KorSA from the *Streptomyces* Integrative Element pSAM2 Is a Central Transcriptional Repressor: Target Genes and Binding Sites

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pSAM2, a 10.9-kb mobile integrative genetic element from *Streptomyces ambofaciens*, possesses, as do a majority of *Streptomyces* conjugal plasmids, a kil-kor system associated with its transfer. The kor function of pSAM2 was attributed to the korSA gene, but its direct role remained unclear. The present study was focused on the determination of the KorSA targets. It was shown that KorSA acts as a transcriptional repressor by binding to a conserved 17-nucleotide sequence found upstream of only two genes: its own gene, korSA, and *pra*, a gene positively controlling pSAM2 replication, integration, and excision. A unique feature of KorSA, compared to Kor proteins from other *Streptomyces* conjugal plasmids, is that it does not directly regulate pSAM2 transfer. KorSA does not bind to the pSAM2 genes coding for transfer and intramyecial spreading. Through the repression of *pra*, KorSA is able to negatively regulate pSAM2 functions activated by Pra and, consequently, to maintain pSAM2 integrated in the chromosome.

A large number of mobile genetic elements, including plasmids and integrative elements capable of site-specific integration into the chromosome, have been identified in *Streptomyces*, *Saccharopolyspora*, *Amycolatopsis*, and other gram-positive bacteria belonging to the order Actinomycetales (12, 18). Most of the *Streptomyces* elements are self-transmissible and able to mobilize chromosomal markers. Transfer in *Streptomyces* is usually associated with pock formation characterized by a local inhibition of growth and development of the recipient strain in contact with the donor strain (1, 12). Bacterial plasmids often contain conditionally lethal genes (reviewed in reference 9). Certain genes (*kil* genes) specify functions lethal to either the host or the plasmid, while others (*kor* genes) encode products that control expression of the *kil* phenotype. It is impossible to obtain transformants with derivatives in which the *kor* gene is inactivated. The presence of a kil-kor system associated with transfer has been identified for all *Streptomyces* plasmids able to form pocks during conjugative transfer. The kil function was attributed to the *tra* genes (transfer genes), whose expression was lethal in the absence of the *kor* gene. Most of the identified *kor* genes code for proteins that belong to the GntR family of transcriptional regulators (8) and contain in their sequences a DNA binding motif. For all known cases, Kor directly regulates the expression of *tra* and also the transcription of its own gene. The best-studied *Streptomyces* plasmid, pIJ101, carries two kil-kor systems, *kilA-korA* and *kilB-korB* (16), and both KorA and KorB directly regulate the expression of the genes involved in transfer as well as their own synthesis (16, 26, 27). The different affinities of the processed form of KorB for its operators situated in the promoters of *kilB* and *korB* (34) allow it to maintain the repression established by KorB (29). For pSN22, the TraR (Kor) binding sites were located in the overlapping promoters of the *traR* gene and the *traA-traB-spdB* operon (14, 15). Conjugative plasmid pSG5 from *Streptomyces ghanaensis*, which is unable to form pocks during its transfer, is the only known example in which the *tra* gene (major transfer gene) does not represent a *kil* function. A gene similar to *kor* was found in its sequence, but its direct role remains unknown (17).

The organization of the *kil-kor* system for integrative elements follows the same pattern, with the *Kor* protein being indispensable for the transcriptional repression of the *tra* gene. The *kil-kor* systems of the integrative element pMEA300 from *Amycolatopsis methanolica* (31) and of SLP1 from *Streptomyces coelicolor* (4) are examples. ImpA (*Kor*) of SLP1, which is thought to be a regulator of SLP1 transfer, can bind to a 16-bp operator situated in the promoter of its own gene (24).

