Veterinary Microbiology 167 (2013) 434–439

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Proteomic approach for identification of immunogenic proteins of *Mycoplasma mycoides* subsp. *capri*

L. Corona, G. Cillara, S. ´

View metadata, citation and similar papers at core.ac.uk

Istituto Zooprofilattico Sperimentale dell

ARTICLE INFO

Article history:
Received 10 June 2013
Received in revised form 26 August 2013
Accepted 30 August 2013

Keywords:
Mycoplasma mycoides subsp. *capri*
2-DE
Immunoblot
Mass spectrometry
Immunoproteomics

ABSTRACT

In this study, an immunoproteomic approach was used to identify immunodominant proteins from *Mycoplasma mycoides* subsp. *capri* isolates. Membrane proteins, extracted through TX-114 phase partitioning, were separated using mono- and two-dimensional electrophoresis and detected by Western blotting with pooled sera from naturally infected goats. A total of 27 immunoreactive spots, corresponding to 13 different proteins, were identified using nanoLC–ESI-MSMS. Function annotation revealed that most of these proteins were metabolic enzymes involved in carbohydrate and energy metabolism. The immunogenic proteins identified in this study: pyruvate dehydrogenase, dihydrolipoamide acetyltransferase, dihydrolipoyl dehydrogenase, phosphate acetyltransferase, phosphopyruvate hydratase, adenine phopshoribosyltransferase, transketolase, translation elongation factor G, translation elongation factor Ts, FMN-dependent NADH-azoreductase, peptide methionine sulfoxide reductase, inorganic diphosphatase and trigger factor may be used as biomarkers for the serological diagnosis of contagious agalactia caused by *M. mycoides* subsp. *capri*.

© 2013 The Authors. Published by Elsevier B.V. Open access under CC BY-NC-SA license.

1. Introduction

The *Mycoplasma mycoides* cluster consists of five closely related mycoplasmas that cause disease in ruminants. The five species belonging to this cluster are: *M. mycoides* subsp. *mycoides* (Mmm), *M. mycoides* subsp. *capri* (Mmc), *Mycoplasma capricolum* subsp. *capricolum* (Mcc), *M. capricolum* subsp. *capripneumoniae* (Mccp), and *Mycoplasma leachii* (Ml) (Manso-Silvan et al., 2009; Thiaucourt et al., 2011). Concerning the species Mmc, it now includes strains previously referred to as *M. mycoides* subsp. *mycoides* Large Colony (MmmLC) (Vilei et al., 2006; Manso-Silvan et al., 2009). Mmc causes the "MAKePS" syndrome in goats, characterized by mastitis, arthritis, keratoconjunctivitis, pneumonia and septicaemia specifically in goats (Thiaucourt and Bolske, 1996). Furthermore, Mmc can also be recovered from the external ear canal of healthy dairy goat herds and from semen samples of asymptomatic goat bucks (Mercier et al., 2007; Tardy et al., 2007; Gómez-Martín et al., 2012). The presence of these asymptomatic carriers represents a potential risk in the transmission and maintenance of the infection (Amores et al., 2011). Therefore, control and eradication of Mmc infection can be obtained through better diagnostic tests and more effective vaccines. Efficient diagnostic methods could also be used to identify asymptomatic carriers in herds. The practical approach in the diagnosis of Mmc infection has been so far the serological detection of antibodies against Mmc using a home-made ELISA based on soluble crude antigens extracted from field isolates (Assunçao et al., 2004) or a latex agglutination test adapted from the one used for *M. capricolum* subsp. *capripneumoniae* (Al-Momani et al., 2011). Nowadays, no serological commercial tests are available. So, it is very important to

* Corresponding author at: Istituto Zooprofilattico Sperimentale, Via Vienna 2, 07100 Sassari, Italy. Tel.: +39 0792892339.
E-mail address: sebastiana.tola@izs-sardegna.it (S. Tola).

0378-1135 © 2013 The Authors. Published by Elsevier B.V. Open access under CC BY-NC-SA license.
http://dx.doi.org/10.1016/j.vetmic.2013.08.024

identify potential diagnostic markers characterizing the risk of infection and vaccine candidates for protection. Immunoproteomics, combining conventional proteomics with serology, is a powerful method to identify antigens with diagnostic and protective values.

