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Production of K88, K99, and 987P Antigens by *Escherichia coli* Cultured on Synthetic and Complex Media†

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A synthetic medium (E agar) containing glucose and citric acid as the only organic nutrients was found superior to blood agar, Minca-IsoVitaleX (BBL Microbiology Systems, Cockeysville, Md.) agar and broth, and tryptic soy broth (without glucose) for promoting expression of K99 antigen by *Escherichia coli* strains isolated from diarrheic calves and piglets.

The K88 and 987P adherence pili of enterotoxigenic *Escherichia coli* which cause diarrhea in neonatal pigs are frequently difficult to demonstrate on cultured bacteria by conventional serotyping procedures. Similar problems are experienced in attempting to identify K99 antigen on the cells of enterotoxigenic *E. coli* strains that infect calves and lambs as well as piglets (1). However, K99 antigen is detectable on the cells of many *E. coli* strains cultured in Minca-IsoVitaleX (MI; BBL Microbiology Systems, Cockeysville, Md.) broth (3) or on MI agar (4). Blood agar promotes antigen expression by many K88 and 987P strains (6).

We compared two synthetic media (E agar and E broth) with blood agar (sheep blood with heart infusion base; Difco Laboratories, Detroit, Mich.), MI agar, MI broth, and tryptic soy broth without dextrose (TSB; Difco) for ability to enhance pilus antigen production. E medium was formulated from essential salts and citric acid (8) plus 1% dextrose. We tested 50 isolates of *E. coli* from diarrheic piglets and calves for each of the three pilus antigens. Each isolate was serotyped, and all but three expressed one of the adherence antigens at the time of isolation. The three isolates that did not were from scouring animals in which large numbers of *E. coli* with K99 antigen were observed by indirect immunofluorescence testing on ileal smears.

Cultures stored in TSB (Difco) stab tubes from several days to 4 months were reisolated on blood agar and inoculated into fresh stab tubes. After an overnight incubation at 37°C, the fresh stab cultures were used to inoculate tubes containing 0.5 ml of E broth enriched with 1% minimal essential medium vitamins (GIBCO Laboratories, Grand Island, N.Y.). Enriched E broth cultures were incubated at 37°C for 4 h and then used to inoculate test media. MI broth, E broth, and TSB (2.5 ml each in 12- by 75-mm plastic test tubes) were each inoculated with 1 drop of enriched E broth culture. MI agar, E agar, and blood agar (15 ml in 100- by 15-mm plastic petri plates) were inoculated with cotton-tipped swabs dipped in the enriched E broth. Plates were divided into six sections, and each section was inoculated with one *E. coli* strain. Plates and tubes were incubated for 24 h at 37°C; the cultures were then tested for adherence antigens. Cultures were removed from agar plates with cotton-tipped swabs and suspended in phosphate-buffered saline. Suspensions were adjusted to match the turbidity of the broth cultures. Tubes were randomized to avoid investigator bias.

Antisera used for antigen detection were obtained by immunizing rabbits with antigenic extracts (7) of K-12:K88ac, K-12:K99, or O101:K103, 987P (strain 1592) *E. coli* cultures. Monospecific K88 and K99 antisera were prepared by absorption with the K-12 parent strain of *E. coli* that lacked K88 and K99 pilus antigens. The 987P antiserum was absorbed with strain 1592 cultured at 20°C, a temperature at which the strain did not express 987P antigen.

Plate agglutination tests were performed by a modification of the procedure of Glantz (2). Briefly, 2 drops of culture suspension were added to each of two wells of a multiwell disposable tray (Flow Laboratories, Inc., McLean, Va.). Two drops of 1:50 dilution antiserum and 1 drop of phosphate-buffered saline were added to the test well, and 3 drops of phosphate-buffered saline were added to the control well. Trays were rotated for 5 min at 180 rpm on a horizontal rotator (Eberbach Co., Ann Arbor, Mich.). Agglutination test results were evaluated with the aid of a desk lamp and were graded strong, intermediate, weak, or negative. Controls for each test were judged for autoagglutination and graded positive, weakly positive, or negative. Strong and intermediate seroagglutination results were considered acceptable only when autoagglutination was weak or absent. Weak

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TABLE 1. Seroagglutination results

Medium[a]	No. of strains reacting[b]																			
	K88						K99						987P							
	S	I	W	N	A	WA	NG	S	I	W	N	A	WA	NG	S	I	W	N	A	WA	NG
BA	17 (1)	20 (1)	1	11	0	1	0	0	0	2	46	1	1	0	2	8	5	30	0	5	0
MIA	12 (6)	14 (4)	10	12	1	0	1	15	13	4	14	2	2	0	0	6	7	35	1	0	1
EA	14 (1)	9	5	17	2	0	3	19	14	4	9	2	2	0	1	7 (1)	4	30	0	7	1
TSB	11	15 (1)	11	13	0	0	0	0	2 (1)	9	34	1	4	0	0	4	3	36	1	6	0
MIB	16 (1)	17 (1)	5	9	3	0	0	5	18 (1)	11	13	1	2	0	5	17 (1)	9	17	1	1	0
EB	6 (2)	6 (1)	1	12	22	0	3	7 (7)	6 (6)	7	18	6	6	0	8 (3)	15 (5)	6	10	9	1	1

