ABC transporters in *Mycoplasma hyopneumoniae* and *Mycoplasma synoviae*: Insights into evolution and pathogenicity

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Abstract

ABC transporters represent one of the largest superfamilies of active membrane transport proteins (MTPs) with a highly conserved ATPase domain that binds and hydrolyzes ATP, supplying energy for the uptake of a variety of nutrients and for the extrusion of drugs and metabolic wastes. The complete genomes of a non-pathogenic (J) and pathogenic (7448) strain of *Mycoplasma hyopneumoniae*, as well as of a pathogenic (53) strain of *Mycoplasma synoviae* have been recently sequenced. A detailed study revealed a high percentage of CDSs encoding MTPs in *M. hyopneumoniae* strains J (13.4%), 7448 (13.8%), and in *M. synoviae* 53 (11.2%), and the ABC systems represented from 85.0 to 88.6% of those CDSs. Uptake systems are mainly involved in cell nutrition and some might be associated with virulence. Exporter systems include both drug and multidrug resistant systems (MDR), which may represent mechanisms of resistance to toxic molecules. No relation was found between the phylogeny of the ATPase domains and the lifestyle or pathogenicity of *Mycoplasma*, but several proteins, potentially useful as targets for the control of infections, were identified.

Key words: ABC transporters, bioinformatics, genomes of microorganisms, membrane transport proteins, *Mycoplasma*, phylogeny of prokaryotes.

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Introduction

ATP-binding cassette systems, also known as ABC transporters or traffic ATPases, represent one of the largest superfamilies of active membrane transport proteins (MTPs). These transporters contain a highly conserved ATPase domain, the ABC (ATP-binding domain or nucleotide-binding domain, NBD), which binds and hydrolyzes ATP, supplying energy for the uptake of a variety of nutrients and for the extrusion of drugs and metabolic wastes from cells and organelles (Schneider and Hunke, 1998; Braibant *et al.*, 2000; Dassa and Bouige, 2001; Higgins, 2001; Davidson and Chen, 2004; Garmory and Titball, 2004; Ren and Paulsen, 2005). In bacteria, the number of ABC transporters correlates with the genome size as well as with the physiological niche in which they live, suggesting that the proteins are likely to be necessary for growth, survival, or competitiveness among microbes for nutrients in different ecological niches (Harland *et al.*, 2005; Ren and Paulsen, 2005). An increased interest in ABC transporters can be explained by their potential as targets for the development of antitumor agents, antibacterial vaccines and antimicrobials (Garmory and Titball, 2004).

The transporter classification system (TC-DB) at the University of California - San Diego (Tran *et al.*, 2003) classifies the ATP-binding Cassette (ABC) Superfamily (TC# 3.A.1) as importers and exporters (Saurin *et al.*, 1999; Saier, 2000; Dassa and Bouige, 2001; Garmory and Titball, 2004), and each type of ABC transporter has different protein structures (Dassa and Bouige, 2001). The basic unit of an ABC transporter (Figure 1) consists of four structural domains: two hydrophobic transmembrane domains (TMDs, or integral membrane domains, IMDs, or membrane-spanning domains, MSDs), and two hydrophilic cytoplasmic domains (Linton and Higgins, 1998). Importers usually have the four domains encoded by independent polypeptides (Figure 1a) and require an extracellular substrate-binding protein, but differences may be found among organisms. In Gram-negative bacteria the importers are usually associated with periplasmic substrate-binding proteins (PBP), whereas in Gram-positive bacteria and in *Mycoplasma* they are generally associated with surface-anchored lipoproteins. Prokaryote exporters may have the four domains separated or fused in many ways...
overview of ABC transporters in Mycoplasmas

