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Characterization of the Basic Replicon of *Rhodococcus* Plasmid pSOX and Development of a *Rhodococcus-Escherichia coli* Shuttle Vector†

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The replication region of a 100-kb desulfurization plasmid (pSOX) from *Rhodococcus* sp. strain X309 was localized to a 4-kb *Kpn*I fragment, and its sequence was determined. The amino acid sequence of one of the predicted open reading frames (ORFs) was related to the putative replication (Rep) protein sequences of the mycobacterial pLR7 family of plasmids. Three of the five predicted ORF products were identified by radiolabelling with the *Escherichia coli* T7 polymerase/promoter system. In *E. coli*, the Rep protein of pSOX was apparently synthesized in a shortened form, 21.3 kDa instead of the predicted 41.3 kDa, as a result of an internal initiation. This situation is reminescent of that for some bacterial Rep proteins. A shuttle plasmid was constructed with the pSOX origin, pBluescript II KS−, and the chloramphenicol resistance (Cm^r) gene from pRF29. This new shuttle plasmid was used to demonstrate expression of the *Bacillus subtilis sacB* gene in a strain of *Rhodococcus*, rendering it sensitive to the presence of sucrose.

Rhodococcus sp. strain X309 was one of the first biodesulfurization strains to be characterized at the molecular level (6–8, 16, 30). Like the prototype *Rhodococcus* sp. strain IGTS8, recently classified as *Rhodococcus erythropolis* (25), these bacteria are endowed with the property of specific cleavage of the carbon-sulfur bonds in model organosulfur compounds such as dibenzothiophene (DBT). By not breaking the carbon-carbon backbone, the biodesulfurization process has advantages including conservation of the calorific value of fuels and elimination of noxious emissions of sulfur oxides into the atmosphere when these sulfur-laden compounds are combusted (12, 22, 27).

A long-recognized shortcoming of the biodesulfurization process is its inhibition by the presence of sulfate (22). Sulfur-containing amino acids (methionine and cysteine) also exert a negative effect on desulfurization carried out by the *dsz* genes (also known as *sox* [6, 8]) in strain IGTS8 (25); that sulfur acts by repression of the native desulfurization promoter was demonstrated (25).

We are interested in promoter replacement as a possible strategy and an alternative to expression of the *sox* genes in a heterologous host as a means of alleviating the sulfur repression problem. We reckoned that a native *Rhodococcus* may contain yet uncharacterized factors or membrane properties necessary for uptake or transport and eventual desulfurization of the sulfur-containing compounds. Toward this goal, the replication region of the *sox*-containing plasmid, pSOX, indigenous to *Rhodococcus* sp. strain X309 (6) was delineated. A shuttle plasmid was then constructed, and the utility of the *sacB* promoter in the expression of the *Bacillus subtilis* levansucrase-encoding gene (37) was demonstrated. Additional impetus for this study was provided by the general lack of information on the characteristics of plasmid replicons indigenous to members of the gram-positive genus *Rhodococcus* (4, 18). This information is not only useful in cataloging known replicons and incompatibility groups but also invaluable as a molecular tool for probing potentially new "environmental" plasmids.

MATERIALS AND METHODS

Bacterial strains and plasmids. *Rhodococcus* sp. strain X309-11-15 was used as the source of the 100-kb pSOX plasmid (6), and *Rhodococcus* sp. strain X309-10-2 (henceforth referred to as strain 10-2), a plasmid-free derivative of the parental *Rhodococcus* sp. strain X309 (6), was used as the rhodococcal host. Growth media and plasmid isolation procedures were as previously described (6, 34). Deletion derivatives of the pSOX plasmid described in this study are represented in Fig. 1. The *Escherichia coli* host for recombinant plasmids was strain DH10B (Gibco-BRL). Plasmids pUM24 (33) and pRF29 (9, 10) were kindly provided by J. Wall (University of Missouri), and K. Young (University of North Dakota), respectively; pBluescript II KS− vector was purchased from Stratagene, LaJolla, Calif. *E. coli* K38(pGP1-2) cells were grown by the method of Tabor (39) for expression of the plasmid-encoded gene(s) cloned in the pT7-5 vector.

