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Research Article

Kinases of two strains of *Mycoplasma hyopneumoniae* and a strain of *Mycoplasma synoviae*: An overview

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Abstract

Mycoplasma synoviae and *Mycoplasma hyopneumoniae* are wall-less eubacteria belonging to the class of Mollicutes. These prokaryotes have a reduced genome size and reduced biosynthetic machinery. They cause great losses in animal production. *M. synoviae* is responsible for an upper respiratory tract disease of chickens and turkeys. *M. hyopneumoniae* is the causative agent of enzootic pneumonia in pigs. The complete genomes of these organisms showed 17 ORFs encoding kinases in *M. synoviae* and 15 in each of the *M. hyopneumoniae* strain. Four kinase genes were restricted to the avian pathogen while three were specific to the pig pathogen when compared to each other. All deduced kinases found in the non pathogenic strain (J[ATCC25934]) were also found in the pathogenic *M. hyopneumoniae* strain. The enzymes were classified in nine families composing five fold groups.

Key words: Mycoplasma, kinases, genomes.

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Introduction

Edmond Nocard and Emile Roux successfully cultivated the agent of the contagious bovine pleuropneumonia, *Mycoplasma mycoides*, over a century ago (Nocard and Roux, 1898). Since that time, approximately 111 species of the genus *Mycoplasma* have been identified in animals. These and other 102 species comprise the class of Mollicutes (Minion *et al*., 2004). These prokaryotes are known as the smallest self replicating organisms (Glass *et al*., 2000; Westberg *et al*., 2004). Most members of this class are pathogenic and colonize a wide variety of hosts, such as animals, plants and insects. Mollicutes represent a group of Low-G+C-content eubacteria that are phylogenetically related to the *Clostridium-Streptococcus-Lactobacillus* branch of the phylum (Woese *et al*., 1980; Rogers *et al*., 1985; Maniloff, 1992). As a consequence of the reduced biosynthetic machinery, Mollicutes live in nature as obligate parasites and depend on the uptake of many essential molecules from their hosts (Papazisi *et al*., 2003). Thus, they have been considered model systems for defining the minimal set of genes required for a living cell (Morowitz, 1984).

Although, Mollicutes have a simple genome, mycoplasma diseases are complex and relatively unknown (Minion *et al*., 2004). One hallmark of these diseases is the chronicity (Ross, 1992), but equally important is the ability to alter or circumvent the immune response and to potentiate diseases caused by other pathogens (Ciprian *et al*., 1988; Thacker *et al*,. 1999; Muhlradt, 2002). A key factor in the ability of mycoplasmas to establish a chronic infection is their genome flexibility, which allows them to produce a highly variable mosaic of surface antigens (Citti and Rosengarten, 1997; Chambaud, *et al*., 1999; Shen *et al*., 2000 Assunção *et al*., 2005).

In the last years, the genomes of ten mycoplasma species have been completely sequenced (Himmelreich *et al*., 1996; Glass *et al*., 2000; Chamabaud *et al*., 2001; Sasaki *et al*., 2002; Berent and Messik, 2003; Papazisi *et al*., 2003; Westberg *et al*., 2004; Jaffe *et al*., 2004; Minion *et al*., 2004). Recently, the complete genomes of a pathogenic (7448) and nonpathogenic (J [ATCC 25934]) strains of *Mycoplasma hyopneumoniae,* as well as the complete genome of a strain (53) of *Mycoplasma synoviae* (Vasconcelos *et al*., 2005) were obtained. Both species cause great adverse impact on animal production. *M. hyopneumoniae* is the causative agent of porcine enzootic pneumonia, a mild, chronic pneumonia of swine, commonly complicated by opportunistic infections with other bacteria (Ross, 1992). Like most other members of the order *Mycoplasmatales*, *M. hyopneumoniae* is infective for a single species, but the mechanisms of host specificity are unknown. *M. synoviae* is the major poultry pathogen

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throughout the world, causing chronic respiratory disease and arthritis in infected chickens and turkeys (Allen *et al*., 2005).

