The typing of *Morganella morganii* by bacteriocin production and sensitivity

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Summary. A typing scheme for *Morganella morganii* based on bacteriocin (morganocin) production and sensitivity is described. These characteristics were determined by testing 160 strains in all combinations and permitted their differentiation into 90 types. Morganocin production was induced with mitomycin C and morganocin sensitivity determined with a diluted inoculum on Lab-lemco agar at 30°C. Most strains (82.5%) produced morganocins and 49 different types were defined. Most strains (97.5%) were sensitive to morganocins and usually to several different types. The scheme is more discriminating than other reported methods. The finding in an epidemiological survey of the carriage of certain strains in the bowel for several weeks suggests that in practice the method is stable and reproducible.

Introduction

Morganella morganii (*Proteus morganii*) is the present name of the organism isolated by Morgan (1906) as the predominant species in the faeces of infants with diarrhoea. Because other known enteric pathogens were not found, he considered the organism to be the cause of diarrhoea. Others (Tribondeau and Fichet, 1916; Thjotta, 1920; Magheru, 1923; Rauss, 1936) supported this view but firm evidence for its aetiological role in enteritis has not yet been presented.

Although *M. morganii* has been isolated from blood, sputum and wounds (Braunstein and Tomasulo, 1978) and is occasionally the cause of urinary tract infections (Sevin and Buttiaux, 1939; Marier *et al.*, 1978) though less frequently than *P. mirabilis* (McMillan, 1972; Senior, 1979) because of its slower growth rate and inability to make urine alkaline rapidly (Senior, 1983), the organism has often been regarded as only a harmless opportunistic pathogen. However, recent reports that *M. morganii* strains frequently carry transmissible antibiotic resistance plasmids (Hedges *et al.*, 1973) and have been associated with large nosocomial outbreaks (Tucci and Isenberg, 1981) and with serious morbidity and mortality (Williams *et al.*, 1983) suggest that this organism should be regarded as a true rather than an opportunist pathogen.

These considerations led me to devise a typing scheme for *M. morganii* with better discrimination than biotyping (Rauss and Vörös, 1959) or phage

typing (Schmidt and Jeffries, 1974), both of which only distinguished 12 types. Such a scheme should be simpler to perform than the O and H serotyping methods of Rauss (1936) and Rauss and Vörös (1959, 1967) which now define 42 serogroups and 75 serovars (Rauss *et al.*, 1975). The scheme chosen was typing by bacteriocin production and sensitivity.

Materials and methods

Strains of *M. morganii*

These were isolated at random over 8 years from specimens (mainly faeces) sent to the laboratory for routine bacteriological investigations. Specimens were plated for single colonies on desoxycholate citrate agar and MacConkey agar. After overnight incubation at 37°C, a representative of each type of non-lactose fermenting colony was subcultured into nutrient broth and incubated at 37°C for a few hours. Various media devised for the identification of *Proteaceae* (Senior and Leslie, 1986) were seeded with the cultures and incubated overnight at 37°C. Isolates that fermented mannose and formed indole, tryptophan deaminase, urease and ornithine decarboxylase but failed to ferment lactose, maltose and inositol or form H₂S were identified as *M. morganii*. These pure cultures were maintained on nutrient agar and Dorset's egg slopes in 7-ml screw-capped bottles at 4°C.

Media

Desoxycholate Citrate Agar (Oxoid, CM 35), MacConkey Agar (Oxoid, CM 7), Lab-lemco Agar (Oxoid,

CM 17) and Nutrient Broth (Oxoid, CM 67) were all prepared and sterilised according to the manufacturer's instructions. Saline was NaCl 8.5 g/L of distilled water. PP3 medium was Proteose Peptone No. 3 (Difco) 1% and NaCl 0.5% in distilled water. These were autoclaved at 121°C for 15 min.

