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The Effect of Different Functional Microbial Agents on the Biological Characteristics and Rhizosphere Enzyme Activity of Hydroponic Cucumber

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Abstract: This study aims to comprehensively explore the effect of functional microbial agents on healthy soil cultivation and attempt to increase cucumber yield. Three different microbial agents were used for cucumber seedlings by virtue of the hydroponic cultivation method in order to study their effects on enzyme activity and seedling growth and reveal the relationship between rhizosphere enzyme activity and carbon, nitrogen, and phosphorus cycling processes. The results showed that microbial agent 1 (BaCillus Mucilaginosus and Bacillus megaterium Preparations) significantly enhanced the enzyme activity in hydroponic cucumber solution and promoted the growth of cucumber plants. In addition, microbial agent 1 increased neutral protease activity by 20%, peroxidase activity by 49.7%, urease activity by 18.03% and cellulose activity by 3.42% in the rhizosphere solution of hydroponic cucumber. Overall, the comprehensive analysis of cucumber growth and enzyme activity in the rhizosphere solution has proved the best effect of a microbial agent on productive cucumber cultivation. This study suggests the promising perspective of functional microbial agents for crop high-yield production in the near future.

Keywords: Functional Microbial Agents, The Rhizosphere of Cucumber, Enzymatic Activity, Biological Characteristics, Nitrogen and Phosphorus Cycling Process

Introduction

The planting of cucumbers has important economic and social benefits in China. As one of the most popular vegetables, the cultivation scale and planting area of cucumber has grown gradually in the past years. However, several problems, e.g. the increase in intensive planting years and the extensive application of farm manure and chemical fertilizers, restricted its healthy cultivation. It is attributed to the compaction, acidification, secondary salinization, excess nitrogen and phosphorus nutrients and dysbiosis of microbial communities in soil. As a result, several production barriers arose, including the decline of soil fertility, low fertilizer utilization rate, retardation in yield and quality and prevailing soil-borne diseases (Kianpoor Kalkhajeh *et al*., 2021; He *et al*., 2021).

To date, the use of functional microbial agents has attracted much attention to address the cucumber production issue effectively (Siddiqui *et al*., 2023; O'Callaghan *et al*., 2022). The employment of agricultural microbial agents improves the soil structure by enhancing soil biological activity and promoting the effective circulation of substances in the soil. This can bring a good growth environment for crops through the enhancement of soil's retention capacity in water and fertilizer, meanwhile reducing harmful substances and pathogens in soil, as well as improving soil nutrient richness and biodiversity (Sivaram *et al*., 2023; Qi *et al*., 2022; Shahwar *et al*., 2023; Li *et al*., 2023). Also, the activity of soil enzymes with the addition of microbial agents has been studied by Huang *et al*. (2012). The enzyme activity plays a vital role in adjusting the physicochemical properties and fertility levels of soil as the related enzymes participate in the decomposition and synthesis of soil organic matter along with the involvement in all material cycling, such as nitrogen and phosphorus in both bound and free states (Liu *et al*., 2023; Huang *et al*., 2012).

Additionally, each enzyme only reflects specifically a certain biochemical or nutrient cycling process in the soil that can be used as a reference for fertility grading (Sivaram *et al*., 2023; Qi *et al*., 2022; Xu *et al*., 2024; He *et al*., 2024; Maxwell *et al*., 2020; Janes-Bassett *et al*., 2022; Mori *et al*., 2024). For instance, catalase participates in the material metabolism of biological respiration; thus, its activity can characterize the intensity of soil humification and the rate of conversion to organic

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matter. In the case of sucrase and amylase, their activity has a critical influence on the rate of organic carbon conversion and cycling. The activity of urease is capable of representing the nitrogen supply capacity of the soil as it can partake in the transformation of soil nitrogen-containing organic compounds (Sadhukhan *et al*., 2024). Succinic acid may reduce phosphorus adsorption in soil by affecting the transformation of inorganic phosphorus components (Caraba *et al*., 2024).

