RESEARCH STUDIES ON THE BIOSYNTHESIS CAPACITY OF *STREPTOMYCES NOURSEI* PHENOTYPES OBTAINED BY CULTIVATION ON MEDIA WITH VARIOUS CARBON SOURCES

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INTRODUCTION

The researchers from the fermentation industry direct their microbiological investigations towards the thorough study of the mechanism of antibiotics biosynthesis by different producers.

Nystatin, a macrolide antifungal antibiotic, is industrially obtained by a biosynthesis process using the microorganism *Streptomyces noursei*.

Antibiotics biosynthesis is the result of the secondary (special) metabolism action. The biosynthesis of secondary metabolites as well as the length of the biosynthesis processes depends primarily on the culture medium composition and particularly on the nature of the carbon source in the medium.

Literature data indicate the inhibitory effect of glucose in the biosynthesis of some antibiotics. Glucose and other metabolizable carbon sources adversely affect antibiotic biosynthesis, phenomenon called catabolic repression of the secondary metabolism.

The improvement of the biosynthesis capacity of the producing strains and the attaining of yields as high as possible can be carried out by the following: extensive use of the biosynthesis capacity, assuring the optimal fermentation parameters and genetic modifications.

The authors aimed at establishing the impact of some sugars such as dextrose and dextrinised corn starch on nystatin biosynthesis.

MATERIAL AND METHOD

For the investigations, a biosynthesis medium specific for nystatin production was used, containing corn starch as sugar source.

The biosynthesis medium, distributed in 500 ml Erlenmeyer flasks containing 100 ml of medium each, was inoculated with a vegetative culture of *Streptomyces noursei* obtained from a strain from the collection of the company Antibiotice S.A. Iaşi.

The culture was incubated at 28°C for 312 hours while shaking continuously. The examination was focused on establishing the effect of an approximately 30% supplementation of the corn starch quantity initially added in the biosynthesis medium as well as the effects of the aliquots of dextrose solution 40% and dextrinised corn starch 20% added throughout the fermentation run.

The impact of the different sugar sources was shown by analyzing the dynamics of the pH, biomass, reducing sugar concentration, the hourly rate of nystatin content increase (rate of growth) and the biosynthesis capacity during the fermentation run.

The following parameters were determined:

- pH: potentiometrically;
- biomass: by centrifuging;
- concentration of reducing sugar: method Schoorl;
- biosynthesis capacity: by spectrophotometry.

REZULTATS AND DISCUSSION

The results of the research studies on the influence of some sugars on the pH, biomass, reducing sugar, hourly rate of nystatin content growth and the biosynthetic capacity during the fermentation process are presented in Figures 1 – 9.

The microscopic appearance of the *Streptomyces noursei* mycelium in idiophase in the culture in which the initial quantity of corn starch was supplemented has the following characteristics: young mycelium with hyphae at the stage of development II – elements III; lax, basophil hyphae, with reduced number of pellets (Photo 1).

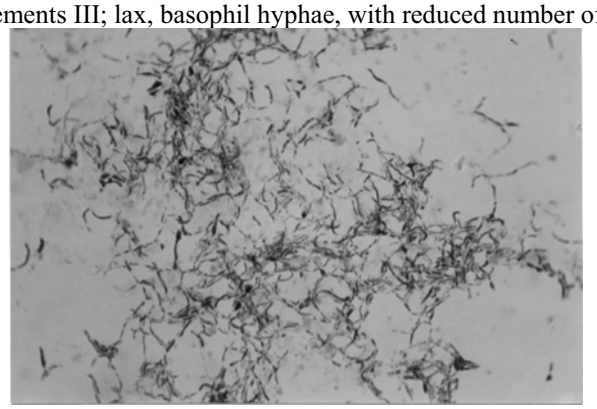

Photo 1 - *Streptomyces noursei* mycelium in idiophase in the culture obtained on biosynthesis medium with initial addition of corn starch

By comparison, the mycelium grown on the control medium exhibits the following: slightly basophil, fragmented hyphae; stage of development II – III (Photo 2).

