NOTES

Conjugal Transfer of an R-Plasmid in *Pasteurella multocida*

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A strain of *Pasteurella multocida* isolated from turkeys during an outbreak of septicemic disease (fowl cholera) was shown to possess the ability to transfer streptomycin and sulphadiazine resistance to *P. multocida* and to *Escherichia coli* by conjugation. The genes necessary for the transfer of the resistance genes appeared to be associated with a plasmid of molecular weight 28.5×10^6 . The resistance genes were shown to be associated with a second plasmid of molecular weight 7.2×10^6 .

Resistance plasmids in animal pathogens belonging to the genus *Pasteurella* (*P. multocida* and *P. haemolytica*) have been shown to be of small molecular weight (2×10^6 to 7×10^6) and nontransmissible (2, 9, 12).

After our initial isolation of an R-plasmid-bearing strain of *P. multocida* from turkeys (2), we undertook a study to determine the plasmid content of *P. multocida* isolated from turkeys throughout the years 1940 to 1975. Of the 75 strains examined, all but 3 possessed plasmids. Of the strains possessing plasmids, all but one, P1552 (isolated from a dead turkey in 1967 in South Carolina), contained a single plasmid of low molecular weight (less than 10×10^6). Strain P1552 contained, in addition to a 7.2×10^6 -dalton plasmid, another of 28.5×10^6 daltons. In this paper, we report the finding that the higher-molecular-weight plasmid may be associated with the conjugal transfer of resistance genes from P1552 to *P. multocida* and to *Escherichia coli*.

P. multocida P1552 was shown by the disk diffusion assay (1) to be susceptible to ampicillin, penicillin, tetracycline, kanamycin, gentamicin, cephalothin, and chloramphenicol. The strain was resistant to streptomycin and sulphadiazine. The minimal inhibitory concentrations (2, 11) of these antimicrobial agents are shown in Table 1.

Agarose gel electrophoresis of covalently closed circular deoxyribonucleic acid obtained from *P. multocida* P1552 by cesium chloride-ethidium bromide gradient centrifugation (4, 5, 7) is shown (Fig. 1, lane A). Two plasmid bands are seen, one of molecular weight 28.5×10^6

(henceforth termed pVM200) and the other of molecular weight 7.2×10^6 (pVM107).

Transformation (3) of *E. coli* C600-1 (nalidixic acid-resistant mutant of strain C600, *lac thr leu thi*) with plasmid deoxyribonucleic acid obtained from *P. multocida* P1552 resulted in transformants resistant to streptomycin and sulphadiazine (Table 1). Agarose gel electrophoresis of deoxyribonucleic acid obtained from these transformants demonstrated that the resistance determinants resided on the 7.2×10^6 -dalton plasmid (pVM107, Fig. 1, lane C). Transformants containing the 28.5×10^6 -dalton plasmid (pVM200) were not found.

Three-hour filter mating (10) of *P. multocida* P1552 with *P. multocida* P1315-1 (nalidixic acid-resistant mutant of a susceptible plasmidless strain of *P. multocida* P1315) resulted in the passage of streptomycin and sulphadiazine resistance, and pVM107, to P1315-1 at a frequency of 6.7×10^{-4} transconjugant per donor cell (Table 1; Fig. 1, lane B). When *P. multocida* P1552 was crossed with *E. coli* C600-1 (using MacConkey agar plus streptomycin to select for transconjugants [*P. multocida* does not grow on MacConkey agar]), streptomycin and sulphadiazine resistance, and pVM107, was transferred at a frequency of 5.9×10^{-3} transconjugant per donor cell (Table 1; Fig. 1, lane D). Attempts to pass pVM107 from *P. multocida* P1315-1 to *E. coli* C600-1 by filter mating were not successful. Transfer was not curtailed in the presence of 2 μ g of deoxyribonuclease per ml, nor was transfer accomplished in the presence of donor suspensions passed through a 0.45- μ m filter.

TABLE 1. Susceptibility to antimicrobial agents

Isolate	Minimal inhibitory concn ($\mu\text{g/ml}$)	
	Streptomycin	Sulphadiazine
<i>P. multocida</i> P1552	>128	>2,048
<i>P. multocida</i> P1315-1 (pVM107)	128	>2,048
<i>P. multocida</i> P1315-1	8	64
<i>E. coli</i> C600-1 (pVM107) transformant	>128	>2,048
<i>E. coli</i> C600-1 (pVM107) transconjugant	>128	>2,048
<i>E. coli</i> C600-1	16	16

The passage of pVM200 from P1552 to P1315-1 was shown to occur when a triparental, replica mating technique was used (6, 8). *P. multocida* P1552 was filter mated to P1315-1 for 3 h. After the 3 h, the filter was shaken vigorously in nutrient broth. Samples of the broth were inoculated onto blood agar plates containing streptomycin (25 $\mu\text{g/ml}$) and nalidixic acid (50 $\mu\text{g/ml}$). After incubation for 18 h at 37°C, the transconjugants were velvet replicated to a nutrient agar plate freshly inoculated with *E. coli* C600. After a 24-h incubation at 37°C, the surface of the nutrient agar plate was velvet replicated to minimal agar with salts and glucose and containing thiamine, leucine, threonine, and streptomycin (*P. multocida* does not grow on minimal medium containing glucose, thiamine, leucine, and threonine). Crosses performed in this manner showed that pVM200 was transferred along with pVM107 approximately 3% of the time. Colonies of P1315-1 possessing the ability to transfer pVM107 to *E. coli* C600 were shown by agarose gel electrophoresis to possess pVM200 (Fig. 1, lane E). Attempts to demonstrate passage of pVM200 from the genus *Pasteurella* to *E. coli* were unsuccessful by the triparental cross technique. Passage of pVM200 without pVM107 to the genus *Pasteurella* or *E. coli* was never detected.

We have demonstrated that a strain of *P. multocida* was able to transfer, by conjugation, antimicrobial resistance determinants to susceptible strains of *P. multocida* and *E. coli*. This finding adds another dimension to the epidemiology of resistance plasmids in the genus *Pasteurella*. Horizontal spread of resistance genes is now shown to be possible and, along with environmental selective pressures exerted by antimicrobial agents, would allow for the efficient spread of resistance determinants throughout the genus *Pasteurella*.

That pVM200 is involved with the transfer of pVM107 there is no doubt. At this point, its

FIG. 1. Agarose gel electrophoresis of plasmid deoxyribonucleic acid. (A) *P. multocida* P1552; (B) *P. multocida* P1315-1 (pVM107); (C) *E. coli* C600-1 (pVM107) transformant; (D) *E. coli* C600-1 (pVM107) transconjugant; (E) *P. multocida* P1315-1 (pVM200, pVM107) transconjugant. The position of the molecular weight (MW) markers was determined by electrophoresis (not shown) of RP4 (34 megadaltons [MDal]), Sa (23 MDal), RSF1010 (5.5 MDal), and pMB8 (3.6, 2.6, and 1.8 MDal). CHR, chromosome.

exact role in this transfer is unclear. Whether pVM200 possesses the structural genes for transfer or whether it possesses regulatory genes for some chromosomal genes is unknown. The fact that pVM200 seems to only promote the transfer of pVM107 when in the genus *Pasteurella* would suggest that the genes are regulatory in nature. Another possibility is that pVM200 is a composite plasmid composed of the genes responsible for transfer and pVM107. When in *E. coli*, pVM200 might become unstable, dissociating into transfer genes and pVM107, the transfer genes being lost. When in the genus *Pasteurella*, pVM200 may be more stable and only a portion dissociating into transfer genes (subsequently lost) and pVM107.

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