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Growth of *Vibrio anguillarum* in Salmon Intestinal Mucus

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The physiological changes of *Vibrio anguillarum* in response to growth in salmon intestinal mucus were investigated. Growth, survival, and changes in protein expression during growth in media supplemented with mucus were compared to growth and starvation in the identical media without mucus. *V. anguillarum* exhibited a rapid decline in CFU following growth in mucus as the sole carbon source. No such decline was observed in Luria broth with a 2% NaCl concentration, in glucose-minimal broth (3M), or during starvation in a carbon-, nitrogen-, and phosphorus-free salt solution (NSS). The changes in protein expression during growth in mucus were examined by labeling cells with [^{35}S]methionine and analyzing the labeled proteins by one- and two-dimensional gel electrophoresis and autoradiography. Comparison of [^{35}S]methionine-labeled proteins from mucus-grown cells with 3M-grown cells and NSS-starved cells revealed four de novo mucus-inducible proteins (Mips). These Mips were localized in the membrane fraction of *V. anguillarum*. Additionally, at least one other membrane protein was found to have increased expression in response to growth in mucus.

Vibrio anguillarum is the causative agent of vibriosis. This disease is one of the major bacterial diseases affecting fish, as well as bivalves and crustaceans (3, 5, 6). As a result, vibriosis can cause great economic losses to the aquaculture industry. This disease is characterized by deep focal necrotizing myositis and subdermal hemorrhages, with the intestine and rectum becoming swollen and filled with fluid (17, 24).

Recently, Olsson (35) demonstrated that the gastrointestinal tract of fish can serve as the port of entry for *V. anguillarum*. Additionally, the gastrointestinal tract of fish appears to be a site of colonization and amplification for pathogenic *Vibrio* species (16, 36). Ransom et al. (36) found *V. anguillarum* and *Vibrio ordalii* primarily in the pyloric ceca and the intestinal tracts of infected Pacific salmon. It has been shown that the gastrointestinal tracts of many animals contain bacterial populations that are relatively stable and consist of hundreds of species (11). This is true of fish. Studies have demonstrated that the digestive tract of marine fish is colonized by several hundred species of bacteria (41). It has been suggested that the presence of large and diverse indigenous populations of bacteria renders the gastrointestinal tract relatively resistant to invasion by nonindigenous species (41). Some bacterial isolates from the turbot gastrointestinal tract appear to secrete substances that inhibit the growth of *V. anguillarum* (35, 41). Further, it appears that populations of bacteria in the intestinal tract are controlled by substrate competition (13). Recently, Burghoff and coworkers have demonstrated that the ability of both *Escherichia coli* and *Salmonella typhimurium* to colonize the mouse colon is dependent upon the ability of each organism to express proteins that allow the utilization of colonic mucus components (7).

The walls of the intestinal tract contain epithelial cells that constantly produce mucus. Gastrointestinal mucus, which contains proteins, lipids, glycoproteins, and glycolipids (8), is rich in nutrients that organisms, including pathogens, may utilize for growth. Many studies have implicated growth in mucus as a critical factor for intestinal colonization by pathogens and identified proteins synthesized that are necessary for establishment of an infection focus (7, 13, 19, 26, 28). Therefore, mucus is a likely nutrient source for invading pathogenic *Vibrio* species, allowing their recovery from starvation during their passage between fish hosts and their proliferation, or the feast part of the feast-and-famine life cycle.

In this investigation, we examined the ability of *V. anguillarum* to grow in salmon intestinal mucus and compared the growth rate obtained in mucus with those obtained in various laboratory media. The changes in protein expression during growth in mucus were also determined and compared to changes observed during growth in a glucose-minimal broth, Luria broth plus 2% NaCl (LB20), and during starvation. Several mucus-inducible proteins (Mips) and mucus-repressible proteins (Mrps) were identified by two-dimensional gel electrophoresis and fluorography. The Mips were shown to be localized in the cell envelope.

