# METHODS IN MICROBIOLOGY

## VOLUME 7B

Edited by
J.R. Norris and D.W. Ribbons

# METHODS in MICROBIOLOGY

*Edited by*

**J. R. NORRIS**
*Borden Microbiological Laboratory,*
*Shell Research Limited,*
*Sittingbourne, Kent, England*

**D. W. RIBBONS**
*Department of Biochemistry,*
*University of Miami School of Medicine,*
*and Howard Hughes Medical Institute,*
*Miami, Florida, U.S.A.*

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# PREFACE

It was inevitable with a Series as large and wide ranging as "Methods in Microbiology" that some topics would fall outside the themes on which the majority of the Volumes were based, and that some contributions intended for inclusion in earlier Volumes would be unavailable at the time they were required. To a certain extent therefore, Volume 7 is a miscellany of disconnected topics.

As with other Volumes, the material has been divided into two parts; a step necessitated primarily by the amount of material presented, but which enabled us to group together related contributions. Thus, Volume 7A contains Chapters dealing with the use of computers in microbiology and a treatment of the mathematical bases of assay methods. Two Chapters concern bacteriophage and one bacteriocins. The rest of the material comprises topics which are of considerable interest and importance but whose themes are unrelated to one another.

Volume 7 completes the initial Series of "Methods in Microbiology" and this preface affords us a welcome opportunity to express our thanks and appreciation to our many contributors whose ready help, co-operation and patience has done so much to make our task of editing an enjoyable one. We are also grateful to numerous of our colleagues who, although not themselves contributing to the Series, have provided valuable advice and comment concerning the subject matter. Our thanks are due to Shell Research Limited, The University of Miami and The Howard Hughes Medical Institute, without whose material assistance in many ways the production of the Series would have been far more difficult, if not impossible. Finally, we would like to acknowledge the assistance of the publishers and that faithful army of typists, secretaries, technicians, research students and sympathetic wives and husbands whose painstaking work and attention to detail earns them little recognition but provides the essential basis for a work of this kind. We would particularly like to mention our appreciation of the co-operation of Dr. C. Booth who edited Volume 4 and enabled the Series to cover techniques in Mycology; an area which was outside our own experience.

The question of continuing the Series comes to the fore at this time. Several contributions have been offered for a further Volume and we have decided to produce one more, a single Volume 8 which should appear early in 1973 following a manuscript date of May 1972. After that we have no plans but we will reconsider the situation from time to time as and when advances in techniques and methodology suggest that the production of a

further Volume will be useful. Of course, not all possible topics have been covered and needless to say we would welcome comments and suggestions for future articles from our colleagues in the field of microbiology.

J. R. NORRIS

D. W. RIBBONS

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CHAPTER I

# Phage-Typing of *Staphylococcus aureus*

M. T. PARKER
*Central Public Health Laboratory, London, England*



## I. INTRODUCTION

Between a quarter and a half of the human population are carriers of *Staphylococcus aureus*. Little progress could therefore be made in studying the sources of infection with this organism, and the routes by which it is spread, until a way had been found of distinguishing between different strains. The absence of a suitable method for the serological typing of

*S. aureus* led medical bacteriologists to develop phage-typing as a means of investigating staphylococcal disease in man. Phage-typing has now been used for this purpose for over 25 years, and the method has been continuously modified in response to changes in the epidemiological situation.

At first phage-typing was used mainly in short-term investigations of limited outbreaks of sepsis or enterotoxic food-poisoning. Fortunately, the workers concerned with developing the method realized the importance of standardizing the typing procedure, and within a short time it became obvious that strains with similar phage-typing patterns were becoming prevalent in hospitals in widely separated parts of the world. Phage-typing thus became an indispensable means of investigating the evolution of endemic strains of *S. aureus* in hospital populations.

Widespread interest in staphylococcus phage-typing developed later among veterinary bacteriologists, and it became apparent that the current method of typing is unsuitable for studying staphylococcal disease in several domestic animals. This, in its turn, has stimulated interest in taxonomic differences between "human" and "animal" staphylococci.

