LIPASE ACTIVITY IN *STREPTOMYCES NOURSEI* DURING NYSTATIN BIOSYNTHESIS

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Abstract: This study aimed at determining the endo- and exolipase activity in the producing microorganism, *Streptomyces noursei*, during nystatin biosynthesis. The results obtained evidenced a exolipase activity reaches peak levels during trophophase, when the metabolism of lipids in the growth medium initiates, and then exhibits levels lower than the endolipase activity and the the fat content in the growth medium impacts on the rate of increase of the nystatin content.

INTRODUCTION

There are many microorganisms which posses the capacity to use natural oils and fats as carbon source (Arpigny J.L., Jaeger K.E., 1999).

The enzymes that break down the oils and fats to be assimilated by microorganisms are the lipases, which catalyze the hydrolysis of triglycerides into free fatty acids, di- and monoglycerides, and glycerin.

Even though the biosynthesis of many antibiotics requires high lipid uptake, literature data show that there is a connection among the lipolytic activity, concentration of carbon hydrates and lipid content of the growth medium (Ettler F., Votruba J., 1980).

Microorganisms have both intracellular lipases (i.e. endolipases), which break down the lipids inside the cell, and extracellular lipases (i.e. exolipases), which break down the lipids in the growth medium (Arpigny J.L., Jaeger K.E., 1999).

This study aimed at determining the endo- and exolipase activity in the producing microorganism, *Streptomyces noursei*, during nystatin biosynthesis.

MATERIAL AND METHOD

For the purpose of this study, the *Streptomyces noursei* strain from the collection of the company Antibiotice SA Iaşi was used. The strain was subjected to submerged cultivation on a specific biosynthesis medium previously sterilized and distributed in 50-mL flasks (100 mL /flask).

The medium preparation involved the use of soybean flour as a protein source, and sunflower oil as a nutritious supplement, administered on a daily basis. The submerged growth process took 336 hours.

Every 24 hours during biosynthesis, the following parameters were determined: endolipases activity, exolipases activity, fat content of the biosynthesis medium, and daily growth rate of the nystatin content.

Lipase activity was determined (Artenie V., Tănase Elvira, 1981) based on the increase in the acidity of the reaction medium as a result of the release of fatty acids from triglycerides, a reaction triggered by the enzyme. The free fatty acids resulted from the action of the lipase were titrated directly using a solution of sodium hydroxide. Pure sunflower oil was used as a substrate.

After centrifuging the culture broth, both the mycelium and the supernatant were used in detecting the enzymatic activity of the lipases. The mycelium (2.5 g) was used to determine the endolipases, while the supernatant (2.5 g) was used to determine the exolipases. In both cases, 1 ml of pure sunflower oil was added.

To determine the activity of acid lipases, 5 ml of acetate buffer solution pH 4.7 were added to each mortar and triturated for 5 minutes.

The suspensions resulted were transferred to 100-mL conical flasks fitted with ground glass stoppers and 5 drops of toluene were added. The flasks were stirred for 5 minutes using a magnetic stirrer, and then incubated at 30^0C for 24 hours. After incubation, 50 mL of a 4:1 mixture of ethyl alcohol and ethyl ether were added to each flask. The resulted solutions were subjected to a titration assay using a 0.1N solution of NaOH in the presence of phenolphthalein. In parallel with the samples to be examined, control samples were prepared. They were not incubated and were titrated immediately after the mixture of ethyl alcohol and ethyl ether was added.

The lipase activity was expressed as ml of NaOH solution 0.1N needed to neutralize the fatty acids released by the action of the lipases calculated for 100 g of mycelium / supernatant:

$X = (Vp – Vm) \cdot F \cdot 100 / g$

where:

- X – lipase activity;
- Vp – mL of NaOH solution 0.1 N used to titrate the sample to be examined;
- Vm – mL of NaOH solution 0.1 N used to titrate the control sample;

- F – titration factor of the Na OH solution 0.1 N determined using oxalic acid;
- g – weight of mycelium / supernatant examined.

The fat content of the growth medium was determined by extraction with petroleum ether, while the daily rate of increase in the nystatin content was determined by spectrophotometry.

RESULTS AND DISCUSSIONS

The endo- and exolipase activities, as well as the fat content of the growth medium, and the daily rate of increase in the nystatin content during biosynthesis are indicated in the Table 1 below and graphically presented in Figures 1, 2, 3.

