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Transfer of Conjugative Plasmids and Mobilization of a Nonconjugative Plasmid between *Streptomyces* Strains on Agar and in Soil†

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The conjugative plasmid pIJ101 and its conjugative nondeletion derivatives pIJ303 and pIJ211 were tested for their transferability between strains of *Streptomyces* on laboratory media and in the soil environment. Their roles in the mobilization of the cloning vector plasmid pIJ702, a nonconjugative deletion derivative of pIJ101, were also examined. Biparental and triparental crosses were performed on agar slants and in sterile soil between the plasmid donor *Streptomyces lividans* and several recipient *Streptomyces* strains previously isolated from soil. Conjugative plasmids were transferred to seven recipients in slant crosses and to three recipients in soil. Plasmids isolated from recipients showed restriction fragment patterns identical to that of the original plasmid in *S. lividans*. Plasmid pIJ303 was transferred less frequently in soil than on slants, and the frequency of transfer was higher at 30°C than at the other temperatures examined. Transconjugant *Streptomyces* strains differed in their ability to maintain pIJ303. The nonconjugative plasmid pIJ702 was mobilized on agar slants into *S. coelicolor* 2708, which already contains a self-transmissible plasmid. Plasmid pIJ702 was also mobilized into *S. flavovirens*, *Streptomyces* sp. strain 87A, and *S. parvulus* on slants and in sterile soil after triparental crosses with two donors, one containing pIJ702 and the other containing either pIJ101 or pIJ211. The presence of a conjugative plasmid donor was required for the transfer of pIJ702 to *S. parvulus* 1234, *S. flavovirens* 28, and *Streptomyces* sp. strain 87A. Plasmid pIJ702 was always transferred in its normal, autonomous form. Chromosomal recombination also occurred in transconjugants after the transfer of pIJ702. This is the first report of gene transfer between *Streptomyces* strains in soil.

Genetically engineered *Streptomyces* species may someday play important roles in the industrial production of useful polypeptides (19, 21) and in the decomposition and bioconversion of organic materials (7, 20). There currently is a high level of interest in the use of *Streptomyces* species in cloning experiments. *Streptomyces* strains express many foreign genes well, and molecular cloning techniques with *Streptomyces* species are well developed (9, 10, 12). In addition, many *Streptomyces* species are important antibiotic producers, and the techniques for their growth in industrial facilities are well known (1).

It is possible that recombinant *Streptomyces* strains may be either purposefully or inadvertently released into the soil environment, from which their parental strains were originally derived. Because of concern over the introduction of genetically altered bacteria into the environment (4, 13), an evaluation of the potential for the transfer of manipulated genes from these recombinant *Streptomyces* strains to other native soil microorganisms is needed. Mobilization of nonconjugative plasmids has been well documented in gram-negative bacteria (6, 17, 22). The transfer of extrachromosomal genetic materials between members of the genus *Streptomyces* has also been shown in laboratory growth media (15, 18). However, little is known of the potential for gene transfer between *Streptomyces* strains in soil.

We examined a system which seems representative of those which might allow environmental gene transfer between *Streptomyces* species. To investigate whether plasmid mobilization might occur, the effects of a conjugative plasmid on the mobilization of a nonconjugative plasmid were assessed. The ability of plasmid DNA to be mobilized between different species of *Streptomyces* on agar and in soil was demonstrated. Chromosomal recombination was also observed.

MATERIALS AND METHODS

Bacterial strains and plasmids. *Streptomyces lividans* 3042, containing plasmid pIJ303 and carrying a thiostrepton resistance gene; *S. lividans* 3063, containing plasmid pIJ211 and carrying a neomycin resistance gene; *S. lividans* 2930, containing plasmid pIJ101; *S. lividans* TK150, containing plasmid pIJ702 and carrying a thiostrepton resistance gene and a tyrosinase gene; *S. lividans* TK23 and TK24; and *S. coelicolor* 2708 and 2709 were kindly provided by D. A. Hopwood (John Innes Institute, Norwich, England). *S. parvulus* 1234 and *S. griseus* 10137 were from the American Type Culture Collection (Rockville, Md.). *S. setonii* 75Vi2; *S. viridosporus* T7A; *S. flavovirens* 28; *S. sioyaensis* P5; *S. albogriseolus* 201; and *Streptomyces* sp. strains 87A, 138, 177, and 523 were isolated from soil in our previous work (2, 3). *Streptomyces* sp. strain SR10 is a protoplast fusion-generated recombinant from our laboratory (20). The strains used in this study and their antibiotic resistances are summarized in Table 1.

