UNIVERSITÀ DEGLI STUDI DI SASSARI

SCUOLA DI DOTTORATO IN SCIENZE BIOMOLECOLARI E BIOTECNOLOGICHE

INDIRIZZO MICROBIOLOGIA MOLECOLARE E CLINICA
XXIII Ciclo

CHARACTERIZATION OF *Mycoplasma agalactiae* MEMBRANE PROTEOME

Direttore:
Prof. Bruno Masala

Tutor:
Dr. Alberto Alberti

Tesi di dottorato della
Dott.ssa Carla Cacciotto

ANNO ACCADEMICO 2009-2010

TABLE OF CONTENTS

1. ABSTRACT

Mycoplasma agalactiae is the etiological agent of contagious agalactia, a serious disease of small ruminants characterized by mastitis, polyarthritis, keratoconjunctivitis, and abortion.

Mycoplasmas totally lack the cell wall, so membrane proteins are directly exposed to environment. To date, a limited number of *M. agalactiae* antigens have been described. The combination of 2-D PAGE and mass spectrometry is a well-established method for proteome studies; however, it is reported that standard 2-D PAGE lacks in resolution of hydrophobic and basic proteins, which are abundant in mycoplasmas membrane. Indeed, membrane proteins are poorly represented in total extracts maps.

In this study, the membrane proteome of *M. agalactiae* $PG2^T$ was characterized. The Triton X-114 fractionation allowed the enrichment for *M. agalactiae* $PG2^T$ membrane proteins. Liposoluble proteins were subjected to 2-D PAGE-MS, leading to the identification of 40 unique proteins. The differential expression of liposoluble proteins, among $PG2^T$ strain and two field isolates, was revealed by 2D DIGE.

The use of GeLCMS/MS allowed to increase the coverage of the liposoluble proteome. A total of 194 unique proteins were identified, (26% of *M. agalactiae* $PG2^T$ genes) and subjected to gene ontology analysis for localization and function. Interestingly, the 11.5% of identified proteins derived from putative horizontal gene transfer events.

This work paves the way for the development of diagnostic and prophylaxis tools.

2. INTRODUCTION

2.1 Mycoplasmas: taxonomy and main biological features

Mycoplasmas (from the Greek, *müces*: fungus; *plasma*: formed) are the smallest and simplest self-replicating organisms being provided only with the minimal machinery required for survival (Carter and Chengappa, 1991); phenotypically, they are distinguished from other bacteria by their small size (0.2-0.8 μm) and total lack of cell wall (Fig. 1) (Razin *et al.*, 1998).

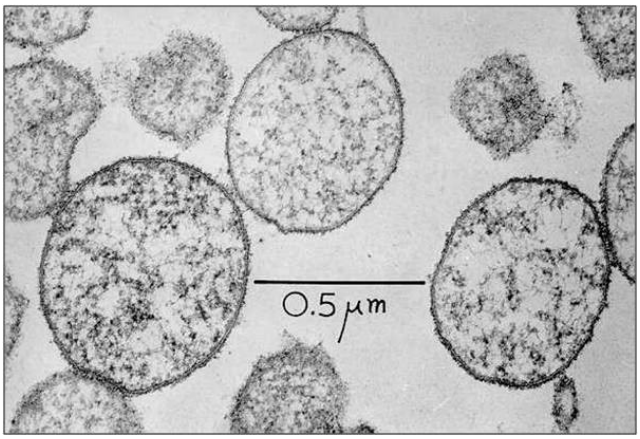


Figure 1: Electron micrograph of thin-sectioned mycoplasma cells

The number of mycoplasmas is continuously increasing, and actually 180 species are known and classified in the phylum *Tenericutes*, class *Mollicutes* (from the Latin, *mollis*: "soft"; *cutis*: skin), order *Mycoplasmatales*, family *Mycoplasmataceae*, genus *Mycoplasma* (Razin and Freundt, 1984; Razin, 1991, 1993). The introduction of 16S rRNA sequencing allowed to extend *Mollicutes* taxonomy. In fact, Haemobartonella and Eperythrozoon genera, historically considered more closed to Rickettsiae, are now included in Mycoplasmataceae. The current classification of *Mollicutes* and the properties distinguishing the currently established taxa are presented in Table 1.

Classification	Current no. of recognized species	Genome size (kb)	Mol% G + C of genome	Cholesterol requirement	Distinctive properties	Habitat
Order I: *Mycoplasmatales*						
Family I: *Mycoplasmataceae*						
Genus I: *Mycoplasma*	102	580-1350	23-40	Yes	Optimum growth at 37°C	Humans, animals
Genus II: *Ureaplasma*	6	760-1170	27-30	Yes	Urea hydrolysis	Humans, animals
Order II: *Entomoplasmatales*						
Family I: *Entomoplasmataceae*						
Genus I: *Entomoplasma*	5	790-1140	27-29	Yes	Optimum growth at 30°C	Insects, plants
Genus II: *Mesoplasma*	12	870-1100	27-30	No	Optimum growth at 37°C; 0.04% Tween 80 required in serum-free medium	Insects, plants
Family II: *Spiroplasmataceae*						
Genus I: *Spiroplasma*	33	780-2220	24-31	Yes	Helical motile filaments; optimum growth at 30-37°C	Insects, plants
Order III: *Acholeplasmatales*						
Family I: *Acholeplasmataceae*						
Genus: *Acholeplasma*	13	1500-1650	26-36	No	Optimum growth at 30-37°C	Animals, some plants, insects
Order IV: *Anaeroplasmatales*						
Family: *Anaeroplasmataceae*						
Genus I: *Anaeroplasma*	4	1500-1600	29-34	Yes	Oxygen-sensitive anaerobes	Bovine/ovine rumen
Genus II: *Asteroleplasma*	1	1500	40	No	Oxygen-sensitive anaerobes	Bovine/ovine rumen
Undefined taxonomic status						
Phytoplasma	Not defined	640-1185	23-29	Not known	Uncultured in vitro	Insects, plants

Table 1: Major characteristics and taxonomy of the class *Mollicutes* (adapted from Razin *et al.*, 1998)

Because of the lack of cell wall, mycoplasmas show a great pleomorphism; the predominant shape is sphere, but many mollicutes exhibit a variety of morphological entities, including pear-shaped cells, flask-shaped cells with terminal tip structures, filaments of various lengths, and helical filaments. The ability to maintain such shapes in the absence of a rigid cell wall has long indicated the presence of a structure similar to cytoskeleton. Moreover, some of the flask-shaped *Mycoplasma* species are capable of gliding on solid surfaces (Razin *et al.*, 1998). The cytoskeleton-like structure is thought to function in modulating cell shape and to participate in cell division, gliding motility, and the proper localization of adhesins (Razin *et al.*, 1998).

Mycoplasmas replicate by binary fission, but often division is not fully synchronized with genome replication, and the cytoplasmic division may lag behind genome replication, resulting in the formation of multinucleate filaments (Fig. 2).

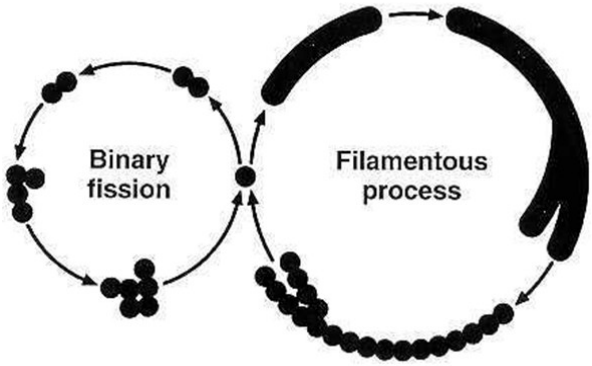


Figure 2: Schematic presentation of mycoplasma reproduction

The transformation of mycoplasma filaments into chains of cocci followed by microcinematography revealed the appearance of constrictions in the cell membrane at about equal distances along the entire length of the filament (Razin, 1978).

Mycoplasmas regressively evolved from gram-positive bacteria by reduction of their genome to an essential minimum, economizing their structural elements, metabolic pathways, and genetic resources (Razin *et al.*, 1998). The mycoplasma genome is typically prokaryotic, consisting of a circular, double stranded DNA molecule. The genome size ranges from 580 kb of *M. genitalium* to 1380 kb of *M. mycoydes* subsp. *mycoydes* LC. Size is variable not only within the same genus but even among strains of

the same species (Citovsky *et al.*, 1988; Robertson *et al.*, 1990; Ladefoged and Christiansen, 1992; Huang *et al.*, 1995). This variability can be explained by the occurrence of repetitive elements or insertion sequences, by the integration into the chromosome of viral sequences, and by horizontal gene transfer (Razin *et al.*, 1998; Sirand-Pugnet *et al.*, 2007).

Mycoplasma genomes are characterized by a very low G+C content, ranging from 24 to 33 mol% (Tully and Razin, 1996). This feature was probably determined by the low specific activity of mycoplasmas uracil-DNA glycosylase, functioning in the removal of uracil residues from DNA arising by spontaneous deamination of cytosine residues (Wieslander *et al.*, 1995). The decreased capacity of mycoplasmas to remove uracil residues from DNA would favor the gradual replacement of G · C base pairs with A · T base pairs, leading to the low G+C content of mollicutes genomes (Razin *et al.*, 1998). The G+C content along the chromosome is not equal. For instance, while *M. genitalium* genome has an average G+C content of 32 mol%, the G+C content of its rRNA genes is 44 mol% and that of its tRNA genes is 52 mol% (Fraser *et al.*, 1995). Moreover, the *M. pneumoniae* adhesin genes, P1 and ORF6, and their repetitive sequences, exhibit a G+C content as high as 56 mol%, while on the other extreme the origin of replication of this mycoplasma has a G+C content of only 26 mol%, compared to 40 mol% of the entire *M. pneumoniae* genome (Himmelreich *et al.*, 1996, 1997). Consequently, many of the mycoplasmal intergenic regions have a higher A+T content than do the coding regions, reaching values as high as 80 to 90 mol% (Muto and Osawa, 1987; Yogev *et al.*, 1991; Dybvig and Yu, 1994). The variable G+C content of coding regions within the mycoplasmal genome indicates the highly conserved nature of the rRNA and tRNA genes and the possible exogenous origin of the adhesin genes (Razin *et al.*, 1998).

The high A+T content in mycoplasma genome established an evolutionary pressure that drove to the preferentially usage of A and T rich codons, and finally to proteins with low Gly, Pro, and Arg content. The A-T pressure led to the reassignment of UGA stop codon to a tryptophan one, as already seen in mitochondria (Osawa *et al.*,

1992). As a consequence, the expression of mycoplasma recombinant proteins in *E. coli* is hampered, and it is necessary the modification of these codons (Razin *et al.*, 1998).

It would be expected that the lack of the cell wall facilitate the introduction of exogenous DNA into mycoplasma cells, but it was demonstrated that the exchange of chromosomal DNA during direct contact of mycoplasma cells and conjugative transposition of transposon Tn916 from *Streptococcus* (*Enterococcus*) *faecalis*, has a very low efficiency. Moreover, the recent work by Sirand-Pugnet and coworkers revealed the occurrence of horizontal gene transfer (HGT) events in *M. agalactiae*. The analysis of genomic data demonstrated the acquisition of entire operons by *M. agalactiae* from *M. capricolum* subsp. *capricolum* and *M: mycoydes* subsp. *mycoides*; it is important to notice that these mycoplasma species belong to different phylogenetic cluster, but they share the same hosts, so the genetic exchange may occour during coinfections (Sirand-Pugnet *et al.*, 2007). The mechanism of this exchange is already unclear, but it was hypothesized that it is mediated by a spontaneous mating process that probably involves transient fusion of the cell membranes at the zone of contact (Roberts and Kenny, 1987; Barroso and Labarère, 1988).

Under laboratory conditions increased transformation and transfection efficiencies have been achieved either in the presence of polyethylene glycol (PEG) or by application of the electroporation procedure, and the application of these methods lead to important genetic and functional studies (Razin *et al.*, 1998).

2.2 Metabolism

During their evolution, mycoplasmas saved only few genes for biosynthetic pathways, and it can be speculated the these are the really essential for a minimal cell survival. Mycoplasmas have lost all the genes for amino acid and cofactors biosynthesis; as a result, mycoplasmas depend on host resources for living and evolved a parasitic lifestyle (Razin *et al.*, 1998). Moreover, because of the lack of the cell wall, mycoplasmas are more sensitive to osmotic shock than other bacteria, and the parasitic mode indirectly protect them because the host provide a constant osmotic environment (Razin *et al.*, 1998).

The reductive evolution also affected the lipid metabolism. In fact, most mycoplasmas depend on host supply for fatty acids, because they cannot synthesize them. Moreover, although mycoplasmas are usually able to synthesize their own membrane phospholipids and glycolipids from the exogenously provided fatty acids, some mycoplasmas incorporate preformed host phospholipids into their membrane. This way, it is not possible to regulate the membrane fluidity with selective fatty acid synthesis; mycoplasmas control membrane fluidity incorporating large amounts of exogenous cholesterol in their membrane (Razin, 1978).

Mycoplasmas preserved the salvage pathways using purines and pyrimidines for the synthesis of ribonucleotides and their conversion to deoxyribonucleotides; despite that, mycoplasmas generally use preformed exogenous nucleotides that they get degrading host DNA and RNA with their potent nucleases (Razin, 1978; Minion *et al.*, 1993; Bendjennat *et al.*, 1997, 1999; Paddenberg *et al*., 1998; Hopfe *et al.*, 2008).

The analysis of the sequenced mycoplasma genomes revealed that the number of genes involved in DNA replication and repair, in transcription, and in translation is smaller than in other bacteria; thus, it can be speculated that mycoplasmas possess the minimal machinery for protein synthesis. The rate of protein synthesis, and consequently of cell replication, is very low. This represents an advantage for a parasite that would lose its safe environment by killing its host; in fact, mycoplasmosis are usually mild and chronic infections (Razin *et al.*, 1998).

The reductive evolution involved also many other cellular processes, such as the cell division, the heat shock response, and the proteins secretion system. For instance, the number of the protein secretion complex is smaller than in *E. coli*; the simpler secretion machinery could be a consequence of the simplicity of mycoplasma cell. Moreover, because of the lack of the periplasmic space, in mycoplasmas protein refolding must be linked to cell surface; they solved this problem anchoring proteins to membrane by long acyl chains (lipoproteins) (Razin *et al.*, 1998).

Despite the importance of nutrients uptake from the host, mycoplasmas do not have many genes for membrane transport. The scarcity of the number of this kind of genes can be explained by the presence of only one barrier between mycoplasmas and the host cells. Another explanation is the apparent low substrate specificity of some mycoplasmal transport systems, such as those for amino acids (Himmelreich *et al.*, 1997). Three types of transport systems were found in *M. genitalium* and *M. pneumoniae* (Fraser *et al.*, 1995; Himmelreich *et al.*, 1996). The first is ABC transporter systems, consisting of two ATP binding domains, two membrane spanning- and one substrate-binding domain; frequently present on separate polypeptides. The second type consists of PTS systems for transporting sugars, resembling homologous systems of gram-positive bacteria. The third is facilitated diffusion by transmembrane proteins functioning as specific carriers.

The reducing process of mycoplasmas evolution involved also the metabolic activities for energy generation. All the mollicutes examined so far have truncated respiratory systems; they lack a complete tricarboxylic acid cycle and have no quinones and cytochromes, ruling out oxidative phosphorylation as an ATP-generating mechanism (Razin, 1978; Miles, 1992; Pollack *et al.*, 1997). On the basis of their ability to metabolize carbohydrates, the mollicutes are divided into fermentative and non-fermentative organisms; the fermentative mycoplasmas produce acids from carbohydrate metabolism, decreasing the pH of the medium (Razin *et al.*, 1998). Most of the non-fermentative mollicutes and some fermentative species possess the arginine dihydrolase pathway, that leads to the production of ornithine, ATP, CO_2, and ammonia, raising the pH of the culture medium (Razin, 1978). The degradation of arginine is

coupled to equimolar generation of ATP by substrate-level phosphorylation. Some mycoplasmas, such as *M. agalactiae*, *M. bovigenitalium*, and *M. bovis* do not use this two metabolic pathways but are able to oxidate organic acids (lactate, pyruvate) to acetate and CO_2 (Miles, 1992; Taylor *et al.*, 1994; Tully and Razin, 1996). Another mollicutes mechanism for energy production is based on ATP generation from acetyl phosphate and ADP by acetate kinase, coupled with acetyl phosphate formation from acetyl-CoA by phosphate acetyl transferase; both enzymes are commonly found in fermentative and non-fermentative mollicutes. Acetyl-CoA can be produced by oxidative phosphorylation of pyruvate by mycoplasmas (Razin, 1978).

2.3 *In vitro* cultivation

The fastidious nature of mycoplasmas hampered the research and laboratory diagnosis of mycoplasmosis. Only a minority of existing species has been cultivated *in vitro* and many mycoplasmas grow very slowly on available media (Razin, 1994). The difficulty of cultivation are explained by scarcity of genes involved in biosynthetic pathways, such as that for amino acid synthesis (Fraser *et al.*, 1995; Himmlreich *et al.*, 1997). As a consequence, mycoplasmas evolved a parasitic lifestyle, and they are totally dependent on the exogenous supply for many substances, that must be provided in complex media, based on beef heart infusion, peptone, yeast extract, and serum with various supplements (Razin, 1991). Serum is very important in mycoplasmas cultivation because it provides, among other nutrients, fatty acids and cholesterol (required for membrane synthesis) in an assimilable non-toxic form. The lipid requirements, particularly for cholesterol, were used as taxonomic criterion distinguishing the sterol-nonrequiring mollicutes, particularly the *Acholeplasma* species, from the sterol-requiring ones (Table 1).

Some *Mollicutes* grow under laboratory conditions only when co-cultivatied with eukaryotic cell lines; in many cases, after co-cultivation, mycoplasmas can be subcultured in cell-free systems (Jensen *et al.*, 1994, 1996).

One of the most useful distinguishing features of mycoplasmas is their peculiar fried-egg colony shape, when cultured in solid media, consisting of a central zone of growth embedded in the agar and a peripheral one on the agar surface (Fig. 3) (Rottem and Kahane, 1993).

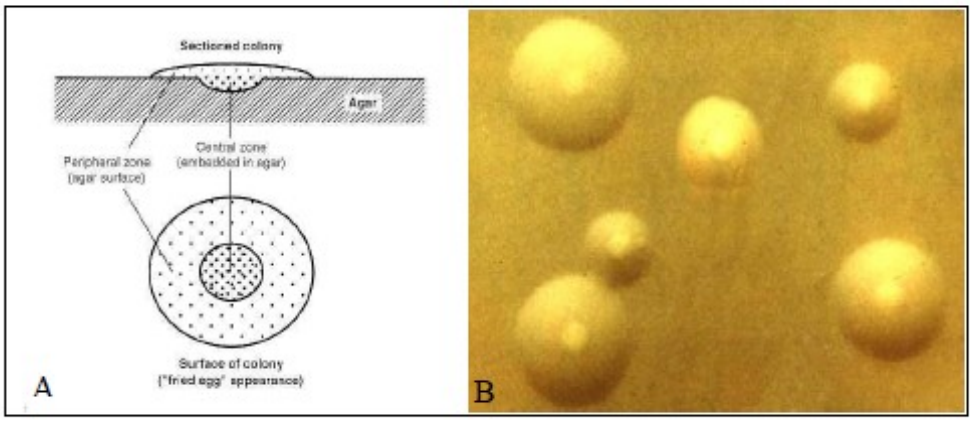


Figure 3: Morphology of a typical "fried-egg" mycoplasma colony. A: schematic representation; B: *Mycoplasma agalactiae* colonies on solid medium

2.4 Mycoplasma lipoproteins

Mycoplasma membrane has a very high protein content, representing over one third of membrane mass and about 50% of total proteins of the cell. Among them, lipoproteins are considered the most interesting class since their abundance is much higher than in eubacteria. Mycoplasma lipoproteins genes code for a signal peptide upstream a cysteine residue (Himmelreich *et al.*, 1996); this residue is modified in the sulfhydryl group by the transfer of a diacylglycerol moiety from glycerophospholipid; the signal peptidase II turns the prolipoprotein into the mature form cleaving the signal peptide, so the modified cysteine becomes the first amino acid. Mycoplasma lipoproteins can be di- or tri-acylated, but the transacylase responsible for the acylation of the amino group of cysteine was never identified (Razin et al., 1998).

Mycoplasmas posses a very high phenotypic plasticity; in fact, lipoproteins, the most immunodominant mycoplasmal antigens, often undergo size and/or phase variation (Fig. 4) (Glew *et al.*, 2000; Chopra-Dewasthaly *et al.*, 2008).

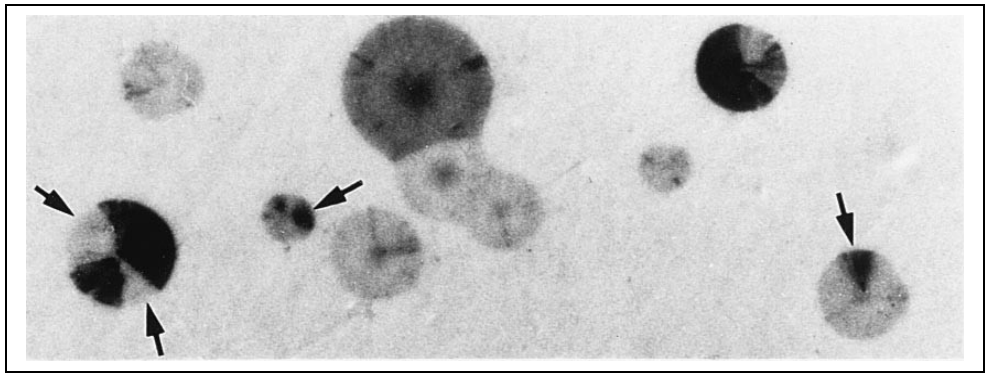


Figure 4: Colony blotting of *M. bovis* PG45^{T} revels differential expression of variable surface lipoproteins

This feature allows mycoplasmas to evade host immunity by constantly changing their antigenic mosaic, and to enhance the colonization of host tissues (Dramsi *et al.*, 1993; Rainey *et al.*, 1993). The major survival strategy of the mycoplasmas seems to depend on random and stochastic processes, consisting of various mutational mechanisms which generate high-frequency phenotypic switching (Razin *et al.*, 1998). There are two main strategies to accomplish surface protein variation. The first is based

on the variation of the number of repeated sequences, by both homologous recombination and slipped-strand mispairing (Levinson and Gutman, 1997; Rasmussen *et al.*, 1992; Robertson *et al.*, 1992); this mechanism leads to the size variation of the antigen The second mechanism is the site-specific inversion of the promoter upstream the coding sequence, that switches ON/OFF the expression of the protein; this hypothesis is validated by the presence of a site-specific tyrosine recombinase gene nearly the genes coding for variable surface lipoproteins (Flitman-Tene *et al.*, 2003).

2.5 Invasivity and pathogenicity

Mycoplasmas are widespread in nature as parasites and commensals of humans, mammals, reptiles, fish, arthropods, and plants. Usually mycoplasmas show a strict host and tissue specificity, probably reflecting their nutritional requirements and their parasitic lifestyle, but there are many reports of the their presence outside of their normal habitats (Razin, 1992; Razin *et al.*, 1998). Human and animal mycoplasmas colonize preferentially the respiratory and urogenital tracts, eyes, alimentary canal, mammary glands, and joints; the obligatory anaerobic anaeroplasmas have been found in bovine and ovine rumen only (see Table 1).

The adhesion of mycoplasma to host cell is essential for colonization and for infection; adhesion-deficient mutants exhibit loss of infectivity and the revertion to wild type is coupled with the gain of infectivity and virulence (Razin *et al.*, 2008). It has been proposed that during contact of mycoplasmas with the host cell membrane there could be a local, perhaps transient fusion of the two membranes. Moreover, there could take place exchange of membrane components and direct "injection" of the mycoplasma cytoplasmic content, such as hydrolytic enzymes, into the host cell cytoplasm (Razin *et al.*, 1998). Mycoplasma adhesion to host cells could be mediated by specific structures or proteins called adhesin, that interact with host surface proteins, such as receptors. It is interesting to notice that many mycoplasma surface proteins undergo size and phase variations; by this mechanism mycoplasmas evade host immune response and, at the same time, and increase the chances to find the right receptors on different tissues of the

host and adapt to the different niches (Razin *et al.*, 1998). Moreover, a recent work highlighted the role of elongation factor tu, a protein with tipically cytoplasmatic localization, in adhesion, by its interaction with fibronectin (Balasubramanian *et al.*, 2008).

Mycoplasmas usually locate on the surface of host cells, but it was already demonstrated that polymorphonuclear leukocytes and macrophages are able to take up human and animal mycoplasmas (Marshall *et al.*, 1995, 1998). The mechanism of cell entry is still unclear. Mycoplasmas such as *M. fermentans* and *M. hominis*, have no dedicated structures (Wise, 1993), while other mycoplasmas with intracellular location, such as *M. penetrans* and *M. genitalium* appear to enter host cells through their specialized tip structure (Fig. 5) (Lo *et al.*, 1993; Jensen *et al.*, 1994).

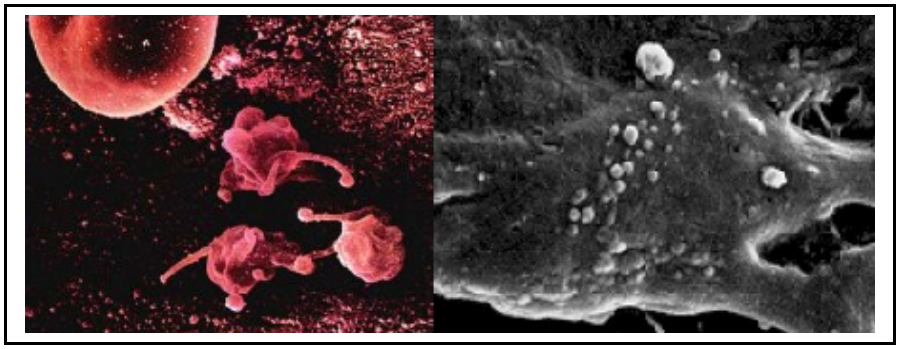


Figure 5: *Mycoplasma hominis* adhesion structures

Following contact of *M. genitalium* with human lung fibroblasts, the host cell membrane appeared to be forced to form a cup or a depression. The membrane pockets resembled clathrin-coated pits, suggesting that the mycoplasma might adhere to and enter the cells by a site-directed, receptor-mediated event, similar to the chlamydias mechanism (Mernaugh *et al.*, 1993; Razin *et al.*, 1998). The presence of mycoplasmas, some of which may not even be enclosed within a vacuole, inside the host cells may expose the cytoplasm and the nucleus to mycoplasmal hydrolytic enzymes, such as proteases, nucleases, and phospholipases. For instance, the potent endonuclease of *M. penetrans* was suggested to cause chromosomal damage and to promote apoptosis (Bendjennat *et al.*, 1997, 1999; Paddenberg *et al*., 1998; Hopfe *et al.*, 2008).

Moreover, intracellular location, if even for a short period, may protect the mycoplasmas from the host immune system and antibiotics and may account to some extent for the difficulty of eradicating mycoplasmas from infected cell cultures. Thus, intracellular residence promotes the establishment of latent or chronic infection states and circumvents bactericidal immune mechanisms and selective drug therapies (Baseman and Tully, 1997).

Toxins were never identified in mycoplasmas and in general their pathogenetic mechanisms are still to be fully clarified. It is known that mycoplasmas can induce oxidative stress in the host because of their metabolism products, such as hydrogen peroxide and superoxide radicals (Fig 6) (Razin *et al.*, 1998).

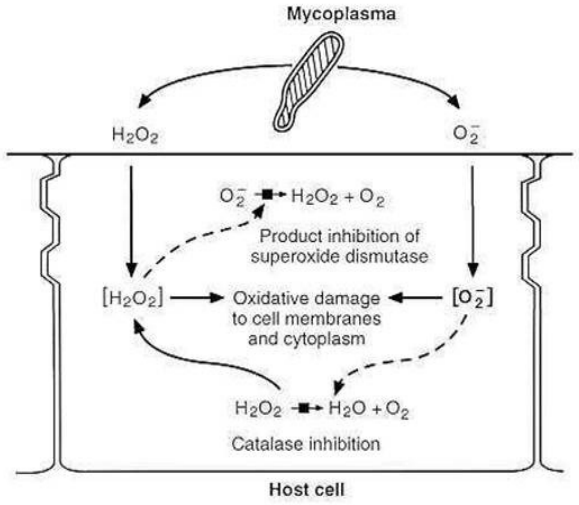


Figure 6: Proposed mechanism of oxidative damage to host cells by adhering *M pneumoniae* by increasing concentrations of H_2O_2 and O^{2-}.

It was reported that *M. orale* can deregulate and reduce the number of Cl^- and of Ca^{2+}-activated K^+ channels of human submandibular gland cell line; these changes *in vivo* could lead to a diminished fluid production and, consequently, to xerostomia (Izutsu *et al.*, 1996; Razin *et al.*, 1998). Similarly, in mycoplasma-infected ciliary cells, such as ciliated bronchial epithelia, it was reported the ciliostasis; the preferential loss of K^+ channels and the consequent depolarization of the membrane, could lead to a similar situation (DeBey and Ross, 1994; Izutsu *et al.*, 1996; Razin *et al.*, 1998).

2.6 Diagnosis of mycoplasmosis

Mycoplasmosis diagnosis is hampered by the great number of variable clinical signs that make necessary the use of laboratory tools.

The classical diagnostic technique used for dectecting and characterizing mycoplamas was their isolation i*n vitro* from swabs, milk or other fluids. The identification with this method can be very difficult because of their fastidious nature. Mycoplasma colonies appear typically after about 72-96 h, and after 5-7 days the characteristic spots and films can be appreciated, a thin layer of lipids and carbohydrates.

The biochemical identification allows mycoplasma classification on the basis of the metabolism of some molecules, such as pyruvate, glucose, arginine; the major disadvantage of these methods is that they are time consuming.

The immunofluorescence and immunohistochemistry are useful to avoid contaminations, but the procedures are very long and expensive (Adegboye *et al.*, 1995) and depends on the availability of specific and sensitive sera.

In the past, many other thecniques were proposed for mycoplasmas diagnosis, but their application was limited by their time-consuming nature, their high costs or their low specificity, such as dot immunoblot, western blotting, complement fixation, DNA hybridation, indirect hemagglutination, and radial hemolysis (Poumarat *et al.*, 1992; Dohms *et al.*, 1993).

Actually, the most used diagnostic tests are ELISA and PCR. ELISA tests allows to reveale both antigens or antibodies, and it is a very simple, fast, and cheap method (Rosati *et al.*, 1999, 2000; Robino *et al.*, 2005; Alberti *et al.*, 2008). PCR is the most sensitive and specific diagnostic tool; it allows the direct identification of the pathogen, and it could be performed after *in vitro* culture or directly on swabs, milk or other fluids (Tola *et al.*, 1996; Alberti *et al.*, 2006).

2.7 *Mycoplasma agalactiae* and Contagious Agalactia

Mycoplasma agalactiae is the aetiological agent of Contagious agalactia (CA), an economically detrimental disease of small ruminants. CA is characterized by mastitis, polyarthritis and keratoconjunctivitis; in lambs, losses due to septicemia and pneumonia can be high. Some outbreaks may affect most of the animals on a farm. Actually, CA was reported in 31 countries, and the only continent CA-free is Oceania (Corrales *et al*., 2007). CA is endemic in the Mediterrean area, Africa, and Asia; in America, exept for the USA, this disease is described as sporadic (Bergonier *et al.*, 1997; Corrales *et al*., 2007).

The control of this disease is very difficult because of the intrinsic characteristics of mycoplasmas. Antibiotic therapy can result in symptomatic improvement, but treated animals may remain carriers due to antibiotic resitence.

Infected animals shed organisms in urine, feces, nasal and ocular discharges, and secretions including milk. Mycoplasmas can be shed during more than one lactation; between lactations, the organisms can survive in the supramammary lymph nodes. Carrier animals can remain infectious for months, and in some cases, for more than a year. Animals become infected by ingestion or occasionally by inhalation, as well as through the teat openings. Young animals are usually infected when they drink contaminated milk or colostrum from the dam. In rare cases, animals can also ingest mycoplasmas shed in other secretions and excretions, either directly or in feed or water. Organisms can enter the teat opening directly during milking, or from fomites such as bedding. Aerosol transmission is possible over short distances, but rare.

Mycoplasma agalactiae survive in the environment thanks to its biofilm, that prevent the death for heat or drying.

Generally, the clinical signs are more severe in goats. Infections with *M. agalactiae* may be asymptomatic, acute or chronic. Acute cases begin with a transient fever followed by malaise, inappetence and mastitis. The udder is hot and swollen, and the milk is usually greenish–yellow or grayish–blue, with a consistency that is watery at first then becomes lumpy. Lactation diminishes and may completely stop. Eventually, the udder atrophies and becomes fibrosed. Polyarthritis is also common, especially in

the tarsal and carpal joints, and may be the major clinical sign in male goats; the severity of this synthom can vary. Keratoconjunctivitis develops in approximately half of all infections. It is usually transient, but occasionally becomes chronic, and can cause blindness in one or both eyes (Fig. 7).

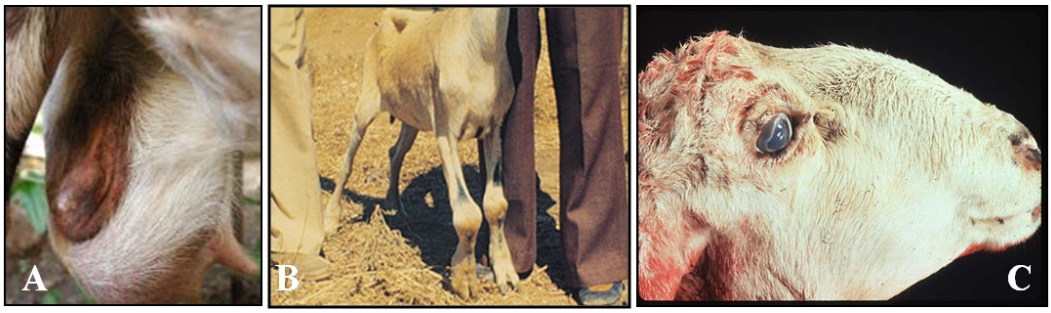


Figure 7: Clinical signs of CA. A: mastitis, B: Arthritis, C: Keratoconjunctivitis

Pneumonia is not consistently seen with *M. agalactiae*, but organisms are occasionally isolated from lesions in the lungs. Abortions can occur in chronically infected animals. Granular vulvovaginitis has also been reported in goats (Farina and Scatozza, 1998).

