



Anais da Academia Brasileira de Ciências

ISSN: 0001-3765

aabc@abc.org.br

Academia Brasileira de Ciências

Brasil

CORDOVA, CAIO M.M.; HOELTGEBAUM, DANIELA L.; MACHADO, LAÍS D.P.N.; DOS  
SANTOS, LARISSA

Molecular biology of mycoplasmas: from the minimum cell concept to the artificial cell

Anais da Academia Brasileira de Ciências, vol. 88, núm. 1, 2016, pp. 599-607

Academia Brasileira de Ciências

Rio de Janeiro, Brasil

Available in: <http://www.redalyc.org/articulo.oa?id=32746362015>

- How to cite
- Complete issue
- More information about this article
- Journal's homepage in redalyc.org

redalyc.org

Scientific Information System

Network of Scientific Journals from Latin America, the Caribbean, Spain and Portugal

Non-profit academic project, developed under the open access initiative



## Molecular biology of mycoplasmas: from the minimum cell concept to the artificial cell

CAIO M.M. CORDOVA<sup>1</sup>, DANIELA L. HOELTGEBAUM<sup>1</sup>, LAÍS D.P.N. MACHADO<sup>2</sup> and LARISSA DOS SANTOS<sup>3</sup>

<sup>1</sup>Departamento de Ciências Farmacêuticas, Universidade de Blumenau, Rua São Paulo, 2171, Campus III, 89030-000 Blumenau, SC, Brasil

<sup>2</sup>Programa de Pós-Graduação em Farmácia, Universidade Federal de Santa Catarina, Rua Delfino Conti, s/n, 88040-900 Florianópolis, SC, Brasil

<sup>3</sup>Programa de Pós-Graduação em Química, Universidade de Blumenau, Rua Antônio da Veiga, 140, Campus I, 89012-900 Blumenau, SC, Brasil

*Manuscript received on May 13, 2015; accepted for publication on June 2, 2015*

### ABSTRACT

Mycoplasmas are a large group of bacteria, sorted into different genera in the *Mollicutes* class, whose main characteristic in common, besides the small genome, is the absence of cell wall. They are considered cellular and molecular biology study models. We present an updated review of the molecular biology of these model microorganisms and the development of replicative vectors for the transformation of mycoplasmas. Synthetic biology studies inspired by these pioneering works became possible and won the attention of the mainstream media. For the first time, an artificial genome was synthesized (a minimal genome produced from consensus sequences obtained from mycoplasmas). For the first time, a functional artificial cell has been constructed by introducing a genome completely synthesized within a cell envelope of a mycoplasma obtained by transformation techniques. Therefore, this article offers an updated insight to the state of the art of these peculiar organisms' molecular biology.

**Key words:** mycoplasma, molecular biology, microbial genetics, synthetic biology, gene transfer techniques.

### INTRODUCTION

It is believed that mycoplasmas originated through degenerative evolution from Gram-positive ancestors, with the loss of non-essential genes (Dybvig and Voelker 1996). The term mycoplasma is often used to refer to all microorganisms in the *Mollicutes* class, regardless of which genus they belong to. In order to prevent confusion with microorganisms specific to the *Mycoplasma* genus,

the term mollicutes has come into use as a generic name for all microorganisms in the class (Razin et al. 1998). The criteria for defining the taxonomy of mollicutes are established by the Subcommittee on the Taxonomy of Mollicutes, linked to the International Committee on Systematics of Prokaryotes, and can be found at its website: <<http://icsp.org/subcommittee/mollicutes/>>.

### MOLECULAR BIOLOGY OF MOLLICUTES

Aside from the fundamental differences between these and other prokaryotes, the molecular biology

Correspondence to: Caio Mauricio Mendes de Cordova  
E-mail: [caio.cordova@gmail.com](mailto:caio.cordova@gmail.com)

of mollicutes is very typical in some respects. Having evolved from Gram-positive bacteria, gene expression and other genetic characteristics of mollicutes are similar to those of their ancestors. On the other hand, because of the limited capacity of their genome, mollicutes do not have many of the enzymatic pathways characteristics of the majority of bacteria (Dybvig and Voelker 1996). These characteristics make mollicutes particularly interesting in molecular and cellular biology studies, as, even with these limitations, they are capable of independent replication and parasitism, causing diseases that lead to losses in agriculture as well as in human health.

#### GENOME STRUCTURE AND ORGANIZATION

Mollicutes have a chromosome made up of circular double stranded DNA, which can vary between 580 and 1,800 kb (Razin et al. 1998). The 580 kb chromosome of the human pathogen *M. genitalium* is currently the smallest known genome (Lucier et al. 1994). The GC content of the majority of mollicutes' chromosomes is around 35%, with 25% being the smallest (Glass et al. 2000). Even though they have such small genomes, some mollicutes have repeated sequences of DNA in their chromosomes. The repeated elements found in mollicutes are homologous to members of the IS3 Family Insertion Sequence (Westberg et al. 2002, Pilo et al. 2003, Ditty et al. 2003, Thomas et al. 2005, Lysnyansky et al. 2009).

