APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Sept. 2000, p. 4124–4127 Vol. 66, No. 9
0099-2240/00/$04.00+0

Murine Monoclonal Antibodies Specific for Lipopolysaccharide of *Escherichia coli* O26 and O111

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Monoclonal antibody (MAb) 12F5 reacted with 35 *Escherichia coli* O26 isolates and cross-reacted with 1 of 365 non-*E. coli* O26 isolates. MAb 15C4 reacted with 30 *E. coli* O111 strains and 8 *Salmonella* O35 strains (possessing identical O antigen) but not with 362 other bacterial strains. Lipopolysaccharide immunoblots confirmed MAb O-antigen specificity.

Escherichia coli O26 and O111 are classic enteropathogenic and emergent enterohemorrhagic *E. coli* (EHEC) serotypes. Among the 13 enteropathogenic serogroups, *E. coli* O26 and O111 are epidemiologically predominant and major causes of pediatric diarrhea, especially in developing countries (22, 29). *E. coli* O111 caused 28% of the 50 U.S. infantile diarrhea outbreaks from 1934 to 1987 (33). Over the past 20 years, Shiga toxin-producing O26 and O111 emerged worldwide as the clinically most important non-O157 EHEC serotypes and were linked with sporadic cases and outbreaks of human disease, including hemorrhagic colitis and hemolytic-uremic syndrome (HUS) (8, 9, 28). Several EHEC O26- and O111-caused outbreaks have occurred in Europe and Australia, and 25% of North American HUS cases may be attributable to non-O157 EHEC (13). In the United States, EHEC O111:NM caused a family cluster of diarrhea and HUS (3), and EHEC O111:H8 caused a gastroenteritis outbreak affecting more than 50 persons (1).

Pathogenic *E. coli* O26 and O111 are typically identified by agglutination, using absorbed polyclonal antibodies (PAb) against lipopolysaccharide (LPS) O antigen generated by immunizing rabbits with *E. coli* reference strains (22, 26). However, absorbed anti-*E. coli* O26 PAb may cross-react with *E. coli* possessing O antigens 4, 13, 25, 32, 100, and 102 (26) and with *Pseudomonas aeruginosa* O12 (34). Although no *E. coli* O antigens cross-react with absorbed anti-*E. coli* O111 PAb (26), this PAb strongly agglutinates *Salmonella* O35 isolates (12, 24) because of the identical chemical structure of the *E. coli* O111 and *Salmonella* O35 O antigens (4, 14, 25). Monoclonal antibodies (MAb) reactive with *E. coli* O26 (15, 27) and O111 (2, 5–7, 21, 23) have been reported but were not characterized for diagnostic sensitivity and specificity. The public-health importance and prevalence of pathogenic *E. coli* O26 and O111 in animals, humans, and foods are probably underestimated due to the lack of available specific serotyping reagents and because most clinical laboratories do not routinely serotype fecal *E. coli* isolates (30). We generated MAb against *E. coli* O26 and O111 in order to have accurate serotyping reagents for use in planned epidemiologic surveys of non-O157 EHEC occurrence in livestock.

