

## REVIEW

of the official opponent

for PhD thesis of **Zhu Hongxia** «**Biocontrol mechanisms of *Streptomyces* spp.**

**HU2014 against *Rhizoctonia* spp. caused wheat sharp eyespot**»

submitted for a for a Doctor Philosophy Degree (PhD)

Field of study: 20 – Agricultural sciences and food Specialty

202 – Protection and quarantine of plants

### **Relevance of the dissertation research topic**

Cereals and their derivative products constitute a huge portion of the human diet, with wheat holding a prominent position among agricultural cereals. The cultivation area for wheat expands annually. Crop yields are influenced by a spectrum of natural factors, such as stable air temperature, regular precipitation, balanced soil microbiota, and anthropogenic factors including crop rotation, watering, fertilization, pest, and disease control, etc. Presently, the predominant methods for controlling weeds, pests, and infectious diseases in agricultural plants involve products derived from natural or chemically synthesized substances.

The excessive chemicalization of agro-ecosystems has resulted in an imbalance in soil microbiota and the emergence of pathogens resistant to existing drugs. This chemical approach also leads to pollution in neighboring areas, negatively impacting natural ecosystems. Furthermore, the application of chemicals to agricultural land contributes to the deterioration of the quality of corresponding food products, adversely affecting human health and, consequently, overall performance. This combination of factors has a detrimental impact on the socio-economic development of any society.

An alternative approach to address this issue involves the development of new biocontrol methods utilizing natural isolates of microorganisms, particularly actinomycetes. These soil microorganisms, notably those belonging to the genus *Streptomyces* and colonizing the rhizosphere of plants, can establish close mutualistic relationships with them. By producing a diverse array of bioactive molecules, they can

contribute to plant nutrition and growth, including the synthesis of phytohormones, and enhance resistance to phytopathogens through antibiotic production.

In view of the above, the chosen research topic is undoubtedly important and relevant, as substantiated by the dissertation to a significant extent.

### **Connection of work with scientific programs, plans, themes**

The research was carried out in accordance with the thematic plans of research works of the department of plant protection of the Sumy National Agrarian University and within the framework of the topics "Optimization of fermentation conditions of *Streptomyces* sp. HU2014 and study on antibacterial active substances" (the Key Science and Technology Program of Henan Province, China. Grant Number 162102210106), "Study on the active components and antibacterial lead compounds from the extracts of *Carpesium abrotanoides* L. (Asteraceae) against phytopathogenic fungi" (the Key Science and Technology Program of Henan Province, China. Grant Number 212102110148), and "Study and application of a beneficial *Streptomyces* strain in disease prevention and growth promotion of wheat" (the Key Science and Technology Program of Henan Province, China. Grant number 232102111015).

### **The main scientific results obtained by the author and their novelty**

The applicant, employing a wide range of modern microbiological, genetic, and agro-technological methodological approaches, has acquired a substantial number of complementary results. These results significantly contribute to the generation of new knowledge in the development of modern biocontrol methods. In particular, a new strain of *Streptomyces* sp. HU2014 was isolated and identified; effective antifungal effect of strain HU2014 against pathogens of wheat rhizoctoniosis has been proven; the mechanisms of induced resistance of wheat by treating *Streptomyces* sp. plants were studied. HU2014; proved that *Streptomyces* sp. HU2014 promotes the growth of wheat by stimulating growth processes, improving soil nutrition of plants by changing the microbial structure of the rhizosphere; optimal cultivation conditions of HU2014 were determined.

## **The practical significance of the obtained results**

The obtained results demonstrate the potential of natural isolates of actinomycetes as a source of a wide range of biologically active compounds for phytostimulation and the control of infectious diseases in agricultural plants. The properties of the *Streptomyces* sp. HU2014 strain serve as a potential basis for creating new systemic biological products to combat infections caused by *Rhizoctonia* spp, was described. The bioactive compounds identified in the extract of the HU2014 strain can also serve as the basis for the development of rapid-response chemicals. Another crucial practical outcome of the study is the detection of phytotoxic activity in the examined streptomycetes against green bristlegrass, which may, in the future, offer an additional effect in weed control. The study further demonstrated the positive impact of the HU2014 strain on soil composition and soil microbiota. This impact is significant for improving plant nutrition and has a positive effect on enhancing plant resistance to infections and other stresses. Additionally, the experimental data and the tested research methods can be applied in research and teaching at Sumy National Agrarian University and other educational and research institutions.

## **Scope and structure of the dissertation**

The dissertation comprises an abstract, introduction, literature review, research materials and methods, research results, conclusions and recommendations, a list of references, and appendices. The work is presented on 154 pages of printed text, featuring 27 figures and 19 tables. The list of references encompasses 158 titles.

The **Introduction** briefly justifies the problematics and relevance of the proposed research, formulates objectives, presents the scientific novelty, practical significance of the results obtained, and provides data on the results' testing at international conferences.

In the **Literature Review** (Section I), the author summarizes the literature on the subject of the study and justifies its relevance. This section includes an overview of the pathogenesis of wheat sharp eyespot, the concept of biological control of infectious plant diseases, and current advances in research on the use of microorganisms in

biocontrol and plant growth improvement. The author also focuses on the prospects for the use of streptomycetes in this area.

The **Materials and Methods** (Section II) describe in detail the research methodology. The work utilized a wide range of modern methods in microbiology, genetics, biochemistry, and agrotechnology, ensuring the successful completion of tasks.

In the **Research Results** (Sections III-VI), the author describes the experimental results step by step, beginning with the phylogenetic characterization of the HU2014 strain, assigned to the genus *Streptomyces*. Biotests confirmed the strong antifungal activity of the investigated strain against *R. cerealis* G11 and *R. solani* YL-3. Extract separation and verification of biological activity identified active fractions - F6 and F8. UPLC-MS on four extracts from the EF of HU2014 identified 10 chemical constituents in the Natural Products Atlas with high match levels (more than 90%).