The above-mentioned results show that during the culture trophophase (0-96 hours) the fat content in the growth medium, maintained at 3-5 ‰ by adding sunflower oil, determined the decrease of both the enzymatic activity of the endolipases and the activity of exolipases.

The rate of increase in the nystatin content was 3-8%.

During the culture idiophase (96-264 hours), a fat concentration maintained at 5-6 ‰ by adding sunflower oil as a nutritive supplement determined an increase in the activity of endolipases and a reduction in the exolipase activity. The rate of increase in the nystatin content was 8-10%.

Table 1. Fat content, lipase activity, and nystatin content increase rate during biosynthesis

Age of culture (hours)	Fat content (‰)	Enzymatic activity (ml of NaOH solution 0.1 N / 100 g)		Rate of increase in nystatin content (%)
		Endolipases	Exolipases	
24	4.1	96	112	3
48	3.3	84	108	5
72	3.9	16	81	7
96	5.4	12	48	8
120	6.0	172	20	8,5
144	6.4	98	11	10
168	6.2	111	25	8,5
192	6.5	138	48	8,5
216	6.7	120	64	8,5
240	5.5	96	24	9
264	5.0	44	4	10
288	4.5	48	2	10
312	3.0	40	0,5	7
336	2.3	64	56	7

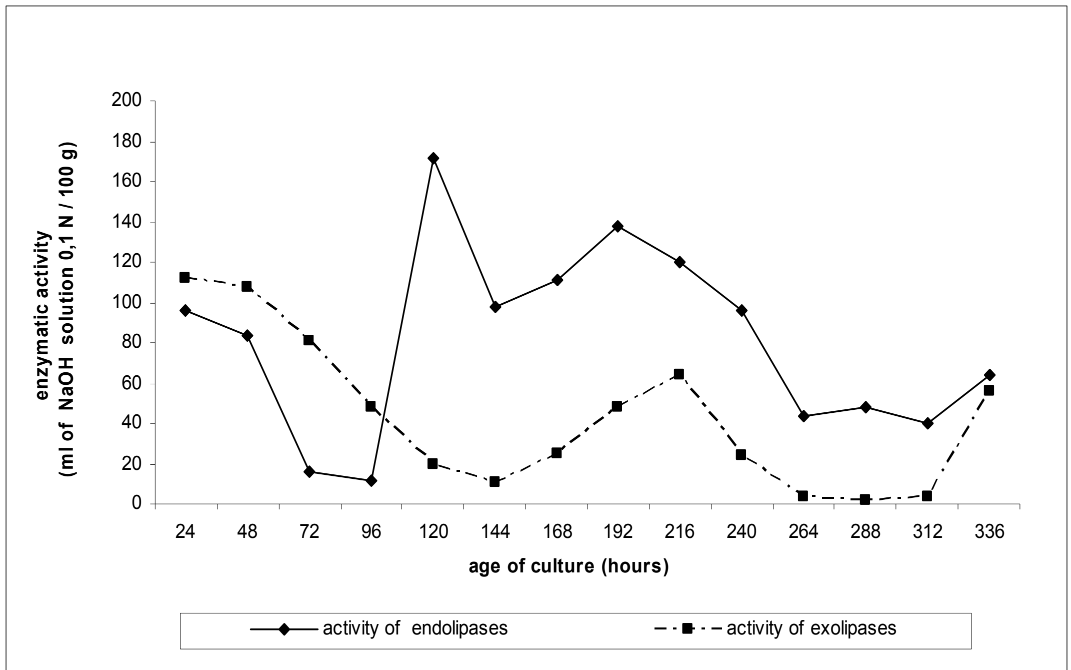

