Area: Microbial Pathogenesis

Microorganism: *Aeromonas trota*

Reference: Takahashi, E., Ozaki, H, Fujii, Y., Kobayashi, H., Yamanaka, H., Arimoto, S,. Negishi T., and Okamoto, K. 2014. Properties of hemolysin and protease produced by *Aeromonas trota*. PloS One 9(3):e91149. doi: 10.1371/journal.pone.0091149.

Bacteria of the genus *Aeromonas* are commonly found in aquatic habitats including fresh water, brackish water, and seawater. Many species can cause wound infections or sporadic diarrhea. *A. trota*, which has been previously called *A. enteropelogenes*, is a mesophilic species that is ampicillin sensitive but difficult to treat because is forms an enzyme called a β-lactamase that can degrade this antibiotic. *A. trota* was previously shown to cause diarrhea in an animal model. Takahashi et al. (PloS One 9(3):e91149, 2014) described the properties of a hemolytic protein (hemolysin) and a protease from this microorganism.

Several strains of *A. trota* were isolated from patients with diarrhea or from environmental water and soil. A type strain (ATCC 49657) was also obtained from the American Type Culture Collection. The bacteria were grown in nutrient broth medium at 37oC with aeration.

1. What are the characteristics of diarrhea as a clinical disease of humans?

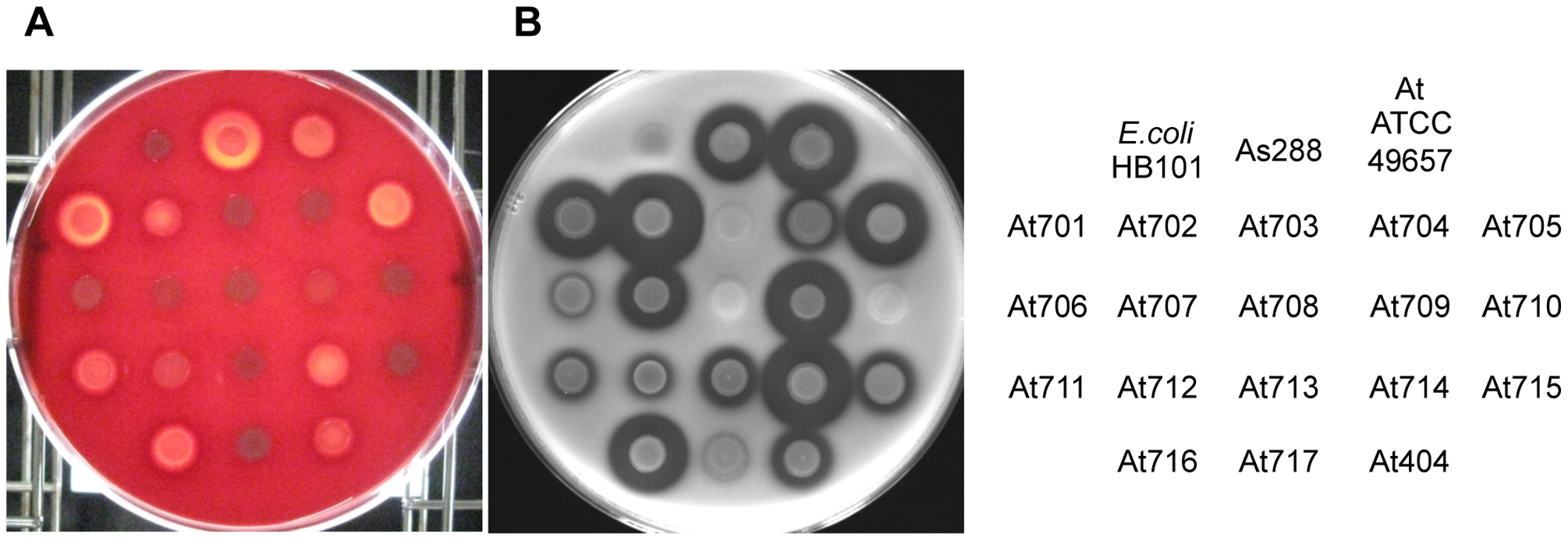
a. the appearance of small red pustules on the surface of the skin.

b. the occurrence of loose, watery, and more frequenct bowel movements.

c. the sudden onset of menory loss or mental confusion.

d. the presence of muscle tremors.

To test for the ability of *A. trota* to form proteins that might lyse red blood cells or breakdown proteins, various strains were grown in liquid nutrient broth for 20 hours and 2 µl portions spotted onto nutrient agar plates supplemented with either 5% sheep erythrocytes or 1% skim milk. The plates were incubated at 37oC for 24 hours the presence or absence of areas of clearing observed around the bacterial spots. *E. coli* HB101 and *A. sobria* 288 were used as controls. The results are shown in the next figure.



