Cryo electron tomography studies of bacterial chemosensory arrays

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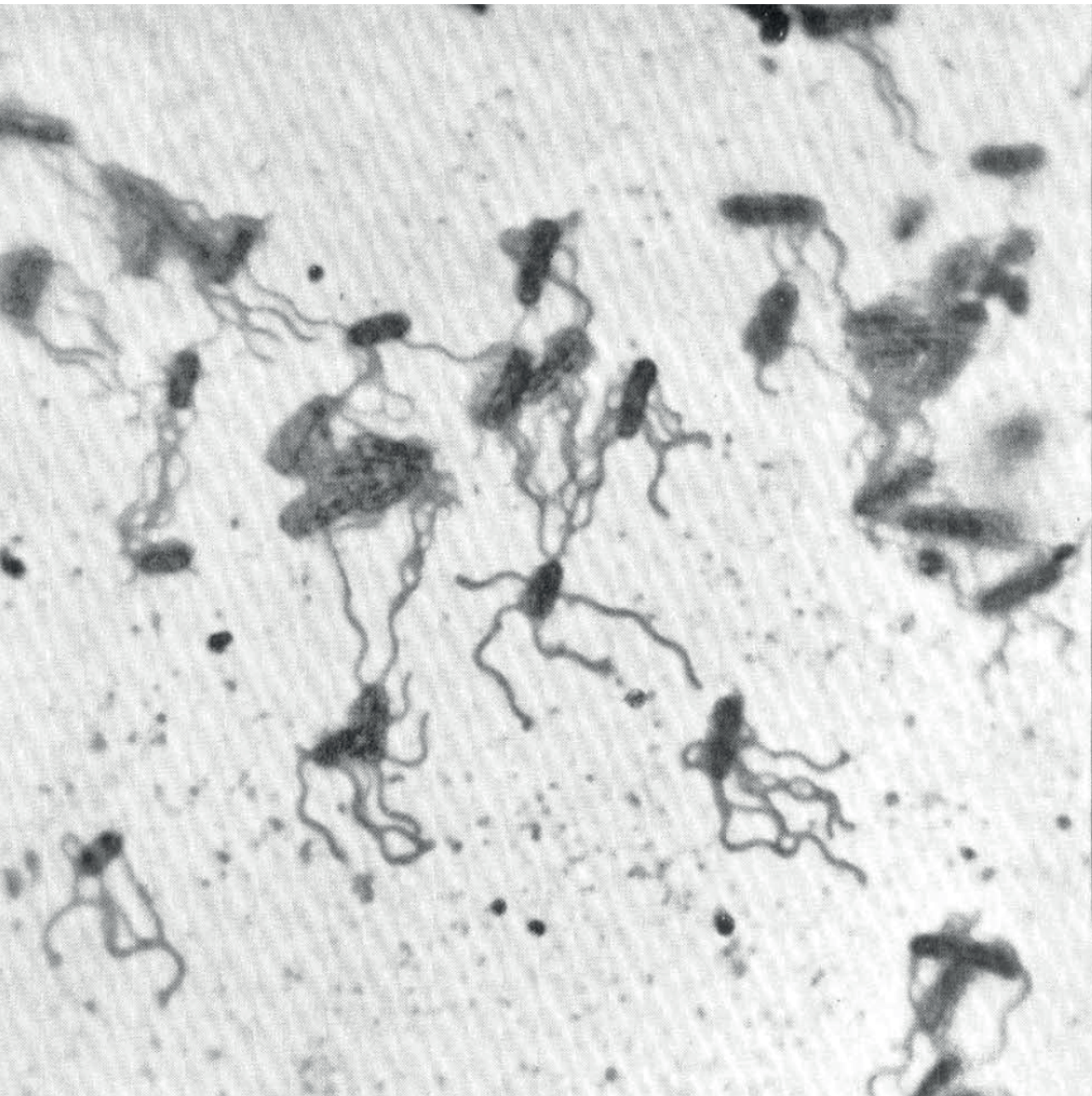
CHAPTER 1



