# Influence of nutrition medium composition on biosynthesis of agglutinins of *Rhizoctonia solani* RS

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**Abstract.** The influence of the composition of the nutrient medium on the yield of biomass and the biosynthesis of agglutinins of the mold fungus strain *Rhizoctonia solani* RS was studied. At the first stage of research, the agglutinin-producing micromycete strain was grown on liquid nutrient media (potato-glucose (PG), Chapek, Sabouraud) at a temperature of 28°C for 8 days. At the next stage of research, the composition of the culture medium was modified in order to select sources of nitrogen ($NH_4NO_3$, $(NH_4)_2SO_4$, $NaNO_3$, arginine, tryptophan, asparagine, threonine, peptone) and carbon (sucrose, glucose, starch) nutrition. Cultivation of the *Rh. solani* RS on a liquid PG medium, containing glucose in an amount of 20.0 g/l led to a maximum yield of fungal biomass of 23.58 ± 1.30 g/l and the production of its agglutinins (titer 16384). The studied micromycete strain was characterized by the ability to actively use the amino acids threonine or asparagine when added to the above nutrient medium in an amount of 0.1 mg/ml. At the same time, the hemagglutination activity of the micromycete agglutinins increased twofold (titer 32768) compared to the activity of agglutinins on the PG medium without the addition of amino acids (titer 16384). Keywords: deep cultivation, micromycete, *Rhizoctonia solani*, agglutinins, activity, nitrogen and carbon sources



## 1 Introduction

The rapid development of research and development in the field of molecular biology, biochemistry and microbiology in recent years has revealed the main role of lectins in carbohydrate-protein and carbohydrate-carbohydrate interactions between the cells of organisms, which has sharply increased the interest of researchers in this group of proteins [1]. Agglutinins (lectins) are glycoproteins with specific biological properties that are able to

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recognize and bind sugars without undergoing any chemical transformations in relation to them [2]. They take part in many biological processes at different levels of organization of a living organism.

In nature, agglutinins are found in animals, plants, bacteria, viruses and fungi [3]. However, domestic and foreign studies show that the most active producers of agglutinins are mold fungi [4. 5]. The ability to form agglutinins has been found in the mycelium and culture liquid of a few micromycetes. The fungi *Sclerotinia sclerotiorum* [6], *Sclerotium rolfsii* [7], *Aspergillus fumigates* [8] synthesize agglutinins in their mycelia and sclerotia, *Macrophomina phaseolina* [9] produces lectin in the culture medium.

Agglutinins are characterized by a different range of biological properties, so in recent years they have been used in the food industry, agriculture and medicine [10-16]. Interest caused by the widespread use of agglutinins in major industries has led lectinologists to search for the most optimal conditions for growing microorganisms to increase the amount of this protein [4, 19]. The process of formation of agglutinins in microorganisms can be influenced by the addition of inorganic and organic compounds to their growth medium. Thus, some lectinologists have shown that the use of various food sources, for example, L-asparagine, L-arabinose [20], sucrose or yeast extract [21], leads to the effective accumulation of agglutinins by producers.

Among the attractive and little-studied biological objects that can help in the intensive development of medicine and agriculture are filamentous fungi of the genus *Rhizoctonia*. They have been shown to form toxic metabolites, hormones, hydrolases and other secondary compounds [17, 20]. In recent years, among the biologically active substances produced by different species of the genus *Rhizoctonia*, agglutinins with antitumor and insecticidal activities have been identified [5, 21].

The purpose of this work is to assess the influence of the composition of the nutrient medium on the yield of biomass and the biosynthesis of agglutinins of the micromycete *Rh. solani* RS.

## 2 Materials and Methods

### 2.1 Objects of research

We used a strain of the filamentous fungus *Rh. solani* RS - a agglutinins producer, which was obtained in 2012 from a vegetable crop and is currently in the collection of microbial cultures of the Department of Biochemistry and Biotechnology of the Institute of Fundamental Medicine and Biology of the Kazan (Volga Region) Federal University. The micromycete culture was stored in cryovials containing a water-glycerol solution (90:10 ratio, respectively) at a temperature of minus 80 °C.

