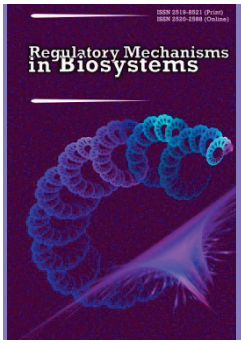

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Multi-objective optimization of nutrient medium composition using model prediction of *Streptomyces recifensis* biosynthesis process

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Secondary metabolites synthesized by the producer *Streptomyces* are widely used in the food, pharmaceutical, cosmetic, textile, and agricultural industries. These industries around the world are developing rapidly, as a result of which they need new engineering solutions that should increase the yield of the final product and optimize the production process. Understanding the dependence of the optimal correlation of the components of the nutrient medium on the increase in the biosynthesis of secondary metabolites by the producer *Streptomyces* will promote the development of these industries in economic and ecological aspects. In this study, we optimized the quantitative correlation of twelve (6+6) components of the nutrient fermentation medium for *Streptomyces recifensis* var. *lyticus* 2P-15. For optimization we used the simplex method of mathematical modeling of the optimization conditions of the biotechnological process. After optimization, a 6.36 times increase in the level of biosynthetic capacity was obtained compared to the control version of the medium. To determine the dynamics of biosynthesis, samples of culture fluid were taken. Sampling was carried out every 24 hours of cultivation, the dynamics of pH diapason, biomass accumulation in mg/ml and amylolytic activity in U/mL were determined. The correlation of amylolytic activity to the level of biomass accumulation was taken as the biosynthetic capacity of the strain. A photocolometric method based on the starch-iodine method was used to determine amylolytic activity. The volume of biomass accumulation was determined by a weighted method. As a result of the optimization, the composition of the nutrient medium was developed, in which the degree of synthesis of amylolytic enzymes increased by 6.11 times, and there was a significant increase in biomass accumulation, while the cost of the optimized medium was reduced by 1.5–2.0 times from the initial one. Positive dynamics were studied when new components were introduced into the environment, such as sodium glutamate and corn extract. Optimum concentrations of monosodium glutamate were established at 0.5% of the volume of the nutrient medium and corn extract at a concentration of 1%, respectively. The significance of the positive effect upon the introduction of heavy metal ions and some vitamins into the medium was also checked, the obtained results provide an opportunity for further research into these aspects of the composition of the nutrient medium. The advantage of biotechnological developments in matters of industrial enzymology is the opportunity to obtain not only raw materials for the pharmaceutical or other industries (where it will only acquire a final form), but also produce as a final product in a ready, convenient form. 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), in connection with which their use in industry will be profitable from the economic and ecological point of view.

Keywords: strains; secondary metabolites; vitamins; heavy metal ions; corn extract; monosodium glutamate; optimization of biosynthesis conditions; duration of cultivation.

Introduction

The global trend of "green engineering" increases with the global support of the United Nations Industrial Development Organization (UNIDO). When looking for new alternative approaches in industry, they turn to biotechnological engineering methods. 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 the economy. To date, about 3,000 enzymes have been described in the scientific literature, and approximately 100 of them are used in industrial production (Iakovlieva et al., 2022). Industry produces more than 50 individual enzymes and twice as many enzyme preparations (Effenberger et al., 2000; Gupta et al., 2003; Fariha et al., 2006). The main part of enzymes entering the world market consists of hydrolases. According to scientists' forecasts, the food industry will remain the main consumer of enzymes in the near future. According to research by Abercade Consulting, the global market for enzyme preparations increases annually by an average of 10%, the European market by approximately 3.5%. The enzyme market for the food industry in Europe is growing by 8% annually. 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 need to be carried out in environments of strictly determined composition, which ensure directed biosynthesis of the required enzyme (Feng et al., 2021). A significant amount of scientific literature is devoted to the issue of the influence of environmental components on the biosynthesis of enzymes, the optimization of nutrient media for the cultivation of various producers, including Streptomycetes, that produce a wide range of enzymes (Feng et al., 2011, Managamuri et al., 2016; Dunia et al., 2019; Souagui et al., 2019). Taking into account the specificity of enzyme biosynthesis processes, the question arises regarding the need for research on cultivation conditions and nutrient media for optimal growth of producer strains and a high level of enzyme synthesis (Patel et al., 2020). The *S. recifensis* strain, when grown on media based on soybean flour, glucose, and starch, synthesizes a lytic enzyme complex, which includes proteases, proteinases, glycosidases, amylases, and mura-

