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Carol A. Hendrick
*University of Nebraska-Lincoln*

William P. Haskins
*University of Nebraska-Lincoln*

Anne K. Vidaver
*University of Nebraska-Lincoln*, avidaver1@unl.edu

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# Conjugative Plasmid in *Corynebacterium flaccumfaciens* subsp. *oortii* That Confers Resistance to Arsenite, Arsenate, and Antimony(III)†

CAROL A. HENDRICK, WILLIAM P. HASKINS, AND ANNE K. VIDAVER*
*Department of Plant Pathology, University of Nebraska-Lincoln, Lincoln, Nebraska 68583-0722*

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**Gene transfer systems for phytopathogenic corynebacteria have not been reported previously. In this paper we describe a conjugative 46-megadalton plasmid (pDG101) found in *Corynebacterium flaccumfaciens* subsp. *oortii* CO101 that mediates resistance to arsenite, arsenate, and antimony(III). Transfer of the plasmid from CO101 to four other strains from the *C. flaccumfaciens* group occurred between cells immobilized on nitrocellulose filters or on agar surfaces. Transconjugant strains expressed the same levels of metal resistance as the donor strain and were able to act as donor strains in subsequent matings. The physical presence of the plasmid was detected by agarose gel electrophoresis. Arsenite-sensitive derivatives of the donor and transconjugant strains were obtained after heat treatment; these were cured of pDG101.**

Studies of the basic genetics and pathogenic mechanisms of corynebacteria have been hampered by the lack of appropriate systems of gene transfer. No systems of gene exchange or rearrangement have been reported for this group of bacteria, except for a generalized transduction system specific for *Corynebacterium renale* (7) and genetic recombination among medically important *C. diptheriae* bacteriophages, some of which convert nontoxigenic strains of the pathogen to toxin production (1).

The phytopathogenic corynebacteria are the most prevalent and economically damaging group of gram-positive plant pathogenic bacteria. As gene transfer systems have not been available, no genetic evidence for determinants of pathogenicity or for other functions of these bacteria has been obtained (17). We have sought a gene transfer system for corynebacteria similar to the conjugation systems described for other gram-positive bacteria (2, 4, 12). Indigenous plasmids have been reported in phytopathogenic corynebacteria (6, 11), but preliminary tests in this laboratory showed no correlation between the presence of plasmids and pathogenicity, bacteriocin production, antibiotic resistance, or ability to grow on various carbon sources (6). In subsequent attempts to identify selectable markers on these plasmids, we screened for resistance to toxic metal ions (3, 15). In this paper, we report the identification of plasmid-borne resistance in the tulip pathogen *C. flaccumfaciens* subsp. *oortii* to arsenite, arsenate, and antimony(III) and describe the conjugal transfer of the metal resistance plasmid pDG101 between strains of phytopathogenic corynebacteria.

## MATERIALS AND METHODS

**Bacterial strains, plasmids, and media.** *Corynebacterium* strains used in this study are listed in Table 1. Strains were maintained on a nutrient broth-yeast extract (NBY) agar medium (16), with antibiotics added as necessary to maintain selection for resistance markers. All broth cultures were grown on a rotary shaker (200 rpm) at 25°C. Selective antibiotic concentrations were: streptomycin sulfate (Sm) (Sigma Chemical Co., St. Louis, Mo.) at 1,000 μg/ml, rifampin (Rf) (Sigma) at 100 μg/ml, fusidic acid–sodium salt (Fus) (Leo Pharmaceutical, Denmark, gift of W. O. Gotfredson) at 20 μg/ml, and sodium arsenite (Asi) (Sigma) at 12 mM. Sodium arsenate (Asa) was obtained from Sigma and potassium antimonyl tartrate (Ant) was obtained from Aldrich Chemical Co., Milwaukee, Wis. Plates containing arsenite were used within 4 to 5 days to avoid problems with toxicity of older media.

Initial screening for metal resistance was done on a semidefined medium (NMCF) consisting of: 6 g of $Na_2HPO_4$, 3 g of $KH_2PO_4$, 1 g of $NH_4Cl$, 0.1 g of L-glutamine, 0.02 g of DL-methionine, 100 μg of nicotinic acid, 10 μg of thiamine, 1 μg of biotin, 1 g of Casamino Acids (Difco), 0.1 g of yeast extract (Difco), 10 g of glucose, 0.12 g of $MgSO_4$, and 13 g of agar per liter of water.

