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A Diffusible Compound Can Enhance Conjugal Transfer of the Ti Plasmid in *Agrobacterium tumefaciens*

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Several octopine strains of *Agrobacterium tumefaciens* were tested for Ti plasmid (pTi) transfer after induction by 400 μg of octopine per ml for 24 h. The strains could be divided into two groups, transfer efficient (Tra^e) and transfer inefficient (Tra^{ie}); the respective rates of transfer were 0.77×10^{-2} to 1.14×10^{-2} and 0.33×10^{-6} to 9.8×10^{-6} plasmid transconjugant per donor cell. Transfer efficiencies of Tra^{ie} strains were greatly increased when the time of induction was 72 h. A diffusible conjugation factor (CF) that can enhance conjugal transfer of pTi in *A. tumefaciens* was discovered when both Tra^e and Tra^{ie} donor strains were induced in the same plate. The evidence indicates that CF is a key factor affecting transfer efficiency of pTi but is not sufficient by itself to induce transfer. Tra^c mutants can produce CF constitutively, and Tra^e strains can produce it after induction by low octopine concentrations. The transfer efficiency of Tra^{ie} strains was greatly increased by adding CF to the induction medium. The thermosensitive strain B6S3, which normally cannot conjugate at temperatures above 30°C, could transfer pTi efficiently at 32 and 34°C in the presence of CF. Production of CF is dependent on the presence of pTi but appears to be common for different opine strains; it was first detected in octopine strains, but nopaline strains also produced the same or a similar compound. CF is very biologically active, affecting donor but not recipient bacterial cells, but CF does not promote aggregation. Data suggest that CF might be an activator or derepressor in the conjugation system of *A. tumefaciens*. CF is a dialyzable small molecule and is resistant to DNase, RNase, protease, and heating to 100°C for 10 min, but autoclaving (121°C for 15 min) and alkaline treatment removed all activity.

There are two DNA transfer processes associated with the Ti plasmid (pTi) of *Agrobacterium tumefaciens*; one is T-DNA transfer from *A. tumefaciens* to a host plant cell (34, 35), and the other is pTi transfer from *Agrobacterium* donor cells to recipient cells (16, 21, 27). Evidence indicates that the basic processes of these two DNA transfers are similar; both involve activation of gene expression in response to external stimuli (21, 22) and, presumably, conjugal transfer of single-stranded DNA from a donor cell to a recipient cell (reviewed in references 24, 33, and 35). Interestingly, these two DNA transfer processes are closely related by their own unique functions; DNA is transferred from bacterial cell to plant cell, and the latter is directed to grow and divide rapidly and to produce opines (reviewed in references 4, 34, and 35), which play a crucial role in the whole process. Most opines are imino acids. Not only do they provide a more or less reserved food supply for the inciting bacteria, but they also induce bacterial conjugation and pTi transfer (11, 15, 27). This was the first example of substrate-induced conjugation in bacteria. Many different opines are synthesized by different strains of *A. tumefaciens*, and conjugation induction is relatively specific. Octopine induces conjugation by octopine strains, but agropine, which is also synthesized in crown gall tissue induced by these strains, does not. Octopine is also synthesized in grapevine crown galls incited by many biovar 3 strains of *A. tumefaciens* (26), but it does not induce their conjugation. Nopaline strains are not induced to conjugate by octopine or even by nopaline but by another opine, agrocinopine A, which is also present in crown gall tissue induced by these strains (7, 27).

Although opine induction of conjugal transfer in *A. tumefaciens* has been known for many years, we still know very little about the process. Early work was mainly on substrate induction of pTi conjugal transfer (reviewed in reference 14). It was found that constitutive opine catabolic (Occ^c) mutants are also transfer constitutive (Tra^c) (9, 17, 27). This suggests a model in which a repressor blocks common operator sites in the promoter region of the two loci and in which the opine derepresses the operon by binding to the repressor (14, 27). However, Klapwijk et al. (16, 17) presented evidence that a single insertion mutant could produce the phenotype Occ^- Tra^-. Therefore, a positive control gene might also be involved in the regulation of pTi conjugal transfer. More recent work with a nopaline strain identified *tra* genes in three separate loci on pTi (3). The combined size of the three loci was only about 9 kb, which seems rather small when compared with about 40 kb in the *vir* region required for T-DNA transfer (23) and with the *tra* region of the *Escherichia coli* F plasmid, which is more than 33 kb (33). Clearly, more work is needed to reveal the mechanism of *Agrobacterium* pTi conjugal transfer.

