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Application and Effect Evaluation of Microbial Fertilizer Strain

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Abstract: In this study, field experiments were carried out on nine crops, including cucumber and rice, using four typical bacilli, including subtilis, amylolytic, lateral spore brevis and gelatinoid, and a compound preparation composed of the above two bacilli. The observed data revealed the following points: (1) the effect of various bactericides on the yield increase of different crops varied. The yield increase of Bacillus amylolytica ranged from 10.4% to 13.2%, while the preparation containing trichoderma showed a gain of 10.5% to 14.8%. (2) On the same crop, the yield increasing effects of different microbial inoculants were also significantly different, ranging from 7.5% to 15.8%, and all the differences were statistically significant, among which trichoderma was the most outstanding. (3) The compound bactericides showed superior efficiency compared with a single strain. Taking rice as an example, the single application of Bacillus subtilis and Bacillus gelatinus resulted in a yield increase of 10.3% and 10.7%, respectively, and the combined yield of the two could reach 16.1%, which was particularly obvious. (4) All tested bactericides significantly improved crop quality characteristics, and verified the potential advantages of multi-strain combination in bactericide formulation. This finding is consistent with the current trend of multi-strain combination research.

1. Introduction

The scientific research work in the field of microbial fertilizer in China covers the basic, application and practical application levels, and there have been significant weak links for a long time[1]. Although some progress has been made in the past decade the existing scientific and technological support system has not fully met the strong demand for industry development, and it is urgent to consolidate and improve. Although China's tradition of naming microbial fertilizers has been more than half a century, the research and development of agricultural microbiology, especially soil microbiology, is relatively lagging behind, which limits the deep understanding of the nature of biological fertility to a certain extent[2]. Although great progress has been made in the

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production process of bacteriological agents, and the density of active strains in the products has been significantly improved, there are still a series of technical problems to be solved in the manufacturing process of actinomycetes and fungal agents. In addition, the solid-state fermentation technology and composite process equipment of bio-organic fertilizer and composite microbial fertilizer still need to be further improved and perfected[3]. The core of the efficacy of microbia fertilizer comes from the active beneficial bacteria that it carries. However, the foundation of this research field is still shallow, coupled with the limited investment in science and technology of enterprises, and the limited knowledge of the characteristics of bacteria and application technology, often make the stability of product performance and application effectiveness face problems. These problems undoubtedly constitute a substantial obstacle to the growth of enterprises and the overall development of microbial fertilizer industry[4] .

Many scholars have discussed this issue. Wu et al. studied the effects of biofertilizer on cucumber growth and yield, as well as the response of soil microbia populations to substrate or soil treated with biofertilizer[5]. We observed that some biofercitic fertilizers significantly promoted cucumber growth and reduced soil-borne pathogens in soil and substrate. The response of rhizosphere microbial communities in soil and substrate to different biofertilizes was different, which also led to significant differences in microbial diversity and taxonomic structure at different periods of the growing season, Biofertilizers increase the prospect of re-using substrates in order to sustainably produce high-guality crops from the same soil each year in a cost-effective manner, while controlling soil-borne diseases. The study by SasPaszt Lidia et al, aimed to determine the effects of biofertilisers containing selected strains off ungi and bacteria on rhizosphere microorganisms of strawberry plants, using the selected microorganisms for enrichment of ore-forming fertilizers[6]. The urea fertilize was mainly composed of Aspergillus Niger and penicillium purpureum. Adam W et al reviewed recent advances in engineered microbial hosts for glycerol production of biofuels, diols, organic acids, biopolymers and specialty chemicals[7]. We begin with an overview of the major pathways and key terminal metabolites involved in the fermentation and respiration of glycerol foreignization, and then focus on four key genera of bacteria for which naturally foreignized glycerol is known. L. Christilda etal studied the effects of organic fertilizers on soil bacterial populations and mulberry yield[8]. FYM, Spirillum azotofixation, phosphorus bacteria and lumbricol were used To fertilize four groups of mulberry trees of the MR2 variety, we utilized biofertilizers enriched with bacteria from the rhizosphere of mulberry plants.

