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Original scientific paper

Optimization of cultivation medium composition for production of bioactive compounds effective against *Penicillium* sp.

Zorana Rončević, Ivana Pajčin*, Dragoljub Cvetković, Siniša Dodić, Jovana Grahovac and Jelena Dodić

University of Novi Sad, Faculty of Technology Novi Sad, Bulevar Cara Lazara 1, 21000 Novi Sad, Serbia
**Corresponding author: paj@tf.uns.ac.rs*

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SUMMARY

Biological control is one of the best alternatives to pesticides as it avoids their weak points in plant disease control. In this study, the composition of cultivation medium for production of bioactive compounds by *Bacillus subtilis* ATCC 6633 was optimized. The produced bioactive compounds were tested against a phytopathogenic *Penicillium* sp. known for infesting different agricultural products and causing substantial crop losses. Antimicrobial activity assaying was carried out using the diffusion-disc method, and inhibition zone diameters were measured as direct indicators of antifungal activity. The response surface methodology (RSM) was used to evaluate the effects of different contents of initial nutrients (glycerol, $NaNO_2$ and K_2HPO_4) in cultivation medium on inhibition zone diameter. Optimization was carried out using the desirability function method in order to maximize bioactive compounds yield and to minimize residual nutrients contents. The optimized concentrations of the selected nutrients in cultivation medium for production of bioactive compounds were: glycerol 20 g/l, $NaNO_2$ 1 g/l and K_2HPO_4 15 g/l.

Keywords: Bioactive compounds; *Bacillus subtilis*; *Penicillium* sp.; Antimicrobial activity

INTRODUCTION

Different phytopathogens infect fruit and vegetables during their growth, harvest, transport or storage. Phytopathogens cause economic losses in terms of yield decrease, and raise human health concerns due to harmful phytopathogen metabolites that remain in fresh fruit and vegetables, as well as in different products obtained by fruits and vegetables processing. One of common fruit and vegetable phytopathogens is the genus *Penicillium*, known for infesting citrus fruits (Nunes et al., 2009), apples (Quagliaa et al., 2011), tomato (Kalyoncu et al., 2005) and other agricultural crops. Crop losses due to diseases caused by *Penicillium* spp. reach up to 50% of crop yield (Mari et al., 2002). Synthetic pesticides are still the most common agents for treatment or prevention of fruit and vegetables diseases (Spadaro & Lodovica Gullino, 2004). Commercial pesticide formulations used

for controlling *Penicillium* plant diseases contain active substances such as thiabenzadole, thiophanate-methyl, pyrimethanil and iprodione (Quagliaa et al., 2011). Along with their high cost, environmental pollution and negative effects on autochthonous organisms in soil, concerns have recently emerged about their insufficiently examined effects on human health (Janisiewicz & Korsten, 2002; Grahovac et al., 2009). Phytopathogens can also develop resistance to these synthetic agents after a period of repeated applications (Joshi et al., 2008), creating a need either for higher concentrations of pesticides or a different strategy of phytopathogen control. All of these listed reasons suggest a need for new methods to be found for fruit and vegetable disease control. One of such methods, avoiding the disadvantages of pesticides, is biological control, which consists of using different antagonistic microorganisms and their metabolites for plant disease control (Droby et al., 2009). The mechanisms by which microbial antagonists suppress plant diseases can be different, and the most common mechanisms include the production of bioactive antimicrobial compounds and competition for nutrients and space (Sharma et al., 2009). Generally, the market share of biopesticides is approximately $2-3 billion, compared to the synthetic pesticides market share of $56 billion, and its projected annual growth rate is more than 15%. Microbial biopesticides account for approximately 85% of biopesticides market. There are several commercial products that can be used for biological control of *Penicillium* spp.: Biosave™ 10LP and Biosave™ 11LP (JET Harvest Solution, Longwood, FL, USA), Serenade™ (AgraQuest, Davis, CA, USA), YeldPlus™ (Anchor Yeast, Cape Town, South Africa), Shemer™ (Bayer CropScience, AG), etc. (Quagliaa et al., 2011).

