JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 1985, p. 1035–1039
0095-1137/85/121035-05$02.00/0

Vol. 22, No. 6

Characterization of a *Yersinia enterocolitica* Antigen Common to Enterocolitis-Associated Serotypes

R. DÍAZ,* E. URRA, J. TOYOS, AND I. MORIYON

Departamento Interfacultativo de Microbiología y Parasitología, Universidad de Navarra, 31080 Pamplona, Spain

Received 7 May 1985/Accepted 3 September 1985

Yersinia enterocolitica **synthesized an exocellular antigen common to the serotypes associated with enterocolitis but absent from other serotypes or from other *Yersinia* species. Both virulent Ca^{2+}-dependent and avirulent Ca^{2+}-independent isogenic pairs derived from the enterocolitis-associated serotypes synthesized the common antigen. Requirements for the synthesis of this common antigen were (i) the presence of metabolizable sugars and (ii) growth on a solid medium at 37°C. The antigen was identified as a 24,000-dalton protein loosely associated with the cell surface but absent from either the cell envelope or the cytoplasmic fraction.**

Yersinia enterocolitica is a gram-negative bacterium that causes several diseases in humans and animals (3). Although this microorganism can be isolated from a variety of sources, only some serotypes have been consistently associated with enterocolitis (20). Up to now, the antigenic differences found between virulent and avirulent serotypes are the temperature-dependent V and W antigens (4) and the plasmid-associated outer membrane proteins (2, 5, 19, 22, 23, 26).

In a previous report (29), we showed that the *Y. enterocolitica* strains belonging to either the O:3 or the O:8 serotypes produce an exocellular common antigen (C-Ag). Such an antigen was synthesized when the cells were grown on a solid medium with a metabolizable carbohydrate at 37°C but not under other conditions. In this report, we present results on the immunological and biochemical characterization of the C-Ag. This antigen was conclusively shown to be a 24,000-dalton (Da) soluble protein, not associated with Ca^{2+} dependence, whose synthesis depended on temperature, the presence of metabolizable sugars, and growth on a solid medium. We also investigated the presence of the C-Ag in other representative serotypes and species of *Yersinia* and found that the 24,000-Da protein was produced by the *Y. enterocolitica* serotypes associated with enterocolitis but not by other serotypes or species of *Yersinia*.

MATERIALS AND METHODS

Bacterial strains. The strains used and their origin and characteristics are given in Table 1. Ca^{2+}-dependent and -independent isogenic pairs were selected by the method of Higuchi and Smith (14).

Preparation of CREX-Ag. Ca^{2+}-dependent or -independent cells from strains 3-89 serotype O:3 and WA serotype O:8 were grown (48 h) in (i) Trypticase soy agar (TSA; BBL Microbiology Systems) at 37°C, (ii) TSA at 26°C, (iii) TSA supplemented with 0.25% glucose and 0.25% sucrose (TSA-DS) at 37°C, and (iv) TSA-DS at 26°C. For the preparation of the CREX-Ag, the growth in 10 Roux bottles was washed off with saline, cells were centrifuged (10,000 × *g*, 20 min), and the supernatant was filtered with a Millipore membrane (0.25-μm pore size) to remove residual cells. The filtered fluid containing the CREX-Ag was ultracentrifuged (86,000 × *g*, 12 h), and the pellet was suspended in distilled water and lyophilized.

All the remaining strains (Table 1) were grown only under conditions iii and iv. The fluid containing the CREX-Ag was obtained as described above, dialyzed against distilled water, and lyophilized directly without being ultracentrifuged.

Cell fractionation. Isogenic Ca^{2+}-dependent and -independent cells obtained from *Y. enterocolitica* serotype O:3 were grown on TSA-DS at 37°C, washed 10 times with saline to remove any loosely attached surface components, and suspended in 20 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES; pH 7.5)–5 mM $MgCl_2$. The cells were disintegrated in an MSK-Braun cell homogenizer, glass beads and unbroken cells were removed by low-speed centrifugation, and the cell envelope and cytosol fractions were separated by ultracentrifugation (85,000 × *g*, 6 h) as described before (21). The cell envelope was fractionated further by sequential detergent extraction, first with Sarkosyl by the method of Filip et al. (11) and then with sodium dodecyl sulfate (SDS; 1% SDS in 10 mM Tris hydrochloride [pH 7.5], 5 min, 100°C).

