Transfer of Drug Resistance to *Myxococcus* from Bacteria Carrying Drug-resistance Factors

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SUMMARY

Resistance to chloramphenicol was successfully transferred from strains of *Escherichia coli* carrying R factors representative of compatibility groups F, W, S and N to strains of *Myxococcus xanthus* and *M. fulvus*. Resistance to kanamycin was transferred from an R factor in group S, and to neomycin from an R factor of group P. Myxobacterial strains differed in their capacity to take up the resistances and also in the stability of the resistance character. Strains of *M. fulvus* were obtained that acquired resistance to chloramphenicol without exposure to R⁺ eubacterial strains. Cell-free preparations of all the chloramphenicol-resistant strains catalysed the acetylation of the drug. Chloramphenicol resistance was successfully transferred from the presumed R⁺ strains of *Myxococcus* and also from the spontaneously occurring chloramphenicol-resistant *M. fulvus* to other *Myxococcus* strains. Moreover, recombinants resistant to both rifampicin and 5-fluorouracil were obtained, though infrequently, by mixing *Myxococcus* strains resistant to rifampicin and chloramphenicol with other myxococci resistant to 5-fluorouracil, both when the chloramphenicol resistance was derived from S-a (group W) and when it was the endogenous *M. fulvus* resistance. Thus it appears that S-a and a new chloramphenicol resistance factor from *M. fulvus* will mobilize a chromosomal genetic marker in *Myxococcus*.

INTRODUCTION

Members of the order Myxobacteriales are Gram-negative rods that demonstrate gliding motility. They form fruiting bodies and also demonstrate morphogenesis of the cell. In the lower myxobacteria (families Myxococcaceae, Archangiaceae and Cystobacteraceae), the morphogenesis consists of the formation of myxospores of grossly different morphology to the vegetative rods. The myxobacteria are thus valuable in studying organism-organism interaction and morphogenesis in prokaryotes (Dworkin, 1973).

Genetic analysis would be of great value in resolving the mechanisms that control these processes. I report the transmission of antibiotic resistance by myxobacteria with a view to establishing the foundation of gene transfer by conjugation.

The organisms used are of the genus *Myxococcus* (Myxococcaceae). These bacteria are the most convenient for experimental study as, unlike most myxobacteria, *M. xanthus* FB (Dworkin, 1962) grows in a dispersed state in liquid culture and moreover can be induced synchronously to produce myxospores in liquid (Dworkin & Sadler, 1966).

METHODS

Media. For experiments involving *Myxococcus*, including mixed growth of *Myxococcus* with other bacterial species, the bacteria were grown in CT medium (1 % w/v, Bactocasitone;

Table 1. *Bacterial strains*

Organism	Strain	Genetic markers*	Origin	Reference
<i>Escherichia coli</i>	J53	K12 F ⁻ lac ⁺ met pro	S. Baumberg	Clowes & Hayes (1968)
	J53-1	nal	S. Baumberg	
	J62	K12 F ⁻ lac his pro trp		Clowes & Hayes (1968)
	J62-1	nal	S. Baumberg	
	J62-2	rif	S. Baumberg	
<i>Serratia marcescens</i>	I 100	K12 F ⁻ T1 ^r T5 ^r m _B ⁺ r _B ⁺	K. Murray	Yoshimori, Roulland-Dussoix & Boyer (1972)
		endo		
<i>Serratia marcescens</i>	K5.8	rif nal	This work	
<i>Pseudomonas fluorescens</i>	P.f.(RP4)	(See Table 2)		
<i>Myxococcus xanthus</i>	A	(See text)	This work	
	A1	rif	This work	
	A44	rif	This work	
	B		NCIB	
	B26	rif	This work	
	B27	flu	This work	
	FB _i	pig	R. P. Burchard	Burchard & Dworkin (1966b)
<i>Myxococcus fulvus</i>	M		This work	
	M26	rif	This work	
	M27	flu	This work	

* *flu*, *nal* and *rif* are resistance to 5-fluorouracil, nalidixic acid and rifampicin respectively; T1 and T5 are bacteriophage T1 and T5; m_B and r_B are the modification and restriction enzymes of *E. coli* B; *endo*, endonuclease; *pig*, pigment.

Difco). For experiments involving exclusively bacteria other than *Myxococcus*, the medium was nutrient broth containing (% w/v): tryptone, 1; yeast extract, 0.5; NaCl, 0.5. Solid media contained 1.5 % (w/v) agar (London Analytical and Biological Media, Ltd). Minimal salts agar was that of Clowes & Hayes (1968) supplemented with the amino acids specified at a concentration of 0.01 %.

