No Correlation Exists between the Conjugative Transfer of the Autotrophic Character and That of Plasmids in *Nocardia opaca* Strains

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A new isolate of *Nocardia opaca* was obtained by enrichment culture for aerobic lithoautotrophic growth on CO₂ and H₂. This strain, MR22, is very similar to *N. opaca* MR11 (formerly 1b) in functioning as a donor for genetic information determining the ability to grow lithoautotrophically (Aut character) in matings with Aut⁻ strains of *N. opaca* or closely related heterotrophic species. The strain contains a plasmid, pHG33 of about 110 kb. A mutant was isolated from strain MR22 which was plasmid-free, and had lost the Aut character, resistance to 50 µM-thallium salt and susceptibility to the nocardia-specific bacteriophage ϕ B1. As a recipient of the Aut character, this plasmid-free mutant was as well suited as plasmid-bearing Aut⁻ strains of *N. opaca*. In matings with the mutant as recipient the frequency of Aut⁺ transconjugants per donor was 3×10^{-4} with *N. opaca* MR11 (pHG31-a, Aut⁺, Tl^r, Str^s, ϕ B1^s) and 2×10^{-3} with *N. opaca* MR22 (pHG33, Aut⁺, Tl^r, Str^s, ϕ B1^r) as donor. Phenotypic characterization of the transconjugants, which had been selected for the Aut marker, revealed that in many cases the Aut marker had been transferred without plasmid transfer. Furthermore, plasmid-free, Aut⁺ transconjugants functioned as donors for the Aut marker. Both plasmid-free and plasmid-bearing transconjugants transferred the Aut marker to the Aut⁻ strains of *N. opaca* with a frequency which was one or two orders of magnitude higher than that of the wild-type strains. The plasmids pHG31-a and pHG33 code for thallium resistance (50 µM-thallium acetate). The frequency of thallium-resistant transconjugants was 10⁻¹ to 10⁻² per donor; all thallium-resistant transconjugants contained the donor plasmid.

We conclude that the plasmids pHG31-a of strain MR11 and pHG33 of strain MR22 of *N. opaca* carry the genetic information for thallium resistance but not the Aut character. As plasmid-free Aut⁺ strains can function as donors the Aut character is assumed to reside on the chromosome and to function as an independent self-transmissible genetic element.

INTRODUCTION

Nocardia opaca is a nutritionally versatile bacterium, able to grow on various sugars, organic and amino acids and long chain hydrocarbons, and lithoautotrophically on H₂ and CO₂ (Aggag & Schlegel, 1973, 1974). *N. opaca* is the only aerobic H₂-oxidizing bacterium known which contains only a cytoplasmic, NAD-reducing hydrogenase and no membrane-bound hydrogenase (Aggag & Schlegel, 1974; Schneider & Schlegel, 1977). The hydrogenase is very similar to the cytoplasmic hydrogenase of *Alcaligenes eutrophus* H16, consisting of four non-identical subunits, and contains FMN, nickel and iron-sulphur clusters (Schneider *et al.*, 1984a, b).

The ability to grow lithoautotrophically on H₂ as sole hydrogen and energy source and CO₂ as sole carbon source, which is in this context called the Aut character, was transferred in numerous experiments by conjugation from *N. opaca* MR11 (formerly known as strain 1b) to Aut⁻ mutants of the same strain and to various heterotrophic strains of the same genus (Reh & Schlegel, 1975, 1981; Reh, 1981). The formation of the enzymes required for lithoautotrophic growth, i.e. hydrogenase, phosphoribulokinase and ribulose-1,5-bisphosphate carboxylase, is

