

QUANTITATIVE CHANGES OF ENZYME ACTIVITY IN WHEAT INDUCED BY *STREPTOMYCES* SP. STRAIN HU2014

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Biocontrol microorganism have a diverse range of activities and they have been focused on potential biocontrol agents in agriculture. They can induce plant defended response and enhance plant disease resistance. *Streptomyces* sp. produce active metabolites that can inhibit the growth of phytopathogens. Induced resistance is usually indicated by the activity of Peroxidase (POD), Polyphenoloxidase (PPO), and Phenylalanine ammonia-lyase (PAL) or other defense enzymes. The related reports mainly focused on disease control or promoting growth of cash crops or vegetables, but less on wheat presently. Moreover, the information about the concentration of fermentation broth and mycelia of *Streptomyces* affected the quantitative changes of defended enzyme activities is limited. In this study, we started from isolating a *Streptomyces* strain, named *S. sp.* strain HU2014, and demonstrated (POD), (PPO), (PAL) enzymes in different concentration of the mycelia (M) and extracellular filtrate (EF) of the strain with the application of soil drench treatment. The enzyme activities were determined by visible spectrophotometry. The results showed that the activities of POD and PAL at the concentration of 10³-fold dilution of the EF increased significantly to some extent in comparison with untreated control, by 173.86 % ($P < 0.05$) and 71.92 % ($P < 0.05$), respectively. In the range of different concentration of the M, POD and PPO activities were enhanced with the increasing of dilution ratio, but the difference was not significant. It is expected to be an excellent resource for the development of new biological preparations.

Key words: Biocontrol microorganism, induced activity, defense enzymes, crops

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Introduction. Many microorganisms as biocontrol agents and their abundant sources of natural substances which have been studied and developed as commercial products for crop protection (Katz et al., 2016), such as *Bacillus thuringiensis* (Bt) from bacterium (Burgerjon et al., 1977; Cannon, 1993), Topshield (*Trichoderma harzianum* T-22) (Lisa, 2001) from fungus, Jing-gangmysin (in China) and streptomycin sulfate from *Streptomyces*.

Microorganisms become an environmentally and economically viable alternatives to the use of synthetic chemicals in plant growing (Rey et al., 2017). Especially, *Streptomyces* spp. constitute a major clade of the phylum Actinomycetes. These Gram-positive, filamentous prokaryotes are abundant in soils, commonly colonize the rhizosphere and marine sediments or can be caused infections in plants (Bignell et al., 2010; Bulgarelli et al., 2013). Over 50 % of them have been known for their role as producers of useful antibiotics (Chater, 2006; Cheng et al., 2014; Law et al., 2017) and the capacity to produce of secondary metabolism (van der Meij et al., 2017). Since *Streptomyces* were found that they have a diverse range of activities these microorganisms were used for a wide range of applications in different

filed. Researchers focus on potential biocontrol agents in agriculture (Schrey et al., 2008; Viaene et al., 2016).

Plant rhizosphere growth promoting bacteria (PGPR) can induce plant systemic disease resistance (ISR) (Abbasi et al., 2019; Farag et al., 2013; Sadeghi et al., 2017). Another pathway is systemic acquired resistance (SAR) which need pathogen or chemical trigger mediated (Kloeppe et al., 1999; Milikisoyants et al., 2017; Pieterse et al., 2002). Most biocontrol microorganisms have shown that they can induce plants to produce SAR or ISR which strengthen the resistance of plants to pathogens.

The enzymatic activities of PPO, PAL, β -1,3-glucanase and chitinase were significantly enhanced in the rice treated with antifungalmycin N2 plus *Rhizoctonia solani* Kühn (Zhang et al., 2020). *Streptomyces rochei* A-1 treatment significantly increased the activities of POD, superoxide dismutase (SOD), catalase (CAT) and PAL, effectively induced the resistance of apple fruit to ring rot (Zhang et al., 2016).

