Published in final edited form as:

Microbes Infect. 2020 ; 22(1): 60–64. doi:10.1016/j.micinf.2019.08.001.

Phosphoglycerate mutase affects *Stenotrophomonas maltophilia* attachment to biotic and abiotic surfaces

Layla Ramos-Hegazy^{a,‡}, Shubham Chakravarty^{b,‡}, Gregory G. Anderson^{a,*}

^aDepartment of Biology, Indiana University Purdue University Indianapolis, Indianapolis, IN USA

^bDepartment of Biochemistry, Université de Sherbrooke, Quebec, Canada

Abstract

Stenotrophomonas maltophilia biofilm formation is of increasing medical concern, particularly for lung infections. However, the molecular mechanisms facilitating the biofilm lifestyle in *S. maltophilia* are poorly understood. We generated and screened a transposon mutant library for mutations that lead to altered biofilm formation compared to wild type. One of these mutations, in the gene for glycolytic enzyme phosphoglycerate mutase (*gpmA*), resulted in impaired attachment on abiotic and biotic surfaces. As adherence to a surface is the initial step in biofilm developmental processes, our results reveal a unique factor that could affect *S. maltophilia* biofilm initiation and, possibly, subsequent development.

1. Introduction

The Gram-negative bacterium *Stenotrophomonas maltophilia* causes numerous human infections that are difficult to treat due to high intrinsic antimicrobial resistance [1]. Moreover, many clinical *S. maltophilia* isolates readily form biofilms on biotic and abiotic surfaces, such as intravascular devices, glass, plastic, and host tissue [2]. Indeed, *S. maltophilia* biofilm infections are increasingly being reported and are associated with augmented patient morbidity and mortality [3]. One of the worst manifestations of *S. maltophilia* is in the airways of individuals with cystic fibrosis (CF), where the pathogen forms persistent biofilms and causes chronic infection [4]. Approximately 10-30% of CF patients in continental Europe and North America experience chronic lung colonization by *S. maltophilia*, and *S. maltophilia* presence indicates a worse prognosis [5]. Inhibiting biofilm formation would greatly reduce these consequences for CF and other *S. maltophilia* biofilm-related infections. However, genetic networks governing biofilm initiation and maintenance in *S. maltophilia* are poorly understood.

*To whom correspondence should be addressed: Indiana University Purdue University Indianapolis, Department of Biology, 723 West Michigan St., SL 320, Indianapolis, IN 46202, Tel 317-278-3896, Fax 317-274-2846, ga2@iupui.edu.

[‡]These authors contributed equally to this work

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

⁵-Conflict of Interest

The authors state that they have no conflicts of interest.

Biofilm formation starts with bacterial attachment to a surface (biotic or abiotic) and/or to adjacent cells [6]. Subsequent or concurrent altered genetic regulation results in the bacteria committing to a sessile lifestyle, markedly different from the planktonic one [7]. Many environmental factors such as pH, temperature, presence of metal ions, and antibiotics greatly effect *S. maltophilia* biofilm formation [8]. Additionally, some polysaccharide synthesis genes (*rmlA*, *rmlC*, and *xanB*) [9], flagellar genes [10], and diffusible signal factor (DSF)-mediated cell communication [11] affect biofilm levels. However, beyond these factors, limited research regarding genes and biochemical cascades associated with *S. maltophilia* biofilm formation has been conducted.

To identify novel *S. maltophilia* biofilm factors on abiotic and biotic surfaces, we generated and screened a transposon mutant library for mutations that lead to altered biofilm levels compared to the wild type (WT) strain. One of these mutations mapped to the gene for glycolytic enzyme phosphoglycerate mutase, *gpmA* [12]. To our knowledge, this gene has not previously been associated with biofilm formation in *S. maltophilia*. Isogenic deletion of *gpmA* revealed adherence defects on abiotic and biotic surfaces, suggesting a role for metabolism in *S. maltophilia* biofilm attachment and/or development.

2. Materials and Methods

2.1 Bacterial strains and culture

We used *S. maltophilia* clinical isolate strain K279a [13]. *Escherichia coli* S17-1 [14] was used for cloning and plasmid maintenance. Bacteria were cultured in LB medium, with gentamicin as needed for maintaining plasmids (10 µg/mL or 70 µg/mL for *E. coli* and *S. maltophilia*, respectively).

