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Electrophoretic Analysis of the Surface Components of Autoagglutinating Surface Array Protein-Positive and Surface Array Protein-Negative *Aeromonas hydrophila* and *Aeromonas sobria*

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The protein and lipopolysaccharide (LPS) compositions of 10 autoagglutinating *Aeromonas hydrophila* and *Aeromonas sobria* strains were studied; one group consisted of five serogroup O:11 strains that contained an S layer, while a second group was composed of diverse serogroups that were S layer negative by transmission electron microscopy. All serogroup O:11 strains were found to contain a predominant 52,000- to 54,000-molecular-weight protein that was present on both whole-cell and outer membrane protein profiles; this protein was found to be glycine extractable under low-pH (pH 4) conditions and was identified as the surface array protein. LPS analysis revealed that all O:11 strains exhibited homogeneous-length O-polysaccharide side chains characterized primarily by two or three major bands. In contrast, S-layer-negative autoagglutinating strains of other serogroups lacked this predominant surface array protein, and silver stain analysis of LPS indicated that such profiles mainly consisted of core antigens and were deficient in or devoid of O-polysaccharide side chains. These collective results offer potential explanations for observed differences between these two groups in virulence, disease spectrum, and pathogenic properties.

Members of the genus *Aeromonas* are increasingly being recognized as important intestinal and extraintestinal pathogens of humans and a variety of other vertebrate and invertebrate species including fish, amphibia, reptiles, and birds (11). Difficulties in determining which factors regulate *Aeromonas* pathogenicity stem from an extremely complicated taxonomy (at least 11 distinct genetic groups) and the failure of most virulence-associated characteristics to be strain and not species specific. In the case of *Aeromonas*-associated gastroenteritis in humans, the lack of an appropriate animal model to faithfully reproduce the diarrheal disease syndrome has been an additional obstacle to conclusively establishing this organism as an etiologic agent (11).

We have recently identified a group of *Aeromonas hydrophila* and *Aeromonas sobria* strains that are associated with systemic infections in humans and share a common phenotypic feature, namely, autoagglutination in liquid media (12). Members of one particular subgroup of this phenotype share several additional features, including a common somatic antigen (serogroup O:11) and enhanced virulence for animals, as determined by mouse pathogenicity studies; to date, most of these phenotypically related O:11 strains obtained from infected humans have been isolated from cases of bacteremia and peritonitis (12, 20). Another linked feature of this subgroup is the presence of a crystalline surface array protein (SAP) in the form of an S layer which lies peripheral to the cell wall (12, 20); other autoagglutination phenotypes lack these linked characteristics (serogroup O:11, mouse pathogenicity, and S layer). At the same time as these studies, Dooley and collaborators (5–7) identified a similar if not identical group of *A. hydrophila* strains responsible for overt pathogenicity in fish (trout) that are S layer positive; these strains exhibit antigenic features identical to our strains (serogroup O:11). The S layer of one of these *A. hydrophila* isolates (strain TF7) has been extensively characterized by Dooley and colleagues (6) and has been found to be a protein of approximately 52,000 molecular weight and to be composed of 520 amino acids, 41% of which are hydrophobic. At present, it is unclear what role (if any) these S layers play in overt pathogenicity, although resistance to complement-mediated lysis is one possibility.

To date, only six S-layer-positive *A. hydrophila* strains have been studied to any degree and only one of these (strain TF7) has been extensively characterized; all of these strains are of environmental origin. From our previous investigations, it is apparent that S-layer-containing O:11 strains occur among other *Aeromonas* species (e.g., *A. sobria*), are involved in infectious processes in humans (both disseminated and localized infections), and vary in their virulence potential. In addition, the marker (autoagglutination) previously used to initially recognize this group is not solely restricted to serogroup O:11, nor does it inherently imply overt pathogenicity; many non-O:11 autoagglutinating (AA^+) strains which to date have received little attention exist. Since the role of the SAP in pathogenesis is presently unknown and other cell-associated proteins are likely to play an important role in virulence, we have studied the surface proteins and lipopolysaccharide (LPS) composition of AA^+ strains that are either SAP positive or SAP negative in an attempt to determine the extent of structural diversity within these groups and to potentially identify unique or important structural features which could help account for observed virulence differences.

MATERIALS AND METHODS

Bacterial strains. Ten AA^+ *Aeromonas* strains were investigated in this study. These strains were selected from previous investigations (13, 20) for further analysis on the basis of their phenospecies designation, source, surface

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TABLE 1. Major characteristics of AA$^+$ *Aeromonas* strains

Strain	Phenospecies	Source	Serogroup[a]	AA$^+$	S layer[b]	Virulence (LD_{50})[c]
LL1	*A. hydrophila*	Trout	O:11	Yes	+	1.5×10^7
AS-28	*A. sobria*	Stool, human	O:11	Yes	+	1.6×10^7
AS-76	*A. sobria*	Blood, human	O:11	Yes	+	1.3×10^7
ATCC 9071	*A. sobria*	Frog	O:11	Yes	+	6.2×10^7
AH-342	*A. hydrophila*	Blood, human	O:11	Yes	+	9.8×10^6
AS-53	*A. sobria*	Site unknown, human	R	Yes	−	2.8×10^8
AH-78	*A. hydrophila*	Urine, human	O:22	Yes	−	2.0×10^8
AS-178	*A. sobria*	Paracentesis, human	O:36	Yes	−	4.8×10^7
ATCC 14715	*A. hydrophila*	Salmon	O:22	Yes	−	3.2×10^8
NCMB 1134	*A. hydrophila*	Trout	O:22	Yes	−	1.6×10^8

[a] R, Rough strain.
[b] Determined by transmission electron microscopy (thin section).
[c] LD_{50}, 50% lethal dose (intraperitoneal injection) in outbred Swiss Webster mice.

properties, and relative pathogenicity in mice. Each strain was identified to the species level (phenospecies) by previously published criteria (13). Working cultures of each strain were maintained on heart infusion agar slants at ambient temperatures and were periodically transferred. The major phenotypic, serologic, and pathogenic properties have been previously described (13, 20) for each strain listed in Table 1.

