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Isolation of a transducing phage forming plaques on *Pseudomonas maltophilia* and *Pseudomonas aeruginosa*

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SUMMARY

A temperate phage, M6, has been isolated from a lysogenic strain of *Pseudomonas maltophilia*. An extended range mutant, M6a, plates on *P. aeruginosa* and is capable of general transduction in this species. This phage may be of value for comparing the genetic maps of different species of *Pseudomonas*.

INTRODUCTION

A number of markers have been mapped in *Pseudomonas aeruginosa* using both transduction and conjugation (Holloway, Krishnapillai & Stanisich, 1971). It is of interest to know whether the gene arrangement in this species is related to that of other species of *Pseudomonas*. Some information on this point has been obtained in *P. putida* by Chakrabarty & Gunsalus (1970) using the transducing phage pf 20. Where comparison was available, strong similarities were shown in the gene arrangement of these two species. In order to extend this work to *P. maltophilia*, transducing phages were sought amongst lysogenic strains of this species with the aim of comparing transduction and gene arrangement in this species to that already described in *P. aeruginosa* and *P. putida*.

MATERIALS AND METHODS

Bacterial strains. *P. maltophilia* strains were isolated from hospital patients (H. Purpan in Toulouse). The *P. aeruginosa* strains used in this study were provided by Professor B. W. Holloway, Monash University, Clayton, Victoria, Australia, and are listed in Table 1. Phages M6 and M6a were isolated in Toulouse in our laboratory.

Media. Difco nutrient broth supplemented with 0·5% Difco Yeast Extract. Nutrient agar was solidified with 1·2% Difco Agar. Minimal medium (Vogel and Bonner, 1956) was solidified with 1·2% Difco Agar and supplemented when necessary with 40 μg/ml of L– aminoacids.

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Table 1

Strain no.	Genotype
PAO1	prototroph
PAO2	*ser-3*, FP⁻
PAO8	*met-28*, *ilv-202*, *str-1*, FP⁻
PAO38	*leu-38*, FP⁻
PAO286	*met-28*, *trp-6*, *chl-2*, FP⁻
PAO406	PAO1 (G101)
PAO1225	PAO2 (F116)
PAO287	*met-28*, *ilv-202*, *arg-1*, *his-12*, *ese-4*, *str-2*,FP⁻

* *ser*, *met*, *ilv*, *leu*, *trp*, *arg* and *his* indicate requirements for serine, methionine, isoleucine and valine, leucine, tryptophane, arginine and histidine respectively; *str* and *chl* and *ese* indicate resistance to streptomycin, chloramphenicol, and phage E79, respectively.