## II. DEVELOPMENT OF THE METHOD

### A. Principle

Cultures of *S. aureus* are classified according to their susceptibility to a set of phages chosen to make as many epidemiologically valid distinctions as possible between strains. It is therefore a method of bacterial classification based on a single class of characters. The one thing it can never do, therefore, is to show that two organisms are "the same". What it can establish, with varying degrees of certainty, is that they are "different". Its use in field investigations is to narrow down the field of enquiry by the exclusion of alternative sources of infection. In this it is most effective when the number of cultures to be considered is limited.

### B. Early typing systems

Fisk (1942a, b) found that temperate phages from *S. aureus* cultures often lysed other members of the same species and that many of them had a relatively narrow host-range. He showed that susceptibility to such phages could be used as the basis of a typing system.

Wilson and Atkinson (1945) obtained a different series of temperate phages from *S. aureus* cultures, made high-titre preparations of them by propagation in broth, and separated them from the bacteria by filtration. To keep the host-range of a phage constant, it was always propagated on the same strain of staphylococcus (the propagating strain). The strength of each phage preparation was measured in terms of the routine test dilution

(RTD)—the highest dilution of a filtrate that produced confluent lysis when a standard loopful was placed on a lawn of the propagating strain. Attempts were also made to adapt phages to cultures on which no plaques were seen at RTD by applying undiluted phage suspensions to them. Some true adaptations resulted, but subsequent serological examination suggest that other phages obtained in this way were unrelated to the original phage.

With 18 phages at RTD, over 80% of cultures from human sources showed some lysis, and 60% could be allotted to one of 21 types or subtypes; but to do this all reactions of less than confluent lysis had to be ignored.

### C. Phage-typing patterns

Williams and Rippon (1952) used Wilson and Atkinson's 18 phages with six others. They confirmed the value of the method, but their conclusions led to substantial changes in the way in which the typing system was to be used. They defined the RTD as the highest dilution of a phage that gave just less than confluent lysis when a 0·02 ml drop was placed on a lawn of the propagating strain, and a strong (+ +) reaction as one of 50 plaques or more. Three-quarters of the cultures examined gave a + + reaction with one or more phages, but when groups of strains from one source were compared there was often a variation in the degree of lysis produced by individual phages. The strict application of a type designation based only upon strong reactions (whether confluent lysis or a + + reaction) would therefore have resulted in related strains being placed in different types. Weak reactions would therefore have to be taken into account, and a limited number of clear cut types could not be defined. Strains could however be characterized by the pattern of their lysis by the phages, if an estimate could be made of the frequency with which epidemiologically related strains showed a variation in typing pattern of a given magnitude. This proved possible (Section IV.A), and it was then found that the comparison of phage-typing patterns was a good guide to the relationships between strains. The number of possible patterns was large.

### D. Use of strong phage preparations

To increase the proportion of typable cultures beyond three-quarters, Williams and Rippon (1952) re-tested with undiluted phages those cultures which gave no strong reactions at RTD. It later became the practice to use a strength of 1000 times the RTD (RTD × 1000) to type strains untypable at RTD. Typable cultures then generally exceed 90%, but the reading of reactions obtained with phages at RTD × 1000 presents difficulties (Sections III.D, 2 and IV.C). For this reason the strength of phage for secondary typing was later reduced to RTD × 100 (Report, 1971).

## E. The basic set of typing phages

By 1953, interest in the method had developed in many countries outside Britain, and the International Subcommittee on Phage-Typing of Staphylococci held its first meeting. The principle was accepted that a common basic set of phages should be used by all. The set agreed upon included 13 of Williams and Rippon's phages (nine of them from the original collection of Wilson and Atkinson), together with six more (see Table IA). A set of 22 additional phages for optional use, either in primary typing or for the examination of untypable cultures, was also defined.

