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Biological characteristics and oxidation mechanism of a new manganese-oxidizing bacteria FM-2

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Abstract. A new manganese-oxidizing strain FM-2 was screened out from biological activated carbon (BAC) filter column and was identified as *Citrobacter freundii*. The results of the systematic study on this species are as follows: At 27℃, the optimum pH for *Citrobacter* sp. FM-2 to remove manganese was 7.0-8.0.The best removal rate of manganese under 27℃, pH 7.0 by FM-2 was reached at 4 d, being 76.2%; Compared with adsorption, biological oxidation played a dominant role in this removing process. Almost 75.7% of manganese was oxidized into oxides by *Citrobacter* sp and there were some particular oxides analogs generated on the bacterial surface; A 296bp DNA fragment amplified from *Citrobacter* sp. FM-2 revealed that this species has multicopper oxidase genes. Meanwhile, the phylogenetic tree indicated that compared with other related species, *Citrobacter* sp. FM-2 has its own evolutional independence.

Key words: manganese-oxidizing strain, *Citrobacter freundii,* multicopper oxidase, evolutional independence

1. Introduction

The contamination of manganese in industrial water, drinking water and groundwater has been a growing problem in many parts of the world recently [1]. At neutral pH, oxidation of soluble Mn(II) is very slow, especially in the absence of catalysts or photochemical enhancement and the conventional treatments have a low removal rate on dissolved Mn (II). Researches have shown good manganese removal performance by biological removal methods [2], which provides a new way to make up for the shortcomings of the traditional methods.

Researches on biological removal have been focused on the mechanism of bacterial manganese oxidation. The genes involved in Mn^{2+} oxidation have been identified to be part of gene operons, such as cumA in *Ps. Putida,* mofA in *Ldiscophora*, mnxG in *Bacillus* and moxA in *Pedomicrobium* sp.ACM 3067 [3]-[5]. The protein encoded by those operons showed significant homology to multicopper oxidases (MCOs), which are a class of copper proteins and have a wide range of substrates, including Fe (II), Mn (II) and phenolic compounds [6].

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However, the MCOs are only known to engage in one-electron transfers from substrate to $O_2$and the specific mechanism and complexity of those reactions are still unclear. What's more, although a wide variety of bacteria are known to catalyze the degradation of manganese, such as *Leptothrix, Crenotrix, Hyphomicrobium, Siderocapsa, Metallogenium, Pedomicrobium, Gallionella, Roseobacter* and other genera[7][9], only four species (*Pseudomonas, Bacillus, Leptothrix and Pedomicrobium*) of them were acknowledged as the model bacteria. Therefore, it is necessary to broaden the manganese-oxidizing species and explore the mechanism of manganese oxide production ulteriorly.

In our previous work, a new manganese-oxidizing strain FM-2 was screened out from the biological activated carbon (BAC) filter column and was identified as *Citrobacter freundii*. In this paper, the influencing factors of culture time, culture temperature, and pH value on its removal efficiency were studied in detail. We explore the mechanism of microbial manganese oxidation by analyzing manganese oxidation properties of FM-2 and the sequence of its oxidation genes. And its evolutionary relationships with other related species were also investigated.

2. Materials and methods

2.1. Bacteria, apparatus and medium

FM-2 was screened out from the biological activated carbon (BAC) filter column; The main apparatus used were Tanon-1600 Gel Image Analysis System (Tanon Science & Technology Co.,Ltd), and TS 5136MM scanning electron microscope (TESCAN); The main culture media used in this study were JFM medium as described by Franciskovic-Bilinski et al.[1] and PYCM medium [10].

2.2. Measurement of removal rate

The concentrations of manganese were measured by phenanthroline spectrophotometry and spectrophotometric oxidation of potassium periodate, respectively [11].After bacterium was cultivated in PYCM culture media at 27℃, 2 eppendorf-tubes of culture media were centrifuged at 12 000 rpm for 10 min. Then the supernatant was collected. The control sample was PYCM culture media with no bacteria. The removal rate was calculated by the following formula:

$$\text{Removal rate (\%)} = \frac{A - B}{A} \times 100\% \quad (1)$$

Where A signifies the manganese concentration in the control group; and B is that in the bacteria-treated sample.

2.3. Biological oxidation mechanism of FM-2

2.3.1. Manganese oxidation properties of FM-2

Citrobacter sp. FM-2 was inoculated into the prepared PYCM medium and cultured at 27 ℃. Several parameters were measured by the methods described in reference [12], including the remaining manganese, the adsorbed manganese and the original manganese concentrations. The PYCM medium without inoculated bacteria was taken as a blank control.