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Protein profile typing—a new method of typing *Morganella morganii* strains

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Summary. A new, simple and stable method for typing *Morganella morganii* strains is described. The 150 strains examined, principally from faeces, contained haemolytic and non-haemolytic representatives of diverse O serogroup, bacteriocin type and biotype. Among the biotypes were some trehalose-fermenting, tetracycline-resistant strains and some non-motile, tetracycline-sensitive, glycerol fermenters. After analysis of cell lysates by sodium dodecyl sulphate-discontinuous polyacrylamide gel electrophoresis, strains could be differentiated into 21 types on the basis of outer membrane proteins (OMP) of 35–40 Kda. The OMP profile was not altered by culture on various common media and was unrelated to either O antigen or morganocin p-type. The finest strain recognition in *M. morganii* can be achieved by application of all three distinct typing methods.

Introduction

Morganella morganii is often regarded as a harmless opportunist pathogen, yet strains frequently carry transmissible antibiotic-resistance plasmids[1] and they have been associated with large nosocomial outbreaks of infection[2] with serious morbidity and mortality.[3] Although *M. morganii* strains are less frequently the cause of urinary tract infection than *Proteus mirabilis*, because of their slower growth rate in urine and their inability rapidly to make it alkaline,[4] they are nevertheless occasionally isolated from urine and blood, sputum and wounds.[5,6] Haemolytic strains may be particularly virulent for man, as they are for mice,[7] because the calcium-dependent haemolysin they secrete[8] is closely related at the molecular level to *Escherichia coli* α haemolysin, a known virulence factor.[9] *M. morganii* strains are frequently isolated from faeces, particularly from people with diarrhoea, and some strains may be the cause of this condition.[10–14]

For this reason, several methods have been developed for typing *M. morganii* strains. Biotyping[15] differentiated strains into 12 groups but 83% of strains were of one type. Phage typing[16] similarly distinguished only 12 types. Typing by bacteriocin production and sensitivity[17] was much more discriminating and permitted the differentiation of 160 strains into 90 types. *M. morganii* strains have also been differentiated by serotyping[14,15,18] and 55 different O-serogroups have been described.[19] The bacteriocin typing and O-serotyping methods are independent of each other[19,20] and, when performed together, give a high rate of discrimination between strains.

Because of the large number of strains and reagents which have to be kept for the latter typing schemes, bacteriocin typing and serotyping have become methods for specialist laboratories and are not very suitable for routine use. In an attempt to provide a discriminating, reliable but simpler typing method for *M. morganii* which might be used on a regular basis, analyses were made of the protein profiles of strains. Protein profile typing has been found to satisfy these criteria. The typing method described here, though independent of O-serotyping and bacteriocin typing, will confirm the identity of some strains found to be indistinguishable by a combination of these typing methods, yet in other instances will discriminate between strains with common O-antigen and bacteriocin type characteristics.

Materials and methods

Bacterial strains

These were 150 of the 160 strains of *M. morganii* which had been collected for the development of the morganocin production (p)/ morganocin sensitivity (s) (p/s) typing scheme.[17] The strains were from 131 patients, mostly

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from their faeces, and pure stock cultures were maintained on both Nutrient Agar (Oxoid, CM3) slopes and Dorset's Egg slopes at 4°C.

Media

Nutrient Broth (Oxoid, CM 67), MacConkey Agar (Oxoid, CM 7), CLED Agar (Oxoid, CM 301), Iso-Sensitest Agar (Oxoid, CM 471) and Blood Agar (Blood Agar Base, Oxoid CM 55, supplemented with horse blood, Oxoid SR 50, 5% v/v) were prepared and sterilised according to the manufacturer's instructions.

Biochemical tests

The strains were speciated and examined by conventional biochemical tests according to methods described previously.[15,17]

O-serotyping of strains

The O-antigenic analysis of the strains was performed by slide agglutination tests with pooled O antisera and subsequently by slide- and tube-agglutination tests with the respective monovalent O-antisera according to the standard methods described previously.[15,18] Strains which agglutinated in saline alone were described as "rough". Strains which did not agglutinate with any of the O-antiserum pools were said to be "non-typable".