pSAM2 is present as one integrated copy in the chromosome of *Streptomyces ambofaciens* strain B2 (pSAM2<sub>12</sub>) and is found simultaneously as one integrated copy and 5 to 10 replicating copies per genome in the independently obtained strains B3 and B4 (pSAM2<sub>B3</sub> and pSAM2<sub>B4</sub>) (19). A kil-kor system associated with transfer seems to be present in pSAM2. The major transfer gene, *traS*, as well as the genes *spdA*, -B, -C, and -D, which are possibly involved in intramyecial spreading, have been identified (7). The hypothesis about the direct role of korSA in the regulation of *kil*, based on the inability of the deletion derivatives lacking korSA and also *pra* to transform was reconsidered after the discovery that *pra* is indispensable for the establishment of pSAM2 in the new host, as it activates pSAM2 replication and integration (22).

In this study we demonstrate that the KorSA protein encoded by pSAM2 is a transcriptional repressor of its own gene, korSA, and of the *pra* gene, which was shown to code for an activator of pSAM2 replication, integration, and excision (22, 23). While the expression of *traS* in pSAM2 seems to be under the control of KorSA, no binding sites could be detected in that region, contrary to the widely adopted model of the organization of the *Streptomyces kil-kor* systems. KorSA seems to code for the pSAM2 major repressor controlling, via the *pra* gene, the main functions of this genetic element.
TABLE 1. Plasmids

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Construction</th>
</tr>
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<tbody>
<tr>
<td>pOS530 ............</td>
<td>ptra gene promoter (a fragment from nt 1766 to 1904 in AJ005260) from pSAM2B3 cloned after PCR amplification as the BamHI-EcoRI fragment in the promoter vector pJ48/Hygro</td>
</tr>
<tr>
<td>pOS556 ............</td>
<td>BamHI-HindIII fragment of pSAM2B3 containing the korSA gene in the integrative vector pTOL1 (22); orf131 present in the fragment was inactivated by deletion of the BamHI-BglII fragment; contains the trs gene conferring resistance to thiostrepton</td>
</tr>
<tr>
<td>pOS699 ............</td>
<td>pSAM2 derivative with the korSA gene inactivated by the NcoI-NcoI fragment; the NcoI site was modified by Klenow polymerase, introducing a frameshift</td>
</tr>
<tr>
<td>pOS572 ............</td>
<td>korSA gene promoter (a fragment from nt 884 to 995 in AJ005260) from pSAM2B3 cloned after PCR amplification as the BamHI-HindIII fragment in the promoter vector pJ48/Hygro</td>
</tr>
<tr>
<td>pIJ48/Hygro......</td>
<td>pIJ48 (32) derivative in which the hyg gene (33) was cloned in the EcoRV site; HygTsr</td>
</tr>
</tbody>
</table>

a All plasmids were constructed in this work.

MATERIALS AND METHODS

Bacterial strains and plasmids. Streptomyces lividans TK24 is the commonly used host strain for cloning experiments (13). S. albusluciens strains have been described elsewhere (19). The plasmids used are listed in Table 1.

Culture and transformation conditions. General culture and genetic techniques for Streptomyces spp. were as described by Hopwood et al. (11), and those for Escherichia coli were as described by Sambrook et al. (21). Streptomyces transformants carrying the thiostrepton resistance (trp) gene (30) were selected using 50 μg of nosophile per ml.

Recombinant clones of S. lividans TK24 containing derivatives of pIJ48/Hygro carrying the aprb resistance gene transcribed from a heterologous promoter (pOS530 and pOS572) were selected on R2YE medium containing 80 μg of hygromycin B per ml. To determine the level of kanamycin resistance, spore suspensions of the recombinant clones were spread on minimal medium (11) containing increasing concentrations of kanamycin monosulphate (Sigma). For strains S. lividans/pIJ48/Hygro and S. lividans/pOS55/pPSS30, the initial transformants were obtained after double selection on medium containing nosophile and hygromycin B.

DNA isolation. Plasmid DNA was isolated from E. coli and from Streptomyces spp. by alkaline lysis (11). Total DNA was isolated as described by Hopwood et al. (11).

