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Plasmid and Transposon Transfer to *Thiobacillus ferrooxidans*

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The broad-host-range IncP plasmids RP4, R68.45, RP1::Tn*501*, and pUB307 were transferred to acidophilic, obligately chemolithotrophic *Thiobacillus ferrooxidans* from *Escherichia coli* by conjugation. A genetic marker of kanamycin resistance was expressed in *T. ferrooxidans*. Plasmid RP4 was transferred back to *E. coli* from *T. ferrooxidans*. The broad-host-range IncQ vector pJRD215 was mobilized to *T. ferrooxidans* with the aid of plasmid RP4 integrated in the chromosome of *E. coli* SM10. pJRD215 was stable, and all genetic markers (kanamycin/neomycin and streptomycin resistance) were expressed in *T. ferrooxidans*. By the use of suicide vector pSUP1011, transposon Tn*5* was introduced into *T. ferrooxidans*. The influence of some factors on plasmid transfer from *E. coli* to *T. ferrooxidans* was investigated. Results showed that the physiological state of donor cells might be important to the mobilization of plasmids. The transfer of plasmids from *E. coli* to *T. ferrooxidans* occurred in the absence of energy sources for both donor and recipient.

Thiobacillus ferrooxidans is a gram-negative, acidophilic, obligately chemolithotrophic bacterium which derives its energy by oxidizing ferrous iron and reduced or partially reduced sulfur compounds and obtains its carbon by fixing carbon dioxide from the atmosphere (34). This organism has been used industrially in metal leaching from ores and decontamination of industrial wastes. There is considerable interest in developing a better understanding of the genetics of this microorganism, for reasons of both fundamental interest in its metabolism and genetic improvement of the bacterial strains used for metal leaching (35).

It has previously been shown that a plasmid isolated from *T. ferrooxidans* was capable of replication in heterotrophic *Escherichia coli* (26), and some of the recombinant plasmids were mobilizable between *E. coli* strains (27). Considerable progress in understanding the molecular genetics of the organism has resulted from cloning genes from *T. ferrooxidans* and studying them in *E. coli* (1, 17, 19, 25, 30). However, introduction of genes from heterotrophic bacteria back into *T. ferrooxidans* cells has not been successful (2, 30, 35). Kusano et al. (18) reported the introduction of plasmids into 1 of 30 *T. ferrooxidans* strains by electrotransformation, but the selective marker used was a *mer* determinant cloned from *T. ferrooxidans*. The introduction of heterogeneic genes into *T. ferrooxidans* will not only provide a basis of improving industrial strains for metal leaching but also open a way to investigate gene expression mechanism and gene regulation in this organism.

Conjugation, one of the main gene transfer processes in bacteria, involves the transmission of genetic material from one bacterium to another and requires cell-to-cell contact. Conjugative gene transfer of other members of the same genus such as *Thiobacillus novellus* (9), *Thiobacillus neapolitanus* (16), *Thiobacillus versutus* (36), and *Thiobacillus thiooxidans* (15) has been reported. Transpositional mutagenesis has been successfully applied to a wide range of gram-negative bacteria, including *T. novellus* and *T. versutus* (8, 33). In this paper, we report the transfer of plasmids by conjugation from *E. coli* to *T. ferrooxidans*. We also demonstrate the introduction of Tn*5* into this bacterium.

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MATERIALS AND METHODS

Bacterial strains and plasmids. The strains and plasmids used in this study are listed in Table 1.

Media and growth conditions. *E. coli* strains were cultured in Luria broth or on Luria agar at 37°C. Strains of *T. ferrooxidans* were cultured in 9K liquid medium (31), in liquid 2:2 medium at 30°C with vigorous shaking, or on solid 2:2 medium. Liquid 2:2 medium was made as follows (per liter). $Na_2S_2O_3 \cdot 5H_2O$ (2 g) was added to 10 ml of H_2O (solution A). $FeSO_4 \cdot 7H_2O$ (2 g) was added to 10 ml of H_2O adjusted to pH 2 with 2 N H_2SO_4 (solution B). A 100-ml portion of 10 × 9K salts was added to 880 ml of H_2O adjusted to pH 4.6 to 4.8 (solution C). Solutions A and B were filter sterilized separately, while solution C was autoclaved. Solutions A, B, and C were mixed when solution C was cooled. The final pH of the medium was 4.6 to 4.8.

