R Factors from *Serratia marcescens*

By R. W. HEDGES, V. RODRIGUEZ-LEMOINE* AND
NAOMI DATTA

*Department of Bacteriology, Royal Postgraduate Medical School,
Hammersmith Hospital, London W12 0HS*

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SUMMARY

R factors transmissible to *Escherichia coli* from naturally occurring strains of *Serratia marcescens* belonged either to compatibility groups (S and L) not represented amongst plasmids reported in other genera or to groups (C, FII, P and M) notable for their wide host range.

INTRODUCTION

In recent years, *Serratia marcescens* has become established in certain localities as an agent of hospital infection and cross-infection (Clayton & von Graevenitz, 1966; Wilfert, Barrett & Kass, 1968; Davis, Foltz & Blakemore, 1970; Wilkowske, Washington, Martin & Ritts, 1970). In general, strains of *S. marcescens* isolated from infective lesions differ from those from other sources in being non-pigmented and antibiotic resistant (Ewing, Johnson & David, 1962; Clayton & von Graevenitz, 1966). Medeiros & O'Brien (1969) and Schaeffer *et al.* (1971) described strains of *S. marcescens*, isolated from hospital patients, which were able to transfer R factors to *Escherichia coli*. Transfer of resistance to *E. coli* has also been reported from strains of *S. marcescens* isolated in France (Grimont & Dulong de Rosnay, 1972; Scavizzi, 1972; Lemosquet-Villemon, Morel & Freymuth, 1973).

We have collected strains of *S. marcescens*, most, but not all, clinical isolates, from widely separate geographical areas; each strain was tested for antibiotic resistance and for R factors transmissible to *E. coli* K12. The R factors were classified by compatibility in K12 (Datta, 1974). Our purpose was to find out how much of the antibiotic resistance observed in *S. marcescens* was determined by such R factors and to what extent the set of R factors found in *S. marcescens* is characteristic of that genus and to what extent it is shared with other bacterial genera (Coetzee, Datta & Hedges, 1972; Datta & Hedges, 1972*a*; Hedges, 1974).

The R factors described by Medeiros & O'Brien (1969) and Lemosquet-Villemon *et al.* (1973) were included in this study.

METHODS

Serratia marcescens. The sources of the 236 strains of *S. marcescens* studied are shown in Table 1.

Antibiotic resistance patterns were scored using a disc method (Hedges, 1974).

Transfer of resistance to Escherichia coli K12. A rifampicin-resistant mutant of *E. coli* K12, J62-2 (Hedges, 1974) was used as recipient in tests for transfer. Methods were as described by Coetzee *et al.* (1972).

* Present address: Department of Botany and Microbiology, University College, London, W.C. 1.

Table 1. *Origin of strains of Serratia marcescens*

Donor	No. of strains	No. which transferred R factors to <i>E. coli</i> K12
Dr W. A. Black, University Hospital, London, Ontario, Canada	19	2
Mrs R. Doherty, Shaughnessy Hospital, Vancouver, B.C., Canada	11	0
Dr Y. Goodman, University of Alberta, Edmonton, Canada	8	0
Dr F. A. Holden, University Hospital, Saskatoon, Sask., Canada	1	0
Mrs C. A. Johnston, Royal Jubilee Hospital, Victoria, B.C., Canada	2	0
Dr B. Brisou, Hôpital d'Instruction des Armées, Toulon, France	33	3
Dr P. A. D. Grimont, Université de Bordeaux, France	12	5
Dr C. Morel, Centre Hospitalier Universitaire, Caen, France	2	2
Dr J. Borowski, University Medical School, Bialystok, Poland	9	0
Mr R. Howden, The Children's Hospital, Sheffield	1	0
Dr E. J. L. Lowbury, Birmingham Accident Hospital	4	0
Dr Soad Tabaqchali, St Bartholomew's Hospital, London	10	1
Dr Donna J. Blazevec, University of Minnesota, Minneapolis, U.S.A.	3	0
Dr J. C. Crowder, Indiana University, Indianapolis, U.S.A.	6	0
Dr G. A. Jacoby, Harvard Medical School, Boston, U.S.A.	27	0
Dr A. A. Medeiros, Peter Bent Brigham Hospital, Boston, U.S.A.	20	16
Dr J. E. Rosenblatt, V. A. Hospital (Wadsworth), Los Angeles, U.S.A.	8	0
Dr J. A. Washington, Mayo Clinic, Rochester, U.S.A.	50	0
Dr J. N. Wilfert, University of Utah, Salt Lake City, U.S.A.	10	0
Total	236	29

Classification of R factors by compatibility was as described by Coetzee *et al.* (1972). Bacterial host strains were J53 and J62 (Clowes & Hayes, 1968). Standard plasmids representing the various compatibility groups were as listed by Hedges (1974).

