

FEEDBACK

from official opponent, candidate of agricultural sciences, associate professor, head of the department of zoology, entomology, phytopathology, integrated protection and quarantine of plants named after B. M. Litvynova,

State Biotechnological University

Serhii Stankevych

for dissertation work of **Zhu Hongxia «Biocontrol mechanisms of *Streptomyces sp.***

HU2014 against *Rhizoctonia spp.* caused wheat sharp eyespot», which was submitted for obtaining the scientific degree of Doctor of Philosophy at the one-time special council at Sumy National Agrarian University, branch of knowledge 20 – «Agricultural sciences and food », on specialty 202 Plant Protection and Quarantine.

1. Relevance of the dissertation topic.

Bacteria, actinomycetes, algae and fungi are ubiquitous microorganisms in soil. They can promote the nutrient absorption of the host and improve the host's resistance to abiotic stress. As an important host of microorganisms, plants have learned how to use microorganisms to protect themselves from diseases in the long-term coexistence relationship. Wheat sharp eyespot (WSE) is a soil-borne disease that affects almost all temperate wheat-growing regions worldwide. In China, it is common in several main provinces for wheat production, such as Jiangsu, Anhui, Henan, Shandong, Shaanxi and Hubei, with the wheat losses from 10% to 40% totally.

This disease is caused primarily by the soil-borne fungus *Rhizoctonia cerealis*

Van der Hoeven and *R. solani* Kuhn. *R. cerealis* anastomosis group CAG-1 and *R. solani* anastomosis group AG- 5 are the main causes of cereal root disease in China.

The current main control against WSE is still chemical fertilizers and pesticides with the advantages of economy and high-efficiency. However, these methods have negative consequences, including environmental pollution and the resistance of virulent strains to chemical treatments. Biocontrol bacteria are considered as a potential method in agriculture production, which plays an important role in ecological balance. *Streptomyces* spp. produce active metabolites that inhibit phytopathogen growth, such as *Rhizoctonia* spp., *Botrytis cinerea* and *Fusarium culmorum*, and promoting plant growth.

Wheat sharp eyespot (WSE) is a serious disease caused by the phytopathogens *R. cerealis* and *R. solani*. The pathogenicity of *R. cerealis* was significantly higher than that of *R. solani* and it is a main pathogen in WSE in China. Currently, the principal method of controlling this disease is by the use of synthetic chemical fungicides. In order to develop sustainable agriculture and protect the ecological environment, biocontrol approach is a possible alternative, which uses beneficial microorganisms to control pathogens and improve plant health. Some species in the genus *Streptomyces* have been identified as potential biocontrol agents against phytopathogens. However, reports on using *Streptomyces* spp. against *R. cerealis* are lacking. Therefore, the study of biocontrol mechanisms of *Streptomyces* sp. HU2014 against *Rhizoctonia* sp. is relevant.

2. Connection with scientific programs, topics, plans.

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).

3. Scientific novelty of the obtained results.

On the basis of analytical and experimental research, the work for the first time:

- 1) a new strain of *Streptomyces* sp. HU2014 was isolated and identified;
- 2) effective antifungal effect of strain HU2014 against pathogens of wheat rhizoctoniosis has been proven;
- 3) the mechanisms of induced resistance of wheat by treating *Streptomyces* sp plants were studied. HU2014;
- 4) 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;

5) optimal cultivation conditions of HU2014 were determined.

The improvement of biological protection of wheat by using a new strain of *Streptomyces* sp. HU2014, which will expand the range of existing bioagents.

4. Theoretical significance of the dissertation.

The dissertation work provides a theoretical foundation and practical solution for screening biocontrol in agriculture. Previous work showed that the strain HU2014 had strong antifungal activities against phytopathogens. To further study this strain, Genome sequencing and average nucleotide identity (ANI) calculation of it were established using the Oxford Nanopore Technologies standard protocol and the JSpecies WS Online Service. The genome size of HU2014 was 8,170,612 bp and the values of ANI_b and ANI_m were 93.30% and 94.01% respectively. The result indicated that HU2014 is a novel *Streptomyces* species most closely related to *Streptomyces albireticuli*. The complete genome sequence of HU2014 has been deposited in GenBank under the accession number CP097123.