In areas that are free of contagious agalactia, infected herds are usually quarantined and euthanized. The premises should be cleaned and disinfected before restocking. Mycoplasmas can be inactivated by many disinfectants including sodium hypochlorite (30 ml of household bleach in 1 gallon of water), 2% sodium hydroxide (pH 12.4), 1% formalin, cresol, sodium carbonate (4% anhydrous or 10% crystalline with 1% detergent), and ionic and nonionic detergents. Eradication has been accomplished in some areas by the euthanasia of infected and exposed flocks or herds.

In endemic areas, good management and hygiene can reduce the transmission of contagious agalactia in a herd. The premises and equipment should be cleaned and disinfected regularly, and sick animals should be isolated. Milking animals should also be separated from young animals. Removal of the newborn from the dam, with feeding of pasteurized colostrum and milk, can be helpful. Regular testing of the flock or herd, with culling or isolation of infected animals, can also help prevent disease introduction

and/or reduce its spread. ELISAs, culture and other tests have been useful in screening programs.

Antibiotics can result in symptomatic improvement, but they may not be effective in chronic joint infections or keratoconjunctivitis. Treatment may not eliminate the infection from carriers. Vaccines may be available for some organisms in some areas. Inactivated vaccines generally provide short-term protection. Live vaccines can prevent symptoms, but do not prevent animals from becoming infected or shedding the organism. Vaccine organisms may also be shed in milk.

3. RESEARCH OBJECTIVES

Mycoplasmas are the smallest and simplest prokaryotes capable of self-replication, being provided only with the minimal machinery required for survival (Razin *et al.*, 1998). During evolution, they have regressively evolved from gram-positive bacteria by reduction of their genome to an essential minimum, economizing their structural elements, metabolic pathways, and genetic resources. Among other consequences, this cost-cutting strategy led to loss of the cell-wall component, and therefore to the lack of a peptidoglycan "shell". Integral and associated membrane proteins are therefore directly exposed at the cell surface and act as the immediate bacterial interface, playing a major role in survival and pathogenesis (Rottem, 2003; You *et al.*, 2006). Gathering information on membrane proteins of such a pathogen might provide novel and interesting insights on its biology, and generate useful information for improving diagnosis, vaccination, and therapy.

Many mycoplasmas are pathogenic for humans, animals, plants, and insects. *M. agalactiae* is the etiological agent of Contagious Agalactia (CA), a serious disease of sheep and goats characterized by mastitis, polyarthritis, keratoconjunctivitis, and abortion (Lambert, 1987; Razin *et al.*, 1998; Corrales *et al.*, 2007). In Europe, the disease has been tentatively controlled either by vaccination or with serological tools based on recombinant surface proteins (Tola *et al.*, 1999; Rosati *et al.*, 2000; Greco *et al.*, 2002; Nicholas, 2005; Fusco *et al.*, 2007; Chessa *et al.*, 2009). At present, the two above mentioned strategies are not actually compatible until proper DIVA (Differentiating Infected from Vaccinated Animals) vaccines will allow discrimination of vaccinated animals from naturally infected ones.

The highly immunogenic, surface-associated membrane proteins represent key antigens for diagnosis and vaccine development. However, the finding of constantly expressed surface proteins in mycoplasmas is complicated by the existence of mechanisms aimed to evade the host immune response (Razin *et al.*, 1998; Glew *et al.*, 2000; Chopra-Dewasthaly *et al.*, 2008). To date, a limited number of constantly expressed surface proteins have been described in *M. agalactiae*. Among them, P30,

P48, and P80 were described as antigens (Rosati *et al.*, 1999; Fleury *et al.*, 2001: Tola *et al.*, 2001); other proteins belong to the variable surface membrane proteins family (Vpma) (Glew *et al.*, 2000; Chopra-Dewasthaly *et al.*, 2008) and P40 was suggested to play an important role in attachment to the host cell (Fleury *et al.*, 2002).

In 2007, the full genome sequence of the *M. agalactiae* $PG2^T$ was published and paved the way for systematic proteomic studies in mycoplasmas (Sirand-Pugnet *et al.*, 2007). The combination of 2-D PAGE and mass spectrometry (MS) is a well-established method for the systematic and comparative study of proteomes, since it allows the simultaneous visualization and identification of the protein complement of a cell. However, it is commonly reported that standard 2-D PAGE lacks in resolution of very hydrophobic and basic proteins, which are particularly abundant in the mycoplasma membrane (Razin *et al.*, 1998; Regula *et al.*, 2000; Sirand-Pugnet *et al.*, 2007). Indeed, membrane proteins are poorly detected in 2-D PAGE maps of mycoplasma total protein extracts (Jores *et al.*, 2009). Triton X-114 fractionation may assist in solving this problem, since it was demonstrated to enable a selective enrichment in hydrophobic proteins (Bordier, 1981; Pittau *et al.*, 1990). Triton X-114 fractionation followed by 2-D PAGE remains the method of choice for proteomic characterization of the membrane protein subset and for differential analysis of membrane protein expression among bacterial strains (Donoghue *et al.*, 2008; Li *et al.*, 2009). However, considering the above mentioned intrinsic limitations of 2-D PAGE, other gel-based proteomic approaches, such as one-dimensional PAGE and Liquid Chromatography-Tandem Mass Spectrometry (GeLCMS/MS). can be combined with the 2-D PAGE/MS in order to mine deeper into a liposoluble proteome.

The major objective of this work is the characterization of *M. agalactiae* membrane proteome, by means of Triton X-114 fractionation, 2-D PAGE-MS, GeLC-MS/MS, and Gene Ontology classification. Differential expression of membrane proteins among *M. agalactiae* strains isalso evaluated by 2D DIGE. The results of this work will allow to develop new diagnostic and prophylaxis for the control of CA.

4. MATERIALS AND METHODS

4.1 Media and buffers

PPLO medium

PPLO broth	21 g/L
Triptone	10 g/L
Yeast extract	5 g/L
Horse serum	20% v/v
H_2OMQ	to volume

Mycoplasma solid medium

Blood agar base	40 g/L
Horse serum	20% v/v
H_2OMQ	to volume

Lysis buffer

Urea	8 M
Tiourea	2 M
CHAPS	2% p/v
ASB-14	2.5% p/v
H_2OMQ	to volume

Running buffer

Tris	3.0275 g/L
Glycine	14.413 g/L
SDS	1 g/L
H_2OMQ	to volume

Transfer buffer

Tris	3.0275 g/L
Glycine	14.413 g/L
Methanol	20% v/v
H_2OMQ	to volume

Equilibrating base buffer

Urea	6 M
Tris-HCl pH 8.8	0.50 mM
Glycerol	20% v/v
SDS	2% p/v

PBS-T

PBS	1 L
Tween-20	0.05%

Laemmli sample buffer

SDS	2% w/v
Glycerol	10% v/v
β- mercaptoethanol	5% v/v
Tris-HCl pH 6.8	0.0625 M
Bromophenol blue	0.002% w/v
H_2OMQ	to volume

4.2 Bacterial strains and culture conditions

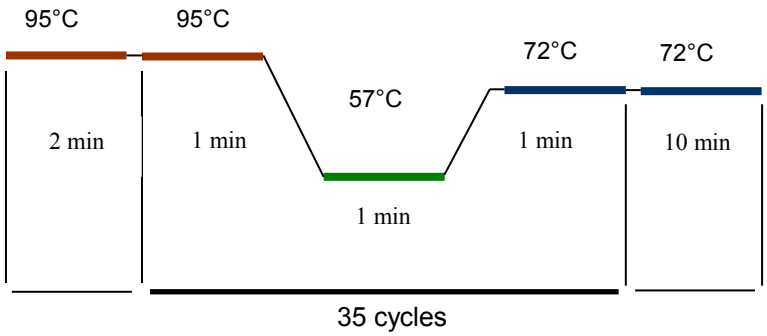
At least three replicate cultures of *Mycoplasma agalactiae* PG2^{T} and two Sardinian field isolates (named Bortigali and Nurri), were grown in PPLO medium supplemented with 20% heat-inactivated horse serum and 500 µg/mL ampicillin, at 37°C with constant agitation. Mycoplasmas were collected by centrifugation (10 min at 10,000 x g at 4°C), and washed three times with ice-cold PBS. At least three mycoplasma pellets were obtained from each bacterial culture replicate, and used for genetic and proteomic analyses.

4.3 Total DNA extraction and PCR

Total DNA was extracted from a set of pellets with DNeasy Blood & Tissue Kit (Qiagen), and subjected to FS1-FS2 PCR for species confirmation (Tola *et al.*, 1996).

FS1 5'-AAAGGTGCTTGAGAAATGGC-3'
FS2 5'-GTTGCAGAAGAAAGTCCAATCA-3'

The PCR reaction was conducted in a final volume of 50 µL using GoTaq DNA polymerase (Promega), in an Eppendorf Mastercycler® with the following amplification program:



The PCR product of 375 bp was displayed on 2% agarose gel stained with ethidium bromide.

4.4 Total proteins extraction

For total protein extracts, bacterial pellets were resuspended in 1% hot SDS, incubated for 3 minutes at 95°C, chilled, and diluted with lysis buffer supplemented with 1% IPG-buffer (GE Healthcare) and protease inhibitors (Sigma), and insoluble materials were discarded by centrifugation (10 min at 10,000 x g at 4°C) (Ferrer-Navarro *et al.*, 2006).

4.5 Triton X-114 fractionation

Hydrophilic and hydrophobic protein fractions were obtained by Triton X-114 fractionation (Bordier, 1981; Pittau *et al.*, 1990), using the following protocol:

1. resuspend bacterial pellet with 900 µL of ice-cold PBS
2. add 100 µL of 10% Triton X-114
3. incubate for 5 minutes at 95°C
4. incubate for 1 h at 4°C under constant agitation
5. spin at 10,000 x g for 10 minutes at 4°C
6. transfer the supernatant into a new tube and incubate for 5 minutes at 37°C (since the solution achieves the cloud point)
7. spin at 8,000 x g for 3 minutes at room temperature
8. separate the two phases

8a **Lower phase (detergent): it carries hydrophobic proteins**	8b. **Supernatant (PBS**): **it carries hydrophilic proteins**
9a add 900 µL of ice-cold PBS	9b add 100 µL of 10% Triton X-114
10a chill the tube on ice for 5 minutes	10b chill the tube on ice for 5 minutes
11a incubate at 37°C for 5 minutes	11b incubate at 37°C for 5 minutes
12a spin at 8,000 x g for 3 minutes at room temperature	12b spin at 8,000 x g for 3 minutes at room temperature
13a discard the supernatant	13b transfer the supernatant into a new

	tube
14a repeat the wash step 3 times	14b repeat the wash step 3 times
15a after the third step, discard the supernatant and recover the detergent phase	15b after the third step, transfer the supernatant into a new tube and discard the detergent phase

Protein concentration was measured with RC DC Protein Assay kit (Bio-Rad) as already described (Ferrer-Navarro *et al.*, 2006).

4.6 SDS-PAGE

Total extracts and hydrophobic and hydrophilic fractions of *M. agalactiae* $PG2^T$ proteins were diluted in loading buffer (Laemmli, 1970) and SDS-PAGE was performed on 8% polyacrylamide gels on a Protean Tetra Cell (Bio-Rad) following the manufacturer instructions. After run, gels were alternatively stained with PageBlue™ Protein Staining Solution (Fermentas), or subjected to Western immunoblotting.

4.7 Western immunoblotting

SDS-PAGE resolved proteins were transferred onto nitrocellulose membranes with a Mini-Trans-Blot Cell (Bio-Rad) at 250 mA for one hour at 4°C After blotting, membranes were blocked with PBS-T containing 3% BSA. Membranes were incubated for one hour with a rabbit hyperimmune serum raised against *M. agalactiae* recombinant P48 (*M. agalactiae* rP48) (Rosati *et al.*, 2000) diluted 1:1000 with blocking solution. Membranes were washed five times with PBS-T and incubated with the α-rabbit-IgG HRP-conjugated secondary antibodies (Sigma) diluted 1:250,000 in blocking solution. After five washes, membranes were developed with Chemiluminescent Peroxidase Substrate (Sigma) and images were acquired with a VersaDoc MP 4000 Imaging System (Bio-Rad).

4.8 2-D PAGE

Prior to 2-D PAGE, Triton X-114 fractions were precipitated with methanol-chloroform (Wessel and Flügge, 1984) with the following protocol:

1. add to sample 4 volumes of methanol and vortex
2. add 1 volume of chloroform and vortex
3. add 3 volumes of H_2OMQ and vortex
4. spin 1 minute at 14,000 x g
5. discard top acqueous phase (proteins are between layers)
6. add 4 volumes of methanol and vortex
7. spin 2 minutes at 14,000 x g
8. discard methanol
9. dry in SpeedVac®

After precipitation protein samples were resuspended in lysis buffer supplemented with 1% IPG-buffer (GE Healthcare) and protease inhibitors (Sigma). Resuspended proteins (150 µg) were then absorbed overnight into 18 cm IPG strips (GE Healthcare, pH 3-10 NL, pH 7-11, and pH 4-7). Strips were focused on an IPGphor (GE Healthcare) for a total of 60,000 Vh with the following program:

No. step	Volt	Gradient	Time
1	250 V	Linear	2h
2	500 V	Linear	2h
3	750 V	Linear	2h
4	1000 V	Linear	2h
5	5000 V	Linear	2h
6	9000 V	Linear	2h
7	9000-60000 V/h	Step'n'hold	
8	200 V	Linear	∞

After focusing, strips were equilibrated in equilibrating buffer supplemented with 2% DTT for 15 min, and then with 2.5% iodoacetamide for 15 min. The second dimension (SDS-PAGE) was conducted on 10% to 18% polyacrylamide gradient gels, on an Ettan DALTsix electrophoresis system (GE Healthcare), following manufacturer's instructions. After run, 2-D gels were silver stained with a mass-compatible method (Chevallet *et al.*, 2006):

1. fix the gel for 30 minutes with 50% methanol,10% acetic acid
2. wash for 15 minutes with 5% methanol
3. wash 3 times with H_2OMQ for 15 minutes
4. sensitivization: 15 minutes with 120 mg/L sodium thiosulfate
5. wash 3 times with H_2OMQ for 30 seconds
6. incubate with 2 g/L silver nitrate for 25 minutes
7. wash 3 times with H_2OMQ for 30 seconds
8. develop with 30 g/L sodium carbonate, supplemented with 2.4 mg/L sodium thiosulfate, and 500 µl/L 37% formaldehyde
9. stop: 14 g/L EDTA, pH 8

All images were digitalized with an Image Scanner (GE Healthcare).

4.9 2D DIGE

For 2D DIGE analysis, the two field isolates Bortigali and Nurri were compared to PG2^T. Triton X-114 Protein extracts were precipitated and resuspended in lysis buffer as described above. Then, samples were labeled with CyDye DIGE Fluors (GE Healthcare) according to the minimal labeling protocol provided by the manufacturer. Briefly, after CyDye reconstitution with dimethylformamide (DMF) and preparation of a working solution (200 pmol/µL), 1 µL of diluted CyDye was added to a volume of protein sample equivalent to 50 µg. Samples were left on ice for 30 minutes in the dark, and then 1 µL of 10 mM lysine was added to stop the reaction. Labeled samples were mixed, IPG buffer corresponding to the desired pH range was added at a 1% final concentration, and DeStreak Rehydration Solution (GE Healthcare) was added to a total volume of 340 µL. 18 cm IPG strips (GE Healthcare) were passively rehydrated for at least 6 hours. IEF was carried out on an Ettan IPGphor II (GE Healthcare) for a total of ~60,000 Vh, as described above. After focusing, strips were equilibrated in equilibrating buffer supplemented with 2% DTT for 10 min, and then with 2.5% iodoacetamide for 10 min. Proteins were then subjected to SDS-PAGE in 10-18% gradient polyacrylamide gels on the Ettan DALTsix system (GE Healthcare), following the manufacturer

instructions. DIGE images were detected with a Typhoon Scanner (GE Healthcare) and processed with DeCyder (GE Healthcare) for image analysis.

4.10 Spot picking and *in situ* tryptic digestion

Protein spots obtained upon 2-D PAGE separation of the Triton X-114 extract from the strain PG2^T were manually excised from gels, destained with 15 mM $K_3Fe(CN)_6$ in 50 mM $Na_2S_2O_3$ and stored in acetonitrile. Spots were then subjected to an O/N tryptic digestion at 37°C in 50 mM $(NH_4)HCO_3$, pH 8.0, using 40 to 80 ng of trypsin depending on spot intensity. Peptide mixtures were collected by elution with acetonitrile followed by centrifugation. Peptides were then acidified with TFA 20%, dried in SpeedVac®, resuspended in 0.2% formic acid and stored at -20°C.

4.11 GeLC-MS/MS.

The Triton X-114 fraction was diluted with 4X Laemmli buffer (Laemmli, 1970), 20 μg of proteins were loaded in an 8% polyacrylamide gel, and SDS-PAGE was performed as previously described. After gel staining, bands were manually excised, destained, reduced, alkylated, and finally subjected to *in situ* tryptic digestion as previously described (Addis *et al.*, 2009). Peptide mixtures were identified by nanoHPLC-nanoESI-Q-TOF-analysis. One-dimensional patterns were analyzed with Quantity One software (Bio-Rad).

4.12 MALDI-MS

Mass spectra were recorded on a MALDI micro (Waters, Manchester, UK) equipped with a reflectron analyser and used in delayed extraction mode, as described previously [56]. Peptide samples were mixed with an equal volume of α-cyano-4-hydroxycynnamic acid as matrix (10 mg/mL in acetonitrile/0.2% TFA) (70:30, v/v), applied to the metallic sample plate, and air dried. Mass calibration was performed by

using the standard mixture provided by manufacturer. Raw data, reported as monoisotopic masses, were then introduced into the in-house Mascot Peptide Mass Fingerprinting software (Version 2.2, Matrix Science, Boston, MA), and used for protein identification. Search parameters were as follows: fixed modifications carbamidomethyl (C), variable modifications pyro-Glu (N-term Q) and oxidation (M), peptide tolerance 80 ppm, enzyme trypsin, allowing up to 2 missed cleavages.

4.13 LC-MS/MS

LC-MS/MS analyses of tryptic digests were performed on a Q-TOF hybrid mass spectrometer equipped with a nano lock Z-spray source, and coupled on-line with a capillary chromatography system CapLC (Waters, Manchester, UK), as described previously (Addis *et al.*, 2009). After loading, the peptide mixture was first concentrated and washed at 20 µL/min onto a reverse-phase pre-column (Symmetry 300, C18, 5 µm, NanoEase, Waters) using 0.2% formic acid as eluent. The sample was then fractionated onto a C18 reverse-phase capillary column (Nanoflow column 5 µm Biosphere C18, 75 µm x 200 mm, Nanoseparations) at a flow rate of 250 nL/min, using a linear gradient of eluent B (0.2% formic acid in 95% acetonitrile) in A (0.2% formic acid in 5% acetonitrile) from 2 to 40% in 27 min. The mass spectrometer was set up in a data-dependent MS/MS mode where a full scan spectrum (m/z acquisition range from 400 to 1600 Da/e) was followed by tandem mass spectra (m/z acquisition range from 100 to 2000 Da/e). Peptide ions were selected as the three most intense peaks of the previous scan. A suitable collision energy was applied depending on the mass and charge of the precursor ion. Argon was used as the collision gas. Mass calibration was conducted on the Glu-fibrino peptide B (Sigma) fragmentation pattern. ProteinLynx software (Version 2.2.5), provided by the manufacturers, was used to analyze raw MS and MS/MS spectra and to generate a peak list which was introduced in the in-house Mascot MS/MS ion search software (Version 2.2, Matrix Science, Boston, MA) for protein identification. NCBI was used as sequence database. Search parameters were as follows: fixed modifications carbamidomethyl (C), variable modifications pyro-Glu (N-term Q) and

oxidation (M), peptide tolerance 30 ppm, MS/MS tolerance 0.3 Da, charge state +2 and +3, enzyme trypsin, allowing up to 1 missed cleavage.

4.14 Data analysis

MS data were subjected to gene ontology analysis with Blast2GO, using default parameters (Götz *et al.*, 2008). Identified proteins were divided into classes for functional and localization analysis; data produced by the software were used for generation of graphs by Microsoft Excel.

5. RESULTS

5.1 Species identification

FS1-FS2 PCR confirmed that all field isolates and strains used in this study belonged to *M. agalactiae* species (Fig.8).

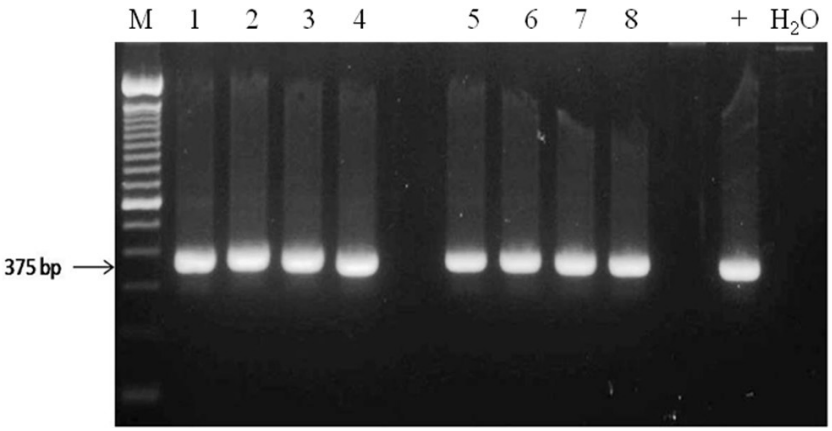


Figure 8: FS1-FS2 PCR on *M. agalactiae* isoletes. 1-4: *M. agalactiae* PG2^T strain; 5-6: *M. agalactiae* Bortigali field strain; 7-8: *M. agalactiae* Nurri field strain.

5.2 Extraction of bacterial proteins and isolation of liposoluble proteins

This study was aimed to the systematic characterization of *M. agalactiae* PG2^T membrane proteins by means of a gel-based proteomic approach. In order to increase coverage for liposoluble proteins, the Triton X-114 fractionation method was chosen. Figure 9A illustrates the hydrosoluble and liposoluble fractions obtained from *M. agalactiae* PG2^T, flanked by the total protein pattern for comparison. The efficiency of the procedure in separating liposoluble proteins was evaluated by Western immunoblotting using a rabbit hyperimmune serum raised against *M. agalactiae* rP48, a previously characterized surface lipoprotein (Rosati *et al.*, 1999, 2000). As expected, presence of P48 was observed only in the total extract and in the Triton X-114 phase (Figure 9B), confirming that the fractionation method enabled separation and enrichment of hydrophobic proteins.

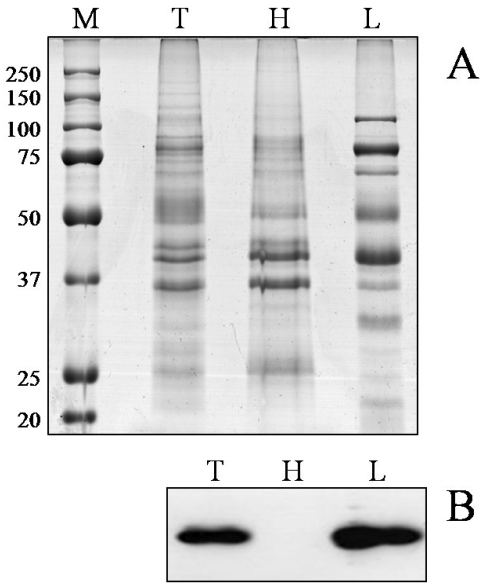


Figure 9: Total protein patterns and Western immunoblotting reactivity of *M. agalactiae* PG2^T proteins. Panel A. Coomassie blue staining. Panel B: Immunoblotting reactivity obtained with antibodies against the P48 lipoprotein. From left to right: M: molecular weight standards (kDa); T: total protein pattern; H: hydrosoluble protein fraction; L: liposoluble protein fraction obtained after Triton X-114 fractionation

5.3 2-D PAGE/MS of *M. agalactiae* PG2^T liposoluble proteins

Total proteins and the Triton X-114 soluble fraction of *M. agalactiae* PG2^T were subjected to 2-D PAGE separation in order to evaluate the extent of enrichment in basic and liposoluble proteins. As illustrated in Figure 10A, a very high number of spots were present in the total protein map of *M. agalactiae* PG2^T but, as expected, basic proteins were poorly represented. Upon comparison, the 2-D PAGE map generated with the Triton X-114 soluble fraction showed a significant enrichment in basic proteins, with an excellent resolution also in high-abundance spots (Figure 10B).

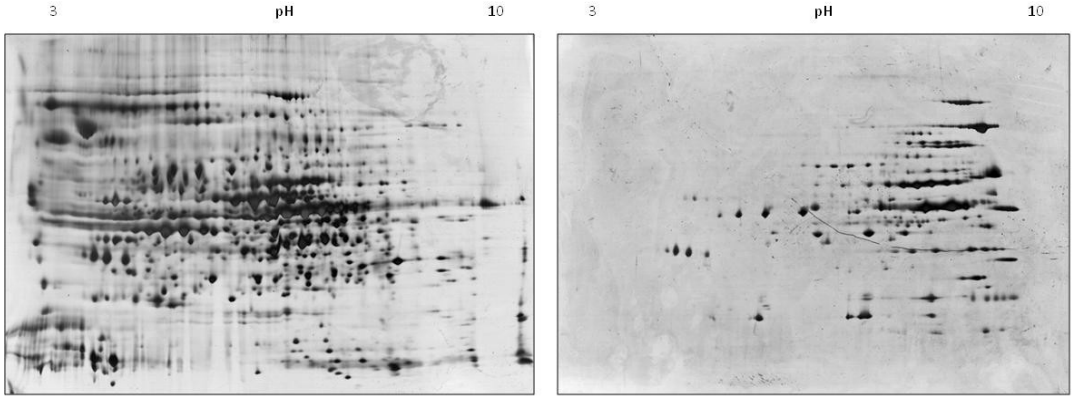


Figure 10: 2-D Page patterns of *M. agalactiae* PG2^T protein extracts. Panel A: 2-D PAGE of a *M. agalactiae* PG2^T total protein extract. Panel B: 2-D PAGE of *M. agalactiae* PG2^T liposoluble proteins obtained upon Triton X-114 fractionation.

In order to attain a systematic characterization of the liposoluble proteome, the Triton X-114 phase fraction of *M. agalactiae* PG2^T was subjected to 2-D PAGE under three different *pI* intervals: 3-10NL, 7-11, and 4-7 (Supplementary data S1, S2, and S3). From these 2D maps, about 300 spots were excised and identified by MALDI-TOF and nanoHPLC-nanoESI-Q-TOF MS. This approach led to the successful identification of 40 unique proteins, corresponding to 5.4% of all *M. agalactiae* PG2^T genes. Figure 11 reports a representative liposoluble protein map summarizing the main protein identifications accomplished on 2-D spots.

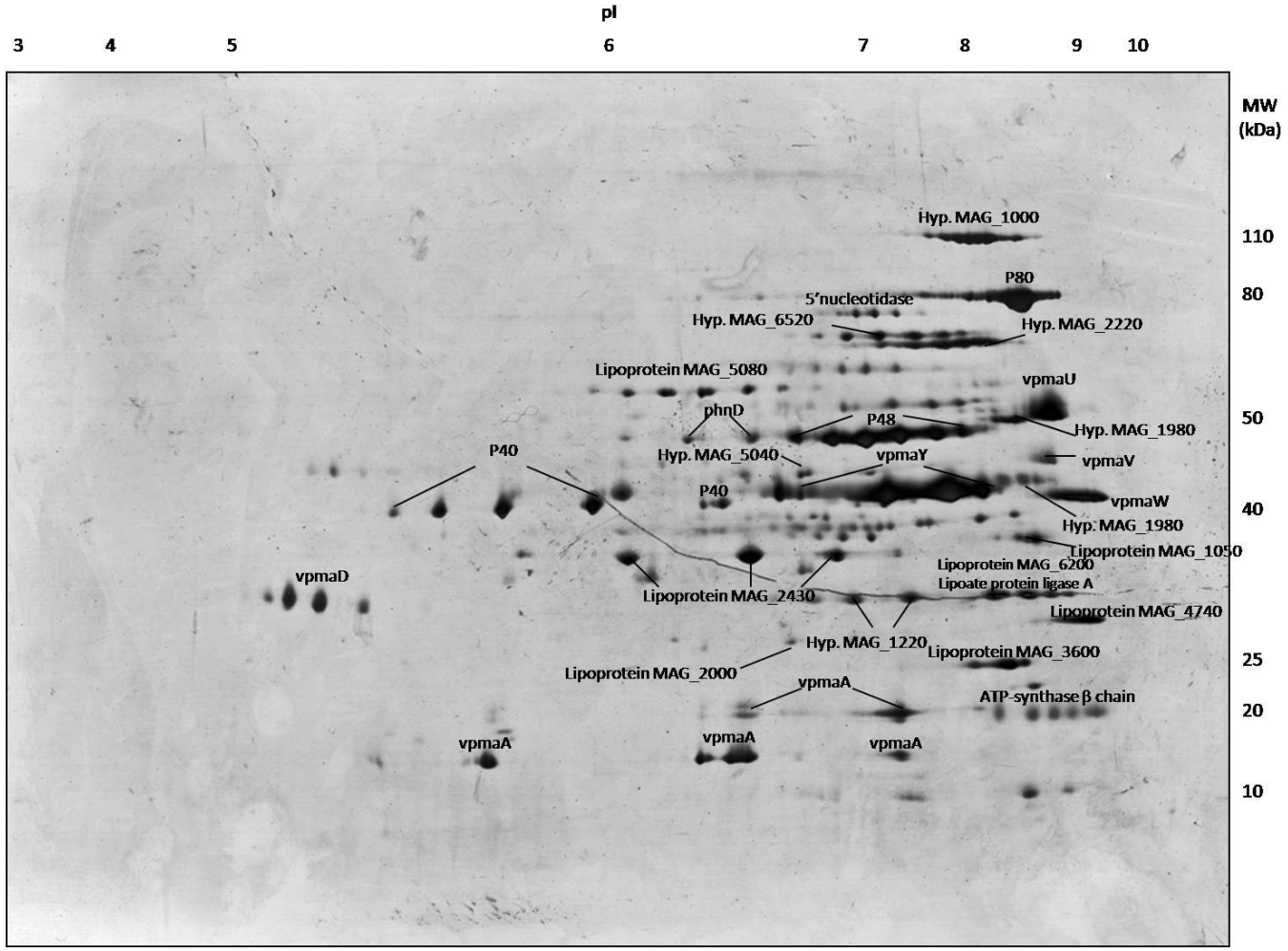


Figure 11: 2-D PAGE map of *M. agalactiae* PG2^T liposoluble proteins illustrating protein identifications obtained by mass spectrometry. Proteins are indicated by grouping all individual identifications corresponding to the same protein in a series of spots.

A detailed description of all protein identifications is given in supplementary data S4. Experimentally deduced molecular weight and *pI* of protein spots were compared with the theoretical parameters obtained from MASCOT, and most experimental data were in accordance with theoretical data. Few proteins, such as VpmA, were detected in multiple spots at different *pI*s and molecular weights, as expected for this class of lipoproteins which undergo size variation. The well-known immunogenic proteins (Tola *et al.*, 1996; Rosati *et al.*, 1999, 2000; Glew *et al.*, 2000; Fleury *et al.*, 2002) were all detected by 2-D PAGE at the expected *pI* and MW. All six variable surface lipoproteins encoded in the *M. agalactiae* $PG2^T$ genome were also detected, some of which (such as VpmaY and VpmaD) with high expression levels, as could be expected considering their relevance in providing variability to the mycoplasmal antigenic mosaic.

5.4 2D DIGE of liposoluble proteins among the type strain and two field isolates of *M. agalactiae*

In order to assess the suitability of 2-D PAGE for comparison of the membrane protein composition, the liposoluble protein profiles of *M. agalactiae* $PG2^T$ and two field isolates were compared by 2D DIGE (Fig 12).

The images generated upon acquisition of the single colour channels enable to evaluate the liposoluble protein profiles separately (Fig. 12, A, B, C), while comparison of two protein profiles can be performed upon superimposition of two colour signals (Fig. 12, D, E, F). In the overlay image, the three proteome 2D maps can be compared. Although many spots are shared among the three profiles (in white), a number of differences in expression can be appreciated. In fact, several spots are present only in one (blue, green, red) or two profiles (purple, yellow, light blue). Many already known antigens (such as P80, P48, P40, and most Vpmas) appear in white, indicating superimposition of the three signals and therefore presence in all three bacterial proteomes.