MATERIALS AND METHODS

Bacterial strains and culture conditions. *V. anguillarum* 2129 (obtained from S. Kjelleberg, University of Göteborg, Göteborg, Sweden) was routinely grown and maintained in LB20 (39). Cells were also incubated in several experimental media, including nine-salt solution (NSS; a carbon-, nitrogen-, and phosphorus-free salt solution) (21), marine minimal medium (3M) (27), NSS supplemented with 50 μg of mucus protein/ml (NSSM), and 3M without glucose supplemented with 50 μg of mucus protein/ml (M3M). To cultivate cells for experiments, *V. anguillarum* was initially grown overnight in LB20 on a rotary shaker at 27°C. An aliquot of the overnight culture was added to a flask of 3M and grown overnight on a rotary shaker at 27°C. Aliquots of the 3M culture were centrifuged at 9,000 × *g* for 10 min and washed twice with NSS. Washed cells were resuspended to the appropriate cell densities in the experimental media. Growth was monitored either by measurement of the optical density (550 nm) or by serial dilution and plate counts.

Preparation of salmon gastrointestinal mucus. Mucus was extracted from Atlantic salmon by a modification of the method suggested by Terence Bradley (Fisheries, Animal and Veterinary Science Department, University of Rhode Island). An incision was made into the peritoneum of the fish, exposing the cecum and the upper and lower intestinal tracts. These were removed and scraped free of the fatty layer covering the organs. The organs were cut open, undigested food and fecal matter were removed by gently washing the open organs with NSS until the wash fluid was clear, and the intestinal and cecal mucus

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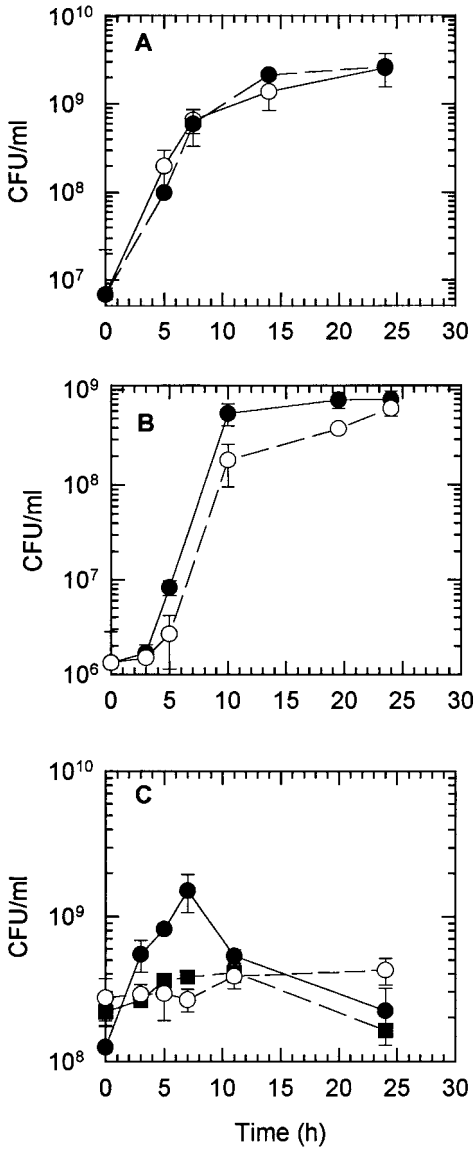

FIG. 1. Growth of *V. anguillarum* in LB20 (A), 3M (B), and NSS (C) in the absence (open symbols) and presence (closed symbols) of salmon intestinal mucus. *V. anguillarum* cells grown overnight in 3M at 27°C were used as the inoculum for all cultures. All cultures were incubated at 27°C with shaking. Salmon intestinal mucus was used at a concentration of 50 μg/ml, except in NSS, where growth was monitored at concentrations of both 50 μg/ml (closed circles) and 2 μg/ml (closed squares). CFU were determined by serial dilution and plating. All data are the result of triplicate samples, and error bars indicate standard deviation.

was scraped from the inner tissues of the organs with a soft rubber spatula. The mucus was then suspended in NSS to preserve its natural osmolarity. Particulate matter was removed by centrifugation (9,000 × *g*, 5°C, 10 min), and the soluble mucus was diluted with NSS to a concentration of 1 mg of mucus protein/ml as determined by a protein assay (Bio-Rad Laboratories, Richmond, Calif.).