The implementation of hydroponic experiments has emerged gradually, which could prevent the inactivation of enzymes in crops. The very first advantage is that it precludes issues such as fertilizer loss and microbial absorption during fertilization. Meanwhile, it is conducive to saving fertilizer conservation and solving several problems, including soil fertility depletion and severe salinization caused by long-term continuous cropping. What's more, the use of pesticides is decreasing as a result of the occurrence of pests and diseases (Zhang *et al*., 2024). Herein, we explored the different effects of three microbial agents on cucumber planting by performing hydroponic experiments and assessed them by determining the enzyme activity in cucumber plants, cucumber roots, and cucumber solutions. Based on the analysis of various enzyme activities, the optimal microbial agent was screened for high-yield cucumber production.

Bacillus mucilaginous is a unique bacterium capable of decomposing silicate minerals. It is widely used in microbial fertilizers due to its multiple beneficial characteristics, including silicate decomposition, phosphorus and potassium solubilization, nitrogen fixation and the enhancement of crop disease resistance. Its application in various crops has shown notable yield improvement effects (Liu *et al*., 2017). Similarly, Bacillus laterosporus is effective in phosphorus and potassium solubilization, making it particularly suitable for microbial fertilizers in dryland crops. It can enhance the content of available phosphorus in soil and improve crop yield to some extent (Chen *et al*., 2017). In addition, Bacillus subtilis is an ideal biocontrol microorganism with a broad-spectrum antibacterial activity, excellent efficacy against plant diseases, a straightforward mass production process, low cost, ease of application and long storage life. These features make it highly promising for widespread agricultural applications (de O. Nunes *et al*. (2023); Olasupo *et al*. (2022); Elsharkawy *et al*. (2022).

Materials and Methods

Experimental Materials

The test cucumber variety is Jinyou 1. The culture medium used in the experiment is the Shanqi cucumber nutrient solution formula, including $Ca(NO_3)_2 \cdot 4H_2O$ (3.5 mm), KNO_3 (6 mm), $NH_4H_2PO_4$ (1 mm), $MgSO_4 \cdot 7H_2O$ (2 mm), NaFe EDTA (54.5 μM), H_3BO_3 (46.3 μM), $MnSO_4 \cdot H_2O$ (9. 5 mm), $ZnSO_4 \cdot 7H_2O$ (0.77 μM), $CuSO_4 \cdot 5H_2O$ (0.32 μM), $(NH_4)\ 6Mo O_{24} \cdot 4H_2O$ (0.02 μM).

Three microbial agents are listed: Microbial agent 1 is a preparation of Bacillus mucilaginous gel, microbial agent 2 is a preparation of Bacillus Laterosporus, and microbial agent 3 is a mixed fermentation fertilizer of Bacillus subtilis and organic fertilizer.

Experimental Setup

The experimental samples were treated with nutrient solution and microbial agents, and CK was treated with pure nutrient solution as the control. The treatment with the addition of microbial 1 is micro 1, the treatment with the addition of microbial 2 is micro 2, and the treatment with the addition of microbial 3 is micro 3. Microbial nutrient solution was uniformly added every two days, 250 mL for each time, with a growth period of 30 days. In summary, there are a total of 4 groups, each with three replicates, in total 12 samples.

Measurement Items and Methods

Measurement of plant biological indicators. During the middle stage of cucumber growth, nine plant samples were selected randomly and marked, then followed by measuring various indicators, including plant height, leaf area, number of flowers and chlorophyll content. The plant height was measured by using a tape measure (from the base of the stem to the highest point of plant growth). The chlorophyll content was determined using the 95% ethanol extraction colourimetric method and quantified using a SPAD-502 analyzer (Wang, 1991).

Measurement of the protein content and enzyme activity of plant leaves, roots and culture solutions, Including catalase, peroxidase, nitrate reductase, protease, superoxide dismutase, cellulase, etc.

Determination of enzyme activity related to nitrogen metabolism: Root samples were collected at 0, 3, 6, 9 and 12 days of treatment, washed, chopped and mixed and placed at 20°C for later use. The activities of GS and NADH-GDH were measured using a reagent kit (Suzhou Keming Biotechnology Co., Ltd.) and an enzyme-linked immunosorbent assay (Thermo Multiskan FC, USA), while the activity of NR (nitrate reductase) was measured *in vivo*.

Data Processing

The obtained data was sorted and plotted using Microsoft Excel, and Duncan's analysis of variance was conducted using SPSS. Various indicators were evaluated using the fuzzy mathematical membership function method on the comprehensive level.