Localization of the replication region of pSOX plasmid. Plasmid DNA was introduced into strain 10-2 by electroporation with a Bio-Rad Gene Pulser apparatus. The conditions for electroporation were as described for *Rhodococcus* sp. strain M5 (24). Plasmid pSOXΔ1, 52 kb in size (Fig. 1), was the first deletion derivative of the pSOX plasmid isolated from the electrotransformed 10-2 strain as a result of repeated selection on DBT-containing plates (6). Digestion of pSOXΔ1 by *Eco*RI produced five fragments. Additional restriction endonuclease digests provided the map shown in Fig. 1; the largest *Eco*RI fragment (30 kb) contains the *soxABC* genes (6–8), since it probed positive with a 4-kb *sox*-containing DNA fragment in a Southern hybridization experiment (6).

Introduction of pSOXΔ1 into strain 10-2 and further selection on DBT plates resulted in a yet smaller plasmid, pSOXΔ2. Since pSOXΔ2 was still quite large (42 kb), a series of defined manipulations which led to the localization of the pSOX replicon to a 4-kb *Kpn*I fragment were carried out (Fig. 1).

First, self-ligation of the 30-kb *sox*-containing *Eco*RI fragment derived from pSOXΔ2 (pSOXΔ3 in Fig. 1) resulted in a transformant that was capable of replication in strain 10-2. On the other hand, similar ligation of the end-filled 12.6-kb *Eco*RI-*Xba*I fragment (leftmost *sox*-containing portion) did not result in any transformants. To test whether the rightmost 15-kb *Xba*I-*Eco*RI fragment could replicate, pSOXΔ3-Cm was constructed by addition of a chloramphenicol resistance (Cm^r) marker gene from pRF29 (13.2 kb) (9, 10) at the unique *Eco*RI

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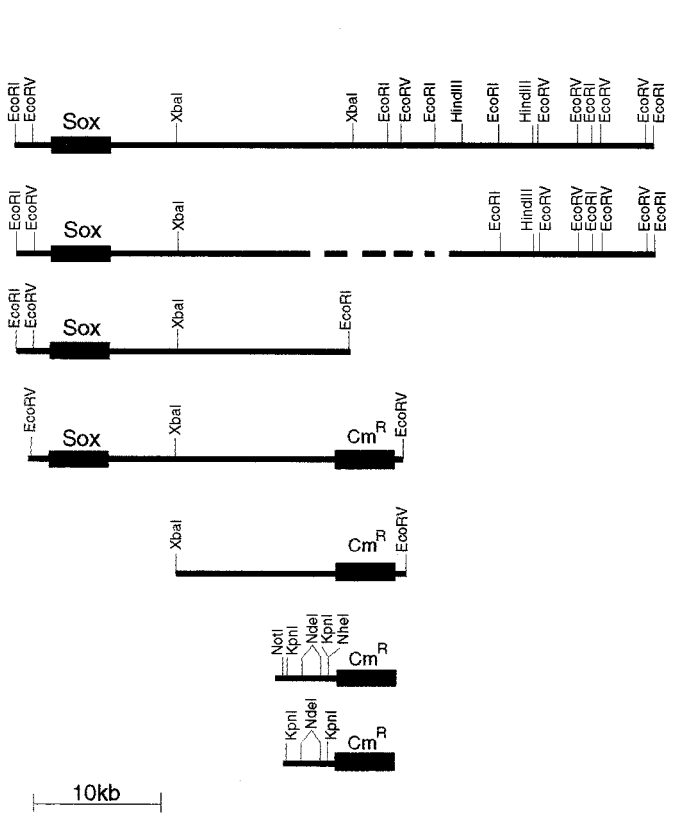

pSOX derivative	Kb	Phenotype *sox*	Phenotype *ori*
Δ1	52	+	+
Δ2	42	+	+
Δ3	30	+	+
Δ3-Cm	33	+	+
Δ4	18.5	−	+
Δ5	9	−	+
Δ6	7.5	−	+