Although the full genome sequence of Mmc strain GM12 is available since 2009 (Lartigue et al., 2009), no proteomic study has been accomplished on expressed immunoproteins. Since lipoproteins are considered to be highly immunogenic due to their membrane surface exposure and their functions in various important cellular processes, we analyzed only the liposoluble proteome of Mmc isolates.

In this study, we applied immunoproteomics to identify proteins extracted by means of Triton X-114 using sera collected from goats with Mmc infection.

2. Materials and methods

2.1. Mycoplasma isolates and growth conditions

In this study, we used the Mmc 49055, 74751, 14349 and 22200 field strains, isolated from infected goats in Sardinia (Italy), during the period 2004–2011. Isolates were grown at 37 °C in 300 ml of modified Hayflick medium containing 8% equine serum. Mycoplasmas were harvested by centrifugation at 20,000 × *g* for 30 min and washed twice with phosphate buffered saline (PBS, 0.1 M phosphate, 0.33 M NaCl, pH 7.4). The final pellet was resuspended in one-tenth of the original volume and used immediately or stored at −80 °C. Protein concentration of washed whole cell suspension was quantified using the Bradford method (Bio-Rad DC Protein Assay Reagent, Bio-Rad, Hercules, CA, USA) according to the manufacturer's protocol.

2.2. Integral membrane proteins

Extraction of integral membrane proteins was carried out through Triton X-114 phase partitioning as described by Bordier (1981) and adapted with some modifications (Reithman et al., 1987). Liposoluble proteins were processed for one- and two-dimensional gel electrophoresis and immunoblotting.

2.3. Antisera

For the present study, we used 4 sera collected from infected goats from different areas of Sardinia. All sera came from goats with clinical symptoms of contagious agalactia (i.e. mammary gland enlargement and altered milk production). We isolated Mmc 49055, 74751, 14349 and 22200 from their milk samples. As negative control serum, we used 5 samples collected from goats belonging to herds without a clinical history of *M. mycoides* subsp. *capri* infection. All sera were tested by immunoblotting. Sera were pooled, aliquoted and stored at −20 °C.

2.4. One-dimensional SDS-PAGE and immunoblotting (IB)

Integral membrane proteins were electrophoresed on 12% (w/v) polyacrylamide gels containing 0.1% SDS. The apparent molecular weight of mycoplasma proteins was determined using appropriate molecular weight markers (Kaleidoscope pre-stained standards, Bio-Rad). After electrophoresis, separated proteins were either stained with 0.25% Coomassie blue R-250 (Bio-Rad), 25% methanol and 10% (v/v) acetic acid or transferred to reinforced nitrocellulose membranes (0.45 μm pore size, Whatman, Germany) in a Trans-Blot-semidry-apparatus (Bio-Rad) as described by the manufacturer. Blots were incubated for 1 h at 37 °C with a pooled serum of infected goats diluted 1:100 in PBS-2% skim milk. After several washings with PBS-2% skim milk, blots were incubated for one more hour at 37° C with anti-goat IgG (whole molecule)-alkaline phosphatase antibody produced in rabbit (Sigma). After five more washes, blots were developed with 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium (BCIP/NBT, Promega, Madison, WI) in alkaline phosphatase buffer (100 mM NaCl, 5 mM $MgCl_2$, 100 mM Tris, pH 9.5).

