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What bacteria want

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Summary

Bacterial signal transduction systems are responsible for sensing environmental cues and adjusting the cellular behavior and/or metabolism in response to these cues. They also monitor the intracellular conditions and the status of the cell envelope and the cytoplasmic membrane and trigger various stress responses to counteract adverse changes. This surveillance involves several classes of sensor proteins: histidine kinases; chemoreceptors; membrane components of the sugar phosphotransferase system; adenylate, diadenylate and diguanylate cyclases and certain cAMP, c-di-AMP and c-di-GMP phosphodiesterases; extracytoplasmic function sigma factors, and Ser/Thr/Tyr protein kinases and phosphoprotein phosphatases. We have compiled a detailed listing of sensor proteins that are encoded in the genomes of *Escherichia coli*, *Bacillus subtilis,* and ten widespread pathogens: *Chlamydia trachomatis*, *Haemophilus influenzae*, *Helicobacter pylori*, *Mycobacterium tuberculosis*, *Mycoplasma pneumoniae*, *Neisseria gonorrhoeae*, *Porphyromonas gingivalis*, *Rickettsia typhi*, *Streptococcus pyogenes*, and *Treponema pallidum* and checked what, if anything, is known about their functions. This listing shows significant gaps in understanding of which environmental and intracellular cues are perceived by these bacteria and which cellular responses are triggered by the changes in the respective parameters. A better understanding of bacterial preferences may suggest new ways to modulate expression of virulence factors and therefore decrease the reliance on antibiotics to fight infection.

In a 2000 Hollywood movie, a self-centered male executive gains the ability to hear what women are thinking, which causes him to re-evaluate his attitudes and adjust his behavior (https://www.wikipedia.org/wiki/What_Women_Want). By studying bacterial signal transduction pathways, microbiologists are trying to acquire a somewhat similar skill: to be able to figure out what the objects of our attention are looking for and, accordingly, how we could make them happy and possibly avoid confrontation.

Similarly to the idea of the ‘bacterial IQ’, introduced several years ago (Galperin, 2005; Galperin *et al.*, 2010), this approach may be perceived as misguided anthropomorphism. However, despite obvious mechanistic differences, sensory logics of bacteria and humans show certain parallels. In bacterial chemotaxis, the sensed compounds are traditionally referred to as attractants and repellents (Szurmant and Ordal, 2004; Hazelbauer, 2012). In diauxic growth, carbon sources are described as ‘preferred’ and ‘non-preferred’ (Monod, 1942; Deutscher, 2008; Buffing *et al.*, 2018). Remarkably, chemotactic attractants are not

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necessarily the best nutrients, the nature of the signaling response is much more complex than that. Hence, the question ‘What do bacteria want?’ seems totally legitimate.

Answering this question becomes ever more important as the pool of effective antibiotics continues to shrink, limiting our choices to counteract bacterial infections. Although the first recipe of using mold extract to cure disease dates back almost 4,000 years (see Galperin and Koonin, 1999), the wide use of antibiotics in the past 75 years has led to the emergence of multidrug-resistant bacteria. In an amazing combination of Darwinian selection (survival of the fittest, i.e. the most resistant, strains) with prokaryotic “altruistic” gene sharing, resistance to commonly used antibiotics is spreading like a wildfire (actually, much faster than that). The recent proliferation of the colistin resistance gene *mcr-1* (Wang *et al.*, 2018) offers just the latest example of this phenomenon. The remarkable success in finding evolution-proof antibacterials (Ling *et al.*, 2015; Bell and MacLean, 2018) might give us a reprieve for the near future. However, another question of using broad-spectrum antibiotics remains: how smart is it to try killing most bacteria in one’s body just to get rid of several bad actors? Development of new vaccines should help (Rappuoli *et al.*, 2017), but the only ready solution to this conundrum is to understand what bacteria really want and how could we dissuade them from harming their human (animal) hosts.

It helps that now we have available the human genome sequence and genome sequences of most bacterial pathogens. In contrast to humans, whose cells encode more than 600 protein kinases and ca. 800 G-protein coupled receptors (GPCRs), bacteria have far fewer signaling proteins, whose functions are gradually being uncovered.