TABLE I

**Constitution of the international basic set of phages for typing *Staphylococcus aureus***

A 1st Meeting of International Subcommittee, 1953
B 5th Meeting of International Subcommittee, 1970

| Lytic group | Phage numbers A 1953 | | | | | Phage numbers B 1970 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| I | 29 | 52 | 52A | 79 | | 29 | 52 | 52A | 79 | 80 |
| II | 3A | [3B] | 3C | 55 | | 3A | 3C | 55 | 71 | |
| III | 6 | [7] | 42E | 47 | 53 | 6 | 42E | 47 | 53 | 54 |
| | 54 | [70] | [73] | 75 | 77 | 75 | 77 | 83A | 84 | 85 |
| IV | 42D | | | | | 42D | | | | |
| Not allotted | | | | | | 187 | 81 | | | |

[ ] Phages removed from the basic set since 1953.
☐ Phages added to the basic set after 1953.
Phages 83A, 84 and 85 are used only at RTD.

## F. The phage groups

Although typing with the basic set of phages revealed numerous patterns of lysis, there were indications of a small number of broad subdivisions. Certain combinations frequently occurred in patterns, and other combinations were rare. For example, lysis by phage 52 was often associated with lysis by phage 29 or phage 52A, but less often with lysis by phages 6 or 7, and very rarely indeed with lysis by phages 3A or 3B. It appeared that the staphylococci belonged to a series of **phage groups** which included strains lysed only by one or more of a restricted set of phages, and that the phages might be classified into corresponding **lytic groups.** Williams, Rippon and Dowsett (1953) recognized three phage groups (I, II and III).

As early as 1946, Macdonald had observed that over half of *S. aureus* cultures isolated from cows' milk in Britain were lysed by phage 42D. Smith (1948a, b) confirmed this, and also showed that "bovine" staphylococci carried a number of other phages that could be used to subdivide strains lysed by phage 42D, and to type other hitherto untypable "bovine" staphylococci. "Human" staphylococci were seldom lysed by phage 42D, and rarely lysed only by it. The only occasion on which such strains are at all commonly found in human material is when they are isolated from victims of staphylococcal food-poisoning due to milk products. A further phage group (IV) was later established to include strains lysed only by phage 42D.

In Table IA the basic-set phages of 1953 are allotted to lytic groups I to IV. Later it became necessary to introduce into the set some phages that did not fall clearly into these groups (Table IB). Phage 81 lyses many strains which otherwise have patterns in group I, but also often forms part of group III patterns. Phage 187, on the other hand, lyses strains that are sensitive only to this phage.

In addition to the strains falling into the four phage groups (and those lysed only by phage 187), there are strains—not numerous among "human" staphylococci—having complex patterns of lysis by phages of more than one group. It has been agreed, however, that lysis by phage 81 in addition to phages of lytic group I or III does not exclude a strain from the corresponding phage group. Complex patterns including phages of lytic groups I and III are more common than I–II or II–III patterns.

## G. International organization

The International Subcommittee for Staphylococcus Phage-Typing was formed in 1953, and has concerned itself with the standardization of the method and its development to meet changing needs. The Staphylococcus Reference Laboratory of the British Public Health Laboratory Service (Central Public Health Laboratory, Colindale, London, NW9 5HT, England) became the international reference centre, and in 1961 was recognized as the World Health Organization Centre for Staphylococcus Phage-Typing.

The Subcommittee consists ordinarily of one representative from each country who takes responsibility for the distribution of materials and information to workers in his own country. The starting materials for propagation used in each country (phages and propagating strains), are drawn from large freeze-dried batches prepared at Colindale. All other laboratories are expected to obtain their supplies from the respective national laboratory.

Acceptable methods for the propagation and testing of phages, and for the typing test, have been agreed upon (Blair and Williams, 1961; see Section III), and regular comparative tests of phage typing in national laboratories have been carried out every 3–4 years since 1955.

Criteria have been laid down for the usefulness of phages. A new phage might be considered for introduction into the typing system if it lysed a significant percentage of otherwise untypable strains, or if it was of value in subdividing a common phage-typing pattern, and if it could be readily propagated to at least RTD × 1000 and was stable in its characteristics. The Colindale laboratory examines such new phages submitted by national laboratories.

