EMBO reports

viewpoint

A new evaluation of our life-support system

Bacterial benefactors—and other prokaryotic pursuits

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After more than 150 years of research in microbiology, new technologies and new insights into the microbial world have sparked a revolution in the field. This is a much needed development, not only to renew interest in prokaryote research, but also to meet many emerging challenges in medicine, agriculture and industrial processes. Although many microbiologists—such as Emil von Behring, Robert Koch, Jacques Monod, François Jacob, André Lwoff, Alexander Fleming, Selman A. Waksman and Joshua Lederberg—grace the list of Nobel laureates, attention moved away from microbiology as biologists focused their interest on eukaryotic cells and higher organisms in the 1970s and 1980s. Furthermore, from the beginning, research on prokaryotes has suffered from an anthropocentric view, regarding as interesting only those organisms that cause disease or that can be exploited for industrial or agricultural use. But the advent of new technologies, some of which have been driven by a need to understand eukaryotes, may change this. We are increasingly realizing how little we know about microbes in general, their diversity, the mechanisms of their evolution and adaptation and their modes of existence within, and communication with, their environment and higher organisms. As bacteria have succeeded in occupying virtually all ecological niches on this planet, ranging from arctic regions to oceanic hot springs, they hold an immense wealth of genetic information that we have barely started to explore and that may provide many useful applications for humans.

The new technologies that allow us to sequence and annotate whole genomes more rapidly and to analyse the expression of thousands of genes in a single experiment are likely to speed up this change, particularly as microbes are well suited for high-throughput analysis. Any microbial genome can now be sequenced within a few hours and, in the near future, new bioinformatics tools will enable scientists not only to assemble and annotate them automatically, but also to infer metabolic pathways and other cellular processes from the sequence data *in silico*. However, the experimental approaches (functional genomics) for testing the veracity of these predictions are likely to be rate-limiting for the foreseeable future. This renewed research will have great usefulness if it means that we understand the microbial world on which all other species on this planet depend for survival.

More than 120 prokaryotic genomes have been completely sequenced (www.ncbi.nlm.nih.gov/genomes/static/eub.html; www.ncbi.nlm.nih.gov/genomes/static/a.html). Genomes are sequenced so quickly that another dozen may become available while this article is being processed for publication. There is already a vast richness to explore in the known genomes. Due to the high-density coding of microbial genomes, 10^9 base pairs of prokaryotic DNA—the equivalent of a eukaryotic genome—represents a million genes or proteins, compared with only about 35,000 for the human genome. Furthermore, the total number of protein families steadily increases with each new genome sequenced, and bacterial genomes clearly contribute more to protein diversity than do eukaryotic genomes; Kunin *et al.* (2003) estimate that the genomes of *Borrelia burgdorferi* and *Xylella fastidiosa* will provide 380 new protein families per million base pairs versus only 1.3 families per million for the whole human genome.

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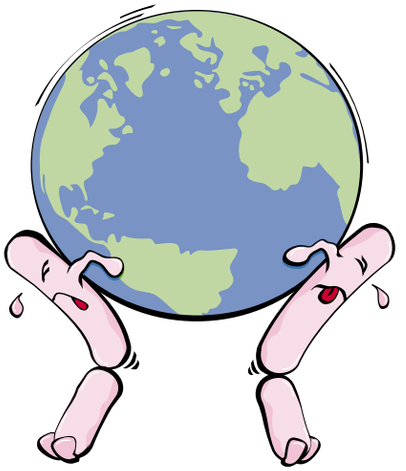
But all of these sequenced prokaryotic genomes are only the tip of the iceberg of total microbial diversity. Of the estimated 10^9 microbial species on Earth, only a minute number is accessible for analysis, as we are not able to cultivate most bacteria in the laboratory (Whitman *et al.*, 1998). Bacteriophages also add to this diversity due to their enormous abundance (estimated to be 10^{31} particles on the globe for tailed bacteriophages alone; Wommack & Colwell, 2000), recycling rate (every second, approximately 10^{25} phages initiate a lytic cycle) and gene product diversity (Pedulla *et al.*, 2003). To circumvent the cultivation bottleneck, microbiologists have started extracting

