IMPACT OF INDUCING CONTROLLED LACTIC FERMENTATION OF CORN EXTRACT ON THE PENICILLIN BIOSYNTHESIS USING THE PELLETIZED FORM OF THE *PENICILLIUM CHRYSOGENUM* STRAIN

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Keywords: *Penicillium chrysogenum*, pelletized form, lactic fermentation, corn extract, penicillin, biosynthesis.
Abstract: The lactic fermentation of the corn extract used in the process of penicillin biosynthesis is considered essential for a high quality extract. Our investigations showed that the inducing controlled lactic fermentation of the corn extract has a significant impact on the evolution of penicillin biosynthesis because it reduces the protein matter which stimulates growth of the cellular mass over the control limits, assures the preservation of the pelletized structure of the mycelium by the end of biosynthesis and increases the final penicillin biosynthesis titer by 20% at 185 hours of growth to maximum 28% at 226 hours.

INTRODUCTION

Corn extract is a by-product resulted from the process of starch extraction from corn. Lactic fermentation is stimulated from the moment the corn grains are prepared for starch extraction if the operation is conducted at 48-50ºC. At this temperature, the associated bacterial flora is inhibited, while the thermophilic lactic bacilli have optimal growth conditions, assuring by the biosynthesis of the lactic acid the preservation of the corn extract at a pH between 3.5 and 4.5 (Demain A.L., 1972). The nutrient value of the corn extract for the antibiotic-producing microorganisms as well as for the other microorganisms is due both to the protein hydrolysates and secondary metabolites resulted from the lactic fermentation process (Bhattcharge J.K., 1985).

MATERIALS AND METHODS

The biosynthesis of biologically active substances of penicillin type require the presence of nourishing compounds which promote biomass growth and the biosynthesis of amino acids, such as the α-aminoadipic acid, cysteine and valine, essential for the formation of the tripeptide δ-(L-α-aminoadipyl)-L-cysteinyl-D-valine (Aharonowitz Y., *et all.,* 1993). The synthesis route of the three amino acids – the α-aminoadipic acid, cysteine and valine – originates in the amphibolic function of the glycolysis pathway and the cycle of the tricarboxylic acids, which not only generates energy, but also supplies precursors for penicillin biosynthesis. The varied composition of the corn extract used to prepare the growth media for the penicillin biosynthesis is a crucial disadvantage. This variability detected by analytical tests conducted on the batches of corn extract depends on the corn variety, quality of the soil, type of fertilizers employed, and storage conditions. Moreover, the starch production process is not uniform due to the fact different extraction technologies are applied; such technologies may generate types of microorganisms other than lactic bacilli, thus influencing the quality of the corn extract (Del Carmen Mateos R., Sanchez S., 1990).

The growth of lactic bacteria and yeasts in the fresh corn extract improves its quality and contributes to the preservation of the corn extract by reducing the pH. The lactic acid synthesized by fermentation leads to the solubilization of the proteins, which break down into polypeptides and amino acids under the action of proteolytic enzymes, thus constituting the main source of organic nitrogen. That is why it is highly important to characterize the corn extract used in penicillin biosynthesis by the ratio of the protein content to the carbohydrates or lactic acid (Benko P.V., *et all*., 1969).

RESULTS AND DISCUSSIONS

The lactic fermentation of the corn extract used in the process of penicillin biosynthesis is considered essential for a high quality extract. The penicillin biosynthesis process using the *Penicillium chrysogenum* strain with pelletized morphological structure involved the use of a corn extract standardized by lactic fermentation induced with a mixed culture of *Bacillus delbrueckii* and *Saccharomyces cerevisiae*. The lactic fermentation was induced for 14 days, using corn extract supplied from Târgul Secuiesc, at pH 4.5 controlled by buffering with calcium hydroxide and a temperature of 48-50ºC. The results were compared to those yields by a corn extract of Italian origin and a control corn extract supplied from Târgul Secuiesc, Romania (Table 1).

Table 1. Description of the corn extract used in penicillin biosynthesis by stimulation of controlled lactic fermentation

Type of extract / Analytical test	Corn extract Source: Italy	Corn extract Source: Târgul Secuiesc – control sample	Corn extract Source: Târgul Secuiesc –subject to lactic fermentation for 14 days
Dry substance %	47	50	42.4
Aminic nitrogen %	1.70	1.98	0.95
Total nitrogen %	6.30	7.32	3.5
Total protein content %	40.6	46	22
Total sugar %	5	5.35	1.60
Reducing sugar %	2	2.46	0.90
Soluble phosphorus mg%	0.475	0.437	0.356
Lactic acid %	9.75	8.65	16.5
Content of amino acids %			
Asparagine	1.108	1.628	0.556
Treonine	0.660	0.934	0.600
Serine	0.791	1.001	0.755
Glutamic acid	3.009	3.061	0.605
Proline	1.864	1.956	1.525
Glycine	0.871	1.036	0.550
Alanine	!.682	1.739	1.540
Valine	0.912	1.086	0.880
Methionine	0.335	0.487	0.475
Isoleucine	1.445	1.680	1.240
Leucine	1.445	1.680	1.540
Tyrosine	0.949	0.545	0.232
Phenylalanine	1.088	2.447	0.885
Histidine	0.758	0.812	0.342
Lysine	0.808	0.984	0.185
Arginine	1.026	0.980	0.256

A good quality corn extract may be obtained also by constantly maintaining the storage temperature at 48-50ºC. This option yields similar results, but increases significantly the storage costs and, implicitly, the penicillin production cost.