The nomenclature of the phages has been standardized. The serial numbers used by the earlier British workers were accepted, and further numbers have been given to phages that have appeared to be useful enough to warrant distribution to other laboratories. This system of numbering is not entirely consistent. At first, phages thought to be adaptations of other phages retained their original number followed by a letter (e.g. phage 29A was obtained by growth of phage 29 on a fresh propagating strain, which is now known as propagating strain (PS) 29A), but it is not always possible to tell from this designation whether or not the new strain resulted from a single adaptation (e.g. phage 42B was an adaptation of phage 42, but phage 42F was an adaptation of phage 42E). More recently, entirely new numbers have been given to apparent adaptations (e.g. phage 80 was adapted from phage 52A, phage 84 from phage 77, and phage 87 from phage 42D).

## H. Later changes in the basic-set phages

Table I shows the present basic set of typing phages in comparison with the set agreed upon in 1953 (see Report, 1959, 1963, 1967, 1971). Seven phages found to be useful over wide geographical areas have been added to the set, and four of the original phages have been removed from it. The objective has been to keep the total number of phages below 25, because this is the largest number of drops that can be conveniently accommodated on a single plate.

In 1953, a total of 22 additional phages were recommended for optional use, but experience showed that this resulted in little increase in the percentage of typable strains. All but 2 of the additional phages have now been discarded, and these (phages 71 and 187) have been upgraded to the basic set. Lysis by phage 71 is characteristic of certain otherwise untypable phage-group II strains that cause vesicular skin lesions in man (Parker *et al.*,

1955), and phage 187 is of value for the recognition of a small but distinct group of strains lysed only by it.

These two phages were added for the purpose of typing strains found in the general population, but the other five additions (phages 80, 81, 83A, 84 and 85) were made in response to the appearance in hospitals of apparently "new" strains of *S. aureus* that were untypable with the basic-set phages. Had these phages not been introduced, the percentage of typable staphylococci in hospital populations would have fallen precipitately.

The first of these additions was phage 80, which was produced by adapting phage 52A to an untypable staphylococcus that had caused outbreaks of sepsis in Australia in 1954 (Rountree and Freeman, 1955). Staphylococci lysed by phage 80 were recognized soon after in many other countries. Phage 81, adapted from phage 42B (Bynoe *et al.*, 1956), lysed most of the strains also lysed by phage 80, but also lysed a number of otherwise untypable but related strains. Later, staphylococci appeared in hospitals which had somewhat similar phage-typing patterns (e.g. 52/52A/80/81, 52/52A/80, 80 etc.) and resembled the 80/81 organisms in pathogenicity and ability to spread. Evidence was soon obtained that these members of what is now called the 52, 52A, 80, 81 complex were really all "the same" staphylococci that had undergone various changes in phage-typing pattern as a result of the loss or gain of phages (Rountree, 1959; Asheshov and Rippon, 1959; Rountree and Asheshov, 1961; Asheshov and Winkler, 1966).

Three further phages have since been introduced into lytic group III to characterize otherwise untypable strains which have appeared in hospitals. The first of these (phage 83A) had been isolated some years earlier (Blair and Carr, 1953) and called VA4. At this time it was used in the U.S.A. for the subdivision of typable group III strains. When untypable strains again became common in Europe after 1958, it was found that they were lysed by this phage (Williams and Jevons, 1961), which was given the number 83. Unfortunately the phage had undergone a good deal of unofficial distribution between 1954 and 1960, and it appeared that two entirely different phages were circulating under the name VA4. The number 83A was therefore given to the original phage VA4, and the mutant was designated 83B (Report, 1963).

In 1961, further untypable strains appeared, and it was shown that they had arisen from 83A strains by lysogenization with a variety of phages (Jevons and Parker, 1964). Phage 84 (adapted from phage 77), and phage 85 were introduced for the recognition of these strains (Report, 1967).

In order to avoid unnecessary complication of the phage-typing patterns given by members of phage-group III, the International Subcommittee agreed that phages 83A, 84 and 85 should be used only in typing at RTD.

Phages 70, 73, 3B and 7 were removed from the basic set between 1958