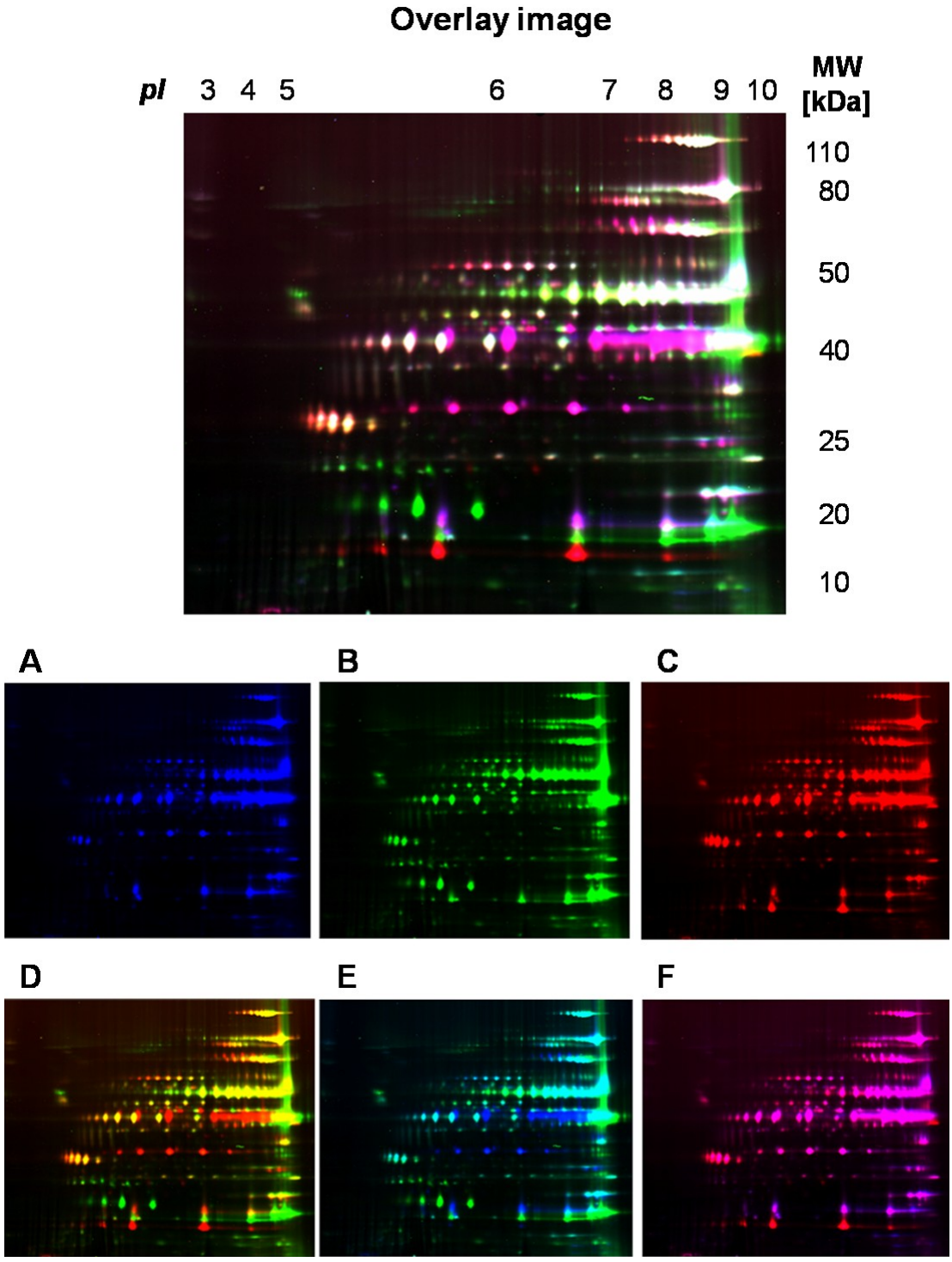


Figure 12: 2D DIGE of liposoluble proteins extracted from *M. agalactiae* PG2^T and two field strains. Overlay image: image generated from the superimposition of the signals generated by the three samples. White indicates presence of the protein spot in all three isolates. Panels A, B, and C represent isolates PG2^T, Nurri, and Bortigali, respectively. Panels D, E, and F represent the superimposition of Nurri/Bortigali, PG2^T/Nurri, and PG2^T/Bortigali, respectively.

Several differences among the three profiles can be easily observed; for example, the series of spots at 40 kDa corresponding to VpmaY (in purple in the overlay image, Fig. 12) is present only in two cases ($PG2^T$ and Bortigali) while the series of spots at 23 kDa (in green) is present only in one case (Nurri). The application of this method to an adequate number of isolates might enable to easily detect constantly expressed proteins that might serve as candidate antigens for development of vaccines and diagnostic tools.

5.5 GeLC-MS/MS of *M. agalactiae* $PG2^T$ liposoluble proteins

Although well suited for lipoprotein analysis, the 2-D PAGE/MS strategy presents drawbacks in analysis of transmembrane proteins, such as permeases or other highly hydrophobic proteins. Moreover, these protein classes may undergo selective loss during precipitation/resolubilization steps. In order to increase the membrane protein coverage and minimize selective protein loss, SDS-PAGE and GeLC-MS/MS analysis were performed on the non-precipitated Triton X-114 liposoluble protein fraction.

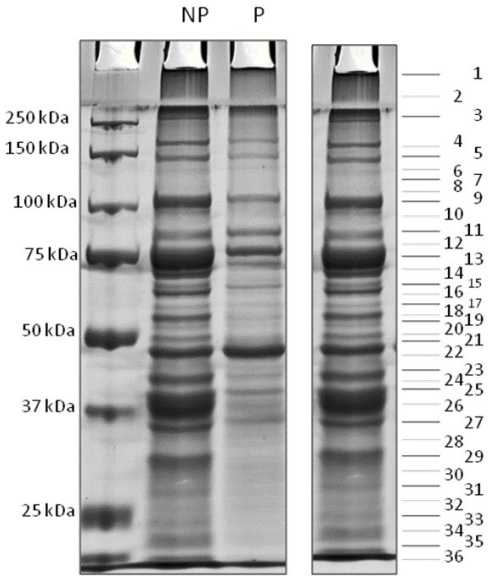


Figure 13: Protein profile of liposolubile proteins before and after precipitation. Right: approach used for GeLC-MS/MS characterization. The bars indicate the regions cut from the PAGE gel and subjected to mass spectrometry characterization. Protein identifications are reported in Supplementary data S6, from top to bottom.

A total of 36 slices were cut from the SDS-PAGE gel lane containing the separated liposoluble proteins (Fig. 12) and subjected to nanoHPLC-nanoESI-Q-TOF-MS/MS identification.

Upon application of this method, 194 mycoplasma proteins were identified in total, corresponding to 26% of all *M. agalactiae* PG2^T genes, 38 of which were also identified by 2-D PAGE/MS (for a detailed list of protein identifications, see Supplemtary data S6; Supplementary data S7 reports a summary table listing all unique protein identifications).

5.6 Data analysis and classification

A gene ontology (GO) classification was carried out on proteins identified by 2-D PAGE/MS and GeLC-MS/MS. For the first method, proteins (n = 40) were mostly classified by the GO software as hypothetical lipoproteins (65%), cytoplasmic proteins (22%), ribosomal proteins (8%), and other membrane-located proteins (5%). When identifications obtained by GeLC-MS/MS were also included in the GO analysis (n = 194), 43% of all identifications were assigned to proteins located on the membrane, either lipoproteins (17%) or other membrane proteins (26%), whereas 36% were classified as cytoplasmic, 17% as ribosomal, and 4% of unknown localization (Fig. 13).

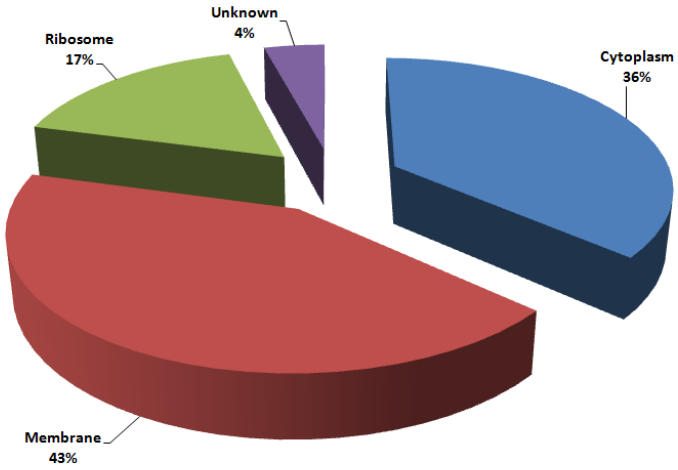


Figure 13: GO graph of proteins identified by 2-D PAGE-MS and GeLC-MS/MS in the Triton X-114 fraction of *M. agalactiae* PG2^T. Protein identifications are classified according to cellular localization.

All protein identifications were then classified according to function (Figure 14, and Supplementary data S7). As expected, a high proportion of the identified proteins perform membrane transport functions (about 16%), and belong mostly to ABC transporters (13%). Transmembrane proteins, such as permeases, were detected only by means of GeLC-MS/MS. Another highly represented functional process was translation (19%), due to the elevated number of ribosomal proteins identified. Hydrolytic enzymes were also significantly represented (6%), highlighting their crucial role for survival of mycoplasmas. Several other functional classes, such as enzymes involved in amino acid, carbohydrate, lipid, and nucleic acid metabolism, were significantly represented in the *M. agalactiae* PG2^{T} liposoluble protein fraction. Secretion/export systems accounted for 4% of all identified proteins; these components are in fact crucial for maturation and release of secreted proteins, but also for positioning/exposing lipoproteins on the outer side of the bacterial cell. About 19% of proteins could not be assigned a specific function by manual searches or GO classification.

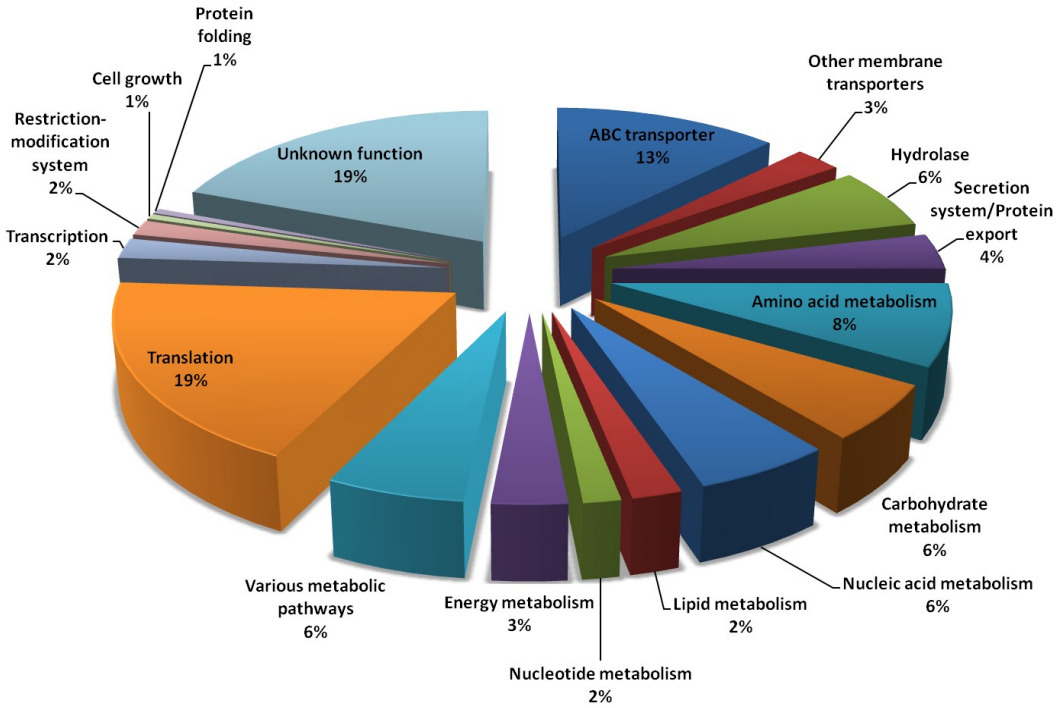


Figure 14: GO graph of proteins identified by GeLC-MS/MS in the Triton X-114 fraction of *M. agalactiae* PG2^{T}. Protein identifications are classified according to function.

Proteomic data were analyzed in order to investigate presence of liposoluble proteins resulting from expression of horizontally-transferred genes (Sirand-Pugnet *et al.*, 2007). Among 194 identified proteins, 15 (7.8%) were acquired by HGT from the *Mycoplasma mycoides* cluster (Supplemtary data S8), while 7 (3.7%) were acquired by HGT from other bacteria (Supplemtary data S9), for a total of 22 proteins, making up to 11.5% of all expressed membrane proteins being derived from putative HGT events.

6. DISCUSSION

Gathering proteomic information on prokaryotic membranes is a challenging task, due to difficulties in cell fractionation and to the intrinsic chemical properties of membrane proteins in general. Therefore, both systematic and differential proteomic information on prokaryotic membranes is generally lacking. In this work, we approached the systematic characterization of what is believed to be one of the simplest bacterial pathogen membranes, in an attempt to move a step forward in our understanding of its composition, complexity, and function. In addition to its lower complexity, investigating membrane composition and plasticity in mycoplasmas is of particular interest since surface proteins are subjected to size and phase variation, and information on the extent and level of such variation is crucial in studies targeting identification of common immunogens, evaluation of immunological escape mechanisms, and adaptation of the bacterium to its host. All 6 variable surface lipoproteins encoded in the $PG2^T$ genome (Sirand-Pugnet *et al.*, 2007) were detected by 2-D PAGE, although one of these (VpmaY) was not expressed in a field isolate examined by 2D DIGE. Triplicate experiments showed that the two-dimensional expression pattern of each field isolate is relatively stable under laboratory conditions, and that there is a reproducible differential expression of several protein spots in the field isolates compared to the type strain PG2. Interestingly, these differences are being detected in bacteria which were grown in culture media, where all protein variants should theoretically be expressed (Sirand-Pugnet *et al.*, 2007). It was already demonstrated that the switching mechanism is so fast that it can be pointed out in a single colony on solid culture (Chopra-Dewasthaly *et al*., 2008). This might suggest that the lack of VpmaY in the isolate Nurri could result from a local genetic mutation. A large-scale study performed on a higher number of field isolates might enable the detection of constantly expressed proteins, which might be useful as targets for the development of vaccines and diagnostic tools for CA.

Mycoplasmas have evolved a parasitic lifestyle, and membrane transporters are consequently very important for uptake of nutrients and growth factors. The genome of

M. agalactiae PG2^T encodes 18 ABC transporters, but proteins from only 10 of these were identified in this study. We also failed to identify all the components of a complete membrane transporter complex; however, it is possible that expression of all sequences encoded by the transporter gene operon may not necessarily take place at the same time. ABC transporters components encoded by different operons may likely interact to form functional transporters, producing the further advantage of creating many different combinations that can help evasion of host defence mechanisms. For instance, the *M. agalactiae* PG2^T genome encodes for two oligopeptide (Opp) ABC transporters, one typical of the hominis group and one probably transferred by means of horizontal gene transfer mechanisms from *M. mycoides* subsp. *mycoides* and *M. capricolum* subsp. *capricolum*. We identified the substrate binding protein (OppA) from one operon, and the permease (OppC) and the ATP-binding protein (OppF) from another operon; notably, these proteins create a functional transporter. Moreover, OppA could be more than a simple substrate binding protein, since it was demonstrated to play an important role in pathogenicity in *M. hominis* by inducing ATP release and cell death of HeLa cells in vitro and by mediating adhesion to host cells (Henrich *et al.*, 1999; Hopfe and Henrich, 2004, 2008). Other authors reported a different pattern of expression of these operons: in the study by Nouvel and co-workers, only OppA, OppF, and OppD were detected (Nouvel *et al.*, 2010). These apparently controversial results could be due to technical issues, or be dependent on variations in expression of Opps within the PG2^T strain. This will need to be elucidated in future studies.

Upon analysis of all MS data, the proteins putatively assigned by the GO software as cytoplasmic accounted to 36%. Among these, many hydrolases were present. However, lipases, peptidases, and nucleases might be associated to the membrane compartment and assist in reducing macromolecules to simple components, enabling their uptake. In fact, mycoplasmas lack many biosynthetic pathways and rely on internalization of nucleotides, amino acids, sugars and lipids from their external environment. Recently, it was reported that hydrolytic enzymes are surface-located in mycoplasmas, and that they can be associated with ABC transporters in order to digest macromolecules before uptake of simpler components, or play major roles in

pathogenicity (Schmidt *et al.*, 2007). Interestingly, in the *M. agalactiae* genome, the genes coding for many of these hydrolases are also located close to ABC transporter operons.

Several other proteins have a predicted cytoplasmic localization, but could be membrane-associated in mycoplasmas, such as the elongation factor tu (EF-Tu) and the E1 beta subunit of the pyruvate dehydrogenase complex. Traditionally, these are considered to be cytoplasmic proteins involved in protein synthesis and energy production, respectively, but it was demonstrated that in *M. pneumoniae* they are surface exposed and interact with host fibronectin, mediating adhesion (Dallo *et al.*, 2002; Balasubramanian *et al.*, 2008). It was also demonstrated that many "cytoplasmic" proteins such as the EF-Tu are strong antigens in many mycoplasma species (Alonso *et al.*, 2002; Bercic *et al.*, 2008; Jores *et al.*, 2009).

Ribosomal proteins represent a significant proportion of the mycoplasma liposoluble proteome. This might appear inconsistent, but in spite of their traditionally cytoplasmic localization, it was already demonstrated that ribosomes interact with the bacterial protein export complex (Johnson, 2009). Moreover, it is well known that in eukaryotes ribosomes are associated with endoplasmic reticulum, where they participate in the protein secretion pathway (White and von Heijne, 2008). Several proteins that take part in other metabolic pathways were also identified in the liposoluble fraction of *M. agalactiae* PG2^{T}. We could speculate that many proteins involved in nutrient metabolism might associate with proteins devoted to internalization of precursors in metabolizing complexes, and be co-purified with these. Nonetheless, a pre-fractionation of membranes was not performed because of inherent technical difficulties, and we cannot rule out that enzymes with high hydrophobicity might be present as cytoplasmic contaminants.

The recent work by Sirand-Pugnet and coworkers revealed the occurrence of horizontal gene transfer (HGT) events in *M. agalactiae*. The expression of proteins acquired by HGT highlights the importance of horizontal gene flow for the evolutionary plasticity of mycoplasmas; for instance, by allowing changes in host and/or tissue tropism through acquisition of traits enabling colonization and survival in new niches

(Marenda *et al.*, 2006; Sirand-Pugnet *et al.*, 2007). In total, an impressing 11.7% of proteins expressed on the *M. agalactiae* membrane are coming from other bacteria, reinforcing the view that an important part in the evolution of mycoplasmas might be driven by genetic exchange with bacteria sharing the same host districts, probably in order to compensate the concurrent process of gene loss (Sirand-Pugnet *et al.*, 2007). Another interesting observation was the detection of MAG_2340, a hypothetical lipoprotein which is apparently the result of an horizontal gene transfer event with mycoplasmas of the mycoides cluster (Supplemtary data S8), which was not detected by Nouvel *et al.* in the $PG2^T$ liposoluble proteome.

Hypothetical proteins were of particular interest; since these did not have an assigned function, similarity searches were conducted with BLAST tools in order to infer their possible role in the biology of mycoplasmas. Among these, the hypothetical lipoprotein MAG_1670 belongs to the *mycoides* cluster LppA/P72 family, and it is an antigen recognized early and persistently in infection (Cheng et al., 1996). The hypothetical protein MAG_0250 has an indigoidine synthase A (IdgA)-like domain similar to *Clostridium* spp. IdgA is involved in the biosynthesis of indigoidine, a blue pigment synthesized by *Erwinia chrysanthemi* and implicated in pathogenicity and protection from oxidative stress by scavenging oxygen radicals (Reverchon *et al.*, 2002). Indigoidine production increases tolerance to oxidative stress and contributes to aggressiveness, and might therefore act as a virulence factor.

7. CONCLUSIONS

2-D PAGE studies might be extremely powerful for comparison of protein expression in different mycoplasma isolates, especially when considering that lipoproteins can be selectively detected with this method, and that size and phase variations can be easily spotted through the application of powerful differential comparison approaches as the 2D DIGE. However, these need to be integrated with traditional Western immunoblotting and GeLC-MS/MS for a deeper coverage and characterization of other mycoplasmal surface immunogens to be used as tools for vaccination, diagnosis, and therapy. This combined approach allowed the identification and characterization of 194 *M. agalactiae* proteins putatively localized on the membrane or associated to it, providing useful insights on its composition. In the future, alternative approaches such as blue native electrophoresis and chemical crosslinking of surface proteins will also enable to elucidate functional and structural aspects of membrane proteins that cannot be accounted for by the traditional gel-based proteomic approaches.

8. SUPPLEMENTARY DATA

S1: 2-D PAGE map of liposoluble proteins from *M. agalactiae* PG2^T illustrating the protein identifications obtained by MS on the 3-10NL pI Interval.

S2: 2-D PAGE map of liposoluble proteins from *M. agalactiae* PG2^T illustrating the protein identifications obtained by MS on the 7-11 pI interval.

S3: 2-D PAGE map of liposoluble proteins from *M. agalactiae* PG2^T illustrating the protein identifications obtained by MS on the 4-7 pI interval

S4: Table listing all protein identifications obtained from 2-D PAGE maps.

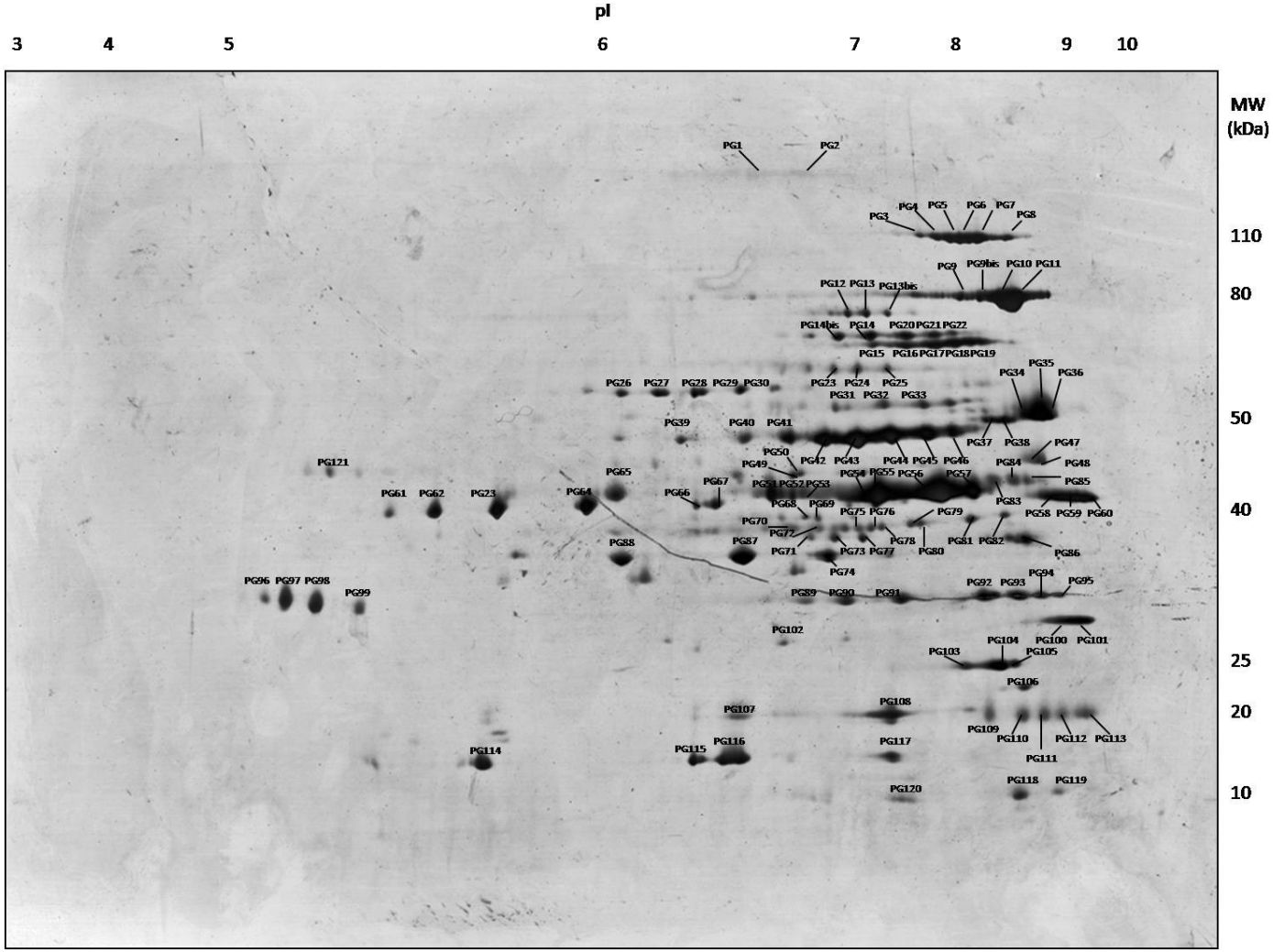
S5: Protein profile of liposolubile proteins before and after precipitation.

S6: Table listing all protein identifications obtained by GeLC-MS/MS of the *M. agalactiae* PG2^T Triton X-114 liposoluble fraction.

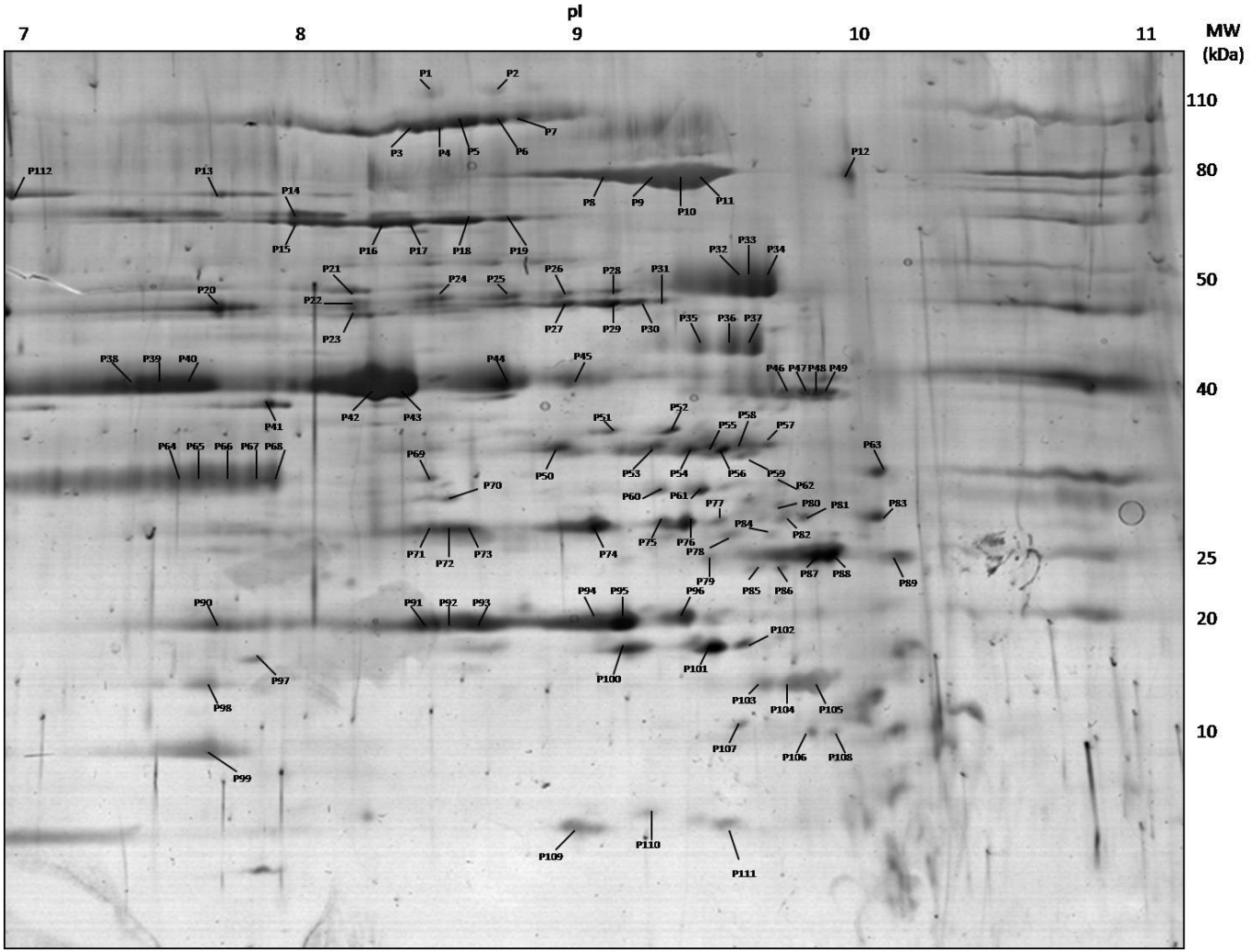
S7: Functional analysis, number of peptide hits, and method of detection of *M. agalactiae* PG2^T liposoluble proteins.

S8: Proteins identified in the *M. agalactiae* PG2^T proteome potentially resulting from Horizontal Gene Transfer events with *M. mycoides* subsp. *mycoides* and *M. capricolum* subsp. *capricolum*.

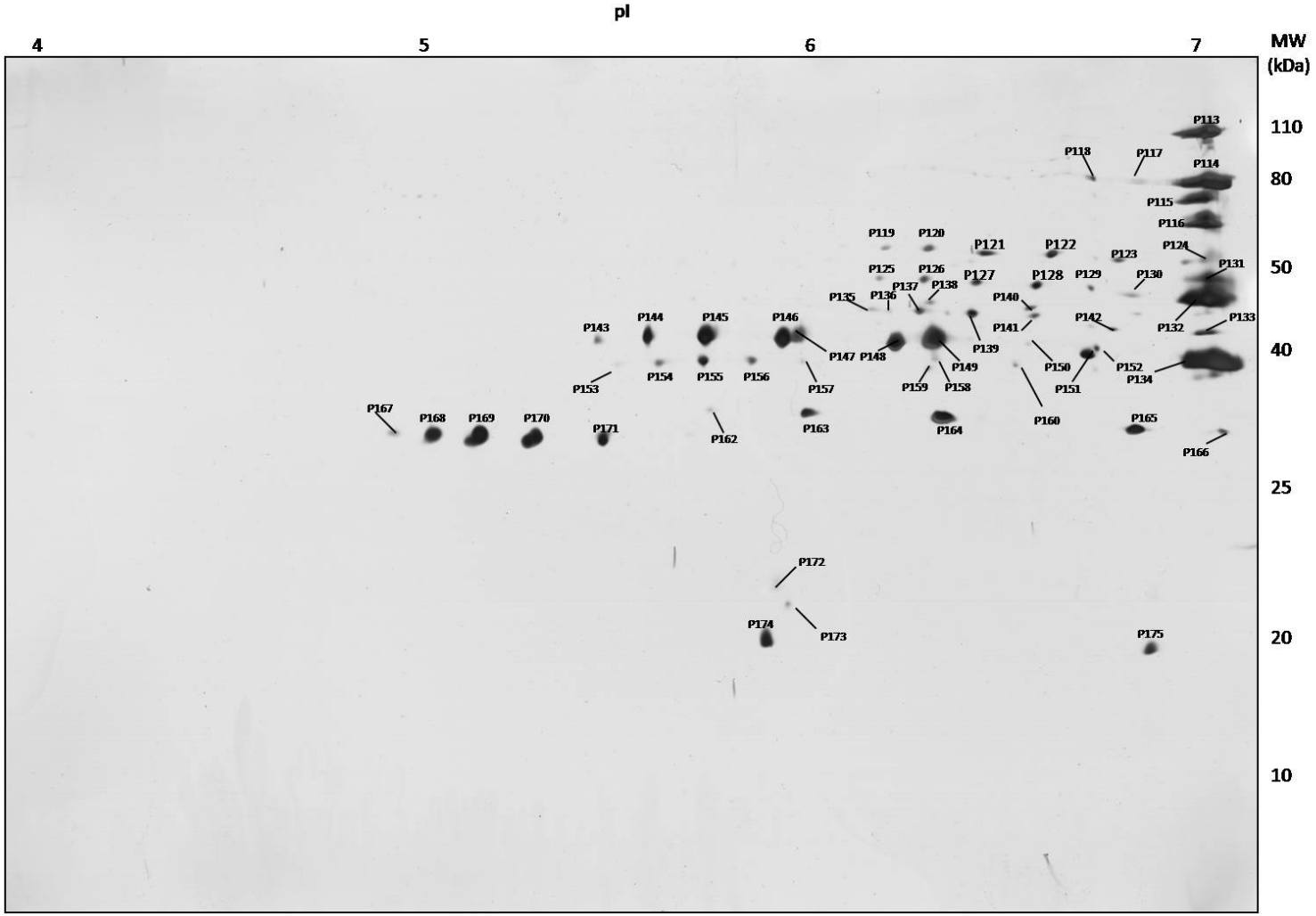
S9: Proteins identified in the *M. agalactiae* PG2^{T}proteome potentially resulting from Horizontal Gene Transfer events with other bacteria.



S1: 2-D PAGE map of liposoluble proteins from *M. agalactiae* PG2^{T} illustrating the protein identifications obtained by MS on the 3-10NL pI Interval.



S2: 2-D PAGE map of liposoluble proteins from *M. agalactiae* PG2^{T} illustrating the protein identifications obtained by MS on the 7-11 pI interval



S3: 2-D PAGE map of liposoluble proteins from *M. agalactiae* PG2^T illustrating the protein identifications obtained by MS on the 4-7 pI interval

S4: Table listing all protein identifications obtained from 2-D PAGE maps. The proteins listed in this table were identified from 2-D PAGE maps of the *M. agalactiae* PG2^{T} Triton X-114 fraction . Maps are represented in supplementary data S1 (pH 3-10NL), S2 (pH 7-11), and S3 (pH 4-7).

