Microbiology Lab Experiment Changes

Experiment #: 5-6

Title: Nitrate Reduction

Live Organisms: *Pseudomonas stutzeri, E. coli, Micrococcus luteus*

Changes: Procedure:
Each person in each group will inoculate a separate nitrate broth tube with a different organism.

Take Home Lesson: We are trying to determine whether a bacterium produces the enzyme **nitrate reductase**. Nitrate (NO_3^-) is in the medium. We will test for the presence of nitrite (NO_2^-). Nitrate reductase catalyzes the conversion of nitrate to nitrite. Some bacteria may possess potent nitrate reductases or additional nitrate reducing enzymes. In this case, nitrate may be reduced completely to ammonia or nitrogen gas. Such organisms are termed **super-reducers**.

Nitrate reductase

NO_3^- $\rightarrow$ **NO_2^-** $\rightarrow$ **NH_3** **or** **N_2**
nitrate nitrite ammonia nitrogen gas

super reducers

How to test?
Solution A (sulfanilic acid) and solution B (alphanaphthylamine) react with **nitrite** to produce a red color.

A + B $\rightarrow$ red color = nitrite (NO_2^-) present, therefore nitrate reductase present

If no red color occurs after the addition of solutions A and B, there are two possibilities:

1. nitrates were not reduced by the organism, therefore it does not possess nitrate reductase

2. the organism reduced nitrate to ammonia or nitrogen gas (if you see a gas bubble in the Durham fermentation tube, then you know this has occurred, but you must continue with the next step)

If you did not get a red color, then add a pinch of zinc metal to the tube. Zinc metal **reduces** nitrate to nitrite. If you get a red color after adding zinc, that means that nitrates were still present. Therefore the organism does not produce the enzyme nitrate reductase. If still no color change occurs, then nitrates have been completely reduced to ammonia or nitrogen gas and the organism is a super-reducer.

If given a set of tubes that have been inoculated, incubated and to which reagents have been added, you must determine whether or not nitrates, nitrites or gas is present, was nitrate reductase produced by the organism, and is the organism a super-reducer.