

## HETEROLOGOUS EXPRESSION OF A GENE ENCODING BIPHENYL 2,3-DIOXYGENASE FROM *BURKHOLDERIA CEPACIA* IN *ESCHERICHIA COLI*

Duong Duc Hoang Sinh<sup>1#</sup>, Nguyen Hoang Tue<sup>1#</sup>, Tran Vu Ngoc Thi<sup>1</sup>, Le Thi Ha Thanh<sup>1</sup>, Nguyen Duc Huy<sup>2</sup>, Nguyen Hoang Loc<sup>1\*</sup>

**Address(es):**

<sup>1</sup>Institute of Bioactive Compounds, University of Sciences, Hue University, 77 Nguyen Hue St., Hue, Thua Thien Hue 530000, Vietnam.

<sup>2</sup>Institute of Biotechnology, Hue University, Phu Vang, Thua Thien Hue 530000, Vietnam.

\*Corresponding author: [nhloc@hueuni.edu.vn](mailto:nhloc@hueuni.edu.vn)

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ABSTRACT

Biphenyl 2,3-dioxygenase (BphA) is an enzyme that participates in initiation step of the pathway of biphenyl degradation in bacteria. Biphenyl is an aromatic hydrocarbon including two benzene rings that causes a serious environmental pollution. This report presents the preliminary results on the cloning and expression of *BphA* gene of *B. cepacia* strains DF2 and DF4 which were isolated from dioxin contaminated soil in Vietnam. The coding DNA sequences of *BphA* genes are 897 bp long and 99% identical. Recombinant BphA enzyme (33 kDa) has strongly expressed in *E. coli* M15 after induction of 0.5 mM IPTG for 4 h at 37°C.

**Keywords:** *B. cepacia*, biphenyl 2,3-dioxygenase, *BphA* gene, heterologous expression

INTRODUCTION

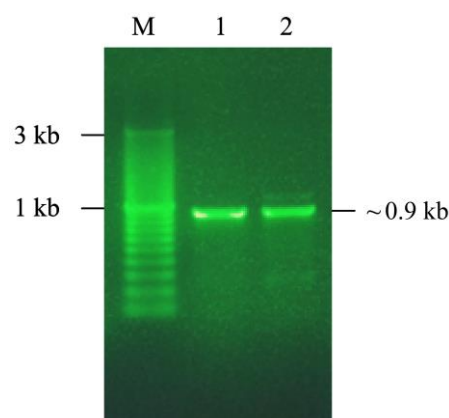
Biphenyl, one of four substrates of biphenyl 2,3-dioxygenase, is an aromatic hydrocarbon in which two benzene rings are connected to each other. Polychlorinated biphenyls have been widely used for various industrial purposes, these compounds are recognized to be some of the most serious environmental pollutants worldwide (Furukawa et al., 2004). Biphenyl 2,3-dioxygenase, also called biphenyl dioxygenase subunit alpha (BphA), is an enzyme that belongs to the family of oxidoreductase participating in initiatory step of the pathway of biphenyl degradation in bacteria (Leewis et al., 2016). Mondello (1989) and Seeger et al. (1995) had cloned and expressed genes encoding biphenyl dioxygenase of *Pseudomonas* strain LB400 in *E. coli*. The *BphA* genes of *Rhodococcus globerulus* P6 were also expressed in *Pseudomonas putida* by McKay et al. (1997). Recently, Ohmori et al. (2011) expressed the *BphA* genes from *Rhodococcus jostii* RHA1 in *E. coli*. In alternative way, Hu et al. (2014) activated the expression of *BphA1* gene in *Pseudomonas fluorescence* P2W and *Ralstonia eutropha* H850 by treatment of these bacteria with salicylic acid and biphenyl. In our knowledge, there is not any report on production of recombinant BphA enzyme from *B. cepacia*. Therefore, in the present study, we report some preliminary results on the expression of *BphA* gene from *B. cepacia* strains DF2 and DF4 in *E. coli*.

Two *B. cepacia* strains DF2 and DF4 (GenBank accession No: MG768914 and MG768915) were isolated from dioxin contaminated soil in former ASo air-base, A Luoi district, Thua Thien Hue province, Vietnam. Total DNA of bacteria was extracted using PowerSoil® DNA Isolation Kit (MO BIO, Qiagen). The 5'- and 3'- untranslated regions of the full-length biphenyl 2,3-dioxygenase gene from *Burkholderia cenocepacia* MSMB384WGS (CP013452.1) were used to design specific primers (forward: 5'-GGATCCATGATCGATG-TCCGTG-3' and reverse: 5'-AAGCTTCCGGTATCCGATGCTG-3') for PCR amplification of the coding DNA sequence (CDS) of *BphA* gene in *B. cepacia* DF2 and DF4. 5'-GGATCC-3' and 5'-AAGCTT-3' are overhang ends for *Bam*HI and *Hind*III, respectively. PCR components were as follows: 50 ng total DNA, 10 pmol each primer, 6 µL 2× GoTaq® Green Master Mix (Promega) in a volume of 12 µL. Amplification was performed in thermocycler Veriti (ABI) with thermal cycles: genomic denaturation at 95°C for 15 min; 30 cycles of 95°C for 30 sec, 55°C for 1 min and 72°C for 1.5 min; finally an extension of 72°C for 10 min.