Soil bacteria of the *Bacillus* genus are well known and have been widely studied as possible agents for biological control of different plant diseases caused by microbial pathogens. *Bacillus subtilis* is one of the most commonly used biocontrol agents with proven antagonistic effect against various phytopathogens (Gisi et al., 2009). Some of these antagonistic microorganisms are naturally present on fruits and vegetables infected by phytopathogens or in soil, but these microorganisms are not normally able to produce sufficient amounts of antimicrobial compounds to suppress pathogen growth. Therefore, after isolating these antagonistic microorganisms they can be used for large-scale bioactive compounds biosynthesis by employing an appropriate production medium under defined conditions.

Cultivation medium composition has great impact on the biomass growth and type and yield of synthesized metabolites (Ibrahim & Elkhidir, 2011), as well as overall process cost. Consequently, optimization of medium composition according to specific productive microorganism nutrition requirements is a critical factor for economically effective production of functional bioactive formulations (Managamuri et al., 2016). Optimization of medium composition, i.e. appropriate selection of nutrient sources (mostly of carbon, nitrogen and phosphorus) and precise defining of their concentrations, is the main method of directing metabolic activity of productive microorganisms towards biomass growth or synthesis of metabolites with antimicrobial activity (Sanchez & Demain, 2002). It is also important to optimize medium composition in terms of nutrient quantities that remain in cultivation broth after the biosynthesis in order to reduce the cost of effluents treatment and environmental pollution (Rončević et al., 2014).

According to literature data, glycerol is a very good carbon source for the biosynthesis of antimicrobial compounds by *B. subtilis* (El-Bana, 2005). Furthermore, as a consequence of increased biodiesel production, a request has emerged in recent years for investigating possible applications of waste glycerol as a carbon source for different microbial bioconversions (Li et al., 2013). Waste glycerol utilization in bioprocesses that result in obtaining value-added products, e.g. antimicrobial compounds, is a good way to reduce production costs and prevent waste glycerol disposal in the environment (Yang et al., 2012). Regarding nitrogen and phosphorus sources, nitrites and phosphates have been shown as appropriate for biosynthesis of antimicrobial compounds by *B. subtilis* (El-Banna & Quddoumi, 2007).

The aim of this study was to optimize the cultivation medium composition regarding glycerol, sodium nitrite and phosphate contents for the production of bioactive compounds with antifungal activity against *Penicillium* sp., using the response surface methodology (RSM) and desirability function method. Biosynthesis of bioactive compounds was carried out by *Bacillus subtilis* ATCC 6633.

MATERIALS AND METHODS

Microorganisms

In this study, *B. subtilis* ATCC 6633 was used as a productive microorganism for the biosynthesis of antifungal compounds which were tested against

a *Penicillium* sp. isolated from the environment. Both microorganisms were stored at 4°C and subcultured at four-weeks interval.

Cultivation media

Inoculum was prepared by using nutrient broth (Torlak, Serbia), and biosynthesis of antimicrobial compounds was performed in media prepared according to the chosen experimental design. The selected nutrients were added to the media at varied concentrations (g/l): glycerol (20, 35, 50), $NaNO_2$ (1, 2, 3) and K_2HPO_4 (5, 10, 15). The media used for biosynthesis also contained (g/l): yeast extract (0.5), $CaCO_3$ (17.0), $MgSO_4 \cdot 7H_2O$ (0.5) and $MnSO_4 \cdot 4H_2O$ (0.05), and their pH was adjusted to 7.0 prior to sterilization performed by autoclaving at 121°C and under pressure of 2.1 bar for 20 min.

Inoculum preparation and biosynthesis conditions

Inoculation was performed by adding 10% (v/v) of inoculum, prepared under aerobic conditions at 28°C over 48 h by mixing on a laboratory shaker (Ika® Werke IKA® KS 4000i control, Germany) at 150 rpm. The production of bioactive compounds with antifungal activity was performed in Erlenmeyer flasks (300 ml) containing 100 ml of appropriate medium according to the experimental design. The biosynthesis of antifungal compounds was carried out under aerobic conditions at the temperature of 28°C and agitation rate of 150 rpm on the laboratory shaker for 96 h.

