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Authors: Ford, Larisa A., and Thune, Ronald L.

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S-LAYER POSITIVE MOTILE AEROMONADS ISOLATED FROM CHANNEL CATFISH[1]

Larisa A. Ford[2,4] and Ronald L. Thune[2,3]

[1] Approved for publication by the Director of the Louisiana Agricultural Experiment Station as Manuscript number 91-64-5044

[2] Department of Veterinary Microbiology and Parasitology, School of Veterinary Medicine, Louisiana State University, Baton Rouge, Louisiana 70803, USA
[3] Department of Veterinary Science, Louisiana Agricultural Experiment Station, Louisiana State University Agricultural Center, Baton Rouge, Louisiana 70803, USA
[4] Corresponding author's present address: National Fish Health Research Laboratory, Box 700, Kearneysville, West Virginia 25430, USA

ABSTRACT: Motile aeromonads are ubiquitous aquatic bacteria that can cause motile aeromonad septicemia (MAS), a disease which affects channel catfish and can produce significant economic loss. Motile aeromonads isolated from commercially-raised channel catfish were screened for production of S-layer protein in order to evaluate its potential role in natural epizootics. The S-layer protein was produced by 14 of 24 (58%) isolates from epizootics evaluated in this study. Concomitant infections with other internal pathogens were detected in 10 of the 24 cases used in this study, and only one of those 10 isolates (10%) produced the S-layer protein. When *Aeromonas* sp. was the only internal pathogen diagnosed, 13 of 14 (93%) isolates produced the S-layer protein.

Key words: S-layer protein, motile aeromonads, motile aeromonad septicemia (MAS), channel catfish, *Aeromonas* sp.

INTRODUCTION

Houwink (1953) first reported "periodic macromolecular monolayers" as a component of the bacterial cell envelope. Since that time, Houwink's monolayers have been referred to as crystalline surface layers or simply, S-layers. Presence of S-layers as a component of the bacterial cell membrane is common. In fact, S-layers are produced by various gram-negative bacteria, gram-positive bacteria, Archaebacteria, Cyanobacteria and *Chlamydia* sp. (Sleytr and Messner, 1983, 1988; Smit, 1987). The S-layer or a structure analogous to this cell wall component is also produced by certain bacterial pathogens of fish, such as the A-layer of *A. salmonicida*. This surface protein imparts a complete and impermeable layer around the cell which enhances virulence by increasing hydrophobicity and resistance to phagocytosis by host macrophages (Kay et al., 1981; Phipps et al., 1983; Udey and Fryer, 1978). Similar proteins are found in *Aeromonas hydrophila* (Dooley and Trust, 1988; Dooley et al., 1986) and *Vibrio salmonicida* (Hjelmeland et al., 1988).

Motile aeromonads, particularly *Aeromonas hydrophila* and *A. sobria*, are considered opportunistic pathogens of a wide variety of animals, including commercially-raised channel catfish (*Ictalurus punctatus*). Although the pathogenicity of motile aeromonads is not well understood, the S-layer may enhance cellular adhesion or function as a physical barrier to host immunity. The S-layer of motile aeromonads is an ~52 *kd* protein that is arranged tetragonally. Motile aeromonads that produce S-layer protein autoaggregate in static cultures and resist the bactericidal activity of normal fish serum (Dooley et al., 1986). The presence of the S-layer protein has been correlated with virulence of motile aeromonads in trout and channel catfish (Dooley et al., 1986; R. L. Thune, unpubl. data).

Many of the *Aeromonas* spp. studied previously were isolated from a wide variety of sources and were often maintained on artificial media for extended periods (R. L. Thune, unpubl. data). Passage on artificial media may result in loss of bacterial production of the S-layer protein, as documented for *Aeromonas salmonicida* (Cipriano et al., 1984; Dooley et al., 1986;

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Trust et al., 1982). Information concerning the production of S-layer by motile aeromonads isolated from diseased channel catfish is lacking. The primary objective of the present study was to evaluate production of the S-layer protein by motile aeromonads isolated from catfish raised in commercial ponds.