Preparation of bacteriocins (morganocins)

Every *M. morgani* strain was seeded into 0.5 ml of PP3 medium in a 30-ml screw-capped bottle. After overnight incubation at 30°C, each culture was diluted by adding 4 ml of PP3 medium and incubated on a rotary shaker at 30°C in the dark for 4 h. Morganocin production was then induced by adding mitomycin C in 0.5 ml PP3 to give a final concentration of 1 µg/ml in each culture and incubation was continued overnight. After removal of bacterial cells and debris by centrifugation for 5 min at 2500 g, each supernate was transferred to a sterile plastic petri dish and left exposed to ultraviolet irradiation for 5 min to inactivate any contaminating bacteriophages. The preparations were then transferred aseptically to sterile 7-ml bottles, to which a few drops of chloroform were added. After shaking, they were stored at 4°C.

Testing for sensitivity to morganocins

Strains for testing were taken from either 2-ml nutrient-broth cultures incubated overnight at 37°C (and subsequently stored at 4°C) or similar cultures which had been stored at 4°C for no more than 1 month. After mixing, a 4-µl loopful of the fresh or stored culture was diluted into 2 ml of saline and a loopful of the diluted culture streaked across a Lab-lemco agar plate. Each plate accommodated about ten streaks. A drop of morganocin preparation from a disposable plastic syringe fitted with a fine needle was placed over each streak. One streak of a strain could normally be tested against seven morganocin preparations. The plates were incubated overnight at 30°C and then examined for zones of inhibited growth.

Results

Each strain of the 160 examined gave the biochemical reactions of *M. morgani*. Most strains (152, i.e., 95%) were from faeces, four were from urine, two from wounds and two from rectal swabs. The strains were isolated from 139 people of whom 62 (45%) were male and 77 (55%) were female; 81 (58%) were in hospital and 58 (42%) at home. Except for two brothers and a husband, wife and son, all the individuals were unrelated.

Each of the 160 strains was tested as a producer of morganocin against all the other strains as indicators of morganocin activity, 25 600 tests in all.

Analysis of strains by morganocin production

Morganocin active against one or more of the

160 strains used as indicators was produced by 132 strains (82.5%). The morganocin preparations retained their activity for many months when stored at 4°C. Twenty-eight strains were recorded as "nonproducers of morganocin" because their preparations failed to inhibit any of the 160 strains used as indicators. These strains were from 22 individuals and, with the exception of one strain from urine, all were isolated from faeces.

The pattern of activity of the 132 morganocin preparations against the 160 indicator strains was complex; 49 distinct patterns of inhibition were observed upon repeated testing. By selecting particular indicator strains, the minimum number which permitted distinction between the 49 different morganocins was 23. The activities of these 49 morganocins together with that of the "nonproducer strains" upon the 23 selected indicator strains, are defined and presented in table I.

Table I shows that some morganocins, such as types 16, 25 and 43, inhibited many—125 (78.1%), 124 (77.5%) and 91 (56.9%) respectively—of the 160 strains, whereas others, such as types 30, 48, 36 and 49 inhibited very few strains—1 (0.6%), 1 (0.6%), 3 (1.9%) and 3 (1.9%) respectively. Morganocin type 1, the commonest, was produced by 56 (35%) of the 160 strains, and there were 28 (17.5%) type O strains, "nonproducers"; type 40 was produced by 11 strains (6.9%) from ten individuals. Six strains from three people produced morganocin type 25, five type 38, three type 21 and two each the morganocin types 2, 8, 10, 23, 30, 33 and 43. All other types of morganocin were produced by one representative strain each. Seven strains (6, 43, 56, 87, 132, 148 and 151) produced inducible morganocins to which they themselves were sensitive.

Analysis of strains by morganocin sensitivity

Of the 160 strains examined, 156 (97.5%) were sensitive to one or more of the 132 morganocin preparations. Most were sensitive to many different morganocins, e.g., strains 30, 49 and 63 were sensitive to 106 (80%), 87 (66%) and 86 (65%) respectively of the 132 morganocin preparations. A small number of strains were sensitive to only a few morganocins, e.g., strains 66 and 148 were each sensitive to only three (2%) of the 132 preparations. There was only one strain, no. 51, which was sensitive to one morganocin only (type 26). Four strains (2.5%), nos. 40, 48, 92 and 133, were not sensitive to any of the morganocin preparations. No strain was both a "nonproducer" and insensitive to morganocins.

