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Qualitative and Quantitative Determination of Enterobacterial Common Antigen (ECA) with Monoclonal Antibodies: Expression of ECA by Two *Actinobacillus* Species

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The presence and quantity of the enterobacterial common antigen (ECA) in several species belonging to the family *Enterobacteriaceae* as well as to other gram-negative families were determined by a solid-phase enzyme-linked immunosorbent assay system and Western blotting by using mouse monoclonal antibodies specific for ECA. Except for *Erwinia chrysanthemi*, previously known to be an exception, all species known or presumed to belong to *Enterobacteriaceae* produced ECA (89 of 90 species). Most species not belonging to *Enterobacteriaceae* did not produce ECA (25 of 28 species), with one already known (*Plesiomonas shigelloides*) and two hitherto unknown (*Actinobacillus equuli* and *Actinobacillus suis*) exceptions. Interestingly, all strains of *P. shigelloides* produced ECA, regardless of the presence of the *Shigella sonnei* cross-reacting O antigen. Quantitation of the amount of ECA in members of the family *Enterobacteriaceae* revealed a remarkable heterogeneity among genera and species as well as within one species. We conclude that the rapid, sensitive, and reliable determination of ECA is a useful aid in taxonomic classification and may help to characterize the relatedness of the family *Enterobacteriaceae* to other families. However, a quantitative analysis of ECA appears to be without value for these purposes.

In addition to the classic O, K, and H antigens, members of the family *Enterobacteriaceae* produce an antigen common to all. This antigen, first described by Kunin et al. (12, 14) and named the enterobacterial common antigen (ECA), is a polymer of the trisaccharide repeating unit 4-, 3-ManNAcUA(1→4)-α-GlcNAc(1→3)-α-Fuc-4-NAc-1 (17), which in part is covalently linked to lipopolysaccharide (LPS) and is part of the bacterial outer membrane (14, 18, 20, 21, 27; for a review, see reference 19). ECA is commonly identified by passive hemagglutination with low-titer polyvalent antisera (13, 29). By definition, ECA is produced by members of the family *Enterobacteriaceae* but not by species belonging to other families (21). Two exceptions are known; *Erwinia chrysanthemi*, which belongs to the family *Enterobacteriaceae*, apparently does not produce ECA (16), and *Plesiomonas shigelloides*, classified as non-*Enterobacteriaceae*, was found to produce ECA (28). To prove the value of this antigen for taxonomic classification, we undertook a large survey investigating several well-known or recently classified (2, 4, 6, 9, 10) members of the family *Enterobacteriaceae* as well as members of other gram-negative families.

In this study, 90 species belonging to the family *Enterobacteriaceae* and 28 species from various other gram-negative families were tested for the presence of ECA by using monoclonal antibodies against ECA (24) in a newly developed enzyme-linked immunosorbent assay (ELISA) system which, in addition, enabled us to quantitate the amount of ECA expressed. In selected cases, the results of the ELISA were verified by Western blotting.

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MATERIALS AND METHODS

All but two strains were obtained from the culture collections of the Enteric Bacteriology Section, Meningitis and Special Pathogens Laboratory Section, or the Nosocomial Infections Laboratory Branch, Centers for Disease Control, Atlanta, Ga. Two *Erwinia tracheiphila* strains (ATCC 27004 and ATCC 33245) were obtained from the American Type Culture Collection, Rockville, Md. All strains were seeded on blood agar and incubated at the appropriate temperature in optimal CO_2 conditions. Suspensions were made by harvesting the colonies and washing in phosphate-buffered saline (PBS). The number of organisms in the suspensions was roughly estimated by a turbidimetric method that determined the optical density at 650 nm (OD_{650}) by comparison to a calibration curve obtained with a strain of *Escherichia coli*. The suspensions were adjusted to 5×10^8/ml. The use of whole bacteria was not favorable, since capsular polysaccharides as well as smooth LPS consistently interfere with the accessibility of ECA to monoclonal antibodies (1, 24). Therefore, the suspensions were heated in a steamer for 10 min at 100°C and centrifuged in an Eppendorf Minifuge at 10,000 rpm; the resulting supernatants were used as antigen. Prolonging the heat incubation up to 30 min did not increase the amount of ECA released into the supernatant (data not shown).