2.5. Two-dimensional gel electrophoresis and immunoblotting

For 2-DE analysis, Triton X-114 detergent was removed from samples by the use of 2-D-Clean-up Kit (GE Healthcare, Freiburg, Germany) according to the manufacturer's instructions. Integral membrane proteins were dissolved in isoelectric focusing buffer (IEF buffer) containing 8 M urea, 4% (w/v) CHAPS, 40 mM dithiothreitol (DTT) and 1% (v/v) ampholytes, pH 3–11. IPG strips (13 cm pH 3–11 NL, Immobiline DryStrips, GE Healthcare) were rehydrated with 300 μl of IEF buffer containing 1X protease inhibitors mix and DeStreak rehydration solution (GE Healthcare). Proteins were focused using a Ettan IPGphor (GE Health) at 16 °C, by applying the following voltages: 30 V (10 h), 500 V (4 h), 1000 V (gradient step, 1 h), 8000 V (gradient step, 2.30 h), 8000 V (3.20 h). After focusing, proteins were reduced by incubation of the IPG strips with 1% (w/v) DTT in 4 ml of equilibration buffer (75 mM Tris–HCl pH 8.8, 6 M urea, 30% (w/v) glycerol, 2% (w/v) SDS and a trace of bromophenol blue) for 15 min, and alkylated with 3% (w/v) iodoacetamide in 4 ml of equilibrated buffer for 15 min. In the second dimension, IPG strips were run vertically onto 12% SDS-PAGE gels using a PROTEAN II xi 2D Cell (Bio-Rad) with 200 V applied for about 4 h, until the dye front reached the bottom of the gel. 2-DE gels were stained with plusOne Silver staining kit (MS compatible, GE Health) according to the manufacturer's instruction. Immunoblotting was carried out as described above.

2.6. Image acquisition and immunogenic proteins identification

Stained gels images were acquired by using GS-800 calibrated densitometer (Bio-Rad) and analyzed using the PDQuest software (Bio-Rad) which allowed automatic spot detection and spot aligning/matching within gels. After comparison with immunoblotting results, 27 protein spots were manually excised from gels and then sent to Proteome Factory (http://www.proteomefactory.com/) for protein identification analysis by nanoLC–ESI-MSMS. The MSMS peptide mass fingerprint (PMF) data were

automatically searched against NCBInr protein database (http://blast.ncbi.nlm.nih.gov/) using MASCOT software package (Matrix Science, UK). In MASCOT searches, protein scores > 83 were significant ($p < 0.05$). Protein scores as well as the original PMF data such as extent of sequence coverage, number of peptide matched were used to accept protein identification. The prediction of protein subcellular localization was searched in the PSORT server (http://www.psort.org/).

3. Results

3.1. One dimensional SDS-PAGE and immunoblotting

Analysis of TX-114 phase fractioned Mmc isolates by SDS-PAGE followed by immunoblotting with pooled serum from infected goats was compared with immunoblot of whole cell proteins (Fig. 1). The lack of any difference in bands number and size between total and liposoluble proteins confirms that, in Mmc too, the most common antigens are membrane proteins. Most proteins was included in the range 46–100 kDa, few immunoreactive proteins were detected with molecular masses lower than 40 kDa. Two proteins of apparent molecular weights of about 90 and 60 kDa were observed within all isolates.

3.2. 2-D electrophoresis and immunoblotting

Proteins extracted with Triton X-114 from Mmc isolates 49055, 74751, 14349 and 22200 were pooled and resolved by 2-DE. Two-dimensional separation profile is shown for separation by isoelectric point (p*I*) on the first dimension