Kinases play indispensable roles in numerous cellular metabolic and signaling pathways, and they are among the best-studied enzymes at the structural, biochemical, and cellular levels. Despite the fact that all kinases use the same phosphate donor (in most cases, ATP) and catalyze apparently the same phosphoryl transfer reaction, they display remarkable diversity in their structural folds and substrate recognition mechanisms, probably due largely to the extraordinarily diverse nature of the structures and properties of their substrates (Cheek *et al*., 2005).

Complete genome sequencing identified 679, 681 and 694 Open Reading Frames (ORF) of *M. hyopneumoniae* strains J (Mhy-J), 7448 (Mhy-P) and *M. synoviae* strain 53 (Msy), respectively. Analysis of these mycoplasma genomes by bioinformatics tools identified 15 Mhy-J ORFs, 15 Mhy-P ORFs and 17 Msy ORFs, all of which encode kinases. Due to the biological importance of these enzymes we expect that their study will improve the comprehension of the reduced biosynthetic pathways in mollicutes.

Methods

By using previous results from the complete genomes of *M. synoviae* and *M. hyopneumoniae,* J and 7448 strains as input to BLAST search tools we obtained 17 ORFs encoding kinase homologues in *M. synoviae* and 15 in both strains of *M. hyopneumoniae*. Putative biological functions of the kinases were deduced by using Pfam interface and InterPro information. The classification of enzymes into fold groups and families was performed by following the scheme described by Cheek *et al*. (2005). In brief, all kinase sequences from the NCBI non-redundant database were assigned to a set of 57 profiles describing catalytic kinase domains by using the hmmsearch program of the HMMR2 package (Eddy, 1998). Sequences from each Pfam/COG profile presenting significant PSI-BLAST (Altschul *et al*., 1997) hits to each other were clustered into the same family. Families in the same fold group share structurally similar nucleotide-binding domains that have the same architecture and topology (or are related by circular permutation) for at least the core of the domain. Multiple sequence alignments were generated using the ClustalX 1.81 software (Thompson *et al*., 1997). The amino acid sequence relationships were generated with the predicted protein sequences obtained from 47 kinase-encoding ORFs identified in the complete genome sequences of *M. synoviae* and *M. hyopneumoniae*. A phylogenetic tree was constructed by multiple sequence alignments (pairwise alignments) using the Clustal X 1.81 program (Thompson *et al*., 1997) and visualized by using the TreeView software. The tree was constructed by using the minimum evolution (neighbor-joining) method (Saitou and Nei, 1987). Robustness of branches was estimated using 100 bootstrap replicates.

Results and Discussion

Mycoplasma kinases

In this study we briefly review the kinase genes of *M. hyopneumoniae* and *M. synoviae*, and we describe a classification and metabolic comparative analysis of kinases of these organisms. In the genome sequences we identified a total of 47 kinase-encoding ORFs which are related to several different biosynthetic pathways, such as purine and pyrimidine metabolism, glycolysis, pyruvate metabolism, as well as cofactor metabolism and others (Table 1). The two *M. hyopneumoniae* strains have equal numbers of the same kinases-encoding ORFs. Three of these are absent in *M. synoviae* (glycerol kinase, glucokinase and 5-dehydro-2-deoxygluconokinase) which has an additional 17 ORFs that encode kinases. Four of them (three ORFs encoding deoxyguanosine kinase and one ORF encoding N-acetylmannosamine kinase) are exclusive to this species when compared to *M. hyopneumoniae* strains J and 7448 (Table 1). These differences between the two species could be related to specific nutritional requirements found by each pathogen in its respective host. All kinases found in the pathogenic strain

Table 1 - Kinases identified in the *M. synoviae* and *M. hyopneumoniae* genomes.