Table 1. *Nocardia opaca* strains

Strain	Relevant phenotype*	Plasmid	Parent strain	Reference or source
MR11 (formerly <i>N. opaca</i> 1b)	Aut ⁺ Tl ^r ϕ B1 ^s	pHG31-a, pHG31-b	Wild-type	DSM 427
MR22	Aut ⁺ Tl ^r ϕ B1 ^r	pHG33	Wild-type	DSM 3346; isolation from soil, this study
MR222	Aut ⁻ Tl ^s ϕ B1 ^s	None	Mutant of MR22	Mitomycin C, this study
MR226	Aut ⁻ Tl ^s ϕ B1 ^s Rif ^r	None	Mutant of MR222	Spontaneous, this study
MR2226	Aut ⁻ Tl ^s ϕ B1 ^s Str ^r Lac ⁻	None	Mutant of MR222	Spontaneous, UV induction, this study
MR2246	Aut ⁺ Tl ^s ϕ B1 ^s Str ^r Lac ⁻	None	MR11 $\times$ MR2226	Transconjugant, this study
MR2247	Aut ⁺ Tl ^r ϕ B1 ^s Str ^r Lac ⁻	pHG31-a	MR11 $\times$ MR2226	Transconjugant, this study
MR2248	Aut ⁻ Tl ^r ϕ B1 ^s Str ^r Lac ⁻	pHG31-a	MR11 $\times$ MR2226	Transconjugant, this study
MR2249	Aut ⁻ Tl ^r ϕ B1 ^r Str ^r Lac ⁻	pHG33	MR22 $\times$ MR2226	Transconjugant, this study

* Aut, autotrophy; Lac, lactose; Tl, thallium; Str, streptomycin; Rif, rifampicin; ϕ B1, behaviour against the nocardia-specific phage.

controlled by derepression and occurs coordinately; the kinetic parameters and other properties of these enzymes in the donor and the transconjugants are equal (C. Ecker, M. Reh & H. G. Schlegel, unpublished observations). So far only the Aut character has been transferred by conjugation.

As the frequency of spontaneous loss of the Aut character was far higher than that of any other character and the set of Aut enzymes was transferred *en bloc*, its localization on a plasmid was assumed. This assumption could not be substantiated as no plasmid-free Aut⁻ recipient strains were available and the plasmid patterns of transconjugants equalled those of the recipients. Furthermore, the analysis of restriction enzyme patterns of Aut⁺ donors, recipients and transconjugants did not show any differences (Reh, 1981).

The present study was aimed at elucidating these controversial conclusions and solving the question of whether the plasmid present in *N. opaca* MR11 is involved in genetic transfer of the Aut character.

METHODS

Bacterial strains and bacteriophages. Sources and references of the strains of *Nocardia opaca* used are listed in Table 1. The bacteriophage ϕ B1 appeared as a contaminant in a culture of *N. opaca* MR11 growing autotrophically in a fermenter (Reh & Schlegel, 1981).

Media and growth conditions. Bacteria were grown autotrophically in mineral salts medium (MM; Schlegel *et al.*, 1961). For heterotrophic growth 0.5% (w/v) fructose plus tryptose and yeast extract (each 0.1%, w/v) were added (FTY). Incubation under autotrophic and heterotrophic conditions was as described by Reh & Schlegel (1981).

In some cases hexadecane agar was used for matings to select for autotrophic transconjugants. Hexadecane (0.5 ml) was supplied to MM agar on a filter via the gas phase. Antibiotics were used at the following concentrations (μ g ml⁻¹): streptomycin sulphate, 300; rifampicin, 20. The thallium acetate concentration was 50 μ M.

Growth measurements. Optical densities were measured in 1 cm cuvettes at 436 nm in a Gilford photometer 250. Cell suspensions were diluted in Triton/saline, containing 9 g NaCl, 2 g MgSO₄ · 7H₂O and 1 ml Triton X-100 l⁻¹.

Isolation of a new wild-type strain of *N. opaca*. *N. opaca* MR22 (DSM 3346) was isolated from soil after a special enrichment procedure. Soil samples were incubated in MM under autotrophic conditions. Part of this first enrichment culture was transferred to liquid MM containing hexadecane (0.25%) and incubated in air. The cell suspension was streaked on mineral agar plates containing cycloheximide (50 μ g ml⁻¹) which were incubated under autotrophic conditions. Colonies which appeared to be similar to those of *Nocardia* were selected and purified.

Isolation of Aut⁻ mutants including a plasmid-free mutant. After treatment of the wild-type strain MR22 with mitomycin C (Reh & Schlegel, 1981), many Aut⁻ mutants were obtained but only one isolate was recognized to be plasmid-free.