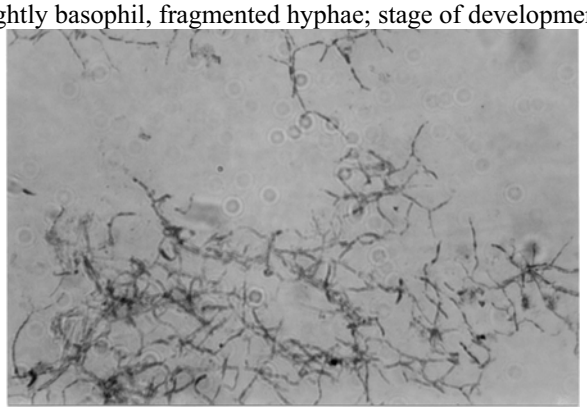

Photo 2 - *Streptomyces noursei* mycelium in idiophase in the culture obtained on control biosynthesis medium

The microscopic appearance of the *Streptomyces noursei* mycelium in idiophase, in the cultures with additions of of dextrose solution 40% and dextrinised corn starch 20% is similar and is characterized by the following: highly basophil hyphae, stage of

development II – elements III; new generations present, appearance of rejuvenated mycelium (Photo 3 and Photo 4).

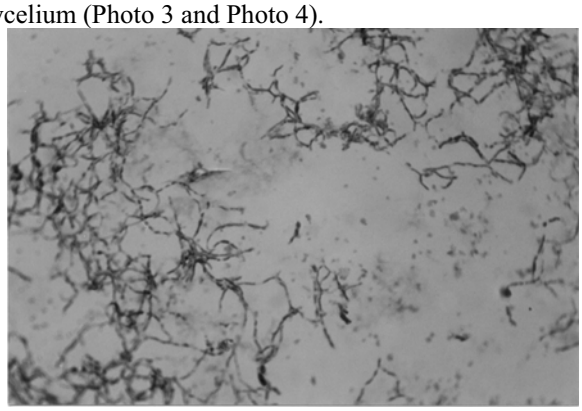

Photo 3 - *Streptomyces noursei* mycelium in idiophase in the culture obtained on biosynthesis medium with additions of dextrose solution 40%

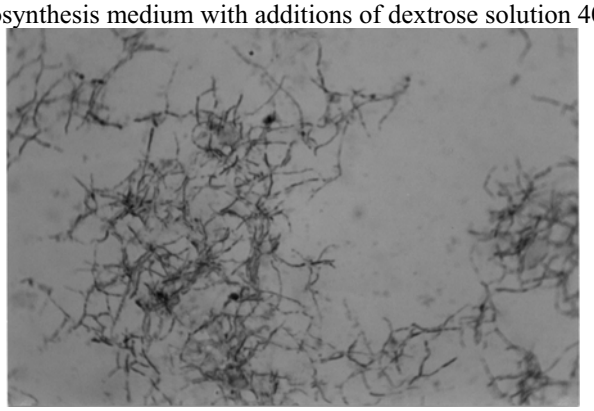

Photo 4 - *Streptomyces noursei* mycelium in idiophase in the culture obtained on biosynthesis medium with additions of dextrinised starch solution 20%

The concentration of hydrogen ions is one of the most important parameters of the biosynthesis process, because the optimal pH for the growth of the microorganism is not always the same with that for the biosynthesis of active metabolite.

The pH dynamics in the nystatin biosynthesis in the control and in the test cultures with additions of dextrinised corn starch 20% and dextrose solution 40% is in general similar, with a decrease in the pH from 7.1 (time 0) to 5.7 (96 hours of growth).

At 144 hours of fermentation an increase of the pH to 6.0 is noted in the tests with addition of dextrinised starch solution 20% and then a decrease to 5.7, maintaining at low values even at 312 hours of fermentation. The test with addition of dextrose solution 40% follows the same curve, but exhibits a series of fluctuations parallel to those of the test with dextrinised starch solution 20%. Interesting to be noted was the fact that contrary to the control sample in which the pH is 7.7 at 312 hours of cultivation, in the two test cultures examined, the pH has rather similar values: 5.9 – 6.0 (Figure 1).

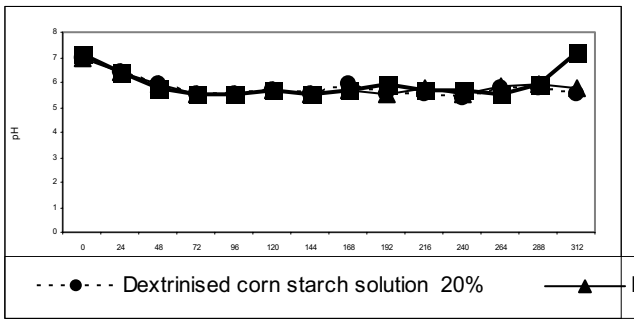

Figure 1 - pH dynamics in nystatin biosynthesis in the control culture and in the cultures with additions of dextrinised corn starch solution 20% and dextrose solution 40%

Examining the pH dynamics in the nystatin biosynthesis in the culture with supplementary concentration of corn starch (Figure 2), we may see that the values of the pH are much higher in the control (7.6 – 7.8) than in the test with addition of dextrinised starch solution (7.1 – 6.7).