Table I. Frequency of occurrence and activity of the different types of morganocin found among 160 strains of *M. morganii* and the sensitivity of these strains to the different morganocin types

Morganocin type	Type producing strain number	Number of strains with same morganocin type	Action of morganocin type on indicator strain number:															Percentage of 160 strains sensitive to the morganocin type	Morganocin sensitivity pattern							
			1	49	63	39	121	27	23	11	122	22	100	77	64	115	3			17	143	130	84	8	126	26
0	24	28	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	...
1	3	56	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	4.4
2	43	2	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	10.0
3	81	1	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	7.5
4	34	1	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	9.4
5	83	1	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	6.9
6	50	1	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	15.6
7	56	1	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	8.1
8	46	2	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	15.6
9	4	1	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	11.2
10	13	2	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	8.8
11	12	1	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	19.4
12	139	1	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	10.6
13	89	1	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	28.8
14	111	1	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	13.1
15	153	1	(+)	(+)	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	6.9
16	97	1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	78.1
17	45	1	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	35.0
18	41	1	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	40.6
19	77	1	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	60.0
20	37	1	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	48.8
21	2	3	—	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3.8
22	155	1	—	(+)	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	21.9
23	133	2	—	(+)	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	38.8
24	102	1	—	(+)	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	25.0
25	57	6	—	(+)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	77.5
26	116	1	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3.1
27	132	1	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	36.9
28	129	1	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	45.6
29	74	1	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	50.6
30	15	2	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.6
31	92	1	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	36.2
32	32	1	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	35.0
33	112	2	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	49.4
34	58	1	—	(+)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	5.6
35	88	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3.1
36	157	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1.9
37	6	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	10.0
38	38	5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	33.1
39	108	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	38.8
40	31	11	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	35.6
41	148	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	31.3
42	32	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	35.6
43	8	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	31.3
44	39	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	35.6
45	160	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	53.1
46	19	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	7.5
47	63	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	41.2
48	17	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.6
49	10	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1.9

+ = Inhibition of growth; (+) = partial inhibition of growth; — = no inhibition of growth; ... not applicable; * see table II.

The typing scheme

There was good discrimination between most strains when they were typed by morganocin production. The method was simple to perform, the preparations stable and the results reproducible. Although nearly all strains were sensitive to morganocins, typing by morganocin sensitivity was complex because most strains were sensitive to many different morganocins. Indeed, only one strain was an indicator for a single morganocin. However, because a large proportion (35%) of the strains belonged to morganocin type 1 when typed by morganocin production, additional discrimination for these strains by including some of their morganocin-sensitivity characteristics was thought necessary. To keep the number of standard typing strains to a minimum, strains which produced morganocin type 1 were retested for sensitivity to the morganocins produced by the 23 strains used as indicators in defining the 50 morganocin types. The results showed that maximum discrimination in sensitivity was achieved by the use of only seven morganocins—those from indicator strains 1 (type 32), 63 (type 47), 39 (type 44), 100 (type 38), 77 (type 19), 8 (type 43) and 126 (type 40).

On this basis the 56 strains that produced morganocin type 1 were differentiated into 14 different morganocin sensitivity patterns (A–N). These are defined and presented in table II. For completeness, all other strains were similarly retyped for sensitivity to the same seven morganocins, and a further 17 patterns (O–e) found. The results are presented in tables I and II. It is important to note that strains which have the same sensitivity pattern to the seven morganocins may in some instances show differences in sensitivity when tested against a larger number of morganocins. Thus while eight of the 28 nonproducing strains (type 0) of 14 sensitivity types were of sensitivity pattern N (see table II) and appeared to be both “nonproducers” and morganocin insensitive, all were sensitive to some morganocins when tested against the other 125 morganocin preparations.

