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 protect 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 crim-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 CO2). 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 aerobic 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 unincubated 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 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.