Media and culture conditions. All of the cultures were grown on yeast extract-malt extract agar (YMA) slants at 30°C until sporulation (5). Spore suspensions were prepared from slants in sterile water. For plasmid isolations, cultures were grown in yeast extract-malt extract-sucrose (YEME) medium containing 5 mM $MgCl_2$ (9). The following antibiotics were used in the media at the indicated concentrations, in

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TABLE 1. Antibiotic sensitivities of the *Streptomyces* strains used for establishment of crosses

Strain	Sensitivity to[a]:		
	Thiostrepton	Neomycin	Clindamycin
S. lividans 3042	R	S	MS
S. lividans 2930	S	S	MS
S. lividans 3063	S	R	MS
S. lividans TK150[b]	R	S	MS
S. lividans TK24[b]	S	S	MS
S. coelicolor 2708 and 2709	S	S	R
S. griseus 10137	S	S	R
S. setonii 75Vi2	MS	MS	R
S. viridosporus T7A	S	S	R
S. albogriseolus 201	S	S	R
S. parvulus 1234	S	S	R
Streptomyces sp. strain SR10	S	S	R
S. sioyaensis P5	S	S	R
Streptomyces sp. strain 177	S	S	R
Streptomyces sp. strain 523	S	S	R
Streptomyces sp. strain 138	S	S	R
Streptomyces sp. strain 87A	S	S	R
S. flavovirens 28	S	S	R

[a] Abbreviations: R, resistant; S, sensitive; MS, most cells were sensitive.
[b] *S. lividans* TK150 and TK24 were also streptomycin resistant.

micrograms per milliliter: streptomycin, 50; thiostrepton, 25; clindamycin, 4; spectinomycin, 100; and neomycin, 10. Thiostrepton and clindamycin were generous gifts from the Squibb Institute for Medical Research (Princeton, N.J.) and The Upjohn Co. (Kalamazoo, Mich.), respectively. Each strain, except *S. lividans* TK150 and 3042, was grown on YMA slants without antibiotics; *S. lividans* TK150 and 3042 were grown on thiostrepton-containing media. Spores were collected and stored frozen at −20°C in 20% glycerol (9). Viability counts were performed routinely on each strain.

Crosses on agar slants. Spores were mixed on YMA slants without antibiotics by the procedures of Hopwood et al. (9). After adequate growth and spore formation, the spores were scraped from the slants and plated onto selective medium. The types of crosses which were established are shown in Tables 2 to 4.

Inoculation of soil with the crosses. A Palouse silt loam soil from the University of Idaho campus was sterilized by the procedure of Trevors and Oddie (26). Sterile soil was inoculated with approximately 10^6 spores of each donor per g of soil and 10^6 or 10^7 spores of plasmid-free recipients. The soil moisture content was adjusted to 60% of its water-holding capacity. The soil samples were then incubated at 30°C unless otherwise specified.

DNA preparations. Plasmid DNA was isolated from mycelia by the alkaline sodium dodecyl sulfate procedure of Hopwood et al. (9), with some modification (16). The mycelia were lysed, first partially with lysozyme and then completely with sodium dodecyl sulfate-alkali. Chromosomal DNA was removed by potassium acetate precipitation; protein was digested with proteinase K (Sigma Chemical Co., St. Louis, Mo.) and removed by phenol-chloroform extraction. Plasmid DNA was precipitated with alcohol. In some cases, the DNA was purified further on cesium chloride-ethidium bromide gradients, precipitated again with alcohol, and dialyzed against Tris hydrochloride buffer (pH 7.5)–1 mM EDTA.

Restriction enzyme digestion and agarose gel electrophoresis. Restriction enzymes were purchased from Bethesda Research Laboratories (BRL; Gaithersburg, Md.), and digestion was carried out under the conditions recommended by BRL. A horizontal system for submerged gels (model 5; BRL) was used for agarose gel electrophoresis at an agarose concentration of 0.8%. After electrophoresis, the DNA bands were stained with ethidium bromide and photographed (16).

DNA labeling. A nonradioactive nucleic acid detection system (BluGENE; BRL) was used to label the plasmid pIJ303 DNA with biotin by nick translation according to the instructions of BRL.