With the exception of spiroplasmas, extrachromosomal DNA is rarely found in mollicutes. Currently, only a few plasmids have been isolated from *M. mycoides* subsp. *mycoides* and from *S. citri* (Bergemann et al. 1989). On the other hand, numerous replicative forms of viral genomes (phages) have been isolated from *Acholeplasma* and other species of *Mycoplasma* and *Spiroplasma* (Maniloff 1992).

In recent years, the greatest advances in the understanding of mollicute biology were obtained

with the sequencing of the complete genome of various species. Currently, the complete genome sequences of 46 species and strains have been published. For a detailed analysis, we suggest visiting MolliGen.org. MolliGen is a database dedicated to the comparative genomics of bacteria belonging to the Mollicutes class. It offers sequence and analysis of data collected on 54 genomes belonging to 37 species. The MolliGen database provides pages with genome and genetic elements, homology relationships defined by various methods, precompiled statistical data, metabolic pathways, and a large collection of tools to explore and compare genomes (Barré et al. 2004). Currently there is an effort, sponsored by the International Organization for Mycoplasmaology (IOM), to encourage genome sequencing of all species of mollicutes.

#### DNA REPLICATION AND REPAIR

Studies in this area have long maintained the idea that mollicutes were very limited with respect to their DNA repair mechanisms. However, at least in *M. pulmonis*, transposon induced mutagenesis in the *recA*, *ruvA*, *ruvB*, and *ruvC* genes produced deficient phenotypes (French et al. 2008). Through *in silico* analysis of sequenced genomes, the only complete repair mechanism found in mycoplasmas is that of repair by nucleotide excision repair (NER), probably complemented by enzymes from the base excision repair (BER) mechanism and the recombination mechanism (Carvalho et al. 2005). The NER mechanism was also identified in *M. genitalium*, in which the RecA protein has an active role in the antigenic variation mechanism but not in DNA repair (Burgos et al. 2012). A histone-like protein that recognizes nucleotide incorporation errors in DNA was identified in *M. gallisepticum*. However, this does not appear to be a rule among mycoplasmas, which, during their evolution, lost the DNA mismatch repair mechanism (MMR) (Kamashev et al. 2011). Nevertheless, despite their

limitations, it is evident that mycoplasmas possess an adequate set of genes and gene products, even if they have not yet been completely identified, to promote DNA repair in a way that makes their development possible, causing significant diseases in a wide range of hosts (Gorbachev et al. 2013).

#### GENETIC RECOMBINATION

Until recently, there was no evidence that homologous recombination could occur in mollicutes' genomes. In *M. agalactiae*, the necessary genes for homologous recombination appear to be reduced, showing only the components involved in the formation of the presynaptic complex (*recD*, *recO*, and *recR*), strand exchange (*recA*), branch migration (*ruvA* and *ruvB*), and the Holliday junction resolution (*recU* and *yqgF*); however, it has already been demonstrated in other bacteria that, with the absence of complete sets of genes, homologous recombination can occur (Rocha et al. 2005). Gene disruption by homologous recombination has already been proven in *M. genitalium* (Dhandayuthapani et al. 1999) and in *S. citri* (Duret et al. 1999), and in *Mycoplasma mycoides* subsp. *capri*, inclusion of *recA* from *E. coli* in a suicide plasmid favored the occurrence of mutants (Allam et al. 2010). Apparently there are various mechanisms that can be explored to increase the effectiveness of this approach, which may be species-dependent.

#### GENETIC MATERIAL TRANSFER

Because of the absence of a cellular wall, it can be expected that the introduction of exogenous genetic material to mollicute cells happens without much difficulty. In fact, the chromosomal DNA exchange occurring during direct contact between mollicute cells (Barroso and Labarere 1988), and the spontaneous conjugal transfer of Tn916 between *Enterococcus faecalis* and *M. hominis* (Roberts and Kenny 1987) is probably due to the momentary fusion of cellular membranes in the contact region.

Nevertheless, the efficiency of DNA transfer in these cases was low.