2.2 Transposon mutagenesis

We generated a transposon mutant library of *S. maltophilia* strain K279a by conjugation of plasmid pBT20 (containing the mariner transposon [15]) from *E. coli* strain S17-1 into K279a. Briefly, overnight cultures of K279a and S17-1 pBT20 were mixed 1:3, 1:1, and 3:1 and spotted on LB agar plates. After 24 hr incubation at 30°C, spots were resuspended in LB and exconjugants were selected for on LB plates with 70 µg/mL gentamicin and 5 µg/mL norfloxacin. For candidate mutants, we performed arbitrary-primed PCR as previously described [16] to identify the location of transposon insertion.

2.3 Strain construction

The suicide vector pMQ30 [17] was used to construct an isogenic *gpmA* deletion strain by allelic replacement, as previously described [16]. Briefly, ~1000bp fragments immediately upstream and downstream of *gpmA* were generated by PCR using primers sets gpmALfor/gpmALrev (TCGACTGAGCCTTTTCGTTTTATTTGATGCCTGGCAGTTCCGGCGCCTGCACGATG ACTTC/CACCAACGGTGGGTATCTACTGCAGGGTCTCCAACGCGAG) and gpmARfor/gpmARrev (CTCGCGTTGGAGACCCTGCAGTAGATACCCACCGTTGGTG/GGAATTGTGAGCGGATAACAATTTCACACAGGAAACAGCTTGAGCGGGTTGTAGC GCTTG), respectively. Fragments were transformed into *Saccharomyces cerevisiae* INVSC1

(Invitrogen; Eugene, OR) along with BamHI-digested pMQ30, as previously described [16, 18]. Recombination in *S. cerevisiae* resulted in plasmid p *gpmA*. This plasmid was isolated as previously described [16], transformed into *E. coli* S17-1 by electroporation, and conjugated into K279a, as described above. Exoconjugants were selected on LB plates containing 70 µg/mL gentamicin and 5 µg/mL norfloxacin. Isolated colonies were grown overnight in LB and then plated on LB agar containing 10% sucrose to select for excision of the plasmid. Deletion mutants were confirmed using primers *gpmA*For/*gpmA*Rev (ATTGGCTGCAGCATCGCATG/CCAGGTTGCGGACGTTTCATG).

Complementation was achieved by PCR-amplifying full length *gpmA*, along with ~500bp upstream, using the primer pairs *GpmA*CompNatForNew/*GpmA*CompRev (TTGTGTGGAATTGTGAGCGGATAACAATTTTCACACAGGAATCGACTTGGCACACCGATG/CTGTATCAGGCTGAAAATCTTCTCTCATCCGCCAAAACAGTTATTTTCGCCTTGCCCC TGGT). Fragments were transformed into *S. cerevisiae*, along with BamHI-digested pMQ132 expression vector [17], as described above, generating plasmid p*gpmA*. This plasmid was then transformed into *E. coli* S17-1 via electroporation, and successful construction was confirmed by PCR using primers p729/p730A (CAGACCGCTTCTGCGTTCTG/TGCTTCCGGCTCGTATGTTG). The *gpmA* strain was then transformed with p*gpmA* by electroporation.

2.4 Biofilm and attachment assays on an abiotic surface

Attachment and biofilm formation on abiotic surfaces was assayed as previously described [16, 19]. Strains were grown overnight in LB, diluted 1:100 into fresh LB, inoculated into individual wells in 96-well polystyrene microtiter plates (Greiner Bio-One; Monroe, NC), and incubated at 37°C for either 1 hr, 4 hr, or 6 hr. Detection of attachment or biofilm formation was achieved by staining the plates with 0.1% crystal violet (CV) for 10 min. CV was solubilized using 30% acetic acid solution and transferred to a new 96-well polystyrene microtiter plate and quantified by measuring optical density at 550 nm (OD₅₅₀) in a SpectraMaxM2 spectrophotometer (Molecular Devices; Sunnyvale, CA).

