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Proteomic Analysis of *Mycoplasma gallisepticum* Vaccine Strain F

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Abstract: The persistence and displacement abilities of the *Mycoplasma gallisepticum* vaccine strain F (F-strain) are well documented. Understanding the mechanism(s) of colonization and persistence of F-strain will aid in the current intervention strategies to diagnose and control MG infections in poultry. In the present study, phase partition and liquid chromatography along with electrospray mass spectrometry were used to evaluate the proteome of F-strain. A total of 586, 478 and 339 proteins were recognized from whole cell lysate (total protein), aqueous (cytosolic) and detergent phases (membrane), respectively. The proteins identified with the database searches were then grouped into three categories: (1) proteins from the membrane phase and found in the total proteins (TM); (2) proteins from the cytosolic phase and found in the total proteins (TC) and (3) proteins derived from the membrane and cytosolic phases and found in the total proteins (TMC). There were a total of 93 (33 as putative membrane proteins predicted by SOSUI), 207(13) and 79(6) proteins in the TM, TC and TMC, respectively. The identified proteins were distributed among John Craig Venter Institute (JCVI) categories, with the majority predicted to be involved in protein synthesis. Investigation of the *Mycoplasma gallisepticum* F-strain proteome may aid in the identification and characterization of F-strain proteins that are important in host colonization.

Key words: *Mycoplasma gallisepticum*, F-strain, mass spectrometry, proteomics

INTRODUCTION

Infection with the poultry pathogen *Mycoplasma gallisepticum* (MG) results in reduced egg production and the condemnation of meat products at poultry processing plants. These infections result in chronic respiratory disease characterized by inflammatory pathology in the trachea, as well as in the pulmonary air-sacs (Branton *et al*., 1988; Yoder, 1991; Whithear, 1996).

The four live attenuated MG vaccine strains used to control losses due to MG include the ts-11 (Merial Select Laboratories, Gainesville, GA) and 6/85 (Intervet Inc., Millsboro, DE) strains as well two related types of the F-strain [Myco F (Zoetis, Florham Park, NJ) and MG F (Lohmann Animal Health Int., Winslow, ME) (Evans *et al*., 2012; Purswell *et al*., 2012)]. The F-strain MG vaccine was the first vaccine developed (Branton *et al*., 1999) and is frequently used by the layer industry in the United States (Abd-el-Motelib and Kleven, 1993).

The F-strain vaccine is not fully avirulent and cannot be used in young chickens or in turkeys (Lin and Kleven, 1984). To that end, two milder MG vaccine strains (ts-11 and 6/85) were developed for broader use in poultry (Whithear *et al*., 1990; Evans and Hafez, 1992; Ley *et al*., 1993). Additionally, the ts-11 and 6/85 vaccines do not demonstrate bird to bird transmission in field settings (Ley *et al*., 1997). Nevertheless, the level of protection afforded by the ts-11 and 6/85 strains appears to be somewhat less than that by the F-strain (Abd-el-Motelib and Kleven, 1993; Ley *et al*., 1997). Also, reports of MG outbreaks in layer flocks previously vaccinated with ts-11 or 6/85 have resulted in revaccination of these flocks with F-strain (Gingerich, 2002). Moreover, the F-strain vaccine has been demonstrated to persist in the host as well as to displace virulent strains of MG (Kleven *et al*., 1990; Kleven *et al*., 1998). These findings may result from the ability of the F-strain to persist for the life of the vaccinated animal (Kleven *et al*., 1998).

We hypothesize that understanding the mechanism(s) of colonization and persistence of F-strain will aid in the current intervention strategies to diagnose and control MG infections in poultry. Therefore, the objective of this study is to identify and characterize the proteome of F-strain. This information can be used in future studies to identify protein candidates important in host colonization. In the present study, mycoplasma proteins separated by detergent phase partition were identified using liquid chromatography along with electrospray mass spectrometry.

MATERIALS AND METHODS

Mycoplasma strain and growth conditions: The F-strain of *M. gallisepticum* was obtained from Dr. S. H. Kleven (University of Georgia) and was inoculated into Frey's

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medium containing 12% swine serum (Frey *et al*., 1968) and grown at 37°C under anaerobic conditions.

Extraction and trypsin digestion of mycoplasma proteins: The isolation of membrane and cytosolic proteins was conducted as described by Wan *et al*. (2004). Briefly, F-strain mycoplasma cells were collected from a 60 mL volume of culture by centrifugation at 3,000 x g for 10 min. The cells were washed 3 times with cold (4°C) phosphate buffered saline (PBS). The final cell pellet was resuspended in 900 uL of PBS, supplemented with 100 mM phenylmethylsulfonyl fluoride (PBS-PMSF). Triton X-114 (10%) was added to the cells (final concentration of 1%) and the mixture incubated on ice for 2.5 h. After incubation, the mixture was centrifuged at 12,000 x g at 4°C for 5 min and the supernatant harvested. An aliquot of the supernatant was collected to designate the whole cell lysate fraction (total protein). The supernatant was then incubated at 37°C for 5 min, followed by centrifugation for 3 min at 8,000 x g to establish the detergent (membrane proteins) and aqueous (cytosolic proteins) phases. The detergent phase was collected and extracted 3 additional times with 9 volumes of PBS-PMSF. The aqueous phase was collected and extracted 3 times with a 1% final concentration of Triton X-114. Equal amounts of mycoplasma protein were collected from each fraction by precipitation with 50% trichloroacetic acid on ice. The proteins from each fraction were then digested overnight at 37°C with 100 ng of trypsin (50:1 ratio of substrate to enzyme). Protein fractions prepared from two separate cultures of the F-strain were digested with trypsin for analysis by mass spectrometry.

Mass spectrometry analysis: The peptides generated in the trypsin digest reactions were purified using a MicroTrap C18 column (Michrom BioResources, Inc., Auburn, CA), dried in a vacuum centrifuge and then solubilized with 0.1% formic acid in preparation for mass spectrometry analysis. The peptides were fractionated on a reverse-phase liquid chromatography column and then analyzed with an electrospray ion trap mass spectrometer as previously described by Collier *et al*. (2006).

Protein identification and data analysis: The turboSEQUEST™ software (Thermo Electron Corporation, San Jose, USA) was used to compare the mass spectra from the F-strain peptides with the mass spectra generated from translated sequences of the MG strain R_{low} genome (Papazisi *et al*., 2003). The peptide amino acid sequences obtained with the turbo SEQUEST™ analysis were also evaluated with BLASTp searches against mycoplasma proteins at the database http://www.ncbi.nlm.nih.gov/Entrez. The proteins identified with the database searches were then grouped into three categories (Wan *et al*., 2010): (1) proteins from the membrane phase and found in the total proteins (TM); (2) proteins from the cytosolic phase and found in the total proteins (TC) and (3) proteins derived from the membrane and cytosolic phases and found in the total proteins (TMC). The proteins present in membrane or cytosolic samples, but not observed in the total protein sample were excluded from further analysis (Wan *et al*., 2010). The John Craig Venter Institute (JCVI) comprehensive microbial resource database (http://cmr.jcvi.org/tigr-scripts/CMR/CmrHomePage.cgi) was used to predict the function of the proteins observed in this study (Davidsen *et al*., 2010). The subcellular location of the proteins was predicted *via* SOSUI (Mitaku *et al*., 2002).

RESULTS AND DISCUSSION

In the present study, a total of 586, 478 and 339 proteins were recognized from the whole cell lysate (total protein), aqueous (cytosolic) and detergent phases (membrane), respectively (Fig. 1). As shown in Fig. 1 and Table 1, there were a total of 93 (33 as putative membrane proteins predicted by SOSUI), 207(13) and 79(6) proteins in the TM, TC and TMC, respectively. As shown in Tables 1 and 2, the identified proteins were distributed among JCVI categories, with the majority predicted to be involved in synthesis of the macromolecular constituents of the cell. This result agrees with the report of Demina *et al*. (2009) wherein of the 446 MG S6 strain proteins identified, most were predicted to be involved in the synthesis of proteins. In addition, Fisunov *et al*. (2011) reported that the core proteome constituents of MG are involved in replication, transcription, translation and minimal metabolism.

