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# Serum Sensitivity of a Fish Pathogenic Strain and Its LPS Mutants of *Aeromonas hydrophila*

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*Keywords* : *Aeromonas hydrophila*, serum sensitivity, LPS mutants, LPS profiles, immunoblot

**Abstract**

Two types of LPS mutants were isolated from a virulent strain, TF7 of *Aeromonas hydrophila* after UV irradiation. LPS profiles of TF7 and its mutants were revealed by using SDS-PAGE and immunoblotting techniques. One type of the mutants was found to lose O-saccharide chain of LPS completely and concomitantly to change to be serum sensitive. The other type of the mutants displayed to produce less amount of O-side chains than TF7 and to be resistant to rabbit serum conditionally. When the cells were grown on TSA containing SDS (TSA-SDS) or MacConkey agars, the former mutants exhibited higher sensitivity, while the latter turned to be serum sensitive considerably. The parent strain TF7 were not significantly different in respect with serum susceptibility even if grown on TSA-SDS agar. The most distinguishable feature of whole cell protein profiles on SDS-PAGE was observed as the decrease of 49 Kd band concomitantly with increase 41 Kd band for both the parent and mutant strains grown on TSA-SDS agar.

*Aeromonas hydrophila* is usually considered as normal inhabitant of the intestinal tract of fresh-water fish as well as environmental waters[1-3]. However, some strains of *A. hydrophila* cause infectious disease in cold and warm blooded animals including human[4,5]. Virulent strains were frequently observed to exhibit serum resistance, autoaggregation and serotypic properties at the same time, suggesting that their surface structure may differ from that of non-virulent strains of *A. hydrophila*[6,7].

Dooley *et al*[7,8]. reported that some virulent strains of *A. hydrophila* display characteristic lipopolysaccharide (LPS) structures with homogeneous O-polysaccharide chains in contrast to those with heterogeneous O-polysaccharide chains in non-virulent strains. These virulent strains also were demonstrated to produce a surface protein array (S-layer), which overlay on outer membrane of the cells and traversed by O-polysaccharide chains of LPS.

Complement system in serum is an important component of the host defense mechanisms against bacterial infection, especially due to gram negative bacteria. Activation of

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complement by gram negative bacteria can occur via the classical or the alternative pathway ; the former is usually initiated by the binding of the first component to antigen-antibody complexes, whereas activation of the latter can be initiated and amplified in the absence of antigen-antibody interaction. The bactericidal activity of serum is effected by deposition on or insertion into the bacterial outer membrane of the membrane attack complex (MAC) of complement system causing lethal damage to the cytoplasmic membrane[9,10]. Recent studies[11-14] indicated that the cell surface structures including outermost proteins, LPS, exopolysaccharides and membrane phospholipid composition are primary determinants for the resistance of bacterial cells to activated complement components.

In the present study, the author examined the susceptibility of a virulent stain, *A. hydrophila* TF7, isolated from trout lesion by Mittal *et al*[6]. and its LPS deficient mutants to rabbit or trout serum. We attempted to elucidate which components or structures of the bacteria are responsible for the resistance of a virulent strain to serum killing activity.

## Materials and methods

*Bacterial strains and culture conditions*

A pathogenic strain used mainly in this study, *Aeromonas hydrophila* TF7, was originally isolated from a moribund trout by Mittal *et al*[6]. Isolation procedure for LPS deficient mutants from a parent strain is as follows ; the bacterial cell suspension of a parent strain ($10^7$ cfu/m*l*) was irradiated under UV-lamp (30 W, 50cm above) for 25 s and spread on TSA plates. Colonies grown on TSA were transferred to fresh TSA plates by using sterile toothpicks and then incubated for 5-7 h at 37℃. Afterwards, 10 $\mu l$ of bacterial suspension ($10^5$ cfu/m*l*) from each colony were mixed with same volume of rabbit serum in MicroTest plates (Falcon 3911) and incubated for 2 h at 37℃. One loop of the incubation mixture was spread on TSA plates and after overnight incubation, bacterial strains which formed no colony or few colonies on TSA were selected as serum sensitive strains. In this experiment, U-1, U-5, U-14, U-21, U-24, U-25 and U-28 were chosen among the strains isolated in the first screening process based on their LPS profiles on SDS-PAGE gels. The other strains of *A. hydrophila* used in this study were isolated from fish, human feces and water by various researchers and kept in the laboratory of Trust, University of Victoria, Canada. Bacterial cells were grown in TSB or on TSA (Difco) at 37℃ unless otherwise indicated.

