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MicroCommentary

Electron cryotomography reveals novel structures of a recently cultured termite gut spirochete

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Summary

Electron cryotromography, a relatively new methodology in the field of microbiology, has been exploited by Murphy *et al*. (in this issue of *Molecular Microbiology*) in their analysis of the recently isolated termite gut spirochete *Treponema primitia*. Unique structures (bowls, arcades of hooks, cones at the cell ends, two layers of wall material) were evident from the analysis of its surface and internal constituents. These results, coupled to video microscopy analysis of swimming cells, allowed the authors to propose a model of cell motility. This highly significant paper highlights the importance of electron cryotomography to the field of microbiology. It also illustrates that newly cultured recalcitrant bacteria from complex environments are likely to possess novel structures not previously seen in other species.

The accompanying paper by Murphy *et al*. (2008) is a harbinger of a new exciting breakthrough in microbiology. This breakthrough is bringing together two recent developments. The first development is related to microbial ecology and nutrition. Over 300 years ago, Antonie van Leeuwenhoek first observed microorganisms with his home-made microscopes (Dobell, 1960). Since that time, a myriad of different species of bacteria have been cultured, and many have been analysed in detail with respect to physiology, structure and genetics. However, we now estimate that between 90% and 99% of the bacterial species in nature have yet to be grown in the laboratory (Leadbetter, 2003). In a real sense, we are just beginning to grasp the diversity of the microbial world.

Recent work has shown that many of those bacteria thought to be uncultivable can now be grown in culture (Leadbetter, 2003; Stevenson *et al*., 2004). We can finally begin to analyse these newcomers to the lab in some detail. These species often grow very slowly, with generation times measured in days. In addition, they often do not reach noticeable turbidities, so new and quite ingenious methods have been and are being developed for both culturing these species and assaying for their multiplication. Besides creativity and a firm background in physiology, one inherent quality of the investigator in isolating and culturing these species is patience. The spirochete *Treponema primitia* is illustrative: *T. primitia* is the first spirochete isolated in pure culture from the complex environment of the termite gut (Leadbetter *et al*., 1999; Graber *et al*., 2004; Graber and Breznak, 2004). The type strain is small (0.2×3–$7\ \mu m$), has a relatively small genome (3.8 kb), is anaerobic and has a generation time of 29 h. It requires complex media and an atmosphere containing H_2 for growth. Its species naming says volumes: As stated in its description (Graber *et al*., 2004), *primitia* stands for 'first fruit (of isolation after long work)'!

The second development relates to new methods for closely examining the overall ultrastructure and substructures of microorganisms. Approximately 180 years ago, Robert Brown looked through a microscope and saw the jittery motion of pollen grains. True, it was not the fundamental life force that Brown originally thought that it might be, but it took less than 80 years for the atomic hypothesis to be widely accepted owing to the behaviour seen in that simple observation. Richard Feynman would later say that this hypothesis, that all things are made of atoms, is the one statement that contains the most scientific knowledge in the fewest amount of words (Feynman *et al*., 1964). A lot can be gained by looking very closely and meticulously at something.

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Only in recent years has cryo-transmission electron microscopy of hydrated cells been used to look closely at bacteria that are *frozen-in-animation* (see for example Matias and Beveridge, 2007; Ting *et al*., 2007; Zhang *et al*., 2007; and a recent review by Jensen and Briegel, 2007). This exciting new methodology allows us to see structures as they are in the living cell. By coupling cryo-transmission electron microscopy with tomography (electron cryotomography or ECT), which is relatively new to the field of microbiology (less than 20 papers published at this time), one is able to examine cells with a three-dimensional perspective at macromolecular resolution. It also allows for observation of nm slices throughout the cells; thus, a clearer image emerges as compared with, for example, frozen cells observed directly on grids. Furthermore, image averaging coupled with ECT allows one to examine structures in exquisite detail. Again, *T. primitia* has emerged at the forefront, primarily because it is so small. Thus, in a recent publication, Murphy *et al*. (2006) characterized its flagellar basal–body complex including the stator in detail; it is first time that this complex has been seen to be associated with the stator *in situ* in any bacterial species.

