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A rapid and simple method for staining of the crystal protein of *Bacillus thuringiensis*

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SUMMARY

A rapid and simple method of staining for the crystal protein (δ-endotoxin or parasporal body) of *Bacillus thuringiensis* has been developed. Changes in colonial morphology were observed when cells lost their ability to form crystal protein or both crystal protein and spore.

INTRODUCTION

Among *Bacillus* species, *Bacillus thuringiensis* is unique for its ability to produce an insecticidal toxin (δ-endotoxin) during sporulation [1,2]. The appearance of spores and crystals is usually detected by phase contrast microscopy [4,6]. However, the method is time-consuming and may be misleading, especially in differentiating crystal protein from inclusion bodies, the latter being frequently encountered in many spore-forming *Bacillus* species.

An interesting point reported by several investigators is the spontaneous occurrence of mutants with altered colonial morphology upon loss of their ability to produce crystal protein [3,4]. Furthermore, Wu and Chang [5] have employed temperature shift for the isolation of such mutants.

Having followed the same procedure, such findings have been reconfirmed by the use of our staining method described in the present paper.

METHODS AND MATERIALS

B. thuringiensis strains (Turkish isolates confirmed by the Faculty of Agriculture in Ankara) were grown on nutrient agar plates and maintained on nutrient agar slants at 4°C.

The method of Wu and Chang [5] was essentially followed for the isolation of mutants with altered colonial morphology.

Differentiation between crystals, spores, and vegetative cells was achieved through light microscopy. Samples were prepared for microscopic examination as follows: smears of bacteria were dipped into a small container containing Coomassie brilliant blue solution (0.25% Coomassie brilliant blue, 50% ethanol, and 7% acetic acid) for 3 min, washed with tap water, dried, and observed under a light microscope.

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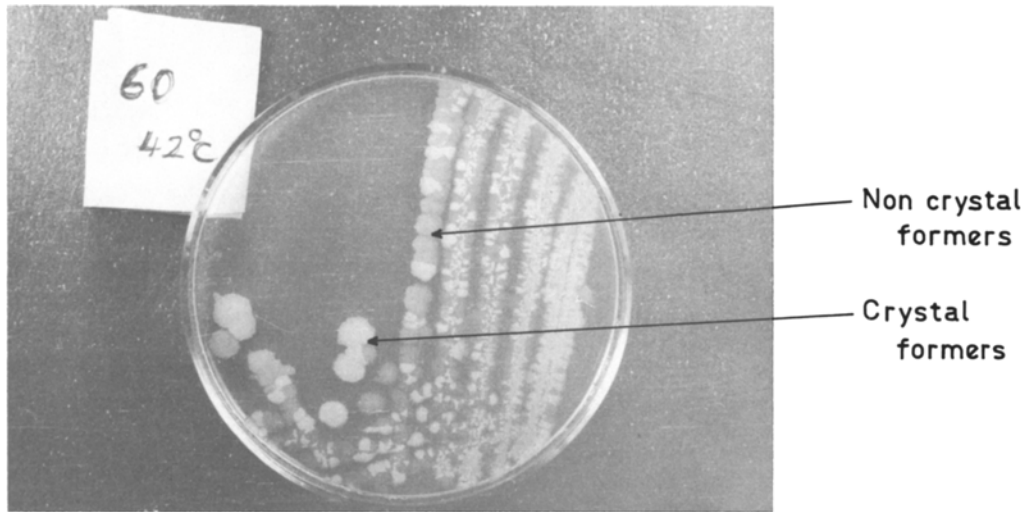

Fig. 1. Change in colony morphology following incubation at 42°C.

RESULTS AND DISCUSSION

Colonies which lost the ability to produce crystals were opaque and raised with smooth margins, those which lost the ability to produce both spores and crystals were translucent, while wild-type colonies (sporulating and crystal producers) were white and depressed with rough edges (Fig. 1).

Upon microscopic observation, released crystals could be distinguished from spores since they stained purple and displayed a unique diamond shape, while spores remained white and elliptical in appearance (Fig. 2). Vegetative cells appeared as purple rods. Crystals and spores appeared as white bodies within purple-stained cells (Fig. 3). Figures presented are of one of the studied strains designated '60'.

