Multicopy Derivative of Pock-Forming Plasmid pSA1 in *Streptomyces azureus*

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*Streptomyces azureus* carried one copy or less of plasmid pSA1, which elicited pocks at 0.1 to 1.0%. Strain PK100 was isolated from the wild-type strain after UV irradiation. PK100 carried approximately 20 to 30 copies of pSA1.1, a derivative of pSA1. Plasmid pSA1.1 elicited pocks at 100% and inhibited spore and thiostrepton production.

Many of the plasmids isolated from the *Streptomyces* species are phenotypically detectable by their ability to elicit zones of growth inhibition or pocks when the plasmid-carrying strains are grown in contact with plasmid-free strains on solid media. Plasmids SCP2 and SCP2* of *S. coelicolor* A3 (2) (2-4) and several other *Streptomyces* plasmids (1, 5-9, 11, 12, 18) are such conjugative plasmids and elicit pocks.

Thiostrepton-producing *S. azureus* ATCC 14921 formed a unique lawn with pocks on agar media (17). These pocks appeared spontaneously during growth of the organisms and increased in number during subculturing. *S. azureus* also formed pocks similar to those of *S. coelicolor* and others at a frequency of approximately 0.1 to 1.0% of the initial spore inoculum on plasmid-free strain PKC, previously called AF-6 (13), obtained from a wild-type strain by acriflavine treatment. A pock-forming plasmid, pSA1, was isolated from *S. azureus*. In previous work, pSA1 was found to be a linear plasmid in a plate culture with confluent pocks (13). Experiments concerning the change from the circular to the linear form of pSA1 are in progress.

When spores of the *S. azureus* wild-type strain were UV irradiated (1,442 μW·erg/cm), 5 to 6% of survivors (survival ratio at 0.1%) formed turbid-type pocks on strain PKC. About 50 pocks were randomly isolated from the centers of these pocks; almost all had the same properties on pock formation and so were named strain PK100. Two strains formed another type of pock. Strain PK100 formed turbid-type pocks at a frequency of 100% on strain PKC. These pocks had morphological features in common with those of *S. coelicolor* and others. Strain PKA100 (Ath⁻ Ade⁻ Thi⁻), obtained from strain PK100 by UV irradiation, also formed pocks at 100% on strain PKC. The properties of these strains and other derivatives are listed in Table 1.

Lack of spore formation and diminished growth of aerial mycelia were noted with strains PK100 and PKA100 (Table 1); however, there was abundant growth of substrate mycelia. Lysis of mycelia was not evident under a scanning electron microscope (data not shown). During subculturing of strains PK100 and PKA100, spore-forming colonies (PK100C from PK100 and PKA100C from PKA100) appeared spontaneously in approximately 0.1 to 1.0% of the colonies. The thiostrepton productivity of all of these strains in liquid culture was determined as previously described (16). Spore-forming strains PKC, PK100C, and PKA100C produced thiostrepton in amounts equivalent to those of the wild-type strain (Table 1). However, thiostrepton productivity was depressed 50 to 75% in non-spore-forming strains PK100 and PKA100.

Plasmid DNA from all strains was isolated by using the sodium dodecyl sulfate method (10). The wild-type strain carried the previously identified pock-forming plasmid pSA1 (13), but with a circular form in liquid culture. Strains PK100 and PKA100 carried a plasmid of the same size as pSA1. The relative copy numbers of plasmids in wild-type and mutant strains were estimated as described by Kieser et al. (8). The wild-type strain contained about one copy or less of pSA1 per host genome equivalent, but strains PK100 and PKA100 carried approximately 20 to 30 copies of the plasmid (Table 1). However, plasmids were absent in strains PKC, PK100C, and PKA100C as determined by Southern blot analysis (Fig. 1) (13). The cleavage sites of various restriction endonucleases in pSA1 and the plasmid from PK100 were determined. The restriction patterns of both plasmids proved to be indistinguishable. The sizes of both plasmids were estimated to be 8.8 kilobases. The homology of both plasmids was also determined by Southern blot analysis (Fig. 1). The plasmid from PK100 was considered to be a derivative of pSA1 and was named pSA1.1. A restriction map of the plasmids is presented in Fig. 2.

Many strains were isolated from the perimeters of pocks produced by strain PK100 on strain PKA100C. Almost all (strain P2) had the same features as PKA100 (Table 1). Therefore, pSA1.1 is probably a self-transmissible plasmid.

