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Demonstration of the K88ac and K88ab Antigens of *Escherichia coli* by Means of Immunoelectrophoresis and Immunodiffusion†

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Five strains of *Escherichia coli* were tested for the presence of the K88ac or K88ab antigens by immunoelectrophoresis and immunodiffusion. The K88ac antigen of ØA2 and Sojka Abbotstown gave an anodic line in the immunoelectrophoresis test and a line in immunodiffusion with homologous K88ac antisera. The K88ab antigens of ØG7, ØE68, and Moon 263 also gave anodic lines in immunoelectrophoresis, and were detectable by immunodiffusions. The O groups of these strains were also demonstrated by immunoelectrophoresis and immunodiffusion with homologous O antisera. Lack of complete inactivation at 100°C of both the K88ac and K88ab antigens was noted in this study.

The K88 antigen of *Escherichia coli*, isolated from swine with enteritis and edema disease in England, was first described in 1961 (13). K88 is an episomally transferred L type of K antigen that is destroyed by heating at 100°C and is not formed at 18°C (11, 13). The K88 antigen exists in at least two serologically different forms, K88ab and K88ac (13). From chemical analysis, K88 was found to be a protein containing all of the common amino acids except cysteine-cystine (16). Electron microscope studies indicated that K88 has a fimbria-like filamentous morphology (15). However, the K88 filaments are finer and more flexible than the fimbria described by Duguid et al. (1-5).

Ørskov et al. demonstrated many of the *E. coli* O and K antigens by immunoelectrophoresis (IEP), but were not able to demonstrate the K88 antigen, probably due to its filamentous nature (10).

This study was undertaken to identify the K88ac and K88ab antigens by the IEP technique. Identification of the K88 antigen could be important as a diagnostic tool because it is often associated with enteropathogenic strains of *E. coli* that cause neonatal diarrhea in piglets (14). (This work was submitted by E.E.C. to the Graduate School, The Pennsylvania State University, in partial fulfillment of the requirements for an M.S. degree in Veterinary Science.)

MATERIALS AND METHODS

Strains. Five strains of *E. coli* were used in this study. Three of the strains, obtained from F. Ørskov (Collaborative Centre for Reference and Research on Escherichia, World Health Organizations, Statens Seruminstitut, Copenhagen, Denmark), were ØA2 (O157:K88ac:H19), ØG7 (O8:K87, K88ab:H19), and ØE68 (O141:K85, K88ab:H4). ØA2 is the standard strain for the K88ac antigen and ØE68 the standard strain for the K88ab antigen. Sojka Abbotstown (O149:K91, K88ac:H10) was received from W. J. Sojka (Central Veterinary Laboratory, Weybridge, Surrey, U.K.). Moon 263 (O8:K87, K88ab:H19) was supplied by H. W. Moon (U.S. Department of Agriculture, Ames, Iowa).

Media. Stock cultures were maintained on Trypticase soy agar (Baltimore Biological Laboratory, Cockeysville, Md.). The strains were cultivated on Trypticase soy agar plates for IEP and immunodiffusion (ID) tests.

Serotyping of *E. coli*. All cultures were examined serologically with their specific O, K, and H antisera by the methods described by Glantz (7). This entailed slide agglutination and tube titer tests for final confirmation of O, K, and H antigens.

Production and absorption of K88 antisera. *E. coli* antisera (O and K specific) were prepared by immunizing rabbits by standard procedures (7). Double-cross-absorbed antiserum was prepared by the method of Ørskov (12). The antigens, prepared as mentioned below, were unheated, heated at 60 or 100°C, or microwaved, then centrifuged for 30 min (20,000 × *g*), and the supernatant was discarded. The antiserum to be absorbed was added to the antigen pellet, mixed, and incubated at 37°C for 2 h. The suspension was then recentrifuged for 30 min (20,000 × *g*), and the antiserum supernatant was transferred to a freshly prepared antigen pellet. The mixture was incubated at 37°C for 2 h, then refrigerated overnight. The suspensions were centrifuged for 30 min (20,000 × *g*), and the absorbed antiserum supernatant was saved for the IEP and ID tests.

