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Full Length Article

Isolation, Identification and the Biological Characterization of *Botrytis cinerea*

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Abstract

Botrytis cinerea (Berk.) Cooke, commonly known as the lily leaf blight, is a major fungus disease and one of the main factors that limit lily cultivation and production. In this study, the pathogen was isolated and purified from typical lesions on the lily. Pathogen sporulation method and the pathogen's biological characteristics were studied systematically. In this investigation we have isolated *B. cinerea* from lesions on lily and identified morphologically and using ITS gene sequencing technology. Results of our investigation of the disease-resistance of different varieties of lily showed that: *Lilium regale* Wilson has the highest disease-resistance index, while Asian lily varieties were most susceptible to disease. The growth rate of mycelium was fast in various media and mannitol and $(NH_4)_2SO_4$ were found most suitable carbon and nitrogen source, respectively. The mycelia growth was observed slowly under full light conditions. The optimal temperature for mycelium growth was 15~20^oC while the lethal temperature of mycelium was 75^oC within 10 min. The mode of the conidial germination was bidirectional. The optimal temperature for conidial germination was 25^oC, while the lethal temperature was 75^oC within 10 min. The Glucose was found as best carbon source for conidia germination under optimum pH 5. Light conditions had no effect on conidia germination. © 2018 Friends Science Publishers

Keywords: Lily; *Botrytis cinerea* (berk.) Cooke; Biological characteristic

Introduction

Grey mould is a common fungal disease that is difficult to prevent and control. The pathogen *Botrytis cinera* has a wide range of hosts (Chen *et al*., 2015). In addition to damage to important economic crops, cereal crops and vegetables, occurrence of grey mould on ornamental plants is also becoming more and more serious (Dong *et al*., 2014). With the development of agriculture, rotation of vegetables and flowers in greenhouses has become much more frequent, allowing the pathogen to cross-infect into different kinds of crops (Zhang *et al*., 2002). Research has reported that the grey mould has occurred in 50 kinds of flowers such as Lily (Zhang *et al.*, 2009), Begonia, Peony, Rose, Rurse, *Primula obconica*, Impatiens, Gerbera and Phalaenopsis (Liu *et al.*, 2004). Grey mould is especially a devastating disease for lily and medicinal and edible Lilium, both of which occur widely throughout the world (Kim *et al.*, 2007).

B. cinerea can make host plants infected with diseases. Different parts of various plants have different symptoms. However, under high humidity conditions, it will grow a remarkable grey mould on the incident locations. Whether *B. cinerea* can infect grey mould is an important symbol (Zhang *et al*., 2015). Regardless of the size of Lily, *B. cinerea* can infect the upper parts of tender tissues. The stems, leaves and flowers of crops all can be infected with the disease - the most severely damaged are the leaves and flowers. The damaged leaves have yellowish brown to reddish brown hygrophanous lesions at an early stage, most are oval or circular spots. When the environment is wet, the lesions expand rapidly and the diseased areas have a grey mould layer. When the environment is dry, the spots crack and the lesions are dry and crisp, appearing light grey or slightly translucent (Du and Xu, 2014). When the plant is severely ill, the leaves wither, like after a fire. Damaged stems of Lily generally have brown or reddish brown elongated patches and mycelium grows in the stem tube, filling it in, which can destroy its conducting tissue. The stalk will be broken or lodging after decay and soft when severely damaged (Zhu *et al.*, 2009).

When Lily petals are infected, they appear as white, brown or light brown hygrophanous lesions, and can even

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decay severely (Yang, 2008). An infected bud, with the progression of the disease, occurs initially with small brown spots and the lesion can cause multiple buds to stick together and later on, decay. Lily grey mould disease, from areas of low temperature and high humidity, is not only a serious disease affecting the production of lily in the world, but is also a typical air borne disease. The pathogenic bacteria and spores can spread widely in the air, water, etc., and there is currently a trend that it is spreading. This paper has researched the characteristics of the symptoms, pathogen isolation, identification of diseases, and the conditions of producing spore, conidia germination and other aspects, in order to make clear the relation between the mycelial growth, conidial germination and environmental conditions. To measure and analyze the relativity between determination of the lethal temperature of conidia and Lily grey mould symptoms in the field, we need to provide a theoretical basis for the comprehensive prevention of the disease.