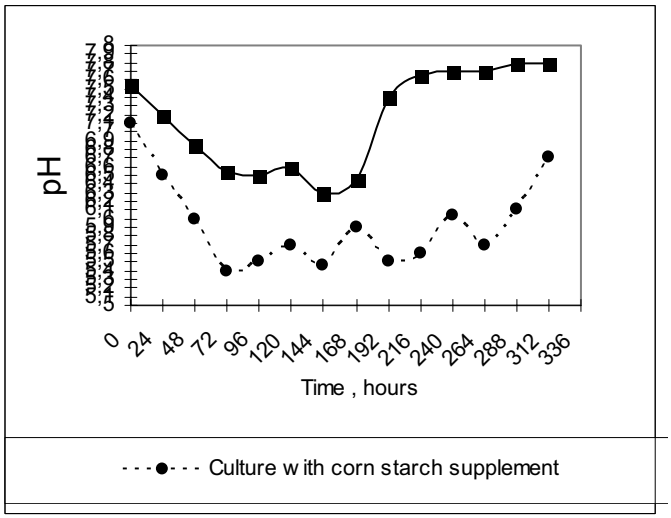

Figure 2 - pH dynamics in nystatin biosynthesis in the culture with supplement of corn starch

In this culture a significant decrease in the pH is noted, starting from the time 0 to 72 hours when the pH value reaches 5.4. Between 192 – 216 hours a decrease in the pH is also noted in the culture with corn starch supplement against the control culture which exhibits a remarkable increase in the pH in the same interval, reaching 7.7. Unlike in the control culture in which the pH maintains high after 192 hours, in the culture with corn starch supplement the pH value starts to increase towards the end of fermentation.

It must be specified that in the cultures examined, nystatin biosynthesis may be carried out using two pH ranges: 6.6 – 7.4 for the control culture and 5.4 – 6.0 for the culture with supplementary addition of corn starch.

In what concerns the dynamics of the biomass, in the control culture and in the cultures with aliquots of dextrose solution 40% and dextrinised starch solution 20%, its increase is noted starting with time 0 until 168 hours, with no significant differences between the test cultures and the control one (Figure 3). After 168 hours, the biomass in the control exhibits a slight decrease, maintaining afterwards constant until the end of fermentation (around 35%).

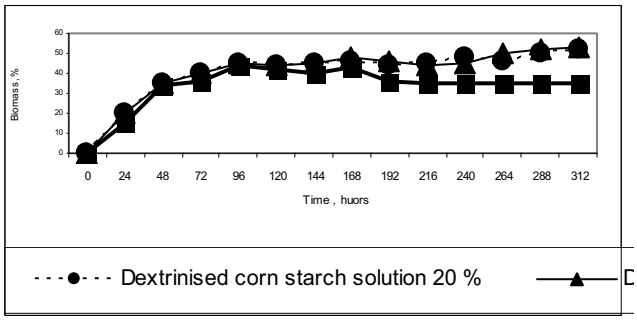

Figure 3 - Biomass dynamics in nystatin biosynthesis in the control culture and in the cultures with additions of dextrinised corn starch solution 20% and dextrose solution 40%

In the two cultures the curve rates are similar, with higher values however in the control culture (i.e. 50.5%). In the culture with corn starch supplement (Figure 4) the increase of biomass parallels the increase in the control culture during the first 144 hours of growth when the maximum value is reached, and then decreases to 30% (in the culture with supplementary addition of corn starch) and to 15% (in the control culture), maintaining constant until the end of fermentation.

The dynamic analysis (0 – 312 hours) of nystatin biosynthesis capacity (Figure 5) in the control culture and in the cultures with additions of dextrose solution 40% and dextrinised starch solution 20% shows similar curve rates, with higher values in the control cultures than in the test ones examined, except for those recorded at 72 hours when both the culture with dextrinised starch solution 20% and that with dextrose solution 40% exhibit biosynthesis capacities superior to that of the control culture. Between 72 – 192 hours the differences between the control and the test cultures are not significant in what concerns nystatin biosynthesis capacity. Starting with 192 hours till the end of the fermentation, the increase of nystatin biosynthesis is higher in the control culture than in the test ones.