This study is dedicated to a detailed analysis of the application of strains and their effectiveness in the field of microbial fertilizers in China over the past decade. It covers the core steps, ranging from the selection, confirmation, preservation, reproduction, implementation strategy, and carrier selection of functional strains[9]. The aim is to gain insight into the current situation and trends in this field. Through in-depth analysis of key technologies, this study intends to lay a solid theoretical and practical foundation for enhancing product performance and ensuring consistent fertilization effects. Field verification experiments were conducted to discuss the interaction between functional strains and cultivated crops, aiming to analyze the similarities and differences in the efficacy of different microbial products within complex agricultural ecosystems[10]. On this basis, a reasonable application strategy will be formulated, thereby providing solid theoretical foundation and practical guidance for predicting the development trend of bacterial culture technology in China's microbial fertilizer industry and ensuring its sustainable development.

The objective of this section is to systematically employ statistical methods to evaluate the effectiveness of microbial inoculants in actual farmland, with a focus on how different strains and crop types influence the effects of microbial inoculants.

2. Arrangement of Trials

2.1. Experimental Preparation and Design

2.1.1. Experiment with Products, Locations, and Crops

Extensive field experiments were carried out in this study, involving the evaluation of the effectiveness of five selected microbial inoculants on nine crops.

The relevant data are detailed in Tab1.

Table 1. List of test products and test crops

The strains contained in the product	Bacteria count (billion cfu/ml)	Trial crop
Bacillus amylolyticus	2.6	Wheat, lettuce, tomatoes, strawberries, grapes, cucumbers
Trichoderma	7.0	Soybeans, apples
Bacillus giganteum	11.6	cucumbers, lettuce, Wheat
Photosynthetic bacteria	5.1	cucumbers, corn, tomatoes
Lactobacillus plantarum	3.6	tomatoes, corn
Bacillus amylolyticus, Bacillus giganteum	3.2	Wheat

2.1.2. Test Site

In this study, Tai 'an City of Shandong Province was selected as the experimental site. The soil in this area belongs to the lignite type and has the characteristics of light loam. Rice cultivation was carried out in the typical paddy soil area, which was clay loam soil. The basic fertility of the soil is shown in Tab2.

Table 2. Basic fertility of soil at the test site

Items	Organic matter g/kg	Alkali-hydrolyzed nitrogen (N) mg/kg	Available phosphorus (P2O5) mg/kg	Quick available potassium (K) mg/kg	pH
Brown soil	13.043	70.3	31.4	101.1	1
Paddy soil	13.589	77.2	34.1	108.6	0

2.1.3. Experimental Design

(1)This study selected nine representative crops, including wheat, lettuce, tomato and strawberry, to carry out detailed field tests. The experimental framework consisted of two unique treatments, each of which carried out four independent repeated experiments. The specific experimental design layout can be seen in Tab3.

Table 3. Lists the design of field trials

Items	Microbial inoculant products
Treatment Design	1.Test inoculant + conventional fertilization 2.Regular fertilization
Test area	1.Each processing area is 30m2; 2. Each treatment area of grapes is 40m2
Number of repetitions	4 repetitions

(2)This study involved a variety of crops, such as grapes, cucumbers, soybeans, apples, cabbages, watermelons and corn, etc. Field experiments were carried out on these crops. The experiment was conFigd with four (or three) different treatment levels, and four independent replicates were performed for each treatment to enhance the statistical reliability of the experimental results. Specifically, the randomized block design layout of the experiment is detailed in Tab4.

Table 4. Lists the design of field trials

Items	Microbial inoculant products
Treatment Design	1.Test inoculant+Regular fertilization 2.Test inoculant+Regular fertilization 3.Test inoculant+Regular fertilization 4.Regular fertilization
Test area	Each treatment area 30m
Number of repetitions	4

2.1.4. Analysis of Application Effect of Microbial Inoculant Products

(1)Yield Effect Evaluation: Detailed crop yield measurements were conducted on the experimental plots after harvest. Further comprehensive analysis and evaluation were carried out by comparing the yield differences among different treatments.

(2)Crop Character and Quality Evaluation: This study encompassed a variety of crops, including wheat, lettuce, tomatoes, strawberries, grapes, cucumbers, soybeans, apples, and corn. An in-depth analysis was conducted for each. Among them, key indicators such as plant height, stalk thickness, number of fruits per plant, and weight of single fruit were measured in detail. Especially for grapes, in order to accurately evaluate their quality, the solid content and sugar content were further tested to comprehensively reflect their inherent quality characteristics.