2.5 Biofilm and attachment assays on a biotic surface

Attachment and biofilm formation of the wild-type, *gpmA*, and complemented strains were assayed on immortalized CF-derived bronchial epithelial (CFBE) cells as previously described [20]. 24-well tissue culture plates (Falcon; Franklin, NJ) were seeded with CFBE cells at a concentration of 2 x 10⁵ cells/well in minimal essential medium (MEM) (Corning Inc.; Corning, NY) supplemented with 10% fetal bovine serum (FBS) (Atlanta Biologicals; Lawrenceville, CA), 50 U/mL penicillin, and 50 U/mL streptomycin (Lonza; Walkersville, MD) [21]. The plates were incubated for 7 to 10 days at 37°C and 5% CO₂. Once confluence was reached, cells were washed with 1X phosphate buffered saline (PBS) solution and inoculated with overnight-grown bacteria at a final concentration of ~1.2 x 10⁷ CFU/mL in 0.5 mL MEM without phenol red, FBS, or antibiotics; this assay was then incubated for the time period specified for each experiment. At each time point, wells were washed 2-3 times with 0.5 mL PBS and treated with 1 mL of 0.1% Triton X-100 for 10 min. Lysates were harvested into micro-centrifuge tubes, vortexed for 3 min, then serially diluted

and plated on LB. Attachment/biofilm level was assessed by CFU determination from colony counts.

2.6 Growth kinetics

Strains were grown overnight in LB and diluted 1:100 into 100 μ L fresh LB or MEM in a polystyrene microtiter plate. Plates were incubated for 12 hr at 37°C in a SpectraMaxM2 spectrophotometer, with 5 seconds of shaking before each OD₆₀₀ reading every 30 min.

2.7 Microscopy of biotic biofilms

For microscopic examination of CFBE cells attachment, wild type and *gpmA* strains were transformed with pMQ400, which constitutively expresses a TdTomato gene, conferring red fluorescence [22]. Attachment of strains was assayed as described above, and after 1 hr, cells were washed twice with PBS and fixed with 4% paraformaldehyde for 15 min at 37°C. Fixed cells were washed twice with PBS and stained with 5 μ g/mL wheat germ agglutinin (WGA) conjugated with Alexa Fluor 350 (Invitrogen; Eugene, OR) and incubated for 10 min at 37°C. Labeling solution was removed and cells were washed twice with PBS. Fluorescence microscopy was performed using a Keyence BZ-X800/810 series all-in-one fluorescence microscope (Osaka; Osaka Prefecture, Japan) to obtain a z-series of images at 20x magnification. 4 images were taken at different fields and stitched together using the microscope software to create a composite image.

2.7 Statistical analysis

At least three independent experiments were performed for each assay, with triplicate or quadruplicate samples each. Statistical significance was determined using Student's *t* test. A *P* value of <0.05 was considered statistically significant. Error bars represent standard deviations.

3. Results

3.1 *pmA strain is defective for attachment*

We created a transposon mutant library (~5,760 mutants) of *S. maltophilia* strain K279a and screened it for defective biofilm formation. Using arbitrary-primed PCR, we mapped the transposon insertion in one of these mutants to *gpmA* (Smlt1430), which codes for the glycolytic enzyme phosphoglycerate mutase [12]. In order to confirm that *gpmA* affects biofilm, we created an isogenic deletion mutant and tested it for biofilm formation. Initial attachment (2 hr time point) and early biofilm formation (4 hr time point) of *gpmA* on polystyrene plates was significantly reduced compared to wild type (Fig. 1A and 1B). Complementation of *gpmA* with a plasmid carrying a full-length copy of *gpmA* restored biofilm production (Fig. 1A and 1B). However after 6 hr, there was no difference in biofilm formation suggesting that *gpmA* is required for early biofilm formation on abiotic surfaces (Fig 1C).

3.2 *gpmA* exhibits the same growth kinetics as wild type

To rule out the possibility that attachment repression in the *gpmA* strain was due to reduction in growth rate, we examined the growth kinetics of planktonic cells in LB medium. The *gpmA* mutant displayed growth equivalent with the wild type and complemented mutant strains (Fig. 2A).

3.3 The *gpmA* strain is defective for attachment to airway epithelial cells

S. maltophilia forms biofilms in the airways of cystic fibrosis patients [23], and we wondered if *gpmA* also affected attachment and biofilm on airway epithelial cells. Thus, we quantified bacteria attached to cystic fibrosis bronchial epithelial (CFBE) [24] cells after incubation with wild type, *gpmA*, and complemented mutant strains. After 1 hr of incubation, *gpmA* exhibited an approximately 100-fold reduction in binding compared to wild type (Fig. 3A). Complementation of the mutant restored the wild type phenotype (Fig. 3A). However, there were no significant differences between wild type and *gpmA* at 4 hr and 6 hr time points (Fig. 3B and 3C). Importantly, there was a significant difference between *gpmA* and the complemented strain at 4 hr and 6 hr (Fig. 3B and 3C). All 3 strains grew equivalently in the MEM used in these assays (Fig. 2B). By fluorescence microscopy, numerous wild type biofilm microcolonies were evident on CFBE cells after 1 hr incubation (Fig. 3D), but fewer *gpmA* clusters were seen at the same time point.

