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ISOLATION AND SCREENING OF METHYL DISULFURON- DEGRADING MICROORGANISMS

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The widespread use of herbicides has caused significant pollution to the soil environment, which has seriously endangered people's life and development. Therefore, there is an urgent need for an effective method to control environmental soil pollution, and the degradation of residual pesticides by microorganisms is an effective bioremediation method developed in recent years. Taking the soil treated with methyl disulfuron as the research subjects, the microbial strains with high degradation effect on methyl disulfuron were isolated by plate gradient dilution method, the isolated strains were enriched and cultured, medicated and cultured, and soil colonization test, so as to determine the effect of methyl disulfuron on the strains. The results showed that a bacterium J20191108-1 and a fungus Tr20191108-E were isolated from soil, and no actinomycetes were isolated. The bacterial colony is milky white, the surface is wet and smooth, and the fungus is preliminarily identified as Trichoderma. The bioaccumulation test of bacteria J20191108-1 showed that there was no significant difference in the external morphology and number of bacterial colonies growing on the two media with and without chemicals, in the soil colonization test, the bacteria grew better in the medium with herbicides. Trichoderma Tr20191108-Ecultured on plates with different drug contents had little effect on colony growth. Fungus grew all over the plate after 2 days of culture. The variance analysis of the spore yield of the fungus by SPSS showed that the p value was 0.163 > 0.05, indicating that there was no significant difference between the concentration of methyl disulfuron and the spore yield of the fungus. It can be seen that the concentration of methyl disulfuron has little effect on the external morphology of bacteria, but the concentration of pesticide has a certain effect on the growth rate of fungi.

Key words: *methyl disulfuron, degrading bacteria, bioremediation, enrichment culture, soil colonization.*

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Introduction. Methyl disulfuron, also known as metsulfuron methyl, is developed by Bayer crop science company in Germany, it has the characteristics of ultra-high efficiency, wide herbicidal spectrum and environmental friendliness, it can be widely used in field weeding of wheat, corn, soybean and other crops (Singh et al., 2018; Vazan et al., 2011; Zhang et al., 2009). It belongs to sulfonylurea high-efficiency herbicide, which acts by inhibiting acetyllactate synthase, is absorbed by weeds' roots and leaves, is conducted in plants, and makes weeds die after stopping growth (Yin et al., 2010; Tang, 2008). However, due to the long-term unscientific application of pesticides, with the widespread use of herbicides, it has caused serious pollution to the soil and environment, which has seriously endangered people's life and development. Therefore, an effective method is urgently needed to control environmental soil pollution.

Pesticide residue degradation technology based on microbial remediation theory is a safe, effective and cheap way to solve residual drug harm, this method has the advantages of non-toxic, no residue and no secondary pollution (Cai et al., 2020; Zhou et al., 2016). This technology is a new technology for soil pollution control developed in recent 20 years (Liang, 2020; Hu, 2020; Dong, 2020), it mainly converts organic pollutants in the environment into small substances such as CO_2 and H_2O with little impact on the environment and no secondary pollution through the action of microorganisms. At present, there are many types of researches on microbial degradation of pesticides at home and abroad, but most of them are on the isolation, degradation characteristics and factors affecting the degradation rate of pesticide degrading bacteria (Wang et al., 2018; Wei et al., 2007; Cheng et al., 2017), most of the work is still limited to the laboratory, and the research is not very in-depth (Zhou et al., 2013; Su et al., 2019; Zabaloy et al., 2014). For example, the research of Thirunarayanan et al. (1985) shows that when the pH is 6.2, the half-life of Chlorsulfuron is 88.5 D, and when the pH increases to 8.1, its half-life is up to 144 days. Xu et al. (2003) showed that the abiotic degradation of pyrazosulfuron was faster at low pH than at neutral conditions, and the degradation of pyrazosulfuron by Pseudomonas was faster at pH 7.0 than pH 9.0. Dinelli et al. (1997) studied the relationship between the degradation rate and temperature of four sulfonylurea herbicides and concluded that the higher the temperature, the shorter the degradation half-life, at the same time, they established the functional relationship between the degradation rate and temperature and humidity to predict their degradation and loss. At present, there is no study on the degradation of methyl disulfuron by microorganisms.

