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Research of *msh* Locus of the Bundle Forming Pilus (Bfp) of *Aeromonas Veronii* bv Sobria

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Abstract

Sequencing *msh* locus of the bundle forming pilus (Bfp) of *Aeromonas veronii* bv sobria and characterization of *mshA* promoter region for further research. A direct genomic sequencing and genomic walking were performed to sequence Bfp. PCR, PCR screen, restriction reaction, and agarose gel electrophoresis were attempted to construct a reporter gene *gus*A contained plasmid. We extended the sequence by 3.4 kb. Some encoded proteins and a promoter between *mshB* gene and *mshA* gene was detected. Work with promoter region did not succeed. The sequence got so far still contained some errors, though 15 encoded proteins and a promoter were found. But the reason that the promoter region project did not work was unknown.

Keywords: *Aeromonas*, Bfp, *mshB*, *mshA*

The genus *Aeromonas* was taxonomically in the family *Vibrionaceae*. However now it has its own family *Aeromondaceae*. To date, only seven of named species have been associated with human disease including: *A. hydrophila, A. veronii biovar sobria, A. caviae, A. veronii biovar veronii, A.jandaei, A. schubertii and A. trota* (Martin-Carnahan, 2005).These purified pili represent a family of typeIV pili(Barnett, 1997). Research of *msh* locus of the bundle forming pilus (Bfp) of *Aeromonas veronii* bv sobria is very rare around the world. Sequencing the genes encoding Bfp from a diarrhoeal isolate of *Aeromona*s *veronii* biovar sobria (strain BC88) paved the way to a further work on how it works.

1. Materials and methods

1.1 Sequencing

1.1.1 Primers

A direct genomic sequencing was originally performed, using a primer designed on the basis of the nucleotide sequence corresponding to the N-terminal amino-acid sequence previous reported by Kirov etal (Kirov & Sanderson, 1996)

-MSHAF2:

5'CTGGTTATCGTGATCATCATTCTG3'

-MSHAR2:

5'CACACGATACGACCACCGTTAGAT3'

-MshORev2:

GGCGACACAGCGGCCATATT3'

1.1.2 Chromosomal extraction

Bacterial strains were grown in BHIB at 37℃ overnight. Then the cells were centrifuged (3,000RPM=1614×g, 15min, room temperature) and resuspended in solution A containing lysozyme. The mixture was incubated at 37℃ for 30 min, and then frozen at -80℃ for 10 min. Solution B was added to the cell suspension, and the tube was inverted till the mixture became clear and viscous. RNAse was added, mixed and incubated at 37℃ for 30 min. An equal volume of phenol was then added to the mixture. The layers were separated by centrifugation (13,200RPM=16100×g, 5min, room

temperature). The upper layer was transferred to a new tube. The same process was repeated for two further times. Precipitation of DNA was done by the addition of sodium acetate and ice-cold absolute ethanol. The mixture was kept for 30 min in -20℃, then centrifuged (13,200RPM=16100×g, 20min, 4℃). Finally the pellet was resuspended in sterile distilled water and left overnight at 4℃, before to be stored at -20℃.

Slution A	Slution B
-10 mM Tris-HCl, PH7.2	-100 mM Tris-HCl, PH8.8
-150 mM NaCl	-1% SDS
-100 mM EDTA	-100 mM NaCL

1.1.3 Sequencing machine and software

Perkin Elmer ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit and AmpliTaq^{r}DNA polymerase were used for performing DNA sequencing. This was then sequenced by a Perkin Elmer ABI PRISM 377 DNA Sequencer. Routine sequence manipulations were carried out using the Gene Jockey II package. Sequence comparisons were carried out using different sequence analysis programmers such as BLASTN, ORF finder,BLASTX.

1.2 Plasmid

1.2.1 Amplify promoter region of *mshA* fragment

Primers MSHAKFOR1 and XYLMSHAR, and Pfx were used.

-MSHAKFOR1

5'CGATACCCAGATCAGCAACGGCGA 3'

-XYLMSHAR

5'CTTACGAGCAGGATCCTGCAGATT 3'

1.2.2 Insert the amplified fragment into plasmid pGJH-Tp*gusA*

Ligate the fragment with *SmaI* cut plasmid pGJH-Tp*gusA.* This new plasmid was designated pGJH-Tp*gusA3.*

1.2.3 Plasmid pUC19 was used for constructing the plasmid pGJH-Tp*gusA3* with right direction. Ligate the fragment with *SmaI* cut plasmid pUC19, and then use *PstI* to choose the new plasmid pUC19PCR3 with right direction. The chosen pUC19, PCR3 and plasmid pGJH-Tp*gusA* were ligated after being both cut with *SacI/Xbal.*

2. Results

2.1 Bfp sequencing

The sequencing result showed we found m*shB* gene, *mshA* gene, *mshC* gene *mshD* gene and part of *mshO* gene in *A.veronii* bv sobria.

