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Characterization of Plasmid-Encoded Di-benzothiophene and Identification of Conserved Protein

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ABSTRACT

Biodesulfurization of organosulfur compounds is mediated by activity of the enzymes DszA, DszB and DszC. Here, we report the nucleotide sequence of an 11.5 kb region, containing the dszABC genes along with 3.3 kb upstream and 4.5 kb down-stream region, from a desulfurizing strain Gordonia sp IITR100. The genes are organized together as dszABC in an operon, and their nucleotide sequence is > 99% identical to those from the *Gordonia* strains IB, RIPI90A & CYKS2. The sequence upstream to *dszA*, is identical to the reported -1 to -1800 bp from the corresponding region of CYKS2, but presence of two transposase genes was identified in the remaining 2.7 kb region. Sequence of the region downstream to dszC was found to be distinct from all the desulfurizing organisms. BLAST analysis suggested that the cloned 11.5 kb region was possibly formed by crossover between the genomes a *Mycobacterium* and a *Gordonia* strain at a site 206 bp downstream to *dszC*. The results have bearing on understanding the organization and horizontal transfer of the desulfurization genes.

***Keywords*—** Biodesulfurization, *dszABC*, *Gordonia* sp. IITR100, Nucleotide sequence

I. INTRODUCTION

Several organisms that mediate desulfurization of the organo-sulfur compounds have been characterized. Many of these have also been shown to be effective in biodesulfurization of the various crude oil fractions (Grossman et al., 2001; Furuya et al., 2003; Yu et al., 2006). The desulfurization activity is mediated by a '4S' pathway, where the sulfur from target molecule is removed by serial activity of the enzymes DszC, DszA and DszB. In addition, the pathway also requires activity of a NADH-FMN oxidoreductase DszD that allows regeneration of $FMNH_2$ co-factor, needed for the reactions catalyzed by DszC and DszA (Gallagher, 1993; Oldfield et al., 1997). Genes for DszA, -B and -C have been studied from many organisms. Broadly, these are organized together as an operon and are coordinately regulated (Denome et al., 1994; Mohebali & Ball, 2008).

Based on their organization and nucleotide sequence, dsz genes from different organisms can be categorized in six groups i.e. *dszABC-type1- dszABC-type6* (Table 1). The *dszABC-type1* was described initially from *Rhodococus* sp. IGTS8 (Denome et al., 1994), and was later found to be present in several other organisms. Here the length of *dszA*, *dszB* and *dszC* is 1362, 1098 and 1254 bp, respectively, and an overlap of 4 bp between 3' end of *dszA* and 5' end of *dszB*, but a space of 13 bp between the genes *dszB* and *dszC* is also present (Table 1). The *dszABC*-type2 has been identified from several strains including *Gordonia alkanivorans* 1B (Alves et al., 2007). It is ~90% identical with *dszABC-type1*. Here, the length of *dszA*, *dszB*, and *dszC* is 1425, 1098 and 1251 bp, respectively. The overlap between *dszA* and *dszB*, and space between *dszB* and *dszC*, is 67 and 10 bp, respectively. The *dszABC-type3* and *dszABC-type4* have been described from thermophilic the bacteria *Paenibacillus* sp. A11-2 (Ishi et al., 2000) and *Bacillus subtilis* WU-S2B (Kirimura et al., 2004), respectively, and exhibit 62 and 50% % identity with *dszABC-type1*. Likewise, the *dszABC*-type5 and *dszABC-type6* have been characterized from the strains *Mycobacterium* sp. G3 (Nomura et al 2005) and *Gordonia amicalis* F.5.25.8 (Kilbane & Robbins, 2007), and are ~70 and 84% identical to *dszABC-type1*, respectively.