The mucus suspension was sterilized by exposure to UV light and stored at −70°C.

Radioactive labeling of cells. *V. anguillarum* cells were labeled with [^{35}S]methionine after a 6- to 12-h incubation in the appropriate experimental medium. Briefly, aliquots (1 ml) were withdrawn from experimental media and labeled with 2 μCi of [^{35}S]methionine (specific activity, 1,174 Ci/mmol; Dupont, Boston, Mass.) for 20 to 30 min at 27°C. Labeling was stopped by the addition of 100 μl of nonradioactive methionine (0.1 M) and incubation for 10 min at 27°C. The labeled cells were centrifuged (16,000 × *g*, 5°C, 2 min), washed twice with NSS, and resuspended in 100 μl of NSS. Incorporation of the radiolabel was determined by scintillation counting of samples.

Gel electrophoresis and fluorography. Following radiolabeling, cells were prepared for either sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (20) or two-dimensional PAGE (34). For SDS-PAGE, the ^{35}S-labeled *V. anguillarum* cells were broken by sonication (sonifier model 350; Branson, Melville, N.Y.). Typically, cells were sonicated for 1 min (50% cycle) on ice. Cell breakage was monitored by phase-contrast microscopy. The sonicated extract was centrifuged (16,000 × *g*, 10 s) to remove unbroken cells. SDS solubilization-reduction mix was added, and the extracts were boiled for 5 min to ensure solubilization. Equal amounts of radioactive samples (10^5 cpm) were loaded onto each gel lane, and the proteins were resolved on 12% polyacrylamide gels. For the analysis of [^{35}S]methionine-labeled *V. anguillarum* proteins by two-dimensional gel electrophoresis, growth, labeling, and lysis were carried out as described above. Samples were prepared and analyzed according to the method of O'Farrell (34). Isoelectric focusing in the first dimension was carried out on a pH 3 to 10 gradient. The pH 3 to 10 gradient was formed by mixing pH 3- to 10-range ampholytes (1 volume) with pH 5- to 7-range ampholytes (4 volumes). The final concentration of ampholytes (Bio-Rad) was 2%. Samples were generally focused for 16 h at 400 V followed by 1 h at 800 V. Following isoelectric focusing, the tube gels were extruded and run in the second dimension through a 12% polyacrylamide–SDS gel. All slab gels were dried and examined by fluorography as previously described (38). Densitometry of the fluorograms was performed with a personal densitometer (Molecular Dynamics, Sunnyvale, Calif.) and Image QuaNT software (Molecular Dynamics). Gel analyses were performed at least three times to confirm reproducibility.

Subcellular fractionation. *V. anguillarum* cells were fractionated to detect the subcellular location of proteins by the technique of Albertson et al. (2). Briefly, following experimental incubation and labeling with [^{35}S]methionine, the cells were resuspended in 100 μl of ultrapure water for 20 min to cause osmotic shock and centrifuged (27,000 × *g*, 5°C, 10 min) to collect the periplasmic fraction (the supernatant). The remaining pellets were lysed by sonication and separated by ultracentrifugation (150,000 × *g*, 1.5 h, 4°C) to yield the cytoplasmic and membrane fractions. The membrane fraction was treated with Sarkosyl to give Sarkosyl-soluble (inner membrane) and -insoluble (outer membrane) fractions. The fractions were analyzed by SDS-PAGE and fluorography, as described above. In some cases, where the radioactivity was low, samples were concentrated by trichloroacetic acid precipitation. Samples were precipitated with trichloroacetic acid (10%, final concentration) for 1 h at 4°C, centrifuged (16,000 × *g*, 5°C, 2 min), and washed twice with ice-cold acetone. The resulting pellets were resuspended in solubilization-reduction mix as previously described and run on the SDS-PAGE gel.

