Report on Enrichment Culture Experiment

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DID YOU ISOLATE *AZOTOBACTER* FROM YOUR ENRICHMENT EXPERIMENT? BRIEFLY OUTLINE THE EVIDENCE IN SUPPORT OF YOUR CONCLUSION.

2 days after initial inoculation from the collected soil sample, the $WINOG_1$ plate displayed substantial growth of large white colonies, with a mucoid appearance - Glistening mucoid colonies are a typical *Azotobacter* macroscopic observation - As the WINOG medium is a nitrogen free medium, substantial growth on the plate implies the presence of nitrogen fixing bacteria; and a subsequent subculture confirmed this (and eliminated the possibility of trace nitrates being carried from the soil) as substantial growth was also displayed on the new plate, $WINOG_2$.

A hanging drop was performed on a singular colony extracted from the $WINOG_2$ plate, which showed bacteria displaying motility - Most, but not all *Azotobacter* are motile.

Observing the $WINOG_2$ singular colony under the microscope showed spherical cells, roughly 2μm in diameter, which stained inconclusively (gram staining variability). Most *Azotobacter* strains are gram negative, although it is known that *Azotobacter* can display gram variability in its staining.

Having observed a motile spherical bacteria, which displayed gram variability in its staining, enriched from soil, with a mucoid colony appearance - I feel confident that a species of Azotobacter has been enriched in this experiment, but not enough evidence was collected for a species identification.

ENRICHMENT CULTURE EXPERIMENT TO ISOLATE *BACILLUS ANTHRACIS*

Bacillus anthracis is a gram positive, aerobic soil bacterium, and is the causative infectious agent of the disease anthrax **?**. *B. anthracis* is an obligate pathogen, and is currently believed to only be able to reproduce inside its host, which in the wild typically consists of herbivorous ungulates **?**. *B. anthracis* is well known for its resilience to stressful physiological conditions, like many *Bacillus* species, *B. anthracis* can form endospores under extreme stress; and within its host *B. anthracis* is able to evade phagocytosis by means of a poly-D-glutamic acid capsule **?**.

Ecologically, *B. anthracis* prefers to live in humus rich, alkaline soils, ideally fortified with high Ca^{2+} concentrations **?**, as it's been shown that Ca^{2+}cations play an important role in both the maintenance of endospore dormancy, as well as in germination **?**. The soil for this experiment will be taken from farmland in Sonora, TX, a site with soil that fits the above conditions, as well as the site of two anthrax outbreaks since 2000 and a site of interest in Blackburn et al, an approximate geographic distribution of *B. anthracis* over the continental US **?**. The sample will taken between the months of November and February, as previous experiments have shown minor, but significant differences in *B. anthracis* endospore concentration in soil between seasons **?**.

To isolate *B. anthracis*, we will first suspend the soil sample in deionised water at a 1:2 (weight/volume [w/v]) ratio and vigorously shake it for 5 minutes. The sample will then be physiologically shocked, to trigger sporulation, by either submerging the sample in a 70°C water bath for 30 minutes, or by ethanol shock, by adding 99% pure ethanol to the sample, at a 1:1 w/v ratio and leaving the sample at room temperature for 30 minutes. After the shock, the sample will be serially diluted into 0.1, 0.01 and 0.001 dilutions, and then individually streaked onto PLET medium plates, which has been used historically used to isolate *B. anthracis* from soil **?**. The medium is complex, and contains digests of animal tissue as the main organic carbon and nitrogen source (as *B. anthracis* is an obligate pathogen and will not grow without the digests) as well as numerous selective agents; chiefly, polymyxin (an antibiotic produced by *Paenibacillus polymyxa*, which disrupts gram negative cell membranes **?***)*, lysozyme (which hydrolyses the 1,4-beta-linkages in peptidoglycan), EDTA and thallous acetate (which in combination select against *B. cereus* **?**). It's important that sodium chloride is also added to maintain the osmotic equilibirum. The full breakdown of the media is listed below, and has a pH of 7.4±0.3 at 25°C.

After streaking, the medium can then be left to incubate at 36°C for 1 day, and then be inspected for roughly 2mm wide, white, or off-white circular colonies, often with curling tails at the edges **?**. Despite the selectivity of the media, some bacteria, including *Staphylococcus* and some *Bacillus* strains are able to survive on the PLET media, so for a full diagnostic result, the colonies will have be to individually tested for traits such as penicillin

Pancreatic Digest of Casein	9.0gm
Peptic Digest of Animal Tissue	5.0gm
Sodium Chloride	5.0gm
Yeast Extract	4.0gm
Beef Heart Infusion	2.0gm
Sodium Carbonate	0.325gm
EDTA	300.0mg
Thallous Acetate	40.0mg
Lysozyme	300,000U
Polymyxin	30,000U
Agar	15.0gm

Figure 1. Ingredients per litre of deionised water for PLET media.

toxicity, lack of motility and susceptibility to γ-phage to confirm species as being *Bacillus anthracis* **?**.