Cloning and sequencing of desulfurization operon from a newly isolated bacterium *Rhodococcus* FMF

Soudabeh Akbarzadeh, Jamshid Raheb and Ferdous Rastegar Jazii

National Research Center for Genetic Engineering and Biotechnology, P.O. Box: 14155-6343, Tehran, I.R. Iran.

Abstract
A native strain of Rhodococcus FMF was isolated from soil samples collected from Tabriz petroleum refinery area in Iran. The presence of sox operon in the genomic DNA and the ability of bacteria to consume dibenzothiophen (DBT) as sulfur source were assessed. DNA was amplified by PCR and subsequently cloned into pTZ57R cloning vector. Diffrent restriction endonucleases; EcoRI, HindIII, EcoRI/HindIII and XhoI were used to prove the accuracy of cloning. Acquired clone was named pTZAB57R. Subsequently, the relevant A and B genes involved in DBT consumption were sequenced and compared with the map of Rhodococcus erythropolis IGTS8 desulfurization pathway genes. Results show that the desulfurization operon in the native isolated Rhodococcus FMF bacterium is completely conserved.
Keywords: *Rhodococcus spp., Biodesulfurization, Dibenzothiophen.*

INTRODUCTION

Microorganisms have evolved diverse biochemical pathways for consuming sulfur as an essential source of nutrient. Recently attentions has been focused on the application of these sulfur-specific pathways to deal with sulfur-related environmental pollutions and refinement of heavy oils or oils which contain much higher level of sulfur containing hydrocarbon (Monticello, 1998; MacFarland *et al*., 1998; Maghsoudi *et al*., 2000).

Microorganisms that selectively desulfurize benzothiophen (BTs) and dibenzothiophen (DBTs) include *Rhodococcus erythropolis* IGTS8 (ATCC 53968), that was patented by the Institute of Gas Technology U.S.A, and licensed for industrial development by Energy Biosystems Corporation in 1991 (Denome *et al*., 1993 and 1994; Piddington *et al*., 1995) and other sulfur-specific desulfurizing microbes including *Rhodococcus erythropolis* D-1 (Izumi *et al*., 1994), *Rhodococcus* ECRD-1 ATCC 55301 (Grossman *et al*., 1999; Denis-Larose *et al*., 1997), *Rhodococcus* B1 (Denis-Larose *et al*., 1997), *Rhodococcus* SY1 (Omori *et al*., 1995), *Rhodococcus* UM3 and UM9 (Purdy *et al*., 1993), *Agrobacterium* MC501 (Constanti *et al*., 1996), *Mycobacterium* G3 (Nekodzuka *et al*., 1997), *Gordona* CYKS1 (Gilbert *et al*., 1998), *Klebsiella* (Dudley and Frost, 1994), *Xanthomonas* (Constanti *et al*., 1994), *Nocardia globelula* (Wang and Krawiec, 1994), thermophilic *Paenibacillus* (Konishi *et al*., 1997) and cytochrome P450-based systems (Schlenk *et al*., 1994).

Rhodococcus erythropolis IGTS8 is mostly used as a model strain in desulfurization studies. In this bacteria three desulfurization genes, collectively known as soxA, B and C (also known as dszA, dszB, and dszC) are organized in tandem, starting with soxC, a 4 kb long operon and as a part of 120 kb linear plasmid that carries out desulfurization reaction. The products of these genes are a set of enzymes responsible for bioconversion of DBT to 2-hydroxybiphenyl (2-HBP) and sulfite (Denome *et al*., 1993 and 1994; Piddington *et al*., 1995 and Li *et al*., 1996). The soxC codes for a 45 kDa protein, which is similar to acyl-CoA dehydrogenase and carries out the initial reaction for the oxidation of DBT to DBT sulfone. The soxA codes for a 50 kDa protein that resembles a secondary flavin monoxygenase, and soxB codes for a 40 kDa protein that acts as a desulfinase which is involved in the conversion of DBT sulfone to 2-hydroxybiphenyl (2HBP)

Correspondence to: **Jamshid Raheb, Ph.D.**
Tel: +98 21 4580387 , Fax: +98 21 4580399
E-mail: jam@nrcgeb.ac.ir

and sulfite (Denome *et al*., 1994 and Piddington *et al*., 1995).

