

# Structural Characterization of *Actinomyces naeslundii* Fimbriae using Mass Spectrometry



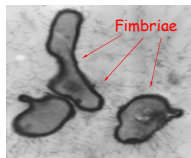
Jenny T.C. Ho<sup>1,3</sup>, John O. Cisar<sup>2</sup> & Sonja Hess<sup>3</sup>

<sup>1</sup>Mass Spectrometry and Proteomics Facility, NIDDK, NIH, DHHS, Bethesda, MD. <sup>2</sup>NICDR, NIH, DHHS, Bethesda, MD. <sup>3</sup>Proteome Exploration Laboratory, California Institute of Technology, Pasadena, CA

## Objectives

• Use sample digestion methods combined with mass spectrometry techniques to probe the presence of amino acid cross-linking in fimbriae of *Actinomyces naeslundii*.

## Actinomyces



• Gram positive Bacteria.

• Found in the oral cavity.

• Involved in early stages of plaque development.

• Have fimbriae/pili protruding from cell wall

## Fimbriae on Actinomyces

• Fimbriae mediate adhesion of bacteria to host cells → colonization → infection.

• Each fimbria is composed of multiple protein subunits covalently linked to form a hair-like structure (fig 2).

• Fimbriae are covalently attached to the peptidoglycan cell wall.

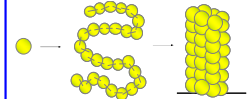


Figure 2: Cartoon depiction of fimbriae on *A. naeslundii*. Polymerisation of subunits forms a 3-dimensional hair-like structure that is covalently anchored to the peptidoglycan cell wall.

• FimP subunits form Type 1 fimbriae, FimA subunits form Type 2 fimbriae.

• Polymerization and attachment to cell wall is proposed to involve a highly conserved LPXTG motif (sorting signal) and the action of sortases.

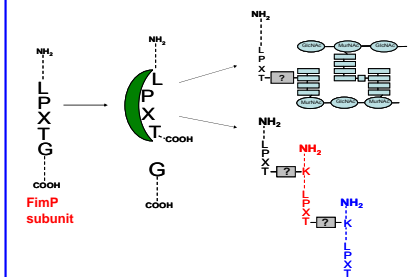


Figure 3: Cartoon depiction of the proposed action of sortases in the polymerization of FimP subunits and the attachment to the cell wall. Briefly, sortase catalyzes cleavage of the amide bond between G and T of the LPXTG motif of FimP followed by amide bond formation either to a peptidoglycan of the cell wall or to a Lys of another FimP subunit.

• Molecular studies supports the proposed mechanism but evidence of the nature and location of cross-linker(s) on *A. naeslundii* is still lacking.

## Experimental

• **FimP**: Type 1 fimbriae from *A. naeslundii* were isolated and purified as previously described (Cisar, J.O *et al.*, Journal of General Microbiology, 1991, 137, 1971-1979).

• **FimP in-solution digestion**: For dilute acid hydrolysis, FimP (400 pmole) was treated with 0.8μL of 6N constant boiling HCl in a glass tube and boiled at 100°C for 1 hour. The resulting mixture was further digested with trypsin in 50mM amm. bic. (25μL), at 37°C for 18 hours.

• **LC-MS/MS analysis**: 1μL of digest mixture was analysed by LC-MS/MS. LC separation was performed on a C18 (MicroTech) column using increasing amounts of 0.1% formic acid in ACN. The eluent was directly electrosprayed into a LTQ-FITICR mass spectrometer (ThermoElectron).

• **Peptide desalting for off-line ES-MS**: digest mixture was desalted using a C8 protein trap column (Michrom Bioresources). Peptides were eluted in 4:1 ACN/H<sub>2</sub>O, containing 0.1% formic acid.

• **ES-MS and MS/MS by direct infusion**: The desalted FimP digest was directly electrosprayed into a LTQ-FT (ThermoElectron) equipped with ECD and IRMPD using a Triversa Nanomate (Advion Biosciences).

## Results: Digestion and LC-MS/MS of Purified FimP

• In-solution digestion of purified FimP by dilute acid hydrolysis followed by trypsin resulted in ~80% sequence coverage when analyzed by LC-MS/MS (fig 4).

APADPNGSTIDPDAATLLTVHKCEQDTNGVKEGTGNEPDQAECQVPSDFEFTIKLNVLDLTYDGW  
KTLADLKGDVVAGALGSTTVYKTTGANGLASFDTAQTEVGAAYLVSERTPDKVIPAEDFVFLPMT  
NPQDTAKWYNNVHYVYKNTLGSVDKQVTKDPAPGSRDITVITTSIPKVDPYGGARIKRYEVDVLD  
KRKKEALTPVVKVGGNEVTLAETTYDLTLAGKDNHWATIQLTEGGRKASEARYNNGETKLGQV  
LNAKFDAAVNLLEGDLNNTAGLIPNDSFNFTWDPNPNPQTDDIPGIPPTVPLSKYGVKLVLTGTDGLD  
ADKTKYNGAOGFVYCEKTAGSAGALLRDSPOSTVDPLDTEGKFTTAAAGGTVFVYLRANDYVNG  
AKKQDLTDDEYCYLVETKAPGVNLAQDPLPFRVLAEKAEKKAATEVTVTDIPXNAGFRPLPLTGANG  
VFILTAGALLVAGGAVVAYANKRRHVAKH

Figure 4: Amino acid sequence of FimP subunit. Conserved LPXTG sorting signal and the lysine proposed to be involved in polymerization is highlighted in red. FimP sample was treated with dilute acid hydrolysis followed by tryptic digestion. The resulting digest was analyzed by LC-MS/MS. Observed FimP peptides are highlighted in blue. Potential cross-linked peptide highlighted in yellow (see fig 6-12).

