"Mechanisms of Recombination and Function of DNA in Bacteria"

PROGRESS REPORT

MASTER

Walter R. Guild
Principal Investigator
Biochemistry Department
Duke University Medical Center
Durham, North Carolina 27710

Period from August 12, 1978 to August 15, 1979

Prepared for The Department of Energy under Contract No. DE-AS05-76EV03941

Contents

Progress in current period	p. 2
Foreign travel	p. 4
Compliance	p. 4
Publications	p. 5
Attachments	p. 5

DISTRIBUTION OF THIS DOCUMENT IS UNLIMITED

SLA

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency Thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

DISCLAIMER

Portions of this document may be illegible in electronic image products. Images are produced from the best available original document.

Progress in Current Period

Most work in the current period has centered on the organization and transfer of two classes of drug-resistance determinants in pneumococcus, a topic that we had just begun at the time of the last Progress Report. The two classes are (a) insertions of foreign DNA in the chromosome and (b) plasmid carried genes. There have been several major findings, as follows:

1. Determinants for chloramphenicol and tetracycline resistance in some clinical isolates of pneumococcus are present as adjacent insertions of heterologous DNA into the normal chromosome. They are not carried on plasmids as are similar determinants in many species. Most studies so far have been on two strains, one from Paris and one from Japan, but work is now under way on analysis of other strains, including some from South Africa. (See DOE/EV/03941-41, attached, for summary).

2. Pneumococcus will support the growth of resistance plasmids from other streptococci, and these can be transformed into pneumococcus from lysates of S. faecalis (group D) or group B streptococcus. The quantitative aspects of the plasmid transformation process are being examined (by graduate student Charles Saunders) along the same general lines as our prior studies of transfection by phage DNA (Porter and Guild, 1978).

3. One of the plasmids (pIP501) that has been transformed into pneumococcus mediates a form of DNase-resistant conjugal transfer among other streptococcal groups (Herhshfield 1979) and we find that it also does so in pneumococcus. The 20 Mdal pIP501 mediates its own transfer and also mobilizes pMV158, a 3.6 Mdal plasmid from a group B streptococcus that carries tetracycline

resistance but cannot mobilize its own transfer, although it transforms pneumococcus well.

4. The insertions of cat and tet genes in the chromosomes of two clinical isolates and in a number of our laboratory derivatives also transfer by a DNase-resistant conjugation-like process to other pneumococcal recipients. Numerous controls show that this transfer is not due to transformation. Further, these determinants (cat tet or tet alone, but not cat alone) from the chromosome of the donor reintegrate into the chromosome of the recipient transconjugant. We do not yet know whether they have integrated at the same locus as they were in the donor or whether they ever carry along part of the normal chromosome. Transconjugants can act as donors in further conjugation experiments.

Thus, what was stated in last year's proposal as "not impossible, though perhaps unlikely" has been found to occur. Conjugation has been demonstrated in pneumococcus not only for plasmids (for which it might have been predicted) but also for chromosomal segments of an unusual class. These results open entirely new fields of study on the genetic mechanisms that operate in pneumococcus, for which only a few years ago transformation was the only known gene transfer system. We have much to do to examine further the basic phenomenology of these systems, how they relate to those we already know about, what they can tell us as tools for studies of molecular mechanisms, and how they have or will influence the further transmission of genes among pneumococcal strains and among streptococci in general.

All of this work is being done with the background and in the context of our ongoing studies on transformation and phage in this species, and this work is continuing as appropriate and as time permits. We need more personnel and are looking for a highly qualified post-doctoral associate to take on

part of these studies. This need is re-emphasized by the fact that we are about to lose Nadja Shoemaker, who has been a major contributor for the past ten years. She will move in late September, 1979, to a research position in John Drake's division at the National Institute of Environmental Health Sciences, to work on basic aspects of mutagenesis and how it is influenced by environmental agents. Her background and experience in our laboratory will be used to excellent advantage in this post and it is a major promotion for her that she could not have obtained in a university setting without a Ph.D. We are happy for her, therefore, but at the same time regret losing her input to this laboratory.

Foreign travel: The P.I. went to France from May 26 to June 3, 1979, to attend a CNRS Molecular Biology Conference at Orsay and to visit laboratories and consult with scientists at several institutions. He presented seminars on the current work at Toulouse University on May 30 (Dr. A.M. Sicard's group) and at the Pasteur Institute (Department of Medical Bacteriology, Professor Chabbert) on June 1. All expenses were paid by the P.I. from personal funds.

Compliance: The work performed is in close relation to that proposed. The P.I. has devoted in excess of 20% of his time and effort to this work and about 50% this summer.

Publications.

- (1) Porter, R.D., N.B. Shoemaker, G. Rampe, and W.R. Guild (1979). Bacteriophage-associated gene transfer in pneumococcus: Transduction or pseudotransduction? J. Bacteriol. 137, 556-567. [ORO-3941-35].
- (2) Smith, M.D., and W.R. Guild (1979). A plasmid in Streptococcus pneumoniae. J. Bacteriol. 137, 735-739. [ORO-3941-37].
- (3) Shoemaker, N.B., M.D. Smith, and W.R. Guild (1979a). Organization and transfer of heterologous chloramphenicol and tetracycline resistance genes in pneumococcus. J. Bacteriol. 139 (⁴³²⁻⁴⁴¹ ~~August issue, 1979~~). [ORO-3941-40, a totally revised manuscript that replaces ORO-3941-36].
- (4) Smith, M.D., N.B. Shoemaker, and W.R. Guild (1979). Chromosomal location of plasmid-type resistance genes in Streptococcus pneumoniae. in Stutard and Rozee (Ed.), "Plasmids" (Proc. of the 4th Annual Symp. on the Scientific Basis of Medicine" Dalhousie Univ.) Academic Press N.Y. (in press). [DOE/EV/03941-41].
- (5) Smith, M.D., N.B. Shoemaker, V. Hershfield, and W.R. Guild (1979). Conjugal transfer of resistance plasmids in Streptococcus pneumoniae. (to be submitted to Plasmid, 1979). [DOE/EV/03941-42].
- (6) Shoemaker, N.B., M.D. Smith, and W.R. Guild (1979b). Conjugal transfer of chromosomal cat and tet determinants in Streptococcus pneumoniae. (to be submitted to Plasmid, 1979). [DOE/EV/03941-43].

Attachments:

Reprints of items (1) and (2) above and a preprint of item (4) are attached.
 Reprints of item (3) and preprints of items (5) and (6) will be sent as soon as available.

Reprints + Preprints Removed -