Protein analysis and purification. Whole-cell (WC) protein extracts of *Aeromonas* spp. were prepared and quantitated as described previously (20). Extraction of S-layer proteins was performed according to a modification of the method of McCoy et al. (17). Cells were harvested from heart infusion agar plates after 18 h of growth, suspended in 15 ml of cold 20 mM Tris buffer (pH 8.0) for 10 min at 4°C, washed, and centrifuged two times in Tris buffer. The bacteria were suspended in 15 ml of cold 0.2 M glycine hydrochloride (pH 4.0) for 30 min at 4°C and mildly vortexed every 10 min. Cells were subsequently removed by centrifugation at 7,000 × *g* for 30 min, and the S-layer protein was recovered by centrifugation at 30,000 × *g* for 30 min, with final resuspension in 1.0 ml of Tris buffer.

Outer membrane proteins (OMP) were prepared by a modification of the method of Filip et al. (8) using *N*-lauroylsarcosine (Sigma Chemical Co., St. Louis, Mo.). *Aeromonas* strains were grown overnight in 100 ml of L broth at 35°C. Bacteria were harvested at 7,000 × *g*, and the cell pellet was suspended in 10 ml cold 0.05 M Tris hydrochloride (pH 7.4). Dilutions and duplicate plate counts revealed an initial bacterial suspension of ca. 10^9 CFU/ml. The cells were placed on ice and disrupted by using a Branson (Danbury, Conn.) Sonifier Cell Disrupter 350 until an optical density at 600 nm of 5 to 10% of the original value was obtained. Sonication resulted in a decrease of ca. 99% of the viable bacteria, as determined by duplicate plate counts. Unbroken cells were removed by centrifugation at 7,000 × *g* for 20 min at 4°C (Beckman J-21 centrifuge and JA-20 rotor; Beckman Instruments, Inc., Palo Alto, Calif.), and the membrane fraction was harvested by centrifugation at 30,000 × *g* for 60 min at 4°C. Membrane pellets were suspended in 500 μl of distilled water and frozen at −70°C. Frozen suspensions were later thawed, and OMP were isolated by differential solubilization of the inner membrane by using 800 μl of sarcosyl-Tris (0.5% [wt/vol] *N*-lauroylsarcosine [Sigma], 10 mM Tris [pH 7.6]) to 200 μl of membrane suspension. The samples were centrifuged at 50,000 × *g* (Beckman L8-80M ultracentrifuge and SW41 Ti rotor with 1.5-ml microfuge tubes), and the outer membrane pellet was suspended in 200 μl of distilled water.

Electrophoresis. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the procedure of Laemmli (15) as described previously (20). The stacking gel was 6%, and the separating gel was either 8.75 or 10% acrylamide. WC protein suspensions (30 μg) and OMP (90 μl) were electrophoresed at 30 mA constant current at room temperature and stained with 0.1% Coomassie brilliant blue R-250. Contact prints were made with Kodak electrophoresis duplicating paper.

LPS. Electrophoretic LPS patterns were determined by using a modification of the procedure of Hitchcock and Brown (9). WC extracts were digested with proteinase K (protease type XI; Sigma) at a ratio of 1 mg of proteinase K to 10 mg of bacterial cell mass at 60°C for 1 h, boiled for 5 min, and cooled to room temperature. DNase I and RNase A, both from bovine pancreas (Sigma) (100 μg of each), were added to 1 ml of the proteinase K-treated sample, and the mixture was incubated at room temperature for 1 h. The samples were boiled for 5 min in sample buffer, centrifuged at 3,000 × *g*, and loaded onto 1.5-mm 10% SDS-PAGE gels. Purified LPS from *Escherichia coli* O111:B4 and *Salmonella typhimurium* TV119 (Ra mutant) (Sigma) were used as controls. After electrophoresis, the gels were stained by the procedure of Merril et al. (18) by using the Bio-Rad silver staining kit (Bio-Rad Laboratories, Richmond, Calif.), with the minor modification of five 10-min washes after application of the oxidizer reagent. Contact prints were made with Kodak electrophoresis duplicating paper.

Isoelectric focusing. The isoelectric point of a glycine-extracted S-layer protein from a selected strain was determined by using precast Ampholine PAGplates (pH 3.5 to 9.5) and the Multiphor II electrophoresis system and Thermostatic Circulator 2219 Multitemp II (Pharmacia LKB, Piscataway, N.J.). An isoelectric focusing calibration kit (Pharmacia LKB) containing pI marker proteins with a pH range of 3 to 10 was used as a control. Sample application strips were placed on the ampholine gel surface at various locations, and 20 μl of the surface protein and the pI-marker control were added to the respective strips. The samples were electrophoresed at prescribed power supply settings for 1.75 h at 15°C.

Virulence studies. The determination of mouse pathogenicity and resulting 50% lethal doses have been described previously (20).

