Mhp107 Is a Member of the Multifunctional Adhesin Family of *Mycoplasma hyopneumoniae*

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*Mycoplasma hyopneumoniae* is the causative pathogen of porcine enzootic pneumonia, an economically significant disease that disrupts the mucociliary escalator in the swine respiratory tract. Expression of Mhp107, a P97 paralog encoded by the gene *mhp107*, was confirmed using ESI-MS/MS. To investigate the function of Mhp107, three recombinant proteins, F1Mhp107, F2Mhp107, and F3Mhp107, spanning the N-terminal, central, and C-terminal regions of Mhp107 were constructed. Colonyization of swine by *M. hyopneumoniae* requires adherence of the bacterium to ciliated cells of the respiratory tract. Recent studies have identified a number of *M. hyopneumoniae* adhesins that bind heparin, fibronectin, and plasminogen. F1Mhp107 was found to bind porcine heparin (KD ~ 90 nM) in a dose-dependent and saturable manner, whereas F3Mhp107 bound fibronectin (KD ~ 180 nM) at physiologically relevant concentrations. F1Mhp107 also bound porcine plasminogen (KD ~ 24 nM) in a dose-dependent and physiologically relevant manner. Microspheres coated with F3Mhp107 mediate adherence to porcine kidney epithelial-like (PK15) cells, and all three recombinant proteins (F1Mhp107–F3Mhp107) bound swine respiratory cilia. Together, these findings indicate that Mhp107 is a member of the multifunctional *M. hyopneumoniae* adhesin family of surface proteins and contributes to both adherence to the host and pathogenesis.

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been identified in Mhp271 (7) and P116 (10). The domain of P116 able to bind fibronectin also contains a C-terminal lysine that mediates adherence to plasminogen (10). Plasmin(ogen) is present in both plasma and basement membranes; active plasmin is a serine protease capable of hydrolyzing extracellular matrix components including fibronectin, collagen, and laminin (21). Damage to cells by plasminogen can also expose extracellular matrix components, increasing the potential for adherence by a colonizing pathogen (22). There is evidence that, like heparin, plasminogen may also function as a bridging molecule to contribute to bacterial attachment (23). All of the proteins in the P97/P102 paralog family studied thus far have been characterized as adhesins. These exhibit a common mechanism of utilizing extracellular matrix components to contribute to the binding of host cilia by M. hyopneumoniae. An ability to recognize and adhere to components of the extracellular matrix is broadly applicable to a wide range of bacterial species, enabling bacteria to anchor to the host, thus establishing colonization (24).

A putative 120-kDa P97 paralog (Mhp107) is encoded by the gene mhp107. The contribution of P97 and related paralogs to adherence and colonization by M. hyopneumoniae is the basis for our interest in establishing whether Mhp107 is a member of this multifunctional adhesin family. This study utilized proteomic analyses to determine expression and surface location of Mhp107. Characterization of the binding of recombinant Mhp107 fragments to heparin, fibronectin, and plasminogen was undertaken. The ability of Mhp107 to interact with porcine kidney epithelial-like (PK15) cells and swine cilia was established, implying a role for Mhp107 in adherence and colonization of M. hyopneumoniae.

EXPERIMENTAL PROCEDURES

Bacterial Strains and Culture Conditions—M. hyopneumoniae strains were grown in Friis medium and harvested as described previously (25). M. hyopneumoniae strain 232 (26) was used throughout this study unless stated otherwise. Additional strains of M. hyopneumoniae were isolated from various locations, including the laboratory-adapted strain J (25) and field strains OMZ407 (25), Hillcrest (isolated from New South Wales, Australia in 2003 and provided by Graeme Eamens at the Elizabeth Macarthur Agricultural Institute, New South Wales, Australia), 95MP1509 (27), 00MP1301 (27), C1735/2 (25), and 2-22421 (provided by J. Forbes-Faulkner, Oonoona Veterinary Laboratory, Queensland, Australia). Escherichia coli TOP10 or BL21 star (DE3) (Invitrogen) were grown on Luria-Bertani agar plates or cultured in Luria-Bertani medium withkanamycin at 25°C for 18–24 h. Escherichia coli was grown on Luria-Bertani agar plates or cultured in Luria-Bertani medium with shaking at 200 rpm. Ampicillin was added to media at 100 μg/ml when required.