Southern blot hybridization. After visualization, denaturation, and renaturation of DNA, the DNA was transferred by capillary transfer to a nylon filter (Hybond-N; Amersham Corp., Arlington Heights, Ill.) for 14 h (23). The DNA was cross-linked to the nylon filter by subjecting the filter to UV irradiation for 3 min. The nylon filter was then incubated in the prehybridization buffer containing formamide at 42°C for 2 h, and then in the hybridization buffer containing biotin-labeled pIJ303 for 12 to 18 h according to the instructions of BRL. After hybridization, the nylon filter containing DNA was washed, treated with streptavidin-alkaline phosphatase conjugate, and then developed with Nitro Blue Tetrazolium and 5-bromo-4-chloro-3-indolylphosphate according to the instructions supplied by BRL for the development of biotin-labeled DNA in the nucleic acid detection (BluGENE) system.

Colony hybridization. The method of Hopwood et al. (9) was used for the transfer of *Streptomyces* colonies to nitrocellulose filters and the subsequent release of the DNA with lysozyme. The filter was baked in a vacuum oven and then hybridized with a biotin-labeled DNA probe. The method for development of hybridized DNA on the filter was the same as that described above for Southern blot hybridization.

RESULTS

Transfer of plasmids. The transfer of plasmids was determined by plating the progeny of crosses on a selective medium containing appropriate antibiotics. Prior to crossing, all of the strains were tested for their sensitivities to the different antibiotics. Most of the *Streptomyces* strains tested were clindamycin resistant and thiostrepton and neomycin sensitive (Table 1). *S. lividans* 3042 and TK150 were partially resistant to clindamycin. Thiostrepton and clindamycin antibiotics were selected for use in plates in which the progeny of most of the crosses were grown.

Although most strains used in this study were thiostrepton sensitive, *S. setonii* produced some thiostrepton-resistant colonies that became evident following exposure to this antibiotic. *S. lividans* TK24 and *S. lividans* TK150(pIJ702) had chromosomal genes encoding streptomycin resistance. This resistance differentiated them from *S. lividans* 3042, which was sensitive to streptomycin. The selective media used for plating the progeny of crosses from Table 2, in which *S. lividans* 3042(pIJ303) (pIJ303 carries a thiostrepton resistance gene) was a plasmid donor, were YMA-thiostrepton, YMA-clindamycin, and YMA-clindamycin-thiostrepton. YMA-clindamycin, YMA-neomycin, and YMA-neomycin-clindamycin plates were used for plating the progeny of some crosses (see Tables 2 and 4) in which *S. lividans* 3063, containing plasmid 211 (carrying a neomycin resistance gene), was used as a plasmid donor. In addition to the media mentioned above, YMA-streptomycin plates were used for plating the progeny of crosses from Tables 3 and 4 in which *S. lividans* TK150 (which has streptomycin and thiostrepton resistance genes on the chromosome and plas-

TABLE 2. Crosses established between *S. lividans* 3042 (pIJ303) carrying the thiostrepton resistance gene or *S. lividans* 3063 (pIJ211) carrying the neomycin resistance gene as donors and recipients that lacked the plasmid[a]

Thiostrepton-sensitive recipients[b]	Development of thiostrepton-resistant transconjugants
S. setonii 75Vi2[c]	+
S. viridosporus T7A	−
S. albogriseolus 201	−
S. parvulus 1234[c]	+
Streptomyces sp. strain SR10	−
S. sioyaensis P5	−
Streptomyces sp. strain 177	−
Streptomyces sp. strain 523[c]	+
Streptomyces sp. strain 138[c]	+
Streptomyces sp. strain 87A[c]	+
S. flavovirens 28[c]	+
S. lividans TK24	+

[a] Crosses with *S. griseus* and *S. coelicolor* were also made but failed because of the lack of growth of one of the strains in the crosses.
[b] All thiostrepton-sensitive recipients were crossed with *S. lividans* 3042, the thiostrepton-resistant donor.
[c] *S. lividans* 3063 containing plasmid pIJ211 was substituted for *S. lividans* 3042 in some crosses.

mid, respectively) was a donor (Table 3) or one of the donors (Table 4). In different combinations, these media allowed the differentiation of donors, recipients, and transconjugants.