Construction of transconjugants. Two mating methods were used. (1) 'Agar mating'. The partners grown on agar slants were cross-streaked on FTY or hexadecane agar as described by Reh & Schlegel (1975). After incubation for 2 d (on FTY agar) or 4 d (on hexadecane agar), the colony pattern was transferred by replica plating to selective

media. For isolating autotrophic transconjugants MM agar containing streptomycin or rifampicin was used. To isolate thallium-resistant heterotrophic transconjugants FTY agar containing thallium and streptomycin (or rifampicin) was used. Autotrophic transconjugants were recognizable after 18–21 d, heterotrophic transconjugants after 3–5 d. (2) 'Filter mating'. For quantitative experiments a modified method of the mating procedure described by Reh & Schlegel (1981) was used. Donor and recipient cells were grown in liquid FTY medium until the stationary phase was reached. Suspensions of donor and recipient cells were mixed in a ratio of 1:50. This mixture (0.1 ml) was sucked onto membrane filters with a hydrophobic rim (0.2 µm pore size and 25 mm filter diameter). The filters carrying the uniformly mixed cells were laid on the agar and incubated on FTY agar for 48 h or on hexadecane agar for 96 h. Subsequently, the bacterial lawn was collected by rinsing the filter with Triton/saline; it was homogenized and diluted, and 0.1 ml samples were plated onto selective media. The results of the mating experiments are based on many repeats, and the numerical data are means of several independent experiments.

Phage propagation. Phages were propagated and their titres determined using the soft agar overlay technique. Cells were grown in FTY medium for 48 h; 0.1 ml of the suspension and 0.1 ml of the diluted phage lysate were dispersed in 3 ml liquid peptone/yeast extract (PY, 0.5% peptone and 0.3% yeast extract) soft agar (0.5%) and laid on top of a PY agar plate.

For the bacteriophage susceptibility plaque test, 5 µl of the diluted phage lysates was placed on the solidified agar plates containing indicator cells. Formation of single plaques indicated the phage sensitivity of the bacteria.

Determination of heavy metal resistance. Cells were grown in FTY medium for 48 h. A PY agar plate was overlaid with a suspension of 0.1 ml of these cells in 3 ml liquid PY soft agar. A paper disk containing 20 µl of one of the heavy metal solutions (for concentrations of the tested heavy metals see Table 2) was placed on the solidified agar plate.

Isolation of plasmid DNA. A 0.1 ml sample of stationary phase culture was inoculated into 5 ml FTY medium and incubated for 16 h. Then 20 ml FTY medium containing 15% (w/v) sucrose and 2% (w/v) glycine was added and incubation was continued for 6 h. This procedure sensitized the cells to lysozyme. For lysis, 10 ml of this cell suspension was harvested by centrifugation. Alkaline lysis of the cells was done according to Kado & Liu (1981). The procedure was modified as follows. The pellet was thoroughly suspended in 0.9 ml E buffer (40 mM-Tris/acetate and 2 mM-EDTA, adjusted to pH 7.9 with glacial acetic acid). Lysis was induced by adding 0.1 ml lysozyme [10 mg (ml E buffer)⁻¹] and incubating for 10 min at 30 °C. Then the cell suspension was mixed with 3 vols lysing solution [50 mM-Tris, 3% (w/v) SDS adjusted to pH 12.6 with NaOH]. Cell lysis was completed by incubating the lysate at 65 °C for 40 min. To purify the plasmid DNA, 0.25 ml 5 M-NaCl and 5 ml phenol/chloroform (1:1, v/v) were added to the warm lysate. The solution was emulsified by inverting the tube 10 times. The aqueous plasmid DNA-containing phase was separated from the phenol phase by centrifugation for 40 min at 2500 g. If the upper phase was not clear, it was mixed with the same volume of phenol/chloroform and centrifuged again for 20 min.

For digestion with *Eco*RI endonuclease, further purification of the lysate was necessary. The method of Hogrefe & Friedrich (1984) was used.

Agarose gel electrophoresis of plasmid DNA. Samples (40 µl) of the clear aqueous phase were mixed with 10 µl of a dye solution [0.07% (w/v) bromophenol blue, 7% (w/v) SDS, 33% (v/v) glycerol] and subjected to electrophoresis in 0.8% agarose (type I, Sigma) dissolved in E buffer. Electrophoresis was done in a horizontal gel apparatus (14 × 11 × 0.6 cm) at 80 mA and 55 V for 5 h. The gel was stained with a solution of ethidium bromide (1 µg ml⁻¹) for 30 min, visualized on a UV transilluminator (254 nm) and photographed on Polaroid type 665 negative film or Polaroid type 667 positive film with an orange filter.

