

Study on bacteriostatic activity of bacillus against penicillium extensus and its application to pear

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Abstract. Currently, research has shown that *Bacillus* plays a crucial role in preserving, storing, and transporting fruit and vegetable products, leading to positive economic outcomes. Through the discussion results, it was found that *Bacillus polymyxoid* HT16 had a remarkable inhibitory effect on *Streptospora nigrospora* of pear. The observations made using scanning electron microscope and it revealed that the spores of the pathogenic bacteria barely germinated after the treatment of crude protein extraction. Additionally, there was a noticeable ablation phenomenon, with the mycelium appearing twisted and atrophied. Furthermore, the outer epidermis of the mycelium was shed and broken. Similarly, the bacteria used for biocontrol also demonstrated a significant impact on suppressing harmful bacteria in grape plants, contributing to the ongoing research on the mechanisms behind grape biocontrol. Providing a theoretical basis for the application of *Bacillus amyloolytica* 22T in pear biological control, the antibacterial activity against *penicillium extensus* was evaluated. This passage provides a review of the inhibitory activity of bacillus against penicillin expansus.

Keywords: *Bacillus*, Fermentation broth, *Penicillium elongatum*, Pear, Biological control

1. Introduction

Pear is known for its natural geographical distribution and economic value. It has the remarkable ability to clear heat and detoxify, promote fluid and quench thirst, and replenish water in the body. The pear is packed with an abundance of fruits, microorganisms, and an array of trace elements. Nevertheless, the issue of pear mold can pose a significant challenge during transportation. There are various factors that can contribute to pear mildew, such as black heart, fruit stem rot, *penicillium*, black skin, brown spot, black spot, and more. Similar to a biochemist, the storage period of pear fruit is greatly impacted by pathogenic bacteria. This disease during the storage process significantly affects the quality and duration of storage, leading to a significant reduction in economic value.

Conventional methods of food sterilization also come with their own limitations. Thus, current research is centered around biological sterilization. The study revealed the significant impact of *Bacillus* on the prevention of corrosion in fruit and vegetable products during transportation, storage, and preservation [1]. Additionally, it plays a crucial role in enhancing the economic value of these products. Typically, *Bacillus* refers to the genus *Bacillus* of the *Bacillus* family, which relies on oxygen to produce spores. It is found in various places in nature and can persist for long periods of time in the form of spores. Currently, *Bacillus* is primarily used to combat biological spoilage caused by grey mold and *penicillium*. Due to the prevalence of *Bacillus* in nature, one of their remarkable characteristics is their

ability to produce spores that are highly resistant to stress. This makes *Bacillus* a valuable tool in production practices for preventing and controlling fungal and bacterial diseases in fruits, vegetables, crops, and more. *Bacillus subtilis* has proven to be effective in preventing peach brown disease and litchi fruit rot. The inhibitory effect of *Bacillus polymyxoides* HT16 on postharvest black spot of pear also demonstrated significant potential for application. This paper thoroughly examines the inhibitory activity of *Bacillus* on penicillium of pear and other related studies. It also includes references and offers suggestions on how to effectively apply bacillus to prevent spoilage during the transportation of agricultural products.

2. Overview

The term “*Bacillus*” typically denotes the genus *Bacillus* within the *Bacillus* family, which is characterized by its ability to produce spores of gram-staining positive bacillus. This bacterium is widely spread in the natural environment and can persist in the form of spores for extended periods of time. Currently, *Bacillus* is mostly employed for the purpose of preventing and managing biological deterioration in griseus and penicillium. As an illustration, the inhibitory effects of *Bacillus subtilis* H110 from Qidongplum on postharvest penicillium and black spot of apple pear were observed [2]. Numerous studies have demonstrated the efficacy of diverse *Bacillus* H110 treatments in mitigating the detrimental impacts of penicillium and black spot on apple pear fruit. *Bacillus* is a significant microbial group that is abundant in nature. Its notable characteristic is its ability to generate highly resistant spores. This makes *Bacillus* suitable for use in production practices to prevent and manage fungal and bacterial diseases in fruits, vegetables, crops, and other crops.