Analytical and immunological methods. Total carbohydrates and protein were determined by standard methods (9, 18). SDS-polyacrylamide gel electrophoresis was performed in the discontinuous buffer system of Laemmli (17). Molecular weight standards were bovine serum albumin (67,000 [67K]), *Escherichia coli* B porin (38K), soybean trypsin inhibitor (21K), and lysozyme (14K). Western blots were performed by the method of Towbin et al. (28) at 250 mA for 8 h. Antigen bands were detected after incubation first with the corresponding antisera and then with peroxidase-conjugated sheep anti-rabbit immunoglobulin G (Nordic Laboratories). 4-Chloro-1-naphthol (E. Merck AG) was used as the substrate (13).

Immunoelectrophoresis was performed in Noble agar (Difco Laboratories) by using barbital buffer (pH 8.6) with or without 2% Triton X-100 in both the reservoirs and the gel. Indirect hemagglutination with lipopolysaccharide (LPS) was performed as described elsewhere (8). LPS was obtained by the method of Westphal et al. (31) from *Y. enterocolitica* O:3 grown in Trypticase soy broth (BBL) at 26°C.

Protein A-containing staphylococci (*Staphylococcus aureus* Cowan 1 [ATCC 12598]) were produced and stabilized as described by Kronwall (16). A 1-ml sample of a 10% (vol/vol) cell suspension was coated with antibodies by incubation for 3 h at room temperature with 0.2 ml of undiluted serum from rabbits immunized with CREX-Ag

* Corresponding author.

TABLE 1. Distribution of the 24,000-Da exocellular antigen among *Y. enterocolitica* serotypes and other *Yersinia* species

Species and strain (source)[a]	Serotype	C-Ag
Y. enterocolitica		
WA (Alonso)	O:8[b]	+
WA/ETBR (Doyle)[c]	O:8	+
Y7N (Doyle)[d]	O:8	+
3-89 (Dorronsoro)	O:3[b]	+
9 (Bottone)	O:3[b]	+
9B (Bottone)[e]	O:3	+
6249-1 (Aulisio)	O:4,32[b]	+
E659 (Doyle)	O:5, 27/2	+
148-69 (Ahvonen)	O:9[b]	+
WC-188 (Aulisio)	O:21[b]	+
5629 (Díaz)	O:5	−
79/Y (Prats)	O:5	−
2387 (Díaz)	O:5,27/1	−
685-C (Prats)	O:7,8	−
81-Y (Prats)	O:13	−
Y. frederiksenii 867 (Brenner)	O:16	−
Y. kristensenii 103 (Brenner)	O:12	−
Y. intermedia 955 (Brenner)	O:17	−
Y. pseudotuberculosis PB1/ + (Brubaker)	IB	−
Y. pestis KIM (Brubaker)	*mediaevalis*	−

[a] Sources are fully identified in the text.
[b] Strains of *Y. enterocolitica* from which Ca^{2+}-dependent and -independent cells were obtained and tested.
[c] Plasmid-cured derivative of WA.
[d] Plasmid-cured derivative of Y7P.
[e] Plasmid-cured derivative of strain 9.