FIG. 1. Localization of the pSOX plasmid replicon from *Rhodococcus* sp. strain X309. The ability of each plasmid to replicate (*ori*) or possess desulfurization activity (*sox*) is shown at the right by the plus and minus signs. Cm^r indicates the chloramphenicol resistance marker gene derived from pRF29 plasmid (10). ---, undefined boundary of deletion.

site of pSOXΔ3. To facilitate cloning, both the linearized vector and the 3.5-kb *Xba*I-*Xho*I DNA fragment containing the Cm^r gene were blunt ended by filling in with deoxynucleoside triphosphate substrates and Klenow DNA polymerase I (34). Subsequent digestion by *Eco*RV and *Xba*I yielded an 18.5-kb fragment which, after end filling, ligation, and transformation in strain 10-2, gave rise to many colonies on chloramphenicol (30 μg/ml) selective media. Surprisingly, when the plasmid content of several transformants was analyzed, two populations of cells were obtained: one harboring the expected 18.5-kb plasmid (designated pSOXΔ4) and the other harboring a 9-kb derivative (designated pSOXΔ5). The pSOXΔ5 plasmid was mapped (Fig. 2) and used as the basis for further constructions.

The pSOXΔ6 plasmid was derived by ligating the 4-kb *Kpn*I fragment to the 3.5-kb Cm^r gene cassette. Because of the incompatible ends of these restriction fragments, repair for blunt-end ligation was carried out accordingly. Similar ligation of the 2.2-kb *Nde*I fragment to the Cm^r cassette did not yield any Cm^r transformants.

When screening for the 7.5-kb pSOXΔ6 plasmid, we observed a deletion of ca. 400 bp in one derivative, designated pSOXΔ6-1. This small deleted region was localized to the end of the 4-kb *Kpn*I fragment that has a *Bam*HI site (Fig. 2). Both pSOXΔ6 and pSOXΔ6-1 were subsequently used for the construction of *Rhodococcus-E. coli* shuttle vectors.

Construction of *Rhodococcus-E. coli* shuttle vectors. Either plasmid pSOXΔ6 or plasmid pSOXΔ6-1, linearized by *Sph*I, was ligated to the *Nae*I site (within the f1 origin) of the pBluescript II KS− plasmid. In each case, it was necessary to end-fill the restriction fragments. The pBluescript vector provides the *col*E1 replication origin, a multiple cloning site, the *lacZ*α reporter gene for screening inserts in *E. coli* (blue-white selection), and ampicillin resistance (Ap). Since *Nae*I digestion disrupts the phage f1 origin, it is anticipated that single-stranded DNA production will be lost in these shuttle plasmids. The resultant plasmids are designated pKSΔ6 and pKSΔ6-1, respectively.

DNA sequencing and analysis. The 4.6-kb *Not*I-*Nhe*I fragment which contains the 4-kb *Kpn*I fragment was cloned at the equivalent sites of pBluescript KS− vector, and its sequence was determined on both strands by primer walking with the automated fluorescence sequencer (Applied Biosystems model 373A) and the T7 sequencing kit. Sequence analysis was performed with the BLAST programs (1) of the National Center for Biotechnology Information (Bethesda, Md.) and the PC/Gene package (IntelliGenetics Inc., Mountain View, Calif.).

Nucleotide sequence accession number. The 4,584-kb sequence of the *Nhe*I-*Not*I fragment of pSOX of *Rhodococcus* sp. strain X309 has been assigned GenBank accession no. AF059700.

RESULTS AND DISCUSSION

Sequence features in the pSOX replication region. In vivo and specific deletion experiments accompanied by appropriate genetic markers led to the localization of the pSOX replication region to a 4-kb *Kpn*I fragment (Fig. 1). A summary of the predicted open reading frames (ORFs) in this sequenced DNA region and expression of three of the ORF products in *E. coli* K38 (pGP1-2) cells (Fig. 3) is described below.