General Introdution

Across all domains of life, seeking favorable environmental conditions is a common behavior. Even the simplest life forms, such as motile bacteria, are capable of detecting the chemicals in their immediate surroundings. They are able to control their movement toward increasing concentrations of beneficial attractants and away from deleterious toxins. This behavior, termed bacterial chemotaxis, has been intensively studied ever since it was first reported back in the late 19th century (1). Chemotaxis was first observed in the organism *Escherichia coli* (*E. coli*). The cells formed visible bands in capillary tubes or rings on agar plates to follow a concentration gradient of nutrients and oxygen (2-4) (Fig. 1). This observation begged the question of how the cells are able to perform this behavior. Decades of extensive research on this topic made the chemotaxis system the best-understood signaling pathway in biology today.

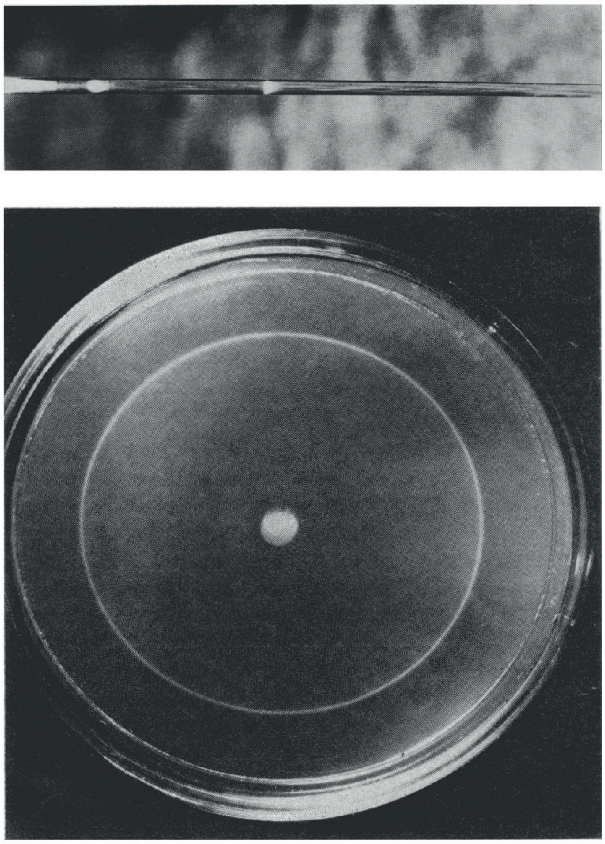


Figure 1. Chemotaxis behavior discovered in *E. coli*. Top panel shows a capillary tube filled with liquid medium. *E. coli* inoculated at the left end prorogate toward the right end and clustered into the bands shown in the capillary tube. Such clustering on a galactose agar plate with *E. coli* inoculated at the center develops as ring pattern shown in the bottom panel. Both pictures are taken from reference (2) .

Chemotaxis allows motile bacteria to modulate their swimming trajectory, which was documented in light microscopy studies in the '70s (5). The swimming trajectory of *E. coli* in an isotropic chemical environment is a random walk consisting of smooth swimming (termed "runs") and intervals (termed "tumbles") where the cells pause and change the direction of swimming. Such random walks can be readily biased by the presence of chemical gradients, in which case the runs lengthen and the tumbles become less frequent. Numerous studies over the past fifty years, gradually unraveled a sophisticated system that is capable of recognizing and responding to certain chemical compounds (6). Indeed, *E. coli* cells are necessarily equipped with complex macromolecular machinery specifically evolved for such a chemotaxis system to function. A detailed introduction to chemotaxis and the intricacies of the chemotaxis system can be found in **Chapter 2.**

In *E. coli*, the chemosensory pathway controls the flagella, which are long filaments that extend outside the cell. They are readily observed and their motor apparatus was first described in the early '70s (11) (Fig. 2A). Additional flagellar components, such as the hook and basal body complexes that anchor the flagellum to the cell wall envelope were also observed in purified samples (8, 9, 12) (Fig. 2B,C). Structural, biochemical and behavior studies collectively reveal that flagellar rotation is powered by a rotary motor (13-15). The 3D structural depiction of intact flagella motors was later achieved via single particle cryo-electron microscopy (cryo-EM) analysis (Fig. 2D) (10, 16, 17). Even today, the flagellar motor remains one of the most popular subjects for structural studies in cryo-EM (18-21).