midases (Todosiychuk et al., 2008; Zhernosekova et al., 2011). It is known that lytic enzymes of microbial origin are inducible, that is, their synthesis depends on different cultivation conditions and the composition of the nutrient medium. The *S. recifensis* strain synthesizes a complex complex of bacteriolysins and growth stimulators on a medium that includes: soy flour, glucose, NH_4NO_3, K_2HPO_4 and a set of metal ions, which, as is known, act as inducers of synthesis processes of various groups (Kilichok et al., 2000; Todosiychuk et al., 2008; Zhernosekova et al., 2011). When choosing a nutrient medium for the cultivation of microorganisms, it is necessary to take into account not only the qualitative composition of the medium, but also the quantitative correlation of its components. An excessive increase in the concentration in the medium of at least one of them leads to suppression of the biosynthetic ability of the cell and slowing down its development and growth. But in some cases, the components of the environment that inhibit the accumulation of biomass can contribute to the active synthesis of secondary metabolites by the *Streptomyces* producer (Khattab et al., 2016; Feng et al., 2021).

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. Analysis of the literature revealed that monosodium glutamate is a growth activator and affects the processes of primary and secondary metabolism, and its effect depends on the concentration in the nutrient medium. However, in high concentration it becomes toxic (Chen et al., 2021). Corn extract is a source of nitrogenous substances, which make up 40-50% of the total content of dry matter in the extract. The extract contains large amounts of phosphorus, potassium and magnesium. The content of ash elements is 15–20% of the dry matter of the extract, and the phosphorus content can reach 5%. The extract contains all the elements necessary for microorganisms, group B vitamins, growth substances and bio stimulants (Abirami et al., 2021). Along with other components, microelements (Fe, Cu, Zn, Mn, Mo, Co and others) play a significant role in the vital activity of microorganisms. They are part of a number of enzymes that take part in metabolic processes. These elements have high catalytic activity in the process of internal exchange. Several authors have investigated the inhibitory effect 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, depending on the concentration, Zn^{2+}, Co^{2+}, Pb^{2+}, Fe^{2+}, Cu^{2+} ions can affect the ability of Streptomycetes to form antibiotics, pigments, lectins, vitamins (Nasser Sahmoune, 2018; Wang et al., 2019; Selvaraj et al., 2023).

The purpose of this study was to determine the effect of the main and alternative components of the nutrient medium on the biosynthesis of the enzyme complex of *S. recifensis* var. *lyticus* 2P-15 with further development of the composition of the optimized nutrient medium based on the amylolytic activity of the product.

Materials and methods

Strain *S. recifensis* var. *lyticus* 2P-15 is the object of research into ways of increasing biosynthetic capacity by optimizing the composition of the nutrient medium. Seeding was done on Chapek's liquid medium in 250 mL flasks containing 100 mL of nutrient medium for 48 hours at t = 28 ± 1 °C. Cultivation was carried out in flasks with a volume of 750 mL containing 200 mL of fermentation medium for 96 hours at t = 28 ± 1 °C. On the medium of the following composition (g/L): soybean flour (Green Agro, Ukraine) – 4.75, glucose (Yuria Farm, Ukraine) – 0.7, NH_4NO_3 (Novochim, Ukraine) – 0.75, K_2HPO_4 (Novochim, Ukraine) – 0.16, $CaCl_2$ (Novochim, Ukraine) – 1.56, $CaCO_3$ (Novochim, Ukraine) – 2.3, $FeSO_4 \cdot 7H_2O$ – $7.6 \cdot 10^{-2}$, $MnCl_2 \cdot H_2O$ – $1.3 \cdot 10^{-2}$, $MgCl_2 \cdot 6H_2O$ – 0.69, $ZnSO_4 \cdot H_2O$ – $1.9 \cdot 10^{-4}$.

