

# [The identification of bacillus subtilis the mystery bacterium biology essay](https://assignbuster.com/the-identification-of-bacillus-subtilis-the-mystery-bacterium-biology-essay/)

Mixed communities of microorganisms consisting of procaryotes such as bacteria, as well as eucaryotes such as fungi, protozoa, algae, and nematodes are found in all sorts of natural environments (Robertson and Egger, 2008). In particular microorganisms are quite abundant in soil environments and are an important part of the soil microbial community (Prescott et al., 2005). However only a fraction of these microbes that make up soil biomass have been cultured. The microbial community which constitutes soils makes significant contributions to biogeochemical cycling as well as the carbon, nitrogen and sulfur cycles (Prescott et al., 2005). Soil microorganisms are also essential for decomposition and the recycling of essential nutrients that are locked in macromolecules of organic material (Robertson and Egger, 2008).

Microbes can be classified based on a wide range of characteristics that allow them to survive and flourish in varying environments. A major classification is based on oxygen requirement; with oxygen dependent microbes being aerobic while anoxygenic microbes being anaerobic. Aerobic microbes are further classified based on the degree of oxygen requirement. This classification has a major impact on where these microbes are distributed with in the soil stratum. Other important environmental conditions and factors that affect microbes include: temperature, pH, and osmotic pressure.

The objective of this experiment is to isolate and identify a bacterium from one of two soil samples provided: coarse woody debris (CWD) or forest soil (FS). This will be achieved by obtaining pure cultures of the bacterium and performing numerous tests to determine Gram stain, colony and cell morphology, biochemical activity and optimal environmental conditions. The information gathered from these tests is crucial in determining the genus of the unknown bacterial isolate.

## Material and Methods:

The Identification of Soil Bacteria experiment was performed over a four-week period and experimental procedures were based on the instructions outlined in the Biology 203 Laboratory Manual (Robertson and Egger, 2008). In week 1, two soil samples, coarse woody debris (CWD) and forest soil (FS) were cultured aseptically on nutrient-containing media.

In week 2, selected bacteria were isolated from the mixed populations by sub-culturing to obtain pure cultures. A compound microscope was used to determine cellular arrangement of the bacteria and colony characteristics described through direct observation. Gram staining was performed on the cultured bacteria to determine whether the bacterium was Gram positive or Gram negative.

In week 3, various biochemical tests were performed to analyze the bacterial isolates for specific biochemical activities involved in the cycling of carbon, nitrogen and sulfur. The bacteria were tested for starch hydrolysis, production of H2S and motility. The bacteria were also tested to reveal their nitrogen cycling capacity. This would determine if the bacterium was able to perform ammonification (release of NH3), nitrification (the oxidation of ammonia), or denitrification (reduction of nitrate). The catalase test was performed to determine whether the bacterium was aerobic or anaerobic.

In the final week, week 4, the bacteria were tested to determine how environmental factors such as temperature, pH and osmotic pressure affect the growth of these soil microbes. The findings of these optimal conditions for each bacterium were recorded and the bacterium was identified based on all observations and results collected over the four-week period which was interpreted using scientific literature (Robertson and Egger, 2008).

## Results:

The bacteria were cultured on agar plate media and observed to describe colony morphology. The following characteristics were found: irregular/filamentous form, raised elevation, undulate margin, dull appearance, opaque optical property, cream color, and rough texture (Table 1). The bacterium turned purple when Gram stained indicating a Gram positive microbe. The bacterium showed single and clustered rods when observed under the compound microscope at 1000X magnification. The rods were ~1. 0µm wide and ~2. 0µm long (Table 1).

Biochemical testing showed that the bacterium was motile and able to hydrolyze starch. The bacterium tested negative for ammonification as no color change was found in the peptone broth after Nessler’s Reagent was added (Table 1). However, the bacterium was capable of nitrification and denitrification. In the denitrification test reagents A & B were added to the nitrate broth and a red color change was observed (Table 1). This is a positive result for denitrification as nitrate (NO3-) is reduced to nitrite (NO2-) by the enzyme nitrate reductase (Robertson and Egger, 2008). For the nitrification test, the nitrite broth turned a deep blue color after the addition of diphenylamine reagent and sulfuric acid (Table 1). This is a positive result for nitrification as ammonia (NH3/NH4+) was oxidized to nitrate (NO3-) by Nitrobacter (Robertson and Egger, 2008). The bacterium was tested for motility and H2S reduction in SIM deeps. Although the bacteria was motile it was however unable to produce a black precipitate which is characteristic of H2S reduction (Table 1). For the catalase test numerous bubbles formed when hydrogen peroxide was added which shows that the bacterium is aerobic. The bacterium grew most optimally at 220C, pH 5 and at all concentrations including over 5% which indicated that the microbe is mesophilic, acidophilic, and halophilic (Table 1).

Table 1. Summary of the numerous tests performed, as well as the observations and findings critical in the identification of the unknown soil bacterium isolate.

