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## NOTE

# Physical Analysis of *nahQ* and *tnpA* Genes from *Pseudomonas fluorescens*

**Ja-Young Seol, Soon-Young Choi[1], and Kyung Hee Min***

*Department of Life Science, Sookmyung Women's University, Seoul 140-742, Korea*
*[1]Division of Molecular Biology, Ewha Women's University, Seoul 120-750, Korea*

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***Pseudomonas fluorescens* SM11 is a naphthalene-degrading strain whose dissimilatory genes are chromosomally encoded. We have cloned the 2.9 kb *Sal* I fragment harboring genes for the naphthalene-degradation upper pathway. The nucleotide sequences were determined to be *nahQ*, *tnpA*, and partial regions of *nahE* genes. The *nahQ* encodes a protein of 188 amino acid residues with a deduced molecular weight of 20.8 kDa. The high homology with other proteins suggests that NahQ may be an active and useful protein which gives a selective advantage to naphthalene degradation. Transposase(TnpA) encodes a polypeptide chain with a molecular mass of 41.8 kDa consisting of 376 amino acid residues. The deduced anino acid sequence of *tnpA* revealed 96% idenitity with putative transposase of *P. stutzeri* OX1. It was assumed that transposase plays an important role in the evolution of the catabolic-pathway in the regulation of *nah* expression.**

***Key words***: *Pseudomonas fluorescens, nahQ, tnpA,* transposase

The degradation of naphthalene has been studied in *Pseudomonas* species with the plasmid NAH7 from *P. putida* G7 (10, 14, 16, 19) and the NAH plasmid pWW60-1 from *P. putida* NCIB9816 (4, 11). In both strains, the dissimilatory genes are organized in two operons: *nah* operon (*nahAaAbAcAdBFCED*) coding for the enzymes involved in the conversion of naphthalene to salicylate in the naphthalene-degradation upper pathway, and *sal* operon (*nahGTHINLOMKJ*) coding for the conversion of salicylate to tricarboxylic acid cycle intermediates (pyruvate and acetyl-CoA) through the *meta*-cleavage pathway in the naphthalene-degradation lower pathway. The two operons seem to be regulated by NahR, acting as a positive regulator for both promoters, and salicylate functions as inducer (14).

*Pseudomonas fluorescens* SM11 was isolated from soil in Korea and was found to degrade naphthalene via upper and low pathways (5). Not much is known about the genetic organization of naphthalene degradation in *P. fluorescens* SM11, unlike other well-stndied *P. putida*.

In an attempt to gain further understanding of the genetic make-up of *P. fluorescens* SM11, we carried out a systematic search for the presence of *nah* genes and mobile elements. Here, we report the genetic organization for naphthalene degradation and nucleotide sequences of *nah* genes and *tnpA* gene, which is the first transposase gene described in *P. fluorescens* SM11. This study aims to elucidate the structure of the trasposase gene and *nahQ* gene.

*Pseudomonas fluorescens* SM11 was isolated from a soil sample (5) and was grown in LB medium. *E. coli* NM522 was used as the host harboring each of the recombinant plasmids. For antibiotic selections, ampicillin with 50 μg/ml as a final concentration was supplemented to LB medium (13). pUC19 was used as the cloning vector in this experiment as shown in Table 1. The plasmid was isolated by the alkali lysis method (13) or by using a kit from Qiagen. DNA cleavage and ligation were accomplished under standard conditions recommended by the supplier, Boehringer Mannheim. DNA was resolved in 0.7% or 1% agarose gel by electrophoresis, and identified by staining with ethidium bromide followed by UV irradiation. Transformation was accomplished by the calcium chloride method (13).

The chromosomal DNA of *P. fluorescens* SM11 was purified as described previously (13). The chromosomal DNA (50 μg) was partially digested with *Sal* I. DNA frag-

* To whom correspondence should be addressed.
(Tel) 82-2-710-9415; (Fax) 82-2-706-3249
(E-mail) khm9415@sookmyung.ac.kr

**Table 1.** Bacterial strains and plasmids used in this study

| Strains or plasmids | Relevant characteristics | References |
|---|---|---|
| Strains | | |
| *Pseudomonas fluorescens* SM11 | naphthalene degradation | Chung *et al.* (5) |
| *E. coli* NM522 | *supE thi* Δ(*lac-pro*AB) Δ*hsd5*(r-,m-) [F' *pro*AB *lacI*$^q$ΔZM15] | Promega |
| Plasmids | | |
| pUC19 | Ap$^r$ and multicloning sites | Novagen |
| pNA1 | pUC19 with 2.9 kb *SalI* fragment encording *nahC*, Ap$^r$ | This study |
| pNA2 | pUC19 with 2.3 kb *SalI-SphI* fragment, Ap$^r$ | This study |
| pNA3 | pUC19 with 1.6 kb *Sca-SalI* fragment, Ap$^r$ | This study |

ments, which were then isolated by agarose gel electrophoresis were ligated to the *Sal* I site of a pUC19 vector.