Plasmid Constructs, Expression, and Purification—Polymerase chain reactions used PfuUltra™ High-Fidelity II DNA polymerase (Stratagene) and a cooled gradient palm cycler GC1–96 (Corbett Research, Australia). Primer sequences are provided in supplemental Table S1. Amplification of mhp107 from M. hyopneumoniae strain 232 chromosomal DNA and subsequent cloning using the pET151/D-TOPO® cloning kit (Invitrogen) to construct pET151:mhp107 was performed following the manufacturer’s instructions. As TGA encodes tryptophan in M. hyopneumoniae, site-directed mutagenesis using the QuikChange site-directed mutagenesis kit (Stratagene) was performed to change in-frame TGA codons to TGG in the pET151:mhp107 construct. Regions of the pET151:mhp107 construct were then amplified and cloned using the pET151/D-TOPO® cloning kit to generate the recombinant polyhistidine fusion proteins described in this study (F1mhp107–F3mhp107). DNA sequencing was performed on the resulting recombinant plasmid constructs to ensure conformity to mhp107. Expression of recombinant proteins was in E. coli strain BL21 star (DE3). Recombinant plasmid constructs were transformed into this strain, and once cell growth reached an A600 of 0.8, protein expression was induced by the addition of 1 mM isopropyl 1-thio-β-D-galactopyranoside. Purification of proteins was performed under denaturing conditions using nickel-nitrilotriacetic acid-agarose in accordance with the manufacturer’s instructions (Qiagen). Purified proteins were dialyzed into 0.01% SDS in PBS (10 mM sodium phosphate, 137 mM sodium chloride, 2 mM potassium phosphate, 2.7 mM potassium chloride (pH 7.4)).

Proteomic Analyses—Detection of Mhp107 expression by M. hyopneumoniae was undertaken using ESI-MS/MS analysis of M. hyopneumoniae proteins separated by SDS-PAGE of M. hyopneumoniae aqueous phase proteins. Extraction of the M. hyopneumoniae aqueous phase proteins using Triton X-114 was performed as described by Wise and Kim (29), with initial overnight cell lysis at 4°C in 1% Triton X-114, 10 mM Tris (pH 8.0), 150 mM NaCl, and 1 mM EDTA. ESI-MS/MS analysis was performed as described previously (10); identified peptides are detailed in supplemental Table S2. Immunoblot analyses were used in addition to ESI-MS/MS analysis to further elucidate Mhp107 expression. Immunoblots were performed with M. hyopneumoniae cell lysates, and aqueous phase proteins were probed with the purified polyclonal antisera. Immunoblot analysis was also employed to investigate variations in the expression and processing of Mhp107 among M. hyopneumoniae field strains. Immunoblots were performed using cell lysates probed with purified polyclonal antisera. The subcellular location of Mhp107 was identified using trypsin-treated intact M. hyopneumoniae as described previously (30). Trypsin treatment was performed with trypsin concentrations of 0–150 μg/ml. Cell lysates were analyzed by immunoblot with the purified polyclonal antisera; a control blot was probed with L7/L12, a ribosomal protein found in the cytosol (18), to verify the integ-