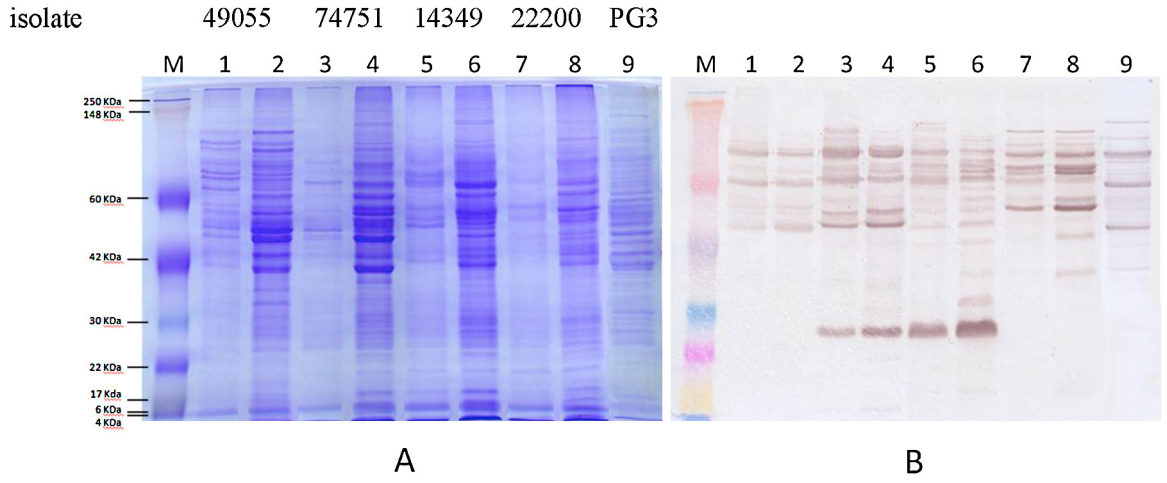

Fig. 1. SDS-PAGE and Western blot analysis of 4 *Mycoplasma mycoides* subsp. *capri* isolates and Reference strain PG3. (A) Comassie G-250-stained gel of whole cell proteins (lanes 1-3-5-7) and TX-114 phase proteins (lanes 2-4-6-8) from Mmc isolate 49055 (lanes 1-2), Mmc isolate 74751 (lanes 3-4), Mmc isolate 14349 (lanes 5-6) and Mmc isolate 22200 (lanes 7-8). Lane 9: whole cell proteins from Reference Strain PG3. (B) Western blot analysis of the same isolates and PG3 against pooled sera from naturally infected goats. The numbers on the left indicate the molecular weight standards (kDa).

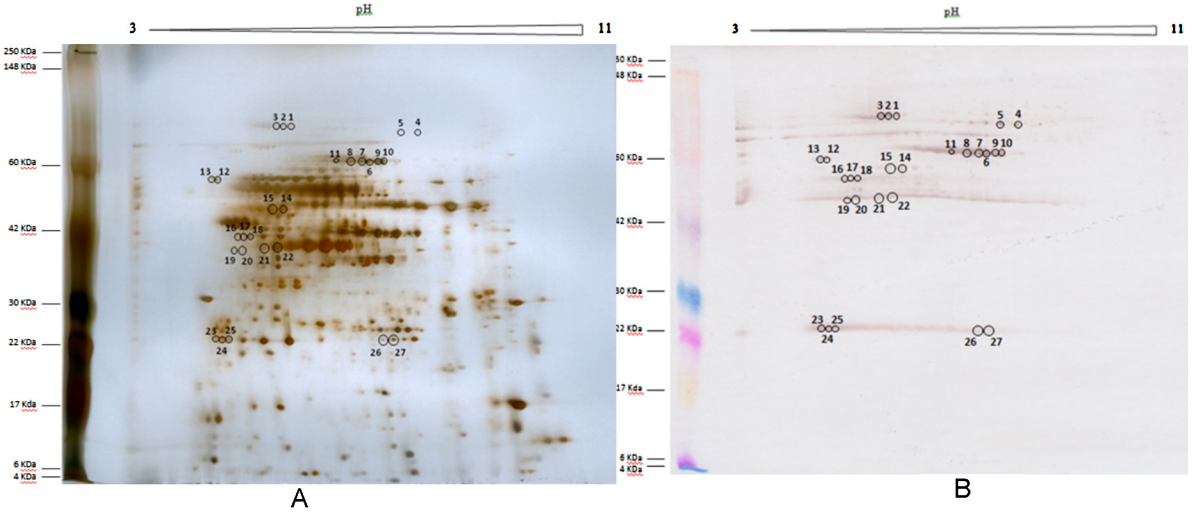

Fig. 2. 2-DE and immunoblot of membrane protein fraction from 4 pooled *Mycoplasma mycoides* subsp. *capri* isolates. (A) Liposoluble proteins were separated by IEF using 13 cm IPG strips, pH 3–11 NL, followed by SDS-PAGE on 12% and stained with plusOne Silver staining kit. Molecular weight markers are on the left in kilodalton. (B) Identification of immunogenic proteins from Mmc isolate 49055: another gel run in parallel was used for Western blotting using a pooled sera from infected goats. Spots identified by nanoLC–ESI-MSMS are circled and indicated by numbers.

using a pH ranges of 3–11 NL (Fig. 2A) The separation profile was highly reproducible in 2-DE followed by Western blot analysis with pooled serum from naturally infected goats (Fig. 2B).