#### VECTORS

Transfection experiments of *S. citri* with the SpV1 virus containing an inserted gene that codes the *M. pulmonis* P1 adhesin resulted in an expression of this spiroplasma fragment. However, this system is inefficient, since there is a rapid loss of the cloned DNA fragment. Transposons have also been used in the transformation of *A. laidlawii* and *M. pulmonis* in antibiotic-resistant mollicutes using the pAM120 plasmid, containing the Tn916 streptococcus transposon, which confers resistance to tetracycline through the expression of the *tetM* gene (Dybvig and Cassell 1987). Other transposons have also been used with various species of mollicutes since then, such as Tn1545 and Tn4001 (Cao et al. 1994, de Barbeyrac et al. 1996, Foissac et al. 1997, Kapke et al. 1994). The transposons undergo excision from the plasmid that contains them, and integrate with the mollicute chromosome in random positions (Foissac et al. 1997). Mutagenesis by transposon is done by inserting it into genes, causing the inactivation of transcription or the formation of truncated products. The genes containing the transposon are then mapped and identified, and the definitive proof of its function is given by the complementation of the mutant with the wild-type gene. This strategy has been used in *M. pneumoniae* (Fisseha et al. 1999), *M. genitalium* (Reddy et al. 1996) and *S. citri* (Jacob et al. 1997). However, in many cases, these elements transpose actively, changing location in the chromosome frequently (Foissac et al. 1997).

Another type of vector was constructed by inserting the *oriC* region of *S. citri* in the commercial plasmid pBS (Stratagene, La Jolla, Ca, USA), and the resulting recombinant plasmid was efficiently introduced into the spiroplasma cells by electroporation, where it was able to replicate

and maintain stability as an extrachromosomal element (Renaudin et al. 1995). Vectors derived from this plasmid show themselves to be capable of inactivating specific *S. citri* genes (Duret et al. 1999). The great advantage of these types of replicative plasmids is the possibility to direct the insertion of the vector to a specific region of the chromosome. In this way interference from the random insertion of the vector in other mollicute genes can be avoided, which can happen with viral particles and transposons. This method also makes it possible to promote complementation of the mollicute genes in mutant strains (Gaurivaud et al. 2000), definitively proving its function. Vectors derived from the pKMK1 plasmid were also used in the transformation of *M. mycoïdes* and *M. capricolum* (King and Dybvig 1993). However, these plasmids produce few copies, they are incapable of transforming other species of mollicutes, and the stability of the inserts in these vectors does not appear to be adequate (Dybvig and Voelker 1996).

The cloning of the fragment containing the DnaA boxes located near the *M. capricolum* subsp. *capricolum* *dnaA* gene, through the pSRT2 vector, initially developed to transform *S. citri* (Lartigue et al. 2002), made possible the construction of two new plasmids, pMCO2 and pMCO3. The pMCO2 plasmid was constructed using the *oriC* region of *M. capricolum* inserted at the *Bam*HI multiple cloning sites oriented in the same direction as the *tetM* gene. Construction of the pMCO3 plasmid was done in a similar manner to that of pMCO2, although the cloned fragment was oriented in the opposite direction of the *tetM* gene. By cloning the amplified fragments of the *spoJ-dnaA* and *dnaN-spoJ* intergenic regions of *M. genitalium* in the pSRT2 vector, it was possible to obtain the pMGO1 and pMGO2 plasmids. In the same way, cloning the fragments containing the putative *M. pulmonis* UAB CTIP DnaA boxes in the pSRT2 vector made it possible to create the new plasmid named pMPO1.

This plasmid contains the *M. pulmonis* *oriC* region inserted at the *Bam*HI site of the multiple cloning regions in the same orientation as the *tetM* gene. By amplifying the 327 bp fragment containing the DnaA boxes located in the *dnaA-dnaN* intergenic regions of *M. pulmonis* and cloning it in the pSRT2 vector, the pMPO2 plasmid was constructed. In the same way, by cloning a 262 bp fragment containing the DnaA boxes located in the *recD-dnaA* intergenic region, the pMPO3 plasmid was constructed. The pMPO4 plasmid was constructed cloning both of these fragments side by side at the *Bam*HI site of the pSRT2 vector. The pMPO5 plasmid was constructed cloning these two fragments with the *tetM* gene in between (Cordova et al. 2002). Further studies using the results of this work were able to produce the specific *oriC* plasmids capable of transforming the *M. mycoïdes* subsp. *mycoïdes* SC and *M. mycoïdes* subsp. *capri* (Lartigue et al. 2003). Suicide plasmids were constructed by amplifying a 717 bp internal fragment of the *M. pulmonis* hemolysin A gene and inserting it in the pSRT2, pMPO1 and pMPO5 plasmids, by which disruption vectors were obtained, with the names pSRT2- $\Delta$ *hlyA*, pMPO1- $\Delta$ *hlyA* and pMPO5- $\Delta$ *hlyA*, respectively (Cordova et al. 2002).