*Serum bactericidal assay*

The bactericidal activity of serum was determined according to a modification of the method described by Schiller *et al.*[14] Namely, 150 $\mu l$ of bacterial suspension ($5\times10^4$ cfu/m*l*) in phosphate buffer saline containing Ca and Mg ions (Oxoid) and 150 $\mu l$ of rabbit or trout serum (50-100% concentration, v/v) were incubated at 37℃ or 30℃ for 3 h. At 0 time and after 1, 2 and 3 h incubation, 10-100 $\mu l$ samples were removed from incubation tubes and spread on TSA plates. After overnight incubation at 37℃, the number of bacterial colony grown on TSA was counted and survival rates were expressed as percentage to the number of colony in PBS solution.

*SDS-PAGE*

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE, 12.5% gel) was carried out according to the method of Laemmli[15]. Whole cell samples (4 mg) or acidic glycine buffer (pH 2.2) extracts[16] were suspended in 200 $\mu l$ of solubilizing buffer and immediately heated at 100℃ for 10 min. Whole cell samples were divided to 2 parts, one part was for protein analysis and the other was for LPS analysis after incubation with same volume of Proteinase K (2 mg/ml, Sigma) at 60℃ for 60 min. After electrophoresis, gels were stained for proteins with Coomassie brilliant blue (CBB), and for LPS by the silver staining procedure of Tsai and Frasch[17].

*Polyclonal antisera to LPS*

Adult New Zealand white rabbits were immunized with 50 $\mu$g of phenol extracted LPS as described by Dooley *et al*[7].

*Immunoblot detection of LPS*

After SDS-PAGE operation, separated LPS components were transferred from the slab gel to 0.45 $\mu$m nitrocellulose paper (NCP, Bio-Rad) according to the procedure described by Towbin *et al*[18]. The transblotted NCP sheet was incubated for 2 h with diluted (1 : 100) rabbit antibody for LPS extraction of strain TF7 and then for 1 h with protein A-horseradish peroxidase (HRP) (1 : 10,000 dilution) according to the Bio-Rad immunoblot assay procedure.

## Results

*Serum sensitivity of natural isolated strains*

Among the laboratory stock strains of *Aeromonas hydrophila* which isolated from fish, human and water by various researchers, a total of 37 strains was examined in respect with susceptibility to normal rabbit serum. Bacterial suspensions were prepared by resuspending the cells grown in TSB for 5 h at 37℃ in PBS containing Ca and Mg salts (pH 7.4). Bactericidal activity was determined by incubating each strain for 60 min at 37℃ in the presence or absence of rabbit serum at final concentration of 50%. As show in Table 1, eighteen strains among the strains tested were resistant to rabbit serum and most of those strains, but not all, showed autoaggregation when they were grown statically in TSB at 37℃. For further experiments, strain TF7, which was isolated from a moribund rainbow trout and shown to have lethal effect to mice at $10^7$ cfu/ml.

*Serum sensitivity of LPS mutants*

Eight strains induced by UV irradiation were selected after the procedure described in *Materials and Methods*. Physiological characterization of these strains is shown in Table 2. As for main characteristics, mutant strains were not distinguishable from the parent strain except no growth ability of U-5 on Rimler-Shotts medium.

Susceptibility of LPS mutants grown under various conditions to normal rabbit serum is summarized in Table 3. The cells grown in TSB exhibited comparatively less sensitivity to rabbit serum than the cells grown on TSA. Although these mutants were isolated as serum sensitive strains in the first screening step, U-1, U-5, U-21, U-25 and U-28 did not display

Table 1. Sensitivity of various strains of *Aeromonas hydrophila* to normal rabbit serum