ORF164. ORF164 (nucleotides 77 to 571, encoding a product of 164 amino acids) encodes a product whose amino acid sequence is 31.4% identical to TrbA (103 amino acids) of the broad-host-range IncPα plasmid RK2 (21). Protein expression of ORF164 was evidenced by the 18.7-kDa labelled band (cf. the predicted M_r of 18,117) derived from three independent clones (pTEN, pTEB, and pTEH) based on the pT7 system (Fig. 3). The ORF164-related TrbA protein functions as a repressor that controls both vegetative replication and conjugative transfer of the RK2 plasmid (21, 28). Like TrbA, ORF164 has a predicted high isoelectric point (pI = 10.6). Among the conserved amino acid sequence is a putative helix-turn-helix motif near the N terminus and a leucine zipper motif at amino acids 68 to 82 (LKQIAQELDVSISVL). The latter motif provides the possible basis for protein dimerization, as noted previously for the TrbA-related proteins (21). There is no evidence yet for a repressor role of ORF164, but its involve-

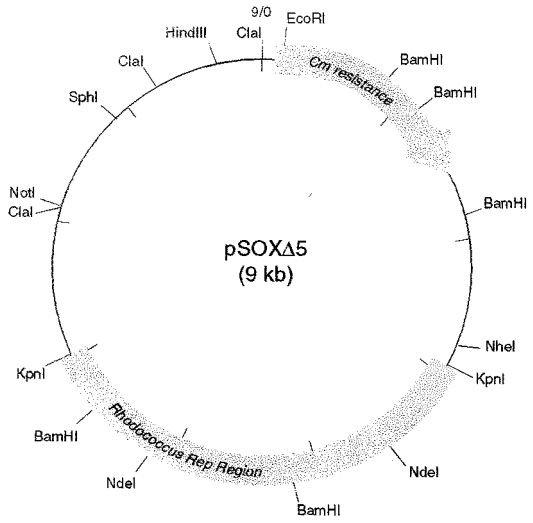

FIG. 2. Restriction map of a replicating pSOXΔ5 derivative. The 4-kb *Kpn*I fragment, but not the internal 2.2-kb *Nde*I fragment, that is sufficient for replication is as outlined. *Nhe*I, *Not*I, *Sph*I, and *Hin*dIII are unique restriction sites that are potentially useful for cloning. Although the *Eco*RI site is also unique, disruption of the plasmid at this site would probably eliminate selection in the presence of chloramphenicol.

ment in plasmid replication and/or maintenance is supported by the fact that the 4-kb *Kpn*I fragment of pSOX, but not the internal *Nde*I fragment, is sufficient for replication (Fig. 3).

ORF81. ORF81 (nucleotides 721 to 966, encoding a product of 81 amino acids with a predicted pI of 4.7) encodes a product that is unusual in not having cysteine, tryptophan, or tyrosine. The amino acid sequence of this polypeptide has no apparent counterpart in the available protein databases; expression of ORF81 as an 11-kDa protein (predicted M_r, 8,787) appeared only in the pTNN clone when the putative initiator codon of ORF81 was placed adjacent to the T7 promoter. Unlike the pTEB and pTEH plasmids, the pTNN DNA is devoid of the ORF164-ORF81 intergenic sequence which can potentially form several secondary structures that may prevent or attenuate the expression of ORF81 (results not shown).

ORF368. ORF368 (nucleotides 1403 to 2509) encodes a product which is most probably a replication (Rep) protein (Fig. 4). Homology to the putative Rep proteins encoded by the mycobacterial plasmids pLR7 of *Mycobacterium avium* (2), pJAZ38 of *M. fortuitum* (14), and pMSC262 of *M. scrofulaceum* (14, 31) is most extensive at the N-terminal portions. Although the putative Rep protein of pMSC262 is shorter (see reference 14 for a discussion), all four proteins have a high arginine, tryptophan, and tyrosine content. The pSOX Rep protein is predicted to be basic (the calculated pI is 11.2).