Plasmids with the *M. capricolum* subsp. *capricolum* *oriC* region are not capable of replicating in *M. mycoïdes* subsp. *mycoïdes* SC or *M. mycoïdes* subsp. *capri*, all phylogenetically related and belonging to the Mycoïdes group. On the other hand, plasmids with the *oriC* region from *M. mycoïdes* subsp. *mycoïdes* SC, *M. mycoïdes* subsp. *capri* or even from *S. citri* are capable of transforming and replicating in *M. capricolum*. *M. mycoïdes* subsp. *mycoïdes* SC and *M. mycoïdes* subsp. *capri* are capable of being transformed with each other's plasmids (Lartigue et al. 2003). The use of plasmids with the *M. mycoïdes* subsp. *capricolum* *oriC* region was shown to be viable for heterologous gene expression in this mollicute, but gene disruption by homologous recombination was

only possible with plasmids containing the *S. citri* oriC region (Janis et al. 2005).

#### PRESENCE OF DNA RESTRICTION-MODIFICATION SYSTEMS

Restriction-modification systems are very common in mollicutes and can obstruct gene transfer. Some studies have shown that the transformation of *S. citri* with isolated *E. coli* plasmids are only successful when the *E. coli* strain used had type I *EcoK* methyltransferase, which suggests that the methylation by *EcoK* is necessary to protect plasmid DNA from the *S. citri* restriction system (Bové 1993). Because of this, transformation errors of certain mollicutes with certain vectors can be due to restriction enzyme activity (Dybvig and Voelker 1996, Sirand-Pugnet et al. 2007).

#### HORIZONTAL GENE TRANSFER

The possibility of horizontal gene transfer between different mollicutes, at least for more phylogenetically related species, has long been investigated, considering the small genome and probable degenerative evolution mechanism, which in theory doesn't contribute to the acquisition of new genes. One of the first studies to demonstrate horizontal gene transfer was between different serotypes of *Ureaplasma* sp., isolated from clinical samples (Xiao et al. 2011). These studies explained the persistent difficulty in relating different levels of pathogenicity and thus different clinical presentations to different microorganism serotypes. In this research, it was demonstrated that not less than 40% of the isolated *U. urealyticum/parvum* strains were genetic mosaics. Evidence of horizontal gene transfer had already been reported in species located in the same ecological site (Sirand-Pugnet et al 2007) and in mycoplasmas of the same species (Teachman et al. 2002). It was also demonstrated that certain strains of *Ureaplasma* sp. are capable of forming biofilms, just as various mollicutes that infect animals, such as *M. pulmonis*,

a characteristic acquired from other bacterial species (Garcia-Castillo et al. 2008). In addition to biofilms, the presence of an integrative conjugative element (ICE) in *M. agalactiae*, as multiple copies in the chromosome or in free circular form, is worthy of attention because it is a microorganism responsible for a disease that can also be caused by *M. capricolum* subsp. *capricolum* and *M. mycoides* subsp. *capri* (Marenda et al. 2006). ICEs can also be called CONSTINS or conjugative transposons and are capable of conferring various metabolic or resistance characteristics to their hosts. They are self-transmissible elements, which carry the genes necessary for excision, conjugative transfer, and integration with the host (Burrus and Waldor 2004). There are indications that at least 18% of the *M. agalactiae* genome was transferred horizontally from mycoplasmas of the Mycoïdes group, since the former, even though it can inhabit the same hosts and ecological sites, is phylogenetically located in the Hominis group (Sirand-Pugnet et al. 2007). Nevertheless, half of these genes' functions are still unknown, and the mass transfer of genes is not believed to have had a very significant role in the evolution of mollicutes. However, this data indicates that, in addition to being able to survive against the host's defenses, mollicutes are also capable of promoting chromosomal exchange to a certain degree, which avoids genome stagnation, making possible the continued adaptation to new sites and even new hosts (Sirand-Pugnet et al. 2007). Nevertheless, the mechanism by which gene transfer occurs between mollicute cells is still not well documented (Dordet-Frisoni et al. 2014).

#### THE MINIMAL GENOME CONCEPT

Because *M. genitalium* has the smallest bacterial genome currently known (Fraser et al. 1995), some authors have investigated the hypothesis that its genome represents the minimum gene set sufficient for life in a microorganism capable of self-replication. Mushegian and Koonin (1996)

