J. Hyg., Camb. (1975), **75**, 121
Printed in Great Britain

Citrobacter koseri. I. An extended antigenic scheme for *Citrobacter koseri* (syn. *C. diversus*, *Levinea malonatica*)

By R. J. Gross and B. Rowe

Salmonella and Shigella Reference Laboratory, Central Public Health Laboratory, Colindale Avenue, London NW9 5HT

(*Received* 30 *January* 1975)

SUMMARY

The names *Citrobacter koseri*, *Levinea malonatica* and *Citrobacter diversus* are synonymous. As a result of the study of representative strains of these organisms an antigenic scheme comprising seven O antigens has been proposed. The examination of a further 165 strains from clinical sources suggests that an additional seven antigens should be included in the scheme. The serological and biochemical reactions of the test strains for all 14 O antigens are described.

INTRODUCTION

Several authors have described bacterial strains resembling *Citrobacter freundii* but differing in their biochemical reactions in several important respects. Frederiksen (1970) described a group of 30 strains belonging to the genus *Citrobacter*, but which differed from *C. freundii* in that adonitol was fermented, malonate was utilized, indole was produced, and there was no growth in Moeller's potassium cyanide medium; H_2S production in ferric chloride gelatin was described as weak. Frederiksen considered that these strains should be regarded as a new species and proposed the name *C. koseri* for them. Booth & McDonald (1971) examined 40 biochemically similar strains and proposed that they should be considered as a new species of *Citrobacter*. Young, Kenton, Hobbs & Moody (1971) studied 108 strains and proposed the establishment of a new genus, *Levinea*, having two species, *L. malonatica* and *L. amalonatica*. The reactions of *L. malonatica* were similar to those of *C. koseri*, but H_2S production was not detected in triple sugar iron agar (TSI). Ewing & Davis (1972) described a strain having the same biochemical reactions as *C. koseri* but considered that the name *C. diversus* (Werkman & Gillen, 1932) was an earlier synonym and should have priority.

Gross & Rowe (1974) examined representative strains from all these authors and showed that Frederiksen's *C. koseri*, Booth and McDonald's 'new species of *Citrobacter*', *L. malonatica* of Young *et al.* and *C. diversus* of Ewing and Davis formed a biochemically homogeneous group and probably all belonged to the same species. The final taxonomic position of this group remains undecided and the use of the name *C. koseri* in the present publication is for convenience only. Strains of *L. amalonatica* were also studied; they differed serologically and biochemically from the other strains and were regarded as a different species. As a