The optimization was carried out by the simplex method of mathematical modeling of the experimental plan (Bezerra et al., 2016). The initial series of experiments corresponds to the vertices of the original simplex. By comparing the results of the experiments at these points, we found the worst among them from the point of view of the selected criterion of optimality, instead of it we introduced a new factor into the experiment and carried out the next optimization step. Thus, we obtain new simplexes that describe the correlation between influencing factors and the response function (Bezerra et al., 2016).

In the first stage of optimization, 6 factors were studied: X_1 – soy flour; X_2 – glucose; X_3 – corn extract; X_4 – sodium glutamate $C_5H_8NO_4Na \cdot H_2O$; X_5 – NH_4NO_3; X_6 – K_2HPO_4. The composition of microelements during the experiment was studied in the second stage of optimization. That is, under these conditions, the process took place in (6+1) – dimensional space against the background of a constant concentration of other components of the environment.

Table 1

The composition of different variants of the fermentation medium according to the content of the main components, calculated according to the coordinates of the initial simplex for the first stage of optimization (pH = 8.0, t = 28 °C, duration of cultivation 76 hours, n = 24)

Variation nutrient medium	Concentration of vital substances in the medium, %					
	X_1 – soy flour	X_2 – $C_6H_{12}O_6$	X_3 – corn extract	X_4 – $C_5H_8NO_4Na$	X_5 – NH_4NO_3	X_6 – K_2HPO_4
1	0.65	1.102	1.020	0.515	0.154	0.028
2	0.55	1.102	1.020	0.515	0.154	0.028
3	0.60	1.090	1.020	0.515	0.154	0.028
4	0.60	1.100	0.930	0.515	0.154	0.028
5	0.60	1.100	1.000	0.497	0.154	0.028
6	0.60	1.100	1.000	0.500	0.131	0.028
7	0.60	1.100	1.000	0.500	0.150	0.020
Control	0.47	0.500	0.500	0.250	0.075	0.016

Note: % composition of nutrient medium components is represented by simplex calculations within the R = (6+1) matrix.

In the second stage of optimization, 6 factors were studied: X_1 – $FeSO_4 \cdot 7H_2O$, X_2 – $MnCl_2 \cdot H_2O$, X_3 – $MgCl_2 \cdot 6H_2O$, X_4 – $ZnSO_4 \cdot H_2O$, X_5 – $CaCO_3$, X_6 – $CaCl_2$. That is, under these conditions, the process took place in (6+1)-dimensional space against the background of a constant concentration of other components of the environment. For the study, the nutrient medium of the composition obtained in the first stage of optimization was used.

Table 2

The composition of different variants of the fermentation medium according to the content of macro- and microelements, calculated according to the coordinates of the initial simplex for the second stage of optimization (pH = 8.0, t = 28 °C, duration of cultivation 76 hours, n = 24)

Variation nutrient medium	Concentration of vital substances in the medium, %					
	X_1 – $FeSO_4 \cdot 7H_2O$	X_2 – $MnCl_2 \cdot H_2O$	X_3 – $MgCl_2 \cdot 6H_2O$	X_4 – $ZnSO_4 \cdot H_2O$	X_5 – $CaCO_3$	X_6 – $CaCl_2$
1	0.0090	$1.5 \cdot 10^{-3}$	0.056	$0.2 \cdot 10^{-4}$	0.28	0.200
2	0.0050	$1.5 \cdot 10^{-3}$	0.056	$0.2 \cdot 10^{-4}$	0.28	0.200
3	0.0070	$1.5 \cdot 10^{-3}$	0.056	$0.2 \cdot 10^{-4}$	0.16	0.200
4	0.0070	$1.5 \cdot 10^{-3}$	0.056	$0.2 \cdot 10^{-4}$	0.25	0.140
5	0.0070	$1.5 \cdot 10^{-3}$	0.018	$0.2 \cdot 10^{-4}$	0.25	0.190
6	0.0070	$1.5 \cdot 10^{-3}$	0.050	$0.11 \cdot 10^{-4}$	0.25	0.190
7	0.0070	$1.2 \cdot 10^{-3}$	0.050	$0.19 \cdot 10^{-4}$	0.25	0.190
Control	0.0076	$1.3 \cdot 10^{-3}$	0.069	$0.19 \cdot 10^{-4}$	0.23	0.156

Note: Table 1.

