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THE INFLUENCE OF SECONDARY EXOMETABOLITES OF *STREPTOMYCES AMBOFACIENS* ONU 561 ON PHYTOPATHOGENIC MICROMYCETES

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Abstract. *The development of microbial preparations based on antagonistically active strains of microorganisms is a very promising direction in the fight against pathogens of fusarium head and grain of wheat and barley. The aim of work was to study the effect of extracted secondary exometabolites of Streptomyces ambofaciens ONU 561 on phytopathogenic micromycetes. Extracted secondary exometabolites of strain S. ambofaciens ONU 561 in concentrations of 25 μg/ml, 50 μg/ml, 100 μg/ml, 250 μg/ml, 500 μg/ml, 1000 μg/ml were applied in 5 μl on sterile discs. To study the effect of extracted exometabolites on collection strains of micromycetes and Fusarium oxysporum strains isolated from infected wheat and barley plants, Petri dishes with Sabouraud's agar medium with glucose were inoculated. After that, disks soaked extracted exometabolites, in working concentrations, were placed on the inoculations. The results were recorded every day for 10 days, measuring the sizes of the zones of no growth of micromycete strains around the disks. Both collection strains of micromycetes and phytopathogenic strains of F. oxysporum were sensitive to extracted secondary exometabolites of S. ambofaciens ONU 561. The zones of no growth of micromycete strains varied widely and depended on the micromycete strain, the exometabolite concentration and the term of registration of the results. The minimum inhibitory concentrations (MIC) of the extracted secondary exometabolites, which inhibited the growth of the micromycete collection strains, were 100 – 250 μg/ml, the phytopathogenic F. oxysporum strains were higher and were at least 250 μg/ml. The presence of compounds with antimycotic activity in the pool of extracted secondary exometabolites allows to recommend strain S. ambofaciens ONU 561 for a more detailed study of the possibility of its use to create antimycotic preparations for agriculture.*

Keywords: *Streptomyces ambofaciens ONU 561, extracted secondary exometabolites, phytopathogenic micromycetes, inhibitory influence.*

Introduction. Currently, among the diseases of cereal crops, the most dangerous is fusarium head and grain blight. Despite the fact that a large number of studies have been devoted to the study of this disease, this problem still cannot be considered solved. Unlike other grain diseases, fusarium head blight leads, in addition to a decrease in yield, to a change in grain quality. The development of pathogens leads to the accumulation of mycotoxins in the grain, which are dangerous to human and animal health [1, 2].

The disease is caused by a complex of fungi of the genus *Fusarium*, which are characterized by high polymorphism, ecological plasticity and rapidly developing resistance to fungicidal drugs [3].

Therefore, the development of strategies to combat fusarium pathogens remains very relevant. One of the promising and perspective direction is the biological method of control, which involves the development and creation of microbial preparations based on strains of microorganisms antagonistically active against *Fusarium* [4].

In this regard, the aim of this work was to study the effect of extracted secondary exometabolites of *Streptomyces ambofaciens* ONU 561 on phytopathogenic micromycetes.

Materials and methods. The object of investigation was the strain *Streptomyces ambofaciens* ONU 561, isolated from mussel shells (*Mytilus galloprovincialis*) in 2020. According to the results of determining the effect on indicator gram-positive and gram-negative bacteria and the yeast-like fungus *Candida albicans* ATCC 18804, this strain was selected as one of the most antagonistically active [5, 6]. In addition, strain *S. ambofaciens* ONU 561 showed high activity against collection strains of micromycetes [6, 7] and phytopathogenic micromycetes isolated from infected wheat and barley plants [7].

For further research, including the study of antagonistic properties, this strain of streptomycetes is supported on Gause 2 medium.