Table 1. *Biochemical reactions of* 14 *test strains of* C. koseri

Test	Strain number: NCTC 10786	E. 1597/71	NCTC 10768	E. 1558/72	NCTC 10769	NCTC 10770	E. 1599/71	E. 2641/72	E. 2567/72	E. 2576/72	E. 2569/72	E. 2577/72	E. 508/74	E.1020/74
Motility (Craigie tube)	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Methyl-red test: 37° C.	$+^{2}$	$+^{2}$	$+^{2}$	$+^{2}$	$+^{2}$	$+^{2}$	$+^{2}$	$+^{2}$	$+^{2}$	$+^{2}$	$+^{2}$	$+^{2}$	$+^{2}$	$+^{2}$
20° C.	$+^{5}$	$+^{5}$	$+^{5}$	$+^{5}$	$+^{5}$	$+^{5}$	$+^{5}$	$+^{5}$	$+^{5}$	$+^{5}$	$+^{5}$	$+^{5}$	$+^{5}$	$+^{5}$
Voges–Proskauer reaction 37° C.	$-^{2}$	$-^{2}$	$-^{2}$	$-^{2}$	$-^{2}$	$-^{2}$	$-^{2}$	$-^{2}$	$-^{2}$	$-^{2}$	$-^{2}$	$-^{2}$	$-^{2}$	$-^{2}$
20° C.	$-^{5}$	$-^{5}$	$-^{5}$	$-^{5}$	$-^{5}$	$-^{5}$	$-^{5}$	$-^{5}$	$-^{5}$	$-^{5}$	$-^{5}$	$-^{5}$	$-^{5}$	$-^{5}$
Simmons citrate	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$
Malonate	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$
Growth in potassium cyanide	$-^{2}$	$-^{2}$	$-^{2}$	$-^{2}$	$-^{2}$	$-^{2}$	$-^{2}$	$-^{2}$	$-^{2}$	$-^{2}$	$-^{2}$	$-^{2}$	$-^{2}$	$-^{2}$
Indole (Kovac's)	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$
H_2S (TSI)	$-^{7}$	$-^{7}$	$-^{7}$	$-^{7}$	$-^{7}$	$-^{7}$	$-^{7}$	$-^{7}$	$-^{7}$	$-^{7}$	$-^{7}$	$-^{7}$	$-^{7}$	$-^{7}$
Gluconate	$-^{2}$	$-^{2}$	$-^{2}$	$-^{2}$	$-^{2}$	$-^{2}$	$-^{2}$	$-^{2}$	$-^{2}$	$-^{2}$	$-^{2}$	$-^{2}$	$-^{2}$	$-^{2}$
Christensen's urea	$+^{2}$	$+^{3}$	$+^{7}$	$+^{1}$	$+^{3}$	$+^{1}$	$+^{4}$	$+^{3}$	$+^{2}$	$+^{2}$	$+^{2}$	$+^{2}$	$+^{1}$	$+^{2}$
Phenylalanine deaminase	$-^{1}$	$-^{1}$	$-^{1}$	$-^{1}$	$-^{1}$	$-^{1}$	$-^{1}$	$-^{1}$	$-^{1}$	$-^{1}$	$-^{1}$	$-^{1}$	$-^{1}$	$-^{1}$
Gelatin (stab)	$-^{28}$	$-^{28}$	$-^{28}$	$-^{28}$	$-^{28}$	$-^{28}$	$-^{28}$	$-^{28}$	$-^{28}$	$-^{28}$	$-^{28}$	$-^{28}$	$-^{28}$	$-^{28}$
Arginine decarboxylase	$+^{1}$	$+^{1}$	$+^{1}$	$+^{3}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{2}$	$+^{2}$	$+^{2}$	$+^{3}$	$+^{2}$	$+^{2}$	$+^{2}$
Lysine decarboxylase	$-^{4}$	$-^{4}$	$-^{4}$	$-^{4}$	$-^{4}$	$-^{4}$	$-^{4}$	$-^{4}$	$-^{4}$	$-^{4}$	$-^{4}$	$-^{4}$	$-^{4}$	$-^{4}$
Ornithine decarboxylase	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$
βgalactosidase (ONPG)	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$
Glucose (acid)	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$
Glucose (gas)	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$
Lactose	$+^{7}$	$+^{4}$	$+^{7}$	$-^{14}$	$+^{7}$	$+^{7}$	$+^{7}$	$+^{3}$	$+^{7}$	$+^{7}$	$+^{7}$	$+^{3}$	$+^{3}$	$-^{14}$
Mannitol	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$
Sucrose	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$-^{14}$	$-^{14}$	$-^{14}$	$-^{14}$	$+^{1}$	$-^{14}$	$-^{14}$	$+^{1}$	$-^{14}$	$-^{14}$
Salicin	$+^{3}$	$+^{3}$	$+^{3}$	$+^{7}$	$+^{2}$	$+^{3}$	$+^{3}$	$+^{3}$	$+^{4}$	$+^{3}$	$+^{3}$	$+^{2}$	$+^{4}$	$+^{3}$
Dulcitol	$+^{1}$	$+^{1}$	$-^{14}$	$-^{14}$	$-^{14}$	$-^{14}$	$+^{1}$	$-^{14}$	$-^{14}$	$-^{14}$	$+^{1}$	$+^{1}$	$-^{14}$	$-^{14}$
Inositol	$-^{14}$	$-^{14}$	$-^{14}$	$-^{14}$	$-^{14}$	$-^{14}$	$-^{14}$	$-^{14}$	$-^{14}$	$-^{14}$	$-^{14}$	$-^{14}$	$-^{14}$	$-^{14}$
Adonitol	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$

Table 1 (*cont.*)

	Strain number													
	NCTC 10786	E. 1597/ 71	NCTC 10786	E. 1558/ 72	NCTC 10769	NCTC 10770	E. 1599/ 71	E. 2641/ 72	E. 2567/ 72	E. 2576/ 72	E. 2569/ 72	E. 2577/ 72	E. 508/ 74	E. 1020/ 74
Raffinose	$-^{14}$	$-^{14}$	$-^{14}$	$-^{14}$	$-^{14}$	$-^{14}$	$-^{14}$	$-^{14}$	$-^{14}$	$-^{14}$	$-^{14}$	$-^{14}$	$-^{14}$	$-^{14}$
Sorbitol	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$
Arabinose	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$
Rhamnose	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$
Xylose	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$
Trehalose	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$
Inulin	$-^{14}$	$-^{14}$	$-^{14}$	$-^{14}$	$-^{14}$	$-^{14}$	$-^{14}$	$-^{14}$	$-^{14}$	$-^{14}$	$-^{14}$	$-^{14}$	$-^{14}$	$-^{14}$
Glycerol	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$
Cellobiose	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$
Sorbose	$+^{3}$	$+^{4}$	$+^{7}$	$+^{7}$	$-^{14}$	$+^{7}$	$+^{7}$	$+^{7}$	$+^{7}$	$+^{7}$	$+^{4}$	$+^{3}$	$+^{7}$	$+^{7}$
Maltose	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{1}$	$+^{2}$	$+^{1}$	$+^{1}$