The ELISA was done by standard methods. A mouse monoclonal antibody specific for ECA and of immunoglobulin G2a subclass, designated 898 (24), was used as the capture antibody. Briefly, U-shaped wells of flexible polyvinyl chloride microtiter plates (Dynatech Germany,

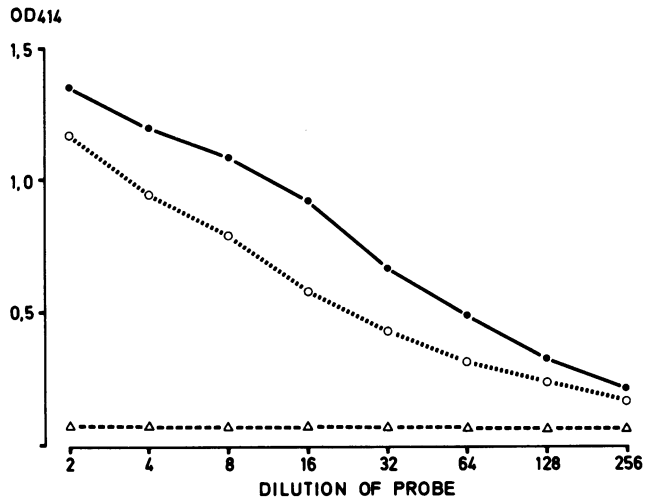

FIG. 1. Graphic representation of a typical ELISA. The OD_{414} is plotted versus the reciprocal of dilution of the probe. Symbols: ●, standard; ○, probe; △, negative control.

Nürtingen, Federal Republic of Germany) were coated with 20 μl of 898 anti-ECA (purified by protein-A Sepharose) in PBS overnight at 4°C. Protein concentration was 7.5 μg/ml, determined by the method of Bradford (5). Plates were then washed with PBS, and 200 μl of PBS-bovine serum albumin (BSA) was added for 1 h at 4°C to cover unreacted sites. Serial twofold dilutions of antigen in PBS (starting with 1:2) were added for 1 h at room temperature. After the wells were washed with PBS and blocked with PBS-BSA, 20 μl of a second monoclonal antibody specific for a different epitope on ECA (24) (designated 865, immunoglobulin M subclass; 15 μg/ml in PBS-BSA) was added and developed with peroxidase-labeled goat anti-mouse immunoglobulin M (20 μl of a 1:1,000 solution in PBS-BSA; Dianova, Hamburg, Federal Republic of Germany) by using 2,2′-azino-di-[3-ethylbenzthiazolin sulfonate (6)]; (Boehringer GmbH, Mannheim, Federal Republic of Germany) as a substrate. OD_{414} values were read in an automatic ELISA reader (Flow Laboratories, Meckenheim, Federal Republic of Germany).

As the standard for ECA analysis, we used the *E. coli* K-12 C600 laboratory strain (24), and we prepared soluble ECA as described above. The quantitative determination of ECA in this standard calculated from a regression curve revealed an amount of 5 μg/ml compared with a highly purified ECA preparation of *Salmonella montevideo* extracted by a combined phenol-water-phenol-chloroform-petrol-ether procedure (19) and kindly provided by H. Mayer, Max Planck Institute, Freiburg, Federal Republic of Germany. For statistical evaluation, we used the model of covariance analysis. The explanatory variable was log dilution, the dependent variable was log OD (we obtained a linear relationship between these two variables). For the analysis, equal slopes were assumed for all regression lines. This assumption was proven to be feasible. Results were expressed as percent ECA compared with the standard. A *Pseudomonas* strain (ATCC 27853) was used as the negative control. Repeated analysis of one strain revealed a variability of plus or minus one dilution step (one standard deviation).