Surface proteins involved in MG colonization: The roles of PvpA, VlhA, MGC2, GapA and CrmA proteins in the cytadherence and virulence of MG is well documented (Hnatow *et al*., 1998; Boguslavsky *et al*., 2000; Papazisi *et al*., 2000; Liu *et al*., 2001; Liu *et al*., 2002; Papazisi *et al*., 2002; Mudahi-Orenstein *et al*., 2003; Winner *et al*., 2003; Indikova *et al*., 2013). This is in contrast to the few documents that describe the comparable role of MslA (Szczepanek *et al*., 2010a; Masukagami *et al*., 2013).

The coexpression of GapA and CrmA are necessary for host colonization and the disease causing properties of the R_{low} strain of MG (Papazisi *et al*., 2000; Papazisi *et al*., 2002). Comparative genomic analysis showed that gapA and crmA were highly divergent between R_{low} and F-strain (Szczepanek *et al*., 2010a). The VlhA and PvpA proteins are phase variable and may therefore contribute to the persistence of MG in the respiratory tract (Boguslavsky *et al*., 2000; Liu *et al*., 2001; Liu *et al*., 2002; Winner *et al*., 2003). Transcriptomic analysis showed that the expression levels of VlhA3.03, PvpA and the immunogenic lipoprotein MslA was higher in R_{low}

Table 1: Proteins identified in membrane, cytosolic and total protein of *Mycoplasma galliseptiucm* F-strain using mass spectrometry

	JCVI cellular role category[a]	------ Peptide hits (PH)[b] -----				
Acession number	Protein	T	M	C	Species[c]	Location[d]
Amino acid biosynthesis						
NP_853074.2	Papain family protease	3		8	MGA	M (2)
NP_110175.1	Cysteine desulfurase	1		1	MPN	S
NP_853072.2	Serine hydroxymethyltransferase	3		5	MGA	S
Cofactor biosynthesis						
NP_975116.1	Riboflavin kinase/FAD synthetase	2		2	MMY	S
NP_853072.2	Serine hydroxymethyltransferase	3		5	MGA	S
Cell envelope						
NP_072726.1	ABC transporter permease	1		1	MGE	M (7)
NP_072733.1	ATPase P transporter	1	3		MGE	M (9)
NP_326338.1	Conserved hypothetical protein	2		2	MPU	S
NP_109998.1	Cytadherence high molecular weight protein 2	1		5	MPN	S
NP_852962.2	Cytadhesin protein GapA	16	2		MGA	M (2)
Q49379.1	Cytadhesin protein GapA	17	3		MGS6	M (2)
NP_852961.2	Cytadhesin protein MGC2	2	8		MGA	M (2)
NP_852963.1	**Cytoadhesin CrmA**	**29**	**15**	**1**	**MGA**	**M (2)**
BAA94278.1	**Cytoadhesin protein CrmA, putative**	**19**	**12**	**1**	**MGF**	**S**
BAA94277.1	**Cytoadhesin protein CrmA, putative**	**17**	**11**	**1**	**MGS6**	**S**
NP_852791.1	Inner membrane protein translocase component YidC, putative	2	2		MGA	M (6)
NP_326436.1	Lipoprotein	2		1	MPU	M (1)
NP_073008.2	**Lipoprotein, putative**	**1**	**1**	**1**	**MGE**	**M (1)**
NP_072961.1	Major facilitator superfamily protein	2		1	MGE	M (11)
NP_852814.2	**Mycoplasma specific lipoprotein A (*mslA*)**	**67**	**181**	**6**	**MGA**	**S**
AAF67108.1	PvpA protein, putative	2	2		MGA	M (2)
AAD53533.1	Variable surface lipoprotein	3	3		MBV	S
NP_853206.1	VlhA.1.03 variable lipoprotein	4	6		MGA	S
NP_853237.2	VlhA.2.01 variable lipoprotein	1	21		MGA	S
NP_853372.2	VlhA.3.02 variable lipoprotein	17	30		MGA	S
NP_853373.1	VlhA.3.03 variable lipoprotein	36	91		MGA	S
AAC69269.1	VlhA.3.03 variable lipoprotein	36	91		MGPG31	S
AAA02996.1	VlhA.3.03 variable lipoprotein	28	61		MGS6	S
NP_853375.1	VlhA.3.05 variable lipoprotein	2	9		MGA	M (1)
AAF91413.1	VlhA.3.07 variable lipoprotein	67	206		MGHS	S
AAF91412.1	VlhA.3.08 variable lipoprotein	2		2	MGHS	S
AAF29523.1	VlhA.3.08 variable lipoprotein, putative	2		2	MGF	S
NP_852977.1	VlhA.4.01 variable lipoprotein	5	38		MGA	S
NP_852982.1	VlhA.4.07 variable lipoprotein	5	13		MGA	S
NP_853122.1	VlhA.5.05 variable lipoprotein	33	73		MGA	S
AAC69274.1	VlhA.5.05 variable lipoprotein	33	72		MGPG31	S
NP_853125.1	VlhA.5.08 variable lipoprotein	8	17		MGA	S
AAO67730.1	**Cytoadhesin protein GapA, putative**	**2**	**7**	**2**	**MGF**	**M (2)**
CAF32691.1	Variable membrane protein precursor	1		3	MHO	S
AAF29525.1	**VlhA.3.07 variable lipoprotein**	**45**	**95**	**1**	**MGF**	**S**
NP_853377.1	**VlhA.3.07 variable lipoprotein**	**67**	**206**	**1**	**MGA**	**S**
NP_853378.1	**VlhA.3.08 variable lipoprotein**	**2**	**1**	**2**	**MGA**	**S**
Cellular processes						
NP_853069.2	**Organic hydroperoxide resistance protein (OsmC)**	**20**	**3**	**24**	**MGA**	**S**
NP_852808.2	Cell division protease FtsH-like protein	7		9	MGA	M (3)
NP_852952.1	Chromosome segregation ATPase SMC	1		3	MGA	M (1)
NP_975109.1	Hemolysin A	2	2		MMY	S
Central intermediary metabolism						
NP_110175.1	Cysteine desulfurase	1		1	MPN	S
NP_853402.1	**HAD-superfamily hydrolase Cof**	**9**	**1**	**9**	**MGA**	**S**
AAN64188.1	**HAD-superfamily hydrolase Cof**	**8**	**1**	**8**	**MGA5969**	**S**
NP_853057.2	Inorganic pyrophosphatase	14		16	MGA	S
NP_757826.1	Inorganic pyrophosphatase	1		1	MPE	S
NP_975265.1	Pyruvate dehydrogenase E1 component beta subunit	2		1	MMY	S
NP_975517.1	N-acetylglucosamine-6-phosphate deacetylase	2	1		MMY	S
NP_853403.2	Phosphate acetyltransferase	24		25	MGA	S
DNA metabolism						
NP_853506.1	DNA gyrase subunit B	8		11	MGA	S
NP_072663.1	DNA gyrase subunit B	2		1	MGE	S
NP_109691.1	DNA gyrase subunit B	2		1	MPN	S
NP_757586.1	DNA polymerase I	2		3	MPE	S
NP_757585.1	DNA polymerase III DnaE	2		1	MPE	S
NP_758303.1	DNA polymerase III subunit alpha	2		1	MPE	S

