A HIGHLY DISCRIMINATORY MULTI-TYPING SCHEME FOR *PROTEUS MIRABILIS* AND *PROTEUS VULGARIS*

B. W. SENIOR AND P. LARSSON*

*Department of Bacteriology, The University of Dundee Medical School, Ninewells Hospital, Dundee DD1 9SY, Scotland and *Departments of Clinical Bacteriology and Clinical Immunology, Institute of Medical Microbiology, University of Gothenburg, S-413 46 Göteborg, Sweden.*

SUMMARY. Strains of *Proteus mirabilis* and *P. vulgaris* isolated in England, Scotland and Sweden were characterised by proticine production-proticine sensitivity (P-S) typing, O serotyping and Dienes typing methods. The determinants of O antigenicity were independent of those determining proticine production and proticine sensitivity. Because of this independence, the combination of P-S typing and O serotyping for the analysis of the 133 serotypable strains separated them into 81 distinct types whereas P-S typing and O serotyping methods alone separated them into only 56 and 19 types respectively. There was a relationship between the Dienes type and the P-S type; the determinants of Dienes compatibility were the proticine production-proticine sensitivity characters. The determinants of O antigenicity appeared to play no role in the Dienes reaction. Some strains that were indistinguishable by P-S typing and O serotyping methods were distinguished by Dienes typing.

INTRODUCTION

Infections of the urinary tract with *Proteus* spp. are common, particularly in young boys (Bergström, 1972; Hallett, Pead and Maskell, 1976) and the elderly (Walkey *et al.*, 1967; Senior, 1979). It is important that these infections are diagnosed rapidly and cured, because it has been suggested that *Proteus* spp. have a special predilection for the upper urinary tract (Fairley *et al.*, 1971). As a result of urease activity (Senior, Bradford and Simpson, 1980) the urine rapidly becomes alkaline. This leads to necrosis of the renal tubular epithelium (Braude, Sieminski and Shapiro, 1960) and the formation of renal stones (Griffith, Musher and Campbell, 1973).

Recurrent urinary tract infections with *Proteus* spp. may arise either by re-infection with a new and different *Proteus* strain or by relapse because of failure to eliminate the original infecting strain. In order to determine which is the case in a specific patient, and for the epidemiological tracing of nosocomial strains, highly specific typing schemes for *Proteus* spp. are needed. Several techniques have been described (Tracy

and Thomson, 1972; Kashbur, George and Ayliffe, 1974; Schmidt and Jeffries, 1974), but few have come into widespread use. Serotyping of *Proteus* spp. is useful for characterising strains of epidemiological interest (Lányi, 1956; de Louvois, 1969; Larsson, Andersson and Norlen, 1978) but certain combinations of O and H antigens can be common and some strains require additional typing procedures.

Serotyping permits identification of 49 O antigens and 19 H antigens among strains of *P. mirabilis* and *P. vulgaris* (Perch, 1948; Kauffmann, 1969). Some additional provisional serotypes have also been reported (Larsson and Olling, 1977; Penner and Hennessy, 1980). The proticine production-proticine sensitivity (P-S) typing method of Senior (1977a) enabled 250 *Proteus* strains to be separated into 90 P-S types. The Dienes typing of 204 swarming strains of *Proteus* in all combinations resulted in the description of 98 distinct Dienes compatibility types (Senior, 1977b).

The aim of this study was to investigate whether these typing schemes were independent of each other so that a combination of the three schemes might permit finer discrimination between strains than is possible by any one typing scheme alone.

