Anaerobic Growth Potentials of *Xenorhabdus nematophila*

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Introduction

Xenorhabdus nematophila is a gram-negative rod bacterium, that is classified as a facultative anaerobe**.** *X. nematophila* colonizes the entomopathogenic nematode Steinernema carpocapsae in a mutualistic association, and is also a pathogen of insects **(1).** The life cycle of *X. nematophila* occurs in two different life cycles: they survive in the gut of the free-living stage of their nematode host, and then are inoculated into and multiply in the body cavity of insects **(2).** Released *X. nematophila* multiply in the insect where they express factors that suppress insect immunity and kill the insect **(2).** Once the insect has died, the bacterium produces antimicrobial compounds that protects the insect cadaver from opportunistic infection and scavengers **(2).**

Although *X. nematophila* is able to grow in the gut of these insects, it is evident that the microbe does undergo stress during colonization and replication. Despite the presence of nutrients to support *X. nematophila* growth in the insect vesicle, the conditions which the microbe experiences are demanding, as well as competition with the insect's normal intestinal microbiota, and growth within the vesicle is discontinuous and causes a significant decrease in bacterial population size **(3).**

Therefore, one factor of *X. nematophila* that may contribute to the survival of this bacterium is its ability to respire or ferment in these different habitats. Yet, little is still known about the anaerobic growth and fermentative abilities of this bacterium.

Purpose

The purpose of this project is to determine the anaerobic (fermentative) growth potentials of *X. nematophila.*

Materials and Methods

Aerobic cultures. *X. nematophila* was initially maintained in aerobic Luria-Bertani (LB; supplemented with 20 mM glucose and 0.1% pyruvate) broth at 25°C. *X. nematophila* was then transferred to a variety of aerobic media such as, Brain Heart Infusion (BHI; supplemented with yeast extract and glucose) broth and an undefined medium (UM; yeast extract, glucose, pyruvate, and minerals).

Anoxic and anaerobic cultures. *X. nematophila* was transferred into anoxic and anaerobic versions of LB broth and UM broth; these media were boil, cooled, and dispense under 100% argon conditions into butyl-rubber stoppered, aluminum crimp-sealed tubes containing 10 ml of medium) and an anaerobic version of BHI broth (supplemented with bicarbonate and cysteine [reducer] and boiled, cooled, and dispensed under CO_2). All cultures were incubated at 25°C, and growth was measured with a spectrophotometer at 600 nm.

Results and Discussion

The three different aerobic media tested were growth supportive for *X. nematophila* **(Figure 1).** When transfers were from aerobic LB broth into anoxic and anaerobic versions of these culture media, only anoxic LB broth was growth supportive; the anoxic version of UM and the anaerobic version of BHI were not growth supportive **(Figure 2).**

Supplementation of anoxic LB with fumarate, nitrate, and a vitamin and amino acid mixture **(Figure 3)** did not enhance the growth of *X. nematophila*. Also, supplementation of anoxic LB broth with glucose slightly stimulated growth, whereas trehalose did not enhance the growth of *X. nematophila* **(Figure 4).** Also, HPLC results indicated that glucose consumption was minimal (data not shown).

However, in each growth supportive aerobic and anoxic media, the final pH reached a range of ~5.2-5.6 at 25°C; significantly lower than the uninoculated media's initial pH.

When transfers were made from the anoxic LB into anaerobic LB (a buffered medium) with the additions of glucose, trehalose, and a vitamin and amino acid mixture, growth was not supported **(Figure 5).**

Studies are currently underway to determine the anaerobic fermentation products of *X. nematophila* as well as to determine whether pyruvate is needed for anaerobic growth of *X. nematophila*.

Resolving the anaerobic growth and fermentation potentials of *X. nematophila* will provide the tools for understanding the respiration and fermentation abilities of this bacterium's survival in different habitats.

