Observing Spores and Crystals of *Bacillus thuringiensis* under Optical Microscope

Yan Zhou¹ ², Feiyan Huang¹ ², Cong Zhao², Jintian Zhu²

1. State Key Laboratory for Conservation and Utilization of Subtropical Agro-bioresources, College of Life Science and Technology of Guangxi University, Nanning, Guangxi, China
2. Hainan Institute of Tropical Agricultural Resources (HITAR), Sanya, Hainan, China

Corresponding author email: crystal@sophiapublisher.com; Author


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**Abstract** In this paper, we reported the experimental protocol of observation *Bt* spores and crystals under optical microscope. Based on the tool of optical microscope, we can effectively observe spores and crystals, which are the key elements to rapidly identify and distinguish *Bt* from other members of subgroup of *Bacillus cereus* in *Bacillus* genus, such as *Bacillus cereus* and *B. anthracis*.

**Keywords** *Bacillus thuringiensis*; Subgroup of *Bacillus cereus*; Optical microscope; Spore; Crystal protein

**Introduction** *Bacillus thuringiensis*, short for *Bt*, a gram-positive and sporulation bacteria, belongs to the *Bacillus cereus* sensu latu subgroup of the *Bacillus*. The *Bacillus cereus* sensu latu subrgroup consists six members, *B. cereus*, *B. anthracis*, *B. thuringiensis*, *B. mycoides*, *B. pseudomycoide* and *B. Weihenstephanensis*. Compared with other subgroup members, *Bt* strain has a remarkable characteristic that can produce insecticidal crystal proteins during sporulation.

Observing spores and crystals by optical microscope is one of the most effective methods to rapidly identify and distinguish *Bt* from other members of subgroup of *Bacillus cereus* in Bacillus genus, such as *Bacillus cereus* and *B. anthracis*. In this paper, we reported the observing protocol for identifying the spores and crystals by using optical microscope in our laboratory. The protocol was proved that it is a simple and effective way to look for parasporal crystal of *Bacillus thuringiensis*.

**1. Equipment and operation**

The OLYMPUS DP70 microscope, made by Japan (Figure 1), was used in this technique. The image resolution is up to 4080 × 3072 pixel. The maximum magnification is up to 1000 times by using the oil immersion lens.

![Figure 1 The Olympus Digital Microscope DP70 Model used in this study](image)

The steps to handle microscope with oil immersion lens:

1. Sample Focus: Firstly, focus the sample under low magnification objective lens.
2. Covered with cedar oil: Add a drop of cedar oil on the glass smear,
3. Focus and observe: Turning the knob to fine adjust,
Observe and record: looking for the appropriate visions, and then record them and take photograph. Whenever finished, get down the object stage, turn out the oil immersion len and remove microscope slide.

Len cleaning: First, wiping the oil on the len with len wiping paper, and then clean the residual oil with some ethanol absolute, finally, clean the detergent and naturally dry.

2 Chemical reagent and preparation

Drawing 10 mL Solution A (10%w/v) (Solution A: basic fuchsin 1 g dissolved in absolute ethyl alcohol 10 mL) and 90 mL Solution B (5%w/v) (Solution B: carbolic acid (phenol) 5 g; dissolved in the mili-Q ultrapured water 100 mL), intensive mixing into the carbol-fuchsin solution, stored at 4 ℃. Dilute 10 folds, and then sterilize by filtration of the Pall Supor® Membrane 0.45 μm before using.

3 Experimental procedure

3.1 Prepare an objective sample

Incubate the fresh activating Bt strain on NB medium at 30 ℃ for more than 72 hours until full sporulation phase.

3.2 Preparation slide

(1) Pipet a drop of double distilled H2O on the middle of the microscope slide, pick a few Bt cultured liquid, then spread on the slide.

(2) Heat the slide on the flames quickly for one second after air-dried completely.

(3) Quickly back and forth the slide on the flames for 2 or 3 times to make the Bt sample sticked strongly.

(4) After cool down, the slide is ready for dye.

3.3 Staining

(1) Pipet the dye solution, cover whole, stain for 30~60s at room temperature.

(2) Pour the dye solution and wash by fresh water.

(3) Cover with coverslip for microscope observation.

3.4 Observation

(1) Observe under microscopy with 100X oil immersion len. adjust the major adjustment knob, let the oil immersion len into cedar oil and approach the slide as close as possible but not touch the glass slide. And then turn the major adjustment len knob slightly and slowly, to make the objective len upward until the image appearing furthermore to adjust the fine knob to bring the image into proper focus.

(2) Under the observation of microscopy, the crystal protein was stained with red dye and the spore was colourless.

3.5 Taking pictures

Set up photograph parameters of the microscopy system, choose a favorite colore for photo background. Please note that each picture needs the scale bar, recommende scale 10 μm.

4 Case study

The specific characteristics of the Bt bacterium is that Bt strain can produce spores and crystals at the later growth stage of sporulation. Therefore, it is the way to observe the crystal in this growth period in order to quickly identify the isolate. In this case, we employed Bt strain S2160-1 (Zhang et al., 2012) and Bt model strain HD-73 as the experimental materials by stained with the carbol-fuchsin solution. The result showed as figure 2, under red or light red background, it can be observed that there are the colorless spores in both strains, bipyramidal crystal in HD-73, as well as spherical crystal in S2160-1.

Figure 2 Bt strains observed by optical microscopy with 100X oil immersion len (S2160-1 and HD-73)

5 Discussions

To obtain high-quality microscopic photographs, there are several points that need to pay attention to.

Firstly, the observed strain must be cultured untill the spores are completely procuced, that is 48 hours to 72 h above different with different strains, and make sure the objectives you want to see available.
Secondly, the observed strain colony should be spread evenly on the slide, do not pick up too much bacteria that will result in difficulty distinguishing spores and crystals since bacteria crowd and overlapand pls avoid to pick up the culture medium that will ruin the photograph background.

Thirdly, the ready slide needs to maintain right moisture. if it is too dry, it is not good for observation; therefore, the best time for observation is under the observation immediately after the slice was fresh made.

Finally, choosing the right microscope is the guarantee to get the good images and operating microscope is the key to acquire high quality pictures as well.

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