Solid 2:2 medium was prepared in four parts. Solutions A and B were identical with that of liquid 2:2 medium; $(NH_4)_2SO_4$ (4.5 g), KCl (0.15 g), and $MgSO_4 \cdot 7H_2O$ (0.75 g) were dissolved in 500 ml of H_2O adjusted to pH 4.6 with 2 N H_2SO_4 (solution C); agar (6.0 g) was added to 480 ml of H_2O (solution D). Solutions A and B were filter sterilized, while solutions C and D were autoclaved. Solutions A, B, C, and D were mixed when solutions C and D were cooled to 45°C. The final pH of the medium was 4.6 to 4.8. About 30 ml of solid 2:2 medium was poured in each 9-cm-diameter plate. When used as a mating medium, the amount of $FeSO_4 \cdot 7H_2O$ in solution B was changed to 0.075 g, and 0.05% (wt/vol) yeast extract was added. When used as a selective medium, kanamycin or streptomycin was added to 300 μg/ml in solid 2:2 medium to select transconjugants.

Conjugation. Crosses between strains were conducted by filter mating. Donor cells were harvested by centrifugation at the late exponential growth phase; recipient cells were harvested at the stationary phase. Iron or sulfur precipitates were removed by low-speed centrifugation (100 × *g*) from 9K or 2:2 liquid culture. Both the donor and recipient cells were washed three times with basal salt solution of mating medium (pH 4.6 to 4.8) and then mixed at a donor-to-recipient ratio of 1:1. Then 0.1 ml of cell suspension (approximate 4×10^{10} cells per

TABLE 1. Bacterial strains and plasmids used

Strain or plasmid	Phenotype or genotype	Reference or source
E. coli K-12 strains		
C600	*thr leu hsd*	21
HB101	*leu pro thi hsd recA*	21
SM10	*thr leu hsd recA* Km^r RP4-2-Tc::Mu integrated	32
T. ferrooxidans strains		
Tf-44	Wild type	This study
Tf-45	Wild type	W. P. Lu
Tf-46	Wild type	This study
Tf-49	Wild type	This study
Tf-50	Wild type	This study
Tf-52	Wild type	This study
Plasmids		
RP4	Ap^r Tc^r Km^r IncP Tra$^+$	7
RP1::Tn*501*	Ap^r Tc^r Km^r Hg^r IncP Tra$^+$	C. Q. Liu
R68.45	Ap^r Tc^r Km^r IncP Tra$^+$	14
pUB307	Tc^r Km^r IncP Tra$^+$	3
pJRD215	Km^r Sm^r IncQ Mob$^+$	J. Davison
pSUP1011	Cm^r Km^r Mob$^+$::Tn*5*	32

TABLE 2. Transfer of IncP plasmids between *E. coli* and *T. ferrooxidans*

Donor	Recipient	Selected marker	Apparent transfer frequency[a] (mean ± SD)
C600(RP4)	Tf-44	Km^r	$(1.6 \pm 0.7) \times 10^{-7}$
	Tf-50	Km^r	$(1.1 \pm 0.6) \times 10^{-7}$
C600(RP1::Tn*501*)	Tf-44	Km^r	$(1.6 \pm 0.8) \times 10^{-7}$
	Tf-50	Km^r	$(3.3 \pm 2.1) \times 10^{-7}$
C600(R68.45)	Tf-44	Km^r	$(7.3 \pm 4.1) \times 10^{-7}$
	Tf-50	Km^r	$(1.1 \pm 0.8) \times 10^{-7}$
C600(pUB307)	Tf-44	Km^r	$(5.6 \pm 2.5) \times 10^{-6}$
	Tf-50	Km^r	$(1.3 \pm 0.4) \times 10^{-5}$
Tf-44(RP4)	HB101	Ap^r	$(2.0 \pm 0.6) \times 10^{-2}$
		Tc^r	$(2.2 \pm 0.7) \times 10^{-2}$
		Km^r	$(1.9 \pm 0.6) \times 10^{-2}$
Tf-50(RP4)	HB101	Ap^r	$(2.5 \pm 0.8) \times 10^{-3}$
		Tc^r	$(2.1 \pm 0.7) \times 10^{-3}$
		Km^r	$(2.2 \pm 0.5) \times 10^{-3}$