2 The abbreviation used is: ESI, electrospray ionization.
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RESULTS

Proteomic Analyses—The hypothetical protein Mhp107 (GenBank™ accession number AAV27416) is a P97 paralog encoded by mhp107. Mhp107 consists of 1032 amino acids with a predicted pl of 8.81. Analysis with SignalP 3.0 server identifies a signal peptide located between amino acids 1–34 with a probability of 0.952. Prediction by TMPred detects a transmembrane domain region between amino acids 17–33 with a score of 2408. MS/MS analysis of M. hyopneumoniae proteins extracted from an SDS-PAGE gel slice containing proteins in the mass range of 79–104 kDa detected peptides that mapped to Mhp107 (Fig. 1A), demonstrating expression of Mhp107. This observation corroborates evidence of in vivo transcription of mhp107 (6). Attempts to express full-length Mhp107 in E. coli were unsuccessful (data not shown); consequently, Mhp107 was expressed as three distinct adjoining polystyrene microspheres (Poly-Sciences) was performed according to Seymour et al. (10). Preparation of recombinant proteins, ligand immobilization, and kinetics assays were performed as described previously (10). Porcine plasminogen was purified using affinity chromatography (31, 32) followed by gel filtration and ultrafiltration. The activity and purity of plasminogen was assessed with Spectrozyme PL (American Diagnostica, Inc.) (10). Fibronectin and plasminogen were used as the ligands in the surface plasmon resonance kinetics assays, and binding of recombinant Mhp107 proteins was performed over a range of concentrations (0–500 nM). Additional cycles were performed with 200 nM F1Mhp107 in the presence of 10, 50, and 100 nM lysine residues were responsible for the observed plasminogen binding. Analysis of F3Mhp107 association kinetics with fibronectin were determined as the sum of two exponentials for a heterogeneous surface as described by Deutscher et al. (7). All other binding data analysis used the 1:1 Langmuir binding model with BioEvaluation software 3.1 (Biacore AB).

PK15 Cell Adherence Assay—The ability of recombinant Mhp107 fragments to promote binding to PK15 cells was determined using protein-coated microspheres. Coating of Fluoresbrite® polychromatic red polystyrene microspheres (Polysciences) was performed according to Seymour et al. (10), and adherence assays were also performed as described previously (10, 18). Briefly, PK15 cells were grown to confluence before varying periods of incubation with protein-coated microspheres. PK15 cells were washed, fixed, and stained before visualization by confocal microscopy. One-way analyses of variance with a Dunnett’s comparison against results from BSA-coated beads were performed with GraphPad Prism version 4 software (GraphPad) to determine significance.

Cilia Binding Assay—The cilia-binding ability of recombinant Mhp107 fragments was investigated with an adherence assay used previously to identify M. hyopneumoniae adhesins. Microtiter plates were coated with purified cilia from tracheal mucosa of specific pathogen-free swine as described previously (16). Mhp107 proteins were incubated with swine cilia, and bound protein was detected colorimetrically as described previously (4). Assays were performed in triplicate and repeated twice. Statistical analysis with an analysis of variance with a Tukey’s comparison was performed. Wells containing no protein were used as negative controls. A cilia-binding protein, P159 (F4p159), (18) was used as a positive control.
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FIGURE 1. Proteomic analyses of Mhp107. A, expression of Mhp107 occurs in vitro. ES1-MS/MS analysis of M. hyopneumoniae proteins extracted from an SDS-PAGE gel identified the amino acids shown in bold. Amino acids in italics and underlined italics indicate the predicted signal peptide sequence and transmembrane sequence, respectively. B, schematic representation of Mhp107, the putative 120-kDa protein encoded by the expression vector. The position of the recombinant Mhp107 protein fragments (F1 Mhp107, F2 Mhp107, and F3 Mhp107) produced for this study are shown with amino acid positions indicated. The recombinant fragments include a 3.8-kDa polyhistidine region encoded by the expression vector. The position of the recombinant Mhp107 protein fragments (F1 Mhp107, F2 Mhp107, and F3 Mhp107) produced for this study are shown with amino acid positions indicated. The recombinant fragments include a 3.8-kDa polyhistidine region encoded by the expression vector. The position of the recombinant Mhp107 protein fragments (F1 Mhp107, F2 Mhp107, and F3 Mhp107) produced for this study are shown with amino acid positions indicated. The recombinant fragments include a 3.8-kDa polyhistidine region encoded by the expression vector. The position of the recombinant Mhp107 protein fragments (F1 Mhp107, F2 Mhp107, and F3 Mhp107) produced for this study are shown with amino acid positions indicated. The recombinant fragments include a 3.8-kDa polyhistidine region encoded by the expression vector. The position of the recombinant Mhp107 protein fragments (F1 Mhp107, F2 Mhp107, and F3 Mhp107) produced for this study are shown with amino acid positions indicated. The recombinant fragments include a 3.8-kDa polyhistidine region encoded by the expression vector. The position of the recombinant Mhp107 protein fragments (F1 Mhp107, F2 Mhp107, and F3 Mhp107) produced for this study are shown with amino acid positions indicated. The recombinant fragments include a 3.8-kDa polyhistidine region encoded by the expression vector. The position of the recombinant Mhp107 protein fragments (F1 Mhp107, F2 Mhp107, and F3 Mhp107) produced for this study are shown with amino acid positions indicated. The recombinant fragments include a 3.8-kDa polyhistidine region encoded by the expression vector.