Gene product	Presence of ORFs encoding kinase in mycoplasmas		
	Msy ORF	Mhy-J ORF	Mhy-P ORF
Deoxyguanosine kinase	MS0380 MS0140 MS0141	-	-
N-acetylmannosamine kinase	MS0195	-	-
Serine/threonine-protein kinase	MS0121	-	-
Pyruvate kinase	MS0648	MHJ0122	MHP0126
Adenylate kinase	MS0580	MHJ0170	MHP0174
Thymidine kinase	MS0521	MHJ0610	MHP0608
Cytidylate kinase	MS0143	MHJ0065	MHP0069
Guanylate kinase	MS0123	MHJ0149	MHP0153
Phosphoglycerate kinase	MS0114	MHJ0487	MHP0490
Uridylate kinase smbA	MS0677	MHJ0536	MHP0535
6-phosphofructokinase	MS0296	MHJ0107	MHP0111
Acetate kinase	MS0652	MHJ0505	MHP0508
Riboflavin kinase / FMN adenylyltransferase	MS0563	MHJ0270	MHP0278
Thymidylate kinase	MS0052	MHJ0251	MHP0259
Ribose-phosphate pyrophosphokinase	MS0150	MHJ0654	MHP0654
Glycerol kinase	-	MHJ0355	MHP0359
Glucokinase	-	MHJ0515	MHP0517
5-dehydro-2-deoxyglucono kinase	-	MHJ0220	MHP0226

of *M. hyopneumoniae* (7448) were also identified in the nonpathogenic strain (J). This finding could be explained by the fact that such enzymatic activities may be essential to Mollicutes which have a reduced metabolism.

Kinase classification

The classification of kinases found in *M. hyopneumoniae* strains J and 7448, as well as in *M. synoviae* was performed according to the description of Cheek *et al*. (2005). Here, the definition of kinase was restricted to enzymes which catalyze the transfer of the terminal phosphate group from ATP to a substrate containing an alcohol, nitrogen, carboxyl or phosphate group as phosphoryl acceptor. The classification scheme lists a total of 25 kinase family homologues which are assembled into 12 groups based on the similarity of the structural fold. Within a fold group, the core of the nucleotide-binding domain of each family has the same architecture, and the topology of the protein core is either identical or related by circular permutation (Cheek *et al*., 2005). In the two *M. hyopneumoniae* strains and in the *M. synoviae* strain the 47 identified ORFs code for 18 different kinases classified in nine families. These were grouped into five fold groups, as shown in Table 2. Fold Group 2 (Rossmann-like) contains 11 enzymes divided into five families, in which all the seven members of the P-loop kinase family are proteins involved in purine and pyrimidine metabolism. The remaining four members of this group are fall into four families which, together with four members of Group 4 and a member of Group 5 (TIM b /a barrel kinase) are involved in the carbohydrate metabolism. Group 1 (Protein S/T-Y kinase) and Group 8 (Riboflavin kinase) are each represented by one enzyme only, which participate in signaling cascades and riboflavin metabolism, respectively.

Nucleotide metabolism and kinases

Mollicutes are unable to synthesize purines and pyrimidines by *de novo* pathways, and guanine, guanosine, uracil, thymine, thymidine, cytidine, adenine and adenosine may serve as precursors for nucleic acids, and nucleotide coenzymes in these organisms (Himmelreich *et al*., 1996). They only synthesize ribonucleotides by the salvage pathway. In the complete genome of *M. hyopneumoniae* and *M. synoviae* we identified six kinases in the first one and seven kinases in the second one, all of which catalyze key steps in the nucleotide salvage pathway. Deoxyribonucleotides are produced from ribonucleotides by a ribonucleoside diphosphate reductase. Adenine, guanine and uracil can be metabolized to the corresponding nucleoside monophosphate by adenine phosphoribosyltransferase, hypoxanthine-guanine phosphoribosyltransferase and uracil phosphoribosyltransferase, respectively. ADP, GDP, UDP and CDP are generated by adenylate, guanylate, uridylate and cytidylate kinases. Only *M. synoviae* has three ORFs encoding deoxyguanosine kinase, which can convert deoxyguanosine to dGMP. However, a nucleotide diphosphate kinase (ndk), the main enzyme for the production of NTP from NDP, was not found in the *M. hyopneumoniae* and *M. synoviae* genomes. This finding is in agreement with data from other Mollicutes genome sequences. It was proposed that the absence of an ndk gene ortholog in Mollicutes could be compensated by 6-phos-

Table 2 - Classification of *M. synoviae* and *M. hyopneumoniae* kinase activities by family and fold group*.