Spot	Map	Protein	Organism	Mw (Da)	pI	Score	Instrument	Queries	Coverage	Acc. No.	Locus tag
PG1	S1	NI									
PG2	S1	NI									
PG3	S1	Hypotetical protein MAG_1000	*M. agalactiae* PG2	109727	8.66	155	MALDI-TOF	21	25%	gi|148377368	MAG_1000
PG4	S1	Hypotetical protein MAG_1000	*M. agalactiae* PG2	109727	8.66	71	MALDI-TOF	10	18%	gi|148377368	MAG_1000
PG5	S1	Hypotetical protein MAG_1000	*M. agalactiae* PG2	109727	8.66	175	MALDI-TOF	24	25%	gi|148377368	MAG_1000
PG6	S1	Hypotetical protein MAG_1000	*M. agalactiae* PG2	109727	8.66	199	MALDI-TOF	23	25%	gi|148377368	MAG_1000
PG7	S1	Hypotetical protein MAG_1000	*M. agalactiae* PG2	109727	8.66	91	MALDI-TOF	13	11%	gi|148377368	MAG_1000
PG8	S1	Hypotetical protein MAG_1000	*M. agalactiae* PG2	109727	8.66	168	MALDI-TOF	20	23%	gi|148377368	MAG_1000
PG9	S1	P80. lipoprotein	*M. agalactiae* PG2	81102	9.08	200	MALDI-TOF	24	37%	gi|148377765	MAG_5030
PG9bis	S1	P80. lipoprotein	*M. agalactiae* PG2	81102	9.08	74	ESI Q-TOF	5	6%	gi|148377765	MAG_5030
PG10	S1	P80. lipoprotein	*M. agalactiae* PG2	81102	9.08	136	ESI Q-TOF	10	11%	gi|148377765	MAG_5030
PG11	S1	P80. lipoprotein	*M. agalactiae* PG2	81102	9.08	181	MALDI-TOF	23	35%	gi|148377765	MAG_5030
PG12	S1	5'nucleotidase	*M. agalactiae* PG2	76195	8.36	72	MALDI-TOF	7	15%	gi|148377854	MAG_5910
PG13	S1	5'nucleotidase	*M. agalactiae* PG2	76195	8.36	96	MALDI-TOF	10	17%	gi|148377854	MAG_5910
PG13bis	S1	5'nucleotidase	*M. agalactiae* PG2	76195	8.36	113	MALDI-TOF	10	18%	gi|148377854	MAG_5910
PG14	S1	Hypothetical protein MAG_6520	*M. agalactiae* PG2	70087	8.58	64	MALDI-TOF	8	14%	gi|148377915	MAG_6520
PG14bis	S1	Hypothetical protein MAG_6520	*M. agalactiae* PG2	70087	8.58	59	MALDI-TOF	6	15%	gi|148377915	MAG_6520
PG15	S1	NI									
PG16	S1	Hypothetical protein MAG_2220	*M. agalactiae* PG2	69573	8.78	73	MALDI-TOF	8	17%	gi|148377488	MAG_2220
PG17	S1	Hypothetical protein MAG_2220	*M. agalactiae* PG2	69573	8.78	141	MALDI-TOF	16	28%	gi|148377488	MAG_2220
PG18	S1	Hypothetical protein MAG_2220	*M. agalactiae* PG2	69573	8.78	149	MALDI-TOF	13	33%	gi|148377488	MAG_2220
PG19	S1	Hypothetical protein MAG_2220	*M. agalactiae* PG2	69573	8.78	110	MALDI-TOF	12	28%	gi|148377488	MAG_2220

Spot	Map	Protein	Organism	Mw (Da)	pI	Score	Instrument	Queries	Coverage	Acc. No.	Locus tag
PG20	S1	Hypothetical protein MAG_6520	*M. agalactiae* PG2	70087	8.58	64	MALDI-TOF	8	18%	gi|148377915	MAG_2220
PG21	S1	NI									
PG22	S1	NI									
PG23	S1	Hypothetical protein MYPU_0730	Mycoplasma pulmonis UAB CTIP	26393	7.77	28	ESI Q-TOF	1	4%	gi|15828544	
PG24	S1	NI									
PG25	S1	NI									
PG26	S1	NI									
PG27	S1	Lipoprotein, MAG_5080	*M. agalactiae* PG2	59415	6.77	94	MALDI-TOF	10	20%	gi|148377770	MAG_5080
PG28	S1	Lipoprotein, MAG_5080	*M. agalactiae* PG2	59415	6.77	65	MALDI-TOF	7	18%	gi|148377770	MAG_5080
PG29	S1	Lipoprotein, MAG_5080	*M. agalactiae* PG2	59415	6.77	63	MALDI-TOF	8	21%	gi|148377770	MAG_5080
PG30	S1	NI									
PG31	S1	NI									
PG32	S1	NI									
PG33	S1	NI									
PG34	S1	Variable surface lipoprotein V	*M. agalactiae* PG2	37362	9.25	56	ESI Q-TOF	2	5%	gi|148377967	MAG_7050
		Variable surface lipoprotein U	*M. agalactiae* PG2	46993	9.26	56	ESI Q-TOF	2	4%	gi|32189693	MAG_7090
PG35	S1	Variable surface lipoprotein V	*M. agalactiae* PG2	37362	9.25	108	ESI Q-TOF	7	14%	gi|148377967	MAG_7050
		Variable surface lipoprotein U	*M. agalactiae* PG2	46993	9.26	108	ESI Q-TOF	7	11%	gi|32189693	MAG_7090
PG36	S1	Variable surface lipoprotein V	*M. agalactiae* PG2	37362	9.25	42	ESI Q-TOF	1	2%	gi|148377967	MAG_7050
		Variable surface lipoprotein U	*M. agalactiae* PG2	46993	9.26	42	ESI Q-TOF	1	2%	gi|32189693	MAG_7090
PG37	S1	Lipoprotein, MAG_1980	*M. agalactiae* PG2	53788	8.98	69	MALDI-TOF	7	15%	gi|148377464	MAG_1980
PG38	S1	Lipoprotein, MAG_1980	*M. agalactiae* PG2	53788	8.98	88	MALDI-TOF	9	19%	gi|148377464	MAG_1980
PG39	S1	Alkylphosphonate ABC transporter substrate-binding protein	*M. agalactiae* PG2	49744	6.99	95	MALDI-TOF	9	22%	gi|148377535	MAG_2690
PG40	S1	Alkylphosphonate ABC transporter substrate-binding protein	*M. agalactiae* PG2	49744	6.99	73	MALDI-TOF	7	17%	gi|148377535	MAG_2690
PG41	S1	P48. lipoprotein	*M. agalactiae* PG2	51232	8.39	62	MALDI-TOF	5	17%	gi|148377280	MAG_0120
PG42	S1	P48. lipoprotein	*M. agalactiae* PG2	51232	8.39	114	MALDI-TOF	10	25%	gi|148377280	MAG_0120

Spot	Map	Protein	Organism	Mw (Da)	pI	Score	Instrument	Queries	Coverage	Acc. No.	Locus tag
PG43	S1	P48. lipoprotein	*M. agalactiae* PG2	51232	8.39	125	MALDI-TOF	12	33%	gi|148377280	MAG_0120
PG44	S1	P48. lipoprotein	*M. agalactiae* PG2	51232	8.39	137	MALDI-TOF	11	33%	gi|148377280	MAG_0120
PG45	S1	P48. lipoprotein	*M. agalactiae* PG2	51232	8.39	98	MALDI-TOF	9	25%	gi|148377280	MAG_0120
PG46	S1	P48. lipoprotein	*M. agalactiae* PG2	51232	8.39	81	MALDI-TOF	7	19%	gi|148377280	MAG_0120
PG47	S1	Variable surface lipoprotein V	*M. agalactiae* PG2	37362	9.25	40	ESI Q-TOF	1	2%	gi|148377967	MAG_7050
		Variable surface lipoprotein U	*M. agalactiae* PG2	46993	9.26	40	ESI Q-TOF	1	2%	gi|32189693	MAG_7090
PG48	S1	NI									
PG49	S1	Hypothetical protein MAG_5040	*M. agalactiae* PG2	44828	8.46	96	MALDI-TOF	8	26%	gi|148377766	MAG_5040
PG50	S1	Hypothetical protein MAG_5040	*M. agalactiae* PG2	44828	8.46	104	MALDI-TOF	8	30%	gi|148377766	MAG_5040
PG51	S1	Variable surface lipoprotein Y	*M. agalactiae* PG2	37528	8.81	81	MALDI-TOF	7	30%	gi|148377970	MAG_7080
PG52	S1	Variable surface lipoprotein Y	*M. agalactiae* PG2	37528	8.81	88	MALDI-TOF	7	25%	gi|148377970	MAG_7080
PG53	S1	Variable surface lipoprotein Y	*M. agalactiae* PG2	37528	8.81	55	MALDI-TOF	5	24%	gi|148377970	MAG_7080
PG54	S1	Variable surface lipoprotein Y	*M. agalactiae* PG2	37528	8.81	55	MALDI-TOF	5	24%	gi|148377970	MAG_7080
PG55	S1	Variable surface lipoprotein Y	*M. agalactiae* PG2	37528	8.81	72	MALDI-TOF	7	28%	gi|148377970	MAG_7080
PG56	S1	Variable surface lipoprotein Y	*M. agalactiae* PG2	37528	8.81	69	MALDI-TOF	7	31%	gi|148377970	MAG_7080
PG57	S1	Variable surface lipoprotein Y	*M. agalactiae* PG2	37528	8.81	56	MALDI-TOF	5	24%	gi|148377970	MAG_7080
PG58	S1	Variable surface lipoprotein W	*M. agalactiae* PG2	35472	9.54	127	MALDI-TOF	10	30%	gi|148377968	MAG_7060
PG59	S1	Variable surface lipoprotein W	*M. agalactiae* PG2	35472	9.54	87	MALDI-TOF	9	27%	gi|148377968	MAG_7060
PG60	S1	Variable surface lipoprotein W	*M. agalactiae* PG2	35472	9.54	135	MALDI-TOF	11	31%	gi|148377968	MAG_7060
PG61	S1	P40, lipoprotein	*M. agalactiae* PG2	39951	8.19	40	ESI Q-TOF	2	5%	gi|148377507	MAG_2410
PG62	S1	P40, lipoprotein	*M. agalactiae* PG2	39951	8.19	80	MALDI-TOF	6	21%	gi|148377507	MAG_2410
PG63	S1	P40, lipoprotein	*M. agalactiae* PG2	39951	8.19	80	MALDI-TOF	6	21%	gi|148377507	MAG_2410
PG64	S1	P40, lipoprotein	*M. agalactiae* PG2	39951	8.19	118	MALDI-TOF	9	28%	gi|148377507	MAG_2410
PG65	S1	NI									
PG66	S1	P40, lipoprotein	*M. agalactiae* PG2	39951	8.19	127	ESI Q-TOF	8	16%	gi|148377507	MAG_2410
PG67	S1	P40, lipoprotein	*M. agalactiae* PG2	39951	8.19	58	MALDI-TOF	6	19%	gi|148377507	MAG_2410

Spot	Map	Protein	Organism	Mw (Da)	pI	Score	Instrument	Queries	Coverage	Acc. No.	Locus tag
PG68	S1	Hypothetical protein MAG_1890	*M. agalactiae* PG2	36683	8.78	118	MALDI-TOF	9	34%	gi|148377455	MAG_1890
PG69	S1	NI									
PG70	S1	Glyceraldehyde-3-phosphate dehydrogenase A	*E. coli*	35681	6.61	55	ESI Q-TOF	1	4%	G3P1_ECO57	
PG71	S1	NI									
PG72	S1	Attachment protein	*M. genitalium*	21972	5.35	30	ESI Q-TOF	1	5%	gi|82906922	
PG73	S1	NI									
PG74	S1	Lipoprotein MAG_2430	*M. agalactiae* PG2	33862	8.29	56	MALDI-TOF	5	24%	gi|148377509	MAG_2430
PG75	S1	ICEF-IIA ORF19	*M. fermentans*	81544	8.20	25	ESI Q-TOF	1	1%	gi|26984117	
PG76	S1	NI									
PG77	S1	NI									
PG78	S1	Hypothetical protein mhp361	*M. hyopneumoniae* 232	38818	7.52	29	ESI Q-TOF	2	3%	gi|54020475	mhp361
PG79	S1	NI									
PG80	S1	NI									
PG81	S1	NI									
PG82	S1	NI									
PG83	S1	Lipoprotein MAG_1980	*M. agalactiae* PG2	53788	8.98	58	MALDI-TOF	5	13%	gi|148377464	MAG_1980
PG84	S1	Variable surface lipoprotein V	*M. agalactiae* PG2	37362	9.25	48	ESI Q-TOF	1	2%	gi|23683074	MAG_7050
PG85	S1	Variable surface lipoprotein Y	*M. agalactiae* PG2	37528	8.81	63	ESI Q-TOF	1	3%	gi|148377970	MAG_7080
PG86	S1	Lipoprotein MAG_1050	*M. agalactiae* PG2	37025	9.26	56	MALDI-TOF	5	23%	gi|148377373	MAG_1050
PG87	S1	Lipoprotein MAG_2430	*M. agalactiae* PG2	33862	8.29	139	MALDI-TOF	9	36%	gi|148377509	MAG_2430
PG88	S1	Lipoprotein MAG_2430	*M. agalactiae* PG2	33862	8.29	149	MALDI-TOF	9	32%	gi|148377509	MAG_2430
PG89	S1	NI									
PG90	S1	Hypothetical protein MAG_1220	*M. agalactiae* PG2	26491	8.69	47	ESI Q-TOF	2	8%	gi|148377389	MAG_1220
PG91	S1	Hypothetical protein MAG_1220	*M. agalactiae* PG2	26491	8.69	97	ESI Q-TOF	5	15%	gi|148377389	MAG_1220
PG92	S1	Lipoprotein MAG_6200	*M. agalactiae* PG2	26952	9.22	86	ESI Q-TOF	7	28%	gi|148377883	MAG_6200
		Hypothetical protein MAG_1220	*M. agalactiae* PG2	26491	8.69	54	ESI Q-TOF	3	12%	gi|148377389	MAG_1220

Spot	Map	Protein	Organism	Mw (Da)	pI	Score	Instrument	Queries	Coverage	Acc. No.	Locus tag
		Transcription Elongation factor GreA	*M. capricolum subsp. capricolum* ATCC 27343	17483	5.84	33	ESI Q-TOF	1	5%	gi|83319925	
		Lipoate-protein ligase A	*M. agalactiae* PG2	37286	6.42	29	ESI Q-TOF	2	2%	gi|148377329	MAG_0600
PG93	S1	Lipoprotein MAG_6200	*M. agalactiae* PG2	26952	9.22	30	ESI Q-TOF	3	11%	gi|148377883	MAG_6200
PG94	S1	Lipoprotein MAG_6200	*M. agalactiae* PG2	26952	9.22	29	ESI Q-TOF	1	3%	gi|148377883	MAG_6200
PG95	S1	Lipoprotein MAG_6200	*M. agalactiae* PG2	26952	9.22	72	ESI Q-TOF	3	13%	gi|148377883	MAG_6200
PG96	S1	Variable surface lipoprotein D	*M. agalactiae* PG2	36608	9.53	129	ESI Q-TOF	8	9%	gi|148377972	MAG_7100
PG97	S1	Variable surface lipoprotein D	*M. agalactiae* PG2	36608	9.53	60	MALDI-TOF	7	18%	gi|148377972	MAG_7100
PG98	S1	Variable surface lipoprotein D	*M. agalactiae* PG2	36608	9.53	77	MALDI-TOF	8	21%	gi|148377972	MAG_7100
PG99	S1	Variable surface lipoprotein D	*M. agalactiae* PG2	36608	9.53	190	ESI Q-TOF	7	11%	gi|148377972	MAG_7100
PG100	S1	NI									
PG101	S1	NI									
PG102	S1	Lipoprotein MAG_2000	*M. agalactiae* PG2	26602	8.39	69	ESI Q-TOF	1	6%	gi|148377466	MAG_2000
PG103	S1	Lipoprotein MAG_3600	*M. agalactiae* PG2	21678	9.13	138	ESI Q-TOF	7	25%	gi|148377626	MAG_3600
PG104	S1	Lipoprotein MAG_3600	*M. agalactiae* PG2	21678	9.13	533	ESI Q-TOF	22	58%	gi|148377626	MAG_3600
		Endonuclease IV	*M. penetrans* HF-2	35632	6.22	33	ESI Q-TOF	1	4%	gi|26553573	
PG105	S1	Lipoprotein MAG_3600	*M. agalactiae* PG2	21678	9.13	142	ESI Q-TOF	9	36%	gi|148377626	MAG_3600
		Endonuclease IV	*M. penetrans* HF-2	35632	6.22	26	ESI Q-TOF	1	4%	gi|26553573	
PG106	S1	Lipoprotein MAG_2400	*M. agalactiae* PG2	38065	8.95	179	ESI Q-TOF	9	20%	gi|148377506	MAG_2400
PG107	S1	NI									
PG108	S1	Variable surface lipoprotein Y	*M. agalactiae* PG2	37528	8.81	831	ESI Q-TOF	26	21%	gi|148377970	MAG_7080
PG109	S1	Variable surface lipoprotein Y	*M. agalactiae* PG2	37528	8.81	153	ESI Q-TOF	3	6%	gi|148377970	MAG_7080
PG110	S1	NI									
PG111	S1	ATP synthase B chain	*M. agalactiae* PG2	21796	9.4	50	ESI Q-TOF	1	6%	gi|148377618	MAG_3520
PG112	S1	ATP synthase B chain	*M. agalactiae* PG2	21796	9.4	29	ESI Q-TOF	1	6%	gi|148377618	MAG_3520
PG113	S1	ATP synthase B chain	*M. agalactiae* PG2	21796	9.4	27	ESI Q-TOF	1	6%	gi|148377618	MAG_3520
PG114	S1	Variable surface lipoprotein A	*M. agalactiae* PG2	24769	8.33	85	MALDI-TOF	5	29%	gi|148377969	MAG_7070

Spot	Map	Protein	Organism	Mw (Da)	pI	Score	Instrument	Queries	Coverage	Acc. No.	Locus tag
PG115	S1	NI									
PG116	S1	Variable surface lipoprotein A	*M. agalactiae* PG2	24769	8.33	60	MALDI-TOF	4	24%	gi|148377969	MAG_7070
PG117	S1	Variable surface lipoprotein A	*M. agalactiae* PG2	24769	8.33	62	MALDI-TOF	4	24%	gi|148377969	MAG_7070
P-1	S2	NI									
P-2	S2	NI									
P-3	S2	Hypotetical protein MAG_1000	*M. agalactiae PG2*	109727	8.66	155	MALDI-TOF	21	25%	gi|148377368	MAG_1000
P-4	S2	Hypotetical protein MAG_1000	*M. agalactiae PG2*	109727	8.66	71	MALDI-TOF	10	18%	gi|148377368	MAG_1000
P-5	S2	Hypotetical protein MAG_1000	*M. agalactiae PG2*	109727	8.66	175	MALDI-TOF	24	25%	gi|148377368	MAG_1000
P-6	S2	Hypotetical protein MAG_1000	*M. agalactiae PG2*	109727	8.66	199	MALDI-TOF	23	25%	gi|148377368	MAG_1000
P-7	S2	Hypotetical protein MAG_1000	*M. agalactiae PG2*	109727	8.66	91	MALDI-TOF	13	11%	gi|148377368	MAG_1000
P-8	S2	P80. lipoprotein	*M. agalactiae PG2*	81102	9.08	219	MALDI-TOF	23	35%	gi|148377765	MAG_5030
P-9	S2	P80. lipoprotein	*M. agalactiae PG2*	81102	9.08	181	MALDI-TOF	23	35%	gi|13992493	MAG_5030
P-10	S2	P80. lipoprotein	*M. agalactiae PG2*	81102	9.08	229	MALDI-TOF	23	35%	gi|13992493	MAG_5030
P-11	S2	P80. lipoprotein	*M. agalactiae PG2*	81102	9.08	245	MALDI-TOF	23	35%	gi|13992493	MAG_5030
P-12	S2	NI									
P-13	S2	5'nucleotidase	*M. agalactiae PG2*	76195	8.26	83	ESI Q-TOF	10	14%	gi|148377854	MAG_5910
P-14	S2	Hypothetical protein MAG_6520	*M. agalactiae PG2*	70087	8.58	62	ESI Q-TOF	4	6%	gi|148377915	MAG_6520
		Hypothetical protein MAG_2220	*M. agalactiae PG2*	69573	8.78	40	ESI Q-TOF	6	8%	gi|148377488	MAG_2220
P-15	S2	Hypothetical protein MAG_2220	*M. agalactiae PG2*	69573	8.78	76	MALDI-TOF	9	22%	gi|148377488	MAG_2220
P-16	S2	Hypothetical protein MAG_2220	*M. agalactiae PG2*	69573	8.78	59	MALDI-TOF	6	17%	gi|148377488	MAG_2220
P-17	S2	NI									
P-18	S2	Hypothetical protein MAG_2220	*M. agalactiae PG2*	69573	8.78	96	MALDI-TOF	11	24%	gi|148377488	MAG_2220
P-19	S2	Hypothetical protein MAG_2220	*M. agalactiae PG2*	69573	8.78	55	MALDI-TOF	6	14%	gi|148377488	MAG_2220
P-20	S2	P48. lipoprotein	*M. agalactiae PG2*	51232	8.39	56	MALDI-TOF	7	21%	gi|148377280	MAG_0120
P-21	S2	NI									
P-22	S2	NI									

Spot	Map	Protein	Organism	Mw (Da)	pI	Score	Instrument	Queries	Coverage	Acc. No.	Locus tag
P-23	S2	NI									
P-24	S2	NI									
P-25	S2	NI									
P-26	S2	NI									
P-27	S2	Lipoprotein MAG_1980	*M. agalactiae PG2*	53788	8.98	73	MALDI-TOF	9	23%	gi|148377464	MAG_1980
P-28	S2	Excinuclease ABC subunit B	*M. mycoides subsp. mycoides SC PG1*	77534	8.03	68	MALDI-TOF	10	15%	gi|42561460	MSC_0944
P-29	S2	NI									
P-30	S2	NI									
P-31	S2	NI									
P-32	S2	NI									
P-33	S2	NI									
P-34	S2	NI									
P-35	S2	NI									
P-36	S2	NI									
P-37	S2	NI									
P-38	S2	NI									
P-39	S2	NI									
P-40	S2	NI									
P-41	S2	Lipoprotein MAG_2350	*M. agalactiae PG2*	40383	8.61		ESI Q-TOF	13	35%	gi|148291547	MAG_2350
		Variable surface lipoprotein Y	*M. agalactiae PG2*	37528	8.81	91	MALDI-TOF	10	27%	gi|148377970	MAG_7080
P-43	S2	Variable surface lipoprotein Y	*M. agalactiae PG2*	37528	8.81	142	MALDI-TOF	13	38%	gi|148377970	MAG_7080
P-44	S2	Variable surface lipoprotein Y	*M. agalactiae PG2*	37528	8.81	121	MALDI-TOF	10	35%	gi|148377970	MAG_7080
P-45	S2	NI									
P-46	S2	Variable surface lipoprotein W	*M. agalactiae PG2*	35472	9.54	82	MALDI-TOF	7	23%	gi|148377968	MAG_7060
P-47	S2	Variable surface lipoprotein W	*M. agalactiae PG2*	35472	9.54	82	MALDI-TOF	7	21%	gi|148377968	MAG_7060
P-48	S2	Variable surface lipoprotein W	*M. agalactiae PG2*	35472	9.54	82	MALDI-TOF	7	21%	gi|148377968	MAG_7060

Spot	Map	Protein	Organism	Mw (Da)	pI	Score	Instrument	Queries	Coverage	Acc. No.	Locus tag
P-49	S2	Variable surface lipoprotein W	*M. agalactiae PG2*	35472	9.54	94	MALDI-TOF	8	23%	gi|148377968	MAG_7060
P-50	S2	Hypothetical protein MAG_1450	*M. agalactiae PG2*	35463	9.15	86	ESI Q-TOF	3	8%	gi|148377412	MAG_1450
		Lipoprotein MAG_1050	*M. agalactiae PG2*	37025	9.26	49	ESI Q-TOF	1	3%	gi|148377373	MAG_1050
P-51	S2	5'nucleotidase	*M. agalactiae PG2*	76195	8.26	52	ESI Q-TOF	3	3%	gi|148377854	MAG_5910
P-52	S2	5'nucleotidase	*M. agalactiae PG2*	76195	8.26	65	ESI Q-TOF	4	4%	gi|148377854	MAG_5910
P-53	S2	Lipoprotein MAG_1050	*M. agalactiae PG2*	37025	9.26	76	MALDI-TOF	8	26%	gi|148377373	MAG_1050
P-54	S2	Lipoprotein MAG_1050	*M. agalactiae PG2*	37025	9.26	233	MALDI-TOF	20	53%	gi|148377373	MAG_1050
P-55	S2	Lipoprotein MAG_1050	*M. agalactiae PG2*	37025	9.26	203	MALDI-TOF	15	45%	gi|148377373	MAG_1050
P-56	S2	Lipoprotein MAG_1050	*M. agalactiae PG2*	37025	9.26	57	MALDI-TOF	6	21%	gi|148377373	MAG_1050
P-57	S2	Putative nicotinate-nucleotide adenylyltransferase	*M. pneumoniae M129*	40168	9.79	55	MALDI-TOF	6	24%	gi|161723288	
P-58	S2	Lipoprotein	*M. agalactiae PG2*	37025	9.26	83	ESI Q-TOF	5	12%	gi|148377373	MAG_1050
P-59	S2	Lipoprotein	*M. agalactiae PG2*	37025	9.26	72	MALDI-TOF	6	26%	gi|148377373	MAG_1050
P-60	S2	NI									
P-61	S2	NI									
P-62	S2	Variable surface lipoprotein W	*M. agalactiae PG2*	35472	9.54	28	ESI Q-TOF	1	3%	gi|148377968	MAG_7060
P-63	S2	50S ribosomal protein L3	*M. agalactiae PG2*	28793	9.75	50	ESI Q-TOF	2	4%	gi|148377809	MAG_5460
P-64	S2	NI									
P-65	S2	NI									
P-66	S2	NI									
P-67	S2	NI									
P-68	S2	NI									
P-69	S2	NI									
P-70	S2	NI									
P-71	S2	Lipoprotein MAG_6200	*M. agalactiae PG2*	26952	9.22	37	ESI Q-TOF	1	3%	gi|148377883	MAG_6200
		Hypothetical protein MAG_1220	*M. agalactiae PG2*	26491	8.69	30	ESI Q-TOF	1	3%	gi|148377389	MAG_1220
P-72	S2	Lipoprotein MAG_6200	*M. agalactiae PG2*	26952	9.22	35	ESI Q-TOF	1	3%	gi|148377883	MAG_6200

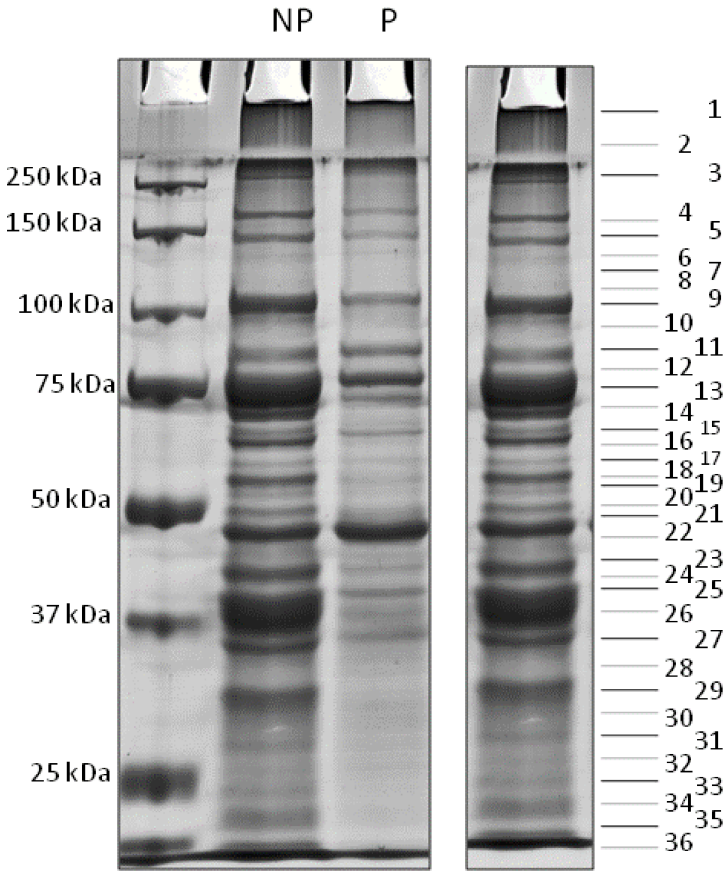
Spot	Map	Protein	Organism	Mw (Da)	pI	Score	Instrument	Queries	Coverage	Acc. No.	Locus tag
	S2	Hypothetical protein MAG_1220	*M. agalactiae PG2*	26491	8.69		ESI Q-TOF	1	3%	gi|148377389	MAG_1220
P-73	S2	Hypothetical protein MAG_1220	*M. agalactiae PG2*	26491	8.69	35	ESI Q-TOF	1	3%	gi|148377389	MAG_1220
P-74	S2	Lipoprotein MAG_6200	*M. agalactiae PG2*	26952	9.22	81	ESI Q-TOF	3	11%	gi|148377883	MAG_6200
P-75	S2	NI									
P-76	S2	Lipoprotein MAG_6200	*M. agalactiae PG2*	26952	9.22	88	ESI Q-TOF	3	11%	gi|148377883	MAG_6200
P-77	S2	NI									
P-78	S2	NI									
P-79	S2	NI									
P-80	S2	NI									
P-81	S2	NI									
P-82	S2	NI									
P-83	S2	50S ribosomal protein L1	*M. agalactiae PG2*	24955	9.68	62	ESI Q-TOF	1	4%	gi|148377349	MAG_0810
P-84	S2	Hypothetical lipoprotein	*M. arthritidis 158L3-1*	77114	8.42	28	ESI Q-TOF	1	1%	gi|193217020	
P-85	S2	NI									
P-86	S2	NI									
P-87	S2	Lipoprotein. MAG_4740	*M. agalactiae PG2*	25452	9.47	71	MALDI-TOF	8	37%	gi|148377737	MAG_4740
P-88	S2	Lipoprotein MAG_4740	*M. agalactiae PG2*	25452	9.47	68	MALDI-TOF	6	27%	gi|148377737	MAG_4740
P-89	S2	30S ribosomal protein S8	*M. agalactiae PG2*	24090	9.91	151	ESI Q-TOF	8	22%	gi|148377803	MAG_5400
P-90	S2	Lipoprotein MAG_3600	*M. agalactiae PG2*	21678	9.13	70	ESI Q-TOF	2	9%	gi|148377626	MAG_3600
P-91	S2	Lipoprotein MAG_3600	*M. agalactiae PG2*	21678	9.13	53	ESI Q-TOF	3	9%	gi|148377626	MAG_3600
P-92	S2	Lipoprotein MAG_3600	*M. agalactiae PG2*	21678	9.13	150	ESI Q-TOF	5	15%	gi|148377626	MAG_3600
P-93	S2	Lipoprotein MAG_3600	*M. agalactiae PG2*	21678	9.13	55	MALDI-TOF	5	35%	gi|148377626	MAG_3600
P-94	S2	Lipoprotein MAG_3600	*M. agalactiae PG2*	21678	9.13	412	ESI Q-TOF	12	52%	gi|148377626	MAG_3600
P-95	S2	Lipoprotein MAG_3600	*M. agalactiae PG2*	21678	9.13	64	MALDI-TOF	6	35%	gi|148377626	MAG_3600
P-96	S2	Lipoprotein MAG_3600	*M. agalactiae PG2*	21678	9.13	57	MALDI-TOF	6	38%	gi|148377626	MAG_3600
P-97	S2	NI									

Spot	Map	Protein	Organism	Mw (Da)	pI	Score	Instrument	Queries	Coverage	Acc. No.	Locus tag
P-98	S2	NI									
P-99	S2	NI									
P-100	S2	Lipoprotein MAG_2400	*M. agalactiae PG2*	38065	8.95	37	ESI Q-TOF	4	11%	gi|148377506	MAG_2400
P-101	S2	Lipoprotein MAG_2400	*M. agalactiae PG2*	38065	8.95	111	ESI Q-TOF	11	15%	gi|148377506	MAG_2400
P-102	S2	NI									
P-103	S2	NI									
P-104	S2	ATP synthase B chain	*M. agalactiae PG2*	21796	9.4	33	ESI Q-TOF	1	6%	gi|148377618	MAG_3520
P-105	S2	NI									
P-106	S2	NI									
P-107	S2	NI									
P-108	S2	NI									
P-109	S2	NI									
P-110	S2	NI									
P-111	S2	NI									
P-112	S2	5'nucleotidase	*M. agalactiae PG2*	76195	8.26	116	ESI Q-TOF	6	8%	gi|148377854	MAG_5910
P-113	S3	Hypotetical protein MAG_1000	*M. agalactiae PG2*	109727	8.66	168	MALDI-TOF	20	23%	gi|148377368	MAG_1000
P-114	S3	P80. lipoprotein	*M. agalactiae PG2*	81102	9.08	200	MALDI-TOF	24	37%	gi|148377765	MAG_5030
P-115	S3	5'nucleotidase	*M. agalactiae PG2*	76195	8.36	91	MALDI-TOF	11	21%	gi|148377854	MAG_5910
P-116	S3	Hypothetical protein MAG_2220	*M. agalactiae PG2*	69573	8.78	85	MALDI-TOF	10	20%	gi|148377488	MAG_2220
P-117	S3	P80. lipoprotein	*M. agalactiae PG2*	81102	9.08	74	ESI Q-TOF	5	6%	gi|148377765	MAG_5030
P-118	S3	P80. lipoprotein	*M. agalactiae PG2*	81102	9.08	136	ESI Q-TOF	10	11%	gi|148377765	MAG_5030
P-119	S3	Lipoprotein	*M. agalactiae PG2*	59415	6.77	58	ESI Q-TOF	7	10%	gi|148377770	MAG_5080
P-120	S3	NI									
P-121	S3	Lipoprotein MAG_5080	*M. agalactiae PG2*	59415	6.77	395	ESI Q-TOF	17	30%	gi|148377770	MAG_5080
P-122	S3	Lipoprotein MAG_5080	*M. agalactiae PG2*	59415	6.77	63	MALDI-TOF	6	17%	gi|148377770	MAG_5080
P-123	S3	Lipoprotein MAG_5080	*M. agalactiae PG2*	59415	6.77	132	ESI Q-TOF	6	8%	gi|148377770	MAG_5080