compared this mycoplasma's genome with that of *Haemophilus influenzae*, identifying 240 common genes. Adding 22 genes related to essential metabolic pathways not identified in these microorganisms, and subtracting 6 redundant genes specific to parasites, the authors came to a total of 256 genes, which they classified as the approximate minimum number of genes necessary for the functioning of a cell. However, *M. genitalium* has double this number of genes. Maniloff (1996) suggests that the difference is probably reflected in that the comparison was made between a minimal genome produced by billions of years of evolution and a minimal gene set produced by a computer. Other authors attempted to determine the minimum number of essential genes through mutagenesis experiments with *M. genitalium* (Hutchinson et al. 1999). In a study involving global transposon mutagenesis, the authors determined that 387 genes is the minimum set necessary for life (Glass et al. 2006). In a comparison of 186 genomes, including *M. genitalium*, other authors determined that 702 is the minimum number for Gram-negative bacteria and 959 for Gram-positive bacteria. Naturally, the necessity for genes in mycoplasma cells is lower, since they maintain a parasitic life in their hosts (Huang et al. 2013). In *M. pneumoniae*, phylogenetically related to *M. genitalium*, 33% of its 816 kpb genome, including 0.9% of intergenic regions, were considered essential (Lluch-Senar et al. 2015). In *M. mycoides*, using gene cloning in yeast cells and carrying out transposon mutagenesis to reduce the genome, making use of yeast's sexual cycle, 368 genes were determined as non-essential gene candidates. From these, 11 of 14 genes were of the DNA restriction-modification system, 3 of 5 were *vlc* genes, and 7 of 22 were integrative conjugative elements (Suzuki et al. 2015).

#### CONSTRUCTION OF AN ARTIFICIAL CELL

With the relative success of the studies attempting to determine the minimal gene set for necessary

for life, at least for a bacterium, the following dream began taking shape, making use of synthetic biology: to construct a cell from the available genetic information. Creating life has not yet been accomplished, as one of the major obstacles is completely synthesizing a viable cell envelope. Nature is still very mysterious. However, constructing a new cell from the genome of a different bacterium has been done, by inserting the genome in the cell envelope (membrane) of the other. It all began with cloning experiments and transposing the entire *M. mycoides* genome into a yeast cell (from which the original DNA was removed) (Benders et al. 2010). Keeping this cloned genome in a plasmid has also been done (Karas et al. 2013), and later transposing it again into the cell membrane of another mycoplasma species (*M. capricolum*) (Lartigue et al. 2009). This mycoplasma to yeast genome transfection technology has also made possible new methods of reducing the genome, in the continuous search for the minimal essential gene set. In this way, it was possible to create strains with reduced genomes but normal growth, without defective phenotypes (Suzuki et al. 2015). The recombination of mycoplasma genomic elements in yeasts may make possible not only the arrangement of deleted regions (non-essential), but also a combination of deletions, insertions and substitutions of genetic elements. It is possible to recombine two entire synthetic genomes and recombine gene cassettes between them, or to recombine synthetic genome segments through homologous recombination (Suzuki et al. 2015).

A major step towards the creation of life was the synthesis of a complete genome from the *M. genitalium* sequence information in genetic databases, first cloned in *E. coli* and *Saccharomyces cerevisiae* (Gibson et al. 2008). The next step, labeled genome synthesis technology, was to construct a new genome from the *M. mycoides* sequence, including 'watermark' sequences, dele-

tions, polymorphisms and mutations, and then inserting this new genome into the cell envelope of *M. capricolum*. This new cell was shown to be completely controlled by the new synthetic genome (Gibson et al. 2010). This created a new microorganism, neither *M. mycoïdes* nor *M. capricolum*, which became widely known as *Mycoplasma laboratorium*.

The cloning of genomes in yeast, in addition to having been recreated with other bacteria, was also demonstrated with eukaryotic chromosomes (Karas et al. 2013).

If, or better yet, when it becomes truly possible to create life in a laboratory from the synthesis of complex molecules using only chemical precursors based on the available genetic information, uniting it all in a synthetic cell envelope, many paradigms will be broken, not only in science, but also in philosophy, theology, ethics and sociology.

#### RESUMO

Os micoplasmas constituem um grande grupo de bactérias, ordenadas em diferentes gêneros na classe Mollicutes, cuja principal característica em comum, além do genoma reduzido, é a ausência de parede celular. São considerados modelos de estudo de biologia celular e molecular. Apresentamos uma revisão atualizada da biologia molecular destes microrganismos modelo e do desenvolvimento de vetores replicativos para a transformação dos micoplasmas. Estudos de biologia sintética inspirados nestes trabalhos pioneiros se tornaram possíveis e ganharam a atenção da grande mídia. Pela primeira vez, um genoma artificial foi sintetizado (um genoma mínimo produzido a partir das sequências de consenso obtidas de micoplasmas). Pela primeira vez, uma célula artificial funcional foi artificialmente construída, por introdução de um genoma completamente sintetizado dentro de um envelope celular obtido de um micoplasma, por técnicas de transformação. Desta forma, este artigo oferece um olhar atualizado sobre estado da arte da biologia molecular destes peculiares microrganismos.

**Palavras-chave:** micoplasma, biologia molecular, genética microbiana, biologia sintética, técnicas de transferência de genes.