Growth conditions. Bacteria were grown in shake culture and incubated (either on agar or as stationary liquid cultures) at 30 °C unless otherwise specified. The rate of expansion of surface colonies of *Myxococcus* was measured by the method of Burchard (1974). The 'fruiting medium' for *Myxococcus* was 0.02 % CT supplemented with the inorganic salts used by Burchard (1974) and solidified with agar (1.5 %, w/v). Plates were inoculated in the centre and incubated at 100 % relative humidity. The organisms form fruiting bodies in concentric rings as described for *Archangium violaceum* by Reichenbach (1965) and for *M. xanthus* by Burchard (1974).

Drugs were used at the following concentrations (µg/ml): rifampicin, 4; nalidixic acid, 25; 5-fluorouracil, 100; streptomycin sulphate, 50; chloramphenicol, usually 25; kanamycin, 25; neomycin, 25; carbenicillin, 500. For certain experiments different concentrations of chloramphenicol were employed; details appear in Results.

Bacterial strains. These are listed in Table 1.

Serratia marcescens K5.8 was obtained from *S. marcescens* K5 (NCIB2847) by selecting spontaneous mutants resistant to rifampicin and selecting from such a mutant a spontaneous mutant resistant to nalidixic acid.

Myxococcus xanthus strains A and B are clones of *M. xanthus* FB (Dworkin, 1962). *Myxococcus xanthus* A was selected on the basis of a very low frequency of phenotypically phage-resistant colonies when plated in the presence of excess (phage/bacterium: 10/1) bacteriophage MX1 (Burchard & Dworkin, 1966a). The *M. xanthus* FB from which the strain was

Table 2. *Plasmids*

Plasmid	Resistance and other markers*	Compatibility group	Reference
R1drd19	<i>chl kan str sul</i>	FII	Lawn, Meynell, Meynell & Datta (1967)
R1drd19-1	<i>chl str sul</i>	FII	S. Baumberg (see text)
R1.19-2	<i>chl str sul tra</i> ⁻	FII	This work
RI	<i>chl str amp</i> r _{RI} ⁺ m _{RI} ⁺	FII	Yoshimori <i>et al.</i> (1972)
RP4	<i>car neo tet</i>	P	Saunders & Grinsted (1972)
R144drd3	<i>kan</i>	I	Lawn <i>et al.</i> (1967)
R387	<i>chl str</i>	K	Hedges, Datta, Coetzee & Dennison (1973)
R57b	<i>chl amp sul gen</i>	C	Witchitz & Chabbert (1972)
R390	<i>chl amp str sul tet hspII</i>	N	Ingram (1973)
S-a	<i>chl kan sul str</i>	W	Hedges & Datta (1971)
R478	<i>chl kan tet</i>	S	Hedges, Rodriguez-Lemoine & Datta (1975)

* Resistance markers are as follows: *chl*, chloramphenicol; *kan*, kanamycin; *str*, streptomycin; *sul*, sulphonamides; *amp*, ampicillin; *car*, carbenicillin; *neo*, neomycin; *tet*, tetracycline; *gen*, gentiomyacin. r_{RI}⁺, m_{RI}⁺ and *hspII* are restriction and modification systems; *tra*, transferability.

selected was obtained from Professor Dworkin's culture collection. *Myxococcus xanthus* B is a clone of *M. xanthus* FB (NCIB9412); the organism produces predominantly tan colonies during the spontaneous segregation of colour variants that is characteristic of *M. xanthus* (Burchard & Dworkin, 1966*b*).

Myxococcus fulvus M is a clone of strain Mittelamerika which was a gift from Dr H. Kühlwein. The original strain did not grow in a disperse form in shake culture. Strain M is a variant that grows as rosettes each composed of approximately 100 organisms; the rosettes are easily dispersed by agitation. This type of behaviour is also characteristic of certain *M. xanthus* strains (R. P. Burchard, unpublished). Certain derivatives of strain M are not disperse-growing (see Results).

All the *Myxococcus* strains are hosts for bacteriophage MX1.

Myxococcus strains resistant either to rifampicin or 5-fluorouracil were obtained by exposing bacteria in 10⁻¹ M-potassium phosphate buffer pH 7.6 to u.v. light to approximately 0.1 % survival, growing the survivors to approximately 10⁸ colony-forming units (c.f.u.)/ml and plating on CT agar containing the drug. Resistant colonies were cloned three times on CT agar containing the drug and were grown in CT. The efficiency of plating (e.o.p.) on CT agar containing the drug was determined. Certain strains (notably those derived from *M. xanthus* A) were unstable and showed low e.o.p. in the presence of the drug. Of those used, A44 *rif*, B26 *rif*, B27 *flu*, M26 *rif* and M27 *flu* all gave e.o.p. of 85 to 100 % on agar containing the drug and were regarded as 'stable'. *Myxococcus xanthus* A1 *rif* was originally unstable (e.o.p. approximately 40 % on rifampicin) at the time the organism A1(R1.19) *chl* (see Results) was obtained. Over a period of six months this organism had become stable with regard to rifampicin resistance and its segregant A1-I (see Results) is also stably rifampicin resistant.