After *S. lividans* 3042 was crossed with *S. lividans* TK24, *S. parvulus*, *S. setonii*, *S. flavovirens*, and *Streptomyces* sp. strain 523 (Table 2), thiostrepton-resistant colonies developed. Plasmid pIJ303 was isolated from thiostrepton-resistant colonies of *S. lividans*, *S. parvulus*, *S. flavovirens*, *Streptomyces* sp. strain 523, and some of the *S. setonii* colonies. Occasionally, thiostrepton-resistant colonies of *S. setonii* developed prior to plasmid transfer. Plasmid DNA from *S. setonii* transconjugants was digested with the restriction enzyme *Sal*I, and in all cases showed a fragment pattern identical to that of the original plasmid pIJ303 from *S. lividans* 3042. The DNA fragments from agarose gels were transferred to a nylon filter (Hybond) and allowed to hybridize with biotin-labeled DNA from pIJ303. The two DNAs shared considerable sequence homology (Fig. 1). The amount of *S. setonii* DNA isolated, and therefore the intensity of the bands, was lower. Since equal amounts of mycelium were used for DNA extraction, pIJ303 in *S. setonii* has a lower copy number.

TABLE 3. Crosses between *S. lividans* TK150 (pIJ702) carrying the thiostrepton resistance gene and various other strains of *Streptomyces*

Thiostrepton-sensitive recipient[a]	Development of thiostrepton-resistant transconjugants containing pIJ702
S. parvulus DLC1 (pIJ303)[b]	−
S. coelicolor 2708	+
S. setonii (pIJ303)[b]	−
S. parvulus 1234	−
S. viridosporus T7A	−
S. setonii 75Vi2	−
S. flavovirens 28	−
Streptomyces sp. strain SR10	−
Streptomyces sp. strain 138	−
Streptomyces sp. strain 523	−

[a] All thiostrepton-sensitive recipients were crossed with *S. lividans* TK150, the thiostrepton-resistant donor.
[b] Strains containing pIJ303 were thiostrepton resistant.

TABLE 4. Triparental crosses between *S. lividans* 2930 containing the conjugative plasmid pIJ101 without a plasmid-encoded antibiotic resistance gene, *S. lividans* TK150 containing pIJ702 with thiostrepton resistance, and plasmid-free *Streptomyces* strains

Thiostrepton-sensitive recipients	Mobilization of plasmid pIJ702
Streptomyces sp. strain 87A[b]	+
Streptomyces sp. strain 138	−
Streptomyces sp. strain 523[b]	−
S. albogriseolus 201	−
S. flavovirens 28[b]	+
S. sioyaensis P5	−
S. parvulus 1234[b]	+

[a] For all crosses, *S. lividans* 2930 was used as the mobilizing strain and *S. lividans* TK150 was used as the thiostrepton-resistant donor.
[b] *S. lividans* 3063 was substituted for *S. lividans* 2930 in similar crosses.

Neomycin-resistant colonies also developed in some strains, including *Streptomyces* sp. strains 87A and 138, when *S. lividans* 3063 was substituted for *S. lividans* 3042 in the cross from Table 2. Similarly, plasmid pIJ211 was isolated from these colonies. The frequency of transfer of plasmids was calculated by comparing the number of colonies of transconjugants that grew on thiostrepton-containing medium with the number of colonies of the recipient strains that grew on clindamycin-containing medium. Thus, we reported transformation frequencies as the proportion of recipients that acquired plasmids after crossing (15). The transfer frequencies are shown in Table 5. The frequency of transfer of plasmids was the highest (83%) for intraspecies transfer between strains of *S. lividans*. *S. lividans* TK24 carries a streptomycin resistance gene on the chromosome which makes it distinguishable from *S. lividans* 3042, which lacks this gene. In *S. parvulus*, 30% of the colonies changed from being plasmid-free to containing transconjugants after it was crossed with *S. lividans* 3042. Since some of the colonies of *S. setonii* naturally developed resistance to

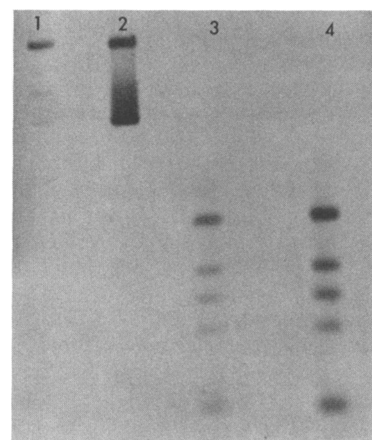

FIG. 1. Southern blot hybridization of plasmid pIJ303 subjected to electrophoresis on an agarose gel and transferred to a filter (Hybond). The filter was probed with biotin-labeled pIJ303. Lanes 1 and 3, Plasmid pIJ303 isolated from *S. setonii* after it was crossed with *S. lividans* 3042 (undigested and digested with *Sal*I, respectively); lanes 2 and 4, control plasmid pIJ303 isolated from *S. lividans* 3042 (undigested and digested with *Sal*I, respectively).