### 2.2 Cultivation conditions

At the first stage of research, three types of liquid nutrient medium were used to grow a microscopic fungus (Czapek medium, PG medium, Sabouraud medium). The potato-glucose medium contained (g/l): potatoes - 200.0, glucose - 20.0. Czapek's medium (g/l): sucrose – 30.0, $K_2HPO_4$ – 1.0, $NaNO_3$ – 3.0, $FeSO_4 \times 7H_2O$ – 0.01? $MgSO_4 \times H_2O$ – 0.5. Sabouraud medium (g/l): peptone – 10.0, glucose – 40.0 [22].

At the second stage of the work, the composition of the nutrient medium was modified in order to select optimal nutrition sources: sucrose, glucose and starch were used for carbon; nitrogen - $NH_4NO_3$, $(NH_4)_2SO_4$, $NaNO_3$, asparagine, threonine, arginine, tryptophan and peptone.

The micromycete strain was grown using a shaker-incubator (180 rpm, 28 °C) in 250 ml flasks containing 50 ml of cultural medium. The growing time was 8 days. To ensure the uniformity of the inoculum, the micromycete culture was added to the nutrient medium in the form of agar disks with a diameter of 5 mm.

After the cultivation period on the specified nutrient media, the mycelium was collected by filtration with a sterile nylon cloth, and its wet and dry weight was determined or homogenized to obtain a fungal mycelium extract according to a previously described method [23]. The supernatants of the micromycete mycelium extract were analyzed for protein content and hemagglutinating activity.

### 2.3 Analysis of lectin (hemagglutinating) activity

Lectin activity was determined by direct hemagglutination reaction (DHR) using a 2% suspension of native erythrocytes of the first human group 0 (I), according to the generally accepted method described previously [24]. A suspension of native erythrocytes for RPGA was obtained according to the method of Lutsik et al. [2]. The titer of agglutinins was taken to be the maximum dilution or the minimum content of lectins in the solution at which hemagglutination of erythrocytes was detected. The activity of agglutinins was determined by the direct DHR using a 2% suspension of native erythrocytes of the first human group 0 (I) according to the method that we described previously [24]. The lectin titer was taken as the minimum agglutinin content (maximum dilution) in the supernatant of the mycelium extract of the fungus under study, at which hemagglutination of erythrocytes was noted.

### 2.4 Statistical Analyses

Statistical processing of the results obtained during the experiments was carried out using Microsoft Office Excel 2016 (USA). To determine the significance of differences between groups, Student's t-tests were used for independent variables (with Bonferroni correction). The level of statistical significance in the work is $p < 0.05$.

## 3 Results

The most used nutrient media for growing micromycetes are Czapek, Sabouraud and PG media, the main difference of which is determined by the presence of various carbon sources in the culture medium (Czapek's medium - sucrose, Sabouraud - glucose, PG - glucose and starch). In this regard, to establish the most favorable environment for the accumulation of crop biomass and agglutinins of the fungus *Rh. solani* RS was deep-cultivated in the above-mentioned media (Figures 1).

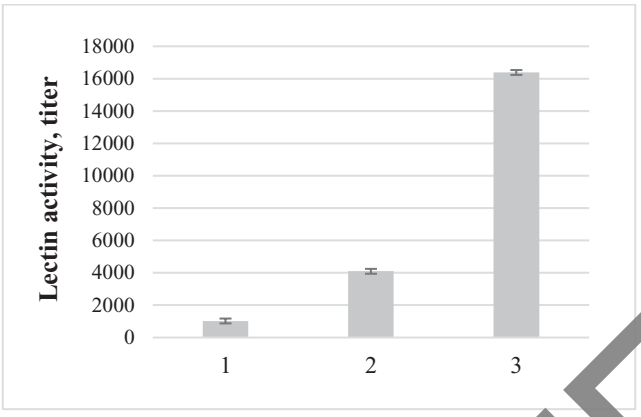


**Fig. 1.** Activity of agglutinins of the mycelium extract of *Rh. solani* RS when growing in liquid media: Sabouraud's medium (1), Czapek's medium (2), PG medium (3). The differences are statistically significant in the version using the PG medium compared to the use of Sabouraud and Chapek media ($p<0.05$)

In the process of selecting a nutrient medium for growing the micromycete *Rh. solani*, it was found that on the 8th day of growth of the fungus, maximum accumulation of biomass 23.58 ± 1.30 g/l and formation of agglutinins (titer 16384) occurs on the PG medium ($p<0.05$). The biomass yield and lectin activity in extracts of *Rh. solani* RS mycelium on Sabouraud and Czapek media were significantly lower.

