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Molecular biology and genomics of *Mollicutes*

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Abstract

A succinct account of recent information about mycoplasmas and *Mollicutes* in general and phytoplasmas in particular is presented. The significant accumulation of knowledge resulting from the extensive application of molecular biology- based methodology is reported and discussed with suggestions for future directions of research expected to promote understanding of the cell biology of the phytoplasmas and the diseases caused by them.

Key words: Phytoplasmas, genomics, mycoplasmas, evolution.

Introduction

There is now solid genetic support for the thesis that *Mollicutes* evolved as a branch of gram-positive bacteria by a process of reductive or degenerative evolution. During this process, the mollicutes lost a considerable portion of their ancestors' chromosomes but retained the genes essential for life. The significant genome compaction that occurred was made possible by adoption of a parasitic mode of life. The supply of many nutrients from their hosts resulted in the loss, during mollicutes evolution, of the genes for many assimilative processes.

The marked dependency on the exogenous supply of nutrients has hampered the *in vitro* cultivation of mollicutes, as reflected by the total inability to grow many mollicutes in axenic cultures, most noticeable in this respect are the phytoplasmas (figure 1) (Razin *et al.*, 1998; Razin and Herrmann, 2002).

There can be little doubt that mollicutes are successful evolutionary creations; their wide distribution in nature points to this conclusion. Animal mycoplasmas are commensals or benign pathogens, causing mostly mild and chronic infections but rarely killing their host. It is possible that mollicutes evolution is advancing toward symbiosis. Thus, spiroplasmas and phytoplasmas usually have little or no pathogenic effects on their insect vectors, although when transmitted to plants, they usually cause disease.

Discussion

A major question to be asked is why can't we cultivate phytoplasmas? Is it related to a particular small genome size? The answer is not so simple. Though Bermuda grass phytoplasma appears to carry the smallest cellular genome of 530 kb (Marcone *et al.*, 1999) the genome size range of other phytoplasmas resembles that of the cultured mycoplasmas. Thus, genome size alone cannot explain the inability to culture phytoplasmas. The explanation most probably depends on the lack of essential genes and functions from the phytoplasmas. The relatively large genome of some phytoplasmas appears to be the result of the occurrence of repeated regions within phytoplasma genomes (Bai *et al.*, 2006). The repeats are organized in large clusters of potential mobile units (PMUs) which contain transposases, insertion sequences and genes for specialized sigma factors. The PMUs appear to be unique to phytoplasmas. They are responsible for genomic variability. The dynamic genome enables the phytoplasmas to adjust to the diverse environments of plants and insects, as well as leading to the marked heterogeneity of genome sizes noticed within closely related phytoplasma strains (Bai *et al.*, 2006; Jomantiene and Davis, 2006).

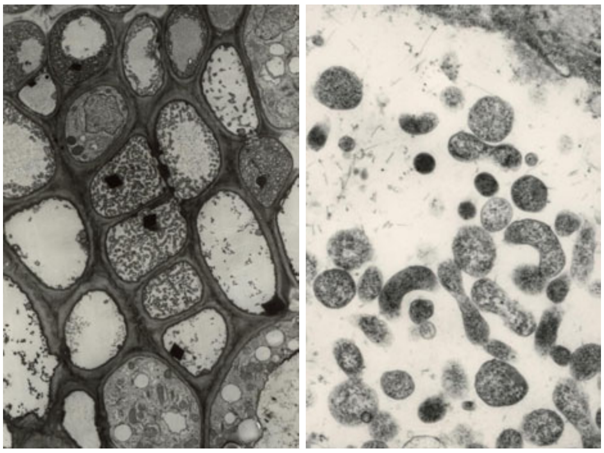

Figure 1. Electron microscope pictures of phytoplasmas in cross section. Magnification: left X 10,000; right X 20,000 (courtesy of A. Bertaccini).

The recent sequencing and annotation of several phytoplasma genomes revealed several significant differences in gene content as compared to animal mycoplasmas. Most pronounced is the lack in phytoplasmas of the F-type ATPase (Oshima *et al.*, 2004) responsible for generating membrane potential and consequently ATP. Whether the phytoplasmas are capable of importing host ATP or use a P-type ATPase (Na+/K+ or H+ pumps) to generate the electrochemical gradient like animals, fungi and plants, is a moot point (Bai *et al.*, 2006). Phytoplasmas lack also the PTS systems for import of sugars essential for glycolysis. However, they have a maltose ABC transport system. The maltose binding protein may have affinity to maltose, trehalose, and sucrose. Trehalose is a major sugar of the insect hemolymph. However, enzymes for converting these sugars to glucose-6-phosphate were not found in the phytoplasmas (Christensen *et al*., 2005). Can the phytoplasmas take up phosphorylated hexoses from the host? Can they use malate as a carbon source by converting it to oxalacetate and pyruvate (Bay *et al*., 2006)? These are possibilities to be investigated further.