RESULTS

Isolation of a new strain of N. opaca and of a plasmid-free Aut⁻ mutant

N. opaca MR22 was enriched from local soil samples under lithoautotrophic growth conditions and compared to *N. opaca* MR11 (formerly strain 1b) and other strains. It is a member of the *Rhodococcus* taxon which is characterized by a chemotype IV cell envelope (Lechevalier & Lechevalier, 1970) and mycolic acids. The *R_F* values of the mycolic acids determined by thin-layer chromatography are identical with those of *Rhodococcus erythropolis* DSM 43200. Concerning the physiological properties tested so far, *N. opaca* MR22 is almost identical to *N. opaca* MR11. Strain MR22 utilizes the same substrates as MR11. However, in contrast to MR11, it contains only a single plasmid, pHG33 (of about 110 kb). Furthermore, strain MR22 is not susceptible to the nocardia-specific phage ϕ B1.

A variant of strain MR22 was isolated after treatment with mitomycin C. This mutant (MR222) differed from the wild-type strain by lack of plasmid pHG33, inability to grow autotrophically and sensitivity to bacteriophage ϕ B1. For use of this mutant as recipient in

Table 2. *Effect of heavy metal salts on N. opaca MR22 and on the plasmid-free derivative MR222*

A PY agar plate was overlaid with 0.1 ml cell suspension, dispersed in 3 ml liquid PYT soft agar. Paper disks each containing 20 µl metal salt solution were placed on the solidified agar. Inhibition zones were detectable after 1–2 d.

Compound	Concn (µmol per filter disk)	Effect*	Compound	Concn (µmol per filter disk)	Effect*
AlCl ₃	2	B	Ag ₂ SO ₄	2	B
K ₂ Cr ₂ O ₇	0.2	B	CdCl ₂	0.2	B
MnCl ₂	20	B	SnCl ₂	2	A
CoCl ₂	2	B	K ₂ TeO ₃	100	B
NiCl ₂	2	B	La(NO ₃) ₃	2	A
CuSO ₄	2	B	Hg(CH ₃ COO) ₂	0.2	B
ZnSO ₄	2	B	Tl(CH ₃ COO)	0.2	C
Na ₂ HAsO ₄	20	B	Pb(NO ₃) ₂	2	A
NaMoO ₄	20	A	Sb ₂ O ₃	2	A

* A, no inhibition zone; B, diameter of inhibition zone of both strains was the same; C, diameter of inhibition zone was different (MR22 1.3 cm, MR222 5 cm).

conjugation experiments, two further selective markers were introduced: resistance to streptomycin (spontaneous mutation) and inability to grow on lactose (UV induction). Besides this mutant (MR2226), a mutant of MR222 was used which had spontaneously acquired resistance to rifampicin (MR226). The availability of the mutant MR222 enabled us to compare the plasmid-bearing strains with the plasmid-free strain in order to detect plasmid-coded markers. Among 18 different heavy metals and 26 different antibiotics only one metal salt, thallium acetate, exerted strain-differentiating effects (Table 2). While strains MR11 and MR22 were resistant to 50 µM-thallium acetate the plasmid-free mutant MR222 was sensitive to this concentration.

Plasmid patterns in wild-type strains and mutants of N. opaca

As nocardia cells do not lyse after growth under usual conditions, the cells grown in FTY medium were incubated for 6 h in the presence of high sucrose and glycine concentrations. This treatment resulted in a moderate susceptibility to lysozyme and hence accessibility to the method of Kado & Liu (1981) for plasmid DNA isolation. *N. opaca* MR11 contained at least two plasmids. Their size as determined by agarose gel electrophoresis was 140 kb for pHG31-a and 17 kb for pHG31-b (Fig. 1). Several preparations contained an additional plasmid of 180 kb. In contrast, in *N. opaca* MR22 only a single plasmid, pHG33 (110 kb), was detectable. Mutant MR222 and its derivatives MR226 and MR2226 were plasmid-free. To avoid artifacts and obtain convincing evidence, the lysis procedure was applied in parallel to mixtures of the mutant cells and MR11 cells. Agarose gel electrophoresis resulted in the appearance of pHG31-a and pHG31-b only, while pHG33 was missing (Fig. 1).