Table 1: Continued

	JCVI cellular role category[a]	------ Peptide hits (PH)[b] -----				
Acession number	Protein	T	M	C	Species[c]	Location[d]
NP_758197.1	DNA polymerase III subunits gamma and tau	1		2	MPE	S
NP_852876.2	**DNA polymerase III, subunit alpha, Gram-positive type**	**1**	**1**	**1**	**MGA**	**S**
NP_853416.1	DNA topoisomerase 1	4		10	MGA	S
AAF19045.2	DNA topoisomerase I	4		10	MGA5969	S
NP_757800.1	DNA topoisomerase I	1		1	MPE	S
YP_016157.1	DNA topoisomerase IV subunit A	1		1	MMO	S
NP_853203.2	**DNA-binding protein HU superfamily**	**5**	**2**	**16**	**MGA**	**S**
NP_758297.1	Excinuclease ABC subunit A	1		1	MPE	S
NP_852869.2	Excinuclease ABC subunit A UvrA	1		2	MGA	S
NP_852820.2	Excinuclease ABC subunit B	1		1	MGA	S
YP_016269.1	Excinuclease ABC subunit B	1		1	MMO	S
AAK94953.1	IS30-like protein, putative	1	4		MBV	S
NP_853464.2	Type I restriction-modification system methyltransferase subunit	2	1		MGA	S
NP_853508.2	**DNA polymerase III, subunit beta**	**7**	**1**	**11**	**MGA**	**S**
NP_975657.1	Primosomal protein	2		2	MMY	S
NP_757678.1	Recombinase	1		1	MPE	S
Energy metabolism						
NP_853262.2	6-Phosphofructokinase (pfkA)	5		10	MGA	S
NP_853269.2	**Acetate kinase (*ackA*)**	**20**	**2**	**24**	**MGA**	**S**
NP_853443.2	Fructose-bisphosphate aldolase	18		34	MGA	S
AAL91129.1	Fructose-bisphosphate aldolase	18		34	MGA5969	S
NP_853418.2	Glucose-6-phosphate isomerase	7		32	MGA	S
AAF36765.1	Glucose-6-phosphate isomerase, putative	7		32	MGA5969	S
NP_853093.1	**Glyceraldehyde-3-phosphate dehydrogenase (gapd)**	**44**	**23**	**35**	**MGA**	**S**
NP_852917.2	Glyceraldehyde-3-phosphate dehydrogenase (putA)	8		20	MGA	S
NP_325955.1	Glycerol-3-phosphate dehydrogenase	2		2	MPU	S
NP_852855.1	**L-lactate dehydrogenase (ldh)**	**54**	**2**	**120**	**MGA**	**S**
NP_853094.1	**Phosphoglycerate kinase**	**67**	**18**	**119**	**MGA**	**S**
NP_853362.1	Phosphoglyceromutase	7		15	MGA	S
NP_853264.1	Pyruvate dehydrogenase E3 component dihydrolipoamide dehydrogenase	15		20	MGA	M (2)
NP_853363.1	Triosephosphate isomerase	13		24	MGA	S
NP_326101.1	ATP synthase F0F1 subunit B	1		1	MPU	M (1)
NP_853088.1	ATP synthase F0F1 subunit beta	6		6	MGA	S
NP_073072.1	ATP synthase F0F1 subunit beta	3		1	MGE	S
NP_757448.2	ATP synthase F0F1 subunit beta	2		1	MPE	S
NP_110287.1	ATP synthase F0F1 subunit beta	3		1	MPN	S
NP_757447.1	ATP synthase F0F1 subunit gamma	1	1		MPE	S
AAB95409.1	**L-lactate dehydrogenase**	**54**	**2**	**124**	**MGA5969**	**S**
NP_853267.1	**Pyruvate dehydrogenase E1 component alpha subunit**	**2**	**1**	**50**	**MGA**	**S**
NP_853266.2	**Pyruvate dehydrogenase E1 component beta subunit**	**47**	**3**	**80**	**MGA**	**S**
YP_016280.1	Pyruvate dehydrogenase E1 component beta subunit	2		1	MMO	S
NP_975265.1	Pyruvate dehydrogenase E1 component beta subunit	2		1	MMY	S
NP_853265.1	**Pyruvate dehydrogenase E2 component, dihydrolipoamide acetyltransferase**	**53**	**8**	**79**	**MGA**	**S**
NP_326514.1	Ribulose-phosphate 3-epimerase	2		1	MPU	S
NP_853415.2	Thioredoxin (trxA)	9		39	MGA	S
AAF19044.1	Thioredoxin (trxA)	4		25	MGA5969	S
NP_853243.2	Thioredoxin reductase (trxB)	3		7	MGA	S
NP_852812.2	Transketolase (tktA)	3		7	MGA	S
NP_853095.1	Amino acid permease	1	4		MGA	M(12)
NP_853084.2	ATP synthase F0F1 subunit B	1	2		MGA	M (2)
NP_853439.2	**ATP synthase F0F1 subunit beta**	**6**	**5**	**3**	**MGA**	**S**
NP_852797.2	Glycerol kinase (glpK)	2	1		MGA	S
NP_853403.2	Phosphate acetyltransferase	24		25	MGA	S
NP_853287.1	Phosphopyruvate hydratase (enolase)	77	2		MGA	S
Metabolism of phospholipids and fatty acid						
NP_853306.2	Azoreductase (acpD)	6		9	MGA	S
NP_853186.2	1-acyl-sn-glycerol-3-phosphate acyltransferase	5	4		MGA	M (1)
NP_853187.2	Holo-[acyl-carrier-protein] synthase (acpS)	4		3	MGA	S
Hypothetical proteins						
NP_852913.1	Beta-galactosidase-like protein	1		3	MGA	S
NP_853188.2	Conserved hypothetical protein	2		2	MGA	S
NP_853013.2	Conserved hypothetical protein	3		8	MGA	S
NP_852955.1	**Conserved hypothetical protein**	**16**	**1**	**25**	**MGA**	**S**
NP_853114.2	**Conserved hypothetical protein**	**3**	**4**	**1**	**MGA**	**S**
NP_325891.1	Conserved hypothetical protein	1		3	MPU	M (4)

Table 1: Continued

	JCVI cellular role category[a]	---------- Peptide hits (PH)[b] ----------				
Acession number	Protein	T	M	C	Species[c]	Location[d]
NP_852915.2	**Conserved hypothetical protein**	**4**	**1**	**5**	**MGA**	**S**
NP_853304.2	Hypothetical protein	6		10	MGA	S
NP_853474.1	Hypothetical protein	1		1	MGA	S
NP_853485.1	Hypothetical protein	1		1	MGA	S
NP_853110.2	Hypothetical protein	4		4	MGA	S
NP_853298.2	Hypothetical protein	8	39		MGA	S
NP_853318.2	Hypothetical protein	11	59		MGA	S
NP_853339.2	Hypothetical protein	4	42		MGA	M (1)
NP_853340.2	Hypothetical protein	9	52		MGA	S
NP_853345.2	Hypothetical protein	3	14		MGA	S
NP_853440.2	Hypothetical protein	1	4		MGA	S
NP_853451.2	Hypothetical protein	1	8		MGA	M (2)
NP_853472.2	Hypothetical protein	2	11		MGA	M (4)
NP_853488.2	Hypothetical protein	2	8		MGA	S
NP_853499.2	Hypothetical protein	2	1		MGA	M (4)
NP_852799.2	Hypothetical protein	3	20		MGA	S
NP_852899.1	Hypothetical protein	14	94		MGA	S
NP_852933.1	Hypothetical protein	7	23		MGA	M (1)
NP_852934.2	Hypothetical protein	2	4		MGA	M (12)
NP_852938.2	Hypothetical protein	2	4		MGA	M (3)
NP_852970.2	Hypothetical protein	4	7		MGA	M (11)
NP_852988.1	Hypothetical protein	26	128		MGA	S
NP_853027.2	Hypothetical protein	3	38		MGA	S
NP_853098.1	Hypothetical protein	1	25		MGA	M (1)
NP_852936.1	**Hypothetical protein**	**2**	**12**	**1**	**MGA**	**S**
NP_853384.1	**Hypothetical protein**	**6**	**6**	**4**	**MGA**	**M (3)**
NP_073087.2	Hypothetical protein	1		1	MGE	M (2)
NP_072908.1	Hypothetical protein	1	1		MGE	M (2)
YP_016070.1	Hypothetical protein	2		2	MMO	S
NP_975443.1	Hypothetical protein	1		2	MMY	M (9)
NP_975512.1	Hypothetical protein	1		2	MMY	S
NP_975053.1	Hypothetical protein	1	1		MMY	S
NP_975132.1	Hypothetical protein	1	1		MMY	S
NP_975150.1	Hypothetical protein	2	3		MMY	S
NP_975930.1	**Hypothetical protein**	**2**	**2**	**3**	**MMY**	**S**
NP_757401.1	Hypothetical protein	1		1	MPE	S
NP_757671.1	Hypothetical protein	1		2	MPE	S
NP_757884.1	Hypothetical protein	1		1	MPE	M (1)
NP_757890.1	Hypothetical protein	2		1	MPE	S
NP_757953.1	Hypothetical protein	1		1	MPE	S
NP_758131.1	Hypothetical protein	1		1	MPE	S
NP_758218.1	Hypothetical protein	2		1	MPE	S
NP_110248.1	Hypothetical protein	2		1	MPN	S
NP_110330.1	Hypothetical protein	1		3	MPN	S
NP_853217.2	Phospholipid-binding protein, putative	8		6	MGA	S
NP_853102.2	Pneumoniae-like protein A (plpA)	17	2		MGA	S
NP_853244.1	Transcriptional regulator, putative	1		2	MGA	S
NP_853049.2	Flavodoxin-like protein	4		5	MGA	S
Extrachromosomal element function						
NP_853457.2	CRISPR-associated protein Cas1	1		1	MGA	S
Protein fate						
CAB96372.1	Heat shock protein 60	2		1	MAA	S
CAB96374.1	Heat shock protein 60	2		1	MAT	S
NP_853259.2	Heat shock protein 60	37		68	MGA	S
NP_073065.2	Heat shock protein 60	2		3	MGE	S
CAB96378.1	Heat shock protein 60	2		1	MHJ	S
NP_853235.2	**Leucyl aminopeptidase**	**21**	**1**	**8**	**MGA**	**S**
NP_853074.2	Papain family protease	3	8		MGA	M (2)
NP_853430.2	Preprotein translocase subunit SecE	1	2		MGA	M (1)
AAF36752.1	Preprotein translocase subunit SecE	1	2		MGA5969	M (1)
NP_852813.1	Protein translocase subunit SecA	4	5		MGA	S
NP_853155.2	**Trigger factor (tig)**	**27**	**1**	**43**	**MGA**	**S**
NP_853156.2	ATP-dependent lon protease	17		26	MGA	S
NP_072905.1	ATP-dependent lon protease	2		3	MGE	S
NP_110020.1	ATP-dependent lon protease	2		3	MPN	S
NP_853274.1	Chaperone protein clpB	1		3	MGA	S
NP_853116.1	Cytadherence-associated molecular chaperone TopJ	18		27	MGA	S