Results and Discussion

Effects of Different Microbial Agents on the Biological Characteristics of Cucumber

As can be seen in Table (1), compared with the CK sample, the treatment of micro one can increase the plant height, leaf area, cucumber flowering number, chlorophyll,

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aboveground fresh weight, root fresh weight, root length and root surface area, by 1.02, 27.272, 21.05, 4.01, 7.17, 56.52, 13.92 and 56.80%, respectively. This indicates that microbes can promote the growth of cucumber seedlings.

Specifically, the treatment with Micro 3 increased the leaf area, root weight, root length and root area of cucumber by 30.54, 74.09, 10.20 and 37.32%, respectively, compared to the control. Furthermore, the application of Micro 3 significantly reduced the number of flowers, whereas the application of Micro 2 had minimal impact on flowering. Additionally, Micro 2 significantly decreased chlorophyll content, while Micro 3 had little effect on this parameter.

Effects of Different Functional Microbial Agents on Cucumber Plants and Rhizosphere Enzyme Activity

Effects of Different Microbial Agents on Enzyme Activity in Cucumber Plants

As shown in Fig. (1), it showed that the protein content from the nutrient solution for cucumber plant absorption had decreased significantly in the presence of different microbial agents, almost 2~3 times reduction from 620 U/g to less than 200 U/g compared to the CK sample. Obviously, the treatments of micro one and micro 2 reduced the catalase activity in hydroponic cucumber leaves by 31.09-26.10%, while a great increase of 48.31% was gained in the treatment of micro 3. We further investigated the three microbial agents' slight, ignorable effect on nitrate reductase activity, as well as a subtle enhancement in peroxidase activity in hydroponic cucumber plants. Additionally, it is worth noting that the treatment with micro three significantly inhibited the activity of Superoxide Dismutase (SOD) in hydroponic cucumber, reducing it by 24.71%.

Effects of Different Microbial Agents on Enzyme Activity in Cucumber Roots

The effects of different microbial agents on enzyme activity in cucumber roots were evaluated. The results are shown in Fig. (2); the protein content increased with an activity sequence compared to the CK sample of micro 1 (2.69 times) >Micro 3 (2.54 times) >Micro 2 (2.43 times) in the presence of three microbial agents, respectively. The catalase activity of micro 1 has an ignorable decrease, but the obvious reduction to micro 2 (22.87%) and micro 3 (58.34%). Three treatments have a slight effect on nitrate reductase in the roots of hydroponic cucumbers. As for the peroxidase activity, it dropped dramatically to 63.58% for the micro 3 sample, 43.17% for micro 1 and 39.64% for micro 2. Regarding the alkaline protease activity, there is a slight decrease to 18.06% for micro 1 and 10.29% for micro 3, but twice as much as CK for micro 2. The three microbial agents significantly reduced the activity of superoxide dismutase in the roots of hydroponic cucumber.

Table 1: Effects of different microbial agents on the biological characteristics of cucumber

Processing number	CK	Micro 1	Micro 2	Micro 3
Plant height	12.755600±1.52ab	12.8000±0.21ab	10.3333±0.70a	9.9056±0.76a
leaf area	9.203300±2.24a	11.7497±0.95a	12.7722±0.48a	12.0144±0.40a
Flower count	12.666700±1.45cd	15.3333±1.20d	13.6667±1.20cd	6.0000±1.15ab
chlorophyll content	29.398300±1.29bc	30.5833±1.55c	22.6833±2.35ab	27.5533±3.15abc
Aboveground fresh weight	3.212600±0.39a	3.4443±0.13a	3.0993±0.17a	2.9326±0.19a
Root weight	0.230400±0.13a	0.3624±0.05a	0.3327±0.03a	0.4011±0.08a
Root length	8.550000±1.79a	9.7389±0.93a	8.8000±0.49a	9.4222±1.81a
Root area	1.685600±0.46a	2.6539±0.24a	2.2753±0.26a	2.3146±0.40a

