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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 1,5-2,4mg/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.

Ключевая слова: меласса, микромицеты, индол уксусная кислота, гибберилиновая 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 phytogormones 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]. Colorometric 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).

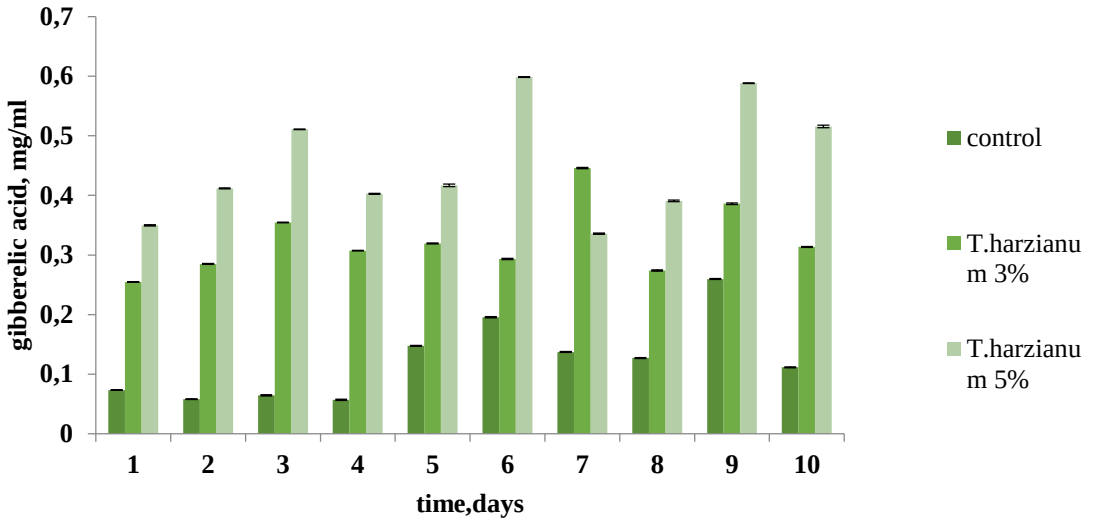

Fig. 1. The amount of GA synthesized by *T.harzianum Uz CF-55* fungus on nutrient medium containing 5% and 3% of sucrose and molasses

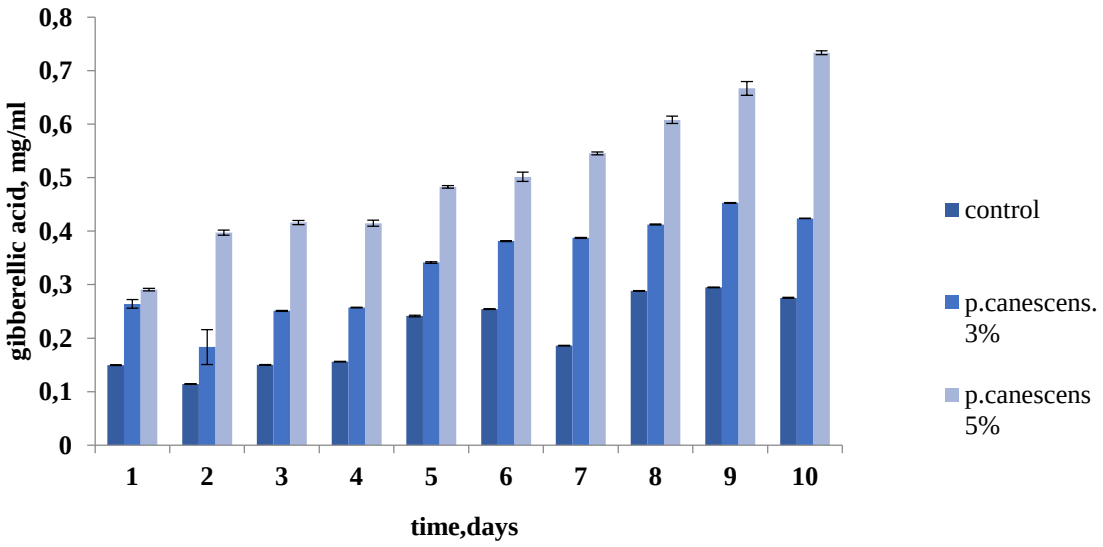

Fig. 2. The amount of GA synthesized by *P. canescens Uz CF-54* fungus on nutrient medium containing 5% and 3% of sucrose and molasses