## Test

## Results

Colony morphology

Form: irregular/filamentous; Elevation: raised; Margin; undulate; Appearance: dull; Optical Property: opaque; Color: cream; Texture: rough

Cell morphology

Cell Shape: rod; Cell arrangement: some single, some cluster; Dimensions: ~1. 0µm wide and ~2. 0µm long

Gram stain

Cells were purple when stained – Gram Positive

Starch hydrolysis

Clear zone around bacterial growth when iodine added. The color of iodine reaction was yellow/caramel – Positive result for Starch Hydrolysis.

H2S reduction

No black precipitate produced therefore sulfur is not reduced and no hydrogen sulfide production – Negative for H2S reduction.

Motility

Bacterial growth away from the stab line – Positive for motility.

Ammonification

No color change observed in peptone broth after addition of Nessler’s Reagent – Negative for Ammonification.

Denitrification

Red color observed after reagents A & B added – Positive for Denitrification: conversion of NO3- to

NO2-.

Nitrification

Deep blue color observed after diphenylamine reagent and sulfuric acid added to nitrite broth – Positive for Nitrification conversion of NH3/NH4+ to NO3-.

Catalase

Lots of bubbles formed when hydrogen peroxide added – Positive for Catalase, thereforeAerobic.

Optimal temperature

Growth greatest when bacterium incubated at 220C – Mesophile

Optimal pH

Growth greatest at pH 5 – Acidophile.

Optimal salt concentration

Great amount of growth at all salt concentrations – Halophile.

## Discussion:

The unknown bacterium was isolated from FS sample diluted to 10-5 with sterile deionized water and identified according to genus and to a species of that particular genus. The unknown bacterium isolate was identified as Bacillus subtilis.

Gram staining and microscopic analysis, revealed that the bacteria were purple Gram positive rods that appeared in single or cluster form. Also, from direct observation it was seen that the bacteria also produced endospores which narrowed our genus possibilities to six: Bacillus, Sporolactobacillus, Clostridium, Desulfotomaculum, Sporosarcina, and Oscillospira (Sneath et al. 1986). Since the bacterium was rod-shaped, motile, aerobic and positive for catalase the genus was determined to be Bacillus since it was the only one of the six which possessed these characterisitics. The remaining data from the tests and observations was used to determine the species of the Bacillus. Positive results for starch hydrolysis, denitrification, growth at pH 5, growth at salt concentrations â‰¥5% and mesophilic temperature requirements narrowed the identity of the bacterium down to two species Bacillus subtilis and B. anthracis (Sneath et al. 1986). B. anthracis was eliminated as possibility because unlike most Bacillus microbes it is not motile, and also colony morphology best supported B. subtilis (Sneath et al. 1986).

The Bacillus genus is capable of forming endospores, structures which allow bacteria to grow and survive in unfavorable environmental conditions, as they are resistant to excessive heat, freezing and desiccation (Robertson and Egger, 2008). The presence of endospores is significant because to gives the microbe the ability to do well in diverse amount of habitats.

B. subtilis is a bacterium commonly found in soil, water sources and in association with plants (Kunst et al. 1997). Bacillus subtilis, can allow the degradation of organic polymers in soil, and therefore play a pivotal role in the biochemical cycling of carbon and nitrogen (Emmert and Handelsman, 1999). It also can promote plant growth and protect against fungal pathogen attack (Harsh et al. 2004). B. subtilis and its close relatives are an important source of industrial enzymes as well (such as amylases and proteases), and much of the commercial interest in these bacteria arises from their capacity to secrete these enzymes at gram per litre concentrations. It has therefore been used for the study of protein secretion and for development as a host for the production of heterologous proteins (Kunst et al. 1997).

Other tests which may have helped to identify the bacterial isolate which were not performed include the Voges-Proskauer test and citrate utilization test. The Voges-proskauer test is a colorimetric procedure that detects the acetoin precursor of butanediol and is positive with butanediol fermenters. Butanediol fermentation is characteristic of Enterobacter and some species of Bacillus (Prescott et al., 2005). The citrate utilization test determines if citrate is used as the sole carbon source resulting in alkalinization of the medium (Prescott et al., 2005). These tests would of have been helpful because only a few of the Bacillus species are positive for these tests and this would have been important in narrowing down the identity of the bacterium (Sneath et al. 1986). Some of the tests performed during the experiment provided limited information regarding bacterial identification because the results were applicable to many microbes. The ammonification and H2S reduction tests could have been excluded as these results were not used in the identification of the bacterium.

Numerous sources of error occurred over the four-week period of this experiment when determining the identity of the unknown bacterium. Contamination may have occurred due to poor aseptic technique including sterilization of laboratory equipment and workbench. Also, some of the chemical tests may have yielded false negative results due to insufficient bacterial growth in some broth cultures. Even though there was a possibility of errors over the course of the experiment, the objectives were met as the bacterium isolated from a forest soil sample was identified as Bacillus subtilis.