PCR product (CDS of putative *BphA* gene) after purification by GeneJET Gel Extraction Kit (Thermo Scientific) was inserted into pGEM®-T-Easy vector (Promega) and was then transformed in *E. coli* TOP10 cells by the heat-shock method (Sambrook et al., 1987). Putative *BphA* gene was sequenced using the dideoxy chain-termination method on ABI 3130 system. Phylogenetic tree was

constructed from the CDS alignment of *BphA* genes in bacterial species using MEGA7 software.

Recombinant pGEM®-T-Easy/*BphA* vector was digested with *Bam*HI and *Hind*III, and pQE30 expression vector (Qiagen) was also opened with the same enzymes. Digested products were purified with MEGA quick-spin™ Total Fragment DNA Purification Kit (iNtRON Biotechnology) which were then ligated together using T4 DNA ligase (Promega), and finally was introduced into *E. coli* M15. Transformed *E. coli* M15 was proliferated in 5 mL of liquid LB medium containing 50 µg/mL ampicillin at 37°C overnight with a shaking speed of 180 rpm. Then, 50 µL of overnight culture was transferred into 50 mL of fresh LB medium and cultured in the same condition until OD at 600 nm reaches 0.5, add 0.5 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) to the medium and culture for another 4 h. Total soluble protein (TSP) content of cell was measured by the Bradford's method (1976), and 10 µg of TSP was used for sodium dodecyl sulfate-12% polyacrylamide gel electrophoresis (SDS-PAGE) to evaluate expression of *BphA* gene.



**Figure 1** PCR amplification of putative *BphA* genes from *Burkholderia cepacia* strains DF2 and DF4. M: DNA size marker, 1: DF2 and 2: DF4.

PCR amplification of putative *BphA* gene (CDS) from *B. cepacia* DF2 and DF4 with specific primers was showed in Figure 1. The amplicons have sizes as expected with approximately 0.9 kb in length. The nucleotide sequences of the amplicons are 99% identical to a gene encoding biphenyl 2,3-dioxygenase of *B.*

cepacia INT3-BP177 (AOI85724.1). Two sequences of *BphA* genes from DF2 and DF4 were deposited in GenBank with accession numbers of MF953295.1 and MF953300.1, respectively. Figure 2 shows the alignment of deduced amino

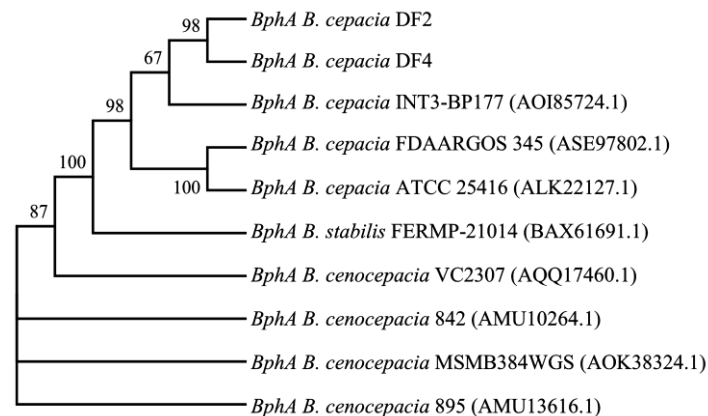
acid sequences between three *BphA* genes from *B. cepacia* DF2, DF4 and INT3-BP177. The identity levels of all proteins are also 99%.

DF2	1	MIDVRLALGYVVVEATRVDARRRYAEDVLGMQALDAPDGALYLKMDERDFRYVIVPGSTDR	60
DF4	1	MIDVRLALGYVVVEATRVDARRRYAEDVLGMQALDAPDGALYLKMDERDFRYVIVPGSADR	60
INT3-BP177	1	MIDVRLALGYVVVEATRVDARRRYAEDVLGMQALDAPDGALYLKMDERDFRYVIVPGSADR	60
DF2	61	YFASGWELPDGAAFDAALAVLQAGVEPIRATPAEAAALRRVQAMAWCTDPSGNRHELYWG	120
DF4	61	YFASGWELPDGAAFDAALAVLQAGVEPIRATPAEAAALRRVQAMAWCTDPSGNRHELYWG	120
INT3-BP177	61	YFASGWELPDGAAFDAALAVLQAGVEPIRATPAEAAALRRVQAMAWCTDPSGNRHELYWG	120
DF2	121	ARCDFFRRFVSP LGVAHFVTGDMGLGHAVLPAPQFDATDAFVRGVLGFELSDIYRVKFTPD	180
DF4	121	ARCDFFRRFVSP LGVAHFVTGDMGLGHAVLPAPQFDATDAFVRGVLGFELSDIYRVKFTPD	180
INT3-BP177	121	ARCDFFRRFVSP LGVAHFVTGDMGLGHAVLPAPQFDATDAFVRGVLGFELSDIYRVKFTPD	180
DF2	181	PAEPEKRIHFHMCNRNARHHSLLALFEMAVPSGCVHVMAEVDSMDEVGRALDRVAAHDKMS	240
DF4	181	PAEPEKRIHFHMCNRNARHHSLLALFEMAVPSGCVHVMAEVDSMDEVGRALDRVAAHDKMS	240
INT3-BP177	181	PAEPEKRIHFHMCNRNARHHSLLALFEMAVPSGCVHVMAEVDSMDEVGRALDRVAAHDKMS	240
DF2	241	ATLGRHCNDQMISFYMKTPGGFDLEYGFGGLTVDWSKHAVFEATKVSQWGHDFSIGYR	298
DF4	241	ATLGRHCNDQMISFYMKTPGGFDLEYGFGGLTVDWSKHAVFEATKVSQWGHDFSIGYR	298
INT3-BP177	241	ATLGRHCNDQMISFYMKTPGGFDLEYGFGGLTVDWSKHAVFEATKVSQWGHDFSIGYR	298