MAb were produced from splenocytes of BALB/c mice immunized with *E. coli* O26:H11 (ECRC DEC 10A) or O111:NM (ECRC 95.0122) whole-bacterium antigen. Immunization, hybridoma and ascites production, and MAb screening and characterization protocols were previously described (10, 11). MAb were isotyped with a commercial kit (Zymed Laboratories, Inc., South San Francisco, Calif.). One anti-O26 MAb (12F5) and one anti-O111 MAb (15C4) were generated and characterized. MAb diagnostic sensitivity and specificity were estimated by enzyme-linked immunosorbent assay (ELISA) reactivity with whole-bacterium lysates from 400 gram-negative bacterial strains: 35 *E. coli* O26 strains, 30 *E. coli* O111 strains, 26 non-*E. coli* O26 strains reported to react with anti-O26 PAb (*E. coli* O4 [$n = 14$], O13, O25 [$n = 6$], O32, O100, and O102; *P. aeruginosa* O12 [$n = 2$]), 225 other *E. coli* strains of various O and H serotypes, 57 *Salmonella* serovars (including O35 [$n = 8$]), and 27 other gram-negative bacterial strains. For ELISA, bacterial-antigen-coated plates were sequentially incubated with MAb (diluted ascites fluid), horseradish peroxidase (HRP)-conjugated antibody against mouse immunoglobulin G (IgG) plus mouse IgM (anti-mouse IgG+IgM), and 2,2′-azino-di-[3-ethylbenzthiazoline sulfonate] solution (ABTS peroxidase substrate) (10). ELISA optical density was measured at dual wavelengths of 405 and 490 nm ($OD_{405/490}$); and $OD_{405/490}$ of >0.200 was considered positive. Dot box plots (16) of MAb ELISA $OD_{405/490}$ values for bacterial-antigen subsets were generated (Prism 3.0; Graph Pad Software Inc., San Diego, Calif.), and MAb diagnostic-sensitivity and -specificity point estimates with exact binomial 95% confidence intervals (CI) were calculated (Epi Info 6.0; Centers for Disease Control and Prevention, Atlanta, Ga.). Sensitivity was defined as the number of MAb ELISA-positive isolates per the total number of isolates tested possessing the target (O111 or O26) antigen. Specificity was defined as the number of MAb ELISA-nonreactive isolates per the total number of isolates tested that did not possess the target antigen.

MAb 12F5 (IgM isotype) reacted strongly by ELISA with 35 *E. coli* O26 isolates (sensitivity, 100%; 95% CI of 90.0 to 100) and cross-reacted with 1 of 369 non-*E. coli* O26 isolates (specificity, 99.73%; 95% CI of 98.5 to 99.99) (Fig. 1). The cross-reactive strain, *E. coli* O4:NM (CDC3377-85), was derived from a sporadic hemorrhagic-colitis case (32) and was subsequently retyped by the *E. coli* Reference Center (ECRC), Pennsylvania State University, University Park, as O negative: NM; the original O4 antigen was apparently lost on passage since its 1983 isolation. MAb 12F5 was later found to cross-react with bovine *E. coli* O-negative:NM field isolates (J. Keen, unpublished data). While the *E. coli* O26 O-antigen structure is known (20), the nature of the MAb-defined O-antigen

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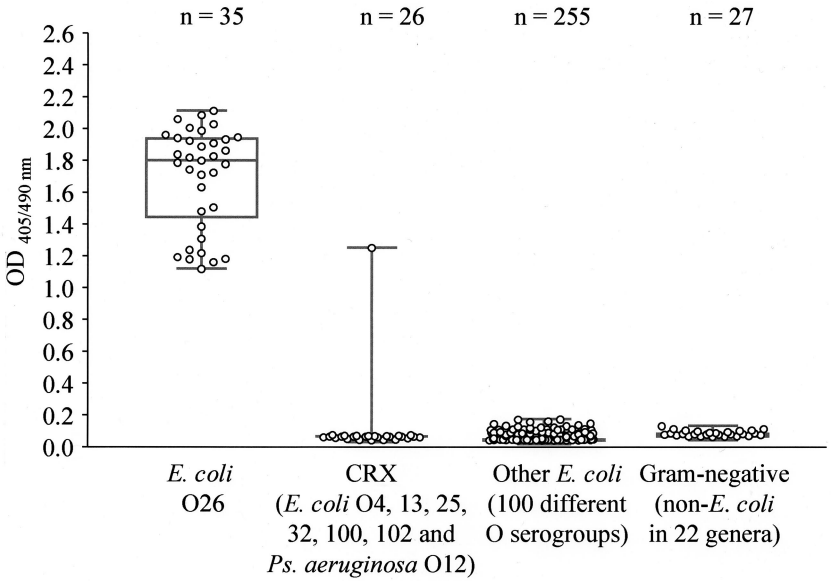

FIG. 1. Dot box plots of ELISA reactivities of 343 whole-bacterium lysates with anti-*E. coli* O26 MAb 12F5. ELISA plates coated with whole-bacterium antigens were incubated sequentially with MAb 12F5 (ascites fluid, 1:7,000), anti-mouse IgG+IgM-HRP, and ABTS solution. A MAb ELISA $OD_{405/490}$ of >0.200 was considered positive. Dots represent means of duplicate OD values. The bottom and top edges of the superimposed box plots are the 25th and 75th distribution percentiles, respectively; the central horizontal line is the median (50th percentile), and the central vertical lines extend from the box as far as the data extend (range). CRX, non-*E. coli* O26 bacteria known to cross-react with anti-*E. coli* O26 PAb. Data for MAb ELISA reactivity with 57 *Salmonella* isolates are not shown.

epitope common to *E. coli* O26 and *E. coli* O-negative:NM isolates is unknown. Importantly, MAb 12F5 did not react by ELISA with any bacteria that cross-react with anti-O26 PAb by agglutination.