Spot	Map	Protein	Organism	Mw (Da)	pI	Score	Instrument	Queries	Coverage	Acc. No.	Locus tag
P-124	S3	NI									
P-125	S3	NI									
P-126	S3	NADH oxidase (NOXASE)	*M. agalactiae PG2*	49980	6.09	39	ESI Q-TOF	2	3%	gi|148377529	MAG_2630
P-127	S3	NADH oxidase (NOXASE)	*M. agalactiae PG2*	49980	6.09	33	ESI Q-TOF	2	3%	gi|148377529	MAG_2630
P-128	S3	P48. lipoprotein	*M. agalactiae PG2*	51232	8.39	46	ESI Q-TOF	2	4%	gi|148377280	MAG_0120
P-129	S3	NI									
P-130	S3	P48. lipoprotein	*M. agalactiae PG2*	51232	8.39	149	ESI Q-TOF	5	10%	gi|148377280	MAG_0120
P-131	S3	NI									
P-132	S3	P48. lipoprotein	*M. agalactiae PG2*	51232	8.39	107	MALDI-TOF	11	26%	gi|148377280	MAG_0120
P-133	S3	NI									
P-134	S3	NI									
P-135	S3	NI									
P-136	S3	NI									
P-137	S3	NI									
P-138	S3	NI									
P-139	S3	P48. lipoprotein	*M. agalactiae PG2*	51232	7.19	149	ESI Q-TOF	5	10%	gi|148377280	MAG_0120
P-140	S3	NI									
P-141	S3	Elongation factor Tu	*M. agalactiae PG2*	43638	5.89	195	MALDI-TOF	14	41%	gi|148377586	MAG_3200
P-142	S3	Hypothetical protein MAG_5040	*M. agalactiae PG2*	44828	8.46	61	MALDI-TOF	9	21%	gi|148377766	MAG_5040
P-143	S3	P40, lipoprotein	*M. agalactiae PG2*	39951	8.2	113	MALDI-TOF	11	33%	gi|148377507	MAG_2410
P-144	S3	P40, lipoprotein	*M. agalactiae PG2*	39951	8.19	126	MALDI-TOF	11	33%	gi|148377507	MAG_2410
P-145	S3	P40, lipoprotein	*M. agalactiae PG2*	39951	8.19	118	MALDI-TOF	10	34%	gi|148377507	MAG_2410
P-146	S3	P40, lipoprotein	*M. agalactiae PG2*	39951	8.19	141	MALDI-TOF	11	37%	gi|148377507	MAG_2410
P-147	S3	NI									
P-148	S3	P40, lipoprotein	*M. agalactiae PG2*	39951	8.19	105	MALDI-TOF	9	30%	gi|148377507	MAG_2410
P-149	S3	Variable surface lipoprotein Y	*M. agalactiae PG2*	37528	8.81	34	ESI Q-TOF	3	10%	gi|148377970	MAG_7080

Spot	Map	Protein	Organism	Mw (Da)	pI	Score	Instrument	Queries	Coverage	Acc. No.	Locus tag
P-150	S3	NI									
P-151	S3	P40, lipoprotein	*M. agalactiae PG2*	39951	8.19	136	ESI Q-TOF	6	16%	gi|148377507	MAG_2410
		Lipoate-protein ligase A	*M. agalactiae PG2*	37286	6.42	25	ESI Q-TOF	1	2%	gi|148377329	MAG_0600
P-152	S3	Pyruvate dehydrogenase E1 component. alphasubunit	*M. agalactiae PG2*	41321	6.13	58	ESI Q-TOF	2	5%	gi|148377361	MAG_0930
P-153	S3	NI									
P-154	S3	Pyruvate dehydrogenase E1 component. betasubunit	*M. agalactiae PG2*	36129	5.44	57	ESI Q-TOF	2	3%	gi|148377362	MAG_0940
P-155	S3	Pyruvate dehydrogenase E1 component. betasubunit	*M. agalactiae PG2*	36129	5.44	97	MALDI-TOF	10	41%	gi|148377362	MAG_0940
P-156	S3	Pyruvate dehydrogenase E1 component. betasubunit	*M. agalactiae PG2*	36129	5.44	120	MALDI-TOF	13	46%	gi|148377362	MAG_0940
P-157	S3	Acyl carrier protein phosphodiesterase	*M. hyopneumoniae 232*	25819	9.52	28	ESI Q-TOF	1	4%	gi|54020205	
P-158	S3	NI									
P-159	S3	Triosephosphate isomerase	*M. hyopneumoniae 232*	26804	8.33	29	ESI Q-TOF	1	3%	gi|54020428	
P-160	S3	D-lactate dehydrogenase	*M. agalactiae PG2*	37021	6.11	34	ESI Q-TOF	1	2%	gi|148377416	MAG_1490
P-162	S3	Lipoprotein MAG_2430	*M. agalactiae PG2*	33862	8.29	36	ESI Q-TOF	2	6%	gi|148377509	MAG_2430
		ICEF-IIA ORF19	*M. fermentans*	81544	8.2	25	ESI Q-TOF	1	1%	gi|26984117	
P-163	S3	Lipoprotein MAG_2430	*M. agalactiae PG2*	33862	8.29	73	MALDI-TOF	7	27%	gi|148377509	MAG_2430
P-164	S3	Lipoprotein MAG_2430	*M. agalactiae PG2*	33862	8.29	120	MALDI-TOF	10	36%	gi|148377509	MAG_2430
P-165	S3	Lipoprotein MAG_2430	*M. agalactiae PG2*	33862	8.29	117	MALDI-TOF	8	34%	gi|148377509	MAG_2430
P-166	S3	Lipoprotein MAG_2430	*M. agalactiae PG2*	33862	8.29	72	MALDI-TOF	6	27%	gi|148377509	MAG_2430
P-167	S3	Variable surface lipoprotein D	*M. agalactiae PG2*	36608	9.53	30	ESI Q-TOF	1	2%	gi|148377972	MAG_7100
P-168	S3	Variable surface lipoprotein D	*M. agalactiae PG2*	36608	9.53	129	ESI Q-TOF	8	9%	gi|148377972	MAG_7100
P-169	S3	Variable surface lipoprotein D	*M. agalactiae PG2*	36608	9.53	60	MALDI-TOF	7	18%	gi|148377972	MAG_7100
P-170	S3	Variable surface lipoprotein D	*M. agalactiae PG2*	36608	9.53	77	MALDI-TOF	8	21%	gi|148377972	MAG_7100
P-171	S3	Variable surface lipoprotein D	*M. agalactiae PG2*	36608	9.53	190	ESI Q-TOF	7	11%	gi|148377972	MAG_7100
P-172	S3	NI									
P-173	S3	NI									

Spot	Map	Protein	Organism	Mw (Da)	pI	Score	Instrument	Queries	Coverage	Acc. No.	Locus tag
P-174	S3	Variable surface lipoprotein A	*M. agalactiae PG2*	24770	8.33	64	MALDI-TOF	5	31%	gi|148377969	MAG_7070
P-175	S3	Variable surface lipoprotein A	*M. agalactiae PG2*	24769	8.33	307	ESI Q-TOF	10	22%	gi|148377969	MAG_7070



S5: **Protein profile of liposolubile proteins before and after precipitation. Right: approach used for GeLC-MS/MS characterization.** The bars indicate the regions cut from the PAGE gel and subjected to mass spectrometry characterization. Protein identifications are reported in supplementary data S6, from top to bottom.

S6: Table listing all protein identifications obtained by GeLC-MS/MS of the *M. agalactiae* PG2^T Triton X-114 liposoluble fraction. The protein profile used and the number of slices are represented in supplementary data S5.

Band	Protein	Organism	Mw (Da)	pI	Score	Queries	Coverage	Acc. No.	Locus
1	Malate permease	*M. agalactiae* PG2	45146	9.75	274	5	9%	gi|148377751	MAG_4890
	Elongation factor Tu	*M. agalactiae* PG2	43638	5.89	261	7	17%	gi|148377586	MAG_3200
	L-lactate dehydrogenase (L-LDH)	*M. agalactiae* PG2	34965	6.13	189	6	18%	gi|148377752	MAG_4900
	Sugar ABC transporter permease	*M. agalactiae* PG2	74657	9.45	179	9	11%	gi|148377283	MAG_0150
	ABC transporter ATP-binding protein	*M. agalactiae* PG2	80105	9.01	176	3	5%	gi|148377767	MAG_5050
	Dihydrolipoamide dehydrogenase (E3 component of pyruvate complex)	*M. agalactiae* PG2	58917	6.36	173	4	6%	gi|148377364	MAG_0960
	NADH oxidase (NOXASE)	*M. agalactiae* PG2	49980	6.09	161	6	15%	gi|148377529	MAG_2630
	Putative inner membrane protein translocase component YidC	*M. agalactiae* PG2	83003	9.48	111	6	7%	gi|148377328	MAG_0590
	Hypothetical protein MAG_0280	*M. agalactiae* PG2	75681	9.03	104	2	2%	gi|148377296	MAG_0280
	Preprotein translocase subunit SecY	*M. agalactiae* PG2	54823	9.7	102	2	3%	gi|148377788	MAG_5260
	Cation-transporting P-type ATPase	*M. agalactiae* PG2	100888	6.32	94	3	3%	gi|148377722	MAG_4590
	ABC transporter. ATP-binding protein	*M. agalactiae* PG2	68597	9.25	94	1	2%	gi|148377862	MAG_5990
	Protein-export membrane protein	*M. agalactiae* PG2	89106	9.52	82	5	5%	gi|148377491	MAG_2250
	ABC transporter permease protein	*M. agalactiae* PG2	39653	9.72	74	1	3%	gi|148377768	MAG_5060
	ABC transporter. ATP-binding protein	*M. agalactiae* PG2	67154	8.52	69	1	1%	gi|148377863	MAG_6000
	ABC transporter. ATP-binding protein. P59	*M. agalactiae* PG2	58997	6.43	67	3	6%	gi|148377282	MAG_0140
	Hypothetical protein MAG_2680	*M. agalactiae* PG2	55645	4.72	65	1	2%	gi|148377534	MAG_2680

Band	Protein	Organism	Mw (Da)	pI	Score	Queries	Coverage	Acc. No.	Locus
	Oligopeptide ABC transporter system. permeaseprotein (OppC)	*M. agalactiae* PG2	47514	8.85	55	4	8%	gi|148377370	MAG_1020
	DNA gyrase subunit A	*M. agalactiae* PG2	103247	5.33	54	2	2%	gi|148377826	MAG_5630
	Pyruvate dehydrogenase E1 component. betasubunit	*M. agalactiae* PG2	36129	5.44	52	1	3%	gi|148377362	MAG_0940
	Pyruvate dehydrogenase E1 component. alphasubunit	*M. agalactiae* PG2	41321	6.13	51	2	5%	gi|148377361	MAG_0930
	ABC transporter permease protein	*M. agalactiae* PG2	37630	9.89	47	1	2%	gi|148377769	MAG_5070
	50S ribosomal protein L1	*M. agalactiae* PG2	24955	9.68	44	2	7%	gi|148377349	MAG_0810
	Glycerol ABC transporter. ATP-binding protein	*M. capricolum* subsp. *capricolum* ATCC 27343	47229	9.59	43	2	6%	gi|83319663	MCAP_0454
	Ascorbate-specific PTS system enzyme IIC	*M. agalactiae* PG2	64257	9.33	42	1	3%	gi|148377901	MAG_6380
	30S ribosomal protein S8	*M. agalactiae* PG2	24090	9.91	38	1	3%	gi|148377803	MAG_5400
	50S ribosomal protein L2	*M. agalactiae* PG2	30773	10.63	38	2	4%	gi|148377806	MAG_5430
	Cation-transporting P-ATPase	*M. agalactiae* PG2	100451	8.6	31	1	1%	gi|148378004	MAG_7420
	Hexosephosphate transport protein	*M. agalactiae* PG2	54592	9.81	30	1	1%	gi|148377759	MAG_4970
	Hypothetical protein MAG_4530	*M. agalactiae* PG2	52907	9.51	29	1	2%	gi|148377716	MAG_4530
	ABC transporter. permease protein	*M. agalactiae* PG2	71089	8.87	27	1	1%	gi|148377723	MAG_4600
	Lipoate-protein ligase A	*M. agalactiae* PG2	37286	6.42	26	2	2%	gi|148377329	MAG_0600
2	L-lactate dehydrogenase (L-LDH)	*M. agalactiae* PG2	34965	6.13	121	5	14%	gi|148377752	MAG_4900
	Hypothetical protein MAG_0280	*M. agalactiae* PG2	75681	9.03	116	2	2%	gi|148377296	MAG_0280
	Malate permease	*M. agalactiae* PG2	45146	9.75	111	2	6%	gi|148377751	MAG_4890

Band	Protein	Organism	Mw (Da)	pI	Score	Queries	Coverage	Acc. No.	Locus
	ABC transporter. ATP-binding protein	*M. agalactiae* PG2	68597	9.25	106	1	2%	gi|148377862	MAG_5990
	ABC transporter permease protein	*M. agalactiae* PG2	39653	9.72	94	2	6%	gi|148377768	MAG_5060
	Cation-transporting P-type ATPase	*M. agalactiae* PG2	100888	6.32	88	4	4%	gi|148377722	MAG_4590
	Sugar ABC transporter permease	*M. agalactiae* PG2	74657	9.45	85	3	4%	gi|148377283	MAG_0150
	ABC transporter ATP-binding protein	*M. agalactiae* PG2	80105	9.01	83	1	1%	gi|148377767	MAG_5050
	Hypothetical protein MAG_2680	*M. agalactiae* PG2	55645	4.72	79	1	2%	gi|148377534	MAG_2680
	XAA-Pro aminopeptidase	*M. agalactiae* PG2	40001	5.88	78	2	6%	gi|148377385	MAG_1180
	ABC transporter. permease protein	*M. agalactiae* PG2	71089	8.87	66	1	1%	gi|148377723	MAG_4600
	Preprotein translocase subunit SecY	*M. agalactiae* PG2	54823	9.7	62	1	2%	gi|148377788	MAG_5260
	Putative inner membrane protein translocase component YidC	*M. agalactiae* PG2	83003	9.48	51	3	3%	gi|148377328	MAG_0590
	Pyruvate dehydrogenase E1 component. alphasubunit	*M. agalactiae* PG2	41321	6.13	50	1	3%	gi|148377361	MAG_0930
	Dihydrolipoamide dehydrogenase (E3 component of pyruvate complex)	*M. agalactiae* PG2	58917	6.36	45	2	3%	gi|148377364	MAG_0960
	ABC transporter permease protein	*M. agalactiae* PG2	37630	9.89	39	1	2%	gi|148377769	MAG_5070
	Elongation factor Tu	*M. agalactiae* PG2	43638	5.89	31	1	2%	gi|148377586	MAG_3200
	Hypothetical protein MYPU_3820	*M. pulmonis* UAB CTIP	140043	8.49	30	1	0%	gi|15828853	MAG_3820
	Protein-export membrane protein	*M. agalactiae* PG2	89106	9.52	30	2	2%	gi|148377491	MAG_2250
	ABC transporter. ATP-binding protein	*M. agalactiae* PG2	67154	8.52	29	1	1%	gi|148377863	MAG_6000
3	Elongation factor Tu	*M. agalactiae* PG2	43638	5.89	299	6	17%	gi|148377586	MAG_3200

Band	Protein	Organism	Mw (Da)	pI	Score	Queries	Coverage	Acc. No.	Locus
	Sugar ABC transporter permease	*M. agalactiae* PG2	74657	9.45	187	10	10%	gi|148377283	MAG_0150
	ABC transporter permease protein	*M. agalactiae* PG2	308764	8.86	178	5	1%	gi|148378006	MAG_7440
	Putative inner membrane protein translocase component YidC	*M. agalactiae* PG2	83003	9.48	158	6	6%	gi|148377328	MAG_0590
	Hypothetical protein MAG_0280	*M. agalactiae* PG2	75681	9.03	155	4	2%	gi|148377296	MAG_0280
	Cation-transporting P-type ATPase	*M. agalactiae* PG2	100888	6.32	154	4	4%	gi|148377722	MAG_4590
	ABC transporter permease protein	*M. agalactiae* PG2	39653	9.72	152	2	3%	gi|148377768	MAG_5060
	Malate permease	*M. agalactiae* PG2	45146	9.75	145	5	9%	gi|148377751	MAG_4890
	L-lactate dehydrogenase (L-LDH)	*M. agalactiae* PG2	34965	6.13	141	4	9%	gi|148377752	MAG_4900
	Protein-export membrane protein	*M. agalactiae* PG2	89106	9.52	103	4	5%	gi|148377491	MAG_2250
	ABC transporter. ATP-binding protein	*M. agalactiae* PG2	68597	9.25	94	1	2%	gi|148377862	MAG_5990
	Preprotein translocase subunit SecY	*M. agalactiae* PG2	54823	9.7	91	2	3%	gi|148377788	MAG_5260
	Prolipoprotein diacylglyceryl transferase	*M. agalactiae* PG2	37843	9.3	87	1	3%	gi|148377358	MAG_0900
	Cation-transporting P-ATPase	*M. agalactiae* PG2	100451	8.6	82	2	2%	gi|148378004	MAG_7420
	ABC transporter. ATP-binding protein	*M. agalactiae* PG2	67154	8.52	81	2	3%	gi|148377863	MAG_6000
	ABC transporter ATP-binding protein	*M. agalactiae* PG2	80105	9.01	78	1	1%	gi|148377767	MAG_5050
	Dihydrolipoamide dehydrogenase (E3 component of pyruvate complex)	*M. agalactiae* PG2	58917	6.36	72	7	7%	gi|148377364	MAG_0960
	ABC transporter permease protein	*M. agalactiae* PG2	37630	9.89	67	1	2%	gi|148377769	MAG_5070
	Hypothetical protein MAG_2680	*M. agalactiae* PG2	55645	4.72	65	1	2%	gi|148377534	MAG_2680
	Pyruvate dehydrogenase E1 component. betasubunit	*M. agalactiae* PG2	36129	5.44	64	2	3%	gi|148377362	MAG_0940

Band	Protein	Organism	Mw (Da)	pI	Score	Queries	Coverage	Acc. No.	Locus
	Pyruvate dehydrogenase E1 component. alphasubunit	*M. agalactiae* PG2	41321	6.13	62	2	5%	gi|148377361	MAG_0930
	Putative phosphoketolase	*M. agalactiae* PG2	89860	6.07	56	3	3%	gi|148377390	MAG_1230
	Oligopeptide ABC transporter system. permeaseprotein (OppC)	*M. agalactiae* PG2	47514	8.85	53	3	4%	gi|148377370	MAG_1020
	DNA-directed RNA polymerase subunit beta'	*M. agalactiae* PG2	167709	6.47	50	1	<1%	gi|148377874	MAG_6110
	ABC transporter. permease protein	*M. agalactiae* PG2	71089	8.87	46	1	1%	gi|148377723	MAG_4600
	P80. lipoprotein	*M. agalactiae* PG2	81102	9.08	45	1	1%	gi|148377765	MAG_5030
	XAA-Pro aminopeptidase	*M. agalactiae* PG2	40001	5.88	44	2	6%	gi|148377385	MAG_1180
	Hypothetical protein MAG_7360	*M. agalactiae* PG2	53119	9.4	42	2	3%	gi|148377998	MAG_7630
	Hypothetical protein MYPU_3820	*M. pulmonis UAB CTIP*	140043	8.49	31	3	1%	gi|15828853	MYPU_3820
	Hypothetical protein	*M. hyopneumoniae*	3320	11.39	30	1	28%	gi|187610428	
	Hexosephosphate transport protein	*M. agalactiae* PG2	54592	9.81	29	3	6%	gi|148377759	MAG_4970
4	ABC transporter permease protein	*M. agalactiae* PG2	308764	8.86	265	14	4%	gi|148378006	MAG_7440
	Putative phosphoketolase	*M. agalactiae* PG2	89860	6.07	252	8	9%	gi|148377390	MAG_1230
	Elongation factor Tu	*M. agalactiae* PG2	43638	5.89	223	7	17%	gi|148377586	MAG_3200
	ABC transporter ATP-binding protein	*M. agalactiae* PG2	80105	9.01	176	4	7%	gi|148377767	MAG_5050
	L-lactate dehydrogenase (L-LDH)	*M. agalactiae* PG2	34965	6.13	103	3	9%	gi|148377752	MAG_4900
	DNA-directed RNA polymerase subunit beta	*M. agalactiae* PG2	135072	5.72	96	3	2%	gi|148377875	MAG_6120
	Dihydrolipoamide dehydrogenase (E3 component of pyruvate complex)	*M. agalactiae* PG2	58917	6.36	95	2	3%	gi|148377364	MAG_0960

Band	Protein	Organism	Mw (Da)	pI	Score	Queries	Coverage	Acc. No.	Locus
	Malate permease	*M. agalactiae* PG2	45146	9.75	77	1	4%	gi|148377751	MAG_4890
	Hypothetical protein MAG_2680	*M. agalactiae* PG2	55645	4.72	70	1	2%	gi|148377534	MAG_2680
	Hypothetical protein MAG_0280	*M. agalactiae* PG2	75681	9.03	66	1	1%	gi|148377296	MAG_0280
	P80. lipoprotein	*M. agalactiae* PG2	81102	9.08	64	2	3%	gi|148377765	MAG_5030
	Sugar ABC transporter permease	*M. agalactiae* PG2	74657	9.45	63	3	4%	gi|148377283	MAG_0150
	Pyruvate dehydrogenase E1 component. alphasubunit	*M. agalactiae* PG2	41321	6.13	60	2	5%	gi|148377361	MAG_0930
	Pyruvate dehydrogenase E1 component. betasubunit	*M. agalactiae* PG2	36129	5.44	51	2	3%	gi|148377362	MAG_0940
	DNA-directed RNA polymerase subunit beta'	*M. agalactiae* PG2	167709	6.47	50	2	1%	gi|148377874	MAG_6110
	Preprotein translocase subunit SecY	*M. agalactiae* PG2	54823	9.7	44	1	2%	gi|148377788	MAG_5260
	Protein-export membrane protein	*M. agalactiae* PG2	89106	9.52	42	1	1%	gi|148377491	MAG_2250
	Hypothetical protein MYPU_3820	*M. pulmonis* UAB CTIP	139998	8.49	41	1	<1%	gi|15828853	MYPU_3820
	Elongation factor G	*M. agalactiae* PG2	77499	5.47	37	1	1%	gi|148377855	MAG_5920
	XAA-Pro aminopeptidase	*M. agalactiae* PG2	40001	5.88	35	1	3%	gi|148377385	MAG_1180
	ABC transporter permease protein	*M. agalactiae* PG2	39653	9.72	34	1	3%	gi|148377768	MAG_5060
	Glycerol ABC transporter. ATP-binding protein	*M. capricolum* subsp. *capricolum* ATCC 27343	47127	9.59	34	1	2%	gi|83319663	MCAP_0454
	Putative inner membrane protein translocase component YidC	*M. agalactiae* PG2	83003	9.48	33	2	3%	gi|148377328	MAG_0590
	Alanyl-tRNA synthetase	*M. agalactiae* PG2	100239	5.37	29	1	1%	gi|148377682	MAG_4160
	Hypothetical protein	*M. hyopneumoniae*	???		27	1		gi|187610428	

Band	Protein	Organism	Mw (Da)	pI	Score	Queries	Coverage	Acc. No.	Locus
5	DNA-directed RNA polymerase subunit beta'	*M. agalactiae* PG2	167709	6.47	1305	54	21%	gi|148377874	MAG_6110
	P80. lipoprotein	*M. agalactiae* PG2	81102	9.08	106	4	5%	gi|148377765	MAG_5030
	Elongation factor Tu	*M. agalactiae* PG2	43638	5.89	104	4	11%	gi|148377586	MAG_3200
	Pyruvate dehydrogenase E1 component. betasubunit	*M. agalactiae* PG2	36129	5.44	97	2	3%	gi|148377362	MAG_0940
	Hypothetical protein MAG_2680	*M. agalactiae* PG2	55645	4.72	76	1	2%	gi|148377534	MAG_2680
	ABC transporter ATP-binding protein	*M. agalactiae* PG2	80105	9.01	73	2	3%	gi|148377767	MAG_5050
	Pyruvate dehydrogenase E1 component. alphasubunit	*M. agalactiae* PG2	41321	6.13	67	4	7%	gi|148377361	MAG_0930
	Sugar ABC transporter permease	*M. agalactiae* PG2	74657	9.45	56	1	1%	gi|148377283	MAG_0150
	Putative phosphoketolase	*M. agalactiae* PG2	89860	6.07	53	2	2%	gi|148377390	MAG_1230
	Preprotein translocase subunit SecY	*M. agalactiae* PG2	54823	9.7	42	2	3%	gi|148377788	MAG_5260
	Malate permease	*M. agalactiae* PG2	45146	9.75	41	2	6%	gi|148377751	MAG_4890
	XAA-Pro aminopeptidase	*M. agalactiae* PG2	40001	5.88	36	2	5%	gi|148377385	MAG_1180
	Dihydrolipoamide dehydrogenase (E3 component of pyruvate complex)	*M. agalactiae* PG2	58917	6.36	27	2	3%	gi|148377364	MAG_0960
	L-lactate dehydrogenase (L-LDH)	*M. agalactiae* PG2	34965	6.13	25	3	9%	gi|148377752	MAG_4900
6	DNA-directed RNA polymerase subunit beta	*M. agalactiae* PG2	135072	5.13	1663	70	28%	gi|148377875	MAG_6120
	Elongation factor Tu	*M. agalactiae* PG2	43638	5.89	119	2	6%	gi|148377586	MAG_3200
	ABC transporter ATP-binding protein	*M. agalactiae* PG2	80105	9.01	117	2	3%	gi|148377767	MAG_5050
	DNA-directed RNA polymerase subunit beta'	*M. agalactiae* PG2	167709	6.47	102	4	2%	gi|148377874	MAG_6110

Band	Protein	Organism	Mw (Da)	pI	Score	Queries	Coverage	Acc. No.	Locus
	Dihydrolipoamide dehydrogenase (E3 component of pyruvate complex)	*M. agalactiae* PG2	58917	6.36	88	2	3%	gi|148377364	MAG_0960
	Pyruvate dehydrogenase E1 component. betasubunit	*M. agalactiae* PG2	36129	5.44	83	3	3%	gi|148377362	MAG_0940
	L-lactate dehydrogenase (L-LDH)	*M. agalactiae* PG2	34965	6.13	68	1	2%	gi|148377752	MAG_4900
	P80. lipoprotein	*M. agalactiae* PG2	81102	9.08	64	1	1%	gi|148377765	MAG_5030
	Pyruvate dehydrogenase E1 component. alphasubunit	*M. agalactiae* PG2	41321	6.13	57	2	5%	gi|148377361	MAG_0930
	Preprotein translocase subunit SecY	*M. agalactiae* PG2	54823	9.7	38	1	2%	gi|148377788	MAG_5260
	XAA-Pro aminopeptidase	*M. agalactiae* PG2	40001	5.88	34	1	2%	gi|148377385	MAG_1180
9	Hypothetical protein MAG_1000	*M. agalactiae* PG2	109727	8.66	1984	76	38%	gi|148377368	MAG_1000
	Hypothetical protein MAG_2680	*M. agalactiae* PG2	55645	4.72	444	14	32%	gi|148377534	MAG_2680
	Alanyl-tRNA synthetase	*M. agalactiae* PG2	100239	5.37	165	3	4%	gi|148377682	MAG_4160
	Elongation factor Tu	*M. agalactiae* PG2	43638	5.89	161	4	8%	gi|148377586	MAG_3200
	Isoleucyl-tRNA synthetase	*M. agalactiae* PG2	103127	6.19	134	7	6%	gi|148377486	MAG_2200
	Preprotein translocase subunit SecA	*M. agalactiae* PG2	96419	5.16	72	2	2%	gi|148377539	MAG_2730
	Pyruvate dehydrogenase E1 component. betasubunit	*M. agalactiae* PG2	36129	5.44	62	1	3%	gi|148377362	MAG_0940
	Pyruvate dehydrogenase E1 component. alphasubunit	*M. agalactiae* PG2	41321	6.13	61	2	5%	gi|148377361	MAG_0930
	Oligopeptide ABC transporter. substrate-bindingprotein (OppA). lipoprotein	*M. agalactiae* PG2	113019	6.24	55	1	1%	gi|148377306	MAG_0380
	DNA gyrase subunit A	*M. agalactiae* PG2	103247	5.33	43	1	1%	gi|148377826	MAG_5630
	XAA-Pro aminopeptidase	*M. agalactiae* PG2	40001	5.88	43	1	3%	gi|148377385	MAG_1180

Band	Protein	Organism	Mw (Da)	pI	Score	Queries	Coverage	Acc. No.	Locus
	Hydrolase (HAD family)	*M. capricolum* subsp. *capricolum* ATCC 27343	5768	5.25	37	1	21%	gi|963067	
10	Hypothetical protein MAG_1000	*M. agalactiae* PG2	109727	8.66	221	10	9%	gi|148377368	MAG_1000
	Preprotein translocase subunit SecA	*M. agalactiae* PG2	96419	5.16	158	6	7%	gi|148377539	MAG_2730
	Alanyl-tRNA synthetase	*M. agalactiae* PG2	100239	5.37	147	3	3%	gi|148377682	MAG_4160
	Modification (methylase) protein of type irestriction-modification system HsdM	*M. agalactiae* PG2	103753	5.07	111	4	5%	gi|148377836	MAG_5730
	Modification (methylase) protein of type irestriction-modification system	*M. agalactiae* PG2	103659	5.02	111	4	5%	gi|148377828	MAG_5650
	Putative phosphoketolase	*M. agalactiae* PG2	89860	6.07	92	1	2%	gi|148377390	MAG_1230
	Valyl-tRNA synthetase	*M. agalactiae* PG2	97424	8.78	87	2	2%	gi|148377404	MAG_1370
	Elongation factor Tu	*M. agalactiae* PG2	43638	5.89	84	1	3%	gi|148377586	MAG_3200
	ABC transporter ATP-binding protein	*M. agalactiae* PG2	80105	9.01	58	1	1%	gi|148377767	MAG_5050
	Pyruvate dehydrogenase E1 component. alphasubunit	*M. agalactiae* PG2	41321	6.13	54	1	3%	gi|148377361	MAG_0930
	Glycerol ABC transporter. ATP-binding protein	*M. capricolum* subsp. *capricolum* ATCC 27343	47127	9.59	37	1	2%	gi|83319663	MCAP_0454
	Dihydrolipoamide dehydrogenase (E3 component of pyruvate complex)	*M. agalactiae* PG2	58917	6.36	31	1	1%	gi|148377364	MAG_0960
11	Putative phosphoketolase	*M. agalactiae* PG2	89860	6.07	1733	48	31%	gi|148377390	MAG_1230
	P80. lipoprotein	*M. agalactiae* PG2	81102	9.08	292	8	9%	gi|148377765	MAG_5030
	Oligopeptide ABC transporter. ATP-bindingprotein (OppF)	*M. agalactiae* PG2	93878	8.99	226	11	15%	gi|148377372	MAG_1040
	Elongation factor Tu	*M. agalactiae* PG2	43638	5.89	211	4	9%	gi|148377586	MAG_3200

Band	Protein	Organism	Mw (Da)	pI	Score	Queries	Coverage	Acc. No.	Locus
	ABC transporter ATP-binding protein	*M. agalactiae* PG2	80105	9.01	112	2	3%	gi|148377767	MAG_5050
	Leucyl-tRNA synthetase (leucine-tRNA ligase)	*M. agalactiae* PG2	91191	7.51	94	5	5%	gi|148377630	MAG_3640
	Elongation factor G	*M. agalactiae* PG2	77499	5.47	85	3	3%	gi|148377855	MAG_5920
	Pyruvate dehydrogenase E1 component. alphasubunit	*M. agalactiae* PG2	41321	6.13	81	3	7%	gi|148377361	MAG_0930
	Endopeptidase O	*M. agalactiae* PG2	75007	8.5	73	1	1%	gi|148377634	MAG_3680
	Ribonuclease R	*M. agalactiae* PG2	84293	6.36	58	1	1%	gi|148377474	MAG_2080
	Hypothetical protein MAG_1000	*M. agalactiae* PG2	109727	8.66	57	1	1%	gi|148377368	MAG_1000
	Hypothetical protein MAG_6230	*M. agalactiae* PG2	82041	6.18	45	2	2%	gi|148377886	MAG_6230
	Glycerol ABC transporter. ATP-binding protein	*M. capricolum* subsp. *capricolum* ATCC 27343	47127	9.59	38	1	2%	gi|83319663	MCAP_0454
12	P80. lipoprotein	*M. agalactiae* PG2	81102	9.08	1144	49	30%	gi|148377765	MAG_5030
	Putative phosphoketolase	*M. agalactiae* PG2	89860	6.07	610	17	12%	gi|148377390	MAG_1230
	Hypothetical protein MAG_6230	*M. agalactiae* PG2	82041	6.18	339	8	14%	gi|148377886	MAG_6230
	ABC transporter ATP-binding protein	*M. agalactiae* PG2	80105	9.01	135	3	6%	gi|148377767	MAG_5050
	Hypothetical protein MAG_1970	*M. agalactiae* PG2	82243	9.03	133	3	5%	gi|148377463	MAG_1970
	ClpB	*M. agalactiae* PG2	82234	5.98	126	2	3%	gi|148377887	MAG_6240
	Elongation factor G	*M. agalactiae* PG2	77499	5.47	125	4	5%	gi|148377855	MAG_5920
	Elongation factor Tu	*M. agalactiae* PG2	43638	5.89	122	3	8%	gi|148377586	MAG_3200

