Identification of unknown soil bacteria

INTRODUCTION

Microorganisms play an extremely important role in soil ecology. Soil bacteria break down organic matter into simpler compounds (Clark). Bacteria in the soil play important roles in different biochemical cycles such as the carbon cycle (Clark). Decomposition is another important role that different bacteria take part in. Without bacteria the ecology of soil would be completely disrupted. The nutrient requirements that different bacteria need to survive decide where each bacteria can be found. Agricultural soil would have a completely different array of microorganisms then soil found in a forest would have. Different conditions such as moisture, nitrogen, oxygen and carbon dioxide levels, temperature and pH of the soil will all determine which types of bacteria will live in thrive in agricultural and forest soil. The objective of this experiment was to try to isolate and identify one specific soil bacteria from either forest or agricultural soil. The bacteria in this report was isolated from agricultural soil and using the morphology of the individual cells and colony along the types of chemicals that would react with the bacteria along with what type of extremes the bacteria could survive in the bacteria was identified.

METHODS

Starting from an original one gram sample of agricultural soil a 10-2 dilution was created and used to create a Tryptic Soy Agar (TSA) streak plate (Robertson and Egger 2010). The cultures that arose from the streak plate were observed and the morphology of a specific colony was described and recorded (Egger 2010). A subculture was created from one colony on a new TSA streak plate. A gram stain was prepared using the original colony of soil

bacteria and whether it was gram negative or gram positive was recorded (Robertson and Egger 2010). Using the new subculture several biochemical tests were performed. A single line of bacteria was streaked onto a starch agar plate and after incubation drops of iodine were added to the culture and if starch is hydrolyzed a color change occurred, results were recorded (Robertson and Egger 2010). On a Sulfide, Indole, and Motility (SIM) deep the bacteria isolate was stabbed into the tube ¾ of the way down (Robertson and Egger 2010). After incubation the deep was observed for movement away from the original line and three drops of Kovac's Reagent was added to see if a color change occurred (Robertson and Egger 2010). The results of the motility and H2S reduction tests were recorded. The bacteria isolate was also mixed in a peptone broth tube and after incubation a drop of Nessler's reagent and the loop of broth solution were mixed in a spot plate and the results were recorded (Robertson and Egger 2010). A loop of bacteria was put into ammonium sulfate and nitrite broth tubes. In a spot plate on loop of the ammonium sulfate broth was mixed with 1 drop of Nessler's reagent and another loop was mixed with three drops of Trommsdorf's reagent and a drop of dilute H2SO4 (Robertson and Egger 2010). Also in the spot plate the nitrite broth was tested with Trommsdorf's reagent and dilute H2SO4 as well as with concentrated H2SO4 and phenylamine (Robertson and Egger 2010). The results for the four tests were recorded. To test for denitrification reagents A and B were added to the nitrate broth tube with the unknown bacteria and the color change of the tube was recorded (Robertson and Egger 2010). The unknown isolate was inoculated in a tube of thioglycollate medium and after incubation any growth in the tube was noted and recorded (Robertson and Egger 2010). To test the bacteria for catalase and oxidase

bacteria was streaked on a TSA plate and after incubation drops of 3% hydrogen peroxide was put on one end of the streak and the formation or lack of bubbles was recorded for catalase, and oxidase was tested on the opposite end of the streak and the results were noted (Robertson and Egger 2010). To test what temperature the unknown bacteria grow best at 4 TSA plates were streaked with the bacteria and each plate was incubated at a different temperature, after incubation any growth on the plates was recorded (Robertson and Egger 2010). To test the optimal pH for the bacteria to grow the bacteria was inoculated into tubes that were each at a different pH (pH 3, 5, 7, & 9) after incubation the pH that the bacteria grew the best in was recorded (Robertson and Egger 2010). TSA plates were also streaked with the unknown bacteria to test in what amount of sodium chloride it could grow in, plates with concentrations of 0, 0. 5, 2 and 5% sodium chloride were used any growth was recorded (Robertson and Egger 2010).

RESULTS

The unknown bacterium is rod shaped and gram positive and the colony formed is a raised white circle (Table 1). Tests showed that the bacterium was positive for ammonification and denitrification from NO3- to NO2- (Table 1). Catalase and oxidase test also were positive for this bacteria (Table 1). The bacterium is also a facultative anaerobe (Table 1). Table 1 also shows that the unknown bacterium does not exhibit motility. The unknown bacteria was found to hydrolyze starch, but was negative for reducing H2S (Table 1). The unknown bacterium also does not exhibit nitrification (Table 1). The optimal temperature of the unknown bacteria was found to be about 37°C

and had an optimal pH of 5 its optimal salt concentrations was also found to be at 0-0. 5% sodium chloride (Table 1).

DISCUSSION

The soil bacteria key created by KN Egger (2010) was used to identify what family the unknown bacteria belongs to. The closest match that could be found using the Common Soil Bacteria Key was Actinomyces. A match could not be made to a genus. Actinomyce are a Gram positive, rod shaped family. Actinomyces are not found to reduce H2S or have significant motility. From the results in Table 1 we can conclude that the bacterium is a mesophile as well as an acidophile. We can also conclude from Table 1 that the bacterium is osmotolerant and a facultative anaerobe. Many different tests could have been used to further help identify the bacterial isolate; the most accurate tests would have been to test the DNA or nucleic acids of the bacteria. Many different biochemical tests could have been used such as lactose and glucose. No test result can be considered completely accurate the bacteria could have been contaminated as well as not every bacteria colony will behave exactly the same. It is extremely hard to classify a specific bacterium without several more tests to safely conclude that it belongs in the chosen group Actinomyces.

REFERENCES

Clark, FE. 1951. Bacteria in the Soil. Cellular and Molecular Life Sciences. 7: 78-80.

Egger, KN. 2010. Common Soil Bacteria Key. UNBC

Robertson, S and Egger, K. 2010. BIOL 203 Microbiology Laboratory Manual. UNBC.