The V76-12 isolate was the most effective treatment tested in reducing leaf spot disease of oil palm seedlings, due to its ability to enhance PAL, POD and PPO activities in the oil palm leaves (Sunpapao et al., 2018). SA from the strain of *Streptomyces diastatochromogenes* KX852460 induced the high activity of

glutathione reductase (GST), catalase (CAD), PAL and PPO in tobacco against *R. solani* AG-3 (Ahsan et al., 2019).

Six endophytes stimulated systemic resistance which was evaluated by seed treatments in pathogen inoculated chickpea (Singh et al., 2017). *Streptomyces rubrogriseus* HDZ-9-47 enhanced the activity of PPO, POD, PAL and SOD in tomato roots (Jin et al., 2016). *S. rochei* D74 and *S. partum* Act12 (Liu et al., 2018) and *S. rochei* strain ZZ-9 (Xie et al., 2019) strongly enhanced the defense activity in wheat leaves. Otherwise, there are few studies investigated that antimicrobials at very low concentrations have high inhibiting effect or eliciting activities (Boukaew et al., 2017; Hennessy et al., 2017; Winding et al., 2004). Balajiu, Kim et al. (2016) studied that paromomycin at lower concentration (1.0 µg/ml) induced the suppression of *Phytophthora capsici* in chili pepper, higher than 100 µg/ml and 1000 µg/ml.

The aim of this study was to reveal the dynamic changes of different enzyme activities in wheat treated with *S. sp.* strain HU2014, and provide the scientific basis for its application in the field.

Materials and methods. 2.1. *Materials.* Soil samples were collected in May 2020 from the field where commonly wheat is grown, in Xinxiang, China (Benton Harbor: N 113.9351°, E 35.3829°), air dried at 25 °C, thoroughly sieved to remove roots and plant residues, and stored until use.

Streptomyces HU2014, kindly provided by Dr. Hu Linfeng of Henan Institute of Science and Technology (HIST), was pre-cultured on potato dextrose agar (PDA) medium at 4 °C before experiment. The mycelia discs were transferred to sterile GPY broth in 250 ml flasks, incubated at 30 °C with shaking at 150 r. min⁻¹ for 28 days. The fermentation broth was centrifuged (8000 r min⁻¹, 4 °C) for 15 minutes to separate the supernatants. The supernatants were filtrated through 0.45 µm candle filters, and then the extracellular filtrate (EF) was stored at 4 °C. The mycelia were incubated for 7 days, filtrated through nylon filter, washed with sterile water, dried with filter paper and stored at 4 °C.

Wheat cultivar of "BN4199" afforded by Breeding Center of HIST was used in this study. The seeds were disinfected with H₂O₂ (30 %) for 2 min and washed thoroughly with sterile distilled water. The seeds were germinated for 12 hours in plastic tray

(30 cm × 20 cm × 3 cm) covered with wet gauze. Subsequently, the germinated seeds were transferred in the pot (12 cm × 12 cm × 9 cm), 15 seeds per pot, placed into growth chamber with the conditions of light (12 h/day) and temperature (27 °C ± 2 °C).

2.2. *Assay of defense enzymes.* The EF was considered as original broth concentration, and five concentrations were set up by adding sterile water to the EF: 10-fold dilution, 10²-fold dilution, 10³-fold dilution, 10⁴-fold dilution and 10⁵-fold dilution. An aliquot was applied by soil drench of 50 ml with 3 replications. The gradient concentration of the M was 0.1 mg/ml, 0.01 mg/ml, 0.001 mg/ml, 0.0001 mg/ml and 0.00001 mg/ml. Sterile water was untreated control. Each experiment had randomized design. Sterile water was untreated control. In ten days after soil drench treatment, leave tissues were collected and weighted, 0.1 g per aliquot for one enzyme activity assay. Then tissues were immediately submerged in liquid nitrogen. Material was ground in mortar with a pestle under liquid nitrogen, transferred into centrifugal tube. The enzyme activity was determined by POD colorimetry (Doerge et al., 1997), PPO colorimetry (Tang et al., 2004) and PAL colorimetry (Aydaş et al., 2013). All detailed steps referred to the instruction of Kit Box (Beijing Solarbio Science & Technology Co., Ltd, in China).