Recent improvements in understanding the biodesulfurization mechanisms were much effective in leading researchers to the industrial applications of biodesulfurization through engineering recombinant strains overexpressing biodesulfurization genes, removal of inhibitory processes and engineering microorganisms which not only carry out a proper biodegradation pathway but also cost effective from the point of industrial applications. The present study is along with such objectives for the improvement and further advancement of such technologies and their applications.

MATERIALS AND METHODS

Materials: Restriction enzymes and InsT/A clone™ PCR product cloning kit were purchased from Fermentas Company (Germany). Molecular weight marker and DIG DNA labeling and detection kit, high pure plasmid purification kit, high pure PCR product purification kit and agarose gel DNA extraction kit were purchased from Roche Company (Germany). All other chemicals were of analytical grade and purchased from E-Merck, Germany.

Bacterial culture and extraction of genomic DNA: *Rhodococcus* FMF was grown in Luria-Bertoni (LB) culture medium containing 60 µg/ml kanamycine for 3 to 4 days in 30°C. The bacteria was separated by centrifugation and washed with EDTA (0.5 M, pH 8). Cells were lysed in 10 ml of lysis buffer containing; lysozyme (10 mg/ml), RNase (100 µg/ml) and proteinase K (30 mg/ml) for 30 minutes at 37°C. The lysate was frozen at -70°C, thawed, and subjected to phenol/chloroform extraction and ethanol precipitation to extract genomic DNA. The extracted DNA was dissolved in TE (10 mM Tris-HCl, 1 mM EDTA; pH 8.0).

PCR amplification: PCR was used to amplify two sox operon genes; dszA and dszB by applying the genomic DNA of R. FMF as template. The sequence of the primers used are as follows:
forward 5´ GAA TTC CGC GAT GAC TCA ACA ACG AC 3´
reverse 5´ AAG CTT CTA TCG GTG GCG ATT GAG GC 3´

The restriction sites for *Eco*RI and *Hind*III at the 5´ ends of forward and reverse primers were also considered for designing the primers. Fast start taq DNA polymerase and high fidelity kit were used for PCR. The amplification steps were comprised primary denaturation at 94°C for 5 min followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 65°C for 1 min, extension at 72°C for 3 min and final extension for 7 min at 72°C. Electrophoresis was carried out in a 1% gel agarose. The 2.46 kb band of PCR product was purified and concentrated by high pure PCR product purification kit.

Cloning: PCR product containing dszA and dszB genes was cloned into pTZ57R plasmid following by purification and concentration. This plasmid contains an ampicillin resistance site and undergoes α-complementation. The cloning was done using Ins T/A clone™ PCR product cloning kit which is used for cloning of of *Taq* amplified PCR fragments and ligation was performed at 22°C overnight using T4 DNA ligase. The ligated product was then transformed into to *E. coli* DH5α. Subsequently, 100 µl of transformed bacteria were spread on LB agar plates containing ampicillin (60 µg/ml), IPTG (0.2 mM), X-Gal (40 µg/ml) and incubated at 37°C. White colonies were selected and grown in LB medium and checked for the presence of dszA and B genes. Such clones were designated as pTZAB57R

Enzymatic digestion: Digestion was carried out by applying different restriction enzyme on 10 µg plasmid DNA in a volume of 20 µl reaction mixture containing 1 unit of each restriction enzyme. Digestion was carried out either by applying a single restriction enzyme or a combination of two enzymes as follows; *Eco*RI, *Hind* III, *Xho*I and *Eco*RI/*Hind*III at 37°C, overnight. Four µl of digested DNA plasmid was mixed with 1 µl of loading buffer and ran on a 1% agarose gel electrophoresis. Staining was done with ethidium bromide and visualized under UV transilluminator.