In this study, the high-efficiency degrading bacteria were isolated from the soil using methyl disulfuron for many years, the degrading bacteria were isolated from the plots containing methyl disulfuron herbicide, enriched and cultured, colonized in the soil and grew in the medicated medium. The purpose was to provide reference for bioremediation and toxic degradation of soil polluted by pesticide methyl disulfuron by microorganisms.

Materials and methods. In July 2021, the surface layer soil of methyldisulfuron (96% lukuan Agricultural Technology Co., Ltd.) was collected from the test field in Xinxiang City, China, and the soil was sampled at 5 points. The soil was air dried at 25 ℃ and sieved at 60 meshes and stored for standby. Fully stir according to soil water 4:5 to remove impurities. The filtered solution was the soil leaching solution at 4 ℃ for standby (Zheng et al., 1990).

10g soil sample was added into a triangular flask of 100 ml water and incubated for 24 hours. Pipette 1 ml of stocked solution, injected it into a 10 ml centrifuge tube, added 9 ml of sterile water, and diluted it to 10^{-8} successively, used a pipette gun to suck the concentration stock solution (fungi: 10^{-3}, 10^{-4}, 10^{-5}, bacteria: 10^{-6}, 10^{-7}, 10^{-8}, actinomycetes: 10^{-3}, 10^{-4}, 10^{-5}) 100 µ L was added drop wise on three kinds of culture media, evenly coated, each concentration was repeated three times, and pure culture was obtained after culture at 28 ℃ (Wang et al., 2011;Ying et al., 2007; Hooda et al., 2015). According to the field application amount (0.06 mg / L), the technical drug of methyldisulfuron was added into a triangular flask with 100 ml LB liquid medium and 1g soil sample, based on the field concentration (0.06 mg / L), diluted to 10^{-6}, 10^{-7}, 10^{-8} and 10^{-1}, 10^{-2} and 10^{-3} respectively by gradient dilution method, and applied them on beef extract peptone medium and PDA plate respectively. The growth of bacteria and fungi (Shen et al., 2020; Li et al., 2020) was observed. Separately packed 50ml of soil leaching solution into a triangular flask, made three dosing and three controls, connected the purified single bacterial colony to the soil leaching solution, and observed its absorbance on the 3th days and 7th days respectively (Sun et al., 2017; Chen., 2018).

Results. Microorganisms were isolated from the soil, where methyl disulfuron was used for many years. In the experiment, the gradient dilution method was used for the separation of degrading bacteria in the soil. The prepared gradient dilution was added to beef extract peptone medium (isolated bacteria), PDA medium (isolated fungi) and Gao's No. 1 medium (isolated actinomycetes) respectively, evenly coated, and single colonies with good growth were selected from the cultured medium. Pure culture was obtained after lineation purification and perforation inoculation. After culture, a bacterium J20191108-1 and a fungus Tr20191108-E were isolated, and no actinomycetes were isolated. It was shown by colony morphology and electron microscope photos. Bacteria were milky white, with smooth and moist surface, the fungus was *Trichoderma*, with white hyphae at first, and then the hyphae turned green from the middle. Diagnosis of these species will be carried later.

Then conducted a study of the effect of the herbicide on the growth of bacterial colonies. The bacteria J20191108-1 were bioaccumulated and cultured, the bacterial diluent was evenly applied to the beef extract peptone medium of methyl disulfuron, and the control was set. It was found that methyl disulfuron did not affect the growth and morphology of bacterial colonies (Fig.1).

It could be seen from Figure 1 that there was no significant difference in the external morphology of bacterial colonies grown on the two media with and without dosing. The colonies were milky white, with wet and smooth surface. It could be seen that the concentration of methyl disulfuron had no effect on the external morphology of bacteria. The bacteria could continue to grow under the action of methyl disulfuron.

Next, in the experiments, the soil colonization test was carried out on the bacterium J20191108-1. The soil leaching solution was sub packed into a triangular flask, 50 ml of soil leaching solution for each bottle, and the control was set. The purified single bacterial colony was picked up and connected to the soil leaching solution, the absorbance on the 3rd and 7th days was observed with a spectrophotometer to compare the effect of the drug on bacterial growth (table. 1).