B. cinerea currently has about 25-30 species and is a humic nutrition pathogen infection of plants. Based on phylogeny it has launched 22 recognized breeds (Staats *et al.*, 2005). For example, some new varieties are: *B pseudocinerea* (Walker *et al.*, 2011), *B. caroliniana* (Li *et al.*, 2012a b), *B. fabiopsis* (Zhang *et al.*, 2010a), *B. sinoallii* (Zhang *et al.*, 2010b), etc. Sclerotia production time and size are affected by ambient temperature and there is a large difference between different strains. Therefore, this article studies the influence of different media - carbon, nitrogen, temperatures, pH value, light, culture days, lethal temperature - on the *B. cinerea* mycelium growth and conidia germination conditions. This is a preliminary study of the biological characteristics of *B. cinerea*, in order to clarify the relationship between occurrence and environmental factors of *B. cinerea* and to provide valid evidence for further study of the incidence rules and conditions of gray mold.

Materials and Methods

Plant Materials

B. cinerea samples were collected from Shenyang, Liaoning Province, China in May 2013 for research purpose.

Pathogenic Fungal Isolation and Purification

Pathogenic fungi were isolated using the Tissue Isolation Method. Leaf that only diseased but symptoms were not obvious. We took intact leaves with scissors the leaves were placed on plat dishes and incubated under humidity conditions. Sporocarps were produced under suitable temperatures and humidity conditions were sustained for 1 day, which were observed and examined under a microscope. The diseased leaves with obvious spots were taken directly as fresh samples, next a typical single diseased spot was selected and the ill/healthy junctions were cut into 3-5 mm tissue pieces with scissors or a scalpel, and the *B.* was isolated on a clean bench.

The isolated strains were placed in an incubator for 3-4 days in darkness, then on a clean bench an inoculating loop was used to pick up the typical *B.* colony and it was streaked on PDA with the streak plate method using a small amount of mycelium. The purified plat dish was placed in an incubator, carried out pure culture at 21°C and conidia were produced after 3-7 days. On the basis of observation of mycelium traits, shape and size of the spores and morphological characteristics of sclerotium by microscopy-examined, the type of fungi were tentatively identified and pure culture of lily gray mold was obtained.

Koch's Postulation to Identify Disease

Following Koch's rule, pure cultures of pathogen were used to identify the disease. After culturing and purifying, the pathogens were inoculated with leaves of *Lilium formolongi*, which have 4-6 leaves, and incubated under humidity conditions for 48 h, then incubated at room temperature normally. Leaf damage symptoms were observed after lesions were grown on the leaves. A conventional tissue isolation method was used to separate the pathogen and the pathogen was observed under a microscope after cultivation for identification.

Morphology Observation of Pathogenic Fungi

Comparative observation was made between inoculated leaves and diseased leaves in the field. Typical spots of infection by *B. cinerea* were selected the mold layer on the back of leaves was scraped, observed and photographed under a light microscope. The number of pathogenic fungi was counted, and the size and morphology of conidia were recorded observed under the light microscope.

Molecular Identification by ITS Gene Sequencing

The separation and purification of pure strains were cultured for 20 days in PDA medium, mycelium scraped and dried in a 60^{o}C oven, then stored at -20^{o}C. Liquid nitrogen was used to grind the above dried mycelia into a fine powder. DNA was extracted by plant genomic DNA extraction kit (Tiangen Biochemical Technology Co. Ltd's China). The extracted DNA was amplified with universal primers ITS1 (5'-TCC GTA GGT GAA CCT GCGG-3') and ITS4 (5'-TCC GTA GGT GAA CCT GCGG-3'). Amplification reaction mixture comprised of DNA template (2 µL), ITS1 (1 µL), ITS 4 (1 µL), Taq polymerase (0.5 µL), Buffer (2.5 µL), dNTP (0.5 µL), ddH_2O (17.5 µL), using 25 µL reaction system. The PCR program was as follows: 3 min pre-denaturation step at 94^{o}C and 40 sec denaturation step at 94^{o}C, annealing at 60^{o}C for 30 sec and extension at 72^{o}C for 1 min. After the 35th cycle, the last step was

incubation at 72°C for 5 min. The resultant product was immediately cooled to 16°C and resolved on 1.0% agarose gel electrophoresis.