The conjugative mechanism of pTi transfer is of interest, not only because opine-induced pTi transfer is important in crown gall disease epidemics, but also because characterization of pTi transfer would help to elucidate whether there is any relationship between it and T-DNA transfer, a suggestion made on several occasions (3, 10, 25, 29, 31). In this paper we describe (i) the discovery of a diffusible conjugation factor (CF) that is important in the regulation of pTi conjugal transfer, (ii) factors influencing the synthesis of CF, (iii) some of the chemical and physical characteristics of CF, and (iv) the way in which CF can influence conjugation.

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TABLE 1. Bacterial strains and plasmids

Strain	Plasmid	Description[a]
A6	pTiA6	Octopine catabolic, supplied by G. Morel
NCPPB1001	pTiNCPPB1001	Octopine catabolic, supplied by NCPPB
K608	pTiB6::Tn*5*	Octopine catabolic, T-DNA::Tn*5*, Tra^{ci} Chl^r, C58 pTi^- background, supplied by J. G. Ellis
K794	pTiB6::Tn*5*	Octopine catabolic, TR-DNA::Tn*5*, Tra^c Rif^r Str^r, C58 pTi^- background, supplied by J. G. Ellis
K804	pTiB6::Tn*5*	Octopine catabolic, TR-DNA::Tn*5*, Tra^c Rif^r Str^r, C58 pTi^- background, supplied by J. G. Ellis
B6	pTiB6	Octopine catabolic, supplied by G. Morel
Ach5	pTiAch5	Octopine catabolic, supplied by J. Schell
B6S3	pTiB6	Octopine catabolic, a phage-sensitive isolate from UV-irradiated and mitomycin C-treated B6 (16), supplied by J. Tempé
T37	pTiT37	Nopaline catabolic, Tra^{ci}, supplied by G. Morel
K323	pTiT37	Nopaline catabolic, Tra^c colony of strain T37 isolated from octopine medium, our laboratory
K749		C58 pTi^- pAt^- Rif^r Str^r, supplied by A. Kondorosi
K749(pTiK608)	pTiB6::Tn*5*	Octopine catabolic, transconjugant from K608 × K749, this study
K608C1		pTiB6::Tn*5* was cured from strain K608, this study

[a] Abbreviations: Chl^r, chloromycetin resistant; Rif^r, rifampin resistant; Str^r, streptomycin resistant; NCPPB, National Collection of Plant Pathogenic Bacteria, United Kingdom.

MATERIALS AND METHODS

Bacterial strains and media. The bacterial strains used in this study are listed in Table 1. Strain K749, a plasmidless derivative of strain C58, was used as the recipient strain in all experiments. Strains were maintained on YM medium (8) and, if necessary, supplemented with antibiotics.

The minimal salts medium of Petit and Tempé (20) was the basis of induction and selective media; 200 μg of octopine per ml and 1 mg of glucose per ml were added except where otherwise stated, and 1 mg of $(NH_4)_2SO_4$ per ml was used in induction medium to replace octopine in control experiments. The selective medium was supplemented with 50 μg of rifampin and 500 μg of streptomycin per ml. The induction and selective media were adjusted to pH 7.0.

Chemicals and enzymes. Opines used in this study were synthesized by M. E. Tate and J. Tempé. L-Proline, D-proline, L-arginine, L-ornithine, protease type XIV, RNase type 1-A, and DNase type III are the products of Sigma. The enzymes were dissolved in sterile distilled water before use.

