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Serological Studies of Antigenic Similarity between Japanese Spotted Fever Rickettsiae and Weil-Felix Test Antigens

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Acute- and convalescent-phase sera obtained from 10 patients infected with a Japanese strain of spotted fever group (SFG) rickettsia were tested by the indirect immunoperoxidase test, the Weil-Felix test, an enzyme-linked immunosorbent assay (ELISA), and immunoblotting. By the Weil-Felix test, the reactivity of these sera to the OX2 antigen was higher than those to the OX19 antigen, as is the case with sera from persons infected with other SFG rickettsiae. By ELISA, the titers of immunoglobulin M (IgM) antibodies against OX2 corresponded to the Weil-Felix test titers of these sera against OX2 but not to the titers obtained with IgG antibodies. The reactivity of the patient sera with the OX2 antigen in the Weil-Felix test was probably due to IgM antibodies against antigens which OX2 and SFG rickettsiae have in common. By immunoblotting tests, both IgG and IgM antibodies from the patient sera reacted with lipopolysaccharides from SFG rickettsiae and *Proteus* strain OX2. These results may show that these lipopolysaccharides contain similar epitopes.

It is well known that whole cells (WC) of *Proteus vulgaris* OX2 react strongly with sera from persons infected with spotted fever group (SFG) rickettsiae, with the exception of Rocky Mountain spotted fever rickettsia. WC of *P. vulgaris* OX19 react with sera from persons infected with typhus group rickettsiae as well as with Rocky Mountain spotted fever rickettsia (26). SFG rickettsiosis in Japan was first observed in 1984 by Mahara et al. (13), who showed that the sera of infected persons reacted against *Proteus* strain OX2 in the Weil-Felix test. This observation was confirmed by several investigators (18, 21), and Uchida et al. (22) isolated the rickettsia causing the disease that Mahara (12) had named Japanese spotted fever (JSF). Uchida et al. (23) demonstrated that the rickettsia was a new species, distinguished serologically from other species of SFG rickettsiae.

In this study, we tried to define the antigens that the JSF rickettsia and *Proteus* strain OX2 have in common by an indirect immunoperoxidase (IP) test, the Weil-Felix test, an enzyme-linked immunosorbent assay (ELISA), and immunoblotting. By these tests, we showed that the most likely common epitopes among these microorganisms are found in their lipopolysaccharides (LPS).

MATERIALS AND METHODS

Bacteria and growth conditions. SFG rickettsia strain Thai tick typhus TT-118 was obtained from D. J. Kelly of the U.S. Army Medical Research Unit, Malaysia. JSF rickettsia strain Katayama was isolated from the blood of a patient infected with JSF rickettsia as described previously (1, 10). The SFG rickettsiae were propagated in cultured green monkey kidney cells and purified by centrifugation in Ficoll density gradients as described previously (19).

The *Proteus* strains OX2, OX19, and OXK used for antigens for the Weil-Felix test were grown, under constant shaking, in liquid medium incubated at 37°C for 16 h (2, 15). After 0.5% phenol was added, the bacterial cells were sedimented by centrifugation (5,000 × *g*, 30 min) and lyophilized.

Sera of patients with JSF. Thirty-one serum samples from 10 patients with JSF were obtained between 1984 and 1991 from Shikoku Island, Japan.

Immunological assays. The IP tests were performed as described by Suto (18). The Weil-Felix tests and ELISA were performed according to methods described previously (2). The secondary antibodies used in the IP test and the ELISA were either horseradish peroxidase-conjugated goat anti-human immunoglobulin G (IgG) or anti-human IgM antibodies (DAKO A/S, Copenhagen, Denmark).

SDS-PAGE and immunoblotting. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting were performed as described previously (2). The secondary antibodies used in the immunoblotting were the same as those used in the IP test. The polyacrylamide gels were stained for protein according to the method of Oakley et al. (16) and were stained for LPS by the method of Hitchcock and Brown (11). The microorganisms were pretreated with proteinase K before being applied to this gel as described by Mandatori and Penner (14).