Myxococcus fulvus M2, M3 and M7 (all *chl*) arose spontaneously on a single occasion (see Results). These, and certain other chloramphenicol-resistant derivatives of strain M obtained from mixed growth with R⁺ strains of *Escherichia coli*, are non-disperse. Such organisms grow in shake culture in the form of spheres, reaching a maximum diameter of approximately 3 mm, similar to a 'rough' variant of *M. xanthus* FB₁ (R. P. Burchard, unpublished).

They were cloned either by crushing the spheres and streaking or by streaking colonies picked from agar plates.

Plasmids. R factors used in this work are listed in Table 2. R1drd19-1 is a kanamycin-sensitive spontaneous segregant of R1drd19, obtained by Drs Baumberg and Dennison. R factors were transferred between *E. coli* hosts by the methods of Datta & Hedges (1972). R1drd19 was transferred from *E. coli* J53 to *S. marcescens* K5.8 by incubating a mixture of the bacteria at 30 °C for 24 h and plating on agar containing rifampicin, nalidixic acid and chloramphenicol. Colonies were tested on CT agar and on CT agar containing kanamycin. (The pink colour of *S. marcescens* is not well developed on the selection plates.) *Serratia marcescens* carrying kanamycin resistance (about 80 % of those tested) was used.

The plasmid R1.19-2 *chl tra* was obtained as follows. *Escherichia coli* J62-1 (R1drd19-1) was plated on nutrient agar in the presence of a high concentration of bacteriophage MS2 (Davis, Strauss & Sinsheimer, 1961). Colonies, shown to be resistant to phage MS2, were grown in nutrient broth, mixed with *E. coli* J53, incubated for 24 h, and plated on minimal salts agar supplemented with chloramphenicol, methionine and proline. Out of 30 colonies tested, five appeared to be transfer-defective (frequency of transfer $< 10^{-6}$) in this screen. These five strains were crossed with *E. coli* J53 (R144drd3) and plated on nutrient agar containing chloramphenicol, nalidixic acid and kanamycin. Colonies growing on this medium were tested on minimal salts agar supplemented with (i) histidine, proline, tryptophan, nalidixic acid, chloramphenicol and kanamycin, and (ii) methionine and proline. Those that grew on (i) but not (ii), i.e. derivatives of *E. coli* J62-1, were crossed with *E. coli* J53 and plated on minimal salts agar supplemented with methionine, proline and chloramphenicol. Kanamycin-resistant colonies were rejected. The others were tested for their ability to transfer chloramphenicol resistance to strain J62-1. After this screen, two of the original five possible *tra* plasmids were found to have a transfer frequency of less than 10^{-6} from J53 to J62-1, and vice versa, in four independent tests in each direction. The fact that the chloramphenicol resistance could be mobilized by R144drd3 shows the independent character of the *chl* gene. One plasmid, R1.19-2, was used as a *tra* mutant of R1drd19-1.

Genetic exchange involving *Myxococcus*. Donor and recipient strains (approximately 10^8 c.f.u./ml in CT medium) were mixed and incubated in tubes overnight. Experiments involving non-disperse strains of *M. fulvus* used 3 or 4 spheres of organism in a total volume of 5 ml. The mixtures were plated on CT agar containing drugs to select against both parents of the cross. When streptomycin was used to select against *E. coli* R⁺ strains the concentration of this drug (50 µg/ml) was higher than the resistance conferred by *str* markers in any of the R factors (15 µg/ml). Colonies of *E. coli* (presumably spontaneous *strA* mutants) were ignored. *Myxococcus* exconjugant colonies appeared after 5 to 6 days (7 to 8 days on plates containing 5-fluorouracil). Drug-resistant strains of *Myxococcus* are referred to as the strain designation with the apparently-transferred R factor in brackets. In the case of R1drd19, the designation for *Myxococcus* is simply (R1.19) as there is no evidence that derepressed transfer capacity is maintained in *Myxococcus*.

In those experiments in which chloramphenicol-resistant *Myxococcus* strains were used as donors, these were grown in CT medium containing chloramphenicol and the bacteria were washed by centrifugation and resuspended in CT before mixing.