In contrast, the sensing part of chemotaxis, which was believed to happen inside the cell, remained only accessible for probing through genetic and biochemical assays. The location and appearance of a chemosensory apparatus had remained elusive for a long time. In fact, the idea that a macromolecular machine underlies the chemotaxis system was not hypothesized until the '90s (22), after the polar clustering of chemotaxis proteins was first confirmed by immune electron microscopy in *E. coli* (23).

Despite the lack of direct structural information, the understanding of the molecular mechanism of chemosensory in *E. coli* progressed rapidly. The attractants and repellents that *E. coli* is capable of sensing in the environment were identified (24-26). Subsequently, mutant strains with chemotactic deficiency were used to identify the *che* (chemotaxis) genes and their respective Che proteins (27-29). Shortly after, the sensing capabilities of specific chemoreceptors, called methyl-accepting chemotaxis proteins (MCPs), were identified (6, 30, 31). The methylation state of the MCPs was then shown to provide a means for modulating the sensitivity towards the ligands, which is termed sensory adaptation, to keep a temporal record of ligand concentration (32-34). A decade later, phosphorylation of a messenger protein was identified as the signaling

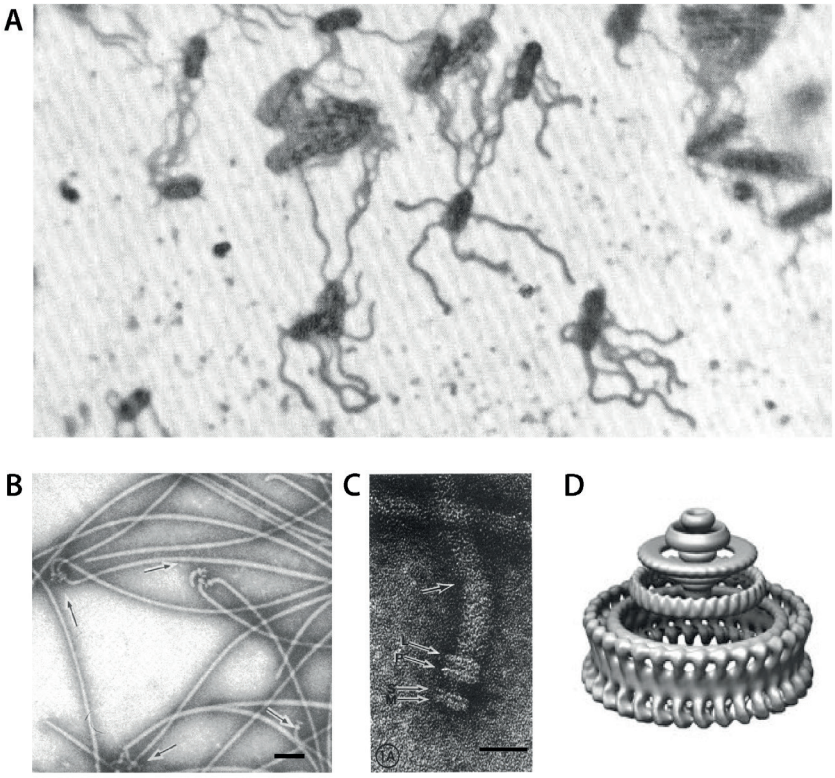


Figure 2. Bacterial flagellum and flagellar motors depicted through electron microscopy studies. (A) A negative stain image of *E. coli* cells. Image is adapted from (7). (B) A negative stain image of flagella purified from *E. coli*. Arrows point at hook-basel bodies. Scale bar is 100 nm. Image is adapted from (8). (C) A close-up negative stain image of the basal end of flagellum from *E. coli*. Arrows point to the location of rings. Scale bar is 30 nm. Panel is adapted from (9). (D) A 3D reconstruction of the isolated flagellar rotor from *Salmonella typgimurium* where the rings are resolved in detail. Image is adapted from (10).

