

TITLE: ANALYSIS OF ENDOTOXIN AND SPORE PRODUCTION BY *Bacillus thuringiensis* SUBSP. *berliner* USING WHEY THROUGH FRACTIONAL FACTORIAL DESIGN

AUTHORS: FREITAS, L. S.; SILVA, T. A. F.; NETO, J. M. W. D.; WANDERLEY, M. C. A.; PORTO, A. L. F.; BEZERRA, R. P.

INSTITUTION: UNIVERSIDADE FEDERAL RURAL DE PERNAMBUCO, RECIFE, PE (RUA DOM MANUEL DE MEDEIROS, S/N, CEP 52171-900, RECIFE – PE, BRAZIL)

ABSTRACT:

The bacterium *Bacillus thuringiensis* (Bt) is known worldwide for its high toxicity against different orders of insects, such as Lepidoptera, Coleoptera, Spodoptera, among others. Generally, Bt is grown in conventional culture media which is expensive. Therefore, alternative culture media have been increasingly used for its cultivation. Among these alternative culture media is WHEY, which has high nutritional value and great potential for Bt growth. Thus, aiming at the production of endotoxins and spores by Bt, the present work tested the effect of some variables besides WHEY from a fractional factorial design 2^{6-2} for the composition of the culture medium for *Bacillus thuringiensis* subsp. *Berliner* strain 370. The variables tested were: lactose concentration of WHEY (0.5%, 1.0% and 1.5%), KH_2PO_4 and K_2HPO_4 (0.0, 0.5, 1.0, g/L), MgSO_4 (0.0, 0.25 0.5 g/L), MnSO_4 and FeSO_4 (0.0 g/L, 0.01 and 0.02 g/L). For the execution of the factorial design, 20 essays were required, including 4 central points for error calculate. Each essay was performed in 250 mL Erlenmeyer flasks containing 50 mL of culture medium with initial cellular concentrations of $0.15 \text{ A}_{600 \text{ nm}}$, kept on a rotary shaker at 30°C , 200 rpm for 72 hours. After this period the endotoxin and spore production were analyzed. It was observed that WHEY, KH_2PO_4 , MgSO_4 presented positive and significant effects which at the highest levels, respectively, an increase of 173, 18.22 and 49.06 mg/L in the production of endotoxins. For spore production, WHEY, KH_2PO_4 and MgSO_4 also were significative variables, but only WHEY and KH_2PO_4 show a positive effect on sporulation, 24.81×10^6 and 10.06×10^6 Spores/mL, respectively. In the presence of MgSO_4 , there was a reduction of sporulation of -8.06×10^6 Spores/mL. Therefore, the increase of lactose concentrations also increases concentrations of proteins presents in WHEY which stimulates microbial growth that consequently promote more sporulation and more endotoxin liberation. Some research explains which presence of phosphates has an important role in metabolic pathways and is a regulator of endotoxin synthesis in Bt. Also was identify which addition of magnesium in Bt culture stimulates its growth and subsequently stimulates endotoxin liberation at the sporulation phase. However, high concentrations of lactose on culture medium supplied by WHEY added with of KH_2PO_4 and MgSO_4 perform a low-cost medium appropriate to endotoxin production by Bt strain 370.

Keywords: *Bacillus thuringiensis*, endotoxins, spores, WHEY, factorial design

Development agency: Fundação de Amparo de Ciência e Tecnologia de Pernambuco (FACEPE).