TABLE 5. Transfer frequency of a conjugative plasmid from *S. lividans* 3042 to different *Streptomyces* species on slants

Strain[a]	Transfer frequency (%)[b]
S. parvulus 1234	30
S. lividans TK24	83
S. flavovirens 28	6
S. setonii 75Vi2	2.54
Streptomyces sp.	3

[a] The strains were crossed with *S. lividans* 3042.
[b] Transfer frequency is reported as the proportion of recipients that carried a plasmid containing the thiostrepton resistance gene at the conclusion of the crossing.

thiostrepton, the frequency of transfer of plasmids to *S. setonii* could not be calculated, but it was less than 2.54%.

The transfer of plasmid pIJ303 from *S. lividans* 3042 to other strains of *Streptomyces* was also studied in sterile soil in a cross similar to those described in Table 2. The frequency of transfer was lower in sterile soil than on slants. Plasmid transfer from *S. lividans* 3042 to a plasmid-free strain, *S. lividans* TK24, occurred in soil at 25, 30, and 37°C. The frequency of transfer was highest at 30°C (Table 6), which was the optimal growth temperature for both the donor and the recipient.

The transfer of plasmids from donors to recipients was confirmed by colony hybridization (Fig. 2) and by isolation of plasmids from streptomycin- and thiostrepton-resistant colonies. In *S. parvulus*, plasmid transfer in sterile soil occurred with a much lower frequency than it did on agar slants. The ratio of transconjugants to recipients for *S. parvulus* was approximately 1% in the sterile soil versus 30% on agar slants. Plasmid pIJ303 was isolated from thiostrepton-resistant *S. parvulus* colonies that developed on thiostrepton-containing medium in crosses with *S. lividans* in sterile soil at 25, 30, and 37°C. The plasmid restriction digest pattern was compared with that of plasmid pIJ303, and the comparison showed that homology was always conserved (Fig. 3). After *S. setonii* was crossed with *S. lividans* in sterile soil, many thiostrepton-resistant colonies developed, but the isolation of plasmids from these colonies always failed. The frequency of transfer, which was less than 2.54% in slant crosses, may have been undetectable in soil. The presence of naturally occurring thiostrepton-resistant colonies made the detection of plasmid-containing colonies difficult. Plasmid pIJ303 was also transferred to *S. flavovirens* 28 in sterile soil.

TABLE 6. Frequency of transfer of conjugative plasmid pIJ303 in sterile soil at different incubation temperatures

Cross[a]	Incubation time (days)	Temperature (°C)	Transfer frequency (%)[b]
10 × TK24 × 3042	12	37	37.5
TK24 × 3042	12	37	12.0
10 × TK24 × 3042	12	30	70.0
10 × TK24 × 3042	12	25	23.0
TK24 × 3042	12	25	16.0
10 × TK24 × 3042	18	30	68.0

[a] Designations: the number of spores added to the soil for the recipients was 10 times greater than the number of spores for the donor; TK24, *S. lividans* TK24; 3042, *S. lividans* 3042.
[b] Transfer frequency is reported as the proportion of recipients that carried a plasmid containing a thiostrepton resistance gene at the conclusion of the crossing.

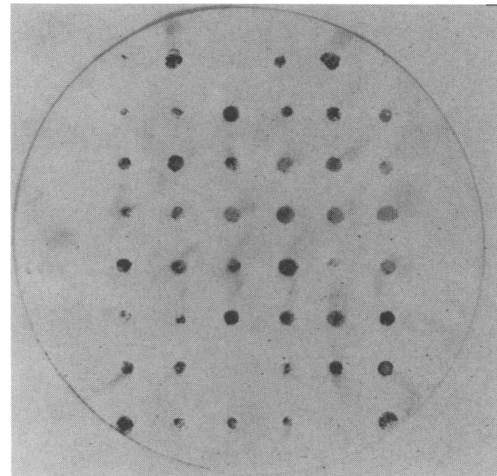

FIG. 2. Colony hybridization of *S. lividans* TK24 after it was crossed with *S. lividans* 3042 in sterile soil. Biotin-labeled plasmid pIJ303 was used as a probe. All of the colonies that were transferred to nitrocellulose paper and that grew had a positive reaction with plasmid pIJ303.

