Evaluation of Amyloid-like FadA Production in Various Strains of Fusobacterium nucleatum

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BACKGROUND

Fusobacterium nucleatum (*Fn*) is a gram-negative oral anaerobe that has been found to play a role in many diseases including colorectal cancer, periodontal disease, and GI disorders. *Fn* encodes Fusobacterium adhesin A (FadA) which is an important virulence factor conserved in *Fn*. Previous studies have shown that *Fn* 12230 produces amyloid like FadA during stationary phase but not in the log phase. This growth dependent amyloid like FadA production may be induced by stress and nutrient deprivation. In vitro and in vivo data showed that amyloid FadA enhances the pathogenicity of *Fn* 12230 and is a key player in biofilm formation, inducing periodontal bone loss and promoting colorectal cancer progression. It is unknown whether amyloid like FadA production is representative for the species and is consistent among the different strains of *Fn*. In this study, we explored the production of amyloid like FadA in *F. nucleatum* strain ATCC 23726 and *F. nucleatum* ATCC 25586.

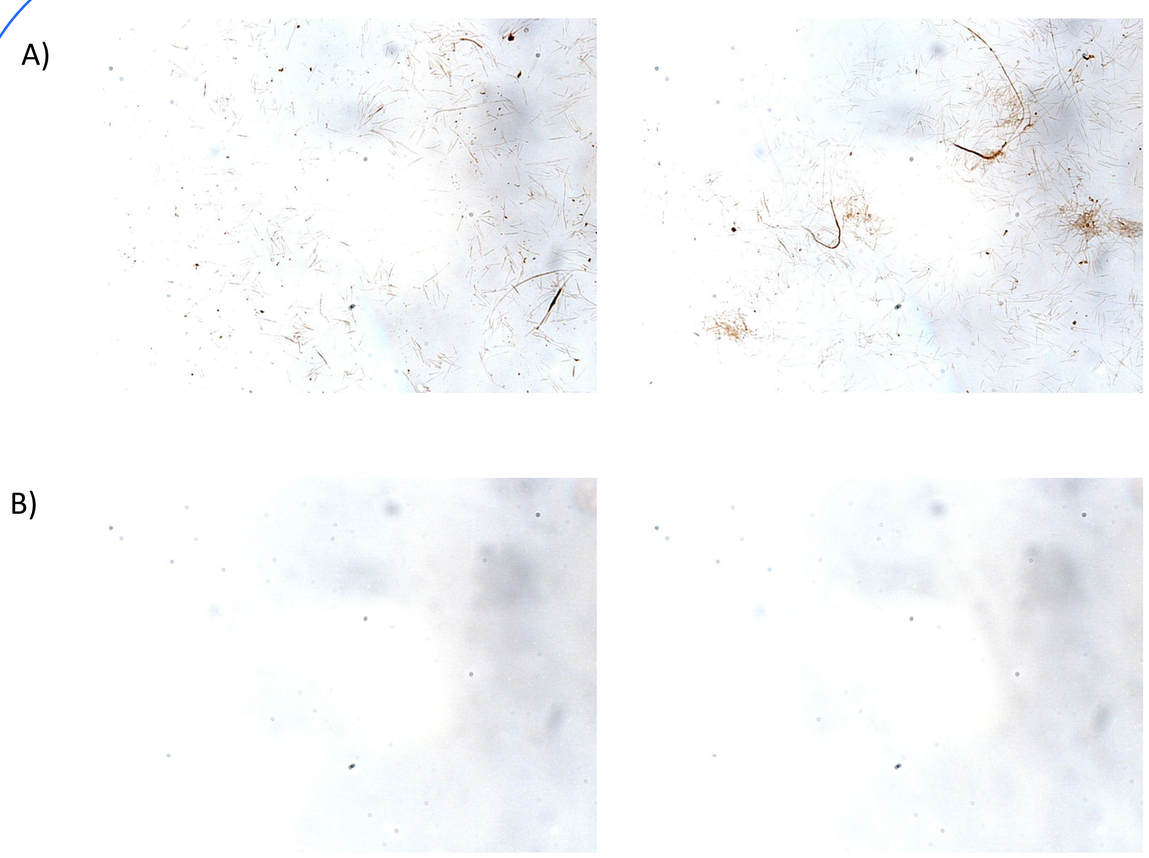
OBJECTIVES

The goal of the study is to evaluate if amyloid FadA production is conserved across different strains of *Fn*

METHODS & MATERIALS

•Wild type *Fn* 12230 and the FadA-deletion mutant designated as US1 (negative control) along with *Fn* 23726 and *Fn* 25586 were grown at 37° C in Columbia broth supplemented with 5 µg/mL hemin and 1 µg/mL menadione under anaerobic conditions. The production of amyloid FadA was then evaluated using immunohistochemistry.
•The bacteria are fixed using 4% paraformaldehyde and resuspended in phosphate buffered saline (PBS) before being fixed on glass slides. The slides are then rehydrated in PBS for 5 minutes and incubated in 3% hydrogen peroxide (H_2O_2) for 30 minutes. After washing in PBS, the slides are blocked in 5% skim milk for an hour at room temperature before incubation with anti-Fn antibody at 1:800 dilution overnight.
•After washing in PBS, the slides are blocked in 2.5% horse serum for an hour at room temperature before incubation with secondary ImmPRESS Horse-Mouse IgG for 30 minutes at room temperature. The slides are then washed with PBS-T (PBS + 0.05% Tween-20) and DAB (3,3'-diaminobenzidine) is added for development. After washing in deionized water, the slides are further washed for 10 seconds each in 70% ethanol (twice), 95% ethanol (twice), 100% ethanol (twice), and xylene (thrice). Finally, permount is added to the slides with a coverslip and allowed to dry for 24 hours before taking images using a light microscope

