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Screening and Characterization of the Bacteria which can Produce Flocculants and Degrade of Phenol

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Abstract: The strain A5 with high flocculating activity was isolated from activated sludge of wastewater treatment system. The flocculation efficiency is more than 80%. Morphological characteristics, physiological and biochemical tests and 16S rDNA sequence alignment identify of the strains were studied. Analyses conclude that A5 belongs to Sphingobacterium. It can be grown in mineral medium containing phenol, the results show that A5 have double effects as producing flocculants and degrading of phenol.

Keyword: Flocculation; Screening; *Sphingobacterium*; Degradation of phenol

1. Introduction

Microbial flocculant (MBF) is a sort of biological molecular polymer secreted by microorganisms, that can flocculate the suspension particles in the water, the main ingredients include RNA, DNA, mucopolysaccharides, lipids and glycoproteins [1]. Because of its non-toxic, pollution-free, biodegradable and other advantages[9], it has been widely used in food and fermentation industry, treatment of drinking water and wastewater, industrial downstream process and other fields[8]. It has become a hot research direction no matter at home or abroad. It has aroused extensive attention from domestic and foreign researchers[10].

MBF treats wastewater [2, 3, 4] had been reported at home and abroad's laboratory, but widespread use of microbial flocculant are few reported [5]. Kurane etc. [2] isolated *Rhodococcus erythropolis*, it was designated as flocculant NOC-1. It was found that NOC-1 was best bioflocculant at present[6, 7].

The study screened out a flocculant producing bacteria strainfrom the activated sludge identifying this strain belongs to the *genus Sphingomonas*. Since *sphingosine genus* species have a very wide metabolism to aromatic compounds, in this study, culturing strains A5 phenol as the sole carbon source in the inorganic culture medium for the development and application of flocculation and degradation of phenol complex functions strains of the foundation.

2. Materials and Methods

2.1 Bacteria source and Medium

For the separation of activated sludge bacteria taken from Hohhot, Inner Mongolia Xinxin board sewage treatment plant's aeration tank.

Beef extract peptone medium: beef extract 3.00g, peptone 10.00g, NaCl 5.00g, pH 7.0~7.5, water is added to 1L, Sterilizing 20min in 120℃. The solid medium supplement with 1.5 to 2% agar.

Enrichment medium: Glucose 55.000g, Urea 1.000g, Potassium dihydrogen phosphate 2.500g, Disodium hydrogen phosphate 0.500g, Magnesium sulphate heptahydrate 1.000g, Ferrous sulfate heptahydrate 0.100g, Yeast extract 0.500g, water is added to 1L, pH 7.0~7.5, Sterilizing 30min in 115℃. The solid medium supplement with 1.5 to 2% agar.

Fermentation medium: Glucose 20.00g, Yeast extract 0.500g, Ammonium sulfate 0.20g, Urea 0.50g, Dipotassium hydrogen phosphate 5.00g, Potassium dihydrogen phosphate 2.00g, Crystalline magnesium

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0.20g, NaCl 0.10g, water is added to 1L, pH 7.5~8.5, Sterilizing 30min in 115℃.

Inorganic salt medium: NaCl 0.10g, $CaCl_2$ 0.05g, Dipotassium hydrogen phosphate 0.05g, Potassium dihydrogen phosphate 0.05g, $FeCl_3$ 0.002g, Ammonium sulfate 0.165g, Crystalline magnesium 0.10g, water is added to 100mL, pH 7.0~7.2. Take 5mL culture medium to tubes tightly stopper.

Inorganic salts culture medium containing phenol: NaCl 0.10g, $CaCl_2$ 0.05g, Dipotassium hydrogen phosphate 0.05g, Potassium dihydrogen phosphate 0.05g, $FeCl_3$ 0.002g, Ammonium sulfate 0.165g, Crystalline magnesium 0.10g, Add appropriate amount phenol liquor, water is added to 100mL, pH 7.0~7.2. Take 5mL culture medium to tubes tightly stopper.