Antigen preparation. The cultures were streaked

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on Trypticase soy agar plates with sterile swabs and incubated overnight at 37°C. The bacterial growth was washed off with 4 ml of Barbitol buffer (Fisher Scientific Co., Pittsburgh, Pa.) with a glass rod. The culture suspensions, divided into three parts, were then heated in a water bath at 60°C for 20 min, at 100°C for 1 h in flowing steam, and microwaved (Hotpoint model RE 910) in glass petri dishes for 15 to 30 s. Then all three antigen preparations were centrifuged for 20 min at 20,000 × g in a refrigerated Lourdes (model A-2) centrifuge. The supernatant, referred to as "extract" (10), was removed and stored at 4°C, and the pellet was saved for slide agglutination studies. Freeze-thawing and disruption by ultrasonic treatment was used to disrupt the bacteria and release the antigens, but no electrophoretic patterns were obtained. Minca medium (8) was used to enhance growth of K88 but did not give any better results than Trypticase soy agar.

IEP. A Gelman immunoelectrophoresis system (Gelman Instrument Co., Ann Arbor, Mich.) was used. Slide preparation and electrophoresis were carried out according to the Gelman manual (6). Washed, Difco Special Noble agar (Difco Laboratories, Detroit, Mich.) was employed as the electrophoretic medium, and Barbitol buffer was used to fill the electrophoretic chamber. The slides were stained with Coomassie brillant blue dye ($C_{45}H_{44}N_3NaO_7S_2$) (Fisher Scientific Co., Pittsburgh, Pa.) for permanent records.

ID. Double diffusion was performed on agar-coated glass slides with the same antigen extract preparations, buffer, agar, and stain as described in the IEP method. The diffusion medium was applied with a 12-ml syringe (20-gauge needle) under a polished lucite block (3 by 2.5 by 0.8 cm). Antigen extract and antiserum were applied to the agar with a TB syringe (27-gauge, 1.27-cm needle) with a lucite diffusion block (2.5 by 2.5 by 0.8 cm) containing seven funneled wells. The slides were refrigerated at 4°C for 4 days, and the results were recorded.

RESULTS

Table 1 lists the IEP, ID, and slide agglutination results of strain ØA2 (O157:K88ac:H19). All of the other strians tested gave results identical to this standard K88ac antigen strain, so for purposes of simplification, only the results of strain ØA2 are listed in this table. The 60°C and microwave extracts of ØA2 agglutinated with OK88ac (K88) antisera prepared with the ØA2 strain but not with O157 antisera in the slide agglutination tests using unabsorbed antisera. The 100°C extract reacted as O157 with unabsorbed OK88ac and O157 antisera. This is evidence of the K masking effect that is removed at 100°C. When these three antigen extracts were tested with K88ac antisera absorbed with unheated K88ac antigen (K88/K88 Unh), no agglutination occurred, because no antibodies should have remained after absorption. Only the 60°C and microwave extracts agglutinated with K88/K88 (100°C) antisera, because these absorbed antisera should contain antibodies only to the K88ac antigen. The other strain tested containing the K88ac antigen, Sojka Abbotstown, agglutinated with O149 antisera, with K88ac antisera prepared with the ØA2 strain, and with K(A1) antisera prepared with O149:K91, K88ac:H19. Strain ØA2 also agglutinated with K(A1) antisera, although it is not included in the table. Neither of these two strains agglutinated with K88ab antisera. Heated (100°C) antigens of Ø67, Moon 263, and OE68 all agglutinated with their respective O group antisera, O8 and O141, and when unheated, with K88ab antisera but not K88ac antisera.