2. Look first at the three controls at the top of the plates. What can you see?

a. HB101, As288, and At49657 all formed distinct zones of clearing on both types of medium.

b. As288 and At49657 both formed distinct zones of clearing on both types of medium.

c. As288 formed distinct zones of clearing on both types of medium but At49657 only showed clearing on the plate with red blood cells.

d. As288 formed distinct zones of clearing on both types of medium but At49657 only showed distinct clearing on the plate with skim milk.

3. Now look at the look the spots for the various *A. trota* isolates. What can you conclude from these data?

a. All of the isolates formed distinct zones of clearing on both types of medium.

b. None of the isolates formed distinct zones of clearing on both types of medium.

c. At701 formed distinct zones of clearing on both types of medium but At708 showed no zones of clearing.

d. A7707 formed a distinct zones of clearing on the blood agar plate but no clearing on the skim milk plate.

To determine if the proteins causing hemolysis could be formed and released when the bacteria were grown in liquid, medium, *A. trota* 701, *A. trota* 49657, and *A. sobria* 288 were cultivated in nutrient broth at 37oC. Samples were removed at 6, 12, and 24 hours and centrifuged at 15,000 x g for 5 min. The supernatant (culture fluid) was decanted and saved. The cell pellet was suspended in buffer and the bacteria disrupted by sonication. Each sample was then tested for hemolytic activity with the results shown in the next figure.

Chart, histogram

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4. What can you conclude from these data?

a. *A. sobria* 288 forms a hemolysin that is released into the medium early during growth but it is not retained in the cells.

b. *A. trota* 49657 forms a hemolysin that is released into the medium early during growth but it is not retained in the cells.

c. *A. trota* 49657 and *A. trota* 701 form more hemolysins when it is grown in liquid medium than when they are grown on agar plates.

d. *A. trota* 701 forms more hemolysis than *A. trota* 49657 when it is grown in liquid medium.

To compare the genes for the hemolysins, the *alh* genes from *A. sobria*, *A. trota* 49657, and *A. trota* 701 were amplified by PCR and sequenced. The genes from *A. trota* 49657 and *A. trota* 701 showed 61.9% sequence identity and 93% sequence similarity to that of *A. sobria*.

5. What does the observation contribute to this analysis?

a. It does contribute at all.

b. It suggests that amino acid replacements in the *A. trota* protein have led to a decrease in activity.

c. It suggests that amino acid replacements in the A. trota protein have prevented its release from the cells.

d. It suggests that amino acids are not important in determining the activity of the protein.

To determine if the hemolysin from *A. trota* 701 has toxin activity, suspensions of the bacteria containing a total of 3 x 107 cells mixed with either preimmune serum or serum containing hemolysin antibodies were injected into loops of mouse intestine. The mice were killed after 3 hours and the weights of the intestinal loops determined. The results are shown in the next figure. The open bars show samples treated with preimmune serum and the filled bar show samples treated with hemolysin antibodies.

Chart, bar chart, histogram

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6. What can you conclude from these data?

a. The protein from *A. trota* 701 in an enterotoxin.

b. The protein from *A. trota* 701 not an enterotoxin but the antibodies against it are.

c. The protein from *A. trota* 701 is an enterotoxin but only when combined with the antibodies against it

d. The mouse model is not a valid test for a toxin.

Takahashi et al. then followed up on the experiment with skim milk agar by testing for the presence of a gene coding for a serine protease. The various *A. trota* isolates, the control strains used before, and another control called *A. hydrophila* 453 were grown on nutrient agar plates at 37oC foer 24 hours. The bacteria were then transferred to a Hybond-N nylon membrane, lysed, and the DNA denatured. The spots were then process and tested using synthetic probes specific to serine protease and metalloprotease genes of *A. sobria*. Hybridization was observed by a chemical assay. The results are shown in the next figure.

Chart, scatter chart

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7. What does these data indicate about the presence of the serine protease and metalloprotease genes in these bacteria?

a. *E. coli* and *A. sobria* contain both genes.

b. None of the *Aeromonas* strains contain both genes.

c. All of the *A. trota* isolates contain both genes.

d. 17 of the 19 *A. trota* isolates and the ATCC 49657 strain contain a serine protease gene but not a metalloprotease gene.

When Takahashi et al. attempted to find the serine protease activity in the liquid cultures of *A. trota* using antibodies against the protein from *A. sobria*, they could not find it either in the cultures supernatants or the cell lysates. They also could not detect a protease activity use azocasein (a colored polymer) as a substrate.

8. Since the many of the *A. trota* strains did show good zones of clearing on skim milk agar plates, what might you want to study next?

a. the genomes of the bacteria in liquid cultures and solid media.

b. the transcription of the hemolysis and protease genes in liquid cultures and solid media.

c. the synthesis of the hemolysis and protease activities in liquid cultures and solid media.

d. the stability of the hemolysis and protease activities in liquid cultures and solid media.

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