To date, in addition to the three genomes from this study, eight complete mycoplasma genomes have been sequenced: M. genitalium G-37 (Fraser et al., 1995), M. pneumoniae M129 (Himmelreich et al., 1996), M. pulmonis UAB CTIP (Chambaud et al., 2001), M. penetrans HF-2 (Sasaki et al., 2002), M. gallisepticum R (Papazisi et al., 2003), M. mobile 163 K (Jaffe et al., 2004), M. mycoides subsp. mycoides PG1T (Westberg et al., 2004), M. hyopneumoniae 232 (Minion et al., 2004) and M. capricolum subsp. capricolum ATCC 27343 (unpublished data). In all but one genome (32.4% in M. mobile strain 163 K, Jaffe et al., 2004), ATP-dependent transporters represented 50% or more of all membrane transport proteins, and ABC proteins represented from 58.8% (M. mycoides subsp. mycoides PG1T, Westberg et al., 2004) to 91.7% (M. mobile strain 163 K) of all ATP-dependent proteins.

In relation to M. synoviae strain 53 and M. hyopneumoniae strains J and 7448, 11.2%, 13.4% and 13.8% of the valid CDSs were transporters, respectively, and ABC systems represented from 85.0% to 88.6% of all ATP-dependent proteins.

Results and Discussion

Overview of ABC transporters in Mycoplasmas

Valid CDSs encoding ABC transporters of M. synoviae strain 53 and of M. hyopneumoniae strains J and 7448 were retrieved from the BRGENE, GENESUL and NCBI databases. Missing genes were confirmed through NCBI tblastn program using known Mycoplasma proteins as queries against the three genomes. The InterProScan and ScanProsite online programs were also used to identify the ABC ATP-binding and transmembrane domains. Online topology prediction programs, such as DAS and Tmpred were applied to recognize transmembrane regions. The ABCISSE database was used to validate the uptake and efflux systems. The transporter numbers (TC#) were defined using TC-DB database nomenclature.

Material and Methods

Valid CDSs encoding ABC transporters of M. synoviae strain 53 and of M. hyopneumoniae strains J and 7448 were retrieved from the BRGENE, GENESUL and NCBI databases. Missing genes were confirmed through NCBI tblastn program using known Mycoplasma proteins as queries against the three genomes. The InterProScan and ScanProsite online programs were also used to identify the ABC ATP-binding and transmembrane domains. Online topology prediction programs, such as DAS and Tmpred were applied to recognize transmembrane regions. The ABCISSE database was used to validate the uptake and efflux systems. The transporter numbers (TC#) were defined using TC-DB database nomenclature.

For the phylogenetic analysis, 14 ATP-binding domains from ABC proteins presented in all three genomes were retrieved from the BRGENE, GENESUL and NCBI databases. The least conserved segments of these sequences were eliminated and multiple sequence alignments were performed with CLUSTAL W version 1.83 (Thompson et al., 1994). Phylogenetic trees were built using MEGA version 3.1 (Kumar et al., 2004) with default parameters and the JTT substitution matrix (Jones et al., 1992). The pairwise deletion criterion was used in order to maximize the number of sites compared between sequences. The bootstrap test (Felsenstein, 1985) was used to calculate the statistical support for individual nodes, with 1,000 replicates and consensus cut-off value of 60%.

The complete genomes of a non-pathogenic (J = ATCC 25934) and of a pathogenic (7448) strain of M. hyopneumoniae, this last one as an infective agent of enzootic pneumonia in swine, as well as of the poultry pathogenic strain 53 of M. synoviae have been recently sequenced (Vasconcelos et al., 2005). Here we have investigated, in greater detail, the ABC transporters in those three genomes, in an effort to obtain more information about the evolution, lifestyle and pathogenicity of these species.
Table 1 - General transporter CDSs present in the \textit{M. synoviae} strain 53 (MS) and \textit{M. hyopneumoniae} strains J (MHJ) and 7448 (MHP) genomes \(^1\).