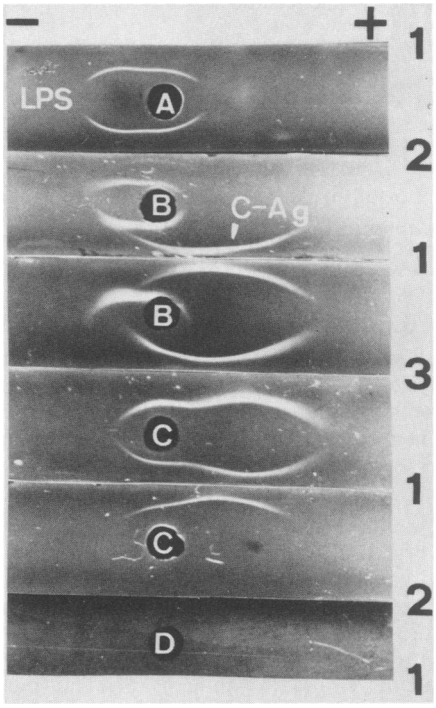

FIG. 1. Immunoelectrophoresis of *Y. enterocolitica* O:3 antigens. Troughs: 1, serum to homologous Ca^{2+}-dependent viable cells; 2, serum to homologous Ca^{2+}-dependent acetone-killed cells grown at 26°C; 3, serum to heterologous Ca^{2+}-dependent viable cells. Antigen preparations: A, homologous LPS; B, CREX-Ag (TSA-DS, 37°C); C, same CREX-Ag preparation but treated with periodate; D, periodate-treated LPS.

treated with periodate. Coagglutination tests were performed as described elsewhere (24).

Antiserum production. All antisera used were raised in rabbits. Antisera to Ca^{2+}-dependent living cells (serotypes O:3 and O:8), grown on TSA at 26°C, were prepared by repeated injection with 10^7 viable cells at weekly intervals (once subcutaneously, then twice intramuscularly, and then twice intravenously). Antisera to Ca^{2+}-independent cells were obtained likewise. In every case, the animals were bled 1 week after the last injection.

Antisera to acetone-killed Ca^{2+}-dependent serotype O:3 cells grown on TSA at 26°C were obtained after four weekly intramuscular injections of 5 mg of cells in Freund incomplete adjuvant.

Two different antigenic preparations were used to obtain antibodies to the C-Ag. One lot of animals was injected intramuscularly with the CREX-Ag of *Y. enterocolitica* O:3 grown on TSA-DS at 37°C (1 mg in Freund incomplete adjuvant per animal), and the injection was repeated 1 week later. The second lot of animals was immunized likewise, but the CREX-Ag had been treated previously with periodate as described by Hurvell and Lindberg (15) to destroy the O antigenic determinants of LPS.

RESULTS

The immunoelectrophoretic analysis of the CREX-Ag of *Y. enterocolitica* O:3 grown on TSA-DS showed two main antigenic components with sera from rabbits infected with homologous cells (Fig. 1, well B, trough 1). The component corresponding to the cathodic precipitin line was sensitive to periodate oxidation and was amphiphilic, as shown by its sensitivity to the presence of Triton X-100 in the gel (Fig. 2, well B). Water-phenol-extracted LPS also showed cathodic mobility (Fig. 1, well A) and was sensitive to periodate oxidation (Fig. 1, well D) and to the presence of the detergent in the gel (Fig. 2, well A). The cathodic precipitin line was detected by the homologous sera (Fig. 1, well B, troughs 1 and 2) but not by the heterologous sera (Fig. 1, well B, trough 3), regardless of the growth conditions of the cells used to obtain the CREX-Ag. Moreover, it gave a line of total identity with the homologous LPS (data not shown).

In contrast, the precipitin line of anodic mobility was

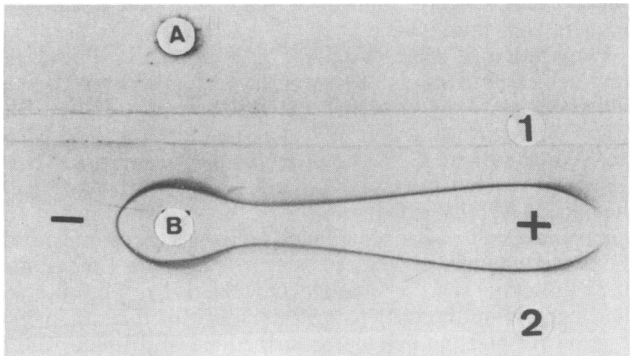

FIG. 2. Triton X-100 immunoelectrophoresis of LPS (well A) and CREX-Ag of *Y. enterocolitica* O:3 (TSA-DS, 37° [well B]). Trough 1, serum to Ca^{2+}-dependent viable homologous cells; trough 2, serum to Ca^{2+}-dependent viable heterologous (O:8) cells.

detected with sera from rabbits infected with either homologous or heterologous cells (Fig. 1, well B, troughs 1 and 3) but not by sera from rabbits immunized with acetone-killed cells grown at 26°C (Fig. 1, well B, trough 2). This component was detected regardless of the presence of Triton X-100 in the gel (Fig. 2). Also, periodate treatment under the conditions that destroyed the cathodic component had only a moderate effect on the shape of the precipitin line (Fig. 1, well C). This component was termed the C-Ag.