Table 1: Continued

	JCVI cellular role category[a]	------ Peptide hits (PH)[b] -------				
Acession number	Protein	T	M	C	Species[c]	Location[d]
NP_110262.1	Heat shock protein 60	2		1	MPN	S
NP_758282.1	Leucyl aminopeptidase	2		1	MPE	S
NP_110261.1	Leucyl aminopeptidase	2		1	MPN	S
P47707.1	Leucyl aminopeptidase, putative	1		1	MSAL	S
NP_853263.1	Lipoate-protein ligase A (lplA)	4		4	MGA	S
NP_326564.1	Molecular chaperone DnaJ	2		3	MPU	S
NP_072972.1	Molecular chaperone DnaK	13		17	MGE	S
NP_975590.1	Molecular chaperone DnaK	9		14	MMY	S
NP_758332.1	Molecular chaperone DnaK	2		2	MPE	S
NP_110122.1	Molecular chaperone DnaK	13		17	MPN	S
NP_326054.1	Molecular chaperone DnaK	6		11	MPU	S
Q2SSB0.1	Molecular chaperone DnaK	9		14	MCP	S
NP_853321.2	**Molecular chaperone Dnak**	**128**	**9**	**167**	**MGA**	**S**
NP_853117.1	Molecular chaperone GrpE	12		5	MGA	S
NP_072863.1	Molecular chaperone GrpE	2	1		MGE	S
NP_853483.2	Peptide methionine sulfoxide reductase msrA	2		7	MGA	S
NP_975100.1	Proline dipeptidase (pepQ)	1	1		MMY	M (1)
Protein synthesis						
NP_852832.2	**30S ribosomal protein S10**	**6**	**1**	**7**	**MGA**	**S**
AAB95386.1	30S ribosomal protein S10	6	1		MGA5969	S
NP_853316.2	30S ribosomal protein S12	10	2		MGA	S
NP_757415.1	30S ribosomal protein S12	1	1		MPE	S
NP_072838.1	30S ribosomal protein S13	2	1		MGE	M (1)
NP_853412.1	30S ribosomal protein S13	2	3		MGA	S
NP_852846.2	**30S ribosomal protein S14 type Z**	**6**	**4**	**1**	**MGA**	**S**
NP_852864.1	30S ribosomal protein S15	1		1	MGA	S
AAF19037.1	30S ribosomal protein S16	4		2	MGA5969	S
NP_853395.1	30S ribosomal protein S18	4		5	MGA	S
NP_852837.1	30S ribosomal protein S19	4		4	MGA	S
NP_853305.1	30S ribosomal protein S2	14		12	MGA	S
NP_072732.1	30S ribosomal protein S2	3		2	MGE	S
NP_109896.1	30S ribosomal protein S2	3		2	MPN	S
NP_852839.2	30S ribosomal protein S3	6	1		MGA	S
O52338.3	30S ribosomal protein S3	6	1		MGA5969	S
NP_853317.1	**30S ribosomal protein S4**	**19**	**12**	**4**	**MGA**	**S**
NP_852850.2	30S ribosomal protein S5	4	2		MGA	S
AAB95404.1	30S ribosomal protein S5	4	2		MGA5969	S
NP_853397.1	30S ribosomal protein S6	6		5	MGA	S
NP_853315.1	30S ribosomal protein S7	7		1	MGA	S
NP_852847.1	30S ribosomal protein S8	3		3	MGA	S
NP_853076.2	30S ribosomal protein S9	4	2		MGA	S
NP_853447.1	**50S ribosomal protein L1**	**15**	**2**	**19**	**MGA**	**S**
NP_852928.3	**50S ribosomal protein L10**	**6**	**3**	**3**	**MGA**	**S**
NP_853446.1	50S ribosomal protein L11	8		21	MGA	S
NP_072743.1	50S ribosomal protein L11	4		6	MGE	S
NP_109907.1	50S ribosomal protein L11	4		6	MPN	S
NP_853075.2	50S ribosomal protein L13	6		4	MGA	S
NP_852851.2	**50S ribosomal protein L15**	**4**	**1**	**4**	**MGA**	**S**
AAB95405.1	**50S ribosomal protein L15**	**4**	**1**	**4**	**MGA5969**	**S**
NP_852840.1	50S ribosomal protein L16	1	1		MGA	S
NP_853406.2	**50S ribosomal protein L19**	**8**	**1**	**3**	**MGA**	**S**
YP_016125.1	50S ribosomal protein L19	2		1	MMO	S
NP_975414.1	50S ribosomal protein L19	1		1	MMY	S
NP_852836.1	50S ribosomal protein L2	8	2		MGA	S
AAB95390.1	50S ribosomal protein L2	8	2		MGA5969	S
NP_852838.1	50S ribosomal protein L22	5		3	MGA	S
NP_852835.2	50S ribosomal protein L23	2		5	MGA	S
NP_852844.1	**50S ribosomal protein L24**	**4**	**1**	**1**	**MGA**	**S**
NP_853151.1	50S ribosomal protein L27	8		5	MGA	S
NP_110015.1	50S ribosomal protein L27	2		1	MPN	S
NP_852841.2	50S ribosomal protein L29	6		2	MGA	S
NP_072822.1	50S ribosomal protein L29	1		1	MGE	S
NP_852833.1	**50S ribosomal protein L3**	**13**	**4**	**8**	**MGA**	**S**
AAB95387.1	**50S ribosomal protein L3**	**9**	**2**	**6**	**MGA5969**	**S**
NP_852918.2	**50S ribosomal protein L31**	**7**	**2**	**1**	**MGA**	**S**
NP_852789.1	50S ribosomal protein L34	3		1	MGA	S
NP_853140.2	**50S ribosomal protein L35**	**5**	**4**	**4**	**MGA**	**S**
P10135.2	50S ribosomal protein L4	5	4		MCP	S