Status of pSAM derivatives in S. lividans. Total DNAs of recombinant strains digested with EcoRI were checked for the presence of free and/or integrated pSAM2 sequences by Southern hybridization with the [α-32P]-dATP-labeled EcoRI-EcoRI fragment of pSAM2 containing the attP site.

RNA isolation, Northern hybridization, and high-resolution S1 mapping. Total RNA from Streptomyces was isolated as described by Hopwood et al. (11). For Northern hybridization, total RNA (40 to 50 μg) was denatured with glyoxal and dimethyl sulfoxide and, after electrophoresis, was transferred to a Hybond-N filter (Amersham). The filter was hybridized with a radiolabeled probe corresponding to the korSA gene (the 0.8-kb PCR fragment synthesized with the oligonucleotides Bam-kor-pr [5′-ACGGAGATCC TGGCGCAGACCCACTTGA] and Hind-ko-pr [5′-TTCAAGCCTTGTT CCAATAGTCTTCCTG]). This 111-bp DNA fragment containing the korSA gene promoter (0.4 pmol) was radiolabeled with Klenow polymerase (BamHI site) and then incubated with the MBP-KorSA protein at 30°C for 10 min in binding buffer. Samples were diluted twofold with DNase I buffer (10 mM HEPES-HCl [pH 7.8], 5 mM MgCl2, 1 mM CaCl2, and 15 mM NaCl) and treated with 5 ng of DNase I for 1 min at room temperature. Samples were precipitated with ethanol in the presence of 0.3 M sodium acetate, resuspended in 0.5% formamide containing 20 mM EDTA, and loaded onto a 6% PAA sequencing gel. The gel was fixed in 10% acetic acid–10% methanol and dried, and radioactive bands were visualized by autoradiography.

RESULTS

Transcriptional analysis of korSA. To obtain data concerning the transcription of the korSA gene in different pSAM2 derivatives, Northern hybridization with S1 low-resolution mapping were carried out with total RNA isolated from the strains S. lividans/pSAM2B12 and S. lividans/pSAM2B3. These experiments gave the same result. The korSA gene is constantly transcribed in both variants of pSAM2, either integrated only (pSAM2B12) or integrated and replicating (pSAM2B3). The presence in both strains of a 1-kb mRNA corresponding to the size of korSA (Fig. 1) indicates that it is a monocistronic transcript (data not shown).

The start point of korSA transcription was localized by high-resolution S1 mapping. Transcription is initiated from the adjacent A or T nucleotide situated 18 and 19 nucleotides (nt) upstream of the putative initiation codon ATG (data not shown) (see Fig. 5). These results allowed us to locate the korSA promoter. Its sequence does not show similarity to the consensus sequence proposed for Streptomyces promoters similar to those recognized by E. coli RNA polymerase containing the σ70 subunit (28).

The KorSA protein binds to two regions in pSAM2. The deduced protein KorSA belongs to the GntR family of transcriptional regulators (8) and contains a helix-turn-helix DNA binding motif (7). Determination of the positions of the KorSA binding sites in the pSAM2 sequence could give direct indications of the targets of this regulator. The protein KorSA was expressed in E. coli as a fusion with the N-terminal part of MBP (MBP-KorSA) and purified as described in Materials and Methods. The MBP-KorSA fusion protein was used for further analysis.

To determine the number and positions of all of the KorSA binding sites present in the sequence of pSAM2, we took advantage of the relatively small size of pSAM2 (10.9 kb) and of the knowledge of its whole nucleotide sequence (references 2, 3, 5–7, and 23 and our unpublished results; the published part of the pSAM2 sequence has been deposited in the EMBL
database under accession number AJ005260). The total number of KorSA binding sites in pSAM2 was determined directly in gel shift experiments using the entire set of unlabeled DNA fragments obtained after digestion of the circular form of pSAM2B3 with restriction enzymes and separation of these DNA fragments on a PAA gel. If these fragments were mixed with the MBP-KorSA protein prior to gel electrophoresis, any fragment whose migration is retarded could be expected to bind to KorSA, and thus the binding region could be localized. The restriction enzymes (AatII, AccI, AvaII, DdeI, HindII, RsaI, StyI, and XhoII) were chosen because each cuts pSAM2 into 15 to 25 fragments that could be identified from the neighboring fragments. Typical results obtained after cutting with DdeI and StyI are shown in Fig. 2.

