Inorganic pyrophosphatase in uncultivable hemotrophic mycoplasmas: identification and properties of the enzyme from *Mycoplasma suis*

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**Abstract**

**Background:** *Mycoplasma suis* belongs to a group of highly specialized hemotrophic bacteria that attach to the surface of host erythrocytes. Hemotrophic mycoplasmas are uncultivable and the genomes are not sequenced so far. Therefore, there is a need for the clarification of essential metabolic pathways which could be crucial barriers for the establishment of an *in vitro* cultivation system for these veterinary significant bacteria. Inorganic pyrophosphatases (PPase) are important enzymes that catalyze the hydrolysis of inorganic pyrophosphate PP\(_i\) to inorganic phosphate P\(_i\). PPases are essential and ubiquitous metal-dependent enzymes providing a thermodynamic pull for many biosynthetic reactions. Here, we describe the identification, recombinant production and characterization of the soluble (s)PPase of *Mycoplasma suis*.

**Results:** Screening of genomic *M. suis* libraries was used to identify a gene encoding the *M. suis* inorganic pyrophosphatase (sPPase). The *M. suis* sPPase consists of 164 amino acids with a molecular mass of 20 kDa. The highest identity of 63.7% was found to the *M. penetrans* sPPase. The typical 13 active site residues as well as the cation binding signature could be also identified in the *M. suis* sPPase. The activity of the *M. suis* enzyme was strongly dependent on Mg\(^{2+}\) and significantly lower in the presence of Mn\(^{2+}\) and Zn\(^{2+}\). Addition of Ca\(^{2+}\) and EDTA inhibited the *M. suis* sPPase activity. These characteristics confirmed the affiliation of the *M. suis* PPase to family I soluble PPases. The highest activity was determined at pH 9.0. In *M. suis* the sPPase builds tetramers of 80 kDa which were detected by convalescent sera from experimentally *M. suis* infected pigs.

**Conclusion:** The identification and characterization of the sPPase of *M. suis* is an additional step towards the clarification of the metabolism of hemotrophic mycoplasmas and, thus, important for the establishment of an *in vitro* cultivation system. As an antigenic and conserved protein the *M. suis* sPPase could in future be further analyzed as a diagnostic antigen.

**Background**

*Mycoplasma suis* belongs to a group of highly specialized uncultivable hemotrophic bacteria within the family *Mycoplasmataceae* that attach to the surface of host erythrocytes [1,2]. In the last few years reports on hemotrophic mycoplasmas in various animal species [1] as well as in humans [3,4] continuously increased. Obviously, hemotrophic mycoplasmas are emerging agents with a zoonotic potential. *M. suis* causes infectious anemia in pigs leading to serious economic loss in the pig industry due to acute anemia as well as chronic persistent infections with increased susceptibility to respiratory and enteric diseases [1,5]. Instead of a clear and long-dated clinical significance of hemotrophic mycoplasmas [6] our knowledge on the physiology and metabolism of hemotrophic mycoplasmas is rather limited. This can primarily led back to their unculturability and the lack of sequence data [6]. Probably, *M. suis* can use glucose as a source of carbon and energy [7,8]. However, detailed energy requirements of *M. suis* are largely unknown and its key enzymes have not been described so far. In previous studies we
successfully screened genome libraries to identify M. suis proteins which are involved in pathogenetic processes of M. suis infections (e.g. adhesion) and the energy metabolism of these rather unexplored pathogens [9,10]. In this paper we identified the soluble inorganic pyrophosphatase (sPPase) of M. suis by applying said strategy. Inorganic pyrophosphate (PPi) is an important by-product of many biosynthetic processes, and sPPases which hydrolyze PPi to inorganic phosphate (P_i), are essential and ubiquitous metal-dependent enzymes providing a thermodynamic pull for many biosynthetic reactions [11-13]. Soluble PPases belong to two non-homologous families: family I, widespread in all types of organisms [14], and family II, so far confined to a limited number of bacteria and archaea [15,16]. The families differ in many functional properties; for example, Mg^{2+} is the preferred cofactor for family I sPPases studied, whereas Mn^{2+} confers maximal activity to family II sPPases [17,18]. Detailed aims of this study were the recombinant production and characterization of the M. suis sPPase and the comparison of its properties to those of other bacteria. Characterization of essential enzymes in the metabolism of hemotrophic mycoplasmas are important steps towards the establishment of an in vitro cultivation system for this group of hitherto uncultivable hemotrophic bacteria.

