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LASER HE-NE EFFECT ON *BACILLUS THURINGIENSIS* VAR. *KURSTAKI* STRAIN LBT-24

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ABSTRACT

Bacillus thuringiensis toxin is one of the world widely used entomopathogen. It presents an strong insecticide activity on Lepidoptera, Coleoptera and Diptera. It was studied the effect of LASER He-Ne on *Bacillus thuringiensis* var. *kurstaki* strain LBT-24. Growing curves were made and were calculated the duplication time and the specific growing speed of each one. The curves were statistically compared. It was also analysed the phage induction with and without LASER red light influence. Also, it was observed the presence of the δ-endotoxin crystal with this treatment. The red LASER He-Ne enhanced the growth of this micro-organism under laboratory conditions and didn't have any effect over the other characteristics analysed.

INTRODUCTION

Chemical insecticides change irreversibly the environment and also damage human health. That's why the production of entomopathogenic micro-organisms have increased (Heimpel, 1972). *Bacillus thuringiensis* produces crystal protein which are toxic to several orders of economically important insects. Many of them are important vectors of human diseases, while others are pests of a number of crops. Its use offer several advantages over chemical control (Aptosoglou, Sivropoulou and Koliais, 1997; Hansen and Hendriksen, 1997) This micro-organism has been studied in great details for more than 30 years. It produces a toxic parasporal crystal identified as δ-endotoxin. The advantages of using endotoxins based insecticides include that it is highly compatible with natural enemies because of a narrow host range, safe to vertebrates, receptive to genetic engineering and biodegrades rapidly (Martínez, Robacker and García., 1997). In our country have been used the HeNe LASER to increase the production of yeast with good results because of its enhancement influence on the growth of micro-organisms (Castro, 1999; Mauro, 1987) With this research we want to study the effect of HeNe LASER on the bioinsecticide *Bacillus thuringiensis*. For this purpose it was determined the effect on the growth of this micro-organism and also the influence over phage production and the presence of crystal protein. Here we show the growing curves and microphotographs of the last two experiments.

MATERIALS AND METHODS

It was used a commercial strain LBT-24 of *Bacillus thuringiensis* var. *kurstaki*. The micro-organism was grown on Triptone Soya Broth and incubated at 30 ± 2°C with shaking (200 rpm).

Procedure

Curves

Curves were made with and without application of HeNe LASER over all the growth up to early stationary phase. The micro-organism was incubated at 30^0 C during five days to get total sporulation and was inoculated up to an initial DO of 0.1. It was measured the optical density every hour and plotted in a graphic DO vs time(h). Also, every hour a millilitre of a dilution was added in three empty plates and later was added between 15-20 ml of Tryptone Soya Agar (TSA) melted at 40-45^0 C for plate. They were carefully mixed and incubated at 30 ± 2°C for 24h. After that the colonies were counted. The curves were made three times for each treatment.

Results analysis were made with more than nine replicas by point. It was applied Bartlet test and Kolmogorov Smirnov test for each dose and it was made a variance analysis to compare the curves of the same treatment and treatments each other.

For each treatment were calculated the growing specific speed and the duplication time.

Growing specific speed:

(1) : $\mu = (\log_{10} Z - \log_{10} Z_0) / (t-t_0)$

where:

Z: Colony former unit by millilitre (cfu/ml) belonging to time t.

Z_0: Colony former unit by millilitre (cfu/ml)belonging to time to.

Duplication time:

(2) : $tg = 0.693 / \mu$. (Pazos et. al., 1980).

Bacteriophages Isolation and Purification

The micro-organism was grown in CSST medium during up to early stationary phase at 30 degrees. One of the erlenmeyers was grown under the influence of HeNe LASER up to stationary phase.

CSST medium composition:

Lab-Lemco Broth	*8 g/l*
$MgSO_4$	*0.2 g/l*
$MnSO_4$	*0.05 g/l*
$CaCl_2$	*0.15 g/l*
Na Cl	*2.5 g/l*
pH = 7,2	

After that 0.5 ml of this suspension was mixed with some drops of spores grown in non induction conditions and 5 ml of solid CSST medium (1%). They were distributed in plates and incubated at 30 ± 2°C 24 hours. The plates were flooded with SM buffer (Sambrook et al., 1989) and shaked carefully during four hours. The buffer was centrifuged at 10 000 rpm for 10 minutes and the supernatant was observed under the electron microscope.

Crystal observation

The micro-organism was grown in TSA up to early stationary phase at 30 degrees One of the erlenmeyers was grew under the influence of HeNe LASER. From each one was taken 1.5 ml and centrifuged at 6000 rpm during 15 minutes and the pellet was observed under the electron.

Electron microscopy

Phage observation

For this purpose the samples were negatively stained with uranyl acetate 2%. Samples were examined in a JEOL JEM 100S electron microscope at magnification of

Crystal observation

The samples were fixed in 3.2% (v/v) glutaraldehyde in 0.1M cacodylate buffer, pH 7.4, for 1 hour. Samples were incubated with osmium tetroxide for 1 . After a brief washing , the cells were dehydrated in a graded series of ethanol before being embedded in EPON. The sectioned material was stained with uranyl acetate and lead citrate. The sections were examined in a JEOL JEM 100S electron microscope at magnification of

RESULTS AND DISCUSSION

Curves with and without the influence of HeNe LASER.

