

Bacillus

In 1876, Ferdinand Cohn published his famous paper on the biology of bacilli. At this time, Pasteur and Tyndall reached conclusions that contradicted those of Bastian and others concerning whether spontaneous generation did or did not occur. Cohn found that microorganisms differ in their degree of heat resistance.

By boiling hay in water, Cohn killed the heat-sensitive vegetative cells of the of bacteria, fungi, and protozoa normally present on hay. Cohn thereby set up an enrichment situation for the aerobic spore-forming bacilli, and discovered the "hay bacillus," which he named *Bacillus subtilis*. By microscopic observations he discovered the heat-resistant bacterial endospores and showed them to be part of the life cycle of the organism.

Instead of using a hay infusion for the isolation of bacillus we will take advantage of their indigenous presence in our air. Using the air plates we will isolate a bacillus, purify it, and then identify it.

Materials required (per group)

- Previously incubated air plates
- Microscope slides and **cover slips**
- Gram stain reagents
- Plates of TSA other nutrient medium
- 1 TSA slant

Procedure

1. Examine isolated colonies from the air plate looking for those colonies that produce endospores. Bacterial endospores appear as brightly refractile structures within the vegetative cells (sporangia) when viewed in phase-contrast microscopy. If a colony contains endospores streak a TSA plate for isolation. Incubate the plate at 30 C until the next laboratory period, and then describe the types of colonies that have developed.
2. After two consecutive transfer without alteration of colony morphology prepare wet mounts and Gram stains of the new axenic culture. Once you have isolated an axenic strain, make a detail description of the vegetative cell, sporulating cell, and spores. Transfer the axenic culture to a slant, incubate it, and store it in cold room.

The Identification of the Bacillus

The new taxonomy of the *Bacillus* is still somewhat uncertain. The rRNA data indicate that they belong to a procaryotic branch that contains nonsporulating bacteria and possibly the mycoplasmas. Many of the nonsporulating bacteria are similar to spore formers except they lack endospores. Currently members of the *Bacillus* genus are being subdivided to form at least five new separate lines.

Instead of following the new rRNA taxonomy, we will revert back to an older taxonomic scheme that is solely dependent on physiological tests. Using this scheme you will be able to create your own identification key for members of the old family *Bacillaceae* (those organisms that produce endospores that are heat resistant).

Procedure

PERIOD X

1. Transfer your *Bacillus* isolate to fresh TSA slant. Inoculate also one glucose-TSA slant, one MBN-glucose stab, and one mannitol stab. Incubate all cultures at 30°
2. Examine your old cultures microscopically in wet mounts noting the following characteristics:
 - a. spore shape (spherical or cylindrical)
 - b. spore size
 - c. spore location in the cell (central, terminal, subterminal)
 - d. obvious swelling of the cell by the spore. Make sure you are not confused by cell shrinkage. Measurement of young cells will indicate whether the cell is smaller than the spore.

PERIOD Y

1. Examine the media inoculated last period, and note the size of young cells on TSA. Examine motility in the glucose TSA stab.
2. Further identification of your isolate will require you to carry out additional appropriate physiological characterizations: (positive reactions)
 - a. catalase - exercise 31
 - b. growth in 7% NaCl - 7% NaCl-nutrient broth
 - c. starch hydrolysis - exercise 24
 - d. V-P reaction - exercise 28
 - e. growth at 50C - growth on TSBY/5 + 0.1% glucose slants
 - f. NO₃ reduced to NO₂ - exercise 37
 - g. acid and gas from glucose - exercise 22
 - h. casein hydrolysis - exercise 29
 - i. growth at 65C - growth on TSBY/5 + 0.1% glucose slants
 - j. anaerobic growth - MBN - 0.1% glucose exercise 20 GasPak
 - k. rods \exists μ m wide - microbial measurements with a microscope calibrated against a stage micrometer
- l. pH in V-P medium < 6.0 - Use pH paper to measure

Tests should be carried out as explained in the laboratory manual. Inoculate the media appropriate to the identification of your isolate and incubate all cultures at 30°. Media will be given out after a requisition is filled with the GA. Continue to incubate the previously made slants.

PERIOD Z

Examine the media inoculated last period and note reactions. Record your results and, if possible, identify the species of your isolate. Turn in a pure culture with proper identification and a summary of characteristics.

Summary of the characters used in the simplified key for *Bacillus* species.^a

	Catalase	V-P reaction	Growth in anaerobic agar	Growth at 50°C	Growth in 7% NaCl	Acid and gas in glucose	NO ₃ reduced to NO ₂	Starch hydrolyzed	Growth at 65°C	Rods 1.0 μm wide or wider	pH in V-P medium < 6.0	Acid from glucose	Hydrolysis of casein	Parasporal bodies
<i>B. megaterium</i>	+	-	-	-	+	-	0	+	-	+	0	+	+	-
<i>B. cereus</i>	+	+	+	-	+	-	+	+	-	+	+	+	+	0
<i>B. thuringiensis</i>	+	+	+	-	+	-	+	+	-	+	+	+	+	+
<i>B. licheniformis</i>	+	+	+	+	+	-	+	+	-	-	0	+	+	-
<i>B. subtilis</i>	+	+	-	+	+	-	+	+	-	-	0	+	+	-
<i>B. pumilus</i>	+	+	-	+	+	-	-	-	-	-	+	+	+	-
<i>B. firmus</i>	+	-	+	-	+	-	+	+	-	-	-	+	+	-
<i>B. coagulans</i>	+	+	+	+	-	-	0	+	-	0	+	+	0	-
<i>B. polymyxa</i>	+	+	+	-	-	+	+	+	-	-	0	+	+	-
<i>B. macerans</i>	+	-	+	+	-	+	+	+	-	-	-	+	-	-
<i>B. circulans</i>	+	-	0	+	0	-	0	+	-	-	0	+	0	-
<i>B. stearothermophilus</i>	0	-	-	+	-	-	0	+	+	0	+	+	0	-
<i>B. alvei</i>	+	+	+	-	-	-	-	+	-	0	+	+	+	-
<i>B. laterosporus</i>	+	-	+	+	-	-	+	-	-	-	-	+	+	+
<i>B. brevis</i>	+	-	-	+	-	-	0	-	-	-	-	+	+	-
<i>B. larvae</i>	-	-	+	-	+ ^b	-	0	-	-	-	-	+	+	-
<i>B. popilliae</i>	-	-	+	-	+ ^b	-	-	-	-	-	-	+	-	+
<i>B. lentimorbus</i>	-	-	+	-	-	-	-	-	-	-	-	+	-	-
<i>B. sphaericus</i>	+	-	-	-	0	-	-	-	-	0	-	-	0	-

^a +, Greater than 85% of strains examined by Gordon, Haynes, and Pang (1973) positive; -, greater than 85% of strains negative; 0, variable character.

^b Growth in 2% NaCl agar.