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Diversity of bacterial chemosensory systems

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Abstract

Chemosensory systems control the most complex, specialized mode of signal transduction in bacteria and archaea. They are composed of several core and auxiliary protein components that are highly organized in order to deliver a fast response to changing environmental conditions. Chemosensory pathways have been studied in-depth in a handful of model organisms and experimentally characterized at least to some degree in approximately thirty species. However, genome-wide analyses have revealed their presence in thousands of sequenced microbial genomes. Both experimental and computational studies uncovered substantial diversity in system design, functional regulation, cellular localization and phyletic distribution of chemosensory pathways. Here, we summarize advances and expose gaps in our current understanding of the diversity of chemosensory systems.

Introduction

Signal transduction pathways control cellular processes in all living organisms. Based on their component design, these pathways in bacteria and archaea can be classified into three major modes: one-component, two-component and chemosensory systems [1]. The chemosensory system (also called chemotaxis system) is a special case of two-component signal transduction. Its designation as a distinct mode is dictated by a complex system design and unique elements that are not seen in classical two-component pathways (Fig. 1). A pathway controlling chemotaxis in *Escherichia coli* is the best understood chemosensory system [2,3]. Similar to classical two-component signal transduction systems, the key components of this pathway are a histidine kinase and its cognate response regulator. The CheA histidine kinase lacks an input domain and receives signals from dedicated chemoreceptors (also called methyl-accepting chemotaxis proteins, MCPs) that share a repertoire of input domains with sensor histidine kinases of classical two-component systems. Chemoreceptors, CheA and the CheW scaffolding protein form signaling complexes that assemble into chemosensory arrays [4]. Upon changes in concentration of attractants and repellents, chemoreceptors modulate CheA activity, which ultimately controls flagellar rotation via phosphorylation of the CheY response regulator. The CheR methyltransferase and the CheB methylesterase that covalently modify MCPs by adding and removing methyl groups, correspondingly, comprise an adaptation pathway. The system

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also involves the CheZ phosphatase, which de-phosphorylates CheY leading to signal termination. Homologous systems were identified and experimentally studied in other bacterial and archaeal species, where they control not only flagellar motility, but also regulate type IV pili (Tfp) based motility, development, biosynthesis, biofilm formation, cell-cell interactions and other cellular functions.

Diversity of system design

A large-scale phylogenomic analysis of chemosensory systems, which was carried out a decade ago, uncovered their remarkable diversity [5]. Merging large volumes of published experimental data with phylogenomic clustering revealed the existence of three principal functional groups of chemosensory systems: those that evolve to control flagellar motility (Fla), Tfp-based motility (Tfp), and alternative (non-motility) cellular functions (ACF). While the Fla group appeared to be very diverse, with seventeen distinct classes (F1-F17), each of the other two groups contained a single class. The core of the chemosensory system includes four essential components (95% occurrence in genomes with at least one chemosensory system gene [5]) comprising the excitation pathway, as it is seen in *E. coli*: MCPs, CheA, CheW, and CheY. Two other core components, CheR and CheB, are dispensable (85% occurrence). For example, they are present in the Tfp system in *Pseudomonas aeruginosa* but missing from the Tfp system in cyanobacteria.

