Nucleotide Sequence of a Desulfurization Operon from a Newly Isolated Rhodococcus FMF.

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Abstract:

Rhodococcus FMF isolated from soil samples of Tabriz refinery in Iran is capable of utilizing dibenzothiophene (DBT) as a sole source of sulfur. This gram positive bacterium is able to catabolize dibenzotiophene (DBT) to 2-hydroxybiphenyl (2-HBP) and sulfite without the cleavage of carbon-carbon bonds. We showed that this phenotype was due to the expression of a chromosomal-encoded desulfurization gene cluster which codes for proteins Dsz A, B and C. The dszABC genes identified, isolated and amplified by PCR technique. Sequencing analysis revealed that high conservation between this chromosomal encoded operon and its plasmid-encoded counterpart in Rhodococcus sp. strain IGTS8 exists.

Introduction:

Microorganisms have evolved diverse biochemical strategies for dealing with sulfur, an essential element. Scientists and engineers are employing these sulfur-specific mechanisms to deal with sulfur related environmental problems. To decrease sulfur-related air pollution, the sulfur content of fossil fuels should be effectively limited. To achieve this goal, sulfur must be removed from crude oil during the refining process [1]. The conventional method used so far to desulfurize organosulfur materials is hydrodesulfurization. Since deep desulfurization according to the newly imposed standard specification is expensive and requires developed chemical catalysts at high pressure and temperature [1]. The decreased availability of low sulfur crude oils has resulted in a need to refine heavier, higher sulfur crude's, which upon combustion produces sulfur oxides that contribute to air pollution. [1-3] consequently, legislation in several countries requires progressive annual reductions in sulfur content of petrochemicals, driving up refining costs [3]. The refractory polynuclear aromatic sulfur heterocyclic such as [benzothiophenes (DBT) and dibenzothiophenes (DBTs)] prevent the current desulfurization technology hydrodesulfurization (HDS), and serve as model compounds in the study of biodesulfurization (BDS) [4]. HDS treatment is a catalytic process converting organic sulfur to hydrogen sulfide gas by reacting crude oil fractions with hydrogen at pressures between150 and 3000 pounds per cubic inch and temperatures between 290 and 455 oC [4]. Stricter regulations on sulfur content in fuels plus the high cost of HDS make alternative desulfurization methods, to use with or in place of HDS, of interest to the petroleum industry. Microbial degradetive desulfurization pathways are not commercially viable for petroleum because a significant amount of carbon is

mineralized, reducing the fuel value [1,5,6].

 Microorganisms that selectively desulfurize DBTs include Rhodococcus erythropolis IGTS8 (ATCC 53968), which was patented by the Institute of a Gas Technology and licensed for commercial development to Energy Biosystems Corporation in 1991. Other sulfur-specific desulfurizing microbes include Rhodococcus erythropolis D-1 [7], Rhodococcus ECRD-1 ATCC 55301[8,9], Rhodococcus B1[9], Rhodococcus SY1[10], Rhodococcus UM3 and UM9 [11], Agrobacterium MC501[12], Mycobacterium G3 [13], Gordona CYKS1 [14], Klebsiella [15], Xanthomonas [16], Nocardia globelula [17], thermophilic Paenibacillus [18] and some cytochrome P450 systems [19]. Recent discoveries related to biodesulfurization mechanisms may lead to commercial applications of biodesulfurization through engineering recombinant strains for over expression of biodesulfurization genes, removal of end product repression, and/or by combining relevant industrial and environmental traits with improvements in bioprocess design [20].

 In this report the dszABC gene from Rhodococcus FMF was identified by southern blot, amplified by PCR technique and cloned into plasmid pTZ57R/T and completely sequenced. Then the sequence of dszABC operon from Rhodococcus FMF was compared with Rhodococcus sp. strain IGTS8 operon. The conservative nature of dszABC gene cluster independent of its origin (chromosomal or plasmid-encoded) was confirmed, since they showed the homology of 99.99%.

Materials and Methods:

a) Enzyme and chemicals: The restriction enzymes and InsT/A cloneTM PCR product cloning kit were bought from Fermentas Company. Molecular weight marker and DIG DNA labeling and detection kit, High pure plasmid purification kit, High pure PCR product purification kit, Agarose gel DNA extraction kit were bought from Roche company. All the chemical material was made by Merck Company.

b) Bacterial culture and extraction of genomic DNA: Rhodococcus FMF was grown at 30 oC for 3-4 days in LB culture contains kanamycine antibiotic, suspension was centrifuged and supernatant discarded, the pellet washed with EDTA (0.5 M, pH 8.0). Cells were solved in 10ml of lysis buffer contain lysozyme, RNase and proteinaze K and after incubation in 37 oC for 30 minutes transferred in freezer -70 oC until the next day. Then proteins were eliminated by two stages of phenol/chloroform and genomic DNA after separation and washing was solved in TE buffer.

c) PCR Method: The PCR method was used for separation and amplification of dszAB genes in designing of primers was supposed restriction sites for EcoRI and HindIII in 5' ends of forward and reverse primers ordinarily. Then was used fast start taq DNA polymerase and High fidelity kit for PCR. The gene amplification was provided by Corbett Research set in annealing temperature 65 oC. After providing a distinct band in 2.48 kb region, PCR product was purified and concentrated by a high pure PCR product purification kit.