MAb 15C4 (IgG3 isotype) reacted strongly by ELISA with 30 *E. coli* O111 strains (sensitivity, 100%; 95% CI of 88.4 to 100) and 8 *Salmonella* O35 isolates (Fig. 2). MAb interaction with *Salmonella* O35 represents true antibody-antigen reactivity, not cross-reactivity, since the *E. coli* O111 and *Salmonella* O35 O antigens are identical (14). MAb 15C4 did not react with 317 non-*E. coli* O111 or 49 *Salmonella* non-O35 isolates (specificity, 100%; 95% CI of 99.0 to 100).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (18), followed by Western immunoblotting (31) using semipurified LPS (17, 19) and whole-bacterium lysates from *E. coli* O26 and O111 and non-*E. coli* O26 and O111 as antigens, confirmed MAb O-antigen specificity. Reactive bands from gels transferred onto polyvinylidene difluoride membranes and incubated with MAb were revealed with HRP-conjugated rabbit anti-mouse IgG+IgM and diaminobenzidine substrate (10).

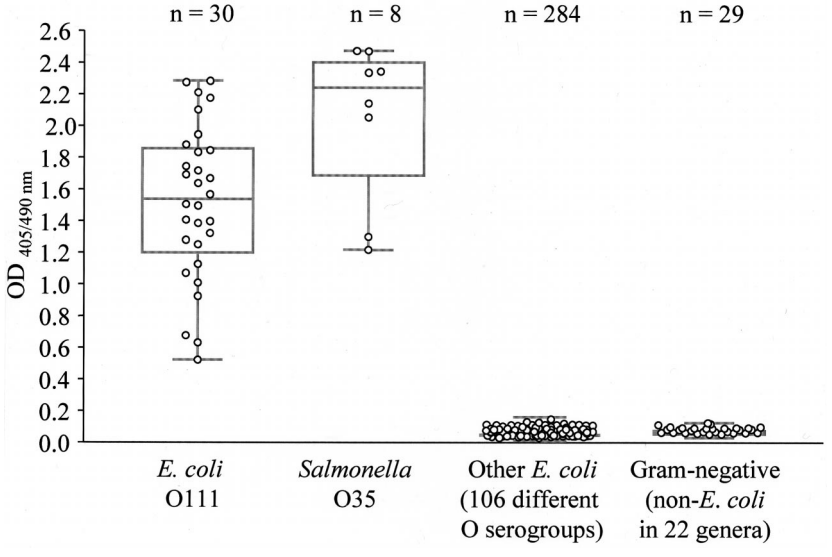

FIG. 2. Dot box plots of ELISA reactivities of 351 whole-bacterium lysates with anti-*E. coli* O111 MAb 15C4. ELISA plates coated with whole-bacterium antigens were incubated sequentially with MAb 15C4 (ascites fluid; 1:64,000), anti-mouse IgG+IgM-HRP, and ABTS solution. OD values represent means of duplicate wells. For dot box plot interpretation, see the legend for Fig. 1. Data for MAb ELISA reactivity with 49 non-O35 *Salmonella* isolates are not shown.