Sequencing: Two bacterial colonies were selected and grown in 100 ml of LB medium; plasmid was purified by applying high pure plasmid purification kit (Roche). The entire cloned genes were sequenced by applying automated DNA sequencer (MWG DNA Biotech Company, Germany) using tandemly designed primer sets for internal parts of dszA and B genes. Each round of sequencing was able to unravel almost 700 base pairs.

RESULTS

In our pervious paper we reported the ability of the *Rhodococcus* FMF strain to specifically desulfurize dibenzothiophene (DBT) (Akbarzadeh *et al*., 2003). In the present study, cloning and characterization of dszA and B genes which constitute a part of 4S pathway responsible for desulfurization of DBT was investigated. Full length desulfurization operon is 3800 nucleotides comprising of three genes namely; dszA, B and C. By applying a set of primers a 2.46 kb region bearing the dszA and B genes located tandemly in the host chromosome was amplified.

Two different types of DNA polymerases were used in amplification reaction, the result of which is shown in figure 1. It is inferred that both types of DNA polymerases were suitable for application in the amplification, however it seems that the high fidelity DNA polymerase has superiority to some extent to that of Taq- DNA polymerase for long range DNA amplification. Following to purification and concentration of the amplified fragment (Fig. 1, lanes 9, 10 and 11) the fragment was cloned in pTZ57R cloning vector (Fig. 2).

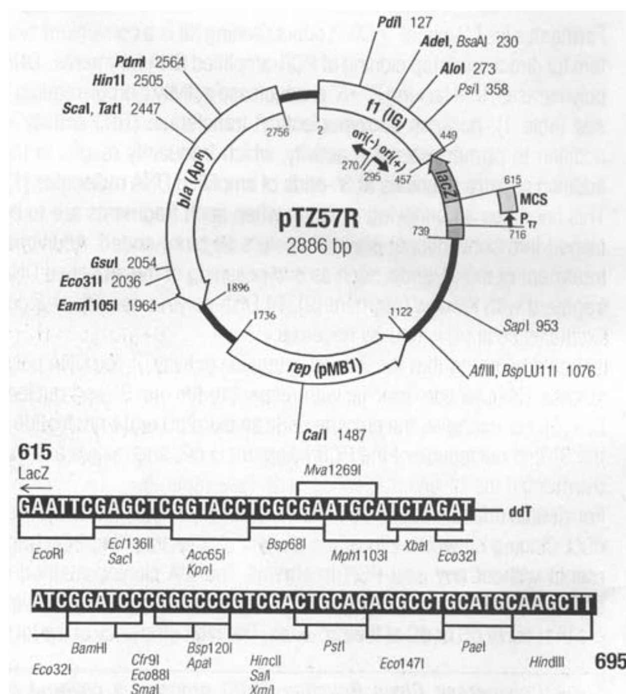

Figure 2: Map and multiple cloning site of the vector pTZ57R used for cloning PCR product of the dszA and B genes.

The circularized vector was transformed into DH5α strain of *E. coli* and transformed bacteria were selected according to blue and white selection procedure and subsequently were screened for the presence of dszA and B genes respectively (Fig. 3). Among the clones confirmed for the presence of dszA and B genes, two colonies were selected, grown and subjected to restriction digestion using restriction enzymes; *Eco*RI, *Hind*III, *Eco*RI/*Hind*III and *Xho*I, the result of which is shown in Figure 4. Presence of two bands of 2.5 and 2.9 kbs following digestion with *Eco*RI, *Hind*III and *Eco*RI/*Hind*III, not only approves a proper cloning but also indicates that the fragment is integrated in reverse direction within the vector (Fig. 4, bands 1, 2 and 3 and bands 5, 6 and 7).

