

REVIEW

of the Official Reviewer **Olha BAKUMENKO** Candidate of Agricultural Sciences, Associate Professor of the Department of Plant Protection for PhD Thesis Чжу Хунся (**Zhu Hongxia**) «Biocontrol mechanisms of *Streptomyces* spp. HU2014 against *Rhizoctonia* spp. caused wheat sharp eyespot», Submitted for a Scientific Degree of a Doctor of Philosophy Field of Study: 20 – Agricultural Sciences and Food Specialty 202 – Plant Protection and Quarantine

Relevance of the dissertation topic. Wheat sharp eyespot (WSE) is a soil-borne disease that is prevalent in wheat in almost all temperate growing regions worldwide. In China, the disease is found in several major wheat-producing provinces, including: Jiangsu, Anhui, Henan, Shandong, Shaanxi and Hubei, with total wheat losses ranging from 10% to 40%. This disease is caused mainly by the soil fungi *Rhizoctonia cerealis* Van der Hoeven and *R. solani* Kuhn. The anastomosing groups *R. cerealis* CAG-1 and *R. solani* AG-5 are the main causes of cereal root diseases in China.

The current main control of wheat rhizoctonia is still chemical pesticides with the advantages of economy and high efficiency. However, this method has negative consequences, including environmental pollution and resistance of virulent strains to chemical treatments.

Biocontrol bacteria are considered as a potential method in agricultural production that plays an important role in ecological balance. *Streptomyces* spp. produce active metabolites that inhibit the growth of phytopathogens such as *Rhizoctonia* spp., *Botrytis cinerea* and *Fusarium culmorum* and promote plant growth.

The invention of a new strain of *Streptomyces* sp. (HU2014) with antifungal efficacy, the study of the mechanisms of its biological action against wheat rhizoctonia pathogens and ways to increase plant resistance is relevant both in theoretical and practical terms.

The purpose and objectives of the study. To get insight into the determinants of the beneficial activity of *Streptomyces* sp. HU2014 and explore its potential as a biocontrol agent, the main objectives of this study were to assess its antifungal activities and ability to promote plant growth. The goal was to solve the following tasks:

1. To identify HU2014 strain with its whole genome sequencing.
2. To study the antifungal mechanism of HU2014 against WSE pathogen.
3. To separate the active compounds produced by HU2014.
4. To determine plant-growth promotion of HU2014 in wheat.
5. To investigate the allelopathy of HU2014 on wheat.
6. To explore the effects of HU2014 inoculation on soil factors and rhizosphere microbial structure.
7. To screen the fermentation conditions of HU2014.
8. To verify the biocontrol effect of HU2014 in field.

Object of study. Investigation the potential of HU2014 as a biocontrol agent with its antifungal activities and ability to promote plant growth.

Subject of study. *Rhizoctonia cerealis* Van der Hoeven and *R. solani*, *Streptomyces* sp. HU2014, active secondary metabolites produced by HU2014, wheat.

Research methods. General scientific methods: analysis, induction, deduction, synthesis; field methods – phenological observations of the collection of winter wheat varieties and biometric parameters of plant growth and development, in particular measuring, height, weight and control effectivity analysis; statistical methods – to generalize and determine the reliability of certain experimental results (variation, correlation, dispersion, cluster, factual).

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).

Scientific novelty and theoretical significance of the thesis. The scientific novelty of the obtained results is the isolation of a new strain of *Streptomyces* sp. HU2014, which will help to effectively regulate soil pathogens of wheat rhizoctonia.

Based on the analytical and experimental studies, for the first time in the work:

- a new strain of *Streptomyces* sp. HU2014 was isolated and identified;
- proved the effective antifungal effect of strain HU2014 against wheat rhizoctonia pathogens;
- the mechanisms of induced resistance of wheat after treatment of plants with *Streptomyces* sp. HU2014
- it was proved that *Streptomyces* sp. HU2014 promotes wheat growth by stimulating growth processes, improving soil nutrition of plants with changes in the microbial structure of the rhizosphere;

- the optimal conditions for cultivation of HU2014 were determined.