[a] Determined in each case from at least three independent experiments; defined as the ratio of recipient colonies scored on selective medium compared with the number of recipient colonies scored on nonselective medium after mating.

ml) was transferred to a filter membrane (0.45-μm pore size; 25-mm diameter) placed on mating medium. After incubation at 30°C for 60 h (*T. ferrooxidans* as recipient) or 6 days (*E. coli* as recipient), the filter was transferred to 3.0 ml of basal salt solution of solid 2:2 medium or sterile saline, diluted, and plated on appropriate selective plates and nonselective plates. As a control for spontaneous mutation, the recipient strain was plated on the same selective plates. The apparent plasmid transfer frequency is defined as the ratio of recipient colonies scored on selective medium compared with the number of recipient colonies scored on nonselective medium after the mating period. The term "apparent transfer frequency" is used instead of "transfer frequency," for growth could occur and secondary conjugation could also take place during the extended mating period.

Plasmid detection and DNA preparation. Plasmids were isolated by the method described by Birnboim and Doly (6), with slight modification (lysozyme was omitted in solution I). *T. ferrooxidans* cells in 100 ml of culture were harvested for each sample at the stationary phase. Cells were washed with solution I at least twice before plasmid isolation. Genomic DNA of *T. ferrooxidans* was prepared by the method described by Yates and Holmes (37), with some modification as described by Liu and Yan (20).

Southern hybridization. Genomic DNA of *T. ferrooxidans* and plasmid DNA of pSUP1011 were digested completely with *Eco*RI. Agarose (0.8%) gel electrophoresis in Tris-acetate buffer was performed as described by Maniatis et al. (21). Southern blotting and hybridizations were conducted by standard protocols (21). The pSUP1011 DNA probe was labeled with biotin-7-dATP, using a nick translation system (BRL Life Technologies, Inc., Gaithersburg, Md.) as instructed by the manufacturer. After hybridization, DNAs were stained with a DNA detection system (BRL Life Technologies, Inc.) as recommended by the manufacturer.

RESULTS

Conjugative transfer of IncP plasmids to *T. ferrooxidans*. Despite the marked differences in their growth requirements, crosses of *E. coli* and *T. ferrooxidans* could be made on the mating medium described above. *E. coli* C600 containing IncP plasmids was used as the donor strain; *T. ferrooxidans* strains Tf-44 and Tf-50 were used as the recipient strains. Plasmids RP4, R68.45, RP1::Tn*501*, and pUB307 were transferred from *E. coli* donors to Tf-44 and Tf-55 recipients. The transfer frequencies of IncP plasmids from *E. coli* C600 to strains Tf-44 and Tf-50, using kanamycin resistance as the selective marker, are given in Table 2. Tetracycline is inactive in solid 2:2 medium, and no ampicillin-resistant transconjugants were obtained. The RP1::Tn*501* transconjugants of Tf-44 and Tf-50 and their original strains were cultured in 9K liquid medium and then streaked on solid medium described by Mishra et al. (22), containing 0.5 to 2.0 μg of $HgCl_2$ per ml. No significant difference was observed between them during 10 days of incubation at 30°C. All *T. ferrooxidans* strains tested (Tf-44, Tf-45, Tf-46, Tf-48, Tf-49, Tf-50, and Tf-52) served as recipients to receive IncP plasmids (data not shown).

Transfer of plasmids back to *E. coli* from *T. ferrooxidans*. To confirm that plasmids had been transferred into *T. ferrooxidans*, mating between *T. ferrooxidans* transconjugant donors and *E. coli* HB101 recipient was conducted on mating medium with double-strength ferrous iron concentration. *T. ferrooxidans* transconjugants containing RP4 were cultured in liquid 2:2 medium and harvested at the late exponential growth phase; *E. coli* HB101 was harvested at the stationary phase. *E. coli* transconjugants were screened on Luria agar containing antibiotics after mating for 2, 4, and 6 days. No transconjugant was detected after mating for 2 days, and fewer than 10^{-7} transconjugants per recipient were obtained after mating for 4 days, but as the mating extended for 6 days, the apparent transfer frequencies were much higher (Table 2). The high apparent transfer frequencies might be due to the secondary conjugation among the recipients. All genetic markers of RP4 were expressed in HB101.