<table>
<thead>
<tr>
<th>CDS</th>
<th>MS</th>
<th>MHJ</th>
<th>MHP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transporter</td>
<td>88</td>
<td>107</td>
<td>109</td>
</tr>
<tr>
<td>ABC transporter (^1)</td>
<td>78</td>
<td>91</td>
<td>94</td>
</tr>
<tr>
<td>Valid</td>
<td>694</td>
<td>679</td>
<td>681</td>
</tr>
<tr>
<td>Transporter in valid CDSs (%)</td>
<td>12.7</td>
<td>15.8</td>
<td>16.0</td>
</tr>
<tr>
<td>ABC transporter in valid CDSs (%)</td>
<td>11.2</td>
<td>13.4</td>
<td>13.8</td>
</tr>
<tr>
<td>ABC transporter in transporter CDSs (%)</td>
<td>88.6</td>
<td>85.0</td>
<td>86.2</td>
</tr>
</tbody>
</table>

\(^1\)In accordance with Vasconcelos \textit{et al.} (2005), but including peripheral membrane proteins of each ABC complex.

quenced. This is due first to the fact that peripheral membrane proteins of each ABC complex were also considered, and second because we searched for the proteins by using a very careful manual procedure, since, as pointed out by Ren \textit{et al.} (2004), it is often difficult to detect these proteins through current primary annotation due to the presence of multiple transporter gene paralogs and the complexity of the ABC family.

Uptake systems

ABC uptake systems are absent in eukaryotes, probably because their function was incorporated into other organelles (Ren and Paulsen, 2005). The comparison of several prokaryotes has indicated that the primary role of ABC uptake systems is the acquisition of nutrients, therefore a greater diversity of transporter types might allow the utilization of a broad range of substrates (ions, amino acids, carbohydrates, peptides, vitamins, polyamines, sulfate, metals, chelators, etc.) (Ren and Paulsen, 2005). Nine types of ABC transporters related to nutrients uptake were found in all three mycoplasma genomes, sharing structures similar to Gram-positive bacterial systems.

The ABC proteins capable of mediating the uptake of sugar and other carbohydrates are included in two families, which are designated as the carbohydrate uptake transporters 1 and 2 (CUT1 or TC# 3.A.1.1 and CUT2 or TC# 3.A.1.2). In general, members of CUT1 can transport diverse di- and oligosaccharides, glycerol, glycerol-phosphate and polyols, while CUT2 transporters can only transport monosaccharides (Schneider, 2001); members of both families are found in the three mycoplasmas (Table 2).

Uptake of glycerol by mycoplasmas can take place by the active system specified by GtsABC. In \textit{M. synoviae}, three homologous genes encoding two transmembrane components (MS0385, MS0386) and one ATP-binding protein (MS0384) compose this ABC transporter, and activity may be mediated by the predicted lipoprotein B (MS0387). The genomic region shows similarity (50-60\%) with operon gtsACB and the \textit{lpb} gene of \textit{M. mycoides} subsp. mycoides. Additionally, there is an overlap of all three CDSs encoding the ABC transporter, and it should be noted that analogous loci found in both \textit{M. mycoides} subsp. mycoides and \textit{M. genitalium} are also composed of three overlapping CDSs. The three components of this ABC system were also found in both the \textit{M. hyopneumoniae} pathogenic strain 7448 (MHP0369, MHP0370 and MHP0371) and the non-pathogenic strain J (MHH0375, MHH0376 and MHH0377). The predicted lipoprotein B was identified in \textit{M. hyopneumoniae} strain J (MHH0374), whereas in the pathogenic strain 7448 other lipoproteins might function in combination with the ABC glycerol transporter (MHP0366, MHP0367 or MHP0368). Vilei and Frey (2001) assumed that the phosphate group sequestered from the ABC transporter GtsA, GtsB and GtsC could be transferred to glycerol (glycerol-3-phosphate, G3P) during the uptake process in \textit{M. mycoides} subsp mycoides. CDSs encoding glycerol kinase (\textit{glpK}) have been identified in \textit{M. hyopneumoniae} strains J and 7448 (MHH0355 and MHH0359), and the only genome where \textit{glpK} gene was not found was that of \textit{M. synoviae} strain 53. In the cytoplasm, G3P might be used as substrate by glycerol-3-phosphate dehydrogenase (MHH0588 and MHP0588), accompanied by massive release of hydrogen peroxide (\textit{H}_2\text{O}_2) (Vilei and Frey, 2001). It has been suggested that the production of \textit{H}_2\text{O}_2 might be involved in the pathogenicity by damaging the host cells (Miles \textit{et al.}, 1991; Rice \textit{et al.}, 2001). Consequently, highly efficient glycerol systems allowing the production of \textit{H}_2\text{O}_2 might represent a virulence attribute of those mycoplasmas. However, although the genes were present in both the pathogenic and non-pathogenic strains of \textit{M. hyopneumoniae}, differences could reside in gene expression, which could be elucidated by simple biochemical assays to evaluate the production of \textit{H}_2\text{O}_2 in the presence of glycerol (Rice \textit{et al.}, 2001).