Acetylation of chloramphenicol. Chloramphenicol acetyltransferase (EC. 2.3.1.28) cannot be assayed by the colorimetric method of Shaw & Brodsky (1968) in crude extracts of *Myxococcus* because of the high background of acetyl CoA hydrolase activity. The following radioactive assay based on the methods of Shaw (1967) was employed. Bacteria were washed, resuspended in 0.01 M-tris-HCl buffer pH 7.8 (10^{12} c.f.u./ml) and sonicated (4 A,

1 min, 0 °C; Dawe Soniprobe). Debris was removed by centrifugation (18000 g for 30 min). For many experiments, the unfractionated supernatant was employed. Protein was assayed by the method of Lowry, Rosebrough, Farr & Randall (1951) using bovine serum albumin for calibration. For some purposes the protein precipitated by ammonium sulphate (50 % saturation) was used. Protein solution and added buffer (protein concentration 5 mg/ml), acetyl CoA (final concentration 0.1 mM) and dichloroacetyl [^{14}C]chloramphenicol (8.8 Ci/mmol; 10 $\mu\text{Ci/ml}$) were incubated at 37 °C. Samples were extracted three times with 2 vol. ethyl acetate; the solvent was removed by rotatory evaporation and the residue was redissolved in a small volume of ethyl acetate and spotted on to a thin-layer chromatographic plate (stationary phase Kieselgel Typ G, 0.5 mm; Merck, Darmstadt, Germany). The chromatograms were developed in methanol and chloroform (5:95, v/v) and exposed to X-ray film. The films were developed and the film blackening was measured at 450 nm in a Pye/Unicam SP1800 spectrophotometer fitted with a gel scanning attachment.

For certain experiments bacteria were grown in the presence of dichloroacetyl [^{14}C]chloramphenicol and the medium was assayed for radioactive chloramphenicol and acetyl chloramphenicols. The medium was extracted twice with 1 vol. ethyl acetate and the ethyl acetate was reduced in volume and the products analysed as above.

Carotenoids. Recombinants of strains B and M were identified by analysis of their carotenoids. Bacteria were streaked on CT agar and incubated under uniform tungsten-light illumination for 6 days. The bacteria were scraped off, extracted with acetone, and spectra of these extracts were recorded between 325 and 600 nm. The spectra showed maxima at 377, 400, 425, 450, 477 and 507 nm. The ratio A_{377} to A_{400} is characteristic of the strains (0.8 to 1.0 for strain B; 1.2 to 1.4 for strain M). Additionally, the carotenoids were analysed by thin-layer chromatography using the method of Kleinig & Reichenbach (1973). Components were resolved with the following R_F values and appearances, the fluorescent spots being visualized under u.v. light: 0.0 (yellow), 0.08 (red), 0.13 (blue fluorescence), 0.20 (orange), 0.90 (diffuse, orange), 0.97 (red), 0.98 (orange). The components with R_F 0.13 and 0.90 are characteristic of *M. fulvus* M.

RESULTS

Transfer of drug resistance to Myxococcus

Chloramphenicol-resistant derivatives of *M. xanthus* A1 were obtained from mixtures of this strain with *E. coli* J53(R1drd19). Chloramphenicol-resistant *E. coli* formed a lawn on the selection plates (rifampicin plus chloramphenicol) due either to spontaneous *rif* mutants or loss of rifampicin during the 6-day incubation. *Myxococcus xanthus* colonies appeared initially as plaques in this lawn. After further incubation (4 days), yellow *M. xanthus* colonies were visible in the plaques. Alternatively, picks from the centres of the plaques yielded viable *M. xanthus* organisms.

An objection to this procedure of scoring 'plaques' in such gene-exchange experiments is that the limited growth of *E. coli* J53(R1drd19) might reduce the effective concentration of chloramphenicol in the agar and thus permit selection of chromosomal mutations in *M. xanthus* to produce chloramphenicol resistance. Subsequent studies involving R factors employed streptomycin to select against *E. coli*. All our *Myxococcus* strains are streptomycin resistant and, at the concentration of streptomycin used, growth of the donor strains was totally inhibited. Although certain plates contained isolated streptomycin-resistant *E. coli* colonies, the numbers (in no case more than 7 colonies) were insufficient to detoxify chloramphenicol in the agar. The results are summarized in Table 3. In addition to chlor-

Table 3. Summary of transfer of *R* factor determinants from eubacteria to *Myxococcus*

Incubations were performed as in Methods with 5×10^9 c.f.u. eubacterial donor and typically 10^9 c.f.u. *Myxococcus* before incubation. Numbers refer to drug-resistant c.f.u. per 10^8 surviving myxococci. Counterselection was achieved by plating on a mixture of the drug plus streptomycin, except when the donor was *P. fluorescens* (RP4) in which case the mixture was plated on CT agar containing the appropriate drug and the plates were incubated at 37 °C as the donor does not grow at this temperature. The control (*Myxococcus* incubated in the absence of R^+ bacteria) showed no colonies (< 1 per 10^8 c.f.u.) except for certain strains plated on either carbenicillin or neomycin. In these cases the transfer is recorded by the symbol '?'. NT, Not tested.