genes from what they call the 'metagenome' (Lorenz *et al*., 2002), that is, from DNA taken directly from environmental samples. This approach should uncover new genes, proteins, enzymes, metabolic compounds and pathways that could be exploited for industrial processes. Such an expanded representative catalogue of microorganisms holds great promise. It may allow us to understand and predict the impact of industrial, agricultural and other activities on prokaryotic diversity. We will also better understand the mechanisms of the evolution of pathogens and of potentially useful bacteria, such as xenobiotic degraders. A metagenomic approach also represents an important first step towards understanding what some have called the second human genome (Relman & Falkow, 2001)—the 10^{13} bacteria that populate our bodies, some of which are essential to our survival. However, before we are able to grasp fully the complex interactions between bacteria and ourselves, and their contribution to our well-being, new analytical techniques are needed.

Bacteria live in habitats where environmental and physiological homeostasis is the exception. Consequently, adaptive ability is a key feature of bacterial life, which necessitates metabolic plasticity. Bacteria need to detect environmental cues in 'real time' and to integrate the resultant signals to trigger appropriate metabolic responses. Evolution has created extremely efficient bacterial sensing and signal transduction systems for the monitoring of diverse environmental factors, such as temperature, pH, osmolarity and the availability of nutrients (Stock *et al*., 2000). One particularly important sensing system is the ability to measure the density of the surrounding bacterial population—both siblings and competitors—in any niche. Now known as 'quorum sensing' (QS), it is the production of one or several molecular signals as intercellular messengers (Whitehead *et al*., 2001). The concentration of these signals indicates population density, and, by sensing these signals, a bacterium may effectively titrate the number of 'self' and 'non-self' cells. The potential advantages of QS for microorganisms are still speculative and depend on the physiological traits that are under QS control in any particular bacterium. For instance, QS-mediated control of bacterial population density might be advantageous for plant and animal pathogens to overwhelm the host defence systems with a massive onslaught of invading bacteria (Whitehead *et al*., 2001). In general, the bacterial QS system can be viewed as a primitive form of multicellularity, allowing bacterial cells to act in concert and thus to gain advantages that are unavailable to single cells. However, there is still very little known about the production, abundance or ecological function of QS signals for most bacterial systems outside the artificial laboratory environment (Whitehead *et al*., 2001; Manefield & Turner, 2002).



The most common QS signals in Gram-negative bacteria are *N*-acyl homoserine lactones (*N*-AHLs), many of which diffuse freely. Another 'signal'—the AI-2 class, or furanosyl borate diester—is widespread in bacteria, but whether these molecules actually have true signalling roles in QS among most of the bacteria that produce them remains controversial (Winzer *et al*., 2002). The most common QS signals in Gram-positive bacteria are small, modified peptides, but γ-butyrolactones are also known to have key roles in morphogenesis and secondary metabolite regulation in some *Streptomyces* species. In general, we do not know how widespread QS is in bacteria (Manefield & Turner, 2002) or how large is the structural spectrum of QS signalling molecules. Nonetheless, it is clear that QS influences diverse phenotypes, including bioluminescence, antibiotic and pigment production, virulence in plant and animal pathogens, symbiosis with plants and animals, and polysaccharide and biofilm formation (Whitehead *et al*., 2001).

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Just as there is a paucity of information on natural *N*-AHL production by bacteria, little is known about its metabolic turnover. *N*-AHLs can be chemically inactivated in an alkaline pH environment and are thermally unstable (Byers *et al*., 2002; Yates *et al*., 2002). Bacterial enzymes have been isolated that are able to break down *N*-AHLs, and this 'quorum quenching' process has already been exploited to engineer plants that are resistant to bacterial pathogenesis (Dong *et al*., 2001). However, whether the natural function of the known *N*-AHL-degrading enzymes is connected with ecologically relevant QS molecule turnover is unknown. *N*-AHLs can interact with the animal immune system (Telford *et al*., 1998), and plants respond to *N*-AHLs by making biomimetic molecules (Teplitski *et al*., 2000). Also, red algae produce halogenated analogues (furanones) that have been implicated in the control of bacterial populations at the site of furanone biogenesis (Kjelleberg *et al*., 1997). It is clear that *N*-AHLs and related molecules affect eukaryotes, and it will be important to understand how biologically and ecologically meaningful are such effects. Equally interesting is how we might exploit them in biotechnology, agriculture and medicine in the future.