4. Discussion

The molecular mechanisms by which *S. maltophilia* initiates and maintains biofilm are poorly understood. In this study, we found that *gpmA* mediates *S. maltophilia* adherence to an abiotic surface and to human airway cells (Figs. 1 and 3). The *gpmA* gene codes for the glycolysis enzyme phosphoglycerate mutase, catalyzing the interconversion of 3-phosphoglycerate and 2-phosphoglycerate [25]. Importantly, *S. maltophilia* contains genes for a complete Entner-Doudoroff alternative glycolysis pathway, and so it is likely to survive disruption of genes in the later stage of “standard” glycolysis. Indeed, our observed adherence phenotype is not due to a growth deficiency (Fig. 2), thus indicating a specific defect in either detecting the surface or synthesis of an adhesion molecule.

Previous studies have shown the importance of GpmA involvement in adherence of other organisms. Interestingly, phosphoglycerate mutase is upregulated in *Staphylococcus aureus* biofilm formation versus planktonic growth, highlighting its importance across different pathogenic species [26]. In *Streptococcus suis*, phosphoglycerate mutase binds to host fibronectin and collagen type I [27]. Similarly, in the yeast *Candida albicans*, phosphoglycerate mutase (Gpmlp) can localize to the cell wall, where it can facilitate binding to host proteins, possibly as a mechanism of immune evasion during infection [28]. It is unknown whether *S. maltophilia* GpmA is similarly localized to the cell surface, though this action could directly explain the attachment defect in the *gpmA* strain. Furthermore, a separate *S. maltophilia* transposon mutagenesis screen identified additional glycolysis/gluconeogenesis enzymes (glyceraldehyde 3-phosphate dehydrogenase and pyruvate dehydrogenase) as involved in biofilm formation, suggesting a unique role for carbohydrate metabolism in *S. maltophilia* biofilm formation, possibly through effects on extracellular

matrix production [10]. Alternatively, *gpmA* might contribute to production of a metabolite important for synthesis of an adhesive molecule. As in other biofilm forming bacteria, pili and flagella have been implicated in *S. maltophilia* adherence [1, 10]. Thus, it is possible that *gpmA* affects fimbrial or flagellar synthesis.

Taken together, this study reports a novel gene that facilitates *S. maltophilia* adherence to epithelial cells and plastic. Attachment to both such surfaces has important implications in nosocomial settings involving patient tissues, surgical equipment, and prosthetic devices. Thus, our finding has significant clinical importance, given the frequency and seriousness of *S. maltophilia* biofilm infections. Moreover, our data link biofilm formation with carbohydrate metabolism in *S. maltophilia*, suggesting a unique avenue for attacking this deadly pathogen.

Acknowledgments

We thank Rob Shanks for kind donation of pMQ400 and Teri Belecky-Adams for use of the Keyence microscope. We also thank Eli Klein, AJ Shih, Cameron Melton, Natalie Collins, Jordan Sterr, Nicole Egbert, Samip Vaidh, and Sian Zhang for technical assistance. This work was supported by 16GRNT30230012 from the American Heart Association and 1R03AR068724-01 from the National Institutes of Health to G.G.A.