RESULTS

Growth of *V. anguillarum* in mucus-supplemented media. The growth of *V. anguillarum* was observed in several different media (Fig. 1), including LB20 (a rich complete medium), 3M (a glucose-minimal medium), and NSS (a starvation medium), and compared to growth in identical media supplemented with salmon intestinal mucus (50 μg of mucus protein/ml). A typical growth curve of *V. anguillarum* in LB20 is illustrated in Fig. 1A.

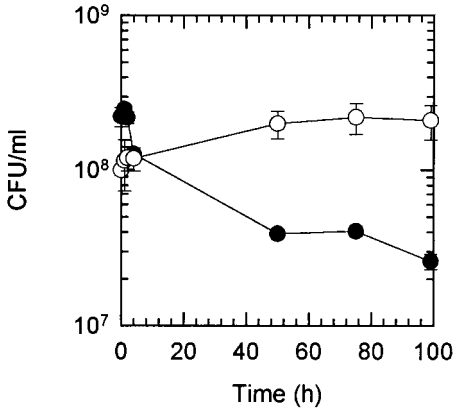

FIG. 2. Effect of incubation in conditioned mucus on the survival of *V. anguillarum*. Cells grown for 10 h in 3M were washed in NSS and resuspended in either NSS (open circles) or conditioned NSSM (closed circles) at 27°C. The conditioned NSSM was prepared by growing cells in NSSM for 24 h and removing the cells by centrifugation and then by filtration through a 0.22-μm-pore-size filter. CFU were determined by serial dilution and plating. All data are the result of triplicate samples, and error bars indicate standard deviation.