d) Cloning: PCR product contains dszAB gene was cloned into PTZ57R plasmid after purification and concentration. This plasmid is comprised of bla marker (ampicillin resistance site) and can do α-complementation. This stage done by InsT/A cloneTM PCR product cloning kit that can clone fragments with soft end. Ligation stage was achieved by adding T4 DNA ligase to solution contains PCR product and PTZ57R vector in 22 oC temp overnight. Ligation product was transformed into propriety competent bacteria (E. coli, DH5α). In the next stage 100µl of transformed bacteria was spread on a LB, Amp, IPTG, X-Gal plate and was incubated in 37 oC. Existence of dszAB gene were studied in white colonies after culturing and plasmid extraction.

d) Sequencing: For achieving to a high purity sample, PTZAB57R was treated after purification with large scale method by high pure plasmid purification kit. Where as used method for sequencing can read 700 nucleotides therefore provided two samples from each plasmid and each of them was sent for

sequencing with a primer (forward or reverse). Sequencing was done in automated sequence method by MWG DNA Biotech Company in Germany. To complete the full length of dszAB gene sequences, a couple of specific primers for 5' and 3' of the obtained sequences from the preliminary primers were used.

Results:

a) Identification, Isolation and sequencing of dszAB genes from Rhodococcus FMF: Rhodococcus FMF native bacterium is a strain, which was picked up from around Tabriz petroleum refinery soil and can desulfurized DBT as a model molecule. After proving of bacterium major enzyme action in desulfurization route (4S pathway) was started studies for operon recognizing and cloning. More studies were followed by molecular cloning of desulfurization operon. Because of operon length (3.8 kb) the forward and reverse primers for AB genes with 2.5 kb length was designed. Digestion sites for EcoRI and HindIII located in 5' end of forward primer and reverse primer respectively. After setting PCR program, amplified AB genes by two kit fast start taq DNA polymerase (Fig. 1, lanes 1, 2) and high fidelity (lanes 3,4). For confirmation of PCR was used CC118 SOX4 as positive control (lanes 5,6) and DH5α as negative control (lanes 7,8). After amplification, AB gene was purified and concentrated by high pure PCR product purification kit (lanes 9-11). Sample absorbance read by a UV spectrophotometer in 260 and 280nm, after determination of concentration, equal with 0.5μg DNA used for cloning. PTZ57R vector (2886 bp) was digested in multiple cloning site (MCS) by Eco321 and have protrude due to adding ddT nucleotide in 3' ends by terminal deoxy nocleotidyl transferase. If in PCR test, the final extension time consider about 20-25 minutes, 3' end of these segments will load by adenine nucleotide. If PCR product is pure and vector to insert proportion be suitable (1:3), ligation efficiency will be up 95%. Ligation was done in

presence of T4 DNA ligase and PEG4000 overnight and its product transformed to competent DH5α cell and cultured on plate. After incubation in 37 oC overnight, colonies were screened. After reculturing of white colonies in LB culture, their plasmid was extracted. Here was seen a super coil band in 3.5 kb region. We due attention to length of vector segment, all white colonies have received insert apparently. Then were selected two colonies for more studies. Plasmid was digested by EcoRI, HindIII, EcoRI/HindIII and XhoI (Fig. 2). After digestion by EcoRI and HindIII appeared two bands in 2.5, 2.9 kb regions. With regard to 3' end of vector and 5' end of insert have digestion site with EcoRI and 5' end of vector and 3' end of insert have digestion site with HindIII, can result the segment have been cloned in reverse direction.

Final confirmation of AB gene cloning was performed by digestion by XhoI enzyme. Since this enzyme haven't digestion site on vector and two digestion sites on AB gene with 1000 bp interspaces, existence of 1000 bp fragment from vector show definitive cloning of AB gene.

PTZAB57R plasmid sequenced by forward and reverse primers that read from beginning of a gene and end of B gene ordinarily. In this part 496 primary nucleotides of a gene and 893 terminal nucleotides of B gene were sequenced. Then two specific primers for the 5' and 3' ends of the genes AB were used to obtain the partial sequences. With regard to this information homology rate of desulfurization operon in Rhodococcus FMF native bacterium to Rhodococcus erythropolis sp. strain IGTS8 is 100% and this rate denote to conserving dszAB genes in mentioned strain.

b) Identification, Isolation and sequencing of dszC gene from Rhodococcus FMF:

Southern blot analysis and detection of dszC gene form Rhodococcus FMF. The chromosomal DNA from Rhodococcus FMF was extracted (Materials and Methods) and then digested with EcoRI, SalI, XbaI, SacI, SmaI, XhoI, KpnI, HindIII and

HincII, loaded on 1% agarose gel stained with Ethidium bromide, run at room temperature under voltage 20 overnight and transferred to a nylon membrane. The probe required for this experiment was prepared using 1280 bp salI digested fragment of SoxC gene from Rhodococcus sp. strain IGTS8. In order to obtain this, E. coli strain CC118 Transformed by pESOX4 plasmid containing the whole operon of desulfurization was employed. Then the hybridization to the nylon membrane was performed (Materials and Methods). According to the southern blot analysis and in contrast with studies on other Rhodococci species, we described that the desulfurization operon in Rhodococcus FMF is located on the chromosomal DNA of this bacterium (data not shown).