2.2 Screening of the Strains

Separation and purification of bacteria: In this study, the plate dilution coating method and the flat crossed separation method combined for Screening of the Strains. Take sewage collected add to the culture medium, 160r/min shaker 48h in 30℃. Take 1ml after 48h enrichment bacterial liquid culture diluted for different concentrations to coat on the enrichment medium plates, 30 ℃ cultured 48h. After the medium plate waiting to dilute and coat grows colonies, according to the different of colony color and morphological characteristics, picking representative individual colonies, streaking repeatedly to observe colony characteristics and using Gram staining to observe whether purebred strains, after to be determined a single bacterium, numbered, slant tube and liquid glycerol.

Flocculant producing bacteria screening method: Screening of microorganisms producing flocculation method is divided into primary and secondary screening. Weigh 5g kaolin beaker, 1000mL water is added to prepare a kaolin suspension.

Primary screening: Accessing screening strain to 5mL sterile fermentation medium, 160r/min Shaker 48h in 30℃, take 1mL bacteria add to the colorimetric tube containing kaolin suspension 20mL, then add 1mL 1% $CaCl_2$, set the volume to 25mL, stand for 5 minutes, visual inspect the effect of the various strains of flocculation, to make the kaolin suspension big block floc settlement rate and clarified supernatant after flocculation strains recorded.

Rescreening: Strain screening records will be preserved flocculation activity and continue to do rescreening. Rescreening culture conditions with screening, after 48h, taking 1mL fermentation broth flocculation activity was measured using a spectrophotometer.

The activity of flocculation' measuring method: Weighing kaolin suspension 20mL add to 25mL colorimetric tube, adding 1mL 1% $CaCl_2$, 48h fermentation broth 1mL, adding distilled water volume to 25mL, fully reversed mix and after let stand 20 minutes, measuring the absorbance at 550nm. Taking 1mL the culture medium to 24mL distilled water to zero adjustment, doing blank with don't add the culture medium' kaolin suspensions. Flocculating rate indicates the level of flocculation strain's activity. Flocculating rate is calculated as:

Flocculating rate= (A-B)/A ×100%

In the formula: A- Control supernatant absorbance; B- Absorbance of the supernatant added Flocculating bacteria.

2.3 Identification of bacteria

Microscopic observation：

After flocculation strains screened repeatedly crossed purified, using an optical microscope Gram staining.

Scanning electron microscopic observation:

Taking flocculent single bacterium screened out to observe by microscope to ensure pure bacteria, and then using scanning electron microscope to observe the form of bacteria.

rDNA sequencing and sequence alignment:

Taking the most active flocculation screened out A5 to perform 16S rDNA sequencing, and 16S rDNA sequences obtained using the BLAST to perform sequence comparison for species classification in NCBI.

2.4 Determination of phenol ability to degrade

Part of the bacteria *Bacillus sphingosine* has a strong ability to degrade phenol. In this study, to detect

strains screened have the capable of degrading phenol, accessing A5 strains 5mL sterilized medium, 30 ℃, 160r/min shaking culture 48h. Taking inorganic salts medium don't inoculate to do blank, measuring the absorbance of phenol containing 100mg/L and medium containing 200mg/L of phenol strains.

3. Results

3.1 Strain screening

After screening get 35 strains have flocculation activity. After rescreening get high flocculation activity of strains 4, flocculation activity can up to 83.87%.

3.2 Identification of bacteria

3.2.1 Morphological characteristics

(1) Individual form of bacteria

A5 strains are Gram-negative bacteria, oval shape.

(2) Colony morphology

Colonies are round, yellow, moist, neat edge, raised, shiny.

rDNA sequencing and sequence alignment:

Taking the most active flocculation screened out A5 to perform 16S rDNA sequencing, sequence length is 1439bp. The 16S rDNA sequences to use the BLAST for sequence comparison in NCBI, and knowing that homology with *mesophilic sphingosine* genus 16S rDNA sequence is 98%.