7. References

- [1]. Sánchez MB. Antibiotic resistance in the opportunistic pathogen *Stenotrophomonas maltophilia*. Front Microbiol 2015;6:658. [PubMed: 26175724]
- [2]. Lemos DR, Franco AR, de Sa Roriz ML, Carneiro AK, de Oliveira Garcia MH, de Souza FL, et al. Measles epidemic in Brazil in the post-elimination period: Coordinated response and containment strategies. Vaccine 2017;35:1721–8. [PubMed: 28256359]
- [3]. Adegoke AA, Stenström TA, Okoh AI. *Stenotrophomonas maltophilia* as an emerging ubiquitous pathogen: looking beyond contemporary antibiotic therapy. Front Microbiol 2017;8:2276. [PubMed: 29250041]
- [4]. Esposito A, Pompilio A, Bettua C, Crocetta V, Giacobazzi E, Fiscarelli E, et al. Evolution of *Stenotrophomonas maltophilia* in cystic fibrosis lung over chronic infection: a genomic and phenotypic population study. Front Microbiol 2017;8:1590. [PubMed: 28894437]
- [5]. Rouf R, Karaba SM, Dao J, Cianciotto NP. *Stenotrophomonas maltophilia* strains replicate and persist in the murine lung, but to significantly different degrees. Microbiology 2011;157:2133–42. [PubMed: 21546584]
- [6]. Costerton JW, Lewandowski Z, Caldwell DE, Korber DR, Lappin-Scott HM. Microbial biofilms. Annu Rev Microbiol 1995;49:711–45. [PubMed: 8561477]
- [7]. Davey ME, O'Toole GA. Microbial biofilms: from ecology to molecular genetics. Microbiol Mol Bio Rev 2000;64:847–67. [PubMed: 11104821]
- [8]. Brooke JS. *Stenotrophomonas maltophilia*: an emerging global opportunistic pathogen. Clin Microbiol Rev 2012;25:2. [PubMed: 22232370]
- [9]. Huang TP, Somers EB, Wong AC. Differential biofilm formation and motility associated with lipopolysaccharide/exopolysaccharide-coupled biosynthetic genes in *Stenotrophomonas maltophilia*. J Bacteriol 2006;188:3116–20. [PubMed: 16585771]
- [10]. Kang X, Wang F, Zhang H, et al. Genome-wide identification of genes necessary for biofilm formation by nosocomial pathogen *Stenotrophomonas maltophilia* reveals that orphan response regulator FsnR is a critical modulator. Appl Environ Microbiol 2015;81: 1200–09. [PubMed: 25480754]
- [11]. Ryan RP, Fouhy Y, Garcia BF, Watt SA, Niehaus K, Yang L, et al. Interspecies signalling via the *Stenotrophomonas maltophilia* diffusible signal factor influences biofilm formation and

polymyxin tolerance in *Pseudomonas aeruginosa*. *Mol Microbiol* 2008;68:75–86. [PubMed: 18312265]