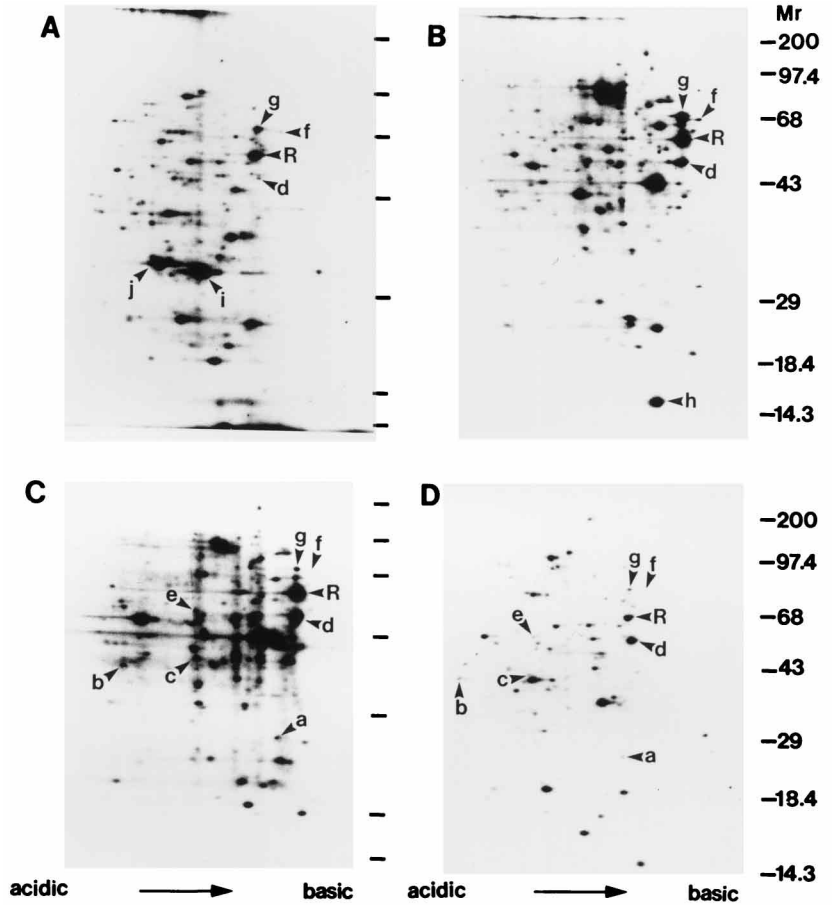

FIG. 3. Two-dimensional fluorograms of [^{35}S]methionine-labeled *V. anguillarum* proteins during incubation in NSS (A), 3M (B), NSSM (C), and M3M (D). *V. anguillarum* cells (1 ml, ~10^8/ml) incubated in the appropriate experimental medium for 13 h at 27°C were labeled with 10 μCi of [^{35}S]methionine for 30 min. Labeled proteins were separated and analyzed by two-dimensional gel electrophoresis and fluorography according to the method of O'Farrell (34). Proteins identified were Mip21 (a), Mip39 (b), Mip40 (c), Mip52 (d), Mip57 (e), Mrp68 (f), Mrp69 (g), Grp15 (h), Sti30.5 (i), and Sti32.5 (j). M_r is given in thousands on the right.

The average growth rate (determined for three trials) ± standard deviation of logarithmic-phase cells in LB20 at 28°C was 0.70 ± 0.14 generation/h. The addition of mucus had virtually no effect upon either the growth rate or the final growth yield. Growth in 3M (Fig. 1B) was characterized by a short lag phase followed by exponential growth at approximately the same rate (0.71 ± 0.20 generation/h; average from four trials) as seen in LB20. The addition of mucus to 3M also had little effect on either the growth rate or the final yield of cells. Cells suspended in NSS exhibited no significant change in CFU over a 24-h period (Fig. 1C). However, cells incubated in NSSM grew rapidly (0.78 ± 0.13 generation/h; average from 10 trials) (Fig. 1C). The growth yield was dependent upon the concentration of mucus added to the medium. Typically, NSSM (50 μg of mucus protein/ml) supported the growth of *V. anguillarum* to concentrations of 10^8 to 10^9 CFU/ml. Lower concentrations of mucus supported the growth of proportionately lower concentrations of cells. Cessation of growth in mucus was always followed by a decline in CFU. In the experiment presented in Fig. 1C, the decline in CFU was 82% from the maximum cell concentration. The addition of mucus to NSS, even at low concentrations (e.g., 2 μg of protein/ml), always resulted in cell growth followed by a decline in CFU (Fig. 1C). Additionally, the pH of the mucus medium (pH 7.4) did not change during the course of growth in the mucus medium.

The above data indicated that the decline in CFU might have been due to a substance secreted into the medium by *V. anguillarum* during growth in mucus. In order to examine this possibility, a culture of *V. anguillarum* was grown in NSSM (50 μg of mucus protein/ml) for 24 h. The cells were removed from the medium by filtration (0.22-μm-pore-size filter). *V. anguillarum* cells grown concurrently in 3M for 10 h were washed in NSS and resuspended either in the conditioned NSSM or, as a control, in NSS without mucus. The cells resuspended in the conditioned medium exhibited a decline in CFU of about 50% within 4 h (Fig. 2). Over the nearly 100 h of incubation in the conditioned mucus medium, the total CFU declined by approximately 1 order of magnitude. In contrast, the control cells starved in NSS did not exhibit a decline in CFU.

Identification of Mips by two-dimensional gel electrophoresis. The patterns of protein expression by *V. anguillarum* during growth in various media were examined by labeling the cells with [^{35}S]methionine during growth in each of the media and analyzing the labeled proteins by two-dimensional gel electrophoresis and fluorography. The proteins expressed by *V. anguillarum* during short-term (6-h) starvation in NSS (Fig. 3A) served as the control to which all other two-dimensional fluorograms were compared. The protein labeled R (reference) indicates the protein used as an internal standard. The expression of this 60-kDa protein varied little under all experimental growth conditions used. The following classes of proteins were observed and grouped according to their patterns of expression

TABLE 1. Relative induction of selected *V. anguillarum* proteins during growth in mucus or glucose or during starvation[a]