The plasmids previously described (6) from strain CO101 have been designated pDG101 (46 megadaltons [Mdal]) and pDG102 (64 Mdal), and that from strain CP1 has been designated pDG103 (44 Mdal). These designations were registered with the Plasmid Reference Center at Stanford University (E. M. Lederberg, personal communication).

**Screening for metal resistance.** Thirty-six strains of corynebacteria were tested for sensitivity or resistance to 32 different metal salts. The strains tested were a representative sample of the 60 strains studied previously by Gross et al. (6). Bacterial suspensions (ca. $10^4$ and $10^7$ cells per ml) were spread on plates of NMCF agar, and 5-μl droplets of metal salt solutions were placed on the plates. The metal salts we tested included: LiCl, $NaBO_2$, $MgCl_2$, $NaCrO_4$, $MnCl_2$, $Na_2MoO_4$, $Na_2HAsO_4$, $SnCl_2$, $ThCl_4$, $Ba(NO_3)_2$, $Pb(CH_3COO)_2$, $UO_2(CH_3COO)_2$ (group I); $CoCl_2$, $NiSO_4$, $CaCl_2$, $AgNO_3$, $AuCl_3$ (group II); $AlCl_3$, $CaCl_2$, CsCl, $Sr(NO_3)_2$, $Sr(CH_3COO)_2$, $CuSO_4$, $FeSO_4$, $FeCl_3$, $ZnSO_4$, $CrCl_3$ (group III); $NaAsO_2$, $CuCl_2$, $K_2TeO_3$, TlCl, $HgCl_2$ (group IV) (groupings reflect differential sensitivity patterns reported below). Metal concentrations varied from 0.002 to 2 M, depending on solubility and previously published toxicity (15). Controls consisted of buffer or water alone. Inhibition zones were measured after 72 h at 25°C. When differential resistance was observed for a particular metal, it was incorporated into NBY agar at several concentrations, (0.002, 0.01, 0.02, 0.1, and 0.2 M), and the resistance of the bacteria was retested. Droplets (5 μl) containing about 200

* Corresponding author.
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TABLE 1. Bacterial strains and properties

| Strain | Chromosomal phenotype | Plasmid | Plasmid mass (Mdal)[a] | Construction |
|---|---|---|---|---|
| *C. flaccumfaciens* subsp. *oortii* | | | | |
| CO101[b] | | pDG101, pDG102 | 46, 68 | From *Tulipa* sp. |
| CO101.1 | | pDG102 | 68 | Spontaneous Asi$^s$ derivative of CO101 |
| CO101.2 | | pDG102 | 68 | Heat-cured CO101 |
| CO101.3 | | pDG102 | 68 | Heat-cured CO101 |
| *C. flaccumfaciens* subsp. *betae* | | | | |
| CB101[b] | | | | From *Beta vulgaris* |
| CB101.0 | Sm$^r$ | | | Sm$^r$, CB101 |
| CB101.1 | Sm$^r$ | pDG101 | 46 | CO101 × CB101.0 |
| CB101.2 | Sm$^r$ | pDG101 | 46 | CO101 × CB101.0 |
| CB101.3 | Sm$^r$ | | | Cured CB101.1 |
| CB101.4 | Sm$^r$ | | | Cured CB101.1 |
| CB101.5 | Rf$^r$ Fus$^r$ | | | Rf$^r$ Fus$^r$, CB101 |
| CB102[b] | | | | From *Beta vulgaris* |
| CB102.0 | Sm$^r$ | | | Sm$^r$ , CB102 |
| CB102.1 | Sm$^r$ | pDG101 | 46 | CO101 × CB102.0 |
| CB102.2 | Sm$^r$ | pDG101 | 46 | CO101 × CB102.0 |
| CB102.3 | Sm$^r$ | | | Cured CB102.1 |
| CB102.4 | Sm$^r$ | | | Cured CB102.1 |
| *C. flaccumfaciens* subsp. *flaccumfaciens* | | | | |
| CV1[c] | | | | From *Phaseolus vulgaris* |
| CV1.0 | Sm$^r$ | | | Sm$^r$, CV1 |
| CV1.1 | Sm$^r$ | pDG101 | 46 | CO101 × CV1.0 |
| CV1.2 | Sm$^r$ | pDG101 | 46 | CO101 × CV1.0 |
| *C. flaccumfaciens* subsp. *poinsettiae* | | | | |
| CP1[b] | | pDG103 | 44 | From *Euphorbia pulcherrima* |
| CP1.0 | Sm$^r$ | pDG103 | 44 | Sm$^r$, CP1 |
| CP1.1 | Sm$^r$ | pDG101, pDG103 | 44, 46 | CO101 × CP1.0 |

