

Turaeva Bakhora

Junior researcher

Institute of Microbiology of Uzbekistan Academy of Sciences

Тураева Бахора

Младший научный сотрудник

Институт микробиологии Академии Наук Узбекистана

Zukhritdinova Nigora

PhD in biology, senior researcher

Institute of Microbiology of Uzbekistan Academy of Sciences

Зухритдинова Нигора

Кандидат биологических наук, старший научный сотрудник

Институт микробиологии Академии Наук Узбекистана

Azimova Nodira

Junior researcher

Institute of Microbiology of Uzbekistan Academy of Sciences

Азимова Нодира

Младший научный сотрудник

Институт микробиологии Академии Наук Узбекистана

Karimov Khusniddin

Junior researcher

Institute of Microbiology of Uzbekistan Academy of Sciences

Каримов Хусниддин

Младший научный сотрудник

Институт микробиологии Академии Наук Узбекистана

Tokhtakhunova Aziza

Junior researcher

Institute of Microbiology of Uzbekistan Academy of Sciences

Тохтахунова Азиза

Младший научный сотрудник

Институт микробиологии Академии Наук Узбекистана

Khamidova Khursheda

PhD in biology, director

Institute of Microbiology of Uzbekistan Academy of Sciences

Хамидова Хуршеда

Кандидат биологических наук, директор

Институт микробиологии Академии Наук Узбекистана

**DYNAMIC STUDY OF PHYSIOLOGICALLY ACTIVE SUBSTANCES
SYNTHESIZED BY MICROMYCETES INCLUDED IN BIOPREPARATION
«MICROUSTIRGICH»**

**ИЗУЧЕНИЕ ДИНАМИКИ ОБРАЗОВАНИЯ ФИЗИОЛОГИЧЕСКИ АКТИВНЫХ
Веществ микромицетами, входящими в состав биопрепарата
«МИКРОУСТИРГИЧ»**

Summary. Molasses, which is considered to be industrial waste, was used as carbon and energy source in Chapeka and Mandels nutrient media. The most active synthesis of indole acetic acid (IAA) by *T. harzianum* Uz CF-55 was 2,4 mg/ml on the 1, 4, 5th days, by *P. canescens* Uz CF-54 was 1,54 mg/ml on the 1st, 2nd days, by *F. moniliforme* Uz GC-12 was 1,5–2,4 mg/ml on the 1, 2, 3, 4th days of cultivation. The maximum activity of gibberellin acid (GA) synthesized by the fungus *T. harzianum* Uz CF-55 on nutrient medium containing 5% sucrose and molasses was 3 mg/ml, 0,67 mg/ml and 0,68 mg/ml on the 3rd, 6th and 9th days of cultivation, respectively. *F. moniliforme* Uz GC-12 fungus cultivated on nutrient medium containing 5% sucrose and molasses was found to be effective on the 3rd day with 0,58 mg/ml, and on the 5th and 6th days with 0,62 mg/ml of GA. *P. canescens* Uz CF-54 cultivated on the same nutrient medium produced the maximum amount of GA which was 0,69 mg/ml and 0,78 mg/ml on the 9th and 10th days, respectively. Enrichment of the nutritional content of these micromycetic strains by cheap raw, such as molasses, allows to increase the amount of biologically active substances stimulating the growth of the plant and to reduce the net cost of the biopreparation.

Key words: molasses, micromycetes, indole acetic acid, gibberellin acid.

Аннотация. Меласса, являющаяся промышленным отходом, использовалась в качестве источника углерода и энергии в питательных средах Чапека и Мандельса. Наиболее активный синтез индолил-уксусной кислоты (ИУК) штаммом гриба *T. harzianum* Uz CF-55 составил 2,4 мг/мл на 1, 4, 5 сутки, *P. canescens* Uz CF-54 – 1,54 мг/мл на 1–2 сутки, *F. moniliforme* Uz GC-12 – 1,5–2,4 мг/мл на 1, 2, 3, 4-сутки культивирования. Максимальное количество гиббереллиновой кислоты (ГК), синтезированной грибом *T. harzianum* Uz CF-55 на питательной среде, содержащей 5% сахарозы и мелассы, составило 3 мг/мл, 0,67 мг/мл и 0,68 мг/мл на 3, 6 и 9-сутки культивирования, соответственно. Было установлено, что гриб *F. moniliforme* Uz GC-12, культивированный на питательной среде, содержащей 5% сахарозы и мелассы, эффективен на 3-сутки, при этом количество ГК составило 0,58 мг/мл, а на 5, 6-сутки – 0,62 мг/мл. *P. canescens* Uz CF-54, культивированный на той же питательной среде, продуцировал максимальное количество ГК, которое составило 0,69 мг/мл и 0,78 мг/мл на 9 и 10-сутки, соответственно. Обогащение питательной среды дешевым сырьем, таким как меласса, для данных штаммов микромицетов позволило увеличить количество синтезируемых биологически активных веществ, стимулирующих рост растений и снизить себестоимость биопрепарата.

Ключевые слова: меласса, микромицеты, индол уксусная кислота, гибберелиновая кислота.