RESULTS AND DISCUSSION

Serratia marcescens isolates

The sources of strains are listed in Table 1; most came from the U.S.A., France and Canada. We do not claim that our sample accurately reflects the world-wide distribution of *S. marcescens* from clinical sources, but it was evident that the distribution was very uneven. In some hospitals, notably in the U.S.A., infection with *S. marcescens* has presented an important clinical problem (see, for example, Davis *et al.* 1970).

At Hammersmith Hospital, in a diagnostic laboratory handling some 60 000 clinical cultures each year, only one or two isolations have been made per annum. A similar low incidence was found at Glasgow Royal Infirmary (Black, 1970). Information from colleagues in various parts of the country and, informally, from the Medical Research Council's Committee on Hospital Infection indicated that this was generally true in the U.K. A similar low incidence was reported to us from hospitals as far apart as Canada (personal communications from Dr F. A. Holden, University Hospital, Saskatoon, Mrs C. A. Johnston, Royal Jubilee Hospital, Victoria, and Dr D. H. Starkey, Queen Mary Veterans Hospital, Montreal) and Hong Kong (Dr C. H. Teoh-Chan, University of Hong Kong, personal communication). The variations in incidence cannot be accounted for solely by differences in methods of identification and classification of bacteria in different laboratories (Black, 1970). Isolates sent to us from the U.S.A. were identified at Hammersmith Hospital as *S. marcescens* by methods in routine daily use. We were told of at least two other cross checks of this kind

Table 2. *R factors from Serratia marcescens arranged by compatibility group*

R factors given the same number but designated a or b (e.g. R476a and R476b) were derived from a single strain of *S. marcescens*. Abbreviations for resistance markers: A, ampicillin; S, streptomycin; T, tetracycline; C, chloramphenicol; K, kanamycin; G, gentamicin; Su, sulphonamide. Abbreviations for origins (see Table 1): B, Dr A. A. Medeiros, Boston, U.S.A.; T, Dr B. Brisou, Toulon, France; C, Dr C. Morel, Caen, France; L, Dr S. Tabaqchali, London; G, Dr P. A. D. Grimont, Bordeaux, France; O, Dr. W. A. Black, Ontario, Canada.

R factor	Resistance pattern	Origin	R factor	Resistance pattern	Origin
Group L			Group FII		
R471	ACK	B	R83ob	STCSu	B
R472	A	B	Group M		
R473	A	B	RS28	ASTCG	C
R474	A	B	R957	A	O
R475	A	B	R958	A	O
R476a	A	B	R930*	A	
R479a	A	B	Group P		
R830a	SK	B	R839	ASTKSu	L
R831	SK	B	R934	ATK	G
R832	A	B	Group C		
Group S			R935	ASTCKSu	G
R476b	STSu	B	R936	ASTCKSu	G
R477	STCKSu	B	R937	ASTCKSu	G
R478	TCK	B	R938	ASTCKSu	G
R479b	STCKSu	B			
R826	ASTCKG	T			
R827	ASTCKG	T			
R828	ASTCKG	T			
R829	STCK	B			

* R930 was derived from a strain of *S. liquefaciens* isolated at Hammersmith Hospital.

(MRC Committee on Hospital Infection and D. H. Starkey, personal communication) with similar results.

Most of the strains (224) were from clinical material; among these only 13 produced pigment. [Pigment was observed in cultures on nutrient agar (Oxoid No. 2 broth, solidified with Oxoid New Zealand agar); special media to encourage pigment production were not used.] The remaining 12 strains, provided by Dr B. Brisou, Hôpital d'Instruction des Armées, Toulon, France, were isolated from river and sea water; all of these were pigmented.