RESULTS

Protein analysis. WC extracts of five serogroup O:11, S-layer-positive aeromonads and of five AA$^+$ isolates belonging to other serogroups were analyzed by SDS-PAGE

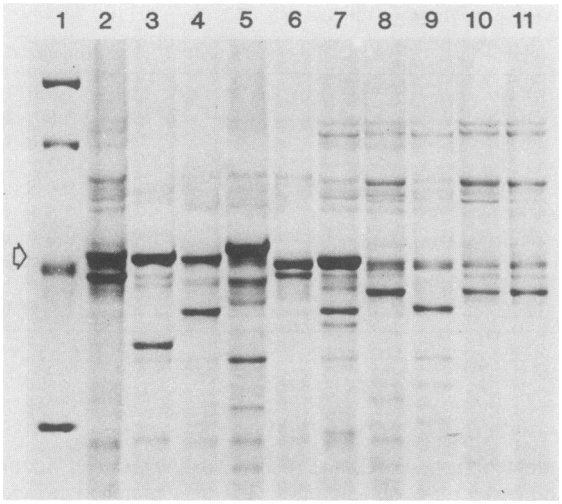

FIG. 1. WC protein profiles of 10 AA^+ *Aeromonas* strains electrophoresed on 10% SDS-PAGE. Shown are protein standards (90, 77, 52, and 18K) (lane 1); LL1 (lane 2); AS-28, serogroup O:11 (lane 3); AS-76, serogroup O:11 (lane 4); ATCC 9071, serogroup O:11 (lane 5); AH-342, serogroup O:11 (lane 6); AS-53, rough strain (lane 7); AH-78, serogroup O:22 (lane 8); AS-178, serogroup O:36 (lane 9); ATCC 14715, serogroup O:22 (lane 10); and NCMB 1134, serogroup O:22 (lane 11). Each lane (excluding protein standards) contains 30 μg of protein. Arrow indicates position of 52,000- to 54,000-molecular-weight SAP.

(Fig. 1). At least 30 resolvable proteins by Coomassie blue staining were exhibited by each of the respective strains. Among serogroup O:11 isolates, a predominant major protein in the molecular weight range of 52,000 to 54,000 was consistently observed on 10% SDS-PAGE gels; previous molecular weights calculated for these proteins in three of these strains on 8.75% polyacrylamide gels were estimated to be between 44,000 and 46,000. Other AA^+ serogroups, with the exception of AS-53, failed to demonstrate a similar 52,000- to 54,000-molecular-weight major protein when equivalent loads of protein were analyzed by 10% SDS-PAGE. In addition to this band, common and unique proteins were observed within the O:11 strains and other serogroups studied. LL1 and AH-342 share a major band with an M_r of ca. 50,000; AS-76, AS-53, and AS-178 share a major band with an M_r of ca. 43,000; AH-78, ATCC-14715, and NCMB 1134 share a major band with an M_r of ca. 46,000 and less prominent bands with M_rs of ca. 68,000 and 53,000; non-O:11 strains also appear to share major protein bands with M_rs of 78,000 and 80,000. All three of the serogroup O:22 strains displayed relatively similar WC protein profiles, as opposed to the greater diversity exhibited by serogroup O:11 strains.

SDS-PAGE analysis of the major OMP of the 10 AA^+ strains indicated that the major proteins detected in WC preparations (excluding SAP) appear to reside in the outer membrane (Fig. 2). Partial OMP purification of the O:11 strains of AS-76 and AS-9071 revealed additional major proteins of M_r ca. 20,000 and 18,000 not detected by WC analysis. Of the non-O:11 strains, ATCC 14715 revealed a major OMP band of M_r ca. 46,000 not present in the other O:22 strains (Fig. 3). In addition, the rough strain AS-53 exhibited the greatest number of major OMP bands when compared with O:11 or O:22 strains; a majority of these bands were not detected by WC analysis (Fig. 1).

Glycine hydrochloride-extracted Tris suspensions of the

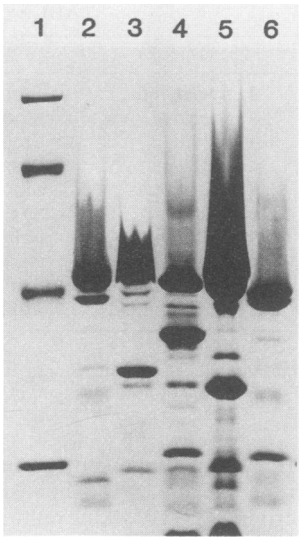

FIG. 2. 10% SDS-PAGE of OMP profiles of serogroup O:11 strains. Shown are protein standards (90, 77, 52, and 18K) (lane 1), LL1 (lane 2), AS-28 (lane 3), AS-76 (lane 4), ATCC 9071 (lane 5), and AH-342 (lane 6). Each lane (excluding standards) contains 90 μl of reconstituted pellet and sample buffer.

five O:11 strains were run on 8.75% SDS-PAGE gels (Fig. 4). From each extract a single major protein of 52,000 to 54,000 molecular weight, consistent with that previously observed in WC protein profiles, was detected; negatively stained preparations of these glycine-extracted suspensions observed by transmission electron microscopy revealed typical sheets of S-layer material composed of tetragonally arranged SAP subunits (Fig. 5). Laser densitometric scanning of the WC lane of ATCC 9071 prior to glycine extraction revealed

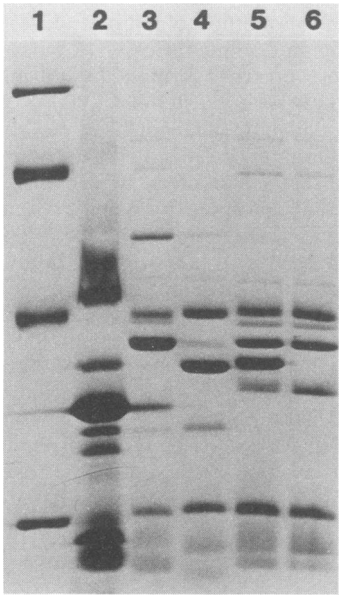

FIG. 3. 10% SDS-PAGE of OMP profiles of non-O:11 strains. Shown are protein standards (90, 77, 52, and 18K) (lane 1), AS-53 (lane 2), AH-78 (lane 3), AS-178 (lane 4), ATCC 14715 (lane 5), and NCMB 1134 (lane 6). Each lane (excluding standards) contains 90 μl of reconstituted pellet and sample buffer.