Bacteriocin typing of strains

Morganocin production (p)/ morganocin sensitivity (s) (p/s) typing of strains was performed as previously described.[17]

Haemolysin production

Strains were examined for haemolysin production on solid medium with sheep erythrocytes and in liquid medium with horse erythrocytes as described previously.[8,20]

Protein-profile typing of strains

In the standard method, 0·5 ml of a 2-ml nutrient-broth culture of a strain incubated statically overnight at 37°C was added to 4·5 ml of nutrient broth and incubated with shaking at 37°C. After 2 h, 1·5 ml of the culture was removed and centrifuged at 11 600 *g* for 2 min, the supernate was decanted and discarded, and the cell pellet was resuspended in 0·5 ml of saline. After further centrifugation as before, the supernate was removed and discarded and the washed cell pellet was resuspended in 50 µl of distilled water and 50 µl of sample buffer—0·125 M Tris-HCl, pH 6·8, sodium dodecyl sulphate (SDS) 4% w/v, glycerol 20% w/v, mercaptoethanol 10% v/v and a trace of bromophenol blue dye.

In the alternative method, a number of colonies sufficient to fill approximately one quarter of a 2·5-mm diameter wire loop, were scraped from blood agar or MacConkey agar or CLED agar plates which had been inoculated with the strain and incubated overnight at 37°C, and were resuspended in 0·5 ml of saline. After centrifugation at 11 600 *g* for 2 min, the supernate was removed and discarded and the washed cell pellet was resuspended in 75 µl of distilled water and 75 µl of sample buffer.

The samples were stored at −20°C and boiled for 5 min before electrophoresis on polyacrylamide gels.

Polyacrylamide gel electrophoresis

Samples were run on polyacrylamide gels after the method of Lugtenberg *et al.*[21] A 15–20-µl sample of the boiled cell extract was loaded on to a stacking gel of acrylamide 4% in 0·125 M Tris-HCl, pH 6·8, SDS 0·1% over a resolving gel of acrylamide 11% in 0·375 M Tris-HCl, pH 8·8, SDS 0·2%. The upper tank buffer was 0·053 M Tris, 0·052 M glycine, SDS 0·1%, pH 8·9. The lower tank buffer was 0·1 M Tris-HCl, SDS 0·1%, pH 8·1. Electrophoresis was performed in the cold at 25 mA/gel until the dye front reached the bottom of the gel. Gels were stained overnight in Coomassie Brilliant Blue R250 0·125% in water containing methanol 50% and acetic acid 10% and destained in several changes of water containing methanol 10% and acetic acid 10%.

Results

Biochemical characteristics of strains

All strains had the typical biochemical characteristics of *M. morganii*. They produced urease, tryptophan deaminase, ornithine decarboxylase and indole and fermented glucose and mannose but not lactose, adonitol or inositol. All gave negative results in H_2S, citrate and Voges-Proskauer tests but positive results in the methyl red test.

In an attempt to determine whether different biotypes were present, strains were tested for their ability to ferment other sugars. All strains fermented galactose and xylose within 2 days at 37°C. However, fucose, raffinose, sorbitol, sorbose or sucrose were not fermented by any strain within 7 days at this temperature. Trehalose was fermented by 16 (10·7%) strains within 1 day at 37°C but the remainder were unable to do this even after incubation for 7 days. All but one of the trehalose fermenters were motile and all but one were tetracycline-resistant and grew on Iso-Sensitest agar up to the edge of a 10-µg antibiotic disk. Four (2·7%) strains fermented glycerol within 1 day and another 50 strains fermented glycerol after prolonged incubation for 4–7 days at 37°C. All four

glycerol rapid fermenters had other unusual characteristics for *M. morganii*—all were non-motile at 22°C, 30°C and 37°C (electronmicroscopy showed that these strains did not form flagella), and sensitive to tetracycline (10-μg disk; zone diameter ⩾15 mm). None of them formed lysine decarboxylase.

Protein profile typing of strains

The protein profiles of the 150 strains were found to be remarkably similar, except for the profiles of proteins of 35–40 Kda which comprise some of the major proteins of the outer membrane. A comparison of the profiles of the proteins in this size range revealed 21 different patterns. These were called outer membrane protein (OMP) types and were given different letters A–U (figure). The standard OMP type strains and the frequency of occurrence of the different OMP types is presented in table I. Approximately half of the strains were of OMP types A or E. The remaining strains were relatively evenly distributed between the other 19 OMP types.