Biosynthetic capacity of strain *S. recifensis* is expressed in the performance of the strain. The influence of one or another component of the medium on the biosynthetic capacity of the culture was assessed by the level of amylolytic activity produced by the strain-producer of the enzyme complex and the level of biomass accumulation.

Determination of biomass was carried out by the weight method. The mycelium was filtered and washed with a 5% solution of 2,2,2-trichloroethane acid (Novochim, Ukraine) and water, dried at 105 °C to constant weight and expressed in mg/mL of medium.

Amylolytic activity was determined by the photocolometric method using a photoelectrocolorimeter (SpectroLab, Ukraine) based on the starch-iodine method (Xiao et al., 2006). The amount of enzyme that breaks down 10 mg of starch in 30 minutes at a temperature of 37 °C was taken as a unit of amylolytic activity. The control and experimental sam-

ples were calibrated with a light filter (670 nm) against the content of the comparative sample. The following were used for the determination: starch solution, acetic-acetate buffer (the solution contains ethylenediaminetetraacetic acid (EDTA) (Novochim, Ukraine), 0.5 M NaCl solution (Novochim, Ukraine), HCl solution (Novochim, Ukraine), iodine reagent (Novochim, Ukraine).

Results are presented in the tables as x ± SD (mean ± standard deviation). Statistical processing was performed using ANOVA with Bonferroni correction. The statistical significance of the results where the differences were considered probable at $P < 0.05$ (taking into account Bonferroni's correction). All experiments were performed in 3 repetitions.

Results

It was established that monosodium glutamate affects not only the vital activity of the studied strain, but also the formation and activity of enzymes ($P < 0.029$). It was found that the corn extract, in turn, in addition to being an additional source of nutrients for biosynthesis, also affects the increase in the biosynthetic capacity ($P < 0.035$) of the strain in terms of the synthesis of amylolytic enzymes. As a result of the conducted experiment, a linear dependence of the amylolytic activity increase on the concentration of corn extract and monosodium glutamate was revealed. As can be seen from the results, the addition of monosodium glutamate and corn extract in all samples brings an increase in amylolytic activity in the range of 155.4–316.8% compared to the control medium. The optimal concentration of monosodium glutamate is 0.5% ($P < 0.029$), after which the amylolytic activity decreases. Regarding the dependence of the concentration of corn extract on amylolytic activity, the optimal value is 1%, higher concentrations of increased amylolytic activity are not as significant as with a concentration of 1% ($P < 0.035$). Therefore, in the process of work, the stimulating effect of corn extract and monosodium glutamate, which were added to the medium during cultivation, was established. Taking this into account, these components were used in the composition of the experimental nutrient media, and in the control variants, the basic nutrient media listed above. After studying the dynamics of the influence of the above-mentioned components, we carried out complex optimization of the composition of the nutrient medium using the optimal concentrations determined in the composition of the nutrient medium.

Table 3

The influence of some components of the nutrient medium in different concentrations on the yield of amylolytic activity of the strain *S. recifensis* (duration 72 hours, n = 3)

Concentration of components nutrient medium, %	Sodium glutamate				Corn extract			
	amylolytic activity, U/mL, x ± SD	correlation of amylolytic activity to control, %	biomass, mg/mL, x ± SD	correlation of biomass to control, %	amylolytic activity, U/mL, x ± SD	correlation of amylolytic activity to control, %	biomass, mg/mL, x ± SD	correlation of biomass to control, %
0.0	7.27 ± 0.16^{a}	100.0	3.60 ± 0.08^{a}	100.0	7.27 ± 0.16^{a}	100.0	3.60 ± 0.08^{a}	100.0
0.5	19.03 ± 0.41^{c}	261.8	4.60 ± 0.09^{b}	127.8	15.23 ± 0.39^{ab}	209.5	4.11 ± 0.11^{b}	114.2
1.0	13.71 ± 0.35^{ab}	188.6	3.76 ± 0.12^{a}	104.4	23.02 ± 0.53^{c}	316.8	5.68 ± 0.06^{c}	157.8
1.5	11.54 ± 0.23^{b}	158.7	3.66 ± 0.14^{a}	101.7	16.77 ± 0.44^{ab}	230.7	4.89 ± 0.13^{ab}	135.8
2.0	11.31 ± 0.21^{b}	155.4	3.52 ± 0.27^{a}	97.8	13.89 ± 0.36^{b}	191.0	3.93 ± 0.09^{a}	109.2

Note: different letters indicate data that are significantly different from each other within one column of the table according to the results, the reliability of the differences was checked out using ANOVA with Bonferroni correction; amylolytic activity – units/mL.