RESULTS

Immunological reaction performed with sera from JSF patients against rickettsiae and *Proteus* WC. Paired serum samples from 10 patients with JSF collected from Shikoku Island, Japan, between 1984 and 1991 were analyzed by the IP test (Table 1). IgG and IgM antibodies were detected in sera collected from day 9 onward after the onset of fever when tested with WC of JSF rickettsia strain Katayama and SFG rickettsia strain Thai tick typhus TT-118. The titers of IgG antibodies were higher than those of IgM antibodies, especially with the sera of patients no. 3, 4, 8, and 10, which gave only low titers with IgM antibodies. By IP tests, the

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TABLE 1. Antibody titers of paired serum samples from patients with JSF measured by the IP test

Patient no.	Day(s) after onset of fever	Antibody titer by IP test against SFG rickettsia strain:			
		Katayama		TT[a]	
		IgG	IgM	IgG	IgM
1	8	40	40	20	20
	18	20,480	5,120	10,240	2,560
2	5	40	40	20	10
	9	320	40	320	40
	22	1,280	1,280	1,280	640
	37	1,280	320	1,280	320
3	5	<10	<10	<10	<10
	12	640	40	640	10
	19	640	40	640	10
	26	320	20	320	<10
4	6	<10	<10	<10	<10
	13	1,280	20	640	<10
	20	2,560	40	2,560	10
	24	2,560	40	2,560	10
5	5	<10	<10	<10	<10
	9	160	80	160	80
	12	160	160	160	160
	18	1,280	320	640	320
	22	640	160	640	160
6	4	<10	<10	<10	<10
	16	160	80	160	80
7	4	<10	<10	<10	<10
	16	160	160	80	160
8	4	<10	<10	<10	<10
	11	320	10	320	10
9	10	320	20	320	<10
	22	1,280	320	1,280	40
10	1	<10	<10	<10	<10
	8	80	<10	80	10
	15	160	40	160	40
	24	160	40	160	40

[a] Thai tick typhus TT-118.

sera did not react with WC from the Gilliam, Karp, or Kato strain of *Rickettsia tsutsugamushi* (data not shown).

Table 2 gives the antibody titers of paired serum samples from JSF patients measured by the Weil-Felix test and the ELISA against various *Proteus* WC and the homologous strain of SFG rickettsia. With the exception of the sera from patients no. 6 and 10, the sera gave higher titers to the OX2 antigen than to the OX19 antigen by the Weil-Felix test. Those results suggest that the JSF rickettsia is similar to SFG rickettsiae. By the ELISA, all sera reacted with WC of JSF rickettsia strain Katayama, specially those obtained 10 days or later after the onset of fever. The titers measured by the ELISA were higher than those obtained by the IP test, because of the difference in the sensitivities of the two techniques. When the sera were tested by the ELISA against OX2 WC, the increase in titers of IgM antibodies in the convalescent-phase sera of eight patients (no. 1, 2, 3, 4, 5, 7, 8, and 9) correlated closely with the increase in titers obtained by the Weil-Felix tests run against *Proteus* strain OX2, while the increase in titers obtained with IgG antibodies in the convalescent-phase sera of only three patients (no. 4, 7, and 9) correlated the same way. The ELISA titers of IgM antibody against OX2 WC in the sera of JSF patients also seemed to correspond to the IP titers of IgM antibody against WC of SFG rickettsia. These data suggest that the reactivity of the sera from JSF patients to the OX2 antigen is mainly against IgM antibodies rather than against IgG antibodies.

Detection of antigenic components by immunoblotting. Immunoblotting tests were performed with the sera of 10 JSF patients obtained at various times after the onset of fever (Fig. 1). When the gels were stained with silver, rickettsial WC showed major bands of 115-, 64-, 48-, 32-, 30-, and 26-kDa polypeptides and several minor bands, while OX2 WC showed major bands of 65-, 55-, 40-, 38-, 35-, and 29-kDa polypeptides and many minor bands (Fig. 1A). All of the sera had antibodies in their IgM that reacted with the 115-kDa band of strain Katayama WC. Some sera also had antibodies to this band in their IgG. Sera obtained 11 days or later after the onset of fever had IgM antibodies that reacted strongly with 115-, 32-, and 30-kDa bands and weakly with 26- and 19-kDa bands. IgM antibodies reacted also with several bands between 19 and 32 kDa and 40 and 60 kDa. When OX2 WC were used as the antigen, IgG antibodies of patients no. 2, 4, 5, 8, and 9 and IgM antibodies of patients no. 1, 2, 3, 4, 5, 7, and 9 reacted with various broad, high-molecular-weight bands. The bands of OX2 WC detected by the serum of patient no. 5, 18 days after the onset of fever, showed a distinct ladder structure similar to what has been seen previously with OX2 LPS (15).