Figure 1 shows the curves obtained with and without treatment

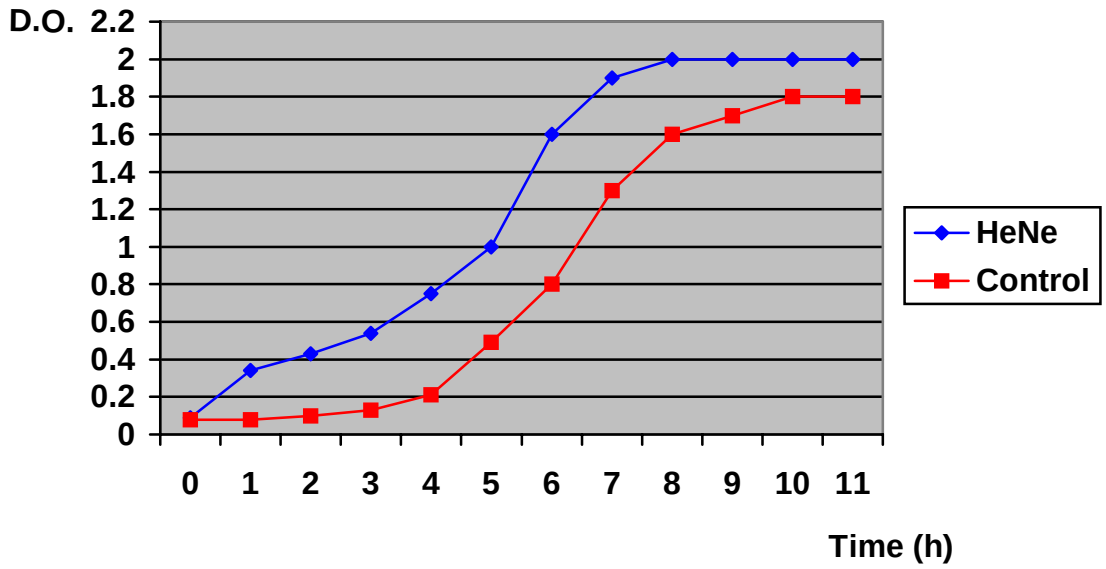

Figure 1: Curves with and without the influence of HeNe LASER.

Using the Bartlet and Kolmogorov-Smirnov tests was demostrated that data have variance homogeneity and normality. Statistic analysis shown significative differences between the treatment and the control. The curve with treatment present a very short latence phase and also the D.O. reached is higher at the stationary phase (2.0) in comparison with the control (1.8).

Table 1 shows the duplication time and growing specific speed for the treatment and the control. The influence of the HeNe LASER decreased significatively the duplication time and increased the growing specific speed in comparison with the control. From the physiological point of view a possible explanation is that at the level of the electron transport chain there are flavoproteins. Between the oxidative state and the

reduce one of these ones there are an special kind of specie called semiquinones and among them the blue semiquinones able to absorb red light with a maximum between 590-640 nm. According to that it is possible that the interaction between these proteins and red light provoke an stimulation in the electron transport chain that give the result of an increase in the growing specific speed. This is in correspondence with the researches made by Mauro, 1997 in *Saccharomyces itálicus*.

The decrease of the latence phase and the D.O. reached at stationary phase can be explaining according to Kaback hypothesis. It means, for each two electron from electronic donor to oxygen a substrate molecule (carbohydrate or aminoacid) should be transported inside the cell through a vesicle in the cell membrane where it is concentrated. Then, a stimulation in the electron chain transport could increase medium substrate utilisation. According to P. Mitchell and F. M. Harold there are a coupling between the electron transport chain and the substrate transport from the medium to inside the cell (Lehninger, 1981).

Table 1: Duplication time and growing specific speed for the treatment and the control.

	tg (h)	μ (h^{-1})
Control	1.95	0.356
HeNe	1.37	0.503

Bacteriophages Isolation and Purification

The treatment and the control shown lysis areas and even the phage presence have been seen by electron microphotography . These results suggest that the HeNe LASER didn't affect the qualitative phage induction in this strain, but it is necessary to do a quantitative study of phage induction for each one.

Crystal observation

The parasporal crystal was observed in both with and without treatment. This result suggests that the HeNe LASER didn't affect the crystal formation, that's why the virulence of this entomopathogen should be no affected and because the quantity of micro-organisms is higher at the stationary phase it should be higher also. Any way it is necessary to do a bioassay to be sure.

CONCLUSIONS

- LASER HeNe with potency 1.4 mW/cm^2 and a λ= 6328 A°, acting over *B. thuringiensis* var *kurstaki* (strain LBT-24), enhanced the growth increasing the specific growing speed and decreasing the duplication time in comparison with the control.
- With this treatment it was possible to decrease the latency phase and it was also obtained a higher D.O. at the stationary phase in comparison with the control.
- In the strain studied phage induction was not different in relation with control.
- Crystal production was not affected by HeNe action

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