For every restriction enzyme used, no more than two fragments of pSAM2 were retarded (Fig. 2). Comparison of the results obtained using different endonucleases allowed us to identify the minimal fragments containing the binding regions. They were relatively short and located in the promoters of the korSA [the 204-bp HindIII(948)/StyI(1152) fragment] and pra promoters (the 106-bp HindIII(1816)/HindIII(1922) fragment) genes. Binding of the MBP-KorSA protein was not observed with the fragments containing the traSA gene or its promoter. This was unexpected, since in Streptomyces plasmids such as pIJ101 (26, 27) or pSN22 (15), Kor was shown to repress directly the expression of the intermycelial transfer genes by binding in their promoter regions.

The fragments corresponding to the regions localized after the preliminary gel shift experiments with the whole DNA of pSAM2 were synthesized by PCR, radioactively labeled, and tested in a new round of gel shift experiments with the MBP-KorSA protein. The first region, upstream of korSA, corresponds to the 125-bp fragment (positions 880 to 999 in Fig. 1 and in the AJ005260 sequence) and additional nucleotides creating the BamHI and HindIII sites at the ends of the fragment. The second region, upstream of pra, corresponds to the 174-bp fragment (positions 1760 to 1922 in the same sequence and additional nucleotides creating the Asp718I and BamHI sites at the ends of the fragment). The results obtained confirmed and refined the first suggestion about the position of the KorSA protein binding sites. As shown in Fig. 3A, MBP-KorSA was able to bind efficiently to the 174-bp fragment corresponding to the pra gene promoter from the variants pSAM2B3, pSAM2B3, and pSAM2B4. This means that the mutations found in the pra promoter in pSAM2B3 and pSAM2B4, while strengthening pra expression, did not abolish the binding of KorSA.

To refine the analysis, the 174-bp fragment from pSAM2B3 corresponding to the pra gene promoter was cut with ApaLI (giving fragments of 88 and 86 bp) and with HindIII (giving fragments of 69 and 105 bp). Both ApaLI subfragments were able to bind MBP-KorSA, but only the 105-bp HindIII fragment was retarded (Fig. 3B). It was concluded that each ApaLI fragment contains at least one site for KorSA and that no functional site is situated on the 69-bp HindIII fragment. It is interesting that the point substitution present in pSAM2B3 and located in the core sequence of binding site 2 (see Fig. 5) significantly decreases but does not abolish the binding of the 88-bp ApaLI DNA fragment to KorSA (data not shown). This indicates that this nucleotide (G) is important for KorSA binding, as predicted by the footprint experiment (see below). In addition it confirms the validity of the KorSA targets determined by gel retardation experiments.

Binding of KorSA to its own promoter was confirmed with the 125-bp fragment corresponding to the korSA gene promoter (Fig. 3C). The protein KorSA has 10 to 20 times more affinity for the pra gene promoter than for its own promoter, since, using equal amounts of DNA, 10 to 20 times more KorSA was required to obtain the same pattern of retardation with the korSA promoter as with the pra promoter (Fig. 3C).
To confirm the absence of binding sites for KorSA in the traSA gene sequence, three fragments corresponding to the promoter and 5′ coding part, to the central part, and to the 3′ coding part of the traSA gene were synthesized by PCR, radioactively labeled, and used in gel shift assays. None of these fragments were retarded in the presence of MBP-KorSA (data not shown). These results could also be considered to be a control of the specificity of the KorSA binding.