Transfer of autotrophy by conjugation

As shown previously, the ability to grow autotrophically can be transferred from Aut⁺ strains of *N. opaca* MR11 to Aut⁻ strains by conjugation (Reh & Schlegel, 1975, 1981). In contrast to previous studies, mating occurred on hexadecane medium because the yield of autotrophic transconjugants was considerably higher than that after mating on FTY agar (Reh & Schlegel, 1981). When the plasmid-free mutant MR2226 was used as recipient and MR11 or MR22 as donors, the transconjugant frequency was about 10 times higher in the latter case (Table 3).

About 70 transconjugants of each cross were isolated and characterized with respect to the non-selective markers (Table 4). All transconjugants bore the recipient markers (Lac⁻Str^r). However, surprisingly not all the transconjugants contained the donor plasmids. Those transconjugants which contained the plasmid were Aut⁺ and thallium-resistant. Transconju-

Fig. 1. Plasmid DNA in wild-type strains and mutants of *N. opaca*. Lysates of the following strains were prepared and subjected to agarose gel electrophoresis as described in Methods. Tracks: A, MR11(pHG31-a, pHG31-b); B, MR22(pHG33); C, MR222(pHG33⁻); D, MR222 mixed with MR11(pHG31-a, pHG31-b); E, MR226(pHG33⁻); F, MR226 mixed with MR11(pHG31-a, pHG31-b); G, MR2226 (pHG33⁻); H, MR2226 mixed with MR11(pHG31-a, pHG31-b); I, four different plasmids of *Rhizobium meliloti* MVII/1 were used as size standards (A. Pühler, personal communication) (from top: pRMVII/1a, pRMVII/1b, pRMVII/1c, pRMVII/1d). LF, linear DNA fragments.

Table 3. Frequency of *Aut*⁺ transconjugants and thallium-resistant transconjugants

2.6×10^6 donor cells (*Aut*⁺*Tl*^r) and 10^8 recipient cells (*Aut*⁻*Tl*^s*Str*^r) were mixed, sucked onto a membrane filter which had a hydrophobic rim and incubated on hexadecane agar for 4 d (autotrophic transconjugants) or on FTY agar for 2 d (thallium-resistant transconjugants) at 30 °C. The bacterial lawn was collected by rinsing with Triton/saline solution, homogenized and diluted, and samples were plated on selective media. *Aut*⁺ transconjugants were visible after 21 d, thallium-resistant transconjugants after 4–5 d.

Cross	Transfer frequency*	
	<i>Aut</i> ⁺ <i>Str</i> ^r	<i>Tl</i> ^r <i>Str</i> ^r
MR11 × MR2226	3×10^{-4}	1.6×10^{-1}
MR22 × MR2226	2×10^{-3}	1.3×10^{-2}

* Transfer frequencies are expressed as number of *Aut*⁺ or *Tl*^r transconjugants per donor. The titre was determined after the incubation on selective agar.

gants lacking the plasmid (3% with MR11 as donor and 43% with MR22 as donor) were thallium-sensitive. These results indicated that plasmids pHG31-a and pHG33, respectively, were not necessarily involved in coding for the autotrophic ability, but their presence correlated rather with thallium resistance.

A small proportion of the transconjugants (2/70) contained a plasmid carrying a large deletion (Fig. 2) and were thallium-sensitive. The *Eco*RI digestion pattern of the plasmids indicated their derivation from pHG31-a.

The transconjugants contained only a single plasmid: pHG31-a from MR11 or pHG33 from MR22. The second plasmid of MR11 was not transferred, except in a single case.

The availability of transconjugants of MR11 which contained only plasmid pHG31-a enabled us to compare the plasmids pHG31-a and pHG33 which apparently code for thallium resistance.