#### REFERENCES

- ALLAM AB, REYES L, ASSAD-GARCIA N, GLASS JI AND BROWN MB. 2010. Enhancement of Targeted Homologous Recombination in *Mycoplasma mycoïdes* subsp. *capri* by Inclusion of Heterologous *recA*. *App Env Microbiol* 76: 6951-6954.
- BARRÉ A, DE DARUVAR A AND BLANCHARD A. 2004. MolliGen, a database dedicated to the comparative genomics of Mollicutes. *Nucleic Acids Res* 32: D307-310.
- BARROSO G AND LABARERE J. 1988. Chromosomal gene transfer in *Spiroplasma citri*. *Science* 241: 959-961.
- BENDERS GA ET AL. 2010. Cloning whole bacterial genomes in yeast. *Nucleic Acids Res* 38: 2558-2569.
- BERGEMANN AD, WHITLEY JC AND FINCH LR. 1989. Homology of mycoplasma plasmid pADB201 and staphylococcal plasmid pE194. *J Bacteriol* 171: 593-595.
- BOVÉ JM. 1993. Molecular features of mollicutes. *Clin Infect Dis* 17: S10-S31.
- BURGOS R, WOOD GE, YOUNG L, GLASS JI AND TOTTE PA. 2012. RecA mediates MgpB and MgpC phase and antigenic variation in *Mycoplasma genitalium*, but plays a minor role in DNA repair. *Mol Microbiol* 85: 669-683.
- BURRUS V AND WALDOR MK. 2004. Shaping bacterial genomes with integrative and conjugative elements. *Res Microbiol* 155: 376-386.
- CAO J, KAPKE PA AND MINION FC. 1994. Transformation of *Mycoplasma gallisepticum* with Tn916, Tn4001, and integrative plasmid vectors. *J Bacteriol* 176: 4459-4462.
- CARVALHO FM, FONSECA MM, BATISTUZZO DE MEDEIROS S, SCORTECCI KC, BLAHA CA AND AGNEZ-LIMA LF. 2005. DNA repair in reduced genome: the *Mycoplasma* model. *Gene* 360: 111-119.
- CORDOVA CMM, LARTIGUE C, SIRAND-PUGNET P, RENAUDIN J, CUNHA RAF AND BLANCHARD A. 2002. Identification of the Origin of Replication of the *Mycoplasma pulmonis* Chromosome and Its Use in oriC Replicative Plasmids. *J Bacteriol* 184: 5426-5435.
- DE BARBEYRAC B, DUPON M, RODRIGUEZ R, RENAUDIN H AND BEBEAR C. 1996. A Tn1545-like transposon carries the tet(M) gene in tetracycline resistant strains of *Bacterioides ureolyticus*, as well as *Ureaplasma urealyticum*, but not *Neisseria gonorrhoeae*. *J Antimicrob Chemother* 37: 223-232.
- DHANDAYUTHAPANI S, RASMUSSEN WG AND BASEMAN JB. 1999. Disruption of gene mg218 of *Mycoplasma genitalium* through homologous recombination leads to an adherence-deficient phenotype. *Proc Natl Acad Sci USA* 96: 5227-5232.
- DITTY SE, LI B, ZHANG S, ZOU N AND LO SC. 2003. Characterization of an IS element in *Mycoplasma orale* that is highly homologous to *M. fermentans* ISLE. *Curr Microbiol* 46: 302-306.