Protein	Relative induction ± SEM in:			
	NSS	3M	NSSM	M3M
R (60 kDa)	1.00	1.00	1.00	1.00
Mucus inducible				
Mip39	0	0	0.02 ± 0.01	0.09 ± 0.03
Mip40	0	0	0.19 ± 0.16	1.21 ± 1.18
Mip57	0	0	0.04 ± <0.01	0.05 ± 0.01
Mip21	0	0	0.09 ± 0.06	0.09 ± 0.02
Mip52	0.11 ± 0.09	0.25 ± 0.11	1.00 ± 0.24	2.23 ± 0.56
Mucus repressed				
Mrp68	1.36 ± 0.67	0.06 ± 0.03	0	0
Mrp69	1.86 ± 1.55	0.40 ± 0.08	0.05 ± 0.01	0.08 ± 0.05
Grp15	0	0.42 ± 0.23	0	0
Starvation induced				
Sti32.5	0.59 ± 0.09	0	0	0
Sti30.5	1.32 ± 0.08	0	0	0

[a] Relative induction of proteins was determined by densitometry of fluorograms from [^{35}S]methionine-labeled proteins separated by two-dimensional gel electrophoresis. The internal protein standard, R, was expressed under all incubation conditions. Expression of all proteins was related to this protein. All densitometry determinations were performed on at least three separate samples obtained from independent experiments.

in response to starvation, glucose, and mucus (see Fig. 3 and Table 1): starvation-induced proteins (Stis), glucose-regulated proteins (Grps), Mrps, and Mips). Two proteins, Sti32.5 and Sti30.5 (32.5 and 30.5 kDa, respectively), were expressed only during starvation and were termed Stis. It should be noted that other Stis were observed during this study. We list only the most prominent and reproducible of these proteins here. Three proteins were consistently found to be repressed during growth in mucus. However, one of these proteins, termed Grp15 (15 kDa), was induced only during growth in glucose and was classified a Grp. Grp15 was not expressed during starvation, nor was it expressed during growth in mucus. The other two proteins, Mrp69 and Mrp68 (69 and 68 kDa, respectively), were expressed during starvation and during growth in glucose. Both Mrp69 and Mrp68 were repressed during growth in mucus. Expression of Mrp69 was decreased five- to eightfold when cells were grown in media containing mucus as the sole carbon source (NSSM and M3M, respectively) compared to levels seen in 3M. Mrp68 was not expressed in mucus-containing media.

We observed five Mips consistently (Fig. 3C and D; Table 1). While all five Mips exhibited increased expression during the growth of *V. anguillarum* in mucus, four (Mip21, Mip39, Mip40, and Mip57) were expressed only in mucus-containing media. In contrast, Mip52 (52 kDa) was observed during starvation and during growth in 3M (Fig. 3A and B; Table 1). Mip52 expression increased 2.3-fold when cells were shifted from NSS to 3M. Mip52 expression increased an additional four- to ninefold when cells were grown in a mucus-containing medium (Table 1). Growth of *V. anguillarum* cells in M3M (3M without glucose, supplemented with mucus) showed the expression of all five Mips at increased levels compared to growth in NSSM (Table 1).

Identification of Mips in cell fractions. The above data demonstrated that *V. anguillarum* cells induced several proteins during growth in salmon intestinal mucus. In order to determine the subcellular location of these Mips, cells were grown in mucus- and non-mucus-containing media for 13 h, radioactively labeled with [^{35}S]methionine, and fractionated to yield cytoplasmic, periplasmic, and membrane fractions. The proteins in each fraction were identified and analyzed by SDS-PAGE and fluorography. The changes in protein synthesis that occurred in the Sarkosyl-soluble membrane fraction (Fig. 4A) showed that a 57-kDa protein was synthesized de novo in mucus. This protein had the same molecular mass as Mip57. The changes in protein synthesis that occurred in the Sarkosyl-insoluble membrane fraction (Fig. 4B) demonstrated that three proteins (~21, 39, and 40 kDa) were expressed specifically in mucus. These proteins had the same apparent molecular masses as Mip21, Mip39, and Mip40, respectively. Another protein (~52 kDa) preferentially expressed in mucus had the same molecular mass as Mip52 (Fig. 4B). No Mips were observed in either the cytoplasmic or the periplasmic fractions (data not shown).