Determination of the KorSA binding site by footprint analysis.

To determine the sequence recognized by KorSA, footprint analysis was performed. In the fragment containing the korSA promoter, the MBP-KorSA protein was able to protect against DNase I the 25- to 27-bp region situated immediately upstream of the korSA transcriptional start point (Fig. 4). The same type of experiment was performed with the fragment containing the pra promoter. In this case the MBP-KorSA protein protected two regions, upstream and downstream of the pra transcriptional start point (Fig. 5B). For this fragment, the position of the protected regions was in good agreement with the results obtained by the gel shift analysis, indicating the presence of two binding sites (Fig. 3B).

When the three protected sequences were aligned, it was possible to deduce a consensus sequence (Fig. 5C). Sequences identical to this 17-bp consensus sequence were searched for in the complete pSAM2 sequence and were only found in the three regions protected by MBP-KorSA. The presence of a 9-nt perfect inverted repeat was noted in the sequence of the KorSA binding site 3. Due to these repeats, the 17-bp consensus is present in both strands for site 3.

KorSA is a transcriptional repressor of the two genes pra and korSA. The KorSA protein, like other known Streptomyces Kor proteins, belongs to the GntR family of transcriptional repressors (8). To determine whether KorSA plays a similar role in pSAM2, the strengths of the pra and korSA promoters, in the presence or absence of KorSA, were compared using the promoter probe vector pIJ487 (32). In this vector the kanamycin resistance gene is transcribed from the inserted promoter. The level of transcription from a tested promoter was estimated by the level of resistance to kanamycin. It was observed that when the korSA gene is present in trans, the pra promoter (PpraB2) is repressed, demonstrating that KorSA acted as a transcriptional repressor of the pra gene. For example, at a kanamycin concentration of 3 μg/ml, the presence of korSA
reduces survival about 10-fold (Fig. 6A). Surprisingly, in the presence of pOS699 with korSA disrupted, PpraB2 was found to be activated, indicating that pSAM2 codes for another, positive, regulator of pra expression. The PpraB2 promoter is much stronger than PpraB3. For instance, with PpraB3 cloned in pIJ487, survival was at least 50% with kanamycin at 100 μg/ml (23), while survival was about 5% with kanamycin at 5 μg/ml with PpraB2 (Fig. 6A). The repression of PpraB2 in the presence of korSA in trans was detectable only at kanamycin concentrations higher than 100 μg/ml (data not shown).

Using the same approach, it was demonstrated that KorSA is also a transcriptional repressor of its own gene (Fig. 6B). The korSA gene promoter is repressed only by KorSA, since in the presence of pOS699 containing the whole pSAM2 sequence, but with the korSA gene disrupted, no repression of the korSA promoter was observed.

**Inactivation of the korSA gene.** A variant, pOS699 (Fig. 1; Table 1), containing an internal deletion in the korSA gene was constructed from a derivative of pSAM2B3, pTS39, which has been used for the functional analysis of pSAM2 (25). pOS699 was able to transform S. lividans with the same efficiency as pTS39. For pTS39 the integrated and replicative forms coexist in S. lividans. However, pOS699 was present only as a replicative form (Fig. 7). The introduction of a copy of the korSA gene in trans in the strain S. lividans/pOS699 restored the presence of the pOS699 integrated form (Fig. 7) but, for unknown reasons, did not restore the usual proportion between the free and integrated forms for pSAM2B3. It should be emphasized that strong activation of pSAM2 excision was previously observed for the derivative pOS548, in which the pra gene was overexpressed under the control of an inducible and very strong heterologous promoter, pitpA (22). These results, together with the binding of KorSA to the pra probe, allow us to conclude that KorSA is still able to repress the pra gene promoter in the mutant pSAM2B3.

