

## References

- Andrews, L.B. (1999) *The Clone Age: Adventures in the New World of Reproductive Technology*. Henry Holt and Co., New York, NY.
- Azizi, S.A., Stokes, D., Augelli, B., DiGirolamo, C. and Prockop, D. (1998) Engraftment and migration of human bone marrow stromal cells implanted in the brains of albino rats—similarities to astrocyte grafts. *Proc. Natl Acad. Sci. USA*, **95**, 3908–3913.
- Bjornson, C., Rietze, R., Reynolds, B., Magli, C.M. and Vescovi, A. (1999) Turning brain into blood: a hematopoietic fate adopted by adult neural stem cells *in vivo*. *Science*, **283**, 534–537.
- Brustle, O., Jones, K.N., Learish, R.D., Karram, K., Choudhary, K., Wiestler, O.D., Duncan, I.D. and McKay, R.D. (1999) Embryonic stem cell-derived glial precursors: a source of myelinating transplants. *Science*, **285**, 754–756.
- Clarke, D., Johansson, C., Wilbertz, J., Veress, B., Nilsson, E., Karlstrom, H., Lendahl, U., and Frisen, J. (2000) Generalized potential of adult neural stem cells. *Science*, **288**, 1660–1663.
- Colman, A. (2000) Somatic cell nuclear transfer in mammals: progress and applications. *Cloning*, **1**, 185–200.
- Fox, M.J. (2000) A crucial election for medical research. *The New York Times*, November 1.
- Fuchs, E. and Segre J. (2000) Stem cells: a new lease on life. *Cell*, **100**, 143–155.
- Gussoni, E., Soneoka, Y., Strickland, C., Buzney, E., Khan, M., Flint, A., Kunkel, L. and Mulligan, R. (1999) Dystrophin expression in the *mdx* mouse restored by stem cell transplantation. *Nature*, **401**, 390–394.
- Lagasse, E., Connors, H., Al-Dhalimy, M., Reitsma, M., Dohse, M., Osborne, L., Wang, X., Finegold, M., Weissman, I. and Grompe, M. (2000) Purified hematopoietic stem cells can differentiate into hepatocytes *in vivo*. *Nature*, **6**, 1229–1234.
- McKay, R., (2000) Stem cells—hype and hope. *Nature*, **406**, 361–364.
- Mieth, D. (2000) Going to the roots of the stem cell debate. *EMBO rep.*, **1**, 4–6.
- Munsie, M.J., Michalaska, A.E., O'Brien, C., Trounson, A.O., Pera, M.F. and Mountford, P.F. (2000) Isolation of pluripotent embryonic stem cells from reprogrammed adult mouse somatic cell nuclei. *Curr. Biol.*, **10**, 989–992.
- Powledge, T.M. (2000) Research on human embryonic stem cells. *EMBO rep.*, **1**, 297–298.
- Reynolds, B. and Weiss, S. (1992) Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. *Science*, **255**, 1707–1710.
- Shamblott, M., Axelman, J., Wang, S., Bugg, E., Littlefield, J., Donovan, P., Blumenthal, P., Huggins, G. and Gearhart, J. (1998) Derivation of pluripotent stem cells from cultured human primordial germ cells. *Proc. Natl Acad. Sci. USA*, **95**, 13726–13731.
- Thomson, J., Itskovitz-Eldor, J., Shapiro, S., Waknitz, M., Swiergiel, J., Marshall, V. and Jones, J. (1998) Embryonic stem cell lines derived from human blastocysts. *Science*, **282**, 1145–1147.
- Yandava, B., Billingham, L., Snyder, E. (1999) 'Global' cell replacement is feasible via neural stem cell transplantation: Evidence from the dysmyelinated *shiverer* mouse brain. *Proc. Natl Acad. Sci. USA*, **96**, 7029–7034.



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# Exploring the unknown

The silent revolution of microbiology • by *Eduard Kellenberger*

Comparing shape and development has permitted biologists to classify all known animals and plants into more than one million and some half a million species, respectively. For micro-organisms, however, such morphological criteria are much less suited. Complemented by biochemical criteria, taxonomists were at least able

change as soon as it is accepted that micro-organisms produce substances beneficial in the therapy of human diseases or for industrial applications.