The high degree of sulfation of fucoidan may disrupt binding between F1 Mhp107 and heparin.

Binding of F3 Mhp107 to Fibronectin—M. hyopneumoniae is a fibronectin-binding pathogen that expresses surface-located fibronectin-binding proteins P116 and Mhp271 (7, 10). The ability to bind fibronectin has been reported in other Mycoplasma species as a key virulence factor (34–36). Investigation into the fibronectin binding ability of Mhp107 fragments revealed only F3 Mhp107, as a fibronectin-binding domain (Fig. 4). Using surface plasmon resonance, it was determined that F3 Mhp107 is able to bind to fibronectin in a dose-dependent manner with a physiologically relevant KD of 174 ± 80 nM; kₐ = (4.8 ± 0.8) × 10⁴ M⁻¹ s⁻¹. Binding of F3 Mhp107 to fibronectin was very stable, resulting in a slow dissociation rate. No previously described fibronectin binding motif was identified; however, analysis of Mhp107 with PONDR VSL1 (37) revealed regions of disorder within F3 Mhp107. Disordered regions in proteins often contain binding sites that become ordered upon binding to target molecules (38).

F1 Mhp107 Binds Plasminogen—M. hyopneumoniae cells are able to bind plasminogen (10). Plasminogen contains five kringle domains with lysine-binding sites that become ordered upon binding to target molecules (38).

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gen-binding proteins, therefore it is anticipated that a novel binding motif exists within the F1Mhp107 region of Mhp107.

**Adherence of PK15 Cells and Swine Cilia by Mhp107 Fragments**—Binding of *M. hyopneumoniae* to swine cilia is a prerequisite for colonization in the host. The PK15 cell line is useful as a model for adherence to swine epithelia (4, 10, 18, 40). Both PK15 cells and swine respiratory cilia were used in this study to investigate the role of Mhp107 in adherence. Protein-coated fluorescent microspheres were used to determine the ability of Mhp107 fragments to promote binding to PK15 cells. It was found that F3Mhp107 was able to promote adherence of microspheres to PK15 cells (Fig. 6A–E). An assay based on that developed by Zhang et al. (16) was employed to investigate the ability of Mhp107 fragments to bind swine respiratory cilia (Fig. 7). A recombinant fragment of a *M. hyopneumoniae* adhesin (F4P159) (18) was used as a positive control protein. Each of the Mhp107 fragments described in this study is able to bind swine respiratory cilia.