• Fig 5 shows the 1D SDS PAGE separation of purified FimP, stained with Coomassie Blue. Band at ~50kDa = monomeric FimP.

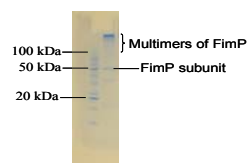


Figure 5: 1D SDS PAGE separation of FimP sample. Proteins stained with Coomassie blue.

• Since fimbriae from *A. naeslundii* are resistant to dissociation into monomeric subunits by biochemical methods and that a majority of the peptides observed in the LC-MS/MS analysis of the digest corresponded to FimP (fig 6), this suggests that the higher molecular weight bands on the gel (fig 5) correspond to covalently linked multimers of FimP.

## Results: Determination of potential cross-linked FimP peptide using MS<sup>n</sup>

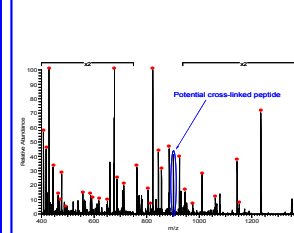


Figure 6: Summed TIC of LC-MS/MS analysis of FimP treated with dilute acid hydrolysis followed by trypsin digestion. • Indicates peptides corresponding to FimP. Highlighted in blue corresponds to a triply charged ion at m/z 901.15, a potential cross-linked FimP peptide (see subsequent figures).

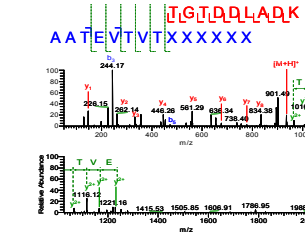


Figure 7: (top and bottom panel): MS2 IRMPD spectrum of triply charged ion at 901.15. The spectrum shows 2 fragment ion series; those labeled in red correspond to peptide TGTDDLADK and labeled in blue corresponds to a peptide beginning with AATEVTVTX. Both peptides correspond to FimP, the latter is upstream from the LPXTG motif (see fig 4). Spectrum acquired by direct infusion using a Nanomate and LTQ-FITICR.

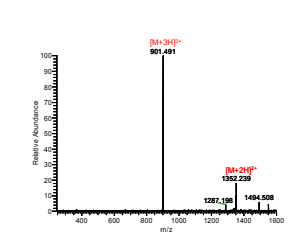


Figure 8: MS2 ECD spectrum of triply charged ion at 901.15. Spectrum acquired by direct infusion using a Nanomate and LTQ-FITICR. Spectrum shows the triply charged precursor and its corresponding double charged ion. No fragment ions were observed.

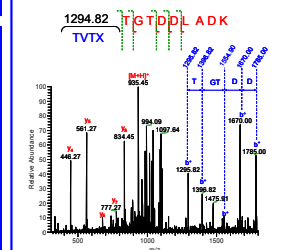


Figure 9: MS3, 901.15<sup>3+</sup> → 1115.2<sup>+</sup> (1115 observed as a y-ion in figure 7). The spectrum shows y- and b-ions from the peptide beginning with TGTDDLADK. The b-ion series has an additional mass of 1294.82 Da, which corresponds to part of the peptide beginning with AATEVTVTX (fig 7). The observation of b<sub>1</sub>-ion of TGTDDLADK indicates that the side chain of Thr is involved in cross-linking. Spectrum acquired by direct infusion using a Nanomate and LTQ.

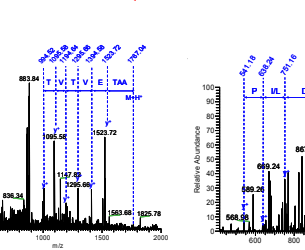


Figure 10: MS3, 901.15<sup>3+</sup> → 884.2<sup>+</sup> (883 corresponds to the entire peptide beginning with AATEVTVTX). Although the remainder of the sequence is known the measured mass of the entire peptide does not correspond to the sequence shown in fig 4. We attribute the mass difference to the crosslinker. Spectrum acquired by direct infusion using a Nanomate and LTQ.

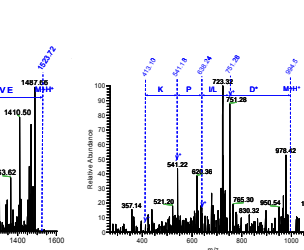


Figure 11: MS3, 901.15<sup>3+</sup> → 1523.5<sup>+</sup> (1523 observed in fig 7 (MS2) and fig 10 (MS3)). Further stages of CAD of fragment ions from the peptide beginning with AATEVTVTX provided further peptide sequence information and also confirmation of the sequence given in fig 4. \* corresponds to an addition of 128 Da of unknown chemical composition. Spectrum acquired by direct infusion using a Nanomate and LTQ.

## Conclusions

• Mass spectrometry provided evidence for the presence of novel cross-linking in fimbriae of *A. naeslundii*.  
 • CID of a triply charged ion at m/z 901.15 showed two fragment ion series, one corresponding to TGTDDLADK and the other ATEVTVTD\*IPX. Both are FimP peptides.  
 • MS2 CID experiments indicated that the side chain of N-terminal threonine in TGTDDLADK is involved in cross-linking to the other peptide.  
 • MSn CID experiments reveals that aspartic acid of ATEVTVTDIPX is covalently modified by an unknown chemical group. The remaining sequence of this peptide is yet to be determined.

## Future work

• Use a combination of chemical derivatization methods and MS techniques to determine the remaining sequence of peptide AATEVTVTD\*IPX. Elucidate the chemical composition of the modification on Asp and the nature of the linkage to TGTDDLADK.

## Acknowledgments

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