The Western blot was performed as previously described (24, 26) with minor modifications. Bacteria were adjusted to 5×10^8/ml, and 1 ml was centrifuged in an Eppendorf

TABLE 1. Determination of ECA expression by members of the family *Enterobacteriaceae*

Species	No. of strains	Reference	ECA present
Budvicia aquatica (formerly Enteric Group 84, 85)	2	2	+
Buttiauxella agrestis	5	6	+
Cedecea			
C. neteri	2	6	+
Cedecea sp. 3	1	6	+
Cedecea sp. 5	1	6	+
Citrobacter			
C. amalonaticus	2	6	+
C. diversus	1	6	+
C. freundii	1	6	+
Edwardsiella			
E. hoshinae	2	6	+
E. ictaluri	2	6	+
E. tarda	2	6	+
E. tarda biogroup I	2	6	+
Enterobacter			
E. aerogenes	11	6	+
E. agglomerans	7	6	+
E. amnigenus biogroup I	6	6	+
E. amnigenus biogroup II	4	6	+
E. cloacae	6	6	+
E. gergoviae	6	6	+
E. intermedium	4	6	+
E. sakazakii	5	6	+
E. taylorae	6	6	+
Erwinia			
E. ananas	1	11	+
E. chrysanthemi	13	11	−
E. cypripedii	1	11	+
E. carotovera	1	11	+
E. herbicola	1	11	+
E. nimipressuralis	1	11	+
E. nigrifluens	1	11	+
E. rhapontici	1	11	+
E. rubrifaciens	1	11	+
E. salicis	1	11	+
E. stewartii	1	11	+
E. tracheiphila	2	11	+
Escherichia			
E. blattae	2	6	+
E. coli	1	11	+
E. coli inactive	2	11	+
E. fergusonii	2	6	+
E. hermannii	2	6	+
E. vulneris	2	6	+
Ewingella americana	2	6	+
Hafnia			
H. alvei	5	6	+
H. alvei biogroup I	1	6	+
Klebsiella			
K. oxytoca	1	6	+
K. planticola	1	6	+
K. terrigena	1	6	+
Klebsiella group 47	1	6	+

Continued on following page

TABLE 1—*Continued*

Species	No. of strains	Reference	ECA present
Kluyvera			
K. ascorbata	2	6	+
K. cryocrescens	2	6	+
Koserella trabulsii (formerly Enteric Group 45)	2	9	+
Leminorella			
L. grimontii	6	10	+
L. richardii	4	10	+
Moellerella wisconensis	8	6	+
Morganella morganii	5	6	+
Obesumbacterium proteus biogroup II	1	6	+
Pragia fortium (formerly Enteric Group 86)	2	Unpublished data	+
Proteus			
P. mirabilis	2	11	+
P. myxofaciens	1	6	+
P. penneri	2	6	+
P. vulgaris	2	11	+
Providencia			
P. alcalifaecens	2	11	+
P. rustigianii	2	6	+
P. rettgeri	2	11	+
P. stuartii	2	11	+
Rahnella aquatilis	2	6	+
Serratia			
S. ficaria	5	6	+
S. fonticola	6	6	+
S. liquefaciens	5	6	+
S. marcescens	6	11	+
S. marcescens biogroup I	3	6	+
S. odorifera biogroup I	6	6	+
S. odorifera biogroup II	6	6	+
S. plymuthica	7	6	+
S. rubidaea	7	6	+
Tatumella ptyseos	2	6	+
Xenorhabdus			
X. luminescens	1	6	+
X. nematophilus	2	6	+
Yersinia			
Y. aldovae	2	4	+
Y. enterocolitica	2	6	+
Y. frederiksenii	2	6	+
Y. intermedia	2	6	+
Y. kristensenii	2	6	+
Y. pseudotuberculosis	3	11	+
Y. ruckeri	2	6	+
Enteric Group			
41	2	6	+
58	2	6	+
59	1	6	+
60	2	6	+
63	2	6	+
64	2	6	+
68	1	6	+

