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Nucleotide Sequence of the Gene for the Hole-Forming Toxin Aerolysin of *Aeromonas hydrophila*

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The gene for the hole-forming toxin aerolysin from *Aeromonas hydrophila* was sequenced. Although most of the sequence seems unrelated to that of *Staphylococcus aureus* alpha-toxin, both proteins are very hydrophilic, and they each contain a nearly identical string of 10 amino acids.

Aerolysin is a cytolytic toxin exported by the gram-negative bacterium *Aeromonas hydrophila* (6–9, 14). There are two precursor forms of the toxin. It crosses the inner bacterial membrane as a preprotoxin containing a typical signal sequence, which is removed cotranslationally (8). The resulting protoxin is exported from the bacteria and activated by proteolytic removal of about 25 amino acids from the carboxy terminus (9). Like *Staphylococcus aureus* alpha-toxin (3), the mature protein binds to eucaryotic cells and aggregates to form holes approximately 3 nm in diameter, leading to destruction of the membrane permeability barrier and osmotic lysis (6). We have recently cloned the structural gene for preproaerolysin into *Escherichia coli* (10). Here we present its nucleotide sequence.

The plasmid pPH4 (10) was digested with the restriction enzymes shown in Fig. 1. The resulting fragments were inserted into the polylinker cloning regions of M13 mp18 and mp19 (15) and sequenced (19). The universal 17-base single-stranded primer was used to begin sequencing from the 3′ end of the inserts. Sequencing was continued with 18-base oligonucleotide primers prepared by using a Sam One DNA synthesizer (Biosearch Inc., San Rafael, Calif.).

The sequence of the region containing the aerolysin gene is shown in Fig. 2. The longest open reading frame extends from base pairs (bp) 532 to 1989. This would produce a protein with a molecular weight of 53,800, very close to the observed molecular weight of preproaerolysin (8). Several features of the region preceding the gene, including the presence of sequences analogous to Shine-Dalgarno (20) and −10 and −35 regions, have been discussed earlier (10). The translation product of the first 69 bp beginning at 532 is a typical 23-amino-acid signal peptide (17). This is followed by a sequence corresponding to the amino terminus of purified aerolysin (10). In addition, the translated amino acid sequence matches the amino-terminal sequences of two of the cyanogen bromide fragments which were isolated and sequenced after cleavage of purified aerolysin with cyanogen bromide (5). There are no extended open reading frames in the 350-bp 5′ untranslated sequence upstream of the gene, which appears to contain a promoter, and there is a palindromic sequence analogous to a Rho-independent termination signal just downstream of the termination codon (18). These observations, which indicate that aerolysin is translated from a monocistronic message, are inconsistent with the results of Chakraborty et al. (1). Those authors have recently cloned and mapped a fragment of *A. hydrophila* DNA containing a gene for a hemolytic toxin, as well as flanking regions downstream and upstream which they suggest modulate its expression and activity. Our earlier observation that hemolytic activity is not affected in recombinants containing plasmids with Tn*5* insertions at or downstream of the *Bam*HI site at bp 2176 (10) also contrasts with their report that hemolysis is changed by transposon insertions in comparable regions of the gene they have cloned. In addition, Chakraborty et al. (1) found that plasmid deletions obtained by digestion with *Pst*I produced nonhemolytic recombinants, yet the sequence reported in Fig. 2 contains no *Pst*I sites (we have confirmed this by restriction analysis of pPH4). Indeed, the restriction map of the aerolysin structural gene in Fig. 2 differs in several other respects from the map reported by Chakraborty et al. (1). This may mean either that the aerolysin genes of the two isolates of *A. hydrophila* have diverged enormously, or that two different hemolysins have been cloned. *A. hydrophila* is known to release a weakly hemolytic phospholipase (14), and it may produce another cytolysin (21).

The experimentally determined amino acid composition of purified aerolysin (Table 1) is very similar to the composition calculated assuming that the site of activation is between Arg-442 and Leu-443. This site was located by digesting the mature toxin with carboxypeptidase Y (12) and determining the amino acids released from the C terminus (not shown here). The nucleotide sequence predicts five cyanogen bromide fragments in both aerolysin and proaerolysin, including an unusually large fragment of 27,900 molecular weight, in good agreement with our experimental observations (9).

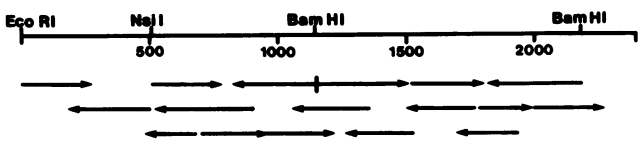

FIG. 1. Strategy for sequencing the aerolysin gene. Arrows represent sequences obtained by using fragments produced by restriction enzyme digestion at the sites indicated or sequences obtained using synthetic oligonucleotides as primers.