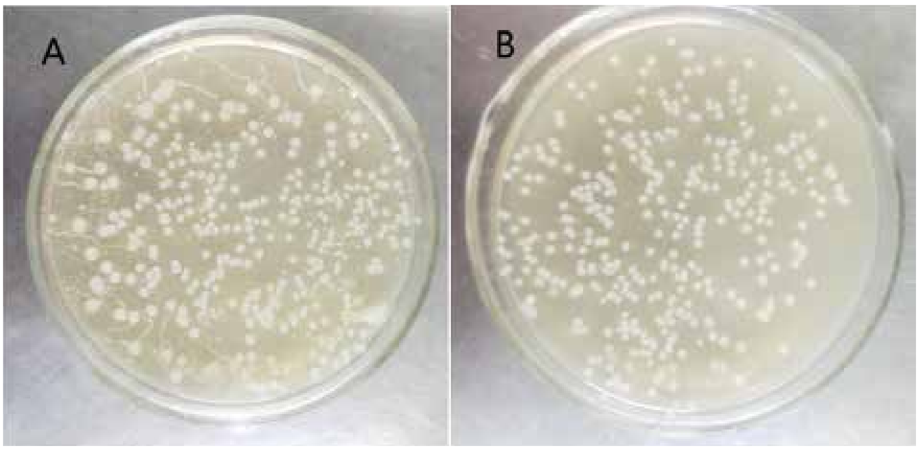

Fig. 1. Morphology of bacterial colonies (J20191108-1):
A – dosing; B – No dosing

It was found from the results of soil colonization test of bacteria J20191108-1 in Table 1, through the analysis of variance by SPSS, the P value on the third day was 0.581 > 0.05, and the p value on the seventh day was 0.254 > 0.05. The difference was not statistically significant, and the bacteria grew better in the environment where the pesticide existed. Therefore, it showed that the bacteria had a certain degradation effect on methyl disulfuron.

The next step in our research was to test the growth of fungus Tr20191108-E on the first and second days medium with and without the addition of herbicide (Fig. 2).

It could be seen from Fig. 2 that there was little difference in the colony diameter of fungi with and without drugs on the first day (Fig. 2-AB). On the second day, all fungi were full of Petri dishes, and the diameter of fungi was 90 mm (Fig. 2-CD). But the fungus grew better on a nutrient medium with the addition of herbicides.

Next we continued to study the growth of fungus Tr20191108-E on the first day, the purified fungi were perforated and connected to the PDA plate, numbered, cultured in the dark in the incubator at 28 ℃, and the diameter of fungi was measured by cross method (Table. 2).

The p value obtained by SPSS analysis of variance was 0.23 > 0.05, indicating that there was no significant difference among treatments. Therefore, the concentration of methyl disulfuron had no effect on the growth rate of fungi.

Next, we studied and observed the features of sporulation Tr20191108-E (Fig. 3).

It could be seen from Fig. 3 that after Trichoderma completely turned green, the spore production of different treatments was measured (Fig. 3-A), and the blood cell count plate was used for detection (Fig. 3-B).

Finally, after all the fungi turned green, all fungi on the culture dish were eluted with 10 ml normal saline, diluted with sterile water in a certain proportion, and the number of spores was observed with a blood cell counting plate (the best number was 5-6 spores per small grid), and then the number of fungal spores per 1 ml was calculated (Table.3).

It could be seen from table 3 that although the spore production decreases with the decrease of concentration, the difference was not particularly obvious. The analysis of variance by SPSS showed that the p value was 0.163 > 0.05, indicating that the difference of pesticide concentration on spore production was not significant. However, the fungus could continue to grow with methyl disulfuron and had a certain growth promoting effect, indicating that the fungus had a certain degradation effect on methyl disulfuron.