2.2. Second Section

2.2.1. Analysis of Application Effect of Microbial Inoculants Containing the Same Strains in Different Crops

The paired design experiment strategy was employed to observe and comprehend the impact of Bacillus amyliquefaciens microbial bactericide on wheat, lettuce, tomatoes, strawberries, grapes, and cucumbers. The yield of each crop increased by an average of 15.2%, 18.1%, 17.4%, 15.6%, 14.3%, and 16.2%, respectively, as depicted in Fig 1. This increasing trend was statistically significant ($p < 0.05$).Conversely, when Trichoderma-containing inoculants were applied, the yields of soybeans and apples increased by 14.8% and 16.7%, respectively (see Fig 2). The statistical significance of these yield differences was further verified through variance analysis. From the data presented in Figs 1 and 2, it can be inferred that different crops exhibit heterogeneity in their response to microbial inoculants containing specific strains (Suying Wang, 2003). These findings underscore the crop-specific nature of microbial inoculants, indicating that selecting strains that are compatible with the target crop is crucial for optimizing yield enhancement.

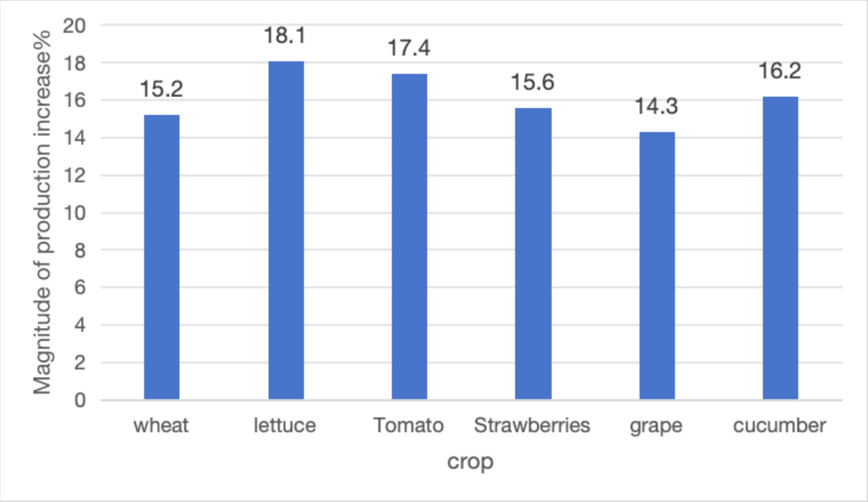

Figure 1. Renderings of bactericide products containing Bacillus amylolyticus on different

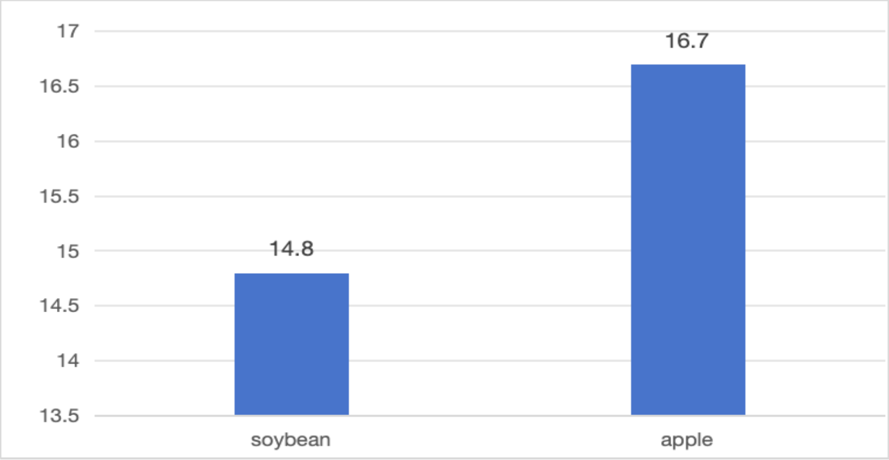

Figure 2. The effect of Trichoderma bactericide product on different crops

Through an in-depth comparison of the application effects of Bacillus amyloliquefaciens microbial bactericide on wheat, lettuce, tomato, strawberry, grape, and cucumber crops with conventional management practices, as shown in Tables 5 to 10, and combined with empirical studies of the effects of Trichoderma-based fungicides on soybean and apple crops, as detailed in Tables 11 and 12, it is evident that microbial inoculants demonstrate significant advantages in promoting crop growth and development. Specifically, when compared with the standard treatment, the core indices of each experimental crop, including plant height, fruit yield, fruit length, and single fruit weight, exhibited a noTabincrease. Notably, in leafy lettuce, plant height, the number of leaves per plant, biomass, and chlorophyll content increased significantly, with statistical significance. These findings robustly support the potential of microbial agents to enhance crop yield and optimize crop quality.

