2011

Investigating the Bacterial Predator Bdellovibrio's Ability to Degrade Aspartate

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University of Puget Sound Summer 2011

INTRODUCTION

Bdellovibrio bacteriovorus is a predatory Gram-negative Deltaproteobacterium that attacks and invades larger Gram-negative bacteria devouring them from within (Sockett, 2004). Enzymatic results obtained in the 1970s suggest that *Bdellovibrio* relies on its tricarboxylic acid (TCA) cycle and the oxidation of prey cell derived amino acids (Hespell, 1976). However, annotation of the published genome of *Bdellovibrio* HD100 revealed that it lacked numerous genes involved with the degradation of amino acids (Rendulic, 2004). Thus it is of great interest to determine if *Bdellovibrio* can degrade amino acids. If it can, new genes related to the degradation of amino acids will be discovered thanks to the unique genome of *Bdellovibrio*. If not, new research into why *Bdellovibrio* is so particular about which amino acids it degrades can be started.

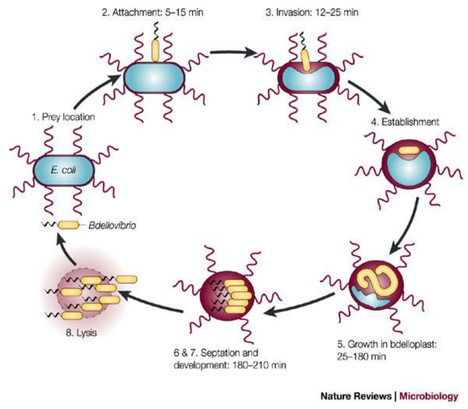

Figure 1. Life cycle of *Bdellovibrio* Bacteriovirus (Sockett, 2004).

ABSTRACT

Enzyme assays were run on a strain of *E. coli* that lacked the gene responsible for making aspartate transaminase. Then a plasmid containing part of the *Bdellovibrio* genome thought to be responsible for creating aspartate transaminase, courtesy of Andrew Collins, was inserted into the same strain of *E. Coli*. Results showed fumarate being produced indicating activity of the Asp A gene. Similar results were shown in HD109J, a host dependent strain of *Bdellovibrio* and HI109J-KAIRf, a host independent strain of *Bdellovibrio*. This implied that *Bdellovibrio* could degrade aspartate. Further research can be done to show how the lifestyle of *Bdellovibrio* is affected by the removal of this gene.

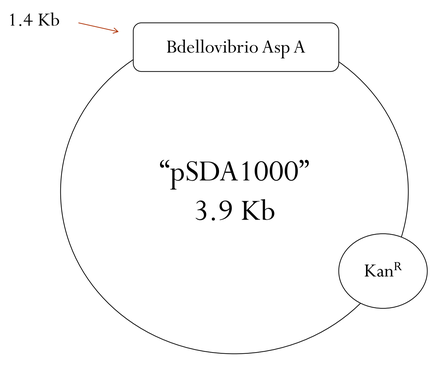

Figure 2. Plasmid containing the gene for aspartate transaminase and kanamycin resistance created by Andrew Collins.

METHODS AND MATERIALS

Aspartase catalyzes the reaction of aspartate to fumarate. The resulting double bond betweem the 2 & 3 carbon of fumarate allows for absorption of UV light at 240 nm. Thus all assays done to study aspartase activity were done at 240 nm and studied the change in fumarate concentration over time. Reaction mixtures were made up of 0.8 mLITRIS Buffer (0.5 M, pH=8), 0.2 ml sodium aspartate (0.5 M), 0.01 ml $MgCl_2$ (0.1 M) and 1 mlof cells grown to saturation and lysed with either toluene or pop culture. Once the reaction mixture was made, the spectrophotometer was blanked with the complete mixture and the first reading was taken. Measurements were recorded every 15 minutes for 1 hour.

Such assays were done with ML35, SDA1000, Asp A KO + pAspA , and two strains of *Bdellovibrio*. Micrograms of product were calculated using Beer's Law ($A=\varepsilon bc$) where $\varepsilon=2530\ M^{-1}\ cm^{-1}$ (Paulsen). The amount of product produced over 1 hour was standardized using a Bradford Assay with a standard curve of 0, 0.25, 0.5, 1.0, 1.4 mg/ml of BSA protein. This curve was compared with the 1 ml cell lysate readings to find the amount of protein in each reaction volume. Standardized results are shown in figure 3. Figure 4 shows absorption over time before standardization by Bradford's.