In order to find ways to further increase the amylolytic activity of the *S. recifensis* strain, we tested vitamins which are known to stimulate the growth and synthesis of enzymes.

Table 4

Dynamics of the effect of vitamins on the biosynthesis of amylolytic enzymes by strain *S. recifensis* (duration 72 hours, n = 3)

No.	Vitamins	Concentration of vitamins in the nutrient medium, μg/L	Amylolytic activity U/mL, x ± SD	Correlation of amylolytic activity to control, %
1	Control	0.00	7.52 ± 0.09^{a}	100.0
2	Biotin (B_7)	1.00	8.27 ± 0.10^{b}	109.9
3	Folate (B_9)	0.05	9.33 ± 0.13^{b}	124.1
4	Thiamine (B_1)	0.10	10.12 ± 0.12^{ab}	134.6
5	Cyanocobalamin (B_{12})	0.20	7.91 ± 0.09^{a}	105.2
6	Pantothenic acid (B_5)	2.00	11.46 ± 0.11^{c}	152.4
7	Riboflavin (B_2)	10.0	15.74 ± 0.14^{d}	209.3

Note: Table 3.

The effect of some vitamins was studied and the best results were obtained with the addition of thiamine, pantothenic acid, and riboflavin. In the best responses, there was an increase in amylolytic activity by 109.3% ($P < 0.021$) compared to the control. This may indicate that the fermentation medium is not optimized in terms of quantitative and qualitative composition and requires further experimental studies aimed at increasing the level of biosynthesis of amylolytic enzymes, which will make it possible to expand the scope of their application in various branches of the economy.

The influence of heavy metals on the manifestation of the amylolytic activity of the indicated strain was investigated. When heavy metal ions were added to the environment, the synthesis of amylolytic enzymes increased slightly, compared to the control.

It should be noted that lower concentrations of metals (0.00001 mg/mL) in all cases to some extent reduced the output of amylases to the medium. Thus, when 0.00001 mg/mL of molybdenum was added to the fermentation medium, there was a decrease in amylolytic activity by 28% compared to the control. When applying higher concentrations of 0.001 mg/mL, a sharp increase in the synthesis of amylolytic enzymes was observed ($P < 0.038$). For example, the introduction of cobalt and molybdenum in the specified concentrations led to an increase in amylase output to the medium to 55.07 and 65.62 U/mL ($P < 0.038$), respectively, which is almost twice as high as the control (24.00 U/mL). The optimal concentration was 0.0005 mg/mL. When it was added to the medium in all cases, a significant increase in the synthesis of amylolytic enzymes was observed. Thus, with the introduction of cobalt in the specified concentration, an increase in amylase output to the medium of up to 229.5% was observed, compared to the control, molybdenum – up to 354.4% ($P < 0.038$), which is the highest indicator. Cadmium in the studied concentrations did 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 increased.

Table 5

The effect of heavy metal ions on the amylolytic activity of the *S. recifensis* strain (duration 72 hours, n = 3)

Concentration of metal ions, mg/mL	Amylolytic activity, U/mL, x ± SD	Correlation of amylolytic activity to control, %
Control (without added metals)	24.0 ± 1.5^{a}	100.0
Co $1 \cdot 10^{-5}$	21.4 ± 1.2^{a}	89.2
Co $5 \cdot 10^{-4}$	61.0 ± 2.8^{b}	254.3
Co $1 \cdot 10^{-3}$	55.1 ± 2.4^{b}	229.4
Mo $1 \cdot 10^{-5}$	17.5 ± 1.8^{ab}	72.7
Mo $5 \cdot 10^{-4}$	85.1 ± 4.1^{c}	354.4
Mo $1 \cdot 10^{-3}$	65.6 ± 2.9^{b}	273.4
Cd $1 \cdot 10^{-5}$	23.3 ± 1.4^{a}	97.0
Cd $5 \cdot 10^{-4}$	25.5 ± 1.7^{a}	106.4
Cd $1 \cdot 10^{-3}$	24.9 ± 1.6^{a}	103.9

Note: Table 3.

