slant culture medium to a 100-mL flask containing inorganic medium, 30 mg/L IOPr and 100 mg/L glucose. The optimum dosage of I-24 was 5%, and this dosage was used during the whole experiment. The flask was incubated on a rotary shaker at 160 rpm and 30 °C for 5 days. The solution containing the IOPr degradation bacterium I-24 was used for the following IOPr degradation test. This solution was called the seed solution.

2.5.1. IOPr measurement

Samples were filtered through a 0.45-μm porous nylon membrane filter (Quandao, Shanghai) and then processed for high performance liquid chromatography (HPLC) measurements. For each measurement, the total run time was 20 min, the flow rate was 0.4 mL/min, and the sample volume was 20 μL. The column (Eclipse XDB C18, 4. 6 × 250 mm, 5 μm) temperature was 25 °C.

2.5.2. Parameters affecting IOPr degradation by strain I-24

The pH of the 100 mL seed solution was adjusted to 7.0. As described above, 5% inoculation amounts were used for the IOPr degradation experiments. Each solution was incubated on a rotary shaker at 160 rpm and 30 °C for 5 days, after which the residual IOPr (or iomeprol) was measured. Experiments were carried out to assess temperature and pH effects on IOPr degradation.

An experiment to assess external carbon source effects was carried out as follows: I-24 was added to 100 mL inorganic salts culture medium containing 30 mg/L IOPr and 10 mg of 4 different types of carbon source (starch, yeast, peptone and glucose) to test the IOPr removal efficiency by the method described above. After 5 days, IOPr degradation were measured.

2.6. Enzymatic activity (EA) analysis

Enzyme which has the IOPr degradation ability is named IOPr enzyme and is called enzyme briefly in the following part of this paper. The enzyme was extracted using the following procedure: 40 mL of the ISC medium containing 5% strain I-24 was centrifuged at 8000 rpm at 4 °C (Anke GL-20G-II) for 30 min. The supernatant was decanted and replaced with 4 mL pH 7.1 Tris–HCl buffer solution to wash the pellet (microorganisms), and the solution was again centrifuged at 8000 rpm at 4 °C, The washing step was repeated using 20 mL Tris–HCl buffer solution. The mixed solution was placed into an ultrasonic cell crusher (Xinzhi, China) to destroy the cells using 300 W input power and cycles of 2 s crushing followed by 1 s rest for 30 min. The resulting solution was called the crude enzymatic solution (CES). Subsequently, 1 mL of a 15 mg/L IOPr solution was added to 1 mL CES and 3 mL Tris–HCl