| Strains tested | | | Aggregation | Serum sensitivity | |
|---|---|---|---|---|---|
| Lab. No. | | Source | | % survival*1 | Type |
| 13 | | trout | − | <0.1 | S *2 |
| 65 | | trout lesion | − | <0.1 | S |
| 108 | | Evelyn | cA*3 | <0.1 | S |
| 109 | | Evelyn | cA | 231 | R |
| 110 | | Evelyn | − | <0.1 | S |
| 189 | | Shotts | − | <0.1 | S |
| 199 | | Udey | pA | 155 | R |
| 200 | | goldfish, Fryer | pA | 1089 | R |
| 218 | | Bullock | − | <0.1 | S |
| 224 | | Lallier | − | 216 | R |
| 225 | | Lallier | − | <0.1 | S |
| 227 | | moribund fish, Lallier | − | <0.1 | S |
| 230 | | moribund fish, Lallier | − | <0.1 | S |
| 250 | | NCMB 74. A. punctata | − | <0.1 | S |
| 271 | | human feces, Atkinson | − | 760 | R |
| 274 | | human lesion, Atkinson | pA | 583 | R |
| 412 | | human feces, Lee | pA | <0.1 | S |
| 413 | | human feces, Lee | pA | <0.1 | S |
| 416 | | human feces, Lee | pA | 122 | R |
| 421 | | animal, Sanyl | − | 227 | R |
| 423 | | Dunsmore | cA | 329 | R |
| 424 | | Dunsmore | − | 401 | R |
| 427 | | ATCC 7966 | − | 94 | R |
| 428 | | ATCC 19570 | − | <0.1 | S |
| 434 | | water, Sanyl | cA | <0.73 | S |
| 435 | | water, Sanyl | cA | <0.1 | S |
| 512 | (TF 2) | moribund trout, Lallier | − | <0.1 | S |
| 413 | (LL 1) | trout lesion, Lallier | cA | 257 | R |
| 514 | (p 82–256) | moribund trout, Lallier | cA | 167 | R |
| 515 | (A 79–115) | Lallier | pA | 249 | R |
| 516 | (TF 7) | trout lesion, Lallier | cA | 144 | R |
| 517 | (A 80–160) | bovine brain, Lallier | cA | 279 | R |
| 518 | (A 80–199) | Lallier | − | 1.33 | S |
| 519 | (Ba 5) | eel, Lallier | − | 1.53 | S |
| 520 | (A 80–140) | piglet liver, Lallier | pA | 272 | R |
| 525 | | human feces, Atkinson | − | <0.1 | S |
| 530 | | human feces, Atkinson | − | 251 | R |

* 1 % survival after 60 min incubation at 37℃.
* 2 S ; sensitive, R; resistant.
* 3 cA ; complete aggregation, pA; partial aggregation.

Table 2. Brief characterization of isolated and mutant strains of *A. hydrophila*

| Strains | Gram | Motility | Oxidase | Catalase | O–F TEST | Hemolys. | Hemaggl. | MacConkey | RS agar |
|---|---|---|---|---|---|---|---|---|---|
| TF2 | – | + | + | + | F *1 | + | ++*2 | +Br *3 | +Gr *4 |
| LL1 | – | + | + | + | F | + | + | +Br | +Y |
| p82–256 | – | + | + | + | F | + | + | +Br | +Y |
| A79–115 | – | + | + | + | F | + | + | +Br | +Y |
| A80–160 | – | + | + | + | F | + | – | +R | – |
| A80–199 | – | + | + | + | F | + | – | +Br | – |
| Ba5 | – | + | + | + | F | + | + | +Br | +Y |
| TF7 | – | + | + | + | F | + | + | +Br | +Y |
| U–1 | – | + | + | + | F | + | + | +Br | +Y |
| U–5 | – | + | + | + | F | + | + | +Br | – |
| U–8 | – | + | + | + | F | + | + | +Br | +Y |
| U–14 | – | + | + | + | F | + | + | +Br | +Y |
| U–21 | – | + | + | + | F | + | + | +Br | +Y |
| U–24 | – | + | + | + | F | + | + | +Br | +Y |
| U–25 | – | + | + | + | F | + | + | +Br | +Y |
| U–28 | – | + | + | + | F | + | + | +Br | +Y |

* 1 F ; fermentative.
* 2 Slide hemagglutination was performed at room temperature by mixing human blood suspension and bacterial suspension grown on TSA for 8 hr at 30℃
* 3 Br ; brown, R ; red.
* 4 Gr ; green, Y ; yellow.

Table 3. Effect of the growth media on sensitivity of LPS mutants to rabbit serum

| Strains | Growth media | | | | |
|---|---|---|---|---|---|
| | TSB | TSA | TSA–CM*2 | TSA–SDS*2 | MacConkey |
| TF7 | 474±10*3 | 571±138 | 47.2 | 374 | 382 |
| U–1 | 506±35 | 293±28 | 1.2 | 13.2 | 72.6 |
| U–5 | 681±204 | 520±73 | 72.2 | 309 | 453 |
| U–8 | 68.5±11.1 | 11.5±4.5 | < 0.1 | < 0.1 | < 0.1 |
| U–14 | 74.9±24.5 | 25.5±2.5 | 1.1 | < 0.1 | 0.7 |
| U–21 | 359±42 | 319±29 | 2.4 | 13.4 | 35.2 |
| U–24 | 66.4±20.1 | 31.1±10.6 | < 0.1 | < 0.1 | 0.3 |
| U–25 | 422±44 | 285±61 | 3.3 | 2.3 | 31.7 |
| U–28 | 559 | 304±85 | 3.7 | 4.5 | 110 |