Table 5. Lists the design of field trials

Crops	Main stem height	Main stem thickness	Number of fruit plants	Fruit transverse diameter	Weight of single fruit
	(cm)	(cm)	(a)	(cm)	(g)
tomatoes	175.5	2.09	12.1	7.17	196.7
CK	171.3	1.98	11.7	6.72	184.5

Table 6. Investigation results of cucumber plant traits

Crops	Main stem height	Main stem thickness	Number of fruit plants	Fruit transverse diameter	Weight of single fruit
	(cm)	(cm)	(a)	(cm)	(g)
cucumbers	194.8	1.02	9.3	30.2	204.7
CK	191.2	0.92	8.4	29.5	199.4

Table 7. Results of strawberry plant traits survey

Crops	Main stem height	Main stem thickness	Number of fruit plants	Fruit length	Weight of single fruit
	(cm)	(cm)	(a)	(cm)	(g)
strawberries	64.9	0.97	25.4	16.7	60.4
CK	62.5	0.89	23.6	15.9	58.2

Table 8. Results of grape plant traits survey

Crops	Ear number of fruit number of fruit	Number of grains per ear of fruit	Grain weight	Solid content	Sugar content
	(ear/plant)	(grain/ear)	(g)	(%)	(%)
grapes	14.2	35.3	10.5	15.0	12.1
CK	13.9	34.2	9.7	14.2	11.5

Table 9. Results of investigation on lettuce plant traits

Crops	Plant height	Stem size	Number of leaves	Weight per plant	Leaf chlorophyll
	(cm)	(cm)	(Piece/plant)	(g)	(mg/g)
lettuce	34.6	1.82	13.4	59.5	1.66
CK	32.4	1.70	12.1	54.3	1.59

Table 10. Results of wheat plant traits survey

Crops	Plant height	Stem size	Number of ripe green leaves	Spike length	Number of grains per spike	Thousand kernel weight
	(cm)	(cm)	(piece/strain)	(cm)	(grain))	(g)
Wheat	224.9	2.27	13.7	18.1	515.3	336.8
CK	221.3	2.22	10.8	17.4	474.9	329.1

Table 11. Results of soybean plant traits survey1

Crops	Main stem height	Main stem thickness	Lateral branch length	Number of fruit plants	Hundred fruit weights
	(cm)	(cm)	(cm)	(a)	(g)
Soybeans	41.7	0.52	43.9	7.4	242.9
CK	40.2	0.43	41.1	7.0	230.7

Table 12. Results of the survey of apple plant traits2

Crops	Main stem height	Main stem thickness	Lateral branch length	Number of fruit plants	Hundred fruit weights
	(cm)	(cm)	(cm)	(a)	(g)
apples	168.4	2.09	9.7	7.09	177.8
CK	164.9	1.94	9.2	6.42	162.3

2.2.2. Analysis of the Application Effect of Microbial Inoculants Containing Different Strains on the Same Crops

According to the data analysis presented in Tab13 and Fig 3, there was evident heterogeneity in the yield-increasing effects of different types of microbial inoculants on cucumbers. Specifically, when compared with the control group, the treatment enriched with Bacillus giganteus resulted in a 12.5% yield increase, the treatment with Bacillus amyloliquefaciens led to a 10.8% increase, and the treatment with photosynthetic bacteria achieved a 7.3% yield boost. All these increases were statistically significant. Notably, the yield difference between the Bacillus amyloliquefaciens and photosynthetic bacteria treatments also reached a statistically significant level. However, no significant difference was observed between the Bacillus giganteus treatment and another comparison group (assuming this refers to a specific or combined treatment not explicitly mentioned earlier, but for clarity, this should be specified or corrected if necessary).This phenomenon may stem from the fact that Bacillus amyloliquefaciens and photosynthetic bacteria belong to similar bacterial groups and share certain ecological functions. In particular, the bactericide containing Bacillus giganteus demonstrated the most prominent performance in enhancing cucumber yield, which is closely tied to its unique biological characteristics. These characteristics are the primary reason for its widespread registration and application in the field of microbial fertilizers (see Tab3 for details).The findings of this study robustly validate the correlation between the efficacy of microbial inoculants and the specific strains they contain. Therefore, in practical agricultural production, the selection of appropriate strain inoculants is crucial for maximizing yield growth.