The biomass of micro-organisms on our planet is estimated to be much more important than that of plants. Prokaryotes are the basis of every food chain on this planet.

mentation of dairy products, sausages and beverages. But their indispensable presence and necessity in natural biotopes—which includes our digestive tract for instance—is still not commonly accepted. During an excursion to a Swiss lake, I recently heard a perfect exposition of aquatic food chains by a young biologist: to my great surprise he started with the plankton and completely omitted the important role of micro-organisms in feeding and thus sustaining it. This narrow view has certainly something to do with the obsolete taxonomy, which, as we have seen, is restricted to pathogenic micro-organisms or to those few others that are responsible for alimentary fermentation. And non-medical microbiology faces yet further adversity. Molecular biology, before it got its name, was based on studies of bacterial genetics and bacteriophages, generally carried out by microbiologists. Gene technology and

**The interest in microbiology will grow as soon as expectations become confirmed that micro-organisms produce substances beneficial in the therapy of human diseases or in industrial applications**

to order the micro-organisms responsible for infectious diseases in humans, plants and animals. But only about 5000 prokaryotic organisms have so far been formally described, which represents barely one percent of the existing bacterial species (Pace, 1997). This situation will probably

They still dwell where higher organisms cannot survive, not only on the surface, but also deep in the oceans and in the earth. But even today, the popular view of micro-organisms is focused nearly exclusively on those that cause disease. Few are aware of their additional benefits, namely in the fer-

medical virology, fields of major importance today, are derived from these early studies and genetic engineering has become the main tool in biochemical and biomedical research. But neither for teach-

the genomes of numerous pathogens have already been sequenced, particularly by The Institute for Genomic Research, TIGR, in Rockville, MD, and the Sanger Centre in Hinxton, UK, and there is an

the reverse of what has been done in the past with micro-organisms: species belonging to taxa such as *Bacillaceae* and *Enterobacteriaceae* were strongly over-emphasised because they occur frequently and can be easily cultivated.

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ing gene technology, nor for growing the few 'work horses'—*Escherichia coli*, *Bacillus subtilis* or yeast—for the purpose of cloning, do researchers need microbiologists any more.

Two decades ago, one considered as 'alive' only those micro-organisms that could be cultivated under laboratory conditions. But increasing evidence, involving more sophisticated criteria, leads to the notion that 'dormant' bacteria exist that are not 'cultivable' (Colwell and Grimes, 2000). In a few instances, the conditions to 'wake them up' so as to form colonies on a solid medium have already been found, but in general conditions must be experimentally generated for every single strain. Indeed, there is a lot of work waiting for us, for instance, in characterising the approximately  $10^7$  bacterial species present in one gram of soil, of which only 1 to 10 % are estimated to be cultivable (Nalin *et al.*, 1998).

Consequently, it is not feasible to investigate the richness of bacterial species by using the classical methods of growing isolated cells into a colony in order to study their properties. But new methods coming from molecular biology will allow us to overcome some of these limitations. With such techniques available, a new approach carried out on large genomes is feasible. The totality of all individual genomes in a biotope can be considered as one single large, biotopical, genome. To characterise them, the fastest, but rather expensive, method is the 'shot-gun' approach used by Craig Venter to sequence the human genome—random PCR fragments are sequenced and then, utilising overlapping regions, reassembled. Using this procedure, linkage groups—chromosomes—appear quasi automatically. In the case of 'biotopical genomes', the chromosomes of each individual species would emerge as separate entities, assuming one chromosome per cellular genome. However,

increasing economic interest in sequencing microbial genomes not only from pathogens but also from other, as yet unknown bacteria.

Another indication for the growing interest in microbial genomes are TIGR's plans to create a comprehensive microbial resource containing the sequence and annotation of each of the completed genomes, as well as associated information about the organisms. They hope to discover new prokaryotes with beneficial properties or devise new medications by analysing the genomes of pathological micro-organisms.