Results of biparental crosses involving donors containing nonconjugative plasmids. The types of crosses made involving donors containing nonconjugative plasmids are shown in Table 3. Some of the strains used in the crosses contained conjugative plasmids; for instance, *S. coelicolor* carries plasmid SCP1 (12). *S. parvulus* DLC1 and one of the *S. setonii* strains carried plasmid pIJ303. Since both plasmids pIJ303 and pIJ702 carried the thiostrepton resistance gene, the selection of transconjugants that mobilized pIJ702 in

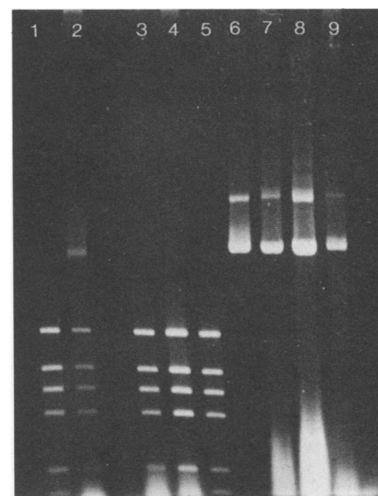

FIG. 3. Agarose gel electrophoresis of plasmid pIJ303 isolated from *S. lividans* 3042 and *S. parvulus* 1234 after it was crossed with *S. lividans* 3042 on agar slants or in sterile soil. Lanes 1 and 6, pIJ303 isolated from *S. lividans* 3042 (digested with *Sal*I and undigested, respectively); lanes 2 through 5, pIJ303 isolated from *S. parvulus* 1234 after crosses with *S. lividans* 3042 on agar medium (lane 2) and in sterile soil incubated at 25, 30, and 37°C and digested with *Sal*I (lanes 3, 4, and 5, respectively); lanes 7 through 9, undigested pIJ303 isolated from *S. parvulus* 1234 after crosses with *S. lividans* 3042 on agar (lane 7) and in sterile soil incubated at 25 and 30°C (lanes 8 and 9, respectively).

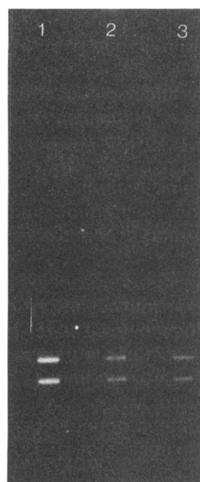

FIG. 4. Agarose gel electrophoresis of plasmid pIJ702 digested with *Sal*I. *S. lividans* TK150, *S. coelicolor* 2708, and *S. flavovirens* 28 were the sources for the plasmids in lanes 1, 2, and 3, respectively. *S. coelicolor* 2708 acquired plasmid pIJ702 after it was crossed with *S. lividans* TK150. *S. flavovirens* 28 acquired plasmid pIJ702 following triparental cross with *S. lividans* 2930 containing plasmid pIJ101 and *S. lividans* TK150 containing plasmid pIJ702.

these two strains was based on the production of dark pigmentation that developed because of the tyrosinase gene on pIJ702. However, after the progeny of the crosses were plated, dark pigment-producing *S. setonii* and *S. parvulus* were not readily detectable. Since the melanin gene is not always expressed (8), the detection of transconjugants with mobilized pIJ702 was difficult.

In the biparental cross between *S. coelicolor* and *S. lividans*, the growth of *S. coelicolor* was suppressed, except for that of a few colonies. These colonies were selected for use in subsequent crosses with *S. lividans* TK150. Thiostrepton-resistant *S. coelicolor* colonies that developed after these crosses carried plasmid pIJ702 (Fig. 4). The frequency of transfer of pIJ702 was lower than that of the conjugative plasmid (10^{-3}). There was no evidence of the transfer of nonconjugative plasmids to any other strains in biparental crosses, since no thiostrepton-resistant colonies developed either on agar slants or in crosses in sterile soil.