Band	Protein	Organism	Mw (Da)	pI	Score	Queries	Coverage	Acc. No.	Locus
	Endopeptidase O	*M. agalactiae* PG2	75007	8.5	67	1	1%	gi|148377634	MAG_3680
	Pyruvate dehydrogenase E1 component. alphasubunit	*M. agalactiae* PG2	41321	6.13	53	1	3%	gi|148377361	MAG_0930
	Glycerol ABC transporter. ATP-binding protein	*M. capricolum* subsp. *capricolum* ATCC 27343	47127	9.59	42	1	2%	gi|83319663	MCAP_0454
	Pyruvate dehydrogenase E1 component. betasubunit	*M. agalactiae* PG2	36129	5.44	42	1	3%	gi|148377362	MAG_0940
	leucyl-tRNA synthetase (leucine-tRNA ligase)	*M. agalactiae* PG2	91191	7.51	29	2	2%	gi|148377630	MAG_3640
13	P80. lipoprotein	*M. agalactiae* PG2	81102	9.08	1913	51	48%	gi|148377765	MAG_5030
	ABC transporter ATP-binding protein	*M. agalactiae* PG2	80105	9.01	600	18	23%	gi|148377767	MAG_5050
	Hypothetical protein MAG_1970	*M. agalactiae* PG2	82243	9.03	424	15	18%	gi|148377463	MAG_1970
	ClpB	*M. agalactiae* PG2	82234	5.98	101	4	6%	gi|148377887	MAG_6240
	Cell division protein ftsH-like protein	*M. agalactiae* PG2	74857	5.94	101	3	6%	gi|148378007	MAG_7450
	DNA gyrase subunit B	*M. agalactiae* PG2	73507	5.85	92	2	2%	gi|148377999	MAG_7370
	Hypothetical protein MAG_6230	*M. agalactiae* PG2	82041	6.18	84	1	2%	gi|148377886	MAG_6230
	Elongation factor G	*M. agalactiae* PG2	77499	5.47	59	1	1%	gi|148377855	MAG_5920
	Glycerol ABC transporter. ATP-binding protein	*M. capricolum* subsp. *capricolum* ATCC 27343	47127	9.59	47	1	2%	gi|83319663	MCAP_0454
	Endopeptidase O	*M. agalactiae* PG2	75007	8.5	47	4	5%	gi|148377634	MAG_3680
	Pyruvate dehydrogenase E1 component. alphasubunit	*M. agalactiae* PG2	41321	6.13	44	1	3%	gi|148377361	MAG_0930
	Putative phosphoketolase	*M. agalactiae* PG2	89860	6.07	43	1	1%	gi|148377390	MAG_1230

Band	Protein	Organism	Mw (Da)	pI	Score	Queries	Coverage	Acc. No.	Locus
	Dihydrolipoamide dehydrogenase (E3 component of pyruvate complex)	*M. agalactiae* PG2	58917	6.36	43	1	1%	gi|148377364	MAG_0960
14	5'nucleotidase	*M. agalactiae* PG2	76195	8.36	1167	35	34%	gi|148377854	MAG_5910
	P80. lipoprotein	*M. agalactiae* PG2	81102	9.08	934	29	33%	gi|148377765	MAG_5030
	Putative inner membrane protein translocase component YidC	*M. agalactiae* PG2	83003	9.48	617	14	21%	gi|148377328	MAG_0590
	Molecular chaperone DnaK	*M. agalactiae* PG2	65263	4.95	459	11	16%	gi|148377413	MAG_1460
	Endopeptidase O	*M. agalactiae* PG2	75007	8.5	394	12	15%	gi|148377634	MAG_3680
	ABC transporter ATP-binding protein	*M. agalactiae* PG2	80105	9.01	310	9	14%	gi|148377767	MAG_5050
	Cell division protein ftsH-like protein	*M. agalactiae* PG2	74857	5.94	204	8	13%	gi|148378007	MAG_7450
	Excinuclease ABC subunit B	*M. agalactiae* PG2	77404	5.78	94	3	4%	gi|148377644	MAG_3780
	Pyruvate dehydrogenase E1 component. alphasubunit	*M. agalactiae* PG2	41321	6.13	58	2	5%	gi|148377361	MAG_0930
	Hypothetical protein MAG_1970	*M. agalactiae* PG2	82243	9.03	54	1	1%	gi|148377463	MAG_1970
	DNA gyrase subunit B	*M. agalactiae* PG2	73507	5.85	52	1	1%	gi|148377999	MAG_7370
	DNA polymerase III subunit gamma and tau	*M. agalactiae* PG2	70644	5.25	51	2	3%	gi|148377949	MAG_6870
	Hypothetical protein MAG_4720	*M. agalactiae* PG2	86074	8.82	47	2	2%	gi|148377735	MAG_4720
	Glycerol ABC transporter. ATP-binding protein	*M. capricolum* subsp. *capricolum* ATCC 27343	47127	9.59	44	2	6%	gi|83319663	MCAP_0454
	Putative transmembrane protein	*M. agalactiae* PG2	83256	9.06	43	1	1%	gi|148377558	MAG_2920
15	Putative inner membrane protein translocase component YidC	*M. agalactiae* PG2	83003	9.48	768	23	23%	gi|148377328	MAG_0590

Band	Protein	Organism	Mw (Da)	pI	Score	Queries	Coverage	Acc. No.	Locus
	5'nucleotidase	*M. agalactiae* PG2	76195	8.36	508	17	17%	gi|148377854	MAG_5910
	P80. lipoprotein	*M. agalactiae* PG2	81102	9.08	421	13	19%	gi|148377765	MAG_5030
	Hypothetical protein MAG_6520	*M. agalactiae* PG2	70087	8.58	376	12	17%	gi|148377915	MAG_6520
	Hypothetical protein MAG_1210	*M. agalactiae* PG2	70044	5.71	234	8	10%	gi|148377388	MAG_1210
	ABC transporter ATP-binding protein	*M. agalactiae* PG2	61006	5.24	200	9	11%	gi|148377859	MAG_5960
	DNA ligase	*M. agalactiae* PG2	74509	6.12	106	3	5%	gi|148377548	MAG_2820
	Endopeptidase O	*M. agalactiae* PG2	75007	8.5	79	3	2%	gi|148377634	MAG_3680
	Dihydrolipoamide dehydrogenase (E3 component of pyruvate complex)	*M. agalactiae* PG2	58917	6.36	43	4	5%	gi|148377364	MAG_0960
	Pyruvate dehydrogenase E1 component. alphasubunit	*M. agalactiae* PG2	41321	6.13	42	1	3%	gi|148377361	MAG_0930
	Hypothetical protein MAG_2360	*M. agalactiae* PG2	75426	6.19	33	1	1%	gi|148377502	MAG_2360
	Topoisomerase IV subunit B	*M. agalactiae* PG2	72252	8.07	31	2	2%	gi|148377448	MAG_1820
16	Hypothetical protein MAG_2220	*M. agalactiae* PG2	69573	8.78	1512	63	32%	gi|148377488	MAG_2220
	ABC transporter ATP-binding protein	*M. agalactiae* PG2	61006	5.24	394	18	19%	gi|148377859	MAG_5960
	Hypothetical protein MAG_6520	*M. agalactiae* PG2	70087	8.58	365	13	13%	gi|148377915	MAG_6520
	Threonyl-tRNA synthetase	*M. agalactiae* PG2	67728	8.77	146	3	3%	gi|148377610	MAG_3440
	Putative inner membrane protein translocase component YidC	*M. agalactiae* PG2	83003	9.48	101	3	5%	gi|148377328	MAG_0590
	P80. lipoprotein	*M. agalactiae* PG2	81102	9.08	97	4	5%	gi|148377765	MAG_5030
	Type III restriction-modification system: methylase	*M. agalactiae* PG2	66381	6.17	96	7	8%	gi|148377420	MAG_1530

Band	Protein	Organism	Mw (Da)	pI	Score	Queries	Coverage	Acc. No.	Locus
	Trigger factor	*M. agalactiae* PG2	56779	5.15	86	6	9%	gi|148377421	MAG_1540
	Pyruvate dehydrogenase E1 component. alphasubunit	*M. agalactiae* PG2	41321	6.13	58	2	5%	gi|148377361	MAG_0930
	Dihydrolipoamide dehydrogenase (E3 component of pyruvate complex)	*M. agalactiae* PG2	58917	6.36	43	2	3%	gi|148377364	MAG_0960
	Hypothetical protein MAG_3240	*M. agalactiae* PG2	72359	8.02	36	2	3%	gi|148377590	MAG_3240
	Hypothetical protein MAG_1210	*M. agalactiae* PG2	70044	5.71	36	2	2%	gi|148377388	MAG_1210
	Protein-export membrane protein	*M. agalactiae* PG2	89106	9.52	34	1	1%	gi|148377491	MAG_2250
	Hypothetical protein MAG_2340	*M. agalactiae* PG2	66023	7.56	28	5	1%	gi|148377500	MAG_2340
	Hypothetical protein MAG_4410	*M. agalactiae* PG2	60649	5.12	26	1	3%	gi|148377704	MAG_4410
17	Hypothetical protein MAG_2220	*M. agalactiae* PG2	69573	8.78	300	12	13%	gi|148377488	MAG_2220
	Threonyl-tRNA synthetase	*M. agalactiae* PG2	67728	8.77	135	8	8%	gi|148377610	MAG_3440
	Hypothetical protein MAG_0280	*M. agalactiae* PG2	75681	9.03	55	1	1%	gi|148377296	MAG_0280
	P80. lipoprotein	*M. agalactiae* PG2	81102	9.08	49	2	3%	gi|148377765	MAG_5030
	Type III restriction-modification system: methylase	*M. agalactiae* PG2	66381	6.17	40	1	1%	gi|148377420	MAG_1530
	Elongation factor Tu	*M. agalactiae* PG2	43638	5.89	26	1	2%	gi|148377586	MAG_3200
18	ABC transporter. ATP-binding protein. P59	*M. agalactiae* PG2	58997	6.43	852	29	18%	gi|148377282	MAG_0140
	F0F1 ATP synthase subunit alpha	*M. agalactiae* PG2	58096	5.95	126	3	7%	gi|148377620	MAG_3540
	Phosphoglyceromutase	*M. agalactiae* PG2	55761	5.87	126	2	2%	gi|148377951	MAG_6890
	CTP synthetase	*M. agalactiae* PG2	60669	7.65	122	4	7%	gi|148377485	MAG_2190

Band	Protein	Organism	Mw (Da)	pI	Score	Queries	Coverage	Acc. No.	Locus
	Hypothetical protein MAG_2220	*M. agalactiae* PG2	69573	8.78	84	4	7%	gi|148377488	MAG_2220
	Pyruvate kinase	*M. agalactiae* PG2	53469	5.81	72	2	3%	gi|148377411	MAG_1440
	Lysyl-tRNA synthetase	*M. agalactiae* PG2	56530	5.56	53	1	2%	gi|148377884	MAG_6210
	Spermidine/putrescine ABC transporter ATP-binding protein	*M. agalactiae* PG2	54920	6.15	53	1	1%	gi|148377392	MAG_1250
	Preprotein translocase subunit SecY	*M. agalactiae* PG2	54823	9.7	48	1	2%	gi|148377788	MAG_5260
	P80. lipoprotein	*M. agalactiae* PG2	81102	9.08	38	2	3%	gi|148377765	MAG_5030
	ABC transporter. ATP-binding protein	*M. agalactiae* PG2	67154	8.52	37	2	3%	gi|148377863	MAG_6000
	Elongation factor Tu	*M. agalactiae* PG2	43638	5.89	31	1	2%	gi|148377586	MAG_3200
	Type III restriction-modification system: methylase	*M. agalactiae* PG2	66381	6.17	29	1	1%	gi|148377420	MAG_1530
	XAA-Pro aminopeptidase	*M. agalactiae* PG2	40001	5.88	25	1	2%	gi|148377385	MAG_1180
19	Pyruvate kinase	*M. agalactiae* PG2	53469	5.81	751	24	35%	gi|148377411	MAG_1440
	Lipoprotein MAG_5080	*M. agalactiae* PG2	59415	6.77	608	30	27%	gi|148377770	MAG_5080
	Lysyl-tRNA synthetase	*M. agalactiae* PG2	56530	5.56	363	13	25%	gi|148377884	MAG_6210
	Hypothetical protein MAG_1430	*M. agalactiae* PG2	56379	8.52	330	8	15%	gi|148377410	MAG_1430
	ABC transporter. ATP-binding protein	*M. agalactiae* PG2	67154	8.52	241	4	8%	gi|148377863	MAG_6000
	Glutamyl-tRNA synthetase	*M. agalactiae* PG2	53870	6.08	239	9	17%	gi|148377841	MAG_5780
	Methionyl-tRNA synthetase	*M. agalactiae* PG2	60263	5.89	207	3	7%	gi|148377472	MAG_2060
	Prolyl-tRNA synthetase	*M. agalactiae* PG2	55488	9.24	198	5	11%	gi|148377386	MAG_1190

Band	Protein	Organism	Mw (Da)	pI	Score	Queries	Coverage	Acc. No.	Locus
	ABC transporter. ATP-binding protein. P59	*M. agalactiae* PG2	58997	6.43	185	8	16%	gi|148377282	MAG_0140
	Elongation factor Tu	*M. agalactiae* PG2	43638	5.89	97	3	6%	gi|148377586	MAG_3200
	Hypothetical protein MAG_2220	*M. agalactiae* PG2	69573	8.78	89	3	5%	gi|148377488	MAG_2220
	Phosphoglyceromutase	*M. agalactiae* PG2	55761	5.87	89	1	2%	gi|148377951	MAG_6890
	Pyruvate dehydrogenase E1 component. alphasubunit	*M. agalactiae* PG2	41321	6.13	56	1	3%	gi|148377361	MAG_0930
	Spermidine/putrescine ABC transporter ATP-binding protein	*M. agalactiae* PG2	54920	6.15	53	1	1%	gi|148377392	MAG_1250
20	Pyruvate kinase	*M. agalactiae* PG2	53469	5.81	243	11	20%	gi|148377411	MAG_1440
	Glycerol kinase	*M. agalactiae* PG2	56853	5.95	223	7	9%	gi|148377710	MAG_4470
	Hypothetical protein MAG_1430	*M. agalactiae* PG2	56379	8.52	153	2	5%	gi|148377410	MAG_1430
	Aminopeptidase (leucine aminopeptidase)	*M. agalactiae* PG2	50393	5.63	91	2	2%	gi|148377964	MAG_7020
	Elongation factor Tu	*M. agalactiae* PG2	43638	5.89	80	2	5%	gi|148377586	MAG_3200
	Lysyl-tRNA synthetase	*M. agalactiae* PG2	56530	5.56	80	4	5%	gi|148377884	MAG_6210
	Replicative DNA helicase	*M. agalactiae* PG2	56206	6.66	71	1	1%	gi|148377504	MAG_2380
	Glutamyl-tRNA synthetase	*M. agalactiae* PG2	53870	6.08	48	1	1%	gi|148377841	MAG_5780
	P80. lipoprotein	*M. agalactiae* PG2	81102	9.08	46	2	3%	gi|148377765	MAG_5030
	Lipoprotein. MAG_5080	*M. agalactiae* PG2	59415	6.77	45	4	6%	gi|148377770	MAG_5080
	XAA-Pro aminopeptidase	*M. agalactiae* PG2	40001	5.88	33	1	2%	gi|148377385	MAG_1180

Band	Protein	Organism	Mw (Da)	pI	Score	Queries	Coverage	Acc. No.	Locus
21	Hypothetical protein MAG_4460	*M. agalactiae* PG2	52483	8.7	658	30	26%	gi|148377709	MAG_4460
	P48. lipoprotein	*M. agalactiae* PG2	51232	8.39	481	17	30%	gi|148377280	MAG_0120
	F0F1 ATP synthase subunit beta	*M. agalactiae* PG2	53330	5.28	371	9	17%	gi|148377622	MAG_3560
	Aminopeptidase (leucine aminopeptidase)	*M. agalactiae* PG2	50393	5.63	367	13	21%	gi|148377964	MAG_7020
	Glycerol kinase	*M. agalactiae* PG2	56853	5.95	238	6	11%	gi|148377710	MAG_4470
	NADH oxidase (NOXASE)	*M. agalactiae* PG2	49980	6.09	235	9	17%	gi|148377529	MAG_2630
	Variable surface lipoprotein V	*M. agalactiae* PG2	37362	9.25	133	6	12%	gi|148377967	MAG_7050
	GTPase ObgE	*M. agalactiae* PG2	46867	5.66	116	4	7%	gi|148377771	MAG_5090
	Signal recognition particle protein	*M. agalactiae* PG2	50358	9.17	95	2	4%	gi|148377845	MAG_5820
	DNA recombination protein	*M. agalactiae* PG2	55552	6.27	80	3	7%	gi|148377514	MAG_2480
	P80. lipoprotein	*M. agalactiae* PG2	81102	9.08	54	2	2%	gi|148377765	MAG_5030
	Hypothetical protein MAG_1430	*M. agalactiae* PG2	56379	8.52	54	1	2%	gi|148377410	MAG_1430
	Pyruvate dehydrogenase E1 component. alphasubunit	*M. agalactiae* PG2	41321	6.13	50	1	3%	gi|148377361	MAG_0930
	Glycyl-tRNA synthetase	*M. agalactiae* PG2	53051	6.02	44	1	2%	gi|148377533	MAG_2670
	Hypothetical protein MAG_1670	*M. agalactiae* PG2	53061	8.58	36	2	4%	gi|148377434	MAG_1670
	Hypothetical protein MAG_1220	*M. agalactiae* PG2	26491	8.69	30	1	3%	gi|148377389	MAG_1220
	Glutamyl-tRNA synthetase	*M. agalactiae* PG2	53870	6.08	26	1	1%	gi|148377841	MAG_5780
22	P48. lipoprotein	*M. agalactiae* PG2	51232	8.39	1501	47	57%	gi|148377280	MAG_0120

Band	Protein	Organism	Mw (Da)	pI	Score	Queries	Coverage	Acc. No.	Locus
	Lipoprotein. MAG_1980	*M. agalactiae* PG2	53788	8.98	176	8	20%	gi|148377464	MAG_1980
	Mg^{2+} transport protein (MGTE)	*M. agalactiae* PG2	54236	4.74	149	3	7%	gi|148377573	MAG_3070
	Hypothetical protein MAG_4460	*M. agalactiae* PG2	52483	8.7	138	6	13%	gi|148377709	MAG_4460
	NADH oxidase (NOXASE)	*M. agalactiae* PG2	49980	6.09	112	3	9%	gi|148377529	MAG_2630
	Aminopeptidase (leucine aminopeptidase)	*M. agalactiae* PG2	50393	5.63	83	1	2%	gi|148377964	MAG_7020
	XAA-Pro aminopeptidase	*M. agalactiae* PG2	40001	5.88	72	2	5%	gi|148377385	MAG_1180
	Pyruvate dehydrogenase E1 component. alphasubunit	*M. agalactiae* PG2	41321	6.13	52	1	3%	gi|148377361	MAG_0930
	P80. lipoprotein	*M. agalactiae* PG2	81102	9.08	47	3	3%	gi|148377765	MAG_5030
	Signal recognition particle protein	*M. agalactiae* PG2	50358	9.17	43	1	2%	gi|148377845	MAG_5820
	PhosphoPyruvate hydratase	*M. agalactiae* PG2	49710	5.43	32	2	3%	gi|148377585	MAG_3190
	Seryl-tRNA synthetase	*M. agalactiae* PG2	48281	5.62	32	2	4%	gi|148377324	MAG_0560
23	Elongation factor Tu	*M. agalactiae* PG2	43638	5.89	1003	23	33%	gi|148377586	MAG_3200
	P48. lipoprotein	*M. agalactiae* PG2	51232	8.39	997	28	49%	gi|148377280	MAG_0120
	XAA-Pro aminopeptidase	*M. agalactiae* PG2	40001	5.88	407	15	28%	gi|148377385	MAG_1180
	Phosphopentomutase	*M. agalactiae* PG2	43893	5.48	336	7	18%	gi|148377546	MAG_2800
	Alkylphosphonate ABC transporter substrate-binding protein	*M. agalactiae* PG2	49744	6.99	272	6	12%	gi|148377535	MAG_2690
	Phosphoglycerate kinase	*M. agalactiae* PG2	42781	5.79	139	4	10%	gi|148377849	MAG_5860
	Hypothetical protein MAG_1810	*M. agalactiae* PG2	52991	9.16	134	2	3%	gi|148377447	MAG_1810

Band	Protein	Organism	Mw (Da)	pI	Score	Queries	Coverage	Acc. No.	Locus
	Amidase	*M. agalactiae* PG2	48487	6.29	121	1	3%	gi|148377979	MAG_7170
	Lipoprotein. MAG_1980	*M. agalactiae* PG2	53788	8.98	67	2	4%	gi|148377464	MAG_1980
	Hypothetical protein MAG_4460	*M. agalactiae* PG2	52483	8.7	63	1	2%	gi|148377709	MAG_4460
	P80. lipoprotein	*M. agalactiae* PG2	81102	9.08	49	3	4%	gi|148377765	MAG_5030
	Pyruvate dehydrogenase E1 component. alphasubunit	*M. agalactiae* PG2	41321	6.13	42	1	3%	gi|148377361	MAG_0930
	Asparaginyl-tRNA synthetase	*M. agalactiae* PG2	51629	7.25	33	2	4%	gi|148377568	MAG_3020
24	Elongation factor Tu	*M. agalactiae* PG2	43638	5.89	2042	45	46%	gi|148377586	MAG_3200
	Phosphoglycerate kinase	*M. agalactiae* PG2	42781	5.79	441	19	25%	gi|148377849	MAG_5860
	Hypothetical protein MAG_5040	*M. agalactiae* PG2	44828	8.46	401	14	26%	gi|148377766	MAG_5040
	XAA-Pro aminopeptidase	*M. agalactiae* PG2	40001	5.88	168	9	16%	gi|148377385	MAG_1180
	Phosphopentomutase	*M. agalactiae* PG2	43893	5.48	111	4	7%	gi|148377546	MAG_2800
	Alkylphosphonate ABC transporter substrate-binding protein	*M. agalactiae* PG2	49744	6.99	106	2	6%	gi|148377535	MAG_2690
	P48. lipoprotein	*M. agalactiae* PG2	51232	8.39	95	2	4%	gi|148377280	MAG_0120
	Tyrosyl-tRNA synthetase 1	*M. agalactiae* PG2	46669	7.59	54	2	5%	gi|148377992	MAG_7300
	Pyruvate dehydrogenase E1 component. alphasubunit	*M. agalactiae* PG2	41321	6.13	43	1	3%	gi|148377361	MAG_0930
	Hypothetical protein MAG_2810	*M. agalactiae* PG2	48061	9.19	40	2	3%	gi|148377547	MAG_2810
	DNA-directed RNA polymerase subunit alpha	*M. agalactiae* PG2	37453	5.98	36	2	5%	gi|148377782	MAG_5200
25	Elongation factor Tu	*M. agalactiae* PG2	43638	5.89	1296	27	39%	gi|148377586	MAG_3200

Band	Protein	Organism	Mw (Da)	pI	Score	Queries	Coverage	Acc. No.	Locus
	Pyruvate dehydrogenase E1 component. alphasubunit	*M. agalactiae* PG2	41321	6.13	581	21	31%	gi|148377361	MAG_0930
	Variable surface lipoprotein Y	*M. agalactiae* PG2	37528	8.81	502	16	33%	gi|148377970	MAG_7080
	Hypothetical protein MAG_5040	*M. agalactiae* PG2	44828	8.46	475	16	33%	gi|148377766	MAG_5040
	DNA-directed RNA polymerase subunit alpha	*M. agalactiae* PG2	37453	5.98	156	5	16%	gi|148377782	MAG_5200
	XAA-Pro aminopeptidase	*M. agalactiae* PG2	40001	5.88	111	4	11%	gi|148377385	MAG_1180
	Alcohol dehydrogenase	*M. agalactiae* PG2	37640	6.31	111	3	10%	gi|148377540	MAG_2740
	Hypothetical protein MAG_6740	*M. agalactiae* PG2	40257	5.55	96	2	3%	gi|148377936	MAG_6740
	Alcohol dehydrogenase	*M. agalactiae* PG2	38157	6.28	92	3	8%	gi|148377697	MAG_4340
	Phosphoglycerate kinase	*M. agalactiae* PG2	42781	5.79	86	5	12%	gi|148377849	MAG_5860
	Alkylphosphonate ABC transporter substrate-binding protein	*M. agalactiae* PG2	49744	6.99	71	1	3%	gi|148377535	MAG_2690
	Phosphopentomutase	*M. agalactiae* PG2	43893	5.48	59	2	5%	gi|148377546	MAG_2800
	Acetate kinase	*M. agalactiae* PG2	44283	6.24	55	1	3%	gi|148377407	MAG_1400
	ABC transporter ATP-binding protein	*M. agalactiae* PG2	40400	9.22	43	1	3%	gi|148378005	MAG_7430
	Hypothetical protein MAG_2810	*M. agalactiae* PG2	48061	9.19	39	1	1%	gi|148377547	MAG_2810
	Variable surface lipoprotein U	*M. agalactiae* PG2	25529	8.62	33	1	3%	gi|148377971	MAG_7090
26	Variable surface lipoprotein Y	*M. agalactiae* PG2	37528	8.81	1004	28	32%	gi|148377970	MAG_7080
	Pyruvate dehydrogenase E1 component. alphasubunit	*M. agalactiae* PG2	41321	6.13	500	14	37%	gi|148377361	MAG_0930
	D-lactate dehydrogenase	*M. agalactiae* PG2	37021	6.11	424	11	31%	gi|148377416	MAG_1490

Band	Protein	Organism	Mw (Da)	pI	Score	Queries	Coverage	Acc. No.	Locus
	P40. lipoprotein	*M. agalactiae* PG2	39951	8.19	380	9	25%	gi|148377507	MAG_2410
	Elongation factor Tu	*M. agalactiae* PG2	43638	5.89	366	10	28%	gi|148377586	MAG_3200
	30S ribosomal protein S7	*M. agalactiae* PG2	35450	9.51	303	9	39%	gi|148377525	MAG_2590
	Alcohol dehydrogenase	*M. agalactiae* PG2	37640	6.31	115	3	15%	gi|148377540	MAG_2740
	Hypothetical protein MAG_5040	*M. agalactiae* PG2	44828	8.46	91	3	9%	gi|148377766	MAG_5040
	Glyceraldehyde 3-phosphate dehydrogenase (GAPDH)	*M. agalactiae* PG2	36871	6.72	67	1	3%	gi|148377323	MAG_0550
	Putative glycerol-3-phosphate acyltransferase PlsX	*M. agalactiae* PG2	37025	8.14	29	2	5%	gi|148377703	MAG_4400
27	D-lactate dehydrogenase	*M. agalactiae* PG2	37021	6.11	649	15	47%	gi|148377416	MAG_1490
	Pyruvate dehydrogenase E1 component. betasubunit	*M. agalactiae* PG2	36129	5.44	634	16	40%	gi|148377362	MAG_0940
	Variable surface lipoprotein Y	*M. agalactiae* PG2	37528	8.81	517	10	24%	gi|148377970	MAG_7080
	Glyceraldehyde 3-phosphate dehydrogenase (GAPDH)	*M. agalactiae* PG2	36871	6.72	428	8	27%	gi|148377323	MAG_0550
	Hypothetical protein MAG_4450	*M. agalactiae* PG2	37490	5.32	258	4	11%	gi|148377708	MAG_4450
	P40. lipoprotein	*M. agalactiae* PG2	39951	8.19	243	6	17%	gi|148377507	MAG_2410
	Variable surface lipoprotein W	*M. agalactiae* PG2	35472	9.54	226	4	7%	gi|148377968	MAG_7060
	Phosphotransacetylase	*M. agalactiae* PG2	34447	6.11	208	4	12%	gi|148377406	MAG_1390
	30S ribosomal protein S7	*M. agalactiae* PG2	35450	9.51	155	6	18%	gi|148377525	MAG_2590
	Lipoprotein. MAG_2350	*M. agalactiae* PG2	40383	8.61	131	4	13%	gi|148377501	MAG_2350
	Hypothetical protein MAG_1780	*M. agalactiae* PG2	37986	6.06	113	4	15%	gi|148377444	MAG_1780

Band	Protein	Organism	Mw (Da)	pI	Score	Queries	Coverage	Acc. No.	Locus
	Pyruvate dehydrogenase E1 component. alphasubunit	*M. agalactiae* PG2	41321	6.13	98	2	7%	gi|148377361	MAG_0930
	Elongation factor Tu	*M. agalactiae* PG2	43638	5.89	77	1	3%	gi|148377586	MAG_3200
	L-lactate dehydrogenase (L-LDH)	*M. agalactiae* PG2	34965	6.13	69	1	3%	gi|148377752	MAG_4900
	Lipoate-protein ligase A	*M. agalactiae* PG2	37286	6.42	60	2	5%	gi|148377329	MAG_0600
	Hypothetical protein MAG_4440	*M. agalactiae* PG2	36488	5.87	33	1	2%	gi|148377707	MAG_4440
28	Pyruvate dehydrogenase E1 component. betasubunit	*M. agalactiae* PG2	36129	5.44	950	29	25%	gi|148377362	MAG_0940
	L-lactate dehydrogenase (L-LDH)	*M. agalactiae* PG2	34965	6.13	567	21	35%	gi|148377752	MAG_4900
	Elongation factor Ts (EF-Ts)	*M. agalactiae* PG2	32763	5.18	539	8	24%	gi|148377526	MAG_2600
	Elongation factor Tu	*M. agalactiae* PG2	43638	5.89	232	5	14%	gi|148377586	MAG_3200
	Hypothetical protein MAG_7400	*M. agalactiae* PG2	37245	9.18	170	5	14%	gi|148378002	MAG_7400
	Phosphotransacetylase	*M. agalactiae* PG2	34447	6.11	152	4	10%	gi|148377406	MAG_1390
	Lipoprotein. MAG_1050	*M. agalactiae* PG2	37025	9.26	129	5	15%	gi|148377373	MAG_1050
	Glyceraldehyde 3-phosphate dehydrogenase (GAPDH)	*M. agalactiae* PG2	36871	6.72	128	3	11%	gi|148377323	MAG_0550
	50S ribosomal protein L4	*M. agalactiae* PG2	32406	10.26	126	6	18%	gi|148377808	MAG_5450
	D-lactate dehydrogenase	*M. agalactiae* PG2	37021	6.11	92	2	6%	gi|148377416	MAG_1490
	Glycerol-3-phosphate dehydrogenase	*M. agalactiae* PG2	36931	7.01	85	5	11%	gi|148377318	MAG_0500
	Hypothetical protein MAG_4440	*M. agalactiae* PG2	36488	5.87	85	4	7%	gi|148377707	MAG_4440
	Hypothetical protein MAG_4450	*M. agalactiae* PG2	37490	5.32	85	2	6%	gi|148377708	MAG_4450

Band	Protein	Organism	Mw (Da)	pI	Score	Queries	Coverage	Acc. No.	Locus
	50S ribosomal protein L2	*M. agalactiae* PG2	30773	10.63	72	4	9%	gi|148377806	MAG_5430
	Hypothetical protein MAG_1450	*M. agalactiae* PG2	35463	9.15	71	4	11%	gi|148377412	MAG_1450
	5'nucleotidase	*M. agalactiae* PG2	76195	8.36	60	1	1%	gi|148377854	MAG_5910
	Pyruvate dehydrogenase E1 component. alphasubunit	*M. agalactiae* PG2	41321	6.13	59	2	5%	gi|148377361	MAG_0930
	Variable surface lipoprotein A	*M. agalactiae* PG2	24769	8.33	56	3	14%	gi|148377969	MAG_7070
	Lipoprotein. MAG_2430	*M. agalactiae* PG2	33862	8.29	52	2	7%	gi|148377509	MAG_2430
	30S ribosomal protein S7	*M. agalactiae* PG2	35450	9.51	46	2	2%	gi|148377525	MAG_2590
	Malate permease	*M. agalactiae* PG2	45146	9.75	42	1	2%	gi|148377751	MAG_4890
	P30. lipoprotein	*M. agalactiae* PG2	29213	9.29	42	1	4%	gi|148377613	MAG_3470
	Lipoate-protein ligase A	*M. agalactiae* PG2	37286	6.42	27	2	5%	gi|148377329	MAG_0600
29	Lipoprotein. MAG_2430	*M. agalactiae* PG2	33862	8.29	1289	31	54%	gi|148377509	MAG_2430
	Elongation factor Ts (EF-Ts)	*M. agalactiae* PG2	32763	5.18	353	8	32%	gi|148377526	MAG_2600
	Variable surface lipoprotein D (Variable surface lipopr. Z)	*M. agalactiae* PG2	36608	8.52	240	8	15%	gi|148377972	MAG_7100
	Lipoprotein. MAG_1050	*M. agalactiae* PG2	37025	9.26	224	4	16%	gi|148377373	MAG_1050
	Pyruvate dehydrogenase E1 component. betasubunit	*M. agalactiae* PG2	36129	5.44	220	7	20%	gi|148377362	MAG_0940
	L-lactate dehydrogenase (L-LDH)	*M. agalactiae* PG2	34965	6.13	219	7	23%	gi|148377752	MAG_4900
	Malate permease	*M. agalactiae* PG2	45146	9.75	216	3	6%	gi|148377751	MAG_4890
	50S ribosomal protein L3	*M. agalactiae* PG2	28793	9.75	194	10	34%	gi|148377809	MAG_5460