The OMP type of most strains was readily determined because the profiles of many OMP types were quite different from others. However, some were very similar. For example, there were only very small variations in the sizes of the proteins and the profiles of the 24 (16%) strains of OMP types B, H, O, K and P. This was also true for the 14 (9·3%) strains of OMP types C, I and S and the 11 (7·3%) strains of OMP types L and T. Care was necessary when typing strains with these OMP profiles.

Deliberate attempts to alter the OMP type by varying the cultural conditions failed. The OMP profile of each one of the standard OMP type strains prepared by the alternative method from growth on blood agar, MacConkey agar and CLED agar and from large and small colony variants on these media was found to be identical to that of the same strain from broth culture when prepared by the standard method. Thus the OMP type of a strain was not altered by these differences in cultural conditions. The OMP type of a strain appeared also to be a stable characteristic because cell lysates of

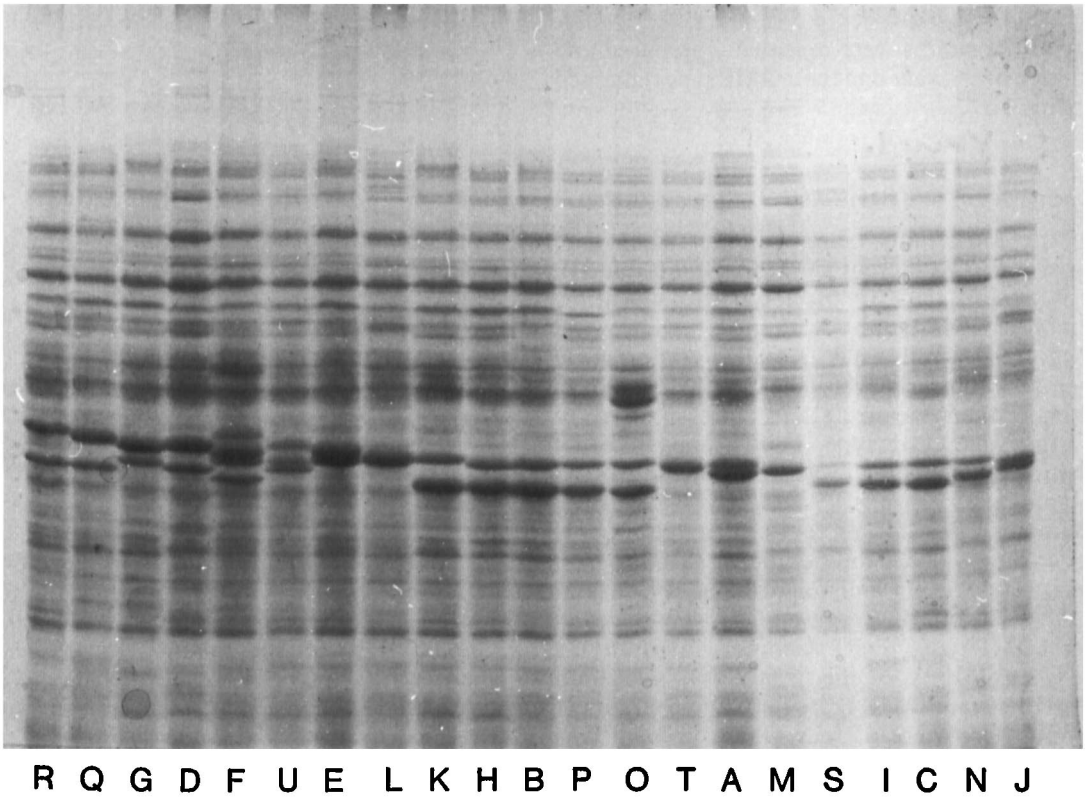

Figure. The protein profiles of the 21 different OMP type strains of *M. morganii* after electrophoresis on polyacrylamide gels.

Table I. The frequency of OMP types among 150 strains of *M. morganii*

OMP type	Type strain	Number of strains with this OMP type	Percentage of total strains
A	Mg 101	64	42·6
B	Mg 11	8	5·3
C	Mg 60	6	4·0
D	Mg 28	4	2·6
E	Mg 48	17	11·3
F	Mg 126	2	1·3
G	Mg 148	2	1·3
H	Mg 134	7	4·6
I	Mg 10	7	4·6
J	Mg 160	2	1·3
K	Mg 151	2	1·3
L	Mg 62	9	6·0
M	Mg 132	3	2·0
N	Mg 26	1	0·7
O	Mg 97	1	0·7
P	Mg 50	6	4·0
Q	Mg 73	2	1·3
R	Mg 30	1	0·7
S	Mg 108	1	0·7
T	Mg 36	2	1·3
U	Mg 75	3	2·0

many of the strains were repeatedly prepared and examined over a year and their OMP type remained unchanged.