Donor	Plasmid	Resistance	Recipient				
			<i>M. xanthus</i>	<i>M. xanthus</i>	<i>M. xanthus</i>	<i>M. xanthus</i>	<i>M. fulvus</i>
			A	A44	FB _t	B	M
<i>E. coli</i> J53	R1drd19	<i>chl</i>	9	< 1	< 1	10	27
		<i>kan</i>	< 1	< 1	< 1	< 1	< 1
<i>S. marcescens</i> K5.8	R1drd19	<i>chl</i>	< 1	NT	NT	< 1	< 1
<i>E. coli</i> 62-1	R1drd19-1	<i>chl</i>	10	NT	NT	30	30
<i>E. coli</i> 62-1		<i>chl</i>	15	NT	NT	27	60
<i>E. coli</i> 1100	RI	<i>chl</i>	2	NT	NT	10	8
<i>E. coli</i> J53	R144drd3	<i>kan</i>	< 1	NT	NT	< 1	< 1
<i>E. coli</i> J53	R387	<i>chl</i>	< 1	< 1	NT	< 1	< 1
<i>P. fluorescens</i>	RP4	<i>car</i>	100	NT	NT	?	< 1
		<i>neo</i>	30	NT	NT	20	?
		<i>chl</i>	3	NT	NT	120	60
<i>E. coli</i> J53	R57b	<i>chl</i>	< 1	NT	< 1	160	160
<i>E. coli</i> J62-2	R478	<i>kan</i>	< 1	NT	< 1	< 1	100
<i>E. coli</i> J53-1	S-a	<i>chl</i>	< 1	NT	< 1	100	140
<i>E. coli</i> J62-2	R390	<i>chl</i>	< 1	NT	NT	16	< 1

Table 4. Summary of *R* factors transferred to *Myxococcus*

+, Transferable and surviving; —, not transferable; a, abortive; ?, cannot be tested (see text).
Blank entries were not tested. *Myxococcus* strains as in Table 1.

Plasmid	Compatibility group	Resistance	Myxococcus strain					
			A	AI	A44	B	FB _t	M
R1drd19	FII	<i>chl</i>	a	+	—	+	—	+
		<i>kan</i>	—	—	—	—	—	—
RI	FII	<i>chl</i>	a			+		+
RP4	P	<i>car</i>	a			?		—
		<i>neo</i>	+			+		?
R144drd3	I	<i>kan</i>	—	—		—		—
R387	K	<i>chl</i>	—		—	—		—
R57b	C	<i>chl</i>	a			+		+
R390	N	<i>chl</i>	—			+		—
R478	S	<i>chl</i>	—			+	—	+
		<i>kan</i>	—			—	—	+
S-a	W	<i>chl</i>	—			+	—	+

amphenicol, attempts were made using suitable donor strains to select for *Myxococcus* strains resistant to neomycin, carbenicillin and kanamycin. Other resistance determinants carried by the *R* factors (see Table 2) are not suitable for study, either because myxobacteria are inherently resistant (ampicillin, streptomycin and sulphonamides) or (in the case of tetracycline) the drug is unstable and might decompose during the prolonged incubations.

Drug-resistant derivatives of *Myxococcus* strains A, B and M were picked and streaked on CT agar containing the appropriate drug. Strains A(RI.19) *chl*, A(RP4) *car*, and A(R57b) *chl* were found to be incapable of further growth in the presence of the drug and are regarded

Fig. 1. Growth of chloramphenicol-resistant *Myxococcus* strains upon transfer from chloramphenicol to CT medium. A culture in CT plus chloramphenicol was diluted $\times 10^8$ into CT and incubated on CT (open symbols) and CT plus chloramphenicol (solid symbols). (a), $\circ$, $\bullet$, AI(R1.19); $\square$, $\blacksquare$, B(R390). (b) $\circ$, $\bullet$, M(R1.19); $\square$, $\blacksquare$, M(R1). (c) $\circ$, $\bullet$, M(R478); $\square$, $\blacksquare$, M(S-a).

tentatively as abortive. Isolates of M(R478) *chl* were tested on kanamycin and 80 % were found to be kanamycin resistant. All strains originally scored as M(R478) *kan* were found to be chloramphenicol resistant. On the basis of these data and those in Table 3, the transfer of R factors to *Myxococcus* is summarized in Table 4.

In addition to these strains, a set of chloramphenicol-resistant derivatives of *M. fulvus* M appeared spontaneously with a frequency of approximately 10^{-7} . The appearance of such mutants was not observed on any subsequent occasion. The chloramphenicol-resistant phenotype has also been obtained in derivatives of *M. xanthus* FB₄ (R. P. Burchard and J. H. Parish, unpublished). This phenotype did not appear in controls with cultures used for the incubations in Table 3, nor has it ever been observed in strains A, AI, A44 or B. All the chloramphenicol-sensitive strains described here plate with 100 % efficiency on CT agar containing 5 μ g chloramphenicol/ml; a concentration of 10 μ g of the drug/ml is inhibitory (no detectable growth in liquid medium and an e.o.p. of less than 5×10^{-8}).