Results
Identification of the M. suis inorganic pyrophosphatase (PPase)
The sPPase of M. suis was identified by screening of genomic libraries of M. suis using short gun sequencing. By means of sequence analysis and database alignments of 300 randomly selected library clones we identified library clone ms262 containing an M. suis insert with highest identity to the gene encoding the M. penetrans sPPase. Since prokaryotic sPPases are known to be essential in energy metabolism [11,12] we selected the ms262 clone for further studies. To confirm the M. suis authenticity of ms262 Southern blot analyses of M. suis genomic DNA were performed using two EcoRI ms262 library fragments as probes. The ms262 EcoRI fragments hybridized with two genomic M. suis fragments of 1.2 and 2.7 kb, respectively (Figure 1A). Detailed sequence analysis revealed that the clone ms262 contains a 2059-bp insert with an average G+C content of 30.11%. Clone ms262 includes two ORFs (Figure 1B); ORF1 showed the highest identity with U. parvum thioredoxin trx (significant BLAST score of 1.3 \times 10^{-7}, overall sequence identity 44.5%). ORF2 with a length of 164-aa protein with a calculated molecular mass of 18.6 kDa and an isoelectric point of 4.72. The ORF2 matched best with M. penetrans ppa (63.7% identity). The overall degrees of identity to the ppa of U. urealyticum, M. mycoides ssp mycoides, and M. capricolum ssp capri- culum were calculated to be 59.7%, 58.7%, and 58.3%, respectively. Figure 2 shows an alignment of sPPases of selected Mycoplasma species. The characteristic signature of sPPase which is essential for the binding of cations was identified at amino acid positions 54 to 60 (Figure 2) using the program PREDICT PROTEIN http://cubic.bioc.columbia.edu/predictprotein/. Possible signatures for sPPases are D[SGDN]D[PE][LIVMF]D[LI]VMGAG. The signature of the M. suis sPPase was determined as DGDPDLV (amino acids are underlined in the universal signature; Figure 2). The 13 conserved residues which build the active site of sPPases could be identified in the M. suis sPPase, too (Figure 2).

Expression of recombinant PPase in E. coli
The entire ORF of the M. suis ppa was assembled as a synthetic gene and one UGA_{Trp} codon at position 274-276 was replaced by UGG. Other changes in the synthetic ppa were done to optimize the sequence for the
heterologous E. coli expression. Induction of E. coli transformants containing the ppa gene resulted in the high-level expression of a 20 kDa-protein as shown in Figure 3A. Recombinant PPase was used to raise a PPase-specific rabbit polyclonal antiserum. The specificity of the rabbit serum was demonstrated by probing an immunoblot containing purified rPPase and a M. suis preparation. The anti-PPase serum reacted clearly with a single band of 20 kDa corresponding to the purified rPPase. In the M. suis preparations a weak band of 20 kDa and a clear band of 80 kDa potentially corresponding to a tetrameric form of the M. suis PPase were detected (Figure 3B). No reaction could be observed neither with the blood control preparation of M. suis negative pigs nor the non-induced E. coli control.

Characterization of PPase in M. suis

In order to prove the conserved existence of the PPase gene in M. suis, 25 M. suis isolates (20 isolates from domestic pigs and five isolates from wild boars) were screened by PCR. All isolates revealed a PCR amplification product of the expected size of approximately 500 bp. Sequence analysis of ten ppa PCR products revealed 100% sequence identity with the determined M. suis ppa sequence (Accession number FN394679).