The author experimentally demonstrated an increase in the activity of major wheat enzymes under conditions of infection with *R. cerealis* G11 and treatment with active fractions of the HU2014 strain extract, indicating the induction of plant resistance to fungal infection. The author also describes the allelopathic effect of HU2014 strain metabolites and proves that some metabolites of this strain, in low concentrations, can promote wheat growth in germination experiments. Meanwhile, other allelochemicals of this strain can inhibit the growth of segetal vegetation. Pot tests showed a positive effect of the HU2014 strain on the chemical and microbiological parameters of rhizosphere soil. Eventually, the author optimized the conditions of fermentation of strain HU2014 using a single-factor analysis, allowing an increase in the production of bioactive metabolites of this strain. Field trials of the strain showed its positive effect on wheat plant growth and its ability to suppress WSE.

Each chapter of the thesis concludes with interim summary conclusions.

**Conclusion and recommendation for production** summarise and generalise the main results of the research.

### **Completeness of presentation of dissertation results and scientific contributions in publications.**

Based on the dissertation materials, six articles were published: two in international journals that included in the Scopus databases and four in professional journals in Ukraine. Additionally, seven materials and abstracts were presented at international and national scientific conferences.

### **Absence (presence) of violations of academic integrity.**

No signs of academic plagiarism, fabrication, or falsification were identified in the work. The dissertation stands as an independent scientific research endeavor, highlighting the original ideas and research findings of the dissertator. The ideas and contributions of other scientists utilized in the study are appropriately referenced and employed to support the author's own ideas.

**Discussion clauses and remarks to the dissertation.** Positively evaluating the dissertation work of Zhu Hongxia, at the same time, it is worth to focus on the following remarks and wishes:

1. The **Introduction** section appears superficial, particularly concerning the relevance of the topic. In addition:
  - There is a deficiency in references to literature sources that underpin the relevance of the chosen research topic.
  - The reference (Law et al., 2017) is absent from the References section.
  - The object and subject of the study are not clearly defined. We propose that the object of research should encompass the properties of the *Streptomyces* sp. HU2014 strain as a potential biocontrol agent and phytostimulant. The subject of the study is the mechanisms that contribute to the realisation of antagonistic and phytostimulating properties of *Streptomyces* sp. HU2014.
  - The personal contribution section should acknowledge the role of the supervisor and clarify which parts of the work were conducted collaboratively and with whom.

2. The author sequenced the whole genome of HU2014 strain but utilized the data solely for determining its taxonomic position, a task achievable through the nucleotide sequence of the 16S rRNA gene alone. While the author mentions future genome analysis, it would have been more advantageous to conduct it as part of this study. Specifically, identifying biosynthetic gene clusters early on could have enabled an assessment of the strain's potential as a biocontrol agent and phytostimulant.
3. In the study of Plant Growth-Promoting (PGP) properties of HU2014 strain, it is noteworthy that the reaction with the Salkowski reagent, while commonly used for Indole-3-acetic acid (IAA), lacks specificity and may indicate the production of various indol-related substances. Therefore, in this context, a more accurate term would be indol-related substances.
4. Using UPLC-MS analysis of metabolites from HU2014 strain and comparative analysis with the NP Atlas, the author identified 10 compounds that share a 90% similarity with previously described ones. However, it is crucial to note that accurate identification of chemical structures requires NMR analysis. Therefore, the percentage of similarity should have been indicated in Table 3.2. Moreover, with the genome sequenced, screening for potential biosynthetic gene clusters of the identified compounds could have provided additional supporting evidence.

The comments and shortcomings presented do not detract from the high scientific level of the work, its novelty and practical significance. The dissertation is a complete work, logically presented, and is distinguished by a large amount of experimental work, crowned with practical implementation, as evidenced by the acts of implementation of the results.

#### **General conclusion.**

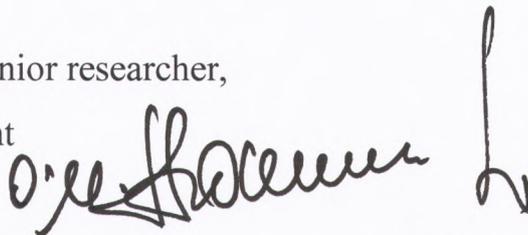
The dissertation work of **Zhu Hongxia** «**Biocontrol mechanisms of *Streptomyces* sp. HU2014 against *Rhizoctonia* spp. caused wheat sharp eyespot**», submitted for defense to the specialized academic council for obtaining the degree of Doctor of Philosophy in the field of knowledge 20 – Agricultural sciences and food in the specialty 202 – Protection and quarantine of plants in terms of its relevance, scientific and theoretical level, main results of validity, main provisions and results

published in professional publications, and novelty of the setting and practical significance meets the requirements of the order of the Ministry of Education and Science of Ukraine No.40 dated January 12, 2017 “On approval of requirements for registration dissertation” and the Decree of the Cabinet of Ministers of Ukraine dated January 12, 2022 No.44 “On approval of the Procedure for awarding the degree of Doctor of Philosophy and cancellation of the decision of the one-time specialized academic council of the higher educational institution, scientific institution on awarding the degree of “Doctor of Philosophy” with amendments made according to the Resolution of the Cabinet of Ministers No.341 dated 03.21.2022.

Based on the mentioned above, I believe that the author of the dissertation, **Zhu Hongxia**, deserves to be awarded the scientific degree of Doctor of Philosophy in the specialty 202 – Protection and quarantine of plants of the field of knowledge 20 – Agrarian sciences and food.

**Official opponent:**

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