The status of pOS699 could be explained by the absence of repression of pra. Nevertheless, it should be stressed that the role of KorSA cannot be limited only to the repression of pra. Inactivation of korSA had other consequences. S. lividans was transformed by pOS699 with the same efficiency as pTS39, but transformants appeared after more than 1 week, compared to 3 days for pTS39 transformants. Moreover, after 2 to 3 weeks, the clones obtained by transformation did not give progeny when picked on new plates. This phenotype was considered to be an attenuated Kil phenotype and was suppressed by complementation in trans with a single copy of the korSA gene in the strain S. lividans/pOS699/pOS556.

**DISCUSSION**

The conjugal S. ambofaciens integrative element pSAM2 possesses a kil-kor system. The korSA gene has been identified as a key element of this system, and the kil locus has been located in the region of traS, the main transfer gene (7, 25), but the direct role of KorSA in the regulation of the kil locus remained unknown. In this study we characterized the targets of the KorSA protein in the pSAM2 sequence.

Considering the relatively small size of the pSAM2 genome and the availability of its complete sequence, gel shift experiments were performed with the totality of the DNA fragments obtained after pSAM2 digestion with restriction endonucleases. Using this new approach, it was possible to demonstrate directly that KorSA binds only to the promoter regions of two pSAM2 genes, pra and korSA, with no other binding sites detected. Unlike other known actinomycete mobile elements, KorSA did not bind either to traS, the main pSAM2 transfer gene, or to the spdA, -B, -C, and -D genes involved in pSAM2 spreading. These conclusions were confirmed after determination by footprint analysis of the sequence recognized by KorSA. This 17-nt consensus sequence was found only upstream of the korSA and pra genes and not elsewhere in pSAM2. The promoters of these genes, cloned from the wild-type pSAM2B3, and studied in a Streptomyces promoter probe vector, were shown to be repressed in vivo in the presence of korSA in trans, thus confirming the in vitro studies.

These data allowed us to conclude that KorSA negatively regulates the expression of another regulator gene of pSAM2, pra, characterized to be essential for the expression of genes involved in integration-excision and replication of pSAM2. The presence of genes similar to pra-like gene is present in the sequence of pSA1 of Streptomyces azureus ATCC 1421 [EMBL accession number AB010724].

Although the presence of functional pSAM2 has never been observed in the S. coelicolor genome, several open reading
frames exhibiting very high similarity with pSAM2 genes were recently found during the sequencing of the complete S. coelicolor chromosome. These sequences could be detected by hybridization of S. coelicolor total DNA with a pSAM2 probe. Nothing is known about the possible expression and role of these open reading frames in S. coelicolor. However, when hybridization of a pSAM2 probe was performed under the same conditions with total DNA of S. lividans, no hybridizing fragment could be detected (data not shown). Therefore, these open reading frames, which are highly similar to pSAM2 genes, are absent from S. lividans and could not interfere with the functional study of pSAM2 performed in this strain.

Functional analysis confirms the role of KorSA as the pra repressor. In the derivative pOS699, in which korSA is inactive, strong activation of pSAM2 excision was observed, a phenomenon very similar to that obtained by overexpression in cis of the pra gene (22).

Another role discovered for KorSA, i.e., as a negative regulator of its own synthesis, is consistent with the data published for the kor genes of other actinomycete conjugative elements (see the introduction). The affinity of KorSA for the korSA promoter, which is weaker than that for pra, probably allows this protein to maintain its concentration at a level sufficient to keep the pra gene constantly repressed. It should be noted that the affinity of the fused protein MBP-KorSA could be slightly different from that of KorSA, but unfortunately, the cleavage of the fused protein was extremely inefficient.

It has been demonstrated that pSAM2B3 is different from the wild-type form pSAM2B2 by the coexistence of replicative and integrated forms, while pSAM2B2 is present in the cells only as an integrated sequence. This difference is a consequence of the point substitution in the pra gene promoter (23). This mutation causes a more efficient transcription of praB3, whereas mRNA of praB2 was not detected. A plausible explanation of this phenomenon might have been the different affinities of KorSA for the mutated and nonmutated sequences. However, the mutation in the pra gene promoter is not located in the KorSA binding sites, and KorSA binds to the praB3 promoter with roughly the same affinity as it does to the praB2 promoter. These results indicate that the mutated praB3 promoter is still regulated by KorSA.