The predicted M_r of pSOX Rep is 41,328, but from two independent clones a protein band of only 21.4 kDa was observed (Fig. 3). Analysis of the nucleotide sequence preceding the presumptive initiator codon of pSOX Rep showed a weak consensus ribosome-binding site (RBS) sequence (gcggtactgcc agATG; potential RBS underlined). Instead, initiation at an internal GTG codon (positions 1949 to 1951), preceded by a strong consensus RBS (GAGG), would produce a 186-amino-acid residue protein of approximately 21.4 kDa. Such internal initiation or in-frame translation has been noted in the broad-host-range plasmid RSF1010, in which RepB was found to exist as either a 70-kDa or a 38-kDa (RepB*) form (36), and in the RK2 plasmid of *Pseudomonas aeruginosa*, where a 44-kDa form of TrfA is required for RK2 replication in *P. aeruginosa* but a 33-kDa form of TrfA is sufficient for replication in many other bacterial species, including *E. coli* (11).

During the preparation of the manuscript, the DNA sequences of three replicons derived from cryptic plasmids of *R. erythropolis*, *R. equi*, and *R. rhodochrous* were determined (5, 23, 40). The pFAJ2600 plasmid of *R. erythropolis* was found to contain two potential Rep proteins; RepA (310 amino acids) and RepB (93 amino acids), both of which are predicted to be basic (5). The replication region of the pKA22 plasmid of *R. rhodochrous* (23) was also predicted to contain two ORFs (encoding the ORF1 protein, of 242 amino acids, and the ORF2 protein, of 296 amino acids). In *R. equi*, a putative protein of 240 amino acids, with no apparent counterpart in the available database, was assumed to be a Rep protein of the pTOS plasmid (40).

Interestingly, RepA of pFAJ2600 and ORF2 of pKA22 are related to one of the two Rep proteins (ORF1 or RepA; 307 amino acids) of *Mycobacterium fortuitum* plasmid pAL5000 (38). A family of pAL5000-like replicons hence emerged (5). By the same token, the Rep protein of pSOX from strain X309 is believed to belong to the family of the pLR7 replicon (2), which now has four members (Fig. 4). In all these cases, further characterization of the Rep proteins, together with their possible binding site(s), is required. There is an 11-bp tandem repeat, 5′-GTCCGCGGGCA, which is 76 bp upstream of the potential start codon of ORF368. It is likely that this tandem

