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Identification of pili on the surface of *Finegoldia magna* – A Gram-positive anaerobic cocci

Elizabeth C. Murphy [a,*], Robert Janulczyk [a,b], Christofer Karlsson [a], Matthias Mörgelin [a], Inga-Maria Frick [a]

[a] Department of Clinical Sciences, Division of Infection Medicine, Lund University, SE-22184, Sweden
[b] Novartis Vaccines and Diagnostics, Via Fiorentina 1, 53100 Siena, Italy

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ABSTRACT

Pili have only been discovered in the major Gram-positive pathogens in the past decade and they have been found to play an important role in colonisation and virulence. Pili have been shown to have many important functions including attachment to host tissues, mediating bacterial aggregation, biofilm formation and binding to proteins in the extracellular matrix. In this study, sortase-dependent pili have been found to be expressed on the surface of *Finegoldia magna* ALB8. *F. magna* is a Gram-positive anaerobic coccus that, primarily, is a commensal of the skin and mucous membranes, but has also been isolated from various clinical infection sites and is associated with soft-tissue abscesses, wound infections and bone and prosthetic joint infections.

In this study, *F. magna* ALB8 was found to harbour three sortases at the pilus locus, two of which bear high similarity to class C sortases in *Streptococcus pneumoniae*. Two putative sortase-dependent pili proteins were found in the locus, with one being identified as the major pilus subunit, Fmp1 (*F. magna* pilus subunit 1), due to its high similarity to other major pilus proteins in prominent Gram-positive pathogens. The presence of sortase-dependent pili was confirmed experimentally through recombinant production of Fmp1 and production of antiserum. The Fmp1 antiserum was used in Western blot to show the presence of a high molecular weight protein ladder, characteristic of the presence of pili, in trypsin released cell wall surface proteins from *F. magna*. The presence of sortase-dependent pili was visually confirmed by transmission electron microscopy, which showed the binding of gold labelled anti-Fmp1 to individual pilus proteins along the pilus. Furthermore, pili could also be found to bind and interact with keratinocytes in the epidermal layer of human skin, suggesting an adhesive role for pili on *F. magna*.

Our work represents the first description of pilus structures in *F. magna*. This discovery further elucidates *F. magna* physiology and allows for additional analysis of host–bacterial interactions in future studies.

1. Introduction

Finegoldia magna (previously *Peptostreptococcus magnus*) is a commensal bacterium colonising human skin, mucous membranes and the gastrointestinal and urogenital tracts [1]. As well as being a commensal, it can also act as an opportunistic pathogen and is a member of the Gram-positive anaerobic cocci (GPAC). Among the Gram-positive anaerobic bacteria associated with clinical infections GPAC are the most prominent group, accounting for 25–30% of all anaerobic bacteria isolated from clinical specimens [1]. *F. magna* has the highest pathogenicity of the GPAC, being the most common GPAC isolated from clinical infection sites [1]. It is associated with soft tissue abscesses, bone and joint infections, wound infections and vaginosis [1–6]. *F. magna* is isolated from both mixed and pure infection sites, where approximately 70% of *F. magna* strains have been found to co-exist with other bacteria, such as group D streptococci, *Staphylococcus*, *Bacteroides* and *Fusobacterium* [7]. Problems in obtaining good quality anaerobic specimens and difficulties in culturing GPAC, mean that their abundance and importance, as both commensals and opportunistic pathogens, is probably highly underestimated.

* Corresponding author. Tel.: +46 46 222 81 68.
E-mail addresses: Elizabeth.Murphy@med.lu.se (E.C. Murphy), janulczyk@yahoo.se (R. Janulczyk), Christofer.Karlsson@med.lu.se (C. Karlsson), Matthias.Morgelin@med.lu.se (M. Mörgelin), Inga-Maria.Frick@med.lu.se (I.-M. Frick).

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Possible reasons for the prominence of *F. magna* in GPAC infections could be due to the expression of proteins that enhance virulence. Previously identified virulence factors in *F. magna* include the superantigen protein L, the albumin binding protein PAB, protein FAF (*F. magna* adhesion factor) and the subtilase-like enzyme SufA [8–12]. Other important pathogenicity factors of *F. magna* include capsule formation and production of the enzymes collagenase and gelatinase [13,14].

In this study, another potential virulence factor was identified – pili on the surface of *F. magna*. Pili are long filamentous structures extending from the cell surface and are present on most bacterial pathogens [15]. Pilus-like structures were first observed in Gram-negative pathogens in the early 1950s, but have only recently been identified in some of the more prominent Gram-positive pathogens [15]. In the past decade, pili have been characterised in the three most significant streptococcal pathogens – group A *streptococcus* (*Streptococcus pyogenes*) [16], group B *streptococcus* (*Streptococcus agalactiae*) [17,18] and *Streptococcus pneumoniae* [19]. Two types of pilus-like structures have thus far been identified in Gram-positive pathogens: short, thin rods or fibrils that extend between 70 and 500 nm from the bacterial surface and longer more flexible rods up to 3 μm long [15].