Mobilization of IncQ cloning vehicle pJRD215 to *T. ferrooxidans*. Using *E. coli* SM10(pJRD215) as the donor strain and five *T. ferrooxidans* strains as recipients, we mobilized the IncQ vector pJRD215 into *T. ferrooxidans* strains with the aid of plasmid RP4 integrated in the chromosome of SM10 (Table 3). Both the kanamycin and streptomycin resistance markers of

TABLE 3. Mobilization of IncQ cloning vector pJRD215 from *E. coli* SM10(pJRD215) to *T. ferrooxidans*

Recipient	Selected marker	Apparent transfer frequency[a] (mean ± SD)
Tf-44	Km^r	$(9.0 \pm 4.3) \times 10^{-6}$
	Sm^r	$(8.7 \pm 4.0) \times 10^{-6}$
Tf-46	Km^r	$(8.1 \pm 3.2) \times 10^{-6}$
	Sm^r	$(8.8 \pm 3.9) \times 10^{-6}$
Tf-49	Km^r	$(2.2 \pm 0.8) \times 10^{-5}$
	Sm^r	$(2.1 \pm 0.7) \times 10^{-5}$
Tf-50	Km^r	$(2.8 \pm 1.2) \times 10^{-5}$
	Sm^r	$(2.3 \pm 1.4) \times 10^{-5}$
Tf-52	Km^r	$(2.1 \pm 0.9) \times 10^{-5}$
	Sm^r	$(2.0 \pm 0.8) \times 10^{-5}$

[a] Determined in each case from three independent experiments; defined as the ratio of recipient colonies scored on selective medium compared with the number of recipient colonies scored on nonselective medium after mating.

pJRD215 were expressed in *T. ferrooxidans*. Results of plasmid analysis of both donor and recipients (Fig. 1) confirmed the presence of pJRD215 in transconjugants.

Stability of pJRD215 in *T. ferrooxidans*. After being incubated at 30°C for 15 days after mating, single colonies of *T. ferrooxidans* transconjugants on the selective plates were transferred to 20 ml of 9K liquid medium; 1/1,000 of the fully grown cultures were transferred to 20 ml of fresh 9K liquid medium and cultured at 30°C with vigorous shaking for 5 days. After five transfers (more than 50 generations) had been made, samples were diluted and plated on solid 2:2 medium with or without kanamycin (300 μg/ml) and cultured at 30°C. Two weeks later, colonies were counted and percentages of plasmid maintenance were calculated as the number of colonies on medium with kanamycin divided by that on medium without kanamycin. The results in Table 4 show that pJRD215 was stable in *T. ferrooxidans*.

Influence of some factors on plasmid transfer from *E. coli* to *T. ferrooxidans*. To determine the influence of the substances in the mating medium on the mating process, crosses between *E. coli* C600(pUB307) or SM10(pJRD215) and *T. ferrooxidans* were conducted in the absence of energy sources for the donor (yeast extract), the recipient ($FeSO_4 \cdot 7H_2O$ and $Na_2S_2O_3 \cdot 5H_2O$), or both the donor and recipient. The results (Table 5) show that the number of the *E. coli* donor increased when the mating medium contained 0.05% yeast extract, while the number of *T. ferrooxidans* recipients showed no significant variation in different mating media. The apparent transfer

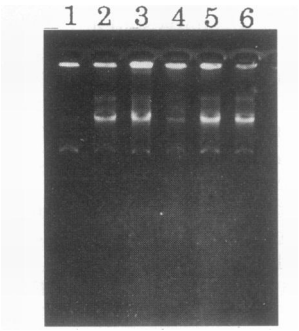

FIG. 1. Agarose gel electrophoresis of plasmid donor and transconjugant strains. Lane 1, donor strain SM10(pJRD215); lane 2, Tf-44(pJRD215); lane 3, Tf-46(pJRD215); lane 4, Tf-49(pJRD215); lane 5, Tf-50(pJRD215); lane 6, Tf-52(pJRD215).