About 20 years ago, Céline Fabret, Victoria Feher and James Hoch published a minireview on two-component signal transduction in *Bacillus subtilis* with a provocative subtitle “How one organism sees its world”. Making use of the just-finished complete genome sequence of *B. subtilis* strain 168, they examined 36 histidine kinases (HKs) and 35 response regulators (RRs) encoded in this genome and classified them into several groups based on the organization of the conserved motifs surrounding phosphoacceptor His residues in HKs and Asp residues in RRs (Fabret *et al.*, 1999). The same year, a joint paper from Saier and Stülke labs provided a careful analysis of the components of the phospho*enol*pyruvate:sugar phosphotransferase system (PTS) encoded in *B. subtilis* (Reizer *et al.*, 1999), while we presented an account of the c-di-GMP-related GGDEF, EAL and HD-GYP domains in several model genomes (Galperin *et al.*, 1999). These and other papers paved the way to the knowledge-based annotation of the signaling machineries in model organisms, such as *Escherichia coli*, *Salmonella enterica*, *Pseudomonas aeruginosa*, *Vibrio cholerae*, and many others. Several groups independently compiled comprehensive lists of the signaling proteins encoded in bacteria with completely sequenced genomes (Galperin *et al.*, 2001; Ashby, 2004; 2006; Galperin, 2004; 2005; 2006; Ulrich and Zhulin, 2010; Letunic *et al.*, 2015; Ortet *et al.*, 2015; Finn *et al.*, 2016) and used these data to analyze trends in microbial genome evolution (Ulrich *et al.*, 2005; Galperin *et al.*, 2010; Williams and Whitworth, 2010). Since then, the list of experimentally characterized signal transduction systems kept growing. It currently includes those transmitting signals from a dozen or so types of sensory proteins: HKs (two-component systems); methyl-accepting chemotaxis proteins (MCPs); membrane components of the PTS; adenylate cyclases and cAMP phosphodiesterases; diguanylate

cyclases and c-di-GMP-specific phosphodiesterases; diadenylate cyclases and c-di-AMP-specific phosphodiesterases; extracytoplasmic function (ECF) sigma factors, and Ser/Thr/Tyr protein kinases and phosphoprotein phosphatases (Table 1). This list is probably incomplete: there are other signaling systems, e.g. those involving ppGpp (Potrykus and Cashel, 2008; Kamarthapu *et al.*, 2016) and cGAMP (Davies *et al.*, 2012; Severin *et al.*, 2018), which appear to be involved primarily in intracellular surveillance but could also be involved in environmental sensing. There is also the most common regulatory mechanism that involves LacI-like transcriptional regulators consisting of a sensory (ligand-binding) and a DNA-binding domain, but these are typically confined to the cytosol.

Each of these sensor types involves a specific kind of signal transduction machinery:

- Two-component systems involve sensor HKs, which respond to the environmental or intracellular cues by autophosphorylation, followed by the phosphoryl group transfer to the REC domain in their cognate RRs. Depending on the nature of the output domains (if present), RRs may serve as transcriptional regulators, control flagellar or twitching motility, or control other signaling systems, for example, by synthesizing or hydrolyzing second messengers cAMP or c-di-GMP (Galperin, 2006; 2010; Gao *et al.*, 2007; Gao and Stock, 2009; Zschiedrich *et al.*, 2016).
- Methyl-accepting chemotaxis sensor proteins (MCPs) respond to their respective signals by interacting with the chemotaxis-specific HK CheA, which causes phosphorylation of the stand-alone REC domain RR CheY. Interaction of CheY~P with the flagellar FliM protein affects the direction of flagellar rotation. (Ortega *et al.*, 2017; Salah Ud-Din and Roujeinikova, 2017; Bi and Sourjik, 2018). Some MCPs serve as inputs for alternative signal transduction pathways (Hickman *et al.*, 2005; Willett and Kirby, 2011). A great majority of MCPs have extracytoplasmic sensor domains, but some MCPs are intracellular.
- Membrane components of the PTS, EIIC proteins (or domains), serve as receptors for a variety of mono- and oligosaccharides. In the absence of their sugar substrates, soluble PTS components are maintained in the phosphorylated state by a complex phosphorylation cascade that typically includes phospho*enol*pyruvate, common PTS proteins EI and HPr, and the sugar-specific proteins (or domains) EIIA and EIIB. Sugar binding to EIIC's leads to the phosphoryl transfer from the respective EIIB~P to this sugar, which causes temporary dephosphorylation of the proteins in that specific cascade. Non-phosphorylated PTS components can bind to a variety of target proteins, such as the gammaproteobacterial (class I) adenylate cyclase, certain membrane permeases and transcriptional regulators, typically inhibiting their activity. In addition, some of them may serve as chemotactic signals (Deutscher *et al.*, 2006; 2014; Deutscher, 2008; Västermark and Saier, 2014).
- Adenylate cyclases, enzymes that produce second messenger 3',5'-cyclic adenosine monophosphate (cAMP) are found in several distinct classes, at least two of which are involved in bacterial signal transduction. Class I enzymes (ACI), found in gammaproteobacteria and several deltaproteobacteria, are