Analytical methods

In vitro assaying for antifungal activity check

Production of bioactive compounds was estimated *in vitro* by the diffusion-disc method (Bauer et al., 1966) and expressed as antifungal activity against the test microorganism presented by inhibition zone diameter (mm). Cultivation broth samples used in each experiment were concentrated by evaporation on a rotary vacuum evaporator (MRC ROVA-100, Israel) to one tenth of their initial mass and then their antifungal activities were tested against the test microorganism. *Penicillium* sp. was grown on a commercial medium (Sabouraud maltose agar, Himedia, India) at 28°C and inhibition zone diameters were measured after 48 h.

Determination of residual nutrients contents in cultivation media samples

After the end of biosynthesis, samples of cultivation media were centrifuged at 10000 rpm for 15 min (Eppendorf Centrifuge 5804, Germany). Only the liquid phase of cultivation media was used in further examination. The obtained supernatants were filtered through a 0.45 μm nylon membrane (Agilent Technologies, Germany) and filtrates were analyzed by the HPLC (Thermo Scientific Dionex UltiMate 3000 series, California, USA) to determine residual glycerol content. The HPLC instrument was equipped with an HPG-3200SD/RS pump, WPS-3000(T)SL autosampler (10 μl injection loop), ZORBAX NH_2 (250 mm x 4.6 mm, 5 μm) column (Agilent Technologies, Germany), and a refractive index detector (ERC RefractoMax520, Germany). Acetonitrile (70%, v/v) was used as eluent at a flow rate of 1.0 ml/min and elution time of 20 min at the column temperature of 30°C. The Kjeldahl method (Herlich, 1990) was used for determining the total nitrogen residual content, while the residual content of total phosphorus was determined by spectrophotometric analysis (Gales et al., 1966).

Experimental design and optimization by RSM

Experiments were carried out according to the Box-Behnken experimental design with three factors at three levels and three repetitions at the central point, as presented in Table 1. The examined factors and their values (g/l) were: X_1 – glycerol content (20-50), X_2 – $NaNO_2$ content (1-3) and X_3 – K_2HPO_4 content (5-15). Experimental results were fitted into the polynomial models of second degree that describe selected responses [Y_1 - inhibition zone diameter (mm), Y_2 - residual glycerol content (g/l), Y_3 - residual total nitrogen content (g/l) and Y_4 - residual total phosphorus content (g/l)]:

$$Y = b_0 + \Sigma b_i X_i + \Sigma bii^2 Xii^2 + \Sigma b_{ij} X_i X_j$$

where b_0 represents the intercept, b_i represents the linear, b_{ii}^2 quadratic and b_{ij} interaction regression coefficients. Statistical analyses of the experimental results were performed using Statistica software v. 12.0. The same software was used for generating response surface plots, drawn for a constant value of one of the factors, while the remaining two factors were varied. Optimization of the examined factors according to the selected optimization aims was performed using the desirability function method (Design-Expert 8.1 software).

Table 1. Box-Behnken experimental plan: factors and their levels

Experiment	Coded levels of factors			Varied values of factors		
	X_1	X_2	X_3	Glycerol [g/l]	$NaNO_2$ [g/l]	K_2HPO_4 [g/l]
1	−1	−1	0	20	1	10
2	1	−1	0	50	1	10
3	−1	1	0	20	3	10
4	1	1	0	50	3	10
5	−1	0	−1	20	2	5
6	1	0	−1	50	2	5
7	−1	0	1	20	2	15
8	1	0	1	50	2	15
9	0	−1	−1	35	1	5
10	0	1	−1	35	1	5
11	0	−1	1	35	3	15
12	0	1	1	35	3	15
13	0	0	0	35	2	10
14	0	0	0	35	2	10
15	0	0	0	35	2	10

RESULTS AND DISCUSSION

Biosynthesis of bioactive compounds was carried out using *B. subtilis* ATCC 6633, and cultivation media prepared according to the Box-Behnken experimental design, under previously defined cultivation conditions. After biosynthesis, the samples of cultivation media were analysed and antifungal activity of each cultivation broth was examined against a *Penicillium* sp. In order to investigate the effects of chosen factors (glycerol, $NaNO_2$ and K_2HPO_4 content) on appropriately selected responses (inhibition zone diameter, residual glycerol content, residual total nitrogen and residual total phosphorus contents), four regression equations were established based on the experimental results. The significance of the obtained models and regression coefficients was

Table 2. Regression equation coefficients and their *p*-values for selected responses