The presence of the components described above in the CREX-Ag of *Y. enterocolitica* O:3 grown under different conditions was examined by immunoelectrophoresis with sera of rabbits infected with either C^{2+}-dependent or -independent cells from both serotypes. The results (Table 2) showed that the C-Ag was synthesized only at 37°C and with glucose and sucrose. Under those conditions, both Ca^{2+}-dependent and -independent isogeneic cells produced the C-Ag. The C-Ag was also synthesized upon transferring cells grown at 26°C to TSA-DS and incubation at 37°C.

The CREX-Ag of *Y. enterocolitica* O:3 grown on TSA-DS at 37°C contained carbohydrate (35%) and protein (59%). SDS-polyacrylamide gel electrophoresis analysis (Fig. 3) showed that all the preparations developing the C-Ag precipitin line contained a major band that was stained by Coomassie blue. The apparent molecular weight of this band was 24,000. The same analysis showed that some of the preparations that had not been ultracentrifuged contained minor additional lines (for example, Fig. 3, lane 8) not detected in preparations from other strains.

To obtain a serum specific for the C-Ag (anodic line), rabbits were immunized with either the CREX-Ag from *Y. enterocolitica* O:3 grown on TSA-DS at 37°C or with the same preparation treated with periodate. Whereas the first serum developed both the LPS and the C-Ag precipitin lines, the second developed only the latter (Fig. 4). The titers of these sera in the indirect hemagglutination test with the homologous LPS were 1:16,384 and less than 1:8, respectively.

With the specific serum, Western blot analysis showed conclusively the identity between the 24,000-Da protein and the C-Ag (Fig. 5, lanes 1 and 2). Also, coagglutination with this serum confirmed that the 24,000-Da protein was present only in CREX-Ag preparations of cells grown at 37°C on TSA-DS.

Absorption experiments with whole living cells confirmed that the antigen was present on the cell surface. Both coagglutination and gel precipitation became negative after absorption with cells of the representative strains of serotypes O:3 and O:8 grown on TSA-DS at 37°C but not with cells of the same strains grown on either TSA-DS at 26°C or TSA at 37°C.

It has been reported (2, 5, 19, 22, 23, 26) that in virulent *Y.*

TABLE 2. Results of immunoelectrophoretic analysis of the CREX-Ag obtained from *Y. enterocolitica* O:3

Serum serotype (infecting cells)[a]	Antigenic components synthesized in the following medium at the indicated temp:			
	TSA-DS		TSA	
	37°C	26°C	37°C	26°C
O:3 (Dep.)	C-Ag/LPS	LPS	LPS	LPS
O:3 (Ind.)	C-Ag/LPS	LPS	LPS	LPS
O:8 (Dep.)	C-Ag			
O:8 (Ind.)	C-Ag			

[a] Dep., Ca^{2+}-dependent cells; Ind., Ca^{2+}-independent cells.