![Image of Southern blot analysis](image_url)

**FIG. 1.** Southern blot analysis of EcoRI-cleaved plasmids isolated from *S. azureus* by using pSA1.1 as a hybridization probe. Lanes: 1, PKC; 2, the wild-type; 3, PK100; 4, PK100C. The applied volume of DNAs isolated from PK100 was one-fifth of that of other strains. The lengths (in kilobases [kb]) and positions of *HindIII*-cleaved lambda phage DNA markers are indicated on the left.

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TABLE 1. Summary of the properties of S. aureus ATCC 14921 and its derivatives

<table>
<thead>
<tr>
<th>Strain</th>
<th>Plasmid</th>
<th>Copy no. of plasmid/host genome equivalent</th>
<th>Pocks/colony size (%)</th>
<th>Spore formation</th>
<th>Thiostrepton production (mg/g of mycelia)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type (ATCC 14921)</td>
<td>pSA1</td>
<td>1</td>
<td>0.1 - 1.0</td>
<td>+</td>
<td>2.2 - 2.8</td>
</tr>
<tr>
<td>PKC</td>
<td>pSA1.1</td>
<td>20 - 30</td>
<td>100</td>
<td>-</td>
<td>0.9 - 1.3</td>
</tr>
<tr>
<td>PK100</td>
<td>pSA1.1</td>
<td>0</td>
<td>&lt; 10^-8</td>
<td>+</td>
<td>2.5 - 2.8</td>
</tr>
<tr>
<td>PKA100C</td>
<td>pSA1.1</td>
<td>20 - 30</td>
<td>100</td>
<td>-</td>
<td>0.6 - 1.1</td>
</tr>
<tr>
<td>T1</td>
<td>pSA1</td>
<td>0</td>
<td>&lt; 10^-8</td>
<td>+</td>
<td>2.2 - 2.5</td>
</tr>
<tr>
<td>T2</td>
<td>pSA1.1</td>
<td>20 - 30</td>
<td>100</td>
<td>-</td>
<td>0.8 - 1.0</td>
</tr>
<tr>
<td>T3</td>
<td>pSA1.1</td>
<td>20 - 30</td>
<td>100</td>
<td>-</td>
<td>ND</td>
</tr>
<tr>
<td>P1</td>
<td>pSA1</td>
<td>1</td>
<td>0.1 - 1.0</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>P2</td>
<td>pSA1.1</td>
<td>20 - 30</td>
<td>100</td>
<td>-</td>
<td>ND</td>
</tr>
</tbody>
</table>

* Frequency of pock formation with respect to the initial size of the cell inoculum on strain PKC.

+ , Approximately 10^5 spores per slant culture; −, no spore formation.

ND, Not determined.

Strain P1, obtained from the perimeters of pocks produced by strain SA2-a (an Arg^- strain obtained from the wild-type strain by UV irradiation) on PKC, had the same properties as the wild-type strain. Plasmid pSA1 also was a self-transmissible plasmid.

Plasmids pSA1 and pSA1.1 were introduced into PKC or PK100C, by the polyethylene glycol-protoplast transformation method we previously reported (14, 15). About 8 × 10^8 CFU protoplasts and about 0.1 μg of plasmid DNA were used for transformation. Pock-forming transformants appeared at a low frequency with pSA1 (approximately 5 × 10^3 pocks per μg of DNA) but at a high frequency with pSA1.1 (approximately 7 × 10^3 pocks per μg of DNA). This difference in transformation frequency appears to be due to the difference in pock-forming ability between pSA1 and pSA1.1. All of the transformants (strain T1) with pSA1 had the same features as the wild-type strain (Table 1). All of the transformants with pSA1.1 (T2 from PKC and T3 from PK100C) had the same properties as PK100, formed pocks at 100% on PKC, and carried about 20 to 30 copies of pSA1.1. Their spore and thiostrepton productivities were depressed. These results indicate that the copy numbers of plasmids do not relate to features of the host strains but to the plasmids. Therefore, pSA1.1 may be a high-copy-number mutant derived from pSA1 with small mutations induced by UV irradiation. There were no evident differences between pSA1 and pSA1.1 in their restriction patterns. The number and site of mutations which occurred on pSA1 were not determined. However, we did isolate many strains (PK100 type) which carried 20 to 30 copies of plasmid pSA1.1 after UV irradiation. Therefore, the increased copy number and the pleiotropic phenotype alternations probably are caused by a single mutation.

Plasmid pSA1.1 elicits pocks at 100% on the plasmid-free strain and simultaneously inhibits spore and thiostrepton production. We are now attempting to use these plasmids to analyze the systems of regulation of spore and thiostrepton production in S. aureus.

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LITERATURE CITED


FIG. 2. Restriction enzyme cleavage map of plasmids pSA1 and pSA1.1.


