

# [A guide to the identification of bacillus cereus biology essay](https://assignbuster.com/a-guide-to-the-identification-of-bacillus-cereus-biology-essay/)

Bacillus cereus is an endospore-forming, gram-positive bacterium that commonly resides in the soil. Because of its location, Bacillus cereus is usually found on a variety of foods that come into close contact with contaminated soil, and can cause two different types of food borne illnesses: emetic and diarrheal. The type of illness that Bacillus cereus can infect a person with depends mainly on the type of contaminated food that is ingested (Cowan and Talaro, 2009). Strains that produce the emetic form of the disease grow mainly in starchy foods like rice and potatoes; especially after they have been cooked and kept warm for a long period of time. After the ingestion of such contaminated foods, Bacillus cereus may incubate inside the infected organism for up to six hours before showing any symptoms that the emetic form displays like nausea, vomiting and abdominal cramps (Drobniewski, 1993). Strains that produce the diarrheal form of the disease grow in a variety of foods ranging from vegetables to even meat products. “ Patients experience profuse diarrhea with abdominal pain and cramps…which begin around eight to sixteen hours after ingestion of the contaminated food” (Drobniewski, 1993). Recovery is usually rapid for both types of diseases; around twelve to twenty four hours after the symptoms have emerged. “ Diagnosis of the emetic form of the disease is accomplished by finding the bacterium in the implicated food source. Microscopic examination of stool samples is usual to diagnose the diarrheal form of the disease…. In both cases, the only prevention is the proper handling of food” (Cowan and Talaro, 2009).

The Bacillus species are gram-positive, “ rod-shaped, and often arranged in pairs or chains, with rounded or square ends. Endospores are oval or sometimes cylindrical” (Harley, 2008). For the case of Bacillus cereus, this rod-shaped bacterium is commonly arranged in chains with square ends and contains terminal endospores. On a regular TSA plate, the colonies of Bacillus cereus appear to have a cream “ dull or frosted glass appearance and often an undulate margin from which extensive outgrowths do not develop” (Sneath, 1986). “ The genus Bacillus is either aerobic or facultatively anaerobic, most members of this group are also motile…and with the exceptions of Bacillus anthracis and Bacillus cereus, are nonpathogenic” (Harley, 2008).

When trying to identify Bacillus cereus, there are several tests and results that can help correctly identify this bacterium (Table 1). The first procedure that should be done is to streak the bacteria for isolation on a TSA plate. In order to be able to view the correct morphology of any bacteria, one must have a fresh 24 hour culture containing individual colonies for proper Gram staining. Gram staining is a technique used to identify the cell morphology of a specific bacterium and differentiates bacteria as gram-positive and gram-negative cells. As stated earlier, Bacillus cereus is a gram-positive rod-shaped bacterium that is arranged in chains. Once the cell morphology of the bacterium is determined, an endospore stain would be the next test used in order to establish whether or not it is an endospore or non-endospore forming bacterium. Bacteria in the genera Bacillus and Clostridium can produce endospores, which are structures “ capable of surviving for long periods in an unfavorable environment and then giving rise to a new bacterial cell” (Harley, 2008). As a result, by being able to classify the bacterium as endospore-forming, many groups of bacteria can be ruled out by narrowing it down it the two different genera previously stated. Since endospores are used as a form of survival when nutrients are being depleted, a four to six day culture may be better to use so that the endospores have had some time to fully develop. Oxygen requirements would be the next test because bacteria in the Bacillus genus can either be obligate aerobes or facultative anaerobes, and bacteria in the Clostridium genus are obligate anaerobes. Bacillus cereus can either grow only at the surface of the thioglycollate broth, meaning that it is an obligate aerobe, or it may display growth throughout the broth with a larger amount on the surface, meaning that it is a facultative anaerobe. Motility is also an important characteristic used to identify microorganisms and since not all Bacillus bacteria possess this trait, the range of possible bacteria can be narrowed again with these results. Bacillus cereus is motile, because when this bacterium is inoculated into the motility media, the growth of this bacterium is not restricted within the stab line of the inoculation but can be seen throughout the media. After determining that the bacterium is positive for motility, fermentation reactions of both glucose and lactose should be done to establish whether or not the bacterium has the ability to ferment either of these carbohydrates, as well as produce gas during the process. True Bacillus cereus can ferment glucose but it cannot ferment lactose; none of the fermentation reactions produce gas as well.

Several problems can be encountered when trying to correctly identify Bacillus cereus because of similar characteristics shared with Bacillus anthracis; both bacteria are gram-positive endospore-forming rods that are arranged in chains. The distinction between Bacillus cereus and Bacillus anthracis and their separation from nonpathogenic species are the most important clinically since both are pathogenic. “ In general, Bacillus cereus is motile, ß-hemolytic on blood agar, and penicillin resistant, whereas B. anthracis is not. Bacillus cereus, unlike Bacillus anthracis, produces ß -lactamases, and so it is resistant to ß -lactam antimicrobial agents, like Penicillin” (Drobniewski, 1993). One method that is used to test for Penicillin sensitivity is the Kirby-Bauer method; Penicillin-filled paper disks are placed on a fully inoculated Mueller-Hinton agar plate of the bacteria to observe zones of inhibition (Harley, 2008). Bacillus anthracis will produce a distinguishable zone of inhibition due to its sensitivity to Penicillin, whereas Bacillus cereus will either produce a minor zone of inhibition or none at all due to its resistance to Penicillin. The table that can be used to identify a bacterium’s sensitivity or resistance to Penicillin, based on the zones of inhibition, can be found on page 265 of the Microbiology Lab Manual by John P. Harley.

Other than the tests previously discussed for the identification of Bacillus cereus, there are several tests that can accompany them to definitively identify the bacterium as Bacillus cereus. One test is a hemolysis test on blood agar. The blood agar plate is a differential media that distinguishes between hemolytic and non-hemolytic bacteria. A hemolytic bacterium produces clear zones around the colonies due to red blood cell destruction; É‘-hemolysis displays a partial clearing around the bacterial colonies, ß-hemolysis displays a total clearing around the bacterial colonies and Î³-hemolysis displays no hemolysis, therefore, no clearing around the colonies. “ Typical isolates of Bacillus cereus on blood agar form large, flat, granular, ground glass, ß-hemolytic colonies” that display a slight green tinged color (Drobniewski, 1993). Another test that can be done to correctly identify the bacterium as Bacillus cereus is its appearance on PEMBA (Polymixin pyruvate egg yolk mannitol bromothymol blue agar) media. On the agar, Bacillus cereus forms crenate or fimbriate to slightly rhizoid colonies. They have distinct turquoise to peacock blue color due to the absence of mannitol fermentation and are usually surrounded by an egg yolk precipitate of similar color due to lecithinase production (Sneath, 1986). Most other members of the Bacillus genera are mannitol positive, appear as yellow colonies and do not produce lecithinase.

Due to its pathogenicity, proper identification of Bacillus cereus in contaminated foods is important to prevent food poisoning. By performing these tests and tracking the results, a strain of Bacillus cereus can be correctly identified.

Table 1. Biochemical tests and the expected results used to correctly identify Bacillus cereus.

## Biochemical Test

## Results

Gram Stain

## (+)

Endospore

## (+)

Thioglycollate

FA

Motility

## (+)

Glucose

A and NG

Lactose

NA and NG

Hemolysis

## (+)

A= Acid production, NA= No acid production, G= Gas production, NG= No gas production, FA= Facultative Anaerobe, (+)= Positive for biochemical test, (-)= Negative for biochemical test

Literature Cited

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