Independence of OMP type and O antigen of strain

Of the 150 strains examined, 126 (84%) were typable into 31 different O serogroups (table II). The 90 strains (60% of the total) with the three commonest OMP types—A, E and L—were found among, respectively, 11, 7 and 6 different O serogroups. In nearly every instance, O serogroups with more than one strain had representatives of more than one OMP type. Thus it appeared that protein profile typing was independent of O serotyping.

In many instances protein-profile typing was found to be of value in differentiating strains which were O serologically indistinguishable. For example, the 23 strains of serogroup O47 were of seven distinct OMP types, the 12 strains of serogroup O25 were of six OMP types, the six strains of serogroup O40 were of four OMP types and the 16 strains of serogroup O48 were of four OMP types. It was noted that the 16 strains of serogroup O46 were unusual in this respect for all were of OMP type A. These strains may represent a local clone of strains.

Table II. The distribution of OMP types among haemolytic and non-haemolytic strains of *M. morganii* of different O serotype

O-serogroup	Number of strains	OMP types (number) among strains of the serogroup that were: haemolytic	non-haemolytic
1	9	A(4)	A(2), P(3)
2	1	...	A(1)
7	1	...	G(1)
13	1	...	M(1)
15	1	...	M(1)
17	1	...	A(1)
21	1	...	T(1)
22	1	...	U(1)
23	1	...	I(1)
24	1	...	I(1)
25	12	A(2), E(1), L(1), U(1)	A(1), C(3), I(2). L(1)
26	2	A(1)	S(1)
27	2	...	B(1), H(1)
29	4	E(1), L(1)	E(2)
30	1	L(1)	...
31	1	A(1)	...
32	2	...	G(1), K(1)
37	4	E(2), L(2)	...
40	6	A(3)	B(1), L(1), P(1)
44	3	...	D(3)
45	3	...	A(1), D(1), T(1)
46	16	A(1)	A(15)
47	23	A(9), U(1)	A(6), B(3), E(1), L(1), P(1), R(1)
48	16	A(2), E(2), F(2)	A(7), B(1), E(2)
49	3	P(1)	C(2)
50	1	E(1)	...
51	2	...	B(1), M(1)
52	2	H(1)	K(1)
53	2	...	H(1), N(1)
54	2	...	I(2)
55	1	...	E(1)
Rough	4	...	A(1), J(1), Q(2)
Non-typable	20	A(4)	A(2), B(1), C(1), E(4), H(4), I(1), J(1), L(1), O(1)
Total	150	45(30%)	105(70%)

OMP type and morganocin type

The 150 strains examined were of 50 different morganocin p-types. However, 96 strains (64%) belonged to only five morganocin types, p0, p1, p35, p38 and p40. The commonest OMP types, namely strains of type A, E and L, were of 18, 12 and 8 different morganocin p types respectively. Therefore, it appeared that OMP type was unrelated to the morganocin p-type of a strain. However, a study of the five most common morganocin p-types showed that protein-profile typing differ-

Table III. The distribution of OMP types amongst haemolytic and non-haemolytic strains of *M. morganii* of common morganocin p-type

Morganocin p-type of strains (number of strains)	Haemolytic activity of strains (number of strains)	OMP types (number of strains) with this morganocin p-type
0 (26)	+ (4)	A(4)
	− (22)	A(5), B(2), D(4), E(1), H(3), I(1), J(1), K(1), L(1), R(1), T(1), U(1)
1 (48)	+ (13)	A(12), P(1)
	− (35)	A(23), B(5), E(1), H(1), P(4), U(1)
40 (11)	+ (6)	E(4), F(1), H(1)
	− (5)	B(1), H(1), K(1), Q(2)
35 (6)	+ (0)	...
	− (6)	C(5), E(1)
38 (5)	+ (3)	F(2), L(1)
	− (2)	E(2)

+, Haemolytic; −, non-haemolytic.

entiated strains indistinguishable by their morganocin p-type (table III). Thus, the 48 strains of morganocin type p1 were of six different OMP types. It was noted that 35 (55%) of the 64 OMP type A strains, 5 (62·5%) of the eight OMP type B strains and 5 (83%) of the six OMP type P strains produced this type of morganocin. Similarly, the 11 morganocin p-type 40 strains were of six OMP types. The six morganocin p-type 35 strains were of two OMP types—C and E—and 5 (83%) of the six OMP type C strains were of this morganocin p type. Five of the morganocin p-type 38 strains were of three OMP types. The 26 strains of morganocin p-type O (i.e., those unable to produce detectable morganocin) were of 12 different OMP types. Thus, although OMP type was unrelated to morganocin p type, certain morganocin p types were more often of some OMP types than others (table III).

