

MYCOPLASMAS

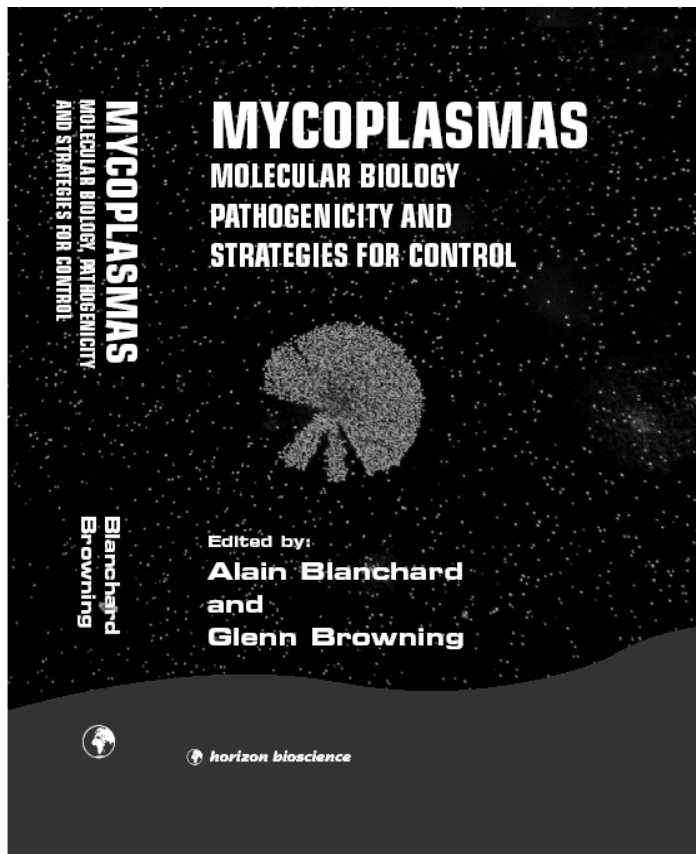
MOLECULAR BIOLOGY PATHOGENICITY AND STRATEGIES FOR CONTROL

The mycoplasmas, or mollicutes, form a large group of bacteria that can infect humans, animals, and plants. Leading international mycoplasmatologists have created this comprehensive and authoritative reference text that focuses not only on the molecular and cell biology of mycoplasmas and related mollicutes, but also on the pathogenesis and emerging strategies for control. The book represents a cutting edge summary of current knowledge in this topical field. Topics covered include genome analysis, gene vectors, genomics, motility, chemotaxis, attachment, molecular epidemiology, immunology, diagnosis, antimicrobial resistance, and vaccine technology.

Essential reading for all scientists with an interest in mycoplasmas and a recommended volume for all microbiology laboratories.



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Mycoplasmas: Molecular Biology, Pathogenicity and Strategies for Control

Chapter Abstracts

Section 1: Molecular Biology

Chapter 1 OriC Plasmids as Gene Vectors for Mollicutes

Joël Renaudin and Carole Lartigue

Genomes of mollicutes have been among the very first to be fully sequenced. However, due to the lack of suitable gene transfer systems, genetic studies have been limited to a few mollicute species. In the plant pathogen *Spiroplasma citri*, artificial *oriC* plasmids were engineered to act as vectors for expression of cloned genes and for specific gene targeting through homologous recombination, leading to the identification of pathogenicity determinants. Putative *oriC* regions have also been identified in the mycoplasma and ureaplasma genomes that have been sequenced. With the exceptions of *Mycoplasma pneumoniae*, *Mycoplasma penetrans* and *Ureaplasma urealyticum* (*parvum* biovar), they all contain several *dnaA* boxes in the vicinity of the *dnaA* gene. The putative *oriC* regions of *Mycoplasma pulmonis*, *Mycoplasma mycoides* subspecies *mycoides* large colony type (MmmLC), *M. mycoides* subspecies *mycoides* small colony type (MmmSC) and *Mycoplasma capricolum* subsp. *capricolum* (*M. capricolum*) have been shown to promote plasmid replication in their respective hosts. Studying host specificity revealed that *S. citri* and *M. pulmonis* could only be transformed by the corresponding *oriC* plasmids. In contrast, *M. capricolum* could be transformed by the *oriC* plasmids from mycoplasmas of the *mycoides* cluster and also by the *oriC* plasmid from *S. citri*. By using disruption vectors based on *oriC* plasmids, the *hlyA* gene of *M. pulmonis* and the *lppA* genes of MmmLC and *M. capricolum* have been successfully targeted. Thus *oriC* plasmids have considerable potential as useful genetic tools in a range of mollicutes.

