Identifying bacterial growth

Introduction:

This experiment was about isolating a single bacterial colony from a soil sample and identifying which genus it belongs in. This is especially important for functions such as agriculture, as knowing whether a specific soil has a high concentration of nutrient producing bacterium is essential to being able to harvest good crops. Another key component of soil bacterium, is that they tend to be high in nutrient recycling organisms. As well there tends to be a high number of varying phylum in soil bacterium that was previously unknown, as in 2003 Joseph et al. managed to isolate 350 different bacterium which were assigned into 9 different phyla. As well approximately 27% of the isolated bacterium belonged to unnamed families, and were located in very poorly studied phyla. (Joseph et al. 2003)

Methods:

The experiment began by subculturing a bacterial colony identified from a myriad of soil bacterium isolated in an agar plate. Then this bacterial colony was observed through a microscope, as well as tested for whether it was gram positive or gram negative. Then the bacterium was re-cultured into varying solutions to test for specific nutrient use. First it was subcultured onto an agar plate rich in starch, incubated, and tested for starch hydrolysis via the use of lugol’s iodine, to see if there was starch remaining in the area of the bacterial colony. Then a deep rich in sulfur was inoculated with the bacterium, and observed for whether motility was displayed, or whether hydrogen sulphide was produced. Then the bacterium was inoculated in a peptone broth, to test for production of ammonia, through the addition of Nessler’s Reagent, an ammonium sulphate broth and a nitrite broth, to test

for the ability to nitrify compounds using Nessler's reagent; Trommdorf's Reagent; diphenylamine; and Sulfuric acid, and a nitrate broth to test for the ability to denitrify compounds using indicated reagents. Then the bacterium was placed into a thioglycollate medium to test the oxygen tolerance of the bacterium. Next the bacterium was subcultured onto a normal agar plate, to test for the presence of catalase and oxidase. Finally the bacterium was subcultured on plates with varying NaCl concentrations, and inoculated in tubes of varying pH's and tubes with varying temperatures. (Robertson and Egger, 2010)

Results:

In this lab our obtained data was collected and summarized (Table 1). This was then used to obtain a possible bacteria genera from the collected data, based on a text of classifications. These include references for whether a microbe genera happens to have a specific enzyme, to which types of electron donors it utilizes throughout its ATP production phase, be it in oxidative phosphorylation, or substrate level phosphorylation, or even a form of fermentation.

The information gathered in table in is well correlated in the assigned manual, and is indicative of the genera Bacillus, which is known for its Rod-like shape, and the ability of this specific genera to grow across a vast array of nutrient types. (Sneath, 1986) Other indicators of this particular Genus, are that Bacilli tend to grow most predominantly within a temperature range characterized by mesophilic organisms, and have a wide range of osmotic pressure, or salt concentration tolerances. Another indicator of the bacillus

genera, is the fact that this unknown bacteria tested positive on the gram test, as most members of the bacillus genera are gram-positive bacteria.

Discussion:

The bacterium is of the genus bacillus, because of the fact that it fits into the category of being able to survive in most conditions. (Sneath, 1986) The main characteristics that helped identify this bacterium were the fact that it is gram positive, that it is rod-shaped, and the determination through chemical testing that it can utilize multiple nutrient types to grow, and reproduce. These characteristics pointed definitively to the bacterium bacillus, and further investigation revealed them to most likely be either Bacillus cereus, or Bacillus licheniformis. These bacterium share almost all in common with the isolated bacterium in terms of chemical use, and similar makeup. This bacterium could have been further identified through other tests, such as looking for other enzymes that may be present, and performing tests to determine the exact makeup of the cell wall. The other tests that could be performed, could be to detect for the presence of chemicals known to be associated with fermentation, such as lactic acid. The limitations of the tests which were performed, are that they tend to test for the same type of thing. For example if something tests positive for nitrification, they may not test positive for ammonification because it is all converted straight to a nitrate form.

The Bacillus strain plays many varied roles in nature, based on its vast adaptability, and the variance of locations it is found in. For example, it ranges from living in soil, to water, from animals, to plants. The Bacillus is even found in a pathogenic form, as Bacillus anthracis in humans, and in

multiple forms in insects and animals. (Sneath, 1986) This means that the Bacillus anthracis strain is of a particular interest to scientists, as it is a reason to research antibiotics, as well as a reason to investigate different strains of this bacterium to see what other pathogenic roles this bacterium can cause in living creatures.

This lab contains many possible sources of error, the main one being cross contamination. As the bacterium was recultured so many times, each time represents a possible instance where another bacterium was added to the mix, or substituted for the bacterium being isolated. Also during testing different bacteriums could have been picked up and added to the testing which could have caused a shift in results, or an increase in the range a specific result is in. Another source of error, is that there was what appeared to be a subculture of yeast intermixed with one of the bacterium subcultures obtained during testing. As this yeast subculture was at one time located on the same plate as the bacterium which was isolated and tested for, it could have also been located in our sample throwing our specific results off. These specific errors could inevitably cause the data gathered to point to the wrong bacterium genus and lead to misclassification. This could be corrected for by taking multiple subcultures of the bacterium, and observing each subculture carefully to determine if there is any presence of different bacterial strains.

In the end, the objectives of this experiment were properly met, as a bacterial sample was subcultured from a soil sample, and the bacterium was adequately identified via a myriad of tests and procedures. Since these objectives were met the experiment could be considered to be successful,

especially as it taught me about different subculturing techniques as well as the methods that a microbiologist uses to determine bacterial identity.

Literature Cited:

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