regulated by the PTS EIIA^{Glc} and possibly other EIIA's. Class III enzymes (ACIII), found in most bacterial phyla, usually contain N-terminal sensor domain and are regulated by the respective environmental and/or intracellular cues. cAMP-mediated signal transduction involves interaction of the cAMP with specific binding domains, found in the transcriptional regulator CRP and in several other proteins. The cAMP-CRP complex serves as a global regulator that binds to the chromosomal DNA and activates transcription from a variety of bacterial promotors. In *P. aeruginosa*, a class III adenylate cyclase with membrane-embedded MASE2 sensor domain (PA3217) proved to be a key regulator of virulence (Smith *et al.*, 2004). Flooding the host cell with cAMP, which inhibits phagocytosis and causes cytolysis and apoptosis of macrophages, may be a common mechanism of pathogenesis involving both sensory (class I, class III) and secreted toxin (class II) adenylate cyclases. Ligands for most receptor adenylate cyclases have not yet been identified (Bassler *et al.*, 2018).

- cAMP-hydrolyzing phosphodiesterases (CPDs) usually represent stand-alone proteins without any sensor domains. Their regulation by environmental or intracellular cues has not yet been demonstrated, but a recent paper described involvement of CpdA in regulation of persistence state in *E. coli* (Nosho *et al.*, 2018).
- Diguanylate cyclases (DGCs, proteins with the conserved GGDEF domains) respond to environmental or intracellular signals by synthesizing the second messenger 3',5'-cyclic dimeric guanosine monophosphate (c-di-GMP). Cyclic di-GMP has been shown to regulate a variety of systems, including motility, protein and polysaccharide secretion, cell division, and biofilm formation. In bacterial pathogens, c-di-GMP is often involved in regulating the expression of virulence factors (Cotter and Stibitz, 2007; Hengge, 2009; Römling *et al.*, 2013; Jenal *et al.*, 2017). Effects of c-di-GMP are mediated by its binding to a variety of receptors that include PilZ and MshEN domains, several types of transcriptional regulators, diguanylate cyclases themselves and other binding proteins (Chou and Galperin, 2016; Krasteva and Sondermann, 2017).
- C-di-GMP-specific phosphodiesterases (PDEs, containing either EAL or HD-GYP domains) hydrolyze c-di-GMP to linear pGpG to reverse the effects of the DGCs and affect a variety of c-di-GMP-regulated systems. Many PDEs have extracytoplasmic or intracellular sensor domains, which allow them to regulate c-di-GMP levels independently of the respective DGCs. *Escherichia coli*, for one, encodes five different PDEs with closely related CSS sensor domains that apparently monitor the redox status in the periplasm (Herbst *et al.*, 2018). Some inactivated EAL and HD-GYP domains function as c-di-GMP-binding proteins (Chou and Galperin, 2016; Jenal *et al.*, 2017).
- Diadenylate cyclases (DACs, containing the conserved the DisA_N domain) produce second messenger 3',5'-cyclic dimeric adenosine monophosphate (c-di-AMP). Cyclic di-AMP has been shown to mediate signaling related to K^+ ion transport, osmotic pressure inside the cell, and cell wall stress (Corrigan and

Gründling, 2013; Gründling and Lee, 2016; Commichau *et al.*, 2015; 2018). In *B. subtilis*, one of its three DACs monitors the integrity of chromosomal DNA and serves as a checkpoint for the cells entering the sporulation process.