Chapter 2 Genome Analysis: Recombination, Repair and Recombinational Hotspots

Eduardo Rocha, Pascal Sirand-Pugnet, and Alain Blanchard

The very small genomes of mycoplasmas lack a substantial part of the standard recombination and repair mechanisms available in the larger genomes of *Escherichia coli* and *Bacillus subtilis*. However, they contain large numbers of repeated DNA sequences. Thus, although these genomes seem to suffer a strong selective pressure for minimizing their genome sizes, they cope with a substantial amount of repeated sequences that may be vital for adaptation and chronic colonization of their hosts. Here, we analyze repair and recombination mechanisms in mycoplasmas as they can be inferred from the genes identified in the complete genomes. This analysis indicates that some of the basic machinery which exist in *B. subtilis* and *E. coli* is missing in mycoplasmas. Since homologous recombination has been described in several mollicutes, there is a clear lack of understanding of how recombination can take place in these genomes. On the other hand, although many standard repair pathways are absent or minimized in mycoplasma, these genomes contain the minimal set of proteins able to repair every major type of DNA lesions or replication errors. Finally, because repeats are targets of recombination processes and a basis for genetic variation strategies in mycoplasma, we also describe the current knowledge of repeats present in the six fully sequenced genomes. We then try to place these results in relation to what is known of mycoplasma molecular biology and pathogenicity.

Chapter 3 Resources for Mining Mollicute Genomes

Aurélien Barré, Antoine de Daruvar, Alain Blanchard, and Pascal Sirand-Pugnet

Since the first bacterial genome sequence was published in 1995, eight genomes of species belonging to the class Mollicutes have been completely sequenced and many others should be released soon in public databanks. Access to complete genome sequences offers new possibilities for formulating and investigating biological questions. Here we present an overview of some of the main bioinformatics resources that can be used by biologists interested in mollicute genomics. These resources are mostly genome sequence databanks and tools dedicated to their exploration. Genome sequences of mollicutes are available from general sequence databanks and, in some cases, from specific web-sites. To manipulate these data, biologists can use the basic exploration functionalities available through standard interfaces to genome databanks. More sophisticated tools have been developed for multi-genome queries or for specific projects such as metabolic pathway reconstruction. In this

chapter, we first describe the possibilities offered on different web-sites for retrieval of sequence and annotations. Possibilities for formulation of single and multi-genomes queries are presented, as well as the data and links accessible from the result page. In addition, tools have been developed for comparative genomics. They are dedicated to graphical visualisation of multiple genomes, whole genome alignments, differential queries of multiple proteomes and comparisons of metabolic and cellular pathways. Finally, we present new web-sites that contain data about some species of mollicutes and we discuss the expected evolution of databanks and associated tools.

Chapter 4 **Phytoplasma Genomics**

Shigetou Namba, Kenro Oshima, and Karen Gibb

The phytoplasmas are a group of plant pathogenic bacteria that cause devastating damage to plants. They can propagate intracellularly in both insect and plant hosts. Despite their economic importance and unique biological features, phytoplasmas remain the most poorly characterized plant pathogens. To shed light on these microorganisms, we obtained a draft sequence of the Onion Yellowings phytoplasma (*Candidatus Phytoplasma asteris*; OY) covering the majority of the genome. The chromosome encodes genes for basic housekeeping functions, such as DNA replication, transcription and translation, but none for amino acid or fatty acid biosynthesis, the TCA cycle, or oxidative phosphorylation. The phytoplasma probably cope with the lack of these genes because it can import many biological substances from its host cells, as is the case with parasitic mycoplasmas. Surprisingly, the phytoplasma genome encodes even fewer genes for metabolic functions than that of mycoplasmas, which are known to possess minimal gene sets; genes for the pentose phosphate cycle, conserved in the genomes of all other reported bacteria, were not found in the phytoplasma genome. Phytoplasmas appear to possess the most minimal set of metabolic pathways identified in an organism to date; this minimalism may be related to the fact that phytoplasmas inhabit the nutrient-rich environment of the phloem. Phytoplasma does not possess the typical genes related to pathogenicity found in other phytopathogenic bacteria. However, many unknown genes exist in the genome may be related to pathogenicity via their unique metabolic properties, such as actively importing host metabolites and/or affecting normal cellular functions. As this manuscript was "in press", we published the complete genomic sequence of the OY phytoplasma.