During the past decade, much attention has concentrated on surface-attached microbial populations, so-called biofilms (Fig. 1). These biofilms, which may account for most prokaryotic biomass, are multicellular and heterospecific matrix-enclosed bacterial communities found in almost all ecosystems (Stoodley *et al*., 2002) that have clearly distinct morphological and physiological properties compared with free-floating, planktonic bacteria. This communal

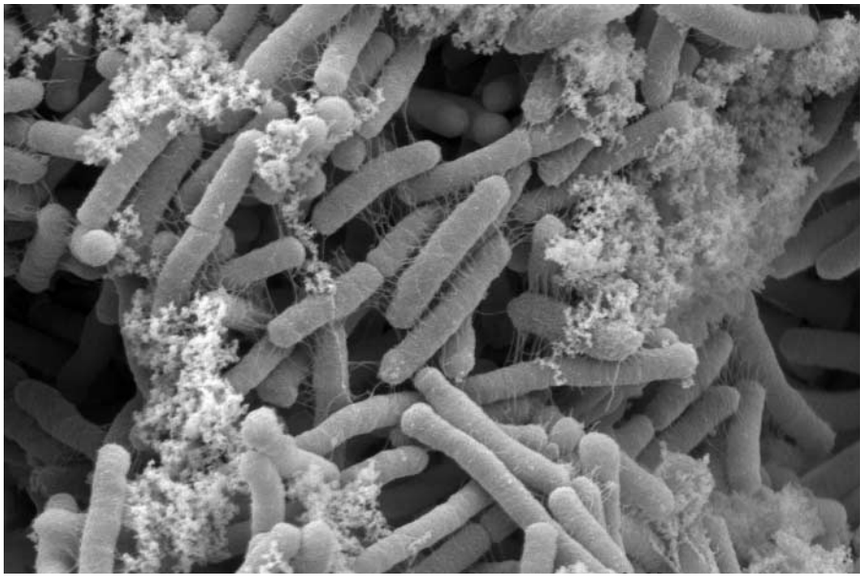

Fig. 1 | Laser-scanning micrograph of an *Escherichia coli* biofilm. The extracellular-matrix material remaining after fixation can be seen between tightly interconnected bacteria. Magnification, × 10,000. Micrograph kindly provided by B. Arbeille, Laboratoire de Biologie Cellulaire et Microscopie Electronique, UFR Médecine, Tours, France.

lifestyle, which has not been addressed by traditional microbiology, may account for the high proportion of genes with unassigned functions in many microbial genomes.

The genetic analysis of the functions leading to the biofilm phenotype *per se* have begun to reveal the expression of new genes, some of which were unsuspected in classical laboratory planktonic cultures (Ghigo, 2003). Studies using DNA arrays or proteomic approaches to compare planktonic and biofilm lifestyles produced a wealth of data, but did not lead to the identification of a common expression pattern for biofilm formation (Sauer, 2003; Schembri *et al.*, 2003; Whiteley *et al.*, 2001). Nevertheless, it is already clear that only small portions of the genomes seem to be differentially expressed during the formation of biofilms. This is surprising considering the dramatic phenotypic changes that occur in biofilms compared with planktonic life. We thus expect to discover many other biological consequences of the biofilm lifestyle, including aspects related to QS, horizontal gene transfer and unexplored microbial activities. Biofilms are ideally suited to studying microbial interactions, mixed microbial communities and the molecular ecology of microbial interactions in their natural and complex environments. However, new molecular approaches will be necessary to address such complex issues and, in particular, to circumvent the need for pure and homogeneous cultures.