2.1. *Bacillus* and its application in agriculture

One potential application of *Bacillus subtilis* is its utilization in the prevention and management of peach brown disease and litchi fruit rot. One of the bacilli, *Bacillus polymyxoides* HT16, has demonstrated significant potential in the inhibition of postharvest black spot in pear. The assessment of the application efficacy of *Bacillus polymyxoides* HT16 on the pear black spot pathogen revealed a highly potent inhibitory impact, with an inhibitory rate of 53.2% [3]. The findings of the study indicate that *Bacillus polymyxoides* HT16 exhibited a noteworthy inhibitory impact on *Streptospora nigrospora* strains found in pear. The utilization of scanning electron microscopy and electron microscope revealed that following the application of crude protein extraction treatment, the germination of pathogenic bacterial spores was minimal. Additionally, an ablation phenomena was seen, accompanied by the twisting and atrophy of mycelia, as well as the detachment and breaking of the outer epidermis of the mycelia. The findings of this study demonstrate the viability of *Bacillus polymyxoides* HT16 as a bacterium for biocontrol purposes. This study aimed to explore the effects of *Bacillus amylus* 22T on colony diameter, spore germination rate, and mycelium content of *Penicillium* expansions, using the prior configuration of LB liquid medium, LB solid medium, and PDA plate medium. For the purpose of assessing the antibacterial efficacy of *Bacillus amylolytica* 22T against *Penicillium extensus*. Furthermore, it aims to establish the theoretical foundation for its implementation in the biological management of pear.

2.2. *To expand the harm of penicillium in pear and its control measures*

The fruit possesses a high nutritional content and an ample water content, thereby establishing a conducive environment for the proliferation of microorganisms, including mold. Once subjected to microbial infection, it becomes susceptible to mildew. *Penicillium* typically occurs subsequent to fruit mildew. *Rhizopus*, *mucor*, *Aspergillus*, and *penicillium* are among the commonly encountered molds. Typically, mold infiltrates the fruit following damage, proliferates within the fruit, and leads to mold growth and decay. The microorganisms create toxins, which are harmful metabolic compounds, during their growth, which can negatively impact human health. Picillin is one of these poisons. Moldy fruits and their derivatives, such as apples, pears, hawthorns, tomatoes, and apple juice, are the primary sources of this substance. Penicillins, which are the predominant toxin found in moldy fruits, are

synthesized by *penicillium diffusum*. Penicillin, a toxic substance, possesses detrimental effects on the gastrointestinal system, as well as exhibiting carcinogenic and teratogenic characteristics. Aflatoxin is the most poisonous. Aflatoxin is widely recognized as the most carcinogenic toxin identified to date. Spoiled peanuts, corn, and dairy products are sources of this substance. Additionally, it can be observed in mildewed raisins and various other dehydrated fruits. Aflatoxin primarily elicits the development of liver cancer and subcutaneous sarcoma, exhibiting a carcinogenic impact that is 75 times higher than that of hazardous substances such as dimethylnitrosamines. Certain types of molds have the potential to induce renal and hepatic impairment, as well as neurological diseases. The presence of mildew in fruit can lead to financial losses during the processes of transportation and storage.

Current research suggests that *Bacillus* exhibits significant promise as a biocontrol agent and a means of preventing some postharvest illnesses affecting fruits. For instance, the research and analysis conducted by Che Jianmei demonstrated that *Bacillus* SPP exhibits the most effective inhibitory impact on saprophytic bacteria and fungi in the Longan region [4]. *Bacillus licheniformis* W10, a recently identified biocontrol bacterium, has the ability to metabolize antibacterial proteins. The research conducted by Ling Zheng reveals that the bacteria and antimicrobial proteins present during the fruit storage period of apple ring spot (*Botryosphaeria berengeriana* f.), anthrax bacteria (*Glomerella Coagulate*), and citrus *Penicillium* germs (*Penicillium Citrinum*) exhibit antagonistic effects [5]. This bacterium demonstrates a favorable preventive effect on postharvest ring spot, anthracnose, and *penicillium* in both apple and citrus fruits. The findings indicated that the W10 bacterial solution, culture filtrate, and antibacterial protein had significant detrimental and inhibitory impacts on the mycelial shape and proliferation of the three pathogens. The compound W10 exhibited the most pronounced impact on the mycelia of the fungus. The presence of W10 was found to exert an influence on the processes of sporulation, spore germination, and bud tube formation in the bacteria. In conclusion, *Bacillus* exhibits significant promise for the preservation of fruit.