MATERIALS AND METHODS

Bacterial strains. The 169 strains of *Proteus* examined were isolated in England (Portsmouth), Scotland (Dundee) and Sweden (Gothenburg) and were maintained as pure cultures either on nutrient agar slopes at 4°C or in deep agar stab cultures at room temperature. Each strain was identified from the results of reactions in glucose, lactose, mannitol and maltose peptone-water-sugar cultures and tests for ornithine decarboxylase and indole formation: 161 strains gave reactions typical of *P. mirabilis* and eight strains gave reactions typical of *P. vulgaris*. The 100 strains from Sweden (97 *P. mirabilis* and three *P. vulgaris*) consisted of 55 isolates from urine and 45 from faeces selected to represent 10 common O serotypes of *Proteus*, as well as non-agglutinating and spontaneously agglutinating strains. The urinary strains were isolated from patients with significant bacteriuria ($\geq 10^5$ organisms/ml.) and the faecal strains were from healthy individuals. The 48 English strains (45 *P. mirabilis* and three *P. vulgaris*) were isolated from the urine of young boys with urinary tract infections. Each strain was present as a pure growth in freshly voided urine in numbers greater than 10^5 /ml. The 21 Scottish strains (19 *P. mirabilis* and two *P. vulgaris*) were selected to represent eight proticine production-proticine sensitivity (P-S) types found commonly among typical urinary types and typical faecal types of *Proteus* (Senior, 1979). Twelve of the strains were isolated from patients with urinary tract infections and nine strains were isolated from the faeces of different people without urinary tract infection. Only one isolate from each patient or subject was included in the study.

O serotyping. *Proteus* O serotyping was performed as previously described (Larsson and Olling, 1977). Briefly, an overnight nutrient broth culture of each strain was autoclaved for 30 min. One drop of this suspension was mixed with one drop of diluted monovalent O antiserum (final dilution 1 in 800–1 in 1600) in wells of Perspex agglutination trays. Agglutinations were read with a plate microscope ($\times 12.5$) after incubation overnight at 50°C. Monovalent O antisera were available against the following O antigens: 1, 3–17, 19, 20, 23–33, 35, 36, 38, 40, 41, 44, 49 and provisional O antigens A–E.

Proticine production and proticine sensitivity (P-S) typing. Each strain was tested for the ability to produce a *Proteus* bacteriocine (proticine)—P type—and also for sensitivity to different proticines—S type—when tested against the standard set of proticine producers and indicator strains previously described (Senior, 1977a). The strains were typed by cross streaking young cultures on 1% (w/v) Proteose Peptone No. 3 agar (Difco) containing NaCl 0.5% (w/v) and 0.2 mM p-nitrophenyl glycerol (PNPG)—an anti-swarming agent without effect on P-S typing (Senior, 1977b).

Dienes typing. For each strain, a loopful of culture was applied to the surface of dried blood agar plates (Oxoid CM55 Blood Agar Base with 5% (v/v) horse blood). Inocula were arranged (Senior, 1977b) so that the Dienes compatibility of each member of a set of strains could be

tested simultaneously against each other member of the set. The plates were incubated at 30°C for 18 h, or longer if necessary, and examined with reflected light for the presence of Dienes lines of inhibited or partially inhibited growth where the spreading haloes of growth of incompatible strains met. The swarming growths of strains of the same Dienes type were compatible and swarmed into one another without the formation of a Dienes line. Care was needed in reading the plates, because if a non-spreading strain was over-run by a spreading neighbouring strain and this was not recognised, the reaction would be wrongly interpreted as indicating compatibility.

RESULTS

All of the 169 strains were independently O serotyped and P-S typed; 19 O serotypes and 56 P-S types were identified among 133 strains. Of the remaining 36 strains, 21 agglutinated spontaneously in saline and 15 were not agglutinated by any of the 41 O sera tested; amongst these, nine P-S types not found among the O agglutinating strains were found. The 100 Swedish strains of 10 serotypes, together with non-agglutinating and spontaneously agglutinating strains, belonged to 46 P-S types. Amongst the 21 Scottish strains of eight common P-S types were found representatives of 10 serotypes. The 48 English strains were of 30 P-S types and 14 serotypes.

Standard proticines were produced by 122 (72%) of the strains. The commonest proticine producers were those that produced proticines 1, 5, 3 and 2 which were formed by 29 (17%), 22 (13%), 18 (10.5%) and 15 (9%) respectively of the strains (table I). None of the strains in this study produced proticines 11 or 13. Generally, several O serotypes, usually in proportion to the number of strains, were found amongst the producers of a given proticine (table I).