Based on immunoblot results using pooled positive and negative serum, twenty-seven protein spots were detected and identified by nanoLC–ESI-MSMS. Four proteins were identified as single spots whereas 9 proteins were represented by two, three and six isoforms, indicating post-translational modifications of these proteins. The thirteen immunoreactive proteins were identified as dihydrolipoyl dehydrogenase, dihydrolipoamide acetyltransferase, transketolase, pyruvate dehydrogenase (lipoamide)-alpha chain, phosphate acetyltransferase, phosphopyruvate hydratase, adenine phosphoribosyltransferase, translation elongation factor G, translation elongation factor Ts, FMN-dependent NADH-azoreductase, peptide methionine sulfoxide reductase, inorganic diphosphatase and trigger factor. Their identities, isoelectric point (p*I*), molecular weight (MW), identification score, number of peptide matches and sequence covered by the peptides are shown in Table 1. The sequence coverage ranged from 25 to 83% and the identification scores were from 513 to 2667. Of 13 identified proteins, 12 matched with *M. mycoides* subsp. *capri* strain GM12 and 1 showed homology to proteins from *M. mycoides* subsp. *mycoides* reference strain PG1. The protein subcellular localization was predicted by PSORTb version 3.0 software: most of proteins were annotated as cytoplasmic proteins, only one as unknown (Table 1). These proteins are metabolic enzymes that are involved in translation, pyruvate and energy metabolism.

4. Discussion

The combination of 2-DE, immunoblotting and mass spectrometric analysis is an effective approach for identification of immunoreactive proteins potentially suited for the development of diagnostic tools. This strategy has been used for some small ruminants mycoplasmas, such as *M. mycoides* subsp. *mycoides*, *Mycoplasma agalactiae* and *M. capricolum* subsp. *capripneumoniae* (Jores et al., 2009; Cacciotto et al., 2010; Zhao et al., 2012). In our study, we applied this approach to identify immunogenic proteins of Mmc isolates collected in Sardinia. The *M. mycoides* subsp. *capri* strains GM12 and 95010 have been sequenced (Lartigue et al., 2009; Thiaucourt et al., 2011) whereas its proteome was not analyzed. In this study, a total of 27 immunoreactive spots, corresponding to 13 different proteins, were identified by IB with sera from infected goats. The majority of these proteins were linked to housekeeping function such as pyruvate metabolism, or, in general, energy production. In this study, all major components of pyruvate deidrogenase complex (PDC): pyruvate dehydrogenase (spots #14-15-16), dihydrolipoamide acetyltransferase (spots #6-7-8-9-10-11) and dihydrolipoyl dehydrogenase (spots #1-2) were strongly immunogenic. The immunogenicity of the PDC has been observed in *M. mycoides* subsp. *mycoides* SC and *M. capricolum* subsp. *capripneumoniae* (Jores et al., 2009; Zhao et al., 2012), belonging the same cluster "*M. mycoides*" and, in other Mycoplasma species such as *M. pneumoniae* (Dallo et al., 2002) and *M. hyopneumoniae* (Pinto et al., 2007). In eukaryotes, the PDC is located on mitochondria where it controls the flow of carbon from the glycolytic pathway into the citric acid cycle. In spite of its usually cytoplasmic localization, confirmed also by pSORT bioinformatics tool, previous studies link mycoplasma pyruvate dehydrogenase with non-cytoplasmic activity (Wallbrandt et al., 1992; Layh-Schmitt et al., 2000). In *M. pneumoniae*, its localization has been observed on the cell surface where it mediates adhesion in close association with the major P1 adhesin (Layh-Schmitt et al., 2000; Dallo et al., 2002). The several p*I* isoforms observed for PDC are

Table 1
Identification of immunoreactive proteins of 4 pooled *Mycoplasma mycoides* subsp. *capri* isolates using serum from naturally infected goats.