To finally confirm the cloning step, the dszA and B genes were further digested with restriction enzyme *Xho*I. This enzyme does not have any restriction site within the vector but has two sites, 1000 bp apart from each other within the cloned dszA and B genes. The result obtained after treatment of the plasmid with *Xho*I restriction enzyme is shown in figure 4; lanes 4 and 8. Appearance of a 1000 bp band on electrophoresis indicates integration of dszA and B genes within

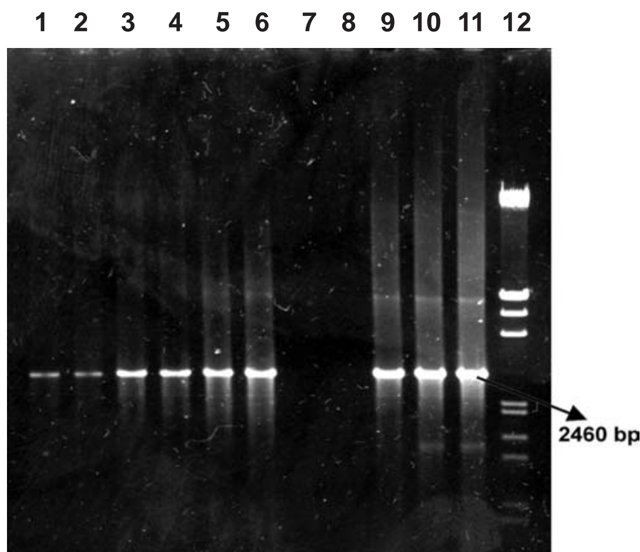

Figure 1: PCR product following amplification of the gene fragment sox (dszA and B) by applying primers designed according to *Rhodococcus* IGTS8 sequence. The 2455 base pairs of PCR product includes the entire dszA and B genes which was further used for cloning (Fig. 3). Lanes 1, 2, 3 and 4; amplified dszA and B genes by applying the DNA source from R. FMF, lanes 1 and 2 are the amplification product by applying High Fidelity Amplification Kit (Roche company) and lanes 3 and 4 are the amplification product by applying Fast Start *Taq*-DNA Polymerase Amplification Kit. Lanes 5 and 6; amplification of the genes dszA and B by applying CC118 pESOX4 (positive control). Lanes 7 and 8; PCR product of DH5α-PUC18 plasmid (negative control). Lanes 9, 10 and 11; amplification product of the genes dszA and B from *Rhodococcus* FMF following purification and concentration which was used for cloning. Lane 12; molecular size marker λ DNA digested by *Eco*RI/*Hind*III.

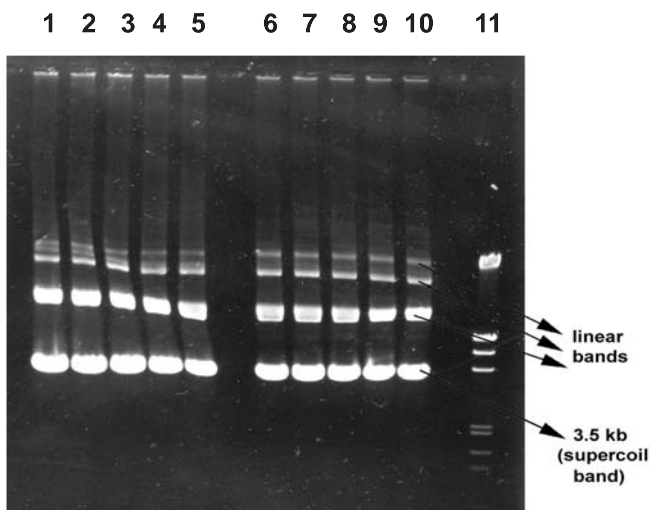

Figure 3: Purified plasmid from two white colonies out of many positive clones for dszA and B genes named as clones T7 and T8. From each clone five overnight cultures was prepared, plasmid extracted and ran on 1% agarose gel electrophoresis. Lanes 1, 2, 3, 4 and 5; extracted plasmid from white colonies of the clone T7. Lanes 6, 7, 8, 9 and 10; extracted plasmid from white colonies of the clone T8. Lane 11; molecular size marker (λ DNA digested by *Eco*RI/*Hind*III).