	Y_1		Y_2		Y_3		Y_4	
Effect	Coefficient	*p*-value	Coefficient	*p*-value	Coefficient	*p*-value	Coefficient	*p*-value
Intercept								
b_0	10.164	0.472	23.588	0.229	−0.002	0.989	0.163	0.660
Linear								
b_1	0.899	0.096	0.428	0.493	−0.002	0.735	**0.045**	0.012*
b_2	5.542	0.404	**−20.777**	0.049*	0.158	0.059	−0.208	0.257
b_3	**−4.362**	0.016*	−4.032	0.053	0.024	0.123	−0.076	0.066
Quadratic								
b_{11}	−0.010	0.128	0.008	0.326	0.000	0.581	−0.001	0.013*
b_{22}	−0.333	0.794	3.704	0.068	0.006	0.668	0.015	0.658
b_{33}	**0.352**	0.001*	0.124	0.110	−0.001	0.209	**0.004**	0.038*
Interaction								
b_{12}	0.058	0.486	−0.108	0.339	−0.001	0.436	−0.002	0.502
b_{13}	−0.037	0.065	0.005	0.831	0.000	1.000	−0.001	0.185
b_{23}	−0.275	0.291	**1.030**	0.02*	−0.004	0.182	**0.028**	0.006*

*Regression coefficients significant at $p<0.05$ level

Table 3. Analysis of variance for selected responses

	Residual			Model					
Response	DF	SS	MS	DF	SS	MS	F-value	*p*-value	R^2
Y_1	5	147.604	29.521	10	6489.646	648.965	21.983	0.002	0.957
Y_2	5	47.036	9.407	10	3819.562	381.956	40.602	0.000	0.968
Y_3	5	0.003	0.001	10	2.043	0.204	331.611	0.000	0.974
Y_4	5	0.019	0.004	10	1.274	0.127	32.847	0.001	0.956

DF – degree of freedom; SS – sum of squares; MS – mean squares

evaluated using their *p*-values. The obtained model for inhibition zone diameter was also graphically presented by generating response surface plots in order to examine interactions between the chosen factors. Thereafter optimization of cultivation medium composition was performed according to the desired aims, and optimal values of the examined factors, as well as predicted values of selected responses, were obtained. An additional experiment with optimized values of the examined factors was performed in order to confirm optimization validity.

Statistical analysis of experimental results

According to the Box-Behnken experimental plan, the following responses were examined: Y_1 – inhibition zone diameter (mm), Y_2 – residual glycerol content (g/L), Y_3 – residual total nitrogen content (g/l), Y_4 – residual total phosphorus content (g/l). Regression coefficients of mathematical models for each response obtained by regression analysis and their *p*-values are given in Table 2. Regression coefficient *p*-values below 0.05 indicate that the regression coefficient is statistically significant at the confidence level of 95%. Significant regression coefficients are bolded in Table 2.

The results of an analysis of variance for the selected responses are presented in Table 3. High values of the coefficients of determination for each response indicate good fit of experimental data to the obtained regression equations. The obtained F-values shown in Table 3 and *p*-values less than 0.05 for each response indicate that the models for selected responses are statistically significant.

Model for inhibition zone diameter response

Inhibition zone diameter is a major indicator of the amount of synthesized bioactive compounds, and it is therefore the most significant response for antifungal compounds production. The ranges of the selected nutrients contents were chosen according to literature data. Various data about carbon source concentrations in cultivation media could be found, ranging from approximately 8 g/l (Cheng et al., 2011) to 50 g/l (de Faria et al., 2011). The range examined in this study (20-50 g/l) was set around the central point of 35 g/l of glycerol, in conformity with a study conducted by El-Banna (2005). Concentration range of the inorganic nitrogen source, i.e. $NaNO_2$ (1-3 g/l), was selected in the same way (El-Banna & Quddoumi, 2007). Regarding phosphorus source concentration, literature data range from very low (Mnif et al., 2012) to very high concentrations (El-Bana, 2005), depending on bioproduct type. Therefore, phosphorus content range in this study was set to 5-15 g/l.

In order to better understand the effects of the examined factors and their interactions on inhibition zone diameter, response surface plots were generated (Figures 1, 2, 3). The response surface plots show the effects of two examined factors on the selected response, while the third factor remains constant and has the value of the central point of experimental design.