**DISCUSSION**

The gene *mhp107* encodes Mhp107, a putative 120-kDa protein and a P97 cilium adhesin paralog. ESI-MS/MS analysis of Mhp107 demonstrated the presence of Mhp107 in the *M. hyopneumoniae* proteome, consistent with evidence of *mhp107* transcription (6). Adams *et al.* (6) reported *mhp107* transcription *in vivo* during *M. hyopneumoniae* infection. Immunoblots of *M. hyopneumoniae* field strains probed with Mhp107 antiserum recognized a ~100-kDa protein comparable in size to that observed in whole cell immunoblots and trypsin digest blots. The surface location and presence of Mhp107 in multiple field strains corroborates evidence for the expression of Mhp107 and a potential role of Mhp107 as an adhesin.

Mhp107 contains a heparin-binding consensus sequence (8) and is able to bind specifically to heparin. Heparin inhibits the adherence of *M. hyopneumoniae* to porcine cilia and PK15 cells (16, 18), and a number of adhesins from the P97/P102 paralog family are heparin-binding proteins (4, 7, 8, 18). A structurally similar proteoglycan, heparan sulfate, is found in the extracellular matrix and on cell surfaces, including the epithelial cells of the swine respiratory tract (41, 42). Heparin is also able to act as a bridging molecule, providing a mechanism to bind extracellular matrix components, including fibronectin, vitronectin, and laminin (43). Mhp107 thus joins the P97/P102 adhesin family, which contains other adhesin proteins with the capacity to bind heparin. As with F1Mhp107, the interaction between each of these other adhesins and heparin is blocked by fucoidan (4, 7, 8, 18). Such inhibition of binding by fucoidan suggests that these proteins bind heparin through electrostatic interactions. The negatively charged sulfate groups of fucoidan compete with the basic amino acids in these *M. hyopneumoniae* adhesin proteins. The basic residues in the identified putative heparin binding motif 204SKKKLKKL211 within...
F1Mhp107 may contribute to this interaction. The ability of heparin to inhibit binding of M. hyopneumoniae to ciliary cells coupled with several M. hyopneumoniae adhesins able to bind heparin provides compelling evidence of the important role for heparin or heparan sulfate binding in the colonization process. Recent reports indicate that M. hyopneumoniae binds both fibronectin and plasminogen (7, 10). The ability to interact with fibronectin promotes virulence in a large number of bacterial species (44), including the evolutionally related streptococci (45, 46). Here we have used surface plasmon resonance to demonstrate that F3Mhp107 binds fibronectin in a dose-dependent manner with a physiologically relevant $K_D$ value. Mhp271 and P116 are also M. hyopneumoniae proteins with a fibronectin binding capability (7, 10); F3Mhp107 binds fibronectin with a similar affinity to F271. Other mycoplasmal species that express fibronectin-binding proteins include Mycoplasma penetrans (20), Mycoplasma pneumoniae (47), and Mycoplasma gallisepticum (34). Fibronectin-binding proteins can provide a mechanism for adherence to host cells and tissues during colonization and enable some bacteria to trigger cytoskeletal rearrangements, thereby facilitating invasion (20, 44, 48, 49). The ability of Mhp271, P116, and Mhp107 to bind fibronectin suggests that the interaction of M. hyopneumoniae with fibronectin...
tin is a key virulence determinant and contributes significantly to the pathogenesis of infection.

F1 Mhp107 is also a plasminogen-binding protein. Inhibition of plasminogen binding by a lysine analog suggests that the internal lysine residues of this protein contribute to the observed plasminogen binding ability of F1 Mhp107. Bound plasminogen is more susceptible to activation to plasmin, a broad-spectrum serine protease (50). Bacterial interactions with plasmin(ogen) have been implicated in host tissue damage (39, 51, 52) and may contribute to tissue damage observed in the respiratory tract of swine during *M. hyopneumoniae* infection. The plasminogen binding affinity of F1 Mhp107 is physiologically relevant, characterized by a relatively slow association and dissociation rate. The plasminogen binding characteristics observed in this study are similar to those reported for the *M. hyopneumoniae* plasminogen-binding protein P116 (10). Damage to host epithelial cells by plasmin(ogen) can expose extracellular matrix components such as fibronectin to enhance adherence by pathogens. Deposition of fibronectin also occurs during wound repair at the interface of epithelial cells and the extracellular matrix (22, 53). Typically, plasminogen-binding proteins are multifunctional surface proteins (39) and have been reported to contribute to virulence in pathogens of the respiratory tract (54–58).

