Chemotaxis of *Vibrio cholerae*

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Abstract

The purpose of this project is to study which methyl-accepting chemotaxis proteins (MCPs) are related to chemotaxis of *Vibrio cholerae.* 44 MCP mutant strains were tested in seven different chemoattractants and the resulting swarm circle size was compared to that of C6706 lac-Z wildtype. Several genes were identified that resulted in a significantly different phenotype compared to the wildtype, suggesting that these genes are involved in sensing and motility in response to these chemicals. Some of the genes, specifically ΔVC 1405, ΔVC 1406, and ΔVCA 1031, were involved in movement in several of the chemoattractants, suggesting that these genes may be related to general motility of *V. cholerae*.

Introduction

Vibrio cholerae is a human intestinal pathogen that has been responsible for several pandemics and continues to cause around 100,000 deaths annually. The expression of its cholera toxin has been shown to be related to its chemotaxis activity (i.e. the controlled movement toward or away from specific chemicals).[2] Chemotaxis in *V. cholerae* is expressed through the use of methyl-accepting chemotaxis proteins (MCPs) on its surface. These proteins are related to the sensors that sense different chemoattractants and send signals to the flagellum to swim towards or away from that chemical. It is through methylation of these methyl-accepting chemotaxis proteins that the bacteria know whether they are going away or towards a chemical. Relatively little is known about MCPs in *V. cholerae*; very few have been characterized. This project aims to study how these proteins are related to chemical sensing, and characterize several MCP mutant strains in various chemoattractants. It is worth noting that *V. cholerae* has 2 chromosomes and it has MCP genes in both of them (the VC and VCA chromosomes, see figure 2).

Methods

45 MCP mutant strains were previously created. This was done by disrupting the genes that punitively make the MCPs and then growing them and comparing them to the wildtype.

Bacterial culture medium:

- LB medium
- Antibiotics streptomycin and kanamycin added as needed
- Grown at 37ºC

Chemotaxis assay medium:

- M9 soft-agar minimal medium (0.3% agar, pH 7.4)
- Succinate carbon source
- Chemoattractants added: serine, arginine, glutamic acid, asparagine, N-acetylglucosamine (NAG), mucin, and bile

V. cholerae C6706 wildtype and MCP mutant strains were inoculated on chemotaxis assay media to compare swarm circle size (Figure 3). Swarm circle diameter was measured, and data was analyzed using various statistical techniques, including standard deviation and paired t-tests.

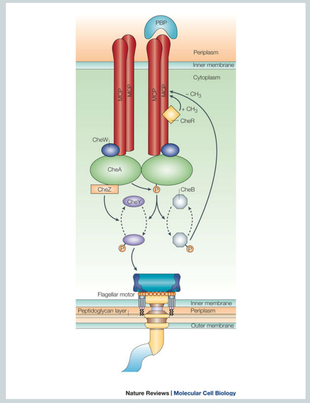

Figure 1. MCP cell signaling pathway

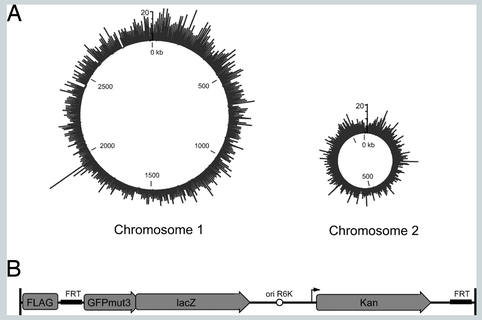

Figure 2. Chromosomes VC and VCA of *V. cholerae*

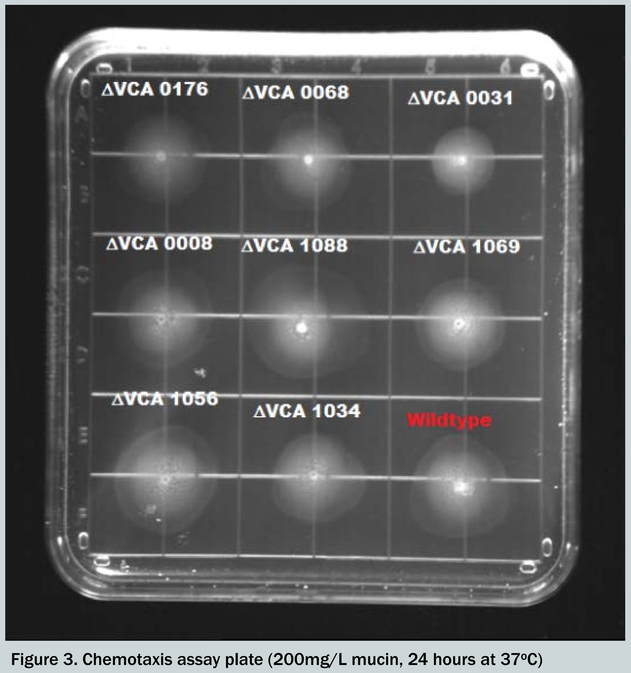

Figure 3. Chemotaxis assay plate (200mg/L mucin, 24 hours at 37ºC)

Results

The following mutant strains were shown to have statistically different phenotypes (p-value of <0.05) in the specified chemoattractant when compared to the wildtype:

Serine	Arginine	Glutamic Acid	NAG	Mucin	Bile
• ΔVC 0216	• ΔVC 1405	• ΔVC 0449	• ΔVC 1248	• ΔVC 1405	• ΔVC 0825
• ΔVC 0825	• ΔVC 1406	• ΔVC 1394	• ΔVC 1406	• ΔVC 1967	• ΔVC 1405
• ΔVC 1289	• ΔVC 1898	• ΔVC 1403	• ΔVC 1643	• ΔVCA 0658	• ΔVC 1406
• ΔVC 1405	• ΔVC 1967	• ΔVC 1405	• ΔVCA 1031	• ΔVCA 1031	• ΔVC 1967
• ΔVC 1406	• ΔVCA 0979	• ΔVC 1406	• ΔVCA 1056		• ΔVCA 1031
• ΔVC 2161	• ΔVCA 0988	• ΔVC 1535			
• ΔVC 2439	• ΔVCA 1056	• ΔVC 1868			
• ΔVCA 0031		• ΔVC 1967			
• ΔVCA 0906		• ΔVC 2439			
• ΔVCA 1031		• ΔVCA 0068			
		• ΔVCA 0176			
		• ΔVCA 0268			
		• ΔVCA 0906			
		• ΔVCA 0974			
		• ΔVCA 1034			
		• ΔVCA 1088			

Discussion

Clearly, several different genes are involved in the expression of MCPs that allow the organism to sense various chemicals in its environment. A few genes stood out in the sense that they showed statistically different phenotypes in several of the different chemoattractants that were tested: specifically, ΔVC 1405, ΔVC 1406, and ΔVCA 1031. Interestingly, ΔVC 1405 was tested in M9 media without any added chemoattractants, and using two different carbon sources, and still showed a significantly different phenotype in each case. This may suggest that this gene is involved in general motility rather than sensing one specific chemical.

Literature Cited

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