Figure 1 presents the effects of initial glycerol and $NaNO_2$ contents on inhibition zone diameter, while the initial content of K_2HPO_4 was 10 g/l. The results presented in the figure show that, with the initial glycerol content remaining constant, increase in initial $NaNO_2$ content leads to inhibition zone diameter increase. Reversely, when the initial $NaNO_2$ content is constant, the initial glycerol content increase of over 40 g/l leads to inhibition zone diameter decrease. As the results presented in Figure 1 show, inhibition zone diameter was at a maximum when the initial content of $NaNO_2$ in cultivation medium was maximal (2.7-3.0 g/l), and initial glycerol content ranged 30-40 g/l. The presented results indicate that a large amount of nitrogen source is essential for biomass growth or bioactive metabolite(s) synthesis under given cultivation conditions. Regarding glycerol content, the presented results are in accordance with the initial carbon source concentration reported by El-Banna (2005) and Issazadeh et al. (2012).

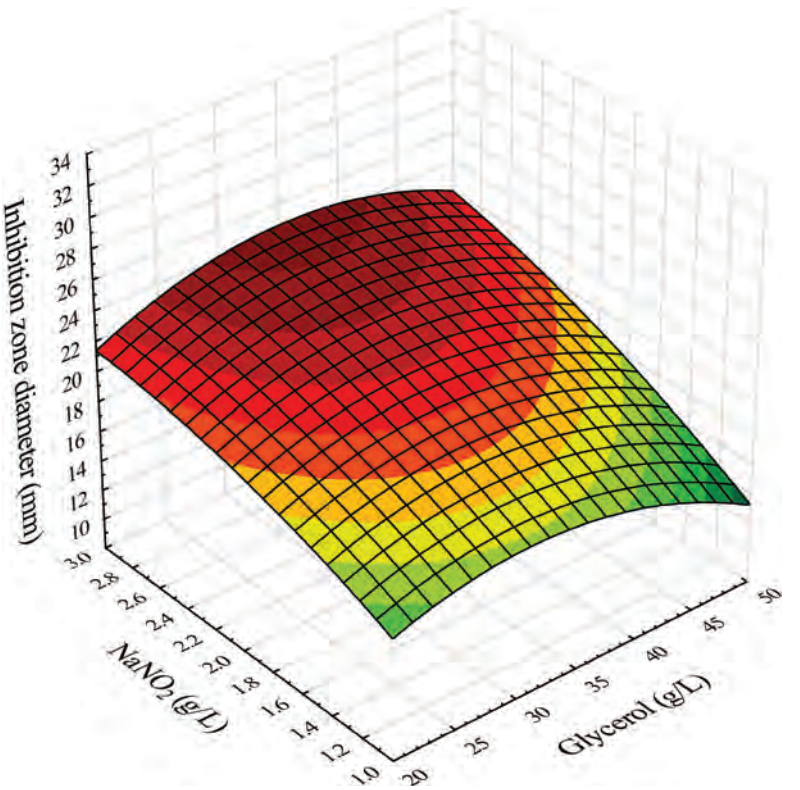

Figure 1. Effects of initial glycerol and initial $NaNO_2$ contents on inhibition zone diameter against *Penicillium* sp.

The effects of initial glycerol and initial K_2HPO_4 contents on inhibition zone diameter under constant initial $NaNO_2$ content of 2 g/l are presented in Figure 2. Under constant initial glycerol content, initial K_2HPO_4 content increase leads to inhibition zone diameter increase. As previously, an increase in initial glycerol content of over 35 g/l leads to inhibition zone diameter decrease when the initial K_2HPO_4 content is constant and near its maximum value. Maximum inhibition zone diameter was obtained at maximum K_2HPO_4 content and glycerol content within a range of 20-35 g/l. An analysis of the inhibition zone diameter model using response surface plots presented in Figures 1 and 2 indicates that initial glycerol contents exceeding approximately 35 g/l inhibit the biosynthesis of bioactive compounds effective against *Penicillium* sp., leading to inhibition zone diameter decrease. The results also indicate that a large amount of phosphorus source is essential for the biosynthesis of antifungal compounds. As limiting phosphorus source content in cultivation medium is one of common ways to direct the metabolism of productive microorganisms towards synthesis of secondary metabolites (Martin & Demain, 1980), the large amount of phosphorus source that *B. subtilis* ATCC 6633 required in this study can indicate that the bioactive agent is not a product of secondary metabolism. Similar initial K_2HPO_4 contents in cultivation media for *B. subtilis* have been used by different researchers (de Carvalho et al., 2010; de Sousa et al., 2014).