Figure 2 shows the reactivities of IgG and IgM antibodies of the same 10 JSF patients to the proteinase K-treated WC of the strains Katayama and OX2. By the silver-staining method, WC of strain Katayama showed several bands in the ladder structure that were weakly stained in both the high-molecular-weight and the low-molecular-weight portions of the gel. The proteinase K-treated WC of strain OX2 showed many ladder bands throughout the gel. These ladder patterns are typical of the wild-type LPS of enteric bacteria (20). IgG and IgM antibodies in the sera of patients no. 1, 2, 3, 4, 5, and 8, who were in the early stage of the disease, did not react with the proteinase K-treated WC of Katayama and OX2. However, IgG antibodies of patients no. 6 and 10, who were also in the early stage, reacted with OX2 LPS, probably because of previous exposure to *Proteus* strain OX2. ELISA data also indicated that the IgG antibodies in the sera from patients no. 6 and 10 reacted strongly with OX2 WC. In the sera obtained late after the onset of fever, IgG antibodies from all sera, except that from patients no. 1 and 2, reacted strongly with Katayama LPS. All sera, except that from patients no. 1, 2, and 3, also reacted with OX2 LPS. Only the serum from patient no. 10 failed to show IgM antibodies against Katayama LPS. The IgM antibodies from patients other than no. 6 and 10 during the late stages of disease also reacted with OX2 LPS.

The immunoblot tests indicate that the sera of all patients scarcely reacted with Katayama LPS and OX2 LPS in the early stage after the onset of fever but reacted strongly in the late stage.

DISCUSSION

The well-known cross-reaction between certain *Proteus* strains and *Rickettsia* spp. has been used as a presumptive diagnosis of rickettsial infection for many years (27). This

TABLE 2. Antibody titer of paired serum samples from patients with JSF measured by Weil-Felix test and ELISA

Patient no.	Day(s) after onset of fever	Antibody titer by:						
		Weil-Felix test against antigen of:			ELISA against WC of:			
					SFG rickettsia[a]		OX2	
		OX2	OX19	OXK	IgG	IgM	IgG	IgM
1	8	20	<20	20	800	800	800	200
	18	160	80	40	102,400	12,800	400	1,600
2	5	40	20	<20	1,600	800	400	400
	9	80	20	20	1,600	1,600	800	400
	22	640	40	20	12,800	12,800	400	6,400
	37	640	20	20	6,400	6,400	200	3,200
3	5	20	<20	<20	1,600	200	1,600	<100
	12	320	40	<20	>102,400	6,400	3,200	3,200
	19	320	40	<20	51,200	6,400	3,200	3,200
	26	160	40	<20	25,600	6,400	1,600	1,600
4	6	<20	<20	<20	1,600	400	1,600	100
	13	160	20	<20	>102,400	12,800	6,400	6,400
	20	160	20	<20	102,400	12,800	6,400	3,200
	24	160	20	<20	>102,400	12,800	6,400	3,200
5	5	20	<20	<20	3,200	400	3,200	200
	9	80	20	<20	12,800	3,200	6,400	1,600
	12	320	80	<20	25,600	6,400	6,400	3,200
	18	160	80	<20	25,600	12,800	6,400	3,200
	22	160	80	<20	25,600	12,800	6,400	3,200
6	4	<20	<20	<20	800	200	6,400	<100
	16	20	40	<20	12,800	800	6,400	200
7	4	20	<20	<20	200	400	400	200
	16	1,280	80	20	3,200	12,800	1,600	1,600
8	4	<20	<20	<20	400	200	3,200	200
	11	40	<20	<20	25,600	1,600	6,400	1,600
9	10	80	20	20	12,800	1,600	6,400	1,600
	22	320	80	20	51,200	12,800	25,600	12,800
10	1	<20	<20	<20	800	<100	25,600	<100
	8	<20	<20	<20	3,200	<100	12,800	<100
	15	<20	20	<20	12,800	200	12,800	<100
	24	20	20	20	12,800	200	25,600	<100

