

Transformation of *Pasteurella novicida*

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Deoxyribonucleic acid from a streptomycin-resistant mutant of *Pasteurella novicida* transformed portions of *P. novicida* streptomycin-sensitive populations to streptomycin-resistant. Similarly, mutants auxotrophic for tryptophan or purine biosynthesis were also transformed to nutritional independence.

Genetic exchange by transformation has been demonstrated for a variety of bacteria, but such a process has not been reported for any members of the genus *Pasteurella*. This report describes gene transfer in *Pasteurella novicida* employing "plate transformation" (7).

The wild-type strain *P. novicida* U112 was obtained from LTC John Marshall, USAMRIID. A spontaneous streptomycin-resistant mutant (NM-1, Sm^r) was obtained from GCBA (5) plates containing 1 mg/ml streptomycin sulfate. Mutants auxotrophic for tryptophan (NM-15, Trp⁻) and purine (NM-38, Pur⁻) were obtained after exposure of wild-type cells to *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine (NTG), employing modifications of the method of Altenbern (1). After treatment of cells with 100 µg/ml NTG, mutants were selected for growth on the defined medium of Chamberlain (4) supplemented with additional amino acids, purines, and pyrimidines for which nutritional dependence was desired, and on unsupplemented defined medium. All cultures were maintained on GCBA slants.

DNA was extracted from *P. novicida* by the method of Marmur (9). DNA concentrations were determined by the method of Burton (3).

Transformations of *P. novicida* from Sm^s to Sm^r were performed essentially as described by Bøvre for *Moraxella* (2). Recipient cells were grown overnight at 37 C on a GCBA plate. The growth was removed with 2 ml of gel-saline (6), diluted to approx 1×10^{10} cells/ml, and 0.1 ml of the cell suspension was mixed with 0.1 ml of NM-1 DNA on GCBA plates (25 ml of medium per plate). The plates were incubated at 37 C and, at hourly intervals, the agar from each plate was transferred to a large petri dish containing 50 ml of GCBA + 1.5 mg/ml streptomycin sulfate. Incubation was continued at 37 C and Sm^r transformants were scored after 48 hr. Control plates contained cells alone, cells + NM-1 DNA + deoxyribonuclease (100 µg), or cells + wild-type DNA.

Transformations of auxotrophic mutants were performed by spreading 0.1 ml of recipient cells, prepared as described above, plus wild-type or NM-1 DNA on Chamberlain's agar plates.

Table 1 shows that when Sm^s cells were incubated in the presence of NM-1 DNA, streptomycin-resistant colonies were produced. That these colonies resulted from DNA-mediated transformation was indicated by the following: (i) very few colonies were produced in the absence of NM-1 DNA; (ii) deoxyribonuclease obliterated the production of virtually all Sm^r colonies; and (iii) only a few spontaneous revertants appeared when the Sm^s recipients were plated in the presence of wild-type DNA.

Further evidence for DNA-mediated marker transfer is presented in Table 2. Transformants for Trp⁺ or Pur⁺ were produced only in the presence of wild-type DNA, and the number of transformants obtained was dependent on the amount of wild-type DNA used.

Because of the inherent limitations of the "plate transformation" (7) technique, we have been unable thus far to obtain quantitative data

TABLE 1. Transformation of *Pasteurella novicida* wild-type on agar plates

Expt no.	Cells per plate	DNA		Deoxy-ribonuclease (µg/plate)	Sm ^r colonies per plate ^a
		Source	µg/plate		
1	1.0×10^9	NM-1	108	0	>1,000
			0	0	11
2	6.5×10^8	NM-1	108	100	2
			0	0	3
3	2.6×10^8	NM-1	108	0	300
			108	0	175
			0	0	2
			wild-type	62	0

^a For each experiment, the cells and DNA were incubated on GCBA plates for 3 hr at 37 C before contact with streptomycin.

TABLE 2. Transformation of *Pasteurella novicida* auxotrophic mutants^a

Expt no.	Recipient strain	Cells per plate	DNA		Deoxyribonuclease (μg/plate)	Transformants per plate
			Source	μg/plate		
1	NM-15	8.4 × 10 ⁸	NM-1	108	0	Trp ⁺
				108	100	>1,000
2	NM-15	1.4 × 10 ⁹	wild-type	62	0	0
				31	0	>1,000
				62	100	>1,000
			NM-15	30	0	0
				NM-1	10.8	0
				1.1	0	153
				0.1	0	17
	0.01	0	5			
3	NM-38	6.0 × 10 ⁷	NM-1	10.8	0	Pur ⁺
				1.1	0	>1,000
				10.8	100	>1,000
						0

^a Control plates without DNA produced no colonies.

for conditions of competence, uptake of DNA, and phenotypic expression (Sm^r). Experiments designed to obtain such data by transforming cells in a liquid medium are currently in progress.

P. novicida is closely related to *Pasteurella tularensis* by gross appearance of cultures, microscopic appearance, pathogenicity (8), and DNA hybridizations (11). However, differences in serological reactions and metabolism support a separate species designation (8, 10). We anticipate that future studies on transformation between *P. tularensis* and *P. novicida* will aid in their taxonomic placement.

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LITERATURE CITED

- Altenbern, R. A. 1966. Apparent genomic mapping of *Staphylococcus aureus* by a new method. *Biochem. Biophys. Res. Commun.* 25:346-353.
- Bövre, K. 1964. Studies on transformation in *Moraxella*. I. A method for quantitative transformation in *Moraxella* and *Neisseria*, with streptomycin resistance as the genetic marker. *Acta Pathol. Microbiol. Scand.* 61:457-473.
- Burton, K. 1956. A study of the conditions and mechanism of the diphenylamine reaction for colorimetric estimation of deoxyribonucleic acid. *Biochem. J.* 62:315-322.
- Chamberlain, R. E. 1965. Evaluation of live tularemia vaccine prepared in a chemically defined medium. *Appl. Microbiol.* 13:232-235.
- Downs, C. M., L. L. Coriell, S. S. Chapman, and A. Klauber. 1947. The cultivation of *Bacterium tularensis* in embryonated eggs. *J. Bacteriol.* 53:89-100.
- Eigelsbach, H. T., and C. M. Downs. 1961. Prophylactic effectiveness of live and killed tularemia vaccines. I. Production of vaccine and evaluation in the white mouse and guinea pig. *J. Immunol.* 87:415-425.
- Gwinn, D. D., and C. B. Thorne. 1964. Transformation of *Bacillus licheniformis*. *J. Bacteriol.* 87:519-526.
- Larson, C. L., W. Wicht, and W. L. Jellison. 1955. A new organism resembling *P. tularensis* isolated from water. *Pub. Health Rep.* 70:253-257.
- Marmur, J. 1961. A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J. Mol. Biol.* 3:208-218.
- Owen, C. R., E. O. Buker, W. L. Jellison, D. B. Lackman, and J. F. Bell. 1964. Comparative studies of *Francisella tularensis* and *Francisella novicida*. *J. Bacteriol.* 87:676-683.
- Ritter, D. B., and R. K. Gerloff. 1966. Deoxyribonucleic acid hybridization among some species of the genus *Pasteurella*. *J. Bacteriol.* 92:1838-1839.