[a] Plasmid sizes determined by Gross et al. (6).
[b] From the International Collection of Phytopathogenic Bacteria, Davis, Calif.
[c] Nebraska strains or obtained from other investigators.

bacteria were placed on plates, and after 72 h at 25°C, the ability of the strains to form single colonies on the metal-containing media was evaluated.

**Mating conditions.** For filter matings between donor and recipient strains, cells were grown overnight in NBY containing the appropriate antibiotic and then subcultured into 10 to 25 ml of NBY in 250-ml sidearm flasks at 25°C. When the cultures reached early log phase (about $3 \times 10^8$ cells per ml), the cells were pelleted by centrifugation (13,800 × $g$), washed once with phosphate buffer (0.0125 M at pH 7.1), and resuspended in an equal volume of buffer. The donor (Asi$^r$) and recipient (Sm$^r$ or Rf$^r$ Fus$^r$) cells were mixed at a ratio of 1:10 (usually 5 ml of recipient per 0.5 ml of donor) and filtered onto a 0.45-μm nitrocellulose filter (25-mm diameter) (Millipore Corp., Bedford, Mass.). The filters were placed cell-side up on NBY agar and, unless otherwise specified, were incubated for 4 h at 25°C. The cells were washed from the filter with 1 ml of NBY broth, vortexed vigorously for 30 s, diluted, and plated on NBY agar containing both selective antibiotics. Donor and recipient populations also were determined independently after 4 h at 25°C. Transfer frequency was expressed as the number of Asi$^r$ transconjugants per milliliter divided by the viable count of recipient cells in the mating mixture after 4 h. Transfer frequency was expressed in terms of the recipient population because of reductions in the donor population during mating and also because of variable plating efficiency of the donor strain. Controls consisting of donor and recipient cells alone were treated similarly to determine the frequency of spontaneous resistant mutants in the population. Transconjugants could be distinguished easily from occasional spontaneous Sm$^r$ or Rf$^r$ donors by colony morphology and color and were purified by at least two serial colony transfers on selective medium. For agar surface matings, the cultures were prepared as described above for membrane matings. A drop (0.1 ml) of the recipient culture was spotted on an NBY plate and allowed to dry, and then 0.1 ml of a 1:10 dilution of the donor culture was spotted on top of the first drop. After 4 h, the cells were washed from the surface of the plate with 1 ml of NBY broth, diluted, and plated on selective medium. For broth matings, donor and recipient cultures were prepared as described above, except that the cells were resuspended in an equal volume of NBY broth. The cultures were mixed at 1:1 and 1:10 ratios, incubated statically for 4 h, and then diluted and plated on the selective medium.

To determine that transfer of resistance was not due to either transformation or transduction, the following experiments were done. Filter matings in the presence of DNase were done as described above, except that cell mixtures contained 10 μg of DNase (Sigma; type I) per ml and 10 mM $MgSO_4$, and the filters were incubated on NBY agar contain-

ing 100 μg of DNase per ml and 10 mM $MgSO_4$. In these experiments, the selective medium also contained 10 μg of DNase per ml and 10 mM $MgSO_4$. To determine the possibility of transduction, the donor and recipient were grown as described above, and then the donor culture was centrifuged, and the supernatant was filtered through a 0.22-μm filter (Millipore). Equal volumes of the cell-free donor filtrate and the recipient culture were mixed and treated as described above.

**MICs.** Cultures to be tested were grown to late log phase in NBY broth. Serial twofold dilutions of the metals were made in NBY broth (100 μl per well) in Microtiter plates. When arsenate and antimony were tested, the basal medium was NBY broth made without phosphate buffer. Five microliters of the test bacteria was added, and the plates were incubated overnight at 25°C. The MIC was taken as the lowest metal concentration that suppressed the growth of the cultures.