[a] WC of SFG rickettsia strain Katayama were used as the antigen.

test employs *Proteus* OX2, OX19, and OXK, which give characteristic agglutination patterns with the sera of patients infected with different rickettsial agents. Castaneda (8) reported that *Proteus* strain OX19 possessed an acid-stable and moderately alkali-stable polysaccharide antigen responsible for the cross-reaction with *Rickettsia* spp. Schramek et al. (17) described the presence of a biologically active LPS fraction in the typhus group rickettsiae, and Anacker et al. (7) demonstrated the patterns of LPS in *R. rickettsii* by SDS-PAGE. Amano et al. also reported the chemical compositions of LPS from *Coxiella burnetii* (5, 6), but LPS was not detected in *R. tsutsugamushi* (4).

We found that LPS from the *Proteus* strains OX2, OX19, and OXK were antigenically and chemically different (2, 15). The antisera from rabbits immunized with WC or LPS from each strain did not react with some rickettsial WC (*R. typhi*, *R. prowazekii*, and *R. tsutsugamushi*) (2). In a preliminary report (3), we suggested that the cross-reactivity between the JSF rickettsia and the Weil-Felix test antigens was probably due to the common antigenicity of their LPS.

The results obtained in the present study indicated that the reactivity of sera from patients infected with the JSF rickettsia to *Proteus* strain OX2 was mainly found with IgM antibodies. According to Cox (9), in murine typhus infections, the *Proteus* agglutinins were probably in the IgM.

The results of immunoblot tests for the detection of the common antigenicity of JSF rickettsia and *Proteus* strains with WC or LPS as the antigens and the sera of JSF patients as the developing reagents showed that the sera reacted strongly with 115-, 32-, and 30-kDa bands and that several bands in the ladder structure were stained in WC of JSF rickettsia strain Katayama. The 115-kDa band, probably a cell wall surface component, reacted with sera taken during the early as well as the late stage after the onset of disease. Uchiyama et al. (24) reported that 145- and 120-kDa proteins are located on the surface of the JSF rickettsia as species-