Figure 1. *Activity of endolipases and exolipases during nystatin biosynthesis*

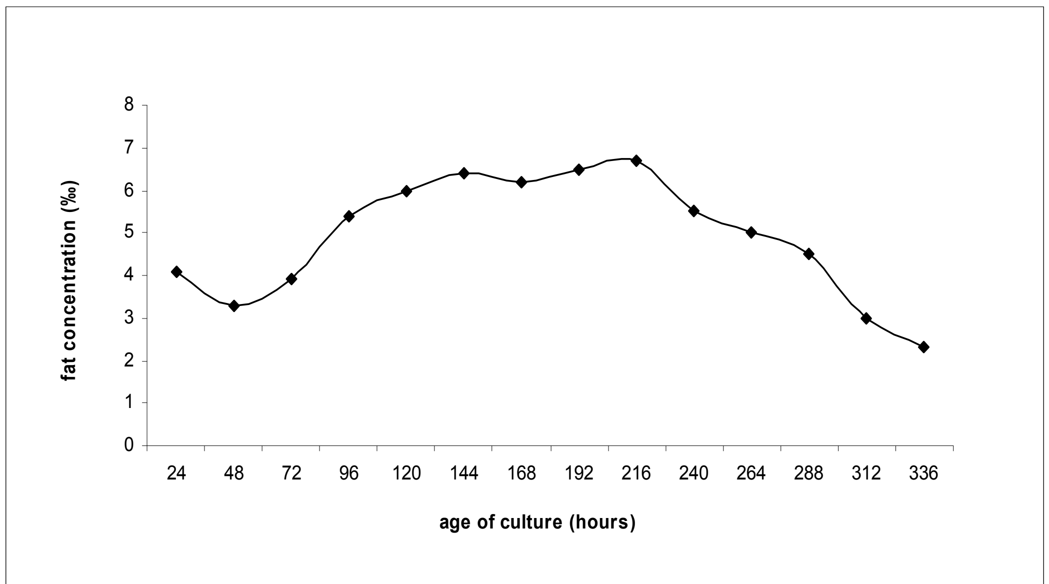

Figure 2. *Evolution of fat content during nystatin biosynthesis*

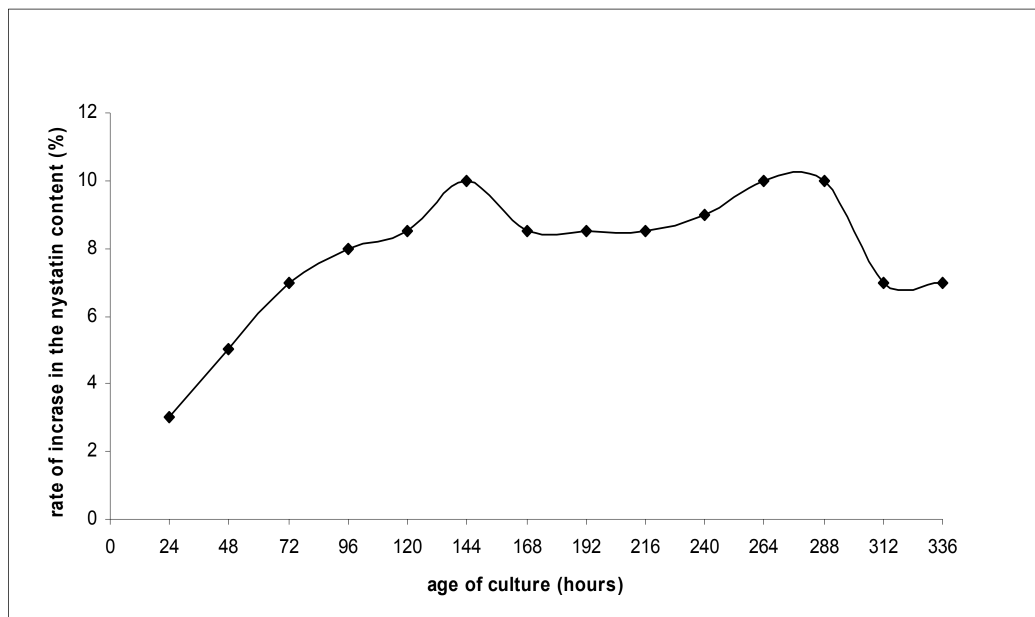

Figure 3. ***Rate of increase in the nystatin content during biosynthesis***

At the end of biosynthesis, when the addition of sunflower oil was reduced and then discontinued (the fat content reduced to 2-3%), both the endolipases and exolipases showed reduced activities. The rate of increase in the nystatin content was also diminished (i.e. 7%).

CONCLUSIONS

The exolipase activity reaches peak levels during trophophase, when the metabolism of lipids in the growth medium initiates, and then exhibits levels lower than the endolipase activity.

The endolipase activity reaches peak levels during idiophase, when the antibiotic synthesis initiates, followed by levels higher than the exolipase activity. Such levels are maintained until the end of the biosynthesis process.

The fat content in the growth medium impacts on the lipase activity and implicitly the rate of increase of the nystatin content.

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