TABLE 4. Stability of pJRD215 in *T. ferrooxidans* strains

Host strain	% of plasmid maintenance[a] (mean ± SD)
Tf-44	100 ± 6
Tf-45	93 ± 4
Tf-49	86 ± 8
Tf-50	86 ± 5
Tf-52	95 ± 5

[a] Determined in each case from three independent experiments; defined as the ratio of colonies scored on medium with kanamycin compared with number of colonies scored on medium without kanamycin.

frequency of pUB307 increased while energy sources for the donor or recipient were present. The increase of the apparent transfer frequency of pUB307 in the presence of energy sources for the donor or recipient suggested that the physiological states of both the donor and the recipient were important for transfer of the large self-transmissible plasmid. In contrast, as for the transfer of pJRD215, the energy source for the donor contributed more to plasmid transfer than that for the recipient. This indicated the physiological state of the donor be more important than that of the recipient during the transfer of a small mobilizable plasmid. The transfer of plasmids from *E. coli* to *T. ferrooxidans* was detected in the absence of energy sources for both donor and recipient.

The influence of the concentration of $FeSO_4 \cdot 7H_2O$ in the mating medium on plasmid transfer from *E. coli* to *T. ferrooxidans* is shown in Fig. 2. Figure 2A shows that the apparent transfer frequency of pUB307 reached the maximum value when the concentration of $FeSO_4 \cdot 7H_2O$ was 150 μg/ml. At this concentration of $FeSO_4 \cdot 7H_2O$, the *E. coli* donor was not inhibited strongly by Fe(III) or Fe(II), while the number of *T. ferrooxidans* recipient began to increase. Figure 2B shows that the transfer frequencies of pJRD215 corresponded to the numbers of surviving donor cells. The data displayed in Fig. 2 also indicate that in the conjugation between *E. coli* and *T. ferrooxidans*, the physiological states of both donor and recipient were important for transfer of the large self-transmissible plasmid, while the transfer frequencies of the small mobilizable plasmid were determined mainly by the physiological state of the donor.

Figure 3 shows that apparent plasmid transfer frequency increased as the mating was prolonged and the number of the surviving donor and recipient cells changed with time.

In all experiments done, we cultured the *T. ferrooxidans* strains in liquid 2:2 medium for 2 weeks, expecting that *T. ferrooxidans* strains adapted to growth in 2:2 medium might grow better in the mating medium. In fact, the transfer frequencies of pJRD215 were higher when *T. ferrooxidans* recipients were cultured in 9K liquid medium. Table 6 shows the results of one test, in which the *T. ferrooxidans* strains had been grown in 9K liquid medium for 6 days and the matings also lasted 6 days. The transfer frequency of pJRD215 to Tf-44 was about 100 times higher than the transfer frequency to Tf-44 grown in liquid 2:2 medium (Fig. 3). The plasmid transfer frequencies of most strains reached relative high levels, with the exception of Tf-50. Other factors affecting transfer frequency such as donor-to-recipient ratio remain to be investigated.

Introduction of Tn*5* into *T. ferrooxidans*. pSUP1011 (32) is a mobilizable plasmid carrying transposon Tn*5*. The plasmid is unable to replicate in strains outside the enteric bacterial group and is useful as a transposon carrier replicon for random

TABLE 5. Influence of substance in mating medium on plasmid transfer[a]

Mating medium[b]	Conjugation between C600(pUB307) and Tf-49			Conjugation between SM10(pJRD215) and Tf-49		
	Donor no. (10^9)	Recipient no. (10^9)	Transfer frequency (10^{-7})	Donor no. (10^9)	Recipient no. (10^9)	Transfer frequency (10^{-6})
O	1.6 ± 0.4	3.2 ± 0.5	2.2 ± 1.0	0.46 ± 0.12	2.7 ± 0.4	0.45 ± 0.22
O+S+F	1.6 ± 0.3	3.2 ± 0.4	8.3 ± 3.3	0.52 ± 0.17	2.9 ± 0.5	1.8 ± 0.7
O+Y	2.7 ± 0.5	3.1 ± 0.5	11 ± 4.2	1.3 ± 0.3	2.7 ± 0.5	87 ± 34
O+S+F+Y	3.2 ± 0.6	3.4 ± 0.6	14 ± 6.2	1.3 ± 0.4	3.5 ± 0.7	46 ± 21

[a] Values were obtained from three independent experiments and are shown as means ± standard deviations.
[b] O, 0.45% $(NH_4)_2SO_4$, 0.015% KCl, 0.075% $MgSO_4 \cdot 7H_2O$, and 0.6% agar; S, 0.2% $Na_2S_2O_3 \cdot 5H_2O$; F, 0.0075% $FeSO_4 \cdot 7H_2O$; Y, 0.05% yeast extract.