The quality of the corn extract subjected to controlled lactic fermentation required to maintain the pelletized structure (Figure 1) and a high penicillin content during biosynthesis should meet the following criteria:

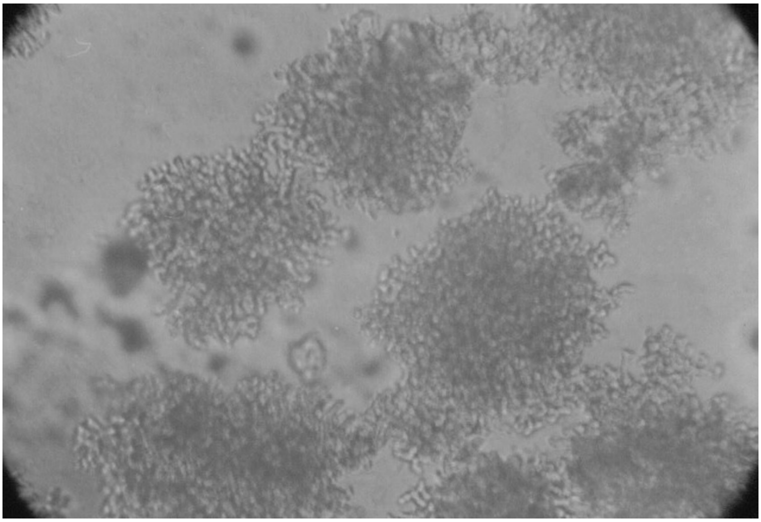

Figure 1. Microscopic appearance of the *Penicillium chrysogenum* mycelium with pelletized morphological structure cultivated on a growth medium containing corn extract subjected to controlled lactic fermentation

1. Appearance: dark yellow to light tan, slightly creamy liquid;
2. Odor: characteristic to advanced lactic fermentation;
3. Settling: 100%, after 24 hours of storage in a 100-mL graduated cylinder;
4. Microscopic appearance: the examination of stained smears shows the presence of bacterial flora specific to thermophilic lactic bacilli, 70% of which is represented by *Bacillus delbrueckii*;
5. Dry substance: not more than 45 g%;
6. pH: 3.5 – 4.5;
7. Content of lactic acid: not less than 15 g%;
8. Total sugar: not more than 2.5 g%;
9. Reducing sugar: not more than 1 g%;
10. Protein content: not more than 25 g%;
11. Aminic nitrogen: not more than 1.25 g%;
12. Soluble phosphorus: not more than 400 mg%;
13. Sulphur dioxide: not more than 0.1 g%;
14. Ash: 16 – 18 g%.

Only the corn extract meeting the above-mentioned quality requirements can be used to prepare the growth medium for the biosynthesis of penicillin using the *Penicillium chrysogenum* strain with pelletized morphological structure.

CONCLUSIONS

Inducing controlled lactic fermentation of the corn extract has a significant impact on the evolution of penicillin biosynthesis because it:

Reduces the protein matter which stimulates growth of the cellular mass over the control limits, thus disturbing the dissolved oxygen transfer at the cellular level. The loss of the glomerated arrangement of the hyphae forming a pellet is due to the anoxia generated by the increase of the biomass and viscosity of the culture broth, which ultimately leads to the death of the cells found in the core of the pellet.

Assures the preservation of the pelletized structure of the mycelium by the end of biosynthesis, eliminating the risk of pellet loosening into filamentous lax hyphae.

Enhances the concentration of lactic acid used in the energy metabolism of penicillin biosynthesis.

Decreases the content of lysine, an amino acid that exerts a negative effect on penicillin biosynthesis. The negative impact is determined by feed-back, as a result of the common biosynthesis pathway of L-lysine and penicillin starting from the α- aminoadipic acid.

Controls the evolution of the biomass and penicillin biosynthesis by addition of mineral nitrogen supplies such as the 17% solution of ammonium sulphate and ammonia water 25%.

Controls the pH at 6.5 ±1 by the addition of the 40% solution of glucose and 25% solution of ammonia water, thus preventing pH increase following the metabolism of the aminic acids in excess.

Maintains the precursors used in the biosynthesis of penicillin G (i.e. the phenylacetic acid) or penicillin V (i.e. the phenoxyacetic acid) within the upper limits, which increases the biosynthesis yield.

Increases the final penicillin biosynthesis titer by 20% at 185 hours of growth to maximum 28% at 226 hours.

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