Various classes of the chemosensory system differ substantially in their repertoire of auxiliary (60% occurrence) components (Fig. 2). Many chemosensory pathways contain an additional scaffolding protein, CheV, which is a fusion of CheW and receiver (CheY-like) domains [6]. It was proposed that CheV might couple CheA with certain types of chemoreceptors that cannot be effectively accommodated by CheW, and its receiver domain might function as a phosphate sink preventing over-stimulation of CheA [7]. In *Vibrio cholerae,* a CheW homolog called ParP incorporates into the chemosensory baseplate to promote array formation and localization near the flagellar pole [8]. The diversity of scaffolding CheW homologs is still not fully understood and current automated annotations cannot distinguish between their biological functions Another common auxiliary component is the CheD deamidase that acts on MCPs to augment CheR-mediated methylation, as shown in such distantly related systems as F1 from *Bacillus subtilis* [9] and F7 (Che2) from *P. aeruginosa* [10]. The *E. coli* chemosensory system utilizes the CheZ phosphatase, however, homologous systems in many other prokaryotes do not have CheZ, but instead contain other phosphatases for signal termination [11]. For example, the *B. subtilis* system has two phosphatases: FliY, which is an integral part of flagellar motor C-ring, and CheC, which localizes with chemoreceptors [9]. Many chemotactic bacteria, including spirochetes *Treponema denticola* and *Borrelia burgdorferi*, encode yet another phosphatase, CheX, which is homologous to CheC, but unlike CheC has only one active site and does not require CheD binding [11]. Apart from modification proteins, additional components are found in some classes of the chemosensory system. A small STAS domain-containing protein, CheS, identified in *Sinorhizobium meliloti,* provides an efficient drainage of the phosphate from CheY1 increasing its binding to CheA more than 100 times [12]. A novel chemotaxis regulator, ChePep, localizes to the flagellar pole and controls swimming direction and switching of flagellar rotation in the *Helicobacter pylori* [13]. A chemosensory

pathway Che7/F13 in *Myxococcus xanthus* involves a HEAT-repeat protein, which couples aggregation and sporulation [14].

The number of individual components per system may also vary. Whereas *E. coli* has one CheY protein, the chemosensory system of the same F7 class in *S. meliloti* has two: CheY1 and CheY2. In this pathway, the level of phosphorylated CheY2 responsible for flagellar control is reset by phospho-transfer back to CheA, and then to CheY1, which plays a role of a phosphate sink [15]. The Frz/ACF pathway in *M. xanthus* contains two CheW proteins with distinct roles in signaling and chemosensory cluster formation [16]. While *Campylobacter jejuni* has one CheV protein, its close relative *H. pylori* has three CheV proteins in its single F3 chemosensory pathway [17]. Another dimension of variability in chemosensory systems is brought about by uncommon domain fusions. Although in the vast majority of chemosensory systems CheW is a single domain protein, its homolog from the F9 system in *V. cholerae* has three CheW domains [18]. The *T. denticola* F2 chemosensory pathway contains a CheW-CheR fusion protein that plays a critical role in maintaining an extremely curved chemoreceptor array [19]. The CheA protein from Che1/F5 system of *Azospirillum brasilense* Sp7 contains the N-terminal TMX domain, which anchors the kinase to the membrane [20].

Chemoreceptors are by far the most diverse components of the chemosensory system. Even their highly conserved cytoplasmic domain displays substantial diversity in the number of its helical heptads comprising several distinct classes [21] that are preferentially associated with certain classes of the chemosensory system [5,22]. Truly remarkable diversity of chemoreceptors is seen in the number of different input domains, with nearly one hundred identified to date [23]. Furthermore, the number of chemoreceptors varies dramatically between organisms and, according to our survey using the MiST database [24], ranges from one chemoreceptor per genome, e.g. in a single ACF system of *Acinetobacter baumannii*, to ninety (!) in *Caryophanon latum,* which has two chemosensory pathways. Finally, the number of chemosensory pathways per genome also varies substantially [5]. While the model organism for chemotaxis, *E. coli*, has a single chemosensory pathway, the genome of *Cystobacter fuscus* encodes twelve such pathways, according to the MiST database survey, which is the largest number of chemosensory pathways identified in a single genome to date.