Conjugation. (i) Drop mating. On day 1, fresh cultures of donor and recipient strains grown on YM plates for 1 or 2 days were suspended in 5 ml of sterile distilled water and adjusted to an optical density at 600 nm of 0.4, and 200 μl of recipient suspension was spread on to selective plates; 30 μl of each donor strain was inoculated into a culture tube that contained 1 ml of liquid induction medium. Donors were incubated with shaking at 25°C. The induction time was 24 h except when the effect of induction time on conjugation was tested. Recipient plates were kept at 28°C for 24 h. On day 2, donor strains were suspended, and a 10-fold dilution series was prepared; 10-μl samples of 10^0 to 10^{-5} dilutions were spotted in triplicate onto a lawn of the recipient growing on selective medium; 10 μl of 10^0 dilution of donor cells was spotted on selective medium as a control. To determine the number of viable donor cells, 10-μl samples of 10^{-5}, 10^{-6}, and 10^{-7} dilutions were spotted onto YM plates. Plates were incubated at 28°C, and then the numbers of donors and transconjugants were recorded after 2 and 5 days, respectively. Since the recipient strain cannot utilize octopine, transconjugants are easily distinguished. Transfer efficiencies of donor strains were calculated as the number of transconjugants observed per donor cell applied.

(ii) Replica plating mating. On day 1, a recipient strain was spread on selective medium as above, and donors were applied as patches to solid induction medium. Both recipient and donors were incubated at 28°C for 24 h. On day 2, induced donors were replica plated onto the recipient lawn. Transconjugants were observed after incubation at 28°C for 4 or 5 days.

Octopine utilization. Test strains were inoculated into YEB (30) and incubated for 24 h at 25°C. The optical density at 600 nm of these fresh inocula was adjusted to 0.4, and 0.5 ml of each inoculum was added to 3 ml of liquid induction medium containing 50 μg of octopine per ml. The presence of octopine in the medium was determined by the colorimetric method of Lippincott et al. (18) at 0 and 30 min and at 1, 2, 5, 10, and 24 h after inoculation.

CF preparation and CF activity bioassay. CF filtrate was prepared as follows: strain K608 was inoculated into 10 ml of liquid induction medium containing 400 μg octopine per ml and incubated at 25°C for 24 h with shaking. The culture was centrifuged, and the supernatant was collected and sterilized by membrane filtration (0.2-μm pore size; Millipore). The presence of octopine was determined. When CF induction by other opines and chemicals was tested, the opines and chemicals also were used at 400 μg/ml, and the uninoculated media were used as controls to make sure that enhanced pTi transfer efficiency was due to CF rather than to the added opines and chemicals.

The same procedure was used to examine the CF-producing ability of all Tra^c and partially transfer constitutive (Tra^{ci}) strains used. For the inducible strains A6, NCPPB 1001, B6, Ach5, K749, and plasmidless strain K608C1, which fail to produce CF in liquid medium, the replica plate mating method was used to determine CF production; the CF-producing strain and CF activity indicator strain (B6S3) were patched side by side at a distance of 1.2 cm (Fig. 1).

Strain B6S3 was selected to indicate CF activity because it cannot transfer pTi when the concentration of octopine in the induction medium is 200 μg/ml (Table 2). Drop mating and replica plate mating methods, respectively, were used in quantitative and qualitative assays of the promoting effect of CF on pTi conjugal transfer. In the qualitative assay, 400 μl of a CF preparation was added to an agar induction plate containing 200 μg of octopine per ml; after drying, strain B6S3 was patched onto the plate, incubated for 24 h at 28°C, and replica plated onto a lawn of the recipient on selective medium. In the quantitative assay, 200 μl of CF filtrate and 30 μl of a cell suspension of strain B6S3 were added to 1 ml of liquid induction medium (200 μg of octopine per ml),

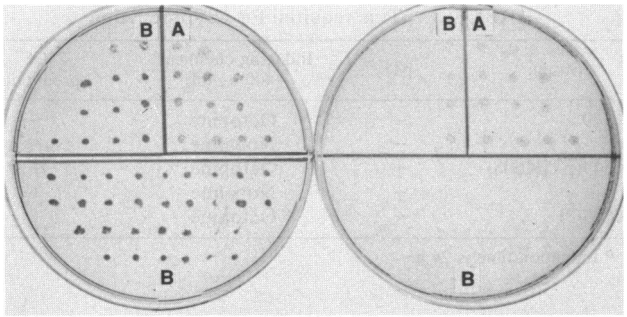

FIG. 1. A diffusible conjugation factor produced by strain K608 (A) promotes pTi conjugal transfer of strain B6S3 (B). Left, Induction plate; right, selective plate showing transconjugants. Double lines indicate the location of the ditch.

incubated for 24 h, and diluted; 10-μl drops were added to a lawn of the recipient on selective medium.