Table 1: Continued

	JCVI cellular role category[a]	------- Peptide hits (PH)[b] -------				
Acession number	Protein	T	M	C	Species[c]	Location[d]
NP_852834.2	**50S ribosomal protein L4**	**10**	**3**	**3**	**MGA**	**S**
NP_975720.1	50S ribosomal protein L4	5	4		MMY	S
NP_852845.2	50S ribosomal protein L5	13	3		MGA	S
AAB95402.1	50S ribosomal protein L6	12		10	MGA5969	S
NP_852848.1	50S ribosomal protein L6	13		12	MGA	S
NP_852929.1	**50S ribosomal protein L7/L12**	**20**	**1**	**11**	**MGA**	**S**
AAN17793.1	50S ribosomal protein L7/L12	6	6		MHO	S
NP_853394.1	50S ribosomal protein L9	8		16	MGA	S
NP_852904.2	Alanyl-tRNA synthetase	3		6	MGA	S
NP_852794.2	Arginyl-tRNA synthetase	2		2	MGA	S
NP_853388.2	Aspartyl/glutamyl-tRNA	3		1	MGA	S
NP_852967.1	Aspartyl-tRNA synthetase	3		6	MGA	S
NP_853495.1	Glutamyl-tRNA synthetase	6		10	MGA	S
NP_853101.2	**Isoleucyl-tRNA synthetase**	**5**	**2**	**1**	**MGA**	**S**
NP_853215.1	Leucyl-tRNA synthetase	4		5	MGA	S
NP_852857.2	Lysyl-tRNA synthetase	6		11	MGA	S
NP_852939.1	**Methionyl-tRNA synthetase**	**5**	**4**	**1**	**MGA**	**S**
NP_853504.2	**Seryl-tRNA synthetase**	**3**	**2**	**2**	**MGA**	**S**
NP_853329.2	Tryptophanyl-tRNA synthetase (trpS)	1		1	MGA	S
AAD10542.1	30S ribosomal protein S13, putative	2	1		MGE	M (1)
Q48979.2	**Aspartyl-tRNA synthetase**	**1**	**1**	**2**	**MCP**	**S**
NP_853314.2	Elongation factor G	28		41	MGA	S
NP_072751.1	Elongation factor G	1		3	MGE	S
NP_975162.1	Elongation factor G	2		2	MMY	S
NP_757417.1	Elongation factor G	2		2	MPE	S
NP_852870.3	**Elongation factor Ts**	**41**	**3**	**85**	**MGA**	**S**
YP_015874.1	Elongation factor Ts	1		2	MMO	S
NP_975587.1	Elongation factor Ts	1		2	MMY	S
NP_326363.1	Elongation factor Ts	1		2	MPU	S
NP_853008.2	**Elongation factor Tu**	**142**	**14**	**210**	**MGA**	**S**
P22679.1	**Elongation factor Tu**	**14**	**7**	**5**	**MHO**	**S**
YP_015921.1	**Elongation factor Tu**	**13**	**7**	**5**	**MMO**	**S**
CAC87988.1	**Elongation factor Tu**	**16**	**9**	**7**	**MMY**	**S**
NP_975163.1	**Elongation factor Tu**	**16**	**9**	**7**	**MMY**	**S**
NP_757418.1	**Elongation factor Tu**	**16**	**7**	**6**	**MPE**	**S**
NP_326236.1	**Elongation factor Tu**	**13**	**7**	**5**	**MPU**	**S**
NP_852872.2	Ribosome-recycling factor	6		9	MGA	S
NP_852957.1	Threonyl-tRNA synthetase	4		12	MGA	S
NP_852897.1	Translation initiation factor IF-2	1		5	MGA	S
NP_853139.2	Translation initiation factor IF-3	6		8	MGA	S
NP_853071.2	**Tyrosyl-tRNA synthetase**	**3**	**3**	**2**	**MGA**	**S**
Nucleotides						
NP_852971.1	Adenine phosphoribosyltransferase	4		5	MGA	S
NP_852853.2	Adenylate kinase	2		9	MGA	S
AAB95407.1	Adenylate kinase	2		8	MGA5969	S
NP_853273.2	Deoxynucleoside kinase (dgk)	1	2		MGA	S
NP_853422.1	Guanylate kinase	1		2	MGA	S
AAF36761.1	Guanylate kinase	1		2	MGA5969	S
NP_853259.2	Heat shock protein 60	37		68	MGA	S
NP_852807.2	**Hypoxanthine-guanine phosphoribosyltransferase**	**2**	**1**	**4**	**MGA**	**S**
NP_853268.1	**NADH oxidase**	**52**	**4**	**76**	**MGA**	**S**
NP_852827.1	Ribonucleoside-diphosphate reductase subunit beta	22		36	MGA	S
NP_072895.1	Ribonucleoside-diphosphate reductase subunit beta	4		4	MGE	S
NP_975825.1	Ribonucleoside-diphosphate reductase subunit beta	5		7	MMY	S
NP_757474.1	Ribonucleoside-diphosphate reductase subunit beta	4		4	MPE	S
NP_110010.1	Ribonucleoside-diphosphate reductase subunit beta	4		4	MPN	S
NP_326370.1	Ribonucleoside-diphosphate reductase subunit beta	4		4	MPU	S
NP_852825.1	Ribonucleotide-diphosphate reductase subunit alpha	4		14	MGA	S
NP_853072.2	Serine hydroxymethyltransferase	3		5	MGA	S
NP_326477.1	Thymidine phosphorylase	2		3	MPU	S
NP_853078.2	Uracil phosphoribosyltransferase	1		3	MGA	S
NP_852871.2	**Uridylate kinase**	**1**	**2**	**1**	**MGA**	**S**
NP_758178.1	Aspartate carbamoyltransferase (pyrB)	1		1	MPE	S
NP_852969.1	CTP synthase	1		2	MGA	S
NP_853365.2	Cytidine deaminase	1		1	MGA	S
NP_853368.2	Purine nucleoside phosphorylase *deoD*-type	2		7	MGA	S
NP_853357.2	Ribose-phosphate pyrophosphokinase (prsA)	5		1	MGA	S