Spot no.	Protein name	NCBI ID	Species	p*I*	MW (kDa)	MASCOT score	Peptide match (sequence coverage, %)	PSORTb localization	PSORTb probability
1-2	Dihydrolipoyl dehydrogenase	gi|256385136	*Mmc* str. GM12	5.53	67.75	1739	30 (50%)	Cytoplasmic	9.97
3	Translation elongation factor G	gi|256383728	*Mmc* str. GM12	5.29	76.10	2202	53 (63%)	Cytoplasmic	9.97
4-5	Transketolase	gi|256384392	*Mmc* str. GM12	7.05	72.90	1338	30 (37%)	Unknown	–
6-7-8-9-10-11	Dihydrolipoamide acetyltransferase	gi|256384401	*Mmc* str. GM12	6.42	46.94	995	17 (36%)	Cytoplasmic	9.67
12-13	Trigger factor	gi|256384284	*Mmc* str. GM12	4.86	49.11	2667	40 (83%)	Cytoplasmic	7.50
14-15-16	Pyruvate dehydrogenase (acetyl-transferring), alpha chain	gi|256384125	*Mmm*SC GM12	5.47	41.87	1377	31 (53%)	Cytoplasmic	9.97
17-18	Phosphate acetyltransferase	gi|256383812	*Mmc* str. GM12	5.46	35.61	729	15 (43%)	Cytoplasmic	7.50
19-20	Phosphopyruvate hydratase	gi|256384154	*Mmc* str. GM12	5.32	49.47	647	11 (25%)	Cytoplasmic	9.97
21	Peptide methionine sulfoxide reductase	gi|42561299	*Mmm*SC PG1	5.45	35.75	762	16 (40%)	Cytoplasmic	7.50
22	Translation elongation factor Ts	gi|256383861	*Mmc* str. GM12	5.47	32.57	986	18 (56%)	Cytoplasmic	9.97
23-24	Inorganic diphosphatase	gi|256384354	*Mmc* str. GM12	4.92	21.44	759	19 (49%)	Cytoplasmic	9.97
25	FMN-dependent NADH-azoreductase	gi|256383765	*Mmc* str. GM12	5.48	22.29	707	14 (53%)	Cytoplasmic	7.50
26-27	Adenine phosphoribosyltransferase	gi|256384269	*Mmc* str. GM12	6.29	19.26	513	10 (37%)	Cytoplasmic	9.97

caused by changes in phosphorylation states; in fact, activation and inactivation of PDC components is regulated by phosphorylation of several serine residues located on their chains (Korotchkina and Patel, 1995).

Among immunoreactive proteins identified in the liposoluble proteome, the elongation factors G (spot # 3) and Ts (spot # 22) may be used for the development of a serological assay. In this study, we did not detect the elongation factor Tu (EF-Tu), reported as highly immunogenic in Mmm, Mcc and other mycoplasma species (Jores et al., 2009; Cacciotto et al., 2010; Zhao et al., 2012). Translation elongation factors are responsible for two main processes during protein synthesis on the ribosome. EF-Tu is responsible for the selection and binding of the cognate aminoacyl-tRNA to the A-site (acceptor site) of the ribosome. EF-G is responsible for the translocation of the peptidyl-tRNA from the A-site to the P-site (peptidyl-tRNA site) of the ribosome. EF-Ts is a nucleotide exchange factor that is required to regenerate EF-Tu from its inactive form (EF-Tu-GDP) to its active form (EF-Tu-GTP) (https://www.ebi.ac.uk/interpro/entry/IPR001816?q=elongation factor). The predicted molecular weight of EF-G (76.10 kDa) matched with the size of *Escherichia coli* EF-G (77 kDa) whereas EF-Ts presented a molecular mass of 37.57 kDa, much smaller than *E. coli* EF-Ts, corresponding to 74 kDa. EF-G has been described as immunogenic protein in *M. synoviae* (Bercic et al., 2008) while this study also showed the immunoreactivity of EF-Ts.

