

INFLUENCE OF SOME PHYSICO-CHEMICAL FACTORS ON THE BIOSYNTHESIS OF AMYLOLYTIC ENZYMES OF STREPTOMYCETE ORIGIN

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Modern enzyme biotechnology is a promising and rapidly developing field that requires the latest research on the conditions of enzyme biosynthesis. Optimizing the composition of the nutrient medium depending on the needs of microorganisms and physicochemical factors directly affect the increase in the efficiency of the biosynthesis of amylolytic enzymes, namely the biosynthetic capacity of the strain *Streptomyces recifensis* var. *lyticus* 2P-15. Modulation of the biosynthetic activity of strains producing amylolytic enzymes will allow to significantly increase their economic yield.

Goal. The purpose of this work was to optimize the biosynthetic capacity of the strain *Streptomyces recifensis* var. *lyticus* 2P-15 in terms of the synthesis of amylolytic enzymes and the study of the dynamics of the influence of physical and chemical factors on optimization.

Methods. The object of the study was the strain *Streptomyces recifensis* var. *lyticus* 2P-15, obtained by three-stage selection of the producer. The simplex method of selecting the composition of the environment was used for the research. The ratio of amylolytic activity to the level of biomass accumulation was taken as the biosynthetic capacity of the strain. A photocolometric method was used to determine amylolytic activity. The level of biomass accumulation was determined by the weight method.

Results. It was established that as a result of optimizing the composition of the simplex nutrient medium by the method of mathematical modeling, the biosynthetic capacity increased by 3.63 compared to the control variant. It was also investigated that the optimal concentration of such a component of the nutrient medium as monosodium glutamate $C_5H_8NO_4Na \cdot H_2O$ was 1.5%, which increased the amylolytic activity by 2.63 and increased the accumulation of biomass. Separately, it should be noted the obtained results of the study of the optimal concentrations of heavy metal ions added to the optimized version of the nutrient medium, which enable further research in this aspect to be continued and the use of Co, Mo, Cd ions in the composition of the nutrient medium. With the obtained results, there was an increase in amylolytic activity in the best response by 3.54. The obtained results have theoretical and practical significance for further research in the biotechnology of enzymes.

Conclusions. The prospect of further research into the optimization of the biosynthesis of actinomycetes by the simplex method of other aspects of its regulation will be to increase the biosynthetic capacity of the studied strain, which will have a positive effect on the economic output of the production of amylolytic enzyme preparations by obtaining microbial synthesis.

Key words: *Streptomyces recifensis* var. *lyticus* 2P-15; monosodium glutamate; heavy metal ions; amylolytic activity.

It is theoretically stated that actinomycetes of the genus *Streptomyces* belong to one of the most widely used producers of biologically active substances. The industrial implementation of the obtained results will allow to increase the output of the target product, to improve the indicators of amylolytic activity of the products that

are already produced in domestic and global industry. Optimizing the composition of nutrient media requires further research, which will allow them to be used for industrial, food or pharmaceutical purposes. An important task of biotechnological laboratories around the world is the development of scientific foundations and engineering

solutions for obtaining products that have wide prospects for practical use. One of the leading places among them belongs to enzymes of microbial origin, which are used in various branches of industry and economy. Enzyme preparations of microbial origin, which can be obtained from the studied strain, have unique properties (efficiency and specificity of action, non-toxicity, ability to work in mild conditions, to process various raw materials of plant and animal origin, including waste), in connection which makes their use in industry profitable from the economic and ecological point of view. To date, about 3,000 enzymes have been described in the scientific literature, and approximately 100 of them are used in industrial production. The industry produces more than 50 individual enzymes and twice as many enzyme preparations. Hydrolases account for the majority of enzymes entering the world market. According to scientists' forecasts, the food industry will remain the main consumer of enzymes in the near future. The functioning of the biosynthetic system is regulated by the composition of the nutrient medium and the dynamics of changes in its individual components during the growth of microorganisms. Cultivation of microorganisms-producers of enzymes is expedient to be carried out in environments of a strictly determined composition, which ensure directed biosynthesis of the required enzyme. All of this presents researchers with the task of optimizing methods of studying cultures, using modern methods and opportunities [1–3].