**Plasmid isolation.** Plasmids were isolated initially from cultures grown to late log phase in 25 ml of NBY broth by a modification of the procedure of Leblanc and Lee (9). After lysozyme (Sigma) treatment for 45 to 60 min at 37°C, the cells were exposed for 10 min at 37°C to 0.5 mg of Pronase (Calbiochem) per ml (predigested for 90 min at 37°C).

In later experiments, plasmids were isolated by a modification of the procedure of Kado and Liu (8). Late log phase NBY cultures (10 ml) were harvested by centrifugation and washed with 0.0125 M phosphate buffer (pH 7.1). Washed cells were suspended in 0.5 ml of 50 mM Tris-hydrochloride (pH 8.0) followed by the addition of 0.5 ml of lysozyme (5 mg/ml in 50 mM Tris-hydrochloride) for 45 to 60 min at 37°C, and then 0.36 ml of 0.25 M EDTA was added, and the suspension was incubated for 5 min at 37°C. Two volumes of lysing solution (3% sodium dodecyl sulfate, 50 mM Tris, pH 12.6) were added, and the suspension was mixed briefly. The lysate was heated at 95°C for 5 min and then cooled rapidly in ice water. The solution was extracted twice with two volumes of phenol-chloroform (1:1 [vol/vol]). Samples were withdrawn for electrophoresis directly from the aqueous phase after extraction.

DNA-containing samples (40 μl) were mixed with 5 μl of gel-loading solution (75% glycerol, 0.125% bromphenol blue) and applied to horizontal 0.7% agarose gels. Electrophoresis was carried out in Tris-borate buffer (89 mM Tris, 89 mM boric acid, 2.5 mM EDTA, pH 8.2 to 8.3) at 100 V for 6 h at 4°C (10).

**Plasmid curing procedure.** Strains were grown overnight in 10 ml of NBY broth at 25, 35, or 37°C, diluted, and plated on

TABLE 2. Transfer of Asi$^r$ from *C. flaccumfaciens* subsp. *oortii* CO101 to other subsp.

| Donor | Recipient | Transfer frequency (per recipient)[a] |
|---|---|---|
| CO101 | CB101.0 | $1.3 \times 10^{-4}$ |
| CO101 | CB102.0 | $3.2 \times 10^{-5}$ |
| CO101 | CV1.0 | $2.7 \times 10^{-7}$ |
| CO101 | CP1.0 | $1.3 \times 10^{-8}$ |
| CV1.1 | CB101.5 | $2 \times 10^{-8}$ |
| CP1.1 | CB101.5 | $5 \times 10^{-9}$ |

[a] Transfer frequency is expressed as the number of Asi$^r$ colonies per recipient CFU. The recipient CFU was determined after mating. Transconjugants were selected for Sm$^r$ or Rf$^r$ and Asi$^r$. Control platings of the donor (Asi$^r$) and recipient (Sm$^r$ or Rf$^r$) produced colonies at $<10^{-10}$ frequency (controls yielded less than one colony for every $10^{10}$ cells plated).

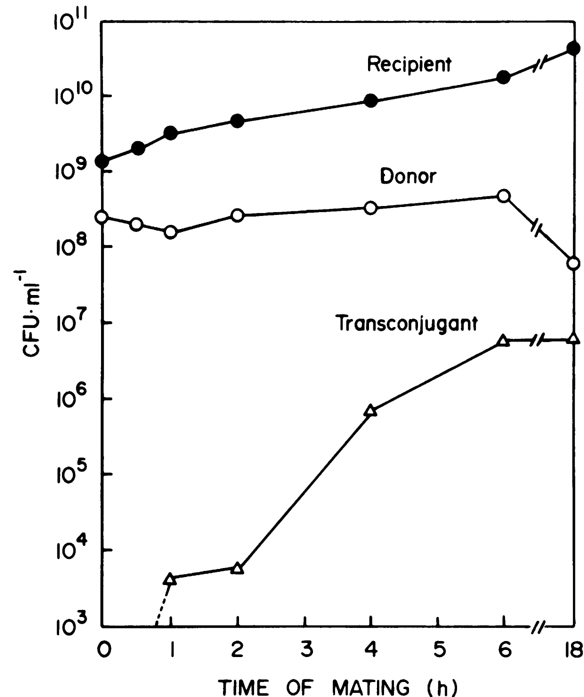


FIG. 1. Time course of transfer of Asi$^r$ from CO101 to CB101.0. Donor and recipient cells were mixed and placed on six filters as described in the text. After incubation at 25°C for 30 min, 1, 2, 4, 6, and 18 h, the cells were resuspended and scored for CFU of the following type: recipient (Sm$^r$) (●), donor (Asi$^r$) (○), and transconjugant (Sm$^r$ Asi$^r$) (△).