Antibiotic resistance. Most strains (75 %) were resistant to polymyxin B, and 70 strains (30 %) were resistant to no other drug besides polymyxin B; 119 strains (50 %) were resistant to ampicillin, and of these, 50 (20 % of the total) were also resistant to carbenicillin; 42 % were tetracycline resistant, 19 % kanamycin resistant and 16 % chloramphenicol resistant.

These figures are from tests in our laboratory: information from the laboratories where the strains were isolated indicated that larger numbers (the proportion cannot be accurately estimated) had been resistant to more antibiotics on first isolation. Spontaneous loss of resistance suggests the presence of plasmids which were unstable in their host bacteria. Medeiros & O'Brien (1969) recognized a high rate of spontaneous loss of multiple resistance in their *S. marcescens* isolates, which we have confirmed both with their strains and others.

R factors transferred to Escherichia coli K12

The 166 strains of *S. marcescens* which were resistant to some drug or drugs other than polymyxin B were tested for transfer of resistance to *E. coli* K12. Twenty-seven R factors were

detected; all came from strains isolated from clinical material. This number includes 15 whose transfer had been demonstrated by Medeiros & O'Brien (1969). In addition, Dr C. Morel provided us with eight R factors, derived from strains of *S. marcescens*, already transferred to *E. coli* K12 (Lemosquet-Villemon *et al.* 1973). The R factors studied are listed in Table 2.

Compatibility groups of R factors

Among the R factors studied were the prototypes of two new groups, S and L. Plasmids of groups S and L have, so far, never been found in naturally occurring bacteria other than *S. marcescens*.

Group L. All the R factors of group L came from one source, the Peter Bent Brigham Hospital, Boston, U.S.A. They conferred three different patterns of resistance and were incompatible with one another but compatible with plasmids of all other defined groups.

Group S. Plasmids of group S were temperature sensitive in some component of their transfer property (Rodriguez-Lemoine, Jacob, Hedges & Datta, 1975). They occurred in strains isolated in Boston and Toulon. They determined various resistance patterns; in those from France, this included resistance to gentamicin.

Other groups. RS28, taken as the exemplar of the R factors identified by Lemosquet-Villemon *et al.* (1973), belonged to group M. R factors from *S. marcescens* from Ontario also belonged to this group. A strain of *Serratia liquefaciens* (Ewing, Davis & Tife, 1972) isolated at Hammersmith Hospital also carried an R factor of group M.

Four R factors from strains of *S. marcescens* sent from Bordeaux belonged to group C. Although conferring chloramphenicol resistance, these plasmids did not determine the production of chloramphenicol transacetylase (Shaw, 1971; Nagai & Mitsuhashi, 1972; Mrs L. C. Sands, personal communication).

One R factor from Boston belonged to group FII and two, one from Bordeaux and one from London, belonged to group P.

The R factor set for S. marcescens

The plasmids from the Peter Bent Brigham Hospital belonged to three compatibility groups, two of which had not previously been described. In conformity with Schaeffler *et al.* (1971), we believe that this indicates that the *S. marcescens* population of this hospital is ecologically isolated from other *Serratia* populations, including that in Boston City Hospital. The ecological isolation, however, is evidently not complete since in the same hospital, one strain of *S. marcescens* carried an R factor of group FII, very similar to plasmids isolated from several other genera (Harada, Suzuki, Kameda & Mitsuhashi, 1960), and R factors of group S were also identified in *S. marcescens* strains from Toulon.

The other R factors identified belonged to groups P, C and M, which are known to have unusually wide host ranges among bacterial genera (Datta *et al.* 1971; Datta & Hedges, 1972*a, b*; Hedges, Datta, Coetzee & Dennison, 1973; Olsen & Shipley, 1973).

Thus the R factor set of *S. marcescens* subdivides into plasmids whose natural range seems to be limited to this species and plasmids with a markedly promiscuous distribution.

We are very grateful to all those who sent us strains of *S. marcescens* and information about its incidence in various environments, and to Mrs L. C. Sands, Department of Medicine, University of Miami, U.S.A., for testing R⁺ strains for production of chloramphenicol transacetylase.

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