Discussion. Microbial degradation had the advantages of low investment, non toxicity, no residue and no secondary pollution, and the treatment effect was relatively obvious. It was recognized as a low-cost and environment-friendly method to remove pollutants (Tatiane et al.,2007; Singh et al., 2010; Das et al., 2011; Wang ,2017). In this experiment, the strains were isolated and screened from the poluted soil treated with methyldisulfuron for a long time

Table 1

Absorbance on the 3th and 7th day of soil colonization

	Dosing		No dosing	
Absorbance (unit: 1)	the 3th day	the 7th day	the 3th day	the 7th day
Average value±standard	0.034±0.026a	0.035±0.026a	0.059±0.042a	0.0003±0.0003a

Note: the data in the table are mean ± standard error. The lowercase letters after the data indicate the difference at the 0.05 level

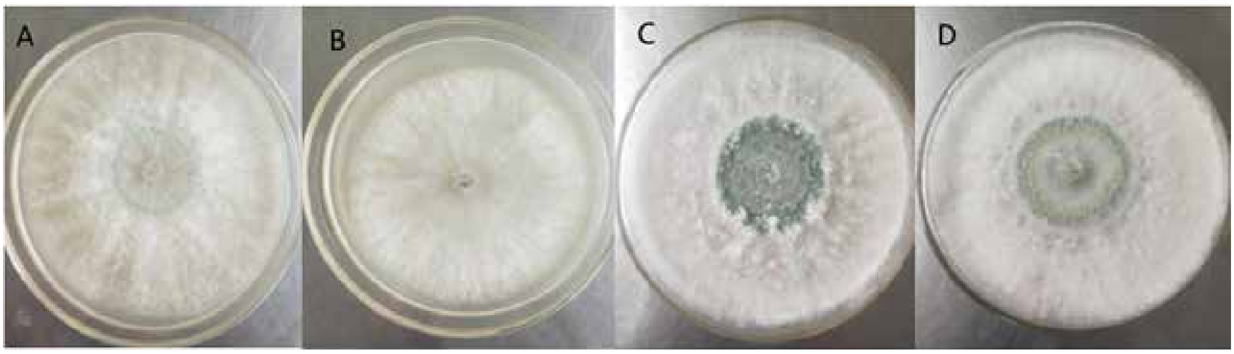

Fig. 2. Morphology of Trichoderma colonies: A – the first day of dosing, B- the first day without dosing, C – the second day after dosing, D – the second day without dosing

Table 2

Growth of fungus Tr20191108-E on the first day

Colony diameter(mm)	Concentration			
	10-1	10-2	10-3	CK
average value±standard	76.00±1.00a	78.67±2.60a	79.33±2.91a	75.33±0.67a

Note: the data in the table are mean ± standard error. The lowercase letters after the data indicate the difference at the 0.05 level

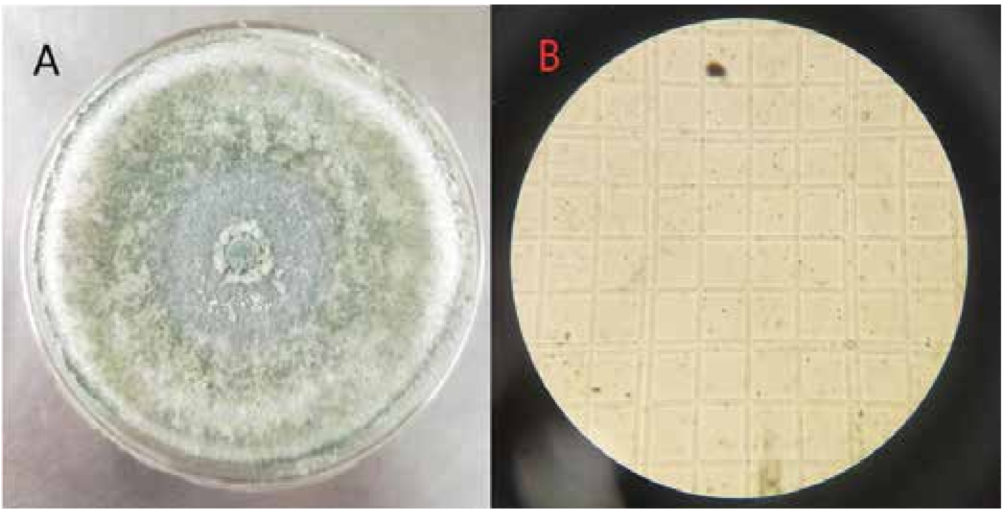

Fig. 3. Counting conidia of the fungus Tr20191108-E

Table 3

Spore production of fungi under different concentrations

Spore yield (10^9/mL)	Concentration			
	10^{-1}	10^{-2}	10^{-3}	CK
average value±standard	2.24±0.06a	1.84±0.06a	1.57±0.02a	1.71±0.38a