NBY agar. Individual colonies then were tested for Asi$^s$ by replica plating onto NBY containing 12 mM arsenite. Metal-sensitive colonies from several independent experiments were purified and examined for plasmids by agarose gel electrophoresis.

## RESULTS

**Screening for metal resistance.** In an attempt to identify selectable markers carried on the indigenous plasmids of phytopathogenic corynebacteria (6), we screened 36 strains for resistance to 32 different metals. The strains varied in plasmid content and represented all of the major groups of phytopathogenic corynebacteria. The metals could be divided into four groups: those to which all strains were either resistant (group I) or sensitive (group II), those to which most strains were resistant (group III), and those to which a relatively few strains were resistant (group IV). We were interested primarily in the latter group of metals, since only within this group were any correlations observed between metal resistance and the presence of one or more plasmids.

When the metals from this group were incorporated in NBY agar and the bacteria were retested for resistance, there was a clear correlation between the presence of a plasmid and Asi$^r$. Strain CO101 of *C. flaccumfaciens* subsp. *oortii* contains two plasmids, designated pDG101 (46 Mdal) and pDG102 (64 Mdal) (6). This strain was Asi$^r$ to 10 mM sodium arsenite, whereas strains which lacked plasmids were Asi$^s$ to 2 mM arsenite. Since high phosphate concentrations can protect against the toxic effect of arsenate (14), we subsequently used a low-phosphate basal medium (NBY made without phosphate buffer) and found that CO101 also was Asa$^r$ (100 mM) and Ant$^r$ (50 mM).

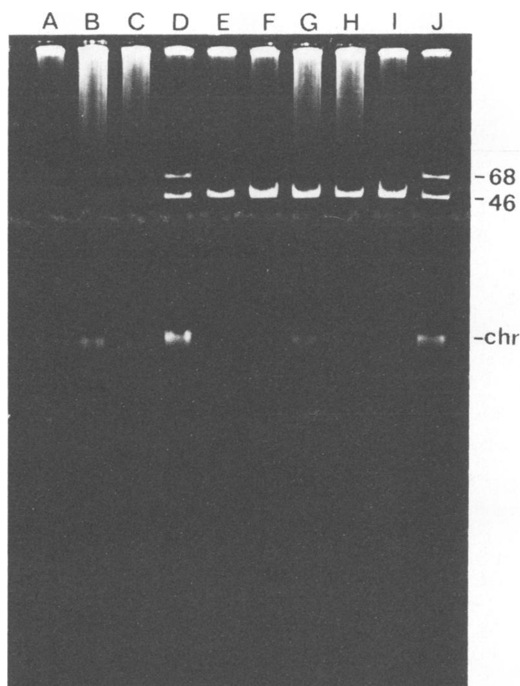


FIG. 2. Agarose gel electrophoresis of lysates of donor, recipient, and transconjugant *Corynebacterium* strains. A, CB101.0; B, CV1.0; C, CB102.0; D and J, CO101; E, CB101.1; F, CV1.1; G, CV1.2; H, CB102.1; I, CB102.2. chr, Chromosomal DNA. Molecular masses of plasmids pDG101 (46 Mdal) and pDG102 (68 Mdal) from strain CO101 were determined previously (6).

**Mating experiments.** To determine whether the arsenite resistance determinant of CO101 was borne on a transmissible plasmid, we conducted filter mating experiments between CO101 and several potential recipient strains. Transfer of Asi$^r$ between CO101 and four strains from the *C. flaccumfaciens* group occurred at frequencies ranging from $1.3 \times 10^{-4}$ to $1.3 \times 10^{-8}$ transconjugants per recipient (Table 2). Two transconjugant strains, CV1.1 and CP1.1, were able to act as plasmid donors in subsequent matings with CB101.5 (Table 2). Transfer to members of the *C. michiganense* group of phytopathogenic corynebacteria was not detected. In all cases, the background frequency of spontaneous Asi$^r$ recipient colonies in control crosses was less than $10^{-10}$ per recipient. Time course experiments (Fig. 1) showed that transfer frequencies in filter matings between CO101 and CB101.0 reached a maximum after 4 to 6 h. Transfer was observed between strains CO101 and CB101.0 at a frequency of $2.5 \times 10^{-5}$ per recipient when cells were mated on the surface of an NBY agar plate, but no transfer was observed when matings were carried out in NBY broth.