Another member of the CUT1 family detected only in \textit{M. synoviae} genome was the multiple sugar porter (MS0102, MS0103, MS0104 and MS0105), showing similarity (53-70\%) with \textit{M. pulmononis}. This system is homologous to \textit{msm} of \textit{Streptococcus mutans}, which is responsible for the transport and utilization of raffinose, melibiose and isomaltotrioses and may be transcribed as a single operon (McLaughlin and Ferretti, 1996). The region upstream of MS0105 includes two CDSs for alpha-amylase (MS0108) and maltose/threhalose hydrolase (MS0107), thus suggesting a role in starch-degradation and sugar transport (Sahm \textit{et al.}, 1996).

A remarkable ABC uptake system annotated in the three mycoplasmas is the oligopeptide transporter OppABCDF, which has been well characterized in \textit{Salmonella typhimurium} and \textit{Escherichia coli} (Table 2). It is known that the transport of peptides is not only related to cell nutrition, but also to several signaling processes (Detmers \textit{et al.}, 2001). Recently, Hopfe and Henrich (2004) demonstrated in \textit{M. hominis} that OppA, the substrate-
A binding protein of the oligopeptide transporter system, has a surface-localized ATP-binding site which functions as the main ecto-ATPase of the bacteria; although the physiological role of that ATPase activity remains unknown, experiments with oppA-deficient mutants have shown that it might affect cell viability. Similarly, Yoshida et al. (1999) reported that OppA is an important protein for cell growth and viability in *E. coli*, and its synthesis is stimulated by polyamines at the transcriptional level.

Several reports have demonstrated that polyamines (putrescine, spermidine and spermine) play regulatory roles in cellular growth processes (Tabor and Tabor, 1984; Igarashi and Kashiwagi, 2000; Yoshida *et al.*, 2002). The polyamines are present at millimolar concentrations, and the intracellular concentration is regulated by synthesis, degradation, efflux and uptake (Igarashi and Kashiwagi, 1999). Orthologs of *potABCD* genes encoding the polyamine uptake system of *E. coli* were found in *M.*

### Table 2 - Description of CDSs related to ABC importers (uptake systems) in the *M. synoviae* strain 53 (MS) and *M. hyopneumoniae* strains J (MHJ) and 7448 (MHP) genomes.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>TC# Description (MS)</th>
<th>(MHJ)</th>
<th>(MHP)</th>
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<tr>
<td></td>
<td></td>
<td>MS0384 [M]</td>
<td>MHO375 [C]</td>
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<td></td>
<td></td>
<td>MS0386 [SB]</td>
<td>MHO377 [M]</td>
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<td></td>
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<td>MHO367 [M]</td>
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<td></td>
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<tr>
<td>Carbohydrate multiple sugar</td>
<td>3.A.1.1.2 (CUT1) Multiple sugar (raffinose/melibiose/somaltotrioses) porter (homolog to MsmEFGK of <em>S. mutans</em>)</td>
<td>MS0102 [C]</td>
<td>NF</td>
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<tr>
<td></td>
<td>Xylose</td>
<td></td>
<td>MS0137 [C]</td>
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<td>MS0140 [SB]</td>
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<td>MS0187 [C]</td>
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<td>MS0349 [SB]</td>
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<td>MS0346 [C]</td>
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<td></td>
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<td>MS0345 [C]</td>
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<tr>
<td></td>
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<tr>
<td>Metal ions cobalt</td>
<td>Cobalt uptake (Co2+) importer (homolog to CbiOQ of <em>S. typhimurium</em>)</td>
<td>MS00699 [C] MS00660 [C]</td>
<td>NF</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>MS0034 [C]</td>
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<td></td>
<td></td>
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<td>MS0061 [M]</td>
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<td></td>
<td></td>
<td></td>
<td>MS0086 [C]</td>
</tr>
<tr>
<td>Amine Polyamine</td>
<td>Polyamine (Putrescine/Spermidine) importer (homolog to PotABCD of <em>E. coli</em>)</td>
<td>MS0508 [C]</td>
<td>MHO544 [C]</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>MS0511 [SB]</td>
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and a model for the utilization of phosphonate was sug-
fors the C-P lyase pathway were identified (to the action of phosphatases. In drolysis, thermal decomposition, and photolysis, as well as carbon-phosphorous (C-P) bond is resistant to chemical hy-
tes, which are also found in herbicides organophos-
valently bound to phosphorous characterizes phosphona-
the three mycoplasmas was a phosphonate transporter, this study (Table 2).

