Fusobacterium Nucleatum Produces Amyloid FadA when Metabolic Activities are Reduced

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INTRODUCTION

- Fusobacterium Nucleatum (*Fn*) is a gram-negative oral commensal implicated in various human diseases. Recent studies have suggested that *Fn* enhances its pathogenicity by controlling the production of amyloid-like FadA under diseased and stressed conditions.
- Amyloid FadA functions to promote acid resistance, biofilm formation, host-cell binding, periodontal bone loss and colorectal cancer progression. In this study we focused on *Fn*'s metabolic activity in relation to amyloid-FadA production.
- We aimed to evaluate amyloid FadA production during various growth phases and assess its relation to bacterial metabolic activities.

METHODS & MATERIAL

- Wild Type *Fn* 12230 and its FadA-deletion mutant US 1 (negative control) were grown at 37°C in Columbia broth supplemented with 5 µg/mL hemin and 1µg/mL menadione under anaerobic conditions for 1 (log phase), 3 (early stationary phase), or 7 (late stationary phase) days.
- Bacterial metabolic activity at different time points were evaluated using Click-It Plus OPP Alexa Fluor 488 Protein Synthesis Assay Kit for protein synthesis following the manufacturer's instructions. The bacteria were then fixed on glass slides and stained with anti-FadA antibody 7H7 and mounted with antifade mounting medium with DAPI. Images were taken under a fluorescence microscope.

RESULTS

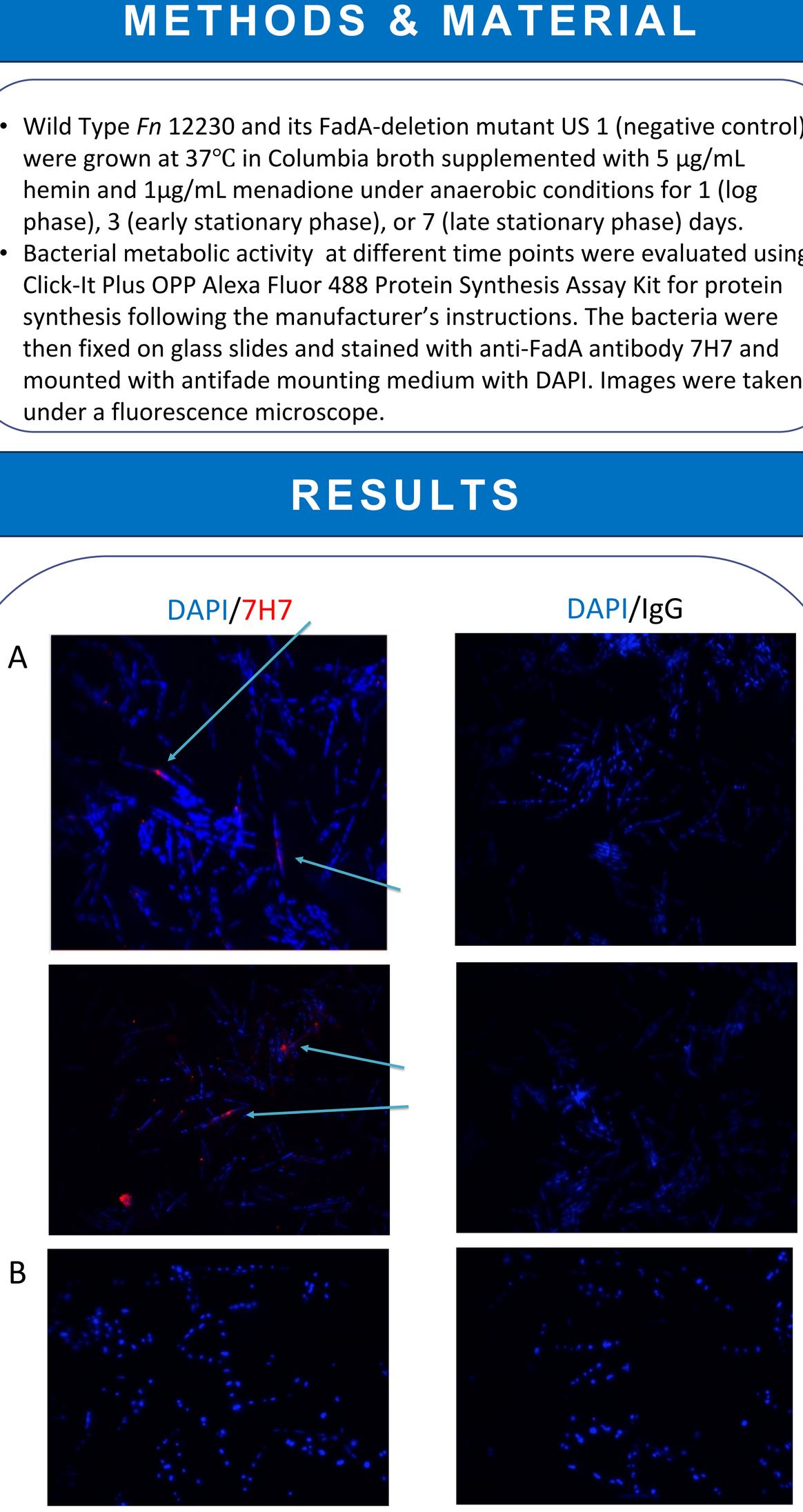

Figure 1: *Fn* produces significant amyloid like FadA in early stationary phase (day 3).
A Fn's nuclei was stained with DAPI (blue), amyloid FadA was stained with anti-FadA 7H7 (red). The images were stacked together to generate where amyloid FadA is produce in relation to the bacteria.
B US1 mutant was treated with DAPI and anti-FadA 7H7, no activity was seen.