Amongst the 133 serotypable strains, 91 (68%) were sensitive to one or more of the standard proticines. Several O serotypes, usually in proportion to the number of strains, were found amongst the strains of a given proticine sensitivity type (table II).

TABLE I

The frequency of different types of proticine-producing strains and the number of O serotypes found amongst the O-serotypable strains

Proticine (P) type* of producer strain	Number (percentage) of producer strains	Number of O-serotypable producer strains	Number of serotypes amongst the O-serotypable producer strains
0	47 (28)	33	15
1	29 (17)	25	7
2	15 (9)	14	4
3	18 (10.5)	15	7
4	6 (3.5)	2	2
5	22 (13)	18	5
6	12 (7)	10	3
7	6 (3.5)	3	2
8	1 (0.6)	1	1
9	5 (3)	5	2
10	1 (0.6)	1	1
12	7 (4.1)	6	5
Total	169 (100)	133	54

* See Senior (1977a); type 0 = proticine not produced.

TABLE II

The frequency of different combinations of proticine-sensitivity and the number of O serotypes found amongst 133 O-serotypable strains

Proticine-sensitivity (S) type* of strain	Number of O-serotypable strains of the S type	Number of O serotypes found
0	42	9
1	16	4
1, 5	3	3
1, 8	8	3
1, 8, 13	2	1
1, 13	2	1
2	1	1
2, 3	1	1
2, 11	1	1
3	1	1
3, 9	1	1
4	8	5
4, 12	4	1
5	8	6
5, 12	1	1
6	1	1
6, 11	1	1
7	13	5
7, 12	1	1
8	1	1
9	8	4
10	2	2
11	6	5
12	1	1

* See Senior (1977a); type 0 = not sensitive to any proticine.

A full cross-match analysis of P-S type and O serotype of the serotypable strains is presented in table III. These results show that the characteristics that determined the P-S type of a strain were independent of those that determined the O serotype. Thus, regardless of the origin of strains, those of a given P-S type could belong to different O serotypes and those of a given O serotype could include representatives of several P-S types. The independence of the P-S typing scheme from the O serotyping scheme meant that a combination of the results of the two schemes gave greater discrimination between strains than either scheme alone. The 133 strains typable by both methods could be separated into 81 types.

Because Dienes reactions of compatibility are given only by strains of the same P-S type (Senior, 1977b) predictions were made and tested of the inter-relationship between P-S type, O serotype and Dienes type. For example, it was predicted that strains of the same P-S type and same O serotype would show Dienes reactions of compatibility with each other. In all of the 30 Dienes tests performed on strains representing seven combinations of O serotype and P-S type, the predictions were found to be correct.

The possibility of a relationship between Dienes type and O serotype was examined by testing the Dienes compatibility of strains of the same O serogroup but of different P-S types. Amongst the Swedish strains examined were six strains of serotype O3, three strains of serotype O10, four strains of serotype O13-30, six strains of serotype O28 and four strains of serotype O30. All strains of the same O serotype but of

different P-S types were incompatible with one another. This indicates that the determinants of Dienes compatibility are independent of the determinants of O antigenicity.

On the basis of this result and the independence of P-S type and O serotype, it was predicted that amongst Dienes-compatible strains of the same P-S type, some should be of the same serotype whereas others should be of different serotypes. Again, these predictions were found to be correct, confirming the validity of the conclusions drawn from the results.

If the determinants of Dienes compatibility are independent of the O serotype of a strain and are determined by the P-S characters, strains of the same P-S type but of different O serotypes should be compatible with each other. This prediction was tested, wherever possible, with strains representative of the three geographical regions. The results, presented in table IV, show that the prediction was generally correct. In a few instances, however, strains that were indistinguishable serologically and appeared to be of the same P-S type were of different Dienes types. Dienes typing was found to be most useful with these strains because it permitted distinction between strains that the other typing methods had failed to separate.