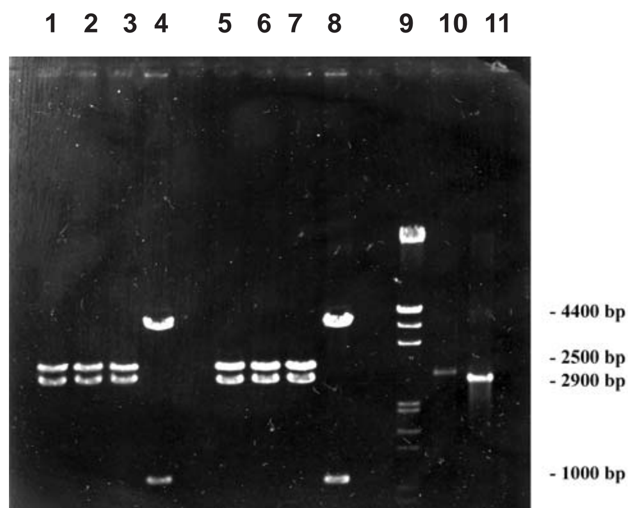

Figure 4: Restriction digestion analysis in order to confirm the presence of dszA and B genes and the direction of cloning following cloning and transformation of pTZAB57R into *E. coli* (DH5α). Lanes 1, 2, 3 and 4; the products of digestion with *Eco*RI, *Hind*III, *Eco*RI/*Hind*III and *Xho*I on the extracted plasmids of the T7 clones. Lanes 5, 6, 7 and 8; digestion products of the extracted plasmids of T8 clone with *Eco*RI, *Hind*III, *Eco*RI/*Hind*III and *Xho*I, resteriction enzymes respectively. Lane 9; molecular size marker (λ DNA digested with *Eco*RI/*Hind*III). Lane 10; pTZ57R vector following purification from a blue colony and digestion with *Eco*RI and *Hind*III restriction enzymes. Lane 11; PCR product of dszA and B genes.

the vector.

The well documented bacterial colonies for the presence of the transformed construct (pTZAB57R) were grown in LB medium, plasmid was extracted and purified. Sequencing was carried out by applying sequentially sets of primers such that to anneal at 5´ end of dszA in forward and dszB gene in reverse direction. By applying such primers it was possible to sequence the entire dszA and B genes as depicted in figure 5 and 6. Figure 5 shows the entire sequence of dszA gene and figure 6 shows the corresponding sequence of dszB gene. Following to sequencing of the entire dszA and dszB genes, the sequences were submitted and deposited in data bank (EMBL) with the entry IDs of RH0514949 and RH0514947.

DISCUSSION

Extraction of sulfur containing oil, removal and clearance of it's sulfur is one of the major problems in the oil refinement industries. Sulfur contamination, not only reduces the value of the refined oil but also is one of the environmental pollution and one of the bio-environmental concerns in many parts of the world. To solve this problem an unique, recent and highly developed technology is being used in the world which is based on biodesulfurization. Many microbial strains have been engineered and applied in this regard and recombinant strains are now available that have drawn much attention in the oil industries.

Since *Rhodococcus* FMF does not carry any plasmid, we focused on the genomic DNA of the *Rhodococcus* FMF and the sox (dszA and B) genes of desulfurization operon to use both as a source and back up of desulfurization genes for further genetic engineering. For this purpose the entire fragment of dszA and B genes was amplified and cloned. After successful cloning of dszA and B genes, the complete sequence of these genes were obtained and then we deposited the entire sequences of the genes in the European data bank; EMBL, with the entry IDs; RH0514949 and RH0514947. Comparing the sequence of this strain with standard strain; *Rhodococcus erythropolis spp.* strain IGTS8 an almost 100% homology was found, therefore, it is inferred that this operon is highly conserved among prokaryotes and further indicates a common biodesulfurization pathway in these organisms.