<Figure 1>

<Table 1>

2.2 Construction of plasmid pGJH-TpgusA

<Figure 2>

<Figure 3>

3. Discussions

The *mshB, A, C, D* genes are thought to be in an operon with a promoter upstream of *mshB* by research of *msh* locus of close related bacterial (Mattick, 2002). Prokaryotic promoter predictions by BCM launcher demonstrated the possibility of a promoter between *mshB* gene and *mshA* gene at nucleotides 1022 and 1067 with a core value of 0.97. Type IV pilus always have the major gene beside the promoter region, and the *mshA* gene usually is the major gene in Bfp locus (Kirov, 1996). All these demonstrate there is a really promoter between *mshB* gene and *mshA* gene. Many of the genes located upstream of the *mshA* pilin subunit gene including *mshB* gene encode homologs of general secretory pathway components. The 15 encoded proteins got in this research have a very important value for understanding how *msh* locus of the Bfp of *Aeromonas veronii* bv sobria causes human desease. We have got 2.3kb long sequence of Bfp of *Aeromonas veronii* bv sobria so far. However it is not good enough to fully understand its translation and regulation mechanism, so further work could be done later.

In order to determine under what conditions the Bfp is expressed in addition to finding what proteins are involved in its express. Construction of transcriptional fusions of the *mshA* promoter region to the reporter gene *gusA* was attempted. Two methods were applied to the construction, but both failed. Firstly, we inserted the amplified promoter region directionally into report gene involved plasmid. PCR screen showed a positive result, but nucleotide sequencing found

the *mshA* promoter region was in inverse direction later. After unsuccessful numerous attempts, another method was used. We got a pUC19PCR3 plasmid that had correct *mshA* promoter region orientation. Clones with the correct orientation of the promoter region was cut with *SacI/XbaI* releasing the fragment that could be directionally cloned into *SacI/XbaI* digested pGJH-Tp*gusA*. But we eventually did not get correct plasmid. We guess such an event could be selected for if the gene product was toxic to cell, an inversion occurred more times. We could use another plasmid afterwards for an idea result.

4. Acknowledgements

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References

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Martin-Carnahan, A . (2005). *Aeromonas infections*.

Mattick, J.S. (2002). Type 4 Pili and Twitching Motility. *Annu. Rev. Microbiol*, 56, 289-314.

Table 1. 15 encoded proteins in *A.veronii* bv sobria analyzed by BLAST

Number of RF	**Encoded proteins**	**Length**	**Amino acid No**	**Molecluar**
1	Urocanate hydratase	568	138AA	14.3K Da
2	Putative MSHA biogenesis protein MshH	647	652AA	74.3KDa
3	MSHA biogenesis protein MshL	560	575AA	62.0KDa
4	ATPase	571	569AA	63.2KDa
5	Histidine ammonialyase	510	510AA	54.0KDa
6	MSHA biogenesis protein MshI	491	287AA	31.2KDa
7	Tetratricopeptide repeat family protein	381	365AA	39.9KDa
8	MshO	256	273AA	29.2KDa
9	Actin-like ATPase	347	346AA	37.0KDa
10	Rod shape-determing protein MreC	286	301AA	32.9KDa
11	MSHA biogenesis protein MshM	310	296AA	33.6KDa
12	MSHA biogenesis protein MshG	406	406AA	45.0KDa
13	Protein VCA0101	518	528AA	59.9KDa
14	Calcium/proton antiporter	365	365AA	39.6KDa
15	COG2515:1-aminocyclopropane-1-carboxylate deaminase	302	315AA	34.0KDa

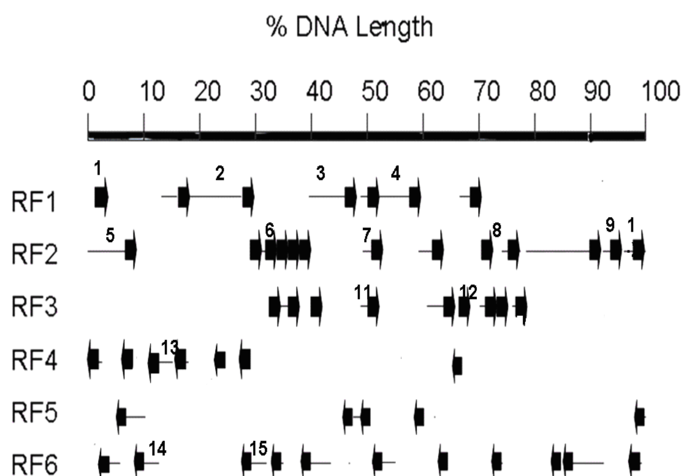
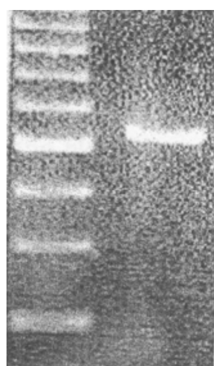


Figure 1. ORFs of A.veronii bv sobria by using ORF finder and Gene Jockey II package. No 1-15 were detected to be capable of encoding proteins



M pGJH-Tp*gusA*3

Figure 2. Agarose (8%) gel electrophoresis analysis of pGJH-TpgusA3 that was direct constructed with pGJH-TpgusA and promoter contained fragment. M was Supercoild DNA ladder. The fragment in the pGJH-TpgusA3 was in wrong direction

M 1 2 3 3

Figure 3. Agarose (8%) gel electrophoresis analysis of the pUC19PCR3 plasmid. M was Supercoild DNA ladder. 1 and 2 were plasmid pUC19 and 3 were pUC19PCR3. The band showed pUC19PCR3 had the right weight