5. Practical significance of the results of the dissertation.

Research results show that a new strain HU2014 has been identified, which has proven to be effective in protecting wheat against *Rhizoctonia* spp. and can be used for the production of new biofungicides. Also, the determination of ten biologically active metabolites in the extracellular filtrate of strain HU2014 will be useful for the

production of biofungicides. The studied strain of *Streptomyces* sp. HU2014 showed a phytotoxic effect on seed germination by the green mouse, so it is promising in the fight against segetal vegetation. The stimulating effect of the new strain on the growth and development of wheat plants due to the improvement of soil nutrition and plant metabolism has been proven. The obtained results are included in the training programs of bachelors in the specialty 202 "Protection and quarantine of plants" at the Sumy National Agrarian University.

6. Number of scientific publications.

Based on the results, a total of 13 academic articles have been published, including 2 Web of Science Core Collection, 4 Ukrainian professional journals, 7 other conference papers.

7. Complete presentation of the dissertation material in scientific publications.

The main material of the dissertation is fully presented in 6 articles published in scientific publications and 7 papers published in international academic conferences with the applicant as the first or corresponding author. The published works effectively reflect and confirm the results of the studies conducted.

8. The degree of validity of scientific provisions.

The research results of this dissertation are not only based on professional scientific research methods, systematic theoretical research, but also through extensive

experimental research and test verification. The validity of scientific regulations is fully reflected in published scientific publications. This dissertation is a detailed study independently completed by the applicant. Full text in English.

Taken together, these fully demonstrate the validity of the applicant's compliance with the scientific provisions of the resolutions of the Cabinet of Ministers of Ukraine (Resolution 283 of April 3, 2019, Resolution 502 of May 19, 2023).⁹ The structure and content of the dissertation, its completeness and compliance with the established requirements for design.

9. The structure and content of the dissertation, its completeness and compliance with the established requirements for design.

The dissertation consists of Introduction, 6 chapter, Chapter 1. Pathogenesis and control progress of wse (literature review); Chapter 2. Materials and methods of research; Chapter 3. Study on antifungal mechanism of HU2014 against *R. cerealis* G11 and *R. solani* YL-3; Chapter 4. Determination of plant-growth promotion; Chapter 5. Study on fermentation processing of HU2014; Chapter 6. Field application of HU2014 in wheat plants; Conclusion; Recommendations for production; References and Appendixes.

The structure of the dissertation is reasonable and scientific, consistent with standards, clear logical thinking, detailed, systematic and complete. It has a meaningful integrity, consistency and completeness that is entirely consistent with the requirements formulated in the design.

10. Discussion clauses, comments and wishes regarding the content.

There are a few points worth discussing regarding the content of the dissertation:

1. Chapter 1, it would be desirable to consider the prevalence of rhizoctoniosis pathogens on other grain ear crops.

2. Chapter 2, four varieties of winter wheat were used in the research: 2 medium and 2 medium-late. It would be desirable to take varieties with other ripening dates for research.

3. Chapter 3, investigating the antifungal mechanism of HU2014 against *R. cerealis* G11 and *R. solani* YL-3 65 and the feasibility of its use, it would be desirable to make a comparison with traditional plant protection measures.

4. Subchapter 4.1, the allelopathy of HU2014 metabolites on the growth of wheat and green stubble was investigated in laboratory conditions, however, in field conditions, different data might have been obtained due to the influence of wheat microbiota and abiotic factors.

5. Chapter 5 provides data on conducting a one-factor experiment using glucose-peptone-yeast extract as the main medium, but it would be desirable to include other common nutrient media in the research scheme as well.

6. Section 6. Field studies of the application of HU2014 on wheat plants were conducted in 2022-2023. It was desirable to conduct at least three-year field studies.

General conclusion

Dissertation work of Zhu Hongxia on the topic «Biocontrol mechanisms of *Streptomyces* sp. HU2014 against *Rhizoctonia* spp. caused wheat sharp **eyespot**» which

was 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 with a specialty 202 - Plant Protection and Quarantine of agricultural production according to its relevance, scientific and theoretical level, main results of validity, main provisions and the results published in professional publications, the novelty of the formulation and the practical significance meet the requirements of the order of the Ministry of Education and Culture of Ukraine No. 40 of January 12, 2017 "On approval of requirements for the preparation of a dissertation" and Resolution of the Cabinet of Ministers of Ukraine of January 12, 2022 No. 44 "On approval of the Procedure for awarding a degree doctor of philosophy and cancellation of the decision of the one-time specialized academic council of the institution of higher education, scientific institution on awarding the degree of doctor of philosophy" with changes introduced in accordance with Resolution of the Cabinet of Ministers No. 341 dated 03.21.2022.

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