Diversity of functional regulation

Chemotaxis, which was studied in different organisms, appears to be the main function controlled by chemosensory systems (Table 1). The classical F7 system in *E.coli* and F1 pathway in *B. subtilis* regulate flagellar motility through opposing mechanisms of CheA activation. Attractant binding inhibits CheA activity in *E. coli* [2], but activates it in *B. subtilis* [9], while leading to the same final result: moving towards attractants and away from repellents. Che4/F7 and Che1/F5 control two aspects of flagellar motility in *A. brasilense*: flagellar motor reversal frequency and transient increases in swimming speed, respectively [25]. Remarkably, in *Rhodobacter sphaeroides* two systems, CheOp2/F8 and CheOp3/F7, together control stop frequency and duration of a single subpolar flagellum rotation [26], while another system, CheOp1/F7, controls chemotaxis mediated by several polar flagella

[27]. In *P. aeruginosa* Che1/F6 controls flagella-driven chemotaxis [22], while Che2/F7 regulates the oxygen stress response unrelated to chemotaxis [10].

Chemosensory systems also control Tfp- based twitching motility across solid surfaces. The Tfp system (Fig. 2) modulating twitching motility is best studied in *P. aeruginosa* [28] but it was also described in other gamma-proteobacteria, including human and plant pathogens [29–31] and in cyanobacteria. Paralogous Tfp systems in *Synechocystis* sp. PCC 6803 and *Nostoc punctiforme* control not only phototaxis [32,33], but also other cellular functions related to development and biosynthesis [32,34]. Interestingly, the Tfp-based motility in *M. xanthus* is regulated by Frz and Dif chemosensory pathways [35] that belong not to Tfp, but to ACF and F1 classes, respectively. Many non-motility functions are regulated by chemosensory systems that belong to the ACF class (Fig. 2). The Che3/ACF pathway regulates formation of stress-resistant cysts in *R. centenum* [36] and the Che3/ACF pathway in the closely-related *A. brasilense* controls flocculation in liquid cultures [37]. Three ACF pathways in *M. xanthus*, namely Che4, Che5 and Che6, interact to form a large chemosensory module regulating fruiting body formation [38], while the fourth member of the ACF class in this bacterium, Che3, regulates expression of genes important for fruiting body formation [35]. In *P. aeruginosa* the Wsp/ACF chemosensory system mediates biofilm formation through modulation of c-di-GMP production [39]. Regulation of biofilm formation has recently emerged as yet another common feature of chemosensory systems [40] and in several instances non-ACF pathways were implicated in this function. For example, in *Comamonas testosteroni*, the Tfp pathway termed Flm regulates biofilm formation [41], and in *Caulobacter crescentus* two pathways that belong to F5 and F7 classes appeared to be involved in this process [42]. Intriguingly, a cross talk between systems controlling chemotaxis and biofilm formation was reported in both organisms [41, 42] as well as in a plant pathogen *P. syringae* [43]. The F5 pathway of *Azorhizobium caulinodans* that is important for plant colonization was also reported to mediate biofilm formation [44]. The exact function of some other classes of chemosensory systems remain poorly understood. The Che2/F9 pathway in *R. centenum* was found to regulate flagella biosynthesis [36]; however, the exact function of orthologous pathways in *A. brasilense* [25] and *V. cholerae* [18] awaits elucidation.

The phylogenomic classification of chemosensory systems [5] should not be taken as an absolute predictor of their function. As shown in several examples here, in some organisms, pathways that originally controlled flagellar motility might have switched their output targets to mediate other functions. During the course of evolution one chemosensory pathway may also "take over" the original function of another pathway to control the same output target [45].

Diversity of cellular localization

Typical MCPs are transmembrane and in the homodimer form assemble into trimers-of-dimers that together with CheA and CheW form functional signaling units [2,3]. A network of such units is arranged into a hexagonal array, as observed in many distantly related bacteria [46], but their cellular localization varies [4]. Chemoreceptor arrays in *E. coli*, *Magnetospirillum magneticum*, *R. sphaeroides*, *T. primitia*, *T. maritima*, and *Listeria*

monocytogenes localize to the poles of the cell. In *H. hepaticus* and *C. jejuni* the arrays are also polar, but the chemoreceptors surround the gap at the apex occupied by the flagellar motor. In *V. cholerae* and *C. crescentus* polar receptors are localized to the convex side of the crescent shaped cells. In *Acetonema longum* and *B. burgdorferi* the arrays were shown to be typically subpolar but inconsistently positioned [4].