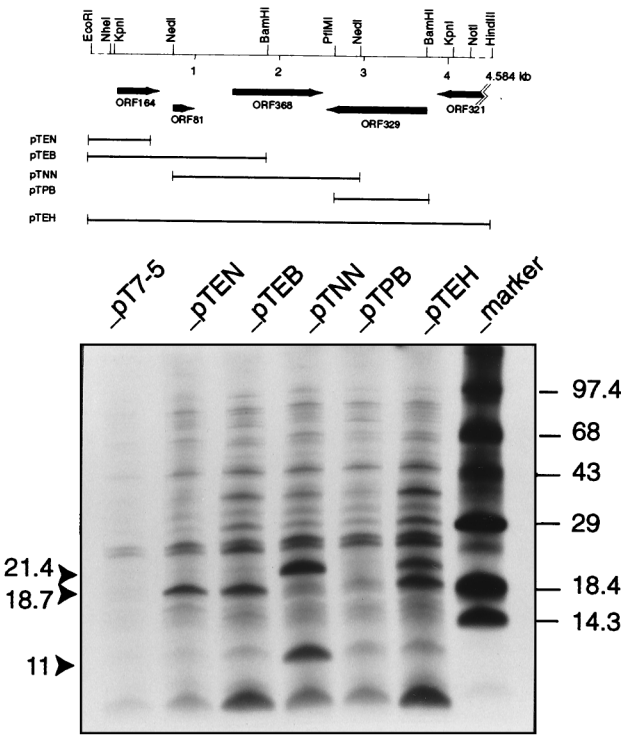

FIG. 3. Predicted ORFs in the pSOX replication region and [^{35}S]methionine identification of proteins. Locations of ORFs and direction of transcription are indicated by arrows. Protein labeling was carried out as described by Tabor (39). Expression plasmids derived from the pT7-5 vector (control) are pTEN (0.72-kb *Eco*RI-*Nde*I [Klenow blunted] cloned in the *Eco*RI-*Sma*I sites), pTEB (1.86-kb *Eco*RI-*Bam*HI cloned in the *Eco*RI-*Bam*HI sites), pTNN (2.2-kb *Nde*I [Klenow blunted] cloned in the *Sma*I site), pTPB (1.05-kb *Pfl*MI [T4-blunted]-*Bam*HI cloned in the *Sma*I-*Bam*HI sites), and pTEH (4.6-kb *Eco*RI-*Hin*dIII cloned in the *Eco*RI-*Hin*dIII sites). The calculated molecular sizes indicated by the solid arrowheads are those of the pSOX truncated Rep (ORF368), ORF164, and ORF81 products in descending order. Since low-molecular-weight proteins were being analyzed, the gel recipe (10% T–3% C) of Schagger and von Jagow (35) was used.

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pSOX      MR-------LDLGDGAYNGIPIWQGAQ-HWVEIAVREAYTAEYK-NIRPA  41
pLR7      MP--------------YAGVPCWTGTQ-RWAQWTVPVAYDLRYDTDVRPH  35
pJA238    MPAPSEFVGLELDADAYAGVPCWSGGPAHWAHVTVAVAYDVHYAM-VRPR  49
pMSC262   V--------LELGEAPYAGVPCWTGRLERWARWTVAVAYDCRYDAQVRPV  42
          .                *.*.* * *   .*.  .*  **.  *   .**

          LVETTGGGISLKTLLAVATVMASVAEFDTGRESRLSLDKTIERTGKGERT  91
          M---GANQISRRALLRIAEARARYADYATGRDCRPSNERLATDTGYDVRT  82
          M---CNGGIARTTLIVIAAAMAQTADWDTGRNCRPTNEQLEAATGFDERT  96
          M---PGNPISRRALLAIARARARYADHATGRNCRPSNERLAADTGYSVRT  89
          .    .. *.  .*. .* ..*  *. .***..* . ..  . ** . **

          VQRARQALKLLRVATEVFRGRLRRKKGERQGSYRVGDKGRGWASVWALHL 141
          IQRASTVLRLLGVATEVLRGR-QRTRIERLASWRVGDRGRGWASVWALHD 131
          IQGAHECLRLLGVATEVLRGR-QRTYTERMPPWRMGDRHRGWPSLWALHG 145
          VQRADTVLRLLGVATEVLRGR-QRTRVERLASWRVGDRGRGWASVWALHD 138
          .* *   *.** *****.*** .*.  **   ..*.**. ***.*.****

          RKPVDKTRVYLDGSIKMAPHPRRGHLLSLPSRREVINTKRSVNKRAAPRR 191
          HRLLN--RVIHKVQSVLSPHPRSGPVRDQHVRQDVVTT--RNRRRTGA-- 175
          NPHIA--RVVHSLSPHL--ERSQATTKNSPLKRLVTTQ--GGRKRPAP-- 187
          NPQLA--RFVQR----LSPHPRSGPVRDQPSGKDVVTT--GA---GGP-- 175
          .  ..  *        .  ... .  . .  . *..        ..

          KESKAEVEKVEAIRKGLLLASKWLSNPRTPVWARRHTPRGWASALTEPAA 241
          -GNRGAARRARPDGYGLALAKTWRAHPQAPPWCHRHSPTAWAAILAAPAA 224
          -K---PARRRAPDEAGRRLATRWRADRHAPPWVRTYAADSWAAMLAAPAA 233
          -AGRRQGRRCAPPS------TRWRGVGAGAGLARRCARPA---------- 208
                .  .        . * .   ..   .  .  .