Section 2: Cell Biology

Chapter 5 **Gliding Motility of Mycoplasmas: the Mechanism Cannot be Explained by Current Biology**

Makoto Miyata

Many mycoplasma species bind to glass surfaces and exhibit movement while maintaining their binding, a process known as gliding motility. They form a small membrane protrusion at a cell pole and move in the direction of the protrusion. Genomic sequencing and analysis has revealed that the mechanism must differ from those of other bacterial motility and motor protein systems. *M. pneumoniae* glides at 0.3-0.4 $\mu\text{m/s}$. The protrusion, known as the attachment organelle, is a large structure with a central rod, into which more than 8 proteins are integrated. *M. mobile* glides at 2.0-4.5 $\mu\text{m/s}$. Its gliding machinery is composed of a complex of three huge proteins coded in tandem on the genome. Four hundred units of this machinery are located around the base of protrusion, an area designated the "neck". Rapid freeze and fracture electron microscopy revealed spikes of 50 nm in length protruding from the neck and bound to the glass surface. Extensive analyses have been performed, including analysis with motility-inhibiting antibodies and examination of mechanical characteristics. The results could be explained by a model in which a spike composed of a gliding protein and powered by a force-generating protein repeatedly binds to and releases from the solid surface.

Chapter 6 **Spiroplasma Motility and Chemotaxis: Molecular Aspects of Cell Behaviour**

Shlomo Trachtenberg

Spiroplasmas are members of the mollicutes, the smallest free-living and self-replicating organisms. These are wall-less, helical bacteria $\sim 0.2 \mu\text{m}$ in diameter. Their helical shape is maintained by an internal cytoskeleton in the form of a flat, monolayered ribbon comprised of six or seven pairs of $\sim 5 \text{ nm}$ fibrils attached to the inner face of the membrane along the shortest helical line. Dynamic helical and non-helical shape changes and consequent motility are achieved by differential length changes of the fibrils. The motility modes of the cells in environments of

different molecular compositions and spatial structures are discussed in terms of the chemotactic response and geometrical shape changes.

Chapter 7

Mycoplasma Attachment Organelle and Cell Division

Mitchell F. Balish and Duncan C. Krause

Mycoplasma pneumoniae and several other mycoplasma species attach to host cells by means of a specialized polar structure known as the attachment organelle. This structure is also the leading end as mycoplasma cells move by gliding motility. The architecture of this organelle is characterized by a cytoskeleton made of novel components. During *M. pneumoniae* cell division, the attachment organelle appears to duplicate in concert with the onset of DNA replication; prior to cytokinesis one attachment organelle migrates to the opposite cell pole. Although the molecular mechanisms regulating both attachment organelle assembly and coordination of function in adherence and motility with the DNA replication process are unclear, analysis of cell division in *M. pneumoniae* and other mycoplasma species has revealed both common features with other bacteria, as well as notably divergent and unique features. __

Chapter 8

Proteomic Analysis of the Mycoplasmas

Jacob D. Jaffe

Protein studies and proteomics have enjoyed a rich and varied history in mycoplasma. Here, several important milestones in mycoplasma proteomics are discussed. Early protein-based studies in the mycoplasmas that served as both platforms for proteomics technology development as well as methods of differentiating species and serotypes of mycoplasmas are described first. These developments have led to more modern proteomics experiments that employ multi-dimensional separation coupled to mass spectrometry, and the rationales and methodologies of these experiments are discussed herein. Recent large-scale proteomics efforts in *Mycoplasma pneumoniae* have led to detection of over 80% of its proteome, and the results from these experiments are conflated to provide a unified picture of the *M. pneumoniae* proteome including subcellular localization information for a number of proteins. This work is described against the background of several smaller proteomic studies that predated complete genome sequences but nonetheless made important inroads for proteomics in the mycoplasmas. Computational methods of proteome analysis including whole-genome annotation strategies and protein-based genome structure prediction (proteogenomic mapping) are also discussed. This work is intended to be a resource for the current generation of proteomic knowledge in the mycoplasmas as well as a springboard into the next, with a discussion of some future prospects for proteomics in mycoplasma.