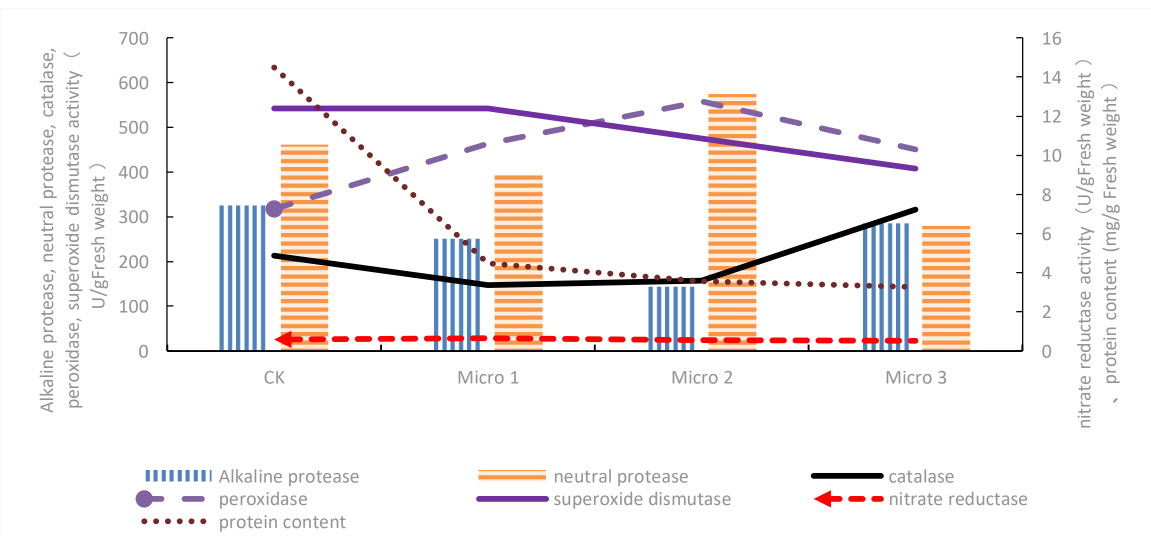

Fig. 1: Effects of different microbial agents on enzyme activity in cucumber plants

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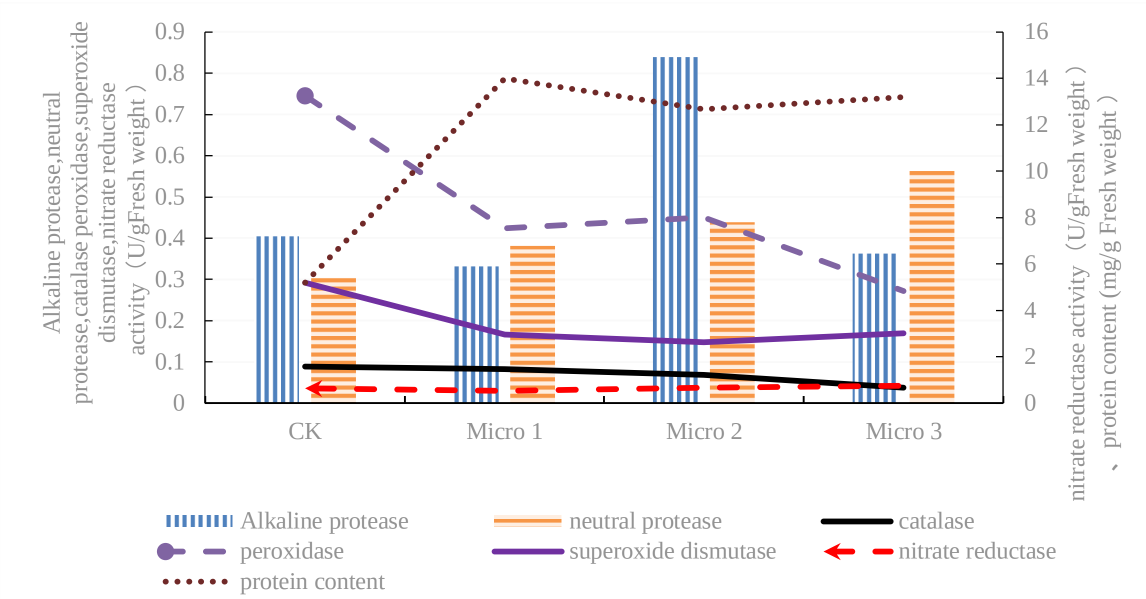

Fig. 2: Effects of different microbial agents on enzyme activity in cucumber roots