Optimization of Various Parameters on the Mycelium Growth and Spore Germination

Various process parameters such as medium (PDA, PSA, Oats, Lily leaf, Lily flower, Czapek, Rechard and Agar) initial medium pH (at 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0 and 11.0), incubation temperature (5, 10, 15, 20, 25 and 30°C), lethal temperature (45, 50, 55, 60, 65, 70, 71, 72, 73, 74 and 75°C), carbon source (maltose, glucose, mannitol and soluble starch), nitrogen source (peptone, yeast extract, glycine and $(NH_4)_2SO_4$) and illumination condition (24 h dark, 12 h dark/light cycle and 24 h light) were optimized for the mycelium growth of *B. cinerea*.

Results

Morphology and Disease Identification of *B. cinerea*

Pathogens from the infected lily leaves were isolated, purified and cultivated. Pathogenic fungi morphology that was grown in the medium was the same as that separated from the field. Pathogenic fungi was observed under the microscope and identified by Professor Liu Zhiheng, College of Plant Protection, and determined to be the pathogen of lily gray mold, *B. cinerea.* Results showed that *B. cinerea* was inoculated in the healthy leaves and after 5-7 days the leaves produced spots. All symptomatic characteristics of the artificial inoculations of lily gray mold, such as Fig. 1, were similar in appearance to those original diseased leaves collected from the field.

All symptomatic characteristics of the artificial inoculations of lily gray mold were similar in appearance to those observed on lily gray mold in the field: tawny to reddish brown water-soaked shape spots in the early stages of the disease which gradually developed into oval or round shaped spots. There was a discernible ill health junction, with the spot growth producing white mycelium on both sides of the lesion area and then gray mold layer forming in cultures. There can be interconnections between the spots which developed into large irregular spots. The leaves became wilted and showed the burning shape in the later stages. Upon inspection, the characteristics of disease symptoms showed typical symptoms of being infected by gray mold, the disease was tentatively identified as *B. cinerea* (Berk.) Cooke.

Artificial inoculations of lily gray mold compared to lily gray mold in the field showed: gray mold layer was produced on both sides of spots including pathogenic fungal mycelium, conidium and conidiophore. Mycelium gathered into a mass or cluster were dark gray, relatively straight, had a few branches and the diaphragm was 20-25 μm; conidium was colorless, oval or round shape, the top was almost obtusely round or slightly pointed, 10-15 × 5-10 μm. The germination of conidium could be from a single side, two ends or center and the germ tube was long and thin, as shown in Fig. 2.

Molecular Identification of *B. cinerea*

For molecular identification, genomic DNA was extracted and PCR amplification was done using ITS region. Sequencing results showed a length of 494 bp as shown in Fig. 3. The target sequence for Blast sequence alignment in the NCBI, and the ITS sequence of *B. cinerea* B05.10 strains ID was gb | KP003819.1 |, sequence homology was 100% between *B. cinerea* B05.10 and target fragment. The results showed that pathogenic fungi of lily grey mould and the ITS of *B. cinerea* B05.10 strain sequence homology was 100%. By Koch's Rule, microscopy, molecular identification, synthesize appraisal results to determine pathogen of lily grey mould was *B. cinerea*.