To determine the antigenicity of the PPase of M. suis we analyzed convalescent serum pools from experimentally infected pigs by immunoblotting. All convalescent serum pools reacted clearly with rPPase. No reaction could be observed with sera taken from M. suis negative pigs. A representative immunoblot is shown in Figure 3C.
The dependency of the \textit{M. suis} PPase activity on the pH value was determined between pH 5 and 10.5. As shown in Figure 4D the optimum pH for the \textit{M. suis} PPase activity was observed at pH 9.0. At conditions below pH 7.5 and above pH 10.0 its activity decreased considerably.

The effect of different Mg\textsuperscript{2+} concentrations on the \textit{M. suis} PPase activity is shown in Figure 4A. High enzyme activity was found between 1 and 100 mM Mg\textsuperscript{2+} with a maximum activity at a concentration of 10 mM Mg\textsuperscript{2+}. Performing the reaction at a pH of 7.5 the maximum PPase activity was found at a concentration of 50 mM. Using an Mg\textsuperscript{2+} depleted reaction buffer the \textit{M. suis} PPase-mediated Pi hydrolysis was nearly abolished. Substitution of Mg\textsuperscript{2+} cations with Mn\textsuperscript{2+} and Zn\textsuperscript{2+} resulted in significantly lower activities of 25.34\% ± 12.1\% and 14.3\% ± 9.5\% respectively of the Mg\textsuperscript{2+} induced activity (Figure 4B).

To further characterize the \textit{M. suis} PPase the effect of inhibitors on the activity was evaluated. Enzymatic activity was inhibited more than 95\%, and 70\% in the presence of 5 mM Ca\textsuperscript{2+} and 5 mM EDTA, respectively (Figure 4C).

**Discussion**

In this study, we identified, for the first time, a gene encoding the sPPase of one representative of the uncultivable hemotrophic mycoplasma group, i.e. \textit{M. suis}. PPase plays an important role in the bacterial energy metabolism [11,12] and is the enzyme responsible for the hydrolysis of pyrophosphate which is formed principally as the product of many biosynthetic reactions that utilize ATP. Since our knowledge on the metabolism of \textit{M. suis} and other hemotrophic mycoplasmas is rather limited enzymes associated with their metabolism are of our special interest.

The \textit{M. suis} ORF encoding the sPPase showed a typically low G+C content of 30.11\% which lies within the normal range of other mycoplasmas [19,20]. The identified \textit{M. suis} sPPase signature sequence which is responsible for the cation binding was identical to those of \textit{M. mycoides} ssp \textit{mycoides} and \textit{M. capricolum} ssp \textit{capricolum}. Furthermore, all functionally important active site residues could be identified in the \textit{M. suis} sPPase. Interestingly, the \textit{M. suis} sPPase is considerably shorter than other mycoplasma sPPases (164 vs. 180-185 amino acid residues) due to differences in the C-terminal region. State-of-the-art knowledge on the uncultivable hemotrophic mycoplasmas does not allow for a statement as to which function the absence of amino acid residues on the C-terminus might incur. There could be a possible relevance for its subcellular localization. Additionally, the \textit{ms}262 clone harbors a second ORF encoding a putative \textit{M. suis} thioredoxin. The thioredoxin system operates via redox-active disulphides and provides electrons for a wide range of metabolic processes in prokaryotic cells. Especially within the genus \textit{Mycoplasma} the thioredoxin complex apparently belongs to the metabolic core reactions [21,22]. Comparison of the
genome structures flanking the ppa ORF with the sequenced Mycoplasma species revealed no homologies (data not shown).