The main requirement for the composition of the nutrient medium is its completeness for the growth of the producer and ensuring the synthesis of the target product. Microorganisms need compounds containing carbon, nitrogenous substances, hydrogen, and oxygen. Domestic and foreign scientists in the field of enzyme biotechnology are constantly searching for new alternative components of the nutrient medium that meet the needs of microorganisms. Mineral nitrogen in the studied environment is represented by ammonium salt. Ammonium salts contain nitrogen in a reduced form, so it is easily and quickly consumed. The drug (aqueous solution of monosodium glutamate ("Synnad", China) is the sodium salt of glutamic acid $C_5H_8NO_4Na \cdot H_2O$. Glutamic acid belongs to vital amino acids, which plays the most important role in plastic, energy, and lipid metabolism. Along with other components, it plays a significant role in microelements (Fe, Cu, Zn,

Mn, Mo, Co, and others) play a role in the vital activity of microorganisms. They are part of a number of enzymes involved in metabolic processes. These elements have high catalytic activity in the process of internal metabolism. A number of authors have studied the inhibitory the influence of heavy metals on the growth and vital activity of microorganisms. Studies have shown that Cu^{2+} Cd^{2+} ions equally inhibit the growth of soil *streptomycetes*, but Co^{2+} Pb^{2+} and Zn^{2+} ions have a positive effect. In addition, Zn^{2+} Co^{2+} Pb^{2+} Fe^{2+} Cu^{2+} ions, depending on the concentration, can affect the ability of *streptomycetes* form antibiotics, pigments, lectins, vitamins [9–11].

Materials and Methods

Amylolytic enzymes of microbial origin are inducible, that is, their synthesis depends on different conditions of cultivation and the composition of the nutrient medium. The strain *Streptomyces recifensis* var. *lyticus* 2P-15 is a producer of a complex complex of bacteriological, yeast-lytic and other extracellular enzymes and glycoprotein growth stimulators. Its metabolites include amylases, glycosidases, lytic endopeptidases, muramidases, and proteases [4].

The strain *Streptomyces recifensis* var. *lyticus* 2P-15 in the process of cultivation on liquid nutrient media accumulates a complex of hydrolytic enzymes in the culture liquid. Deep cultivation of the indicated producer with the aim of obtaining amylolytic enzymes was carried out in flasks with a capacity of 250 ml, which contained 100 ml of medium for 72 hours at 28 °C. The mother medium had the following composition (per 100 ml): soy flour (GreenAgro, Ukraine) — 0.48%, $CaCl_2$ (NovoChim, Ukraine) — 0.1%, $CaCO_3$ (NovoChim, Ukraine) — 0.2%, H_2O (dist.), NH_4NO_3 (NovoChim, Ukraine) — 0.075%, K_2HPO_4 (NovoChim, Ukraine) — 0.01%, starch (Nordic) — 4.0%, and the composition of metal ions, which are known to act as inducers of synthesis processes of various groups of enzymes [4, 6].

For the optimization process, the enzyme medium with the following composition (per 50 ml) was used: soy flour (GreenAgro, Ukraine) — 0.47%, glucose — 0.5%, corn extract-1%, monosodium glutamate (aqueous solution ("Synnad", China) $C_5H_8NO_4Na \cdot H_2O$ — 0.25%, NH_4NO_3 (NovoChim, Ukraine) — 0.075%, K_2HPO_4 (NovoChim, Ukraine) — $1.6 \cdot 10^{-2}\%$, $CaCl_2$ (NovoChim, Ukraine) — 0.156%, $CaCO_3$ (NovoChim,

Ukraine) — 0.23%, H₂O (dist.), FeSO₄·7H₂O (NovoChim, Ukraine) — 7.6·10⁻³%, MnCl₂·H₂O (NovoChim, Ukraine) — 1.3·10⁻³%, MgCl₂·6H₂O (NovoChim, Ukraine) — 6.9·10⁻²%, ZnSO₄·H₂O (NovoChim, Ukraine) — 1.9·10⁻⁵%. Accumulation of amyolytic enzymes was carried out in flasks with a capacity of 250 ml for 72 hours at a temperature of 28 °C [4].