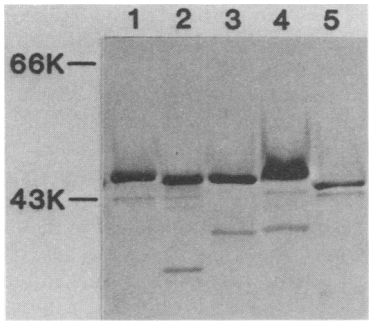

FIG. 4. SDS-PAGE (8.75%) of glycine hydrochloride extraction of SAP from serogroup O:11 strains. Shown are LL1 (lane 1), AS-28 (lane 2), AS-76 (lane 3), ATCC 9071 (lane 4), and AH-342 (lane 5). Each lane contains 5 μg of protein.

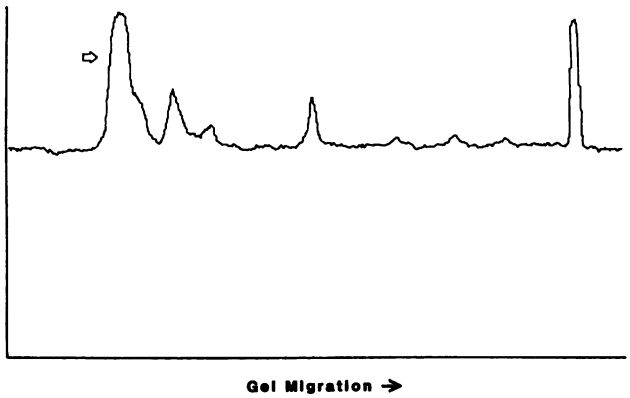

FIG. 6. Soft laser densitometric scanning of WC protein profile of *A. sobria* ATCC 9071. ◊, position of SAP.

that the S layer composes approximately 48% of the total protein (Fig. 6). Subsequent densitometric scanning of its corresponding S layer after glycine extraction and electrophoresis by SDS-PAGE revealed that the S layer represents approximately 97% of the total protein present. This surface-layer protein of ATCC 9071 was isoelectrically focused in an ampholine-containing gel (pH 3.5 to 9.5) and was found to have an isoelectric point of 4.70, compared with the internal protein standards (Fig. 7).

SDS-PAGE analysis of glycine hydrochloride extracts from four of the five non-O:11 S-layer-negative strains failed to show any detectable S-layer protein (data not shown); only strain AS-53 contained a major glycine-extractable protein of similar molecular weight to that observed in S-layer-containing strains. However, this strain was repeatedly confirmed to be S layer negative upon ultrastructural analysis. Since AS-53 possessed a major 52K protein that was glycine extractable, we further characterized this isolate by analyzing the protein composition of this strain after

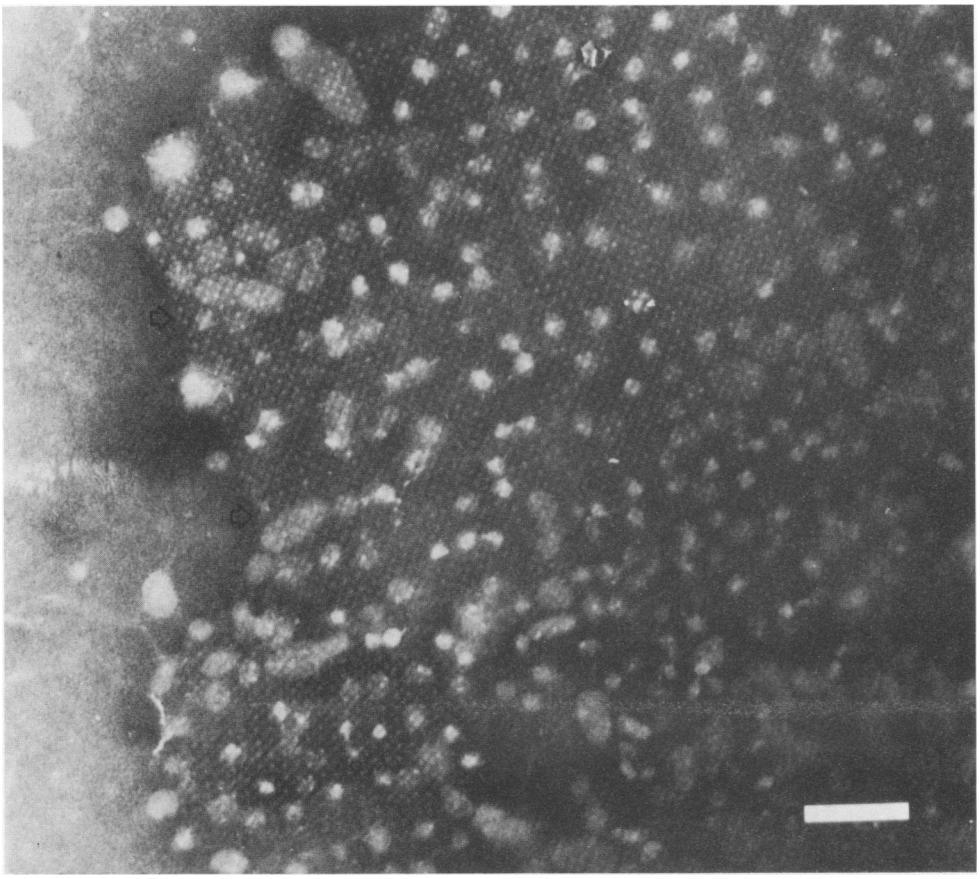

FIG. 5. Negative stain (3% phosphotungstic acid) of glycine extracts from S-layer-containing strains observed by transmission electron microscopy. Arrows indicate edge of S-layer sheet. Bar, 100 nm.