          HGWTAADLNDTIDDWANAQNMVP-TPKHPIAF----IRWLMKQQDLAFAP 286
          AGWTPRDLNQLITDWLGVGHWIPDTPHKPIGLLGAILAWHGPEN-LAERP 273
          AGWTPADLTALVRDWLSTGHWVPDVPARPIALLGTMLAWHTSHNSLEDRP 283
          ---------------------.--VGAPAHRWSLGGAV------------- 222
                               .* .. ..

          HVLAQIAADQEKAERER------QSAALEMERERYASAAPEDSPGRQAAR 330
          AALDEAREAQARAANEQLRRAESATSHRAHLAGRAAAQAAQSGPGRAEAF 323
          AALDEAREAEELAAARRCVRDQF-RAHDEYATDRATAQAALDGPGHAAAR 332
          -----GRAGRVWLDAARSQRAHYRLGRGDRPAHPRSPAQADRTAGRDAGL 267
                ..   .  .       .  .    . .....  ..*....

          LVARRAADTARCRKVDTSARENAAQPVWITHLRDLGPQ 368
          AALAAARQRSAQRRTAQAAAEQARIDALIERART-PRR 360
          QAVAEAVRRAACKRTVVVAAETAQFAAMVQTARS-PR- 368
          -----AWPRA----------------LGRASG-RPG 281
               *   .                .  ..  .
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FIG. 4. Amino acid sequence alignment of the predicted replication proteins from plasmids pSOX (this study), pLR7 (*M. avium* [2]), pJAZ38 (*M. fortuitum* [14]), and pMSC262 (*M. scrofulaceum* [31; see reference 14 for a discussion]). The CLUSTAL program of PC/Gene was used. Asterisks indicate invariant residues. Dots indicate chemically similar residues. Pairwise alignment of either the pLR7 or the pJAZ38 Rep sequence with that of ORF368 gave 34.5% identity and 47% overall similarity. Note that the relatively short sequence of the putative Rep protein of the *M. scrofulaceum* pMSC262 plasmid skews the sequence similarity and identity at the C termini of the first three proteins.

repeat will be essential for binding to initiate replication (4, 18). Alternatively, inverted-repeat sequences found in the noncoding sequence of ORF368 are potential binding sites (4, 18).

ORF329. ORF329 (nucleotides 2520 to 3509) encodes a protein whose predicted amino acid sequence in the complementary strand was found to have 39.2% overall similarity to a protein of an equivalent size (345 amino acids) in the *Mycobacterium tuberculosis* cosmid sequence (GenBank accession no. Z95436; PID: e316540). The functions of these proteins, which are predicted to be acidic, are unknown. The C terminus of the ORF329 product has a segment (positions 229 to 245) that is sufficiently hydrophobic to qualify it as a transmembrane segment. The molecular size of this protein has not been verified, but the complementary strand (clone pTPB, Fig. 3) appeared not to encode any protein.

ORF321. ORF321 (nucleotide 3619 to the *Not*I site) encodes a protein whose sequence is homologous to the hemoprotein domains of various bacterial cytochrome P-450 systems (17). The highest score in the BLAST search (43.3% identity and 58.9% overall similarity) came from the hemoprotein domain of the cytochrome P-450 of *Bacillus megaterium* (32). This is an interesting finding since it suggests a possible cytochrome P-450 function in the pSOX plasmid.

Sucrose sensitivity as a positive selection marker in *Rhodococcus* sp. strain X309. The production of levansucrase, encoded by *sacB* of *Bacillus subtilis* (37), is lethal in the presence of sucrose in numerous bacteria (15, 19, 20, 29). By screening for *sacB* inactivation, this positive selection system has been found useful in the isolation of insertion sequence elements from numerous bacteria including *R. fascians* (15, 19, 20, 29).

TABLE 1. Effect of sucrose on growth of *Rhodococcus* sp. strain X309-10-2 transformed with pKSΔ6-1 or pKSΔ6-1 containing *B. subtilis sacB*

Strain	×309 growth (CFU) in:		
	LB	Selective medium[a]	
		Without sucrose	Plus 10% sucrose
10-2 (pKSΔ6-1)	>500	>500	>500
10-2 (pKSΔ6-1*sac*)	326	305	0
10-2 (pKSΔ6-1*sac*)[b]	232	236	0

[a] LB medium with chloramphenicol (30 μg/ml). (cfu) are from 10 μl of a 10^{-4} dilution.
[b] An independent clone.