Control matings did not support transformation or transduction as mechanisms for the transfer of resistance. Filter matings between strains CO101 and CB101.0, conducted in the presence of 100 μg of DNase I per ml, resulted in normal frequencies of transfer. No transconjugants were observed when cell-free supernatant from the donor culture was incubated with strain CB101.0.

**Plasmid analysis.** To confirm that a plasmid was transferred from CO101 to the transconjugant strains, lysates of the donor, recipients, and transconjugants were examined by agarose gel electrophoresis (Fig. 2). The gel patterns showed that whereas the recipient strains CB101.0, CV1.0, and CB102.0 (lanes A, B, and C, respectively) contained no resident plasmids, transconjugants derived from these strains (lanes E through I) all contained a single plasmid with a mobility identical to that of the smaller plasmid (pDG101, 46 Mdal) from strain CO101 (lanes D and J). Strain CP1.0 contained a single resident plasmid of ca. 44 Mdal. Three of the six CP1 transconjugants tested (including CP1.1) contained two plasmid bands of 44 and 46 Mdal, whereas the other three contained only the 46-Mdal plasmid (not shown).

**Curing of pDG101.** To confirm the association of Asi$^r$ with the presence of pDG101, we attempted to cure the plasmid from the donor strain and two of the transconjugant strains. After overnight growth of strain CO101 at 25 and 35°C, no Asi$^s$ segregants were observed; at 37°C, Asi$^s$ colonies occurred at a frequency of 10% (2/20). An additional spontaneous cured derivative of CO101 was obtained after several weeks of repeated transfer on NBY agar without arsenite. Overnight growth of strain CB101.1 at 25°C produced Asi$^s$ segregants at a frequency of 7.8% (6/77); at 35°C the frequency was 17.8% (34/191). Similar studies with strain CB102.1 gave frequencies of 0.75% (4/534) at 25°C, 3.1% (12/387) at 35°C, and 12.6% (14/111) at 37°C.

Lysates of the Asi$^s$ derivatives of CO101, CB101.1, and CB102.1 were examined by gel electrophoresis. All of the derivatives lacked the 46-Mdal plasmid band corresponding to pDG101 (not shown).

**MIC testing.** MICs of arsenite, arsenate, and antimony(III) were tested to determine whether or not the plasmid was expressed similarly in the donor and transconjugant strains and to determine whether Asa$^r$ and Ant$^r$ also could be associated with the presence of pDG101 (Table 3). In general, transconjugant strains expressed the same levels of Asi$^r$ as did the donor strain. Ant$^r$ and Asa$^r$ also apparently were transferred to the transconjugant strains, and resistance was at the same levels found in the donor strain.

Cured derivatives of transconjugant strains CB101.1 and CB102.1 were Asi$^s$, Asa$^s$, and Ant$^s$ at essentially the same concentrations as the wild-type parental strains. The cured

TABLE 3. MICs of arsenite, arsenate, and antimony(III) for corynebacteria

| Strain | Plasmid(s) | MIC (mM) | | |
|---|---|---|---|---|
| | | Arsenite | Arsenate | Antimony(III) |
| CO101 | pDG101, pDG102 | 25 | 200 | >100 |
| CO101.1 | pDG102 | 3.13 | 12.5 | 0.78 |
| CO101.2 | pDG102 | 12.5 | 25 | >100 |
| CO101.3 | pDG102 | 12.5 | 25 | >100 |
| CB101.0 | | 1.56 | 0.39 | 0.098 |
| CB101.1 | pDG101 | 25 | 100 | >100 |
| CB101.2 | pDG101 | 25 | 200 | >100 |
| CB101.3 | | 1.56 | 0.39 | 0.098 |
| CB101.4 | | 1.56 | 0.39 | 0.098 |
| CB102.0 | | 0.78 | 0.39 | 0.098 |
| CB102.1 | pDG101 | 25 | 200 | >100 |
| CB102.3 | | 1.56 | 0.39 | 0.098 |
| CB102.4 | | 1.56 | 0.39 | 0.098 |
| CV1.0 | | 0.39 | 0.39 | 0.098 |
| CV1.1 | pDG101 | 25 | 200 | >100 |
| CP1.0 | pDG103 | 3.13 | 0.195 | 0.098 |
| CP1.1 | pDG101, pDG103 | 25 | 200 | >100 |
| CP1.2 | pDG101, pDG103 | 25 | 200 | >100 |