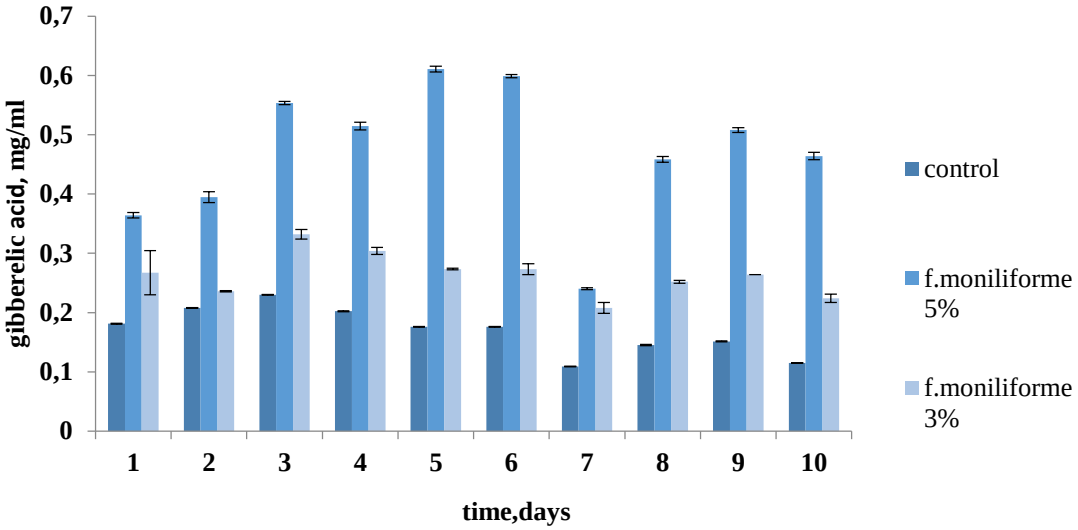

Fig. 3. The amount of GA synthesized by *F. moniliforme Uz GC-12* fungus on nutrient medium containing 5% and 3% of sucrose and molasses.

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

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±001
4	2,4±0	0,4±0,05	1,5±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

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 means that phytogormones 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.

References

1. Зухритдинова Н.Ю., Хамидов Д. М., Абдуллаев Т., Тураева Б. И.Влияние ростстимуляторов, продуцируемых микромицетами, на развитие сельскохозяйственных культур / Узбекский Биологический Журнал. Специальный выпуск: Микробиология. Ташкент, 2012. с 25-28.
2. Муремцев Г.С., Агнистикова В.И. Гиббереллины. М.:Наука,1984-208 с.
3. Алимова Ф. К. Промышленное применение грибов рода Trichoderma / Монография Казань: Казанский государственный университет им.В.И.Ульянова-Ленина, 2006- 209 с.
4. Бондарь П. Н. Штаммы грибов рода *Trichoderma* Pers (Fr.) как основа для создания препаратов защиты растений и получения кормовых добавок / Автореферат. Москва – 2011.с 8-11.
5. Муромцев Г.С., Нестюк М.Н. Количественное колориметрическое определение гиббереллловой кислоты / Вестник сельскохозяйственной науки, 1960, №2- С. 119-122.
6. Gordon, S.A., Weber, R.P. Colorometric estimation of indoleacetic acid / *PlantPhysiol*.-1951.-№26. P.192-195.

7. Хамидова Х.М, Байбаев Б.Г., Абдуллаев Т., Зухритдинова Н.Ю., Тураева Б.И. / Штамм гриба *T. harzianum* Uz CF – 55 синтезирующий ферменты целлюлазного комплекса, белок, гибберелловую кислоту, гетероауксин и витамины группы В. № IAP 04900 20.05. 2014.
8. Царенко И. Ю., Рой А. А., Курдыш И. К.. Оптимизация питательной среды для культивиревания Bacilus subtilis ИМВ. В-7023 / IISN 0201-8462, Мікробіол журн. Украина. 2011Т,73,№ 2. с 13-15.
9. Хамидова Х.М., Байбаев Б.Г., Паттаева М.А., Зухритдинова Н.Ю., Тураева Б.И. / Штамм *Fusarium moniliforme* Uz GC -12-продуцент фитогормонов гиббереллинов А4 и А7. № IAP 05002 30.12.2014г.
10. Байбаев Б.Г., Хамидова Х.М., Абдуллаев Т. / Биоўғитларни олиш учун фосфор ташувчи целлюлолитик фаол *P.canescens UzCF-54* микромицет штамми. № IAP 04211.21.07.2010.