Aggregation assay. The method of Dunny et al. (6) was followed to determine whether CF could induce aggregation between donor cells, recipient cells, or combined donor and recipient cells; 1 ml of CF preparation plus 800 μl of fresh liquid induction medium plus 200 μl of late-log-phase cultures of donor cells, recipient cells, or a 1:1 mixture of donor and recipient were mixed, incubated at 28°C, and monitored for aggregation.

RESULTS

Sensitivity of strains to octopine induction. Octopine concentrations from 200 to 1,400 μg/ml were tested on several strains for induction of pTi conjugal transfer. The sensitivity of strains to octopine induction of conjugation was variable (Table 2). Transfer-efficient (Tra^e) strains were very sensitive; octopine at a concentration of 200 μg/per ml induced strain A6 to transfer pTi at a rate of 0.52×10^{-2} per donor cell. Other strains were transfer inefficient (Tra^{ie}) and much less sensitive; the transfer efficiency of strain B6S3 was only 0.39×10^{-4} transconjugant per donor at an octopine concentration of 1,400 μg/ml. Rates of octopine utilization by strains K608 and B6S3 were measured, but no significant difference was detected (data not shown).

Influence of induction time. The influence of time of induction by octopine on pTi conjugal transfer was pronounced. For the Tra^{ie} strains B6, Ach5, and B6S3, induction for 24 h by 200 μg of octopine per ml did not result in any pTi transfer (Table 3), but when the induction time was increased to 72 h, the transfer efficiencies increased markedly.

Tra^e strains are fully competent after 24 h induction by octopine. Shorter periods were tested with strain A6; no pTi transfer occurred after 5 h of induction ($<1 \times 10^{-7}$ transconjugant per donor). However, when after 5 h of induction, bacteria were grown on a noninducing substrate for another 19 h before plating on selective medium, pTi transfer occurred (0.59×10^{-6} transconjugant per donor). The corresponding rates following 10 h of induction followed by 0 or 14 h of growth on noninducing substrate were 0.16×10^{-6} and 0.23×10^{-4} transconjugant per donor. This compared with a rate of 0.83×10^{-2} transconjugant per donor after induction for 24 h.

TABLE 3. Effect of time of induction by 200 μg of octopine per ml on pTi conjugal transfer

Strain	Period of induction (h)	Transfer efficiency (transconjugants per donor)
B6	24	$<1 \times 10^{-7}$
	72[a]	0.19×10^{-3}
Ach5	24	$<1 \times 10^{-7}$
	72[a]	0.35×10^{-3}
B6S3	24	$<1 \times 10^{-7}$
	72[a]	0.57×10^{-5}
A6	24	0.50×10^{-2}

[a] The transfer efficiencies in control experiments were $<1 \times 10^{-7}$ transconjugant per donor.

A new CF. In our preliminary experiments to investigate the effect of temperature on pTi transfer by different strains, it was noted that, under some conditions, transconjugant colonies of Tra^{ie} strains appeared only when adjacent to Tra^e transconjugants. It appeared that Tra^e strains were producing a diffusible compound that could stimulate pTi transfer. This was confirmed by a "ditch plate" experiment in which a Tra^{ie} strain was separated from a Tra^e strain on an agar induction plate by removing a thin slice of agar. Figure 1 shows the effect of chemical diffusion from strain K608 to strain B6S3. When the agar slice was not removed, transconjugant colonies of strain B6S3 appeared only when adjacent to strain K608. When the two strains were separated by a ditch in the induction medium, no transconjugants of strain B6S3 could be seen on the selective medium. The existence of this diffusible conjugation factor was confirmed by chromotography and high-voltage paper electrophoresis (data not shown).

Influence of CF on pTi transfer efficiency. Several octopine strains were tested for synthesis of CF after induction by various chemicals for various periods. There was a strong correlation between CF production and pTi conjugal transfer efficiency (Table 4). Tra^e strains A6 and NCPPB1001 produced CF after being induced for 24 h by 200 μg octopine per ml on agar plates, but Tra^{ie} strains Ach5 and B6 could only