Section 3: Pathogenicity and Control

Chapter 9

New Developments in Human Diseases Due to Mycoplasmas

Ken Waites and Deborah Talkington

There are at least 16 different species of organisms in the Class *Mollicutes* for which humans are the primary host. Three in the genus *Mycoplasma* are well-known pathogens: *Mycoplasma pneumoniae*, *M. hominis*, and *M. genitalium*. *Ureaplasma* spp. have also been proven to cause disease in humans. *M. fermentans* can also be pathogenic for humans in some settings, as an opportunist in persons with HIV infection and AIDS, and it also may be associated with chronic arthritic conditions. *M. amphoriforme* is a newly identified species that has been detected in the lower respiratory tract secretions of several immunocompromised persons in association with chronic bronchitis, and investigations are now underway to determine if it has a pathogenic role in human disease. The remaining mollicute flora of humans exist primarily as commensals in the respiratory and/or urogenital tracts. Most of the more than 100 species of mycoplasmas are host-specific for other vertebrates, including fish, reptiles, birds, and mammals, and have never been detected in humans. However, there are occasional reports of invasive disease in humans due to commensal species and a few reports of accidental infections of humans by mycoplasmas that are usually associated with animals. Such isolations almost always occur in persons who are immunosuppressed. The primary focus of this chapter is discussion of newer information compiled on a variety of conditions in humans for which mycoplasmas are now acknowledged or suspected to play a role. Even though most mycoplasmal and ureaplasma infections involve the respiratory or

urogenital tracts, systemic diseases are becoming better recognized as the ability to detect these infections improves. Reports of unusual mycoplasmal infections in susceptible hosts are also summarized.

Chapter 10 **Diagnosis of Mycoplasmosis in Animals**

Philip F. Markham and Amir H. Noormohammadi

There have been a number of advances in antibody and DNA based techniques for detection of mycoplasma infections in animals over the past decade. The production and use of recombinant antigens has been a major improvement in ELISA technology with concomitant increased detection of host antibody to the infecting mycoplasma. The expression and purification of mycoplasma antigens in *Escherichia coli* has provided a means to individually tailor ELISA antigens for improved sensitivity and specificity. These antigens may be produced in relatively large quantities at reasonable cost, free from cross-reactive antigens that may otherwise be found in whole cell preparations. Detection of mycoplasma DNA using specific probes has mostly been superseded or combined with polymerase chain reaction (PCR) based tests. PCR detection of mycoplasmas has typically used oligonucleotide primers designed to the 16S rDNA gene sequence and in some cases species identification is aided by restriction endonuclease fragment length polymorphism analysis of the amplicon. There have been several PCR based formats used employing targets other than rRNA sequences, including nested or semi-nested PCR assays that greatly enhance sensitivity and specificity. APD/RAPD PCR assays have been used successfully for avian mycoplasma strain identification and for epidemiological studies. In the future the use of specific gene targets will allow rapid and simple identification of most mycoplasma species and strains without the need for cultivation of the organism.

Chapter 11 **Emerging Mycoplasmoses in Wildlife**

Daniel R. Brown, Laurie A. Zacher, Lori D. Wendland and Mary B. Brown

Mycoplasma probably evolved from ancestral mollicutes beginning about 4×10^8 years ago by processes that resulted in obligate commensalism or parasitism of host cells. For more than 99.9% of mycoplasma evolutionary history, non-human, undomesticated animals must have been the hosts that helped to shape the mycoplasmoses of all modern vertebrates. The epidemiology of mycoplasma in free-ranging wildlife is scanty because of resource allocation priorities and limited access to sampling, but recent examples of emerging mycoplasmoses are known from reptiles, ungulates, and birds. Wildlife mycoplasmoses are often regarded with a narrowly utilitarian view as reservoirs or models of domesticated animal or human infections, but it is increasingly apparent that wildlife hosts also harbor mycoplasma with greater than previously conceived genetic, phenotypic, and ecologic diversity. This survey of mycoplasmoses of vertebrate wildlife aims to renew attention to their probable central significance in the evolution of mycoplasma-host interactions, and to broaden the perspective of current mycoplasmaology that is otherwise dominated by mycoplasmoses of domesticated animals and humans.