After heterologous expression of the sPPase in E. coli the protein was found in the cytoplasm with a molecular weight of 20 kDa. In M. suis whole cell preparations the sPPase was detected as a 20 kDa band to a minor degree. Predominantly the enzyme was found to have a molecular weight of approx. 80 kDa indicating that the M. suis sPPase obviously consists of four subunits. Since the inference that the M. suis sPPase is tetrameric is solely based on the results of an immunoblot using anti-rPPase antibodies (Fig 3B) the final proof of the tetrameric form has to be provided as soon as an in vitro cultivation of M. suis is possible. For other Mycoplasmas nothing is known about the protein properties of sPPase since they have only been identified via their DNA sequences. However, other studies report that most eubacterial PPases are homohexamers [23,24], and, as is unusual, sometimes homotetramers e.g. Aquifex aeolicus [25,26] or Rhodospirillum rubrum [27]. Where molecular phylogeny is concerned the Mycoplasma sPPases are clustered with the cyanobacteria within the prokaryotic Family I PPase lineage [27]. The M. suis sPPase showed characteristic properties in terms of cation requirement: Mg²⁺ confers the highest efficiency in activating the M. suis sPPase in a concentration-dependent manner. Other cations (Zn²⁺ and Mn²⁺) could replace Mg²⁺, but the effectiveness of the latter cations was significantly lower. Furthermore, Ca²⁺ and EDTA inhibited the enzyme for catalysis. These results support the conclusion that the M. suis sPPase belongs to the Family I PPases. Family I PPase has shown strong metal cation-dependency, with Mg²⁺ conferring the highest efficiency [14] and sensitivity to inhibition by Ca²⁺ [28]. In contrast, Family II PPase prefers Mn²⁺ over Mg²⁺ [17]. The most notable characteristic of the M. suis recombinant sPPase was its pH activity profile with an optimum at pH 9.0 since (i) optimal pH of most bacterial sPPases ranged from pH 5.0 to 8.0 [25], and (ii) the physiological blood pH value of pigs is 7.4 ± 0.4. Therefore, it is ambiguous which role the unusual pH optimum could play with regard to the pathogenesis of M. suis induced diseases. Moreover, no statement is possible about optimal pH ranges for other mycoplasmal sPPases since this study is the first functional characterization of a sPPase of a Mycoplasma species. For M. suis it is known that experimental induced acute diseases lead to severe hypoglycemia and blood acidosis with a mean pH value of 7.13 [29]. All these changes were considered to result from the high glucose consumption of M. suis during maximum bacteremia [1]. However, nothing is known about the changes in blood parameters during natural M. suis infections and especially during the chronic

Figure 4 Functional characterization of the recombinant M. suis sPPase. (A) Activation of M. suis rPPase by Mg²⁺. The rPPase (10 ng/µl) was incubated for 5 min in the same buffer containing different concentrations of MgCl₂. Values represent mean values ± standard deviation of five independent experiments. (B) Differences in the activation of rPPase by Mg²⁺, Mn²⁺, or Zn²⁺. Recombinant PPase (10 ng/µl) was incubated for 5 min in the same buffer containing 5 mM MgCl₂, 5 mM MnCl₂ and 5 mM MgCl₂, respectively. Activation of M. suis rPPase by MgCl₂ was set as 100%. Values represent mean values ± standard deviation of triplicates. (C) Inhibition of M. suis rPPase activity by Ca²⁺ and EDTA. Recombinant PPase (10 ng/µl) was incubated for 5 min in buffer containing 5 mM MgCl₂ alone and with 5 mM CaCl₂ and 5 mM EDTA, respectively. Activity value of M. suis rPPase with MgCl₂ alone was set as 100%. Values represent mean values ± standard deviation of triplicates. (D) pH value dependency of the M. suis rPPase activity. PPase activity was measured using 50 mM MgCl₂ and buffers with increasing pH values. Data represent mean values ± standard deviation from five independent experiments. (E) Activity of M. suis rPPase using different PPI concentrations. Activity was measured with fixed concentrations of rPPase (10 ng/µl) and 50 mM MgCl₂ at a pH of 9.0. Values represent mean values ± standard deviation of five independent experiments.
course of persistent infections with nearly physiological glucose metabolism. It has been reported from other infections, e.g. *Streptococcus pneumoniae*-infections in rats that infections could lead to significantly increased blood pH values [30].

Notably, infected pigs showed antibodies against recombinant sPPase. This may result from the sPPase being an ectoenzyme which might be located on the external surface. Alternatively, anti-*Ms* PPase antibodies could be an outcome of bacterial lysis in the animal host. The first possibility is rather unlikely since no signal peptide was found in any *Mycoplasma* PPase and all other Family I PPases are clearly soluble and not secreted [27]. Probably sPPase could be one of the eight *M. suis* specific antigens which we have described recently [9].