TABLE 2. Transfer efficiency of octopine strains

Strain	Transfer efficiency (no. of transconjugants per donor) at the following concn of octopine (μg/ml):					Classification based on sensitivity
	0	200	400	1,000	1,400	
A6	$<1 \times 10^{-7}$	0.50×10^{-2}	0.77×10^{-2}	0.83×10^{-2}	0.97×10^{-2}	Tra^e
NCPPB1001	$<1 \times 10^{-7}$	NT[a]	1.14×10^{-2}	NT	NT	Tra^e
K608	0.97×10^{-6}	NT	0.79×10^{-2}	NT	NT	Tra^e
B6S3	$<1 \times 10^{-7}$	$<1 \times 10^{-7}$	0.33×10^{-6}	0.28×10^{-5}	0.39×10^{-4}	Tra^{ie}
Ach5	NT	$<1 \times 10^{-7}$	0.98×10^{-5}	NT	0.27×10^{-3}	Tra^{ie}
B6	NT	$<1 \times 10^{-7}$	0.57×10^{-5}	NT	0.14×10^{-3}	Tra^{ie}

[a] NT, Not tested.

TABLE 4. CF production by different octopine strains of *A. tumefaciens*, with and without induction

Strain	Transfer system	Inducing chemical (per liter)	Induction time (h)	CF activity[a]
K794	Tra^c	$(NH_4)_2SO_4$ (1 g)	24	+
K804	Tra^c	$(NH_4)_2SO_4$ (1 g)	24	+
K608	Tra^{ci}	$(NH_4)_2SO_4$ (1 g)	24	−
		Octopine (200 mg)	24	+
A6	Tra^e	Octopine (200 mg)	24	+
NCPPB1001	Tra^e	Octopine (200 mg)	24	+
B6	Tra^{ie}	Octopine (400 mg)	24	−
		Octopine (400 mg)	72	+
Ach5	Tra^{ie}	Octopine (400 mg)	24	−
		Octopine (400 mg)	72	+

[a] CF production was determined by using a CF filtrate preparation for strains K794 and K804 and by the ditch plate method for the other strains.

synthesize CF after being induced for 72 h. Tra^c strains could produce CF constitutively, but in the Tra^{ci} strain K608 CF production was detected only after induction by octopine.

Strain B6S3 was induced by a wide range of octopine concentrations with and without the addition of CF. The presence of CF had a marked influence on conjugation over the whole range of octopine concentrations (Fig. 2). However, when no octopine was present, CF did not induce conjugation.

Several strains were tested for production of CF in both liquid and solid induction media. All Tra^c and Tra^{ci} mutants tested (K608, K794, K804, T37, and K323) produced CF in both solid and liquid media, but with the wild-type inducible strains A6, NCPPB1001, B6, and Ach5, CF production was detected only in solid medium.

Relationship of CF to nopaline strains. pTi can be divided into octopine, nopaline, and agropine types based upon the opines they produce and catabolize (14, 28). Although different opine strains need specific opines for induction of pTi conjugal transfer (7, 14, 28), strong homology between Tra regions of these strains has been observed by several groups (5, 12, 13). This indicates that there are some *tra* genes conserved in different opine-type Ti plasmids. When the nopaline Tra^c and Tra^{ci} strains K323 and T37 were grown for 24 h in liquid induction medium with 1 g of $(NH_4)_2SO_4$ per liter, a substance was produced that promoted conjugal transfer in the octopine strain B6S3.

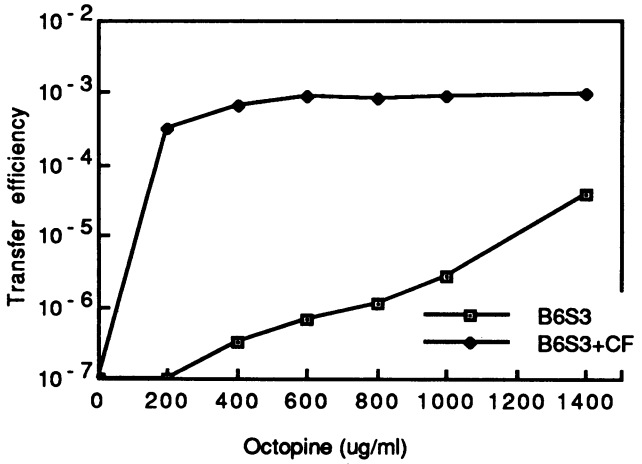

FIG. 2. CF promoting pTi transfer efficiency by strain B6S3.