Table 13. Effects of microbial inoculants containing different strains on cucumber yield3

Treatment	Average yield	Each treatment yielded more than the control (kg)	Each treatment yielded more than the control (%)
Bacillus giganteum	148.0aA	16.4	12.5
Bacillus amylolyticus	147.3aAB	14.4	10.8
Photosynthetic bacteria	141.7bB	10.0	7.3
CK	132.9cC		

Note: The data in the mean column of the above tables marked with different lowercase letters indicate a significant difference at the level of 0.05, and those marked with different uppercase letters indicate a significant difference at the level of 0.01.

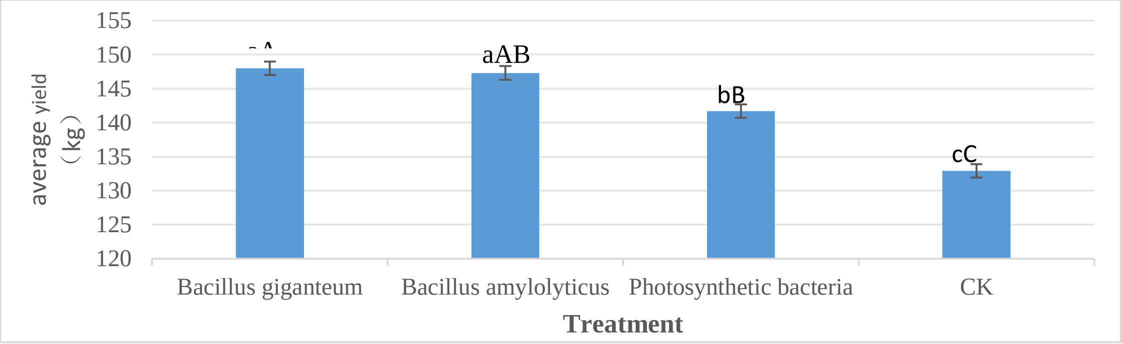

Figure 3. Effect diagram of the application of bactericide products containing different strains on cucumber

Data analysis in Tables 14, 15 and Figs 4 and 5 revealed that, compared with the control group, the yield of corn and tomatoes, was significantly increased by the Treatment with the agent rich in different microbial strains. The yield of corn was increased by 12.9% and 7.3%, while that of tomatoes was increased by 15.6% and 11.4%, respectively. In particular, inocicants containing inocicant bacteria showed significant advantages in improving corn yield due to their ability to efficiently decompose soil silicate minerals and promote the absorption of potassium fertilizer, thus providing plants with key potassium elements. It was better than Lactobacillus plantarum containing bacterial agent. Similarly, in tomato cultivation, the effect of the agent containing bacteria was also better than that of the agent containing Bacillus amylolyticus. These findings strongly demonstrate that the effects of microbial fertilizers are significantly affected by the strain species, and highlight the scientific rationality and urgency of selecting appropriate strains according to crop characteristics and needs when designing microbial fertilizers.

Table 14. Effects of microbial inoculants containing different strains on maize yield

Treatment	Average yield	Each treatment yielded more than the control	Each treatment yielded more than the control
	(kg)	(kg)	(%)
Photosynthetic bacteria	92.3aA	11.2	12.9
Lactobacillus plantarum	88.1bB	6.4	7.3
CK	81.0cC		

Note: The data in the mean column of the above tables marked with different lowercase letters indicate a significant difference at the level of 0.05, and those marked with different uppercase letters indicate a significant difference at the level of 0.01.