In order to determine the optimal extension of the cultivation of the *S. recifensis* strain under the specified fermentation conditions, namely the

initial level of pH – 8.0, t = 28 °C, sampling was carried out every 24 hours of cultivation. The pH change, biomass accumulation in mg/mL and amylolytic activity in U/mL were determined. It was determined that the maximum accumulation of biomass occurred at 48–60 hours of cultivation, and the maximum biosynthesis of amylolytic enzymes at 76 hours. Therefore, the length of cultivation during the experiment was equal to 76 hours. The dynamics of changes in the pH level in the control and researched variants of the medium during the fermentation process were also investigated. According to the results of the experiment, it is possible to draw conclusions about the variation of pH in the range of 8.0–7.3.

The nutrient medium was optimized for the content of sources of carbon, nitrogen and phosphorus nutrition, as well as for the content of trace elements. The study was conducted in two stages, taking into account the large number of investigated factors influencing biosynthetic capacity. With the calculated plan of the initial simplex, 8 optimization steps were carried out. The results of the conducted experiments show that already at the third step of variation, a stationary phase of cultivation was reached, because the 4th, 5th and 6th steps of variation did not give results higher than the 3rd step. As a result of the conducted research, amylolytic activity increased by 20.8–393.1%, which indicates a significant effect of optimizing the composition of the nutrient medium on the synthesis of amylolytic enzymes. As a result of the optimization process, the best response was obtained (Table 6). For comparison, the control composition of the medium and the composition of the optimized medium with the maximum value of amylolytic activity after optimization were assessed. Amylolytic activity increased from 7.6 to 39.9 U/mL, that is, there was an increase by +32.3 U/mL ($P < 0.021$).

Table 6
Quantitative composition of the fermentation medium after the first stage of optimization (based on the content of the main components)

Component	Quantity in control nutrient medium, %	Quantity after optimization, %	Composition difference, %
Soy flour	0.470	0.580	+0.11
Glucose	0.500	0.930	+0.43
Corn extract	1.000	0.850	–0.15
Sodium glutamate	0.250	0.453	+0.20
Ammonium nitrate	0.075	0.108	$+3.3 \cdot 10^{2}$
Dipotassium phosphate	0.016	0.018	$+2.0 \cdot 10^{3}$

With the calculated plan of the initial simplex, 8 optimization steps were carried out. The results of the conducted experiments show that already at the 2nd step of variation, a stationary phase of cultivation was reached, because the 3rd, 4th and 5th steps of variation did not give more than the 2nd step result. The results of the conducted experiments indicate that the change in the ratio of ions of mineral nutrition in most variants of the media did not significantly affect the amylolytic activity of the studied strain, which ranged from 83.7% to 128.1% compared to the control. Amylolytic activity increased from 36.3 to 46.46 U/mL, that is, there was an increase of +10.16 U/mL ($P < 0.027$).

Table 7
Quantitative composition of the nutrient medium after the second stage of optimization (by the content of macro- and microelements)

Component	Quantity in control nutrient medium, %	Quantity after optimization, %	Composition difference, %
$FeSO_4 \cdot 7H_2O$	0.008	0.005	$-2.6 \cdot 10^{-3}$
$MnCl_2 \cdot H_2O$	$1.3 \cdot 10^{-3}$	$1.5 \cdot 10^{-3}$	$+2 \cdot 10^{-4}$
$MgCl_2 \cdot 6H_2O$	0.069	0.056	–0.013
$ZnSO_4 \cdot H_2O$	$1.9 \cdot 10^{-5}$	$2.0 \cdot 10^{-5}$	$+1 \cdot 10^{-6}$
$CaCO_3$	0.230	0.28	+0.050
$CaCl_2$	0.156	0.20	+0.044

As a result of the optimization of the composition of macro- and microelements, it was determined that the content of these components led to an increase in the biosynthetic capacity of the studied strain, but due to very low concentrations in the composition, it did not have a significant effect on amylolytic activity. There is a positive dynamic, but it does not have such an influence on the biosynthetic capacity as the main components of the nutrient medium.