When tested by immunodiffusion, the 60°C and microwave antigen extracts of OA2 gave two lines with K88ac unabsorbed antisera (Fig. 1A). The lines close to the antigen (outer) wells were considered to be K88ac, and the lines closer to the antiserum (center) well were due to O157. All three antigen extracts gave one line (O157) when tested with O157 antisera (Fig. 1B). What appears to be a second line close to outer wells

TABLE 1. *IEP, ID, and slide agglutination results of the K88ac antigen by using unabsorbed and double-cross-absorbed antisera*[a]

Antigen treatment			Slide agglutination				ID			
			Unabsorbed serum		Absorbed serum		Unabsorbed serum		Absorbed serum	
Strain	Antigen	Temp	OK88ac	O157	Unh[b] K88/K88	100°C[c] K88/K88	OK88ac	O157	Unh K88/K88	100°C K88/K88
ØA2	K88ac	60°C	+	–	–	+	L(2)[d]	L[e]	–	L
ØA2	K88ac	100°C	+	+	–	–	L	L	–	–
ØA2	K88ac	MW, 15 s	+	–	–	+	L(2)	L	–	L

[a] 60°C, 100°C, and microwave extracts (MW) gave anodic and cathodic lines with unabsorbed OK88ac antisera. When tested with absorbed antisera K88/K88 (Unh) in IEP, no lines were observed. Only anodic lines (K88ac) observed with all extracts when tested in IEP with K88/K88 (100°C) antisera.

[b] K88ac antisera were absorbed with unheated (Unh) K88ac antigen.

[c] Antigen was heated at 100°C for 1 h for absorption.

[d] L(2), Two lines observed.

[e] L, One line observed.