Figure 3 shows the effects of initial $NaNO_2$ and initial K_2HPO_4 contents on inhibition zone diameter under constant initial glycerol content of 35 g/l. The results presented in the figure show that when initial $NaNO_2$ content is constant, increase in initial K_2HPO_4 content leads to an inhibition zone diameter increase. Also, increase in initial $NaNO_2$ content under the constant initial content of K_2HPO_4 leads to an inhibition zone diameter increase. Therefore, simultaneous increase in initial $NaNO_2$ and initial K_2HPO_4 contents leads to maximum inhibition zone diameter. The fact that phosphorus and nitrogen sources are required in large amounts for bioactive compounds biosynthesis support an assumption that either the biomass of *B. subtilis* ATCC 6633 or the primary metabolite(s) were the antifungal agent(s) since phosphorus and nitrogen are the key nutrients required by the productive microorganism during the exponential growth phase (Martin & Demain, 1980; Sanchez & Demain 2002).

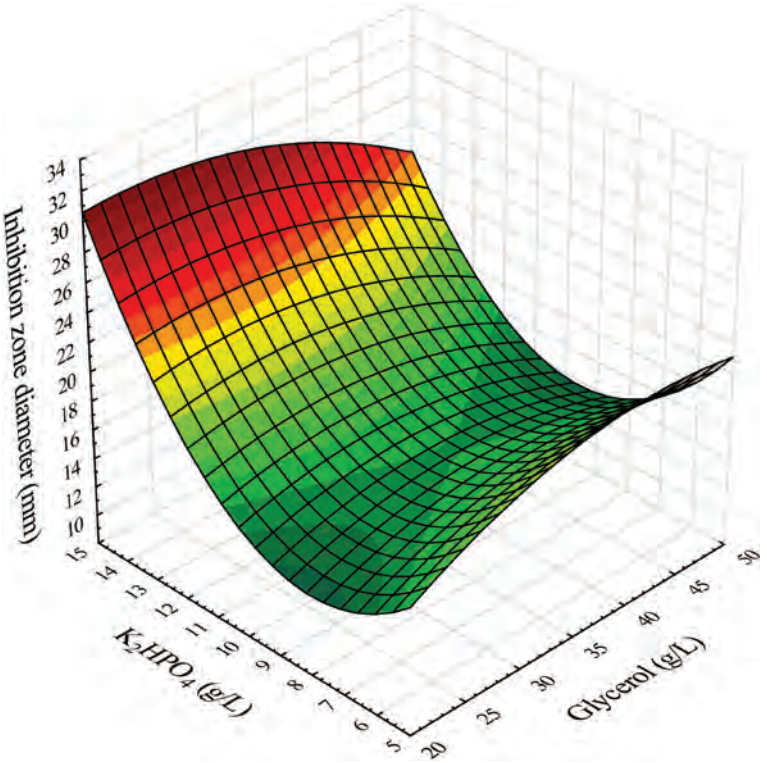

Figure 2. Effects of initial glycerol and initial K_2HPO_4 contents on inhibition zone diameter against *Penicillium* sp.

