NEW LIMITATION CHANGE

TO
Approved for public release, distribution unlimited

FROM
Distribution authorized to U.S. Gov’t. agencies and their contractors; Administrative/Operational Use; AUG 1969. Other requests shall be referred to Department of the Army, Fort Detrick, MD.

AUTHORITY
BDRL ltr, 29 Sep 1971
TECHNICAL MANUSCRIPT 549

TRANSFORMATION OF PASTEURELLA NOVICIDA

Franklin J. Tyeryar, Jr.
William D. Lawton

AUGUST 1969

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland
Reproduction of this publication in whole or in part is prohibited except with permission of the Commanding Officer, Fort Detrick, ATTN: Technical Releases Branch, Technical Information Division, Fort Detrick, Frederick, Maryland, 21701. However, DDC is authorized to reproduce the publication for United States Government purposes.

DDC AVAILABILITY NOTICES

Qualified requesters may obtain copies of this publication from DDC.

Foreign announcement and dissemination of this publication by DDC is not authorized.

Release or announcement to the public is not authorized.

DISPOSITION INSTRUCTIONS

Destroy this publication when it is no longer needed. Do not return it to the originator.

The findings in this publication are not to be construed as an official Department of the Army position, unless so designated by other authorized documents.
TRANSFORMATION OF PASTEURELLA NOVICIDA

Franklin J. Tyeryar, Jr.
William D. Lawton

Medical Bacteriology Division
BIOLOGICAL SCIENCES LABORATORY

Project 1B061102871A
August 1969
ACKNOWLEDGMENT

We thank John D. Boyer for excellent technical assistance.

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." 1

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, SmR) was obtained from glucose cysteine blood agar (GCBA) 2 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. 3 After treatment of cells with 100 μg NTG/ml, 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. 5 DNA concentrations were determined by the method of Burton. 6

Transformations of P. novicida from SmR to SmS were performed essentially as described by Bövre for Moraxella. 7 Recipient cells were grown overnight at 37°C on a GCBA plate. The growth was removed with 2 ml of gel-saline 8 and diluted to approximately 1 x 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 SmS 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 SmR 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 SmR colonies; and (iii) only a few spontaneous revertants appeared when the SmR 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

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>Cells per Plate</th>
<th>DNA Source</th>
<th>DNA µg/Plate</th>
<th>Deoxyribo-nuclease, µg/plate</th>
<th>Sm&lt;sup&gt;+&lt;/sup&gt; Colonies per Plate&lt;sup&gt;2/&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.0 x 10&lt;sup&gt;9&lt;/sup&gt;</td>
<td>NM-1</td>
<td>108</td>
<td>0</td>
<td>&gt;1,000</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>2</td>
<td>6.5 x 10&lt;sup&gt;8&lt;/sup&gt;</td>
<td>NM-1</td>
<td>108</td>
<td>100</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>108</td>
<td>300</td>
</tr>
<tr>
<td>3</td>
<td>2.6 x 10&lt;sup&gt;8&lt;/sup&gt;</td>
<td>NM-1</td>
<td>108</td>
<td>0</td>
<td>175</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Wild-type</td>
<td>62</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

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" technique, we have been unable thus far to obtain quantitative data for conditions of competence, uptake of DNA, and phenotypic expression (Sm<sup>+</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, and DNA hybridizations. However, differences in serological reactions and metabolism support a separate species designation. 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\(^a\)/

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>Recipient Strain</th>
<th>Cells per Plate</th>
<th>DNA Source</th>
<th>DNA μg/Plate</th>
<th>Deoxyribonuclease, μg/plate</th>
<th>Transformants per Plate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NM-15</td>
<td>(8.4 \times 10^8)</td>
<td>NM-1</td>
<td>108</td>
<td>0</td>
<td>&gt;1,000</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>108</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>NM-15</td>
<td>(1.4 \times 10^9)</td>
<td>Wild-type</td>
<td>62</td>
<td>0</td>
<td>&gt;1,000</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>31</td>
<td>0</td>
<td>&gt;1,000</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>62</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>NM-15</td>
<td></td>
<td></td>
<td>30</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>NM-1</td>
<td>10.8</td>
<td>0</td>
<td>1.1</td>
<td>0</td>
<td>153</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.1</td>
<td>0</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.01</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>NM-38</td>
<td>(6.0 \times 10^7)</td>
<td>NM-1</td>
<td>10.8</td>
<td>0</td>
<td>&gt;1,000</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.1</td>
<td>0</td>
<td>&gt;1,000</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>10.8</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

\(\text{Trp}^+\)

\(\text{Pur}^+\)

\(a\). Control plates without DNA produced no colonies.
LITERATURE CITED


TRANSFORMATION OF PASTEURELLA NOVICIDA

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.

Key Words

Pasteurella novicida
Gene transfer
Transformation
Deoxyribonucleic acid
Streptomycin resistance
Auxotrophic mutants