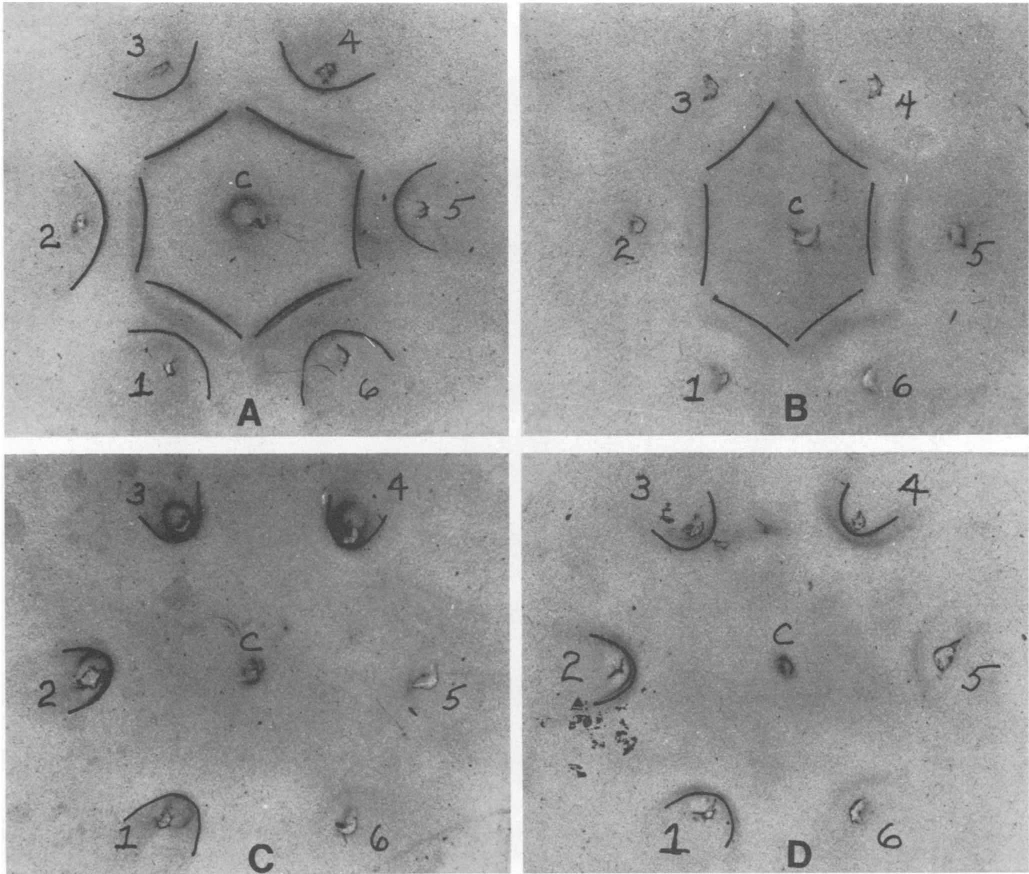

FIG. 1. *ID patterns obtained with E. coli OK88ac and O157 antigens and antisera. (A) Center well, K88ac antiserum. K88ac antigen extracts in outer wells: (1) and (2), microwave, 15 s; (3) and (4), microwave, 30 s; (5) and (6), 60°C, 20 min. 0157 and K88ac lines are evident. (B) Center well, O157 antiserum. K88ac antigen extract in outer wells: (1) and (2), microwave, 15 s; (3) and (4), microwave, 30 s; (5) and (6), 100°C, 1 h. Lines are O157. (C) Center well, absorbed antisera, K88ac/K88ac 100°C, 1 h. K88ac antigen extract in outer wells: (1) and (2), unheated; (3) and (4), 60°C, 20 min; 5 and 6, 100°C, 1 h. Lines are K88ac antigen. (D) Center well, absorbed antisera, K88ac/K88ac microwave, 30 s. K88ac antigen extract in outer wells: (1) and (2), unheated; (3) and (4), 60°C, 20 min; (5) and (6) and 100°C, 1 h. Lines are K88ac antigen.*

5 and 6 may be an unknown antigen released at 100°C.

Only the 60°C and unheated antigen extracts produced a line with absorbed K88/K88 (100°C) antisera, denoting the presence of K88ac (Fig. 1C). The K88ac lines were apparent (Fig. 1D) with absorbed K88/K88 (microwave, 30 s) antisera, and unheated or heated (60°C, 20 min) antigen extracts. Very faint lines were observed with the 100°C, 1-h antigen extract. The K88ac antigen is released into the supernatant after microwave treatment, and possibly the amount retained in the centrifuged precipitate was not sufficient to absorb out all of the K antibody, but did remove the O157 antibody.

Lines were not observed when K88ac antiserum was absorbed with K88ac unheated antigen, with microwave 15-s or with 60°C, 20-min antigens and tested with all antigen extracts. As mentioned above, the microwave treatment at 30 s may release more K88ac antigen into the supernatant than the other two heating methods.

When tested by IEP (Table 1), ØA2 gave anodic and cathodic lines with OK88ac unabsorbed serum. The anodic line represents the K88ac antigen, and the cathodic line is due to the O157 antigen (Fig. 2). All three extracts of ØA2 did not form lines with K88/K88 (unheated)-absorbed antisera, as expected. An anodic line, apparent when the 60°C, 100°C, and microwave extracts were tested in IEP with K88/K88 (100°C)-absorbed antisera (Fig. 2), confirmed the presence of the K88ac antigen.