Stability and growth of chloramphenicol-resistant strains of Myxococcus

The e.o.p. of chloramphenicol-resistant strains on chloramphenicol was measured during growth in the presence and absence of chloramphenicol, in the light of the observation (R. P. Burchard and J. H. Parish, unpublished) that after one generation FB₄ *chl* organisms showed a loss of chloramphenicol resistance. Strain AI(R1.19) *chl* has changed during the period of one year since its original isolation. Initially, chloramphenicol-grown bacteria demonstrated an e.o.p. on chloramphenicol of 20 %. Recent experiments show an e.o.p. of 100 %. All other chloramphenicol-resistant strains have plated with consistently high

Fig. 2. Acetylation of chloramphenicol by extracts of chloramphenicol-resistant strains of *E. coli* and *M. xanthus*. Experimental details are described in Methods. Equal concentrations of protein were used in each case. Tracings of film blackening of autoradiographs of thin-layer chromatographic plates are shown. O, origin; C, chloramphenicol; A1, 1-acetylchloramphenicol; A3, 3-acetylchloramphenicol; AA, 1,3-diacetylchloramphenicol. Results were obtained after incubations with extracts of (a) *E. coli* 353(R1drd19) for 10 min and (b) *M. xanthus* A1(R1.19) *chl* for 2 h.

efficiency (approximately 100 %) on CT agar containing chloramphenicol when grown in the presence of chloramphenicol from the time of their original isolation. The effects of transferring such strains to CT medium are shown in Fig. 1. The anomalous growth of M-strains is due to aggregation and dispersion which show apparent phase variation. The data show that, with the exception of M(R1.19) *chl*, none of the organisms show loss of resistance from the formerly resistant population although certain strains, such as B(R390) *chl*, show a rapid segregation of chloramphenicol-sensitive organisms during division. After 15 generations, the e.o.p. on CT plus chloramphenicol was: A1(R1.19), 1.7×10^{-6} ; M(R1), 4.5×10^{-5} ; M(S-a), 7.5×10^{-1} ; M(R478), 5×10^{-7} ; M(R1.19), $< 10^{-7}$; B(R390), $< 10^{-7}$. Such experiments are not possible with totally non-disperse strains. These were cloned by streaking on either CT alone or CT plus chloramphenicol. Of 12 M7 clones on CT, 11 were chloramphenicol resistant; of 12 M(R57b) clones on CT, 10 were chloramphenicol resistant. In both cases 12 out of 12 clones on chloramphenicol were chloramphenicol resistant upon transfer to medium containing chloramphenicol.

An alternative to studying exponential growth in *Myxococcus* is to measure the rate of increase in colony diameter. This function, which is primarily a measure of motility, is linear in *M. xanthus* FB_t (Burchard, 1974). Strains derived from *M. fulvus* showed a lag of 50 to 100 h during which colony spreading was very slow, before linear growth developed. In calculating the rate of spreading (Table 5) these lag periods have been neglected and the rates are those achieved after the lag. The motility of *M. xanthus* A1(R1.19) *chl* was severely limited by chloramphenicol despite its stable genotype and good growth (Fig. 1). The motility of other chloramphenicol-resistant strains was only partially limited. The limitation may have been due to motility being sensitive to low intracellular concentrations of the drug remaining in these strains, despite the presence of acetylase activity (next section).

On fruiting agar, all the strains formed normal fruiting bodies in the absence of chloramphenicol. In the presence of chloramphenicol, strains A1(R1.19), B(R1.19), B(R390), M2, M(R1.19), M(R1), M(S-a) and M(R478) formed mature fruiting bodies, M(R57b), M3 and

Fig. 3

Fig. 4

Fig. 3. Acetylation of chloramphenicol by extracts of strains derived from *M. xanthus* A1. Extracts were fractionated by ammonium sulphate precipitation. Incubations were as in Fig. 2, for 2 h. (a) A1(R1.19) *chl* plus acetyl CoA; (b) A1(R1.19) *chl* without acetyl CoA; (c) A1 *chl* plus acetyl CoA.

Fig. 4. Acetylation of chloramphenicol by extracts of *M. fulvus* M and its derivatives. Conditions for extraction and incubation (2 h) were as in Fig. 2. (a) Strain M, (b) M2 *chl*, (c) M(S-a) *chl*, all plus acetyl CoA; (d) M(S-a) *chl* without acetyl CoA.

M7 formed immature fruiting bodies containing few myxospores, and B(R1.19-2), B(R1) and B(S-a) did not form fruiting bodies.

