Auxotrophy of *Pseudomonas aeruginosa* in cystic fibrosis

Rowena F.H. Taylor †, Margaret E. Hodson † and Tyrone L. Pitt ‡

† Royal Brompton and National Heart Hospital, London, and ‡ The Central Public Health Laboratory, London, UK

Received 23 January 1992
Accepted 15 February 1992

Key words: Auxotrophy; *Pseudomonas aeruginosa*; Cystic fibrosis

1. SUMMARY

Seventy-four of 403 (18.4%) sputum isolates of *Pseudomonas aeruginosa* from 49 of 136 (36.0%) adults with cystic fibrosis (CF) were auxotrophic mutants. Two of 11 (18.2%) isolates of *P. aeruginosa* taken from patients with non-CF bronchiectasis were also auxotrophic. All 99 strains taken from non-bronchiectatic sources were prototrophic. Forty-six of 35 (83.6%) CF auxotrophs required one or more of 36 growth factors tested; the requirements for the remaining 9 isolates were not identified. Methionine was the sole factor required by 17 of 22 (77.3%) isolated which depended on a single factor. We conclude that auxotrophy is a feature of *P. aeruginosa* infection in cystic fibrosis.

2. INTRODUCTION

Pulmonary infection with *Pseudomonas aeruginosa* in patients with cystic fibrosis (CF) is associated with significant morbidity and mortality [1]. Once acquired, the organism is seldom eradicated from the lungs of CF patients [2], suggesting that this environment favours the survival and persistence of the organism. Strains of *P. aeruginosa* isolated from the sputum of CF patients often exhibit in vitro a wide range of altered phenotypic properties which are not characteristic of isolates from the environment and other clinical sources. These include the production of alginate polysaccharide [3], loss of lipopolysaccharide (LPS) constituents, increased sensitivity to serum complement [4] and hypersensitivity to beta-lactam antibiotics [5].

A feature of most pseudomonas species, including *P. aeruginosa*, is their ability to utilise a wide variety of compounds as single carbon sources for growth [6] thus permitting survival in diverse nutrient-limited conditions. In 1975 Govan [7] observed that the conversion of isolates of *P. aeruginosa* from the mucoid to non-mucoid state was restrained by culture in minimal broth. Indeed, Speert et al. [8] proposed that in the CF lung, *P. aeruginosa* probably grows in nutrient-limited conditions and they showed that phenotypic conversion of strains to mucoid production and LPS alterations could occur when isolates were grown in vitro in a single carbon source. We
speculated that one way in which *P. aeruginosa* persists in the CF lung might be by adaptation to the CF mucosal environment; mutants of *P. aeruginosa* with specific requirements for nutrients which are present in relative excess may be selected. We therefore examined the growth requirements of isolates of *P. aeruginosa* from patients with CF and non-CF bronchiectasis and compared these with isolates from other clinical sources.

3. METHODS

3.1. Bacterial cultures

A collection of 513 isolates of *P. aeruginosa* which had all been stored on agar slopes at room temperature for up to 2 years were examined for auxotrophy. Four hundred and three were from the sputum of 136 CF patients from six different CF centres, 11 from 11 non-CF bronchiectatic patients from the Royal Brompton Hospital, London, and 91 were from other clinical non-CF sources taken from 73 patients as follows: urinary tract (27, 15 from 14 patients with in-dwelling catheters); respiratory tract (16); skin (11); wound sites (7); stool (7); blood (5); burns (4); drains (4); central lines (3). Strains from eight environmental sites, such as sinks and bed-liners, isolated from four wards (non-CF) of three hospitals were also tested. The 403 CF isolates were selected at random from a group of strains under evaluation for antibiotic susceptibility and genotyping studies. The non-CF bronchiectatic strains were taken from all patients with non-CF bronchiectasis from whom *P. aeruginosa* was isolated from the sputum during a four-week period in 1989. The non-bronchiectatic clinical and environmental isolates were obtained from 20 hospitals distributed throughout the UK from whom requests for strain identification had been received by the Gram Negative Unit, Division of Hospital Infection, C.P.H.L., London.

3.2. Test for auxotrophy

Isolates were grown overnight on King's 'A' agar [9] at 37°C and 5–10 colonies were dispersed in 5 ml of distilled water to give an opacity corresponding to Macfarland 0.5 standard, containing approximately 10⁸ cfu/ml. This suspension was diluted 1:100 in distilled water and 0.3 µl was spotted onto a minimal salt medium (MSM) [10] and a nutritionally complete medium, King's 'A' agar, with the aid of a multipoint inoculator (Mast Laboratories, Merseyside, UK); the final inoculum on the agar being approximately 10² to 10³ cfu. MSM contained 75 ml of 2% agar to which, when molten, 6 ml of distilled water, 1 ml of 20% glucose (final concentration 0.2%) and 18 ml of a sterile solution of salts were added. Plates were examined after 48 h at 37°C; prototrophic isolates grew on both media and auxotrophs grew on the complete medium only.