Effects of Different Microbial Agents on Enzyme Activity in Cucumber Solution

Furthermore, we complemented this experimental work with the research on the effect of different microbial agents on enzyme activity in cucumber solution. As shown in Fig. (3), the three microbial agents had no significant effect on the alkaline protease and nitrate reductase activities compared to the CK sample. The addition of microbial agents increased the neutral protease activity with a sequence of micro 2 (88.57%) >Micro 1 (20%) >micro 3 (1.43%), showing the highest improvement with the treatment of micro 2. On the contrary, the Micro 2 treatment resulted in reduced peroxidase activity (20.36%), while the Micro 1 and Micro 3 treatments increased it by 49.7-9.58%. As for the catalase activity, the treatment of micro 3 reduced the catalase activity dramatically by 61.29%, but the micro one and micro two treatments had a slight growing effect. It is worth noting that the urease activity was raised by 18.03% in the presence of Micro 1, which is obviously different from the reduction by 57.09-58.89% with the addition of Micro 2 and Micro 3. Moreover, the micro one treatment also increased the cellulose activity in the rhizosphere solution of hydroponic cucumber by 3.42%. Besides, the three microbial agents did not show any impact on the activity of sucrose, β-1, 3-glucanase and chitinase in the rhizosphere solution of hydroponic cucumber. Overall, micro two and micro three treatments significantly reduced the protein content in the rhizosphere solution of hydroponic cucumber by 25.35-19.47%, respectively.

Compared with CK, the three microbial agents had a vital impact on the succinic acid content in the rhizosphere solution of hydroponic cucumber, showing a significant increase with the sequence of 8.47% (Micro 2) >7.91% (Micro 1) >4.33% (Micro 3), respectively.

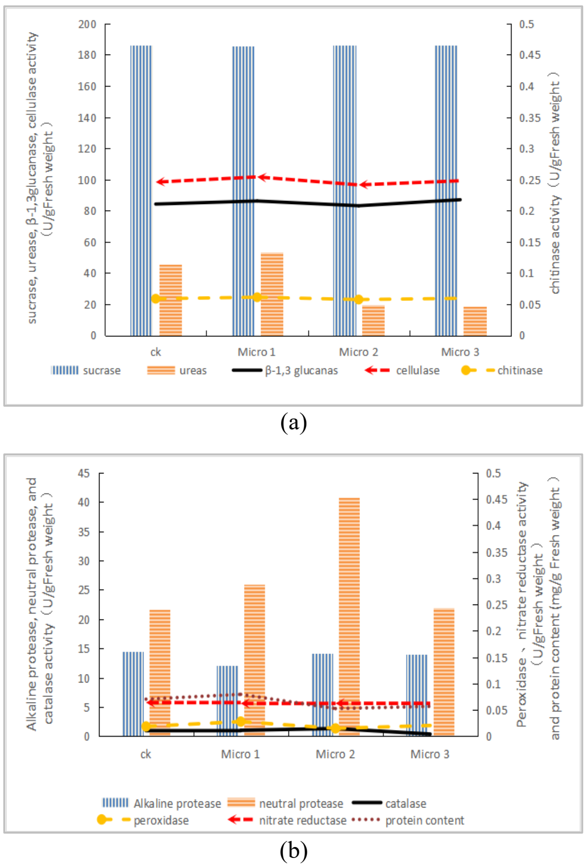

Fig. 3: Effect of different microbial agents on enzyme activity in cucumber solution

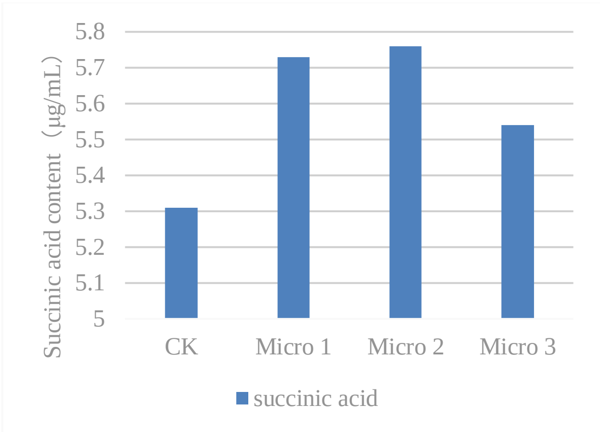

Fig. 4: Effect of different microbial agents on succinic acid content in cucumber solution

Compared with CK, the three bacteria agents significantly affected the succinate content in the rhizosphere solution; the micro 2 treatment increased by 8.47%, and the micro 1 and micro three treatments increased by 7.91-4.33%.