The staining method thus developed, beside its

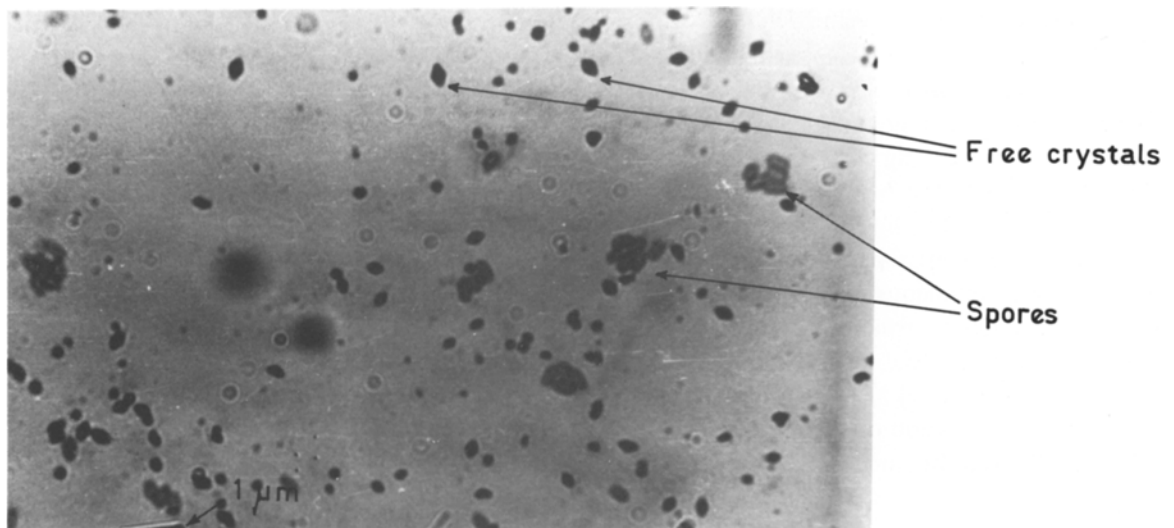

Fig. 2. Light microscopy indicating crystals and spores in free form. Magnification: 100 ×.

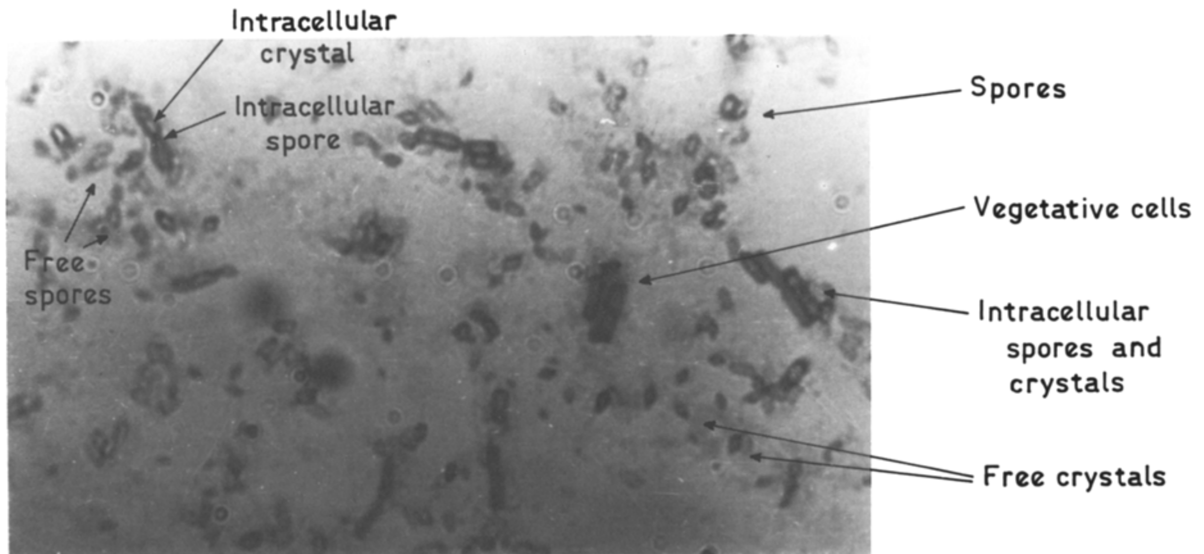

Fig. 3. Light microscopy indicating intracellular spores and crystals, free spores and crystals, and vegetative cells. Magnification: 100 ×.

simplicity and rapidness, proved to be reliable, especially in differentiating *B. thuringiensis* from the closely related species *B. cereus*. The latter may have spores, along with several inclusions which are sometimes indistinguishable from the crystal protein of *B. thuringiensis* by the use of a phase-contrast microscope.

Our staining method worked efficiently in the ten *B. thuringiensis* strains that were available to us and can be employed as a method for rapid identification of crystals in other strains.

REFERENCES

1 Bechtel, P.B. and L.A. Bulla Jr. 1976. Electron microscope study of sporulation and parasporal crystal formation in *Bacillus thuringiensis*. J. Bacteriol. 127: 1472–1481.

2 Bulla, L.A., Jr., R.A. Rhodes and E. St. Julian. 1975. Bacteria as insect pathogens. Annu. Rev. Microbiol. 29: 163–190.

3 Gonzalez, J.M., Jr., H.T. Dulmage and B.C. Carlton. 1981. Correlation between specific plasmids and delta-endotoxin production in *Bacillus thuringiensis*. Plasmid 5: 351–365.

4 Kamdar, H. and K. Jayaraman. 1983. Spontaneous loss of a high molecular weight plasmid and the biocide of *Bacillus thuringiensis* var. *israelensis*. Biochem. Biophys. Res. Commun. 110: 477–482.

5 Wu, D. and F.N. Chang. 1985. Location and characterization of *Bacillus thuringiensis* subsp. *israelensis* mutants deficient in the production of delta-endotoxin. Fed. Proc. 44 (S.5): 1800–1801.

6 Yousten, A.A. and M.H. Rogoff. 1969. Metabolism of *Bacillus thuringiensis* in relation to spore and crystal formation. J. Bacteriol. 100: 1229–1236.