Statement of the problem

Analysis of recent researches and publications

Microorganisms of the rhizosphere are of great importance in growth and development of plants [1, 3, 4]. They help to meet certain requirements of plants in nutrients, hormones, vitamins and other physiologically active compounds. In addition, they have a positive impact on the growth of plants, prevent the various plant diseases and increase their productivity [4, 8].

Mycelial fungi synthesize auxin (AU), gibberellin (GA) and Indole Acetic Acid (IAA) during their growth. These biologically active substances are the main components of biological preparations, which are prepared to accelerate growth and development of plants as well as the crops' yield [1].

The chemical composition of the selected nutrient media for mycelial fungi not only greatly influences on their growth and development, but also on the formation of the above mentioned biologically active substances. Up to this day, sucrose containing nutrient media have been used for GA and IAA synthesis by microorganisms. However, in order to achieve cost-effectiveness in the production of biological preparations, there is a need to use cheap carbon source, which can replace sucrose. At present time, molasses is used as a major source of carbon and energy for production of nutrient media [8].

Formulation purposes of article (problem)

In this study, molasses which is the waste of sugar production from sugar beet was used for the purpose of enriching the content of the nutrient medium and reducing its net cost. The aim of this work was to study the synthesis of phytohormones by micromycetes while using molasses as a carbon source in the nutrient medium.

The main material

MATERIALS AND METHODS

Trichoderma harzianum UzCF-55 [7], *Penicillium canescens* UzCF-54 [10] and *Fusarium moniliforme* GC-12 [9] being basis of biopreparation "Microustirgich" created in Institute of Microbiology and protected by patents of the Republic of Uzbekistan were the objects of the study. These local strains were isolated from the rhizosphere of plant's root [7, 9, 10].

In this study, 3% and 5% concentrations of sucrose and molasses were used as carbon source. Culture suspension with a titer of 10^6 – 10^7 spores/ml cultivated for 6 days was used as inoculum. The strains were cultivated on traditional Chapek and Mandels nutrient media in 250 ml of Erlenmeyer tubes on the shaker at 180 revs/min and 28–30 °C for 10 days.

The amount of GA was determined by the conventional method of Muromtsev and Nestyuk [5]. Coloro-

metric estimation of indoleacetic acid was carried out due to method by Gordon and Weber [6].

The amount of IAA and GA produced by micromycetes was determined by the spectrophotometer (Spekol 1300 Analytic Jena (4613)).

RESULTS AND DISCUSSION

Several laboratory and field experiments have been conducted on these micromycetic strains. Coleoptiles of wheat and maize plants were used as biotest to determine the active cultivation properties of the cultural broth of *Trichoderma harzianum*, *Penicillium canescens* and *Fusarium moniliforme* strains. The highest activity was observed in *Trichoderma harzianum* cultural broth, which was 2,5 times more active than the control [2].

The maximum amount of gibberellin acid (GA) synthesized by the fungus *T. harzianum* Uz CF-55 on nutrient medium containing 3% sucrose and molasses was 0,43 mg/ml, 0,50 mg/ml and 0,45 mg/ml on the 3rd, 6th and 9th days of cultivation, respectively. The highest synthesized indexes of indole-3-acetic acid were found on the 1st and 4th days, which was 1,6 mg/ml and 0,7 mg/ml, respectively (Fig. 1).

GA synthesis by *T. harzianum* Uz CF-55 fungus on 5% sucrose + 5% molasses nutrient medium was at the maximal levels on the 3th, 6th and 9th days and reached 3,75 mg/ml, 0,67 mg/ml and 0,88 mg/ml, respectively (Fig. 1).

Besides, *P. canescens* Uz CF-54 fungus cultivated on Chapek nutrient medium containing 3% saccharose and molasses, produced the highest amount of GA, 0,49 mg/ml on the 8–9th days. The highest synthesis of indole-3 acetic acid were found on the 1st day, i.e. 1,5 mg/ml and the 2nd day, which was 1,0 mg/ml (Table 1).

The highest synthesis of GA by *P. canescens* Uz CF-54 fungus grown on 5% saccharose and molasses was found on the 9th, 10th days and was 0,69 mg/ml, 0,78 mg/ml, respectively (Fig. 2). The highest synthesis of Indole-3-acetic acid was detected on the 8–9th days (0,6 mg/ml) and the 10th day (0,7 mg/ml) (Table 1).

F. moniliforme Uz GC-12 fungus was also found to synthesize GA on nutrient medium with 3% sucrose and molasses. Its amount reached 0,48 mg/ml and 0,37 mg/ml on the 2nd and 3rd day of cultivation, respectively. The highest synthesis of Indole-3-acetic acid were found to be 1,0 mg/ml on the 1st day and 1,1 mg/ml on the 2nd day of cultivation (Table 1).

F. moniliforme Uz GC-12 fungus cultivated on nutrient medium containing 5% sucrose and molasses was found to be effective on the 3rd day with 0,58 mg/ml, and on the 5th and 6th days with 0,62 mg/ml of GA (Fig. 3). The highest synthesis of Indole-3-acetic acid were found to be 2,4 mg/ml on the 1st–2nd days and 2,3 mg/ml on the 3rd day of cultivation (Table 1).