Metal ions as cobalt are essential for optimal activity of some enzymes, such as the coenzyme B_{12} and nitrile hydratase (Kobayashi and Shimizu, 1999). Synthesis of en-
zymes is dependent on a high-affinity uptake of cobalt from the environment, where the nutrient is available in trace amounts only. Cobalt uptake transporters in microorgan-
isms are mediated by secondary transporters and by ABC transporters (Eitinger et al., 2005). The ABC cobalt transport-
er CbiOQN of \textit{S. typhimurium} is well characterized and may be associated with a substrate-binding lipoprotein. A similar complex was found in all three genomes analyzed in this study (Table 2).

Another interesting ABC uptake system annotated in the three mycoplasmas was a phosphonate transporter, which is homolog to PnhCDE of \textit{E. coli}. A carbon atom co-
valently bound to phosphorous characterizes phosphona-
tes, which are also found in herbicides organophospho-
nates, insecticides, and other chemicals. This stable carbon-phosphorous (C-P) bond is resistant to chemical hy-
drolysis, thermal decomposition, and photolysis, as well as to the action of phosphatases. In \textit{E. coli}, genes responsible for the C-P lyase pathway were identified (pnhGHIJKLM) and a model for the utilization of phosphonate was sug-
gested (Yakovleva et al., 1998). CDSs showing homology with the pnhCDE operon were found in all three strains an-
alyzed in this report (Table 2); however, there was no evidence of genes showing homology to those encoding the enzyme of dephosphonation of phosphonic acids.

**Efflux systems**

ABC export systems in bacteria are also involved in the efflux of substances including surface components of the cell (such as capsular polysaccharides, lipopolysaccha-
rides, and techoic acid), proteins involved in pathogenesis (such as hemolysin, heme-binding protein, and alkaline-
protease), peptide antibiotics (bacteriocins), heme, drugs and siderophores (Davidson and Chen, 2004).

The active efflux of antibiotics and other drugs repres-
ts a major mechanism developed by microbial species to acquire resistance to the toxicity of these molecules, and the process may be mediated by both drug and multidrug efflux systems (Putman et al., 2000). The drug efflux systems mediate the extrusion of a specific drug or class of drugs, while the multidrug resistance (MDR) systems mediate the extru-
sion of a variety of structurally unrelated compounds (Putman et al., 2000).

There are few MDR efflux systems characterized in prokaryotes: LmrA in \textit{Lactococcus lactis}, MsbA in \textit{E. coli}, HorA in \textit{Lactobacillus brevis}, VcaM in \textit{Vibrio cholerae}, and the heterodimeric ABC transporter EfrAB in \textit{Enterococcus faecalis} (Raherison et al., 2005). Putative ABC MDR genes were identified in the genomes of both \textit{M. genitalium} and \textit{M. pneumoniae} (van Veen and Konings, 1998;Paulsen et al., 2000; Raherison et al., 2005), and in \textit{M. hominis}, two MDR ATP-binding cassette transporters were identified (md1 and md2) that mediate the active efflux of both ciprofloxacin and ethidium bromide (Raherison et al., 2002, 2005).