Superscript numerals represent days of incubation.

result of these studies an antigenic scheme comprising seven O antigens was proposed for *C. koseri*.

The present study concerned 165 strains from clinical sources. All were shown to have the biochemical reactions of *C. koseri* and 155 could be serotyped with the existing scheme (Rowe, Gross & Allen, 1975). Further examination of the remaining strains suggested that a further seven O antigens should be added to the scheme. The biochemical and serological reactions of test strains for all 14 O antigens are described below.

MATERIALS AND METHODS

Strains

Fourteen strains of *C. koseri* were selected as test strains. Strains NCTC 10786, NCTC 10768, NCTC 10769 and NCTC 10770 were obtained from the National Collection of Type Cultures, Colindale, London. NCTC 10786 had been deposited as a representative of *C. koseri* as described by Frederiksen (1970) and NCTC 10768, NCTC 10769 and NCTC 10770 were representatives of a new *Citrobacter* sp. described by Booth & McDonald (1971). Strains E. 1597/71, E. 1558/72, E. 1599/71, E. 2641/72, E. 2567/72, E. 2576/72, E. 2569/72, E. 2577/72, E. 508/74 and E. 1020/74 were received at the Salmonella and Shigella Reference Laboratory, Colindale, London, having been isolated from clinical specimens in the United Kingdom.

Biochemical tests

The biochemical reactions of the 14 test strains were determined using the methods of Cowan & Steel (1965).

Preparation of antisera

O antigen suspensions were prepared by harvesting the growth from two nutrient agar slopes in 0·9 % saline and heating to 100° C. for 2½ hr. After centrifugation the heated organisms were resuspended in 15 ml. saline and commercial formalin was added to a final concentration of 0·3 %. Rabbits were immunized by intravenous injections of 0·5 ml., 1·0 ml., 2·0 ml., 2·0 ml., and 2·0 ml. at five day intervals; 40 ml. blood was taken at 5 and 10 days after the final injection, and the rabbits were exsanguinated 5 days later. The products of all three bleedings were pooled.

Bacterial agglutination

Agglutination tests were performed in perspex agglutination trays and were incubated at 50° C. for 16 h. O antigen suspensions for agglutination tests were prepared by heating overnight broth-cultures at 100° C. for 30 min.; commercial formalin was added to a final concentration of 0·3 %. Antisera for all 14 strains were tested against all 14 O antigen suspensions and their homologous and heterologous titres determined.

Table 2. *Serological reactions of* 14 *test strains of* C. koseri

Serum prepared against strain no.	Agglutination titre against suspension of strain number													
	NCTC 10786	E. 1597/71	NCTC 10768	E. 1558/72	NCTC 10769	NCTC 10770	E. 1599/71	E. 2641/72	E. 2567/72	E. 2576/72	E. 2569/72	E. 2577/72	E.508/74	E.1020/74
NCTC 10786	12800	200	1600	3200	—	—	—	100	—	—	—	—	—	—
E. 1597/71	6400	800	200	6400	—	—	—	—	—	—	—	—	—	—
NCTC 10768	800	—	25600	800	—	—	—	—	—	—	—	—	—	—
E. 1558/72	25600	—	400	25600	—	—	—	—	—	—	—	—	—	—
NCTC 10769	—	—	—	—	25600	—	—	—	—	—	—	—	1600	—
NCTC 10770	—	—	—	—	—	12800	—	1600	—	—	—	—	—	—
E. 1599/71	—	—	—	—	—	—	1600	—	400	—	400	—	—	—
E. 2641/72	—	400	—	—	—	12800	—	3200	—	—	—	—	—	—
E. 2567/72	—	—	—	—	—	—	1600	—	1600	—	800	—	—	—
E. 2576/72	—	—	—	—	—	—	—	—	—	1600	—	—	—	—
E. 2569/72	—	—	—	—	—	—	800	—	800	—	1600	—	—	—
E. 2577/72	—	—	—	—	—	—	—	—	—	—	—	1600	—	—
E. 508/74	—	—	—	—	3200	—	—	—	—	200	—	—	3200	—
E. 1020/74	—	—	—	—	—	—	—	—	—	—	—	—	—	6400