- DORDET-FRISONI E, SAGNÉ E, BARANOWSKI E, BRETON M, NOUVEL LX, BLANCHARD A, MAREND A, TARDY F, SIRAND-PUGNET P AND CITTI C. 2014. Chromosomal transfers in mycoplasmas: when minimal genomes go mobile. *mBio* 5(6): e01958-14.
- DURET S, DANET JL, GARNIER M AND RENAUDIN J. 1999. Gene disruption through homologous recombination in *Spiroplasma citri*: an scm1-disrupted motility mutant is pathogenic. *J Bacteriol* 181: 7449-7456.
- DYBVG K AND CASSELL GH. 1987. Transposition of Gram-positive transposon Tn916 in *Acholeplasma laidlawii* and *Mycoplasma pulmonis*. *Science* 235: 1392-1394.
- DYBVG K AND VOELKER LL. 1996. Molecular biology of mycoplasmas. *Annu Rev Microbiol* 50: 25-57.
- FISSEHA M, GOHLMANN HW, HERRMANN R AND KRAUSE DC. 1999. Identification and complementation of frameshift mutations associated with loss of cytoadherence in *Mycoplasma pneumoniae*. *J Bacteriol* 181: 4404-4410.
- FOISSAC X, SAILLARD C AND BOVÉ JM. 1997. Random insertion of transposon Tn4001 in the genome of *Spiroplasma citri* strain GII3. *Plasmid* 37: 80-86.
- FRASER CM ET AL. 1995. The minimal gene complement of *Mycoplasma genitalium*. *Science* 270: 397-403.
- FRENCH CT, LAO P, LORAIN AE, MATTHEWS BT, YU H AND DYBVG K. 2008. Large-scale transposon mutagenesis of *Mycoplasma pulmonis*. *Mol Microbiol* 69: 67-76.
- GARCIA-CASTILLO M, MOROSINI MI, GÁLVEZ M, BAQUERO F, DEL CAMPO R AND MESEGUER MA. 2008. Differences in biofilm development and antibiotic susceptibility among clinical *Ureaplasma urealyticum* and *Ureaplasma parvum* isolates. *J Antimicrob Chemother* 62: 1027-1030.
- GAURIVAUD P, LAIGRET F, GARNIER M AND BOVE JM. 2000. Fructose utilization and pathogenicity of *Spiroplasma citri*: characterization of the fructose operon. *Gene* 252: 61-69.
- GIBSON DG ET AL. 2008. Complete chemical synthesis, assembly, and cloning of a *Mycoplasma genitalium* genome. *Science* 319: 1215-1220.
- GIBSON DG ET AL. 2010. Creation of a bacterial cell controlled by a chemically synthesized genome. *Science* 329: 52-56.
- GLASS JI, ASSAD-GARCIA N, ALPEROVICH N, YOOSEPH S, LEWIS MR, MARUF M, HUTCHISON CA 3<sup>rd</sup>, SMITH HO AND VENTER JC. 2006. Essential genes of a minimal bacterium. *Proc Natl Acad Sci USA* 103: 425-430.
- GLASS JI, LEFKOWITZ EJ, GLASS JS, HEINER CR, CHEN EY AND CASSELL GH. 2000. The complete sequence of the mucosal pathogen *Ureaplasma urealyticum*. *Nature* 407: 757-762.
- GORBACHEV AY ET AL. 2013. DNA repair in *Mycoplasma gallisepticum*. *BMC Genomics* 23: 726.
- HUANG CH, HSIANG T AND TREVORS JT. 2013. Comparative bacterial genomics: defining the minimal core genome. *Antonie van Leeuwenhoek* 103: 385-398.
- HUTCHISON CA, PETERSON SN, GILL SR, CLINE RT, WHITE O, FRASER CM, SMITH HO AND VENTER JC. 1999. Global transposon mutagenesis and a minimal *Mycoplasma genome*. *Science* 286: 2165-2169.
- JACOB C, NOUZIERES F, DURET S, BOVE JM AND RENAUDIN J. 1997. Isolation, characterization, and complementation of a motility mutant of *Spiroplasma citri*. *J Bacteriol* 179: 4802-4810.
- JANIS C, LARTIGUE C, FREY J, WRÓBLEWSKI H, THIAUCOURT F, BLANCHARD A AND SIRAND-PUGNET P. 2005. Versatile Use of oriC Plasmids for Functional Genomics of *Mycoplasma capricolum* subsp. *capricolum*. *Appl Environ Microbiol* 71: 2888-2893.
- KAMASHEV D, OBERTO J, SEREBRYAKOVA M, GORBACHEV A, ZHUKOVA Y, LEVITSKII S, MAZUR AK AND GOVORUN V. 2011. *Mycoplasma gallisepticum* produces a histone-like protein that recognizes base mismatches in DNA. *Biochemistry* 50: 8692-8702.
- KAPKE PA, KNUDTSON KL AND MINION FC. 1994. Transformation of Mollicutes with single-stranded Tn4001 DNA. *Plasmid* 32: 85-88.
- KARAS BJ ET AL. 2013. Direct transfer of whole genomes from bacteria to yeast. *Nat Methods* 10: 410-412.
- KING KW AND DYBVG K. 1993. Mycoplasma cloning vectors derived from plasmid pKMK1. *Plasmid* 31: 49-59.
- LARTIGUE C, BLANCHARD A, RENAUDIN J, THIAUCOURT F AND SIRAND-PUGNET P. 2003. Host specificity of mollicutes oriC plasmids: functional analysis of replication origin. *Nucleic Acids Res* 31: 6610-6618.
- LARTIGUE C, DURET S, GARNIER M AND RENAUDIN J. 2002. New plasmid vectors for specific gene targeting in *Spiroplasma citri*. *Plasmid* 48: 149-159.
- LARTIGUE C ET AL. 2009. Creating bacterial strains from genomes that have been cloned and engineered in yeast. *Science* 325: 1693-1696.
- LLUCH-SENAR M ET AL. 2015. Defining a minimal cell: essentiality of small ORFs and ncRNAs in a genome-reduced bacterium. *Mol Syst Biol* 11: 780.
- LUCIER TS, HU PQ, PETERSON SN, SONG XY, MILLER L, HEITZMAN K, BOTT KF, HUTCHISON CA 3<sup>rd</sup> AND HU PC. 1994. Construction of an ordered genomic library of *Mycoplasma genitalium*. *Gene* 150: 27-34.
- LYSNYANSKY I, CALCUTT MJ, BEN-BARAK I, RON Y, LEVISOHN S, METHÉ BA AND YOGEV D. 2009. Molecular characterization of newly identified IS3, IS4 and IS30 insertion sequence-like elements in *Mycoplasma bovis* and their possible roles in genome plasticity. *FEMS Microbiol Lett* 294: 172-182.
- MANILOFF J. 1992. Mycoplasma viruses. In: Maniloff J, McElhaney RN, Finch LR and Baseman JB (Eds), *Mycoplasmas: molecular biology and pathogenesis*, Washington: ASM Press 1: 41-59.
- MANILOFF J. 1996. The minimal cell genome: "on being the right size". *Proc Natl Acad Sci USA* 93: 10004-10006.
- MAREND A, BARBE V, GOURGUES G, MANGENOT S, SAGNE E AND CITTI C. 2006. A new integrative