c) Amplification of dszC gene: Based on the conservation of desulfurization operon through different strains of Rhodococci, two primers were designed using the sequence of dszC gene from Rhodococcus sp. strain IGTS8 to employ by PCR technique. Two primers alongside the genomic DNA of Rhodococcus FMF were employed. In order to facilitate the cloning process, the forward and reverse primers were designed respectively with restriction sites for EcoRI and HindIII at 5´ends.Forward primer C1 and the reverse primer C2 were as follows; C1: 5´GAATTCGCCTCAATGCCCACCGATAC 3´and the reverse

primer C2:   5´AAGCTTTCAGGAGGTGAAGCCGGGAA 3´. Amplification was carried out using high fidelity PCR Master Kit (from Roche) and a Perkin-Elmer, Norwalk, U.S.A. thermocycler. Amplification conditions were 94°C for 5 min (1 cycle), 94 oC for 1 min, 70 oC for 1 min, 72 oC for 2 min repeated for 30 cycles in addition to an extra 72 oC for 4 min. Then the contents of the PCR vial was run on 1% agarose gel and after staining by Ethidium bromide, as it was expected ,a 1280 bp fragment was detected indicating the existence of complete translated region of SoxC gene from Rhodococcus FMF (Fig. 3).

d) Purification and cloning of PCR product: The purification of PCR product was performed using high pure PCR product purification kit (manufactured by Roche Company). It was then cloned into a plasmid vector using InsT/A clone™ PCR product cloning kit # K1213 (Fermentas): A 1280-bp purified PCR product was ligated into the plasmid vector pTZ57R/T. In order to perform transformation procedure, competent cells of E. coli strain DH5α on LB-agar plates containing X-Gal, IPTG, and required amounts of Ampicillin were selected according to white-blue reaction based on the Lac ZΔM15 mutation in E. coli strain DH5α.Transformed bacteria were differentiated based on α-complementation phenomenon as white colonies. These colonies in concert with a blue one (as a negative control) were selected from plates, transferred to distinct freshly made LB medium and incubated at 37˚C to be collected for plasmid extraction according to plasmid minipreparation protocol. Since the forward and reverse primers were respectively equipped with EcoRI and HindIII recognition sites at their 5′ ends, we managed to show that the orientation of dszC gene cloned into pTZ57R is reverse. Furthermore, to examine the insertion of PCR fragment, an approximately 500 bp fragment (Fig. 4, lanes 2,4) consisting of two restriction sites for enzyme PstI was selected as a representative of the total PCR product cloned into the vector. The plasmid designated pTZC57K was then digested with PstI at 37 oC overnight. After loading on 1% agarose gel, the desirable fragment was clearly detectable confirming the proper insertion of concerned dszC gene into the newly constructed vector (Fig. 4).

In this study, for the first time we showed that the desulfurization operon is located on chromosomal DNA in Rhodococcus FMF (data not shown). To isolate large plasmids from Rhodococcus FMF, we employed the procedure modified (9). However there was no large plasmid found in this strain.

e) Sequencing analysis: The plasmid pTZC57k was selected for the insert fragment to be sequenced. This was done by MWG DNA Biotech Company using

dye terminators using the ABI 3700 sequencer (Germany) automatically. Required sequencing primers was obtained by this company and prepared according to the manufacturer's instructions. The dszC gene in Rhodococcus FMF was completely sequenced and compared with that of Rhodococcus sp. strain IGTS8. The sequences were aligned with the sulfide/sulfoxide monooxygenase gene from Rhodococcus Erythropolis strain IGTS8. The existence of only one changed nucleotide between the compared dszC genes confirmed that the sulfide/sulfoxide monooxygenase gene in these two strains is highly conserved. The dszAB genes and dszC gene sequences were joined by DNAstar software to demonstrate the complete dszABC gene cluster sequences of Rhodococcus FMF (Fig. 5) compared to Rhodococcus IGTS8 (Fig. 6).

Discussion:

A dszAB proteins gene were amplified by PCR from a newly isolated Rhodococcus FMF (Isolated from Tabriz refinery soil in Iran) using the primers GAATTCCGCGATGACTCAACAACGAC and AAGCTTCTATCGGTGGCGATTGAGGC specific for the 5´ and 3´ ends of the genes. The genomic DNA was used as the target for these primers. dszAB genes were amplified After providing a distinct band in 2.48 kb region, PCR product was purified and concentrated by a high pure PCR product purification kit. PCR product containing dszAB gene was cloned into PTZ57R plasmid (InsT/A cloneTM PCR product cloning kit from Fermentas Company). The same specific primers were used for sequencing analysis of both strands of this 2.4 kb fragment. Other primers CTATGACCGCGCCGATGAGT and CCGAGTAGACGCGTGCGCCC specific for the 5´ and 3´ of the obtained sequence from the preliminary primers were used to complete the full length of dszAB genes sequences. Sequencing was done in an automated sequence apparatus by MWG DNA Biotech Company in Germany. The sequences were aligned with the

dszAB gene from Rhodococcus Erhytropolis strain IGTS8 [2] and demonstrated 100% homology.