* 1 TSA–CM ; TSA grown cells at 37℃ for 5 h, and assay mixture contained chloramphenicol at final concentration of 5 μg/ml.
* 2 TSA–SDS ; TSA supplemented with 0.2% SDS.
* 3 Figures represent the mean plus or minus standard error of the mean based on three experiments. The percent survival was determined after 3 h incubation at 37℃ with serum solution.

sensitivity to rabbit serum at all in this experimental condition. It can be inferred that bactericidal activity varied depending on serum samples taken from individual rabbit and furthermore that bacterial growth rates in the assay mixture containing serum were different between the parent and mutant strains. In fact, these parent strain and U-5 grew more rapidly in the assay mixture than mutants which displayed sensitivity to rabbit serum.

On the other hand, U-1, U-21, U-25, and U-28 exhibited higher serum sensitivity after grown on TSA supplemented with 0.2% SDS (TSA-SDS) or MacConkey plates and U-8, U-14 and U-24 were much more sensitive to rabbit serum, whereas the parent strain and U-5 did not show significant change in respect with susceptibility to rabbit serum. The addition of chloramphenicol (5 $\mu$g/ml) to the assay mixture also increased the rate of bactericidal activity of serum as accompanied by inhibition of bacterial growth in the assay mixture.

In Figs. 1 and 2, kinetics of bactericidal activity of rabbit and trout serum are shown for the parent and two mutant strains grown on TSA or TSA-SDS plates. Although it is difficult to compare the bactericidal activity directly between rabbit and trout serum due to different assay temperature, both sera showed almost identical bactericidal kinetics.

*Electrophoretic profiles of LPS*

Fig. 3 shows SDS-PAGE profiles of whole cell LPS from natural isolates and mutant strains. The high virulent and serum resistant strains including TF7 (Fig. 3, A ; lanes 2-6) displayed the characteristic LPS profiles composed of a slow migrating band, a small

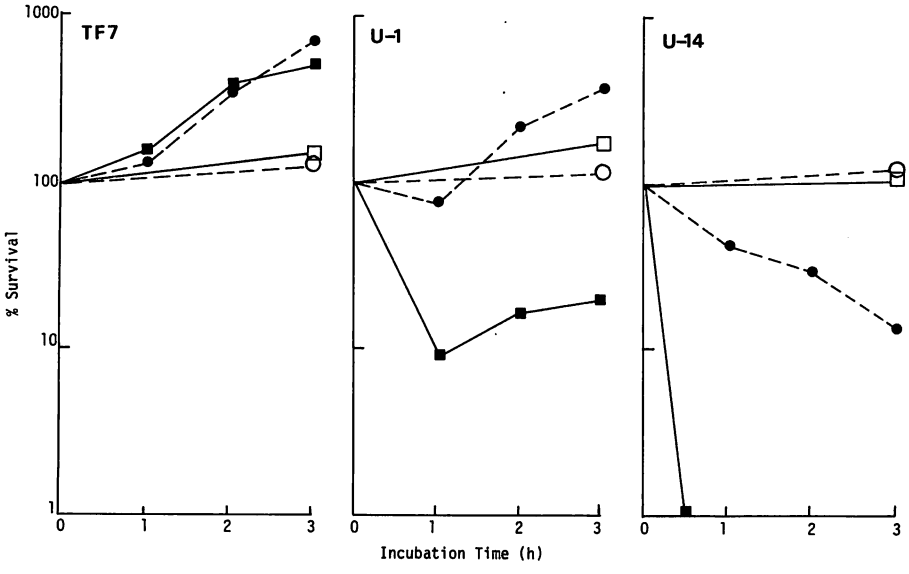


Fig. 1. Survival of a parent and mutant strains during incubation with rabbit serum at 37℃.
○---○ ; TSA grown cells incubated with PBS,
●---● ; TSA grown cells incubated with rabbit serum,
□——□ ; TSA-SDS grown cells incubated with PBS,
■——■ ; TSA-SDS grown cells incubated with rabbit serum.

number of well isolated faster migrating bands and the fast migrating lipid A-core oligosaccharide fraction on a SDS-PAGE gel. As described by Dooley *et al.*[7,8], the slow migrating thick band was composed of LPS dimers of faster migrating O-saccharide chains. The appearance of dimer bands was depending on the loading amount of samples to SDS-PAGE gels. On the other hand, serum sensitive strains such as TF2, A80-199 and Ba5 (Fig. 3, A ; lanes 1,7 and 8) showed regularly spaced bands composed of heterogeneous O-polysaccharide chains.