Fold Group	Family	PFAM members+	Kinase activity (EC)
Group 1: protein S/T-Y kinase/ atypical protein kinase/ lipid kinase/ ATP-grasp	Protein S/T-Y kinase	PF00069	2.7.1.37 Serine/threonine protein kinase
Group 2: Rossmann-like	P-loop kinases:	PF00406	2.7.4.3 Adenylate kinase
		PF00265	2.7.1.21 Thymidine kinase
		PF01712	2.7.1.113 Deoxyguanosine kinase
		PF02224	2.7.4.14 Cytidylate kinase
		PF00625	2.7.4.8 Guanylate kinase
		PF00696	2.7.4.- Uridylate kinase
		PF02223	2.7.4.9 Thymidylate kinase
	Phosphoglycerate kinase:	PF00162	2.7.2.3 Phosphoglycerate kinase
	Phosphofructokinase-like:	PF00365	2.7.1.11 6-phosphofructokinase
	Ribokinase-like:	PF00294	2.7.1.92 5-dehydro-2-deoxygluconokinase
	Thiamin pyrophosphokinase	PF00156	2.7.6.1 Ribose-phosphate pyrophosphokinase
Group 4: ribonuclease H-like	Ribonuclease H-like	PF00480	2.7.1.60 N-acetylmannosamine kinase
		PF00871	2.7.2.1 Acetate kinase
		PF00370	2.7.1.30 Glycerol kinase
		PF02685	2.7.1.2 Glucokinase
Group 5: TIM β/α? barrel kinase	TIM β/α? barrel kinase	PF00224	2.7.1.40 Pyruvate kinase
Group 8: riboflavin kinase	Riboflavin kinase	PF01687	2.7.1.26 Riboflavin kinase

*The classification was based on Cheek *et al*. (2005).

phofructokinases, phosphoglycerate kinases, pyruvate kinases, and acetate kinases. In addition, besides reactant ADP/ATP, these organisms could use other ribo- and deoxyribo-purine and pyrimidine NDPs and NTPs (Pollack *et al*., 2002).

Like in *M. penetrans*, important enzymes such as uridine kinase and pyrimidine nucleoside phosphorylase, which convert cytosine in CMP, are also missing in the two species. The synthesis of CTP from UTP by CTP synthetase is possible only in two *M. hyopneumoniae* strains. The production of deoxythymidine diphosphate from thymidine may be performed by thymidine and thymidylate kinases. A gene encoding ribose-phosphate pyrophosphokinase is present and this enzyme would produce 5-phosphoribosyl diphosphate, a crucial component in nucleotide biosynthesis. All kinases involved in the nucleotide salvage pathway are fall into fold Group 2. Moreover, only ribose-phosphate pyrophosphokinase is not in the P-loop kinases family of this group.

Kinases involved in the metabolism of carbohydrates

Both *M. hyopneumoniae* and *M. synoviae* have the entire set of genes responsible for glycolysis. Like in *M. pulmonis* (Chambaud *et al*., 2001), *M. hyopneumoniae* strain 232 (Minion *et al*., 2004), and *M. mobile* (Jaffe *et al*., 2004), glycolysis in *M. hyopneumoniae* J and 7448 can begin by direct phosphorylation of glucose by glucokinase (Group 4; ribonuclease H-like family) activity. Alternatively, as described for other Mollicutes (Fraser *et al*., 1995; Himmelreich *et al*., 1996; Glass *et al*., 2000), *M. synoviae* produces glucose 6-posphate only by the action of phosphoenolpyruvate-dependent sugar phosphotransferase system. The two species *M. hyopneumoniae* and *M. synoviae* have a 6-phosphofructokinase (Group 2; phosphofructokinase-like family), phosphoglycerate kinase (Group 2; phosphoglycerate kinase family) and pyruvate kinase (Group 5; TIM β/α? barrel kinase family). These three key enzymes also participate in the glycolysis pathway, like in other Mollicutes. In addition, they have an acetate kinase (Group 4; ribonuclease H-like family), an essential enzyme in the production of acetyl-CoA from acetate.