Chapter 12 **Biodiversity of Mycoplasmas and Molecular Epidemiology**

François Thiaucourt and François Roger

Molecular epidemiology can be defined as an integrated approach that combines epidemiology and biotyping using molecular biology tools. As such it is a hybrid discipline and any molecular epidemiology study should obey the basic principles of both epidemiology and molecular typing. The sampling frames and gathering of epidemiological data should allow a statistical analysis of the results as the molecular biology tools should have a good repeatability as well as a good discriminating power below the species level. The choice of one or multiple techniques will naturally depend on the biodiversity that exists among the species to be considered. This biodiversity is the result of the evolution of populations of strains with bacterial species being seen as "condensed nodes" in a cloudy space. In the long term, it is expected that whole genome sequencing will become the ultimate tool for strain characterization. Until then, suboptimal techniques have to be used and the choice of the technique will have to be tailored for each mycoplasma species and for the specific objective of the study. Among the various techniques that can be used for the molecular typing of Mycoplasmas, some require the isolation of the strains and DNA extraction. This is the case for pulsed field gel electrophoresis, restriction endonuclease analysis, Southern blotting, random amplification of polymorphic DNA or amplified fragment length polymorphism. Other techniques do not require the isolation of the causative organism as they are based on PCR followed by an enzymatic restriction analysis or sequencing. Ultimately multiple loci can be amplified and sequenced in order to increase the discriminatory power of the technique. It is hoped that the integration of epidemiological studies with fine molecular typing will induce a better knowledge on the dynamics of mycoplasma strains evolution and spread and, finally, allow a better evaluation of risk and better definition of disease control strategies.

Chapter 13

Phenotypic Diversity and Cell Invasion in Host Subversion by Pathogenic Mycoplasmas

Christine Citti, Glenn F. Browning and Renate Rosengarten

Despite an apparent structural and genomic simplicity, a number of mycoplasma species are successful pathogens of humans and a wide range of animals. Over the past decade, the mechanisms used by these organisms to colonize their hosts and avoid or subvert host defences have begun to emerge. These include a variety of sophisticated genetic systems that elegantly combine high frequency reversible mutational events associated with subsets of structural genes encoding cell surface components. In propagating mycoplasma populations, these mutations spontaneously generate a highly variable surface coat, providing these pathogens with a means to adapt to and survive within complex, immunocompetent hosts. The recent discovery that some mycoplasmas can reside and persist intracellularly also offers new insights into the potential strategies employed by these simple prokaryotes. The ability of these organisms to cycle between the extra- and intracellular compartments in the host and to diversify their surface architecture during infection may, independently or in concert, be the major contributors to persistent infections and chronic disease. This chapter highlights the common and unique strategies evolved by mycoplasma pathogens to enable colonization and survival during infection.

Chapter 14

Immune Responses Following Mycoplasma Infection

Jerry W. Simecka

Mycoplasmas are responsible for many human and animal respiratory diseases and have a tremendous economic and health impact worldwide. Because of the chronic nature of these infections, it is likely that almost every component of the host immune system is involved in the response to mycoplasma disease. Innate immunity is critical in the early clearance and control of infection. Adaptive immune responses against mycoplasma have contrasting impacts on infection and the pathogenesis of infection. Vaccines can induce immune responses that resist infection. In addition, immune responses also minimize the potential spread of infection to other tissues, which can lead to arthritis and other diseases. However, the hallmark of many mycoplasma diseases is the persistence of the organism, and the provocation of frustrated and ineffective immune responses against the infection results in the development of chronic inflammation. Despite the vast amount of research, the mechanisms that control hosts' resistance and susceptibility to mycoplasma infection remains unclear. Immune responses are believed to be critical players in the pathogenesis of mycoplasma disease. In this review, we will highlight the potential roles of innate and adaptive immunity as influential mediators in animal and human mycoplasma pathogenesis and resistance infection.