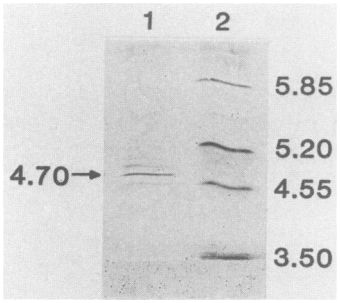

FIG. 7. Isoelectric focusing gel of SAP from ATCC 9071 (glycine hydrochloride extracted). Shown are SAP of ATCC 9071 (lane 1) and standards (lane 2). Each lane contains a 20-μl load.

overnight growth in brain heart infusion broth (BHIB). When AS-53 was grown on heart infusion agar, PAGE of WC extracts revealed the same predominant 52K protein (Fig. 8). However, when cell pellets (7,000 × *g*) of AS-53 grown in BHIB were similarly analyzed, this protein was almost entirely absent (Fig. 8, lane BP). When the pelleted (12,000 to 18,000 × *g*) protein obtained from the BHIB supernatant was additionally analyzed by PAGE, only the 52K protein was detected, which indicated that most if not all of this synthesized molecule was excreted into the medium and was not cell associated (Fig. 8, lane BS). Transmission electron microscopy analysis of this 52K protein by negative staining (Fig. 9) revealed that this molecule was a SAP with an architecture similar to that previously described (20).

LPS profiles. Given the multiple functions ascribed to LPS and the speculation by Dooley and Trust (7) concerning the possible function of OMP and LPS with regard to S-layer production in strains of *Aeromonas salmonicida* and *A. hydrophila* TF7, we analyzed the LPS profiles of selected strains from each of our different serogroups. We observed three different LPS patterns on 10% SDS-PAGE (Fig. 10). Serogroup O:11 S-layer-positive strains exhibited O-antigenic polysaccharide side chains of similar or homogeneous chain length with only two to three predominant bands (pattern A). At least two major homogeneous side chain types could be detected among O:11 strains. Type I profiles, which included *A. hydrophila* LL1 and 342 and *A. sobria* 76, were exemplified by LPS side chain bands that occurred as closely migrating doublets in SDS gels. Type II profiles, which were represented by *A. sobria* 28 and ATCC 9071, exhibited LPS bands that had significantly different M_r (Fig. 10, lane 3); minor LPS variations were noted with other O:11 strains analyzed (data not shown). By increasing the concentration of LPS loaded into the respective SDS-PAGE wells for O:11 strains, additional LPS bands of decreasing molecular weight of repeating O-antigen side chains could be observed. In contrast, non-O:11 strains failed to exhibit a similar electrophoretic LPS profile but, rather, presented as a population devoid of or deficient in O-antigenic polysaccharide side chains and predominantly contained only core antigen (LPS pattern C). Only AS-178 and AS-53 produced a diffuse fast-migrating band which may consist of a lipid A-core oligosaccharide fraction. The ability of AS-178 and O:22 strains to react with specific polyclonal somatic antisera suggests that these are not deep rough strains but, rather, those of the Ra or SR chemotype (9). LPS pattern B consisted of a heterogeneous (ladderlike) population of molecules of varying O-antigenic chain lengths similar to that exhibited by *E. coli* O111:B4 (Fig. 10). While this pattern was not observed in any of the 10 strains, to date it has been the predominant pattern observed in other mesophilic aeromonads (data not shown). A summary of the major protein and LPS patterns of the 10 strains is presented in Table 2.

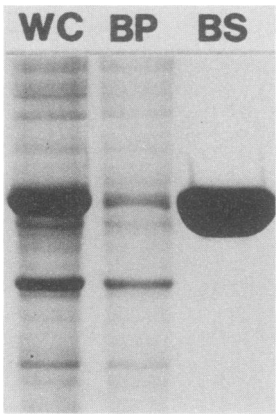

FIG. 8. 10% SDS-PAGE protein profiles of AS-53. Shown are AS-53 (WC) grown on heart infusion agar (lane WC), AC-53 (cell pellet) grown in BHIB (lane BP), and 52K SAP protein of AS-53 recovered by centrifugation (12,000 × *g*) from the BHIB supernatant (lane BS).

DISCUSSION

Recent studies have indicated that a number of bacterial species produce a SAP that is arranged in an orderly fashion on the outermost surface of the bacterium as a paracrystalline S layer (14, 23). Groups of bacteria which have been found to possess such a structure and are pathogenic for either humans or animals include *A. salmonicida*, *A. hydrophila*, *A. sobria*, *Campylobacter fetus*, and, most recently, *Mycobacterium bovis* (16). For *Aeromonas* spp., a common phenotype, namely, autoagglutination (autoaggregation) in broth, is useful for screening for S-layer-containing strains; however, we have identified a number of AA^+ aeromonads that are S layer negative and are relatively avirulent in animal models. While the S layer of *A. salmonicida* has been demonstrated by Trust and collaborators (10, 19) to be essential to the virulence of *A. salmonicida* for fish (salmon), little information currently exists regarding the function and potential pathogenic role that surface array proteins play in other S-layer-containing bacteria.