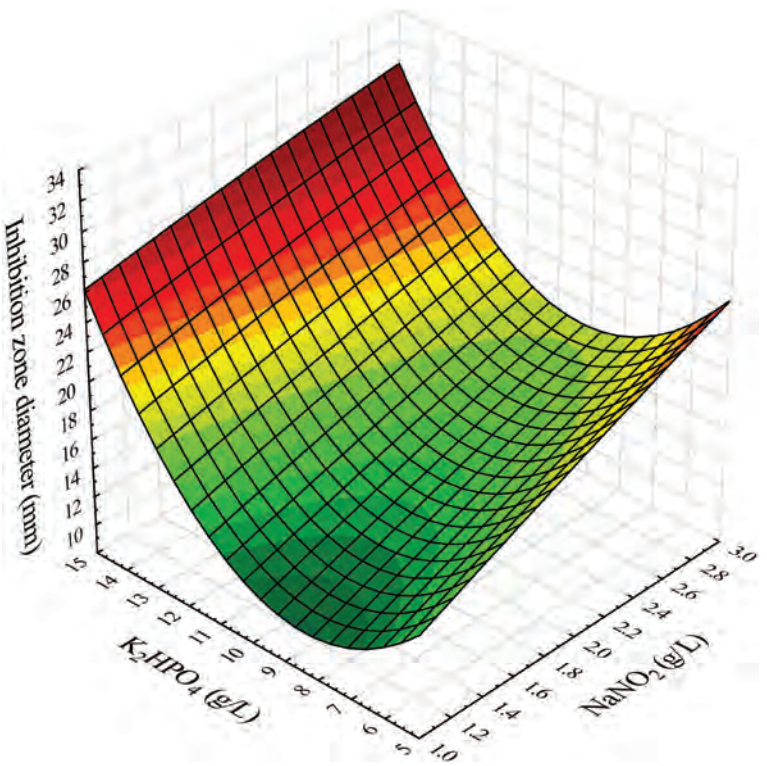

Figure 3. Effects of initial $NaNO_2$ and initial K_2HPO_4 contents on inhibition zone diameter against *Penicillium* sp.

Optimization of cultivation medium composition for biosynthesis of antifungal compounds

The main aim of optimization of cultivation medium composition is to obtain an economically viable medium that completely satisfies the nutritive requirements of a productive microorganism for biosynthesis of large amounts of a desired product along with maximum nutrients utilization and minimum environmental pollution. The main goal of biosynthesis of antifungal compounds effective against *Penicillium* sp. is to maximize the desired product yield. Since the main indicator of the amount of synthesized bioactive compounds is inhibition zone diameter, the first optimization set had maximal inhibition zone diameter as the goal. Optimization results, optimal values of the examined factors and predicted values of analyzed responses are presented in Table 4.

In the first optimization set, maximum inhibition zone diameter of 32.11 mm was predicted at the initial glycerol content of 26.74 g/l and maximum initial $NaNO_2$ and K_2HPO_4 contents (3 g/l and 15 g/l, respectively). The desirability function value of 0.96 shows that the obtained results are in a very good agreement with the assigned optimization aim. On the other hand, these initial nutrients contents in cultivation media resulted in very high predicted residual contents of nutrients after biosynthesis, i.e. 18.50 g/l for glycerol, 0.47 g/l for residual total nitrogen and 0.74 g/l for residual total phosphorus.

Another objective of optimization of cultivation medium composition is to formulate a medium with optimal contents of carbon, nitrogen and phosphorus sources, and to achieve maximal utilization of nutrients by the productive microorganism. Minimal amounts of unutilized nutrients reduce the process cost, as well as the cost of necessary effluent treatment and environmental pollution. Another optimization set was made in order to minimize residual contents of the main nutrients (glycerol, $NaNO_2$ and K_2HPO_4) after biosynthesis.

The second set of optimization results shows that the optimum values of the examined factors were glycerol 20 g/l, $NaNO_2$ 1 g/l and K_2HPO_4 15 g/l (Table 4). The second optimization data set shows that minimization of initial glycerol content from 26.74 g/l to 20 g/l, as well as initial $NaNO_2$ content to its minimum examined value, led to a reduction in predicted residual glycerol, total nitrogen and total phosphorus contents of 98.59%, 44.68% and 71.62%, respectively, and to an inhibition zone diameter decrease of 9.16%, compared to the first optimization set. The results show that optimization of cultivation medium composition can significantly contribute to a reduction in bioprocess costs. Optimal values of the initial nutrients contents near minimum values of the examined range reduce the cost of cultivation medium, while the cost of necessary effluent treatment could be decreased by minimizing the residual nutrients contents that remain in cultivation broth after cultivation. The desirability function value of 0.88 in the second optimization set indicates that the obtained results fulfill the assigned optimization aims.