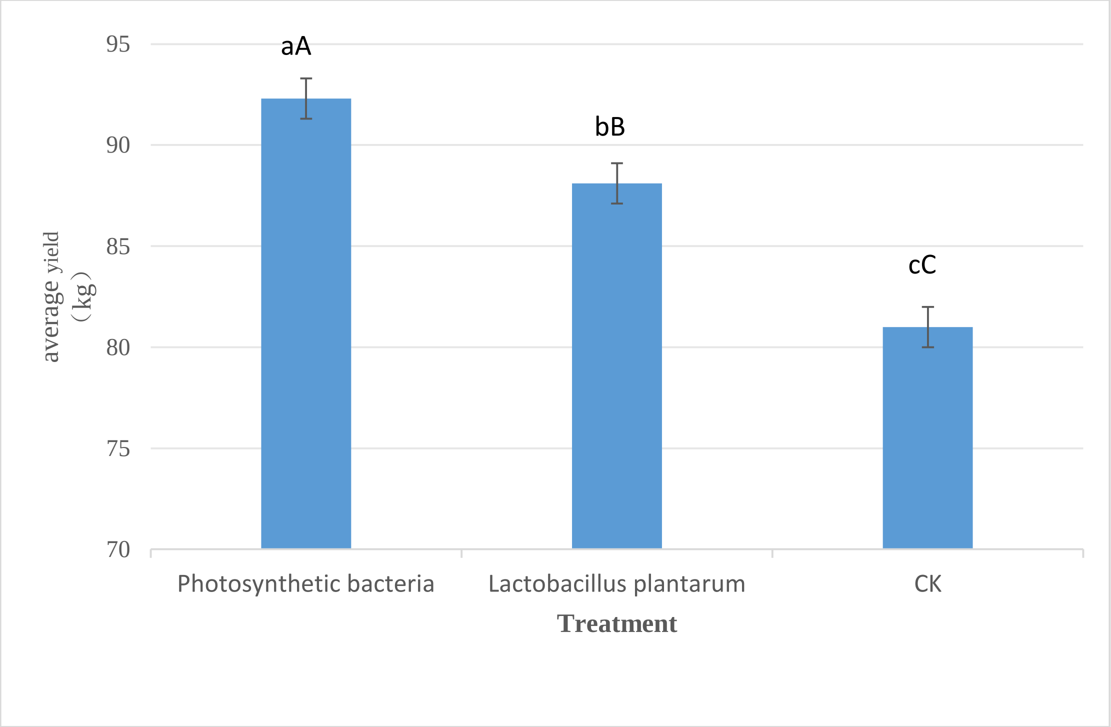

Figure 4. Effect diagram of application of inoculant products containing different strains on Corn

Table 15. Effects of microbial inoculants containing different strains on tomato yield

Treatment	Average yield	Each treatment yielded more than the control	Each treatment yielded more than the control
	(kg)	(kg)	(%)
Photosynthetic bacteria	170.1aA	24.4	15.6
Bacillus amylolyticus	169.9bA	17.3	11.4
CK	153.2cB		

Note: The data in the mean column of the above tables marked with different lowercase letters indicate a significant difference at the level of 0.05, while those marked with different uppercase letters indicate a significant difference at the level of 0.01.

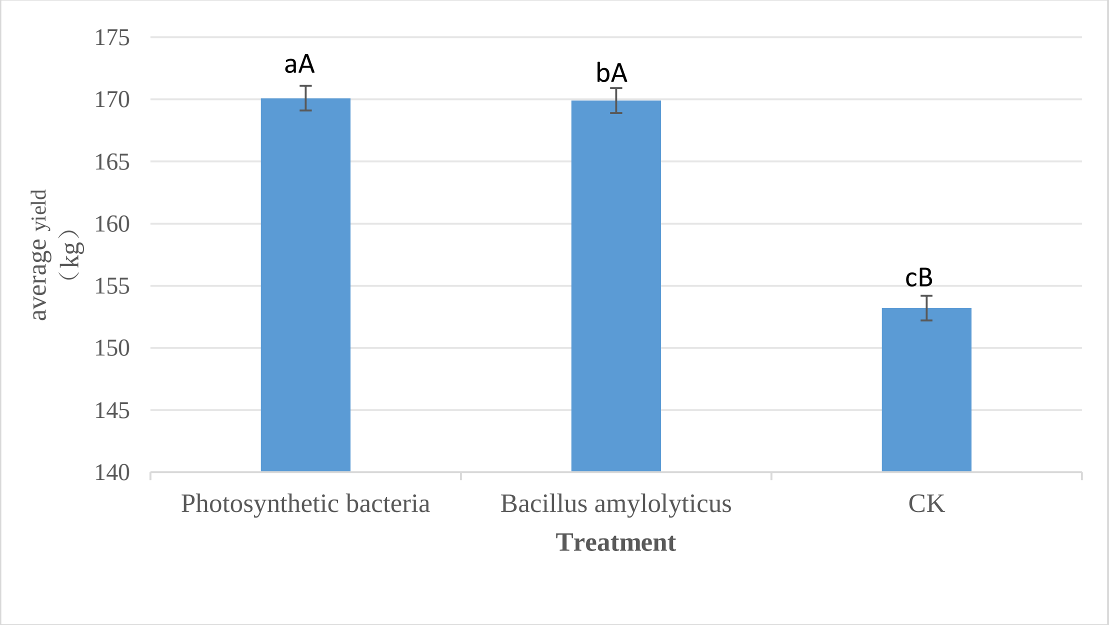

Figure 5. Effect diagram of the application of bactericide products containing different strains on tomatoes