Since the PG medium was the most favorable nutrient medium for the accumulation of biomass and *Rh. solani* RS agglutinins, we selected this medium as a base for further analysis of the effect of different amounts (concentrations) of carbohydrate and nitrogen sources on the accumulation of biomass and lectins by the micromycete strain.

A study of the effect of different amounts of carbohydrates in a PG medium showed the advantage of a medium with glucose on the process of agglutinin formation and the yield of fungal biomass of *Rh. solani* RS (Table 1).

**Table 1.** The influence carbon nutrition sources on agglutinin biosynthesis and biomass yield of mycelium of *Rh. solani* RS*

| Concentration of carbon sources, g/l | | Activity of agglutinins, titer | Specific activity of agglutinins, U/mg | Biomass, g/l |
|---|---|---|---|---|
| glucose | 15.0 | 4096 | $3.69 \times 10^2$ | 15.72 ± 1.30 |
| | 17.0 | 8192 | $6.66 \times 10^2$ | 20.56 ± 1.03 |
| | 20.0 | 16384 | $1.06 \times 10^3$ | 24.29 ± 1.64 |
| | 23.0 | 16384 | $1.03 \times 10^3$ | 26.10 ± 1.21 |
| sucrose | 27.0 | 4096 | $3.44 \times 10^2$ | 15.84 ± 1.37 |
| | 30.0 | 8192 | $6.25 \times 10^2$ | 19.18 ± 1.23 |
| | 33.0 | 8192 | $6.72 \times 10^2$ | 20.20 ± 1.09 |
| starch | 8.0 | 1024 | $1.53 \times 10^2$ | 9.35 ± 1.12 |
| | 10.0 | 2048 | $2.33 \times 10^2$ | 11.56 ± 1.28 |
| | 12.0 | 1024 | $1.44 \times 10^2$ | 10.21 ± 1.16 |
| glucose syrup | 15.0 | 8192 | $6.21 \times 10^2$ | 16.89 ± 1.05 |
| | 17.0 | 8192 | $6.35 \times 10^2$ | 18.66 ± 1.47 |
| | 20.0 | 16384 | $1.05 \times 10^3$ | 26.02 ± 1.39 |
| | 23.0 | 16384 | $1.01 \times 10^3$ | 25.78 ± 1.71 |

* Note: differences between options are statistically significant ($p < 0.05$);

in terms of biomass yield: the differences are unreliable between the options using glucose in quantities of 20.0 g/l, 23.0 g/l and glucose syrup in the indicated concentrations; glucose in an amount of 15.0 g/l and sucrose in a concentration of 17.0 g/l; glucose in quantities of 17 g/l, glucose syrup in the same concentration, sucrose in quantities of 30 and 33 g/l; starch at concentrations of 8, 10 and 12 g/l ($p > 0.05$)

The most reliably significant accumulation of biomass and agglutinins by the *Rh. solani* RS was observed on a nutrient medium containing glucose and glucose syrup from 20.0 to 23.0 g/l ($p<0.05$). With these amounts of glucose syrup and glucose, the acidity (pH) of the micromycete growth medium ranged from 6.0 to 7.0, and the titer of lectin activity in the fungal mycelium was 16384. A decrease in the content of glucose syrup and glucose in the PG medium (from 15 to 17 g/l) led to a decrease in the hemagglutinating activity of micromycete agglutinins by an average of 3 times. A decrease ($p<0.05$) in the formation of agglutinins by the studied fungal strain and the accumulation of its biomass was detected when replacing the glucose monosaccharide in the PG medium with an equivalent amount of sucrose or starch. The addition of starch polysaccharide to the medium led to a decrease ($p < 0.05$) in the biomass of the fungus and the activity of its agglutinins.

Our experiments established that the introduction of inorganic nitrogen in various concentrations into the culture medium led to a statistically significant (2–32 times) decrease ($p < 0.05$) in the activity of agglutinins in the mycelium extract (Table 2), compared with the hemagglutinating activity of agglutinins from fungal mycelium grown on the original medium (control).