To obtain secondary exometabolites, *S. ambofaciens* ONU 561 was grown in TSB medium on a rotary shaker at 180 rpm for 3 days at 28 °C. After that, the SG medium was inoculated with the grown culture in a seed dose of 1 – 2 ml. Cultivated on a rotary shaker at 180 rpm at 28 °C for 7 days. Further manipulations for the isolation (extraction) of secondary exometabolites were carried out according to [6, 8].

The extracted secondary exometabolites were dissolved in dimethylsulfoxide (DMSO, Gaylord Chemical Corp., USA) at a concentration of 100 mg/ml to transfer from the dried state to the liquid state. Working solutions in concentrations of 25 μg/ml, 50 μg/ml, 100 μg/ml, 250 μg/ml, 500 μg/ml, 1000 μg/ml were prepared using sterile distilled water. After that, they were sterilized using membrane filters (pore diameter 0.22 μm, Millex Syringe Filter Unit, Millipore Corp.) and applied 5 μl to sterile discs (5 mm) to

determine their inhibitory effect on micromycetes collection strains and on *Fusarium oxysporum* strains (n=30) isolated from infected wheat and barley plants. Micromycete strains were prepared for the experiment as described in [7]. Disks soaked extracted exometabolites in working concentrations were placed on Petri dishes with Sabouraud's glucose agar medium, inoculated with the appropriate micromycetes strain. The study of the effect of extracted exometabolites was carried out by cultivating inoculations at 28 °C. The results were recorded every day for 10 days, measuring the sizes of the zones of no growth of micromycetes strains around the disks. It was considered that the concentration at which the growth of the studied micromycetes strain was not observed is the minimum inhibitory (suppressive) concentration (MIC). The controls were the inoculation of micromycetes strains on Sabouraud's glucose agar medium, as well as the inoculation of these strains on the specified medium, on which disks soaked DMSO at a concentration of 500 μg/ml were placed.

The study was conducted three times. To analyze the obtained results, descriptive statistics were carried out using the Microsoft Office Excel-2016 program.

Results and discussion. The *S. ambofaciens* ONU 561 strain exhibits pronounced antagonistic activity against a wide range of proto- and eukaryotic microorganisms when pre-cultivated on nutrient media of different composition [5 – 7]. However, as evidenced by the data of many publications of specialized scientists, extracts from the biomass of actinobacteria can show different antagonistic activity, which is explained by the unequal composition of exo- and endometabolites [9, 10]. Therefore, in this study determined the antagonistic effect of extracted secondary exometabolites on micromycetes collection strains and on *Fusarium oxysporum* strains isolated from infected plants.

Both collection strains and strains of *F. oxysporum* isolated from infected plants were sensitive to extracted secondary metabolites of *S. ambofaciens* ONU 561. The zones of no growth of micromycete strains varied widely and depended primarily on the micromycetes strain, the exometabolite concentration and the term of registration of the results, which does not contradict the data of similar studies [11 – 13].

So, for example, the collection strain *F. oxysporum* UKM F-54201 showed sensitivity to streptomycete exometabolites at a concentration of 250 μg/ml already on the 3rd day of observation, the zone of no growth of its growth was 6.33 ± 0.02 mm. At the same time, increasing the concentration to 1000 μg/ml did not lead to an earlier manifestation of the activity of exometabolites, which is associated with the growth rate of this micromycete strain, but affected the size of the zone of no growth, which was 7.66 ± 0.02 mm per 3 days. At the end of the observation period (10 days), under the influence of this

concentration of exometabolites, the zone of no growth increased and reached 10.0 ± 0.01 mm.

Among the collection strains, the most sensitive to exometabolites of *S. ambofaciens* ONU 561 was the strain *Alternaria alternata* UKM F-16866. Already on the second day of observation, the zone of absence of its growth was 6.66 ± 0.02 mm under the action of extracted substances at a concentration of 100 μg/ml.

The MIC of exometabolites of the studied streptomycete was 100 μg/ml against *Aspergillus flavus* UKM F-3023, *Alternaria alternata* UKM F-16866 and *Penicillium expansum* UKM F-575. As for other collection strains of micromycetes, this indicator was 250 μg/ml.