mechanism that facilitates the communication between the chemoreceptors and the flagellar motor (35-38). In 1992, based on protein interaction studies *in vitro*, Gegner *et al.* proposed the initial model of the sensory apparatus which is a ternary protein complex consisting of MCPs, the histidine kinase CheA, and the adaptor protein CheW which physically couples the MCPs to CheA (39) (Fig. 3A). This model implied that the formation of a ternary protein complex is instrumental for proper chemotaxis.

Visualizing the chemosensory complex became necessary in order to understand the molecular mechanism underlying the system. Although it was clear where the chemotaxis proteins were located (cell poles) and what proteins comprise the system (ternary complex form by MCPs/CheW/CheA), few techniques allowed direct visualization of protein complexes inside living bacterial cells. Nevertheless, atomic-

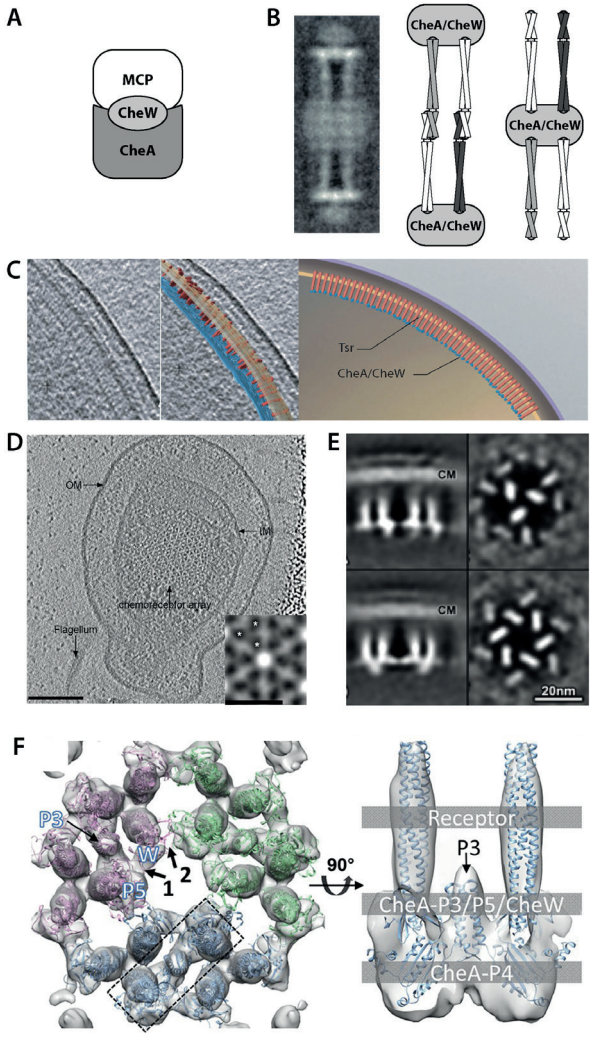


Figure 3. Models proposed for the ternary chemosensory complexes. (A) An early model proposed for receptor/CheW/CheA complex in chemotaxis pathway in reference (39). (B) Cryo-electron microscopy image of chemoreceptor-kinase complex and two different schemes describing potential molecular arrangements. Image modified from (40, 41). (C) Cryo-tomographic image of the cell pole of intact wild-type *E. coli* (left panel) and a segmented representation (middle panel) of the image, and a schematic representation of the arrays structure (right panel). Images are adapted from (42). (D) A tomographic slice of a *Salmonella enterica* minicell showing the native order of the chemoreceptor arrays in the top-view (Scale bar is 100 nm), and an inset shows the subtomogram average of the arrays (Scale bar is 12 nm). Images are adapted from (43). (E) Side-view (left column) and the top-view (right column) of subtomogram averaging results of two different kinase CheA arrangements (one in each row) of the chemosensory arrays in *E. coli*. Images are adapted from (44). (F) Density map (translucent gray) with molecular models fit within the density, with three core signaling complexes colored differently. Images are adapted from (45).

level structural information of the individual chemotaxis proteins gradually became available by X-ray crystallography (46-48). In the meanwhile, a significant amount of effort was invested into visualizing the recombinant protein complexes *in vitro* through cryo-EM (40, 41, 49). Due to limited image quality and means for analysis, the molecular architecture of the complex remained largely unknown. In fact, almost completely opposing interpretations could be derived from the same *in vitro* preparation of the ternary complex (Fig. 3B) (40, 41, 49).