Other immunoreactive proteins identified in this study and potentially suited for the development of serological assays are: transketolase (spots # 4-5), enolase (spots # 19-20), trigger factor (spots # 12-13) and FMN-dependent NADH azotoreductase (spot # 25). Transketolase (TKT) is a key enzyme in the non-oxidative branch of the pentose phosphate pathway that transfers a two-carbon glycoaldehyde unit from ketose-donor to aldose-acceptor sugars. It is typically cytosolic enzymes with a molecular mass of 70–75 kDa. Recent studies have demonstrated the immunogenicity of the transketolase in *M. mycoides* subsp. *mycoides* small colony type (Jores et al., 2009) and *M. capricolum* subsp. *capripneumoniae* (Zhao et al., 2012). Enolase (phosphopyruvate hydratase) is one of the most abundantly expressed cytosolic proteins in many organisms. It is an essential enzyme for the degradation of carbohydrates via glycolysis even if it was also found on the cell surface of pathogenic streptococci (Pancholi and Fischetti, 1998). Prior to this study, the immunogenicity of enolase has been observed in *Mycoplasma synoviae* (Bercic et al., 2008) and *M. capricolum* subsp. *capripneumoniae* (Zhao et al., 2012). Trigger factor (TF) is a very abundant ribosome-associated chaperone that assists early folding steps of nascent polypeptides in bacteria. In *E. coli*, the N-terminal domain of TF mediates ribosome binding, whereas the middle domain harbors peptidyl-prolyl isomerase activity (Hesterkamp et al., 1997). The fact that these cytosolic proteins elicit a specific antibody response may depend on (1) by their abundance and (2) since their release during infection, due to the death of Mmc cells. Anyhow, a cell surface localization cannot be excluded for these antigens, as it has been demonstrated for PDH and elongation factor Tu (Dallo et al., 2002). In this study, we did not detect any lipoproteins although we analyzed liposoluble proteome of Mmc isolates. This could be attributed to the fact that (1) we did not identify all immunoreactive spots on blots, (2) we lost the integral membrane proteins because of low solubility, their tendency to aggregate and precipitate in aqueous solutions and their difficulty to transfer from first to second dimension (Gorg et al., 2009), and/or (3) we did not identify other immunoreactive spots because of the denaturing conditions of the technique 2-DE with loss of conformational structure of proteins; as a consequence, most antibodies directed against conformational epitopes may have lower specificity when tested against denatured proteins.

In conclusion, we reported herein a total of 13 immunogens of *M. mycoides* subsp. *capri* identified by an immunoproteomics approach. These immunogenic proteins may represent candidate markers for the diagnosis of "MAKePS" syndrome caused by *M. mycoides* subsp. *capri* in small ruminants.

Acknowledgment

This research was supported by a grant from the Istituto Zooprofilattico Sperimentale of Sardinia.

References

Al-Momani, W., Abo-Shehada, M.N., Nicholas, R.A.J., 2011. Seroprevalence of and risk factors for *Mycoplasma mycoides* subspecies *capri* infection in small ruminants in Northern Jordan. Trop. Anim. Health Prod. 43, 463–469.

Amores, J., Gomez-Martin, A., Corrales, J.C., Sanchez, A., 2011. Presence of contagious agalactia causing mycoplasmas in Spanish goat artificial insemination centres. Theriogenology 75, 1265–1270.

Assunçao, P., De la Fe, C., Ramirez, A.S., Andrada, M., Poveda, J.B., 2004. Serological study of contagious agalactia in herds of goats in the Canary islands. Vet. Rec. 154, 684–687.

Bercic, R.L., Slavec, B., Lavric, M., Narat, M., Bidovec, A., Dovc, P., Bencina, D., 2008. Identification of major immunogenic proteins of *Mycoplasma synoviae* isolates. Vet. Microbiol. 127, 147–154.

Bordier, C., 1981. Phase separation of integral membrane proteins in Triton X-114 solution. J. Biol. Chem. 256, 1604–1607.

Cacciotto, C., Addis, M.F., Pagnozzi, D., Chessa, B., Coradduzza, E., Carcangiu, L., Uzzau, S., Alberti, A., Pittau, M., 2010. The liposoluble proteome of *Mycoplasma agalactiae*: an insight into the minimal protein complement of a bacterial membrane. BMC Microbiol. 10, 225–237.

Dallo, S.F., Kannan, T.R., Blaylock, M.W., Baseman, J.B., 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.

Gómez-Martín, A., Corrales, J.C., Amores, J., Sánchez, A., Contreras, A., Paterna, A., De la Fe, C., 2012. Controlling contagious agalactia in artificial insemination centers for goats and detection of *Mycoplasma mycoides* subspecies *capri* in semen. Theriogenology 77, 1252–1256.

Gorg, A., Drews, O., Luck, C., Weiland, F., Weiss, W., 2009. 2-DE with IPGs. Electrophoresis 30, 122–132.

Hesterkamp, T., Deuerling, E., Bukau, B., 1997. The amino-terminal 118 amino acids of *Escherichia coli* trigger factor constitute a domain that is necessary and sufficient for binding to ribosomes. J. Biol. Chem. 272, 21865–21871.