Note: the data in the table are mean ± standard error. The lowercase letters after the data indicate the difference at the 0.05 level

in the experimental field of Xinxiang City, China. By using the plate gradient dilution method, a bacterium J20191108-1 and a Trichoderma Tr20191108-E were finally obtained. Through the observation of individual morphology and electron microscope, the bacteria were milky white, the surface was smooth and moist, and the fungus was *Trichoderma* fungi.

This study showed that the higher the concentration of methyl disulfuron degrading bacteria, the stronger the degradation characteristics, which showed that the degradation change of the strain was basically consistent with the growth trend, that was, the higher the degradation rate, the greater the growth, which was consistent with the results of Chen et al. (2011), Liang et al. (2019) and Ye et al. (2017). In the crop soil where pesticides were widely used for a long time, the degradation ability of bacteria and fungi to pesticides was greater than that of crop soil where pesticides were less used, and the long-term use of a single pesticide may lead to bacterial gene mutation, so as to improve the degradation characteristics of pesticides. At the same time, the large number of bacteria and fungi with different degradation ability in soil further proved that soil bacteria and fungi played an irreplaceable role in environmental remediation (Wu et al., 2007; Zhu et al., 2012; Zhou et al., 2008). However, the application of microbial degradation in the environment needs to be improved. At present, the research on microbial degradation was mainly carried out under pure culture conditions, which was very different from the environmental conditions and pesticide treatment load in natural ecology (Li et al., 2008; Tian et al., 2016). Therefore, its application research in the environment needs to be further strengthened.

Conclusions. In this study, high-efficiency degrading bacteria were screened and isolated from cultivated soil using methyl disulfuron for many years. Through gradient acclimation, separation and purification, a bacterium J20191108-1 and a *Trichoderma* Tr20191108-E were finally obtained. Through the study on the effect of methyl disulfuron on the growth of bacteria J20191108-1, it was found that the addition of herbicide did not affect the external morphology and quantity of bacterial colonies, and the bacteria could continue to grow. The results showed that the bacteria grew better in the presence of herbicide environment and had a certain degradation effect on methyl disulfuron. The cross method was used to measure the diameter of fungus Tr20191108-E. The experiment showed that the concentration of methyl disulfuron basically had no effect on the growth rate of fungi. After *Trichoderma* turned green, the spore yield was measured. The results showed that fungi could continue to grow with methyl disulfuron and had a certain biodegradation of herbicide.

References:

1. Andersen, S. M., Hertz, P. B., Holst, T., Bossi, R., & Jacobsen, C. S. (2001). Mineralisation studies of 14C-labelled metsulfuron-methyl,tribenuron-methyl,chlorsulfuron and thifensulfuron-methyl in one Danish soil and groundwater sediment profile. Chemosphere, 45(6–7), 775–782. doi: 10.1016/s0045-6535(01)00132-1

2. Brown, H. M., Joshi, M. M., Van, A. T., Carski, T. H., & Doughty, J. (1997). Degradation of Thifensulfuron Methyl in soil:Role of Microbial Carboxyesterase Activity. Journal of Agricultural&Food Chemistry, 45(3), 955–961. doi: 10.1021/jf9604145

3. Cai, D. M., Huang, W., Ren, Y. L., Xue, Y. Z., Ren, Z. Z., Liu, H. J., & Shao, H. X. (2020). Study on Mitigation Measures of Wheat after Spraying SigMa.Tillage and Cultivation. 40(1), 24–26. doi: 10.13605/j.cnki.52-1065/s.2020.01.007

4. Cheng, J., Du, H. L., Zhang, D. B., & Wang, W. (2017). Isolation and Identification of tetracycline degrading bacteria. Journal of Nuclear Agricultural Sciences, 31(5), 884–888. doi: 10.11869/j.issn.100-8551.2017.05.0884

5. D. Chen., & G. A. Zentmyer. (2018). Production of Sporangia by Phytophthora Cinnamomi in Axenic Culture, Brief Articles, 62(2), 397–402. doi: 10.1080/00275514.1970.12018977

6. Das, N., & Chandran, P. (2011). Microbial Degradation of Petroleum Hydrocarbon Contaminants:An Overview. Biotechnology Research International, 10, 1–13. doi: 10.4061/2011/941810.