Minifuge at 10,000 rpm. The pellet was suspended in 50 μl of sample buffer (0.0625 M Tris hydrochloride [pH 6.8], 5% 2-mercaptoethanol, 2% sodium dodecyl sulfate, 12.5% glycerol) and incubated for 5 min at 100°C. A 50-μl portion of a proteinase K solution (2.5 mg/ml; Sigma Chemie, Taufkirchen, Federal Republic of Germany) in sample buffer was added, and the incubation was continued for 150 min at 65°C. A sample of 20 μl (equivalent to 10^8 bacteria) was used for each lane on a sodium dodecyl sulfate gel (stacking gel, 3%; running gel, 12% [16]). Electrophoresis was performed until the bromphenol blue tracking dye had reached the bottom of the gel. The blotting procedure was done by previously described methods (24) by using 100 V for 3 h. The nitrocellulose sheet was saturated with PBS-BSA overnight. As the detecting antibody, we used the monoclonal antibody 898 (15 μg/ml in PBS-BSA), which is specific for ECA, developed with peroxidase-conjugated rabbit anti-mouse immunoglobulin (1:500 in PBS-BSA; Dakopatts, Hamburg, Federal Republic of Germany). The substrate was 4-chloro-1-naphthol (Sigma).

RESULTS

Enterobacteriaceae. A graphic representation of a typical ELISA is shown in Fig. 1, and Table 1 gives the results of the determination of ECA expression for different bacterial species. All 90 species known or presumed to be members of *Enterobacteriaceae* were positive for ECA with one exception, i.e., *E. chrysanthemi*, which was expected (16, 21). It appeared that the amount of ECA expressed by *Enterobacteriaceae* was not fixed but rather variable. Therefore, the genera *Enterobacter*, *Serratia*, *Moellerella*, *Morganella*, and *Leminorella* were investigated in more detail (Table 2). It should, however, be noted that the quantitative analysis was rather rough; estimation of the number of organisms by a turbidimetric method is not exact, and the variability of the test system itself was plus or minus one dilution step. Therefore, the ELISA system was not used to determine the amount of ECA in absolute terms but rather to get a hint for possible variability in the production of ECA. Taking *Enterobacter* species as an example, we found a large variation in ECA expression, roughly 50-fold, among different *Enterobacter* species as well as within a single *Enterobacter* species, e.g., *Enterobacter aerogenes*. This observation demonstrates the variability of ECA production in different strains belonging to the same species. However, it appears that certain genera produce only limited amounts of ECA as a general genus characteristic, e.g., *Moellerella*.

Visualizing ECA in a Western blot (Fig. 2) showed the typical ladder phenomenon (24, 26). Interestingly, within a species on a strain-specific level, e.g., *E. aerogenes*, it appeared that the amount of ECA expressed was connected with differences in the ladder phenomenon in the Western blot; i.e., a limited amount of ECA, as tested in the ELISA, seemed to be connected with an unequal expression of the different bands. Low-molecular-weight bands were maintained, whereas higher-molecular-weight bands disappeared (Fig. 2). In contrast, the genus *Moellerella* showed faint low-molecular-weight as well as high-molecular-weight bands, although it only expressed limited amounts of ECA (Fig. 3).

Non-*Enterobacteriaceae*. The results for 28 species not belonging to the family *Enterobacteriaceae* are shown in Table 3. As expected, these strains generally did not produce ECA, but there were three exceptions, i.e., *P. shigelloides*, *Actinobacillus equuli*, and *Actinobacillus suis*. This result is

TABLE 2. ECA expression by species of *Enterobacter*, *Serratia*, *Leminorella*, *Morganella*, and *Moellerella*

Species	No. of strains	Mean relative amount (%) of ECA (range)[a]
Enterobacter		
E. aerogenes	11	150 (5–300)
E. agglomerans	7	150 (30–250)
E. amnigenus biogroup I	6	250 (150–350)
E. amnigenus biogroup II	4	200 (125–300)
E. cloacae	6	200 (100–300)
E. gergoviae	6	125 (80–250)
E. intermedium	4	250 (125–400)
E. sakazakii	5	100 (50–50)
E. taylorae	6	300 (200–400)
Leminorella		
L. grimontii	6	20 (10–40)
L. richardii	4	20 (10–40)
Moellerella wisconensis	8	30 (10–40)
Morganella morganii	5	40 (30–60)
Serratia		
S. ficaria	5	170 (100–200)
S. fonticola	6	70 (40–90)
S. liquefaciens	5	50 (20–120)
S. marcescens	6	40 (30–50)
S. marcescens biogroup I	3	80 (50–150)
S. odorifera biogroup I	6	60 (50–100)
S. odorifera biogroup II	6	60 (50–100)
S. plymuthica	7	60 (50–100)
S. rubidea	7	100 (50–150)

[a] Expressed as percentage of the standard.

expected (28) for *P. shigelloides*. Since it is known that certain, although not all, strains of *P. shigelloides* cross-react with *Shigella sonnei* (3, 7), it was of interest whether a connection exists between the presence of the O antigen cross-reacting with *S. sonnei* and the presence of ECA. As suggested previously (28), our data established that these two antigens were expressed independently, i.e., ECA was produced by all strains tested belonging to the species *P. shigelloides* independent of the presence of the O-antigen.