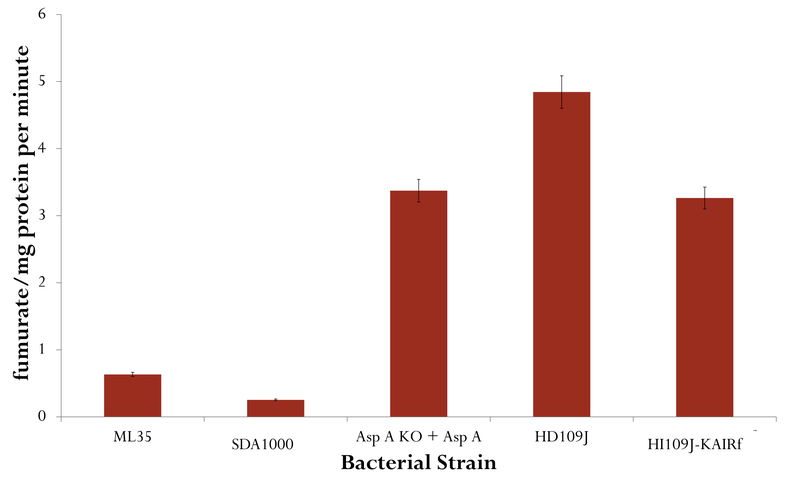

Figure 3. Total productivity of cell lysates in nm of fumarate/mg protein per minute. The strain of *E. Coli* containing the aspartase construct had about three times the activity of a wild type strain of *E. Coli* (ML35) and similar actiivity to the host independent strain of *Bdellovibrio* (HI109J-KAIRf). Error bars show standard deviation with n=3.

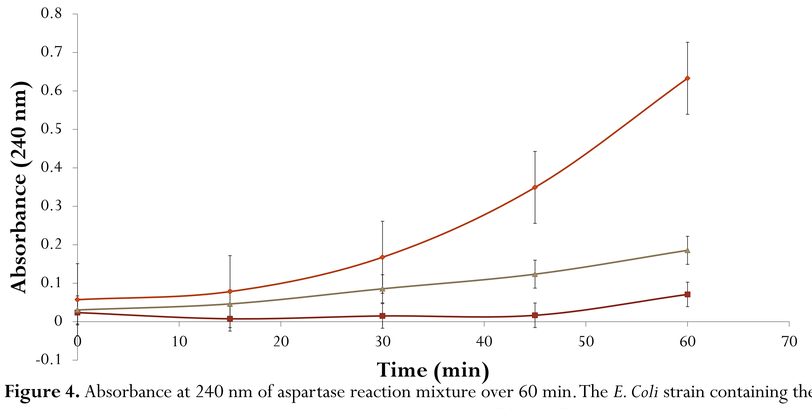

Figure 4. Absorbance at 240 nm of aspartase reaction mixture over 60 min. The *E. Coli* strain containing the aspartase construct (diamonds) exhibited the most change. The wild type *E. Coli* (ML35, triangles) showed the next highest activity, and as expected, the strain without aspartase activity (squares) showed relatively no change. Errors bar show standard deviation with n=3.

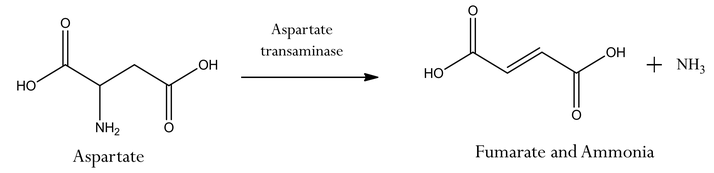

Figure 5. The basic reaction facilitated by aspartate transaminase.

RESULTS & CONCLUSION

In the assays, each strain performed as expected. The wild type (ML35) showed very mild aspartase activity. The SDA1000 strain had practically no activity whatsoever, and the AspA KO + pAspA strain showed similar activity to that of host independent *Bdellovibrio*. These results can be seen in figures 3 and 4.

This research gives solid evidence that *Bdellovibrio* has the capacity to degrade aspartate in spite of bioinformatic data. This begs the question, does bioinformatics reflect what amino acids *Bdellovibrio* can degrade. If not, what genes are responsible for the creation of the enzymes.

Future research can be taken in multiple directions. One such direction would be looking at how the lack of an aspartase gene affects *Bdellovibrio* and its lifestyle. This could lead to insights about how *Bdellovibrio* hunts, replicates, and enters the periplasmic space of its prey.

Another avenue of future research could be studying what other amino acids can be degraded by *Bdellovibrio*. If more amino acids can be degraded, then new genes can be discovered and identified for future bioinformatic studies. Much more can be unveiled about this little-studied microbial predator's metabolism and habits.

Cannot be Degraded	Cannot be Synthesized
Cysteine, leucine, lysine, tyrosine, Alanine, arginine, methionine, phenylalanine, tryptophan, valine	Asparagine, histidine, isoleucine, Alanine, arginine, methionine, phenylalanine, tryptophan, valine

Table 1. Ability for *Bdellovibrio* to degrade amino acids based on structural genomics (Rendulic, 2004)

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Acknowledgements

My deepest thanks go out to my advisor, Dr. Mark O. Martin who was always supportive, helpful, and enthusiastic throughout my whole project. I would also like to thank the UPS biology department for trusting me with their equipment and showing me the ropes, and Andrew Collins for supplying the pSDA1000. Finally, I would like to express disappointment in my lab partner Kat Schmidt because she said I failed to entertain her, when I did splendidly each and every day.