Results of triparental crosses. By using *S. lividans* 3042, *S. lividans* 2930, or *S. lividans* 3063 as a donor of a conjugative plasmid; *S. lividans* TK150 as a donor of a nonconjugative plasmid; and plasmid-free recipients, several crosses were established (Table 4). Spores from these crosses were plated onto four different media. In these crosses, we expected to see not only the transfer of the self-transmissible plasmid but also the mobilization and transfer of pIJ702. *S. lividans* 2930 and 3063 carried plasmids pIJ101 and pIJ211, respectively. pIJ101 is a native plasmid without an antibiotic resistance gene, and pIJ211 was constructed by adding a neomycin resistance gene to pIJ101. These two plasmids have all of the genes except the thiostrepton resistance gene and the melanin gene present in pIJ702. If plasmid pIJ101 or plasmid pIJ211 mobilized plasmid pIJ702, the new cells carrying plasmid pIJ702 would become thiostrepton resistant.

Thiostrepton-resistant colonies developed in triparental crosses between *S. setonii*, *S. lividans* TK150, and *S. lividans* 3042, but none of these colonies yielded a plasmid. *S. flavovirens* 28 produced a black diffusible pigment, which is characteristic of this species, on YMA agar. In the triparental crosses *S. lividans* 2930(pIJ101) × *S. lividans* TK150 × *S. flavovirens*, some colonies, at a frequency of 3 × 10^{-5}, developed on YMA containing thiostrepton and produced black pigment. These colonies were transferred to YMA-thiostrepton and were then grown in YEME broth containing thiostrepton. Plasmid DNA was isolated from these colonies and then digested with *Sal*I. The plasmids had fragment patterns identical to that of plasmid pIJ702 after restriction (Fig. 4). This indicates that pIJ702 is mobilized into *S. flavovirens* by plasmid pIJ101. Using the same procedures, we have also shown the mobilization of pIJ702 into *Streptomyces* sp. strain 87A by plasmid pIJ211. Strain 87A was isolated from soil in our laboratory (2).

In the cross of *S. lividans* 3063 × *S. lividans* TK150 × *S. parvulus* in sterile soil, one colony with the yellow pigment characteristic of *S. parvulus* grew on thiostrepton-containing medium after the progeny were plated. This colony was picked and inoculated into YEME broth containing 25 μg of thiostrepton per ml. Plasmid pIJ702 was isolated from the culture derived from this colony. In this three-way cross, neomycin-resistant colonies of *S. parvulus* containing pIJ211 also developed. The frequency of mobilization of plasmid pIJ702 by plasmid pIJ101 in *S. parvulus* in soil was 3 × 10^{-5}.

Following the transfer of plasmid pIJ303 or pIJ702 to *S. flavovirens*, colonies that developed on thiostrepton-containing medium had pigments characteristic of both parents in the cross. This probably indicates chromosomal recombination between the *Streptomyces* strains in the crosses (12).

DISCUSSION

The transfer of conjugative plasmids and the mobilization of nonconjugative plasmids has been shown in gram-negative bacteria (17, 22, 24, 26). Plasmid-mediated conjugation in the genus *Streptomyces* is genetically and perhaps structurally simpler than that in gram-negative bacteria such as *Escherichia coli* (15). In the present study, the presence of a thiostrepton or neomycin resistance gene on plasmids pIJ303, pIJ702, and pIJ211 provided a selection method for the recognition of plasmid-carrying transconjugants. These selections were employed for the determination of plasmid transfer. The presence and the identity of plasmids in confirmed transconjugants were always verified by plasmid isolation and comparison of the restriction patterns of the plasmids from the transconjugants with those of plasmids from donors. An identical restriction pattern of a plasmid isolated from a transconjugant and a plasmid isolated from the donor indicated that the plasmid was not modified after transfer to the transconjugant, at least for the restriction enzyme employed. Thus, this could not include base deletions, inversions, or additions that may have occurred in sections of DNA other than recognition sites for *Sal*I. Hybridization of plasmids from donors with those from recipients showed that homology was conserved. Kieser et al. (15) also noted the stability of *Streptomyces* plasmids but reported an occasional deletion in the derivative of plasmid pIJ101 on transfer.

A high copy number of pIJ303 was maintained in transconjugants after transfer of the plasmid for most, but not all, of the recipients. The lower intensity of DNA bands on the agarose gel for *S. setonii* transconjugants indicated a lower yield of plasmid DNA in *S. setonii* than in *S. lividans*. This difference could be interpreted as being due to the fact that pIJ303 has a low copy number in *S. setonii*. Plasmid pIJ303 was unstable in *S. setonii* and was lost on transfer. This is in agreement with the finding of Kieser et al. (15), who noted that plasmid stability after transfer varies in different hosts.

The frequency of transfer of plasmids from *S. lividans* 3042 varied in different strains and was the highest in *S. lividans* TK24. Kieser et al. (15) previously reported that the frequency of transfer of conjugative plasmids ranges between 2 and 100%.