Table 3. *Antigenic scheme for* C. koseri

O antigen	Test strain	Absorptions required to prepare pure antiserum
O1	NCTC 10786	O2 and O4
O2	E 1597/71	O1
O3	NCTC 10768	O1 and O2
O4	E 1558/72	O1
O5	NCTC 10769	O13
O6	NCTC 10770	O8
O7	E 1599/71	O9
O8	E 2641/72	O2 and O6
O9	E 2567/72	O11
O10	E 2576/72	none
O11	E 2569/72	O9
O12	E 2577/72	none
O13	E 508/74	O5
O14	E 1020/74	none

Absorption studies

Where cross-reactions were found among the test strains, reciprocal absorptions were performed to determine the relations between the strains. Where necessary specific antisera were prepared by absorbing with suspensions of the cross-reacting strains.

RESULTS

Biochemistry

All the 14 test strains were gram-negative rods. They were oxidase negative, catalase positive, fermentative in the Hugh and Liefson test, and reduced nitrate. Their remaining reactions are summarized in Table 1.

Agglutination reactions

There were extensive serological cross-reactions among the 14 strains (Table 2) but specific antisera could be prepared as shown in Table 3.

Proposed O antigen scheme

It is proposed that the specific O antigen components of the 14 test strains be designated as *C. koseri* O1 to O14 and form the basis of a serotyping scheme for this species (Table 3).

DISCUSSION

The widespread use of serotyping techniques in the study of the family Enterobacteriaceae has contributed greatly to the understanding of the epidemiology and taxonomy of these organisms. Kauffmann's (1947) antigenic scheme for *Escherichia coli* included 25 O groups; the scheme has now been expanded to include more than 150 O groups and has been invaluable in the understanding of the ecology of *E. coli* in man and animals. The Kauffmann–White scheme for

Salmonella contains numerous serotypes and has made possible the detailed study of the epidemiology of this organism throughout the world. There are already some indications that *C. koseri* may be of clinical importance; Gross, Rowe & Easton (1973) and Gwynn & George (1973) have described outbreaks of neonatal meningitis associated with this species. The development and application of an antigenic scheme for *C. koseri* is a necessary step towards a better understanding of its pathogenic role.

REFERENCES

Booth, E. V. & McDonald, S. (1971). A new group of enterobacteria, possibly a new *Citrobacter* sp. *Journal of Medical Microbiology* **4**, 329.

Cowan, S. T. & Steel, K. J. (1965). *Manual for the Identification of Medical Bacteria.* Cambridge University Press.

Ewing, W. H. & Davis, B. R. (1972). Biochemical characterisation of *Citrobacter diversus* (Burkey) Werkman and Gillen and designation of the neotype strain. *International Journal of Systematic Bacteriology* **22**, 12.

Frederiksen, W. (1970). *Citrobacter koseri*, a new species within the genus *Citrobacter*, with a comment on the taxonomic position of *Citrobacter intermedium* (Werkman and Gillen). *Publications of the Faculty of Science of the University J. E. Purkinye, Brno* **47**, 89.

Gross, R. J. & Rowe, B. (1974). The serology of *Citrobacter koseri*, *Levinea malonatica* and *Levinea amalonatica*. *Journal of Medical Microbiology* **7**, 155.

Gross, R. J., Rowe, B. & Easton, J. A. (1973). Neonatal meningitis caused by *Citrobacter koseri*. *Journal of Clinical Pathology* **26**, 138.

Gwynn, C. M. & George, R. H. (1973). Neonatal citrobacter meningitis. *Archives of Disease in Childhood* **48**, 455.

Kauffmann, F. (1947). The serology of the coli group. *Journal of Immunology* **57**, 71.

Rowe, B., Gross, R. J. & Allen, H. A. (1975). *Citrobacter koseri*. II. Serological and biochemical examination of *Citrobacter koseri* strains from clinical specimens. *Journal of Hygiene* **75**, 129.

Werkman, C. H. & Gillen, G. F. (1932). Bacteria producing trimethylene glycol. *Journal of Bacteriology* **23**, 167.

Young, V. M., Kenton, D. M., Hobbs, B. J. & Moody, M. R. (1971). *Levinea*, a new genus of the family Enterobacteriaceae. *International Journal of Systematic Bacteriology* **21**, 58.