Validation experiment

Cultivation media with optimal contents of the selected factors from the second optimization set (glycerol 20 g/l, $NaNO_2$ 1 g/l and K_2HPO_4 15 g/l) were prepared and biosynthesis of antifungal compounds by *B. subtilis* ATCC 6633, as well as antifungal activity assaying against *Penicillium* sp. and analysis of cultivation broth samples after biosynthesis, were performed under the same conditions as in the optimization experiments. The experiment was conducted in triplicate tests and the mean value of each response was calculated along with standard deviation determination. The observed inhibition zone diameter was 28.83±1.04 mm, which

Table 4. Goals and results of optimization of cultivation medium composition for biosynthesis of antifungal compounds

	First set		Second set	
Factors	Goal	Value	Goal	Value
Glycerol (g/l)	is in range	26.74	is in range	20
$NaNO_2$ (g/l)	is in range	3	is in range	1
K_2HPO_4 (g/l)	is in range	15	is in range	15
Responses				
Inhibition zone diameter (mm)	maximize	32.11	maximize	29.17
Residual glycerol (g/l)	is in range	18.50	minimize	0.26
Residual N (g/l)	is in range	0.47	minimize	0.26
Residual P (g/l)	is in range	0.74	minimize	0.21
Desirability	0.96		0.88	

is a very good agreement with the predicted value of 29.17 mm. Residual glycerol, total nitrogen and total phosphorus contents of 0.29±0.04 g/l, 0.28±0.03 g/l and 0.21±0.01 g/l, respectively, also showed very good agreement with the predicted values of selected responses. The experimental results presented above show that the established models for selected responses are statistically significant and could be used for further development of the bioprocess for production of antifungal compounds effective against *Penicillium* sp. by *B. subtilis* ATCC 6633.

CONCLUSIONS

The results of this study indicate that the RSM can be successfully employed for optimization of cultivation medium composition aimed for the production of bioactive compounds by *B. subtilis* with antifungal activity against *Penicillium* sp. Glycerol (carbon source) content is optimized to the minimum value of a selected range, which indicates that, in case of biomass synthesis, the optimal ratio between carbon and nitrogen source contents would be 20:1, and optimal ratio between carbon and phosphorus source contents 1.33:1. The results of this study indicate a need for further examination of mechanisms by which bioactive compounds of *B. subtilis* affect *Penicillium* sp. Also, this study presents a basis for further development of commercial biocontrol products based on microbial antagonists, and a need for determining the optimal concentration of the antagonistic microorganism, as well as an appropriate formulation of the final product.

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Optimizacija sastava hranljive podloge za proizvodnju bioaktivnih jedinjenja koja deluju protiv *Penicillium* sp.

REZIME

Biološka kontrola predstavlja jednu od najpogodnijih alternativa kojom se eliminišu nedostaci primene pesticida u kontroli biljnih bolesti. U ovom istraživanju izvršena je optimizacija sastava hranljive podloge za proizvodnju bioaktivnih komponenti od strane *Bacillus subtilis* ATCC 6633. Proizvedene bioaktivne komponente su testirane protiv fitopatogenog izolata *Penicillium* sp., poznatog po izazivanju bolesti različitih poljoprivrednih proizvoda i uzrokovanju značajnih gubitaka u prinosu useva. Ispitivanje antimikrobne aktivnosti je izvedeno primenom disk-difuzionog metoda, pri čemu su mereni prečnici zona inhibicije,

kao direktni pokazatelji antifungalne aktivnosti. Metodologija odzivne površine je primenjena za procenu uticaja inicijalnih sadržaja nutrijenata (glicerola, $NaNO_2$ i K_2HPO_4) u hranljivoj podlozi na prečnik dobijenih zona inhibicije. Optimizacija sastava hranljive podloge je izvršena primenom metoda željene funkcije, sa ciljem maksimizacije prinosa bioaktivnih komponenti i minimizacije rezidualnog sadržaja nutrijenata. Optimizovane koncentracije odabranih nutrijenata u hranljivoj podlozi za proizvodnju bioaktivnih komponenti bile su: glicerol 20 g/l, $NaNO_2$ 1 g/l i K_2HPO_4 15 g/l.

Ključne reči: Bioaktivna jedinjenja; *Bacillus subtilis*; *Penicillium* sp.; Antimikrobno delovanje