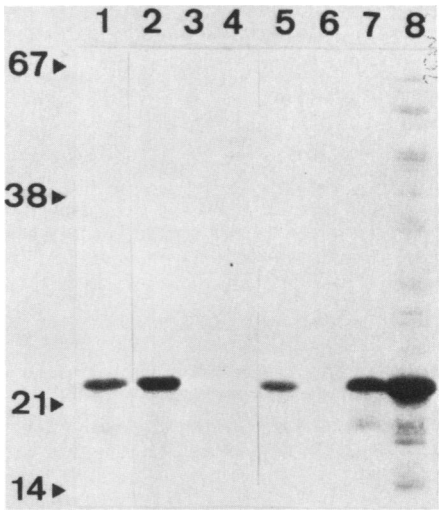

FIG. 3. SDS-polyacrylamide gel electrophoresis of *Y. enterocolitica* CREX-Ag from cells as follows. Lanes: 1, serotype O:3 Ca^{2+}-dependent cells grown on TSA-DS at 37°C; 2, O:3 Ca^{2+}-independent cells grown on TSA-DS at 37°C; 3, O:3 Ca^{2+}-dependent cells grown on TSA-DS at 26°C; 4, O:3 Ca^{2+}-dependent cells grown on TSA at 37°C; 5, O:8 Ca^{2+}-dependent cells grown on TSA-DS at 37°C; 6, O:5,27/1 Ca^{2+}-independent cells grown on TSA-DS at 37°C; 7, O:9 Ca^{2+}-dependent cells grown on TSA-DS at 37°C; 8, O:5/27,2 Ca^{2+}-independent cells grown on TSA-DS at 37°C. Molecular weight markers ($\times 10^3$) are shown at the left.

enterocolitica some outer membrane proteins are expressed at 37°C but not at 26°C. Although the water solubility of the 24,000-Da protein did not support the possibility of its being an outer membrane protein, we investigated its presence in the cell envelope. Neither the Sarkosyl-soluble proteins nor the Sarkosyl-resistant, SDS-solubilized fraction contained a 24,000-Da band that could be detected by Western blot. Likewise, the C-Ag could not be detected in the cytosol fraction by similar methods. In contrast, coagglutination with the 24,000-Da-protein monospecific serum and either whole live cells or acetone-killed cells grown at 37°C on TSA-DS was positive, demonstrating that the antigen was on the cell surface. However, when the cells were washed with saline, the C-Ag was found in the washings, and coagglutination with the cells became negative after the fifth washing.

The presence of the C-Ag on the cells of other serotypes and species of *Yersinia* was studied by coagglutination with the monospecific serum, and the results were confirmed in every case by immunoelectrophoresis and Western blot of the corresponding CREX-Ags (Table 1). Only the serotypes

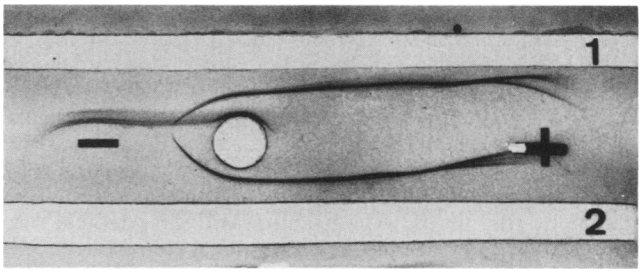

FIG. 4. Immunoelectrophoresis of CREX-Ag from *Y. enterocolitica* O:3 grown on TSA-DS at 37°C. Sera were from rabbits immunized as follows: 1, with CREX-Ag; 2, with periodate-treated CREX-Ag.

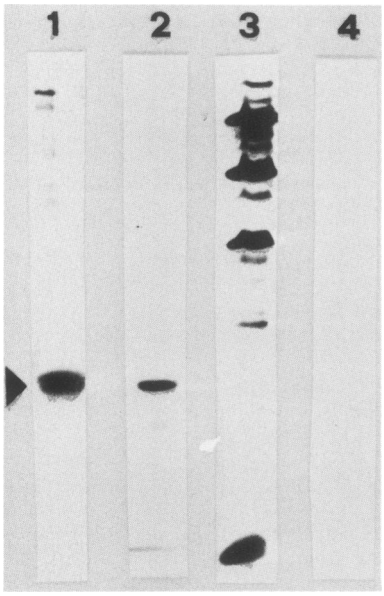

FIG. 5. Western blot analysis of *Y. enterocolitica* O:3 preparations. Lanes: 1, TSA-DS at 37°C with serum to viable homologous cells; 2, same preparation as in lane 1 but with serum to periodate-treated CREX-Ag; 3, supernatant of Trypticase soy broth (37°C) with serum to viable homologous cells; 4, supernatants of Trypticase soy broth (37°C) with serum to periodate-treated CREX-Ag.

associated with enterocolitis produced the C-Ag. In contrast, the serotypes isolated from normal stools, as well as other species of *Yersinia*, did not produce the C-Ag. In all positive serotypes, the C-Ag was synthesized only on TSA-DS at 37°C.