**Conclusion**

By using a screening of genomic libraries of uncultivable bacteria *M. suis* we were able to identify so far unknown components of the energy metabolism. We identified and characterized the inorganic pyrophosphatase of *M. suis*. Knowing the functional characteristics of such an essential enzyme may help to establish an in vitro cultivation system for hemotrophic mycoplasmas. Furthermore, as an antigenic and conserved protein *M. suis* sPPase could in future be further analyzed as a diagnostic antigen.

**Methods**

**Bacterial strains and isolates, plasmids, and experimental porcine sera**

*M. suis* cells were obtained from experimentally infected pigs as previously described [31,32]. *E. coli* K12 strains were Top10 and LMG194 (Invitrogen, Basel, Switzerland). For DNA manipulation and protein expression the plasmids pUC19 (Roche-Diagnostics, Rotkreuz, Switzerland) and pBadMycHis (C-terminal His- and Myc-tag, Invitrogen) were used. Experimental sera and *M. suis* isolates were available from previous studies [33,34].

**DNA extraction, library construction and sequence analysis**

DNA extraction of *M. suis* was performed as previously described [31]. Customized DNA library construction was performed by Medigenomix ( Martinsried, Germany). *M. suis* DNA fragments averaging from 1.5 kb to 3.0 kb were ligated into the pUC19 vector. In order to detect *M. suis* sequences 300 clones were randomly selected for DNA-sequencing. Customized sequencing was performed by Medigenomix. Nucleotide sequences were analyzed by using the FASTA algorithm (Biocomputing service, University Zurich, http://www.bio.unizh.ch). For determination of putative open reading frames we used an ORF finder program http://www.ncbi.nlm.nih.gov/projects/gorf/. Translation of ORFs to amino acid sequences was performed by taking into account the alternative genetic codon usage of mollicutes (UGA encodes tryptophan instead of stop).

**Hybridization analysis**

Hybridization was performed as previously described [31]. Briefly, *M. suis* genomic DNA was digested with EcoRI, analyzed on a 0.8% agarose gel and transferred to Hybond-N nylon membranes by capillary transfer using 1.5 M NaCl, 0.25 M NaOH as transfer buffer. The ppa-containing library clone ms262 was digested with the restriction enzymes HindIII and EcoRI. Due to an internal EcoRI digestion site the insert was divided into two fragments of approx. 1200 bp and 800 bp. Both fragments were labeled with digoxigenin-dUTP (Roche-Diagnostics) and used as probes.

**Cloning, expression of *M. suis* ppa and purification of the recombinant enzyme**

To account for the *Mycoplasma* specific use of the UGA codon as tryptophan the ppa sequence was adapted to the codon usage of *E. coli* and de novo synthesized (Medigenomix). The de novo ppa was ligated into the pBadMycHis vector (pBad-ppa) and transformed into *E. coli* LMG194.

Recombinant pBad-ppa *E. coli* clones were grown to an OD600 nm of 0.6 at 37°C. Protein expression was induced by the addition of 0.02% arabinose. *E. coli* cultures were further incubated for 2 h at 37°C. His-tagged proteins were purified by nickel affinity chromatography (Qiagen, Hombrechtikon, Switzerland) as previously described [9,10]. The purification of 2 liter culture yielded a total of 1 mg recombinant protein. Purity of protein was estimated as 90%. Non-induced cultures were prepared accordingly as controls for immunoblots and enzyme activity assays.