7. Dinelli, G., Vicari, A., Bonetti, A., & Catizone, P. (1997). Hydrolytic Dissipation of Four Sulfonylurea Herbicides. J.Agric. Food Chem, 45, 1940–1945. doi: 10.1021/jf960649g

8. Dong, H. F. (2020). Research status and trend of contaminated soil remediation technology. Shanxi chemical industry, 1, 163–164, doi: 10.16525/j.cnki.cn14-1109/tq.2020.01.59

9. Hooda, R., Bhardwaj, N. K., & Singh, P. (2015). Screening and Identification of Ligninolytic Bacteria for the Treatment of Pulp and Paper Mill Effluent. Water Air Soil Pollut, 226(9), 305. doi:10.1007/s11270-015-2535-y

10. Hu, X. (2020). Research status and trend of contaminated soil remediation technology. New technologies and products in China, 1, 124–125. doi: 10.13612/j.cnki.cntp.2020.02.054

11. Li, S. G., & Li, R. Q. (2008). Research Progress on biodegradation of organophosphorus pesticides. Scientific and technological information development and economy, 18(2), 123–124. doi: 10.3969/j.issn.1005-6033.2008.02.071

12. Li, Y., Li, H. P., & Yang, Z. G. (2020). Study on the growth and degradation characteristics of a strain of methylparaben degrading bacteria. Science and technology wind, 3(10), 176–177. doi: 10.19392/j.cnki.1671-7341.202010158

13. Liang, Z. D. (2020). Research status and contaminated technology. Environment and development, 2, 79–80. doi:10.16647/j.cn15-1369/x.2020.02.045

14. Paporisch. A., Laor, Y., Rubin, R., & Eizenberg, H. (2020). Effect of Repeated Application of Sulfonylurea Herbicides on Sulfosulfuron Dissipation Rate in Soil. Agronomy, 10(11), 1724. doi: 10.3390/agronomy10111724

15. Shen, Z.H., Wang, Y., Li, H. C., Mao, X. X., Feng, F. Y., Ren, L. Y., & Yu, X. Y. (2020). Isolation,identification and degradation characteristics of a fluconazole degrading strain. Jiangsu Agricultural Sciences, 48(4), 273–278. doi: 10.15889/j.issn.1002-1302.2020.04.049

16. Singh, A., Kewat, M. L., & Sondhia, S. (2018). Studies on the effect of day time application of herbicide mesosulfuronmethyl on soil microbial communities of wheat rhizosphere. Journal of envirenmental Biology, 39(1), 59–65. doi: 10.22438/jeb/39/1/mrn-562

17. Singh, B. K., & Walker, A. (2010). Microbial degradation of organophosphorus compounds. Fems Microbiology Reviews, 30(3), 428–471. doi: 10.1111/j.1574-6976.2006.00018.x

18. Su, Z. J., Liu, Y. T., Xu, C. J., Ding, H., Yu, L. X., & Ai, X. H. (2019). Isolation,identification and degradation characteristica of a deltamethrin-degrading bacterial strain. Freshwater Fisheries, 49(1), 98–106. doi: 10.13721/j.cnki.dsyy.2019.01.018

19. Sun, Z. N., Cui, H. C., Wang, H. L., Ren, X. B., & Liu, H. R. (2017). Screening of sporangium producing medium and induction method of Phytophthora. Science Technology and Engineering, 17(19), 18–23. doi: 10.3969/j.issn.1671-1815.2017.19.004

20. Tang, J. M. (2008). Malignant gramineous weeds and their suitable herbicides in wheat field of Jiangsu Province. Weed science, 3, 75–77. doi: 10.19588/j.issn.1003-935x.2008.03.026