The results described above were obtained with cells grown on solid media. When the same strains were grown in broth, the C-Ag was not found on the cell surface or in either the cell envelope or the cytosol fraction, regardless of the presence of sugars and of the incubation temperature. Likewise, coagglutination, immunoelectrophoresis, and Western blots of the supernatants of the growth medium, concentrated by lyophilization, were negative for the presence of the C-Ag (Fig. 5, lanes 3 and 4).

DISCUSSION

The CREX-Ag of *Y. enterocolitica* O:3 contained two main antigenic components. The component of cathodic mobility was amphiphilic, sensitive to periodate, detected by homologous but not by heterologous sera, and produced under all growth conditions tested. It was identified as LPS. In contrast, the anodic component was not sensitive to either Triton X-100 or periodate and was produced only at 37°C on a solid medium with sucrose and glucose. Both homologous and heterologous cells, either Ca^{2+}-dependent or -independent, produced the C-Ag under these conditions. The C-Ag was also synthesized in vivo, because specific antibody was produced when rabbits were inoculated with viable cells grown at 26°C but not by immunization with acetone-killed cells grown at the same temperature. This cannot be due to the effect of the acetone on the C-Ag, because the antigen was detected on acetone-killed cells grown at 37°C on TSA-DS but not on living cells grown at 26°C. On the other hand, that observation indicates that when injected into the experimental animal, the cells start synthesizing the C-Ag because of the rise in temperature. This interpretation is consistent with the fact that in vitro the C-Ag was produced on TSA-DS only when the temperature was shifted from 26 to 37°C.

The C-Ag was detected in the washings of the cells but not in either the cytoplasmic or the cell envelope fractions, and it was identified as a soluble protein of 24,000 Da. This protein is unlikely to be the temperature-dependent antigen of Doyle et al. (10). This conclusion is supported by the following lines of evidence. First, in contrast to the 24,000-Da protein, the antigen of Doyle et al. is not produced by the plasmid-cured strains WA/ETBR and Y7N. Second, whereas the antigen described by Doyle et al. (10) is produced by cells grown in broth, the C-Ag described here was produced only on solid media. Third, it was shown recently (6) that the synthesis of the antigen of Doyle et al. is associated with the presence of a 42.2 ± 1.1-MDa plasmid, and, as indicated above, both WA/ETBR and Y7N are plasmid cured. Furthermore, both Ca^{2+}-dependent and -independent cells produced the C-Ag, and it is known that Ca^{2+} requirements are related to a 42.2 ± 1.1-MDa plasmid (12).

Because the 24,000-Da protein could not be detected in the cell envelope fraction, it is also different from the temperature- and plasmid-dependent outer membrane proteins described by other researchers (2, 5, 19, 22, 23, 26).

The 24,000-Da protein was produced on solid media with metabolizable sugars but could not be produced in broth. Although we have no data to support it, a possible explanation is that the sugars are required for the synthesis of some sort of glycocalyx or other surface structure containing polysaccharide, for it is known that glycocalyx structures are seldom produced in liquid media (7). In turn, such a structure would be necessary for the 24,000-Da protein to be expressed and retained on the cell surface. In fact, our results suggest some kind of loose association between the C-Ag and the LPS, a major surface structure, because both sediment together upon ultracentrifugation despite their widely different molecular weights.

Our results also show that the *Y. enterocolitica* serotypes O:3, O:8, O:9, O:4,32, O:5,27/2, O:21 (1, 20, 27), and O:13,7 (1), all associated with enterocolitis, produced the C-Ag. In contrast, the *Y. enterocolitica* serotypes O:5, O:5,27/1, O:1,6, O:7,8, and O:13 isolated from normal feces or from feces from which other enteropathogenic bacteria had been isolated and the representative strains of other species did not synthesize the C-Ag.