**Enzyme activity assay**

Protein content was determined by the method of Bradford (BioRad, Reinach, Switzerland) using bovine serum albumin as a standard. The recombinant *M. suis* sPPase activity was assayed as described by Saheki and coworkers [35] using a reaction mixture containing 5 mM Mg2+, 100 mM Tris, pH 7.5 and 1 mM PPi (Na2P2O7) at 55°C in a final volume of 200 μl. Reactions were started by adding 10 μl diluted *M. suis* tPPase (100 ng) and stopped by adding 1 ml 200 mM Glycin/HCl, pH 3.0. Then, 125 μl of 1% ammonium molybdate (in 25 mM H2SO4) and 125 μl of 1% ascorbic acid (in 0.05% KHSO4) were added to the mixtures and incubated for 30 min at 37°C. Yeast sPPase (Sigma, Buchs, Switzerland) was used as positive control. Preparations derived from non-induced pBad-ppa (purified accordingly to recombinant PPhase) were used as negative controls. To determine the Mg2+.
and pH dependency individual assay components were varied. Activity was also measured using 5 mM Mn$^{2+}$, Zn$^{2+}$ instead of Mg$^{2+}$ cations. For inhibition assays 5 mM Ca$^{2+}$ and EDTA, respectively, were added to the reaction mixture. The amount of $P_i$ liberated from the hydrolysis of $PP_i$ was measured using a spectrophotometer (Shimadzu 160-UV-A) and a standard $P_i$ curve. The $PP_i$ase activity was defined as μmol $P_i$ min$^{-1}$ mg$^{-1}$ protein.

Preparation of an anti-PPase rabbit immune serum

A rabbit immune serum was prepared as previously described [10] using 0.4 mg recombinant PPase for each immunization. Immunizations were conducted under the registration number 156/2002 with the legal prescriptions.

SDS PAGE and immunoblots

SDS PAGE and immunoblots were performed according to standard protocols. The $M. suis$ cells were prepared from the blood of experimentally infected pigs as previously described [32]. Negative controls were accordingly prepared from the blood of $M. suis$-negative pigs.

PCR and sequencing

PCR amplification of the $ppa$ gene was performed using the primers: $ppa_{for}$: ATGTCAAAAATAATATAGTGGA; $ppa_{rev}$ TTAAATATTGTGGTTATCCTCC, and the HotStarTaq Polymerase Master Mix (Qiagen). PCR conditions were: 15 min at 95°C for activation of Taq polymerase, 30 cycles of denaturation at 95°C for 30 s, annealing at 60°C for 30 s, and extension at 72°C for 1 min. Amplified fragments were purified using the Qiаquick PCR Purification Kit (Qiagen) and sequenced (Medigenomix).

The $ppa$ sequence was deposited in the EMBL Nucleotide Sequence Database under accession number FN394679.

Authors’ contributions

KH-planned, developed and co-coordinated the project, analyzed the data, wrote the manuscript; SP-functional characterization; did the enzyme activity assays; MG-screened the $M. suis$ genomic libraries, performed the hybridization experiments; MK-expressed the inorganic pyrophosphatase in E.coli, performed SDS PAGE and immunoblots; MMW-contributed to the data analysis and manuscript preparation; KMF-performed enzyme activity assays, protein purification procedures, SDS PAGE and immunoblots; LEH-project design, manuscript preparation and project oversight.