21. Tatiane, M. S., Maria, I. S., Ander, M. M., Fabiana, D. A., Sonia, A. V. P., Paulo, R. F., Marcelo, D. C., Emanuel, C., Paulo, I. B. C., & Marcos, P. (2007). Degradation of 2, 4-D herbicide by microorganisms isolated. Brazilian Journal of Microbiology, 38, 522–525. doi: 10.1590/S1517-83822007000300026

22. Thirunarayanan, K., Zimdahl, R. L., & Smika, D. E. (1985). Chlorsulfuron adsorption and degradation in soil. Weed Science, 33(4), 558–563. doi: 10.1111/j.1365-3180.1985.tb00668.x

23. Tian, C. Y., Xu, J., Dong, F. S., Liu, X. G., Wu, X. H., & Zheng, Y. Q. (2016). Research Progress on microbial degradation of triazole fungicides. Journal of Pesticide Science, 18(2), 141–150. doi: 10.16801/j.issn.1008-7303.2016.0019.

24. Vazan, S., Oveisi, M., & Baziar, S. (2011). Efficiency of mesosulfuron-methyl and clodinafop-propargyl dose for the control of Lolium perenne in wheat. Crop Protection, 30(6), 592–597. doi: 10.1016/j.cropro.2011.01.004

25. Wang, J. W., Zhen, J., Xie, B. E., Liu, Y. Y., & Li, G. J. (2011). Isolation,screening and preliminary identification of organophosphorus degrading bacteria. Henan Science, 29(1), 31–34. doi: 10.3969/j.issn.1004-3918.2011.01.010

26. Wang, X. W. (2017). Research Progress on degradation modes of sulfonylurea herbicides in soil. Journal of Shandong Agricultural Engineering College, 34(10), 164–165. doi: 10.15948/j.cnki.37-1500/s.2017.10.081

Wang, X., Sun, S. Y., & Zhang, H. W. (2018). Advances in the research on microbial degradation of sulfonylurea herbicides. Chinese Journal of Ecology, 37(11), 3449–3457. doi:10.13292/j.1000-4890.2018.11.021

Wei, M., & Li, Y. M. (2007). Advance and Prospect of Microorganism Degrading Soil Pesticide. Shandong chemical industry, 3, 15–18. doi: 10.19319/j.cnki.issn.1008-021x.2007.06.006

27. Wu, H. P., & Zheng, F. C. (2007). Research progress of microbial degradation of organophosphorus pesticides. Guangxi Agricultural Science, 38(6), 637–642. doi: 10.3969/j.issn.1004-874X.2008.01.018

28. Xu, J., Li, Y. X., Qiu, L. H., & Pan, C. P. (2009). Biodegradation of pyrazosulfuron-ethyl by three strains of bacteria isolated from contaminated soils. Chemosphere, 74(5), 682–687. Doi: 10.1016/j.chemosphere.2008.09.078

29. Yin, L. B., Liu, Y., Zhang, D. Y., Zhang, S. B., & Zhang, G. M. (2010). Research Progress on Microbial Degradation of Sulfonylurea Herbicides.Microbiology bulletin, 37 (4), 594–600. doi: 10.13344/j.microbiol.china.2010.04.014

30. Ying, F., Xie, M., Wan, F. H., Wang, R., Qiu, W. L., & Li, L. (2007). Isolation and screening of atrazine degrading bacteria. Chinese Journal of Biological Control, 23(4), 368–372. doi: 10.16409/j.cnki.2095-039x.2007.04.017

31. Zabaloy, M. C., & Gomez, M. A. (2014). Isolation and characterization of indigenous 2,4-D herbicide degrading bacteria from an agricultural soil in proximity of Sauce Grande River, Argentina.Ann Microbiol, 64, 969–974. doi: 10.1007/s13213-013-0731-9

32. Zhang, S. J., Yang, G. L., Zheng, Z. S., Chen, Y. D., Wang, L., & Qian, X. (2009). Residue dynamics of methyl disulfuron in wheat and soil,Journal of Agricultural University of Hebei. 32(3), 5. doi: 10.3969/j.issn.1000-1573.2009.03.003

33. Zheng, X. B., & Lu, J. X. (1990). A preliminary study on the stimulation of soil extract to Phytophthora producing Ascomycetes. Journal of Nanjing Agricultural University, 13(4), 125. doi: 10.7685/j.issn.1000-2030.1990.04.025

