DETERMINATION OF INCUBATION CONDITIONS FOR PRODUCING SUBTILISIN FROM *Bacillus subtilis*

Luis Alejandro Ángeles Mendoza, Beatriz Ortega Escamilla, Florencia del Carmen Salinas Pérez and Jesús Alarcón Bonilla. División de Biotecnología, Universidad Tecnológica de Tecámac. Tecámac, Estado de México. C.P. 55740

jalarconbonilla@yahoo.com.mx

Bacillus subtilis, subtilisin, microbial protease

Introduction. Currently, enzymes are used in many industrial activities, such as in the production of detergents, food production, textile industry or health area. (1). Proteases are a group of enzymes belonging to the hydrolases, which can be obtained from animal, plant or microbial sources. Subtilisin is an alkaline protease produced by *Bacillus subtilis*, a bacterium which is indigenous to the soil. It is a Gram positive bacillus, isolated, mobile, aerobic and it produces heat resistant spores. It grows on a range of temperatures and it is capable of producing extracellular hydrolytic enzymes, as well as a variety of antibiotics. It is a catalase positive, Voges-Proskauer positive. It cannot growth in anaerobic conditions and the hydrolysis of starch is positive (2).

The aim of this work was to establish the optimal incubation conditions for subtilisin production by *B. subtilis* in Schaeffer culture medium enriched with glucose

Methods. The experimental strategy was: **A**. Microscopic morphology identification of *Bacillus subtilis* strain ATCC 6633 grown in trypticase soy broth (TSB). **B**. Reseeding of strain in trypticase soy agar (TSA); **C**. Fermentation in Schaeffer culture medium enriched with glucose, under two conditions: a shaking water bath and a shaking incubator, both at 37 ° C at 150 rpm for 18 hours **D**. Enzyme obtaining by centrifugation at 3000 rpm/10 min. **E**. Evaluation of the potency of the subtilisin from binary dilutions (2^2, 2^4, 2^8, 2^{16} and 2^{32}) for measuring the proteolytic activity of hydrolysis zone on Nutrient Agar plates supplemented with the sample protein (casein, egg albumin and hemoglobin) and **F**. Protein quantification by the Bradford method.

Results. Subtilisin activity was more efficient in the hydrolysis of ovalbumin generating a diameter hydrolysis zone of 3.8 cm (see Fig.1). It must be mentioned that the hydrolysis activity was increased to a higher dilution (see Table 1). The amount of protease obtained from the incubated sample in water bath at 37 ° C/150 rpm for 18 hours was 2 mg/mL being more efficient in this incubation condition compared to shaking incubation which subtilisin production was 1.8 mg/mL.

Table 1. Diameters hydrolysis halos of three kinds of proteins

	1st. Sampling (cm)			2nd. Sampling (cm)		
Dil.	NA+C	NA+O	BAP	NA+C	NA+O	BAP
2^2	Neg.	2.2	2.2	Neg.	2.4	2.0
2^4	Neg.	2.9	2.5	Neg.	2.5	2.2
2^8	1.9	3.2	2.8	1.7	2.7	2.5
2^{16}	2.2	3.6	3.0	1.9	2.9	2.8
2^{32}	2.4	3.8	3.1	2.0	3.1	3.0

NOTE: NA+C: Nutrient Agar with Casein, NA+O: Nutrient Agar with ovalbumin, BAP: Blood Agar Plate

Fig.1 Hydrolysis halos in nutrient agar plates with casein

Conclusions. The best incubation conditions for the subtilisin production were 37 ° C/18 h in water bath at 150 rpm, obtaining 2mg/mL of subtilisin concentration. The hydrolytic activity of subtilisin was increased to a greater dilution. Subtilisin increased proteolytic activity on ovalbumin.

References.

1. Corales, Z. M. (2008). *Metogenómica y sus aplicaciones industriales*. Universidad de Puerto Rico, Puerto Rico.
2. Espinosa, J. J. (2005). *Caracterización del proceso de crecimiento de Bacillus subtilis bajo condiciones anaerobias.* UNAM, México.