**Table 2.** The influence of inorganic nitrogen sources on agglutinins biosynthesis and biomass yield of mycelium of *Rh. solani* RS*

| Concentration of nitrogen sources, g/l | | Activity of agglutinins, titer | Specific activity of agglutinins, U/mg | Biomass, g/l |
|---|---|---|---|---|
| control | - | 16384 | $1{,}04 \times 10^3$ | 23.58 ± 1.30 |
| $NH_4NO_3$ (ammonium nitrate) | 1.0 | 2048 | $1{,}65 \times 10^2$ | 22.01 ± 1.08 |
| | 2.0 | 1024 | $0{,}91 \times 10^2$ | 19.12 ± 1.14 |
| | 3.0 | 512 | $0{,}52 \times 10^2$ | 14.86 ± 1.09 |
| $NaNO_3$ (sodium nitrate) | 1.0 | 8192 | $5{,}89 \times 10^2$ | 23.14 ± 1.55 |
| | 2.0 | 8192 | $5{,}94 \times 10^2$ | 22.80 ± 2.02 |
| | 3.0 | 4096 | $3{,}18 \times 10^2$ | 18.57 ± 1.33 |
| $(NH_4)_2SO_4$ (ammonium sulfate) | 1.0 | 2048 | $1{,}61 \times 10^2$ | 23.06 ± 1.50 |
| | 2.0 | 1024 | $0{,}86 \times 10^2$ | 22.11 ± 1.79 |
| | 3.0 | 1024 | $0{,}89 \times 10^2$ | 21.94 ± 1.42 |

* Note: the differences between the options are statistically significant ($p < 0.05$);
in terms of biomass yield: the differences are not significant between the options using ammonium nitrate in an amount of 1.0 g/l, sodium nitrate in an amount of 1.0 and 2.0 g/l, ammonium sulfate in an amount of 1.0, 2.0 and 3 .0 g/l compared to control ($p > 0.05$)

A study of the growth of the micromycete strain studied in the work and its ability to form mycilial agglutinins on nutrient media with organic sources of nitrogen nutrition showed that the addition of asparagine to the PG medium at a concentration of 0.05 to 0.1 mg/ml and threonine at a concentration of 0.1 to 0.2 mg/ml leads to an increase ($p < 0.05$) in fungal biomass, protein content and activity of agglutininis (2 times) and compared to control (Table 3). At the same time, we noted a slight increase (1.2 times) in the protein content in the mycelium extract of the studied producer and the increase in the biomass of the latter. The addition of peptone to the medium increased the growth (biomass) of the micromycete (1.3 times), however, the activity of agglutinins was significantly lower (16 times) than in the control.

**Table 3.** The influence of various organic nutrition sources on agglutinins biosynthesis and biomass yield of mycelium of *Rh. solani* RS**

| Amino acid concentration, mg/ml | | Activity of agglutinins, titer | Specific activity of agglutinins, U/mg | Biomass, g/l |
|---|---|---|---|---|
| control | - | 16384 | $1{,}04 \times 10^3$ | 23.58 ± 1.30 |
| arginine | 0.05 | 8192 | $7{,}19 \times 10^2$ | 21.11 ± 2.23 |
| | 0.1 | 16384 | $1{,}20 \times 10^3$ | 22.42 ± 1.69 |
| | 0.2 | 4096 | $3{,}63 \times 10^2$ | 16.78 ± 1.02 |
| asparagine | 0.05 | 32768 | $1{,}77 \times 10^3$ | 26.90 ± 1.06 |
| | 0.1 | 32768 | $1{,}65 \times 10^3$ | 28.67 ± 1.51 |
| | 0.2 | 8192 | $6{,}72 \times 10^2$ | 22.09 ± 2.10 |
| tryptophan | 0.05 | 16384 | $1{,}27 \times 10^3$ | 22.86 ± 1.45 |
| | 0.1 | 16384 | $1{,}21 \times 10^3$ | 24.81 ± 1.24 |
| | 0.2 | 8192 | $6{,}72 \times 10^2$ | 20.73 ± 1.02 |
| threonine | 0.05 | 16384 | $1{,}17 \times 10^3$ | 24.02 ± 1.47 |
| | 0.1 | 32768 | $1{,}46 \times 10^3$ | 27.08 ± 0.50 |
| | 0.2 | 32768 | $1{,}56 \times 10^3$ | 29.84 ± 2.03 |
| peptone | 5.0 | 1024 | $0{,}66 \times 10^2$ | 30.75 ± 1.01 |
| soy flour | 5.0 | 512 | $0{,}56 \times 10^2$ | 18.16 ± 1.07 |
| feed yeast | 5.0 | 16384 | $1{,}12 \times 10^3$ | 23.33 ± 1.28 |