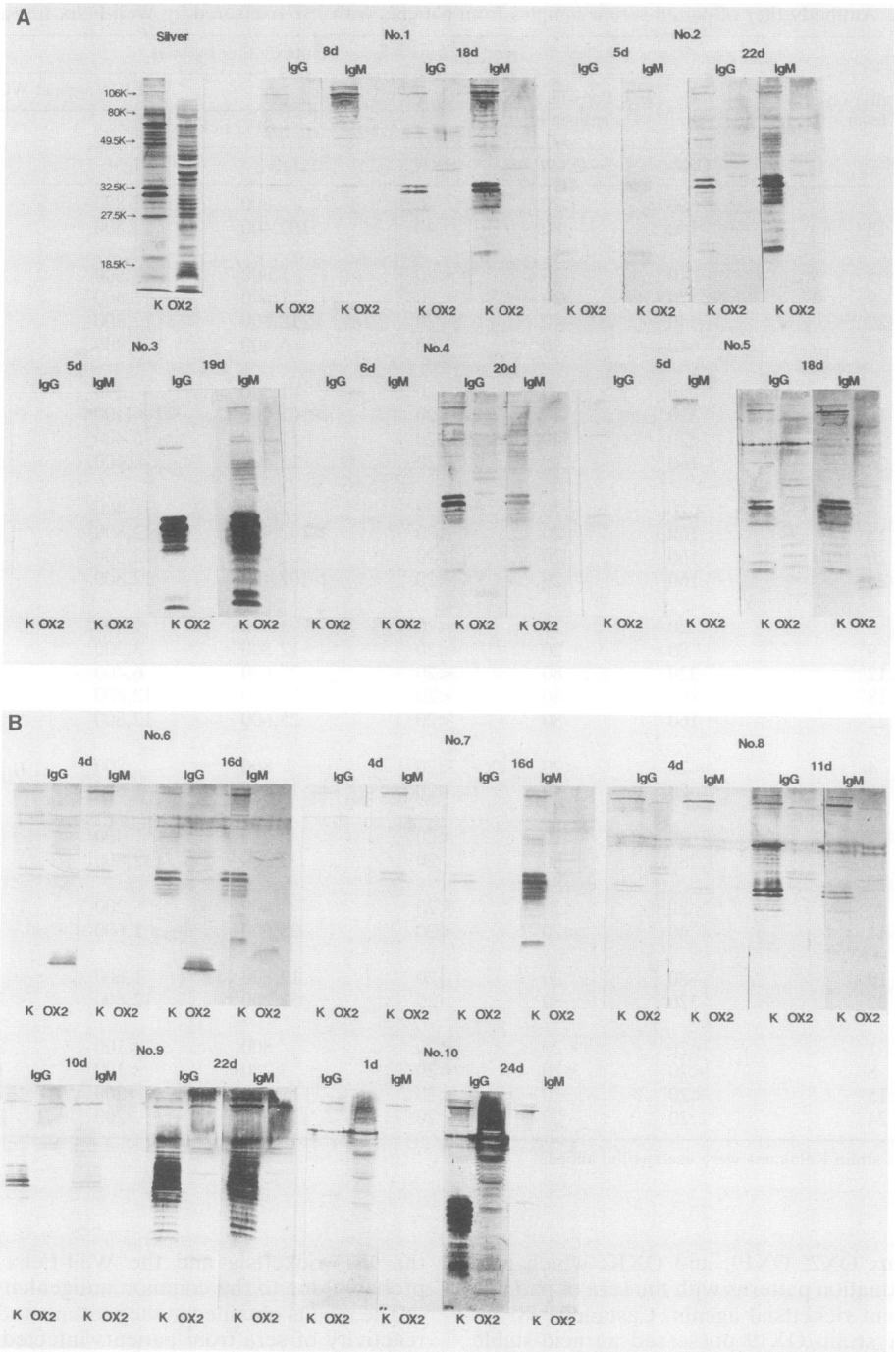

FIG. 1. Antigenic materials detected in WC of JSF rickettsia strain Katayama and *Proteus* strain OX2 by immunoblotting with sera from JSF patients. K, strain Katayama WC; OX2, OX2 WC. As a control, WC of two organisms were stained with silver after SDS-PAGE. Molecular weights (in thousands) of standard proteins are indicated to the left of this gel.

specific epitopes. Our results suggested strongly that the 115-kDa component is identical to the 120-kDa protein. We do not know whether 32- and 30-kDa antigenic bands are proteins or LPS, because these two bands had the same migration as some of the ladder bands from LPS. When treated with proteinase K, LPS fractions of the rickettsia reacted with the IgG and IgM antibodies from most of the JSF patient sera. LPS from *Proteus* strain OX2 reacted with IgM antibodies from all of the patient sera, except for patients no. 6 and 10, who had antibodies mainly in their IgG. On the basis of the immunological assays of sera from these two patients, we speculate that sera from persons previously exposed to *Proteus* strains similar to OX2 and then subsequently infected with the JSF rickettsia do not give a positive agglutination with OX2 antigens and rarely react with OX2 LPS in IgM antibodies. Therefore, it is