In addition, the dszC gene responsible for the conversion of DBT-as a model sulfur-containing heterocyclic present in middle distillate petroleum fractions [21] to DBT-sulfone in sulfur specific pathway in Rhodococcus FMF. Previous DNA sequencing and molecular subcloning experiments in Rhodococcus sp. strain IGTS8 revealed that the desulfurization pathway consists of three genes, namely dszA, dszB, and dszC [22]. In fact, the conversion of DBT to HBP is catalyzed by a multienzymatic pathway which includes two monooxygenases and a desulfinase. Subcloning experiments [22-24] confirmed the activity of DszC (DBT-monooxygenase or DBT-MO) in the conversion of DBT to dibenzothiophene5,5-dioxide (DBTO2), DszA (DBTO2-monooxygenase or BTO2-MO) in the conversion of DBTO2 to 2-(2-hydroxyphenyl) benzenesulfinate (HPBS) and DszB (2-(2-hydroxyphenyl) benzenesulfunate desulfinase or HPBS desulfinase in the last step with the production of HBP and inorganic sulfur. No other products besides the sulfoxide and dioxide were detected suggesting that DBT-MO is specific for sulfoxidation. Various mono and dioxygenases can carry out sulfoxidation reactions [25-27], however unlike the DBT-MO activity, the major product from other oxygenases is the sulfoxide, and the yield of dioxide is very low. So, DBT-MO seems to be unique among the oxygenases because of its ability to stoicheiometrically convert aromatic sulfide to the dioxide. DBTO2-MO catalyzes the hydroxylation of DBTO2 to form HPBS without any detectable intermediate. Based on previous studies, the reaction rates for DBTO2-MO were 5-6 folds higher than for DBT-MO resulting in no accumulation of DBTO2 in the cell free desulfurization. All three enzymes had no tightly associated chromophores since they were colorless as isolated. According to size exclusion chromatography and elusion patterns, it was suggested that DBT-MO is a tetramer in its native state (Mr. of 180 kDa), DBTO2-MO a dimmer (Mr. of 100 kDa), and HPBS desulfinase a monomer.

The organization of these genes initially suggested that they act as an operon based on the same direction of their transcription and the fact that the termination codon for dszA and the initiation codon for dszB overlap and there is only a 13 bp gap between dszB and dszC. Moreover, expression studies revealed that dszA and dszB had to be co-expressed in order to observe their relevant activities. In order to determine the functions of the three genes in Rhodococcus sp. strain IGTS8, Piddington et al. [22] subcloned various fragments of the complete cluster, transformed them into deletion mutants, and determined the resulting phenotypes by performing a HPLC analysis in which DBT and DBT-sulfone were used as the substrates. Those deletion experiments clarified that dszC is responsible for conversion of DBT to DBT-sulfone and that dszA and dszB are responsible for the conversion of DBT-sulfone to 2-HBP [22].

Rhodococcus FMF desulfurizes DBT by a modification of the 4S pathway. The first step in this pathway is catalyzed by a sulfide/sulfoxide monooxygenase encoded by dszC gene. In Rhodococcus sp. strain IGTS8, dszC encodes a protein of 417 amino acids with a molecular weight of 44,977 Dalton [28].

The desulfurization genes are located on a plasmid in Rhodococcus sp. strain IGTS8 Endogenous plasmids in Rhodococcus IGTS8 were separated from chromosomal DNA by hexagonal pulsed–field gel electrophoresis. Ethidium bromide staining revealed the existence of three large plasmids in IGTS8 50, 120 and 400 kb respectively [24]. Previous southern blot analysis revealed that the DBT-modifying genes are located on the 120 kb plasmid. No hybridization to the chromosomal DNA band or the 50 and 400 kb plasmid bands was detected. Furthermore, hybridization of the same probe to restriction -digested IGTS8 DNA indicated that there was only one set of sox genes in the Rhodococcus genome. The sulfide/sulfoxide monooxygenase in Rhodococcus IGTS8 [29] does not exhibit any significant flavin reduction activity and relies on a separate enzyme species to reduce the flavin and the preferred flavin is FMN for this enzyme. One flavin site per dimmer has been detected for it. Among different

substrates, the liver microsomal flavin-containing monooxygenase catalyses the monooxygenation of organic sulfides (such as dimethyl sulfoxide) to the corresponding sulfoxides and subsequently to sulfone [30]. Thus, the sulfide/sulfoxide monooxygenase provides first example of a bacterial enzyme resembling the microsomal flavin-containing monooxygenase. The latter monooxygenase is distinct from the sulfide /sulfoxide monooxygenase in being a monomer, having a bound FAD cofactor and being able to reduce the bound flavin by its own catalysis.