DISCUSSION

In this report, we show that *V. anguillarum* grows rapidly and efficiently in salmon intestinal mucus. Interestingly, cells grown in mucus do not enter stationary phase but rather begin to decline when mucus nutrients are depleted. This decline in CFU is observed at all concentrations of mucus used in our experiments (2 to 200 μg of mucus protein/ml) but not when cells are grown in any other medium (data not shown). Additionally, *V. anguillarum* cells grown in another medium (LB20

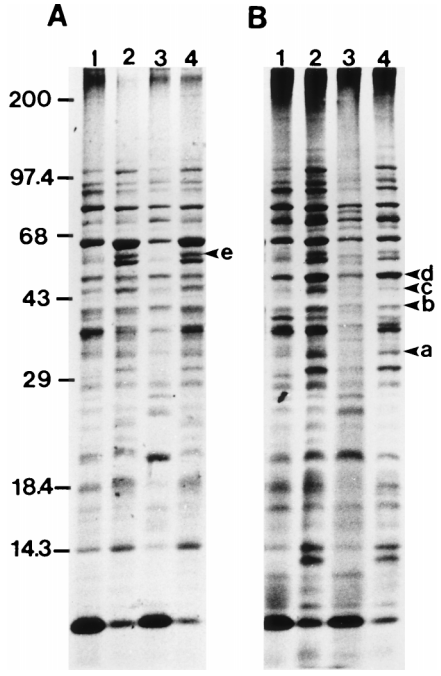

FIG. 4. Fluorogram of *V. anguillarum* membrane fractions showing the subcellular location of Mips. *V. anguillarum* cells grown and labeled with [^{35}S]methionine as described in the text were treated with distilled water to release the periplasm and then lysed by sonication. The lysate was separated by ultracentrifugation (150,000 × *g*, 1.5 h) to yield membrane and cytoplasmic fractions. The membrane fractions were washed and extracted to yield Sarkosyl-soluble (cytoplasmic membrane) (A) and Sarkosyl-insoluble (outer membrane) (B) fractions. The [^{35}S]methionine-labeled proteins of the fractions were separated by SDS-PAGE (14) and analyzed by fluorography. No Mips were detected in the periplasmic or cytoplasmic fractions (data not shown). Lanes: 1, NSS; 2, NSSM; 3, 3M; 4, M3M. Proteins are identified as follows: a, Mip21; b, Mip39; c, Mip40; d, Mip52; e, Mip57. Sizes are given on the left in kilodaltons.

or 3M) that are resuspended in spent or conditioned mucus also exhibit a decline in CFU comparable to that seen in cells grown in mucus (Fig. 2). These data suggest that cells grown in mucus secrete a compound toxic to *V. anguillarum* cells.

Our data also demonstrate that *V. anguillarum* cells express a number of different proteins in response to specific nutritional conditions. The conditions that specifically affect protein expression include starvation, growth in glucose, and growth in mucus (Table 1). For example, two proteins are routinely found to be expressed after 12 h of starvation (Sti32.5 and Sti30.5). Neither protein is seen in cells growing in either 3M or mucus-containing media. Five proteins (Mip57, Mip52, Mip40, Mip39, and Mip21) are induced and three proteins (Mrp69, Mrp68, and Grp15) are repressed during growth in mucus. Four of the five Mips are expressed only in mucus-containing media. Mip52 is expressed at all times, including during starvation, but is induced up to 20-fold during growth in mucus over levels seen during starvation.

All five Mips are associated with the cell envelope of *V. anguillarum* (Fig. 4). Subcellular fractionation demonstrated that Mip57 was in the Sarkosyl-soluble (cytoplasmic) membrane fraction. Mip52, Mip40, Mip39, and Mip21 were all in the Sarkosyl-insoluble (outer) membrane fraction. These data suggest that the ability of *V. anguillarum* to grow in intestinal mucus involves the expression of envelope proteins that may be important in the uptake of mucus nutritional components. Our observations are similar to those reported by Burghoff et al. (7) for *E. coli*. Those investigators showed that at least three outer membrane proteins were specifically expressed by mucus-grown *E. coli* strains able to colonize the mouse large intestine. Noncolonizing *E. coli* strains did not express these outer membrane proteins. These observations are also consistent with the observation that mucus-grown cells use very specific nutrients in mucus in order to successfully compete in the mucus environment. For example, Krivan et al. (19) have shown that *E. coli*, *Salmonella choleraesuis*, and *S. typhimurium* use phosphatidylserine when growing in mouse colonic mucus.