There are other important fundamental microbiological questions, such as the nature of commensalism. The genomes of commensals and pathogens can be compared (Dobrindt *et al.*, 2003; Welch *et al.*, 2002), and DNA arrays can be used to investigate how bacteria interact within complex flora and eukaryotic hosts (Hooper *et al.*, 2001). Another neglected issue is the molecular basis of probiotic effects. It has long been known that mucosal flora have the capacity to limit the growth of, or kill, certain transient microbial pathogens by 'bacterial interference' (Reid *et al.*, 2001). The alarming rise of antimicrobial resistance has spurred a renewed interest in the therapeutic use of this competition between enteric bacteria, a promising approach to protecting and controlling human bacterial flora. However, the methods used to identify, select and evaluate bacterial colonization factors are limited and little is known about the molecular basis of this phenomenon, even if ill-defined 'beneficial flora' strains are now being widely commercialized.

Another challenge is the nature of 'viable but non-cultivatable' organisms (VNCs). Some VNCs are suspected to be dependent on metabolic cooperation (Kaeberlein *et al.*, 2002), possibly within biofilms. Thus, learning more about biofilms and bacterial co-cultivation may allow us to understand their metabolic interactions and eventually cultivate these VNCs, which by far outnumber the known cultivatable prokaryotes. As mentioned earlier, metagenomics is one way to investigate the microbial diversity of populations that cannot be cultivated. Other VNCs may be bacteria in a state of metabolic quiescence due to the biochemical status of their environment. We know that spent culture media from some bacterial cultures can, when added to another inoculated culture, stimulate the growth of bacteria and allow rapid resuscitation of very slow growing cultures. In the case of some Gram-positive bacteria, the resuscitation-promoting factor (Rpf)—a bacterial cytokine—has been identified as a small essential protein (Kell & Young, 2000). A recently discovered structural component of a mycobacteriophage may be able to mimic host Rpf (Pedulla *et al.*, 2003). If this hypothesis turns out to be correct, it will mean that the phage can 'wake up' its quiescent host so that it may replicate within it, another stunning example of phages' evolutionary ingenuity in exploiting host functions as biochemical 'Trojan horses' for their own propagation. Perhaps we could take advantage of this strategy to awaken VNC bacteria in the laboratory.

Any discussion about the future of microbiology cannot ignore gene transfer between bacterial species. Understanding this phenomenon will probably be one of the greatest challenges of this century. The 50-year long 'experiment' of the massive—not to say abusive—use of antibiotics gave us the first hints about exchange between bacteria of mobile genetic elements (MGEs) that allow rapid adaptation to cope with deadly compounds in the environment. Many of these elements, which carry various combinations of genes that enable bacteria to degrade or detoxify a wide range of compounds, have been identified and characterized. More recently, the complete sequencing of several strains of the same bacterial species revealed a new

vision of lateral gene transfer in prokaryotes. *Escherichia coli* and *Salmonella* share around 70% of their genes (Parkhill *et al*., 2001). A similar level of divergence is seen between the genomes of two *E. coli* strains (the laboratory strain K12 and the pathogen O157-H7), which differ by as much as 20–30% of their genomes. Strikingly, much of the difference is accounted for by prophages (Hayashi *et al*., 2001; Perna *et al.*, 2001). All of the major gaps in the alignment between the genomes of *Listeria monocytogenes* and *Listeria innocua* correspond to the prophages that are integrated into the latter (Glaser *et al*., 2001). The main differences between the pathogenic *Bacillus anthracis* and the closely related *Bacillus thuringiensis*, which is the source of Bt toxins, do not reside in their chromosomes but in the nature of the plasmids that they host (Read *et al*., 2003). The number of similar observations is increasing, begging a reassessment of the importance of horizontal gene transfer in speciation and subspeciation in bacteria (De la Cruz & Davies, 2000; Hacker & Carniel, 2001; Gogarten *et al.*, 2002).