Although both monooxygenases (dszC and dszA) require NADH and FMN, the purified enzymes could not oxidize NADH. Thus it was presumed that another enzyme (dszD) is present and acts as a NADH-FMN oxidoreductase in order to supply both enzymes with reduced flavin. It has an apparent molecular weight of 25 kDa and is not a flavoprotein since it does not contain tightly bound FMN. It seems to use the flavin as a dissociable substrate [28]. The employment of an NADH-FMN oxidoreductase to supply free FMNH2 to the oxygenases is one of the characteristics of the dsz monooxygenases in comparison with the well-known di- and monooxygenases [31-33]. The flavin reductase from Rhodococcus reduces FMN in the absence of DBT-MO and DBTO2-MO and the presence of these enzymes and their substrates does not enhance the rate of NADH oxidation. The Ec apparently uses FMNH2 as a preferred cosubstrate in Rhodococcus IGTS8 [29]. One molecule of homodimeric enzyme binds one FMNH2 and the binding is tight. Ec significantly interacts with FMN but the interaction is not as tight as that for FMNH2 binding. Probably, Ec can catalyze the oxidation of both DBT and more efficiently DBTO. The results suggested that DBTO is an intermediate in the oxidation of DBT to DBTO2. The DBT oxidation activity of Ec requires exogenously added FMNH2. Inclusion of a FRP-dependent FMNH2-generating system in the lysate of Rhodococcus sp. strain IGTS8 [34] and the addition of NADH and FMN to a partially purified enzyme from Rhodococcus erythropolis D-1 [35] enhance the DBT oxidation activity. However no significant activity of reduced FAD for Ec was

detected, in contrast to earlier reports in which DBT oxidation activity could be enhanced by the inclusion of FAD in the lysate of E. coli cells that express Ec [24] or by the addition of FAD and NADH to a partially purified enzyme from Rhodococcus erythropolis D-1 [35]. Kilbane [36] previously proposed a theoretical pathway for desulfurization of DBT which includes a stepwise conversion of DBT to DBTO and subsequently to DBTO2. However the intermediacy of DBTO in such a process has never been experimentally established. By several experiments, it was found that Ec is capable of catalyzing both the oxidation of DBT to DBTO and the subsequent conversion of DBTO to DBTO2. Ec functions as a monooxygenase as showed below:

$$DBT + FMNH_2 + O_2 \rightarrow DBTO + FMN + H_2O$$

$$DBTO + FMNH_2 + O_2 \rightarrow DBTO2 + FMN + H_2O$$
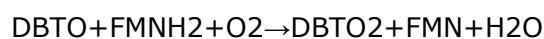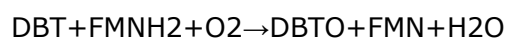
Mrachko et al. [37] reported that Ec functions as a monooxygenase in converting DBT to DBTO2 in two steps. Both oxygen atoms in DBTO2 originated from molecular oxygen. Also it was shown that the single oxygen in DBTO is also derived from O_2 in its formation from DBT. Therefore, Ec can be identified as a monooxygenase capable of forming DBTO from DBT and, subsequently producing DBTO2 from DBTO. Also benzyl sulfide and benzyl sulfoxide was tested with the purified Ec and found that both were active substrates for the formation of the sulfone product. Therefore, the monooxygenase activity of Ec is not limited to thiophene and thiophene sulfoxide as the only substrates. Thus sulfide/sulfoxide monooxygenase proposed as a general name for Ec. In the absence of SoxC, only DBTO2 was desulfurized to 2HBP by cells expressing the remaining two enzymes in the pathway, namely SoxA and SoxB. Thus, DBTO does not accumulate as the product of a single enzyme, nor is it metabolized further if SoxC is absent. Experiments with DBT as a substrate revealed that DBTO can appear in cultures of bacteria that are desulfurization negative, for instance, in the supernatants of wild-type E. coli. There is some evidence that higher levels of DBTO may actually inhibit the metabolism of DBTO2 to 2HBP in E. coli (Denome, Unpublished results). This could explain why Rhodococcus sp. strain IGTS8 grows very poorly [38] or not at all [23] when supplied with DBTO as the sole source of sulfur.

The SoxC protein is closely related to the family of acyl-CoA dehydrogenases. The similarity of SoxC to this family is distributed throughout the first four-fifths of the protein and included a large proportion of amino acids that are strictly

conserved in the consensus sequence of these enzymes. The acyl-CoA dehydrogenases bind FAD [34], and consistent with its relationship to this family, the activity of SoxC was increased by the addition of FAD to lysates.

In contrast with previous studies on Rhodococci species, the desulfurization genes in Rhodococcus FMF are positioned on the chromosomal DNA according to southern analysis (data not shown). In a previous report, Denome et al. [24] had also described the cloning of the Rhodococcus sp. strain IGTS8 desulfurization cluster which codes for three proteins DszA, DszB and DszC under the control of a single promoter (19 mine). In this work we demonstrated that the dszC desulfurization gene exhibited significant levels of sequence similarity with the sequence of dszC gene in Rhodococcus IGTS8.

Based on our unpublished results, an additional copy of desulfurization genes causes an increase in desulfurization activity. So it is expected that Rhodococcus FMF which appears to possess an extra copy of dsz genes, could show high desulfurization activity. According to the recognition sites of the related restriction enzymes used in southern blot technique, we expected to observe just one blot, but unexpectedly two blots were detected in concerned areas. On the basis of our previous experiments it was supposed that these blots could be associated with insertion elements IS1166/IS1295 homologous to Sox/dszA,B genes. However our findings suggest that it can be the result of existing more than one copy of dsz operon on chromosomal DNA from Rhodococcus FMF.