Table 3 shows the CDSs related to both drug and multidrug exporter ABC systems found in the three geno-
mes analyzed in this study, categorized by their substrate-
type transport. The characterization of these ABC exporter systems can provide a rationale for the use of resistance-blocking agents helpful for the control of bacterial infec-
tions.

In \textit{M. synoviae} and \textit{M. hyopneumoniae}, specific export-
ers of the antibiotic sublancin (homologs to SunT of \textit{Bacillus subtilis} and LagD of \textit{Lactococcus lactis}) were found. Bacteriocins are extracellular-released bioactive-
peptide complexes that have a bactericidal or bacteriostatic effect on other (usually closely related) bacterial species. Also, in the \textit{M. synoviae} genome two CDSs were found, which show homology to the ABC multidrug/lipid efflux system MsbA of \textit{E. coli}, and are capable of exporting a vari-
ety of antibiotics and lipids.

Interestingly, several CDS homologs to a putative ABC MDR efflux system (Pr1 and Pr2-like) were identi-
fied in all three mycoplasma genomes analyzed: six in strain 7448, five in strain J and just one in strain 53. There-
fore, the characterization of the substrate associated to these ABC efflux systems and their gene expression in the pathogenic strains may help to identify new strategies to control bacterial infections.

Also related to efflux systems are the CDSs of a puta-
tive heme (homolog to CcmABC) group of ABC exporters. They were found in the genomes of both pathogenic and non-pathogenic strains of \textit{M. hyopneumoniae}. The CcmABC is an ATP-binding cassette encoding a trans-
porter related to the maturation of c-type cytochromes in \textit{E. coli}. The CcmA and CcmB are probably the subunits of an ABC exporter; the first is a peripheral membrane protein with a well conserved ATP binding cassette that associates with CcmB, a membrane protein (Meyer, 2002). It is still controversial whether CcmC is another subunit of the trans-
porter, related to the transport of heme to the periplasm of \textit{E. coli}, where it is attached to a c-type cytochrome precur-
sor in a post-translational maturation process (Meyer, 2002).

In addition, we found uncharacterized ABC trans-
porters with several CDSs encoding ATP-binding domains
fused to transmembrane domains, which were exclusively detected in the *M. hyopneumoniae* genomes (Table 4).

**Pathogenicity and ABC transporters**

In relation to the ABC uptake systems identified in all three sequenced mycoplasma genomes, these were mainly involved in cell nutrition, but some might be indirectly associated with virulence, *i.e.*, H₂O₂ production from imported glycerol. In addition, virulence of pathogenic bacteria may also be attributed to the uptake of critical nutrients. Apparently, the number and families of ABC uptake systems identified in the genomes of the non-pathogenic strain J, of the pathogenic strain 7448 of *M. hyopneumoniae*, and of the pathogenic strain 53 of *M. synoviae* were similar. However, one must consider that there are reports of signature-tagged mutants (STM) in *ABC* transporter genes (*e.g.* oppC, oppD, oppF, potA and potD) in *Staphylococcus aureus* and in *Streptococcus pneumoniae* that display attenuated virulence phenotypes in animal infection models (Mei *et al.*, 1997; Polissi *et al.*, 1998).

Altogether, the findings described here suggest a potential use of these ABC uptake transporters as targets for novel antimicrobial compounds for pneumonia caused by mycoplasmas of swine and poultry. The successful development of such antimicrobial ABC transporters would rely on their absence in mammalian cells and also on the knowledge of their structure and molecular modes of action. For example, Smith and Payne (1990) have suggested the use of OppA (oligopeptide-binding protein) in combination with manufactured drugs, in which an antibacterial compound would be linked to the natural substrate (oligopeptide) of this ABC transporter. In this study we identified some ABC uptake transporters in pathogenic strains 7448 and 53 that might represent interesting targets for antimicrobial therapies, such as OppA, PotA and PhnC. Furthermore, since some ABC substrate-binding proteins may be immunogenic, they might also be considered as putative candidates for the development of vaccines.