Characterization of the protein and LPS compositions of AA^+ O:11 and non-O:11 strains revealed several notable structural differences. First, all O:11 strains evaluated to date, irrespective of taxonomic designation, possessed a single major glycine-extractable 52,000- to 54,000-molecular-weight protein that was identified as the SAP. This SAP was the predominant band observed on all O:11 protein profiles, and in two strains (ATCC 9071 and AS-76) analyzed by soft laser scanning densitometry, it represented 45 to 50% of the total analyzable protein. These data are consistent with the known facts regarding S layers, in that they are produced by bacteria in larger amounts than any other class of proteins, accounting for 5 to 10% of the total cell protein. Dooley et al. (5–7) have characterized a number of SAPs from virulent *A. hydrophila*; the SAP from one strain (TF7) has been extensively analyzed and has been found to be a 52,000-dalton protein composed of 41% hydrophobic amino acids and with a pI of 4.6 (6). This latter value is similar to the pI of 4.7

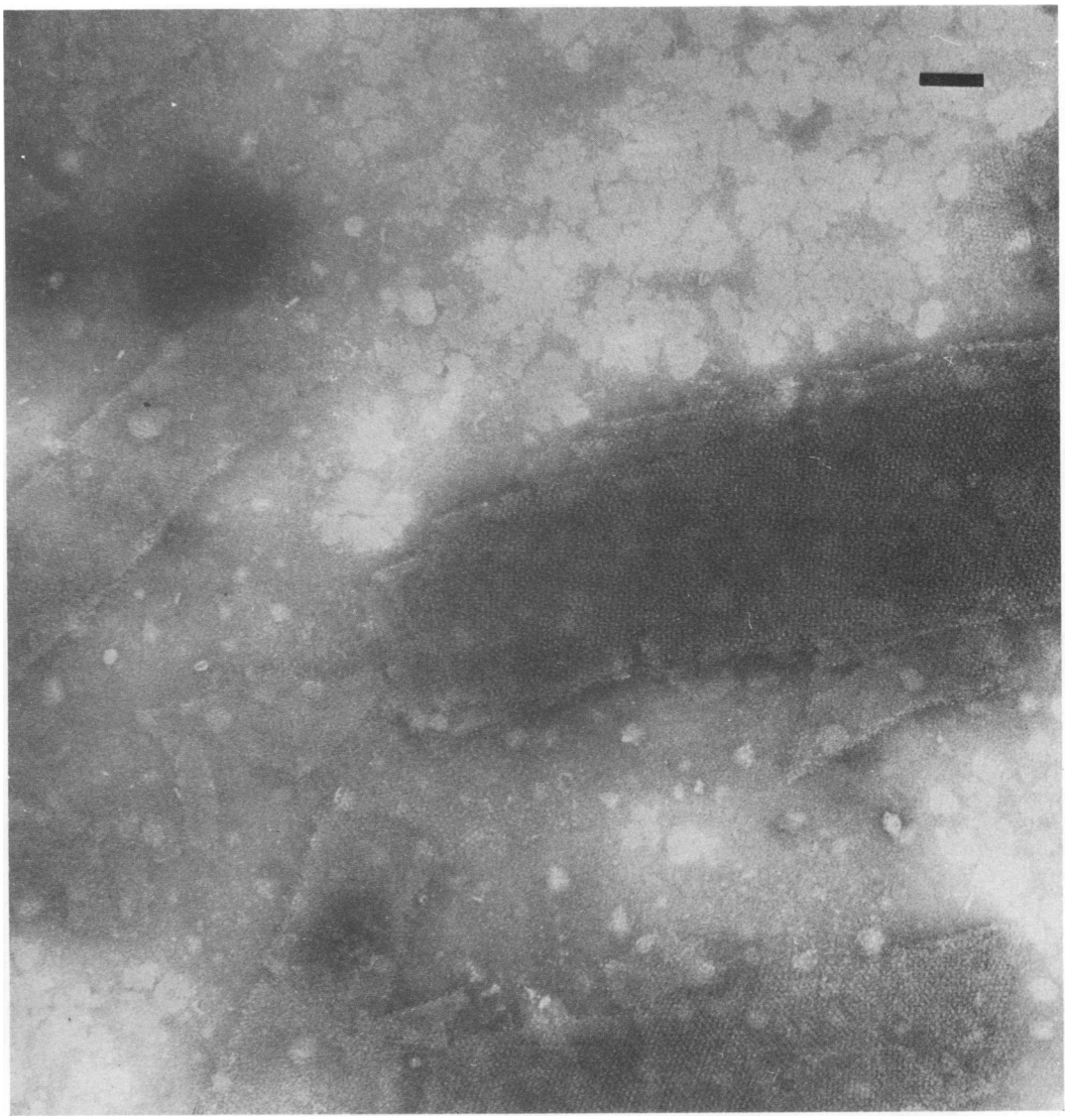

FIG. 9. Transmission electron microscopy of recovered S-layer sheet from supernatant of *A. sobria* 53 grown in L broth. Bar, 100 nm.

determined for the SAP of *A. sobria* ATCC 9071, suggesting that these molecules from distinct species share many structural, biochemical, and physiological features.

In contrast, non-O:11 strains of serogroups O:22 and O:36 did not contain a similar predominant protein and, as previously reported, were S layer negative; only AS-53, a rough strain, released a similar-size protein upon low-pH extraction, but it did not contain an S layer, as determined by repeated transmission electron microscopy analysis. Further studies conducted on this strain revealed that this molecule was a SAP which, upon synthesis, was not anchored to the cell surface of the bacterium but was excreted preferentially into the culture supernatant. Among O:22 strains, an apparently unique major 46K protein was noted; this protein was associated with the OMP fraction and was not detected in any of the other seven strains analyzed. This 46K protein may be serogroup specific or, alternatively, linked to some other phenotypic feature, such as autoagglutination, present in these three strains. Further studies are in progress to investigate these possibilities.