2.2.3. Analysis of the Application Effect of Microbial Inoculants Containing Single and Multiple Strains

Tab16 and Fig 6 present the significant effects of various strain combinations in microbial inoculants on wheat yield. All treatment groups exhibited statistically significant yield differences compared to the control groups. Notably, treatment with Bacillus gigantis or Bacillus amyloliquefaciens alone did not significantly increase wheat yield, and their effects were comparable. However, none of these single-strain treatments achieved the same yield increase as the multi-strain inoculants. This phenomenon may suggest the advantage of multi-strain inoculants in enhancing crop yield, potentially due to the functional synergy among different strains (Liang Yunjiang, 2001; Shi Zhenyun, 1999). Specifically, Bacillus amyloliquefaciens can activate soil phosphorus, while Bacillus gigantis aids in potassium mineralization. The combined effect of these two strains enhances the crop's access to essential nutrients, thereby improving wheat production efficiency (Ge Cheng, 2007). This finding aligns with the current trend towards developing multi-strain compounds in microbial fertilizers. Looking ahead, multi-strain compounds are expected to play a pivotal role in fertilizer production and agricultural production. However, given the complex interactions between strains, such as potential competition or collaboration mechanisms, and optimizing their effectiveness, these are crucial issues that require attention from both industry and academia.

Currently, the evaluation of microbial fertilizer effectiveness has primarily focused on increased yield. However, the fundamental benefits relate to improved crop quality, pest control, and enhanced crop stress resistance. Studies of wheat plant characteristics, as shown in Tab17, reveal significant differences between plants treated with microbial fertilizers and untreated controls in terms of plant height, ear length, number of grains per ear, and 1000-grain weight. Notably, the improvement in wheat traits was more pronounced when treated with the compound strain inoculant compared to single-strain treatments. These positive physiological changes not only provide a solid foundation for increased yield but also strongly demonstrate the practical advantages of composite strain inoculants over single strain inoculants.

Table 16. Effects of microbial inoculants containing different strains on wheat yield

Treatment	Average yield (kg)	Each treatment yielded more than the control (kg)	Each treatment yielded more than the control (%)
Bacillus amylolyticus, Bacillus giganteum	28.4aA	3.9	16.3
Bacillus amylolyticus	26.9bA	2.8	10.5
Bacillus giganteum	26.8bA	2.7	10.4
CK	24.3cB		

Note: The data in the mean column of the above tables marked with different lowercase letters indicate a significant difference at the level of 0.05, while those marked with different uppercase letters indicate a significant difference at the level of 0.01.

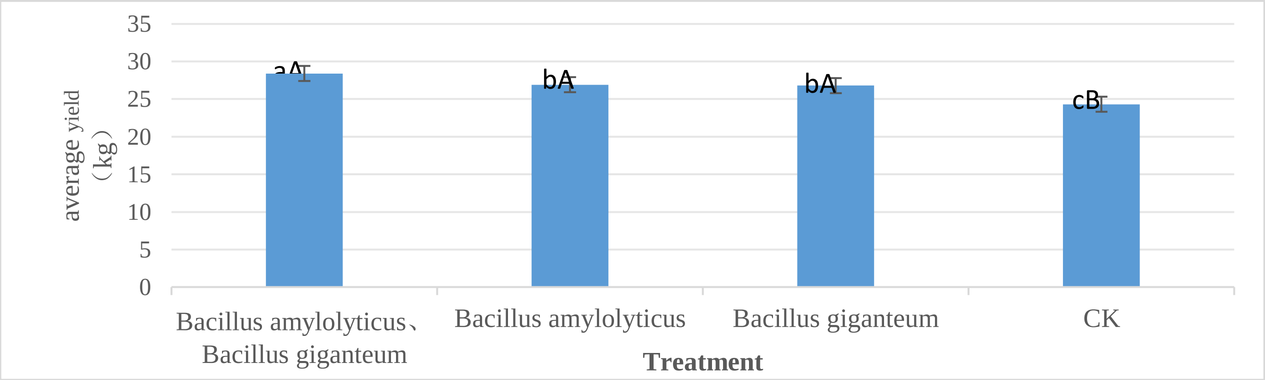

Figure 6. Effect diagram of the application of bactericide products containing different strains on wheat1

Table 17. Statistics of plant traits of single strain and multi-strain strains on wheat