The amount of ECA produced by *A. equuli* and *A. suis* was tiny but clearly present (1 to 10% of the standard). In contrast, other species of *Actinobacillus*, i.e., *Actinobacillus lignieresii* and *Actinobacillus actinomycetemcomitans*, were clearly negative in our assay. Interestingly, the Western blots of the species *A. equuli* and *A. suis* showed only low-molecular-weight bands (Fig. 4).

DISCUSSION

Our results confirm the definition of ECA as an antigen produced by all species of the family *Enterobacteriaceae* but not by species belonging to other families (25). With one exception, the 90 species tested, known or presumed to belong to *Enterobacteriaceae*, all produced ECA. This is particularly gratifying for the several species classified only recently (2, 4, 6, 9, 10), e.g., *Budvicia*, *Buttiauxella*, *Koserella*, *Leminorella*, *Moellerella*, and *Pragia*. The expected exception was *E. chrysanthemi* (16). The rough quantitative estimation of ECA produced by different genera, species, and strains of *Enterobacteriaceae* revealed a remarkable heterogeneity even within one species. Although

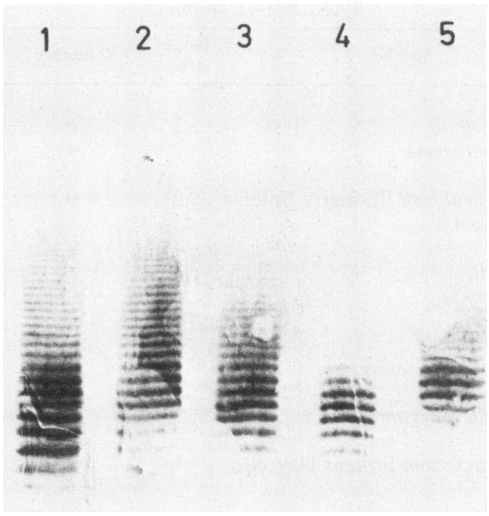

FIG. 2. Western blot of different *E. aerogenes* strains. Lanes (Centers for Disease Control code number is given, and percent ECA as determined by the ELISA is indicated in parentheses): 1, 1437-81 (300%); 2, 1942-81 (125%); 3, 4782-84 (45%); 4, 4601-84 (5%); 5, control *E. coli* K-12 strain. The other *E. aerogenes* strains expressing 100 to 250% ECA (compared with the standard) showed the same pattern as strains 1437-81 or 1942-81.

it appeared that certain genera and species exhibited only a limited amount of ECA, e.g., *Moellerella*, the large heterogeneity commonly found excluded the quantitation of ECA as a useful species specific marker.

The Western blot analysis of the ECA ladder phenomenon revealed that in a species expressing variable amounts of ECA, the variability of the different strains in ECA expression seemed to be connected with different ladders in the Western blot. *E. aerogenes* strains expressing large amounts of ECA showed low-molecular-weight as well as high-molecular-weight bands, whereas strains expressing limited amounts of ECA seemed to miss several high-molecular-weight bands. In contrast, for a genus generally expressing only limited amounts of ECA, e.g., *Moellerella*, the complete and broad spectrum of low-molecular-weight as well as high-molecular-weight bands were observed. The reason for this interesting divergence is not known and needs to be further analyzed. It should be stressed that ECA occurs in two different forms; one is haptenic, and the other is