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Many mobile elements reside in the host genome in a latent state. In this state, MGEs can undergo mutation and recombination with transient integrated and extrachromosomal elements. These changes eventually create new elements that may get transferred to another cell where they confer a greater survival fitness. As bacteria live in complex communities, new MGE–host combinations could invade new niches and continue their evolution there. Bacterial genomes are not as stable as we thought. Sequencing of some human pathogens has revealed that they tend to have reduced genome size compared with their non- or less pathogenic relatives. Some have undergone mutations that inactivate genes that are inessential in their new environment, where survival depends on

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dealing with host defences rather than synthesizing nutrients that are already abundant (Sebaihia *et al*., 2001). This evolutionary commitment encourages some pathogens to become extreme specialists that are unfit to cope with other environmental conditions—for example, *Pseudomonas* strains from the lungs of cystic fibrosis patients can often not be grown in the laboratory. It has become clear that MGEs, many of which carry the enzymatic machinery to integrate and excise DNA segments, are a driving force for the constant reshuffling of prokaryotic genetic information. New pathogens are thus more likely to emerge from environments where they can benefit from a gigantic horizontal gene pool. Therefore, an assessment of the diversity in this environment becomes crucial for any understanding of the emergence of new pathogens. The plastic nature of prokaryotic genomes is central to the continuing debate on the nature of species in prokaryotes, and calls for new methods to measure diversity and assess the basic concept of species and subspecies (Cases & De Lorenzo, 2002).

Given the enormous diversity of phages in the biosphere, the abundance of cryptic prophages—including some that carry pathogenicity determinants—and a large variety of genomic islands and plasmids in most bacterial genomes, it is reasonable to assume that MGEs have a central role in driving the adaptive evolution of their bacterial hosts. We need to understand more about the interactions between different MGEs inside bacteria. Antibiotic-resistance-encoding plasmids have attracted the most interest so far, simply because of the clinical importance of multi-drug resistance. However, various other plasmid-encoded systems are also important in determining bacterial life-and-death issues, notably after phage infection. These include, for example, the restriction/modification (R/M) systems and the abortive infection (Abi) systems that block productive replication of an invading viral genome and thus protect against lethal infections (Bickle & Kruger, 1993; Garvey *et al.*, 1995). The R/M systems degrade invading phage DNA that is not modified with the signature of the domestic genome to ensure survival of the attacked bacterium. But, by contrast, most of the plasmid-encoded Abi systems halt the replication of the invading virus at different stages depending on the system, from viral genome transcription to viral genome replication, capsid morphogenesis, assembly and burst (Snyder, 1995; Garvey *et al.*, 1995). The phage is trapped in the infected cell—a molecular and evolutionary *cul-de-sac*—and the infected cell dies without releasing active phages. This strategy is akin to apoptosis and could represent bacterial altruism, in which the infected cell dies to protect the rest of the population. Some plasmids and cryptic genomic islands may thus have an important population-based function for the host bacteria—surviving the effects of intermittent viral invasion could be just one of them.

The increasing volume of genomics research, coupled with information from metagenomics, may uncover new proteins, enzymes, pathways and metabolic products that could become useful tools for microbiology research and, if we are fortunate, novel chemotherapeutic agents. Advances in genomics, transcriptomics and proteomics will also help to elucidate the nature and functions of gene products that have unknown functions at present. From a utilitarian perspective, the biotechnological spin-off could be even more exciting. It will allow us to address many current problems in agriculture, nutrition and medicine, which are all domains relying on complex microbial flora that have been, so far, used largely empirically. These new approaches will clearly reshape many aspects of human activities, when we finally have the tools to explore the fantastic reservoir of biochemical know-how in the microbial world. Bacteria were on this planet long before we arrived and, no doubt, will be here long after we have disappeared. Learning more about the prokaryotic world by 'smart' modern microbiology methods may help to extend our tenure here; a tenure that is inextricably linked to a fascinating, dynamic—but ill-understood—prokaryotic life-support system.

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doi:10.1038/sj.embor.embor930