- C-di-AMP-specific phosphodiesterases (CDAs, containing either GGDEF-DHHA-DHHA1 or HD-type domains) hydrolyze c-di-AMP. Most, albeit not all of them do not contain any sensor domains and apparently simply reverse the effects of DACs.
- Extracytoplasmic function (ECF) sigma factors comprise a vast signal transduction machinery that regulates transcription primarily in response to intracellular cues but can also respond to environmental factors, such as envelope stress (Helmann, 2016; Asai, 2018), blue light (Gaidenko *et al.*, 2006), or extracellular polysaccharides (Kahel-Raifer *et al.*, 2010; Yaniv *et al.*, 2014), see (Mascher, 2013; Paget, 2015; Sineva *et al.*, 2017) for reviews.
- Signal transduction from Ser/Thr/Tyr protein kinases (STYKs) involves direct or indirect phosphorylation of various (mostly unknown) targets. The few experimentally characterized targets of Ser/Thr/Tyr protein phosphorylation include metabolic (e.g. glycolytic) enzymes and transcriptional regulators. However, Ser/Thr/Tyr phosphorylation affects hundreds of diverse proteins (Macek *et al.*, 2007; 2008) and the full scope of its effects on the cell behavior remains obscure (Kennelly, 2002; Grangeasse *et al.*, 2012; Hansen *et al.*, 2013; Wright and Ulijasz, 2014).
- Ser/Thr/Tyr phosphoprotein phosphatases (PP2Cs) dephosphorylate Ser, Thr, and/or Tyr residues, both in STYK protein kinases and in their targets and reverse the effects of Ser/Thr/Tyr phosphorylation (Wright and Ulijasz, 2014).

A tally of these types of proteins in two model organisms, *E. coli* and *B. subtilis*, and in several widespread pathogens is presented in Table 1 with detail listings provided in Supplementary Tables S1–S3. Similar lists for many other organisms are available at https://www.ncbi.nlm.nih.gov/Complete_Genomes/SignalCensus.html as well as at several other web sites (Ulrich and Zhulin, 2010; Ortet *et al.*, 2015).

This analysis showed that the range of compounds sensed by *E. coli* provides a reasonable coverage of the entire metabolic map (Galperin, 2009a; 2009b). Indeed, although each sensory system displays certain bias (the PTS only senses sugars, MCPs sense only a small number of compounds), taken in its totality, the signal transduction machinery of *E. coli* pretty much covers the key physicochemical parameters (t^o, pH, oxygen levels) and nutrients (amino acids, peptides, mono- and disaccharides, nucleobases) that this bacterium encounters in its environment. In *B. subtilis*, many receptors still remain uncharacterized (Table S2) but the list of sensed compounds again appears to cover most, if not all, bases. A remarkable number of HKs, DGCs, PDEs, and DACc in both organisms are sensing general parameters of cellular well-being, such as envelope and osmotic stress, the redox state of the cell, and the availability of terminal electron acceptors. Obviously, these parameters greatly influence the lifestyle choices (growth vs. persistence, motility vs. sessility, planktonic vs. biofilm state) in both *E. coli* and *B. subtilis.*

Table 1 shows that bacterial pathogens typically encode fewer environmental sensors and have a biased distribution of those that they do encode. *Mycobacterium tuberculosis*, whose genome is similar in size to those of *E. coli* and *B. subtilis*, does not have any chemotaxis or PTS sensors, codes for fewer HKs and c-di-GMP turnover proteins, but has dramatically expanded sets of adenylate cyclases and Ser/Thr/Tyr kinases. *Streptococcus pyogenes* encodes 12 HKs and 14 EIIC components of the PTS but few, if any, other receptors.

Table S3 presents the lists of sensory proteins encoded in the genomes of ten widespread bacterial pathogens ranging in size from 816 kb in *Mycoplasma pneumoniae* to 4,412 kb in *M. tuberculosis*. Each such sensor protein is characterized with respect to (i) which signal (ligand) it is sensing, and (ii) which genes and/or systems it regulates; there are also hyperlinks to the respective entries in UniProt and PubMed (The UniProt Consortium, 2017). This listing clearly shows that, although signal transduction pathways in pathogenic bacteria are streamlined compared to those in free-living *E. coli* and *B. subtilis* (not to mention the highly sophisticated signaling machineries of *P. aeruginosa* or *V. cholerae*), only few of them have been studied in sufficient detail. There is not a single organism for which all sensed signals have been characterized and all genes or pathways regulated by these signals have been identified. *Haemophilus influenzae* and *Mycoplasma pneumoniae,* the first bacteria with completely sequenced genomes, fare somewhat better than the others, owing to the long history of research and relatively small sets of sensor molecules.