Unidirectional deletion mutants were constructed from the fragment in the polycloning site of the pUC19 by using an Erase-a-Base system (Promega Co., Madison, WI. USA). Both strands of the DNA segments containing the *nahQ* gene and *tnpA* gene were sequenced with an automated-sequencing apparatus (Pharmacia Biotech Inc. Piscataway, NJ. USA). The nucleotide sequences obtained were analized by using the BLAST program.

### *Molecular cloning of nahQ and tnpA genes*

*Pseudomonas fluorescens* SM11 degrades naphthalene to the tricarboxylic acid cycle (5). In order to study the structure and function of *nah* operons at the molecular level, we have cloned about 2.9 kb DNA fragment containing the *nahC* gene from the genomic DNA of the strain SM11 (unpublished data). A *SalI* fragment of genomic DNA was cloned into the polycloning site of pUC19, and a ligated fragment was introduced into *E. coli* NM522, and subjected to selection by ampicillin. The selected plasmid, pNA1 (5.7 kb), carried a 2.9 kb *SalI* fragment carrying *nahC* gene and other ORFs. The *nahC* gene was selected by spraying of a solution of 100 mM 3-methylcatechol to develop yellow colonies.

### *Genetic structure of nahQ and tnpA gene*

In order to map accurately the *nah* genes, and to determine the nucleotide sequence, subclones of the pNA1 were constructed. Then restriction enzyme digestion and electrophoresis analysis of the plasmid pNA1 were used to generate a restriction map of the cloned fragment of the *nah* genes (Fig. 1).

According to the nucleotide sequence analysis, a genetic organization of *nah* genes were found to be *nahC*, *nahQ*, *tnpA*, and *nahE* genes as shown in Fig. 1.

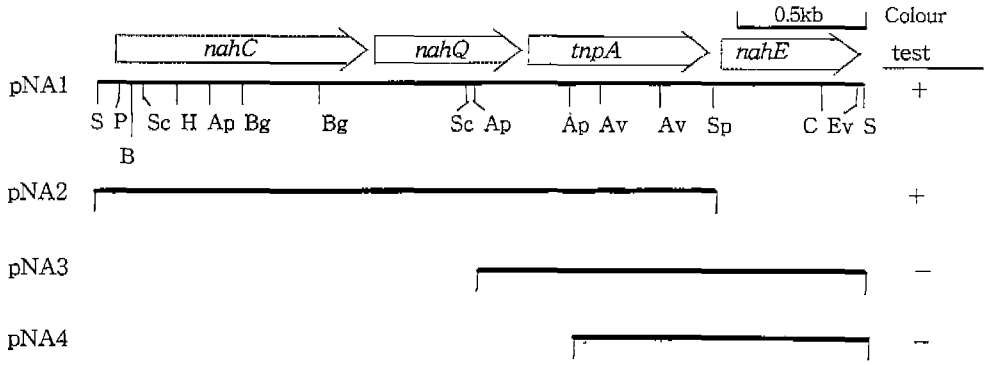


**Fig. 1.** Physical maps of pNA1 and its subclones. Abbreviations: S, *SalI*; P, *PstI*; B, *BamHI*; Sc, *ScaI*; H, *HindIII*; Ap, *ApaI*; Bg, *BglI*; Av, *AvaI*; Sp, *SphI*; C, *ClaI*; Ev, *EcoRV*.

**Table 2.** Identity and similarity to amino acid sequences of NahQ from *P. fluorescens*

| Protein | Organism | Identity | Similarity | Reference and GenBank accession number |
|---|---|---|---|---|
| PahQ | *P. putida* OUS82 | 166/176 (94%) | 167/176 (94%) | Takizawa *et al.* (17) |
| PahQ | *P. aeruginosa* PaK1 | 150/172 (87%) | 155/172 (89%) | Unpublished; D84146 |
| DoxH | *Psedomonas* sp. C18 | 150/172 (87%) | 155/172 (89%) | Denome *et al.* (6) |
| NagQ | *Ralstonia* sp. U2 | 120/164 (73%) | 142/164 (86%) | Zhou *et al.* (22) |
| NahQ | *P. putida* plamid NPL-41 | 69/92 (75%) | 72/92 (78%) | Unpublished; Y14173 |