2.3. A review on the antibacterial activity of *Bacillus* against *Penicillium expansum*

The results indicate that *Bacillus* has a significant impact on the prevention and control of spoilage in the majority of fruits and vegetables. An experiment conducted by Du Hongyan demonstrated the impact of *Bacillus amylolityca* on the prevention and control of grape powdery mildew. The results indicated that *Bacillus amylolityca* was successful in effectively managing grape powdery mildew and enhancing photosynthesis in grape leaves [6]. The data is presented in the following manner: During the initial peak period of grape powdery mildew, the control effect of *Bacillus amylolityca* was found to be significantly lower than that of fungicides ($P < 0.05$) after the third application. However, it still achieved a control rate of 70.00%. In the second peak of grape powdery mildew, after three applications, the control effect of *Bacillus amylolityca* was 65.00%, which was significantly higher than that of the fungicide ($P < 0.05$). Thus, *Bacillus amylolityca* has the potential to serve as an effective method for managing grape powdery mildew in Yunnan's agricultural regions. The study conducted by Zhang Lili investigated the indoor inhibition effect of *Bacillus subtilis* on pear fruit rot prevention and control. The results indicated that the culture of *Bacillus subtilis* Sf-19 exhibited the most effective comprehensive inhibition effect. It achieved a plate inhibition rate of 87.33% for mycelium growth and spore germination inhibition rate. The study on the inhibition effect of *fructus verticillaris* on fruit was conducted in two parts: protection (I) and treatment (II). The protection test (I) of the Sf-19 cultured stock solution resulted in an inhibition rate of 71.97% for pear *verticillaris*. Similarly, the treatment test (II) showed an inhibition rate of 64.92%. The inhibitory effect of the Sf-28 culture solution on pear ring fungus was evaluated comprehensively. The plate inhibition rate of mycelia growth of pear ring fungus was found to be 87%, while the inhibition rate of spore germination was 80%. In the fruit pear ring fungus protection test (I), the Sf-28 culture solution exhibited an inhibition rate of 64.54%. Similarly, in the treatment test (II), the inhibition rate was measured to be 59.21%. The test conducted yielded a significant effect, with an observed inhibition rate of approximately 40% on pear mold. In conclusion, it can be observed that the practical implementation of *Bacillus* for biological control is supported by a strong theoretical basis.

Additionally, the utilization of bacillus for the control of corrosion in vegetables and fruits exhibits significant potential.

3. Review of relevant Experimental results

3.1. *Penicillium expansum*

The colonies of *Penicillium expansum* exhibit small blotchy, grassy green appearance with a cinnamon-colored back and small red spots in the center. The tip of the conidial peduncle consists of 1-2 brush-like branches, while the peduncle itself has a slender, bottle-shaped structure. The conidia exhibit an oblate or round shape and possess a greenish coloration when they are clustered together. The conidia are characterized by their small size, measuring $1.8 - 2.2 \times 1.8 - 2.2 \mu\text{m}$, as depicted in figure 1.

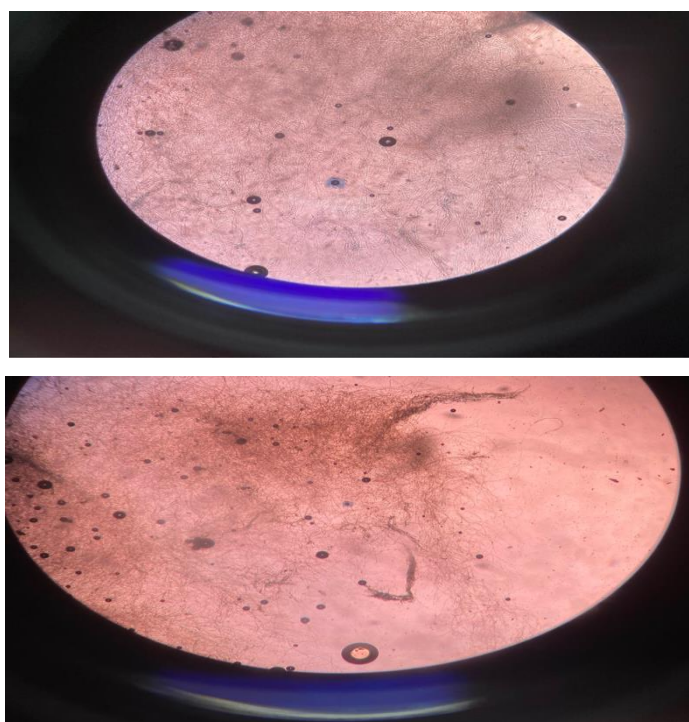


Figure 1. Mycelium imaged under a microscope from Zhejiang University

3.2. *In vivo inhibition test*

The strain *Bacillus* 22T exhibits significant potential for the biological control of pears. The experimental data, depicted in Figure 2, reveals that the average incidence of pear fruit treated with 22T within a 48-hour timeframe is 66.6%. This percentage is significantly lower compared to the incidence observed in pear fruit treated with normal saline. The average bacteriostatic zone of the 22T fermentation solution was measured to be 0.5 units.