From the results, however, it appeared that the type of morganocin a strain produced and its sensitivity to morganocin were independent characteristics, and therefore both were useful for the typing of strains. The 160 strains were differentiated into 90 different types by morganocin production and limited morganocin sensitivity testing, whereas typing by morganocin production alone only distinguished 50 types.

Epidemiological studies

The typing of strains from patients submitting several specimens provided useful epidemiological information which is presented in table III. The repeated isolation of strains of the same type from three specimens taken at different times on the same day from patient 1 indicated the reliability and reproducibility of the typing method. Among other patients of different ages and sex it was found that a strain of a given type could persist in the bowel for longer periods—1 day (case 2), 3 days (case 3), 5 days (case 4a), 9 days (case 5), 11 days (case 6), 17 days (case 4b) and even up to 1 month (case 7).

The fine discrimination of the typing method is illustrated with cases 8 and 9. In the former, two brothers in the same household had diarrhoea, one 6 weeks after the other. The strains isolated were of different types, 11B and 10H, yet both the morganocin types and sensitivity patterns of these isolates have many common features which suggest that they may be closely related. In case 9, strains of types 18R and 17R were isolated from stools taken 1 week apart from a healthy man seeking employment in the water industry and from whom no pathogens were isolated. Their morganocins were distinct—but with many common features—and their sensitivity patterns were identical.

The transmission of infection is illustrated in case 4 which involved a mother with a *Salmonella* group B infection who carried, for a short period, a *M. morgani* type 25R strain, later to be replaced with a type 0Q strain. Her baby son carried only the 25R strain throughout this period and was free from infection with *Salmonella* until his last specimen was received. The father of the family also finally became infected with both the *Salmonella* and the 25R strain of *M. morgani* 14 days after both organisms first appeared together in the baby.

Discussion

The typing of bacterial strains through the determination of both their bacteriocin production and bacteriocin sensitivity characteristics was the scheme originally developed by Senior (1977a) for *P. mirabilis* and *P. vulgaris*. It has been found to be a useful technique, simple to perform, stable and of considerable discriminating potential (Senior, 1977b; Senior and Larsson, 1983). However, when this method was first applied to *M. morgani* to detect bacteriocin (morganocin) producing and sensitive strains, none were found, despite the use of various media and times and temperatures of incu-

Table II. Sensitivity patterns found amongst 160 strains of *M. morgani* producing morganocins of different types when tested against seven morganocins

Strain no. of morganocin producer	Sensitivity pattern of strains to morganocins																										Number of different sensitivity patterns						
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z		a	b	c	d	e	
1	+	+	+	+	+	+	+	+	-	-	+	-	-	-	+	+	-	-	+	-	-	-	-	+	-	-	+	-	-	+	+	-	13
63	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-	-	-	-	+	-	+	+	+	-	-	-	+	+	-	-	-	-	14
39	+	+	-	+	+	-	+	+	+	-	-	-	-	-	+	+	-	-	-	+	+	-	+	+	-	+	+	+	-	+	-	-	2
100	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	+	+	-	-	-	+	-	-	-	+	-	+	+	-	+	-	-	2
77	+	+	+	+	+	+	+	+	+	-	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	1
8	+	+	+	+	+	+	+	+	+	-	-	+	+	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	3
126	+	+	+	+	-	-	+	+	-	-	-	-	-	-	+	+	-	-	+	-	-	-	-	+	-	+	+	+	-	+	+	+	2
Morganocin type	Number of strains with the given sensitivity pattern																																
0	4	2	0	0	0	0	1	0	0	0	0	1	1	8	2	1	3	1	2	1	1	1	0	0	0	0	0	0	0	0	0	0	13
1	21	2	1	1	1	1	1	1	3	1	1	3	18	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14
2	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	2
8	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	2
10	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
21	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
23	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
30	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
38	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1
40	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	6	1	0	1	0	0	1	0	0	0	0	0	0	0	0	0	5
43	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2

+ = Inhibition of growth of strain; - = no inhibition of growth of strain.