Table 4. *Characterization of Aut⁺ transconjugants and thallium-resistant transconjugants resulting from crosses MR11 × MR2226 and MR22 × MR2226*

Cross	Selective agar	Type of trans-conjugant	Relevant phenotype*	Plasmid	Frequency
MR11 × MR2226	MM + Str	1a	Aut ⁺ Tl ^r Lac ⁻ Str ^r ϕ B1 ^s	pHG31-a	0.94‡
	MM + Str	1b	Aut ⁺ Tl ^s Lac ⁻ Str ^r ϕ B1 ^s	None	0.03‡
	MM + Str	1c	Aut ⁺ Tl ^s Lac ⁻ Str ^r ϕ B1 ^s	54 kb plasmid†	0.03‡
	FTY + Str + Tl	1d	Aut ⁺ Tl ^r Lac ⁻ Str ^r ϕ B1 ^s	pHG31-a	> 0.95§
MR22 × MR2226	MM + Str	2a	Aut ⁺ Tl ^r Lac ⁻ Str ^r ϕ B1 ^r	pHG33	0.57‡
	MM + Str	2b	Aut ⁺ Tl ^s Lac ⁻ Str ^r ϕ B1 ^s	None	0.43‡

* Abbreviations as in Table 1.

† The size of the deletion plasmid was calculated after digestion with *Eco*RI. λ DNA cut by *Eco*RI served as a size standard for calculations according to the equation of Southern (1979).

‡ Frequency related to 70 transconjugants of each cross which were characterized.

§ Frequency related to 20 transconjugants which were characterized.

The *Eco*RI restriction patterns (Fig. 3) show that these are different. Thus, plasmid pHG33 is not a deletion product of plasmid pHG31-a. A further argument for the basic difference of both plasmids is provided by the difference in their transfer frequencies (Table 3).

Plasmid-free transconjugants as donor strains for the Aut marker

The availability of transconjugants which are able to grow autotrophically but lack the plasmid provided the means for solving the question of whether the plasmids pHG31-a or pHG33 have a mobilizing function for the transfer of the Aut character. Crosses were done using the plasmid-free Aut⁻ mutant MR226 (Aut⁻ Rif^r) as recipient and the diverse types of transconjugants obtained in the previous experiments (Table 4) as donors. The frequencies of Aut⁺ transconjugants per donor, which were obtained with the diverse types of transconjugants (as categorized in Table 4) as donors, are presented in Table 5.

Thus, the conjugation experiments show that transfer of the autotrophic character was independent of the presence of a plasmid in the donor. Furthermore, the relatively high transfer frequency of plasmid-free donors compared to plasmid-bearing donors may have been a consequence of the restriction-modification system which was apparently affected by the plasmid (see Table 6 and last paragraph of Results).

Conjugative transfer of thallium resistance

Thallium resistance was an excellent marker in mating experiments and, furthermore, was transferred with high frequency (Table 3). The transfer frequency of the Aut marker was dependent on the kind of mating medium, hexadecane providing the highest frequency. In contrast, the frequency of thallium resistance transfer was independent of the kind of medium. All thallium-resistant transconjugants contained the plasmids of the respective donors, and plasmid-free thallium-resistant transconjugants were not detected. Furthermore, transfer of thallium resistance under heterotrophic conditions was not accompanied by transfer of the Aut character (Table 4, type 1d). This again indicated that the transfer of autotrophy and the transfer of the plasmids were completely independent of each other.

Examination of the autotrophic transconjugants showed that all those that had received the plasmid from the donor were resistant to thallium. Plasmid-free Aut⁺ transconjugants were all thallium-sensitive. Aut⁺ transconjugants of the cross MR11 × MR2226, which carried the 54 kb plasmid pHG31-a2 (Table 4, type 1c) were thallium-sensitive, too. These results provided conclusive evidence that resistance to thallium was coded by the plasmids and that the Aut character was not.

The degree of thallium resistance of strain MR22 was somewhat lower than that of strain MR11; on agar media containing 50 μ M-thallium acetate only 13% of the MR22 cells formed

Fig. 2. Plasmid DNA from *Aut*⁺ transconjugants of *N. opaca*. Lysates were prepared as described in Methods. Tracks: A, MR11; B, MR22; C, MR2226; D, transconjugant of MR11 × MR2226 which harbours a deletion plasmid; E–G, transconjugants of MR11 × MR2226 harbouring only pHG31-a; H, I, transconjugants of MR22 × MR2226. LF, linear DNA fragments; ccc, covalently closed circular DNA.

Table 5. Frequency of *Aut*⁺ transconjugants obtained from plasmid-free strains as mating partners

The procedure used was the same as described in the legend to Table 3.