The simplex method was used to optimize the cultivation system. A simplex is a regular polyhedron with $n + 1$ vertices, where n is the number of influencing factors. The initial series of experiments corresponded to the vertices of the original simplex. The results of experiments at these points were compared among themselves, and the “worst” version of the composition of the nutrient medium was found among them. We removed this experiment from the simplex, instead we introduced a new version of the environment into the matrix. Thus, we obtained new simplexes describing the relationship between influencing factors and response functions. When the extremum of the optimality criterion was reached, further passage of the simplex ended [8].

To determine the biosynthetic capacity of the strain *Streptomyces recifensis* var. *lyticus* 2P-15 at optimal concentrations of metal ions, fractionation was carried out on Sephadex G-100 (the amount of protein applied — 10 mg). Elution was carried out with 0.02 M Na-acetate buffer, pH — 5.4; elution rate — 12 ml/h).

Determination of biomass was carried out by the weight method. The mycelium was filtered and washed with a 5% solution of TCA (trichloroacetic acid) and water, dried at 105 °C to a constant weight and expressed in mg/ml of medium and determined by the formula:

$$M = \frac{A - B}{V},$$

M — dry biomass, mg/ml; A — weight of filter with biomass, mg; B — the weight of the filter without biomass, mg; V — is the amount of culture fluid taken for filtration, ml.

Determination of amyolytic activity was carried out by the photocolometric method using a photoelectrocolorimeter (SpectroLab, Ukraine). The amount of enzyme that cleaves 10 mg of starch in 30 minutes at a temperature of 37 °C was taken as a unit of amyolytic activity of AE. Amyolytic activity was determined by the formula:

$$AO = \frac{(D_{\text{control}} - D_{\text{experiment}}) \cdot 60}{D_{\text{experiment}} \cdot 10},$$

AO — amyolytic activity, U/ml; D_{control} — optical density of the sample from the control tube; $D_{\text{experiment}}$ — optical density of the sample from the test tube; 60 — recalculation per unit of time; 10 — amount of starch, mg.

The obtained results were processed by the methods of mathematical statistics, the differences at $P < 0.05$ were considered reliable. All experiments were performed in 3 repetitions. Calculation of all indicators and construction of graphs and histograms were carried out using Microsoft Office Excel software (USA).

Results and Discussion

The analysis of changes in the influence of the composition of the medium on the growth indicators of the studied actinomycete strain in the simplex method allowed to obtain several different versions of the composition of the medium for cultivation. During the comparative analysis of different options for the cultivation environment, it was established that the optimized environment allows for more effective culture development

In the process of research on the optimization of cultivation conditions of *Streptomyces recifensis* var. *lyticus* 2P-15 in terms of amyolytic capacity, the biosynthetic capacity of the optimized version of the medium was also determined. Biosynthetic capacity is characterized by the amount of accumulated biomass in the fermentation process of the studied strain to amyolytic activity.

One of the most important factors that determines the intensity of the development of microorganisms and is reflected in all their physiological functions is the concentration of substances that are added to the environment. The swelling of the colloidal substances that contain the outer shell of the cell, the change in the permeability of the protoplasm and the entry of substances into the cell, as well as the amount of biomass that will be formed, depend on the concentration in the environment. The concentration of C₅H₈NO₄Na·H₂O affects not only the vital activity of organisms, but also the formation and activity of enzymes (Table 1).