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References

1. Hoelzle LE. Haemotrophic mycoplasmas: recent advances in Mycoplasma suis. Vet Microbiol 2008, 130:215-226.
2. Zachary JF, Bagail EI. Erythrocyte membrane alterations associated with the attachment and replication of Eperythrozoon suis: a light and electron microscopic study. Vet Pathol 1988, 25:164-170.
3. Hu Z, Yin J, Shen K, Kang W, Chen Q. Outbreaks of hemotrophic mycoplasma infections in China. Emerg Infect Dis 2009, 15:1139-1140.
4. dos Santos AP, dos Santos RP, Biondo AW, Dora JM, Goldani LZ, de Oliveira ST, de Sa Guimaraes AM, Timenetsky J, de Morais HA, Gonzalez FH, Messick JB. Hemoplasma infection in HIV-positive patient, Brazil. Emerg Infect Dis 2008, 14:1922-1924.
5. Messick JB. Hemotrophic mycoplasmas (hemoplasmas): a review and new insights into pathogenic potential. Vet Clin Pathol 2004; 33:2-13.
6. Neimark H, Johansson KE, Rikihisa Y, Tully JG. Proposal to transfer some members of the genera Haemobartonella and Eperythrozoon to the genus Mycoplasma with descriptions of Candidatus Mycoplasma haemofelis, Candidatus Mycoplasma haemomuris, Candidatus Mycoplasma haemosuis and Candidatus Mycoplasma wenyonii. Int J Syst Evol Microbiol 2001, 51:891-899.
7. Nonaka N, Thacker BJ, Schilhorn van Veen TW, Bull RW. In vitro maintenance of Eperythrozoon suis. VetParasitol 1996, 61:181-199.
8. Smith JE, Cipriano JE, Hall SM. In vitro and in vivo glucose consumption in swine eperythrozoonosis. Zentralbl Veterinarmed B 1990, 37:587-592.
9. Hoelzle LE, Hoelzle K, Harder A, Ritzmann M, Schoon HA, Heintzki K, Wittenbrink MM. First identification and functional characterization of an immunogenic protein in unculturable haemotrophic Mycoplasmas (Mycoplasma suis HspA1). FEMS Immunol Med Microbiol 2007, 49:215-223.
10. Hoelzle LE, Hoelzle K, Hellinging M, Aupepper H, Schoon HA, Ritzmann M, Heintzki K, Felder KM, Wittenbrink MM, MSG1, a surface-localised protein of Mycoplasma suis is involved in the adhesion to erythrocytes. Microbes Infect 2007, 9:466-474.
11. Chen J, Brevet A, Fromant M, Leveque F, Schmitter JM, Blanquet S, Charpentier C, Fromentin J, Herbrecht E, Lefebre A, Lebbe C, Montagne P, Nuber B, Ollivier S, Puchelle E, Rambaud L, Reynes C, Simon P, Tordjman R, Vandenbrand L, Veuthey JL, Vidal MC, Zotti M. Immunization with a recombinant Haemobartonella felinea 156-kDa protein for the prevention of infection in cats. Infect Immun 1999, 67:5688-5694.
12. Lundin M, Balschefsky H, Rome H. Yeast PPA2 gene encodes a mitochondrial inorganic pyrophosphatase that is essential for mitochondrial function. J Biol Chem 1991, 266:12268-12272.
13. Sonnewald U. Expression of E. coli inorganic pyrophosphatase in transgenic plants alters photosynthetic assimilation. Plant J 1992, 25:7-81.
14. Cooperman BS, Baykov AA, Lahti R. Evolutionary conservation of the active site of soluble inorganic pyrophosphatase, Trends Biochem Sci 1992, 17:262-266.
15. Shintani T, Uchihmi T, Yonezawa T, Salminen A, Baykov AA, Lahti R, Hachmoni A. Cloning and expression of a unique inorganic pyrophosphatase from Bacillus subtilis: evidence for a new family of enzymes. FEBS Lett 1998, 439:263-266.
16. Young TW, Kuhn NL, Wadson A, Ward S, Burges D, Cooke GD. Bacillus subtilis ORF ybbQ encodes a manganese-dependent inorganic pyrophosphatase with distinctive properties: the first of a new class of soluble pyrophosphatase? Microbiology 1998, 144(Pt 9):2563-2571.
17. Parfyniev AN, Salminen A, Hakonen P, Hachmoni A, Baykov AA, Lahti R. Quaternary structure and metal ion requirement of family II pyrophosphatases from Bacillus subtilis, Streptococcus gordoni, and Streptococcus mutans. J Biol Chem 2001, 276:24511-24518.
18. Zuytynov AB, Vener AV, Salminen A, Goldman A, Lahti R, Baykov AA. Rates of elementary catalytic steps for different metal forms of the family II pyrophosphatase from Streptococcus gordoni. Biochemistry 2004, 43:1065-1074.
19. Chambaud I, Heilig R, Ferris S, Barber V, Samson D, Galisson F, Moszer I, Dybvig K, Wroblewski H, Vian A, et al. The complete genome sequence of the murine respiratory pathogen Mycoplasma pulmonis. Nucleic Acids Res 2001, 29:2145-2153.
20. Himmelreich R, Bilich H, Flagens H, Pirkil E, Li BC, Hermann R. Complete sequence analysis of the genome of the bacterium Mycoplasma pneumoniae. Nucleic Acids Res 1996, 24:4400-4409.
21. Pollack JD, Williams MV, McElhaney RN. The comparative metabolism of the mollicutes (Mycoplasmas): the utility for taxonomic classification and the relationship of putative gene annotation and phylogeny to enzymatic function in the smallest free-living cells. Crit Rev Microbiol 1997, 23:269-354.
22. Weinert VA, Soongling D, Holmqvist A, Ollingro R, Ottenhoff T, Thole J. Unique gene organization of thioredoxin and thioredoxin reductase in Mycobacterium leprae. Mol Microbiol 1995, 16:921-929.
23. Oliva G, Romero J, Ayala G, Barrios-Jacobo J, Celis H. Characterization of the inorganic pyrophosphatase from the pathogenic bacterium Helicobacter pylori. Arch Microbiol 2000, 174:104-110.
24. Shimizu T, Imai M, Araki S, Kishida K, Terasawa Y, Hachimori A: Some properties of inorganic pyrophosphatase from Bacillus subtilis. *Int J Biochem Cell Biol* 1997, 29:303-310.

