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Proteolytic Enzyme Activity of *Bacillus thuringiensis* Berliner in Two Different Culture Media

There is an increasing interest in substances formed by microorganisms that are toxic to insects. These substances are not only employed for the study of the mechanism responsible for the pathogenicity for insects, but also can be applied in microbial control. Proteolytic activity of bacterial pathogens, especially the potential pathogens and obligatory crystalliferous pathogens like *Bacillus thuringiensis* is of importance as it hydrolyses the host proteins and supplies the pathogen with suitable form of nitrogen. Although there are reports about the proteolytic enzyme activity of *B. thuringiensis* by Bucher (1960) and Krieg (1961), there has been no substantial evidence about the production of this enzyme with quantitative data. Hence, the present study on the proteolytic activity of *B. thuringiensis* in two different culture media was made.

To obtain the enzyme preparation, the bacterium was grown in nutrient broth and egg albumin media which have been autoclavad at 15 lb pressure for 20 minutes. The media were inoculated with *B. thuringiensis* and kept for incubation at room temperature. The enzyme activity was measured by the casein digestion method described by Kunitz (1947).

The reaction mixture contained 1.0 ml of culture filtrate and 1.0 ml of 1.0 per cent casein in 0.1 M phosphate buffer at pH 7.5. The enzyme substrate mixture was incubated at 35°C. The residual protein was precipitated at 'zero' and 40 minutes with 3 ml of 5 per cent trichloro acetic acid. After the preparation had remained at room temperature for one hour or longer, it was centrifuged and the optical density was measured at 280 mμ with Beckman DU Spectrophotometer against a blank that consisted of the supernatant fluid from the reaction mixture precipitated with trichloro acetic acid at 'zero' time. The observation was made up to 72 hr at an interval of 24 hr.

The results obtained in this study showed the maximum activity in nutrient agar medium when compared with the egg albumin (Table 1). It is reported that the difference in medium will have certin effect on the growth, multiplication and sporulation of the bacterium (Steinhaus, 1951). He further stated that sporulation can occur during 48 hr in nutrient agar whereas it will be completed after 72 hr in the nutrient broth. So it is all the more possible to get a difference in the proteolytic enzyme activity in different media. Since *B. thuringiensis* is said to complete its sporulation within a period of 48 and 72 hr (Heimpel, 1967) this may be the reason for the decrease in activity of the proteolytic enzmes as the time lapses.

TABLE 1. Proteolytic enzyme activity of *Bacillus thuringiensis* in two different culture media

Medium	Enzyme activity in absorption units			Initial pH	Final pH
	24 hr	48 hr	72 hr		
Nutrient broth	1.05	0.08	0.010	6.94 (6.7—7.0)	4.9
Egg albumin	0.015	0.005	—	6.94 (6.7—7.0)	4.9—5.2

The probable role of this proteolytic enzyme as one of the factors in causing the mortality of the insect is further supported by Bucher (1960) in potential pathogens like *Pseudomonas aeruginosa*. Further evidence for the production of such enzymes and their relation in the pathogenicity was given by Patel and Cutkomb (1961) in *Bacillus larvae*, the causal organism of American Foul Brood of honey bee. Evidence given by Lysenko (1967) about the role of proteolytic enzyme activity of *P. aeruginosa* and LD_{50} in greater wax moth, *Galleria mellonella*, larvae is further strengthened by the present findings.

Thus it may be expected that proteolytic enzyme produced by this baterium may have a role in pathogenicity of the test insect. Observation on the pH of the medium before and 72 hr after incubation showed a reduction of pH value from neutral to acid range. This may be attributed to the activity of bacteria and casein which in turn resulted in the remarkable decrease in the pH value to the acid level. Such a trend was observed in the growth and production of proteinase of *P.aeruginosa* by Lysenko (1967). Further it was also suppored by Heimpel (1955) that bacteria grow well over a fairly wide range of pH but the activity of the enzyme they produce is generally restricted to a narrower range.

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