The use of *in vitro* cellular models to investigate adherence has underpinned the characterization of P97, P216, P159, and P116 as *M. hyopneumoniae* adhesins (4, 10, 18, 59). During disease, *M. hyopneumoniae* adheses to and colonizes ciliated cells of the swine respiratory tract (1). In *in vitro* *M. hyopneumoniae* is able to bind the porcine kidney epithelial-like cell line PK15, prompting the use of PK15 cells as an eukaryotic cell binding model (40). We have demonstrated the ability of Mhp107 fragments to adhere to swine cilia and to promote adherence to PK15 cells. These findings support a role for Mhp107 in the adherence of the bacterium to the host. In recent years since the initial discovery of P97 as the cilium adhesin, P216, P159, Mhp271, and P116 from the P97/P102 paralog family have been described as multifunctional adhesins (4, 7, 10, 18, 59). The genetic relationships among these protein paralogs indicate that they are likely to have similar or related functions. Ongoing research has provided mounting evidence of the importance of these proteins in mediating *M. hyopneumoniae* adherence to and colonization of the host. Here, we have described Mhp107 as a multifunctional ciliary adhesin found on the surface of *M. hyopneumoniae*. Furthermore, the ability of this adhesin to interact with heparin, fibronectin and plasminogen indicates that Mhp107 may be an important factor in the virulence and disease potential of *M. hyopneumoniae*.