In the new paper by Murphy *et al*. (2008), ECT was used to examine the overall structure of *T. primitia*. These results were then related to its mechanism of cell movement. Members of the spirochete phylum are best known for their helical or flat-wave morphologies and their ability to swim though highly viscous gel-like media that slow down or stop other species of bacteria (Charon and Goldstein, 2002). These ubiquitous bacteria, which vary tremendously in size, are present in salt and fresh water, soil, in termite and mammalian guts, oral cavities of mammals, in clams, ticks, lice and in annelids of hyperthermal vents. (Holt *et al*., 1994; Saier and Garcia-Lara, 2001; Charon and Goldstein, 2002). Some cause disease, others are parasitic, and still others are even commensal. Some of the *Treponema* species in the termite gut fix atmospheric nitrogen and help contribute this essential nutrient to its arthropod host (Graber *et al*., 2004). Spirochetes are also recognized for the unique placement of their flagella, which is inside the periplasmic space. These flagella (referred to as periplasmic flagella) are structurally similar to the flagella of other bacteria, and are anchored subterminally to the cell wall-inner membrane by attachment to motors. Rotation of these periplasmic flagella within this space drives their motility (Charon and Goldstein, 2002).

Murphy *et al*. report some remarkable structures in *T. primitia* (see, for example, fig. 2B and D in Murphy *et al*., 2008). It has a more complex array of structures – both internally and on its surface – than has been reported for any other spirochete. One of the most intriguing features is a porous cone-like structure located in the periplasmic space at the tip of the cell. While the authors are unclear about the physiological significance of these structures, they propose an interesting hypothesis. As the flagella are anchored subterminally, they do not enter into the cone region. Therefore, the cone could act to stabilize the periplasm at the ends of the cell, thereby allowing channels to form between the inside and outside of the cell without interference with the rotating flagella.

Treponema primitia was also found to have an ornately adorned surface. Polar fibrils are seen at its tips, and bowl-shaped structures and arcades of hooks are evident along its surface. These structures have not been previously reported for any spirochete, nor to our knowledge, have identical bowl-shaped structures and arcades of hooks been observed in other bacterial species. *T. primitia* is unique among the spirochetes, as the surface of spirochetes is typically an unadorned outer membrane. The functions of these various structures can only be speculated upon at this point. However, the hindgut of termites is a remarkably complex ecosystem, and as the decorated surface of *T. primitia* suggests, complex interactions occur between this spirochete and its environment.

Another striking feature reported for the first time is the presence of a second cell wall just inside the outer membrane. The periplasmic flagella are observed to reside between the two cell wall regions. The composition and function of this second cell wall is not clear; however, the video light microscopy of swimming cells presented in the supplemental information suggests a possible role of this material. Rotation of the flagella in a number of spirochetes, such as *Borrelia burgdorferi* and *Leptonema* (formerly *Leptospira*) *illini*, induces deformations of the cell cylinder that are important for the motility of these species (Charon and Goldstein, 2002). *T. primitia*, however, was observed to swim as a rigid helix, without noticeable deformations or gyrations. The additional cell wall in these cells might provide added structural integrity that prevents rotation of the flagella from inducing deformation of the cell cylinder. In addition, it might help stabilize the lipid outer membrane sheath so the flagella can roll against this structure, producing counter-rotation of the secondary cell wall and outer membrane sheath. Thus, the proposal by Murphy *et al*. for *T. primitia*, which is similar to one proposed several years ago for *Spirochaeta aurantia* (Berg, 1976), postulates that the cell cylinder rotates in one direction, while the outer membrane sheath and secondary wall rotate in the opposite direction.