Regarding LPS analysis, all S-layer-positive serogroup O:11 strains possessed a homogeneous-length O-polysaccharide side chain similar to that reported by Dooley et al. (4) for a high-virulence group of *A. hydrophila* strains recovered from fish, porcine, cow, and otary infections; a similar situation has previously been shown to exist for S-layer-containing *A. salmonicida* and *Campylobacter fetus* subsp. *fetus* (3, 20, 21). On the basis of UV-induced LPS mutants of *A. hydrophila* TF7, Dooley and Trust (7) have shown that homogeneous O-polysaccharide side chains are not required for the anchoring of intact S layers to the cell surface; however, a deep rough mutant (TF7/B) failed to bind the surface layer to the cell wall in a similar fashion, suggesting that a minimum-size LPS oligosaccharide is required for structural integrity. In our studies of a large number of S-layer-containing O:11 strains by transmission electron microscopy analysis, we have failed to observe an isolate which upon electrophoretic analysis did not exhibit a homogeneous-length O-polysaccharide side chain, thus supporting the concept of Dooley and Trust (7) that the LPS structure is intimately involved in some fashion with the expression and the structural integrity of intact S layers. Further support for

TABLE 2. Cumulative structural analysis of *Aeromonas* strains on the basis of protein and LPS profiles

Strain	Serogroup[a]	Phenospecies	Mol wt (10^3)			LPS pattern (type)[b]
			WC proteins	OMP	SAP	
LL1	O:11	*A. hydrophila*	54, 50	54, 50	54	A (I)
AS-28	O:11	*A. sobria*	53, 35	53, 35	54	A (II)
AS-76	O:11	*A. sobria*	53, 43	53, 43, 20	53	A (I)
ATCC 9071	O:11	*A. sobria*	53, 48, 32	53, 48, 32, 18	53	A (II)
AH-342	O:11	*A. hydrophila*	52, 50	52, 50	52	A (I)
AS-53	R	*A. sobria*	52, 43	52, 43, 36, 34, 30, 15, 11	52	C
AH-78	O:22	*A. hydrophila*	68, 53, 46	68, 53, 46, 20		C
AS-178	O:36	*A. sobria*	53, 43	53, 43, 20		C
ATCC 14715	O:22	*A. hydrophila*	68, 53, 46	53, 48, 46, 41, 20		C
NCMB 1134	O:22	*A. hydrophila*	68, 53, 46	53, 46, 41, 20		C

[a] R, Rough strain.

this concept stems from the inability of a rough strain, AS-53, to anchor its SAP to the cell surface.

In contrast, all AA^+ non-O:11 strains investigated lacked this homogeneous pattern but, rather, displayed a profile similar to that of rough strains, in which specific O-polysaccharide side chains were either partially or completely absent. These results, therefore, help explain our former observations regarding the autoagglutinability, the high surface hydrophobicity, and the susceptibility to complement-mediated lysis of this group, since organisms deficient in O-specific polysaccharide side chains (rough strains) are generally hydrophobic, more susceptible to lysis in normal serum, and less virulent than their counterparts (homogeneous and heterogenous LPS) (22).

Regarding the role of S-layer-containing *A. hydrophila* and *A. sobria* in human infections, it is striking to note the similarities in disease presentation and biochemical characteristics of these aeromonads and surface-layer-containing isolates of *C. fetus* subsp. *fetus*. In both groups, a number of common or identical features are shared, including the predilection for causing systemic infection (e.g., bacteremia), resistance to complement-mediated lysis, and a common homogeneous O-polysaccharide side chain pattern (1, 2, 21). *C. fetus* subsp. *fetus* has also been shown to resist phagocytosis by decreased opsonization in the presence of normal serum (2, 17). These collective facts suggest that genetically distinct groups of bacteria causing systemic disease have evolved common structural, biochemical, and pathogenic properties to aid them in overt disease potential.

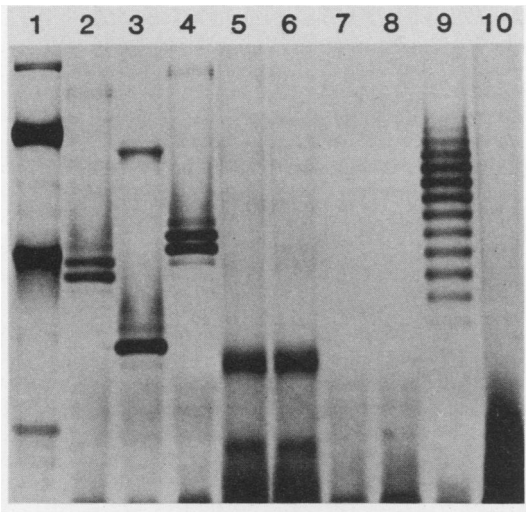

FIG. 10. Silver stain of LPS (proteinase K treated) on 10% SDS-PAGE from selected AA^+ strains. Shown are protein standards (90, 77, 52, and 18K) (lane 1), LL1 (lane 2), AS-28 (lane 3), AS-76 (lane 4), AS-178 (lane 5), AS-53 (lane 6), ATCC 14715 (lane 7), NCMB 1134 (lane 8), *E. coli* O111:B4 (control) (lane 9), and *S. typhimurium* TV 119 Ra mutant (control) (lane 10).

S layers are present on a number of bacteria pathogenic to humans, as well as on many nonpathogenic bacteria (14, 23). Their exact function in most instances is unknown; however, in one case, that of *A. salmonicida*, the S layer has been linked to the overt virulence of this bacterium for fish. An additional related observation was made in the present investigation, namely that AS-53, a strain that functionally fails to bind its SAP to the cell surface, was avirulent in animal model studies. Surface layers may provide protection for microorganisms in their natural environment or may provide strains with a selective advantage in causing infection in a susceptible host; additionally, S layers may be involved in strain dissemination from intestinal body sites by providing a mechanism for microbes to resist phagocytosis and the lytic activity of complement. The increasing list of S-layer-containing bacteria that can cause serious infections in humans, coupled with the remarkable similarities previously detailed between *A. hydrophila*/*A. sobria* and *C. fetus* subsp. *fetus*, points to these structural elements as important determinants in microbial pathogenicity.