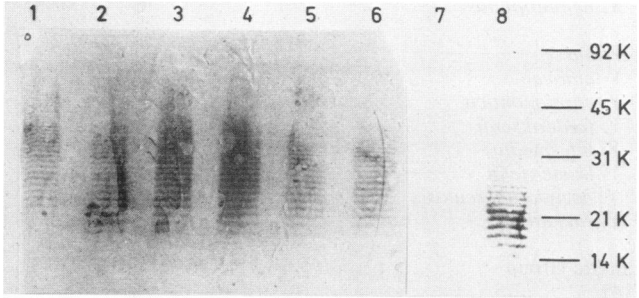

FIG. 3. Western blot of different *M. wisconensis* strains. Lanes (Centers for Disease Control code number is given, and percent ECA as determined by the ELISA is indicated in parentheses): 1, 2552-77 (20%); 2, 3065-75 (10%); 3, 2252-80 (25%); 4, 1626-81 (25%); 5, 2397-81 (30%); 6, 52-83 (15%); 7, control *Pseudomonas* strain; 8, control *E. coli* K-12 strain.

TABLE 3. ECA expression in different gram-negative species not belonging to the family *Enterobacteriaceae*

Species[a]	No. of strains	ECA present
Aeromonas		
A. caviae	1	−
A. hydrophila	1	−
A. salmonicida subsp. *masoncida*	1	−
Actinobacillus		
A. actinomycetemcomitans	6	−
A. equuli	3	+
A. lignieresii	7	−
A. suis	5	+
Alcaligenes		
A. denitrificans	1	−
A. faecalis	1	−
Bordetella		
B. bronchiseptica	1	−
B. parapertussis	1	−
Campylobacter		
C. coli	1	−
C. fecalis	1	−
C. fetus subsp. *fetus*	1	−
C. jejuni	1	−
C. sputorum subsp. *bubulus*	1	−
C. sputorum subsp. *sputorum*	1	−
Cardiobacterium hominis	10	−
Chromobacterium violaceum	1	−
Eikenella corrodens	2	−
Flavobacterium		
F. meningosepticum	1	−
F. odoratum	1	−
Gardnerella sp.	1	−
Haemophilus sp.	1	−
Kingella kingae	1	−
Moraxella		
M. bovis	1	−
M. phenylpyruvica	1	−
Pasteurella multocida	1	−
Plesiomonas		
P. shigelloides		
O-antigen positive	3	+
O-antigen negative	4	+

[a] The reference for all species is *Bergey's Manual of Systematic Bacteriology*, vol. 1 (11).

covalently bound to LPS (8, 12, 19, 22, 23). However, the nature of the ladder phenomenon in the Western blot is as yet unknown. It was suggested that it was due to size heterogeneity or to binding to repetitive LPS chains (24). Therefore, the molecular form of ECA visualized in the Western blot was vague; on the other hand, it is conceivable that the ELISA system probably detected the haptenic form as well as LPS-bound ECA.

Gram-negative species not members of the family *Enterobacteriaceae* in general did not produce ECA (25 of 28 species tested), but certain exceptions existed, i.e., *P. shigelloides*, *A. equuli*, and *A. suis*. *P. shigelloides* is a well-known exception (28), and our results confirm that the expression of ECA in this species is irrespective of the simultaneous presence of the cross-reacting O antigen (only 3 of 7 tested strains had the O antigen, whereas all were positive for ECA).

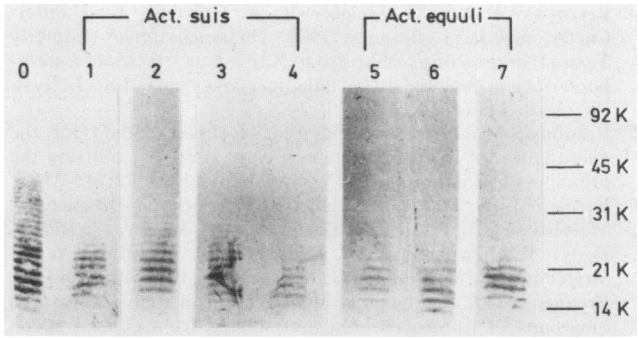

FIG. 4. Western blot of different *A. equuli* and *A. suis* strains. Lanes (Centers for Disease Control code number is given): 0, control *E. coli* K-12; 1, *A. suis* D 1067; 2, *A. suis* A 8479; 3, *A. suis* KC 561; 4, *A. suis* A 2866; 5, *A. equuli* A 9510; 6, *A. equuli* KC 607; 7, *A. equuli* KC 377.