- [12]. Crossman LC, Gould VC, Dow JM, Vernikos GS, Okazaki A, Sebaihia M, et al. The complete genome, comparative and functional analysis of *Stenotrophomonas maltophilia* reveals an organism heavily shielded by drug resistance determinants. *Genome Biol* 2008;9:R74. [PubMed: 18419807]
- [13]. Ryan RP, Monchy S, Cardinale M, Taghavi S, Crossman L, Avison MB, et al. The versatility and adaptation of bacteria from the genus *Stenotrophomonas*. *Nat Rev Microbiol* 2009;7:514–25. [PubMed: 19528958]
- [14]. Kuchma SL, Connolly JP, O'Toole GA. A three-component regulatory system regulates biofilm maturation and type III secretion in *Pseudomonas aeruginosa*. *J Bacteriol* 2005;187:1441–54. [PubMed: 15687209]
- [15]. Simon R, Quandt J, Klipp W. New derivatives of transposon Tn5 suitable for mobilization of replicons, generation of operon fusions and induction of genes in gram-negative bacteria. *Gene* 1989;80:161–9. [PubMed: 2551782]
- [16]. Alshalchi SA, Anderson GG. Involvement of stress-related genes *polB* and PA14_46880 in biofilm formation of *Pseudomonas aeruginosa*. *Infect Immun* 2014;82:4746–57. [PubMed: 25156741]
- [17]. Shanks RM, Caiazza NC, Hinsa SM, Toutain CM, O'Toole GA. *Saccharomyces cerevisiae*-based molecular tool kit for manipulation of genes from gram-negative bacteria. *Appl Environ Microbiol* 2006;72:5027–36. [PubMed: 16820502]
- [18]. Redelman CV, Chakravarty S, Anderson GG. Antibiotic treatment of *Pseudomonas aeruginosa* biofilms stimulates expression of the magnesium transporter gene *mgtE*. *Microbiology* 2014;160:165–78. [PubMed: 24162608]
- [19]. Coffey BM, Anderson GG. Biofilm formation in the 96-well microtiter plate. *Methods Mol Biol* 2014;1149:631–41. [PubMed: 24818938]
- [20]. Anderson GG, Moreau-Marquis S, Stanton BA, O'Toole GA. *In vitro* analysis of tobramycin-treated *Pseudomonas aeruginosa* biofilms on cystic fibrosis-derived airway epithelial cells. *Infect Immun* 2008;76:1423–33. [PubMed: 18212077]
- [21]. Bruscia E, Sangiuolo F, Sinibaldi P, Goncz KK, Novelli G, Gruenert DC. Isolation of CF cell lines corrected at F508-CFTR locus by SFHR-mediated targeting. *Gene Ther* 2002;9:683–5. [PubMed: 12032687]
- [22]. Melvin JA, Gaston JR, Phillips SN, Springer MJ, Marshall CW, Shanks RMQ, et al. *Pseudomonas aeruginosa* contact-dependent growth inhibition plays dual role in host-pathogen interactions. *mSphere* 2017;2.
- [23]. Wu K, Yau YCW, Matukas L, Waters V. Biofilm compared to conventional antimicrobial susceptibility of *Stenotrophomonas maltophilia* Isolates from cystic fibrosis patients. *Antimicrob Agents Chemother* 2013;57:1546–8. [PubMed: 23295930]
- [24]. Moreau-Marquis S, Bomberger JM, Anderson GG, Swiatecka-Urban A, Ye S, O'Toole GA, et al. The F508-CFTR mutation results in increased biofilm formation by *Pseudomonas aeruginosa* by increasing iron availability. *Am J Physiol Lung Cell Mol Physiol* 2008;295:L25–37. [PubMed: 18359885]
- [25]. Britton HG, Clarke JB. The mechanism of the phosphoglycerate mutase reaction. *Biochem J* 1969;112:10P–1P.
- [26]. Becker P, Hufnagle W, Peters G, Herrmann M. Detection of differential gene expression in biofilm-forming versus planktonic populations of *Staphylococcus aureus* using micro-representational-difference analysis. *Appl Environ Microbiol* 2001;67:2958–65. [PubMed: 11425708]
- [27]. Zhang H, Zheng J, Yi L, Li Y, Ma Z, Fan H, et al. The identification of six novel proteins with fibronectin or collagen type I binding activity from *Streptococcus suis* serotype 2. *J Microbiol* 2014;52:963–9. [PubMed: 25359271]
- [28]. Poltermann S, Kunert A, von der Heide M, Eck R, Hartmann A, Zipfel PF. Gpm1p is a factor H-, FHL-1-, and plasminogen-binding surface protein of *Candida albicans*. *J Biol Chem* 2007;282:37537–44. [PubMed: 17959597]

Figure 1. Isogenic deletion of *gpmA* leads to reduced biofilm levels at early stages on abiotic surfaces.

(A) Attachment efficiency was quantified by measuring the intensity of CV staining of *gpmA* compared to that of wild type (WT) and *gpmA* complemented with *pgpmA*. pMQ132 is the empty vector, used as a control. Absorbance was measured at 550 nm (OD₅₅₀) as described in Materials and Methods. (B and C) Biofilm formation was measured after 4 hr and 6 hr respectively. Data are representative of three independent experiments performed in triplicate (n = 3). Error bars represent standard deviations. * $p < 0.05$ compared to WT. ‡ $p < 0.001$ compared to *gpmA*.

Figure 2. Mutant strains grow equally as well as the wild type in LB and MEM.

No differences were observed in the growth kinetics of wild type (WT) pMQ132, *gpmA* pMQ132, and *gpmA pgpmA* in both LB (A) and MEM (B). Absorbance was measured at 600 nm (OD_{600}) over the course of 12 hr. The results are representative of three independent experiments carried out in triplicate ($n=3$) and error bars represent standard deviations. No statistically significant difference was found between strains in either medium.

Fig. 3. *gpmA* is defective for attachment on CFBE airway cells.

(A) Attachment efficiency was quantified by measuring CFU/mL after 1 hr incubation at 37 °C. (B and C) Biofilm levels were measured at 4 hr and 6 hr respectively. The results are representative of three independent experiments carried out in triplicate (n = 3) and error bars represent standard deviation. * $p < 0.05$ compared to WT. ‡ $p < 0.05$ compared to *gpmA*. (D and E) Composite images from fluorescence microscopy of CFBE cells incubated with WT (D) or *gpmA* (E) for 1 hr. pMQ400 contains a constitutive TdTomato gene, conferring red fluorescence to the bacteria. CFBE cell membranes were stained with WGA-Alexa Fluor 350 (blue).