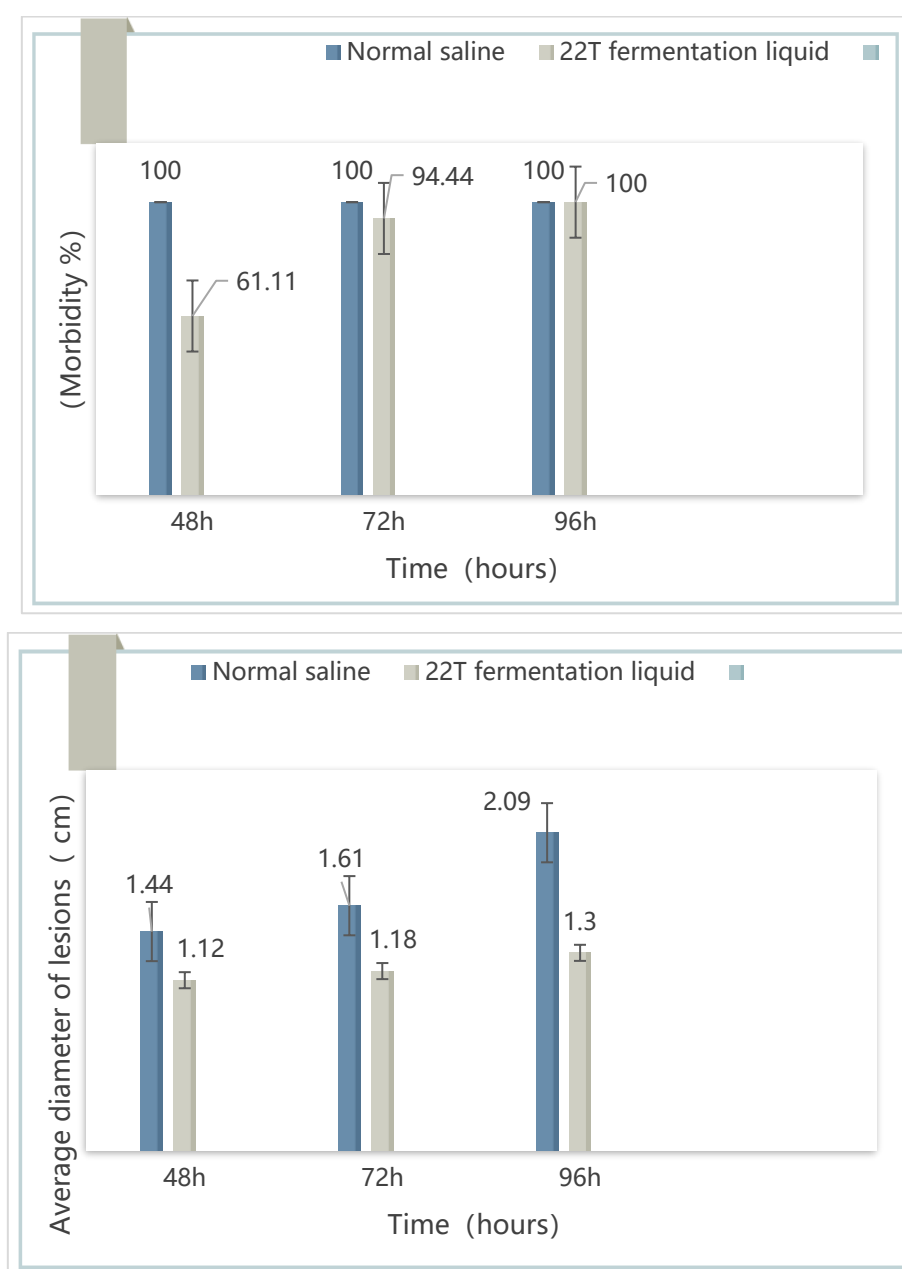


Figure 2. Inhibitory effect of Bacillus 22T on Penicillium

4. Conclusion

The bacteriostatic effect of Bacillus 22T on pear fruit is readily apparent. The application of 22T has a clear and noticeable impact on the eradication of penicillium extensus, making it a promising candidate for biocontrol against pear rot disease. The potential of Bacillus 22T in preventing and controlling pear penicillium disease is promising. Additionally, it has demonstrated a notable ability to inhibit pathogenic bacteria. Therefore, there is a possibility for its practical application. However, this paper is deficient in comprehensive research and fails to engage in thorough investigation, providing only superficial experimental results. Thus, it is recommended to postpone this experiment until the conditions have been further enhanced and stabilized in order to conduct a more comprehensive study, enhance the accuracy of the results, and obtain more precise experimental reports and data.

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