Band	Protein	Organism	Mw (Da)	pI	Score	Queries	Coverage	Acc. No.	Locus
	50S ribosomal protein L2	*M. agalactiae* PG2	30773	10.63	172	8	23%	gi|148377806	MAG_5430
	phosphate acetyltransferase (Phosphotransacetylase)	*M. agalactiae* PG2	35626	6.75	110	2	7%	gi|148377754	MAG_4920
	Elongation factor Tu	*M. agalactiae* PG2	43638	5.89	97	2	6%	gi|148377586	MAG_3200
	Hypothetical protein MAG_0250	*M. agalactiae* PG2	32658	6.55	67	1	3%	gi|148377293	MAG_0250
	Hypothetical protein MAG_1450	*M. agalactiae* PG2	35463	9.15	58	1	3%	gi|148377412	MAG_1450
	Pyruvate dehydrogenase E1 component. alphasubunit	*M. agalactiae* PG2	41321	6.13	37	1	3%	gi|148377361	MAG_0930
	50S ribosomal protein L1	*M. agalactiae* PG2	24955	9.68	34	2	9%	gi|148377349	MAG_0810
	50S ribosomal protein L4	*M. agalactiae* PG2	32406	10.26	30	2	8%	gi|148377808	MAG_5450
30	Lipoprotein. MAG_2430	*M. agalactiae* PG2	33862	8.29	1053	35	42%	gi|148377509	MAG_2430
	50S ribosomal protein L1	*M. agalactiae* PG2	24955	9.68	354	15	30%	gi|148377349	MAG_0810
	50S ribosomal protein L3	*M. agalactiae* PG2	28793	9.75	256	10	20%	gi|148377809	MAG_5460
	Variable surface lipoprotein D	*M. agalactiae* PG2	36608	8.52	227	16	19%	gi|148377972	MAG_7100
	50S ribosomal protein L2	*M. agalactiae* PG2	30773	10.63	174	12	12%	gi|148377806	MAG_5430
	L-lactate dehydrogenase (L-LDH)	*M. agalactiae* PG2	34965	6.13	149	3	6%	gi|148377752	MAG_4900
	Elongation factor Ts (EF-Ts)	*M. agalactiae* PG2	32763	5.18	62	1	5%	gi|148377526	MAG_2600
	Cobalt transporter ATP-binding subunit	*M. agalactiae* PG2	29354	6.03	51	1	3%	gi|148377780	MAG_5180
	Pyruvate dehydrogenase E1 component. betasubunit	*M. agalactiae* PG2	36129	5.44	51	1	3%	gi|148377362	MAG_0940
	Lipoprotein. MAG_6200	*M. agalactiae* PG2	26952	9.22	42	2	9%	gi|148377883	MAG_6200

Band	Protein	Organism	Mw (Da)	pI	Score	Queries	Coverage	Acc. No.	Locus
	Methionyl-tRNA formyltransferase	*M. agalactiae* PG2	31227	9.21	34	1	2%	gi|148377891	MAG_6280
	F0F1 ATP synthase subunit gamma	*M. agalactiae* PG2	32874	9.01	31	1	2%	gi|148377621	MAG_3550
31	Hypothetical protein MAG_1220	*M. agalactiae* PG2	26491	8.69	323	13	34%	gi|148377389	MAG_1220
	Lipoprotein. MAG_6200	*M. agalactiae* PG2	26952	9.22	299	4	9%	gi|148377883	MAG_6200
	50S ribosomal protein L1	*M. agalactiae* PG2	24955	9.68	201	9	26%	gi|148377349	MAG_0810
	Uridylate kinase	*M. agalactiae* PG2	26309	6.64	154	3	16%	gi|148377314	MAG_0460
	30S ribosomal protein S5	*M. agalactiae* PG2	25266	10.25	151	10	28%	gi|148377792	MAG_5300
	Lipoprotein. MAG_2430	*M. agalactiae* PG2	33862	8.29	120	5	13%	gi|148377509	MAG_2430
	L-lactate dehydrogenase (L-LDH)	*M. agalactiae* PG2	34965	6.13	101	4	9%	gi|148377752	MAG_4900
	30S ribosomal protein S68	*M. agalactiae* PG2	12097	10.2	86	1	15%	gi|148377544	MAG_2780
	ABC transporter permease protein	*M. agalactiae* PG2	39653	9.72	73	1	3%	gi|148377768	MAG_5060
	Triosephosphate isomerase	*M. agalactiae* PG2	29382	6.18	62	1	5%	gi|148377776	MAG_5140
	HAD superfamily hydrolase	*M. agalactiae* PG2	31183	9.45	57	1	3%	gi|148377295	MAG_0270
	Variable surface lipoprotein D	*M. agalactiae* PG2	36608	8.52	56	3	9%	gi|148377972	MAG_7100
	Cobalt transporter ATP-binding subunit	*M. agalactiae* PG2	29354	6.03	52	1	3%	gi|148377780	MAG_5180
	50S ribosomal protein L2	*M. agalactiae* PG2	30773	10.63	48	3	9%	gi|148377806	MAG_5430
	Pyruvate dehydrogenase E1 component. betasubunit	*M. agalactiae* PG2	36129	5.44	48	1	3%	gi|148377362	MAG_0940
	Translation initiation factor IF-3	*M. agalactiae* PG2	23332	9.19	39	2	7%	gi|148377738	MAG_4750

Band	Protein	Organism	Mw (Da)	pI	Score	Queries	Coverage	Acc. No.	Locus
	Methionyl-tRNA formyltransferase	*M. agalactiae* PG2	31227	9.21	35	1	2%	gi|148377891	MAG_6280
	Alanyl-tRNA synthetase	*M. agalactiae* PG2	100239	5.37	32	1	<1%	gi|148377682	MAG_4160
	50S ribosomal protein L3	*M. agalactiae* PG2	28793	9.75	26	2	7%	gi|148377809	MAG_5460
32	30S ribosomal protein S5	*M. agalactiae* PG2	25266	10.25	384	17	38%	gi|148377792	MAG_5300
	Hypothetical protein MAG_1220	*M. agalactiae* PG2	26491	8.69	320	13	30%	gi|148377389	MAG_1220
	Lipoprotein. MAG_6200	*M. agalactiae* PG2	26952	9.22	178	3	9%	gi|148377883	MAG_6200
	Uridylate kinase	*M. agalactiae* PG2	26309	6.64	136	4	16%	gi|148377314	MAG_0460
	Prolipoprotein diacylglyceryl transferase	*M. agalactiae* PG2	37843	9.3	90	1	3%	gi|148377358	MAG_0900
	50S ribosomal protein L1	*M. agalactiae* PG2	24955	9.68	90	3	18%	gi|148377349	MAG_0810
	30S ribosomal protein S68	*M. agalactiae* PG2	12097	10.2	85	1	15%	gi|148377544	MAG_2780
	30S ribosomal protein S8	*M. agalactiae* PG2	24090	9.91	67	2	8%	gi|148377803	MAG_5400
	Lipoprotein. MAG_2000	*M. agalactiae* PG2	26602	8.39	51	1	6%	gi|148377466	MAG_2000
	Triosephosphate isomerase	*M. agalactiae* PG2	29382	6.18	50	1	15%	gi|148377776	MAG_5140
	ABC transporter permease protein	*M. agalactiae* PG2	39653	9.72	46	1	3%	gi|148377768	MAG_5060
	Dihydrolipoamide acetyltransferase component of pyruvate deshydrogenase complex	*M. agalactiae* PG2	26767	7.68	33	1	5%	gi|148377363	MAG_0950
	ABC transporter. ATP binding protein	*M. agalactiae* PG2	27496	8.58	31	1	3%	gi|148377430	MAG_1630
	Pyruvate dehydrogenase E1 component. alphasubunit	*M. agalactiae* PG2	41321	6.13	28	1	2%	gi|148377361	MAG_0930

Band	Protein	Organism	Mw (Da)	pI	Score	Queries	Coverage	Acc. No.	Locus
	Pyruvate dehydrogenase E1 component. betasubunit	*M. agalactiae* PG2	36129	5.44	25	1	3%	gi|148377362	MAG_0940
33	30S ribosomal protein S8	*M. agalactiae* PG2	24090	9.91	452	13	29%	gi|148377803	MAG_5400
	30S ribosomal protein S5	*M. agalactiae* PG2	25266	10.25	147	6	25%	gi|148377792	MAG_5300
	P40. lipoprotein	*M. agalactiae* PG2	39951	8.19	132	3	9%	gi|148377507	MAG_2410
	Hypothetical protein MAG_1220	*M. agalactiae* PG2	26491	8.69	119	4	18%	gi|148377389	MAG_1220
	3-keto-L-gulonate-6-phosphate decarboxylase	*M. agalactiae* PG2	24126	6.64	88	3	5%	gi|148377898	MAG_6350
	Pyruvate dehydrogenase E1 component. betasubunit	*M. agalactiae* PG2	36129	5.44	64	1	3%	gi|148377362	MAG_0940
	Lipoprotein. MAG_2000	*M. agalactiae* PG2	26602	8.29	35	2	6%	gi|148377466	MAG_2000
34	Lipoprotein. MAG_3600	*M. agalactiae* PG2	21678	9.13	579	14	35%	gi|148377626	MAG_3600
	30S ribosomal protein S8	*M. agalactiae* PG2	24090	9.91	228	9	23%	gi|148377803	MAG_5400
	1-acyl-SN-Glycerol-3-phosphate acyltransferase	*M. agalactiae* PG2	28539	9.95	184	8	33%	gi|148377984	MAG_7220
	P40. lipoprotein	*M. agalactiae* PG2	39951	8.19	162	2	3%	gi|148377507	MAG_2410
	30S ribosomal protein S4	*M. agalactiae* PG2	22616	10.21	121	14	27%	gi|148377824	MAG_5610
	Transcription antitermination protein NusG	*M. agalactiae* PG2	22639	6.22	61	1	7%	gi|148377312	MAG_0440
	Variable surface lipoprotein Y	*M. agalactiae* PG2	37528	8.81	52	1	7%	gi|148377970	MAG_7080
	Hypothetical protein MAG_1220	*M. agalactiae* PG2	26491	8.69	46	1	3%	gi|148377389	MAG_1220
	Pyruvate dehydrogenase E1 component. betasubunit	*M. agalactiae* PG2	36129	5.44	39	1	3%	gi|148377362	MAG_0940
	Thymidine kinase	*M. agalactiae* PG2	21731	8.45	29	1	4%	gi|148377963	MAG_7010

Band	Protein	Organism	Mw (Da)	pI	Score	Queries	Coverage	Acc. No.	Locus
	Acyl carrier protein phosphodiesterase	*M. agalactiae* PG2	22409	9.32	26	3	5%	gi|148377731	MAG_3680
35	Lipoprotein. MAG_3600	*M. agalactiae* PG2	21678	9.13	221	9	42%	gi|148377626	MAG_3600
	Variable surface lipoprotein Y	*M. agalactiae* PG2	37528	8.81	177	5	14%	gi|148377970	MAG_7080
	L-lactate dehydrogenase (L-LDH)	*M. agalactiae* PG2	34965	6.13	101	3	9%	gi|148377752	MAG_4900
	50S ribosomal protein L6	*M. agalactiae* PG2	19288	9.88	96	2	5%	gi|148377794	MAG_5320
	Acyl carrier protein phosphodiesterase	*M. agalactiae* PG2	22409	9.32	62	2	5%	gi|148377731	MAG_3680
	30S ribosomal protein S4	*M. agalactiae* PG2	22616	10.21	43	3	13%	gi|148377824	MAG_5610
	Variable surface lipoprotein A	*M. agalactiae* PG2	24769	8.33	37	2	15%	gi|148377969	MAG_7070
	Lipoprotein. MAG_2400	*M. agalactiae* PG2	38065	8.95	36	2	5%	gi|148377506	MAG_2400
36	Variable surface lipoprotein Y	*M. agalactiae* PG2	37528	8.81	608	14	23%	gi|148377970	MAG_7080
	Variable surface lipoprotein A	*M. agalactiae* PG2	24769	8.33	353	6	25%	gi|148377969	MAG_7070
	30S ribosomal protein S7	*M. agalactiae* PG2	17810	10.24	327	12	39%	gi|148377856	MAG_5930
	30S ribosomal protein S68	*M. agalactiae* PG2	12097	10.2	168	3	24%	gi|148377544	MAG_2780
	50S ribosomal protein L13	*M. agalactiae* PG2	16160	10.07	161	4	18%	gi|148377718	MAG_4550
	50S ribosomal protein L5	*M. agalactiae* PG2	20778	9.85	125	8	23%	gi|148377797	MAG_5350
	Hypothetical protein MAG_6920	*M. agalactiae* PG2	20745	5.99	116	2	13%	gi|148377954	MAG_6920
	50S ribosomal protein L18	*M. agalactiae* PG2	12978	10.25	94	3	14%	gi|148377793	MAG_5310

Band	Protein	Organism	Mw (Da)	pI	Score	Queries	Coverage	Acc. No.	Locus
	50S ribosomal protein L10	*M. agalactiae* PG2	18901	9.26	91	3	17%	gi|148377882	MAG_6190
	30S ribosomal protein S9	*M. agalactiae* PG2	14863	11.42	91	4	20%	gi|148377719	MAG_4560
	30S ribosomal protein S60	*M. agalactiae* PG2	12101	10.24	86	1	13%	gi|148377810	MAG_5470
	30S ribosomal protein S69	*M. agalactiae* PG2	10395	9.94	82	3	8%	gi|148377805	MAG_5420
	50S ribosomal protein L17	*M. agalactiae* PG2	13876	10.53	81	5	25%	gi|148377781	MAG_5190
	50S ribosomal protein L32	*M. agalactiae* PG2	7922	10.31	79	1	21%	gi|148377396	MAG_1290
	50S ribosomal protein L11	*M. agalactiae* PG2	16890	9.37	70	1	8%	gi|148377348	MAG_0800
	50S ribosomal protein L23	*M. agalactiae* PG2	16678	9.63	67	2	12%	gi|148377807	MAG_5440
	30S ribosomal protein S63	*M. agalactiae* PG2	14057	10.49	66	4	28%	gi|148377784	MAG_5220
	Lipoprotein. MAG_2400	*M. agalactiae* PG2	38065	8.95	65	2	5%	gi|148377506	MAG_2400
	30S ribosomal protein S62	*M. agalactiae* PG2	15141	11.09	61	3	8%	gi|148377857	MAG_5940
	50S ribosomal protein L14	*M. agalactiae* PG2	13262	9.88	52	1	8%	gi|148377799	MAG_5370
	50S ribosomal protein L27	*M. agalactiae* PG2	10149	10.91	44	1	9%	gi|148377817	MAG_5540
	50S ribosomal protein L22	*M. agalactiae* PG2	12716	10.88	40	1	6%	gi|148377804	MAG_5410
	30S ribosomal protein S6	*M. agalactiae* PG2	16627	9.87	39	1	5%	gi|148377542	MAG_2760
	50S ribosomal protein L6	*M. agalactiae* PG2	19288	9.88	39	1	5%	gi|148377794	MAG_5320
	50S ribosomal protein L15	*M. agalactiae* PG2	15751	10.81	39	2	11%	gi|148377791	MAG_5290
	30S ribosomal protein S61	*M. agalactiae* PG2	14244	10.35	34	1	7%	gi|148377783	MAG_5210
	50S ribosomal protein L24	*M. agalactiae* PG2	12043	10.3	33	1	7%	gi|148377798	MAG_5360

Band	Protein	Organism	Mw (Da)	pI	Score	Queries	Coverage	Acc. No.	Locus
	50S ribosomal protein L19	*M. agalactiae* PG2	13446	11.13	33	1	6%	gi|148377890	MAG_6270
	Hypothetical protein MAG_3830	*M. agalactiae* PG2	17288	9.3	32	1	7%	gi|148377649	MAG_3830

S7: Functional analysis, number of peptide hits, and method of detection of *M. agalactiae* PG2^T liposoluble proteins. The results of 2D DIGE with the two field strains Nurri and Bortigali are also reported (TPH, total peptide hits; NA, not applicable).

Name	Locus	Function	TPH	2D-PAGE-MS	GeLC-MS/MS	PG2^T	Nurri	Bortigali
P48, lipoprotein, MAG_0120	MAG_0120	ABC transporter	94	X	X	X	X	X
ABC transporter, ATP-binding protein P59, MAG_0140	MAG_0140	ABC transporter	40		X	NA	NA	NA
Sugar ABC transporter permease, MAG_0150	MAG_0150	ABC transporter	26		X	NA	NA	NA
Hypothetical protein MAG_0250	MAG_0250	Indigoidine sinthase A superfamily, putative virulence factor	1		X	NA	NA	NA
HAD superfamily hydrolase, MAG_0270	MAG_0270	Hydrolase	1		X	NA	NA	NA
Hypothetical protein MAG_0280	MAG_0280	ABC transporter	10		X	NA	NA	NA
Oligopeptide ABC transporter, substrate-bindingprotein (OppA), lipoprotein, MAG_0380	MAG_0380	ABC transporter	1		X	NA	NA	NA
Transcription antitermination protein NusG, MAG_0440	MAG_0440	Transcription	1		X	NA	NA	NA
Uridylate kinase, MAG_0460	MAG_0460	Nucleotide metabolism	7		X	NA	NA	NA
Glycerol-3-phosphate dehydrogenase, MAG_0500	MAG_0500	Lipid metabolism	5		X	NA	NA	NA
Glyceraldehyde 3-phosphate dehydrogenase (GAPDH), MAG_0550	MAG_0550	Carbohydrate metabolism	12	X	X	X	X	X
Seryl-tRNA synthetase, MAG_0560	MAG_0560	Amino acid metabolism	2		X	NA	NA	NA
Putative inner membrane protein translocase component YidC MAG_0590	MAG_0590	Secretion system/Protein export	57		X	NA	NA	NA
Lipoate-protein ligase A, MAG_0600	MAG_0600	Metabolism of cofactors/Protein modification	6	X	X	NA	NA	NA
50S ribosomal protein L11, MAG_0800	MAG_0800	Translation	1		X	NA	NA	NA
50S ribosomal protein L1, MAG_0810	MAG_0810	Translation	31	X	X	X	X	X
Prolipoprotein diacylglyceryl transferase MAG_0900	MAG_0900	Secretion system/Protein export	2		X	NA	NA	NA
Pyruvate dehydrogenase E1 component, alphasubunit, MAG_0930	MAG_0930	Carbohydrate metabolism	72	X	X	X	X	X
Pyruvate dehydrogenase E1 component, betasubunit, MAG_0940	MAG_0940	Carbohydrate metabolism	69	X	X	X	X	X
Dihydrolipoamide acetyltransferase component of pyruvate dehydrogenase complex, MAG_0950	MAG_0950	Carbohydrate metabolism	1		X	NA	NA	NA

Name	Locus	Function	TPH	2D-PAGE-MS	GeLC-MS/MS	PG2^T	Nurri	Bortigali
Dihydrolipoamide dehydrogenase (E3 component of pyruvate complex), MAG_0960	MAG_0960	Carbohydrate metabolism/Amino acid metabolism	27		X	NA	NA	NA
Hypotetical protein MAG_1000	MAG_1000	ABC transporter	87	X	X	X	X	X
Oligopeptide ABC transporter system, permeaseprotein (OppC), MAG_1020	MAG_1020	ABC transporter	7		X	NA	NA	NA
Oligopeptide ABC transporter, ATP-bindingprotein (OppF), MAG_1040	MAG_1040	ABC transporter	11		X	NA	NA	NA
Lipoprotein, MAG_1050	MAG_1050		9	X	X	NA	NA	NA
XAA-Pro aminopeptidase, MAG_1180	MAG_1180	Hydrolase	41		X	NA	NA	NA
Prolyl-tRNA synthetase, MAG_1190	MAG_1190	Amino acid metabolism	5		X	NA	NA	NA
Hypothetical membrane protein MAG_1210	MAG_1210		10		X	NA	NA	NA
Hypothetical transmembrane protein MAG_1220	MAG_1220	LemA family	32	X	X	X		X
Putative phosphoketolase, MAG_1230	MAG_1230	Carbohydrate metabolism/Energy metabolism	80		X	NA	NA	NA
Spermidine/putrescine ABC transporter ATP-binding protein, MAG_1250	MAG_1250	ABC transporter	2		X	NA	NA	NA
50S ribosomal protein L32, MAG_1290	MAG_1290	Translation	1		X	NA	NA	NA
Valyl-tRNA synthetase, MAG_1370	MAG_1370	Amino acid metabolism	2		X	NA	NA	NA
Phosphotransacetylase, MAG_1390	MAG_1390	Carbohydrate metabolism/Amino acid metabolism	8		X	NA	NA	NA
Acetate kinase, MAG_1400	MAG_1400	Carbohydrate metabolism/Amino acid metabolism	1		X	NA	NA	NA
Hypothetical protein MAG_1430	MAG_1430		11		X	NA	NA	NA
Pyruvate kinase, MAG_1440	MAG_1440	Carbohydrate metabolism	37		X	NA	NA	NA
Hypothetical lipoprotein MAG_1450	MAG_1450		5	X	X	NA	NA	NA
Molecular chaperone DnaK, MAG_1460	MAG_1460	Nucleic acid metabolism/Membrane ion channel	11		X	NA	NA	NA
D-lactate dehydrogenase MAG_1490	MAG_1490	Carbohydrate metabolism	28	X	X	X	X	X
Type III restriction-modification system: methylase, MAG_1530	MAG_1530	Restriction-modification system	9		X	NA	NA	NA

Name	Locus	Function	TPH	2D-PAGE-MS	GeLC-MS/MS	PG2^{T}	Nurri	Bortigali
Trigger factor, MAG_1540	MAG_1540	Protein folding	6		X	NA	NA	NA
ABC transporter, ATP binding protein, MAG_1630	MAG_1630	ABC transporter	1		X	NA	NA	NA
Hypothetical lipoprotein MAG_1670	MAG_1670	Mycoides cluster P72 family, putative virulence factor	2		X	NA	NA	NA
Hypothetical protein MAG_1780	MAG_1780		4		X	NA	NA	NA
Hypothetical transmembrane protein MAG_1810	MAG_1810		2		X	NA	NA	NA
Topoisomerase IV subunit B, MAG_1820	MAG_1820	Nucleic acid metabolism	2		X	NA	NA	NA
Hypothetical transmembrane protein MAG_1970	MAG_1970	M. synoviae P80 antigen-like	19		X	NA	NA	NA
Lipoprotein, MAG_1980	MAG_1980	M. synoviae P60 antigen-like	10	X	X	X	X	X
Lipoprotein, MAG_2000	MAG_2000		3	X	X	X	X	X
Methionyl-tRNA synthetase, MAG_2060	MAG_2060	Amino acid metabolism	3		X	NA	NA	NA
Ribonuclease R, MAG_2080	MAG_2080	Nucleic acid metabolism	1		X	NA	NA	NA
CTP synthetase, MAG_2190	MAG_2190	Nucleotide metabolism	4		X			
Isoleucyl-tRNA synthetase , MAG_2200	MAG_2200	Amino acid metabolism	7		X			
Hypothetical lipoprotein MAG_2220	MAG_2220		82	X	X	X	X	X
Protein-export membrane protein, MAG_2250	MAG_2250	Secretion system/Protein export	13		X	NA	NA	NA
Hypothetical lipoprotein, MAG_2340	MAG_2340		5		X	NA	NA	NA
Lipoprotein, MAG_2350	MAG_2350		4	X	X	X	X	X
Hypothetical transmembrane protein MAG_2360	MAG_2360	Hydrolase, DHH superfamily	1		X	NA	NA	NA
Replicative DNA helicase, MAG_2380	MAG_2380	Nucleic acid metabolism	1		X	NA	NA	NA
Lipoprotein, MAG_2400	MAG_2400		4	X	X	X	X	X
P40, lipoprotein, MAG_2410	MAG_2410		20	X	X	X	X	X
Lipoprotein, MAG_2430	MAG_2430		73	X	X	X		X
DNA recombination protein, MAG_2480	MAG_2480	Nucleic acid metabolism	3		X	NA	NA	NA
30S ribosomal protein S7, MAG_2590	MAG_2590	Translation	17		X	NA	NA	NA
Elongation factor Ts (EF-Ts), MAG_2600	MAG_2600	Translation	17		X	NA	NA	NA
NADH oxidase (NOXASE), MAG_2630	MAG_2630	Energy metabolism	18	X	X	NA	NA	NA

Name	Locus	Function	TPH	2D-PAGE-MS	GeLC-MS/MS	PG2^{T}	Nurri	Bortigali
Glycyl-tRNA synthetase, MAG_2670	MAG_2670	Amino acid metabolism	1		X	NA	NA	NA
Hypothetical transmembrane protein MAG_2680	MAG_2680		14		X	NA	NA	NA
Alkylphosphonate ABC transporter substrate-binding protein, MAG_2690	MAG_2690	ABC transporter	9	X	X	X	X	X
Preprotein translocase subunit SecA, MAG_2730	MAG_2730	Secretion system/Protein export	8		X	NA	NA	NA
Alcohol dehydrogenase, MAG_2740	MAG_2740	Carbohydrate metabolism/Amino acid metabolism/Lipid metabolism/Xenobiotics Biodegradation and Metabolism	6		X	NA	NA	NA
30S ribosomal protein S6, MAG_2760	MAG_2760	Translation	1		X	NA	NA	NA
30S ribosomal protein S68, MAG_2780	MAG_2780	Translation	5		X	NA	NA	NA
Phosphopentomutase, MAG_2800	MAG_2800	Carbohydrate metabolism/Nucleotide metabolism	13		X	NA	NA	NA
Hypothetical protein MAG_2810	MAG_2810		3		X	NA	NA	NA
DNA ligase, MAG_2820	MAG_2820	Nucleic acid metabolism	3		X	NA	NA	NA
Putative transmembrane protein, MAG_2920	MAG_2920		1		X	NA	NA	NA
Asparaginyl-tRNA synthetase, MAG_3020	MAG_3020	Amino acid metabolism	2		X	NA	NA	NA
Mg2+ transport protein (MGTE), MAG_3070	MAG_3070	Membrane transport	3		X	NA	NA	NA
Phosphopyruvate hydratase, MAG_3190	MAG_3190	Carbohydrate metabolism/RNA degradation	2		X	NA	NA	NA
Elongation factor Tu, MAG_3200	MAG_3200	Translation	150	X	X	NA	NA	NA
Hypothetical lipoprotein MAG_3240	MAG_3240		2		X	NA	NA	NA
Threonyl-tRNA synthetase, MAG_3440	MAG_3440	Amino acid metabolism	11		X	NA	NA	NA
P30, lipoprotein, MAG_3470	MAG_3470		1		X	NA	NA	NA
ATP synthase B chain, MAG_3520	MAG_3520	Energy metabolism	4	X		X	X	X
F0F1 ATP synthase subunit alpha, MAG_3540	MAG_3540	Energy metabolism	3		X	NA	NA	NA
F0F1 ATP synthase subunit gamma, MAG_3550	MAG_3550	Energy metabolism	1		X	NA	NA	NA
F0F1 ATP synthase subunit beta, MAG_3560	MAG_3560	Energy metabolism	9		X	NA	NA	NA

Name	Locus	Function	TPH	2D-PAGE-MS	GeLC-MS/MS	PG2^T	Nurri	Bortigali
Lipoprotein, MAG_3600	MAG_3600		23	X	X	X	X	X
Leucyl-tRNA synthetase (leucine-tRNA ligase), MAG_3640	MAG_3640	Amino acid metabolism	7		X	NA	NA	NA
Endopeptidase O, MAG_3680	MAG_3680	Hydrolase	26		X	NA	NA	NA
Excinuclease ABC subunit B, MAG_3780	MAG_3780	Nucleic acid metabolism	3	X	X	NA	NA	NA
Hypothetical protein MAG_3830	MAG_3830	Hydrolase	1		X	NA	NA	NA
Alanyl-tRNA synthetase, MAG_4160	MAG_4160	Amino acid metabolism	8		X	NA	NA	NA
Alcohol dehydrogenase, MAG_4340	MAG_4340	Carbohydrate metabolism/Lipid metabolism/Amino acid metabolism/Xenobiotic biodegradation and metabolism	3		X	NA	NA	NA
Putative glycerol-3-phosphate acyltransferase PlsX, MAG_4400	MAG_4400	Lipid metabolism	2		X	NA	NA	NA
Hypothetical protein MAG_4410	MAG_4410		1		X	NA	NA	NA
Hypothetical protein MAG_4440	MAG_4440	Hydrolase, DHH family	5		X	NA	NA	NA
Hypothetical protein MAG_4450	MAG_4450	Hydrolase, DHH family	6		X	NA	NA	NA
Hypothetical protein MAG_4460	MAG_4460	Hydrolase, HAD superfamily	37		X	NA	NA	NA
Glycerol kinase, MAG_4470	MAG_4470	Lipid metabolism	13		X	NA	NA	NA
Hypothetical transmembrane protein MAG_4530	MAG_4530		1		X	NA	NA	NA
50S ribosomal protein L13, MAG_4550	MAG_4550	Translation	4		X	NA	NA	NA
30S ribosomal protein S9, MAG_4560	MAG_4560	Translation	4		X	NA	NA	NA
Cation-transporting P-type ATPase, MAG_4590	MAG_4590	ABC transporter	11		X	NA	NA	NA
ABC transporter, permease protein, MAG_4600	MAG_4600	ABC transporter	3		X	NA	NA	NA
Hypothetical lipoprotein MAG_4720	MAG_4720	ABC transporter	2		X	NA	NA	NA
Lipoprotein MAG_4740	MAG_4740		14	X		NA	NA	NA
Translation initiation factor IF-3, MAG_4750	MAG_4750	Translation	2		X	NA	NA	NA
Malate permease, MAG_4890	MAG_4890	Membrane transport	19		X	NA	NA	NA
L-lactate dehydrogenase (L-LDH), MAG_4900	MAG_4900	Carbohydrate metabolism/Amino acid	61	X	X	NA	NA	NA

Name	Locus	Function	TPH	2D-PAGE-MS	GeLC-MS/MS	PG2^{T}	Nurri	Bortigali
		metabolism						
Phosphate acetyltransferase (phosphotransacetylase), MAG_4920	MAG_4920	Carbohydrate metabolism/Amino acid metabolism	2		X	NA	NA	NA
Hexosephosphate transport protein, MAG_4970	MAG_4970	Membrane transport	4		X	NA	NA	NA
P80, lipoprotein, MAG_5030	MAG_5030	ABC transporter	176	X	X	X	X	X
Hypothetical protein MAG_5040	MAG_5040	Hydrolase, SNc superfamily	33	X	X	X	X	X
ABC transporter ATP-binding protein, MAG_5050	MAG_5050	ABC transporter	46		X	NA	NA	NA
ABC transporter permease protein, MAG_5060	MAG_5060	ABC transporter	8		X	NA	NA	NA
ABC transporter permease protein, MAG_5070	MAG_5070	ABC transporter	3		X	NA	NA	NA
Lipoprotein MAG_5080	MAG_5080		34	X	X	X	X	X
GTPase ObgE, MAG_5090	MAG_5090	Hydrolase	4		X	NA	NA	NA
Triosephosphate isomerase, MAG_5140	MAG_5140	Carbohydrate metabolism	2	X	X	NA	NA	NA
Cobalt transporter ATP-binding subunit, MAG_5180	MAG_5180	ABC transporter	2		X	NA	NA	NA
50S ribosomal protein L17, MAG_5190	MAG_5190	Translation	5		X	NA	NA	NA
DNA-directed RNA polymerase subunit alpha, MAG_5200	MAG_5200	Transcription	5		X	NA	NA	NA
30S ribosomal protein S61, MAG_5210	MAG_5210	Translation	1		X	NA	NA	NA
30S ribosomal protein S63, MAG_5220	MAG_5220	Translation	4		X	NA	NA	NA
Preprotein translocase subunit SecY, MAG_5260	MAG_5260	Secretion system/Protein export	10		X	NA	NA	NA
50S ribosomal protein L15, MAG_5290	MAG_5290	Translation	2		X	NA	NA	NA
30S ribosomal protein S5, MAG_5300	MAG_5300	Translation	33		X	NA	NA	NA
50S ribosomal protein L18, MAG_5310	MAG_5310	Translation	3		X	NA	NA	NA
50S ribosomal protein L6, MAG_5320	MAG_5320	Translation	3		X	NA	NA	NA
50S ribosomal protein L5, MAG_5350	MAG_5350	Translation	3		X	NA	NA	NA
50S ribosomal protein L24, MAG_5360	MAG_5360	Translation	1		X	NA	NA	NA
50S ribosomal protein L14, MAG_5370	MAG_5370	Translation	1		X	NA	NA	NA
30S ribosomal protein S8, MAG_5400	MAG_5400	Translation	25	X	X	X	X	X

Name	Locus	Function	TPH	2D-PAGE-MS	GeLC-MS/MS	PG2^T	Nurri	Bortigali
50S ribosomal protein L22, MAG_5410	MAG_5410	Translation	1		X	NA	NA	NA
30S ribosomal protein S69, MAG_5420	MAG_5420	Translation	3		X	NA	NA	NA
50S ribosomal protein L2, MAG_5430	MAG_5430	Translation	29		X	NA	NA	NA
50S ribosomal protein L23, MAG_5440	MAG_5440	Translation	2		X	NA	NA	NA
50S ribosomal protein L4, MAG_5450	MAG_5450	Translation	8		X	NA	NA	NA
50S ribosomal protein L3, MAG_5460	MAG_5460	Translation	22	X	X	X	X	X
30S ribosomal protein S60, MAG_5470	MAG_5470	Translation	1		X	NA	NA	NA
50S ribosomal protein L27, MAG_5540	MAG_5540	Translation	1		X	NA	NA	NA
30S ribosomal protein S4, MAG_5610	MAG_5610	Translation	13		X	NA	NA	NA
DNA gyrase subunit A, MAG_5630	MAG_5630	Nucleic acid metabolism	3		X	NA	NA	NA
Modification (methylase) protein of type I restriction-modification system, MAG_5650	MAG_5650	Restriction-modification system	4		X	NA	NA	NA
Modification (methylase) protein of type I restriction-modification system HsdM, MAG_5730	MAG_5730	Restriction-modification system	4		X	NA	NA	NA
Glutamyl-tRNA synthetase, MAG_5780	MAG_5780	Amino acid metabolism	11		X	NA	NA	NA
Signal recognition particle protein, MAG_5820	MAG_5820	Secretion system/Protein export	3		X	NA	NA	NA
Phosphoglycerate kinase, MAG_5860	MAG_5860	Carbohydrate metabolism	28		X	NA	NA	NA
5'nucleotidase, MAG_5910	MAG_5910	Hydrolase	53	X	X	X	X	X
Elongation factor G, MAG_5920	MAG_5920	Translation	9		X	NA	NA	NA
30S ribosomal protein S7, MAG_5930	MAG_5930	Translation	12		X	NA	NA	NA
30S ribosomal protein S62, MAG_5940	MAG_5940	Translation	3		X	NA	NA	NA
ABC transporter ATP-binding protein, MAG_5960	MAG_5960	ABC transporter	27		X	NA	NA	NA
ABC transporter, ATP-binding protein, MAG_5990	MAG_5990	ABC transporter	3		X	NA	NA	NA
ABC transporter, ATP-binding protein, MAG_6000	MAG_6000	ABC transporter	10		X	NA	NA	NA
DNA-directed RNA polymerase subunit beta', MAG_6110	MAG_6110	Nucleic acid metabolism	61		X	NA	NA	NA
DNA-directed RNA polymerase subunit beta, MAG_6120	MAG_6120	Nucleic acid metabolism	73		X	NA	NA	NA

