Production of Enterobacterial Common Antigen as an Aid to Classification of Newly Identified Species of the Families *Enterobacteriaceae* and *Vibrionaceae*

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A study on the production of enterobacterial common antigen by recently described or as-yet-undescribed species belonging to the families *Enterobacteriaceae* and *Vibrionaceae* was carried out by hemagglutination and hemagglutination inhibition tests with rabbit antisera specific for this antigen. All 28 strains known or presumed to belong to *Enterobacteriaceae* produced this antigen, and none of the seven strains belonging to *Vibrionaceae* did so. The results obtained with *Tatumella ptyseos* and *Xenorhabdus* species are particularly noteworthy, since they represent species which are atypical for *Enterobacteriaceae*. It is suggested that the determination of the production of enterobacterial common antigen is a significant aid to classification, particularly when the assignment of a new genus or species to *Enterobacteriaceae* or to another family presents a problem.

Enterobacterial common antigen (ECA), which is common to members of the family *Enterobacteriaceae*, was first described by Kunin et al. (16, 17). As reviewed by Mäkelä and Mayer (22), this antigen is produced by numerous members of the family *Enterobacteriaceae* but not by species belonging to other families. A few strains belonging to the family *Enterobacteriaceae* do not produce the antigen because of a documented or presumed mutation. The only species of *Enterobacteriaceae* which apparently does not produce ECA is *Erwinia chrysanthemi* (20). As far as species belonging to other families are concerned, a possible exception to the rule is strains previously identified as *Aeromonas shigelloides* and presently listed as *Plesiomonas shigelloides* which do produce ECA (20, 29). In view of the possible usefulness of ECA production in the classification of organisms at the family level, recently described and undescribed species belonging to the families *Enterobacteriaceae* and *Vibrionaceae* were investigated.

In this study, strains of 35 newly described or as-yet-unnamed species of *Enterobacteriaceae* and *Vibrionaceae* were tested for the presence of ECA.

MATERIALS AND METHODS

All strains were from the culture collection of the Enteric Diseases Reference Laboratories in the Enteric Bacteriology Section, Enteric Bacteriology and

Epidemiology Branch, Centers for Disease Control. Type strains or deoxyribonucleic acid (DNA) reference strains were used for all species. The sources of the strains are given in Table 1. Strains were coded at Atlanta, Ga., and tested blind in Buffalo, N.Y. The codes given to these strains were not in the order shown in Table 1. References to the recently described species are given in Table 1. Enteric groups refer to potential new species now under study in the Enteric Diseases Reference Laboratories. DNA groups refer to new species that have not yet been published or named.

All strains were seeded onto chocolate agar and incubated at either 20 or 36°C, depending upon the temperature optima of individual species. Since all strains produced heavy confluent growth on chocolate agar, suspensions were made from this culture medium incorporating the growth of three agar plates each in 6 ml of phosphate hemagglutination buffer (Difco). Based on serial dilution, the number of organisms in each suspension was approximately 10^{12} cells per ml. The suspensions were heated in boiling water for 1 h and centrifuged at $23,500 \times g$ for 10 min. The supernatants were used as antigens. For control purposes, supernatants of an ECA-positive *Escherichia coli* strain and an ECA-negative *Pseudomonas aeruginosa* strain were used, and these preparations yielded the expected results.

The hemagglutination test was carried out as described previously (23). Briefly, to the sediment of three-times-washed human erythrocytes (blood group O; Rh-negative; 2.5% suspension) was added antigen in a dilution of 1:10. The suspension was incubated at 37°C and washed three times to remove excess antigen. The antigenically modified erythrocytes (0.2 ml)

TABLE 1. Production of ECA in 35 recently described species or groups of *Enterobacteriaceae* and *Vibrionaceae*

Organism	Reference	Source ^a	ECA present
<i>Cedecea davisae</i> 3278-77 (CIP 80.34, ATCC 33431)	7	CDC	+
<i>Cedecea lapagei</i> 485-76 (CIP 80.35, ATCC 33432)	7	CDC	+
<i>Edwardsiella hoshinae</i> 2169-80 (CIP 78.56, ATCC 33379)	9	P. A. D. Grimont	+
<i>Edwardsiella ictaluri</i> 1976-78 (GA-77-52, ATCC 33202)	11	J. P. Hawke	+
<i>Enterobacter amnigenus</i> 1325-79 (CUETM 78-118, ATCC 33072)	15	H. Leclerc	+
<i>Enterobacter gergoviae</i> 604-77 (CIP 76.01, ATCC 33028)	1	C. Richard	+
<i>Enterobacter intermedius</i> 2992-79 (Leclerc 77-148)	14	H. Leclerc	+
<i>Escherichia blattae</i> 9005-74	2	N. R. H. Burgess	+
<i>Hafnia</i> DNA group 2 3226-74	26	CDC	+
<i>Kluyvera ascorbata</i> 648-74 (ATCC 33433)	5	CDC	+
<i>Kluyvera cryocrescens</i> 2065-78 (ATCC 33435)	5	CDC	+
<i>Obesumbacterium proteus</i> 1496-74 (Priest 502, NCIB 8771)	25	F. G. Priest	+
<i>Proteus</i> DNA group 1808-73		CDC	+
<i>Providencia</i> DNA group 132-68		CDC	+
<i>Serratia ficaria</i> 1165-79 (CIP 79.23, ICPB 4050, ATCC 33105)	10	P. A. D. Grimont	+
<i>Serratia fonticola</i> 2988-79 (ATCC 29844)	6	H. Leclerc	+
<i>Serratia odorifera</i> 1979-77 (Grimont 1073, ICPB 3995)	8	P. A. D. Grimont	+
<i>Tatumella ptyseos</i> H36 (9591-78, D6168, ATCC 33301)	13	CDC	+
<i>Xenorhabdus luminescens</i> 9016-80 (Thomas Hb, ATCC 29999)	28	G. M. Thomas	+
<i>Xenorhabdus nematophilus</i> 1184-80 (ATCC 19061)	28	G. M. Thomas	+
<i>Xenorhabdus</i> DNA group 3 1195-80 (Thomas NC-19, ATCC 29304)	28	G. M. Thomas	+
<i>Xenorhabdus</i> DNA group 4 9014-80 (Thomas DN)	28	G. M. Thomas	+
Enteric group 1 875-72		CDC	+
Enteric group 10 1350-81		CDC	+
Enteric group 11 980-72		CDC	+
Enteric group 19 1042-80		CDC	+
Enteric group 45 329-73		CDC	+
Enteric group 46 2896-78		CDC	+
<i>Aeromonas sobria</i> 9538-76 (Popoff 278, CIP 7433)	24	M. Popoff	-
<i>Vibrio damsela</i> 2588-80 (ATCC 33539)	21	M. Love	-
<i>Vibrio fluvialis</i> 9555-78 (VL 5125, NCTC 11327)	19	J. V. Lee	-
" <i>Vibrio hollisae</i> " 75-80 (ATCC 33564) ^b	12	CDC	-
<i>Vibrio metschnikovii</i> 1316-78 (NCTC 8443)	18	NCTC	-
<i>Vibrio mimicus</i> 1721-77 (ATCC 33653)	3	CDC	-
<i>Vibrio vulnificus</i> 9107-79 (ATCC 27562)	4	ATCC	-