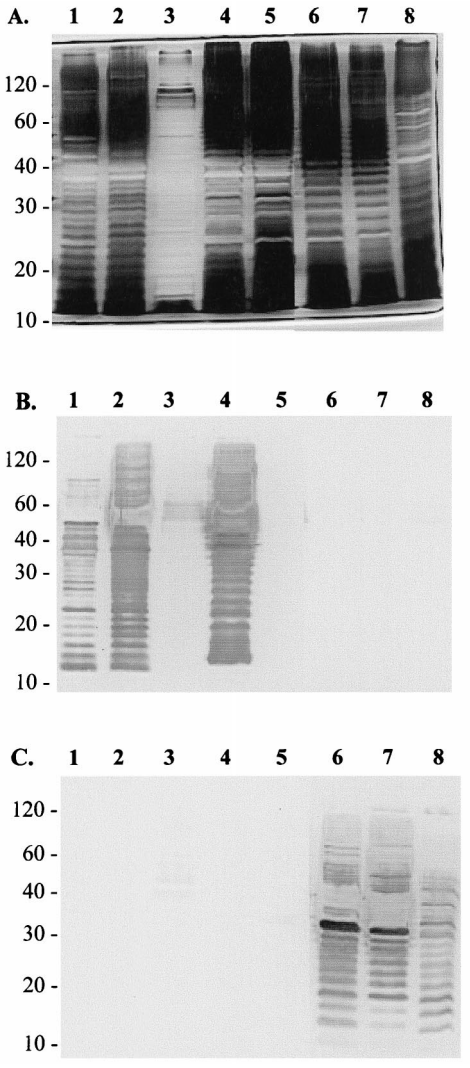

FIG. 3. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Western blots of MAb 12F5 (anti-*E. coli* O26) and 15C4 (anti-*E. coli* O111) on purified LPS. Lanes 1, *E. coli* O26:H11 H311b; lanes 2, *E. coli* O26:H11 DEC 10A; lanes 3, *P. aeruginosa* O12; lanes 4, *E. coli* O4:NM CDC3377-85; lanes 5, *E. coli* O4:H5 U4-41; lanes 6, *E. coli* O111:NM Stoke W; lanes 7, *E. coli* O111:NM ECRC 95.1022; lanes 8, *Salmonella* serovar Adelaide O35 ATCC 10718. After electrophoresis, one gel was silver stained (A). The other two gels were transferred onto polyvinylidene difluoride membranes and incubated with cell culture medium of MAb 12F5 (B) or MAb 15C4 (C). MAb-reactive bands were revealed with rabbit anti-mouse IgG+IgM-HRP (1:1,000) and diaminobenzidine substrate. Molecular mass (in kilodaltons) is indicated on the left.

The ladder pattern characteristic of LPS was observed on silver-stained gels and MAb-incubated membranes (Fig. 3). MAb 12F5 reacted with LPS from *E. coli* O26:H11 and O4:NM CDC3377-85 but not with LPS from five other bacterial strains. MAb 15C4 reacted only with *E. coli* O111 and *Salmonella enterica* serovar Adelaide O35 LPS. Similar immunoblots resulted when whole-bacterium lysates were used in place of LPS (data not shown).

In summary, we produced and characterized MAb against *E. coli* O26 and O111 that were sensitive and specific as assessed by ELISA against 400 target and nontarget isolates. Immunoblots against purified LPS confirmed MAb O-chain specificity. These MAb have potential use as immunodiagnostic reagents whether used alone or in combination with other phenotypic or genotypic markers.

We thank the following individuals for providing bacterial strains: R. A. Wilson, ECRC, Pennsylvania State University; J. R. Johnson, University of Minnesota, St. Paul; E. G. Sowers, CDC; E. Nystrom and R. A. Schneider, National Animal Disease Center, Ames, Iowa; K. Ferris, National Veterinary Services Laboratories, Ames, Iowa; and J. Lam, University of Guelph, Guelph, Ontario, Canada. We also acknowledge S. Fryda-Bradley, K. Thomas, and R. Mlejnek for technical assistance and J. Rosch for manuscript preparation.