- conjugative element occurs in *Mycoplasma agalactiae* as chromosomal and free circular forms. *J Bacteriol* 188: 4137-4141.
- MUSHEGIAN AR AND KOONIN EV. 1996. A minimal gene set for cellular life derived by comparison of complete bacterial genomes. *Proc Natl Acad Sci USA* 93: 10268-10273.
- PILO P, FLEURY B, MAREDA M, FREY J AND VILEI EM. 2003. Prevalence and distribution of the insertion element ISMag1 in *Mycoplasma agalactiae*. *Vet Microbiol* 92: 37-48.
- RAZIN S, YOGEV D AND NAOT Y. 1998. Molecular biology and pathogenicity of mycoplasmas. *Microbiol Mol Biol Rev* 62: 1094-1156.
- REDDY SP, RASMUSSEN WG AND BASEMAN JB. 1996. Isolation and characterization of transposon Tn4001-generated, cytoadherence-deficient transformants of *Mycoplasma pneumoniae* and *Mycoplasma genitalium*. *FEMS Immunol Med Microbiol* 15: 199-211.
- RENAUDIN J, MARAIS A, VERDIN E, DURET S, FOISSAC X, LAIGRET F AND BOVÉ JM. 1995. Integrative and free *Spiroplasma citri* oriC plasmids: expression of the *Spiroplasma phoeniceum* spiralin in *Spiroplasma citri*. *J Bacteriol* 177: 2870-2877.
- ROBERTS MC AND KENNY GE. 1987. Conjugal transfer of transposon Tn916 from *Streptococcus faecalis* to *Mycoplasma hominis*. *J Bacteriol* 169: 3836-3839.
- ROCHA EP, CORNET E AND MICHEL B. 2005. Comparative and evolutionary analysis of the bacterial homologous recombination systems. *PLoS Genet* 1: e15.
- SIRAND-PUGNET P ET AL. 2007. Being pathogenic, plastic, and sexual while living with a nearly minimal bacterial genome. *PLoS Genet* 3: e75.
- SUZUKI Y ET AL. 2015. Bacterial genome reduction using the progressive clustering of deletions via yeast sexual cycling. *Genome Res* 25(3): 435-444.
- TEACHMAN AM, FRENCH CT, YU HX, SIMMONS H AND DYBVIK K. 2002. Gene transfer in *Mycoplasma pulmonis*. *J Bacteriol* 184: 947-951.
- THOMAS A, LINDEN A, MAINIL J, BISCHOF DF, FREY J AND VILEI EM. 2005. *Mycoplasma bovis* shares insertion sequences with *Mycoplasma agalactiae* and *Mycoplasma mycoides* subsp. *mycoides* SC: Evolutionary and developmental aspects. *FEMS Microbiol Lett* 245: 249-255.
- WESTBERG J, PERSSON A, PETTERSSON B, UHLÉN M AND JOHANSSON KE. 2002. ISMmy1, a novel insertion sequence of *Mycoplasma mycoides* subsp. *mycoides* small colony type. *FEMS Microbiol Lett* 208: 207-213.
- XIAO L, PARALANOV V, GLASS JI, DUFFY LB, ROBERTSON JA, CASSELL GH, CHEN Y AND WAITES KB. 2011. Extensive Horizontal Gene Transfer in *Ureaplasmas* from Humans Questions the Utility of Serotyping for Diagnostic Purposes. *Clin Microbiol* 48: 2818-2826.