TABLE 5. pTi is required for CF production

Strain	pTi	Inducing chemical[a] (400 μg/ml)	CF production
K749	−	Octopine	−
	−	Nopaline	−
K749(pTiK608)	+	Octopine	+
	+	Nopaline	+
K608C1	−	Octopine	−

[a] Induction time is 24 h.

Requirement of pTi for CF production. pTi is required for the production of CF. When pTi was cured from strain K608, it lost CF production ability; conversely, strain K749 gained this ability along with pTi from strain K608 (Table 5).

Imino acids induce CF production in strain K608. Because nopaline strains can produce a compound that promotes pTi transfer by an octopine strain, it appeared possible that CF induction also was not highly specific. A wide range of chemicals was tested by using the Tra^{ci} strain K608 as a producer. All of the imino acids tested could induce strain K608 to produce CF, although some of them could not be utilized by strain K608 or were utilized only poorly (Table 6). Octopine was the most efficient inducer, followed by nopaline and noroctopinic acid. L- and D-proline, which are not opines, could also efficiently induce production of CF. But L-arginine and pyruvate, the components of octopine (19), had no inducing effect.

Effect of temperature on CF production by strain B6S3. Tempé et al. (29) found that strain B6S3 could not transfer its pTi at a temperature above 30°C. The data in Table 7 confirm their observation. When the induction temperature was higher than 30°C, transfer efficiency of strain B6S3 was less than 1×10^{-7} transconjugant per donor. This was not due to a difference in octopine catabolism at the various temperatures (data not shown). However, when CF was added to the induction medium, strain B6S3 could transfer pTi at 32 and 34°C at rates of 0.93×10^{-3} and 0.55×10^{-4} transconjugant per donor, respectively.

Properties of the diffusible CF. Aliquots (1 ml) of CF

TABLE 6. CF production by strain K608 after induction by various imino acids

Chemical	Catabolism by strain K608	CF activity[a]
Octopine	+	0.83×10^{-3}
Norooctopinic acid[b]	Very poor	0.87×10^{-4}
Homooctopine[b]	−	0.61×10^{-5}
Agropine	+	0.35×10^{-4}
Deoxymannityl glutamic acid[b]	Poor	0.42×10^{-5}
Nopaline[b]	−	0.97×10^{-4}
Allo-nopaline[b]	−	0.36×10^{-5}
L-Proline	+	0.72×10^{-4}
D-Proline	+	0.54×10^{-4}
L-Arginine and pyruvate	+	$<1 \times 10^{-7}$
Control[c]	+	$<1 \times 10^{-7}$

[a] CF activity is expressed as pTi transfer efficiency (transconjugants per donor) by strain B6S3.

[b] When the inducing chemical could not be catabolized by strain K608, the medium was supplemented with 1 mg of $(NH_4)_2SO_4$ per ml to provide a source of nitrogen.

[c] Control treatment consisted of 1 mg of $(NH_4)_2SO_4$ per ml.

TABLE 7. pTi conjugal transfer by strain B6S3 at a range of temperatures, with and without CF

Induction treatment	Transfer efficiency (transconjugants per donor) at the following temp (°C):					
	24	28	30	32	34	37
Octopine (2.4 mg/ml)	1.01×10^{-3}	0.94×10^{-3}	0.37×10^{-3}	$<1 \times 10^{-7}$	$<1 \times 10^{-7}$	$<1 \times 10^{-7}$
Octopine (2.4 mg/ml) + CF	1.04×10^{-3}	1.12×10^{-3}	0.88×10^{-3}	0.93×10^{-3}	0.55×10^{-4}	$<1 \times 10^{-7}$

preparation were treated (i) with 2 N NH_4OH at 45°C for 2 h, (ii) by autoclaving at 121°C for 15 min, (iii) with DNase (500 μg/ml), (iv) with RNase (500 μg/ml), or (v) with protease (50 μg/ml). All enzymatic treatments were at 37°C for 20 min followed by 100°C for 10 min. After treatments, 400 μl from each treatment was used to assay CF activity. Only treatments (i) and (ii) removed activity. CF is a small molecule; the filtrate obtained after passing CF through Centricon 10 had lost no activity. CF is also dialyzable; when we dialyzed a CF preparation overnight against liquid induction medium lacking octopine, CF activity was lost from the solution within the membrane but could be detected in the external liquid after concentration.