The frequency of transfer also varied depending on the temperature of incubation. This may be attributed to the better growth of donors and recipients at 30°C. Some of the *Streptomyces* species that were crossed with *S. lividans* 3042 (pIJ303) have, so far, not been shown to acquire conjugative plasmids. This finding is in agreement with the observation of Kieser et al. (15) that this self-transmissible plasmid does not transfer to all *Streptomyces* species in crosses with *S. lividans*. However, transfer followed by the instability of plasmids in transconjugants may also be a reason for the low transfer frequency observed in our study.

The mobilization of the nonconjugative plasmid pIJ702 on slant crosses was observed at a much lower frequency than was the transfer of conjugative plasmids. Mobilization was expected to occur in all of the species of *Streptomyces* that acquired the conjugative plasmid; but it was only observed in *S. flavovirens*, *S. parvulus*, *S. coelicolor*, and *Streptomyces* sp. strain 87A. Mobilization of pIJ702 may have occurred without detection in crosses between *S. parvulus*, *S. setonii* containing pIJ303, and *S. lividans* containing pIJ702. Selection of colonies containing mobilized genes in these crosses was based on the expression of the melanin gene of pIJ702, which is not always adequately expressed (8).

We found that the presence of a conjugative plasmid is necessary for transfer of the nonconjugative plasmid pIJ702. Crosses between plasmid-free recipients and donors containing nonconjugative plasmids yielded no transconjugants. We agree with Kieser et al. (15) and Hopwood et al. (12) that the wild-type pIJ101, or as was seen in our study, its nondeleted derivatives and several unrelated plasmids, promotes the transfer of transfer-defective pIJ101 derivatives. The frequency of mobilization on slants was similar to values reported for plasmid pIJ350 (5×10^{-3}) (15) on slants. Plasmid pIJ350 is identical to pIJ702, except for the presence of the thiostrepton resistance gene in pIJ702 (14). The frequency of mobilization of pIJ702 into *S. parvulus* 1234 in sterile soil (3×10^{-5}) was much lower than that on agar for *S. flavovirens* 28 (5×10^{-3}).

After mobilization, pIJ702 was present in its normal autonomous form rather than as a recombinant intermediate with a conjugative plasmid. Kieser et al. (15) reported in vivo recombination between plasmid pIJ303 and plasmid pIJ24, a nonconjugative derivative of pIJ101. However, they also noticed that nontransferable derivatives of pIJ101 were transferred, under the influence of a mobilizing plasmid, in their normal autonomous form. In addition to the mobilization of plasmid pIJ702, chromosomal transfer also occurred. In the present study, the presence of a red pigment in some of the colonies of otherwise yellow pigment- or black pigment-producing *S. parvulus* and *S. flavovirens* after plasmid transfer from *S. lividans* indicated chromosomal fertility. This is also in agreement with the observation that transferable *Streptomyces* plasmids promote fertility when they are present in one parent in a cross with a plasmid-free recipient (11). A specific region of the plasmid is involved in promoting chromosomal fertility. The integration of plasmid DNA into the donor chromosome and the subsequent spread of the donor marker has been well established in *Streptomyces* species (11, 12).

In this study we have also shown the transfer of pIJ303 and the mobilization of pIJ702 from one strain of *Streptomyces* to another in sterile soil. The mobilization of pIJ702 in soil indicates that recombinant *Streptomyces* strains, if released into soil, may in turn transfer nonconjugative plasmids containing foreign genes to *Streptomyces* strains native to soil. Previously (25, 26), the transfer of plasmids in soil has been reported only in gram-negative bacteria. This is the first report of the transfer of genetic materials between *Streptomyces* strains in soil. The frequency of transfer of plasmids was always lower in sterile soil than on agar slants. One reason for the higher frequency of transconjugants on agar slants may be the relatively better growth and hyphal interaction of the *Streptomyces* strains on agar medium. It has been postulated (11) that the transfer of plasmids from donors to recipients requires mycelial fusion between the donor and the recipient. The physical separation of mycelia in soil makes plasmid transfer less frequent than that on agar slants. In some cases, plasmid transfer was observed in crosses on agar slants but not in sterile soil. The lack of detection of transconjugants in the soil is attributed to the low frequency of transfer of plasmids from *S. lividans* to these strains. Studies of transfer in the more competitive environment of nonsterile soil remain to be completed.

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