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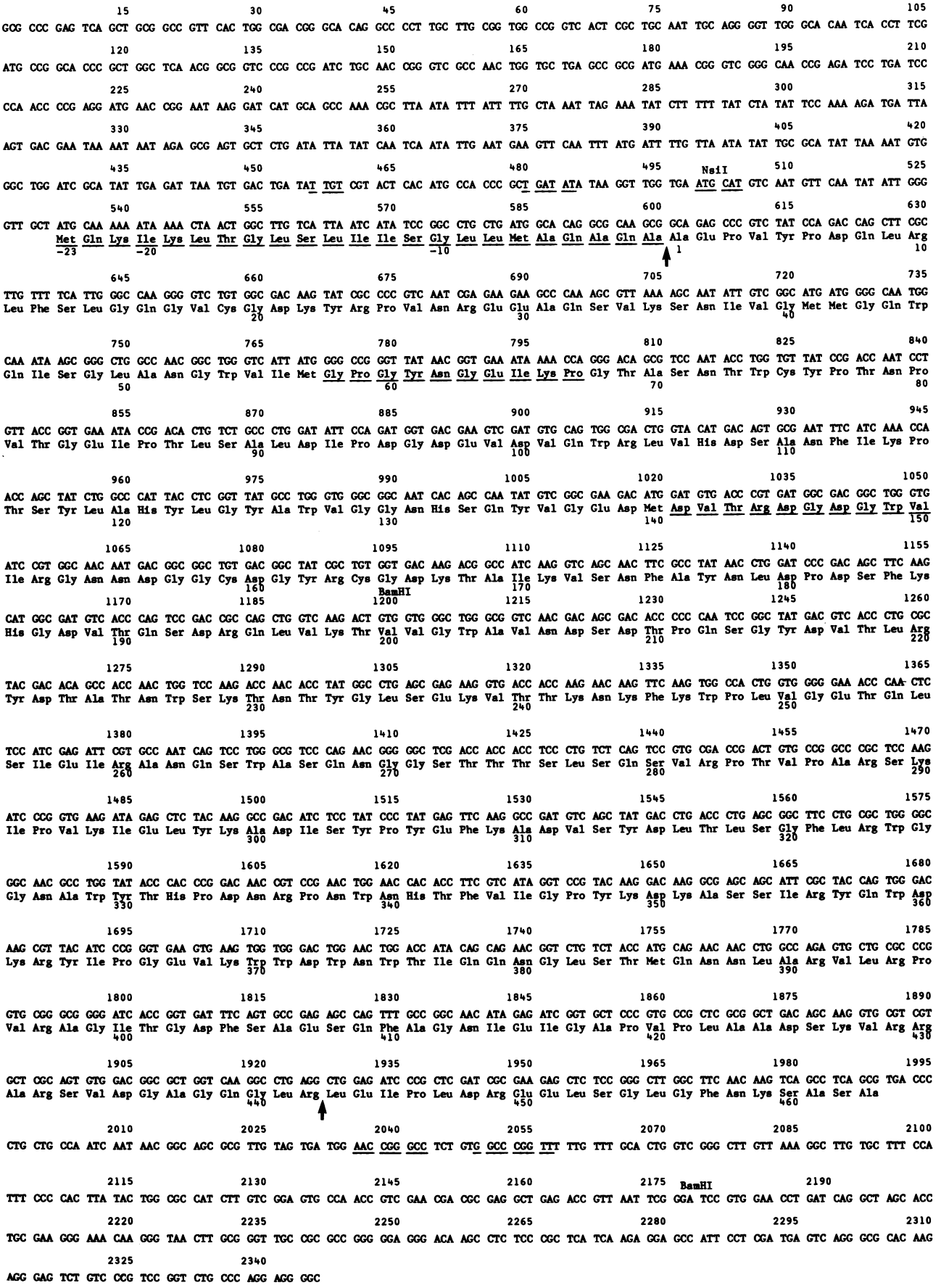

FIG. 2. DNA sequence of the aerolysin gene. Sequences which could be part of a promoter region and a Rho-independent terminator are underlined, as is the amino acid sequence of the signal peptide. The experimentally determined N-terminal amino acid sequences of two cyanogen bromide fragments are also underlined. Sites of proteolytic processing of preproaerolysin and proaerolysin are marked with arrows.

TABLE 1. Comparison of the amino acid composition of aerolysin derived from the DNA sequence with that obtained by analysis of the purified protein

Amino acid(s)	Composition (mol/mol of protein) determined by:	
	Derivation[a]	Protein analysis[b]
Asp + Asn	59	63
Thr	29	29
Ser	34	33
Glu + Gln	35	39
Pro	23	23
Gly	44	41
Ala	28	26
Cys	4	ND
Val	34	33
Met	5	5
Ile	21	21
Leu	25	24
Tyr	21	21
Phe	10	10
His	6	6
Lys	22	23
Arg	24	20
Trp	18	ND

[a] These calculations are based on the assumption that proaerolysin is processed at Arg-442.
[b] Average of two determinations. ND, Not determined.

	223	232
Aerolysin	**Thr.Ala.Thr.Asn.Trp.Ser.Lys.Thr.Asn.Thr.**	
Alpha toxin	**Thr.Ser.Thr.Asn.Trp.Lys.Gly.Thr.Asn.Thr.**	
	302	311

FIG. 3. Comparison of regions of potential sequence homology between aerolysin and alpha-toxin.

Analysis of the predicted amino acid sequence (2, 11) indicates that the protein is remarkably hydrophilic [average (H)19 hydropathy of −0.57] with no obvious hydrophobic stretches long enough to span a membrane. Because aerolysin forms holes in biological membranes, this is at first surprising; however, the pore-forming outer membrane porins also lack hydrophobic sequences (16). Like these proteins, aerolysin may aggregate to accomplish hole formation. It should also be noted that *S. aureus* alpha-toxin is even more hydrophilic than aerolysin (4).

A search of the National Biomedical Research Foundation protein sequence library (13) did not reveal any sequences homologous to aerolysin, but a direct comparison of aerolysin and alpha-toxin (4) uncovered similar strings of 10 amino acids in each protein. Seven of the 10 are identical, including a Trp in the middle of each sequence (Fig. 3). Another position is occupied by Ser in one protein and homologous Ala in the other. Because the two toxins are functionally so similar, it is tempting to speculate that this small area of similarity is significant.

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