OMP type and haemolysin production and biotype

Of the 150 strains which had been examined previously for haemolysin production,[19] 105 (70%) were non-haemolytic and 20 of the 21 OMP types were found among these strains. Among the 45 (30%) haemolytic strains, only seven OMP types, A, L, U, E, F, P and H, were found. The only OMP type found exclusively among the haemolytic strains was type F which was found in two strains.

Among the 16 trehalose-fermenting strains, seven OMP types were found, 75% of these strains being of OMP types I, D and M. However, the four non-motile, tetracycline-resistant *M. morganii* strains that fermented glycerol in 1 day were all of OMP type E. These strains comprised 25% of the strains of this OMP type.

Discussion

The method of typing *M morganii* strains by their protein profiles is a suitable and convenient method for laboratories without facilities to perform serotyping and bacteriocin typing of this organism.[15,17–20] It is more discriminating than phage typing[16] and biotyping.[15] Attempts to improve the discriminating power of the scheme for biotyping *M. morganii*, by testing for the fermentation of some sugars reported to be useful for this purpose, were largely unsuccessful. Our results substantiate those of Siboni[22] who reported that the only carbohydrate useful in the typing of *M. morganii* was trehalose. Some 10% of our strains fermented this sugar—a proportion similar to the 14% reported by Ewing[23] with a larger collection of strains.

Most (88%) strains of *M. morganii* are motile[23] and show varying susceptibilities to tetracycline. The finding that all trehalose-fermenting strains were motile and tetracycline-resistant supports the work of Siboni[22] who reported that trehalose fermentation by *M. morganii* correlates with non-transmissible tetracycline resistance and increased flagellar wavelength. On the other hand, the *M. morganii* strains which fermented glycerol within 24 h were all non-motile and lacked flagella, yet were tetracycline sensitive. Hickman *et al.*[24] described *M. morganii* strains with these unusual features, which, in addition, decarboxylated lysine. They were shown by DNA hybridisation studies not to be a distinct species within the genus, but rather a biogroup of *M. morganii*—biogroup 1.[24,25] The non-motile, tetracycline-sensitive, rapid glycerol fermenters in our study were unable to decarboxylate lysine. It is possible that the *M. morganii* biogroup 1 strains of Hickman *et al.*[24] carried plasmids that determine lysine decarboxylase formation because others have shown that the formation of this enzyme in *M. morganii* is a plasmid-determined trait.[26]

Protein-profile typing of *M. morganii* strains is relatively simple. Repeated use of the method over a year on isolates of *M. morganii* well characterised by other typing methods, has confirmed that it is a stable typing technique. As the typing results were unaffected by growth in and on different commonly used culture media, the typing method could be used routinely for the direct analysis of strains from clinical material isolated on conventional media.

Although protein-profile typing is less discriminating that either O serotyping[19] or morganocin typing,[20] it readily differentiated the 150 strains examined into 21 different OMP types. Most strains were evenly distributed among 20 of the OMP types. The most frequent OMP type was type A, to which 64 (42·6%) strains belonged. This finding may be a feature only of local strains because 40 of the 64 OMP type A strains were of serogroups O46, O47 and O48. These are new serotypes which to date have been detected only in local isolates.[19]

The protein profile of a strain appeared to be independent of its O serotype and morganocin p-type. Since the latter typing methods are also independent of one another,[19,20] the finest strain recognition is given by the combined use of all three methods. Protein-profile typing was able to confirm the identity of strains found to be identical by O serotyping and morganocin typing, yet, as an independent third method, in some instances it could differentiate between strains previously thought to be identical because they were of the same O serotype and morganocin type.

Protein-profile typing was equally applicable to the typing of unusual biotypes of *M. morganii* strains. Generally, it appeared that these strains were of a limited number of OMP types. This was also true for the potentially virulent haemolytic strains. However, it will be necessary to examine more of these types of isolates before firm conclusions on these matters can be drawn.

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