Table 1. Details of the various reported *dszABC* operons

Gene Family	Organism	Accession No.	*dszA* (bp)	Overlap *dszA/B*	*dszB* (bp)	Space/ overlap	*DszC* (bp)	% identity With

						dszB/C		*dsz-type1*
-type1	*Rhodococcus Erythropolis* IGTS8	U08850.1	1362	4	1098	10 (space)	1254	100
-type2	*Gordonia alkanivorans* 1B	AY678116.1	1425	67	1098	10 (space)	1251	~90
-type3	*Paenibacillus* sp. A11-2	AB033997.2	1365	4	1062	15 (space)	1245	~55
-type4	*B. Subtilus* WU-S2B	AB076745.1	1362	4	1071	4 (overlap)	1248	~71
-type5	*Mycobacterium* sp. G3	AB070603.1	1371	1	1071	4 (overlap)	1248	~70

Sequence of the regions, present upstream and downstream to *dszABC-type1*, has also been determined. Thus, sequence of a 9.7 kb DNA from *Rhodococus* sp. IGTS8, containing *DszABC* genes along with 1544 bp upstream and 4498 bp downstream region, has been described (Denome et al., 1994). Presence of a *Rhodococcus* promoter and at least three *dsz* regulatory regions were identified (Li et al., 1996) in the 1-385 bp regions, upstream to *dszA.* In the region downstream to *dszC*, presence of two sequences that were similar to the insertion sequence IS6120 orfB and IS1166 orfA, respectively, was observed. Similarly, in the 1-1800 bp region, upstream to *dszA* from the desulfurizing strain *Gordonia* sp. CYKS2, sequences similar to insertion elements IS1533 OrfA and IS1534 istB (AY396519.1) were present. In the strain RIPI90A, whose sequence in the upstream region is identical to that from CYKS2, a *dsz* promoter was identified in the region 50-156 bp, upstream to the *dszA* (Shavandi et al., 2010). The sequence showed only 52.5% identity to the promoter sequence of *R. erythropolis* IGTS8.

We have earlier reported the characterization of a bacterium *Gordonia* sp. IITR 100 that mediates the desulfurization of both thiophenic and non-thiophenic organosulfur compounds (Ahmad et al., 2014). In the present study, nucleotide sequence of an 11554 bp DNA, which includes the genes *dszA*, *dszB* and *dszC*, along with 3357 bp upstream and 4481 bp down-stream region, was determined and analyzed.

II. MATERIALS AND METHODS

The bacterium *Gordonia* sp. IITR100 was grown in medium-1(Na_2HPO_4, 2.0 g; KH_2PO_4, 1 g; $MgCl_2.6H_2O$, 0.4 g; NH_4Cl, 0.4 g; $Al(OH)_3$, 0.1 g; $SnCl_2.2H_2O$, 0.5 g; KI, 0.05 g; LiCl, 0.01 g; $MnCl_2.4H_2O$, 0.8 g; H_3BO_3, 0.05 g; $ZnCl_2$, 0.1 g; $CoCl_2.6H_2O$, 0.1 g; $NiCl_2.6H_2O$, 0.1 g; $BaCl_2$, 0.05 g; $(NH_4)_6Mo_7O_{24}.4H_2O$, 0.05 g, per liter) that contained 17.1 g sucrose and 50 mg dibenzothiophene (DBT) as carbon and sulfur source, respectively. All the DNA based procedures were done by the standard methods, as described earlier (Macwan et al., 2012).

Table 2. Primers used in the study

Primers	Primer sequence	Primer details

F1	GGAATTCCATATGGCTCAACGGCGACA ACTGCATCTGGCCGGTTTC	Contains 1-36 bases of 5'end of dszA and site for NdeI (underlined)
F2	GGAATTCCATATGACTCTGTCCGTTGA AAAGCAGCACGTTCG	Contains 1-32 bases of 5'end of dszC and site for NdeI
R1	CCGCTCGAGTCAGTGTGTCGAGGATG CCGGTATCAAGTTCTG	Contains 1-33 bases of 3'end of dszA and site for XhoI
R2	CCGCCCAAGCTTCTAGGAGGTGAAGC CGGGAATCGGGTA	Contains 1-27 bases of 3'end of dszC and site for HindIII