To keep the parasitic mode of life, mycoplasmas have developed rather sophisticated mechanisms to colonize their hosts and resist the host immune system. A genomic price had to be paid for these functions, as reflected by the significant number of genes in animal mycoplasmas devoted to adhesion, attachment organelles and generation of antigenic variation systems. In most phytoplasmas a subset of membrane proteins, referred to as immunodominant membrane proteins (IDPs) constitutes a major portion of the total membrane proteins. These proteins have one or two hydrophobic transmembrane regions and a hydrophilic region on the phytoplasma cell surface. These proteins apparently function as adhesins, responsible for the attachment of the phytoplasma to the host cell. Adhesion is a prerequisite for colonization and infection as is the case with animal mycoplasmas (Razin *et al*., 1998; Christensen *et al*., 2005; Kakizawa *et al*., 2006a; 2006b). The phytoplasmal adhesins seem to attach to a ligand in the gut of the insect vector built of a complex made of actin, and light and heavy myosin chains (Suzuki *et al*., 2006). Resembling mycoplasmal adhesins, the phytoplasmal IDPs show significant antigenic variability, suggesting the occurrence of selective adaptation by the parasite to avoid the insect vector immune system.

The relative structural simplicity and lack of a cell wall have been major factors favouring the use of mycoplasmas as models in studies on membrane structure and function. The easy manipulation of the composition of mycoplasmal membrane lipids has been exploited most effectively to elucidate the mode of organization and physical state of membrane lipids, including cholesterol (Razin and Herrmann, 2002). During their evolution and adaptation to a parasitic mode of life, the mycoplasmas have developed various genetic systems providing a highly plastic set of variable surface proteins. The uniqueness of the mycoplasmal systems is manifested by the presence of highly mutable modules combined with an ability to expand the antigenic repertoire by generating structural alternatives, all compressed into limited genomic sequences. In the absence of a cell wall and periplasmic space, the majority of surface proteins involved in generating antigenic variation in animal mycoplasmas are lipoproteins. These surface components, anchored to the cell membrane via acyl chains, are the most dominant antigens, and their abundance in the mycoplasma membrane is remarkable. As mentioned above, the phytoplasmal adhesins studied thus far are integral membrane proteins and not lipoproteins.

Do mycoplasmas represent minimal cells? It appears that *Mycoplasma genitalium* is, thus far, the organism closest to the theoretical minimal cell capable of self-replication. It has been of great interest to define the minimal set of essential genes in *M. genitalium* by selective inactivation or deletion of genes, testing the effects of each of these manipulations on survival and replication under defined conditions. According to Craig Venter and his associates the minimal set of genes essential for life of *M. genitalium* consists of 382 genes (Glass *et al*., 2006). Craig's idea is to insert this minimal set of genes into ghosts of *M. genitalium* cells depleted of their original genomic DNA. This would result in a new, still hypothetical organism, named by Venter *Mycoplasma laboratorium*. This hypothetical organism was recently even patented. An interesting approach to reach this goal was taken by this group very recently (Lartigue *et al*., 2007). They succeeded in replacing the genome of *Mycoplasma mycoides* with the genome of *Mycoplasma capricolum* using polyethylene glycol mediated transformation.

Clearly, definition of the minimal set of essential genes depends to some extent on growth conditions, e. g., whether the minimal cell is growing *in vitro* or in the host, where adhesion and antigenic variation genes are expected to play an essential role. It should be emphasized that even with the smallest sequenced genomes of *M. genitalium* and *M. pneumoniae*, there is still a great deal to do in order to identify the unclassified ORFs that have no data base match, prove the DNA-based predictions experimentally, and assign functions to proposed ORFs with hitherto unknown functions. Cell protein analysis, applying the proteome approach, and extensive tests of metabolic activities, are obviously some of the directions to take.

An important point to be emphasized in conclusion: data on the molecular biology and genomics of phytoplasmas are still rather scanty and fragmentary being based on analysis of only few sequenced phytoplasma genomes. Thus, one should be careful at the present time of drawing sweeping generalizations concerning the entire Phytoplasma clade.

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