2.3. *Statistical methods.* Statistically significant differences (p < 0.05) in enzyme activity assay were evaluated by an analysis of variance (ANOVA) using SPSS version 16.0 (SPSS Inc. Chicago, IL, United States). All data shown are average value of three (in vitro experiments) biological replicates ± SD.

Results. Induced resistance is usually indicated by the activity of POD, PPO and PAL or other defense enzymes. To estimate the quantitative changes in the plant defense-related enzyme activities of POD, PPO, and PAL in wheat leaves, our pot experiment was conducted under different concentrations of the M and EF with soil drench treatment. With the treatment of the EF (Table 1, Fig. 1), the POD and PAL activities at the concentration of 10³-fold dilution of the EF increased significantly to some extent compared to control, by 173.86 % (P < 0.05) and 71.92 % (P < 0.05), respectively. It was shown that EF of *S. sp.* strain HU2014 can significantly induced the activity of these enzymes in wheat at low concentrations.

Table 1

Effect of the EF of *S. sp.* strain HU2014 on the activity of disease defense enzymes in wheat seedlings

Concentration	POD activity (U/g)	PPO activity (U/g)	PAL activity (U/g)
untreated control	3476.61 ± 273.37d	74.51 ± 3.08abc	7.3 ± 0.72c
Original broth	6870.61 ± 219.65b	83.43 ± 1.40ab	8.38 ± 0.66bc
10-fold dilution	6567.13 ± 135.63b	64.58 ± 6.60c	8.91 ± 1.15b
10 ² -fold dilution	5619.70 ± 145.51c	87.17 ± 7.55a	9.33 ± 0.62b
10 ³ -fold dilution	9522.38 ± 106.33a	69.3 ± 3.85bc	12.55 ± 1.30a
10 ⁴ -fold dilution	3340.89 ± 216.28d	86.79 ± 4.15a	5.78 ± 0.37d
10 ⁵ -fold dilution	2768.14 ± 152.48e	67.23 ± 5.30c	7.99 ± 0.39bc*

*Data in the table are means ± SD. Different lowercase letters in the same column show values that are significantly different at the P < 0.05 level by least significant difference (LSD) test.