In addition to transmembrane MCPs, many bacteria have cytoplasmic chemoreceptors [47]. In the *V. cholerae* F9 chemosensory pathway, an unusual receptor DosM containing two signaling domains forms two cytoplasmic hexagonal arrays of trimers-of-dimers sandwiched between two CheA and CheW baseplates thus stabilizing the complex [48]. Transmembrane arrays are curved as they follow the curvature of the cell membrane whereas the cytoplasmic arrays can be either flexible as seen in *R. sphaeroides* and *Methanoregula formicicum* [49] or rigid as in *V. cholerae* due to the stabilizing effect of DosM [48]. In *M. xanthus,* chemoreceptor arrays of the Frz pathway assemble at the nucleoid [16]. Their exact arrangement with CheA and CheW is unknown, but it is speculated that similarly to transmembrane receptors they may form a monolayer running parallel to the nucleoid surface.

Phyletic diversity

Chemosensory systems have been studied experimentally in approximately 30 bacterial species, according to our literature analysis. These organisms represent only eight bacterial phyla (Fig. 3) out of more than one hundred bacterial phyla defined by the latest release of the Genome Taxonomy database [50]. The majority of bacteria with experimentally studied chemosensory systems are Proteobacteria and systems from several major classes, namely F5, F6, F7, and F9, are described only for bacteria in this phylum (Table 1, Fig. 3). Overall, there is a tendency for chemosensory systems to occur most commonly in certain taxonomic groups [5]. For example, F1 system is found in Firmicutes, Thermotogota, and Archaea, but not in Proteobacteria, whereas F6 system is found exclusively in Proteobacteria. F2 and F3 systems are found in Spirochaetota and Campylobacterota, respectively (Fig. 3). The F5 system emerges as the second major chemosensory system in Alphaproteobacteria, in addition to F7 system [25, 36, 42, 44, 51]. Except for Gammaproteobacteria, the twitching motility controlling Tfp system is described only in Cyanobacteria. The F4 system has only been studied in *Desulfovibrio vulgaris* [52], and F10 and F13 systems have been explored exclusively in *M. xanthus* and their function remains largely unknown. Flagellar systems F11 (*Ruminiclostridium thermocellum*), F12 (*Geobacter uraniireducens*), and F17 (*Thermodesulfovibrio yellowstonii*) have never been studied experimentally.

Conclusions

A tremendous amount of information on bacterial chemosensory systems was generated in the past twenty years but most of the in-depth knowledge comes from a handful of model bacteria. The precise evolutionary history of these pathways is poorly understood. What was the original role of the ancestral chemosensory system: regulation of swimming motility, twitching motility or some other cellular function? What is the extent of horizontal gene transfer in chemosensory systems? Can a function of a chemosensory system be predicted

from genome information? Can efficient computational approaches be developed to distinguish CheY from other one domain response regulators and CheW from other CheW domain-containing proteins? These and other questions still await to be answered. The latest improvements in bacterial taxonomy together with comparative genomic approaches and newly generated experimental evidence should enable further advances in our understanding of function and evolution of these remarkable regulatory pathways.