MATERIALS AND METHODS

Collection of bacterial isolates

Bacteria were obtained from commercially-raised channel catfish submitted to the Aquatic Animal Diagnostic Laboratory (School of Veterinary Medicine at Louisiana State University in Baton Rouge, Louisiana 70803, USA) and to the Mississippi Cooperative Research Laboratories (Stoneville, Mississippi 38776, USA and Belzoni, Mississippi 39038, USA). Fish were necropsied by routine procedures. Bacteria isolated from internal organs were identified using standard biochemical tests and reaction on API-20E® strips (Analytab Products, Plainview, New York 11803, USA). *Aeromonas* sp. isolates were inoculated on blood agar base (Difco Laboratories, Detroit, Michigan 48232, USA) supplemented with 5% bovine red blood cells and single colonies transferred to brain heart infusion (BHI) agar (Difco Laboratories, Detroit, Michigan 48232, USA) slants for 24 hr at 30 C. Cells were washed from the agar slant and suspended in 0.85% NaCl + 20% glycerol for subsequent storage at −70 C. Isolates were not transferred on artificial media more than two times prior to storage.

Detection of the S-layer protein

Each isolate was grown in 50 ml of BHI broth at 30 C for 18 hr. Bacteria were pelleted by centrifugation at 1500 × *g*, washed twice with distilled water and resuspended in 10 ml of 0.2 M glycine-HCl buffer (pH 2.2) per gm of pelleted cells. The suspension was mixed by inversion for 15 min at room temperature and then centrifuged for 15 min at 11,000 × *g*. Supernatant was collected and pH adjusted to 4.5 with HCl. Adjusted S-layer extract was prepared for sodium-dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Extracts were mixed (1:1 vol/vol) in 0.125 M tris buffer (pH 6.8) containing 4% SDS, 20% glycerol and 10% 2-mercaptoethanol. Electrophoresis was conducted as described by Laemmli (1970) using 4% stacking and 12% resolving gels. Polyacrylamide gels were stained in 0.125% Coomassie Brilliant Blue and destained with a 50% methanol/10% acetic acid solution to visualize the electrophoresed protein.

RESULTS

Presence of a protein band at ~52 *kd* in polyacrylamide gels was used to confirm S-layer production by a given isolate. For example, isolates S87-164 and S87-166 had the S-layer protein but isolate B87-885 did not (Fig. 1).

Isolates from 24 epizootics were screened for the production of the S-layer protein (Table 1). Production of an S-layer protein was demonstrated in 58% (14 of 24) of the *Aeromonas hydrophila* and *Aeromonas sobria* isolates. In 10 of the epizootics, however, concomitant infections with other internal pathogens were detected including *Flexibacter columnaris* (FLEX), Channel Catfish Virus (CCV), *Edwardsiella ictaluri* (ESC) and *Edwardsiella tarda*. Only one *Aeromonas* sp. isolate (LA87-278) produced the S-layer protein when isolated with another pathogen. Isolates were positive for the production of the S-layer protein in 13 of the 14 remaining cases (93%) in which no other internal pathogens were reported (Table 2).

DISCUSSION

Motile aeromonads are ubiquitous in aquatic environments and can be routinely isolated from dead, moribund and asymptomatic fish. These bacteria are opportunistic pathogens and the clinical significance of the isolation of an aeromonad from a diseased fish is often difficult to determine. Other pathogens are often concurrently diagnosed with motile aeromonad infections. In fact, 42% of the cases included in this study had concurrent infections which further compounds the problem of determining the etiological significance of the aeromonads in the disease process.

The mortality rate of channel catfish infected in a MAS epizootic may vary from a few sporadic deaths to a fulminating septicemia in which 100% of the affected population may die. Severity of disease is often associated with the physiological condition of the fish, environmental and seasonal condition of the ponds and virulence of

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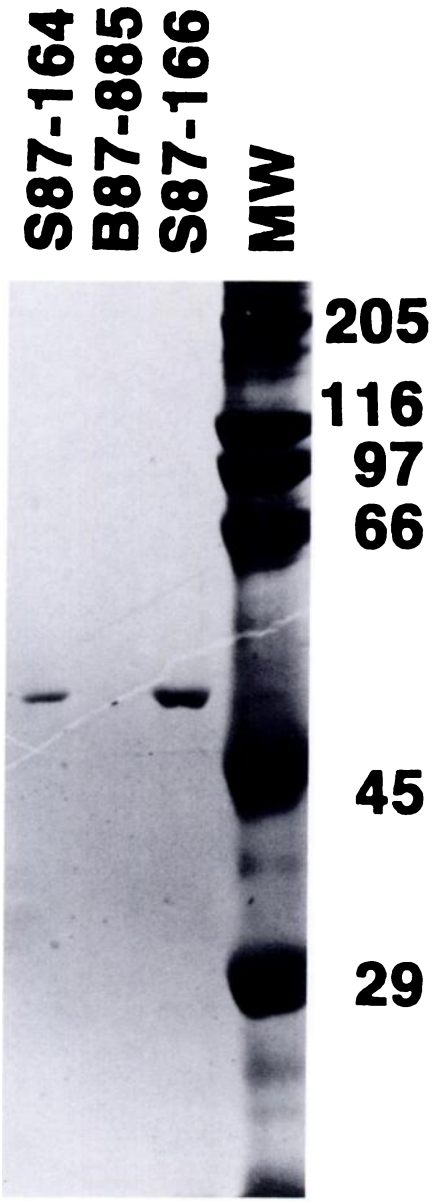