Among mutant strains derived from TF7, U-8, U-21 and U-24 (Fig. 3, B ; lanes 3-6) had lost the band corresponding to O-saccharide chain of LPS completely, whereas U-1, U-25 and U-28 (Fig. 3, B ; lanes 1,7 and 8) displayed less amount of O-polysaccharide chains that of the parent strain. Strain U-5 (lane 2), which were resistant to serum as same as the parent strain but did not grow on RS medium, showed a LPS profile identical to that of the parent strain (lane 9).

*Immunochemical analysis of LPS on SDS−PAGE*

The antigen cross-reactivity to rabbit antiserum raised against LPS fraction from TF7 between whole cell LPS of natural isolates and mutants of *A. hydrophila* was examined by using immunoblotting technique. As shown in Fig. 4, serum resistant strains among natural isolates reacted strongly with rabbit polyclonal antiserum prepared to TF7 LPS fraction, whereas no reaction was seen in the case of serum sensitive strains which displayed LPS

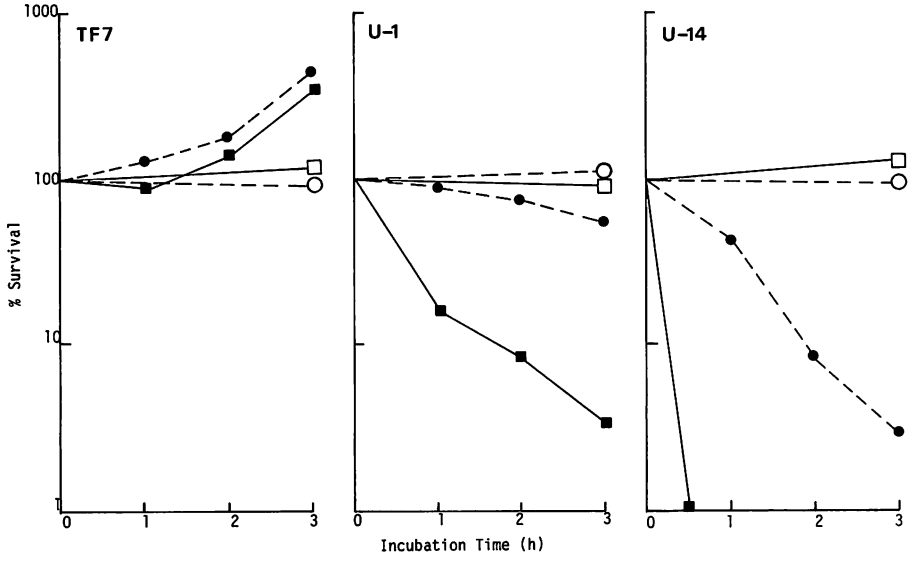


Fig. 2. Survival of a parent and mutant strains during incubation with trout serum at 30℃.
○−−−○ ; TSA grown cells incubated with PBS,
●−−−● ; TSA grown cells incubated with trout serum,
□——□ ; TSA-SDS grown cells incubated with PBS,
■——■ ; TSA-SDS grown cells incubated with trout serum.