Table 1: Continued

	JCVI cellular role category[a]	------ Peptide hits[b] ------				
Acession number	Protein	T	M	C	Species[c]	Location[d]
Regulatory functions						
NP_853328.2	Regulatory protein spx	2		1	MGA	S
NP_853289.1	Histidine triad (HIT) hydrolase-like protein	1		1	MGA	S
NP_853498.2	HPr kinase/phosphorylase (*hprK*)	2		3	MGA	S
YP_015708.1	Truncated phosphate ABC transporter protein PhoU	1		1	MMO	S
Transcription						
NP_853410.1	**DNA-directed RNA polymerase subunit alpha**	**26**	**5**	**12**	**MGA**	**S**
NP_852995.2	**DNA-directed RNA polymerase subunit beta**	**26**	**1**	**13**	**MGA**	**S**
NP_325984.1	Ribonuclease P protein component (protein C5) (RNase P)	1		1	MPU	S
NP_975697.1	DNA-directed RNA polymerase subunit alpha	2		2	MMY	S
NP_853442.2	DNA-directed RNA polymerase subunit delta	9		9	MGA	S
NP_853240.2	Exoribonuclease R (rnr)	5		9	MGA	S
NP_325994.1	Ribonuclease III	1	1		MPU	S
AAB40951.1	**RNA polymerase subunit beta**	**26**	**1**	**13**	**MGA5969**	**S**
NP_852974.1	Transcription elongation factor *greA*	6		7	MGA	S
NP_852895.2	Transcription elongation factor NusA	1		5	MGA	S
Transport and binding proteins						
NP_853371.2	ABC transporter ATP-binding protein	1	2		MGA	S
NP_975321.1	ABC transporter ATP-binding protein	1		2	MMY	S
NP_852884.2	ABC transporter protein, putative	1	1		MGA	M (7)
NP_853301.2	Dipeptide/oligopeptide/nickel ABC transporter permease DppC/OppC	2	6		MGA	M (5)
NP_852800.1	Ferritin-like protein	7	1		MGA	S
NP_072961.1	Major facilitator superfamily protein	2		1	MGE	M (11)
NP_853068.2	Multidrug ABC transporter permease component, putative	4	8		MGA	M (5)
NP_853067.2	**Multidrug-like ABC transporter ATP-binding protein**	**7**	**9**	**3**	**MGA**	**S**
NP_975934.1	Oligopeptide ABC transporter ATP-binding protein	2	2		MMY	S
NP_853303.2	Oligopeptide ABC transporter solute binding protein OppA, putative	13	58		MGA	M (1)
NP_852862.2	**Phosphoenolpyruvate-protein kinase**	**9**	**1**	**17**	**MGA**	**S**
NP_975272.1	**Phosphoenolpyruvate-protein phosphotransferase**	**1**	**2**	**3**	**MMY**	**S**
NP_852916.2	**PTS system glucose-specific transporter subunit IIABC**	**39**	**64**	**9**	**MGA**	**M (11)**
NP_853248.2	Spermidine/putrescine ABC transporter PotD, putative	1	21		MGA	M (1)
NP_072726.1	ABC transporter permease	1		1	MGE	M (7)
NP_072733.1	ATPase P transporter	1	3		MGE	M (9)
NP_853295.2	Dipeptide/oligopeptide/nickel ABC transporter ATP-binding protein	3		2	MGA	S
NP_853296.2	**Dipeptide/oligopeptide/nickel ABC transporter ATP-binding protein**	**1**	**1**	**1**	**MGA**	**S**
NP_326470.1	Maltodextrin ABC transporter permease MALC	1	1		MPU	M (10)
NP_852811.2	Phosphocarrier protein HPr	7		9	MGA	S
NP_109741.1	Phosphocarrier protein HPr	3		4	MPN	S
NP_853450.2	**PTS system fructose-specific enzyme IIABC component**	**13**	**19**	**3**	**MGA**	**M (9)**
NP_853251.2	Spermidine/putrescine transport ATP-binding protein PotA	12		14	MGA	S
NP_758245.1	Spermidine/putrescine transport ATP-binding protein PotA	5		3	MPE	S
NP_853063.1	SufB-like protein, putative transport protein	2		1	MGA	S
NP_853059.1	SufC-like ABC transporter ATP-binding protein	2		1	MGA	S
NP_853214.2	Trk system potassium uptake proteinTrkA	1		1	MGA	S
NP_110148.1	Trk-type K+ transport systems membrane protein	1		1	MPN	M (11)
NP_975044.1	ABC transporter permease	1		1	MMY	M (8)
NP_853300.1	Dipeptide/oligopeptide/nickel ABC transporter ATP-binding protein DppD/OppD	2	3	1	MGA	S
Unclassified						
NP_853456.2	**CRISPR-associated protein, Csn1 family**	**5**	**1**	**16**	**MGA**	**S**
NP_853103.1	Cytadherence-associated protein Hlp2	2		1	MGA	S
NP_853285.1	**Cytadherence-associated protein, putative**	**42**	**1**	**2**	**MGA**	**S**
NP_852943.2	Glutamine amidotransferase domain-containing protein	2		2	MGA	S
NP_853380.1	Macrophage-activating lipoprotein-like protein	22	98		MGA	S
AAL58981.1	Macrophage-activating lipoprotein-like protein	15	77		MGS6	S
NP_853333.2	**Cytadherence-associated, putative**	**40**	**1**	**1**	**MGA**	**S**
NP_852877.2	Helicase superfamily protein, putative	2		1	MGA	S
NP_326486.1	Subtilisin: serine protease	2		1	MPU	S
Unknown function						
NP_853218.2	Aldo/keto reductase family protein	4		4	MGA	S
NP_853277.2	**Dihydroxyacetone kinase-like protein (DAK2)**	**5**	**2**	**1**	**MGA**	**S**
AAT27605.1	**GTP-binding protein, putative**	**2**	**1**	**2**	**MMO**	**S**
NP_853192.1	LemA-family protein	4	16		MGA	M (1)
P43041.1	RNase M5; Ribosomal RNA terminal maturase M5	1	3		MCP	S
NP_758030.1	ATP/GTP-binding protein	2		2	MPE	S
NP_853092.2	DegV-like protein	5		4	MGA	S
NP_852819.2	Exopolyphosphatase-related protein	1		4	MGA	S
NP_853335.2	GTP-binding protein LepA	1		2	MGA	S

Table 1: Continued

	JCVI cellular role category[a]	------- Peptide hits[b] --------				
Acession number	Protein	T	M	C	Species[c]	Location[d]
NP_853444.1	GTP-dependent nucleic acid-binding protein EngD	3		11	MGA	S
NP_853331.2	HAD superfamily hydrolase Cof	1		1	MGA	S
NP_852802.2	Hydrolase	2		1	MGA	S
NP_853311.2	Osmotically inducible protein C (OsmC)	4		13	MGA	S
NP_852824.2	Phosphate ABC transporter protein PhoU	2		3	MGA	S
NP_853421.2	PP2C-like serine/threonine protein phosphatase	2		2	MGA	S
AAF36762.1	Serine/threonine phosphatases, putative	2		2	MGA5969	S

[a]JCVI category provides the function of the protein

[b]No. of peptides identified in the total (T), membrane (M) and cytosolic (C) phases of the Triton X-114 extraction. The proteins identified in TM and TMC are highlighted in gray and bold, respectively

[c]The mycoplasma species in which homologs of the F-strain proteins were found. MAA: *Mycoplasma agalactiae*; MAT: *Mycoplasma arthritidis*; MBV: *Mycoplasma bovis* PG45; MCP: *Mycoplasma capricolum*; MGA5969: *Mycoplasma gallisepticum* A5969; MGF: *Mycoplasma gallisepticum* F; MGHS: *Mycoplasma gallisepticum* HS; MGPG31: *Mycoplasma gallisepticum* PG31; MGA: *Mycoplasma gallisepticum* R; MGS6: *Mycoplasma gallisepticum* S6; MGE: *Mycoplasma genitalium*; MHO: *Mycoplasma hominis*; MHJ: *Mycoplasma hyopneumoniae* J; MMO: *Mycoplasma mobile*; MMY: *Mycoplasma mycoides* subsp. *mycoides* SC PG1; MPE: *Mycoplasma penetrans*; MPN: *Mycoplasma pneumonia*; MPU: *Mycoplasma pulmonis*; MSAL: *Mycoplasma salivarium*

[d]Subcellular location of the identified proteins; membrane (M) or cytosolic (C). Listed in parenthesis is the predicted helix number for each membrane protein

than F-strain (Szczepanek *et al*., 2010b). In addition, MslA mutants of MG R_{low} demonstrated a lower ability to colonize the trachea of birds (Szczepanek *et al*., 2010b). More recently, studies conducted by Masukagami *et al*. (2013) suggested that MslA is a nucleic acid binding protein that delivers oligonucleotides to an exonuclease that produce nucleotides for transport by an ABC transporter; and is necessary for colonization by some attenuated MG strains only when nucleotide concentrations become limiting. In the present study, F-strain expresses homologs to GapA and CrmA proteins found in the R_{low}, S6 and F strains (Table 1). The homologs GapA and CrmA were identified in both TC and TMC (Table 1). In addition, the F-strain expresses homologs to the VlhA proteins found in the R_{low}, PG31, S6, HS and F strains (Table 1). Homologs to the PvpA and MslA proteins in the R_{low} strain of MG were identified. The homologs PvpA and MslA were identified in TC and TMC, respectively (Table 1). Using gene sequencing and comparative genomic hybridization Szczepanek *et al*. (2010b) showed that the F-strain contained fewer intact *vlhA* genes than the R_{low} and that extreme variation existed between gene complements. As shown in Table 1, 11 homologs of VlhA proteins were identified among the TM, TC and TMC. However, in the detergent phase 19 homologous VlhA proteins were identified (data not shown). The VlhA gene products expressed by the F-strain could represent an important source of antigenic variation and this may play an important role in host colonization (Glew *et al*., 2000).

