SHORT COMMUNICATION

Isolation of Alginate-producing Mutants of *Pseudomonas fluorescens*, *Pseudomonas putida* and *Pseudomonas mendocina*

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Spontaneous alginate-producing (*muc*) variants were isolated from strains of *Pseudomonas fluorescens*, *P. putida* and *P. mendocina* at a frequency of 1 in 10⁸ by selecting for carbenicillin resistance. The infrared spectrum of the bacterial exopolysaccharide was typical of an acetylated alginate similar to that previously described in *Azotobacter vinelandii* and in mucoid variants of *P. aeruginosa*. Mucoid variants were not isolated from *P. stutzeri*, *P. pseudoalcaligenes*, *P. testosteroni*, *P. diminuta*, *P. acidovorans*, *P. cepacia* or *P. maltophilia*.

INTRODUCTION

Only two species of bacteria, *Azotobacter vinelandii* (Gorin & Spencer, 1966) and *Pseudomonas aeruginosa* (Evans & Linker, 1973), are known to produce alginic acid, a (1→4)-linked linear copolymer of β -D-mannuronic and α -L-guluronic acids. Alginate production in *P. aeruginosa* is restricted to mucoid variants which are rarely encountered except in association with chronic pulmonary infection in patients with cystic fibrosis (Doggett, 1969).

We have previously reported that mucoid mutants of *P. aeruginosa* can be isolated in vitro from wild-type, non-mucoid strains by selecting for carbenicillin resistance (Govan & Fyfe, 1978) or resistance to a virulent phage (Martin, 1973; Govan, 1975). Mutations responsible for this change in phenotype can be mapped on the chromosome (Fyfe & Govan, 1980). This evidence suggested that wild-type, non-mucoid strains of *P. aeruginosa* carry the genetic information necessary for alginate production but it is normally repressed.

The aim of the present study was to determine whether alginate-producing mutants occur in other species of *Pseudomonas*. We report for the first time the isolation of mutant strains of *P. putida*, *P. fluorescens* and *P. mendocina* producing an alginate-like exopolysaccharide similar to that obtained from *A. vinelandii* and mucoid *P. aeruginosa*.

METHODS

The strains used (Table 1) were obtained from the National Collection of Industrial Bacteria, Torry Research Station, Aberdeen. The species selected represent the five rRNA homology groups of pseudomonads described by Stanier *et al.* (1977).

Media and methods used to determine minimum inhibitory concentrations of carbenicillin and to isolate mucoid mutants were those described previously (Govan & Fyfe, 1978; Fyfe & Govan, 1980) with the following modifications. The temperature employed for growth was 30 °C and *Pseudomonas* Isolation Agar (Difco) was

Table 1. *Strains of Pseudomonas used in this study*

Species	Strain number and reference		
<i>P. testosteroni</i>	NCIB 8893	Strain 79	Stanier <i>et al.</i> (1966)
<i>P. cepacia</i>	NCIB 9135	Strain 425	Stanier <i>et al.</i> (1966)
<i>P. acidovorans</i>	NCIB 9681	Type strain	Stanier <i>et al.</i> (1966)
<i>P. pseudoalcaligenes</i>	NCIB 9946	Type strain	Stanier <i>et al.</i> (1966)
<i>P. putida</i>	NCIB 10007	Strain C1-B	Stanier <i>et al.</i> (1966)
<i>P. fluorescens</i>	NCIB 10525	Strain 12	Stanier <i>et al.</i> (1966)
<i>P. stutzeri</i>	NCIB 9040		Wilkinson (1970)
<i>P. maltophilia</i>	NCIB 9203	Type strain	Hugh & Ryschenkow (1961)
<i>P. diminuta</i>	NCIB 9393	Type strain	Leifson & Hugh (1954)
<i>P. mendocina</i>	NCIB 10541	Strain CH-50	Palleroni <i>et al.</i> (1970)

replaced by medium A for growth of strains NCIB 10541, NCIB 9040, NCIB 9946, NCIB 8893 and NCIB 9393. Medium A comprised 20 g Difco Bacto-peptone, 1.4 g $MgCl_2$, 10 g K_2SO_4 , 20 ml glycerol, 9 g Oxoid agar no. 1 and 980 ml water. Peptone gluconate broth was 1 % (w/v) Difco Bacto-peptone supplemented with 2 % (w/v) sodium gluconate.

Infrared spectroscopy of the sodium salt of the exopolysaccharides was carried out by the KBr disc method (Fillipov & Kohn, 1974).