Protein translocation to the extracellular space is essential for the invasion, colonization, and survival of pathogenic bacteria within a host organism. The ABC efflux systems are involved in the secretion of a variety of exoproteins including RTX (repeats-in-toxin) toxins, cell surface layer proteins, proteases, lipases, bacteriocins and

**Table 3** - Description of the CDSs related to ABC exporters (efflux systems) in the *M. synoviae* strain 53 (MS) and *M. hyopneumoniae* strains J (MHJ) and 7448 (MHP) genomes.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>TC# Description</th>
<th>MS</th>
<th>MHJ</th>
<th>MHP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heme group</td>
<td>Putative heme exporter (homolog to CcmA of <em>M. gallisepticum</em> R and <em>E. coli</em>)</td>
<td>MS0130 [C]</td>
<td>MHJ0466 [C]</td>
<td>MHP0469 [C]</td>
</tr>
<tr>
<td>Multidrug</td>
<td>(Putative MDR efflux system, homolog of Pr1 and Pr2-like)</td>
<td>MS0658 [M]</td>
<td>MHJ0624 [C-M]</td>
<td>MHP0623 [C-M]</td>
</tr>
<tr>
<td>Sublancin and Lactococcin G</td>
<td>Sublancin exporter, SunT (homolog to SunT of <em>Bacillus subtilis</em> and LagD of <em>Lactococcus lactis</em>)</td>
<td>MS0391 [C-M]</td>
<td>MHJ0156 [C-M]</td>
<td>MHP0160 [C-M]</td>
</tr>
<tr>
<td>Phospholipid LPS lipid A and multidrug</td>
<td>Phospholipid, LPS, Lipid A and drug exporter (flippase) (homolog to MsbA of <em>E. coli</em>)</td>
<td>MS0513 [C-M]</td>
<td>MS0514 [C-M]</td>
<td>NE4 NF</td>
</tr>
</tbody>
</table>

1[C] ATP-binding domain; 2[M] Transmembrane domain; 3[ATP-binding domain fused to transmembrane domain; 4Not found.
lipases (Omori and Idei, 2003). Hence, proteins secreted through ABC exporters are deeply involved in the pathogenicity of bacteria (Omori and Idei, 2003). In the genome of the three sequenced Mycoplasma, a CDS involved in efflux systems related to the exportation of bacteriocins (such as lactococcin and sublancin) was reported. Also, in the genomes of both pathogenic and non-pathogenic strains of M. hyopneumoniae and a pathogenic strain of M. synoviae, a set of CDSs related to putative MDR systems was found. However, the efflux systems developed by bacteria may also release toxic compounds and damage the cells (Raherison et al., 2002). One possible mechanism of self-protection would be the removal of the toxic molecules preventing their accumulation in the membrane (Young and Holland, 1999). The MDR transporters may utilize a mechanism of interception of hydrophobic compounds in the bi-layer wall followed by their elimination from the inner leaflet by “flippase” or a “vacuum cleaner” mechanism (Young and Holland, 1999). This is the case for the multidrug/lipid exporter system found in M. synoviae (Table 3).

Finally, ABC efflux systems may be effective as antibacterial targets in the development of inhibitors capable of preventing efflux activity (Li and Nikaido, 2004). Some target candidates are shown in Table 3.

### Phylogeny of ABC transporters

Using the sequence of one of the first representatives of bacterial ABC transporters, the MalK protein, Saurin et al. (1999) analyzed 197 sequences of putative ABC transporters. An unrooted tree displayed two major branches, one grouping the uptake and the other the export system, suggesting that the divergence between these two functionally different types of ABC systems occurred from a common ancestor of living organisms, probably before the divergence of prokaryotes and eukaryotes (Saurin et al., 1999). Clustering of the three protein/domain constituents is similar, therefore each of the three constituents of the ABC transporters has probably arisen from a common ancestral transporter system with minimal shuffling of constituents between systems. However, today they are functionally diverse and play roles in a wide range of cellular functions (Saier and Paulsen, 2001; Garmory and Titball, 2004).