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**REFERENCES**

1. Razin, S. (1999) *Biosci. Rep.* 19, 367–372.
2. Zhang, Q., Young, T. F., and Ross, R. F. (1994) *Infect. Immun.* 62, 4367–4373.
3. Minion, F. C. (2002) *Front. Biosci.* 7, d1410–1422.
4. Wilton, J., Jenkins, C., Cordwell, S. J., Falconer, L., Minion, F. C., Oneal, D. C., Djordjevic, M. A., Connolly, A., Barchia, I., Walker, M. J., and Djordjevic, S. P. (2009) *Mol. Microbiol.* 71, 566–582.
5. Hsu, T., and Minion, F. C. (1998) *Gene* 214, 13–23.
6. Adams, C., Pitzer, J., and Minion, F. C. (2005) *Infect. Immun.* 73, 7784–7787.
7. Deutscher, A. T., Jenkins, C., Minion, F. C., Seymour, L. M., Padula, M. P., Dixon, N. E., Walker, M. J., and Djordjevic, S. P. (2010) *Mol. Microbiol.* 78, 444–458.
8. Jenkins, C., Wilton, J. L., Minion, F. C., Falconer, L., Walker, M. J., and Djordjevic, S. P. (2006) *Infect. Immun.* 74, 481–487.
9. Hsu, T., Artiushin, S., and Minion, F. C. (1997) *J. Bacteriol.* 179, 1317–1323.
10. Seymour, L. M., Deutscher, A. T., Jenkins, C., Kuit, T. A., Falconer, L., Minion, F. C., Crosett, B., Padula, M. P., Dixon, N. E., Djordjevic, S. P., and Walker, M. J. (2010) *J. Biol. Chem.* 285, 33971–33978.
11. Baseggio, N., Glew, M. D., Markham, P. F., Whithear, K. G., and Browning, G. F. (1996) *Microbiology* 142, 1429–1435.
12. Dalo, S. F., and Baseman, J. B. (1991) *Microb. Pathog.* 10, 475–480.
13. Reddy, S. P., Rasmussen, W. G., and Baseman, J. B. (1995) *J. Bacteriol.* 177, 5943–5951.
14. Hsu, T., and Minion, F. C. (1998) *Infect. Immun.* 66, 4762–4766.
15. Zhang, Q., Young, T. F., and Ross, R. F. (1995) *Infect. Immun.* 63, 1013–1019.
16. Zhang, Q., Young, T. F., and Ross, R. F. (1994) *Infect. Immun.* 62, 1616–1622.
17. Duensing, T. D., Wing, J. S., and van Putten, J. P. (1999) *Infect. Immun.* 67, 4463–4468.
18. Burnett, T. A., Dinkla, K., Rohde, M., Chhatwal, G. S., Uphoff, C., Srivastava, M., Cordwell, S. J., Geary, S., Liao, X., Minion, F. C., Walker, M. J., and Djordjevic, S. P. (2006) *Mol. Microbiol.* 60, 669–686.
19. Chauveau, M. S., Cole, R. L., and van Putten, J. P. M. (2000) *Infect. Immun.* 68, 3226–3232.
20. Girón, J. A., Lange, M., and Baseman, J. B. (1996) *Infect. Immun.* 64, 197–208.
21. Dubreuil, J. D., Giudice, G. D., and Rappuoli, R. (2002) *Microbiol. Mol. Biol. Rev.* 66, 617–629.
22. Scarselli, M., Serruto, D., Montanari, P., Cacepchi, B., Adu-Bobie, J., Veggi, D., Rappuoli, R., Pizza, M., and Aricò, B. (2006) *Mol. Microbiol.* 61, 631–644.
23. Pancholi, V., Fontan, P., and Jin, H. (2003) *Microb. Pathog.* 35, 293–303.
24. Pattij, M. J., Allen, B. L., McGavin, M. J., and Höök, M. (1994) *Annu. Rev. Microbiol.* 48, 585–617.
25. Scaran, A. L., Chin, J. C., Eamens, G. J., Delaney, S. F., and Djordjevic, S. P. (1997) *Microbiology* 143, 663–673.
26. Bereiter, M., Young, T. F., Joo, H. S., and Ross, R. F. (1990) *Vet. Microbiol.* 25, 177–192.
27. Madsen, M. L., Oneal, M. J., Gardner, S. W., Strait, E. L., Nettleton, D., Thacker, E. L., and Minion, F. C. (2007) *J. Bacteriol.* 189, 7977–7982.
28. Cole, J. N., Djordjevic, S. P., and Walker, M. J. (2008) *Methods Mol. Biol.*
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425, 295–311
29. Wise, K. S., and Kim, M. F. (1987) *J. Bacteriol.* 169, 5546–5555