Since sucrose sensitivity was demonstrated only once in the genus *Rhodococcus*, we wished to confirm this in strain X309 as a prelude to exploring the possibility of using the promoter elements of *sacB* to drive *sox* gene expression. To test this, *Rhodococcus-E. coli* shuttle vectors (pKSΔ6-1 and pKSΔ6 [10.1 and 10.5 kb, respectively]) were constructed (see Materials and Methods), and both plasmids were found to be capable of replication in *E. coli* and *Rhodococcus* by selection on Luria-Bertani (LB) plates containing ampicillin (50 μg/ml) or chloramphenicol (25 to 40 μg/ml). Figure 5 outlines the cloning of the *sacB* gene in plasmid pKSΔ6-1. Selection in *E. coli* for Ap^r and Km^r produced many white colonies, two of which were electrotransformed into strain 10-2 and plated on selective media (containing chloramphenicol at 30 μg/ml). Subsequently, plasmids were isolated and their size was determined by restriction endonuclease digestion. No deletion was apparent in these plasmids. The orientation of the *sacB* gene was from left to right, the same direction as that of Ap^r (Fig. 5).

The effect of 10% sucrose on the growth of two clones and a control strain containing the shuttle vector alone in LB selective medium is presented in Table 1. This result demonstrated that sucrose sensitivity is a screenable phenotype in strain X309. Like the previously described *sacB*-sensitive systems, the basis for toxicity of levansucrase action on sucrose is

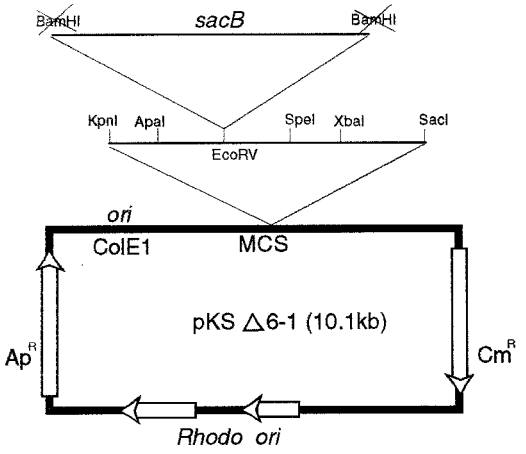

FIG. 5. Construction of the pKSΔ6-1 *Rhodococcus-E. coli* shuttle vector for *sacB* expression in *Rhodococcus* strain 10-2. MCS, multiple-cloning site (expanded region). Blunt-end cloning of the end-filled 3.8-kb *Bam*HI fragment of pUM28 plasmid containing *sacB* (33) at the *Eco*RV site is indicated.

unknown. The fact that not all gram-positive bacteria are sensitive to sucrose (20) suggests that perhaps a different "sucrose-sensing" pathway or device exists in these organisms. Sucrose was recently shown to act as a signal molecule in the control of resource allocation between plant tissues (3).

Preliminary experiments in our laboratory, using the *sacB* promoter to drive *soxABC* expression in cells of *Rhodococcus* sp. strain 10-2 grown in LB broth and added DBT, have produced some of the expected products of DBT metabolism (26). Further experiments are needed to show the possible advantages of the present approach. The feasibility of *sox* gene expression alleviating the sulfate inhibition problem was recently demonstrated in *Pseudomonas* (13).

Finally, the relatedness of at least one of the putative Rep proteins of the rhodococcal replicons to those of the *Mycobacterium* plasmids appears to be an emerging feature in these two genera. Like pLR7 and pJAZ38 (2, 14), the pSOX replicon may expand the transformation spectrum of mycobacteria, an important genus in medicine. Thus far, the majority of vectors available for use in mycobacteria are based on the pAL5000 system (5, 38). Isolation of a new rhodococcal replicon also offers new potentials for complementation studies.

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