**Figure 2** The alignment of deduced amino acid sequences from three *BphA* genes of *Burkholderia cepacia* strains DF2 (AXL14076.1), DF4 (AXL14081.1) and INT3-BP177 (AOI85724.1). Black boxes are different amino acids.

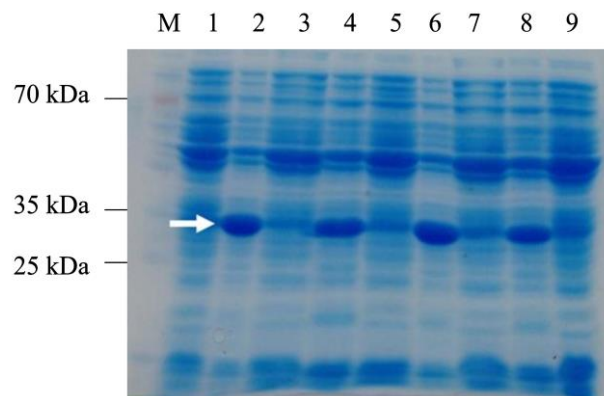
The *BphA* genes from two strains *B. cepacia* DF2 and DF4 were subjected to phylogenetic analysis using MEGA7 software. The phylogenetic tree was created using Maximum Likelihood method (Tamura and Nei, 1993; Kumaret et al., 2015) with bootstrap support of 1000 replicates. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. From results are shown in Figure 3, it can be seen that corresponding genes cluster with very high bootstrap values. This proves that the *BphA* genes are highly preserved in *Burkholderia* genus. The tree helps show orthologs and paralogs of these genes in *B. cepacia*, *B. cenocepacia* and *B. stabilis*.

Expression of *BphA* genes from DF2 and DF4 in *E. coli* M15 was induced with IPTG. SDS-PAGE showed that *BphA* enzyme was produced in high content and they have molecular weight (MW) as expected (approximately 33 kDa) corresponding to deduced amino acid sequences (298 aa) (Fig 4).



**Figure 3** Molecular phylogenetic analysis of *BphA* genes encoding biphenyl 2,3-dioxygenases from strains *Burkholderia cepacia*, *Burkholderia cenocepacia* and *Burkholderia stabilis*. The percentage of trees in which the associated taxa clustered together is shown next to the branches.

Several previous reports showed the *BphA* gene has been expressed in various host cells. For example, McKay et al. (1997) heterologously expressed biphenyl dioxygenase genes from *R. globerulus* P6 in *P. putida* cells. Ohmori et al. (2011) obtained *BphAc* enzyme (MW of 11.5 kDa) of *R. jostii* RHA1 from recombinant *E. coli* Rosetta (DE3) pLacI cells. According to Haddock and Gibson (1995), biphenyl 2,3-dioxygenase from *Pseudomonas* sp. LB400 is composed of a large (alpha) subunit (approx. 53.3 kDa) and small (beta) subunit (approx. 27.3 kDa). While biphenyl 2,3-dioxygenase alpha subunit from *Cupriavidus* sp. WS (G-44364, MetaCyc) has a molecular weight of 51.649 kDa that estimated from the nucleotide sequence.



**Figure 4** SDS-PAGE of total soluble protein from recombinant *Escherichia coli* M15 containing pEQ30/*BphA* vector. M: protein molecular weight marker. 1: non-transformed *Escherichia coli* M15. 2: *BphA* gene from DF2 was induced with IPTG. 3: *BphA* gene from DF2 was non-induced with IPTG. 4, 6 and 8: *BphA* gene from DF4 was induced with IPTG. 5, 7 and 9: *BphA* gene from DF4 was non-induced with IPTG. Arrow indicates bands of recombinant biphenyl 2,3-dioxygenase in *Escherichia coli* M15.

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