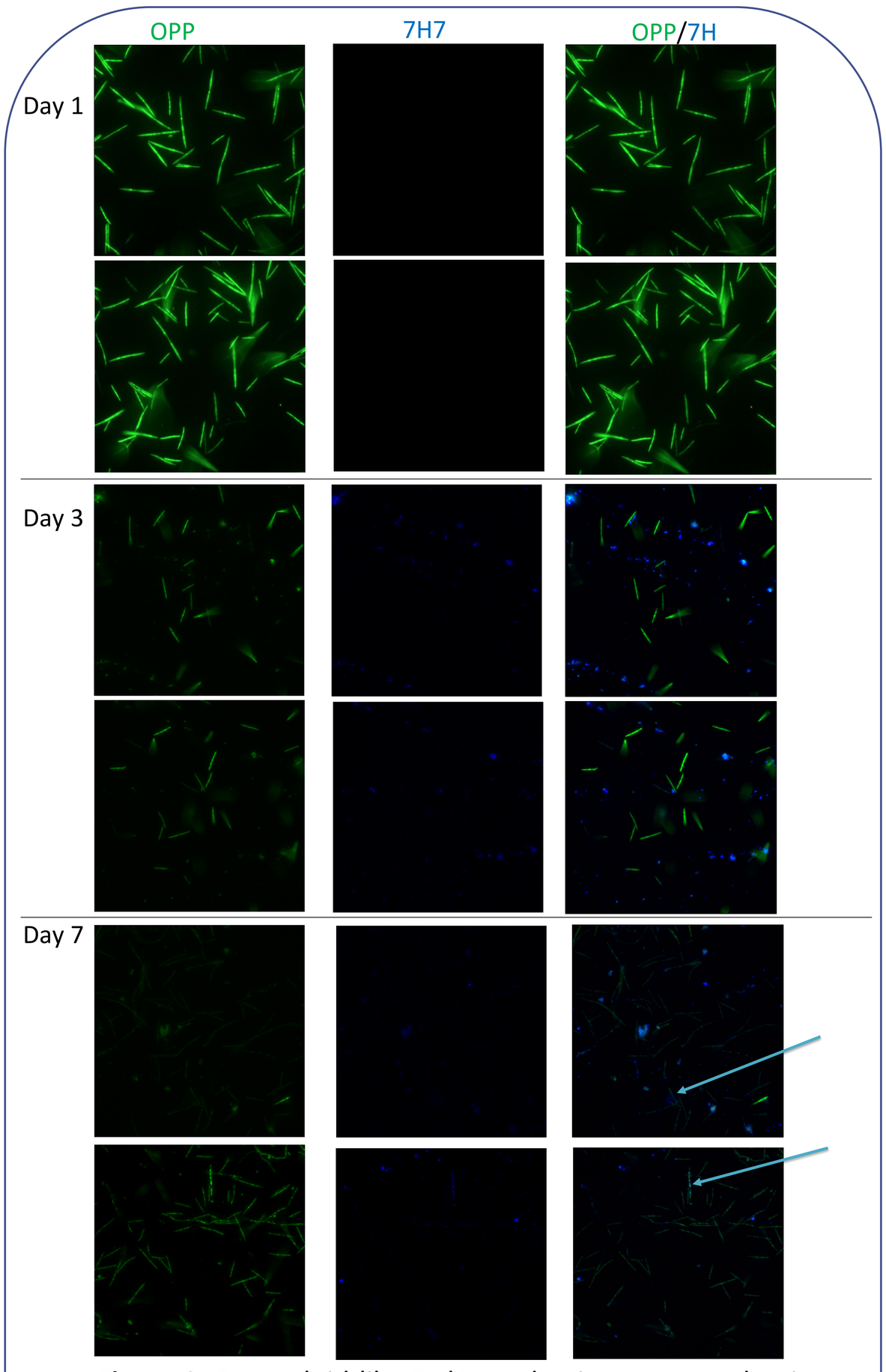

Figure 2: *Fn* amyloid-like FadA production compared to its metabolic activity.
Fn was analyzed during three different phases: log phase, early and late stationary phase. Images shows that only the stationary phase produces significant amount of amyloid like FadA while its metabolic activity was reduced.

DISCUSSION

Fn pathogenicity is regulated through the production of amyloid-like adhesin FadA under diseased and stressful conditions. *Fn* amyloid-like FadA production was initially analyzed with DAPI and Anti-FadA antibody during early stationary phase, showing significant activity. We then analyzed amyloid-like FadA production in comparison to *Fn*'s metabolic activity during various growth phases with the use of OPP and anti-FadA antibody. It was noted that log phase *Fn* showed significant metabolic activity but did not produce amyloid like FadA. However, as *Fn* reached early and late stationary phase, its metabolic activity decreased while amyloid-like FadA production increased.

CONCLUSION

Fn protein synthesis activity was more active during log phase compared to early and late stationary phase. However, secreted amyloid FadA was only detected in *Fn* 12230 in both stationary phases. We concluded that Amyloid FadA is synthesized in bacteria with significant reduced metabolic activities.

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