Many questions remain unanswered. Will these organisms be amenable to genetic manipulation? As a corollary, can the functions of the numerous novel structures on and within these spirochetes be identified? How do the relatively large (45 nm in diameter) bowl-shaped structures assemble on the surface? Do the outer surface and cell

cylinder of *T. primitia* rotate in opposite directions as predicted by the motility model? In more general terms, will the newly cultivated bacteria also open new vistas relating to bacterial structure? And finally, will ECT emerge as the prevailing technology for bacterial ultrastructural and substructural analysis? Clearly, microbiology is again starting anew.

References

Berg, H.C. (1976) How spirochetes may swim. *J Theor Biol* **56:** 269–273.

Charon, N.W., and Goldstein, S.F. (2002) Genetics of motility and chemotaxis of a fascinating group of bacteria: the Spirochetes. *Annu Rev Genet* **36:** 47–73.

Dobell, C. (1960) *Antony van Leeuwenhoek and His Little Animals*. New York: Dover.

Feynman, R.P., Leighton, R.B., and Sands, M. (1964) *The Feynman Lectures on Physics.* Reading, MA: Addison-Wesley.

Graber, J.R., and Breznak, J.A. (2004) Physiology and nutrition of *Treponema primitia*, an H_2/CO_2-acetogenic spirochete from termite hindguts. *Appl Environ Microbiol* **70:** 1307–1314.

Graber, J.R., Leadbetter, J.R., and Breznak, J.A. (2004) Description of *Treponema azotonutricium* sp. nov. and *Treponema primitia* sp. nov., the first spirochetes isolated from termite guts. *Appl Environ Microbiol* **70:** 1315–1320.

Holt, J.G., Krieg, N.R., Sneath, P.H.A., Staley, J.T., and Williams, S.T. (1994) The *Spirochetes*. In *Bergey's Manual of Determinative Bacteriology.* Baltimore: Lippincott Williams & Wilkins, pp. 27–31.

Jensen, G.J., and Briegel, A. (2007) How electron cryotomography is opening a new window onto prokaryotic ultrastructure. *Curr Opin Struct Biol* **17:** 260–267.

Leadbetter, J.R. (2003) Cultivation of recalcitrant microbes: cells are alive, well and revealing their secrets in the 21st century laboratory. *Curr Opin Microbiol* **6:** 274–281.

Leadbetter, J.R., Schmidt, T.M., Graber, J.R., and Breznak, J.A. (1999) Acetogenesis from H_2 plus CO_2 by spirochetes from termite guts. *Science* **283:** 686–689.

Matias, V.R., and Beveridge, T.J. (2007) Cryo-electron microscopy of cell division in *Staphylococcus aureus* reveals a mid-zone between nascent cross walls. *Mol Microbiol* **64:** 195–206.

Murphy, G.E., Leadbetter, J.R., and Jensen, G.J. (2006) *In situ* structure of the complete *Treponema primitia* flagellar motor. *Nature* **442:** 1062–1064.

Murphy, G.E., Matson, E.G., Leadbutter, J.R., Berg, H.C., and Jensen, G.J. (2008) Novel ultrastructures of *Treponema primitia* and their implications for motility. *Mol Microbiol* (in press).

Saier, M.H. Jr and Garcia-Lara, J. (2001) *The Spirochetes, Molecular and Cellular Biology*. Norfolk: Horizon Press.

Stevenson, B.S., Eichorst, S.A., Wertz, J.T., Schmidt, T.M., and Breznak, J.A. (2004) New strategies for cultivation and detection of previously uncultured microbes. *Appl Environ Microbiol* **70:** 4748–4755.

Ting, C.S., Hsieh, C., Sundararaman, S., Mannella, C., and Marko, M. (2007) Cryo-electron tomography reveals the comparative three-dimensional architecture of *Prochlorococcus*, a globally important marine cyanobacterium. *J Bacteriol* **189:** 4485–4493.

Zhang, P., Khursigara, C.M., Hartnell, L.M., and Subramaniam, S. (2007) Direct visualization of *Escherichia coli* chemotaxis receptor arrays using cryo-electron microscopy. *Proc Natl Acad Sci USA* **104:** 3777–3781.