It is interesting to note that a series of reports concerning the growth and survival of *E. coli* in mouse mucus (40), the mouse large intestine (26), and piglet ileal mucus (4) all suggest that both pathogenic and nonpathogenic *E. coli* cells that are successful in colonization of the mucus environment enter either stationary or a stationary-like phase. Newman and coworkers have shown that specific genes, such as *leuX*, are necessary for both colonization of the mouse large intestine and survival during stationary phase (28). Additionally, Blomberg et al. (4) have recently shown that the metabolism of *E. coli* K88 is altered in mucus when it enters stationary phase to enable the cells to specifically utilize mucus lipids. In contrast to those reports, *V. anguillarum* 2129 and at least two other strains examined in our laboratory (14) do not enter stationary phase when grown in mucus (Fig. 1C). Instead, as pointed out above, the cells exhibit a decline in CFU which begins as exponential growth ends. All these *V. anguillarum* strains have been observed to enter stationary phase when grown in LB20 or in 3M.

Finally, it should be pointed out that *V. anguillarum* appears to be ubiquitous in coastal marine environments and is found generally within the cultivable fraction of marine waters, along with other *Vibrio* species (25). In this environment, many types of bacteria, including *Vibrio* species, achieve a physiological state that enables survival despite the constant challenge of stressful conditions. Survival of adverse conditions, in all microorganisms, depends upon the initiation of a successful emergency response, which results in protection for the cell and allows recovery once favorable conditions are experienced. Several species of nondifferentiating bacteria, including *Vibrio* sp. strain S14, *S. typhimurium*, and *E. coli* exhibit survival during long periods of starvation (2, 12, 15). Unlike differentiating microbes, which produce spores that are stress resistant and dormant, these nondifferentiating microbes adapt to a resistant state that is metabolically active. This trait is documented in *Vibrio* sp. strain S14, which form ultramicrocells that are resistant to autolysis, sonic lysis, hydrostatic pressure, and heat or cold shock (29, 30, 32). Stress responses of this type involve a time-dependent, sequential modification of protein synthesis (31). These occur from the onset of starvation and are observed as dramatic physiological and morphological changes which allow protease excretion, synthesis of critical proteins, and substrate transport (1, 10, 22, 33). Thus, adaptation to starvation results in the development of enhanced resistance to multiple environmental stresses. *Vibrio* species are able to persist as free-living organisms in nutrient-poor waters for long periods, quickly recovering from this starvation-adapted state to proliferate when nutrient concentrations are no longer limiting (3). Marouga and Kjelleberg (23) have recently demonstrated that recovery from starvation in *Vibrio* sp. strain S14 also involves a temporal program of gene expression. This program of starvation recovery is likely to be important during the entry of *V. anguillarum* into a new host fish. Such a life cycle of feast and famine is well documented in *V. anguillarum*, imparting success as a pathogen of fish (37).

The critical role played by the starvation response in dictating the biochemical and morphological changes allowing survival in nondifferentiating bacteria has been investigated previously (18). Furthermore, this role has recently begun to be elucidated in *V. anguillarum* (37). This is an important aspect to the pathogenesis of the organism, as it may establish itself in the sediment of hatchery tanks and remain viable several months after an outbreak (9). In this study we have presented data which demonstrate that intestinal mucus is an excellent medium for the recovery from starvation and the proliferation of *V. anguillarum*. We have also documented changes in protein synthesis that occur in *V. anguillarum* during growth in mucus. We are now in a position to begin to understand how the various Mips identified in *V. anguillarum* enable this pathogen to grow in the mucus environment.

ACKNOWLEDGMENTS

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