It was determined that when sodium glutamate is added to the nutrient medium, the pH level in the nutrient medium decreases to the acidic side not significantly, and the accumulation of biomass of the *Streptomyces recifensis* var. *lyticus* 2P-15 strain increases by 27% compared to the control. It was established that the addition of monosodium glutamate C₅H₈NO₄Na·H₂O with a concentration of 1.5%

Effect of different concentrations of monosodium glutamate $C_5H_8NO_4Na \cdot H_2O$ on the biosynthetic capacity of *Streptomyces recifensis* var. *lyticus* 2p-15, during fermentation for 72 h ($n = 3$, $X \pm SD$)

Sodium glutamate concentration (%)	Amylolytic activity, U/ml $X \pm SD$	% to control	pH at the end of fermentation	Biomass, mg $X \pm SD$	% to control
Control (without adding monosodium glutamate)	7.2 ± 0.16	100	7.8	3.60 ± 0.08	100
0.5	11.3 ± 0.21	156	7.7	4.60 ± 0.09	127
1.0	11.5 ± 0.23	159	7.4	3.76 ± 0.12	104
1.5	19.0 ± 0.41	263	7.2	3.66 ± 0.14	110
2.0	13.7 ± 0.35	190	7.4	3.52 ± 0.27	98

Note. Reliability assessment of sample differences ($P < 0.05$).

is optimal for increasing the biosynthetic capacity of the producer *Streptomyces recifensis* var. *lyticus* 2P-15, after which the amylolytic activity decreases.

The composition of the nutrient medium was optimized using sodium glutamate $C_5H_8NO_4Na \cdot H_2O$ in the determined optimal concentration. As a result, a new composition of the nutrient medium was obtained, the biosynthetic capacity of which increased by 5.54. The optimized version of the medium has the following composition: soy flour — 0.58%, glucose — 0.93%, corn extract — 0.85%, monosodium glutamate $C_5H_8NO_4Na \cdot H_2O$ — 1.35%, NH_4NO_3 — 0.108%, K_2HPO_4 — 0.018%, $CaCl_2$ — 0.2%, $CaCO_3$ — 0.28%, H_2O (dist.), $FeSO_4 \cdot 7H_2O$ — $5 \cdot 10^{-3}\%$, $MnCl_2 \cdot H_2O$ — $1.5 \cdot 10^{-3}\%$, $MgCl_2 \cdot 6H_2O$ — 0.056%, $ZnSO_4 \cdot H_2O$ — $2 \cdot 10^{-5}\%$.

The influence of heavy metals on the manifestation of amylolytic activity in an optimized version of the environment was studied. When heavy metal ions are added to the environment, the synthesis of amylolytic enzymes increases slightly, compared to the control (Table 2).

It should be noted that lower concentrations of metals (0.00001 mg/ml) in all cases to some extent reduce the output of amylases to the environment. Thus, when 0.00001 mg/ml of molybdenum is added to the fermentation medium, there is a 28% decrease in amylolytic activity compared to the control. When introducing higher concentrations of 0.001 mg/ml, a sharp increase in the synthesis of amylolytic enzymes is observed. For example, the introduction of cobalt and molybdenum in the specified concentrations leads to an increase in amylase output to the medium to 55.07 and 65.62 U/ml, respectively,

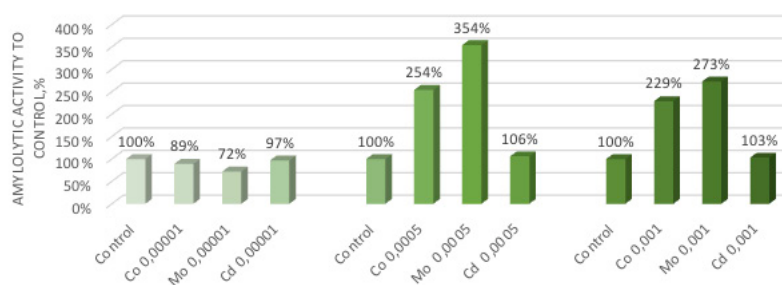
which is almost twice as high as the control (24.0 U/ml). The optimal concentration is 0.0005 mg/ml, when it is added to the medium in all cases, a significant increase in the synthesis of amylolytic enzymes is observed. Thus, with the introduction of cobalt in the specified concentration, an increase in amylase output to the environment is observed up to 229%, compared to the control, molybdenum — up to 354%, which is the highest indicator. Cadmium in the studied concentrations does not cause significant changes in the synthesis of amylolytic enzymes, as the obtained data are close to the control indicators, but with an increase in concentration, the output of amylases to the environment also increases (Figure).