Briefly, the genomic DNA was isolated by phenol: chloroform extraction, and genes dszA and dszC were amplified by using primer sets F1-R1 (Table 2) and F2-R2, respectively. These were cloned in E.coli DH5α cells, after their ligation with the pGEM-T Easy (Promega Madison, USA) vector. Insert DNA were cut from the plasmids by restriction digestion, labeled with DIG-DNA Labeling Kit (Roche, Mannheim, Germany), and used as probes for Southern hybridization. In EcoR1- digested IITR100 total DNA, presence of two fragments (4.6 & 2.1k) and other two fragments (4.8 & 2.1 kb) was observed, after Southern analysis by using DszA and dszC probes, respectively (data not shown). DNA from the gel pieces that contained these fragments was eluted and cloned in DH5α cells. The transformants, carrying the dsz cross reactive fragments, were selected for sequencing. Briefly, the insert DNA from these clones was limit digested with Sau3A1. Fragments (~700 bp) were ligated with the BamH1 treated pGEM-T Easy vector, and cloned in DH5α cells. Nucleotide sequencing was done by using universal M13 primers (Macwan et al., 2012). Likewise, nucleotide sequence of a 3.7 kb PCR fragment that was obtained by using primers F1&R2 was also determined. The obtained 11554 bp sequence has been deposited under accession number GenBankKC693733.1under accession number GenBankKC693733.1

III. RESULTS AND DISCUSSION

Nucleotide sequen ces of larg e subsets of the geno mic DNA from an organism have been extrem ely useful i n providing informatio n about the structure, organization and regu lation of the genes (Denom e et al., 1994; Li et al., 1996). These have also been useful in understanding the past events th at might ha ve occurred during their acquisi tion, assembly, evolution, and transfer amongst d ifferent or ganisms (Yano et al., 2010). Results of this study revealed the sequen ce of an 11.5kb region of a strain IITR100. It contains *dszABC* ge nes along with the 3357 bp upstream and 4483 bp down-stream regi on. Southern hybridiz ation of the EcoR1 digest o f the total I ITR100 D NA, by usin g the dszA probe, reve aled the presence of t wo cross reactive fragments. One, the 4593 bp fragment that contained 1236 bp of *dszA* & 3357 bp upstream to it, and other the 2136 bp frag ment that contained p art of *dszA*, all of *dszB* and part of *dszC* (Figure 1). Similarly, of the two *dszC*-cross reactive fr agments, the 4825 bp fragment contained 344 bp o f *dszC* and 4481 bp d ownstream to it, and the 2136 bp f ragment w as same, which s howed hybridization with *dszA* probe also. T hus, the si ze of the genes *dszA*, *d szB* and *dszC* were 1425, 1098 and 1251 bp, respectively, and these we re present t ogether in the order *dszABC* as an operon (Figure 1). Moreove r, an overla p of 64 bp between *dsz A* & *dszB* and a gap of 11 bp between *dszB* and *d szC*, was also observe d. The sequence was > 99% identical to *dszABC -type2* genes (Table 1), reported earlier from the *Gord onia* strains 1B (AY678116.1), RIPI90 (EU36483 1.1), and C YKS2 (AY 396519.1). The result suggests that this gene is spread widely in different parts of th e the world, as shown earlier for *d szABC-typ e1* (Denis-L arose et al., 1997).