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

The theoretical significance of the work is to substantiate the modern approach to the discovery and disclosure of the effectiveness of a new biological object in the protection of wheat from root phytopathogens.

Practical significance of the thesis results. The results of the research show that a new strain HU2014 was identified, which proved to be effective in protecting wheat from *Rhizoctonia* spp. and can be used for the production of new biofungicides. Also, the identification 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 the germination of green mouse seed, so it is promising in the control of segetal vegetation.

The stimulating effect of the new strain on the growth and development of wheat plants due to improved soil nutrition and plant metabolism was proved.

The results are included in the curriculum for bachelor's degree programs in Plant Protection and Quarantine 202 at Sumy National Agrarian University.

Number of scientific publications. The results of the dissertation are presented in six articles (four in scientific professional journals of Ukraine, two articles in scientific professional journals included in the international database Scopus) and tested at seven international and Ukrainian conferences. A total of 13 scientific papers:

1. Zhu H., Zhou F., Rozhkova T. Quantitative changes of enzyme activity in wheat induced by *Streptomyces* sp. strain HU2014. Bulletin of Sumy National

Agrarian University. The series: Agronomy and Biology. 2020. Vol. 3. P. 57-62. DOI: 10.32845/agrobio.2020.3.7.

2. Zhu H., Cao Z., Rozhkova T., Hu L. Study on antifungal activity of hyphae extract of a *Streptomyces* strain HU2014 against four phytopathogenic fungi. Bulletin of Sumy National Agrarian University. The series: Agronomy and Biology. 2021. Vol. 3. P. 87-92. DOI: 10.32845/agrobio.2021.3.11.

3. Zhu H., Rozhkova T. Induction of wheat resistance by *Streptomyces* sp. HU2014 strain. Quarantine and Plant Protection. 2023. P. 38-43. DOI: 10.36495/2312-0614.2023.1.38-43.

4. Zhu H., Rozhkova T. Allelopathic effect of metabolites produced by *Streptomyces* sp. HU2014 on wheat and green bristlegrass. Bulletin of Sumy National Agrarian University. The series: Agronomy and Biology. 2023. Vol. 50. P. 8-13. DOI: 10.32845/agrobio.2022.4.2

5. Zhu H., Hu L., Hu H.-Y., Zhou F., Wang S., Wu L., Rozhkova T., Li C. Identification of a novel *Streptomyces* sp. strain HU2014 showing growth promotion and biocontrol effect against *Rhizoctonia* spp. in wheat. Plant Disease. 2023. P. 1-15. DOI: 10.1094/pdis-06-22-1493-RE. *Scopus*

6. Zhu H., Hu L., Rozhkova T., Wang X., Li C. Spectrophotometric analysis of bioactive metabolites and fermentation optimisation of *Streptomyces* sp. HU2014 with antifungal potential against *Rhizoctonia solani*. Biotechnology & Biotechnological Equipment. 2023. Vol. 37. P. 231-242. DOI: 10.1080/13102818.2023.2178822. *Scopus*

7. Zhu H., Rozhkova T., Hu L., Li Ch. Biocontrol of main diseases and advance in antifungal mechanism of *Streptomyces* spp. in wheat. Problems of ecology and ecologically oriented plant protection: materials of the international science and practice conference of the Faculty of Plant Protection of the Kharkiv National Agrarian University named after V.V. Dokuchaeva, October 17-18, 2019. Kharkiv: Madrid Printing House, 2019. P. 122 –124.

8. Zhu H., Rozhkova T., Li Ch. Interaction between plant and beneficial microorganisms in agriculture. Problems of ecology and ecologically oriented

plant protection. Materials of the International scientific and practical conference of plant protection of the Kharkiv National Agrarian University named after V.V. Dokuchaeva, dedicated to the 130th anniversary of the birth of academician VASGNIL, corresponding member of NASU, doctor of biological sciences, professor and first dean of the faculty T.D. Strahov (October 29-30, 2020). Kharkiv, "Planeta-print", 2020. P. 169-170.