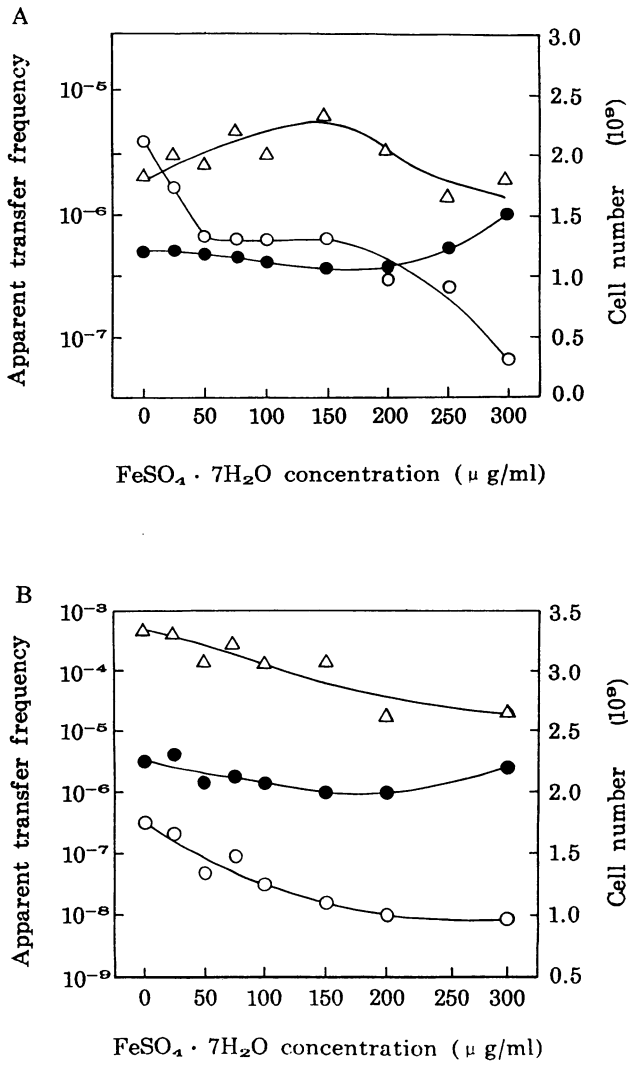

FIG. 2. Influence of $FeSO_4 \cdot 7H_2O$ concentration in mating medium on plasmid transfer. Mating between C600(pUB307) and Tf-50 (A) or SM10(pJRD215) and Tf-50 (B) was done as described in Materials and Methods, with the variation of $FeSO_4 \cdot 7H_2O$ concentration in the mating medium. Three independent matings were performed. Values were reproducible within 1 order of magnitude. Symbols: △, apparent plasmid transfer frequency; ○, cell number of donor; ●, cell number of recipient.

transposon insertion mutagenesis in any strain into which it can be mobilized but not stably maintained. To introduce Tn*5* into *T. ferrooxidans*, mating between the *E. coli* SM10(pSUP1011) donor and *T. ferrooxidans* recipients cultured in 9K liquid medium lasted for 6 days (Table 7). Kanamycin-resistant colonies on solid 2:2 medium with 300 μg of kanamycin per ml required 20 to 25 days, which was about a week longer than the