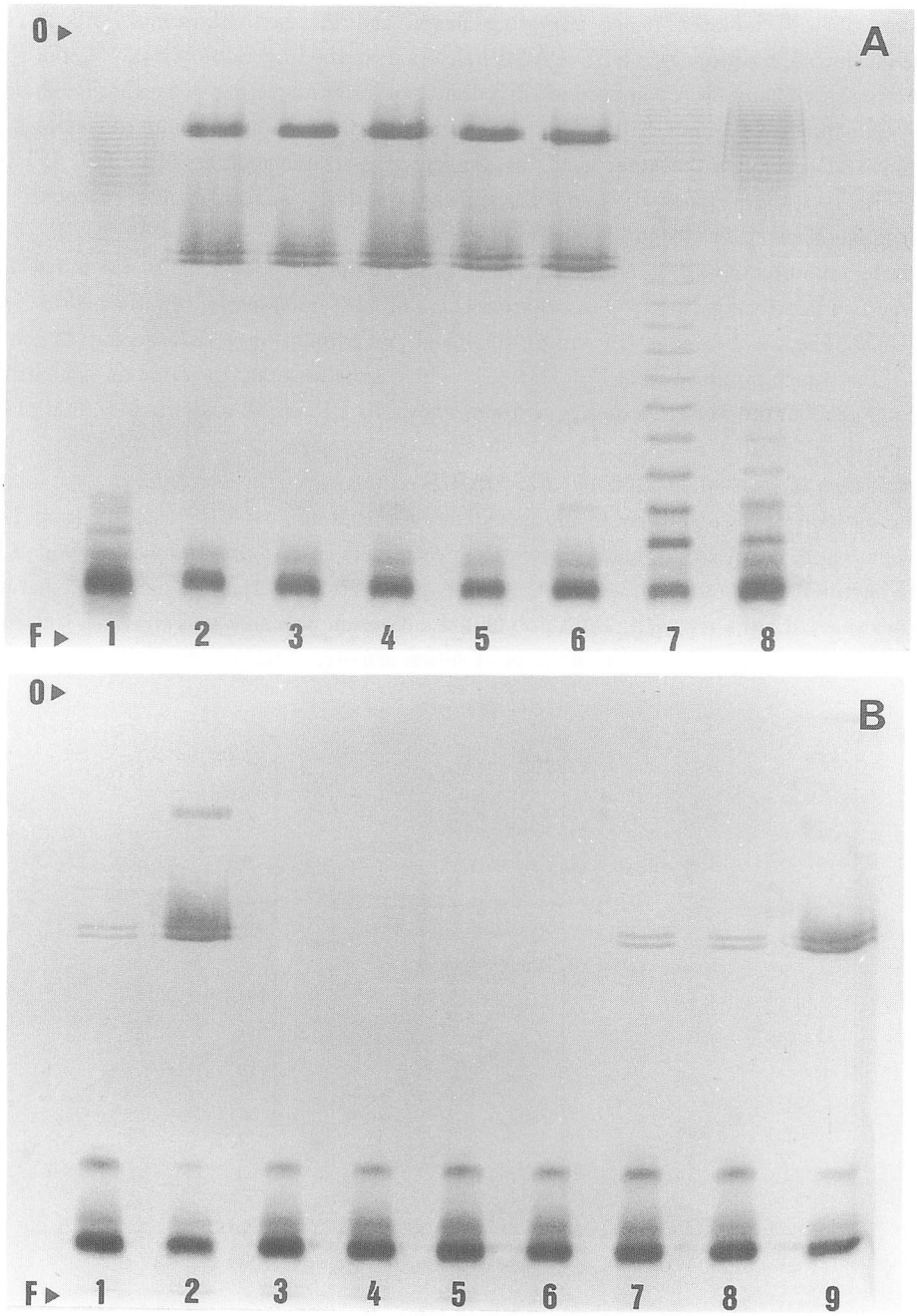


Fig. 3. The silver-stained gel profiles of whole cell lysates from natural isolates (A) and mutant strains (B).
(A) lane 1 ; TF2, 2 ; LL1, 3 ; p82-256, 4 ; A79-115, 5 ; TF7, 6 ; A80-160, 7 ; A80-199, 8 ; Ba5.
(B) lane 1 ; U-1, 2 ; U-5, 3 ; U-8, 4 ; U-14, 5 ; U-21, 6 ; U-24, 7 ; U-25, 8 ; U-28, 9 ; TF7.

profiles with heterogeneous O-polysaccharide chains. Rabbit polyclonal antiserum to LPS from TF7 reacted with slower migrating bands but not with fast migrating bands on SDS-PAGE gel of LPS, indicating that sero-determinants on LPS are attributed to O-polysaccharide chains of LPS molecules.

Immunoblotting profiles obtained with mutants derived from TF7 were shown in Fig. 4. Serum resistant strain U-5 (lane 2) gave a immunoblot reaction identical to TF7 (lane 9), whereas serum sensitive mutants such as U-8, U-14 and U-24 (lanes 3, 4 and 6) did not reacted with this antiserum at all and the conditionally sensitive mutants U-1, U-25 and U-28 (lane 1, 7 and 8) gave less reactive bands corresponding to O-saccharide chains. Exceptionally, U-21 did not display reactive band at all in spite of a conditionally sensitive strain.