Even though, *M. synoviae* and *M. hyopneumoniae* strains have glycerol transporter-related proteins, only the second species presents a glycerol kinase (Group 4; ribonuclease H-like family) enzyme which could directly convert glycerol to glycerol 3-phosphate. This product is then converted into glyceraldehyde 3-phosphate.

In their amino sugar metabolism, mycoplasmas can produce fructose 6-phosphate (F6P) also from N-acetyl-D-glucosamine. In this pathway, *M. synoviae* N-acetylmannosamine kinase (Group 4; ribonuclease H-like family) catalyzes a key reaction in the production of F6P from N-acetyl-neuraminate. Even though both species lack the inositol metabolism pathway, only *M. hyopneumoniae* presents a 5-dehydro-2-deoxygluconokinase (Group 2; Thiamin pyrophosphokinase family), an enzyme which catalyzes a step in this pathway. The presence of specific kinases in the *M. synoviae* and *M. hyopneumoniae* (strain J and 7448) genomes shows the possibility for the use of different metabolic routes by each mycoplasma in response to the specific nutritional conditions found by each pathogen in its respective host environment.

Riboflavin metabolism and kinases

M. hyopneumoniae and *M. synoviae* lack enzymes that synthesize many coenzymes and cofactors. However, they produce Flavine Adenine Dinucleotide (FAD) from riboflavin. This process is performed in two steps where, in the first step, riboflavin kinase phosphorylates riboflavin to form flavin mononucleotide (FMN). Next, FMN is converted to flavin adenine dinucleotide (FAD) by a FMN adenylyltransferase (Karthikeyan, *et al*., 2003). FAD is an enzyme cofactor used in several metabolic pathways. In *M. synoviae* and *M. hyopneumoniae,* the two steps are performed by a single bifunctional enzyme riboflavin kinase/FMN adenylyltransferase, as occurs also in bacteria (Manstein *et al*., 1986; Mack *et al*., 1998). It is a unique enzyme and the only representative for fold Group 5.

Amino acid sequence relationships

In order to investigate the phylogenetic relationships of the kinase families of *M. synoviae* 53, *M.hyopneumoniae* J and *M.hyopneumoniae* 7448, the 47 deduced amino acid sequences of the ORFs encoding kinases were aligned using the ClustalX 1.81 program. Robustness of branches was estimated by using 100 bootstrap replicates.

Figure 1 shows the phylogenetic tree for kinases as calculated from the neighbour-joining method. The tree was rooted with Group 1 since it has only one representative. The kinase sequences were well resolved into clades. The P-loop kinase family of Group 2 (Rossmann-like) was clustered into four subclades (Figure 1, letters A, B, C and D). The subclades B and C comprise sequences from *M. synoviae*, *M. hyopneumoniae* J and *M. hyopneumoniae* 7448 implicated in phosphorylation of the monophosphate nucleotides. Thymidylate kinase and deoxiguanosine kinase convert TMP to TDP and deoxiguanosine to dGMP, respectively. Although these enzymes have different functions, they have structurally similar nucleotide-binding domains following the classification described by Cheek *et al*., (2005). The other members of the Rossmann-like Group, which are the phosphoglycerate kinase, ribokinase-like and thiamine pyrophosphokinase families, clustered in individual groups. The sequences from Group 4 formed four clades. Although belonging to the same fold group they are implicated in different metabolic pathways.