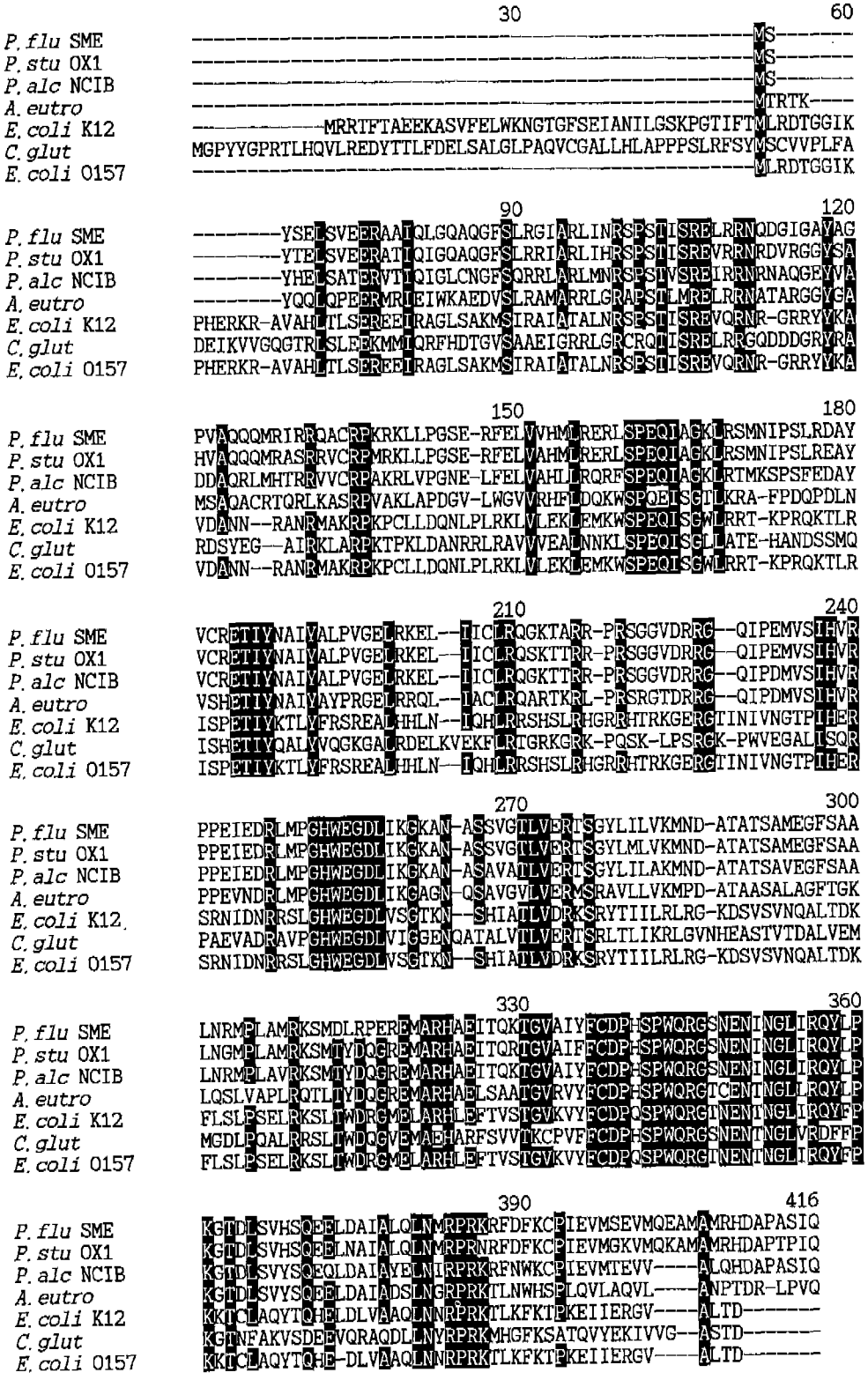


**Fig. 2.** Alignment of amino acid sequences of transposase (*tnpA*). The numbers above the sequences refer to positions in the alignment. The amino acids in positions with more than seven identical sequences are considered identical (highlighted). Accession numbers and references in order of the aligned sequences are as follows : *P. flu*; *Pseudomonas fluorescens* SM11 (AY048765), *P. stu* OX1; *P. stutzeri* OX1 (AJ005663) (1), *P. alc* NCIB; *P. alcaligenes* NCIB (U37284) (20), *A. eutro*; *Alcaligenes eutrophus* (X58441) (7), *E. coli* K12; *E. coli* K12 (AE000133) (2), C. *glut*; *Corynebacterium glutamicum* ATCC13032 (AF189147) (12), and *E. coli* O157; *E. coli* O157 : H7 (AP002559) (21).