Name	Locus	Function	TPH	2D-PAGE-MS	GeLC-MS/MS	PG2^T	Nurri	Bortigali
50S ribosomal protein L10, MAG_6190	MAG_6190	Translation	3		X	NA	NA	NA
Lipoprotein, MAG_6200	MAG_6200	Transferase, choline-bindine protein	9	X	X	X	X	X
Lysyl-tRNA synthetase, MAG_6210	MAG_6210	Amino acid metabolism	18		X	NA	NA	NA
Hypothetical protein MAG_6230	MAG_6230	Transcription	11		X	NA	NA	NA
ClpB, MAG_6240	MAG_6240	Hydrolase	6		X	NA	NA	NA
50S ribosomal protein L19, MAG_6270	MAG_6270	Translation	1		X	NA	NA	NA
Methionyl-tRNA formyltransferase, MAG_6280	MAG_6280	Amino acid metabolism	2		X	NA	NA	NA
3-keto-L-gulonate-6-phosphate decarboxylase, MAG_6350	MAG_6350	Carbohydrate metabolism	3		X	NA	NA	NA
Ascorbate-specific PTS system enzyme IIC, MAG_6380	MAG_6380	Membrane transport/Carbohydrate metabolism	1		X	NA	NA	NA
Hypothetical lipoprotein MAG_6520	MAG_6520		25	X	X	X		X
Hypothetical transmembrane protein MAG_6740	MAG_6740	Secretion system/Protein export	2		X	NA	NA	NA
DNA polymerase III subunit gamma and tau, MAG_6870	MAG_6870	Nucleic acid metabolism	2		X	NA	NA	NA
Phosphoglyceromutase, MAG_6890	MAG_6890	Carbohydrate metabolism	3		X	NA	NA	NA
Hypothetical transmembrane protein MAG_6920	MAG_6920		2		X	NA	NA	NA
Thymidine kinase, MAG_7010	MAG_7010	Nucleotide metabolism	1		X	NA	NA	NA
Aminopeptidase (leucine aminopeptidase), MAG_7020	MAG_7020	Amino acid metabolism, hydrolase	16		X	NA	NA	NA
Variable surface lipoprotein V, MAG_7050	MAG_7050		6	X	X	X	X	X
Variable surface lipoprotein W, MAG_7060	MAG_7060		4	X	X	X	X	X
Variable surface lipoprotein A, MAG_7070	MAG_7070		11	X	X	X	X	X
Variable surface lipoprotein Y, MAG_7080	MAG_7080		74	X	X	X		X
Variable surface lipoprotein U, MAG_7090	MAG_7090		1	X	X	X	X	X
Variable surface lipoprotein D, MAG_7100	MAG_7100		27	X	X	X	X	X
Amidase, MAG_7170	MAG_7170	Amino acid metabolism	1		X	NA	NA	NA
1-acyl-SN-glycerol-3-phosphate acyltransferase, MAG_7220	MAG_7220	Lipid metabolism	8		X	NA	NA	NA

Name	Locus	Function	TPH	2D-PAGE-MS	GeLC-MS/MS	PG2^{T}	Nurri	Bortigali
Tyrosyl-tRNA synthetase 1, MAG_7300	MAG_7300	Amino acid metabolism	2		X	NA	NA	NA
Hypothetical transmembrane protein MAG_7360	MAG_7360		2		X	NA	NA	NA
DNA gyrase subunit B, MAG_7370	MAG_7370	Nucleic acid metabolism	3		X	NA	NA	NA
Hypothetical protein MAG_7400	MAG_7400	Transcription	5		X	NA	NA	NA
Cation-transporting P-ATPase, MAG_7420	MAG_7420	Energy metabolism	3		X	NA	NA	NA
ABC transporter ATP-binding protein, MAG_7430	MAG_7430	ABC transporter	1		X	NA	NA	NA
ABC transporter permease protein, MAG_7440	MAG_7440	ABC transporter	19		X	NA	NA	NA
Cell division protein ftsH-like protein, MAG_7450	MAG_7450	Cell growth	11		X	NA	NA	NA
Glycerol ABC transporter, ATP-binding protein, MCAP_0454	MCAP_0454	ABC transporter	9		X	NA	NA	NA
Hypothetical protein MYPU_3820	MYPU_3820		5		X	NA	NA	NA

S8: Proteins identified in the *M. agalactiae* PG2^T proteome potentially resulting from Horizontal Gene Transfer events with *M. mycoides* subsp. *mycoides* and *M. capricolum* subsp. *capricolum*.

Name	Locus	Homolog in *M. mycoides* subsp. *mycoides*	Homolog in *M. capricolum* subsp. *capricolum*	Other homologies	Comments
Oligopeptide ABC transporter, substrate-bindingprotein (OppA), lipoprotein, MAG_0380	MAG_0380	MSC_0964	MCAP0116	Several homologs from the Hominis group	MAG having homolog in the Hominis group
D-lactate dehydrogenase MAG_1490	MAG_1490	MSC_0034	MCAP0460	Homolog in *M. penetrans* also highly similar	MAG having homolog in the mycoides cluster and other bacteria but not in the Hominis group
Hypothetical protein MAG_1670	MAG_1670	MSC_0240	MCAP0033	No homolog outside of the mycoides cluster	MAG CDS having no homolog outside of the mycoides cluster
Hypothetical protein MAG_2220	MAG_2220	MSC_0519	MCAP0451	Several homologs	MAG having homolog in the Hominis group
Hypothetical protein MAG_2340	MAG_2340	MSC_0519	MCAP0451	Several homologs	MAG having homolog in the Hominis group
Lipoprotein, MAG_2430	MAG_2430	MSC_1005	MCAP0268	No homolog outside of the mycoides cluster	MAG CDS having no homolog outside of the mycoides cluster
Alkylphosphonate ABC transporter substrate-binding protein, MAG_2690	MAG_2690	MSC_0790	MCAP0731	Homolog from the Hominis group probably lost	MAG having homolog in the Hominis group
Putative transmembrane protein, MAG_2920	MAG_2920	MSC_0620	MCAP0357	Homolog from the Hominis group probably lost	MAG having homolog in the Hominis group
Endopeptidase O, MAG_3680	MAG_3680	MSC_0696	MCAP0466	Homolog in non mollicute bacteria	MAG having homolog in the mycoides cluster and other bacteria but not in the Hominis group
Glycerol kinase, MAG_4470	MAG_4470	MSC_0258	MCAP0218	Homolog from the Hominis group probably lost	MAG having homolog in the Hominis group
ABC transporter, permease protein, MAG_4600	MAG_4600	MSC_0324	MCAP0798	No paralog	MAG having homolog in the Hominis group
Malate permease, MAG_4890	MAG_4890	MSC_0035	MCAP0780	Homolog in *M. penetrans*	MAG having homolog in the mycoides cluster and other bacteria but not in the Hominis group
Hexosephosphate transport protein, MAG_4970	MAG_4970	MSC_0118	MCAP0814	Homolog from the Hominis group probably lost	MAG having homolog in the Hominis group
Hypothetical protein MAG_6520	MAG_6520	MSC_0519	MCAP0451	Several homologs	MAG having homolog in the Hominis group
Glycerol ABC transporter, ATP-binding protein, MCAP_0454	MAG_2310	MSC_0516		Several homologs	MAG having homolog in the Hominis group

S9: Proteins identified in the *M. agalactiae* PG2^{T}proteome potentially resulting from Horizontal Gene Transfer events with other bacteria.

Name	Locus	Putative HGT with organisms of	Comments
Hypothetical protein MAG_0250	MAG_0250	Firmicutes/Proteobacteria	No paralog, no homolog in Mollicutes
Putative phosphoketolase, MAG_1230	MAG_1230	Firmicutes (Lactobacillales)	No paralog, no homolog in *Mollicutes*
Alcohol dehydrogenase, MAG_2740	MAG_2740	Firmicutes/Proteobacteria	Paralog MAG_4280, MAG_4340 (also HGT acquired), no homolog in the Hominis group
Alcohol dehydrogenase, MAG_4340	MAG_4340	Firmicutes/Proteobacteria	Paralog MAG_4280, MAG_2740 (also HGT acquired), no homolog in the Hominis group
Hypothetical protein MAG_4460	MAG_4460	Pneumoniae group	No homolog in the Hominis group, homolog only in U. urealyticum
Modification (methylase) protein of type irestriction-modification system, MAG_5650	MAG_5650	Firmicutes	Paralog MAG_5730 (also HGT acquired), paralog from the Hominis group probably lost
Modification (methylase) protein of type irestriction-modification system HsdM, MAG_5730	MAG_5730	Firmicutes	Paralog MAG_5730 (also HGT acquired), paralog from the Hominis group probably lost

9. REFERENCES

1. **Addis, M.F., A. Tanca, D. Pagnozzi, S. Crobu, G. Fanciulli, P. Cossu-Rocca, and S. Uzzau.** 2009. Generation of high-quality protein extracts from formalin-fixed, paraffin-embedded tissues. Proteomics. **9:**3815-3823.
2. **Adegboye, D.S., P.G. Hallbur, D.L. Cavanaugh, R.E. Werdin, C.C. Chase, D.W. Miskimins, and R.F. Rosenbusch.** 1995. Immunohistochemical and pathological study of *Mycoplasma bovis*-associated lung abscesses in calves. J Vet Diagn Invest. **7:**333-7.
3. **Alberti, A., M.F. Addis, B. Chessa, T. Cubeddu, M. Profiti, S. Rosati, A. Ruiu, and M. Pittau.** 2006. Molecular and antigenic characterization of a *Mycoplasma bovis* strain causing an outbreak of infectious keratoconjunctivitis. J Vet Diagn Invest. **18:**41-51.
4. **Alberti, A., P. Robino, B. Chessa, S. Rosati, M.F. Addis, P. Mercier, A. Mannelli, T. Cubeddu, M. Profiti, E. Bandino, R. Thiery, and M. Pittau.** 2008. Characterisation of *Mycoplasma capricolum* P60 surface lipoprotein and its evaluation in a recombinant ELISA. Vet Microbiol. Vet Microbiol. **128:**81-9.
5. **Alonso, J.M., Prieto M., and F. Parra.** 2002. Genetic and antigenic characterization of elongation factor Tu from *Mycoplasma mycoides* subsp. *mycoides* SC. Vet. Microbiol. **89:**277-289.
6. **Balasubramanian, S., T.R. Kannan, and J.B. Baseman.** 2008. The surface-exposed carboxyl region of *Mycoplasma pneumoniae* elongation factor Tu interacts with fibronectin. Infect. Immun. **76:**3116-3323.
7. **Barroso, G., and J. Labarère.** 1988. Chromosomal gene transfer in *Spiroplasma citri*. Science **241:**959-961.
8. **Baseman, J.B., and J.G. Tully.** 1997.Mycoplasmas: sophisticated, reemerging, and burdened by their notoriety. Emerging Infect Dis. **3:**21-32.
9. **Bendjennat, M., A. Blanchard, M. Loutfi, L. Montagnier, and E. Bahraoui.** 1997. Purification and characterization of *Mycoplasma penetrans* Ca^{2+}/Mg^{2+}-dependent endonuclease. J Bacteriol. **179:**2210-2220.

10. **Bendjennat, M., A. Blanchard, M. Loutfi, L. Montagnier, and E. Bahraoui.** 1999. Role of *Mycoplasma penetrans* endonuclease P40 as a potential pathogenic determinant. Infect Immun. **67:**4456-62.
11. **Bercic, R.L., B. Slavec, M. Lavric, M. Narat, A. Bidovec, P. Dovc, and D. Bencina.** 2008. Identification of major immunogenic proteins of *Mycoplasma synoviae* isolates. Vet. Microbiol. **127:**147-54.
12. **Bergonier, D., X. Berthelot, and F. Poumarat.** 1997. Contagious agalactia of small ruminants: current knowledge concerning epidemiology, diagnosis and control. Rev Sci Tech. **6:**848-73.
13. **Bordier, C.** 1981. Phase separation of integral membrane proteins in Triton X-114 solution. J. Biol. Chem. **25:**1604-1607.
14. **Carter, G.R., and M.M. Chengappa.** 1991. Essentials of veterinary bacteriology and mycology. Lea & Febiger. Philadelphia, London.
15. **Cheng, X., J. Nicolet, R. Miserez, P. Kuhnert, M. Krampe, T. Pilloud, E.M. Abdo, C. Griot, and J. Frey.** 1996. Characterization of the gene for an immunodominant 72 kDa lipoprotein of *Mycoplasma mycoides* subsp. *mycoides* small colony type. Microbiology **142:**3515-3524.
16. **Chessa, B., M. Pittau, M. Puricelli, R. Zobba, E. Coradduzza, P. Dall'Ara, S. Rosati, G. Poli, and A. Alberti.** 2009. Genetic immunization with the immunodominant antigen P48 of *Mycoplasma agalactiae* stimulates a mixed adaptive immune response in BALBc mice. Res Vet Sci. **86:**414-20.
17. **Chevallet, M., S. Luche, and T. Rabilloud.** 2006. Silver staining of proteins in polyacrylamide gels. Nat. Protoc. **1:**1852-1858.
18. **Chopra-Dewasthaly, R., C. Citti, M.D. Glew, M. Zimmermann, R. Rosengarten, and W. Jechlinger.** 2008. Phase-locked mutants of *Mycoplasma agalactiae*: defining the molecular switch of high-frequency Vpma antigenic variation. *Mol. Microbiol.* **67:**1196-1210.
19. **Citovsky, V., S. Rottem, O. Nussbaum, Y. Laster, R. Rott, and A. Loyter.** 1988. Animal viruses are able to fuse with prokaryotic cells. Fusion between Sendai or influenza virions and *Mycoplasma*. J Biol Chem. **263:**461-467.

20. **Corrales, J.C., Esnal C. De la Fea, A. Sanchez, P. Assuncao, J.B. Poveda, and A.Contreras** 2007. Contagious agalactia in small ruminants. Small Ruminant Research **68:**154-166.
21. **Dallo, S.F., Kannan T.R., Blaylock M.W., and J.B. Baseman.** 2002. Elongation factor Tu and E1 beta subunit of pyruvate dehydrogenase complex act as fibronectin binding proteins in *Mycoplasma pneumoniae*. Mol. Microbiol. **46:**1041-1051.
22. .**DeBey, M.C., and R.F. Ross.** 1994. Ciliostasis and loss of cilia induced by Mycoplasma hyopneumoniae in porcine tracheal organ cultures. Infect Immun. **62:**5312-5318.
23. **Dohms, J.E., L.L. Hnatow, P. Whetzel, R. Morgan, C.L. Jr Keeler.** 1993. Identification of the putative cytadhesin gene of *Mycoplasma gallisepticum* and its use as a DNA probe. Avian Dis. **37:**380-8.
24. **Donoghue, P.M., C. Hughes, J.P. Vissers, J.I. Langridge, M.J. Dunn.** 2008. Nonionic detergent phase extraction for the proteomic analysis of heart membrane proteins using label-free LC-MS. Proteomics. **8:**3895-905.
25. **Dramsi S, P. Dehoux, and P. Cossart.** 1993. Common features of gram-positive bacterial proteins involved in cell recognition. Mol Microbiol. **9:**1119-1121.
26. **Dybvig, K., and H. Yu.** 1994. Regulation of a restriction and modification system via DNA inversion in *Mycoplasma pulmonis*. Mol Microbiol. **12:**547-560.
27. **Ferrer-Navarro, M., A. Gómez, O. Yanes, R. Planell, F.X. Avilés, J. Piñol, J. Pérez, A. Pons, and E. Querol.** 2006. Proteome of the bacterium *Mycoplasma penetrans*. J. Proteome Res. **5:**688-694.
28. **Fleury, B., D. Bergonier, X. Berthelot, Y. Schlatter, J. Frey, and E.M. Vilei.** 2001. Characterization and analysis of a stable serotype-associated membrane protein (P30) of *Mycoplasma agalactiae*. J Clin Microbiol. **39:**2814-22.
29. **Fleury, B., D. Bergonier, X. Berthelot, E. Peterhans, J. Frey, and E.M. Vilei.** 2002. Characterization of P40, a cytadhesin of *Mycoplasma agalactiae*. Infect. Immun. **70:**5612-5621.

30. **Flitman-Tene, R., S. Mudahi-Orenstein, S. Levisohn, and D. Yogev.** 2003. Variable lipoprotein genes of *Mycoplasma agalactiae* are activated in vivo by promoter addition via site-specific DNA inversions. Infect Immun. **71:**3821-30.

31. **Fraser, C.M., J.D. Gocayne, O. White, M.D. Adams, R.A. Clayton, R.D. Fleischmann, C.J Bult., A.R. Kerlavage, G. Sutton, J.M. Kelley, J.L. Fritchman, J.F. Weidman, K.V. Small, M. Sandusky, J. Fuhrmann, D. Nguyen, T.R. Utterback, D.M. Saudek, C.A. Phillips, J.M. Merrick, J-F. Tomb, B.A. Dougherty, K.F. Bott, P-C. Hu, T.S. Lucier, S.N. Petterson, H.O. Smith, C.A. Hutchison III, and J.C. Venter.** 1995. The minimal gene complement of *Mycoplasma genitalium*. Science. **270:**397-403.

32. **Fusco, M., L. Corona, T. Onni, E. Marras, C. Longheu, G. Idini, and S. Tola.** 2007. Development of a sensitive and specific enzyme-linked immunosorbent assay based on recombinant antigens for rapid detection of antibodies against *Mycoplasma agalactiae* in sheep. Clin Vaccine Immunol. **14:**420-5.

33. **Glew, M.D., L. Papazisi, F. Poumarat, D. Bergonier, R. Rosengarten, and C. Citti.** 2000. Characterization of a multigene family undergoing high-frequency DNA rearrangements and coding for abundant variable surface proteins in *Mycoplasma agalactiae*. Infect. Immun. **68:**4539-4548.

34. **Götz, S., J.M. García-Gómez, J. Terol, T.D. Williams, M.J. Nueda, M. Robles, M. Talón, J. Dopazo, and A. Conesa.** 2008. High-throughput functional annotation and data mining with the Blast2GO suite. Nucleic Acids Res. **36:**3420-3435.

35. **Greco, G., M. Corrente, D. Buonavoglia, A. Aliberti, and A Fasanella.** 2002. Inactivated vaccine induces protection against *Mycoplasma agalactiae* infection in sheep. New Microbiol. **25:**17-20.

36. **Henrich, B., M. Hopfe, A. Kitzerow, and U. Hadding.** 1999. The adherence-associated lipoprotein P100, encoded by an opp operon structure, functions as the oligopeptide-binding domain OppA of a putative oligopeptide transport system in *Mycoplasma hominis*. J. Bacteriol. **181:**4873-4878.

37. **Himmelreich, R., H. Hilbert, H. Plagens, E. Pirkl, B-C Li, and R. Herrmann.** 1996. Complete sequence analysis of the genome of the bacterium *Mycoplasma pneumoniae*. Nucleic Acids Res. **24:**4420-4449.

38. **Himmelreich, R., H. Plagens, H. Hilbert, B. Reiner, and R. Herrmann.** 1997. Comparative analysis of the genomes of the bacteria *Mycoplasma pneumoniae* and *Mycoplasma genitalium*. Nucleic Acids Res. **25:**701–712.

39. **Hopfe, M., and B. Henrich.** 2004. OppA, the substrate-binding subunit of the oligopeptide permease, is the major Ecto-ATPase of *Mycoplasma hominis*. J. Bacteriol. **186:**1021-1028.

40. **Hopfe, M., and B. Henric.** 2008. OppA, the ecto-ATPase of *Mycoplasma hominis* induces ATP release and cell death in HeLa cells. BMC Microbiol. **8:**55.

41. **Huang, Y., J.A. Robertson, and G.W. Stemke.** 1995. An unusual rRNA gene organization in *Mycoplasma fermentans* (incognitus strain) Can J Microbiol. **41:**424-427.

42. **Izutsu, K.T., S. Fatherazi, C.M. Belton, D. Oda, F.D. Cartwright, and G.E. Kenny.** 1996. *Mycoplasma orale* infection affects K^+ and Cl^- currents in the HSG salivary gland cell line. In Vitro Cell Dev Biol Anim. **32:**361-365.

43. **Jensen, J.S., J. Blom, and K. Lind.** 1994. Intracellular location of *Mycoplasma genitalium* in cultured Vero cells as demonstrated by electron microscopy. Int. J. Exp. Pathol. **75:**91-98.

44. **Jensen, J.S., H.T. Hansen, and K. Lind.** 1996. Isolation of *Mycoplasma genitalium* strains from the male urethra. J Clin Microbiol. **34:**286-291.

45. **Johnson, A.E.** 2009. The structural and functional coupling of two molecular machines, the ribosome and the translocon. J. Cell. Biol. **185:**765-767.

46. **Jores, J., Meens J. Buettner F.F., Linz B., Naessens J., and G.F. Gerlach.** 2009. Analysis of the immunoproteome of *Mycoplasma mycoides* subsp. *mycoides* small colony type reveals immunogenic homologues to other known virulence traits in related *Mycoplasma* species. Vet. Immunol. Immunopathol. **131:**238-245.

47. **Ladefoged, S.A., and G. Christiansen.** 1992. Physical and genetic mapping of the genomes of five *Mycoplasma hominis* strains by pulse-field gel electrophoresis. J Bacteriol. **174:**2199-2207.
48. **Laemmli, U.K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. **227:**680-685.
49. **Lambert, M.** 1987. Contagious agalactia of sheep and goats. Rev Sci Tech OIE. **6:**699-711, Mycoplasmoses of ruminants.
50. **Levinson, G., and G.A. Gutman.** 1987. Slipped-strand mispairing: a major mechanism for DNA sequence evolution. Mol Biol Evol. **4:**203-221.
51. **Li, Y.Z., Ho YP, Chen ST, Chiou TW, Li ZS, Shiuan D.** 2009. Proteomic comparative analysis of pathogenic strain 232 and avirulent strain J of *Mycoplasma hyopneumoniae*. Biochemistry (Mosc). **74:**215-20.
52. **Lo, S.C., M.M. Hayes, H. Kotani, P.F. Pierce, D.J. Wear, P.B. Newton III, J.G. Tully, and J.W. Shih.** 1993. Adhesion onto and invasion into mammalian cells by *Mycoplasma penetrans*: a newly isolated mycoplasma from patients with AIDS. Mod Pathol. **6:**276-280.
53. **Marenda, M., V. Barbe, G. Gourgues, S. Mangenot, E. Sagne, and C. Citti.** 2006. A new integrative conjugative element occurs in *Mycoplasma agalactiae* as chromosomal and free circular forms. J. Bacteriol. **188:**4137-4141.
54. **Marshall, A.J., R.J. Miles, and L. Richards.** 1995. The phagocytosis of mycoplasmas. J Med Microbiol. **43:**239-50.
55. **Marshall, A., B. Afshar, J. Pacy, D. Pitcher, and R. Miles.** 1998. Intracellular location of mycoplasmas. Microbiology, **144:**3240-1.
56. **Mernaugh, G.R., S.F. Dallo, S.C. Holt, and J.B. Baseman.** 1993. Properties of adhering and nonadhering populations of *Mycoplasma genitalium*. Clin. Infect. Dis. **17:**S69-S78.
57. **Miles, R.J.** Catabolism in mollicutes. 1992. J Gen Microbiol. **138:**1773-1783.
58. **Minion, F.C., K.J. Jarvill-Taylor, D.E. Billings, and E. Tigges.** 1993. Membrane-associated nuclease activities in mycoplasmas. J Bacteriol. **175:**7842-7847.

59. **Muto, A., and S. Osawa.** 1987. The guanine and cytosine content of genomic DNA and bacterial evolution. Proc Natl Acad Sci USA. **84:**166-169.
60. **Nicholas, R.A.J.** 2005. Contagious agalactia and other mycoplasmal mastitides of small ruminants. In The Merck Veterinary Manual. Edited by: Kahn CM, Line S. Merck , 9 2005:1114-1116.
61. **Nouvel, L.X., P. Sirand-Pugnet, M.S. Marenda, E. Sagné, V. Barbe, S. Mangenot, C. Schenowitz, D. Jacob, A. Barré, S. Claverol, A. Blanchard, and C. Citti.** 2010.Comparative genomic and proteomic analyses of two *Mycoplasma agalactiae* strains: clues to the macro- and micro-events that are shaping mycoplasma diversity. BMC Genomics **2:**11-86.
62. **Osawa, S., T.H. Jukes, K. Watanabe, and A. Muto**. 1992. Recent evidence for evolution of the genetic code. Microbiol Rev. **56:**229-264.
63. **Paddenberg, R., A. Weber, S. Wulf, and H.G. Mannherz.** 1998. Mycoplasma nucleases able to induce internucleosomal DNA degradation in cultured cells possess many characteristics of eukaryotic apoptotic nucleases. Cell Death Differ. **5:**517-28.
64. **Pittau, M., M. Fadda, P. Briguglio, S. Farina, A.Q. Carboni, and A. Contini.** 1990. Triton X-114 phase fractionation of *Mycoplasma agalactiae* membrane proteins and affinity purification of specific antibodies. Atti Soc. Ital. Sci. Vet. **44**:925-928.
65. **Pollack, J.D., M.V. Williams, R.N. McElhaney.** 1997. The comparative metabolism of the mollicutes (mycoplasmas): the utility for taxonomic classification and the relationship of putative gene annotation and phylogeny to enzymatic function. Crit Rev Microbiol. **23:**269-354.
66. **Poumarat, F., D. Longchambon, and J.L. Martel.** 1992. Application of dot immunobinding on membrane filtration (MF dot) to the study of relationships within "*M. mycoides* cluster" and within "glucose and arginine-negative cluster" of ruminant mycoplasmas. Vet Microbiol. **32:**375-90.
67. **Razin, S.** 1978. The mycoplasmas. Microbiol Rev. **42:**414-470.

68. **Razin, S., and E.A. Freundt.** 1984. The Mollicutes, Mycoplasmatales, and Mycoplasmataceae, p. 740-742. *In* N.R. Krieg and J.G. Holt (ed.) Bergey's manual of systematic bacteriology, vol. I The Williams & Wilkins, Baltimore, Md.

69. **Razin, S.** 1991. The genera *Mycoplasma*, *Ureaplasma*, *Acholeplasma*, *Anaeroplasma*, and *Asteroplasma* p.1937-1959. *In* A. Balows, H.G. Truper, M. Dworkin, W.Harder, and K.H. Schleifer (ed.), The prokaryotes, vol. II, 2ª ed. Springer-verlag, New York, N.Y.

70. **Razin, S.** 1992. Mycoplasma taxonomy and ecology, p. 3-22. *In* J. Maniloff, R.N. McElhaney, L.R. Finch, and J.B. Baseman (ed.), Mycoplasmas molecular biology and pathogenesis. American Society for Microbiology, Washington, D.C.

71. **Razin, S.** 1993. Mycoplasma membranes as models in membrane research. Subcell. Biochem. **20:**1-28. Plenum Press, New York, N.Y.

72. **Razin, S.** 1994. DNA probes and PCR in diagnosis of mycoplasma infections. Mol Cell Probes. **8:**497-511.

73. **Razin, S., D. Yogev, and Y Naot.** 1998. Molecular biology and pathogenicity of mycoplasmas. Microbiol. Mol. Biol. Rev. **62:**1094-1156.

74. **Rainey, P.B., E.R. Moxon, I.P. and Thompson.** 1993. Intraclonal polymorphism in bacteria. Adv Microb Ecol. **13:**263-300.

75. **Rasmussen, O.F., M.H. Shirvan, H. Margalit, C. Christiansen, and S. Rottem.** 1992. Nucleotide sequence, organization, and characterization of the *atp* genes and the encoded subunits of *Mycoplasma gallisepticum* ATPase. Biochem J. **285:**881-888.

76. **Regula, J.T., B. Ueberle, G. Boguth, A. Görg, M. Schnölzer, R. Herrmann, and R. Frank.** 2000. Towards a two-dimensional proteome map of *Mycoplasma pneumoniae*. Electrophoresis. **21:**3765-80.

77. **Reverchon, S., Rouanet C., Expert D., and W. Nasser.** 2002. Characterization of Indigoidine Biosynthetic Genes in *Erwinia chrysanthemi* and Role of This Blue Pigment in Pathogenicity. J. Bacteriol. **184:**654-665.

78. **Roberts, M.C., and G.E. Kenny.** 1987. Conjugal transfer of transposon Tn916 from *Streptococcus faecalis* to *Mycoplasma hominis*. J Bacteriol. **169:**3836-3839.

79. **Robertson, J.A., L.E. Pyle, G.W. Stemke, and L.R. Finch.** 1990. Human ureaplasmas show diverse genome sizes by pulse-field electrophoresis. Nucleic Acids Res.**18:**1451-1455.

80. **Robertson, B.D., and T.F. Meyer.** 1992. Genetic variation in pathogenic bacteria. Trends Genet. **8:**422-427.

81. **Robino, P., A. Alberti, M. Pittau, B. Chessa, M. Miciletta, P. Nebbia, D. Le Grand, and S. Rosati.** 2005. Genetic and antigenic characterization of the surface lipoprotein P48 of *Mycoplasma bovis*. Vet Microbiol. **109:**201-9.

82. **Rosati, S., S. Pozzi, P. Robino, B. Montinaro, A. Conti, M. Fadda, and M. Pittau.** 1999. P48 major surface antigen of *Mycoplasma agalactiae* is homologous to a malp product of *Mycoplasma fermentans* and belongs to a selected family of bacterial lipoproteins. Infection and Immunity. **67**:6213-6216.

83. **Rosati, S., P. Robino, M. Fadda, S. Pozzi, A. Mannelli, and M. Pittau.** 2000. Expression and antigenic characterization of recombinant *Mycoplasma agalactiae* P48 major surface protein. Vet. Microiol. **71:**201-210.

84. **Rottem, S., and I. Kahane**. 1993. Mycoplasma cell membranes. Subcell. Biochemistry. **20:** 1-314.

85. **Rottem, S**. 2003. Interaction of mycoplasmas with host cells. Physiol Rev. **83:**417-32.

86. **Schmidt, J.A., Browning G.F., and P.F. Markham.** 2007. *Mycoplasma hyopneumoniae* mhp379 is a Ca^{2+}-dependent, sugar-nonspecific exonuclease exposed on the cell surface. J. Bacteriol. **189:**3414-3424.

87. **Sirand-Pugnet, P., C. Lartigue, M. Marenda, D. Jacob, A. Barré, V. Barbe, C. Schenowitz, S. Mangenot, A. Couloux, B. Segurens, A. de Daruvar, A. Blanchard, and C. Citti.** 2007. Being pathogenic, plastic, and sexual while living with a nearly minimal bacterial genome. *PLoS Genet.* **3:**e75.

88. **Taylor, R.R., H. Varsani, and R.J. Miles.** 1994. Alternatives to arginine as energy sources for the non-fermentative *Mycoplasma gallinarum*. FEMS Microbiol Lett. **115:**163-168.

89. **Tola, S., G. Idini, D. Manunta, G. Galleri, A. Angioi, A.M. Rocchigiani, and G. Leori.** 1996. Rapid and specific detection of *Mycoplasma agalactiae* by polymerase chain reaction. Vet. Microbiol. **51:**77-84.

90. **Tola, S., D. Manunta, S. Rocca, A.M. Rocchigiani, G. Idini, P.P. Angioi, G. Leori.** 1999. Experimental vaccination against *Mycoplasma agalactiae* using different inactivated vaccines. Vaccine. **17:**2764-2768.

91. **Tola, S., S. Crobeddu, G. Chessa, S. Uzzau, G. Idini, B. Ibba, and S. Rocca.** 2001. Sequence, cloning, expression and characterisation of the 81-kDa surface membrane protein (P80) of *Mycoplasma agalactiae*. FEMS Microbiol. Lett. **202:**45-50.

92. **Tully, J.G., and S. Razin,** editors. 1996. Molecular and diagnostic procedures in mycoplasmology. II. Diagnostic procedures. San Diego, Calif: Academic Press, Inc.

93. **Wessel, D., and U.I. Flügge.** 1984. A method for the quantitative recovery of protein in dilute solution in the presence of detergents and lipids. Anal Biochem. **138:**141-3.

94. **Wieslander, A., S. Nordström, Dahlquist A, L. Rilfors, and G. Lindblom.** 1995. Membrane lipid composition and cell size of *Acholeplasma laidlawii* strain A are strongly influenced by lipid acyl chain length. Eur J Biochem. **227:**734-744

95. **Wise, K.S.** Adaptive surface variation in mycoplasmas. 1993. Trends Microbiol. **1:**59-63.

96. **White, S.H., and G. von Heijne.** 2008. How translocons select transmembrane helices. Annu. Rev. Biophys. **37:**23-42.

97. **Yogev, D., R. Rosengarten, R. Watson-McKown, and K.S. Wise.** 1991. Molecular basis of mycoplasma surface antigenic variation: a novel set of divergent genes undergo spontaneous mutation of periodic coding regions and 5′ regulatory sequences. EMBO J. **10:**4069-4079.

98. **You, X.X., Y.H. Zeng, and Y.M. Wu.** 2006. Interactions between mycoplasma lipid-associated membrane proteins and the host cells. J Zhejiang Univ Sci B. **7:**342-50.