^a CDC, Centers for Disease Control, Atlanta, Ga.; ATCC, American Type Culture Collection, Rockville, Md.; NCTC, National Collection of Type Cultures, London, England.

^b Not validly published to date.

were added to equal volumes of ECA antiserum in twofold serial dilutions. The mixtures were incubated for 30 min in a 37°C water bath, and hemagglutination was read grossly after centrifugation at $1,300 \times g$ for 2 min.

The hemagglutination inhibition test was performed as follows. To ECA antiserum in twofold serial dilutions (0.2 ml) were added equal amounts of undiluted antigen. The mixtures were incubated for 30 min in a 37°C water bath. Erythrocytes modified with ECA obtained from *E. coli* were added, and the hemagglutination test was completed as described above.

ECA antiserum was obtained by immunization of rabbits with either *E. coli* O14 or the ethanol-soluble ECA preparation from *Salmonella typhimurium*, as described previously (27).

The hemolysis test was done as described above except that sheep erythrocytes instead of human erythrocytes were used, and guinea pig complement in a dilution of 1:10 was added. Hemolysis was read grossly after incubation for 1 h at 37°C.

RESULTS

Table 1 shows the results of independently executed hemagglutination tests on organisms belonging to 35 bacterial species. Positive and negative control preparations yielded appropriate results. All species known or presumed to be members of the *Enterobacteriaceae* were positive for the production of ECA, and all species known or presumed to be members of the *Vibrionaceae* (genera *Vibrio* and *Aeromonas*) were negative. Hemagglutination occurred with all positive strains with *E. coli* O14 and ECA antisera in dilutions of 1:800 to 1:1,600; all other strains yielded negative results even when the antisera were used in a dilution of 1:50. Additional experiments using the hemolysis test yielded concordant results. To make certain that negative hemagglutination and hemolysis tests were due to the absence of ECA and not to its failure to become attached to erythrocytes, hemagglutination inhibition tests were carried out. The results clearly indicated that ECA was not produced by any of the negative strains. These observations suggest that production of an antigen essentially characteristic of a family may be one of many useful indicators for classification.

DISCUSSION

Seven of the species tested belonged to either *Vibrio* or *Aeromonas*. As expected, these organisms were all negative for ECA. The 28 species known or presumed to belong to the *Enterobacteriaceae* all produced ECA. The positive results were particularly gratifying for *Tatumella ptyseos* and *Xenorhabdus* species, because these species are quite atypical for *Enterobacteriaceae*. *T. ptyseos* is a polar flagellate (13), *Xenorhabdus nematophilus* is catalase negative, and *Xenorhabdus* species do not reduce nitrates (28).

These and previously obtained results (16, 17, 20, 22, 23, 27, 29) establish ECA as a powerful taxonomic tool with which to separate the *Enterobacteriaceae* from other families. The only exceptions (other than a rare negative strain of *Enterobacteriaceae*) are the negative results obtained with all six tested strains of *Erwinia chrysanthemi* (20) and the positive results obtained with six *Plesiomonas shigelloides* strains (20, 29). It is our opinion that ECA production is a valuable addition to the description of the family *Enterobacteriaceae* and should be required in those instances where the assignation of a new genus to *Enterobacteriaceae* or to another family creates a problem.

ACKNOWLEDGMENTS

We are grateful to Mary Alyce Asbury, Geraldine P. Huntley-Carter, Betty R. Davis, Frances W. Hickman, and Alma C. McWhorter for providing the strains and to Frances W. Hickman for coding the strains and decoding the results. Thanks are expressed also to Helga von Langendorff for assistance with the antigen tests.

This research was supported in part by Public Health Service grant AI 00658 from the National Institute of Allergy and Infectious Diseases (to E.N.).

REPRINT REQUESTS

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