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LITERATURE CITED

1. **Blaser, M. J., P. F. Smith, J. A. Hopkins, I. Heinzer, J. H. Bryner, and W.-L. Wang.** 1987. Pathogenesis of *Campylobacter fetus* infections: serum resistance associated with high-molecular-weight surface proteins. J. Infect. Dis. **155**:696–706.
2. **Blaser, M. J., P. F. Smith, J. E. Repine, and K. A. Joiner.** 1988. Pathogenesis of *Campylobacter fetus* infections: failure of encapsulated *Campylobacter fetus* to bind C3b explains serum and phagocytosis resistance. J. Clin. Invest. **81**:1434–1444.
3. **Chart, H., D. H. Shaw, E. E. Ishiguro, and T. J. Trust.** 1984. Structural and immunochemical homogeneity of *Aeromonas salmonicida* lipopolysaccharide. J. Bacteriol. **158**:16–22.
4. **Dooley, J. S. G., R. Lallier, D. H. Shaw, and T. J. Trust.** 1985. Electrophoretic and immunochemical analyses of the lipopolysaccharides from various strains of *Aeromonas hydrophila*. J. Bacteriol. **164**:263–269.
5. **Dooley, J. S. G., R. Lallier, and T. J. Trust.** 1986. Surface antigens of virulent strains of *Aeromonas hydrophila*. Vet. Immunol. Immunopathol. **12**:339–344.

6. **Dooley, J. S. G., W. McCubbin, C. M. Kay, and T. J. Trust.** 1988. Isolation and biochemical characterization of the S-layer protein from a pathogenic *Aeromonas hydrophila* strain. J. Bacteriol. **170**:2631–2638.
7. **Dooley, J. S. G., and T. J. Trust.** 1988. Surface protein composition of *Aeromonas hydrophila* strains virulent for fish: identification of a surface array protein. J. Bacteriol. **170**:499–506.
8. **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.
9. **Hitchcock, P. J., and T. M. Brown.** 1983. Morphological heterogeneity among *Salmonella* lipopolysaccharide chemotypes in silver-stained polyacrylamide gels. J. Bacteriol. **154**:269–277.
10. **Ishiguro, E. E., T. Ainsworth, T. J. Trust, and W. W. Kay.** 1985. Congo red agar, a differential medium for *Aeromonas salmonicida*, detects the presence of the cell surface protein array involved in virulence. J. Bacteriol. **164**:1233–1237.
11. **Janda, J. M., and P. S. Duffey.** 1988. Mesophilic aeromonads in human disease: current taxonomy, laboratory identification, and infectious disease spectrum. Rev. Infect. Dis. **10**:980–997.
12. **Janda, J. M., L. S. Oshiro, S. L. Abbott, and P. S. Duffey.** 1987. Virulence markers of mesophilic aeromonads: association of the autoagglutination phenomenon with mouse pathogenicity and the presence of a peripheral cell-associated layer. Infect. Immun. **55**:3070–3077.
13. **Janda, J. M., M. Reitano, and E. J. Bottone.** 1984. Biotyping of *Aeromonas* isolates as a correlate to delineating a species-associated disease spectrum. J. Clin. Microbiol. **19**:44–47.
14. **Koval, S. F.** 1988. Paracrystalline protein surface arrays on bacteria. Can. J. Microbiol. **34**:407–414.
15. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
16. **Lounatmaa, K., and E. Brander.** 1989. Crystalline cell surface layer of *Mycobacterium bovis* BCG. J. Bacteriol. **171**:5756–5758.
17. **McCoy, E. C., D. Coyle, K. Burda, L. B. Corbeil, and A. J. Winter.** 1975. Superficial antigens of *Campylobacter* (*Vibrio*) *fetus*: characterization of an antiphagocytic component. Infect. Immun. **11**:517–525.
18. **Merril, C. R., D. Goldman, and M. L. Van Keuren.** 1982. Simplified silver protein detection and image enhancement methods in polyacrylamide gels. Electrophoresis **3**:17–23.
19. **Munn, C. B., E. E. Ishiguro, and T. J. Trust.** 1982. Role of surface components in serum resistance of virulent *Aeromonas salmonicida*. Infect. Immun. **36**:1069–1075.
20. **Paula, S. J., P. S. Duffey, S. L. Abbott, R. P. Kokka, L. S. Oshiro, J. M. Janda, T. Shimada, and R. Sakazaki.** 1988. Surface properties of autoagglutinating mesophilic aeromonads. Infect. Immun. **56**:2658–2665.
21. **Perez Perez, G. I., and M. J. Blazer.** 1985. Lipopolysaccharide characteristics of pathogenic campylobacters. Infect. Immun. **47**:353–359.
22. **Porat, R., M. A. Johns, and W. R. McCabe.** 1988. The role of lipopolysaccharide of gram-negative bacilli in sensitivity and resistance to serum, p. 103–114. *In* J. Levin, H. R. Buller, J. W. ten Cate, S. J. H. van Deventer, and A. Sturk (ed.), Bacterial endotoxins: pathophysiological effects, clinical significance, and pharmacological control. Alan R. Liss, Inc., New York.
23. **Sleytr, U. B., and P. Messner.** 1983. Crystalline surface layers on bacteria. Annu. Rev. Microbiol. **37**:311–339.