Strain A5 phylogenetic tree's construction: After the 16S rDNA sequences measured compare to the homologous sequence, transferring of strain 16S rDNA sequence the highest homology with the strains, choosing *E. coli* as outgroup selection, using Jukes-Cantor model in MEGA software to compute evolutionary distance is 0.098, can use distance method of Neighbor-Joining (NJ) to construct unrooted phylogenetic tree, as shown in Figure 1.

Identification conclusion：By 16S rDNA sequence similarity analysis, identification A5 flocculant-producing bacteria belonging to the *genus sphingosine* (*Sphingobacterium*) and with *sphingosine mesophilic* bacterium (*Sphingobacterium thalpophilum* strain DSM 11723) has closest relationship.

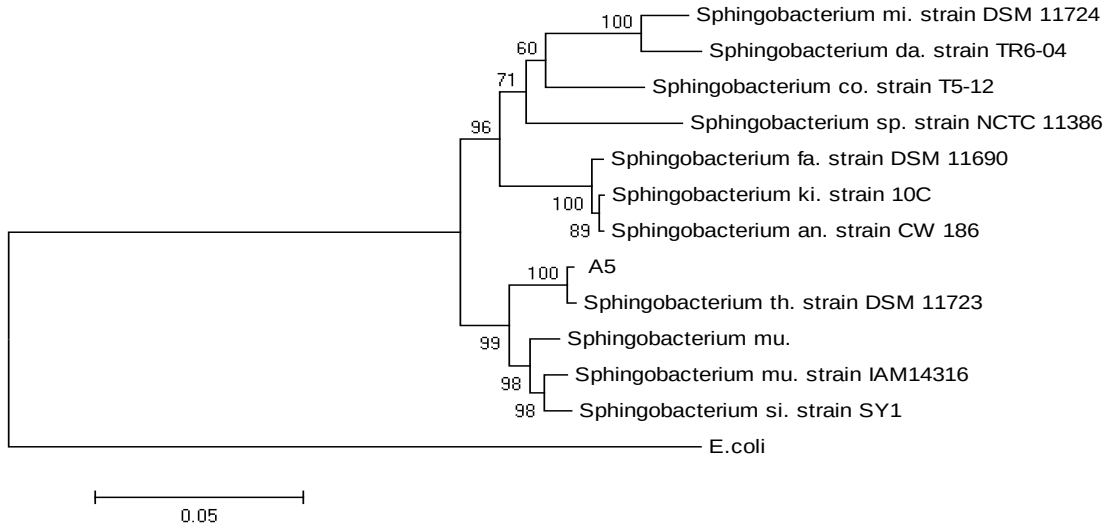

Figure 1 16S rDNA NJ phylogenetic tree for strain A5

3.3 A5 strain determination of phenol degrading capability

After culturing 48 hours in inorganic salt medium containing phenol, determining the OD_{600} of the strain A5, the result is shown below in table 1:

Table 1 OD_{600} of the strain A5 in inorganic salt medium containing phenol

100mg/L		200mg/L	
without bacteria	plus bacteria	without bacteria	plus bacteria

0.244	0.410	0.244	0.394

As can be seen from the table, the strain A5 can grow in inorganic salts medium containing phenol as the sole carbon source, showing that it has the ability to degrade phenol.

4. Discussion

The study on microbial flocculant has more than 20 years, there are few researches in the area which have double effects as producing flocculants and degrading of phenol. In this study, the flocculant-producing strains A5 was isolated from activated sludge samples, flocculation of kaolin suspension is higher than 80%, and to phenol has a certain tolerance, so this is a strain that can produce flocculants and degrading phenol. Next we will focus on improving the flocculation activity of strains A5, exploring flocculation and degradation mechanism and other aspects. With further research, flocculation and degradation complex functions with flocculant-producing bacteria will have broad research and application prospects.

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