Treatment	Plant height(cm)	Ear length (cm)	Acre spike length(cm)	Number of grains per spike (cm)	Thousand grain weight (cm)
Bacillus amylolyticus, Trichoderma	100	14	19.6	107.9	27.1
Bacillus amylolyticus	100.8	13.9	19.5	108.1	27.2
Trichoderma	101.5	14.7	20.1	114.3	28.6
CK	98.1	13.4	18.3	105.3	25.4

3. Conclusions and Outlook

3.1. Conclusion

3.1.1. Analysis of Strain Application Progress

Over the past decade, China's microbial fertilizer industry has undergone significant shifts in its strain application strategy. Initially reliant on external strains, the industry has gradually transitioned to a phase of independent research and development, strain screening, and preservation. During this period, the application scope of spores has expanded, and research on novel functional strains, such as fungi and non-spore bacteria, has become increasingly vigorous. In the realm of strain identification technology, the integration of traditional methods with modern science and technology, the widespread adoption of automation equipment, and the in-depth exploration of molecular biology techniques have collectively enhanced the accuracy and efficiency of identification processes. Consequently, a multi-dimensional, complementary verification and identification framework has been established. In this context, the microbial fertilizer industry has witnessed an increase in bacterial diversity, functional integration of composite strains, standardization of preservation and rejuvenation techniques, the flourishing of new functional strains, modernization of strain propagation methods, and exploration of innovative carrier applications. These technological advancements have provided a robust foundation for improving the quality and efficacy stability of microbial fertilizer products over the past decade.

3.1.2. Field Effect Analysis

A series of paired design experiments were conducted, wherein inoculants containing the same microbial community were applied to crops such as tomatoes, cucumbers, and peppers. Compared to conventional management practices, significant differences in the effects of these inoculants on various crop species were observed, highlighting the importance of selecting crop-specific microbial inoculants to enhance their effectiveness. Subsequent randomized block design experiments, extended to cucumbers, grapes, and tomatoes, examined bactericidal products containing diverse strains. These studies revealed that bactericidal efficacy was strongly correlated with the diversity of the strains present and varied within the same crop type. Additionally, a randomized block design experiment involving rice compared the application of single-strain and composite-strain bactericides. The results unequivocally confirmed the superiority of composite-strain bactericides in terms of effect, aligning with the current trend in the microbial fertilizer industry towards developing multi-strain composite products.

The findings indicated that the performance of microbial inoculants was intimately linked to the types and relative abundance of microorganisms included. Differences in the characteristics and quantities of individual strains could significantly impact the efficacy of the preparation. An analysis of the physiological characteristics of experimental crops further revealed that the bactericide could effectively enhance crop quality, accelerate growth and development, optimize plant morphology, and create favorable conditions for increased crop yield.

3.1.3. Correlation Analysis Between Strain Application and Field Effect

The efficacy of microbial fertilizer is fundamentally contingent upon its strain composition, which directly determines the product's performance. Different strains exert heterogeneous effects on the same crop, leading to varied application performances of fertilizers across different crops, even when the strains are identical. These observations underscore the strategic significance of strain selection in microbial fertilizer design. Therefore, to maximize crop growth, the production and application stages must involve selecting appropriate strains for specific crop types and subsequently addressing diverse agricultural needs through tailored microbial fertilizer solutions. This indicates that the future industry trend will emphasize professional product development and application practices.

3.2. Discussion and Outlook

1. Given that the efficacy of microbial fertilizer is significantly influenced by environmental factors such as light, temperature, and moisture, and considering China's vast geographical expanse with marked variations in soil characteristics, climate conditions, and moisture states, it is imperative to conduct experimental research in several representative regions. These experiments aim to establish a robust theoretical and practical foundation for the scientific production and efficient application of microbial fertilizers by amassing extensive data.
2. Although the fertilizer effect was investigated for one year in this study, its long-term and comprehensive impact requires further verification over an extended period. While the existing experimental framework offers certain streamlining advantages, achieving higher research accuracy and scientific reliability necessitates a more systematic and enduring experimental design in future explorations. Inevitably, this will require increased investments, including human and material resources, to accumulate substantial data and conduct rigorous statistical analysis.
3. The understanding of microbial functional mechanisms remains in its nascent stage, and the analysis of key scientific issues necessitates deeper and more refined exploration. This situation clearly constrains the advancement of microbial fertilizer research. Consequently, future research must escalate to a more in-depth and specific evel to achieve breakthroughs in these areas.

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