* Note: the differences between the options are statistically significant ($p<0.05$);

in terms of biomass yield: the differences are not significant between the options using arginine, tryptophan in an amount of 0.05, 0.1 and 0.2 mg/ml, feed yeast at a concentration of 5.0 g/l, asparagine in an amount of 0.2 mg/ ml, threonine in an amount of 0.05 mg/ml compared to control; asparagine in an amount of 0.05, 0.1 mg/ml, threonine in an amount of 0.1, 0.2 mg/ml and peptone ($p > 0.05$)

## 4 Discussion

In the laboratory of the Department of Biochemistry and biotechnology of the Institute of Fundamental Medicine and Biology of the Kazan Federal University, lectins were obtained and characterized from the mycelium of microscopic fungal strains [23. 24], including the galactose-specific agglutinin *Rh. solani*, which has cytotoxic and insecticidal activities [25. 26].

Changes in metabolic processes when environmental conditions change is a biological feature of microscopic fungi, which makes it possible to control the growth of the latter and increase the yield of the necessary product, including protein [5. 18. 27]. A necessary element in the technology for obtaining stable drugs from microscopic fungi is a nutrient medium [19. 28], since the accumulation of crop biomass and the formation of industrially valuable products of their metabolism directly depend on its composition. It should be noted that for each microorganism strain used in production, the composition of the nutrient medium is individual.

Our studies have shown that optimal growth and formation of agglutinins of *Rh. solani* RS occurs when it is grown on PG medium containing glucose. The process of active formation of agglutinins in the mycelium of the studied micromycete strain on the PG medium is most likely the result of the influence of potato as a (plant) inducer of their biosynthesis. However, the addition of starch to the medium reduced the ability of the fungus to form agglutinins in its mycelium.

When assessing the effect of nutrient media components on the yield of biomass of microscopic fungi, various forms of nitrogen in the medium are important [15, 16], since nitrogen is an essential element of all living systems. In addition, nitrogen takes part in the formation of peptide bonds and the structure of protein molecules, thus being part of the

amino acids of proteins, including agglutinins. We found a decrease in the activity of agglutinins in the mycelium extract of *Rh. solani* RS when inorganic nitrogen was introduced into the culture medium (Table 2), compared with the hemagglutinating activity of agglutinins from fungal mycelium grown on the original medium. The results obtained indicate the possible effect of nitrogen catabolite repression on the expression of the producer agglutinin gene.

A 2-fold increase in activity of agglutinins was observed when *Rh. solani* RS grew in a medium containing threonine or asparagine at concentrations of 0.1 mg/ml. Domestic and foreign authors note that amino acids such as threonine and asparagine, penetrating into the cell of a microorganism, are able to quickly be involved in the processes of biosynthesis of various organic compounds [17. 29]. The literature also shows that one of the additional sources of nitrogen nutrition, which contributes to the highest production by fungi of the genus *Ganoderma* and *Rhizocronia*, is the amino acid L-asparagine [5. 22. 30].

## 5 Conclusions

Thus, as a result of the research, a nutrient medium for growing the microscopic fungus *Rh. solani* RS was selected and optimized, carbon and nitrogen sources were identified at which the maximum biomass yield and biosynthesis of strain agglutinins with insecticidal activity occur. The most effective formation of agglutinin by the *Rh. solani* strain RS and its accumulation of biomass is observed on PG medium with glucose at a concentration of 20.0 g/l. The micromycete studied in this work is characterized by the ability to actively use (assimilate) asparagine or threonine when added to the PG medium in an amount of 0.1 mg/ml. An increase in protein concentration in the mycelium of the micromycete strain under study led to an increase in the yield of agglutinins. At the same time, the hemagglutinating activity of *Rh solani* RS agglutinins increased 2 times relative to the lectin activity values in the PG medium. Our results can be further applied to the process of optimizing the nutrient medium and methods of growing *Rhizoctonia solani* to obtain the required amount of mycelial biomass and industrially valuable metabolic products of the micromycete, including agglutinins.

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