FIGURE 1. A single protein band (the S-layer protein) in acid-extracted preparations demonstrated by SDS-PAGE from motile aeromonad strains (S87-164 and S87-166). Note that isolate B87-885 does not contain S-layer protein. Lane 4 contains the molecular weight standards.

the particular *Aeromonas* sp. isolate (MacMillian, 1985). Differential virulence among isolates of motile aeromonads is well documented. For example, De Figueiredo and Plumb (1977) reported that motile aeromonads isolated from pond water are less virulent than isolates from diseased fish. Also, *Aeromonas hydrophila* isolates from diseased fish are more virulent for rainbow trout than *A. sobria* isolated from healthy fish (Lallier et al., 1980).

Mechanisms and factors involved in de-

TABLE 1. S-layer production of *Aeromonas* sp. isolates from commercially-raised channel catfish and other concurrently diagnosed internal pathogens.

Aeromonas sp. isolate	S-layer production	Other internal pathogens
S87-477	−	*Flexibacter columnaris*
S87-480	−	None
S87-481	−	*Edwardsiella tarda*
B87-821	−	Channel catfish virus*
B87-822	−	Channel catfish virus*
B87-842	−	Channel catfish virus
B87-880	−	Channel catfish virus*
B87-881	−	Channel catfish virus*
B87-884	−	*Flexibacter columnaris*
B87-885	−	*Flexibacter columnaris*
S86-1129	+	None
S86-1326	+	None
S87-74	+	None
S87-153	+	None
S87-157	+	None
S87-164	+	None
S87-166	+	None
S87-316	+	None
S87-532	+	None
S87-693	+	None
S87-749	+	None
LA87-1	+	None
LA87-278	+	*Edwardsiella tarda*
LA87-282	+	None

* Diagnosed by clinical signs only.

TABLE 2. Percentage of S-layer positive *Aeromonas* isolates grouped according to the internal pathogens detected during necropsy of commercial channel catfish.

Internal pathogen	Number of diagnostic cases	Number of S-layer positive isolates
Motile aeromonad (only)	14	13 (93%)
Motile aeromonad + others	10	1 (10%)
Flexibacter columnaris	3	0 (0%)
Edwardsiella ictaluri	1	0 (0%)
Channel catfish virus	5	0 (0%)
Edwardsiella tarda	1	1 (100%)

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termining the virulence of motile aeromonads are not clearly understood. The degree of virulence of motile aeromonad isolates, however, has been associated with variations in the quality and quantity of extracellular toxins produced by the bacterium (Allan and Stevenson, 1981; Thune et al., 1986, 1982a, b) and has also been correlated with the presence of S-layer protein (Dooley et al., 1986).

Although recent studies have described the role of S-layer in virulence of motile aeromonads, this is the first report of S-layer positive motile aeromonads from epizootics involving commercially reared channel catfish. Production of the S-layer in 93% of the cases, in which *Aeromonas* sp. was the sole pathogen, further substantiates the theory that the S-layer is important to virulence.

The majority of isolates that did not produce an S-layer protein were from epizootics in which other internal pathogens were also diagnosed. Lack of S-layer production by these aeromonads may indicate that the isolates are either less virulent, secondary invaders, or incidental isolations of non-pathogenic aeromonads. Production of the S-layer protein by *Aeromonas* sp. might also be influenced by the presence of other pathogens or by seasonal conditions at times when other pathogens are predominant. Further studies to clearly elucidate the significance of the S-layer protein in the virulence of motile aeromonads are indicated.

Herein, the use of trade names does not imply U.S. government endorsement of commercial products.

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