Discussion

In their pioneering work, Cao and coworkers discovered that the employment of agricultural microbial agents can significantly increase cucumber yield, with an increased yield rate of up to 18.04% (Cao *et al*., 2016). Lv *et al*. (2020) have conducted a study on the fertilizer efficiency of agricultural microbial agents on cucumbers, revealing that the application of agricultural microbial agents can improve the plant height, stem diameter, chlorophyll content, yield, vitamin C content, soluble protein content, soluble sugar content and cucumber seedling survival rate of cucumbers. Beyond this, in this context, we demonstrated that both micro two and micro three treatments slowed down the growth of cucumber plants, having a controlling and/or inhibitory effect during the seedling stage, considering the flowering number of cucumbers as the important fertility indicator. Importantly, the utilization of Micro 1 in cucumber plants enabled an increase in the fresh weight of aboveground parts, as the fresh weight of aboveground parts is an essential sign for measuring the quality of cucumber.

During the last decades, much attention has been paid to the application of beneficial microorganisms to the rhizosphere of plants in order to balance soil microbial community structure, improve the soil environment and prevent soil-borne diseases (Wang *et al*., 2019; Zhang *et al*., 2021; Liang *et al*., 2022). However, most researchers have focused on the role of one single microbial agent. For example, Yu has found that microbial agents can improve the soil microenvironment by continuously cropping watermelon roots and increasing yield and fruit quality (Yu *et al*., 2022). The improved soil sucrose activity in watermelon by AMF microbial agents was explored in Xie's study (Xie *et al*., 2018). Also, Xiao-Jie has demonstrated that more soil phosphorus activators resulted in stronger urease and sucrase activities in rice soil (Xiao-Jie *et al*., 2015). Thus, it is of remarkable interest that different microbial agents may lead to discoverable effects on soil enzyme activity in cucumbers.

Herein, the results obtained in this study have indicated that all three microbial agents play a role in increasing the activities of catalase, sucrose, amylase, and urease in cucumber and its environment to a certain extent. The treatment of three bacterial agents had a slight effect on nitrate reductase activity in hydroponic cucumber plants. Thus, it will not increase the content of highly carcinogenic nitrite by accelerating the conversion of nitrate ions into nitrite ions in the roots and aboveground plants of hydroponic cucumber. The participation of three microbial agents significantly reduced the peroxidase activity in the roots of hydroponic cucumber but increased the content of succinic acid in the rhizosphere solution. Specifically, in the rhizosphere solution of hydroponic cucumber, micro one and micro two treatments increased the neutral protease activity, and micro one and micro three treatments increased the peroxidase activity. Moreover, the micro one treatment can significantly upgrade the urease and cellulose activity. It is worth noting that micro 1 displayed the most effective effect on the hydroponic cucumber planting.

Conclusion

In conclusion, microbial agent 1 (Bacillus mucilaginous) was identified as the best microbial agent for enhancing the enzyme activity in hydroponic cucumber solution and promoting the growth of cucumber plants. We postulate that the rapid reproduction of functional bacteria, which can optimize the population structure of the solution microorganisms, is required for an increase in enzyme activity in the solution. As suggested above, the different performances of three microbial agents may contribute to the diversity of functional bacteria.

In this study, the experiments discussed above were conducted under hydroponic conditions. Further research will be conducted in complex soil ecological environments in the field for the application of microbial agents treated with micro 1, micro two and micro 3; the yield-increasing effect on cucumber and the relevant mechanisms of soil physicochemical properties will be clarified.

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Author's Contributions

Ce Wang, Ouya Zhao, Shiyou Sun and Shuhua Ru: Designed and performed the experiments, analyzed the data and prepared the paper.

Lei Liu, Guangmin Xiao, Xueqing Wang and Limin Hou: Participated to collect the materials related to the experiment.

Jing Gao, Pin Li, Shiqin Sun and Ling Wang: Designed the experiments and revised the manuscript.

Conflict of Interest

The authors declare that they have no competing interests.

Ethics

The authors declare their responsibility for any ethical issues that may arise after the publication of this manuscript.

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