Cross*	Transfer frequency†
Transconjugant type 1a × MR226‡	1.2×10^{-4}
Transconjugant type 1b × MR226	6.0×10^{-2}
Transconjugant type 1c × MR226	5.5×10^{-2}
Transconjugant type 2a × MR226‡	3.0×10^{-4}
Transconjugant type 2b × MR226	1.8×10^{-1}
MR11 × MR226‡	7.3×10^{-3}
MR22 × MR226‡	4.5×10^{-3}

* For characterization of the transconjugant types see Table 4.

† Results are expressed as the number of *Aut*⁺ *Rif*^r transconjugants obtained per donor. The titre was determined after incubation on selective agar.

‡ Control matings.

colonies compared to MR11 (Fig. 4). The plasmid-free, thallium-sensitive strain MR2226 formed thallium-resistant mutants at a frequency of about 10^{-4} . The reasons for these findings are not yet understood.

Plasmid-coded change of the restriction-modification system for phage ϕ B1

The bacteriophage ϕ B1, which was isolated from a fermenter culture of *N. opaca* MR11, is not propagated on strain MR22. In contrast, it is propagated on the *Aut*[−], plasmid-free strain MR2226. On lawns of this strain clear plaques of at least 2 mm diameter were formed. This observation posed the question whether the plasmids pHG31-a or pHG33 or an autotrophic system affects the propagation of phage ϕ B1.

If phage ϕ B1, propagated on MR2226, was plated on transconjugants of this strain containing the plasmid pHG31-a, such as MR2247 or MR2248 (see Table 6), the efficiency of plating was

Fig. 3. Patterns of DNA fragments after digestion of plasmids from strains of *N. opaca* with *Eco*RI and agarose gel electrophoresis. Tracks: A, λ DNA, restricted with *Eco*RI, as size standard; B, pHG31-a, isolated from a transconjugant resulting from the cross MR11 $\times$ MR2226; C, pHG33, isolated from MR22; D, pHG33, isolated from a transconjugant resulting from the cross MR22 $\times$ MR2226.

Table 6. *Effect of the propagating host on the efficiency of plating of phage ϕ B1*

Plating cells	Properties	Efficiency of plating of ϕ B1 after propagation on:	
		MR2226	MR2248
MR2226	Aut ⁻ , plasmid-free	1	7×10^{-5}
MR2247	pHG31-a, Aut ⁺	4×10^{-2}	1
MR2246	Aut ⁺ , plasmid-free	1	2×10^{-4}
MR2248	pHG31-a	4×10^{-2}	1
MR22	pHG33, Aut ⁺	0	0
MR2249	pHG33	1×10^{-5}	0

about 10^{-2} times lower than on MR2226. This indicated a restrictive effect of plasmid pHG31-a on phage propagation. In contrast, the Aut marker did not affect the efficiency of plating. On lawns of hosts containing pHG33 no plaques appeared or only a few, scarcely visible, turbid ones.

Fig. 4. Colony counts of the wild type strains, of a plasmid-containing transconjugant and of a plasmid-free mutant of *N. opaca* MR22 on thallium agar. Cells were grown in FTY medium for 48 h and diluted in Triton/saline. Samples were plated on FTY agar plates containing thallium acetate at various concentrations. ○, MR11; ●, MR22; □, MR2226; ■, transconjugant MR11 × MR2226 (pHG31-a).

When phage ϕ B1 was picked from one of the few plaques formed on MR2248 and propagated on the same strain, a lysate of high titre was obtained. The efficiency of plating of this phage ϕ B1 × MR2248 was high on those hosts which contained plasmid pHG31-a (see Table 6, column 2) such as MR2247 and MR2248, but it was low (10^{-5} to 10^{-6}) on plasmid-free hosts (MR2226 and MR2246). The result of the latter experiment, i.e. the decrease of the efficiency of plating on the original, plasmid-free host, indicated that plasmid pHG31-a was certainly involved in a restriction-modification system, but not one that was independent of the chromosomal system. Plasmid pHG31-a could apparently switch off the chromosomal modification system or change its specificity.

