

## Characterization of *Pseudomonas* sp. BCNU 106, 154 and 171 tolerant to organic solvents

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### Abstract

BCNU 106, 154 and 171 was isolated in the areas contaminated by high concentrations of organic solvents. These strains had the tolerance to almost all kinds of organic solvents used in this study. Especially, it was interested that BCNU 106 and 171 had the tolerance to *meta*-, *para*- and *ortho*-xylene. And 3 strains had high MIC (minimal inhibition concentrations).

### Introduction

Alkylbenzenes having toxic and carcinogenic characteristics contaminate surroundings of petrochemistry complexes with high concentration. It is needed to treat these compounds with microorganisms having ability to degrade organic solvents. Especially it is intensively reported that benzene, toluene, ethylbenzene and xylene (BTEX) are degraded by aerobic microorganisms (Chang *et al.* 1992, Davis *et al.* 1968, Gibson *et al.* 1974, Jeong *et al.* 1998, Lee *et al.* 1994, Worsey *et al.* 1975), and also there are many reports about the degradation of *m*- and *p*-xylene by microorganisms. However it is reported microorganisms degrading *o*-xylene are few. Microorganisms degrading *m*- and *p*-xylene can not use *o*-xylene as a sole carbon source, and the opposite fact is same. Even though these microorganisms has the tolerance to organic solvents, several factors like heavy metals, antibiotics *etc.* affect the degrading abilities of microorganisms.

In this study, we isolated the strains having tolerance to virulent organic solvents and degrading not only *m*- and *p*-xylene but also *o*-xylene simultaneously and investigated the characteristics of degrading organic solvents.

## Materials and Methods

### Culture media

Used medium to investigate the tolerance to several organic solvents was LB medium, and the components were tryptone (DIFCO) 10.0g, NaCl (Junsei Chemical Co., Ltd.) 10.0g and yeast extract (DIFCO) 5.0g in 1 l distilled water. Mineral salts (MS) medium was used to certify whether organic solvents were used or not as a sole carbon source.

### Analysis of BTEX degradation

To analyse the amounts of BTEX, 150 $\mu$ l from headspace of ampule with 250 $\mu$ l gas-tight syringe was injected to gas-chromatography (dsChrom, Donam Systems Inc. Korea). The amount of BTEX in the sample was detected by flame ionization detector (FID) connected with DB<sup>TM-1</sup> capillary column (30 m  $\times$  0.32 mm I.D., 3.0 $\mu$ m). Conditions of analysis were as follows; 250 $^{\circ}$ C of injector temperature, 300 $^{\circ}$ C of detector temperature, programs of column temperature were set with 5 min at 35 $^{\circ}$ C and risen with 10 $^{\circ}$ C/min from 35 $^{\circ}$ C to 245 $^{\circ}$ C, and maintained 5 min at 245 $^{\circ}$ C. Final temperature was maintained 3 min at 250 $^{\circ}$ C and flow rate of N<sub>2</sub> gas was controled with 30.0ml/min.

### Tolerance on organic solvents

Several kinds of organic solvents; toluene, *m*-xylene, *o*-xylene, *p*-xylene, *n*-hexane, phenol, heptanol, cyclohexane, propylbenzene and cresol isomers (*m*-, *o*- and *p*-cresol), were used for the test of tolerance to organic solvents. Used medium was LB agar medium. The organic solvents were poured on each agar plate for the test of tolerance, then the growth on agar plate soaked in organic solvents was checked with colony forming.

### Degrading ability on organic solvents

Organic solvents described above were added for a sole carbon source to mineral salts agar plate. Precultured BCNU 106 was streaked on MS agar plate in which durham tube containing organic solvents was placed on the lid of petri dish. Colony forming was the indicator of tolerance to organic solvents. In the case of cresol, MS broth was used to verify the tolerance and the degrading ability because cresol denaturalize the agar.

### Tolerance on antibiotics

Antibiotics used in this study were ampicillin, kanamycin, chloramphenicol, oxytetracycline, amoxicillin, cefatrizin, cephradine, lincomycin, tobramycin and ribostamycin.

### Tolerance on heavy metals

Heavy metals used in this study were nickel, copper, lead, cobalt, zinc, ferrus, barium, mercury, cadmium and silver.

## Results

### Tolerance on organic solvents

To investigate the tolerance on organic solvents, 11~14 kinds of organic solvents were selected. It is revealed that 3 strains had the tolerance on almost organic solvents used in this study.

### Degrading ability on organic solvents

To test the degrading ability on the above 11~14 kinds of organic solvents and *m*-, *o*- and *p*-cresol especially, MS medium was used. It is interesting that BCNU 106 and 171 degraded *m*-, *p*-xylene and *o*-xylene simultaneously.

### Tolerance on antibiotics

These 3 strains had high tolerances on antibiotics, especially in cephradine; MIC was 40000, in lincomycin; 15000~20000, ampicillin; 4000~5000 $\mu$ g/ml.

### Tolerance on heavy metals (units: mM)

Heavy metals \ Strains	BCNU 106	BCNU 154	BCNU 171
NiCl <sub>2</sub> · 6H <sub>2</sub> O	10	11	11
CuSO <sub>4</sub>	5	4	4
Pb(CH <sub>3</sub> CO <sub>2</sub> ) <sub>2</sub> · 3H <sub>2</sub> O	5	4	5
Co(NO <sub>3</sub> ) <sub>2</sub> · 6H <sub>2</sub> O	1	5	6
ZnSO <sub>4</sub> · H <sub>2</sub> O	7	8	5
FeSO <sub>4</sub> · 7H <sub>2</sub> O	*	*	*
BaCl <sub>2</sub> · 2H <sub>2</sub> O	50	25	20
HgCl <sub>2</sub>	0.05	0.05	0.05
CdCl <sub>2</sub> · 2.5H <sub>2</sub> O	3	2	2
AgNO <sub>3</sub>	0.01	0.01	0.05

## 요약

유기용매 내성 균주를 분리하기 위해서 석유화학 공업단지 부근의 폐수 및 토양을 채취하여 각종 유기용매에 적응시킨 후 순수 분리를 통하여 내성을 가진 균주를 분리하였으며 그 중에서 가장 내성이 강한 균주 BCNU 106, 154 and 171을 분리하였다. 이 균주를 이용하여 유기용

매 내성 및 분해능 검사를 하였다. 그 결과 이 세 균주는 본 실험에 사용한 유기용매 대부분에 대해서 내성을 나타내었으며, 또한 이를 탄소원으로도 이용하였다. 특히 BCNU 106과 171은 xylene isomer인 *meta-*, *para*-xylene을 탄소원으로 이용할 뿐아니라 특히 독성이 강한 것으로 알려져 있는 *ortho*-xylene도 동시에 분해하는 것으로 알 수 있었다. 또한 항생제와 중금속에 대해서도 강한 내성을 나타내었는데, 이는 유기용매 내성 기작과 밀접한 관련이 있는 것으로 판단한다.

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