RESULTS

Spontaneous mucoid mutants resembling the colonial type 5 of Phillips (1969) were isolated, by selecting for enhanced carbenicillin resistance, at a frequency of approximately 1 in 10^8 cells from *P. putida* NCIB 10007, *P. fluorescens* NCIB 10525 and *P. mendocina* NCIB 10541. In further studies with strain NCIB 10541 this frequency was increased 40-fold following mutagenesis with ethyl methanesulphonate. Despite repeated attempts, including mutagenesis, no mucoid variants were obtained from *P. stutzeri* NCIB 9040, *P. pseudoalcaligenes* NCIB 9946, *P. maltophilia* NCIB 9203, *P. acidovorans* NCIB 9681, *P. cepacia* NCIB 9135, *P. diminuta* NCIB 9393 and *P. testosteroni* NCIB 8893.

The mucoid variants of strains NCIB 10007, NCIB 10525 and NCIB 10541 did not require the continued presence of carbenicillin for mucoid colonial growth. All of the 90 mucoid variants isolated in this study produced mucoid colonial growth on minimal agar (see Methods) within 24 h. When the variants were grown for 18 h in peptone gluconate broth, cell-free supernates contained an exopolysaccharide which precipitated on addition of 3 vol. 95 % (v/v) ethanol and redissolved in water to form a viscid solution which rapidly gelled on addition of 0.1 % (w/v) $CaCl_2$. No such exopolysaccharide was obtained from similar cultures of the wild-type parent strains. Infrared spectroscopy of the exopolysaccharide from mucoid mutants of each of the three species gave spectra typical of acetylated alginate similar to that previously reported for mucoid *P. aeruginosa* and *A. vinelandii* (Evans & Linker, 1973).

DISCUSSION

As a result of this study the number of bacterial species known to possess alginate-producing potential has been increased. The isolation of alginate-producing or *muc* mutants (Fyfe & Govan, 1980) in *P. fluorescens*, *P. putida* and *P. mendocina* provides a valuable aid to studies of the biosynthesis and regulation of *Pseudomonas* alginates in species less virulent than *P. aeruginosa* and in which alginate synthesis has more favourable carbon conversion efficiencies than in *A. vinelandii* (Jarman *et al.*, 1978).

Taxonomically, the three *pseudomonas* species from which *muc* mutants were isolated belong to the same RNA homology group, designated group I by Palleroni (1978). The isolation of *muc* mutants in *P. mendocina* is particularly interesting. On the basis of DNA

homology this species is the nearest genetic neighbour to *P. aeruginosa* (Stanier *et al.*, 1977). *Pseudomonas mendocina* was not isolated during an extensive investigation of pseudomonads causing infection in humans (Gilardi, 1972) and all strains of the species investigated by Palleroni *et al.* (1970) were obtained from non-clinical sources.

Our failure to isolate *muc* mutants from the other pseudomonads examined is not definitive evidence that such mutants do not exist. The occurrence of *muc* mutants in some species could be strain dependent. This was not found to occur, however, with *muc* mutants of *P. aeruginosa* (Govan & Fyfe, 1978). In addition, no *muc* mutants were found when a further eight strains of *P. cepacia* were investigated whilst *muc* variants were isolated from a single additional strain of *P. putida* (strain NCIB 9494) (J. R. W. Govan & J. A. M. Fyfe, unpublished results).

We have previously reported (Fyfe & Govan, 1980; Govan *et al.*, 1981) that the mucoid strains of *P. aeruginosa* isolated in vitro or from cystic fibrosis patients are heterogeneous with respect to the nutritional factors necessary for alginate production. The *muc* mutants isolated in this study from *P. fluorescens*, *P. putida* or *P. mendocina* produced alginate within 24 h on minimal medium and thus resembled group I variants of *P. aeruginosa* (Fyfe & Govan, 1980).

Similarities in the instability of *muc* variants to form non-mucoid revertants and in the biosynthesis and rheological properties of the bacterial alginate have been noted in *muc* variants of *P. mendocina* (A. Hacking, personal communication) compared to previous reports for *P. aeruginosa* (Govan, 1975; Mian *et al.*, 1978). Further studies are necessary in this area and to determine if the mutations responsible for alginate synthesis in *P. fluorescens*, *P. putida* and *P. mendocina* involve at least one chromosomal locus as in *P. aeruginosa* (Fyfe & Govan, 1980).

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