RESULTS



Immunohistochemical staining:
A) *Fn* 12230 showed amyloid-like FadA aggregates in stationary phase
B) No FadA was detected in US1

RESULTS

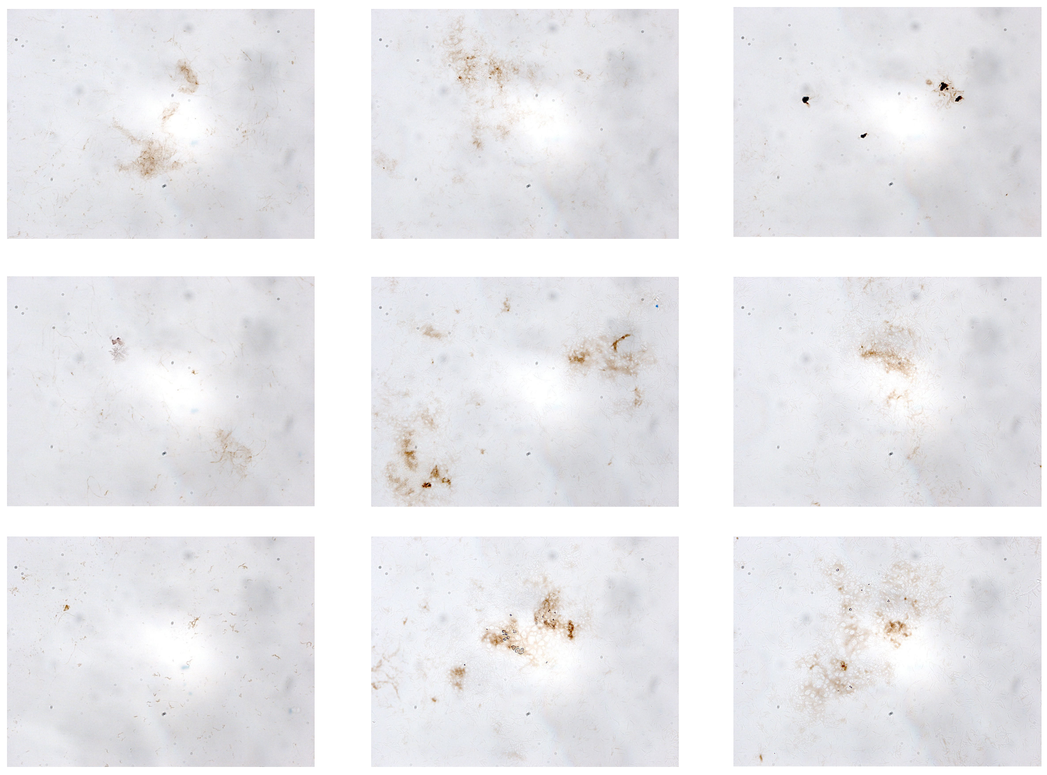


Figure 2: *Fn* 12230 (OD of 0.604) and *Fn* 23726 (OD of 0.481) both produced amyloid-like FadA during the stationary phase. However, *Fn* 25586 (OD of 0.455) did not show significant amyloid-like FadA production. More research is needed to make a conclusive statement.

DISCUSSION

FadA is an important virulence factor in *Fn* and plays an important role in its pathogenicity. As previous studies showed, the production of amyloid FadA in *Fusobacterium nucleatum* 12230 is regulated by stress response and metabolic activity. *Fn* is able to control its virulence and enhance its pathogenicity through regulating the production of amyloid FadA by growth phase. *Fn* 12230 had the greatest amyloid FadA secretion during stationary phase. Building off of this, the experiments with IHC showed that *Fn* 23726 had a similar pathogenic mechanism and also produced amyloid FadA under stressful conditions. This is important because it shows that there are similarities amongst the various strains of *Fn* and gives us a better understanding on how *Fn* can be targeted to improve the outcome of various afflictions like periodontal disease and colorectal cancer. Due to its virulence, amyloid FadA can be a therapeutic target and paves the way towards discovering new ways to inhibit the production of amyloid FadA. Unfortunately, a connection was not able to be made between *Fn* 12230 and *Fn* 25586. Future studies will investigate how production of amyloid FadA affects the virulence potential of different *Fn* strains.

CONCLUSIONS

Our results showed that *Fn* 23726 produced amyloid like FadA in the stationary phase similar to *Fn* 12230 in comparison to FadA deletion mutant (US1). It appears that both *Fn* 12230 and *Fn* 23726 share similar pathogenic mechanisms and secrete amyloid FadA when metabolic activities are reduced during the stationary phase. *Fn* 25586 did not appear to secrete amyloid FadA. Further optimization on growth conditions may be required to make a conclusive statement on the amyloid like FadA production in *Fn* 25586.

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