Acknowledgement:

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Figures
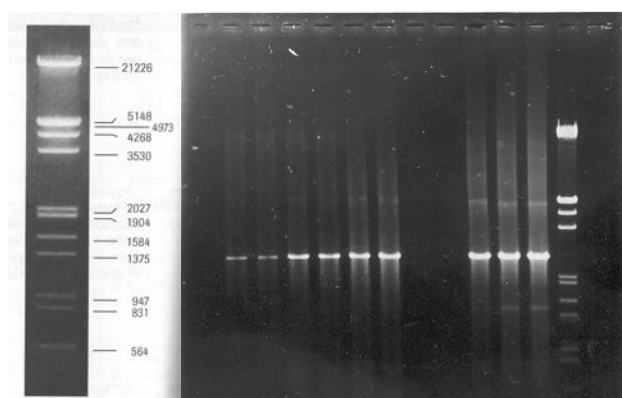
Fig. 1. Amplification of gene fragments sox (dsxAB) by PCR: lanes 1- 4, Gene fragments A,B from Rhodococcus FMF; lanes 5 and 6, Gene fragments A,B from pESOX4 as positive control; lanes 7 and 8, DH5α (negative control); lanes 9 - 11, Gene fragment AB from Rhodococcus FMF after purification and concentration; Lane 12, Molecular weight Marker III.
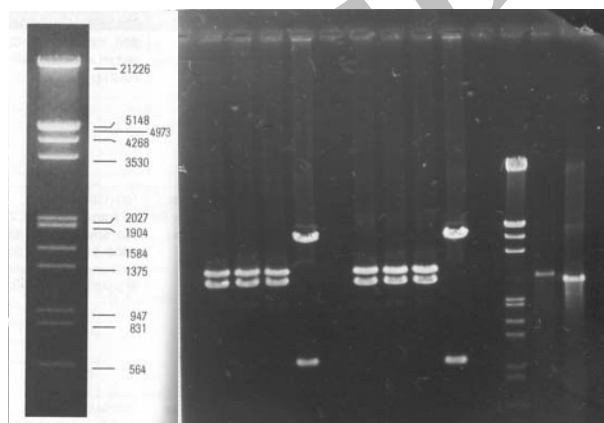
Fig. 2. Confirmation of cloning by enzymatic digestion: lanes1- 4, plasmid of T7 digested with EcoRI, HindIII, EcoRI/HindIII and Xho1; lanes 5 – 8, plasmid of T8 digested with EcoRI, HindIII, EcoRI/HindIII and Xho1; lane 9, Molecular weight markerIII; lane 10, PTZ57R vector after purification from a blue colony and digestion by EcoRI and HindIII; lane 11, PCR product of AB gene.
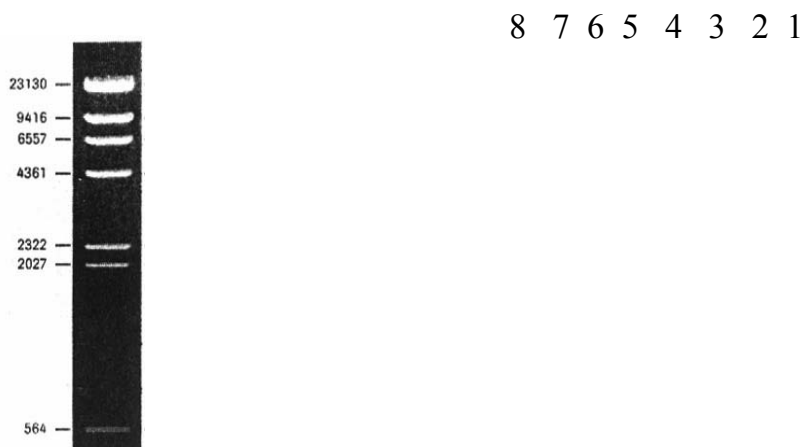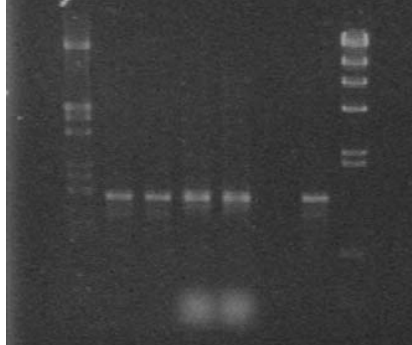
Fig. 3. PCR amplification of dszC gene from Rhodococcus FMF: lane 1, Molecular weight marker III; lanes 2 and 3, Purified PCR product of dszC gene; lane 4 and 5, Double digestion of PCR product with EcoRI/HindIII; lane 6, Distilled water as a negative control; lane 7, dszC gene from Rhodococcus erythropolis obtained by SOX4 digestion with SalI used as a positive control; lane 8, Molecular weight marker II.
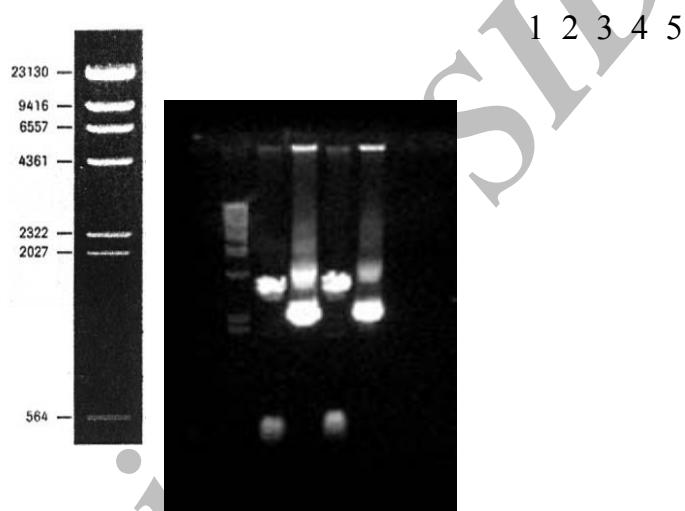
Fig. 4. Minipreparation of pTZC57K: lane1, Molecular weight marker II; lane 2, Digestion of pTZC57K with EcoRI; lane 3, Non-cut pTZC57K; lane 4, Digestion of pTZC57K with HindIII; lane 5, Non-cut pTZC57K.