Figure 1. Sequenced DNA fragments; (a) EcoRI fragments th at showed h ybridization with *dsz* probes and (b) PCR product. Nu cleotides nu mber 1 and 3716 of the PCR pro duct correspon d to 3357 a nd 7073 of the 11554 bp sequence

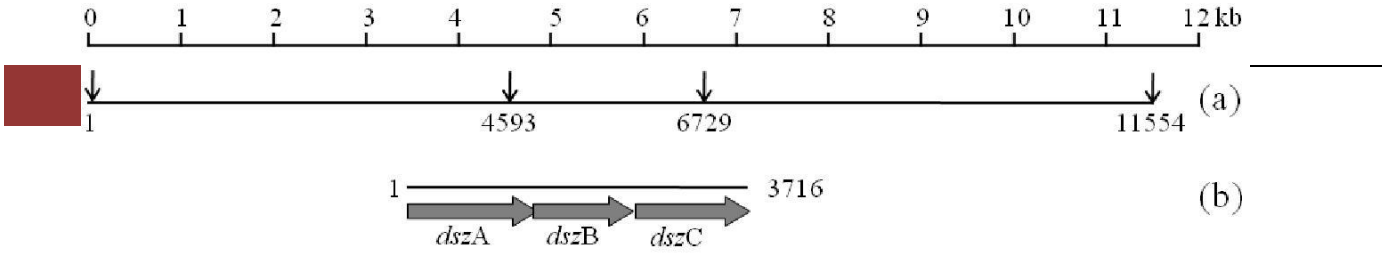

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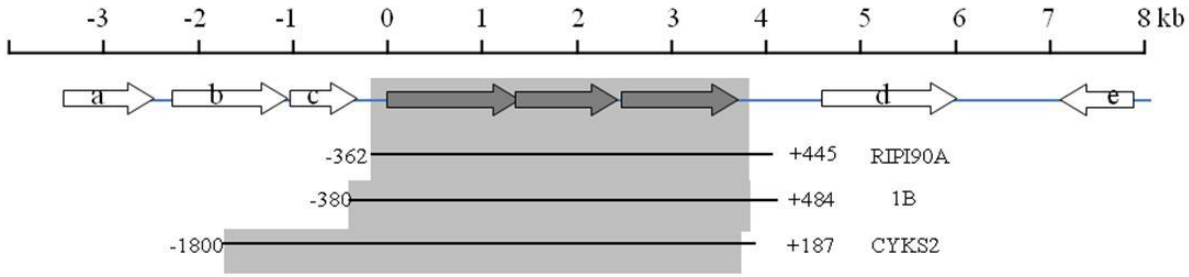
Figure 2. Characterization of the sequenced 11.5kb region, and its identity with known *dsz* genes. Broad arrows indicate the genes identified (a) Transposase IS1533 (AFJ34716.1), (b) Transposase IS1533 (AET20231.1), (c) Transposase IS1xx1 (CAM01140.1) (d) cytosine permease (ABL93443.1) and (e) short-chain ehydrogenase/reductase (ACY19419.1). Shaded regions denote sequences that are >99% identical to RIPI 90A (EU364831.1), IB (AY678116.1) and CYKS2 (AY396519.1)



In IITR 100, sequ ence up to -362, -380 & -1800 bp in the r egion upstream to dszA, was identica l to the rep orted sequences of th e corresponding region s from the strains RIP I90, 1B and CY KS2, respe ctively. Further analysis of the upstream re gion identified the presence of three transposase g enes at the sites 1-96 7 bp, 1047-2291 bp and 2334-309 4 bp that w ere >80% identica l to Tran sposase IS 1533 (AFJ34716.1), Transposas e IS1533 (AET20231 .1) and Transp osase IS1x x1 (CAM01140.1), respectively. Similarly, in the reg ion downstream to dszC, t he sequence of 204, 20 4 and 139 bp region as identica l to the corresponding regions of the strains RIPI90, 1B and CYKS2, respectively, but was distinct in the remaining region (Figure 2). Furthermore, presence of a cytosine permease (ABL93443.1) and a short-chain dehydrogenase/reductase (ACY19419.1) was identified at the sites 7985-9424 bp and 10529-11326 bp, respectively. While the presence of transposaes in the upstream region suggests that the dsz genes might have been imported in the past by their horizontal transfer from some other organism (Ochman et al., 2000), role of genes for permease and dehydrogenase enzymes in the downstream region is not clear at present

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