Internal proteins involved in MG colonization: A number of studies suggest that internal proteins, such as those involved in energy metabolism (Lpd and enolase) and cellular processes (OsmC) may play a role in the colonization and virulence of MG (Hudson *et al*., 2006; Jenkins *et al*., 2007; Chen *et al*., 2011).

Table 2: Number of proteins found in the different functional categories as predicted with the JCVI comprehensive microbial resource database

JCVI cellular role category	Triton 114 soluble proteins	Triton 114 insoluble proteins
Amino acid biosynthesis	2	1
Cofactors biosynthesis	2	0
Cell envelope	17	29
Cellular processes	3	2
Central intermediary metabolism	7	3
DNA metabolism	20	5
Energy metabolism	32	14
Metabolism of phospholipid and fatty acid	2	1
Hypothetical proteins	30	30
Extrachromosomal element function	1	0
Protein fate	27	9
Protein synthesis	73	46
Nucleotides	24	4
Regulatory functions	4	0
Transcription	9	4
Transport and binding proteins	19	17
Unclassified	7	5
Unknown function	14	4

Hudson *et al*. (2006) showed using signature sequence mutagenesis that insertion of a transposon in the coding sequence of Lpd resulted in reduced virulence of the MG mutant Mg7. The cytosolic proteins enolase and OsmC have been shown to mediate adherence by binding to host plasminogen and heparin, respectively (Jenkins *et al*., 2007; Chen *et al*., 2011; Furnkranz *et al*., 2013). In addition, the hypothetical protein PlpA has been shown to bind the extracellular matrix protein fibronectin (May *et al*., 2006). Also, studies conducted by Jenkins *et al*. (2008) suggested that OsmC may protect mycoplasma from reactive oxygen species produced by innate immune cells during infection. It is possible that these proteins may not only aid in the colonization of host cells, but also in the spreading of MG during infection (May *et al*., 2006; Jenkins *et al*., 2007; Chen *et al*., 2011; Tulman *et al*., 2012; Furnkranz *et al*., 2013). In the present study F-strain expresses homologs of the Lpd,

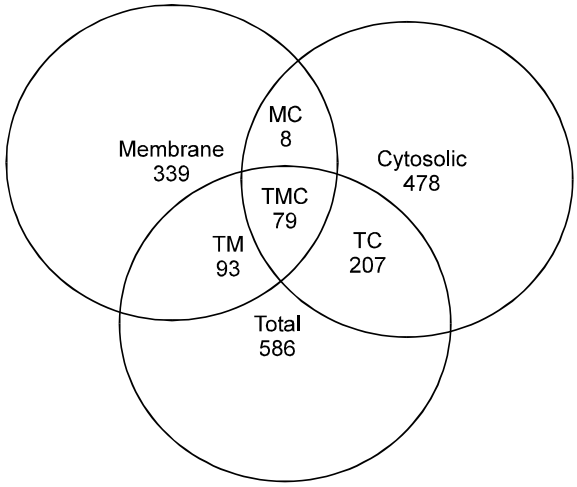

Fig. 1: Number of total proteins identified that partitioned into the membrane (M) or cytosolic (c) phases with Triton X-114 extraction

enolase, OsmC and PlpA proteins found in R_{low} (Table 1). The homologs Lpd, enolase, OsmC and PlpA were identified in TC, TM, TMC and TC, respectively (Table 1). These results are not surprising, Collier *et al.* (2006) showed using western blotting and proteomics that F-strain expressed homologs of the internal proteins fructose-bisphosphate aldolase and MsrA peptide methionine protein, which are also found in R_{low}. Furthermore, these proteins were also found in the TC phases of the present study (Table 1).

Conclusions: In the present study, the surface and internal proteins of F-strain were identified using proteomic analysis. These findings, taken together with the results of others (Collier *et al.*, 2006; Hudson *et al.*, 2006; Jenkins *et al.*, 2007; Jenkins *et al.*, 2008; Chen *et al.*, 2011) show that some proteins which would be predicted to be expressed intracellularly, could also be associated with the surface of the cell. In addition, some of the proteins identified in the F-strain that are important in cellular metabolism may also have a role in host colonization (Chen *et al.*, 2011). In summary, this study has identified a number of gene products which would be candidates for mediating the colonization and host adaptation properties of F-strain.

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REFERENCES

Abd-el-Motelib, T.Y. and S.H. Kleven, 1993. A comparative study of Mycoplasma gallisepticum vaccines in young chickens. Avian Dis., 37: 981-987.

Boguslavsky, S., D. Menaker, I. Lysnyansky, T. Liu, S. Levisohn, R. Rosengarten, M. Garcia and D. Yogev, 2000. Molecular characterization of the Mycoplasma gallisepticum pvpA gene which encodes a putative variable cytadhesin protein. Infect. Immun., 68: 3956-3964.

Branton, S.L., B.D. Lott, J.W. Deaton, J.M. Hardin and W.R. Maslin, 1988. F-strain Mycoplasma gallisepticum vaccination of post-production-peak commercial Leghorns and its effect on egg and eggshell quality. Avian Dis., 32: 304-307.

Branton, S.L., B.D. Lott, J.D. May, W.R. Maslin, G.T. Pharr, J.E. Brown and D.L. Boykin, 1999. The effects of F-strain Mycoplasma gallisepticum, Mycoplasma synoviae and the dual infection in commercial layer hens over a 44-week laying cycle when challenged before beginning of lay. II. Egg size distribution. Avian Dis., 43: 326-330.

Chen, H., S. Yu, X. Shen, D. Chen, X. Qiu, C. Song and C. Ding, 2011. The Mycoplasma gallisepticum alpha-enolase is cell surface-exposed and mediates adherence by binding to chicken plasminogen. Microb. Pathog., 51: 285-290.

Collier, S.D., G.T. Pharr, S.L. Branton, J.D. Evans, S.A. Leigh and B. Felfoldi, 2006. Initial proteomics analysis of differentially expressed proteins from mycoplasma gallisepticum vaccine strains ts-11 and f detected by Western blotting. Int. J. Poult. Sci., 5: 330-336.

Davidsen, T., E. Beck, A. Ganapathy, R. Montgomery, N. Zafar, Q. Yang, R. Madupu, P. Goetz, K. Galinsky, O. White and G. Sutton, 2010. The comprehensive microbial resource. Nucleic Acids Res., 38: D340-345.

Demina, I.A., M.V. Serebryakova, V.G. Ladygina, M.A. Rogova, V.G. Zgoda, D.A. Korzhenevskyi and V.M. Govorun, 2009. Proteome of the bacterium Mycoplasma gallisepticum. Biochem. (Mosc.), 74: 165-174.

Evans, J.D., S.A. Leigh, J.L. Purswell, R. Jacob, E.D. Peebles, S.D. Collier and S.L. Branton, 2012. A comparative study of live attenuated F strain-derived Mycoplasma gallisepticum vaccines. Avian Dis., 56: 396-401.

Evans, R.D. and Y.S. Hafez, 1992. Evaluation of a Mycoplasma gallisepticum strain exhibiting reduced virulence for prevention and control of poultry mycoplasmosis. Avian Dis., 36: 197-201.

Fisunov, G.Y., D.G. Alexeev, N.A. Bazaleev, V.G. Ladygina, M.A. Galyamina, I.G. Kondratov, N.A. Zhukova, M.V. Serebryakova, I.A. Demina and V.M. Govorun, 2011. Core proteome of the minimal cell: comparative proteomics of three mollicute species. PLoS One, 6: 21964.

Frey, M.L., R.P. Hanson and D.P. Andrson, 1968. A medium for the isolation of avian mycoplasmas. Am. J. Vet. Res., 29: 2163-2171.

Furnkranz, U., K. Siebert-Gulle, R. Rosengarten and M.P. Szostak, 2013. Factors influencing the cell adhesion and invasion capacity of Mycoplasma gallisepticum. Acta Vet. Scand., 55: 63.

Gingerich, E., 2002. Vaccine and vaccination for Mycoplasma gallisepticum (MG). In, Proc. Cornell Poultry Conference Ithaca, NY, pp: 2-5.

Glew, M.D., G.F. Browning, P.F. Markham and I.D. Walker, 2000. pMGA phenotypic variation in Mycoplasma gallisepticum occurs *in vivo* and is mediated by trinucleotide repeat length variation. Infect. Immun., 68: 6027-6033.