REFERENCES

1. **Anonymous.** 2000. *Escherichia coli* O111:H8 outbreak among teenage campers—Texas, 1999. Morb. Mortal. Wkly. Rep. **49:**321–324.
2. **Appelmelk, B. J., J. J. Maaskant, A. M. Verweij-van Vught, N. M. van der Meer, B. G. Thijs, and D. M. MacLaren.** 1993. Antigenic and immunogenic differences in lipopolysaccharides of *Escherichia coli* J5 vaccine strains of different origins. J. Gen. Microbiol. **139:**2641–2647.
3. **Banatvala, N., M. M. Debeukelaer, P. M. Griffin, T. J. Barrett, K. D. Green, J. H. Green, and J. G. Wells.** 1996. Shiga-like toxin-producing *Escherichia coli* O111 and associated hemolytic-uremic syndrome: a family outbreak. Pediatr. Infect. Dis. J. **15:**1008–1011.
4. **Bastin, D. A., L. K. Romana, and P. R. Reeves.** 1991. Molecular cloning and expression in *Escherichia coli* K-12 of the *rfb* gene cluster determining the O antigen of an *E. coli* O111 strain. Mol. Microbiol. **5:**2223–2231.
5. **Coughlin, R. T., and W. C. Bogard, Jr.** 1987. Immunoprotective murine monoclonal antibodies specific for the outer-core polysaccharide and for the O-antigen of *Escherichia coli* O111:B4 lipopolysaccharide (LPS). J. Immunol. **139:**557–561.
6. **Evans, M. E., M. Pollack, N. J. Hardegen, N. L. Koles, G. Guelde, and J. K. Chia.** 1990. Fluorescence-activated cell sorter analysis of binding by lipopolysaccharide-specific monoclonal antibodies to gram-negative bacteria. J. Infect. Dis. **162:**148–155.
7. **Evans, M. E., M. Pollack, N. L. Koles, N. J. Hardegen, and D. Panopoulos.** 1992. Lipopolysaccharide heterogeneity in *Escherichia coli* J5 variants: analysis by flow cytometry. J. Infect. Dis. **166:**803–811.
8. **Goldwater, P. N., and K. A. Bettelheim.** 1998. New perspectives on the role of *Escherichia coli* O157:H7 and other enterohaemorrhagic *E. coli* serotypes in human disease. J. Med. Microbiol. **47:**1039–1045.
9. **Griffin, P. M., and R. V. Tauxe.** 1991. The epidemiology of infections caused by *Escherichia coli* O157:H7, other enterohemorrhagic *E. coli*, and associated hemolytic uremic syndrome. Epidemiol. Rev. **13:**60–98.
10. **He, Y., J. E. Keen, R. B. Westerman, E. T. Littledike, and J. Kwang.** 1996. Monoclonal antibodies for detection of the H7 antigen of *Escherichia coli*. Appl. Environ. Microbiol. **62:**3325–3332.
11. **Hockfield, S., S. Carlson, C. Evens, P. Levitate, J. Pinnate, and L. Silberstein (ed.).** 1993. Selected methods for antibody and nucleic acid probes, p. 59–109. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
12. **Hornstein, L.** 1972. A study of the O and protein antigens of *Salmonella adelaide* and *Escherichia coli* O111:B4 by agar diffusion techniques. Isr. J. Med. Sci. **8:**1901–1905.
13. **Johnson, R. P., R. C. Clarke, J. B. Wilson, S. C. Read, K. Rahn, S. A. Renwick, K. A. Sandhu, D. Alves, M. A. Karmali, H. Lior, S. A. McEwen, J. S. Spika, and C. L. Gyles.** 1996. Growing concerns and recent outbreaks involving non-O157:H7 serotypes of verotoxigenic *Escherichia coli*. J. Food Prot. **59:**1112–1122.
14. **Kenne, L., B. Lindberg, E. Söderholm, D. R. Bundle, and D. W. Griffith.** 1983. Structural studies of the O-antigens from *Salmonella greenside* and *Salmonella adelaide*. Carbohydr. Res. **111:**289–296.
15. **Kerr, P., H. Ball, B. China, J. Mainil, D. Finlay, D. Pollock, I. Wilson, and D. Mackie.** 1999. Use of a monoclonal antibody against an *Escherichia coli* O26 surface protein for detection of enteropathogenic and enterohemorrhagic strains. Clin. Diagn. Lab. Immunol. **6:**610–614.
16. **Krieg, A. F., J. R. Beck, and M. B. Bongiovanni.** 1988. The dot plot: a starting point for evaluating test performance. JAMA **260:**3309–3312.
17. **Laegreid, W., M. Hoffman, J. Keen, R. Elder, and J. Kwang.** 1998. Development of a blocking enzyme-linked immunosorbent assay for detection of serum antibodies to O157 antigen of *Escherichia coli*. Clin. Diagn. Lab. Immunol. **5:**242–246.
18. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **227:**680–685.
19. **Leive, L., V. K. Shovlin, and S. E. Mergenhagen.** 1968. Physical, chemical, and immunological properties of lipopolysaccharide released from *Escherichia coli* by ethylenediaminetetraacetate. J. Biol. Chem. **243:**6384–6391.
20. **Manca, M. C., A. Weintraub, and G. Widmalm.** 1996. Structural studies of the *Escherichia coli* O26 O-antigen polysaccharide. Carbohydr. Res. **281:**155–160.
21. **Miner, K. M., C. L. Manyak, E. Williams, J. Jackson, M. Jewell, M. T. Gammon, C. Ehrenfreund, E. Hayes, L. T. Callahan III, H. Zweerink, and N. H. Sigal.** 1986. Characterization of murine monoclonal antibodies to *Escherichia coli* J5. Infect. Immun. **52:**56–62.
22. **Nataro, J. P., and J. B. Kaper.** 1998. Diarrheagenic *Escherichia coli*. Clin. Microbiol. Rev. **11:**142–201.