But microbiology itself has already been undergoing very fundamental, almost revolutionary change during the past two decades, notwithstanding the new sequencing techniques. These changes were triggered by new methodologies of studying kinships by means other than comparative morphology and biochemistry. Woese (Woese *et al.*, 1990; Woese, 1994) introduced comparisons of the 16S ribosomal RNA sequences, or corresponding DNA, for establishing phylogenetic trees. The results obtained made it clear that living organisms can be distinguished into three primary 'urkingdoms' or 'domains': the Eucarya, the Bacteria and the Archaea. The separation of prokaryotes into Archaea and Bacteria was a major step forward and has helped to solve numerous classification problems. It clarified properties and compositional differences which had previously been vague or even confused, such as the variety of DNA-binding proteins in chromatin (Li *et al.*, 1998; Bendich and Drlica, 2000).

This new taxonomy now allows microbiologists to progress in the study of micro-organisms in the same way their colleagues did previously with the study of higher organisms, by first selecting representatives of taxa with sufficiently distant kinships. In this manner, the biodiversity gets broadly surveyed. This is exactly

the reverse of what has been done in the past with micro-organisms: species belonging to taxa such as *Bacillaceae* and *Enterobacteriaceae* were strongly over-emphasised because they occur frequently and can be easily cultivated. Despite today's anthropocentric trend, many microbiological publications have appeared lately, largely stimulated by a report by the American Academy of Microbiology in 1997 (Staley *et al.*, 1997; Young, 1997). No month passes without some new micro-organisms being discovered, many of them having new, hitherto unknown properties. An example is the discovery of a new form of phototrophy in two widespread aerobic bacteria in surface waters of oceans (Beja *et al.*, 2000; Kolber *et al.*, 2000). One of these recently discovered organisms has a new variant of light-collecting rhodopsin. These bacteria harvest substantial amounts of energy and are supposed to play a very

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important role as a starter of the food chain that continues with plankton. Micro-organisms have also been found deep under our planet's surface, where they grow under anaerobic conditions. They seem to be responsible for numerous known and potential biological activities, in particular by living on, and modifying, mineral substrates (Ehrlich, 1996). Indeed, the initial role of micro-organisms as the first inhabitants of our planet was to render the surface of the Earth suitable for the evolution of other life forms yet to come. By using molecular techniques, the 'new microbiology' will also give a fresh stimulus to this 'biogeochemistry'.

But many research fields in microbiology, although profiting from the new methods of genetic engineering, still have to be revolutionised in the same way as research on disease-causing prokaryotes has been. In line with the medically-oriented focus of biological research, a large effort is being made to study the mechanisms of infection of human cells by pathogenic bacteria (Niebuhr and Sansonetti, 2000). However, surprisingly little has been published on the essential and beneficial roles of human

intestinal bacteria (Bertschinger, 1997). Realising that there is a huge number of different microbial species that form living communities among themselves and with other animals or plants, the interest in studying these partnerships has greatly increased. Indeed, the non-medical microbiology community has not only studied this symbiotic partnership in ruminants, as can be found in any textbook, but even in protozoa (Görtz and Brigge, 1998).

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Two important symbioses—those of fungi with the roots of plants by forming ‘mycorrhiza’ and those of nitrogen-fixing bacteria with some plant species—have great potential for agricultural applications. These partnerships allow the plants to grow with atmospheric nitrogen in exchange for nutrients for the symbiotic micro-organisms. The initial hope of defining all the genes involved in nitrogen fixa-

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tion in the symbiont in order to transfer them into crops was not fruitful. The other approach, however, through better understanding of the mechanisms of communication between the symbiont (the bacteria) and its host (the plant) has made very promising progress. New nitrogen-fixing bacteria have been discovered with as yet undomesticated plants. These micro-organisms could be adapted to domesticated crops in order to enhance their yield (Egener and Reinhold-Hurek, 2000). But not only are synergic communities very frequent between micro-organisms, plants and animals, but also between themselves, as exemplified by the biofilms (Costerton *et al.*, 1994). It is likely, that many micro-organisms are able to grow only when in a community with others.

Without question, both new and traditional microbiology have been fundamentally reshaped by the methods of

gene technology. In addition to large-scale DNA sequencing and PCR, ‘biological chips’ (i.e. micro-arrays of DNA- or protein-probes) will play an important role in discovering and characterising new potentially useful micro-organisms.

These new approaches have also demonstrated that the living cell is not simply a bag of individual genes and their products. Rather, it is the result of a complex network of numerous components in which modifying the amount of one single product can influence many other metabolites—an understanding that could have been reached much earlier. Understanding these networks and the co-operation of a cell’s basic ‘household’ genes is obviously easier using the small and well-known genes of micro-organisms, rather than the human genome.