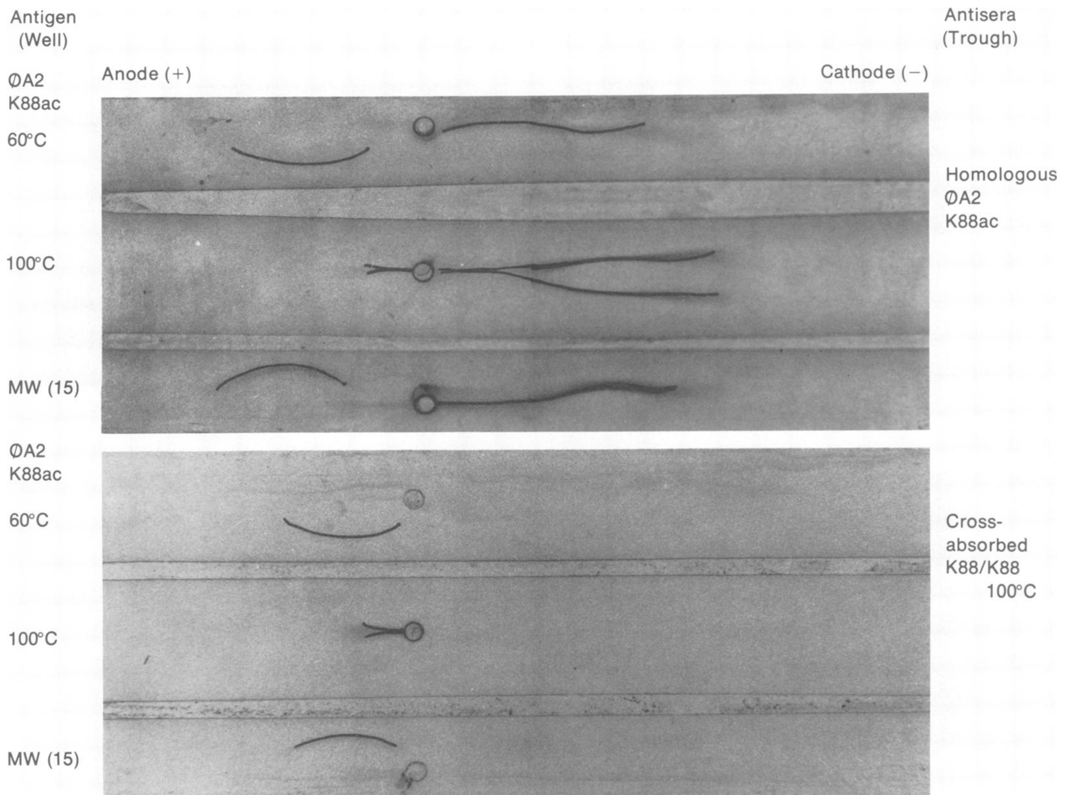

FIG. 2. *IEP patterns obtained when K88ac (ØA2) antigen heated at 60°C, 100°C, and microwaved for 15 s [MW (15)] were tested with homologous OK88ac antisera and OK88ac antisera absorbed with OK88ac antigen heated at 100°C for 1 h (K88/K88, 100°C). Anodic lines appear as an arc parallel to trough, or a line directly from well. The two faint lines at top of slide (K88/K88, 100°C) are artifacts.*

The very short anodic line evidenced with the 100°C extract, with the same serum, indicated that some K88ac antigen was not totally inactivated at 100°C, and also explains why a line was evident with ID under these same conditions. The other strains were also tested with homologous O antisera and gave lines identical to those observed by Ørskov et al. in their IEP studies (9, 10). Both the K88ac and K88ab antigen strains gave anodic lines with homologous K antisera, but no lines were observed when a K88ac strain was tested with K88ab antisera and vice versa. As with the ØA2 strain, the K88ac and K88ab lines were confirmed by cross-absorption studies, and again there was evidence that not all of the K88ac or K88ab antigen was destroyed by heating at 100°C.

DISCUSSION

In this study both the K88ac and K88ab antigens were demonstrated by testing five strains of *E. coli* known to possess these antigens. The demonstration of these antigens by IEP has not been previously reported by other investigators. These antigens were identified by IEP, ID and slide agglutination, and confirmed by cross-absorption studies. Both the 60°C and microwave extracts of K88ac and K88ab antigens gave an anodic line when tested by IEP. A short anodic line was also observed when the 100°C extract was tested. Because the K88 antigen is supposed to be inactivated at this temperature, it is possible that some of the K88 antigen has not been destroyed, or this line may be due to a thermostable K antigen that is released at 100°C. The latter was noted by Ørskov and Ørskov (12). The line observed with the 100°C extract in IEP does not move through the agar gel like the lines obtained with the 60°C and microwave extracts. The immunodiffusion tests also gave the same results but were more difficult to interpret due to the occurrence of multiple lines (12).