The molecular arrangement of the chemosensory complex was eventually revealed by cryo-electron tomography (cryo-ET) (42, 50). This technique allows a three dimensional view of the chemosensory complexes *in vivo* preserved in a near-native state. The EM images revealed large patches of chemotaxis arrays at the cell poles. The receptors are oriented perpendicularly to the inner membrane. Their sensing domains were visible in the periplasmic space, while their other end in the cytoplasm is clearly associated with the CheA/CheW proteins that form a dense layer parallel to the inner membrane (Fig. 3C). With ever-improving resolution limits and image processing methods such as subtomogram averaging, both the hexagonal packing order of the receptors and the ordered arrangement of CheA and CheW underneath the chemoreceptor lattice were revealed (43, 44, 51, 52) (Fig. 3 D,E). Combined with atomic models from X-ray crystallography data, the molecular architecture of the chemotaxis arrays was finally solved for *E. coli*: Here, two receptor trimers-of-dimers, two monomeric CheWs, and one dimeric CheA form the signaling core unit that has also been shown to be the minimal structural unit for full modulation of CheA activity (45, 53) (Fig. 3F).

Cryo-ET provided crucial structural information for resolving the molecular architecture of chemosensory arrays. It has further provided insight to understanding the structure and function of the arrays (54). Studies of chemosensory systems in a wide variety of bacteria and archaea revealed a universal hexagonal packing order of the receptors (51, 55). Cryo-ET continues to provide new structural insights and continuously contributes to our understanding of the array assembly process and the conformational dynamics of both the receptors and the kinase CheA (54, 56-59). Cryo-ET has been an essential method to visualize this macromolecular machinery *in situ*. New developments in microscopy hardware and imaging processing software will produce even higher quality structural data and will continue to provide new insights into understanding the molecular mechanism of signal transduction and kinase activation in bacterial chemosensory (60).

Thesis outline

This thesis comprises several studies where I used cryo-ET to gain insight into the architecture and function of the chemosensory arrays. In **Chapter 2**, an in-depth review covers the basics of chemotaxis behavior, the chemotaxis signaling pathway, the chemotaxis proteins, and their arrangement in chemosensory arrays. Substantial emphasis is given to the fact that chemosensory arrays are an ingenious structure that manifests highly conservative structural features while simultaneously exhibiting a great tolerance for compositional diversity. Most strikingly, the chemoreceptors arrays observed thus far, even across far evolutionary distance, are hexagonally packed trimers-of-dimers with conserved lattice spacing. Still, great variability of chemosensory arrays exists in terms of the exact protein composition and stoichiometry, molecular architecture in the baseplate (where the receptors bind the kinase CheA and coupling proteins), the cellular localization, and the physiological relevance. More importantly, as prompted in this review, little is known about such diversified systems beyond the model organism *E. coli*, which has only one simplified chemotaxis pathway. Despite the wealth of information available today, several outstanding questions and challenges remain regarding the structure and function of the chemosensory arrays.

To gain insights into the architecture of chemosensory arrays in different bacteria, **Chapter 3** describes a study that reveals the compositional variability of chemosensory arrays in *Vibrio cholerae*. Arrays in *V. cholerae* have the potential to tailor their chemoreceptor composition in order to sense different targets. The chemotaxis proteins in the baseplate also exhibit a high degree of compositional variability, which potentially facilitates the incorporation of new receptors into the already existing chemosensory arrays. This high variability of chemosensory arrays is proposed for bacteria with a larger repertoire of chemoreceptors, more extensive auxiliary chemotaxis proteins, and multiple chemotaxis pathways. These attributes are distinctively divergent from the chemotaxis paradigm driven by studies in *E. coli*.