**Table 3.** Identity and similarity to amino acid sequences of transposase

| Protein | Organism | Identity | Similarity | Reference |
|---|---|---|---|---|
| TnpA | *P. stutzeri* OX1 | 309/342 (90%) | 323/342 (94%) | Bertoni *et al.* (1) |
| TnpA | *P. alcaligenes* NCIB | 275/342 (80%) | 304/342 (88%) | Yeo and Poh (20) |
| TnpA | *A. eutrophus* | 188/325 (57%) | 235/325 (71%) | Dong *et al.* (7) |
| TnpA | *E. coli* K12 | 137/326 (42%) | 184/326 (56%) | Blattner *et al.* (2) |
| TnpA | *C. glutamicum* ATCC13032 | 133/329 (40%) | 184/329 (55%) | Quast *et al.* (12) |
| TnpA | *E. coli* O157:H7 | 135/326 (41%) | 183/326 (55%) | Yokoyama *et al.* (21) |

The nucleotide sequence of *nahQ* gene was deposited in GenBank under accession number (AY048761). The *nahQ* gene is 564 bp in length. The GC content of *nahQ* is 49%. The ORF of *nahQ* is capable of encoding a protein of 188-amino acid residues with a deduced molecular weight of 20.8 kDa.

A *tnpA* gene was found close to the naphthalene-degradation upper pathway, just downstream from *nahQ*. The nucleotide saquence of *tnpA* gene from the strain SM11 was found to be 1028 bp in length, as shown in GenBank accession number (AY048765). The GC content of *tnpA* was 71%, reflecting the GC-rich genomic characteristics of pseudomonads. Transposase encodes a polypeptide chain with a molecular mass of 41.8 kDa consisting of 376 amino acid residues.

***Amino acid homology of NahQ and transposase***

The amino acid sequence of the NahQ deduced from the nucleotide sequence was compared with six corresponding enzymes reported in other bacterial strains, as shown in Table 2. The product of the *nahQ* gene showed high sequence similarities to known corresponding bacterial enzymes.

The deduced amino acid sequence of NahQ produced by *P. fluorescens* SM11 exhibited a 94% similarity with that of PahQ by *P. putida* OUS82, GenBank accession number AB004059(17), 89% with that of PahQ from *P. aeruginosa* PaK1 (D84146) (unpublished data), and 89% with that of DoxH from *Pseudomonas* sp. C18 (M60405) (6). However, the homology of the sequences was less than 90% when compared to the corresponding NagQ of *Ralstonia* sp. U2 (AF036940) (22), and NahQ of *P. putida* plasmid NPL-41 (Y14173) (unpublished data), respectively.

The high degree of sequence conservation suggests that they may be genes encoding active and useful proteins which provide a selective advantage to naphthalene- degradation. However, the role of the *nahQ* gene product in the strains remains unknown.

The amino acid sequence of the transposase deduced from the nucleotide sequence was aligned with seven corresponding enzymes reported in other bacterial strains, as shown in Fig. 2. The product of the *tnpA* gene showed high sequence similarities to known bacterial enzymes.

The deduced amino acid sequence of the transposase produced by *P. fluorescens* SM11 exhibited a 94% similarity with that of the enzyme by *P. stutzeri* OX1 (1), 88% with that of the enzyme from *P. alcaligenes* NCIB (20), and 71% with that of the enzyme from *Alcaligenes eutrophus* (7) as shown in Table 3. However, the homology of the sequence was less than 60% when compared to the corresponding enzymes of *E. coli* K12 (2) and *Corynebacterium glutamicum* ATCC13032 (12). Therefore, this result shows high sequence homology in the strains or species of *Pseudomonas*, whereas the similarity of the sequences was less than that in other genera strains as in *E. coli* and *C. glutamicum* .

The genetic organization for the partial naphthalene-catabolic upper-pathway of *P. fluoresence* SM11 have been determined. The genetic organization of *nahQ-tnpA-nahE* in the naphthalene-catabolic upper-pathway of *P. fluorescens* SM11 is similar to that found in other well-characterized naphthalene-degradation upper-pathways, as in *P. aeruginosa* PaK1 (unpublished data).

The transposition of the catabolic clusters might be the mechanism whereby these DNA rearrangements were recruited (9, 18). Bosch *et al.* (3) reported that the presence of *tnpA* in close proximity to the upper naphthalene-degradation pathway of *P. stutzeri* AN10 could support the putative transposition events. In addition, short DNA regions of high homology to the *tnpA* gene of the strain AN10 and its flanking regions are found in other catabolic pathways such as *dmp* of *P. putida* CF600 (15), *nah* of *P. putida* G7 (16), *pah* of *P. putida* OUS82 (17), and *dox* of *Pseudomonas* sp. C18 (6). This conserved DNA region that flanks the entire *nah* upper pathway of *P. putida* G7, has been suggested to be involved in the mobilization and further rearrangement of this entire catabolic module (8).

In this study, the downstream region at position 330 to 360 of *tnpA* gene was found to have high homology in *tnpA* genes from *P. stutzeri* OX1, *P. alcaligenes* NCIB, and *Alcaligenes eutrophus*. Therefore, we propose that transposons carrying a *tnpA* are involved in the tranposition of the catabolic modules in degradation of naphthalene.

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