The importance of tuberculosis as health hazard in many countries and the relative resilience of *M. tuberculosis* to most standard treatments made this organism the subject of several research projects aimed at better understanding its signaling systems and their roles in virulence (Shenoy *et al.*, 2004; Bretl *et al.*, 2011; Parish, 2014; Prisic and Husson, 2014). It is also one of very few bacteria for which the researchers were prepared to get away from wide-spectrum antibiotics and consider organism-specific treatments and drugs. Indeed, several HKs (Parish *et al.*, 2003; Rybniker *et al.*, 2014), adenylate cyclases (Agarwal *et al.*, 2009; Shleeva *et al.*, 2017), and Ser/Thr/Tyr protein kinases and phosphatases (Wong *et al.*, 2013; Sherman and Grundner, 2014) have been experimentally characterized and shown to contribute to virulence. As a result, targeting these systems has been proposed as a viable method of controlling *M. tuberculosis* infection (Bai *et al.*, 2011; Prisic and Husson, 2014; Dey *et al.*, 2015).

The chemotaxis machinery of *Helicobacter pylori* has been comprehensively studied due to the early recognition that *H. pylori* relies on chemotaxis for colonization of gastric mucosa (Foynes *et al.*, 2000; Andermann *et al.*, 2002), which leads to the stomach infection and inflammation of the gastric epithelial tissue (Croxen *et al.*, 2006; Williams *et al.*, 2007; Rolig *et al.*, 2012; Huang *et al.*, 2015). Although all four MCPs (TlpA-TlpD) of *H. pylori* have been experimentally characterized (see Table S3), this bacterium exhibits chemotactic responses to various amino acids and bile acids, which have not yet been assigned to any of these chemoreceptors (Machuca *et al.*, 2017). There is also some understanding of the functions of the *H. pylori*'s HKs (Waidner *et al.*, 2005; Joseph and Beier, 2007; Marcus *et al.*, 2012; Tsang *et al.*, 2015) but not of its Ser/Thr/Tyr protein kinase and phosphatase.

For most other organisms, understanding of signaling mechanisms is very limited (Table S3). We do not know which environmental parameters these organisms sense, let alone why they choose these parameters and not others. The cases of *Chlamydia trachomatis*, the causative agent of trachoma, venereal lymphogranuloma and other diseases, and *Treponema pallidum*, the causative agent of syphilis, are particularly frustrating. The first of them encodes just a single HK, three Ser/Thr/Tyr kinases and three phosphoprotein phosphatases. The second one encodes just four chemoreceptors and a single chemotactic HK, CheA, single copies of an adenylate cyclase, diadenylate cyclase and diguanylate cyclase, and several phosphodiesterases (Table S3). *Chlamydia* is an obligate intracellular pathogen, while *T. pallidum* only grows properly in animal tissues, so investigating their signaling capacities *in vivo* is very challenging. Further, bioinformatics studies, including now-popular "Big Data" approaches, are of limited value: an alignment of *Chlamydia*-specific sensor domains of Ser/Thr/Tyr kinases and phosphatases would not help when none of the respective proteins has been experimentally characterized. Even when these sensor domains belong to known protein families (e.g. GAF, dCache, 7TMR_HDED, as is the case with several *T. pallidum* proteins), these assignments are not specific enough to pinpoint the exact ligand. This means that identification of these signals (ligands) will have to be done experimentally, by cloning and heterogenous expression of sensor domains from these organisms. The fact that this has rarely been undertaken for any common pathogens demonstrates the prevailing attitude towards bacterial signaling systems as something only marginally relevant to pathogenesis.