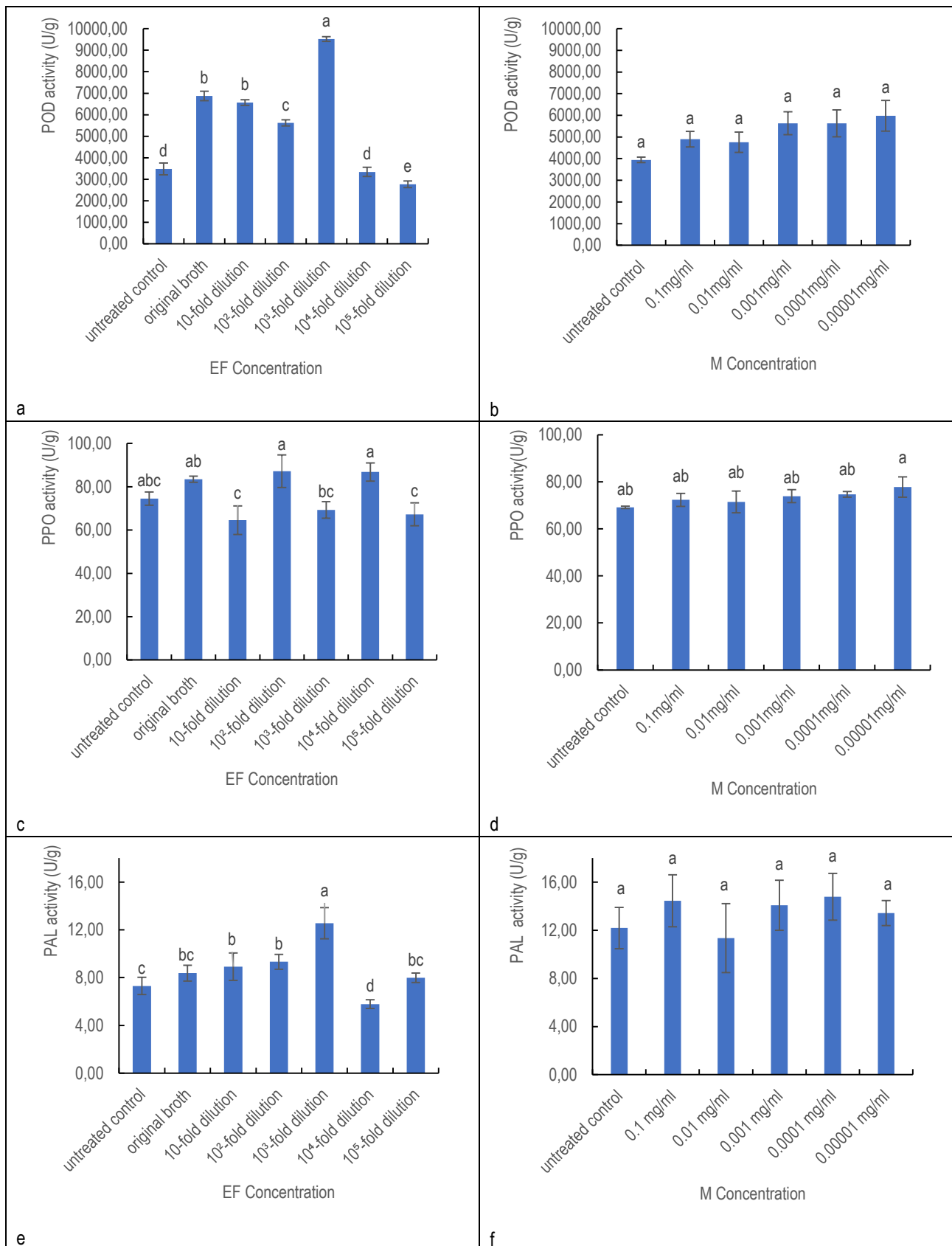


Fig. 1. Quantitative changes in wheat defense enzymes by the EF and M of *S. sp.* strain HU2014.

Although the activity of PPO at the concentrations of 10²-fold dilution and 10⁴-fold dilution increased compared to untreated control, by 16.99 % ($P < 0.05$) and 16.48 % ($P < 0.05$) respectively, the difference was non-significant. With the treatment

of the M (Table 2, Fig. 1), the activities of POD and PPO enhanced with the increasing of dilution ratio in the range of different concentration, but the difference was not significant.

Table 2

Effect of the M of *S. sp.* strain HU2014 on the activity of disease defense enzymes in wheat seedlings

Concentration (mg/ml)	POD activity (U/g)	PPO activity (U/g)	PAL activity (U/g)
untreated control	3940.10 ± 125.56a	69.19 ± 0.50ab	12.20 ± 1.72a
0.1	4903.48 ± 360.60a	72.32 ± 2.75ab	14.45 ± 2.16a
0.01	4759.23 ± 472.73a	71.50 ± 4.63ab	11.35 ± 2.86a
0.001	5635.42 ± 526.09a	73.91 ± 2.79ab	14.08 ± 2.07a
0.0001	5630.14 ± 625.98a	74.69 ± 1.18ab	14.79 ± 1.94a
0.00001	5978.90 ± 704.20a	77.78 ± 4.28a	13.44 ± 1.04a*

*Data in the table are means ± SD, Different lowercase letters in the same column show values that are significantly different at the $P < 0.05$ level by least significant difference (LSD) test.

The activity of PAL (with the exception of 0.01 mg/ml) increased compared to untreated control by 10.16 % ~ 21.23 % ($P < 0.05$), but the difference was non-significant as well.