It is interesting to note that the C-Ag was produced by strain E659 serotype O:5,27/2 but not by strain 2387 serotype O:5,27/1. Although strain E659, originally reported as Ca^{2+} independent (25), could be an avirulent isogenic derivate, the strains of serotype O:5,27 biotype 2 have been consistently associated with enterocolitis (30). In contrast, strain 2387 serotype O:5,27 belongs to biotype 1, which is considered nonpathogenic for humans (20).

Altogether, our observations suggest that the C-Ag is related to the ability to produce enterocolitis. However, the fact that Ca^{2+}-independent cells were able to produce it indicates that the C-Ag cannot be the only virulence factor. It will be necessary to investigate the biological properties of the C-Ag to identify precisely its role in the pathogenicity of *Y. enterocolitica*.

ACKNOWLEDGMENTS

We thank J. M. Alonso, Institut Pasteur, Paris, France; P. Ahvonen, Aurora Hospital, Helsinki, Finland; C. C. G. Aulisio,

Division of Microbiology, U.S. Food and Drug Administration, Washington, D.C.; E. J. Bottone, Mount Sinai Hospital, New York, N.Y.; D. J. Brenner, Centers for Disease Control, Atlanta, Ga.; R. R. Brubaker, Michigan State University, East Lansing; I. Dorronsoro, Hospital Provincial de Navarra, Pamplona, Spain; M. P. Doyle, University of Wisconsin, Madison; and G. Prats, Hospital de la Santa Cruz y San Pablo, Barcelona, Spain, for providing some of the strains used in this work. The valuable assistance of M. A. Iriarte and E. Ardanaz is also gratefully acknowledged.

This work was supported in part by grant 4411/79 from the Spanish CAICYT. J.T. was supported by a grant from the Asociación de Amigos de la Universidad de Navarra.