Aside from the compositional variability determined in **Chapter 3**, I further examined the molecular architecture of the chemosensory array baseplate in *V. cholerae* in **Chapter 4**. The hexagonal packing order of the receptor trimers-of-dimers are conserved among different species as reported previously, but the molecular composition and the architectural arrangement of chemotaxis protein in the baseplate is now considered species-specific. In *V. cholerae*, the histidine CheA exhibits an even distribution across the baseplate but without any distinctive order. Such a kinase distribution is expected to reduce the rigidity of the arrays and facilitate a more dynamic variability of chemoreceptors and baseplate components. It also raises intriguing questions about

how proper functionality of arrays and high cooperativity can be achieved when many of the receptor trimers-of-dimer units are not in direct contact with the kinase.

Chapter 5 presents a study carried out in the model organism *E. coli*. Here I explored the molecular mechanism of chemotaxis within chemosensory signaling core units, the minimal structural and functional repeats of the arrays. Through careful design and engineering, modified signaling core units are biased into either a kinase-on or kinase-off output state. The conformational dynamics of the serine chemoreceptors is visualized on a trimers-of-dimers level, and the corresponding confromational changes in CheA are observed. Such obersavtions suggests that chemoreceptors achieve kinase control through changing its packing compactness of the receptor trimers-of-dimers. This result echoes several classic theories proposed for describing the signaling mechanism in chemoreceptors. The results further suggest why the trimers-of-dimers arrangement of the receptors may be necessary for carrying out its function in the chemosensory arrays.

The chapters listed above are constructed with a focus on the scientific subject of this thesis, namely, the structural and functional studies of chemosensory arrays. **Chapter 6** shifts the focus to the primary scientific technique used throughout the studies included in this thesis, cryo-ET, which is currently the only method that permits high-resolution structural studies of extended arrays in its near-native condition. This chapter provides an overview of how to practice cryo-ET to visualize chemosensory arrays *in situ* using transmission electron microscopy in cryogenic conditions. A brief introduction of cryo-ET is included in this chapter, as well as a step-by-step description of the standard workflow including cryo specimen preparation; 2D tilt-series image acquisition, and 3D tomographic data reconstruction.

The key findings included in this thesis and their implications in a broader context are discussed in each chapter. **Chapter 7** discusses the cryo-ET technique. Particularly, the specific limitations of this technique when applied to studies of bacterial chemosensory arrays, and how improvements of this technique can lead towards an even better understanding of bacterial chemotaxis.

Overall, the studies presented in this thesis were made possible by recent technical advances in both hard- and software that increased data collection speed and achievable resolution of the cryo electron microscopy. This allowed me to achieve two major advances in our understanding of bacterial chemotaxis. While the arrangement of the chemoreceptors in highly ordered hexagonal arrays was already known, insight into the structural changes accompanying a change in receptor activation state inside the arrays had so far been lacking. Furthermore, the detailed architecture of array core components in species other than *E. coli* remained also unknown. In this thesis, I combined cryo-ET and subtomogram averaging methods to investigate the chemotaxis arrays both in the model system of *E. coli* as well as other, less well-understood systems such as Vibrio cholerae. This allowed me, for the first time, to determine conformational dynamics of the *E. coli* chemoreceptors correlated to the signaling states in situ. This study further gave insight on how the receptors function in a trimers-of-dimers packing arrangement. This discovery contributes to a fundamental understanding of why the hexagonal packing order is universal across all investigated species so far. Equally important for the chemotaxis filed were the discovery of a different stoichiometry of chemotaxis proteins and a direct visualization of kinases in situ in a non-model organism. These new insights highlight that a structural diversity of chemoreceptor arrays does exist, and that is the norm and not an exception. This so far under-appreciated structural diversity in chemosensory arrays may be essential for adapting the chemotaxis system to the changing environments and may be essential for surviving a special niche or play a crucial role in pathogenicity.