Hnatow, L.L., C.L. Keeler, Jr., L.L. Tessmer, K. Czymmek and J.E. Dohms, 1998. Characterization of MGC2, a Mycoplasma gallisepticum cytadhesin with homology to the Mycoplasma pneumoniae 30-kilodalton protein P30 and Mycoplasma genitalium P32. Infect. Immun., 66: 3436-3442.

Hudson, P., T.S. Gorton, L. Papazisi, K. Cecchini, Jr. S. Frasca and S.J. Geary, 2006. Identification of a virulence-associated determinant, dihydrolipoamide dehydrogenase (lpd), in Mycoplasma gallisepticum through *in vivo* screening of transposon mutants. Infect. Immun., 74: 931-939.

Indikova, I., P. Much, L. Stipkovits, K. Siebert-Gulle, M.P. Szostak, R. Rosengarten and C. Citti, 2013. Role of the GapA and CrmA cytadhesins of Mycoplasma gallisepticum in promoting virulence and host colonization. Infect. Immun., 81: 1618-1624.

Jenkins, C., S.J. Geary, M. Gladd and S.P. Djordjevic, 2007. The Mycoplasma gallisepticum OsmC-like protein MG1142 resides on the cell surface and binds heparin. Microbiol., 153: 1455-1463.

Jenkins, C., R. Samudrala, S.J. Geary and S.P. Djordjevic, 2008. Structural and functional characterization of an organic hydroperoxide resistance protein from Mycoplasma gallisepticum. J. Bacteriol., 190: 2206-2216.

Kleven, S.H., H.H. Fan and K.S. Turner, 1998. Pen trial studies on the use of live vaccines to displace virulent Mycoplasma gallisepticum in chickens. Avian Dis., 42: 300-306.

Kleven, S.H., M.I. Khan and R. Yamamoto, 1990. Fingerprinting of Mycoplasma gallisepticum strains isolated from multiple-age layers vaccinated with live F strain. Avian Dis., 34: 984-990.

Ley, D.H., A.P. Avakian and J.E. Berkhoff, 1993. Clinical Mycoplasma gallisepticum infection in multiplier breeder and meat turkeys caused by F strain: identification by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, restriction endonuclease analysis and the polymerase chain reaction. Avian Dis., 37: 854-862.

Ley, D.H., J.M. McLaren, A.M. Miles, H.J. Barnes, S.H. Miller and G. Franz, 1997. Transmissibility of live Mycoplasma gallisepticum vaccine strains ts-11 and 6/85 from vaccinated layer pullets to sentinel poultry. Avian Dis., 41: 187-194.

Lin, M.Y. and S.H. Kleven, 1984. Evaluation of attenuated strains of Mycoplasma gallisepticum as vaccines in young chickens. Avian Dis., 28: 88-99.

Liu, L., V.S. Panangala and K. Dybvig, 2002. Trinucleotide GAA repeats dictate pMGA gene expression in Mycoplasma gallisepticum by affecting spacing between flanking regions. J. Bacteriol., 184: 1335-1339.

Liu, T., M. Garcia, S. Levisohn, D. Yogev and S.H. Kleven, 2001. Molecular variability of the adhesin-encoding gene pvpA among Mycoplasma gallisepticum strains and its application in diagnosis. J. Clin. Microbiol., 39: 1882-1888.

Masukagami, Y., K.A. Tivendale, K. Mardani, I. Ben-Barak, P.F. Markham and G.F. Browning, 2013. The Mycoplasma gallisepticum virulence factor lipoprotein MslA is a novel polynucleotide binding protein. Infect. Immun., 81: 3220-3226.

May, M., L. Papazisi, T.S. Gorton and S.J. Geary, 2006. Identification of fibronectin-binding proteins in Mycoplasma gallisepticum strain R. Infect. Immun., 74: 1777-1785.

Mitaku, S., T. Hirokawa and T. Tsuji, 2002. Amphiphilicity index of polar amino acids as an aid in the characterization of amino acid preference at membrane-water interfaces. Bioinformatics, 18: 608-616.

Mudahi-Orenstein, S., S. Levisohn, S.J. Geary and D. Yogev, 2003. Cytadherence-deficient mutants of Mycoplasma gallisepticum generated by transposon mutagenesis. Infect. Immun., 71: 3812-3820.

Papazisi, L., S. Frasca, Jr., M. Gladd, X. Liao, D. Yogev and S.J. Geary, 2002. GapA and CrmA coexpression is essential for Mycoplasma gallisepticum cytadherence and virulence. Infect. Immun., 70: 6839-6845.

Papazisi, L., T.S. Gorton, G. Kutish, P.F. Markham, G.F. Browning, D.K. Nguyen, S. Swartzell, A. Madan, G. Mahairas and S.J. Geary, 2003. The complete genome sequence of the avian pathogen Mycoplasma gallisepticum strain R (low). Microbiol., 149: 2307-2316.

Papazisi, L., K.E. Troy, T.S. Gorton, X. Liao and S.J. Geary, 2000. Analysis of cytadherence-deficient, GapA-negative Mycoplasma gallisepticum strain R. Infect. Immun., 68: 6643-6649.

Purswell, J.L., J.D. Evans, S.A. Leigh, S.D. Collier, H.A. Olanrewaju, E.J. Kim, G.T. Pharr, E.D. Peebles and S.L. Branton, 2012. Mycoplasma gallisepticum transmission: comparison of commercial F-strain vaccine versus layer complex-derived field strains in a tunnel ventilated house. Poult. Sci., 91: 3072-3079.

Szczepanek, S.M., S. Frasca, Jr. V.L. Schumacher, X. Liao, M. Padula, S.P. Djordjevic and S.J. Geary, 2010a. Identification of lipoprotein MslA as a neoteric virulence factor of Mycoplasma gallisepticum. Infect. Immun., 78: 3475-3483.

Szczepanek, S.M., E.R. Tulman, T.S. Gorton, X. Liao, Z. Lu, J. Zinski, F. Aziz, S. Frasca, Jr. G.F. Kutish and S.J. Geary, 2010b. Comparative genomic analyses of attenuated strains of Mycoplasma gallisepticum. Infect. Immun., 78: 1760-1771.

Tulman, E.R., X. Liao, S.M. Szczepanek, D.H. Ley, G.F. Kutish and S.J. Geary, 2012. Extensive variation in surface lipoprotein gene content and genomic changes associated with virulence during evolution of a novel North American house finch epizootic strain of Mycoplasma gallisepticum. Microbiol., 158: 2073-2088.

Wan, X., S.L. Branton, L.A. Hanson and G.T. Pharr, 2004. Identification and initial characterization of a putative Mycoplasma gallinarum leucine aminopeptidase gene. Curr. Microbiol., 48: 32-38.

Wan, X.F., S.L. Branton, S.D. Collier, J.D. Evans, S.A. Leigh and G.T. Pharr, 2010. Proteomics inference of genes involved in host adaptation of Mycoplasma gallinarum. Vet. Microbiol., 145: 177-184.

Whithear, K.G., 1996. Control of avian mycoplasmoses by vaccination. Rev. Sci. Tech., 15: 1527-1553.

Whithear, K.G., E. Ghiocas, P.F. Markham and D. Marks, 1990. Examination of Mycoplasma gallisepticum isolates from chickens with respiratory disease in a commercial flock vaccinated with a living M. gallisepticum vaccine. Aust. Vet. J., 67: 459-460.

Winner, F., I. Markova, P. Much, A. Lugmair, K. Siebert-Gulle, G. Vogl, R. Rosengarten and C. Citti, 2003. Phenotypic switching in Mycoplasma gallisepticum hemadsorption is governed by a high-frequency, reversible point mutation. Infect. Immun., 71: 1265-1273.

Yoder, H.W. Jr., 1991. Mycoplasma gallsepticum infection. In: B. W. Calnek, H. J. Barnes, C. W. Beard and H. W. Yoder, Jr. (Eds.), Diseases of poultry. Iowa State University Press, Ames, IA: pp: 198-212.