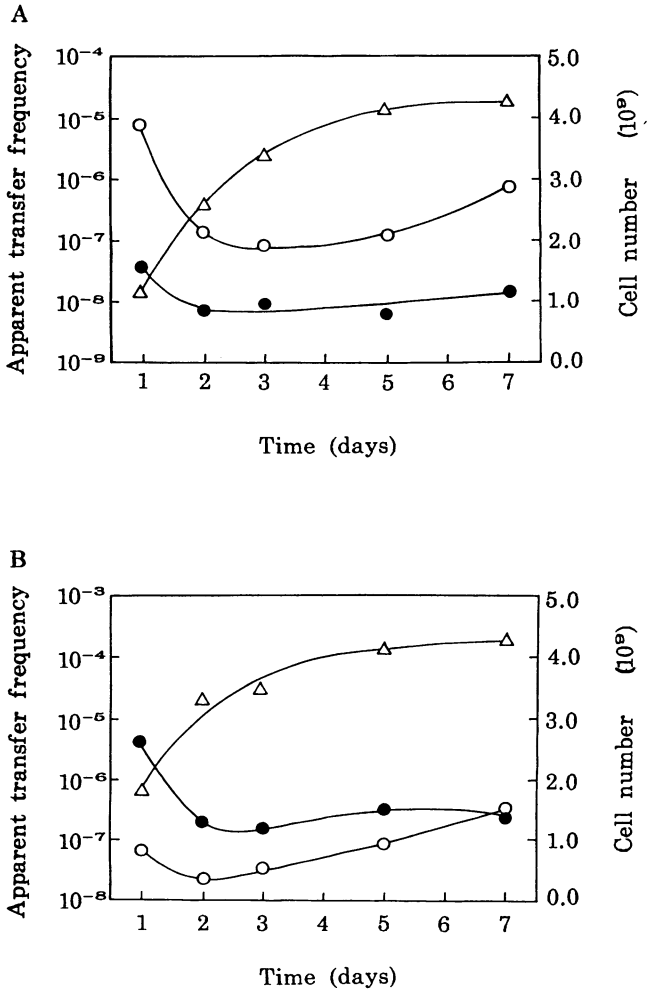

FIG. 3. Change of apparent plasmid transfer frequency (△) and cell numbers of donor (○) and recipient (●) with mating time. Three independent matings were performed. Values were reproducible within 1 order of magnitude. (A) Mating between C600(pUB307) and Tf-44; (B) mating between SM10(pJRD215) and Tf-44.

TABLE 6. Results of mating in a suitable condition[a]

Recipient	Apparent transfer frequency
Tf-44	3.0×10^{-2}
Tf-45	2.0×10^{-2}
Tf-46	8.5×10^{-1}
Tf-49	1.1×10^{-2}
Tf-50	1.2×10^{-5}

[a] The matings [SM10(pJRD215) as the donor] lasted for 6 days, and recipient cells were cultured in 9K medium before mating. The selected marker was Km^r.

colony-forming time for pJRD215 transconjugants. pJRD215 is a multicopy plasmid (10, 13), while Tn*5* inserted in the chromosome of *T. ferrooxidans* might be single copy (4, 5). This would account for the poor resistance to kanamycin of *T. ferrooxidans* strains carrying Tn*5*. Result of Southern hybridization (Fig. 4) confirmed the insertion of Tn*5* in the chromosome of *T. ferrooxidans*.

DISCUSSION

T. ferrooxidans is an obligately autotrophic bacterium for which the optimal pH for growth is 1.5 to 2.5, whereas *E. coli* is a heterotrophic bacterium whose optimal pH for growth is 7.0 to 7.5. The growth requirements of the two bacterial species are so different that it was considered impossible to devise a medium in which both bacteria could meet their energy needs required by the conjugation process at the same time (35). The mating medium described here provides energy sources for *E. coli* with 0.05% yeast extract and for *T. ferrooxidans* with 0.2% $Na_2S_2O_3 \cdot 5H_2O$ and 0.0075% $FeSO_4 \cdot 7H_2O$. The pH of the medium (4.6 to 4.8) permits the growth of both bacteria. The mating between *E. coli* and *T. ferrooxidans* can be conducted on this medium as described in Results.