Mode of action. Dunny et al. (6) reported that recipient strains of *Streptococcus faecalis* produce a diffusible clumping-inducing agent that causes strains carrying certain conjugative plasmids to aggregate. The fact that CF can diffuse from bacterial donor cells prompted us to consider that CF might have some effect on recipient cells. Therefore, 400 μl of CF preparation was added to the selective medium before the recipient was added; the culture was incubated for 24 h at 28°C and then drop mated with strain B6S3 that had been induced for 24 h by 200 μg of octopine per ml. However, the result showed no difference in transfer efficiency between that treatment and the negative control. CF did not induce any measurable aggregation between donor cells, recipient cells, or a mixture of donor and recipient cells.

Although quantitative measurement of CF concentration is impossible at this stage, there can be little doubt that CF is a highly active molecule. A 50-fold dilution of CF preparation can still enhance pTi transfer efficiency in Tra^{ie} strain B6S3 by more than 1,000 times when coinduced by 200 μg of octopine per ml.

DISCUSSION

Our results show that octopine strains of *A. tumefaciens* can be placed in two distinct groups, based on their conjugal transfer efficiency. The distinction is also apparent in their response to different concentrations of octopine and in the time required for induction. However, the distinction appears to be eliminated, or at least reduced, when inefficient strains are exposed to a diffusible CF produced by the efficient strains. The discovery of CF explains the difference in pTi transfer efficiency of Tra^{e} and Tra^{ie} strains and might also help to elucidate the mechanism of opine-induced pTi conjugal transfer.

Tempé and Petit (27) proposed a model to explain regulation of genes controlling opine catabolism and Ti plasmid transfer. In their model, opines induce conjugal transfer by binding to the repressor of *tra* genes. The fact that CF can significantly enhance pTi transfer efficiency in the presence of a low concentration of octopine but is not able by itself to induce transfer indicates that the regulation of pTi transfer is more complicated than that model. Presumably CF is an activator or derepressor of the system, but at present the mode of action is unclear. Its production clearly involves pTi. When the CF-producing strain K608 was cured of its pTi, it lost CF production ability; conversely, strain K749, which is a plasmidless strain, gained this ability after it received the pTi from strain K608.

A temperature greater than 30°C has been reported to inhibit both *vir* gene induction (1, 2) and pTi transfer (29), and therefore it was suggested that T-DNA transfer and pTi conjugal transfer might share a common thermosensitive step. But our results show that temperatures from 24 to 34°C have no significant effect on conjugal transfer by all strains we tested (data not shown) except strain B6S3. Strain B6S3 is a thermosensitive strain; when induction temperature is above 30°C, no pTi transfer is detected. This agrees with the observation of Tempé et al. (29). However, when CF was added to the induction medium, the pTi transfer efficiencies of strain B6S3 were 0.93×10^{-3} and 0.55×10^{-4} transconjugant per donor at 32 and 34°C, respectively. These data suggest that there is a thermosensitive step involved in CF production by strain B6S3. Strain B6S3 is a phage-sensitive isolate from UV-irradiated and mitomycin C-treated strain B6 (32). It is likely that a mutation has also resulted in thermosensitivity in conjugal transfer in this strain.

CF is probably a common factor among different opine strains. Besides octopine strains, nopaline Tra^{c} and Tra^{ci} strains K323 and T37 were able to produce CF or at least a very similar compound. Other evidence supports this hypothesis; all opines tested were able to induce strain K608 to produce CF, although their effect varied. Imino acids L-proline and D-proline could also induce CF production. But the amino acid L-arginine, a substituent of octopine (19), had no effect on CF induction. Results suggest that the imino group might be the basic structure needed for CF induction in strain K608. But at this stage, we cannot determine whether imino acids other than octopine can also induce CF production in wild type-inducible strains, because these strains do not exude CF into liquid induction medium under the culture conditions we used.

CF is a low-molecular-weight compound, as indicated by its dialyzable property and because its activity is not affected by passage through a Centricon 10 membrane, which is a microconcentrator designed to retain molecules with molecular weights higher than 10,000. CF was resistant to nuclease and protease, but activity was destroyed by autoclaving and by alkaline treatment. So it is clearly not a nucleic acid or protein; at present, its nature is not clear.

ACKNOWLEDGMENT

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