The occurrence of further gram-negative species not belonging to *Enterobacteriaceae* but expressing ECA, i.e., *A. equuli* and *A. suis* was hitherto unknown. It should be noted that the amount of ECA expressed by these species was limited. Interestingly, the Western blot revealed only low-molecular-weight ladders for these two species. In the genus *Actinobacillus*, the presence of ECA is not a general characteristic but rather restricted, since the species *A. lignieserii* and *A. actinomycetemcomitans* did not produce ECA. This observation raises taxonomic questions and indicates that the determination of ECA may be useful for examining the relationship of the family *Enterobacteriaceae* to other families.

In summary, our data confirm the usefulness of ECA as a taxonomic tool with which to separate *Enterobacteriaceae* from other families. The only exceptions so far known are *E. chrysanthemi* (15), *P. shigelloides* (26), *A. equuli*, and *A. suis* (confirmed by this study). In addition, our data indicate that the presence of ECA may be a useful hint in elucidating the pathways of bacterial evolution.

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LITERATURE CITED

1. **Acker, C., G. Schmidt, and H. Mayer.** 1982. Accessibility of enterobacterial common antigen to antibodies in encapsulated and non-capsulated S and R forms of *Escherichia coli*. J. Gen. Microbiol. **128**:1577–1583.
2. **Aldova, E., O. Hausner, and M. Gabrhelova.** 1984. *Budvicia*—a new genus of *Enterobacteriaceae*: data on phenotypic characters. J. Hyg. Epidemiol. Microbiol. Immunol. **28**:234–237.
3. **Bader, R. E.** 1954. Uber die Herstellung eines agglutinierenden Serums gegen die Rundform von *Shigella sonnei* mit einem Stamm der Gattung Pseudomonas. Z. Hyg. Infektionskr. Med. Mikrobiol. Immunol. Virol. **140**:450–456.