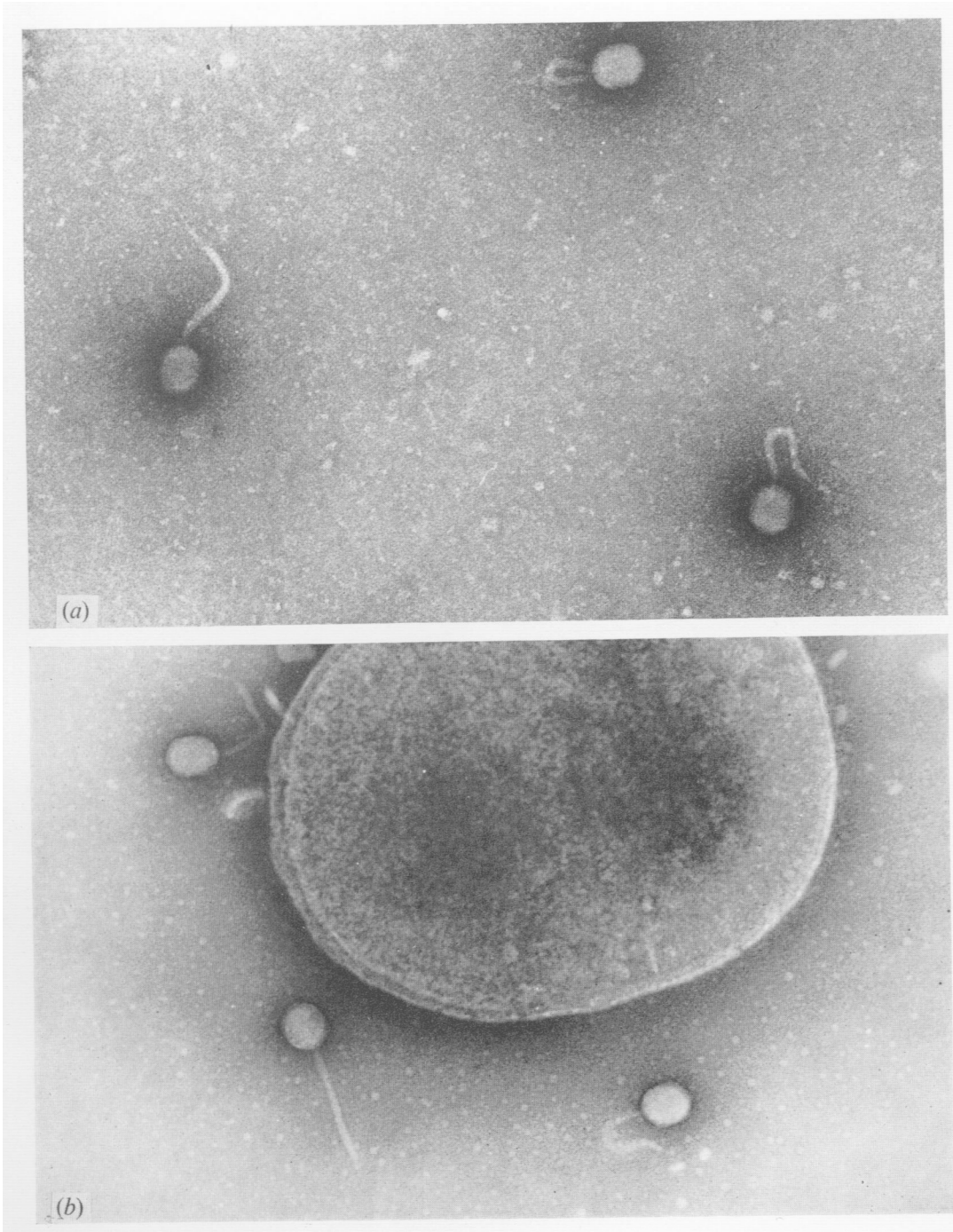
RESULTS

Fifty strains of *P. maltophilia* were isolated from hospital patients, and used both as indicator strains and as potential lysogenic strains. One of these, strain number 6, was found to be lysogenic for a phage giving plaques on four other strains of *P. maltophilia*. This phage did not form plaques on *P. aeruginosa*. Phage M6 can be induced in *P. maltophilia* strain 6 by ultraviolet irradiation and by mitomycin C, although high titre lysates are difficult to obtain using UV induction. By plating high titre preparations on *P. aeruginosa* a host range mutant was obtained which will form few plaques on this species. The phage so obtained (called M6a) plates only poorly on to *P. maltophilia* strain 6 perhaps because of restriction. The morphology of M6a, propagated in PAO1, is shown in Plate 1*a*.

The relationship of this phage to other *P. aeruginosa* phages was studied. The plating efficiency of M6a on PAO1225 (a strain lysogenic for F116) is the same as that on PAO1 suggesting that there is no immunity relationship between M6a and F116. On the other hand, M6a does not form plaques on PAO406, which is lysogenic for G101, and reciprocally, phage G101 does not form plaques on PAO1 made lysogenic for phage M6a, suggesting an immunity relationship between these two phages. It is interesting to note that the morphology of M6a is somewhat similar to that of G101 (Plate 1*b* and Holloway, personal communication). M6a does not adsorb to *P. aeruginosa* PAO1 strains made resistant to E79.

The transducing ability of M6a in *P. aeruginosa* PAO strains was studied using the techniques and procedures described by Fargie & Holloway (1965). A variety of markers can be transduced, indicating that M6a is a general transducing phage, and the levels of transduction obtained were close to those obtained with other general transducing phages in PAO1 such as F116 and G101 (Holloway, 1969).

Phage M6a shows cotransduction of linked markers; for example, using strain PAO8 as recipient, the markers *met-28*⁺ and *ilv-202*⁺ showed 20 % cotransduction with M6 as compared to 11 % cotransduction with F116 and 30 % cotransduction with G101. These two markers are approximately 2 min apart on the chromosome as measured by conjugation (Stanisich & Holloway, 1969).



Electron micrographs of phage M6a (*a*) and G101 (*b*), negative staining, PTA 1%. × 100000.

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Preliminary experiments aimed at overcoming the problems of restriction when M6a is grown in *P. aeruginosa* and plated on *P. maltophilia* have not been successful and this difficulty must be resolved before interspecific transductions can be attempted.

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