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Fig. 5. The nucleotide sequences of dszABC genes (translation reagon) from
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Plus

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Query: 2101  gcaatgcctacgccagtgtgtggacggtcagcagcgggctggttcgccagcgacctggcc  2160
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Sbjct: 2101  gcaatgcctacgccagtgtgtggacggtcagcagcgggctggttcgccagcgacctggcc  2160


Query: 2161  ttgttcaacgactggtcgacgcggccgtcgacgccgggctgtgggcacgcgatcattccg  2220
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Sbjct: 2161  ttgttcaacgactggtcgacgcggccgtcgacgccgggctgtgggcacgcgatcattccg  2220


Query: 2221  acgcggtgaccagcctgcacgccgcgaacctgggcgtatcgaccggagcagtaggccagg  2280
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Sbjct: 2221  acgcggtgaccagcctgcacgccgcgaacctgggcgtatcgaccggagcagtaggccagg  2280


Query: 2281  gcttcggcgccgacttccagcagcgtctggttccacgcctggatcacgacgccctcgccc  2340
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Sbjct: 2281  gcttcggcgccgacttccagcagcgtctggttccacgcctggatcacgacgccctcgccc  2340


Query: 2341  tcctggagcgcacacagcaattcctgctcaccaacaacttgctgcaggaacccgtcgccc  2400
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 2341  tcctggagcgcacacagcaattcctgctcaccaacaacttgctgcaggaacccgtcgccc  2400


Query: 2401  tcgatcagtgggcggctccggaatttctgaacaacagcctcaatcgccaccgataggaac  2460
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```

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Sbjct: 2401 tcgatcagtgggcggctccggaatttctgaacaacagcctcaatcgccaccgataggaac 2460




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Query: 2521 gatcccgtcgcggttgcccgtgggctagccgaaaagtggcgagccaccgccgtcgagcgt 2580
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Sbjct: 2521 gatcccgtcgcggttgcccgtgggctagccgaaaagtggcgagccaccgccgtcgagcgt 2580




Query: 2581 gatcgcgccgggggttcggcaacagccgagcgcgaagacctgcgcgcgagcggcctgctg 2640
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Sbjct: 2581 gatcgcgccgggggttcggcaacagccgagcgcgaagacctgcgcgcgagcggcctgctg 2640




Query: 2641 tcgctcctcgtcccgcgcgaatacggcggctggggcgcagactggcccaccgccatcgag 2700
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Sbjct: 2641 tcgctcctcgtcccgcgcgaatacggcggctggggcgcagactggcccaccgccatcgag 2700




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Sbjct: 2761 accaacgccccgatgatcgaactgatcggctcgcaggaacaagaagaacacctgtacacc 2820




Query: 2821 cagatcgcgcagaacaactggtggaccggaaatgcctccagcgagaacaacagccacgtg 2880
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Sbjct: 2821 cagatcgcgcagaacaactggtggaccggaaatgcctccagcgagaacaacagccacgtg 2880
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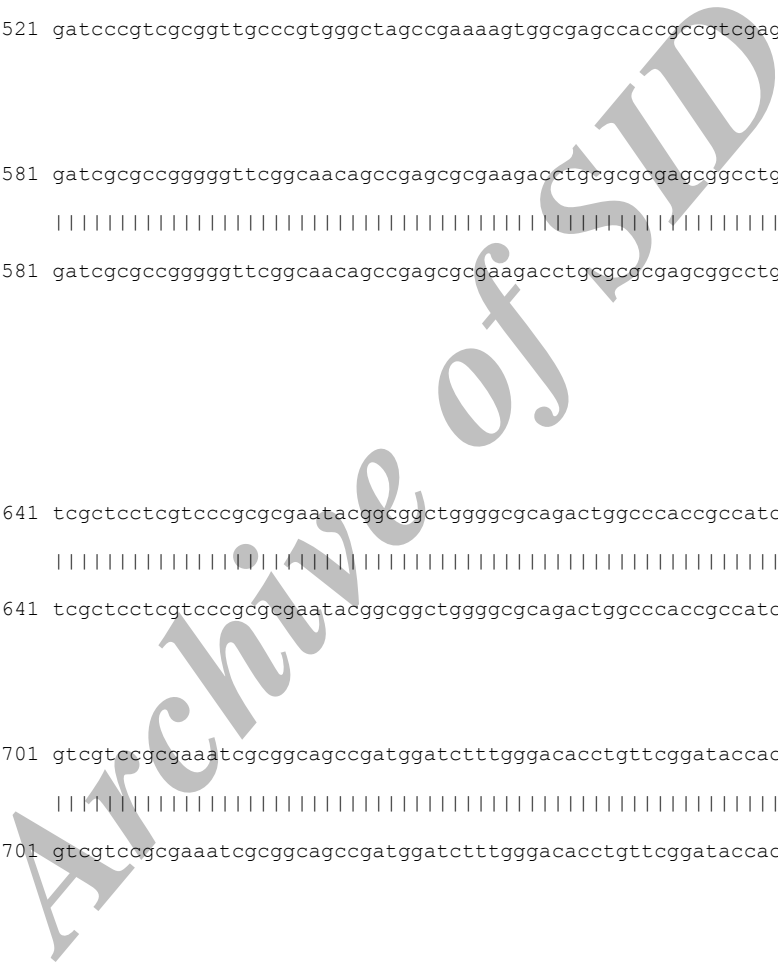
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Query: 2881  ctggactggaaggtcagcgccaccccgaccgaagacggcggctacgtgctcaatggcacg 2940
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 2881  ctggactggaaggtcagcgccaccccgaccgaagacggcggctacgtgctcaatggcacg 2940