3.3. Identification of specific growth factors

The experimental design described by Holliday [11] was used in which 36 growth factors, including 23 amino acids (L-forms, Sigma, St. Louis, MO) were evaluated. Individual stock solutions of each factor were prepared aseptically, at the concentrations indicated by Holliday, by dissolving the constituents in either sterile distilled water or the appropriate organic solvents [12]. All solutions were filter-sterilised, and six factors were combined aseptically in equal volumes to form 12 different pools [11]. The MSM plates were made similarly with 6 ml distilled water replacing 6 x 1 ml growth factors. Isolates were inoculated as above onto: a) all 12 pools, b) King's 'A' agar, c) MSM alone. The control strains of *Escherichia coli*, M254<sup>met</sup>, J53.2<sup>pro/met</sup> and J62<sup>pro/his/tryp</sup> were inoculated similarly. Growth was assessed after 48 h at 37°C. A single growth factor requirement is indicated by growth on two of the 12 pools only; growth on one pool alone implies a need for two or more factors in that pool; an alternative requirement is indicated by growth on more than two pools [11].

3.4. Confirmation of growth requirements

Isolates were inoculated onto: a) minimal medium supplemented with a single growth factor (20 µg/ml), b) King's 'A' agar, and c) minimal medium alone.
4. RESULTS

4.1. Auxotrophic screen

Table 1 shows that 74 of 403 (18.4%) CF sputum isolates were auxotrophic and were harboured by 49 and 136 (36.0%) patients. Two isolates from 11 patients with non-CF bronchiectasis were auxotrophic and all 99 isolates from other clinical and environmental sources were prototrophic.

4.2. Phenotypic appearance of auxotrophs

Both mucoid and non-mucoid strains grew equally well on MSM and King’s ‘A’ agar, although pigment (pyocyanin) was not produced on MSM. Auxotrophs expressed both mucoid and non-mucoid phenotypes.

4.3. Specific growth factors

Twenty-two of 55 (40.0%) isolates grew on only two pools of growth factors, i.e. required a single growth factor (Table 2). Of these, 17 (77.3%) isolated from 13 CF patients were methionine-dependent. Sixteen of 55 (29.1%) grew on only one pool, i.e. required two or more growth factors and eight (14.6%) required multiple factors. The factors necessary for growth were not identified for 9 of the 55 (16.4%) auxotrophs studied.

All control strains grew on the appropriate pools, and 17 methionine-dependent isolates grew on both the minimal medium supplemented with methionine (20 μg/ml) and also the nutritionally complete medium.

5. DISCUSSION

It is unclear why P. aeruginosa persists in the lungs of patients in vast numbers despite aggressive anti-pseudomonal therapy. The factors which enable the organism to grow and multiply within the pulmonary environment have received little attention in the literature. The requirement for particular growth factors by some and not all members of a bacterial species suggests that an underlying specific, biosynthetic defect has developed. We report for the first time that auxotrophy of pulmonary isolates of P. aeruginosa is a feature of CF and chronic lung sepsis. Growth factors may differ between patients, and methionine is an important single factor required by many strains for growth. It is of interest that the methionine-dependent strains did not grow in a medium supplemented with those amino acids which may be converted to methionine, namely serine and cysteine. This suggests that the interruption in the bacterial methionine synthetic pathway is located distal to both these amino acids.

Auxotrophic bacteria may be selected in vivo by antibiotic therapy, [13] and indeed, both thymidine auxotrophy in S. aureus in CF [14] and methionine auxotrophy in Neisseria gonorrhoeae have previously been reported [13]. Having described auxotrophy of P. aeruginosa in CF and defined some of the specific growth requirements in vitro, it may be important to investigate the mechanisms which give rise to these mutants and
to establish the clinical significance of these findings.

ACKNOWLEDGEMENTS

We thank Dr. H. Gaya, Consultant Microbiologist and the staff of the Microbiology Laboratory at the R.B. & N.H. Hospital and Mrs. P.S. Nicholson from the C.P.H.L., London, for their help. We are also grateful to Dr. B.D. Cookson, Director of the Division of Hospital Infection, C.P.H.L., for his helpful comments, and the Frances and Augustus Newman Research Foundation for financial support (R.F.H.T.).

REFERENCES