Rod-like pili were first described in *Corynebacterium diphtheriae* and were found to consist of three covalently linked protein subunits, each consisting of a variant of the cell wall sorting sequence (canonically LPXTG where X denotes any amino acid), which is the target of sortase enzymes. Sortases act as important virulence factors, effectively helping Gram-positive bacteria interact with the host and environment by covalently attaching proteins to the cell wall or polymerising proteins in the biosynthesis of sortase-dependent pili [20–22]. They function as cysteine transpeptidases that connect proteins with a cell wall sorting signal (CWSS) to an amino group of peptidoglycan cross-bridges at the cell surface [23,24]. Variants of the cell wall sorting sequence, with their cognate sortases, lead to a diverging enzymatic reaction. For example, the prototypical reaction involves sortase class A targeting the substrate motif LPXTG of surface proteins and anchors the proteins to the cell wall envelope [25,26]. However, sortase class B targets the NP(Q/K)TN motif of haem transport factors and cross-links the anchored haem-containing products near membrane transporters [27–29]. Pilus proteins have the substrate motif (I/L)(P/A)XTG and are generally targeted by class C sortases, which catalyse a transpeptidation reaction forming covalent links between individual pilus proteins [27,30]. There is also a class D sortase, which targets the LPNTA motif of mother cell and endospore envelope proteins [24]. In order for pilus formation to occur, specific sortases catalyse the covalent binding of pilus subunits to each other by non-disulphide covalent linkages [31,32]. Usually, sortase-dependent pili consist of one main (backbone) protein and one or two ancillary proteins, which can occur at the tip of the pilus, along the side or at the bacterial surface, anchoring the pilus to the bacterial surface [15]. One of the most important functions of sortase-dependent pili is the adhesion and attachment to host cells during colonisation and invasion. Furthermore, pilus components from invasive streptococci have a significant homology to the microbial surface components recognizing the adhesive matrix molecules family of proteins (MSCRAMMs) [15,33]. Sortase-dependent pili have also been shown to mediate bacterial aggregation, which could contribute to the colonisation of tissues, increase the bacterial resistance to the host immune response and aid beneficial interactions between different bacterial species [15,34–36].

In this study, we describe the identification of sortase-dependent pili on the surface of *F. magna*, which sheds further light into its molecular mechanisms for both infection and commensalism and paves the way for further analysis.

2. Methods

2.1. Bacteria and growth conditions

F. magna strain ALB8 isolate was from Lund University Hospital, Lund, Sweden and has been described earlier [10]. *F. magna* ALB8, from which the virulence factors, SufA and FAF have been produced, was originally isolated from a scrotal abscess [11,12]. *F. magna* strain 505, which does not express FAF, but does express SufA, was isolated from a urethra infection site. *F. magna* strains 312, 1458, 1459, 1462, 1468, ATCC 29328, 17.30 and 2186 were also from Lund University Hospital, Lund, Sweden and were isolated from various clinical infection sites. All strains were a kind gift from Dr. Elisabet Holst at the Department of Clinical Microbiology, Lund University Hospital, Sweden and have been described before [11]. No ethical approval was required. *F. magna* was grown in Todd-Hewitt broth (TH) (Difco) supplemented with 0.5% Tween-80 at 37 °C under strict anaerobic conditions.

2.2. Proteins, antibodies and reagents

Antibodies against recombinant protein Fmp1 were raised in rabbits (Biogenes). Horseradish peroxidase (HRP) conjugated goat anti-rabbit IgG was purchased from Pierce.

2.3. Cloning, expression and purification of protein Fmp1

For the cloning and sequencing of *fmp1*, primers were designed based on the genome sequence of *F. magna* ALB8 (NCBI accession number: BA93_05025). Primers were designed to contain the *Bam*HI and *Xho*I restriction sites for later insertion into a pGEX_6p_1 vector. The 5′ forward primer 5′-CAGCAGGGATCC-GAACACAACTGGAATGCT-3′ and 3′ reverse primer 5′-CAGCAGCTC-GAGTGATTCAAGTTTTGCTTCGTA-3′ were used in PCR to amplify a 2209 bp fragment (bp 130-2339, removing the signal peptide and cell wall sorting signal) of *fmp1* from *F. magna* ALB8 chromosomal DNA. The gene was cloned into pGEX_6p_1 (Amersham Pharmacia) and positive clones were sequenced (Eurofins MWG Operon). The Fmp1 protein was expressed in *E. coli* BL21 (Life Technologies), fused to GST, by growing to an OD of 0.6 and induced to express Fmp1 by the addition of 0.5 mM IPTG for 3 h at 37 °C. Bacteria were lysed by the addition of 50 μg/ml lysozyme and freeze thawing of the cell pellet. Soluble proteins were clarified through centrifugation and analysed on a 12% SDS-PAGE gel. Fmp1 was later purified on glutathione Sepharose according to the manufacturer's instructions. The GST-tag was cleaved off using PreScission protease (Amersham Pharmacia).

2.4. Slot blot, SDS-PAGE and western blot analysis

SDS-PAGE was performed as described by Neville et al. [37]. Samples were prepared for boiling in sample buffer containing 2% SDS and 5% β-mercaptoethanol for 5 min and 12% SDS-PAGE were used to study Fmp1 expression. Separated proteins were visualised by PageBlue protein staining solution (Thermo Scientific). To examine for the presence of sortase-dependent pili, samples from trypsin digestion (see method below) were concentrated by trichloroacetic acid precipitation and resuspended in NuPAGE LDS sample buffer (Life Technologies). Concentrated protein samples were separated on 3–8% NuPAGE Tris-Acetate gels (Life Technologies). For Western Blot analysis, proteins were electrophoretically transferred to a polyvinylidene difluoride (PVDF) membrane (Amersham Biosciences). Membranes were blocked in a phosphate buffered saline-Tween (PBS containing 0.1% Tween 20 (PBS-T)) solution containing 5% (wt/vol) skim milk powder at 37 °C for