*Electrophoretic profiles of whole cell proteins*

The CBB stained SDS-PAGE profiles of whole cell lysates from TF7, U-1 and U-14 were shown in Fig. 5 (A and B). In Fig. 5A, whole cell lysates from three strains gave similar polypeptide profiles except for 52 Kd band that was diffused or distorted in proportion to the amount of O-polysaccharide chains of LPS.

When the cells were grown on TSA-SDS, the amount of 49 Kd polypeptide decreased and at same time 41 Kd polypeptide increased in common with all three strains as compared with polypeptide profiles from the cells grown on TSA. The polypeptide with 52 Kd has been considered to constitute the surface protein array, which may contribute to resistance to host defense mechanisms, and shown to be extracted from the cells by acidic glycine buffer (pH 2.2). However, apparent difference of 52 Kd polypeptide band from serum sensitive mutants was not observed in both cultural conditions. Similar results were obtained for protein profiles of other mutants used in this study.

## Discussion

Complement mediated bactericidal activity is considered to be a most important factor in host defense mechanisms. Recent studies[9,10] indicated that membrane attack complex (MAC) of complement system is integrated into the lipid-containing bilayers of bacterial envelope and then a channel that destroys the integrity of the attached membrane, resulting in bacterial death. However, MACs formed on the surface of serum resistant strains are not found to be inserted effectively into the bacterial membrane. Consequently, it is suggested that some macromolecular components located in the outermembrane such as lipopolysaccharide (LPS), acidic exopolysaccharide and outermost protein layer can protect the cells from complement killing. For example, the evidence that the length and number of LPS O-saccharide chains play a critical role of determining the susceptibility of gram negative bacteria to serum was been obtained by many investigators[13,14]. The resistance of virulent strains of *A. salmonicida* to serum was shown to be conferred by a 49 Kd surface protein, the A-protein[12,20].

In this study, two types of LPS mutants were isolated after a virulent strain TF7 of *A. hydrophila* was treated with UV irradiation. One type of UV mutants (U-8, U-14 and

U-24) was found to lack O-saccharide chain of LPS completely as far as shown in LPS profiles and its immunoblotting profile on SDS-PAGE, accompanied by the transition to serum sensitivity when grown on TSA or in TSB. The other type of mutants (U-1, U-25 and U-28) was shown to produce still less amount of O-side chains than a parent strain, TF7 and to be resistant to rabbit serum when grown on TSA, while the cells grown on TSA-SDS or MacConkey plates became serum sensitive. Furthermore, the cells grown on TSA were shown to be more sensitive to rabbit serum in comparison with the cells grown in TSB, especially for apparently sensitive mutants (U-8, U-14 and U-24). Taylor *et al*[21]. described that many surface changes such as LPS content and protein composition occurred in response to alterations in the growth condition resulting in change in serum sensitivity of *E. coli*. It is likely that the culture condition on TSA brings about sufficient supply of $O_2$ to increase growth rates of mutants and followed by the production of lower amounts of O-side chains compared with the cells grown in TSB. Exceptionally, one of mutants, U-21, was shown to lack O-side chain completely but still to be resistant to serum when grown on TSA or in TSB. It was suggested that another surface determinant(s) besides LPS molecules

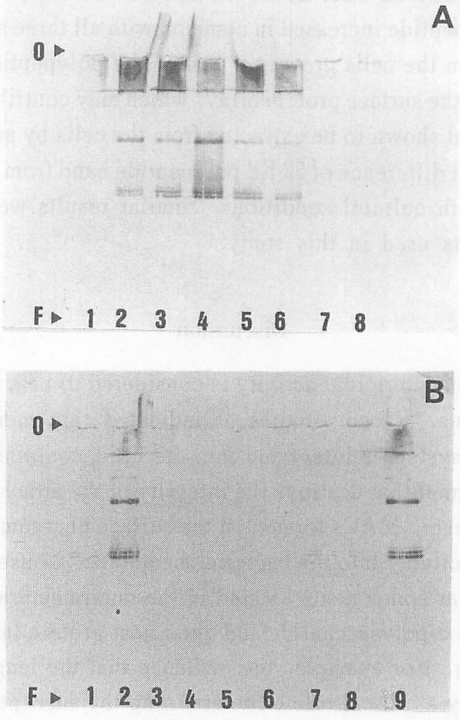


Fig. 4. Immunoblot profiles of SDS-PAGE of whole cell LPS from natural isolates (A) and mutants (B) with polyclonal rabbit antisera raised against purified LPS preparation from TF7.
(A) lane 1 ; TF2, 2 ; LL1, 3 ; p82-256, 4 ; A79-115, 5 ; TF7, 6 ; A8O-160, 7 ; A80-199, 8 ; Ba5.
(B) lane 1 ; U-1, 2 ; U-5, 3 ; U-8, 4 ; U-14, 5 ; U-21, 6 ; U-24, 7 ; U-25, 8 ; U-28, 9 ; TF7.

played a more important role for resistance of U-21 to serum.