Acknowledgements

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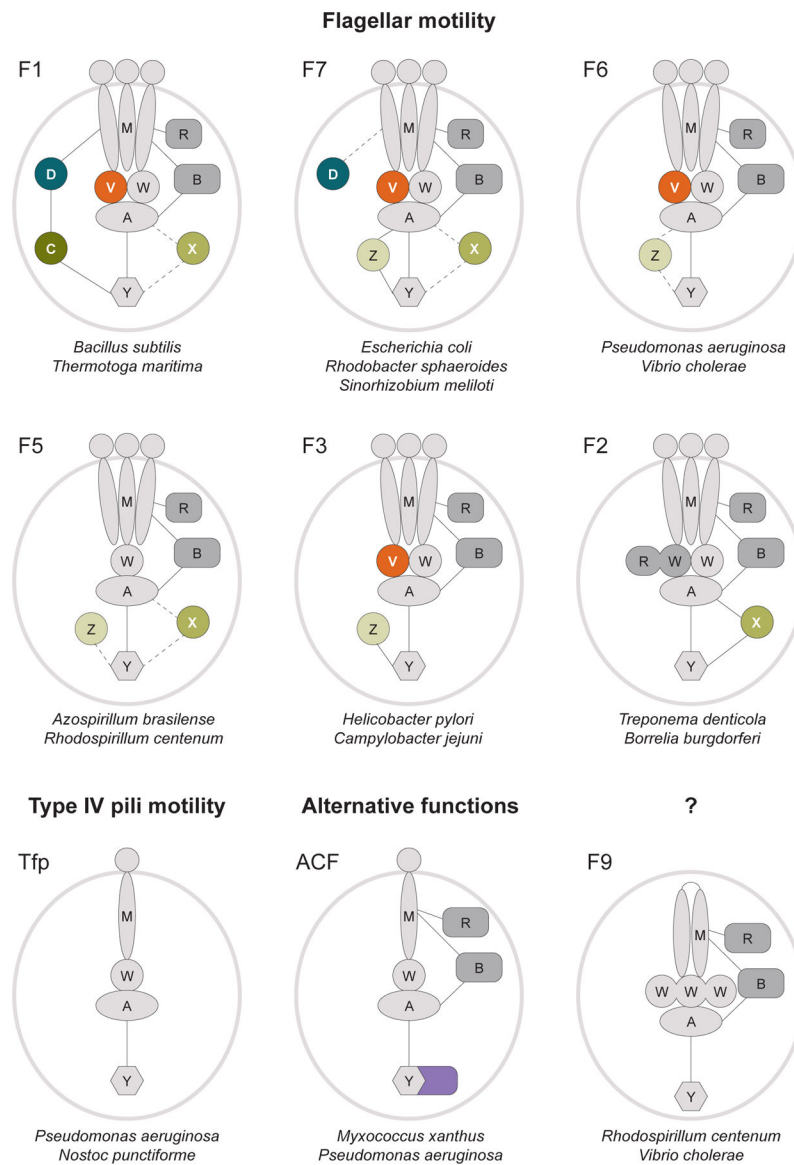
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Two-component system   **Chemosensory system**
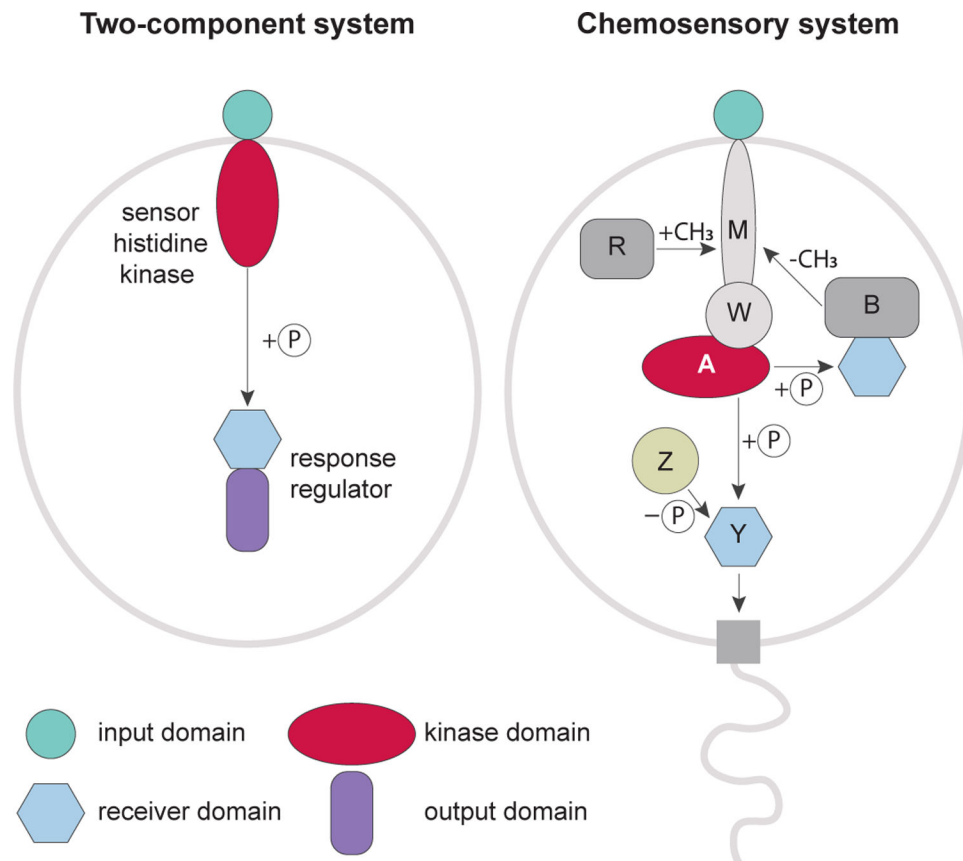
Figure 1. Chemosensory systems as complex type of two-component signal transduction.
Prototypical two-component systems consists of two proteins: a transmembrane sensor histidine kinase, which contains an input (e.g. ligand-binding) domain and a cognate response regulator, which contains an output (e.g. DNA-binding) domain. A chemosensory system of *E. coli* has the sensor-less (no input domain) histidine kinase CheA and its response regulator CheY, which lacks an output domain and directly interacts with the flagellar motor. Abbreviations: M, MCP; W, CheW; A, CheA; B, CheB; R, CheR; Y, CheY; Z, CheZ; P, a phosphate group.