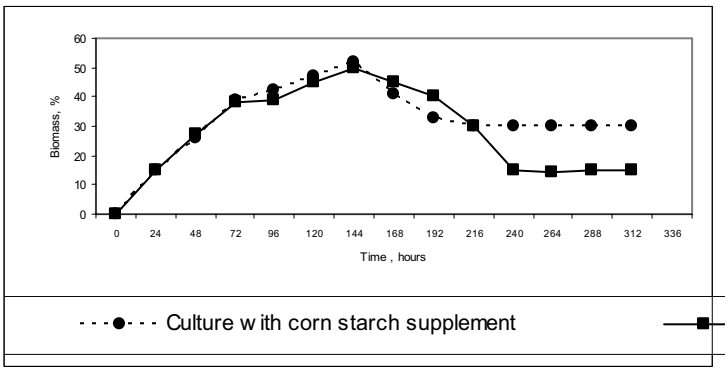

Figure 4 - Biomass dynamics in nystatin biosynthesis in the culture with corn starch supplement

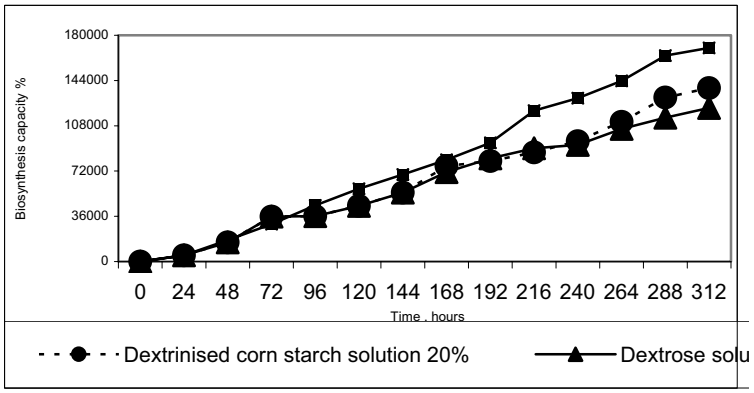

Figure 5 - Dynamics of nystatin biosynthesis capacity in the control culture and in the cultures with additions of dextrinised corn starch solution 20% and dextrose solution 40%

While in the culture with corn starch supplement nystatin biosynthesis capacity (Figure 6) is almost identical to that of the control in the first 144 hours, after 168 hours it becomes higher (100% at 312 hours).

The dynamics of reducing sugar in the cultures with additions of dextrinised starch solution 20% and dextrose solution 40% (Figure 7) during the 312 hours differs from that of the control culture. The highest values are recorded in the culture with dextrose solution 40% addition at 144 hours of growth (1.6% of reducing sugar). A decrease in the content of reducing sugar is noted at 312 hours in the control culture (0.2%) as compared to the two test cultures in which the values maintain relatively high (0.8% of reducing sugar in the culture with dextrose solution 40% and 1.5% of reducing sugar in the culture with corn starch solution 20%).

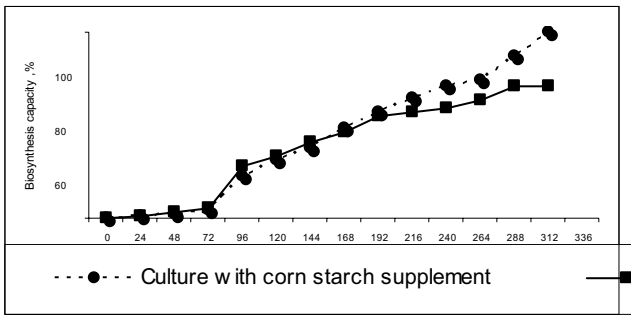

Figure 6 - Dynamics of nystatin biosynthesis capacity in the culture with corn starch supplement