Ren and Paulsen (2005) have identified 41 transporter families present in all three domains of life, suggesting that they might represent ancient families shared by the last common ancestor. In addition, clustering of the transporters was strongly correlated with phylogenetic profiles, indicating that the types of transporters utilized by organisms are related to their evolutionary history. Throughout the evolutionary process, multicellular organisms have an apparent trend towards specialization, as there are redundant transporter paralogs, while in single-cell organisms there are fewer paralogs but several families of transporters (orthologs), suggesting diversification. One exception in the study of Ren and Paulsen (2005) was the cluster of obligate intracellular pathogens/symbionts, including a group of phylogenetically diverse organisms and Mycoplasma.

### Table 4 - Description of CDSs related to uncharacterized ABC transporter in the M. synoviae strain 53 (MS) and M. hyopneumoniae strains J (MHJ) and 7448 (MHP) genomes

<table>
<thead>
<tr>
<th>Substrate Description</th>
<th>(MS)</th>
<th>(MHJ)</th>
<th>(MHP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABC transporter with ATP binding duplicated component (homolog to ybiT of E. coli)</td>
<td>MS0080 2[C]</td>
<td>MHJ0206 2[C]</td>
<td>MHP0210 2[C]</td>
</tr>
<tr>
<td>Uncharacterized ABC transporter</td>
<td>MS0041 [C-M]</td>
<td>MHJ0333 [C-M]</td>
<td>MHP0383 [C-M]</td>
</tr>
<tr>
<td></td>
<td>MHJ0297 [C-M]</td>
<td>MHJ0298 [C-M]</td>
<td>MHP0340 [C-M]</td>
</tr>
<tr>
<td></td>
<td>MHJ0018 [C-M]</td>
<td>MHJ0019 [C-M]</td>
<td>MHP0019 [C-M]</td>
</tr>
<tr>
<td></td>
<td>MHJ0020 [C-M]</td>
<td>MHP0020 [C-M]</td>
<td>MHP0021 [C-M]</td>
</tr>
<tr>
<td></td>
<td>MHJ0021 [C-M]</td>
<td>MHP0023 [C-M]</td>
<td>MHP0024 [C-M]</td>
</tr>
<tr>
<td></td>
<td>MHJ0023 [C-M]</td>
<td>MHP0024 [C-M]</td>
<td>MHP0314 [C-M]</td>
</tr>
<tr>
<td></td>
<td>MHJ0024 [C-M]</td>
<td>MHP0315 [C-M]</td>
<td>MHP0315 [C-M]</td>
</tr>
<tr>
<td>Uncharacterized ABC transporter, probable anti-biotic efflux</td>
<td>MS0612 [C]</td>
<td>MHJ0449 [C]</td>
<td>MHP0452 [C]</td>
</tr>
<tr>
<td></td>
<td>MS0613 [M]</td>
<td>MHJ0450 [M]</td>
<td>MHP0452 [M]</td>
</tr>
</tbody>
</table>

1[C] ATP-binding domain; 2[M] Transmembrane domain; 3ATP-binding domain fused to transmembrane domain.
The 14 ATP-binding components of ABC systems used in this study (Figure 2) reflect the diversity found by Saurin et al. (1999). The most striking feature observed in the phylogenetic tree is that most of the gene families clustered the *M. hyopneumoniae* strains J and 7448, with *M. synoviae* strain 53 as a sister-group. This indicates that ABC protein family diversity is not the only factor responsible for the pathogenicity of *M. hyopneumoniae* strain 7448 and of *M. synoviae* strain 53. This result is compatible with the hypothesis that virulence in mycoplasmas can be attenuated by successive passage in laboratory media, a process that is mediated by genes encoding adhesin proteins (Collier et al., 1985). This is the case of strain J of *M. hyopneumoniae*, which is a high-passage strain showing a decreased adherence to the host cells and thus unable to induce pneumonia (Zielinski and Ross, 1990; Zielinski et al., 1993).

In conclusion, the analysis of the three genomes of *Mycoplasma* has not provided major differences in ABC transporters between pathogenic and non-pathogenic strains; however, several proteins potentially useful as targets for the control of infections were identified.

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Internet Resources


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