Flagellar motility

F1   F7   F6

Bacillus subtilis
Thermotoga maritima

Escherichia coli
Rhodobacter sphaeroides
Sinorhizobium meliloti

Pseudomonas aeruginosa
Vibrio cholerae

F5   F3   F2

Azospirillum brasilense
Rhodospirillum centenum

Helicobacter pylori
Campylobacter jejuni

Treponema denticola
Borrelia burgdorferi

Type IV pili motility   **Alternative functions**   **?**

Tfp   ACF   F9

Pseudomonas aeruginosa
Nostoc punctiforme

Myxococcus xanthus
Pseudomonas aeruginosa

Rhodospirillum centenum
Vibrio cholerae

Figure 2. Major classes of the chemosensory system.
Model organisms in which corresponding chemosensory pathways were studied experimentally are shown for each class. Typical architectures are displayed, but protein repertoire within each class may vary. Tfp, ACF and F9 pathways typically contain a single MCP, whereas multiple MCPs are associated with all other classes. Core essential components (M, MCP; A, histidine kinase CheA; W, scaffolding protein CheW; Y, response regulator CheY) are in light grey; core dispensable components (R, methyltransferase CheR; B, methylesterase CheB) are in dark grey; the auxiliary proteins (D, deamidase CheD; V, scaffolding protein CheV; C, Z and X, phosphatases CheC, CheZ and CheX, respectively) and output domain of response regulator protein from ACF system are shown in color. Experimentally confirmed protein-protein interactions are represented by solid lines; dashed lines indicate predicted interactions.
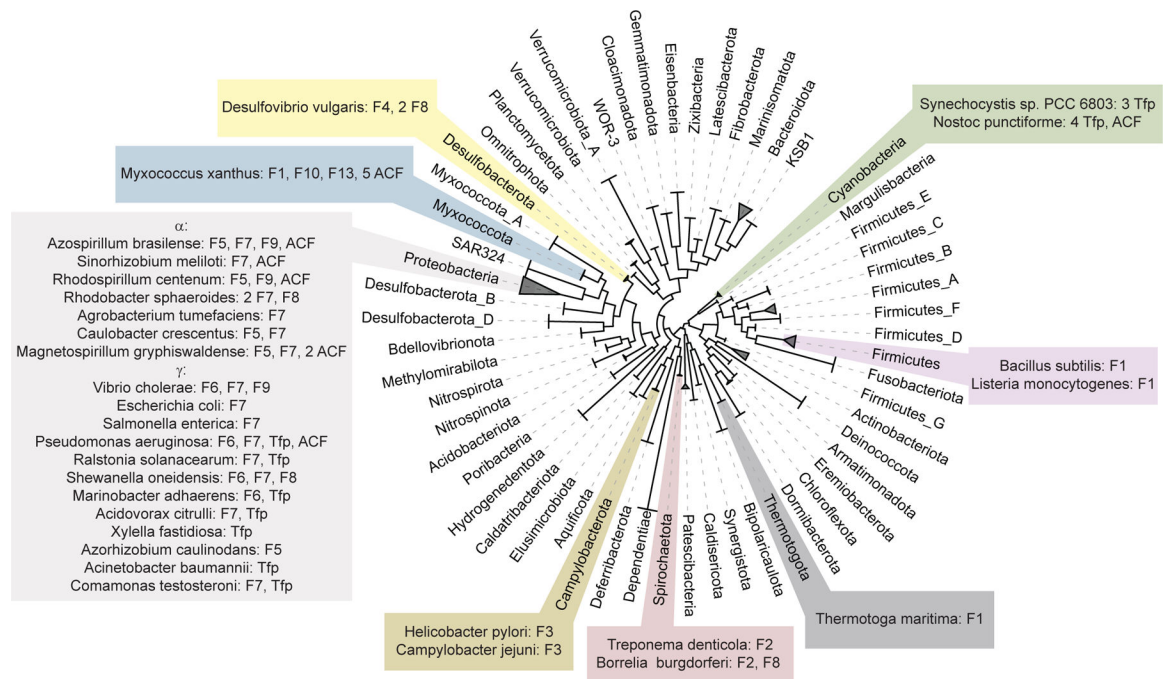
Figure 3. Phyletic distribution of experimentally studied bacterial chemosensory systems.
The genome tree showing all bacterial phyla with at least 10 sequenced genomes was adapted from the Genome Taxonomy Database [49]. Organisms with experimentally studied chemosensory systems are mapped to their respective phyla. All known types of chemosensory systems are listed for each organism.