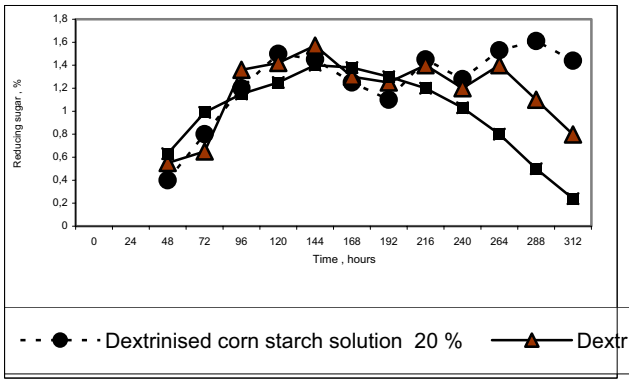

Figure 7 - Dynamics of reducing sugar in the control culture and in the cultures with additions of dextrinised corn starch solution 20% and dextrose solution 40%

In the culture with supplementary concentration of corn starch (Figure 8) there is an increase of reducing sugar as compared to the control culture, the maximum being reached in the former at 144 hours of growth (1.7% of reducing sugar as compared to 0.4% in the control culture).

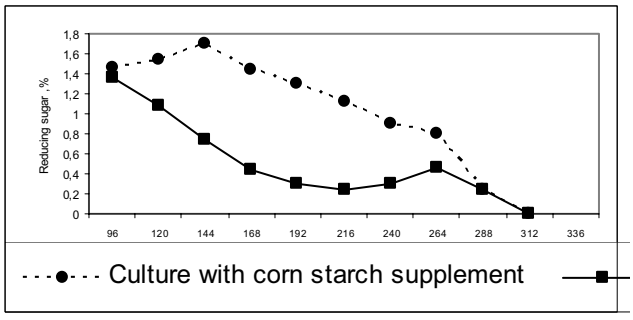

Figure 8 - Dynamics of reducing sugar in the culture with corn starch supplement

Between 96 – 192 hours, the content of reducing sugar drops from 1.4% to 0.3% in the control. In both types of cultures, the value of this parameter decreases after 264 hours of growth. Even if in the culture with corn starch supplement there is also a decrease in the reducing sugar content after 144 hours, like in the control culture, the differences related to the content of reducing sugar maintain considerable.

The hourly rate of the nystatin content increase (Figure 9) exhibits variations in the control culture and in the cultures with aliquotes of dextrinised starch solution 20% and dextrose solution 40%.

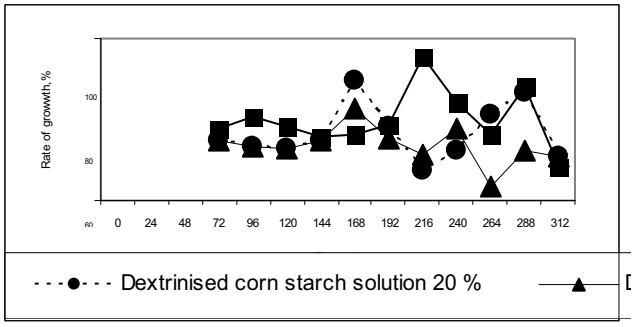

Figure 9 - Dynamics of hourly rate of nystatin content increase in the control culture and in the cultures with additions of dextrinised corn starch solution 20% and dextrose solution 40%

Unlike in the control sample, the other two test cultures examined exhibit lower increase rates. Thus, the maximum increase rate (90%) is reached in the control culture at 216 hours of growth; in the same interval, the increase recorded in the culture with dextrose solution 40% addition is 30% and in the culture with dextrinised corn starch solution 20% is 20%. Superior values of increase rate as compared to the control sample were recorded in the two test cultures at 168 hours, while the values recorded for all the three cultures were almost identical at 312 hours of growth.

The corn starch added initially in the culture medium determines a prolongation of the trophophase and the stimulation of antibiotic synthesis in the culture idiophase, resulting in an increase of the nystatin content with approximately 40%.

The additions of dextrose solution 40% and dextrinised corn starch solution 20% between 72 and 264 hours result in a biomass increase of approximately 20%, a prolongation of the trophophase, but inhibit the antibiotic synthesis in the culture idiophase which results in a decrease of the nystatin content in the biosynthesis broth of approximately 25%.

CONCLUSIONS

In nystatin biosynthesis under laboratory conditions, the addition of portions/aliquotes of glucose and dextrinised corn starch result in the stimulation of biomass formation inhibiting at the same time the antibiotic biosynthesis.

The glucose and other carbon hydrates sources such as dextrinised corn starch, being carbon sources readily metabolizable, have a negative impact on nystatin biosynthesis by the catabolic repression of the secondary metabolism.

The increase in the initial sugar concentration results in a stimulation of the antibiotic biosynthesis and a prolongation of the fermentation cycle.

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