LUMINESCENT

Microbial Ecology

The purpose of this experiment was to test the hypothesis that bioluminescence can be advantageous under microaerophilic conditions. B. harveyi and B. luteum were used as the luminescent and non-luminescent strains. In cells the bacterial bioluminescent system can be viewed as a branch of the electron transport pathway in which electrons from reduced substrates are shunted via reduced flavins to O₂. The affinity of the system for O₂ is very high, at very low O₂ concentrations, where cytochrome c oxidase may be completely blocked. Luminescence can occur without appreciable decrease. Under microaerophilic conditions, such a pathway might be advantageous.

Substrate + O₂ → H₂O
In such a system under microaerophilic conditions, where the cytochrome chain is blocked, reduced NAD can be re-oxidized via the diaphorase system. Under strict anaerobic conditions, E. harnyi presents glucose to succinate, ETH, acetate + CO₂. Finally, instead of producing ETH and CO₂, the diaphorase system can be used todespose of X-ene e-a.

To test this possibility, both strains were to be grown in a chemostat anaerobically and allowed to reach a steady state. Then a ratio of glucose or glucose + succinate was introduced into the chemostat at a level where the diaphorase system could consume it, but the cytochrome chain could not. Finally, the system was to be made totally aerobic, and a final ratio taken. Oxygen would be lost by plating in solid media and counting diminutive colonies in ethanol.

Materials + Methods
A defined medium was used to grow the cells in the chemostat consisting of:
<table>
<thead>
<tr>
<th>Day</th>
<th>Concentration (cells/ml)</th>
<th>Ratio</th>
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</thead>
<tbody>
<tr>
<td>4</td>
<td>5.6 x 10^8</td>
<td>3.3</td>
</tr>
<tr>
<td>5</td>
<td>2.7 x 10^8</td>
<td>3.6</td>
</tr>
<tr>
<td>6</td>
<td>1.2 x 10^8</td>
<td>3.1</td>
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</tbody>
</table>
Day 7: $6.5 \times 10^8$ cells/mL 4.3

Day 8: $1.2 \times 10^9$ 3.0

Day 9: $8.4 \times 10^8$ 3.8

Day 10: $1.6 \times 10^8$ 2.6

Discussion:

The increase in growth between days 7 and 8, however, is due to the free fall rate change due to growth on the media inlet. Since no oxygen electrode was available, it was uncertain whether the system was totally aerobic. The indicator always remained slight pink, showing not a totally reduced medium. Based on the results of a specific antibody assay, glucose was limiting with levels below 0.2 mM detected.

No trend in luciferase apparent in the reaction. The non-luminescent tracer was never totally outcropped. It did survive in numbers after the system was made aerobic.

One other fact that should be brought up is that B. licheniformis growth and analysis...
It has been a depressed luciferase content. There is one or a dependent control mechanism of the luciferase system. This, however, may not be a factor in the experiment where anaerobic condition would be present, no light production was seen essentially. In her made at cell low levels of light were seen. Reductase is produced by the non-lumenar actinonin as well as the hemoglobin from so that should not be a factor in luciferase synthesis. It is not known why no light was produced by samples taken from anaerobic condition unless the luciferase content was decreased to very low levels.

References