Table 1.

Experimentally studied chemosensory systems in Bacteria

Organism	Taxonomy[a]	System class/CheA accession number	Function controlled
Escherichia coli	Gammaproteobacteria	F7 NP_416402.1	Flagellar motility (chemotaxis)
Salmonella enterica serovar *Typhimurium*	Gammaproteobacteria	F7 NP_460878.1	Flagellar motility (chemotaxis)
Bacillus subtilis	Firmicutes	F1 NP_389525.2	Flagellar motility (chemotaxis)
Thermotoga maritima	Thermotogota	F1 NP_228511.1	Unknown
Helicobacter pylori	Campylobacterota	F3 WP_108169127.1	Flagellar motility (chemotaxis)
Campylobacter jejuni	Campylobacterota	F3 YP_002343725.1	Flagellar motility (chemotaxis)
Myxococcus xanthus	Myxococcota	F1 (Dif) WP_011556617.1	Tfp-mediated social (S) motility, EPS production
		ACF (Frz, che1) WP_011554143.1	Adventurous (A) and social (S) motility
		ACF (che3) WP_011555118.1	Gene expression, S motility
		ACF (che4) WP_011552754.1	S motility
		ACF (che5) WP_011555978.1	S motility, development
		ACF (che6) WP_011556872.1	S motility, development
		F10 (che8) WP_011554745.1	unknown
		F13 (che7) WP_020480737.1	S motility
Pseudomonas aeruginosa	Gammaproteobacteria	F6 (Che1) NP_250149.1	Flagellar motility (chemotaxis)
		F7 (Che2) NP_248868.1	Stress response, unrelated to chemotaxis
		ACF (Wsp) NP_252393.1	c-di-GMP levels, biofilm formation
		Tfp (Chp) NP_249104.1	Tfp motility, cAMP levels
Vibrio cholerae	Gammaproteobacteria	F6 (cluster II)	Flagellar motility (chemotaxis)
		F7 (cluster III)	Unknown
		F9 (cluster I)	Unknown
Rhodobacter sphaeroides	Alphaproteobacteria	F7 (cheOp1) WP_002719591.1	Flagellar motility (chemotaxis)
		F7 (cheOp3) WP_002720190.1 WP_002720197.1	Flagellar motility (chemotaxis)
		F8 (cheOp2) WP_002722331.1	Flagellar motility (chemotaxis)