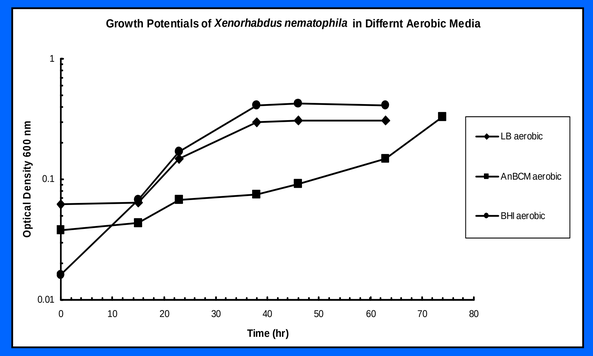

Figure 1: Growth obtained in aerobic media

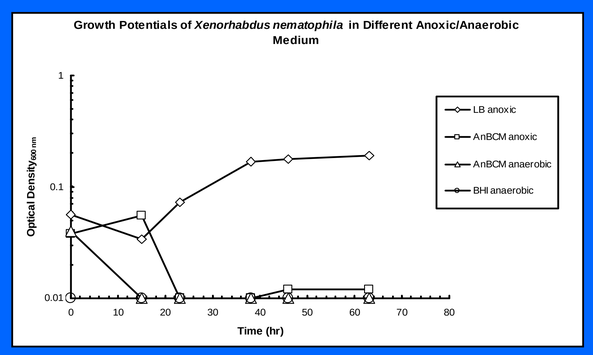

Figure 2: Growth obtained in anaerobic media

Literature Cited

1. **Herbert, E.E., and H. Goodrich-Blair.** 2007. Friend and foe: the two faces of Xenorhabdus nematophila. Nature Publishing Group **5:** 634-646.
2. **Boemare, N., and R. Akhurst**. 2006. The genera *Photorhabdus* and *Xenorhabdus*. Prokaryotes **6:** 451-494.
3. **Goodrich-Blair, H.** 2007. They've got a ticket to ride: *Xenorhabdus nematophila-Steinernema carpocapsae* symbiosis. Current Opinion in Microbiology **10:** 225-230.

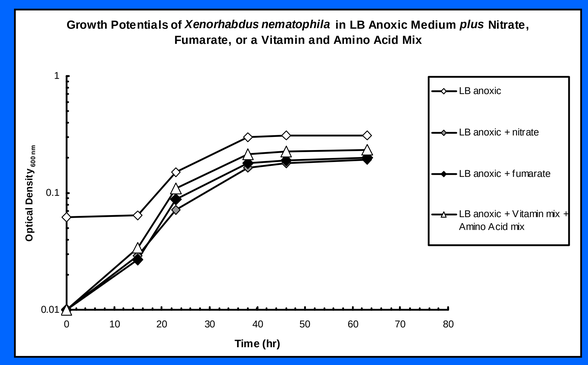

Figure 3: Supplementation of fumarate (10 mM) and nitrate (10 mM) and a vitamin and amino acid mixture into anoxic LB broth medium

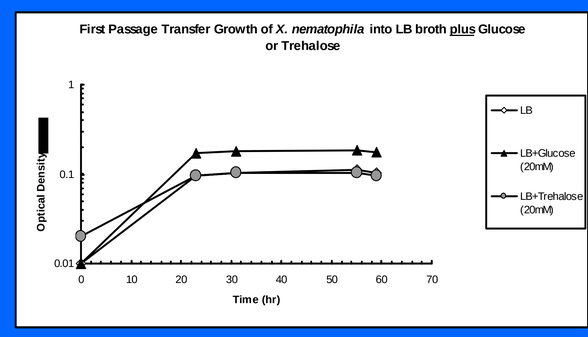

Figure 4: Supplementation of glucose (10 mM) and and trehalose (10 mM) into anoxic LB medium

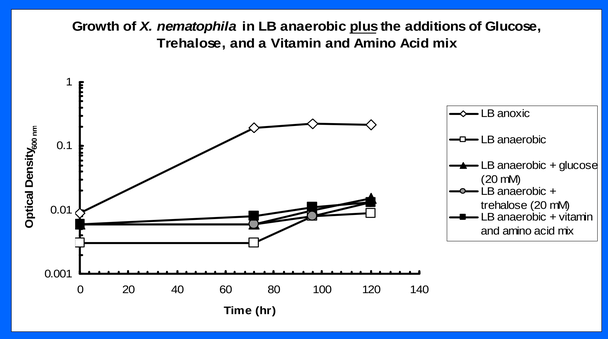

Figure 5: Supplementation of glucose (10 mM), trehalose (10 mM), and a vitamin and amino acid mixture into anaerobic LB medium

Acknowledgements

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