Table III. Epidemiological survey of the types of *M. morgani* strains isolated from patients submitting several specimens

Case no.	Sex	Age	Date of sampling	Strain no. of <i>M. morgani</i> isolated	<i>Morganocin</i> type and sensitivity of isolate
1	M	1 year	10th Sep	20	1M
			10th Sep	21	1M
			10th Sep	22	1M
2	F	29 years	2nd Nov	72	40Q
			3rd Nov	73	40Q
3	M	3 months	12th Sep	24	0N
			14th Sep	25	0N
			15th Sep	28	0N
			15th Sep	29	0N
4a	M	6 months	22nd Oct	57	25R
			22nd Oct	60	25R
			28th Oct	65	25R
			8th Nov	76	25R
4b	F	26 years	22nd Oct	59	25R
			28th Oct	66	0Q
			2nd Nov	69	0Q
			2nd Nov	70	0Q
4c	M	...	22nd Nov	93	25R
5	M	75 years	10th Nov	78	0A
			19th Nov	90	0A
6	F	...	15th Nov	86	1M
			26th Nov	98	1M
			26th Nov	99	1M
7	F	8 months	5th May	5	1A
			5th Jun	14	1A
8	M	...	18th Jun	12	11B
	M	...	30th Apr	13	10H
9	M	55 years	29th Sep	41	18R
			6th Oct	45	17R

bation. These findings corroborated those of Coetzee (1967) who found that only 12 (12.7%) of 94 strains of *M. morgani* formed morganocin on MacConkey agar and none produced morganocin on nutrient agar or in broth. These findings may have discouraged others from investigating the method further and suggest that the optimum conditions for producing morganocins and detecting them are different.

By growing strains in the presence of mitomycin C in conditions whereby morganocin production could be induced it was found that 82.5% of strains were morganocinogenic. Various liquid media, times and temperatures were examined and the method given was the one which consistently gave the highest yields of morganocins. Irradiation of preparations successfully inactivated any concomitantly induced phage and on only one occasion was

there a suspicion of phage activity which was not recorded as indicating morganocin activity.

To make detection of morganocin as sensitive as possible it was found necessary to use the minimum number of indicator cells required to give a semi-confluent streak and grow them under conditions where morganocin receptor production was not so profuse as to cause a small number of cells to absorb most of the morganocin and leave some sensitive cells unaffected by the remaining sub-lethal amounts of morganocin. This would make sensitive cells appear to be resistant. Rich media such as MacConkey agar and optimum growth temperatures of 37°C were therefore found to give unsatisfactory and misleading results. The method and conditions adopted—a diluted inoculum on the nutritionally poorer Lab-lemco agar plates incubated at 25–30°C—were satisfactory for detecting

morganocin activity because morganocin sensitivity was found to be a characteristic of 97.5% of the strains.

The application of these methods to the typing of strains by morganocin production was successful in that most (82.5%) of the 160 strains produced morganocins that remained active over many months. Through the use of only 23 of the 160 strains as indicators, 50 different morganocin types could be distinguished.

It was disappointing to find that 35% of the strains belonged to morganocin type 1. This may be a characteristic of the local strains but it suggested that typing by morganocin production alone might not be sufficiently discriminating in some instances. Typing of strains by morganocin sensitivity, possible with nearly all (97.5%) of the strains, was difficult because of its extreme complexity on account of the sensitivity of most strains to many

different morganocins. However, the compromise of determining the sensitivity of strains to a selected range of seven different morganocin preparations provided the extra discrimination needed, particularly by strains that produced type 1, without increasing the number of strains required for the typing scheme. The typing scheme permitted 160 strains to be differentiated into 90 types and it provided greater discrimination than that offered by other schemes.

The application of the method in a limited epidemiological survey showed it to be stable, reproducible and discriminating and indicated that, in certain instances, strains of *M. morgani* can persist in the bowel for several weeks. The typing method is now to be used to investigate whether certain types of *M. morgani* are the cause of diarrhoea as Morgan (1906) believed.

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