Physiological purpose of proposed Chondroitin binding proteins in *Methanobrevibacter smithii*

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Introduction

Bacteroides thetaiotaomicron (B. theta) and *Methanobrevibacter smithii (M. smithii)* are two organisms that are dominant within the gut of humans (1). The two microbes are predicted to have a commensal relationship. *M. smithii* was recently discovered to have proposed chondroitin binding proteins. Understanding this relationship is important because microbial degradation of polysaccharides leads to increased absorption of nutrients and organic acids that were previously unavailable to the host cells (2).

Purpose Statement: We want to understand whether these binding proteins allowed *M. smithii* to metabolize chondroitin or co-localize with *B. theta*.

Figure 1. *Methanobrevibacter smithii*(3)

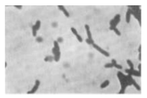


Figure 2. *Bacteroides thetaiotaomicron*(4)

Metabolism and Syntrophy

B. theta is a common dominant bacteria found in all human intestines and is able to break down a wide variety of polysaccharides(5). *M. smithii* is a methanogenic archaeon, which is the dominant archaeal species within the human gut(1). When *M. smithii* is present, *B. theta* experiences a significant rise in population due to the removal of its fermentation end products. Additionally, this produces an increase in acetate and formate production, resulting in both higher *M. smithii* populations and higher uptake of nutrients by the host cells(6).

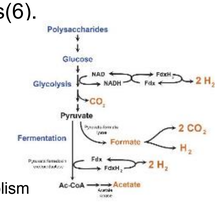


Figure 3. *B. theta* polysaccharide metabolism

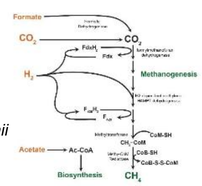
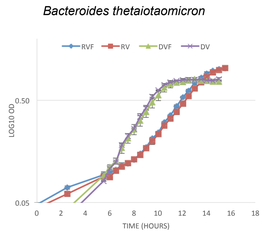


Figure 4. *M. smithii* hydrogenotrophic methanogenesis

B. theta growth on defined media

Figure 5. *B. theta* growth curves. *B. theta* was grown on both complex and defined media to see how doubling times would be affected. *B. theta* grew faster in defined media, but grew at a higher OD in complex media.

- RVF- Rich containing glucose, histidine-hematin and waste products
- RV- Rich containing glucose and histidine-hematin
- DVF- Defined containing glucose, histidine-hematin, and waste products
- DV- Defined containing glucose, histidine-hematin



Co-Culture Forms Clumps

A co-culture between *M. smithii* and *B. theta* was produced. Co-localization can be seen through the clumps shown above. These clumps are entirely blue, yet the outline of the much brighter fluorescing *M. smithii* can be seen below.

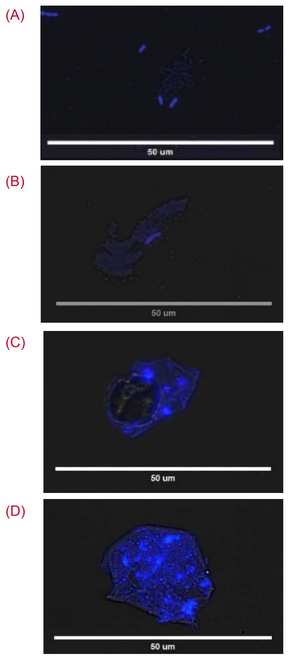
Figure 6. Various co-culture clumps of *M. smithii* and *B. theta*.

(A). Co-culture grown in a rich medium with chondroitin sulfate as sugar. Proposed first stage of clumping

(B). Co-culture grown in a rich medium with glucose as sugar. Proposed second stage of clumping

(C). Co-culture grown in a rich medium with glucose as sugar. Proposed final stage of clumping

(D). Co-culture grown in a defined medium with glucose as sugar. Proposed final stage of clumping



Coenzyme F_{420}

Coenzyme F_{420} is an electron donor used in the methanogenesis pathway of all methanogens(7). This fluoresces under UV, and allows us to create the microscopy images above. While *B. theta* is not known to utilize chondroitin, it has a few genes that are putative $F_{420}H_2$ dehydrogenases. However, the fact that they are present gives substance to the idea that they may be sharing the coenzyme when clumped together.

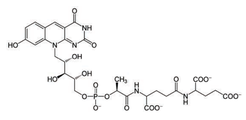


Figure 8. Coenzyme F_{420}

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Minimal Requirements

Variable media experiments were done using a defined media and creating 128 unique compositions of the following 7 additives:

- Histidine-Hematin
- Glucose
- Vitamin K

Necessary for *B. theta*

- Vitamin B_{12}

Necessary for both

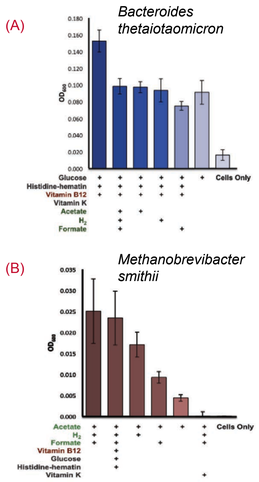
- H_2+CO_2
- Acetate
- Formate

Necessary for *M. smithii*

Figure 7. Variable media graphs

(A). *B. theta* only requires glucose in order to grow. When any of the waste products acetate, formate, and H_2+CO_2 is added into the media, growth is impeded. Also, while histidine-hematin can improve growth but are not necessary

(B). *M. smithii* does not grow to near the same turbidity as *B. theta*. Glucose and histidine-hematin have no effect on the growth of *M. smithii*. However, vitamin K inhibits growth.



Future Directions

- Create knock-out mutants of *B. theta*
- SEM/FiSH microscopy of clumps
- Test out different sugars and starches to see if it creates different effects
- Create a quantitative biding assay to measure biding to chondroitin
- Microscopy of co-culture at exponential phase
- Continue growth curves for Co-culture and *M. smithii*

Acknowledgements

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