As mentioned above, the studied strain produces a complex complex of different groups of enzymes. In order to reveal the heterogeneity of the enzyme complex of the culture liquid of the strain grown with the addition of molybdenum and cadmium ions to the liquid medium at a concentration of 0.0005 and 0.001 mg/ml, respectively, enzymes were separated on Sephadex G-100 (column 16 cm, the amount of protein, which was applied — 10 mg). Elution was carried out with 0.02 M Na-acetate buffer, pH 5.4; elution rate — 12 ml/h). The obtained fractions were studied for the biosynthetic capacity of the strain *Streptomyces recifensis* var. *lyticus* 2P-15. After analyzing the results, it was found that with the addition of Molybdenum ions, 11 peaks are formed, in contrast to the control, which forms 8 peaks. Peaks 1, 2, 3, 4, 5, 6, 9 and 11 are highly active. Their activity ranges from 7.393 to 15.94 ml, which is 2 times higher than the control value. Highly active peaks occupy 11 fractions, while in the control 14 fractions. Peaks 7.8 and 10

Table 2
Effect of heavy metal ions on amylolytic activity of *Streptomyces recifensis* var. *lyticus* 2p-15, during fermentation for 72 h (n = 3, X ± SD)

Concentration of metal ions. mg/ml	Amylolytic activity. U/ml X±SD	% to control
Control (without added metals)	24.0±1.5	100
Co 0.00001	21.4±1.2	89
Co 0.0005	61.0±2.8	254
Co 0.001	55.07±2.49	229
Mo 0.00001	17.46±1.8	72
Mo 0.0005	85.05±4.1	354
Mo 0.001	65.62±2.9	273
Cd 0.00001	23.29±1.4	97
Cd 0.0005	25.53±1.7	106
Cd 0.001	24.94±1.6	103

Note. Reliability assessment of sample differences ($P < 0.05$).



Effect of metal ions Co, Mo, Cd in different concentrations on the yield of amylolytic activity of the strain *Streptomyces recifensis* var. *lyticus* 2P-15

are weakly active, the activity of which is 3.589–5.798 ml, they occupy 6 fractions. Therefore, the yield of active amylases is observed in 17 fractions. Thus, the release of amylolytic enzymes occurs already from the first fractions and continues until the end of elution. The maximum value of amylase yield is observed already in the 12th fraction and is 15.94 ml, which is half the maximum of the control sample, which is observed in the 11th fraction. Fraction 28 (0.439 ml) has the lowest value, while fraction 40 (0.113 ml) has the minimum value of amylolytic activity in the control sample. Therefore, molybdenum in the specified concentration contributes to a sharp increase in the synthesis of individual enzymes.

Thus, we investigated the effect of different concentrations of monosodium glutamate $C_5H_8NO_4Na \cdot H_2O$ on the biosynthetic capacity of the strain *Streptomyces recifensis* var. *lyticus* in terms of the biosynthesis of amylolytic enzymes. It was established that

the optimal concentration of monosodium glutamate, which increases the biosynthetic capacity of the producer, is 1.5%, while the level of amylolytic activity increases by 163%. The obtained results have both theoretical and practical significance. We proposed a sodium glutamate concentration of 1.5%, when added to the fermentation medium, directed biosynthesis of enzymes with a high level of amylolytic enzymes can be conducted. Experimental data showed that heavy metal ions have different effects on the biosynthetic capacity of the strain. Both optimal and inhibiting concentrations of metals were determined. The obtained results have both theoretical and practical significance and may contribute to the production of biological preparations with certain properties.

Conclusions

The composition of the nutrient medium for the strain *Streptomyces recifensis* var.

lyticus 2P-15 complex by the method of mathematical planning of the experiment. Eight steps of optimization were carried out in three repetitions each, and as a result, a 5-fold increase in the accumulation of amylolytic enzymes was obtained.