30. Wilton, J. L., Scaman, A. L., Walker, M. J., and Djordjevic, S. P. (1998) *Microbiology* 144, 1931–1943
31. Sanderson-Smith, M., Batzloff, M., Sriprakash, K. S., Dowton, M., Ranson, M., and Walker, M. J. (2006) *J. Biol. Chem.* 281, 3217–3226
32. Andronicos, N. M., Ranson, M., Bognacki, J., and Baker, M. S. (1997) *Biochim. Biophys. Acta* 1337, 27–39
33. Djordjevic, S. P., Cordwell, S. J., Djordjevic, M. A., Wilton, J., and Minion, F. C. (2004) *Infect. Immun.* 72, 2791–2802
34. May, M., Papazisi, L., Gorton, T. S., and Geary, S. J. (2006) *Infect. Immun.* 74, 1777–1785
35. Yavlovich, A., and Rottem, S. (2007) *FEMS Microbiol. Lett.* 266, 158–162
36. Balasubramanian, S., Kannan, T. R., and Baseman, J. B. (2008) *Biochim. Biophys. Acta* 1337, 27–39
37. Obradovic, Z., Peng, K., Vucetic, S., Radivojac, P., and Dunker, A. K. (2005) *Proteins* 61, 176–182
38. Uversky, V. N., and Dunker, A. K. (2010) *Biochim. Biophys. Acta* 1804, 1231–1264
39. Lähteenmäki, K., Kuusela, P., and Korhonen, T. K. (2001) *FEMS Microbiol. Rev.* 25, 531–552
40. Zielinski, G. C., Young, T., Ross, R. F., and Rosenbusch, R. F. (1990) *Am. J. Vet. Res.* 51, 339–343
41. Erlinger, R. (1995) *Cell Tissue Res.* 281, 473–483
42. Rabenstein, D. L. (2002) *Nat. Prod. Rep.* 19, 312–331
43. Jackson, R. L., Busch, S. J., and Cardin, A. D. (1991) *Physiol. Rev.* 71, 481–539
44. Joh, D., Wann, E. R., Kreikemeyer, B., Speziale, P., and Höök, M. (1999) *Matrix Biol.* 18, 211–223
45. Ramachandran, V., McArthur, J. D., Behm, C. E., Gutzeit, C., Dowton, M., Fagan, P. K., Towers, R., Currie, B., Sriprakash, K. S., and Walker, M. J. (2004) *J. Bacteriol.* 186, 7601–7609
46. Delvecchio, A., Currie, B. J., McArthur, J. D., Walker, M. J., and Sriprakash, K. S. (2002) *Epidemiol. Infect.* 128, 391–396
47. Dall, S. F., Kannan, T. R., Blaylock, M. W., and Baseman, J. B. (2002) *Mol. Microbiol.* 46, 1041–1051
48. Dziewanowska, K., Patti, J. M., Deobald, C. F., Bayles, K. W., Trumble, W. R., and Bohach, G. A. (1999) *Infect. Immun.* 67, 4673–4678
49. Secott, T. E., Lin, T. L., and Wu, C. C. (2004) *Infect. Immun.* 72, 3724–3732
50. Plow, E. F., Herren, T., Redlitz, A., Miles, L. A., and Hoover-Plow, J. L. (1995) *FASEB J.* 9, 939–945
51. Sanderson-Smith, M. L., Dinkla, K., Cole, J. N., Cork, A. I., Maamary, P. G., McArthur, J. D., Chhatwal, G. S., and Walker, M. J. (2008) *FASEB J.* 22, 2715–2722
52. McKay, F. C., McArthur, J. D., Sanderson-Smith, M. L., Gardam, S., Currie, B. J., Sriprakash, K. S., Fagan, P. K., Towers, R. J., Batzloff, M. R., Chhatwal, G. S., Ranson, M., and Walker, M. J. (2004) *Infect. Immun.* 72, 364–370
53. Coraux, C., Roux, J., Jolly, T., and Birembaut, P. (2008) *Proc. Am. Thorac. Soc.* 5, 689–694
54. Pancholi, V., and Fischetti, V. A. (1998) *J. Biol. Chem.* 273, 14503–14515
55. Sanderson-Smith, M. L., Dowton, M., Ranson, M., and Walker, M. J. (2007) *J. Bacteriol.* 189, 1435–1440
56. Bergmann, S., Rohde, M., and Hammerschmidt, S. (2004) *Infect. Immun.* 72, 2416–2419
57. Yavlovich, A., Higazi, A. A., and Rottem, S. (2001) *Infect. Immun.* 69, 1977–1982
58. Cork, A. J., Jergic, S., Hammerschmidt, S., Kobe, B., Pancholi, V., Benesch, J. L., Robinson, C. V., Dixon, N. E., Aquilina, J. A., and Walker, M. J. (2009) *J. Biol. Chem.* 284, 17129–17137
59. Minion, F. C., Adams, C., and Hsu, T. (2000) *Infect. Immun.* 68, 3056–3060