IAA synthesis was observed since the second day of the experiment. The most active synthesis by *T. harzianum* was 2,4 mg/ml and detected on the 1st, 4th, and 5th days, *P. canescens* was 1,54 mg/ml on the 1st, 2nd days, *F. moniliforme* was 1,50–2,40 mg/ml on the 1st, 2nd, 3rd and 4th days (Table 1).

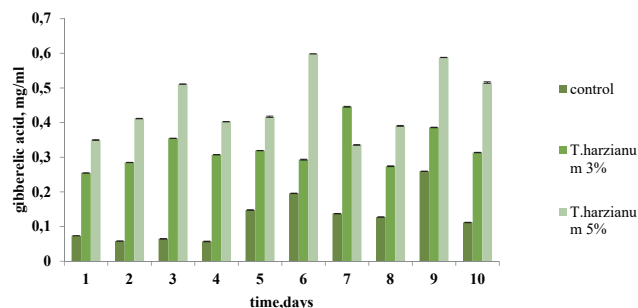


Fig. 1. The amount of GA synthesized by *T. harzianum* Uz CF-55 fungus on nutrient medium containing 5% and 3% of sucrose and molasses

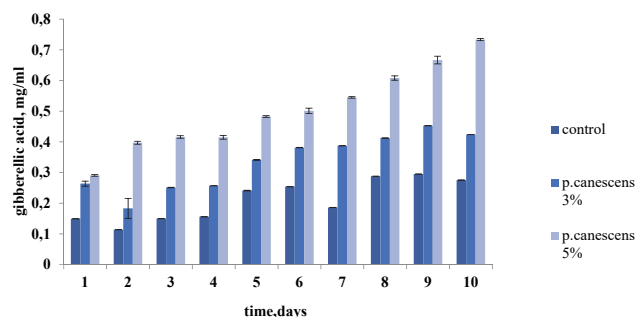


Fig. 2. The amount of GA synthesized by *P. canescens* Uz CF-54 fungus on nutrient medium containing 5% and 3% of sucrose and molasses

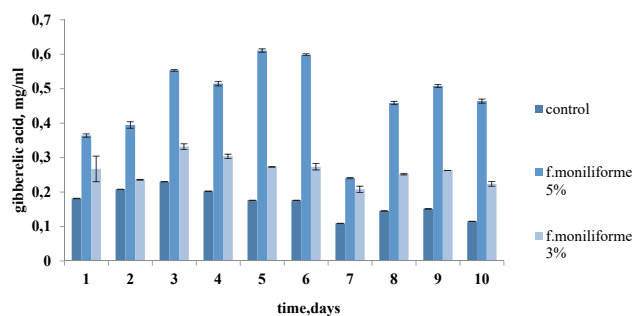


Fig. 3. The amount of GA synthesized by *F. moniliforme* Uz GC-12 fungus on nutrient medium containing 5% and 3% of sucrose and molasses.

The quantities of IAA synthesized by the studied strains at different concentrations of several carbon sources were following:

CONCLUSIONS

The results show that the GA and IAA amounts increase while cultivation of micromycetic strains on nutrient media enriched with sucrose and molasses, which

Table 1

The amount of IAA synthesized by the fungal strains on nutrient medium containing 5% and 3% of sucrose and molasses

days	5% sucrose + 5% molasses			3% sucrose + 3% molasses		
	IAA amount			IAA amount		
	T. harzianum	P. canescens	F. moniliforme	T. harzianum	P. canescens	F. moniliforme
1	2,4±0	0,4±0,03	2,4±0	1,6±0,08	1,5±0,2	1,0±0,01
2	0,5±0,01	0,4±0,05	2,4±0	0,2±0,05	1,0±0,2	1,1±0,01
3	0,6±0,004	0,4±0,04	2,3±0,01	0,3±0,05	0,4±0,1	0,4±0,01
4	2,4±0	0,4±0,05	1,5±0,03	0,7±0,24	0,2±0,01	0,1±0,03
5	2,4±0	0,4±0,03	1,0±0,01	0,5±0,01	0,2±0,01	0,1±0,03
6	0,5±0,01	0,5±0,01	0,3±0,04	0,5±0,01	0,1±0,2	0,1±0,06
7	1,6±0,11	0,5±0,03	0,6±0,01	0,1±0,06	0,2±0,05	0,6±0,01
8	0,9±0,12	0,6±0,01	0,04±0,01	0,5±0,01	0,2±0,05	0,5±0,01
9	0,12±0	0,6±0,01	0,03±0,01	0,5±0,01	0,04±0,01	0,4±0,01
10	0,21±0,01	0,7±0,04	0,7±0,01	0,5±0,09	0,2±0,01	0,2±0,01

means that phytohormones synthesis is activated. Enrichment of the nutritional content of these micromycetic strains by cheap raw, such as molasses, allows to increase

the amount of biologically active substances stimulating the growth of the plant and to reduce the net cost of the created biopreparation.

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