The tolerance of certain strains to higher concentrations of chloramphenicol was examined by picking colonies from CT agar containing 25 $\mu\text{g/ml}$ on to CT agar containing 50, 75 and 100 $\mu\text{g/ml}$. Strain M7 was inhibited by 50 $\mu\text{g/ml}$. Strains A1(R1) *chl*, M(R1) *chl*, M(R1.19) *chl*, M(S-a) *chl* and M(R478) *chl kan* were all tolerant to a concentration of 50 $\mu\text{g/ml}$. With strains A1(R1) *chl* and M(S-a) *chl*, limited growth was discernible on 75 $\mu\text{g/ml}$ after 2 weeks' incubation. The other strains did not grow on this concentration and all the strains were totally inhibited by 100 $\mu\text{g/ml}$.

Acetylation of chloramphenicol by Myxococcus strains

Chloramphenicol resistance in R^+ strains of *E. coli* is due to chloramphenicol acetyltransferase (Shaw, 1967). The enzyme activity has been demonstrated in *Myxococcus* strains by

Table 5. Effect of chloramphenicol on the spreading of *Myxococcus* colonies

Myxococcus xanthus AI *chl* is a segregant from AI(RI.19). Strain IM is a non-disperse clone of *M. fulvus* M; 2M is a disperse growing clone of strain M; M2 and M(R57b) are both non-disperse.

Strain	Rate of increase of colony diameter (mm/h)	
	CT agar	CT agar plus chloramphenicol
AI-1	1.8	0
AI(RI.19) <i>chl</i>	2.1	0.3
B	1.4	0
B(R478) <i>chl</i>	1.9	0.9
B(R390) <i>chl</i>	1.1	0.8
IM	3.1	0
2M	2.5	0
M2 <i>chl</i>	1.2	1.1
M(R478) <i>chl</i>	2.4	0.8
M(S-a) <i>chl</i>	2.1	1.0
M(R57b) <i>chl</i>	1.2	1.4
M(RI) <i>chl</i>	2.8	1.6
M(RI.19) <i>chl</i>	2.3	1.4

Table 6. Transfer of chloramphenicol resistance to *M. xanthus* B26 *rif* and *M. fulvus* M26 *rif*

Mixtures initially contained 5×10^7 c.f.u. of each strain/ml except for the non-disperse strains which were inoculated as 2 to 3 spheres (see Methods). The numbers refer to *rif chl* recombinants per 10^5 recipients (or in the case of controls, per 10^6 organisms) after incubation. *dis*, Non-disperse strain.

Donor	Recipient		
	B26 <i>rif</i>	M26 <i>rif</i>	None
M(R57b) <i>chl dis</i>	4.5	60	—
M(S-a) <i>chl</i>	0.8	17.5	<0.04
M(R478) <i>chl</i>	9.9	14.7	<0.04
M(RI) <i>chl</i>	5.7	8.7	<0.05
M(RI.19) <i>chl</i>	2.2	<0.3	<0.2
M7 <i>chl dis</i>	25.0	2.6	—
B(R390) <i>chl</i>	11.6	<0.05	<0.04
None	<0.03	<0.3	

measuring the conversion of [14 C]chloramphenicol to acetyl [14 C]chloramphenicol (see Methods). The bacteria were grown in CT medium containing chloramphenicol (25 μ g/ml), harvested, washed twice with CT and assayed for the enzyme. Results of such assays are summarized in Figs. 2 to 4. In *E. coli* the enzyme acetylates the 3-hydroxyl of chloramphenicol. A non-enzymic rearrangement to the 1-acetate is followed by a second acetylation yielding some 1,3-diacetyl chloramphenicol (Shaw, 1967). Figure 2 compares the activity of unfractionated extracts of *M. xanthus* AI(RI.19) *chl* and *E. coli* J53(RI Δ rd19). The *Myxococcus* extract is substantially less than that from *E. coli*. By using ammonium sulphate-precipitated protein (Fig. 3) the activity in *M. xanthus* AI(RI.19) *chl* was shown to be totally dependent upon acetyl CoA. The activity (which in partially fractionated preparations yields no 1,3-diacetyl chloramphenicol) is entirely absent from *M. xanthus* AI-1. The activity was examined in *M. fulvus* strains (Fig. 4): little activity was found in strain M (Fig. 4a); *M. fulvus* M2 *chl* showed slight activity (Fig. 4b); *M. fulvus* M(RI) *chl* (not shown) showed comparable activity to strain M2 and this activity was acetyl CoA-dependent; *M. fulvus*

Table 7. *Transfer of rifampicin resistance between strains derived from M. xanthus B and M. fulvus M*

Crosses were performed and scored as in Table 6. The numbers are recombinants per 10^5 recipients. 'Controls' refer to all the components of the crosses. All donor strains were *rif*; those carrying either S-a or m7 were also *chl*. The recipients were *flu*.