Along with these works further amplification and

```
ATGACTCAAC  AACGACAAAT  GCATCTGGCC  GGTTTCTTCT  CGGCCGGCAA  TGTGACTCAT    60
GCACATGGGG  CGTGGCGGCA  CACGGACGCG  TCGAATGACT  TTCTGTCGGG  GAAGTACTAC   120
CAACACATCG  CCCGTACTCT  GGAGCGCGGC  AAGTTCGATC  TGTTGTTTCT  GCCTGACGGG   180
TTGGCCGTCG  AGGACAGCTA  CGGGGACAAC  CTGGACACCG  GTGTCGGCCT  GGGCGGGCAG   240
GGTGCAGTCG  CCTTGGAGCC  GGCCAGTGTG  GTCGCAACCA  TGGCCGCGGT  GACCGAGCAC   300
CTGGGTCTTG  GGGCAACCAT  TTCGGCGACC  TACTATCCCC  CGTATCACGT  TGCTCGGGTG   360
TTCGCGACGC  TCGATCAGTT  GTCAGGGGGT  CGGGTGTCCT  GGAACGTCGT  CACCTCGCTC   420
AACGACGCTG  AAGCGCGCAA  CTTCGGCATT  AATCAGCATC  TGGAACACGA  CGCCCGCTAT   480
GACCGCGCCG  ATGAGTTCTT  GGAAGCGGTC  AAGAAACTCT  GGAACAGCTG  GGACGAGGAC   540
GCCCTCGTGC  TGGACAAGGC  GGCCGGCGTG  TTCGCCGATC  CCGCGAAGGT  GCACTACGTC   600
GATCACCACG  GGGAGTGGCT  GAATGTGCGC  GGACCTCTGC  AGGTACCGCG  TTCACCTCAG   660
GGTGAGCCGG  TGATCCTGCA  GGCCGGCCTG  TCGCCCCGGG  GTCGGCGCTT  CGCCGGGAAG   720
TGGGCCGAGG  CCGTCTTCAG  TCTTGCACCC  AACCTCGAGG  TGATGCAGGC  CACCTACCAG   780
GGCATCAAAG  CCGAGGTCGA  CGCTGCGGGG  CGCGATCCCG  ATCAGACGAA  AATCTTCACC   840
GCCGTGATGC  CGGTACTCGG  CGAAAGCCAG  GCGGTGGCAC  AGGAACGACT  GGAATATCTC   900
AACAGTCTGG  TCCATCCGGA  AGTGGGACTG  TCGACGCATC  CAGTCACACC  GGCATCAACC   960
TGGCGGCGTA  CCCTCTCGAC  ACTCCGATCA  AGGACATCCT  GCGGGATCTG  CAGGATCGGA  1020
ATGTCCCGAC  GCAACTGCAC  ATGTTCGCCG  CCGCAACGCA  CAGCGAAGAG  CTCACGCTGG  1080
CGGAAATGGG  TCGGCGCTAT  GGAACCAACG  TGGGGTTCGT  TCCTCAGTGG  GCCGGTACCG  1140
GGGAGCAGAT  CGCTGACGAG  CTGATCCGCC  ACTTCGAGGG  CGGCGCCGCG  GATGGTTTCA  1200
TCATCTCTCC  GGCCTTCCTG  CCGGGCTCCT  ACGACGAGTT  CGTCGACCAG  GTGGTTCCGG  1260
TTCTGCAGGA  TCGCGGCTAC  TTCCGCACCG  AGTACCAGGG  CAACACTCTG  CGCGACCACT  1320
TGGGTCTGCG  CGTACCACAA  CTGCAAGGAC  AACCTTCATG  A                       1361
```

