

**Keywords:** PlasmidnahRgene; Phyllosphere bacteria; Phenanthrene; Naphthalene; Alcaligenes sp. 11SO

### Introduction

Polyaromatic hydrocarbon (PAH) pollution is a highly concerned environmental problem in the world. Naphthalene and phenanthrene are the highly abundant PAHs in the ambient air due to the vehicular emission, industrial processes and oil refining processes. Naphthalene is

### **Selection of efficient PAH degrading bacteria**

The best PAH degrading bacterial strains were selected based on the results obtained from the colorimetric and HPLC methods indicated below.

#### **Colorimetric assay**

Each bacterial strain was inoculated into Bacto Bushnell-Haas broth incorporated with PAH compound (1%v/v) and Methylene blue (2%v/v), the redox indicator and incubated at room temperature (28°C-30°C) with constant shaking at 180 rev/min, for 14 days with a control without bacterial inoculation. From broth culture 5 ml sample was centrifuged at 6000 rev/min for five minutes. The recovered supernatant was assayed spectrophotometrically by measuring absorbance at 609 nm for the residual hydrocarbon. Six replicates were done for each bacterial strain and PAH degradation percentage was determined using the following equation [16].

$$\text{Percentage of PAH degradation} = \frac{\text{Absorbance of sample}}{\text{Absorbance of Control}} \times 100$$

#### **HPLC determination of PAH degradation**

Each bacterial strain was inoculated into Bacto Bushnell-Haas broth incorporated with PAH (phenanthrene and naphthalene) compound (100 ppm). Then it was incubated at room temperature for 6 (h in) 20 (ur) 13 (e 5 m)-ET EMC /Span <</Lang (en-US)/MCID 239 >>

ability (Figure 1). *Alcaligenes* sp. 11SO (KT356809) also had higher naphthalene (81.32%) and phenanthrene (79.24%) degradation ability compare to other bacterial strains. According to the literature [20] most of the naphthalene degraders were *Pseudomonas* sp. and *Alcaligenes* sp. were the predominant phenanthrene degraders. But the present investigation showed significantly high efficiencies of the two isolated *Alcaligenes* sp. in degrading both naphthalene and phenanthrene.

These two bacterial strains harbor an approximately 23 kb plasmid. Upon transformation of these plasmids into *Escherichia coli* JM109 strain, its PAH degradation ability was similar to that of original organism. Further, after curing of plasmids, the two *Alcaligenes* sp. lost their PAH degradation ability. These results revealed that PAH degradation ability of *Alcaligenes faecalis* and *Alcaligenes* sp. 11SO was a plasmid encoded character. Therefore, these plasmids should harbor naphthalene and phenanthrene catabolic genes *nahR* and *phn* respectively.

*nahR*

(Figure 5). us, phnG gene exists as two different alleles in these two strains enabling them to degrade phenanthrene.

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)XMLL 7 7DNHR 0 0DHGD < 3ODVPLG HQFR 2HGQG Q1HV \*XSHFDLXLQJ&DQLLOLQHDQVPLVVLEOH SODV  
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21. 3DUN -: .QRNH ./ 1RJXHUD '5 )R[ %\* &KDPEOLVV \*+ ZUDQVIRUPDWLRQ 6PLWKLHV 2 \$FRPSU  
RI WULQLWURWROXHQH E\ SXULPseudomonas RELRWLF UHGXFWDVH %HURP3  
ÀXRUH VPH Environ Microbiol 66: 4742-4750.

23. 6DLWR \$ ,ZDEXFKL 7 +DUD\DPD 6 \$ QRYHO S  
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24. =KRD 1< )XHQP D\RU 6/ :LQgenes of *Blastonia* (formerly *Pseudomonas*) sp. strain U2 encoding enzymes for gentisate catabolism. *J EDFWHULDO*

25. HYHUHXI -DHEHUOI  
DQDO\VLV SURJUDPV IRU WKH 9\$; 1XFOHLF \$FLGV