Cross	Recombinants	
	<i>flu chl</i>	<i>flu rif</i>
Controls	<0.2	<0.2
B26/m7 × M27	<0.2	<0.2
B26/m7 × B27	3.0	1.0
B26(S-a) × B27	<0.2	2.0
B26(S-a) × M27	5.0	10.6
M26(S-a) × B27	3.0	5.0
M26(S-a) × M27	1.7	6.0
B26 × M27	—	<0.2
B26 × B27	—	<0.2
M26 × M27	—	<0.2
M26 × B27	—	<0.2

(S-a) *chl* showed greater activity (Fig. 4c); *M. fulvus* M(R478) *chl kan* (not shown) demonstrated high activity, comparable with strain M(S-a) *chl*.

The instability of the Chl phenotype and the difficulties of quantitative colorimetric enzyme assay have prevented satisfactory experiments to establish whether the enzyme is constitutive (as in R⁺ strains of *E. coli*).

Strains A, B, FB₆ and M were grown for 15 h in the presence of non-inhibitory concentrations of [¹⁴C]chloramphenicol to a density of 10^8 c.f.u./ml. No acetylated chloramphenicols were detectable in the supernatant. The limits of the assay would detect a conversion of approximately 2 % of chloramphenicol to acetylated products. These strains do convert acetyl chloramphenicols to chloramphenicol, presumably as a result of esterase activity (R. P. Burchard and J. H. Parish, unpublished).

Transfer of drug resistance between Myxococcus strains

Chloramphenicol resistance was transferred to strains of *M. xanthus* B and *M. fulvus* M when chloramphenicol-resistant myxococci were incubated with rifampicin-resistant recipients. The *rif chl* recombinants were allocated to strains B and M on the basis of their carotenoid spectra and thin-layer chromatograms (see Methods). Results of such crosses are summarized in Table 6. In every case in which the cross involved one B-strain and one M-strain, carotenoid analysis of the recombinants confirmed that they were derived from the recipient.

Recombinant strains are designated as, for example, M26(R478) *chl rif*. The recombinants from the cross M7 × B26 are referred to as B26/m7 *chl rif*. The stability of chloramphenicol resistance in the strains examined [B26/m7, B26(R390), M26(S-a) and B26(S-a)] is qualitatively similar to that in the donors. After overnight growth in CT medium the e.o.p. on chloramphenicol was 10^{-1} to 10^{-4} . The e.o.p. for chloramphenicol-grown bacteria was approximately 100 %; both types of culture showed an e.o.p. of 80 % on rifampicin.

Crosses were attempted using B26, M26 and every type of *rif chl* recombinant in Table 7 as donor and M27 *flu* and B27 *flu* as recipients. Recombinants *chl flu* were obtained with comparable frequencies to those shown in Table 6. The mixtures were also assayed for

flu rif recombinants. The only successful crosses employed B26(S-a), M26(S-a) or B26/m7 as donor (Table 7). Strain M26 (R478) is probably a successful donor of rifampicin resistance but data are not presented and there was a low background of fluorouracil-resistant colonies produced by the donor. Strains of B26 or M26 carrying chloramphenicol resistance derived from R57b, R1, R1.19 or R390 were not successful donors (comparable with R⁻ B26 and M26, Table 7).

DISCUSSION

The transfer of drug resistance to *Myxococcus* strains from eubacterial R factors extends the number of Gram-negative bacteria between which plasmid transfer is possible. The mechanism of the transfer remains unclear. The data on R1*drd19* and its derivatives (Table 3) imply that the transfer function that operates in *E. coli* conjugation is not involved, but the species of the donor is significant (transfer from *S. marcescens* did not occur). There are no data clearly indicating the nature of the chloramphenicol-resistance determinant in strains such as *M. xanthus* A1(R1.19) *chl* and *M. fulvus* (S-a) *chl*, and analysis of the DNA content of such cells is likely to be complicated by the presence of cryptic plasmids in *Myxococcus* strains (N. L. Brown and J. H. Parish, unpublished). However, the instability of the chloramphenicol resistance is suggestive of a plasmid. The R⁺ *Myxococcus* strains do not transfer resistance to R⁻ strains of *E. coli* under the conditions of incubation used here.

The data imply that there is a difference between the abilities of *Myxococcus* strains productively to assimilate extrachromosomal DNA from other bacteria. It is possible that strains FB₁ and A are either restrictive or have different conditions of competence. Strains B and M were used in attempts to transfer a chromosomal marker (*rif*) in *Myxococcus* (Table 7). These results provide preliminary evidence that genetic exchange is possible. The genetic element m7 *chl* is potentially valuable for further studies on gene transfer.

The transfer of genes (including m7 *chl*) between strains B and M and the fact that strain M acts as host to *M. xanthus* phage MX1 suggests that these two strains are closely related and raises the question of the taxonomic status of the strain M. For the present, I propose that the designation *M. fulvus* for strain M be tentatively retained.

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