Figure 5: Sequence of DszA gene following to amplification and cloning in pTZ57R as deposited into EMBL gene bank (RH0514949) and used for homology assessment.

```
ATGACAAGCC  GCGTCGACCC  CGCAAACCCC  GGTTCAGAAC  TCGATTCCGC  CATCCGCGAC    60
ACACTGACCT  ACAGCAACTG  CCCGGTACCC  AACGCTCTGC  TCACGGCATC  GGAATCGGGC   120
TTCCTCGACG  CCGCCGGCAT  CGAACTCGAC  GTCCTCAGCG  GCCAGCAGGG  CACGGTTCAT   180
TTCACCTACG  ACCAGCCTGC  CTACACCCGT  TTTGGGGGTG  AGATCCCGCC  ACTGCTCAGC   240
GAGGGGTTGC  GGGCACCTGG  GCGCACGCGT  CTACTCGGCA  TCACCCCGCT  CTTGGGGCGC   300
CAGGGCTTCT  TTGTCCGCGA  CGACAGCCCG  CGACAGCCCG  CCGCCGACCT  TGCCGGACGT   360
CGAATCGGCG  TCTCGGCCTC  GGCAATTCGC  ATCCTGCGCG  GCCAGCTGGG  CGACTACCTC   420
GAGTTGGATC  CCTGGCGGCA  AACGCTGGTA  GCGCTGGGCT  CGTGGGAGGC  GCGCGCCTTG   480
TTGCACACCC  TTGAGCACGG  TGAACTGGGT  GTGGACGACG  TCGAGCTGGT  GCCGATCAGC   540
AGTCCTGGTG  TCGATGTTCC  CGCTGAGCAG  CTCGAAGAAT  CGGCGACCGT  CAAGGGTGCG   600
GACCTCTTTC  CCGATGTCGC  CCGCGGTCAG  GCCGCGGTGT  TGGCCAGCGG  AGACGTTGAC   660
GCCCTGTACA  GTTGGCTGCC  CTGGGCCGGG  GAGTTGCAAG  CCACCGGGGC  CCGCCCAGTG   720
GTGGATCTCG  GCCTCGATGA  GCGCAATGCC  TACGCCAGTG  TGTGGACGGT  CAGCAGCGGG   780
CTGGTTCGCC  AGCGACCTGG  CCTTGTTCAA  CGACTGGTCG  ACGCGGCCGT  CGACGCCGGG   840
CTGTGGGCAC  GCGATCATTC  CGACGCGGTG  ACCAGCCTGC  ACGCCGCGAA  CCTGGGCGTA   900
TCGACCGGAG  CAGTAGGCCA  GGGCTTCGGC  GCCGACTTCC  AGCAGCGTCT  GGTTCCACGC   960
CTGGATCACG  ACGCCCTCGC  CCTCCTGGAG  CGCACACAGC  AATTCCTGCT  CACCAACAAC  1020
TTGCTGCAGG  AACCCGTCGC  CCTCGATCAG  TGGGCGGCTC  CGGAATTTCT  GAACAACAGC  1080
CTCAATCGCC  ACCGATA                                                     1097
```

Figure 6: Sequence of the dszB gene which was amplified and cloned in pTZ57R and submited to EMBL gene bank (RH0514947).

cloning of the dszC gene (data not shown) was done which needs further analysis for its functionality either singly or in combination with dszA and B under appropriate promoters such as tac and T7. Furthermore cloning of dszD (oxido-reductase gene) or application of strains containing it, either on plasmid or chromosomes. Transforming such strains with the above mentioned constructs should also be considered under control of the same promoter or as a compatible plasmid.

The host bacterium used for transformation of the present engineered vector was *E. coli* strain, DH5α. This bacterium was chosen due to being easy to work and as a good source for back up of dszA and B genes. However it should be kept in mind that this strain is

not well compatible with hydrophobic nature of the growth media in refinement due to the presence of aromatic hydrocarbons, thus for oil refinement and industrial applications the vector has to be re-transformed in a suitable host such as *Rhodococcus* or *Pseudomonas* (previously used as host but recently replaced with *Rhodococcus*).

In summery, in the present study, specific sets of genes in newly isolated *Rhodococcus* bacteria were identified. These genes were successfully cloned and sequenced. The homology of these genes to those previously reported in other strains suggests possible involvement of these strains in oil refinery. Furthermore, it is suggested that expression of such genes under the control of strong promoters may be implicated in removal of oil contaminations or sulfur-containing pollutants in the environment.

Acknowledgment

We would like to thank to Dr. Abdolamir Allameh for his helpful comments on this article and to Dr. M. Mazaheri for donating the *Rhodococcus* FMF strain. This work was financed by the grant No.138 allocated by the National Research Center for Genetic Engineering & Biotechnology of IR Iran.

References

Akbarzadeh S, Raheb J, Aghaei A, Karkhane AA. (2003) Study of desulfurization rate in *Rhodococcus* FMF native bacterium. *Iranian J Biotech* 1: 36-40.