9. Zhu H., Rozhkova T. O., Zhu Y. The promoted effect of *Streptomyces* sp. in wheat planting. Fundamental and applied problems of modern ecology and plant protection. Materials of the International Scientific and Practical Conference dedicated to the 100th anniversary of the birth of doctor of biological sciences, professor B.M. Lytvynov (Kharkiv, October 21-22, 2021). Kharkiv: Ivanchenko I. Publishing House. P. 55–57.

10. Zhu H., Rozhkova T. O., Zhu Y. Common weed species in wheat fields in Henan province, China. Fundamental and applied problems of modern ecology and plant protection. Materials of the International Scientific and Practical Conference dedicated to the 100th anniversary of the birth of doctor of biological sciences, professor B.M. Lytvynov (Kharkov, October 21-22, 2021). Kharkiv: Ivanchenko I. Publishing House. P. 57-59.

11. Zhu Hongxia, Wang Xinfu, Rozhkova T. Preliminary study on antifungal activity of a *Streptomyces* sp. strain HU2014 against phytopathogenic fungi. Topical issues of modern science, society and education. Proceedings of the 3rd International scientific and practical conference. SPC – Sci-conf.com.ua. Kharkiv, Ukraine. 2021. Pp. 21–25.

12. Hongxia Zhu, Yinghui Zhu, Rozhkova T. O. Research progress on active components of metabolites of *Streptomyces* sp. Effectiveness of agricultural technologies of Zhytomyr region: materials of the All-Ukrainian scientific and practical conference (November 10–12, 2021). Zhytomyr: ZHATFK, 2021. P.130-133.

13. Zhu H., Wang X., Rozhkova T. Study the allelopathy of the fermentation extracts from *Streptomyces* sp. HU2014 on cucumber. «Honcharivski chytannya»:

Materials of the International Scientific and Practical Conference dedicated to the 93rd anniversary of the birth of the Doctor of Agricultural Sciences, Professor Mykola Goncharov (May 25, 2022). Sumy, 2022. P. 165-166.

Discussion clauses, comments and wishes regarding the content.

These remarks do not diminish the scientific value of the dissertation and some of them only reflect the reviewer's scientific position, which can also serve as a reason for discussion. There are a few points worth discussing regarding the content of the dissertation:

1. The first chapter is based mainly on the publications of European, Asia and Chinese researchers. The works of domestic scientists remained underexposed;

2. Research methods are formed in a general way. It is desirable to indicate the used scientific research methods and to note exactly which phenomena and parameters were studied by each of the methods.

3. The work was carried out with extensive use of mathematical statistics methods, in particular, molecular calculations, analysis of variance. However, the results of these calculations should have been presented in a more detailed form in the appendices.

4. Recommendations would be advisable to make more detailed, as they reflect the prospect of using the results obtained by the researcher in the further work of the author himself and other scientists.

5. The proposals for production are too optimized, it would be desirable to disclose them in more detail.

6. Some language errors and formatting errors need to be polished.

These remarks do not reduce the scientific value of the thesis, and some of them only reflect the scientific position of the reviewer, which may also serve as an occasion for discussion.

General conclusion. The dissertation work of **Zhu Hongxia** intitled «*Biocontrol mechanisms of Streptomyces spp. 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» in the specialty 202 – «Plant Protection and Quarantine» 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. The PhD candidate **Zhu Hongxia** deserves to be awarded the scientific degree of Doctor of Philosophy in specialty 202 – «Plant Protection and Quarantine».

Official Reviewer:

Candidate of Agricultural Sciences,
Associate Professor of the
Department of Plant Protection,
Sumy National Agrarian University

Olha BAKUMENKO