When the mutants including U-21 were grown in TSA-SDS, TSA supplemented with 0.2% deoxycholate (TSA-DC) or MacConkey plates, serum sensitivity of these strains increased intensively, whereas TF7 and U-5, which had higher contents of O-side chains of LPS, were not significantly different in respect with serum susceptibility. The whole cell protein profiles on SDS-PAGE indicated that the detergent such as SDS decreased 49 Kd polypeptide band concomitantly with increase of 41 Kd polypeptide band. The alteration of protein composition or loss of protein components of bacterial membrane by SDS treatment was reported by many workers[22,23]. Therefore, it was assumed that the protein unit of 49 Kd, which was deprived from outer membrane by SDS in the medium, might conferred serum

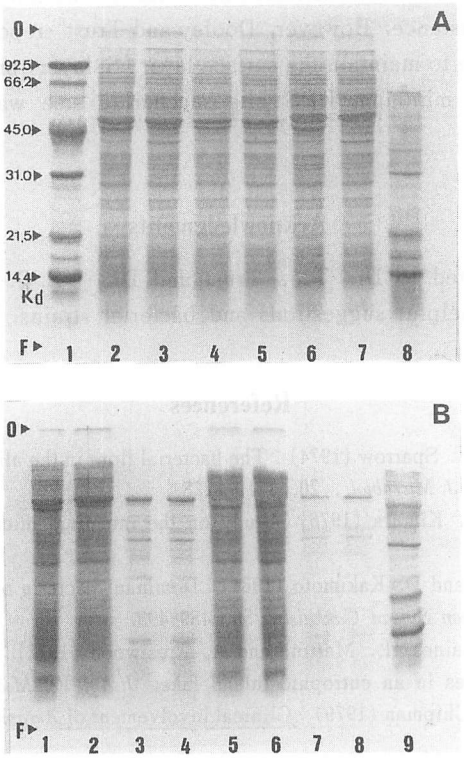


Fig. 5. SDS-PAGE profiles of whole cell proteins (A) and glycine buffer extracts (B) from TF7 and mutant strains.
(A) lane 1 ; standard proteins, 2 ; U-1, grown on TSA, 3 ; U-1 grown on TSA-SDS, 4 ; U-14 on TSA, 5 ; U-14 on TSA-SDS, 6 ; TF7 on TSA, 7 ; TF7 on TSA-SDS, 8 ; standard proteins.
(B) lane 1 ; whole cell proteins(WCP) of U-14 on TSA, 2 ; WCP of U-14 on TSA-SDS, 3 ; glycine buffer extract(GBE) of U-14 on TSA, 4 ; GBE of U-14 on TSA-SDS, 5 ; WCP of TF7 on TSA, 6 ; WCP of TF7 on TSA-SDS, 7 ; GBE of TF7 on TSA, 8 ; GBE of TF7 on TSA-SDS, 9 ; standard proteins.
Kd ; kilodalton, O ; origin, F ; front line of SDS-PAGE.

resistance to TF7 along with LPS molecules.

Similarly to 46 Kd protein extracted from a serum resistant strain of E. coli described by Taylor and Parton[11], 49 Kd protein which was shown to be recovered from the outer membrane fraction, is assumed to be partly responsible for the serum resistance of TF7 although the additional factors including LPS play more important role on complete serum resistance. If sufficient amount of O-saccharide chains of LPS is present on the surface layer, 49 Kd protein might be not essential for serum resistance. On the other hand, when O-side chain were produced less than critical amount or not at all, 49 Kd protein can be functional for serum resistance to some extent.

In virulent strains of *A. salmonicida*[12], 49 Kd surface protein, the A-protein was shown to contribute to virulence and serum resistance principally. In the case of *A. hydrophila* TF7, 52 Kd polypeptide was exhibited to constitute surface lattice structure but not to be associated directly with serum resistance. However, Dooley and Trust[8] reported that the deep rough LPS mutant was unable to maintain the surface layer (S-layer) protein on the cell surface and concluded that a minimum LPS oligosaccharide size was required for S-layer anchoring.

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