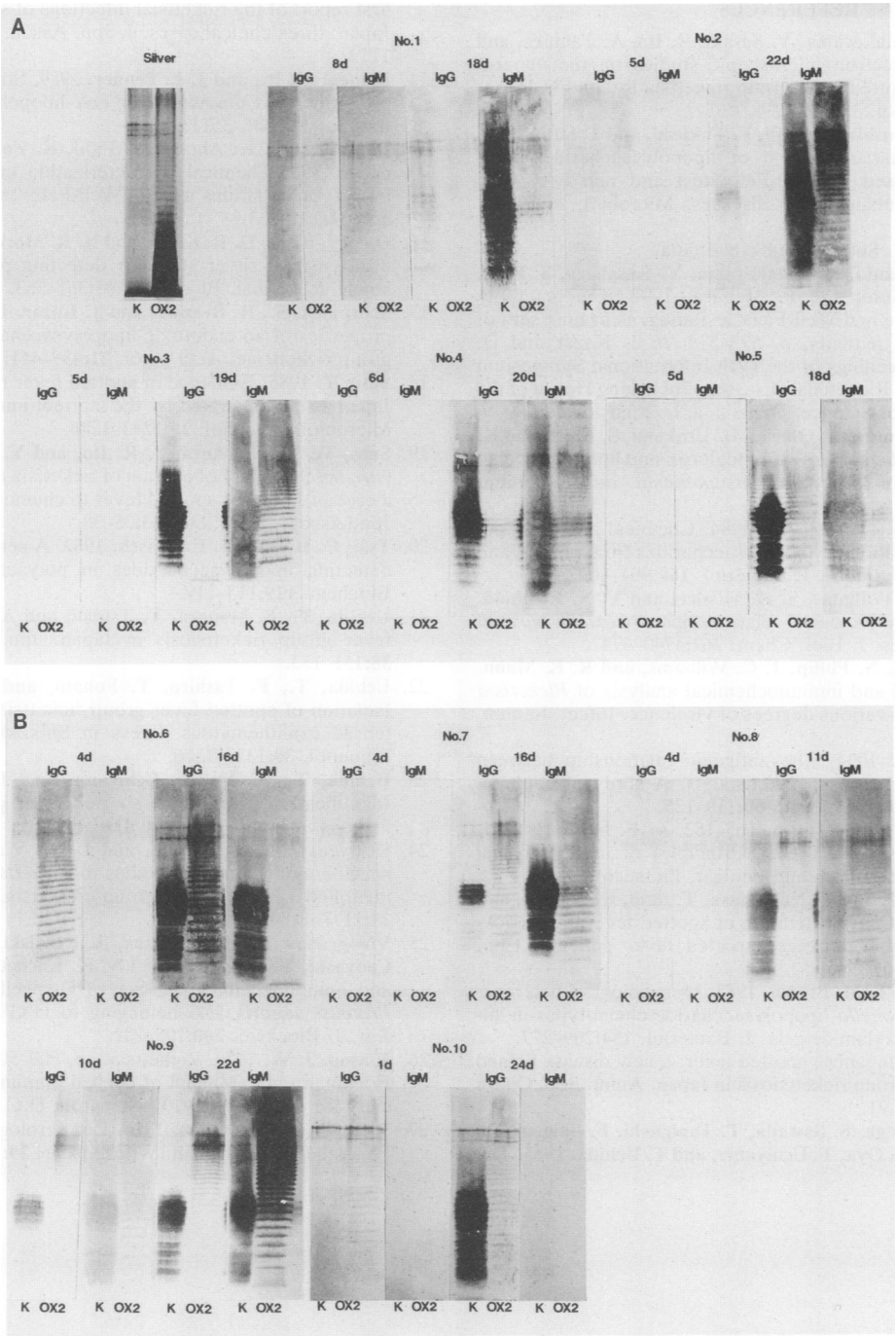

FIG. 2. Antigenic materials detected in proteinase K-treated WC of JSF rickettsia strain Katayama and *Proteus* strain OX2 by immunoblotting with sera from JSF patients. K, proteinase K-treated WC of strain Katayama; OX2, proteinase K-treated WC of OX2. As a control, proteinase K-treated WC of two organisms were stained with silver after SDS-PAGE.

possible that *Proteus* LPS can be used as a vaccine to prevent infection by JSF as well as other spotted fevers.

Recently, Vinogradov et al. (25) reported that the O-specific polysaccharide of LPS in *P. vulgaris* 5/43, belonging to OX19 (O variants), contained glucose, *N*-acetylglucosamine, and *N*-acetylquinovosamine. We also reported the presence of quinovosamine in the OX2 and OX19 LPS (2, 15). On the basis of these reports and our preliminary data (2a) that LPS from SFG rickettsiae contains this amino sugar, we suggest that quinovosamine may be responsible for the cross-reactivity between SFG rickettsiae and *Proteus* strain OX2.

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