According to the results obtained in this study, apparent plasmid transfer frequencies could reach relatively high levels as the requirement of the *E. coli* donor was met. In fact, plasmid transfer can take place with a relatively wide range of conditions (12, 24, 28). For example, Fernandez-Astorga et al. (12) had detected *E. coli* transconjugant formation in the absence of nutrients, even at a mating temperature of 8°C. As reported in this paper, plasmid transfer from *E. coli* to *T. ferrooxidans* also could be detected in the absence of energy sources for both donor and recipient. Therefore, the previous failure to demonstrate plasmid transfer between *E. coli* and *T. ferrooxidans* might be due mainly to lack of a proper selective solid medium. Solid 2:2 medium described in this report is efficient to serve as such a medium in which at least kanamycin and streptomycin resistances can be used as selective markers and the spontaneous mutation rates are lower than 10^{-7}. We will report the growth pattern of *T. ferrooxidans* on this medium elsewhere.

TABLE 7. Introduction of Tn*5* into *T. ferrooxidans*

Strain	Apparent transposition frequency[a] (mean ± SD)
Tf-44	$(8.2 \pm 4.2) \times 10^{-6}$
Tf-45	$(1.0 \pm 0.6) \times 10^{-5}$
Tf-49	$(2.7 \pm 1.3) \times 10^{-4}$
Tf-50	$(6.0 \pm 2.7) \times 10^{-5}$
Tf-52	$(4.7 \pm 2.8) \times 10^{-6}$

[a] Determined in each case from three independent experiments; defined as the ratio of recipient colonies scored on selective medium compared with the number of recipient colonies scored on nonselective medium after mating.

FIG. 4. Southern blot analysis of genomic DNA of Tf-49 carrying Tn*5* by using biotin-7-dATP-labeled pSUP1011 as a probe. Lane 1, genomic DNA of Tf-49 carrying Tn*5* digested with *Eco*RI; lane 2, genomic DNA of Tf-49 digested with *Eco*RI; lane 3, pSUP1011 DNA digested with *Eco*RI.

T. ferrooxidans is an acidophilic obligately autotrophic bacterium, but genes originated from heterotrophic bacteria, including the kanamycin resistance and *tra* gene of RP4, neomycin/kanamycin resistance gene and genes involved in the transposition of Tn*5*, and the streptomycin resistance gene of RSF1010 (10, 13), were expressed in this bacterium. Jin et al. (15) had demonstrated expression of some genes of RP4 (kanamycin resistance, tetracycline resistance, and *tra* genes) in *T. thiooxidans*, another acidophilic obligately autotrophic bacterium of the same genus. Despite the great physiological differences between these bacteria, their gene expression systems might be similar.

The presence of pili (11) and broad-host-range mobilizable plasmids in some *T. ferrooxidans* strains, and the results of our experiments, imply the existence of a genetic transfer process between *T. ferrooxidans* and bacteria sharing its habitat. However, no self-transmissible plasmid was found in *T. ferrooxidans*. If a mobilizable plasmid such as pJRD215 is transferred to *T. ferrooxidans*, and the ability to mobilize the plasmid back to *E. coli* is examined, it may be helpful to find a native self-transmissible plasmid in *T. ferrooxidans*.

Introduction of Tn*501*, Tn*1721*, and Tn*5* into *T. novellus* and *T. versutus* (8, 33) had been reported. Several mutant phenotypes of the two species were obtained by this means. For example, mutants with reduced rates of oxidizing sulfur compounds were obtained from Tn*501* mutagenesis of *T. novellus* (8). If such mutants can be obtained by transposon mutagenesis of *T. ferrooxidans*, using the transposon as a probe, it might be possible to isolate its sulfur oxidation genes, which cannot be cloned by complementation in *E. coli*. Since Tn*5* is widely used as a genetic tool because of its intrinsic properties (5), the introduction of Tn*5* into *T. ferrooxidans* may help in the genetic study of this bacterium.

Studies on the molecular basis for the broad-host-range properties of the IncQ plasmid RSF1010 indicate that its particular mode of replication renders it independent of host replication functions, which are normally required by other plasmid replicons (29). For this reason, the RSF1010-based vector pJRD215 was stable in *T. ferrooxidans*. Therefore, such plasmids can serve as vectors to introduce genes into *T.*

ferrooxidans, avoiding construction of shuttle vectors. In fact, we have introduced arsenic resistance genes derived from plasmid R773 (23) into *T. ferrooxidans* strains, and the resistance to $NaAsO_2$ of *T. ferrooxidans* strains was enhanced significantly.

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