Constanti M, Giralt J, Bordons A. (1996) Degradation and desulfurization of debenzothiophene sulfone and other sulfur compounds by *Agrobacterium* MC501 and a mixed culture. *Enzyme Microb Technol* 19: 214-219.

Constanti M, Giralt J, Bordons A. (1994) Desulfurization of debenzothiophene by bacteria. *World J Microb Biotech* 10: 510-516

Denis-Larose C, Labbe D, Bergeron H. (1997) Conservation of plasmid-encoded dibenzothiophene desulfurization genes in several *rhodococci*. *Appl Environ Microb* 63: 2915-2919.

Denome SA, Oldfield C, Nash LJ, Young KD. (1994) Characterization of the desulfurization genes from *Rhodococcus spp*. strain IGTS8. *J Bacteriol* 176: 6707-6716.

Denome SA, Olson ES, Young KD. (1993) Identification and cloning of genes involved in specific desulfurization of dibenzothiophene by *Rhodococcus spp*. strain IGTS8. *Appl Environ Microb 59*: 2837-2843.

Dudley MW, Frost JW. (1994) Biocatalytic desulfurization of arylsulfonates. *Bioorgan Med Chem* 2: 681-690.

Gilbert SC, Morton J, Buchanan S. (1998) Isolation of a unique benzothiophene-desulfurizing bacterium, *Gordona spp*. strain 213E (NCIMB 40816), and characterization of the desulfurization pathway. *Microbiology* 144: 2545-2553.

Grossman MJ, Lee MK, Prince RC. (1999) Microbial desulfurization of a crude oil middle-distillate fraction: analysis of the extent of sulfur removal and the effect of removal on remaining sulfur. *Appl Environ Microb* 65: 181-188.

Izumi Y, Ohshiro T, Ogino H. (1994) Selective desulfurization of dibenzothiophene by *Rhodococcus erythropolis* D-1. *Appl Environ Microb* 60: 223-226.

Konishi J, Ishii Y, Onaka T. (1997) Thermophilic carbon-sulfur bond targeted biodesulfurization. *Appl Environ Microb* 63: 3164-3169.

Li MZ, Squires CH, Monticello DJ. (1996) Genetic analysis of the dsz promoter and associated regulatory region of *Rhodococcus erythropolis* IGTS8. *J Bacteriol* 175: 6409-6418.

MacFarland BL, Boron DJ, Deever WR. (1998) Biocatalytic sulfur removal from fuel: applicability for producing low sulfur gasoline. *Crit Rev Microbiol* 24: 99-147.

Maghsoudi S, Kheirolomoom A, Vossoughi M. (2000) Selective desulfurization of dibenzothiophene by newly isolated *Corynebacterium spp*. strain P32C1. *Biochem Eng J* 5: 11-16.

Monticello DJ. (1998) Ridding the fossil fuel biodesulfurization wave. CHEMTECH 28: 38-45.

Nekodzuka S, Toshiaki N, Nakajima-Kambe T. (1997) Specific desulfurization of debenzothiophene by Mycobacterium strain G3. *Biocatal Biotransfor* 15: 21-27.

Omori T, Monna LS, Kodama T. (1995) Desulfurization of alkyl and aromatic sulfides and sulfunates by dibenzothiophene desulfurizing *Rhodococcus spp*. strain SY-1. *Appl Environ Microb* 59: 1195-1198.

Piddington CS, Kovacevich BR, Rambosek J. (1995) Sequence and molecular characterization of a DNA region encoding the dibenzothiophene desulfurization operon of *Rhodococcus spp*. strain IGTS8. *Appl Environ Microb* 61: 468-475.

Purdy RF, Lepo JE, Ward B. (1993) Biodesulfurization of organic-sulfur compounds. *Curr Microbiol* 27: 219-222.

Schlenk D, Beverse RJ, Vertino AM. (1994) P450 catalyzed oxidation of dibenzothiphene by *Cunninghamella elegans*. *Xenobiotica* 24: 1077-1083.

Wang P, Krawiec S. (1994) Desulfurization of debenzothiophene to 2-hydroxybiphenyl. *Arch Microbiol* 161: 266-271.