Despite this general attitude, there has been certain progress in harnessing bacterial signaling systems to elicit favorable responses. The natural inclination is to search for compounds (waldiomycin, signermycin B) that target the essential genes involved in signal transduction, e.g. (in Gram-positive bacteria) histidine kinase WalK (YycG), and to measure success by the efficiency of killing *B. subtilis*, *Staphylococcus aureus* and *Streptococcus mutans* (Watanabe *et al.*, 2012; Fakhruzzaman *et al.*, 2015). However, managing – or even preventing – disease does not necessarily require eradication of the bacteria. For example, an HK inhibitor walkmycin C showed the ability to suppress biofilm formation at sub-MIC levels, without killing the bacteria (Eguchi *et al.*, 2011). There is a long list of potential HK inhibitors (Bem *et al.*, 2015); it might make sense to examine them for the ability to decrease virulence at sub-MIC levels. Biofilm formation could also be repressed by modulating c-di-GMP polls with spermine and spermidine (Sobe *et al.*, 2017). Further, relatively low levels of nitric oxide trigger biofilm dispersal in multiple bacteria (Barraud *et al.*, 2009; Cutruzzola and Frankenberg-Dinkel, 2016), apparently also acting through c-di-GMP signaling. Future studies of the bacterial signaling pathways will likely uncover more simple and non-toxic compounds that could be used for taming the infection and preventing secretion of virulence factors.

While 'fooling' bacterial pathogens offers a number of advantages over killing them, there is obviously no guarantee that it will always work, not only because we do not have the required depth of knowledge. Single-approach tricks, such as anti-biofilm treatment alone, may not work simply because bacteria readily adapt to changes that do not involve essential processes (and mutate if the affected process is essential). That is why one may have to utilize multi-target approaches, simultaneously 'tricking' all bacterial senses,

Summing up, what do bacteria want? Obviously, they like their amino acids, peptides, mono- and disaccharides, nucleobases and vitamins and get stressed when nutrients become scarce. They usually like ambient pH and often perceive membrane-penetrating acids as repellents. They differ in their preferred redox environments but tightly monitor the redox conditions around them and inside the cell. They really care about the integrity of their domicile and possess multiple systems to detect envelope stress. In general, bacteria seem to be able to adjust to a variety of conditions but do not like abrupt changes; this may provoke them to respond by secreting virulence factors. A better understanding of their preferences could go a long way towards allowing a more amicable co-existence with our bacterial neighbors.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1.

Distribution of environmental sensors in selected bacteria

Organism, strain name	Genome size, kb	HK	MCP	PTS EIIC	cAMP		c-di-GMP signaling				c-di-AMP signaling			ECF sigma	Ser/Thr/Tyr~P	
					ACI, ACIII	CPD	GGDEF	GGDEF +EAL	EAL	HD-GYP	DisA_N	GbpP	PgpH		STYK	PP2C
Escherichia coli K-12	4,639	30	5	23	1	1	12	7	10	–	–	–	–	2	5	1
Bacillus subtilis 168	4,216	36	10	16	–	–	3	1	2	–	3	1	1	7	4	5
Mycobacterium tuberculosis H37Rv	4,412	14	–	–	16	1	–	1	1	–	1	1	–	10	13	2
Porphyromonas gingivalis W83	2,343	6	–	–	–	–	–	–	–	–	1	1	1	6	–	–
Neisseria gonorrhoeae FA 1090	2,154	4	–	–	–	–	–	–	–	–	–	–	–	1	1	–
Streptococcus pyogenes M1 GAS	1,852	12	–	14		–	–	–	–	–	1	2	–	2	1	1
Haemophilus influenzae Rd KW20	1,830	4	–	1	1	1	–	–	–	–	–	–	–	2	1	–
Helicobacter pylori 26695	1,668	4	4	–	–	–	–	–	–	–	–	–	–	–	1	1
Treponema pallidum str. Nichols	1,138	1	4	–	1	–	1	–	–	3	1	1	1	1	1	3
Rickettsia typhi str. Wilmington	1,111	4	–	–	–	–	1	–	1	–	–	–	–	–	1	–
Chlamydia trachomatis D/UW-3/Cx	1,043	1	–	–	–	1	–	–	–	–	1	–	–	–	3	3
Mycoplasma pneumoniae M129	816	–	–	4	–	–	–	–	–	–	1	2	–	1	1	1

[a]Abbreviations are as defined in the text. The numbers are from the COG database (Galperin *et al.*, 2015), where available, or from the results of iterative PSI-BLAST searches of domain-specific profiles against the protein sets encoded in each organism (see Galperin, 2005, for details). A hyperlinked version of this table is available in the Supplementary Materials; an earlier version is on the NCBI web site: https://www.ncbi.nlm.nih.gov/Complete_Genomes/SignalCensus.html.