Organism	Taxonomy[a]	System class/CheA accession number	Function controlled
Sinorhizobium meliloti	Alphaproteobacteria	F7 (Che1) WP_014528988.1	Flagellar motility (chemotaxis)
		ACF (Che2) WP_014531470.1	Unknown
Azospirillum brasilense	Alphaproteobacteria	F5 (Che1) WP_035678528.1	Flagellar motility (chemokinesis), cell length
		F7 (Che4) WP_059399028.1	Flagellar motility (chemotaxis)
		F9 (Che2) WP_059399521.1	Unknown; not expressed under laboratory conditions
		ACF (Che3) WP_059399396.1	Flocculation
Rhodospirillum centenum	Alphaproteobacteria	F5 (Che1) WP_012566940.1	Flagellar motility (chemotaxis)
		F9 (Che2) WP_012565576.1	Lateral flagellum biosynthesis
		ACF (Che3) WP_012567300.1	Cyst development
Caulobacter crescentus	Alphaproteobacteria	F7 NP_419252.1	Flagellar motility (chemotaxis), biofilm formation
		F5 NP_419412.1	Biofilm formation
Azorhizobium caulinodans	Alphaproteobacteria	F5 WP_012169192.1	Flagellar motility (chemotaxis), biofilm formation
Borrelia burgdorferi	Spirochaetota	F2 (CheA2) NP_212803.1	Flagellar motility (chemotaxis)
		F8 (CheA1) NP_212701.1	Unknown
Treponema denticola	Spirochaetota	F2 NP_972097.1	Flagellar motility (chemotaxis)
Synechocystis sp. PCC 6803	Cyanobacteria	Tfp (tax1/pix) WP_010874009.1	Tfp motility (phototaxis)
		Tfp (tax2) WP_010871827.1	Unknown
		Tfp (tax3/Pil) WP_010873252.1	Pilus biogenesis, Tfp motility
Nostoc punctiforme	Cyanobacteria	Tfp (hmp/cl 1) WP_012412198.1	Tfp motility, EPS secretion
		Tfp (pix) WP_012412241.1	Unknown
		Tfp/ptx WP_012408770.1	Tfp motility (phototaxis)
		Tfp (cl 3) WP_012411886.1	Morphogenesis
		ACF WP_012407058.1	Unknown
Comamonas testosteroni	Gammaproteobacteria	F7 (che) WP_012836878.1	Flagellar motility, biofilm formation

Organism	Taxonomy[a]	System class/CheA accession number	Function controlled
		Tfp (Flm) WP_012839726.1	Biofilm formation
Magnetospirillum gryphiswaldense	Alphaproteobacteria	F5 (cheOp1) WP_106003281.1	Flagellar motility (chemotaxis)
		ACF (cheOp2) WP_106001668.1	Unknown
		ACF (cheOp3) WP_106002789.1	Unknown
		F7 (cheOp4) WP_106001879.1	Unknown

[a]According to the Genome Taxonomy Database [50].