Discussion

The latest research in recent years is devoted to the prospects of practical use of *Streptomyces* producer as a synthesis of antibiotic enzymes, growth stimulants for plant and animal husbandry. *Streptomyces* has its main industrial application in the production of antibiotics. However, several studies have also reported that *Streptomyces* spp. are promising for agriculture. Several strains of *Streptomyces* can promote plant growth and biocontrol of pests, diseases, weeds, and phytopathogenic microorganisms by producing phytohormones, siderophores, enzymes, volatile organic compounds, antibiotics, and other secondary metabolites. In addition, *Streptomyces* spp. can increase nutrient bioavailability and mitigate abiotic stresses such as salinity, drought, and organic and inorganic contaminants in soil (Nazari et al., 2023). In recent years, the optimization of the composition of the nutrient medium for *Streptomyces* has been actively researched and discussed in order to obtain secondary metabolites synthesized by this producer. The benefits associated with various compounds produced by *Streptomyces* are reported, with particular emphasis on the production of exopolysaccharides, antibiotics and other secondary metabolites and the potential innovative use of *Streptomyces* spp. as probiotics (Cuozzo et al., 2023). Most often, mathematical and statistical models of process forecasting are used to optimize the composition of the nutrient medium (Selvaraj et al., 2023). Optimal components for the synthesis of planned metabolites are selected. It is reported that for *Streptomyces* synthesis of antibiotics it is best to use as components starch, urea, $MgSO_4$ and NaCl in optimal concentrations (Selvaraj et al., 2023). The search for optimal conditions for the greatest synthesis of *Streptomyces* secondary metabolites has been the goal of many studies in recent years. Factors such as pH and temperature of the culture medium are reported to significantly affect the synthesis of secondary metabolites. In studies Al-Ansari et al. (2020) it was established that the production of secondary metabolites was maximal at pH 7.5, and a slight decrease in the production of antibiotics was observed at pH 8.0. A high production of antibiotics was found at 38 °C, and the zone of inhibition was 32 °C. Among the tested carbon sources, the addition of glucose and starch enhanced antibiotic production. Among proven nitrogen sources, sodium nitrate enhanced antibiotic production. The basis of the current study was the establishment of the effect of individual components of the environment on the biosynthetic capacity of the producer and their optimal ratio, which makes it possible to increase the yield of the product and generally increase the efficiency of the biotechnological process of its production. A similar study is presented (Feng et al., 2011; Managamuri et al., 2016; Al Farraj et al., 2019; Souagui et al., 2019) in the optimization of the conditions of biosynthesis of an antimicrobial compound from *Streptomyces* sp. by changing the incubation period, temperature, pH, salinity, carbon sources and nitrogen sources through OVAT. In the current work, it was also found that the ability of *Streptomyces* sp. CMSTAAHL-3 to produce an antibiotic is maximal under such conditions as pH = 7, temperature 30 °C, incubation period of 7 days, nitrogen source was urea, mineral source was $MgSO_4$, –3% NaCl starch was the main component of the environment and a source of carbon.

Wang et al. (2011, 2017), Srivastava et al. (2018), Al Farraj et al. (2019) found that antibiotic production was enhanced when starch was used as a carbon source. In this study, in addition, during the OVAT study, starch enhanced the synthesis of antibiotics, which was confirmed by optimization studies. Complex carbon sources, such as polysaccharides, such as starch, are slowly absorbed and often induce the synthesis of secondary metabolites. Al Farraj et al. (2019), determined the optimal temperature (t = 30 °C) for the antibiotic activity of *Streptomyces* sp. Biosynthesis of secondary metabolites (antibiotics) and cellular metabolism in *Streptomyces* sp. are under the influence of pH. This study found that a pH level close to neutral is ideal for maximal metabolite synthesis (pH = 7).