LITERATURE CITED

1. **Aulisio, C. C. G., W. E. Hill, J. T. Stanfield, and R. L. Sellers, Jr.** 1983. Evaluation of virulence factor testing and characteristics of pathogenicity in *Yersinia enterocolitica*. Infect. Immun. **40**:330–335.
2. **Bölin, I., L. Norlander, and H. Wolf-Watz.** 1982. Temperature-inducible outer membrane protein of *Yersinia pseudotuberculosis* and *Yersinia enterocolitica* is associated with the virulence plasmid. Infect. Immun. **37**:506–512.
3. **Bottone, E. J.** 1977. *Yersinia enterocolitica*: a panoramic view of a charismatic microorganism. Crit. Rev. Microbiol. **5**:211–241.
4. **Carter, P. B., R. J. Zahorchak, and R. R. Brubaker.** 1980. Plague virulence antigens from *Yersinia enterocolitica*. Infect. Immun. **28**:638–640.
5. **Chang, M. T., and M. P. Doyle.** 1984. Identification of specific outer membrane polypeptides associated with virulent *Yersinia enterocolitica*. Infect. Immun. **43**:472–476.
6. **Chang, M. T., J. Schink, J. Shimaoka, and M. P. Doyle.** 1984. Comparison of three tests for virulent *Yersinia enterocolitica*. J. Clin. Microbiol. **20**:589–591.
7. **Costerton, J. W., and R. T. Irvin.** 1981. The bacterial glycocalyx in nature and disease. Annu. Rev. Microbiol. **35**:299–324.
8. **Diaz, R., L. M. Jones, D. Leong, and J. B. Wilson.** 1967. Differences between *Brucella* antigens involved in indirect hemagglutination tests with normal and tanned red blood cells. J. Bacteriol. **94**:499–505.
9. **Dische, Z.** 1955. New color reagents for the determination of sugars in polysaccharides. Methods Biochem. Anal. **2**:313–359.
10. **Doyle, M. P., M. B. Hugdahl, M. T. Chang, and J. T. Beery.** 1982. Serological relatedness of mouse-virulent *Yersinia enterocolitica*. Infect. Immun. **37**:1234–1240.
11. **Filip, C., G. Fletcher, J. L. Wulff, and C. F. Earhart.** 1973. Solubilization of the cytoplasmic membrane of *Escherichia coli* by the ionic detergent sodium-lauryl sarcosinate. J. Bacteriol. **115**:717–722.
12. **Gemski, P., J. R. Lazere, and T. Casey.** 1980. Plasmid associated with pathogenicity and calcium dependency of *Yersinia enterocolitica*. Infect. Immun. **27**:682–685.
13. **Hawkes, R., E. Niday, and J. Gordon.** 1982. A dot-immunobinding assay for monoclonal and other antibodies. Anal. Biochem. **119**:142–147.
14. **Higuchi, K., and J. L. Smith.** 1961. Studies on the nutrition and physiology of *Pasteurella pestis*. VI. A differential plating medium for the estimation of the mutation rate to avirulence. J. Bacteriol. **81**:605–608.
15. **Hurvell, B., and A. A. Lindberg.** 1973. Serological cross-reactions between different *Brucella* species and *Yersinia enterocolitica*. Acta Pathol. Microbiol. Scand. Sect. B **81**:113–119.
16. **Kronwall, G.** 1973. A rapid slide-agglutination method for typing pneumococci by means of specific antibody absorbed to protein A containing staphylococci. J. Med. Microbiol. **6**:187–190.
17. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
18. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
19. **Martinez, R. J.** 1983. Plasmid-mediated and temperature-regulated surface properties of *Yersinia enterocolitica*. Infect. Immun. **41**:921–930.
20. **Mollaret, H. H.** 1976. Contribution à l'etude epidemiologique des infections à *Yersinia enterocolitica*. III. Bilan provisoire des connaissances. Med. Mal. Infect. **6–10**(bis):442–448.
21. **Moriyon, I., and D. T. Berman.** 1982. Effects of nonionic, ionic, and dipolar ionic detergents and EDTA on the *Brucella* cell envelope. J. Bacteriol. **152**:822–828.
22. **Portnoy, D. A., S. L. Moseley, and S. Falkow.** 1981. Characterization of plasmids and plasmid-associated determinants of *Yersinia enterocolitica* pathogenesis. Infect. Immun. **31**:775–782.
23. **Portnoy, D. A., H. Wolf-Watz, I. Bolin, A. B. Beeder, and S. Falkow.** 1984. Characterization of common virulence plasmids in *Yersinia* species and their role in the expression of outer membrane proteins. Infect. Immun. **43**:108–114.
24. **Roig, J. M., I. Dorronsoro, and R. Diaz.** 1983. Rapid identification of *Serratia marcescens* by coagglutination. J. Clin. Microbiol. **18**:741–742.
25. **Schiemann, D. A., J. A. Devenish, and S. Toma.** 1981. Characteristics of virulence in human isolates of *Yersinia enterocolitica*. Infect. Immun. **32**:400–403.
26. **Straley, S. C., and R. R. Brubaker.** 1981. Cytoplasmic and membrane proteins of yersiniae cultivated under conditions simulating mammalian intracellular environment. Proc. Natl. Acad. Sci. USA **78**:1224–1228.
27. **Toma, S., and L. Lafleur.** 1981. *Yersinia enterocolitica* in Canada, p. 183–191. *In* E. J. Bottone (ed.), *Yersinia enterocolitica*. CRC Press, Inc., Boca Raton, Fla.
28. **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.
29. **Toyos, J., E. Urra, L. Fernandez-Lago, and R. Diaz.** 1983. *Yersinia enterocolitica*: influencia de la temperatura en la síntesis de un antígeno de superficie detectable por coaglutinación. Rev. Diagn. Biol. **32**:73–77.
30. **Wauters, G.** 1981. Correlation between taxonomy, serology and biochemistry of *Yersinia enterocolitica*, p. 401–403. *In* T. A. Roberts, G. Hobbs, J. H. B. Christian, and N. Skovgaad (ed.), Psychrotropic microorganisms in spoilage and pathogenicity. Academic Press, Inc. (London), Ltd., London.
31. **Westphal, O., O. Lüderitz, and F. Bister.** 1952. Uber die Extraction von Bacterien mit Phenol/Waser. Z. Naturforsch. Teil B **7**:148–149.