25. Hoe HS, Kim HK, Ryon ST: Expression in Escherichia coli of the thermostable inorganic pyrophosphatase from the Aquifex aeolicus and purification and characterization of the recombinant enzyme. *Protein Expr Purif* 2001, 23:242-248.

26. Verhoeven JA, Schenck KM, Meyer RR, Trela JM: Purification and characterization of an inorganic pyrophosphatase from the extreme thermophile Thermus aquaticus. *J Bacteriol* 1986, 168:316-321.

27. Gomez-Garcia MR, Losada M, Serrano A: Comparative biochemical and functional studies of family I soluble inorganic pyrophosphatases from photosynthetic bacteria. *Febs J* 2007, 274:3948-3959.

28. Yang Z, Wensel TG: Molecular cloning and functional expression of cDNA encoding a mammalian inorganic pyrophosphatase. *J Biol Chem* 1992, 267:24641-24647.

29. Heinritzi K, Plank G, Peteranderl W, Sandner N: The acid-base equilibrium and carbohydrate metabolism during infection with *Eperythrozoon suis*. *Zentralbl Vetmed B* 1990, 37:412-417.

30. Elwell MR, Sammons ML, Liu CT, Beisel WR: Changes in blood pH in rats after infection with *Streptococcus pneumoniae*. *Infect Immun* 1975, 11:724-726.

31. Hoelzle LE, Adelt D, Hoelzle K, Heinritzi K, Wittenbrink MM: Development of a diagnostic PCR assay based on novel DNA sequences for the detection of *Mycoplasma suis* (*Eperythrozoon suis*) in porcine blood. *Vet Microbiol* 2003, 93:185-196.

32. Hoelzle LE, Hoelzle K, Ritzmann M, Heinritzi K, Wittenbrink MM: Mycoplasma suis antigens recognized during humoral immune response in experimentally infected pigs. *Clin Vaccine Immunol* 2006, 13:116-122.

33. Ritzmann M, Grimm J, Heinritzi K, Hoelzle K, Hoelzle LE: Prevalence of Mycoplasma suis in slaughter pigs, with correlation of PCR results to hematological findings. *Vet Microbiol* 2009, 133:84-91.

34. Hoelzle K, Doser S, Ritzmann M, Heinritzi K, Palzer A, Elicker S, Kramer M, Felder KM, Hoelzle LE: Vaccination with the Mycoplasma suis recombinant adhesion protein MSG1 elicits a strong immune response but fails to induce protection in pigs. *Vaccine* 2009, 27:5376-5382.

35. Saheli S, Takeda A, Shimazu T: Assay of inorganic phosphate in the mild pH range, suitable for measurement of glycogen phosphorylase activity. *Anal Biochem* 1985, 148:277-281.

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