Chapter 15

Antimicrobial Therapy and Antimicrobial Resistance

Cécile M. Bébéar and Isabelle Kempf

The lack of a cell wall in mycoplasmas makes them intrinsically resistant to β -lactams and to all antimicrobials which target the cell wall. Intrinsic resistance related to specific mycoplasma species concerns essentially the macrolide-lincosamide-streptogramin-ketolide (MLSK) antibiotic group. The most widely antibiotic classes used for the treatment of mycoplasmal infections include tetracyclines, MLSK group, and fluoroquinolones. Both target alterations and efflux mechanisms implicated in acquired antibiotic resistance have been described in mycoplasmas either by genetic mutation or transfer of new genes carried by transposons. Acquired resistance has been reported in clinical or field isolates of human and animal mycoplasmas, respectively, and linked to therapeutic failure. It concerns mainly tetracyclines for human urogenital species, and is due to the presence of the *tet(M)* gene. Acquired resistance to macrolides and fluoroquinolones, rarely observed in human mycoplasmas, mainly in immunosuppressed patients, is linked to mutations in the antibiotic target. For animal mycoplasmas, prevalence of antibiotic resistance is less documented and molecular studies of resistance mechanisms are still lacking.

Chapter 16

Vaccines to Control Mycoplasmosis

Glenn F. Browning, Kevin G. Whithear and Steven J. Geary

The significance of mycoplasmas as pathogens of animals has led to the evaluation of a variety of vaccines for control of the diseases they cause. The earliest vaccines used virulent isolates, which were delivered by an

abnormal route. Attenuated vaccines have been developed for some of the major pathogens of domestic animals, either by laboratory passage or by deliberate mutagenesis and inactivated vaccines have also been developed, although in most cases attenuated vaccines offer superior protection. There are currently effective attenuated vaccines available to control contagious bovine pleuropneumonia, caused by *Mycoplasma mycoides* subspecies *mycoides*, diseases of poultry caused by *M. gallisepticum* and *M. synoviae*, an effective inactivated vaccine to control contagious caprine pleuropneumonia, caused by *M. capricolum* subspecies *capripneumoniae*, and inactivated vaccines of limited efficacy to control enzootic pneumonia in pigs, which is caused by *M. hyopneumoniae*. Experimental attenuated vaccines have been shown to have some efficacy in preventing disease caused by *M. capricolum* subspecies *capripneumoniae*, *M. agalactiae* and *M. hyopneumoniae* and experimental inactivated vaccines have been shown to reduce disease caused by *M. mycoides* subspecies *mycoides* Small Colony Type, *M. agalactiae*, *M. bovis*, *M. mycoides* subspecies *mycoides* Large Colony type, *M. pulmonis* and *Ureaplasma diversum*. However attempts to develop attenuated vaccines to control pneumonia due to *M. pneumoniae* in humans have been frustrated by the difficulty of attenuating strains sufficiently to be safe while still retaining immunogenicity, while attempts to develop inactivated vaccines have been thwarted by the suggestion that the vaccines exacerbate disease in some individuals. In recent years investigations of novel approaches to vaccination against mycoplasmosis have included protein subunit vaccines, DNA vaccination, recombinant protein vaccines, and use of vaccine vectors expressing mycoplasma genes. However, the use of the cytoadherence protein of *M. pneumoniae* as a vaccine exacerbated disease in guinea pigs, and a recombinant cytoadherence protein of *M. hyopneumoniae* failed to induce protection in pigs. DNA vaccines against *M. hyopneumoniae* showed some capacity to induce immune responses in mouse models, but as yet have not been tested in pigs. Expression of *M. hyopneumoniae* genes in *Erysipelothrix rhusiopathiae* or *Salmonella typhimurium* have also shown some promise. Recent studies have also investigated the use of mycoplasmas as vaccine vectors, with the most promising results coming from studies on *M. gallisepticum*. Thus current investigations on mycoplasma vaccines are likely to result not only in improved protection from mycoplasmosis, but also in improved protection from other infectious diseases as well.