[a] BA, Blood agar; MIA, MI agar; EA, E agar; TSB, TSB without dextrose; MIB, MI broth; EB, E broth.
[b] S, strong; I, intermediate; W, weak; N, negative; A, autoagglutination; WA, weak autoagglutination; NG, no growth. Numbers in parentheses are numbers of strains that showed strong or intermediate reactions but were weakly autoagglutinated.

seroagglutination results were considered equivocal when any autoagglutination had occurred.

The test results are summarized in Table 1. Neither E agar nor E broth was as effective in promoting expression of K88 antigen as the other media tested. *E. coli* cultured on blood agar showed the greatest proportion of strong and intermediate seroagglutination reactions.

Test strains cultured on E agar showed the greatest number of positive K99 seroagglutinations. Most of these positive reactions were either strong or intermediate. Large numbers of positive reactions were also obtained from strains cultured on MI agar or in MI broth. Only 2 of 50 K99 test strains cultured on blood agar seroagglutinated, both weakly.

Approximately twice as many 987P test strains agglutinated with 987P antiserum after being cultured in MI broth or E broth than after being cultured on any of the solid media. However, many strains cultured in E broth autoagglutinated. Few strains cultured in TSB gave positive seroagglutination reactions.

More K88-, K99-, and 987P-antigen producing strains were identified after being cultured on several media than would have been possible had only one medium been selected for production of each pilus antigen. For example, only 31 strains cultured in MI broth agglutinated with 987P antiserum, whereas 41 strains from at least one of the six media agglutinated with this antiserum. One K88 strain consistently produced pilus antigen when cultured on E agar but was never shown to produce K88 antigen when cultured on blood agar. Therefore, we recommend that several media such as blood agar, E agar, and MI broth be used when culturing enterotoxigenic *E. coli* for pilus typing.

Others have successfully used synthetic or semisynthetic media in promoting pilus antigen production, but the studies of these researchers were more limited in the number of strains tested and media compared. DeGraaf et al. (1) reported that a minimal medium containing various salts plus glucose was comparable to MI broth in promoting K99 expression. Middeldorp and Witholt (5) recently reported abundant K88 antigen production by a single strain when they used a medium similar to E broth except for having added tissue culture vitamins.

E agar has promise as a medium for promoting K99 expression. It appears at least as efficacious as MI medium. In addition, growth on E agar is much more luxuriant than it is on MI agar, and the medium is less expensive and less chemically complex. E broth was nearly as effective as MI broth in promoting 987P production, but autoagglutination was frequently a problem. However, we found that autoagglutination could

usually be circumvented if cultures were vigorously agitated before being serotyped.

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LITERATURE CITED

1. **DeGraaf, F. K., F. B. Wientjes, and P. Klaasen-Boor.** 1980. Production of K99 antigen by enterotoxigenic *Escherichia coli* strains of antigen groups 08, 09, 020, and 0101 grown at different conditions. Infect. Immun. **27**:216–221.
2. **Glantz, P. J.** 1971. Serotypes of *Escherichia coli* associated with colibacillosis in neonatal animals. Ann. N.Y. Acad. Sci. **176**:67–79.
3. **Guinee, 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.
4. **Isaacson, R. E., H. W. Moon, and R. A. Schneider.** 1978. Distribution and virulence of *Escherichia coli* in the small intestines of calves with and without diarrhea. Am. J. Vet. Res. **39**:1750–1755.
5. **Middeldorp, J. M., and B. Witholt.** 1981. K88-mediated binding of *Escherichia coli* outer membrane fragments to porcine intestinal epithelial cell brush borders. Infect. Immun. **31**:42–51.
6. **Moon, H. W., E. M. Kohler, R. A. Schneider, and S. C. Whipp.** 1980. Prevalence of pilus antigens, enterotoxin types, and enteropathogenicity among K88-negative enterotoxigenic *Escherichia coli* from neonatal pigs. Infect. Immun. **27**:222–230.
7. **Stirm, S., F. Ørskov, I. Ørskov, and B. Mansa.** 1967. Episome-carried surface antigen K88 of *Escherichia coli*. J. Bacteriol. **93**:731–739.
8. **Vogel, H. J., and D. M. Bonner.** 1956. Acetylorinthase of *Escherichia coli*: partial purification and some properties. J. Biol. Chem. **218**:97–106.