derivatives of the donor strain CO101, however, had a higher residual level of resistance to all three metals than the other wild-type recipients. Two of the three derivatives, CO101.2 and CO101.3, were only slightly less Asa$^r$ and Asi$^r$ than their parent strain and appeared to be completely Ant$^r$.

## DISCUSSION

Our results demonstrate that an indigenous plasmid, coding for metal resistance, is transferable between subspecies of *C. flaccumfaciens*. Plasmid transfer apparently occurred by conjugation. Several lines of evidence support such a mechanism: the addition of DNase I to mating mixtures did not reduce transfer frequencies, no Asi$^r$ colonies were detected when filtrates from the donor strain were mixed with recipient cells, and close physical contact between cells was required for transfer, since transfer was not observed in broth matings. It is unlikely, therefore, that transfer occurred either by transduction or transformation.

Plasmid DNA from lysates of transconjugant strains selected for Asi$^r$ was identical in mobility on agarose gels to the 46-Mdal plasmid from the donor strain (Fig. 2). Cured derivatives of transconjugant and donor strains selected for inability to grow on 12 mM arsenite lost the 46-Mdal plasmid. Transconjugant strains selected for Asi$^r$ also were Asa$^r$ and Ant$^r$ (Table 3). Therefore, these results are analogous to those reported in enteric bacteria and *Staphylococcus aureus*, in which resistance to these three metals is plasmid mediated, genetically linked, and coordinately induced (3, 14). The arsenate resistance determinant is separable genetically from the arsenite and antimony resistance determinants (3). Asa$^r$ is due to a specific efflux mechanism, whereas the basis of Asi$^r$ and Ant$^r$ is not known (3, 14). The relationship of pDG101 to metal resistance plasmids from other genera is currently under investigation.

Plasmid-cured derivatives differed in Asi$^s$, Asa$^s$, and Ant$^s$. Those derived from transconjugant strains became Asi$^s$, Asa$^s$, and Ant$^s$ at the same concentrations as did the wild-type recipient strains (Table 3). The three cured derivatives of the donor strain behaved differently, however. Strain CO101.1 was a spontaneous Asi$^s$ derivative of CO101 that had been transferred on a nonselective medium for several weeks. Although this strain became Asi$^s$, Asa$^s$, and Ant$^s$, it was more resistant to these metals than were wild-type recipient strains. Strains CO101.2 and CO101.3 were derived after heat treatment from a culture of CO101 maintained on arsenite-containing medium for several weeks. These two derivatives had higher levels of Asa$^r$ and Asi$^r$ than did CO101.1 (although they were more sensitive than the donor strain). They also were Ant$^r$, although these cells never were exposed to antimony. These observations suggest that additional determinants for Asi$^r$, Asa$^r$, and Ant$^r$ may be located on the chromosome or the other resident plasmid of strain CO101 and that they may be amplified when the strain is grown under selective conditions. In this regard, it is interesting to note that heteroduplex analysis of the highly conserved arsenate, arsenite, antimony resistance region of *S. aureus* penicillinase plasmids shows structural similarity to transposable elements, although there is as yet no evidence for its transposition (5, 13).

Many questions remain unanswered concerning pDG101 and its transfer between strains of corynebacteria. Experiments are in progress to determine whether transconjugant strains can act as efficient donors of the plasmid to strains outside of the *C. flaccumfaciens* group. Crosses between *C. flaccumfaciens* subsp. *oortii* CO101 and several members of the *C. michiganense* group thus far have been unsuccessful. Other unresolved questions are whether the plasmid can mobilize other plasmids or chromosomal markers and whether the plasmid codes for pathogenicity factors.

To our knowledge, we have described the first conjugal gene transfer system for corynebacteria. The ability to transfer indigenous (or introduced) plasmids within corynebacteria should ultimately lead to a better understanding of their genetics and facilitate study of taxonomic relationships and mechanisms of pathogenicity.

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