23. **Oishi, K., N. L. Koles, G. Guelde, and M. Pollack.** 1992. Antibacterial and protective properties of monoclonal antibodies reactive with *Escherichia coli* O111:B4 lipopolysaccharide: relation to antibody isotype and complement-fixing activity. J. Infect. Dis. **165:**34–45.
24. **Olarte, J., and G. Varela.** 1952. A complete somatic antigen common to *Salmonella adelaide*, *Escherichia coli*-Gomez, and *Escherichia coli* O111:B4. J. Lab. Clin. Med. **40:**252–254.
25. **Ørskov, F., and I. Ørskov.** 1992. *Escherichia coli* serotyping and disease in man and animals. Can. J. Microbiol. **38:**699–704.
26. **Ørskov, I., F. Ørskov, B. Jann, and K. Jann.** 1977. Serology, chemistry, and genetics of O and K antigens of *Escherichia coli*. Bacteriol. Rev. **41:**667–710.
27. **Padhye, N. V., and M. P. Doyle.** 1991. Production and characterization of a monoclonal antibody specific for enterohemorrhagic *Escherichia coli* of serotypes O157:H7 and O26:H11. J. Clin. Microbiol. **29:**99–103.
28. **Paton, J. C., and A. W. Paton.** 1998. Pathogenesis and diagnosis of Shiga toxin-producing *Escherichia coli* infections. Clin. Microbiol. Rev. **11:**450–479.
29. **Senerwa, D., Ø. Olsvik, L. N. Mutanda, K. J. Lindqvist, J. M. Gathuma, K. Fossum, and K. Wachsmuth.** 1989. Enteropathogenic *Escherichia coli* serotype O111:HNT isolated from preterm neonates in Nairobi, Kenya. J. Clin. Microbiol. **27:**1307–1311.
30. **Tarr, P. I., and M. A. Neill.** 1996. Perspective: the problem of non-O157:H7 shiga toxin (verocytotoxin)-producing *Escherichia coli*. J. Infect. Dis. **174:** 1136–1139.
31. **Towbin, H., T. Staehelin, and J. Gordon.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA **76:**4350–4354.
32. **Tzipori, S., K. I. Wachsmuth, J. Smithers, and C. Jackson.** 1988. Studies in gnotobiotic piglets on non-O157:H7 *Escherichia coli* serotypes isolated from patients with hemorrhagic colitis. Gastroenterology **94:**590–597.
33. **Wang, L., H. Curd, W. Qu, and P. R. Reeves.** 1998. Sequencing of *Escherichia coli* O111 O-antigen gene cluster and identification of O111-specific genes. J. Clin. Microbiol. **36:**3182–3187.
34. **Yokota, S., S. Kaya, and H. Noguchi.** 1990. Antigenic epitope in *Pseudomonas aeruginosa* lipopolysaccharide immunologically cross-reactive with *Escherichia coli* O26 lipopolysaccharide. FEMS Microbiol. Lett. **56:**245–248.