Concluding Remarks

In the complete genomes of *M. synoviae* strain 53, *M. hyopneumoniae* strains J and 7448 we identified kinases in-

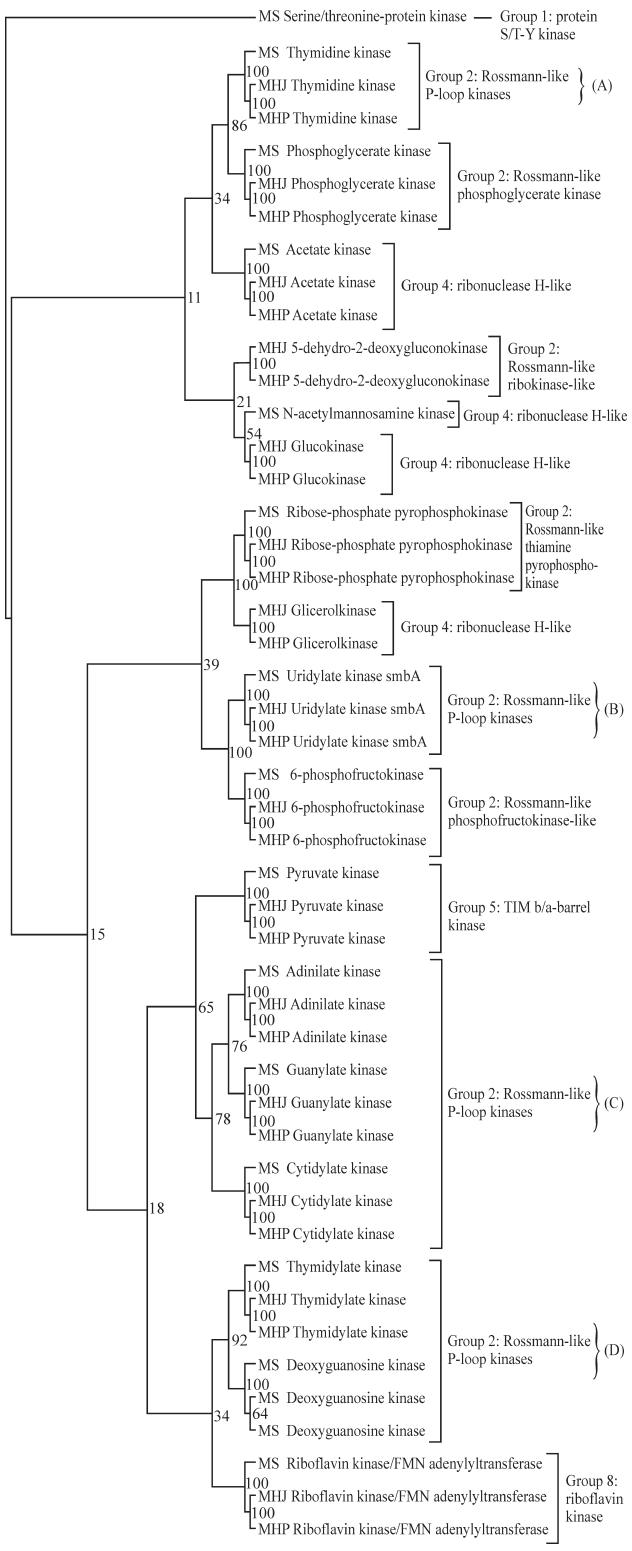

Figure 1 - Phylogenetic tree obtained from kinase amino acid sequence relationships. The kinase fold groups and families are shown in brackets on the right side. The Group 2: Rossmann-like P-loop kinases were clustered into four sub-groups (A, B, C and D). The numbers on the branches are bootstrap values obtained with 100 replications. The kinase encoding ORFs are represented by MSkinase (*M. synoviae*), MHJkinase (*M. hyopneumoniae* J) and MHPkinase (*M. hyopneumoniae* 7448).

volved in many essential metabolic pathways such as carbohydrates, purine, pyrimidine and cofactors metabolism. The presence of those enzymes evidenced the metabolic machinery utilized by these organisms which are considered minimalist models.

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Internet Resources

M. synoviae complete genome database, http://www.brgene.lncc.br/finalMS/.

M. hyopneumoniae strain J and *M. hyopneumoniae* strains 7448 complete genomes databases, http://www.genesul.lncc.br.

BLAST tools, http://www.ncbi.nlm.nih.gov/blast.

Database of protein families (Pfam), http://www.sanger.ac.uk/Software/Pfam/.

InterProScan software, http://www.ebi.ac.uk/InterProScan/.

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