F. oxysporum strains isolated from infected wheat and barley plants also showed different sensitivity to the extracted secondary metabolites of *S. ambofaciens* ONU 561. As in the case of the collection strain *F. oxysporum* UKM F-54201, the antagonistic effect of exometabolites was noted starting from the 3rd day. Zones of no growth ranged from 6.33 ± 0.02 mm to 15.0 ± 0.01 mm, depending on the strain of isolated fusarium, the concentration of exometabolites, and the term of registration of the results.

Compared to the collection strains, the MICs that inhibited the growth of phytopathogenic *Fusarium* were higher and amounted to at least 250 μg/ml. For half of the isolated strains, the MIC was 500 μg/ml. In most cases, the best inhibitory effect, manifested in the size of the zones of no growth, was recorded on the 4th – 5th day of registration of the results without significant changes at the end of the observation term.

Conclusions. The extracted secondary exometabolites of *S. ambofaciens* ONU 561 show a pronounced inhibitory influence on micromycetes of collection strains and *F. oxysporum* strains isolated from infected wheat and barley plants, which indicates the presence of substances with antimycotic activity in the pool of extracts. Strain *S. ambofaciens* ONU 561 can be recommended for further research on the possibility of using it to create antimycotic preparations for agriculture.

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ВПЛИВ ВТОРИННИХ ЕКЗОМЕТАБОЛІТІВ *STREPTOMYCES AMBOFACIENS* ONU 561 НА ФІТОПАТОГЕННІ МІКРОМІЦЕТИ

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Анотація. *Розробка мікробних препаратів на основі антагоністично активних штамів мікроорганізмів є досить перспективним напрямком у боротьбі зі збудниками фузаріозу колоса та зерна пшениці та ячменю. Метою роботи було вивчити вплив екстрагованих вторинних екзометаболітів Streptomyces ambofaciens ONU 561 на фітопатогенні мікроміцети. Екстраговані вторинні екзометаболіти штаму S. ambofaciens ONU 561 у концентраціях 25 мкг/мл, 50 мкг/мл, 100 мкг/мл, 250 мкг/мл, 500 мкг/мл, 1000 мкг/мл наносили по 5 мкл на стерильні диски. Для дослідження впливу екстрагованих екзометаболітів колекційними штамами мікроміцетів і штамами Fusarium oxysporum, виділеними із уражених рослин пшениці і ячменю, інокулювали чашки Петрі із агаризованим середовищем Сабуро з глюкозою. Після цього на посіви накладали диски, просочені екстрагованими екзометаболітами у робочих концентраціях. Облік результатів проводили щодня протягом 10 діб, вимірюючи розміри зон відсутності росту штамів мікроміцетів навколо дисків. І колекційні штами мікроміцетів, і фітопатогенні штами F. oxysporum виявилися чутливими до екстрагованих вторинних екзометаболітів S. ambofaciens ONU 561. Зони відсутності росту штамів мікроміцетів коливалися у широкому діапазоні і залежали від штаму мікроміцету, концентрації екзометаболіту і терміну обліку результатів. Мінімальні інгібуючі концентрації (МІК) екстрагованих вторинних екзометаболітів, які пригнічували ріст колекційних штамів мікроміцетів, становили 100 – 250 мкг/мл, фітопатогенних штамів F. oxysporum були вищими і становили не менше 250 мкг/мл. Наявність у пулі екстрагованих вторинних екзометаболітів речовин з антимікотичною активністю дозволяє рекомендувати штам S. ambofaciens ONU 561 для подальших досліджень щодо можливості використання його для створення антимікотичних препаратів для сільського господарства.*

Ключові слова: *Streptomyces ambofaciens ONU 561, екстраговані вторинні екзометаболіти, фітопатогенні мікроміцети, пригнічувальний вплив.*

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