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

Korotchkina, L.G., Patel, M.S., 1995. Mutagenesis studies of the phosphorylation sites of recombinant human pyruvate dehydrogenase. Site-specific regulation. J. Biol. Chem. 270, 723–732.

Layh-Schmitt, G., Podtelejnikov, A., Mann, M., 2000. Proteins complexed to P1 adhesin of *Mycoplasma pneumoniae*. Microbiology 146, 741–747.

Lartigue, C., Vashee, S., Algire, M.A., Chuang, R.Y., Benders, G.A., Ma, L., Noskov, V.N., Denisova, E.A., Gibson, D.G., Assad-Garcia, N., Alperovich, N., Thomas, D.W., Merryman, C., Hutchison III, C.A., Smith, H.O., Venter, J.C., Glass, J.I., 2009. Creating bacterial strains from genomes that have been cloned and engineered in yeast. Science 325, 1693–1696.

Manso-Silvan, L., Vilei, E.M., Sachse, K., Djordjevic, S.P., Thiaucourt, F., Frey, J., 2009. *Mycoplasma leachii* sp. nov. as a new species designation for *Mycoplasma* sp. bovine group 7 of Leach, and reclassification of *Mycoplasma mycoides* subsp. *mycoides* LC as a serovar of *Mycoplasma mycoydes* subsp. *capri*. Int. J. Syst. Evol. Microbiol. 59, 1353–1358.

Mercier, P., Pellet, M., Morignat, E., Calavas, D., Poumarat, F., 2007. Prevalence of mycoplasmas in external ear canal of goats: influence of the sanitary status of the herd. Small Rumin. Res. 73, 296–299.

Pancholi, V., Fischetti, V.A., 1998. α-Enolase, a novel strong plasmin(ogen) binding protein of the surface of pathogenic streptococci. J. Biol. Chem. 273, 14503–14515.

Pinto, P.M., Chemale, G., de Castro, L.A., Costa, A.P., Kich, J.D., Vainstein, M.H., Zaha, A., Ferreira, H.B., 2007. Proteomic survey of the pathogenic *Mycoplasma hyopneumoniae* strain 7748 and identification of novel post-translationally modified and antigenic proteins. Vet. Microbiol. 121, 83–93.

Reithman, H.C., Boyer, M.J., Wise, K.S., 1987. Triton X-114 phase fractionation of an integral membrane surface protein mediating monoclonal antibody killing in *Mycoplasma hyorhinis*. Infect. Immun. 55, 1094–1100.

Tardy, F., Mercier, P., Solsona, M., Saras, E., Poumarat, F., 2007. *Mycoplasma mycoides* subsp. *mycoides* biotype large colony isolates from healthy and diseased goats: prevalence and typing. Vet. Microbiol. 121, 268–277.

Thiaucourt, F., Bolske, G., 1996. Contagious caprine pleuropneumonia and other pulmonary mycoplasmoses of sheep and goats. Rev. Sci. Tech. 15, 1397–1414.

Thiaucourt, F., Manso-Silvan, L., Salah, W., Barbe, V., Vacherie, B., Jacob, D., Breton, M., Dupuy, V., Lomenech, A.M., Blanchard, A., Sirand-Pugnet, P., 2011. *Mycoplasma mycoides*, from "*mycoides* Small Colony" to "*capri*" A microevolutionary perspective. BMC Genomics 12, 114–133.

Vilei, E.D., Korczak, B.M., Frey, J., 2006. *Mycoplasma mycoydes* subsp. *capri* and *Mycoplasma mycoides* subsp. *mycoides* LC can be grouped into a single subspecies. Vet. Res. 37, 779–790.

Wallbrandt, P., Tegman, V., Jonsson, B.H., Wieslander, A., 1992. Identification and analysis of the genes coding for the putative pyruvate dehydrogenase enzyme complex in *Acholeplasma laidlawii*. J. Bacteriol. 174, 1388–1396.

Zhao, P., He, Y., Chu, Y., Gao, P., Zhang, X., Zhang, N., Zhao, H., Zhang, K., Lu, Z., 2012. Identification of novel immunogenic proteins in *Mycoplasma capricolum* subsp. *capripneumoniae* strain M1601. J. Vet. Med. Sci. 74, 1109–1111.