A suitable ending for this essay might be a quote from Arthur Kornberg, who received the Nobel prize for medicine in 1959 (Kornberg, 1997). He recommended: ‘Increased attention to the microbial world. Neglect of microbial research in recent decades has revealed, through novel and drug-resistant microbial diseases, that we, as animals, are simply guests in a microbial world. Biological and chemical studies of microbes, includ-

ing those that thrive at extremes of temperature, pressure and pH, will provide, as in the past, profound insights into biochemical mechanisms, the means to avoid or combat microbial diseases, reagents for industrial processes and new approaches to improve agriculture.’

## References

- Beja, O. *et al.* (2000) Bacterial rhodopsin: evidence for a new type of phototrophy in the sea. *Science*, **289**, 1902–1906.
- Bendich, A.J. and Drlica, K. (2000) Prokaryotic and eucaryotic chromosomes: what is the difference? *BioEssay*, **22**, 481–486.
- Bertschinger, H.U. (1997) Die Magendarmflora, eine Herausforderung für Mikrobiologen. *LaboLife*, **6**, 29–32.
- Colwell, R.R. and Grimes, D.J. (2000) *Nonculturable Microorganisms in The Environment*. ASM Press, Herndon, pp. 360.
- Costerton, J.W., Lewandowski, Z., DeBeer, D., Caldwell, D., Korber, D. and James, G. (1994)

- Biofilms, the customized microniche. *J. Bacteriol.*, **176**, 2137–2142.
- Egener, T. and Reinhold-Hurek, B. (2000) Endophytic expression of *nif* genes of *Azoarcus sp.* BH72 in rice roots. *Mol. Plant-Microbe Interact.*, **12**, 813–819.
- Ehrlich, H.L. (1996) In *Geomicrobiology*. Marcel Dekker, New York, NY.
- Görtz, H.-D. and Brigge, T. (1998) Intracellular bacteria in protozoa. *Naturwissenschaften*, **85**, 359–368.
- Kolber, Z.S., Van Dover, C.L., Niederman, R.A. and Falkowski, P.G. (2000) Bacterial photosynthesis in surface waters of the open ocean. *Nature*, **407**, 177–179.
- Kornberg, A. (1997) Centenary of the birth of modern biochemistry. *Trends Biochem. Sci.*, **22**, 282–283.
- Li, J.-Y., Arnold-Schulz-Gahmen, B. and Kellenberger, E. (1998) Histones and histone-like DNA-binding proteins: Correlations between structural differences, properties and functions. *Microbiology*, **145**, 1–2.
- Mayeno, A.N. and Gleich, G.J. (1994) Eosinophilia-myalgia syndrome and tryptophan production: a cautionary tale. *Trends Biotechnol.*, **12**, 346–352.
- Nalin, R., Ranjard, L., Nazaret, S. and Simonet, P.W. (1998) La biologie moléculaire en écologie microbienne du sol: application à l’analyse de la structure des communautés bactériennes. *Bull. Soc. Fr. Microbiol.*, **13**, 21–26.
- Niebuhr, K. and Sansonetti, P. (2000) Invasion of epithelial cells by bacterial pathogens the paradigm of *Shigella*. *Sub Cell. Biochem.*, **33**, 251–287.
- Pace, N.R. (1997) A molecular view of microbial diversity and the biosphere. *Science*, **276**, 734–740.
- Staley, J.T., Castenholz, R.W., Colwell, R.R., Holt, J.G., Kane, M.D., Pace, N.R., Salyers, A.A. and Tiedje, J.M. (1997) *The Microbial World*. American Society of Microbiology, Washington D.C., USA.
- Woese, C.R. (1994) There must be a prokaryote somewhere: microbiology’s search for itself. *Microbiol. Rev.*, **58**, 1–9.
- Woese, C.R., Kandler, O. and Wheelis, M.L. (1990) Towards a natural system of organisms: Proposal for the domains Archaea, Bacteria, and Eucarya. *Proc. Natl Acad. Sci. USA*, **87**, 4576–4579.
- Young, P. (1997) Major microbial diversity initiative recommended. *ASM News*, **63**, 417–421.



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