As part of a complex study, the optimal concentration of monosodium glutamate was determined to be 1.5%, which results in a 163% increase in amylolytic activity and biomass accumulation compared to the control variant, thus increasing the biosynthetic capacity of the strain *Streptomyces recifensis* var. *lyticus* 2P-15.

The optimal concentrations of heavy metal ions, which are components of the nutrient medium and also have positive dynamics of influence on increasing amylolytic activity, and the best responses, optimal concentrations of molybdenum and cobalt increase amylolytic

activity by 254% and 154%, respectively, have been established.

Further comprehensive study of the factors affecting the biosynthetic capacity in terms of amylolytic enzymes and the search for ways to increase it will have a large economic yield and within the framework of the actively developing enzyme market will have a positive impact and further appropriate scientific research and prospects for the application of its results.

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ВПЛИВ ДЕЯКИХ ФІЗИКО-ХІМІЧНИХ ФАКТОРІВ НА БІОСИНТЕЗ АМІЛОЛІТИЧНИХ ЕНЗИМІВ СТРЕПТОМІЦЕТНОГО ПОХОДЖЕННЯ

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Сучасна біотехнологія ензимів є перспективною галуззю, яка швидко розвивається і потребує новітніх досліджень щодо умов біосинтезу ензимів. Оптимізація складу поживного середовища залежно від потреб мікроорганізмів та фізико-хімічні фактори напряму впливають на зростання ефективності біосинтезу амілолітичних ензимів, а саме на біосинтетичну спроможність штаму *Streptomyces recifensis var. lyticus 2P-15*. Модулювання біосинтетичної активності штамів продуцентів амілолітичних ензимів уможливить значно збільшити їх економічний вихід.

Мета. Метою роботи є оптимізація біосинтетичної спроможності штаму *Streptomyces recifensis var. lyticus 2P-15* за умов синтезу амілолітичних ензимів та дослідження динаміки впливу фізико-хімічних факторів на оптимізацію.

Методи. Об'єкт дослідження — штам *Streptomyces recifensis var. lyticus 2P-15*, одержаний шляхом триступіневої селекції продуценту. Для виконання досліджень було застосовано симплекс-метод добору складу середовища. За біосинтетичну спроможність штаму приймали співвідношення амілолітичної активності до рівня накопичення біомаси. Для визначення амілолітичної активності використовували фотоколометричний метод. Рівень накопичення біомаси визначали вагомим методом.

Результати. Встановлено, що в результаті оптимізації складу симплексного поживного середовища методом математичного моделювання біосинтетична ємність зросла на 3,63 порівняно з контрольним варіантом. Досліджено також, що оптимальна концентрація такого компонента живильного середовища, як глутамат натрію $C_5H_8NO_4Na \cdot H_2O$ становила 1,5%, що підвищувало амілолітичну активність на 2,63 та збільшувало накопичення біомаси. Окремо слід зазначити отримані результати дослідження оптимальних концентрацій іонів важких металів, що додавалися до оптимізованого варіанту поживного середовища, що дозволяють продовжувати дослідження в цьому аспекті і використовувати іони Co, Mo, Cd у складі поживного середовища. За отриманих результатів спостерігалось підвищення амілолітичної активності в найкращому відгуку у 3,54 рази. Отримані результати мають теоретичне і практичне значення для подальших досліджень біотехнології ензимів.

Висновки. Перспективою подальших досліджень оптимізації біосинтезу актиноміцетів симплекс-методом інших аспектів його регуляції буде підвищення біосинтетичної спроможності досліджуваного штаму, що позитивно вплине на економічну ефективність виробництва амілолітичних ензимних препаратів шляхом отримання мікробного синтезу.

Ключові слова: *Streptomyces recifensis var. lyticus 2P-15*; глутамат натрію; іони важких металів; поживне середовище; амілолітична активність; біосинтетична спроможність штаму; оптимізація умов біосинтезу.