Grigorieva et al. (2008) investigated the effect of the main and alternative components of food products on the biosynthetic activity of the lysozyme complex producer strain *Streptomyces recifensis* var. *lyticus* 2435/M. On the basis of the previously obtained results, as an alternative source of the main food, soybean flour specially treated with IR irradiation was used, and as an additional component, A-300 aerosol. As a result of the optimization, we established the concentration of the components of the research medium and the composition of the fermentation nutrient

medium for the biosynthesis of the lysozyme complex by the strain *Streptomyces recifensis* var. *lyticus* 2435/M, which led to increase in the level of synthesis of the target product by an average of 15%. Cultivation of the producer on the nutrient media proposed as a result of the optimization results will increase the yield of the product while simultaneously reducing the cost of the nutrient medium and the process of its preparation.

Conclusions

A comprehensive assessment of the composition of the nutrient medium for the *S. recifensis* strain was carried out. The dynamics of the impact of new variations on some of the main components of the nutrient environment were studied. An increase in the biosynthetic capacity of *S. recifensis* in terms of the synthesis of amylolytic enzymes of corn extract as part of the nutrient medium was established. As a result, there was an increase in the productivity of the strain by 3.64 ($P < 0.035$), which was expressed in increased amylolytic activity and biomass accumulation. It was established that the optimal concentration is 1%, higher concentrations did not have such a high result. A study of the dynamics of the influence of monosodium glutamate in the nutritional medium was also conducted. The optimum concentration is set at 0.5% ($P < 0.029$), at this concentration there was an increase in the productivity of the strain in terms of biomass, an increase of +4.60 mg and an increase in amylolytic activity by +263.9% ($P < 0.029$), compared to the control sample. The dynamics of the influence of vitamins on the biosynthetic capacity were studied and as a result, it was established that, of the studied vitamins, all of them increased the volume of accumulation of streptomycete biomass, but the largest amount of biomass at the end of the process was accumulated when folic acid and riboflavin were added to the fermentation optimized medium in concentrations of Folate (B_9) –0.05 μg/L and Riboflavin (B_2) – 10.00 μg/L. The best indicators for increasing amylolytic activity are Pantothenic acid (B_5) at a concentration of 2.0 μg/L and Riboflavin (B_2) at a concentration of 10.00 μg/L. The next step of the study was the study of the dynamics of the influence of heavy metal ions Co, Mo, Cd in different concentrations (0.00001, 0.0005, 0.0010 mg/mL), respectively. It was established that the ions of heavy metals Co, Mo, and Cd helped to increase both the quantitative and acidic composition of enzymes synthesized by the *S. recifensis* strain. Adding Co, Mo, Cd in concentrations of 0.00001 and 0.00050 mg/mL inhibited the growth of biomass, but increased the protein content by 13.2–57.1% ($P < 0.038$), which indicates the use of catabolic energy for the synthesis of protein compounds. The best response in terms of the synthesis of amylolytic enzymes is Mo at a concentration of 0.0005 mg/mL, increasing the amylolytic activity by 354.4% compared to the control. The amylolytic activity increased by 3.54 times from 24.00 to 85.05 U/mL ($P < 0.038$). Taking into account the above research results, the composition of the nutrient medium was optimized using the simplex method of mathematical modeling. As a result, a new optimized composition of the nutrient medium was obtained, which significantly increased the biosynthetic capacity of *S. recifensis*. The optimization was carried out in two steps according to the composition of the main components and the composition of macro- and microelements. Ten steps of optimization (8+2) were carried out in three repetitions each, and as a result of the optimization, a significant increase in amylolytic activity by 6.46 times was obtained, as well as an increase in biomass accumulation, which indicates an increase in the productivity of the strain *S. recifensis* 2P-15. During the optimization process, the optimal mode of cultivation under the specified fermentation conditions (pH = 8.0, t = 28 ºC) was determined to be 76 hours. The determined concentrations of the components of the studied environments and the composition of the fermentation nutrient medium for the biosynthesis of the lysozyme complex by the *S. recifensis* strain make it possible to increase the amount of synthesis of the target product. In this way, the research results contribute to increasing the yield of the product and the efficiency of the biotechnological process of its production. In the future, it will be advisable to use an optimized nutrient medium at the stage of biosynthesis in technologies for obtaining enzyme preparations. The use of an optimized environment in the industrial biotechnology of obtaining enzyme preparations will have economic feasibility, especially in the conditions of the development of domestic industrial biotechnology.

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