DISCUSSION

The conjugative transfer of genetic information determining the ability to grow lithoautotrophically on H_2 and CO_2 (Aut character) has so far been studied in *Alcaligenes eutrophus* and *Nocardia opaca* only. In *A. eutrophus* H16 the genes coding for H_2 oxidation (Hox) are localized on a plasmid (Andersen *et al.*, 1981); this plasmid, pHG1 (465 kb), is self-transmissible and can be transferred to plasmid-free mutants of the same strain or closely related strains of the same genus (Friedrich *et al.*, 1981, 1984). As discovered recently (Bowien *et al.*, 1984), the megaplasmid pHG1 is also involved in the formation of the CO_2 -assimilating enzyme system. The conjugative megaplasmids of various H_2 -oxidizing bacteria, such as several strains of *A. eutrophus*, *Alcaligenes hydrogenophilus*, *Paracoccus denitrificans* and *Pseudomonas facilis* have been isolated and their *Eco*RI restriction endonuclease patterns compared (Hogrefe & Friedrich, 1984).

The conjugative transfer of the Aut character in *N. opaca*, which was first described by Reh & Schlegel (1975) has with the present system been well established. Transfer of the Aut character is independent of the plasmid residing in the wild-type donor strains as well as of vector or helper plasmids. Aut is a complex character and comprises the information for hydrogenase,

phosphoribulokinase and ribulose biphosphate carboxylase; in Aut⁺ strains and transconjugants these enzymes are derepressed and formed coordinately (C. Ecker, M. Reh & H. G. Schlegel, unpublished observations). Aut transfer, therefore, concerns *en bloc* transmission of a set of at least eight genes. However, Aut transfer has not been shown to effect transfer of typical chromosomal markers such as growth on lactose or propane, resistance to streptomycin or auxotrophy.

Two assumptions lend themselves to explain these results. The Aut marker may reside on a plasmid which can not be made visible by the methods so far applied. Our methods and that of Rosenberg *et al.* (1982), which resulted in detecting plasmids larger than 680 kb, have failed so far. Alternatively, the Aut character may reside on a genetic element which exists in an integrated state in the chromosome. The inducible loss as well as the specific transfer of the Aut character support this assumption. This explanation is in accordance with reports on the conjugative transfer of genetic markers in which no extrachromosomal plasmid DNA is involved (Franke & Clewell, 1981; Gawron-Burke & Clewell, 1982; Inamine & Burdett, 1985). Our attempts to detect a DNA molecule originating from mitomycin C- or ethylmethane-sulphonate-induced excision were not successful (G. Kruppa & M. Reh, unpublished observations).

Furthermore, plasmids of various sizes appeared in transconjugants of crosses between apparently plasmid-free species of *Streptomyces*. These plasmids are assumed to be present in the donor DNA in the form of silent replicons (Bibb *et al.*, 1981; Hopwood *et al.*, 1984). In the case of *N. opaca*, attempts to detect plasmid ccc-DNA in the transconjugants from matings of plasmid-free partners were not successful. Moreover, it is conceivable that the Aut character of *N. opaca* exists as a kind of transposon which can be inserted not only into the chromosome at any location but also into plasmids. The isolation of plasmids which differ from the present plasmids pHG31-a and pHG33 with respect to their size has not been achieved. The present results do not allow localization of the Aut genes in *N. opaca*. It is highly probable that these genes are integrated into the chromosome. As soon as DNA probes of the hydrogenase and the ribulose biphosphate carboxylase are available, DNA hybridization experiments will clarify this question.

Resistance to thallium of plasmid-bearing wild-type strains MR11 and MR22 of *N. opaca* is coded by their plasmids pHG31-a and pHG33, respectively. Both plasmids are transferred with high frequency to the plasmid-free mutant MR2226. All thallium-resistant transconjugants carried the plasmid of the donor. Aut⁺ transconjugants selected for autotrophic growth were thallium-resistant if they carried the plasmid and thallium-sensitive if they lacked the plasmid. All Aut⁺ and Aut⁻ transconjugants, which had received the plasmid pHG33 from MR22, were also resistant to the nocardia-specific phage ϕ B1.

Therefore, with the exception of coding for thallium resistance, the plasmids of *N. opaca* are still cryptic. They share this lack of recognized genes with plasmids of sizes between 120 and 180 kb detected in actinomycetes (Dobritsa, 1984).

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Dedicated to Professor N. Pfennig on the occasion of his 60th birthday.

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