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TECHNICAL MANUSCRIPT 549

TRANSFORMATION OF PASTEURELLA NOVICIDA

Franklin J. Tyeryar, Jr.  
William D. Lawton

AUGUST 1969

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DEPARTMENT OF THE ARMY  
Fort Detrick  
Frederick, Maryland

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DEPARTMENT OF THE ARMY  
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TECHNICAL MANUSCRIPT 549

TRANSFORMATION OF PASTEURELLA NOVICIDA

Franklin J. Tyeryar, Jr.

William D. Lawton

Medical Bacteriology Division  
BIOLOGICAL SCIENCES LABORATORY

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#### ABSTRACT

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

TRANSFORMATION OF PASTEURELLA NOVICIDA\*

Genetic exchange by transformation has been demonstrated for a number 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."<sup>1</sup>

The wild-type strain P. novicida U112 was obtained from LTC John Marshall, U.S. Army Medical Research Institute of Infectious Diseases. A spontaneous streptomycin-resistant mutant (NM-1, Sm<sup>r</sup>) was obtained from glucose cysteine blood agar (GCBA)<sup>2</sup> plates containing 1 mg streptomycin sulfate per ml. 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.<sup>3</sup> After treatment of cells with 100 µg NTG/ml, mutants were selected for growth on the defined medium of Chamberlain<sup>4</sup> 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.<sup>5</sup> DNA concentrations were determined by the method of Burton.<sup>6</sup>

Transformations of P. novicida from Sm<sup>s</sup> to Sm<sup>r</sup> were performed essentially as described by Bøvre for Moraxella.<sup>7</sup> Recipient cells were grown overnight at 37 C on a GCBA plate. The growth was removed with 2 ml of gel-saline<sup>8</sup> and diluted to approximately  $1 \times 10^{10}$  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 streptomycin sulfate per ml. Incubation was continued at 37 C and Sm<sup>r</sup> transformants were scored after 48 hours. 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<sup>s</sup> 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<sup>r</sup> colonies; and (iii) only a few spontaneous revertants appeared when the Sm<sup>r</sup> recipients were plated in the presence of wild-type DNA.

\* This report should not be used as a literature citation in material to be published in the open literature. Readers interested in referencing the information contained herein should contact the senior author to ascertain when and where it may appear in citable form.

TABLE 1. TRANSFORMATION OF WILD-TYPE PASTEURELLA NOVICIDA  
ON AGAR PLATES

| Exp. No. | Cells per Plate       | DNA       |          | Deoxyribo-<br>nuclease,<br>μg/plate | Sm <sup>r</sup> Colonies<br>per Plate <sup>a</sup> / |
|----------|-----------------------|-----------|----------|-------------------------------------|------------------------------------------------------|
|          |                       | Source    | μg/Plate |                                     |                                                      |
| 1        | 1.0 x 10 <sup>9</sup> | NM-1      | 108      | 0                                   | >1,000                                               |
|          |                       |           | 0        | 0                                   | 11                                                   |
| 2        | 6.5 x 10 <sup>8</sup> | NM-1      | 108      | 100                                 | 2                                                    |
|          |                       |           | 0        | 0                                   | 3                                                    |
|          |                       |           | 108      | 0                                   | 300                                                  |
| 3        | 2.6 x 10 <sup>8</sup> | NM-1      | 108      | 0                                   | 175                                                  |
|          |                       |           | 0        | 0                                   | 2                                                    |
|          |                       | Wild-type | 62       | 0                                   | 1                                                    |

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

Further evidence for DNA-mediated marker transfer is presented in Table 2. Transformants for Trp<sup>+</sup> or Pur<sup>+</sup> 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"<sup>11</sup> technique, we have been unable thus far to obtain quantitative data for conditions of competence, uptake of DNA, and phenotypic expression (Sm<sup>r</sup>). 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,<sup>9</sup> and DNA hybridizations.<sup>10</sup> However, differences in serological reactions and metabolism support a separate species designation.<sup>9,11</sup> We anticipate that future studies on transformation between P. tularensis and P. novicida will aid in their taxonomic placement.

TABLE 2. TRANSFORMATION OF PASTEURELLA NOVICIDA AUXOTROPHIC MUTANTS<sup>a/</sup>

| Exp. No. | Recipient Strain | Cells per Plate   | Source    | DNA                        |                            | Deoxyribo-nuclease, $\mu\text{g}/\text{plate}$ | Transformants per Plate    |
|----------|------------------|-------------------|-----------|----------------------------|----------------------------|------------------------------------------------|----------------------------|
|          |                  |                   |           | $\mu\text{g}/\text{Plate}$ | $\mu\text{g}/\text{Plate}$ |                                                |                            |
| 1        | NM-15            | $8.4 \times 10^8$ | NM-1      | 108                        | 0                          |                                                | Trp <sup>+</sup><br>>1,000 |
|          |                  |                   |           | 108                        | 100                        |                                                | 0                          |
|          |                  |                   |           | 62                         | 0                          |                                                | >1,000                     |
|          |                  |                   |           | 31                         | 0                          |                                                | >1,000                     |
| 2        | NM-15            | $1.4 \times 10^9$ | Wild-type | 62                         | 100                        |                                                | 0                          |
|          |                  |                   |           | 30                         | 0                          |                                                | 0                          |
|          |                  |                   |           | 10.8                       | 0                          |                                                | >1,000 to <2,000           |
|          |                  |                   |           | 1.1                        | 0                          |                                                | 153                        |
| 3        | NM-38            | $6.0 \times 10^7$ | NM-1      | 0.1                        | 0                          |                                                | 17                         |
|          |                  |                   |           | 0.01                       | 0                          |                                                | 5                          |
|          |                  |                   |           |                            |                            | Pur <sup>+</sup>                               |                            |
|          |                  |                   |           | 10.8                       | 0                          |                                                | >1,000                     |
|          |                  |                   |           | 1.1                        | 0                          |                                                | >1,000                     |
|          |                  |                   |           | 10.8                       | 100                        |                                                | 0                          |

a. Control plates without DNA produced no colonies.

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| 14. Key Words<br><br><u>Pasteurella novicida</u><br>Gene transfer<br>Transformation<br>Deoxyribonucleic acid<br>Streptomycin resistance<br>Auxotrophic mutants                                                                                                                                                                              |                                                                             |                                                                                                        |

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