DISCUSSION

The combination of three typing methods permitted fine discrimination between *P. mirabilis* and *P. vulgaris* strains from different countries. When 133 strains were typed by O serotyping alone, 19 types were found. When these strains were typed by P-S typing alone, 56 types were found. However, when the strains were typed by both P-S typing and O serotyping, 81 types were found and even more types could be distinguished if the strains were also Dienes typed.

Because colicines A, K and V are intimately associated with the O antigen of the producing strain (Goebel and Barry, 1958; Hutton and Goebel, 1962; Barry *et al.*, 1965) and bacteriocine receptors are located on the outer surface of the bacterial cell, it was possible that a particular P-S type might have been associated with a particular O serotype. However, this was not found to be the case. Apart from a few clusters of several representatives of a particular P-S type in one serotype, no association was found between O serotype and either production of a particular proticine or sensitivity to proticine(s). The clusters of strains are thought to be repeated isolations of the same strain included by the selection of individuals who, by chance, had all been infected with the same strain. Moreover, the fact that P-S types were found amongst spontaneously agglutinating (rough) strains also lends support to the view that P-S types are not associated with the O side chain of the bacterial cell wall. Because the P-S typing method is based on characteristics that are independent of those that determine O antigenicity, the two typing methods can be used together to provide greater strain discrimination than either method alone can provide.

In a previous survey of Scottish isolates, proticine 3-producing strains were found frequently and were associated with patients who had severe urinary tract infections (Senior, 1979). The frequency of occurrence of the different proticine-producers amongst the English and Swedish strains was similar to that found amongst the Scottish strains in an earlier survey (Senior, 1977a). It is unlikely, therefore, that the observed proticine 3-virulence association was a local phenomenon.

TABLE IV

The Dienes compatibility reactions of strains of the same P-S type but different O serotypes

P-S types of strains tested for Dienes compatibility	O-serotypes of strains in test	Number of Dienes-compatibility types found
0/1	O10, O28	2
0/4	O3, O6, O13, O19	2
0/5	O13, O13-30, O30	1
0/7	O6, O8, O41, OA	1
0/9	O23, O28	1
1/0	O3, O6, O27, O30	4
1/11	O3, O13, O29	3
2/1	O23, OB	1
2/1, 5	O23, OB	1
3/1, 8	O3, OB	2
5/0	O3, O30	1
12/0	O13-30, O27, O28	3

The determinants of Dienes compatibility, according to Cunliffe and Krikler (1953), are H antigens. However, others have found a greater number of Dienes types than can be explained on the basis of differences in H antigenicity (Senior, 1977*b*) and serologically identical strains may be of different Dienes types (De Louvois, 1969). Skirrow (1969) showed that strains with the same O antigen but different H antigens could be of the same Dienes type. Our results confirm those of De Louvois (1969) and Skirrow (1969). We found no evidence to support the suggestion that the determinants of O antigenicity play any role as determinants of Dienes compatibility. Many examples were found of O-antigenically distinct strains that belonged to the same Dienes compatibility group and O-antigenically identical strains that were incompatible with each other in a Dienes test.

Our results support the findings of Senior (1977*b*) that the determinants of Dienes compatibility are the proticine production and proticine sensitivity characters. Regardless of whether strains were of the same O serotype or not, if their P-S types were different they were incompatible. If their P-S types were identical most strains were compatible. A very small number of strains of the same P-S type were incompatible. This was reported by Senior (1977*b*) and is explained on the basis that there are differences between the P-S types of the incompatible strains but that the typing strains available at present cannot detect these differences.

The aim of this study was to investigate the suitability of combining different typing methods to provide a highly discriminating scheme for identifying particular types of *Proteus*. The scheme can be used for strains of *P. mirabilis* or *P. vulgaris* regardless of their origin. It is suggested that if the need is only to determine whether or not there is a common type amongst a small number of isolates, the rapid and simple Dienes typing method should be adequate. However, if a large number of strains have to be examined, Dienes typing of all combinations of strains becomes very complicated and P-S typing or O serotyping methods would be more suitable. The finest discrimination between strains is given by combining P-S typing and O serotyping. Strains that are of the same P-S type and O serotype may then, in some cases, be differentiated by Dienes typing.

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