Discussion. In plant defense system, POD, PPO and PAL are the major defense enzymes, they are used as physiological indexes to identify plant disease resistance (Jinal et al., 2020; Peng et al., 2019). It has been shown in many studies that activities of defense-related enzymes could be altered by microorganisms and their metabolites (Sakineh Abbasi et al., 2019; Van Loon, 1997; Zhao et al., 2012). In our study, the activities of POD and PAL at the concentration of 10³-fold dilution of EF increased significantly to some extent compared to control. In other words, the EF of *S. sp.* strain HU2014 can significantly induce the activity of defense enzymes in wheat at low concentration. From this point of view, the performance of POD and PAL activity coincides with the results of Xie et al. (2019) and Liu et al. (2018). Moreover, in this experiment the increase of induced enzyme activity was higher. Therefore, it was determined that the *S. sp.* strain HU2014 could significantly improve the resistance of

wheat seedlings. The level of enzyme activity with the M was lower than that of the EF. The main reason would be that the mycelia need a certain time to colonize in the rhizosphere and gradually metabolize the active components.

Conclusion. It was found that POD and PAL enzymes had high level of activity at a low dilution of the EF of *S. sp.* strain HU2014 within certain concentration range. Activities of POD and PPO were enhanced with the increasing of dilution ratio in the range of different concentration of M, but the difference was not significant. Characteristics of *S. sp.* strain HU2014 growth in soil need further research. The changes of defense enzymes are related to the induction of plant disease resistance.

In our experiment, *S. sp.* strain HU2014 could induce the enzyme activity without plant pathogenic fungi, so the induction of the strain would follow Jasmonate signaling pathway. Otherwise, the enzyme activities measured at the physiological level of plants were easily affected by some factors, which need to be further verified at the molecular level. Further studies should be focused on wheat disease resistance with RT-PCR assay, metabolic pathways and transcriptomics research.

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КІЛЬКІСНІ ЗМІНИ ЕНЗИМНОЇ АКТИВНОСТІ ПШЕНИЦІ, ІНДУКОВАНІ STREPTOMYCES SP., ШТАМ HU2014

Бактерії для біоконтролю мають різноманітний спектр дії й активно застосовуються як потенційні агенти біологічного контролю шкідливих організмів у сільському господарстві. Вони можуть викликати захисну реакцію рослин і підвищувати стійкість рослин до хвороб. *Streptomyces* sp. продукують активні метаболіти, які можуть пригнічувати ріст фітопатогенів. Індукована резистентність, зазвичай, базується на активності пероксидази (POD), поліфенолоксидази (PPO) та аміакліази фенілаланіну (PAL) або інших захисних ферментів. Відповідні дослідження здебільшого були зосереджені на контролі хвороб комерційних культур або овочів, що сприяло зростанню їх врожайів, меншою мірою пшениці. Більше того, інформація про концентрацію ферментативного рідкого середовища та міцелію *Streptomyces*, що впливали на кількісні зміни активності захисних ферментів, обмежена. У цьому дослідженні спочатку було виділено штам *Streptomyces*, якому дали назву HU2014. Згодом було продемонстровано активність ферментів POD, PPO, PAL з різною концентрацією M і EF за обробки штамом ґрунтової сировини. Активність ферментів визначили за допомогою видимої спектрофотометрії. Результати показали, що активність POD та PAL за концентрації 10^{-3} розведення EF в деякій мірі значно зросла, порівняно з необробленим контролем, відповідно на 173,86 % ($P < 0,05$) та 71,92 % ($P < 0,05$). У діапазоні різних концентрацій M, активність POD та PPO посилювалась зі збільшенням коефіцієнта розведення, але різниця не була значною. З результатів видно, що HU2014 в умовах низької концентрації має очевидний вплив на захисні ферменти пшениці, і, як очікується, він може бути активним ресурсом для розробки нових біологічних препаратів.

Ключові слова: бактерії для біоконтролю, індукована активність, захисні ферменти, зернові культури.

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