34. Zhou, H., Wang, D. M., Tan, H. J., Meng, N. C., & Liao, Y. J. (2017). Isolation and screening of a naphthalene degrading bacterium. Journal of Northwest University of Nationalities, 38(4), 36–40. doi: 10.14084/j.cnki.cn62-1188/n.2017.04.009

35. Zhou, J. H., Huang, R. X., Fan, H. X., Tian, S. N., Li, Z. X., Yang, W., Li, T., & Gao, Q. (2016). A Review on the Progresses of Remediation Technologies for Contaminated Soils.Reserach of Soil and Water Conservation, 23(3), 366–372. doi: 10.13869/j.cnki.rswc.2016.03.064

36. Zhou, J. H., Sun, X. W., Hu, F., & Li, H. X. (2013). Isolation,Screening and Identification of Prometryne-Degrading Bacteria and Their Characteristics. Environmental Science, 34(7), 2894–2898. doi: 10.13227/j.hjkx.2013.07.005

37. Zhu, X. K., Yu, A. L., Zhu, Y. Z., & An, L. J. (2012). Isolation and screening of 114 strains of pesticide degrading bacteria from agricultural cultivated soil. Shandong Agricultural Science, 44(6), 88–92. doi: 10.14083/j.issn.1001-4942.2012.06.002

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Виділення та скринінг мікроорганізмів, які розкладають метилдисульфурон

Широке використання гербіцидів спричинило значне забруднення ґрунтового середовища, що серйозно загрожує життю та розвитку людства. Тому існує нагальна потреба в ефективному методі контролю забруднення ґрунту, а деградація залишкових пестицидів мікроорганізмами ефективний метод біоремедиації, розроблений в останні роки. Метилдисульфурон, також відомий як метсульфурон-метил, розроблений компанією Bayer Crop Science у Німеччині, активно використовується у КНР і призводить до його накопичення у ґрунті з наступною післядією на сільськогосподарські культури. Для дослідження використали зразки ґрунту з метилдисульфуроном (96% Iukuan Agricultural Technology Co., Ltd.), які зібрали у липні 2021 р. з випробувального поля у місті Сіньсян, провінції Хенань. Беручи за предмети дослідження ґрунт, забруднений метилдисульфуроном, штами мікробів з високим ефектом деградації на гербіцид, провели виділення методом градієнтного розведення, ізольовані штами збагачували та культивували, обробляли та культивували, а також тестували на колонізацію ґрунту, щоб визначити вплив метилдисульфурону на штами. Приготоване градієнтне розведення додавали до пептонного середовища яловичого екстракту (для виділення бактерій), середовища PDA (для ізоляції грибів) та середовища Гао № 1 (для виділення актиноміцетів). З культивованих середовищ відбирали поодинокі колонії з хорошим зростанням. Результати показали, що бактерія J20191108-1 та гриб Tr20191108-E були виділені з ґрунту, а очікувані актиноміцети не виділили. Колонії бактерій виявились молочно-білими з вологою та гладкою поверхнею. Гриб попередньо ідентифікували як Trichoderma. Тест на біоакумуляцію бактерій J20191108-1 показав, що не було суттєвої різниці у зовнішній морфології та кількості бактеріальних колоній, які виростили на двох середовищах з хімічними речовинами та без них. У тесті на колонізацію ґрунту бактерії краще росли на середовищі з гербіцидами. Trichoderma Tr20191108-вирощування на чашках з наявністю на відсутністю препарату мало вплинуло на ріст колонії, гриб зайняв усю чашку через 2 дні культивування. Візуально відмітили краще спороношення гриба у варіанті з гербіцидом, тому провели підрахунок конідій Trichoderma. Але дисперсійний аналіз утворення спор гриба показав, що значення р було 0,163 > 0,05, що вказує на відсутність суттєвої різниці між концентрацією метилдисульфурону та кількістю конідій гриба. Визначили, що концентрація гербіциду мало впливає на зовнішню морфологію бактерій, але цей показник має певний вплив на швидкість росту грибів.

Ключові слова: *метилдисульфурон, бактерії-деструктори, біоремедиація, збагачувальна культура, колонізація ґрунту.*