The absence of a direct connection between KorSA and the expression of the main transfer gene, traSA, presents a significant difference between the genetic organizations of transfer control in pSAM2 and in other well-studied conjugative elements of actinomycetes. The derivative of pSAM2B3 lacking functional korSA expressed an attenuated Kil phenotype. This raises the question of the mechanism by which KorSA regulates this determinant. Generally, the kil function is attributed to a transfer gene(s). KorSA did not bind to any region in the

![Fig. 5. Positions of the KorSA binding sites upstream of the korSA and pra genes. (A) DNA sequence upstream of the korSA gene translation initiation codon. (B) DNA sequence upstream of the praB gene translation initiation codon. (C) Alignment of the KorSA binding sites found in the pSAM2 sequence. The positions of the sequences corresponding to the KorSA binding sites are boxed. The positions of the point mutations found upstream of the pra gene in pSAM2B3 and pSAM2B4 are indicated as T (B3) and T (B4).]
may be responsible for the silent status of the pSAM2 integra-
tional regulator of pSAM2. This suggested that KorSA alone
traSA integration, excision, and replication through the control of
traSA expressed, even in pSAM2B2 which was found only integrated.
KorSA, but in the presence of intact
relieving KorSA repression under conditions favorable for
indispensable for transfer, should include a mechanism for
Activation of the excision and replication of pSAM2, which are
gene (pOS556).
FIG. 6. KorSA is a repressor of the
pra
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negro-
ciens plasmid, pSN22: genetic organization and correlation with genetic
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ciens plasmid, pSN22: genetic organization and correlation with genetic
FIG. 7. Effect of korSA gene disruption on the status of pSAM2. Total DNA,
restricted with EcoRI, was analyzed by Southern hybridization with the
32P-
labeled 4.2-kb EcoR1-EcoRI fragment of pSAM2 containing the attP integra-
tion site. The two DNA bands of 6.7 and 5.2 kb correspond to integrated pSAM2,
while the 4.2-kb band corresponds to the free form of pSAM2. DNA was
extracted from S. lividans TK24 containing pOS699 clones 1 and 2 (lanes 2 and
3), pOS699 plus pOS556 clones 1 and 2 (lanes 4 and 5), pSAM2
2Ag (lane 6), and pTS39 (pSAM2
2Ag derivative) (lane 7). Lane 1, 1-kb ladder DNA.
FIG. 6. KorSA is a repressor of the korSA gene and
pra
promoters. The
sensitivity to kanamycin (Km) of S. lividans TK24 harboring the plasmids
pOS530 (A) and pOS702 (B) increases in the presence of the functional korSA
gene (pOS556).

traSA gene. traSA is not cotranscribed with pra (23), and over-
expression of pra from the tip4 promoter, not controlled by
KorSA, but in the presence of intact
korSA (pOS548 [22]), does not cause the Kil phenotype. It could be supposed
that either Pra or KorSA needs an unidentified partner to regulate
traSA expression or that another regulatory gene is involved.
In pSAM2, KorSA exhibits unique properties: it regulates
integration, excision, and replication through the control of
pra but does not directly regulate the main pSAM2 transfer gene
traSA. KorSA could be considered the main negative transcrip-
tional regulator of pSAM2. This suggested that KorSA alone
may be responsible for the silent status of the pSAM2 integra-
ted form, and it should be noted that korSA was always
expressed, even in pSAM2
2Ag which was found only integrated.
Activation of the excision and replication of pSAM2, which are
indispensable for transfer, should include a mechanism for
relieving KorSA repression under conditions favorable for transfer.