4. **Bercovier, H., A. G. Steigerwalt, A. Guiyoule, G. Huntley-Carter, and D. J. Brenner.** 1984. *Yersinia aldovae* (formerly *Yersinia enterocolitica*-like group X2): a new species of *Enterobacteriaceae* isolated from aquatic ecosystems. Int. J. Syst. Bacteriol. **34**:166–172.
5. **Bradford, M.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of proteins, utilizing the principle of protein-dye binding. Anal. Biochem. **72**:248–254.
6. **Farmer, J. J., III, B. R. Davis, F. W. Hickman-Brenner, A. McWhorter, G. P. Huntley-Carter, M. A. Asbury, C. Riddle, H. G. Wathen-Grady, C. Elias, G. R. Fanning, A. G. Steigerwalt, C. M. O'Hara, G. K. Morris, P. B. Smith, and D. J. Brenner.** 1985. Biochemical identification of new species and biogroups of *Enterobacteriaceae* isolated from clinical specimens. J. Clin. Microbiol. **21**:46–76.
7. **Ferguson, W. W., and N. D. Henderson.** 1947. Description of strain C27: a mobile organism with the major antigen of *Shigella sonnei* phase I. J. Bacteriol. **54**:179–181.
8. **Hammarström, S., H. E. Carlsson, P. Perlmann, and S. Svensson.** 1971. Immunochemistry of the common antigen of *Enterobacteriaceae* (Kunin) J. Exp. Med. **134**:565–576.
9. **Hickman-Brenner, F. W., G. P. Huntley-Carter, G. R. Fanning, D. J. Brenner, and J. J. Farmer III.** 1985. *Koserella trabulsii*, a new genus and species of *Enterobacteriaceae* formerly known as Enteric Group 45. J. Clin. Microbiol. **21**:39–42.
10. **Hickman-Brenner, F. W., H. P. Vohra, G. P. Huntley-Carter, G. R. Fanning, V. A. Lowery III, D. J. Brenner, and J. J. Farmer III.** 1985. *Leminorella*, a new genus of *Enterobacteriaceae*: identification of *Leminorella grimontii* sp. nov. and *Leminorella richardii* sp. nov. found in clinical specimens. J. Clin. Microbiol. **21**:234–239.
11. **Krieg, N. R., and J. G. Holt (ed.).** 1984. Bergey's manual of systematic bacteriology, vol. 1. The Williams & Wilkins Co., Baltimore.
12. **Kunin, C. M.** 1963. Separation, characterization, and biological significance of a common antigen in *Enterobacteriaceae*. J. Exp. Med. **118**:565–586.
13. **Kunin, C. M., and M. V. Beard.** 1963. Serological studies of O antigens of *Escherichia coli* by means of the hemagglutination test. J. Bacteriol. **85**:541–548.
14. **Kunin, C. M., M. V. Beard, and N. E. Halmagyi.** 1962. Evidence for a common hapten associated with endotoxin fractions of *E. coli* and other *Enterobacteriaceae*. Proc. Soc. Exp. Biol. Med. **111**:160–166.
15. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
16. **Le Minor, L., A.-M. Chalon, and M. Véron.** 1972. Reserche sur la présence de l'antigène commun des *Enterobacteriaceae* (antigène Kunin) chez les *Yersinia*, *Levinea*, *Aeromonas* et *Vibrio*. Ann. Inst. Pasteur (Paris) **123**:761–774.
17. **Lugowski, C., E. Romanowska, L. Kenne, and B. Lindberg.** 1983. Identification of a trisaccharide repeating-unit in the enterobacterial common antigen. Carbohydr. Res. **118**:173–181.
18. **Mäkelä, P. H., and H. Mayer.** 1974. Participation of lipopolysaccharide genes in the determination of the enterobacterial common antigen: analysis in *Salmonella* groups B and C_1. J. Bacteriol. **119**:765–770.
19. **Mäkelä, P. H., and M. Mayer.** 1976. Enterobacterial common antigen. Bacteriol. Rev. **40**:591–632.
20. **Mäkelä, P. H., H. Mayer, H. Y. Whang, and E. Neter.** 1974. Participation of lipopolysaccharide genes in the determination of the enterobacterial common antigen: analysis of R mutants of *Salmonella minnesota*. J. Bacteriol. **119**:760–764.
21. **Männel, D., and H. Mayer.** 1976. Isolation and chemical characterization of the enterobacterial common antigen. Eur. J. Biochem. **86**:361–370.
22. **Marx, A., and M. Patcovici.** 1976. Immunochemical studies on purified common enterobacterial antigen. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A **233**: 486–494.
23. **McLaughlin, I. C., and G. J. Dominique.** 1974. The immunologic role of the ethanol-soluble enterobacterial common antigen versus experimental renal infection. Immunol. Commun. **3**:51–75.
24. **Peters, H., M. Jürs, B. Jann, K. Jann, K. N. Timmis, and D. Bitter-Suermann.** 1985. Monoclonal antibodies to enterobacterial common antigen and to *Escherichia coli* lipopolysaccharide outer core: demonstration of an antigenic determinant shared by enterobacterial common antigen and *E. coli* K5 capsular polysaccharide. Infect. Immun. **50**:459–466.
25. **Ramia, S., E. Neter, and D. J. Brenner.** 1982. Production of enterobacterial common antigen as an aid to classification of newly identified species of the families *Enterobacteriaceae* and *Vibrioneceae*. Int. J. Syst. Bacteriol. **32**:395–398.
26. **Rick, P. D., H. Mayer, B. A. Neumeyer, S. Wolski, and D. Bitter-Suermann.** 1985. Biosynthesis of enterobacterial common antigen. J. Bacteriol. **162**:494–503.
27. **Rinno, J., J. R. Golecki, and H. Mayer.** 1980. Localization of enterobacterial common antigen: immunogenic and nonimmunogenic enterobacterial common antigen-containing *Escherichia coli*. J. Bacteriol. **141**:814–821.
28. **Whang, H. Y., M. E. Heller, and E. Neter.** 1972. Production by *Aeromonas* of common enterobacterial antigen and its possible taxonomic significance. J. Bacteriol. **110**:161–164.
29. **Whang, H. Y., and E. Neter.** 1962. Immunochemical studies of a heterogenetic enterobacterial antigen (Kunin). J. Bacteriol. **84**:1245–1250.