Query: 2941  aagcacttctgcagcggcgccaaggggtcggacctgctgttcgtgttcggcgtcgtccag 3000
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 2941  aagcacttctgcagcggcgccaaggggtcggacctgctgttcgtgttcggcgtcgtccag 3000


Query: 3001  gatgattctccgcagcagggtgcgatcattgctgccgctatcccgacatcgcgggctggc 3060
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 3001  gatgattctccgcagcagggtgcgatcattgctgccgctatcccgacatcgcgggctggc 3060


Query: 3061  gttacgcccaacgacgactgggccgccatcggcatgcggcagaccgacagcggttccacg 3120
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 3061  gttacgcccaacgacgactgggccgccatcggcatgcggcagaccgacagcggttccacg 3120


Query: 3121  gacttccacaacgtcaaggtcgagcctgacgaagtgctgggcgcgcccaacgccttcgtt 3180
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 3121  gacttccacaacgtcaaggtcgagcctgacgaagtgctgggcgcgcccaacgccttcgtt 3180


Query: 3181  ctcgccttcatacaatccgagcgcggcagcctcttcgcgcccatagcgcaattgatcttc 3240
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 3181  ctcgccttcatacaatccgagcgcggcagcctcttcgcgcccatagcgcaattgatcttc 3240


Query: 3241  gccaacgtctatctggggatcgcgcacggcgcactcgatgccgccagggagtacacccgt 3300
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Sbjct: 3241  gccaacgtctatctggggatcgcgcacggcgcactcgatgccgccagggagtacacccgt 3300


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                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 3301  acccaggcgaggccctggacaccggccggtattcaacaggcaaccgaggatccctacacc  3360


Query: 3361  atccgctcctacggtgagttcaccatcgcattgcagggagctgacgccgccgcccgtgaa  3420
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Sbjct: 3361  atccgctcctacggtgagttcaccatcgcattgcagggagctgacgccgccgcccgtgaa  3420


Query: 3421  gcggcccacctcctgcagacggtgtgggacaagggcgacgcgctcacccccgaggaccgc  3480
             |||||||||| |||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 3421  gcggcccacctgctgcagacggtgtgggacaagggcgacgcgctcacccccgaggaccgc  3480


Query: 3481  ggcgaactgatggtgaaggtctcgggagtcaaagcgttggccaccaacgccgccctcaac  3540
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Sbjct: 3481  ggcgaactgatggtgaaggtctcgggagtcaaagcgttggccaccaacgccgccctcaac  3540


Query: 3541  atcagcagcggcgtcttcgaggtgatcggcgcgcgcggaacacatcccaggtacggtttc  3600
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Sbjct: 3541  atcagcagcggcgtcttcgaggtgatcggcgcgcgcggaacacatcccaggtacggtttc  3600


Query: 3601  gaccgcttctggcgcaacgtgcgcacccactccctgcacgacccggtgtcctacaagatc  3660
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Sbjct: 3601  gaccgcttctggcgcaacgtgcgcacccactccctgcacgacccggtgtcctacaagatc  3660


Query: 3661  gccgacgtcggcaagcacaccttgaacggtcaatacccgattcccggcttcacctcctga  3720
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 3661  gccgacgtcggcaagcacaccttgaacggtcaatacccgattcccggcttcacctcctga  3720


Query: 3721  ggatctgaggcgctga  3736
             ||||||||||||||||
```

```
Sbjct: 3721 ggatctgaggcgctga 3736
CPU time:      0.13 user secs.      0.06 sys. secs      0.19 total
sec
```

Fig. 6. The homology of dszABC genes from Rhodococcus FMF and Rhodococcus erythropolis IGTS8 using the blast search in database. In this scheme the query refers to Rhodococcus FMF and the subject refers to Rhodococcus erythropolis IGTS8.