The use of the microwave oven to release the K88 antigens into the supernatant, with subsequent identification of the released antigen extract by IEP, was very efficient. Exposure to

microwaves for 15 or 30 s was equivalent to heating at 60°C for 20 min in a water bath. The supernatants of unheated, freeze-thawed, and ultrasonic-disrupted bacterial suspensions did not produce any visible lines in the IEP method.

Identification of the K88 antigen by IEP as demonstrated in this paper is of practical importance in evaluating pig enteropathogenic strains of *E. coli.*

LITERATURE CITED

1. **Duguid, J. P.** 1959. Fimbriae and adhesive properties in *Klebsiella*. J. Gen. Microbiol. **21**:271-286.
2. **Duguid, J. P., E. S. Anderson, and I. Campbell.** 1966. Fimbrial and adhesive properties in Salmonella. J. Path. Bacteriol. **92**:107-138.
3. **Duguid, J. P., and R. R. Gillies.** 1957. Fimbriae and adhesive properties in dysentery bacilli. J. Path. Bacteriol. **74**:397-411.
4. **Duguid, J. P., and R. R. Gillies.** 1958. Fimbriae and haemagglutinating activity in *Salmonella, Klebsiella, Proteus,* and *Chromobacterium*. J. Path. Bacteriol. **75**:519-520.
5. **Duguid, J. P., I. W. Smith, G. Dempster, and P. N. Edmunds.** 1955. Non-flagellar filamentous appendages ('fimbriae') and haemagglutinating activity in bacterium coli. J. Path. Bacteriol. **70**:335-348.
6. **Gelman Instrument Co.** Gelman electrophoresis manual, p. 2-35. Gelman Instrument Co., Ann Arbor, Mich.
7. **Glantz, P. J.** 1969. Serological identification of *Escherichia coli*, p. 1-58. The Pennsylvania State University, University Park, Pa.
8. **Guinée, P. A. M., J. Veldkamp, and W. H. Jansen.** 1977. Improved Minca medium for the detection of K99 antigen in calf enterotoxigenic strains of *Escherichia coli*. Infect. Immun. **15**:676-678.
9. **Ørskov, F., and I. Ørskov.** 1972. Immunoelectrophoretic patterns of extracts from *Escherichia coli* O antigen test strain O1 to O157 examinations in homologous OK sera. Acta Pathol. Microbiol. Scand. Sect. B **80**:905-910.
10. **Ørskov, F., I. Ørskov, B. Jann, and K. Jann.** 1971. Immunoelectrophoresis patterns of extracts from all *Escherichia coli* O and K antigen test strains. Correlation with pathogenicity. Acta Pathol. Microbiol. Section B **78**:142-152.
11. **Ørskov, I., and F. Ørskov.** 1966. Episome-carried surface antigen K88 of *Escherichia coli*. I. Transmission of the determinants of the K88 antigen and influence on the transfer of chromosomal markers. J. Bacteriol. **91**:69-75.
12. **Ørskov, I., and F. Ørskov.** 1970. The K antigens of *Escherichia coli*. Acta Pathol. Microbiol. Scand. Sect. B **78**:593-604.
13. **Ørskov, I., F. Ørskov, W. J. Sojka, and W. Wittig.** 1964. K antigens K88ab(L) and K88ac (L) in *E. coli*. Acta Pathol. Microbiol. Scand. **62**:439-447.
14. **Sojka, W. J.** 1971. Enteric diseases in newborn piglets, calves and lambs due to *Escherichia coli* infection. Vet. Bull. (London) **41**:509-522.
15. **Stirm, S., F. Ørskov, I. Ørskov, and A. Birch-Andersen.** 1967. Episome-carried surface antigen K88 of *Escherichia coli*. III. Morphology. J. Bacteriol. **93**:740-748.
16. **Stirm, S., F. Ørskov, I. Ørskov, and B. Mansa.** 1967. Episome-carried surface antigen K88 of *Escherichia coli*. II. Isolation and chemical analysis. J. Bacteriol. **93**:731-739.