Optimization of Various Process Parameters for Growth of *B. cinerea* Mycelium and Spore Germination

Influence of different media on the mycelium growth: Different media were used to study the growth of *B. cinerea* and results showed that Oats, Lily leaf, Lily flower, Rechard and Czapek medium had relatively fast growth rate and the largest diameter of the colony. After five days of incubation, Oats, Lily leaf, Lily flower, Rechard and Czapek medium, the colonies filled the whole petri dish. Colonies on PDA grew 7.84 cm in diameter as compared to the above five kinds of medium, the mycelium growth rate was slower, and the mycelium growth rate on Agar medium was slower than on PDA. The mycelium growth rate on PSA was slowest and 6.18 cm in diameter. From the Fig. 4 it can be seen that the color and morphology of gray mold colonies in every medium showed significant difference: colonies on Rechard medium were most dense; colonies on PDA, PSA, Czapek, Oats, and Lily flower medium were relatively dense; colonies on Lily leaf medium were relatively sparse; colonies on Agar medium were more sparse than others. The color of all colonies was close to gray, except the tawny colonies on PSA and white colonies on Agar medium.

Influence of Different Carbon Source on the Mycelium Growth

When different carbon sources were used, pathogenic fungal growth was slowest on the mannitol medium having 8.29 mm in diameter, but pathogenic fungal growth was faster in the other three kinds of medium. After five days incubation in soluble starch medium, the colonies filled the whole dish. The growth of gray mold was obviously fastest when soluble starch was used as carbon source. The color and morphology of gray mold colonies on these media had significant differences: colonies on soluble starch medium

Fig. 1: The symptom of *Botrytis elliptica* (Berk.) Cooke. (A) The field serious onset conditions, (B) The field symptoms (C) Field typical lesion, (D) Laboratory inoculation onset of symptoms

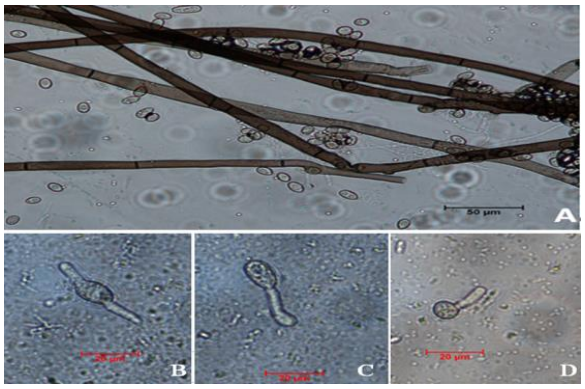

Fig. 2: The shape of *B. cinerea* (A) mycelium (B) Two ends germination of conidia (C) Top germination of conidia (D) Center germination of conidia

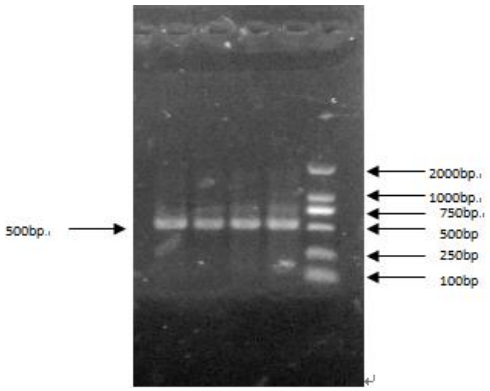

Fig. 3: Gel electrophoresis of amplification products by ITS

were denser than others; colonies on glucose, mannitol and maltose medium were relatively dense; the color of colonies on soluble starch medium and glucose medium were all close to off white. The color of colonies on mannitol medium became dark and close to ash black. The color of colonies on maltose medium had a little bit of yellow and was close to yellow-grey as shown in Fig. 5.

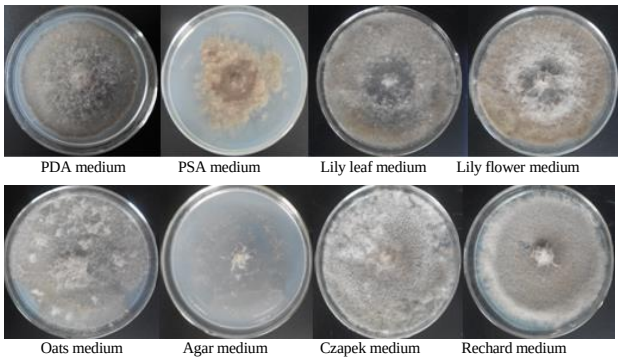

Fig. 4: Mycelial growth pattern on different media (30 d)

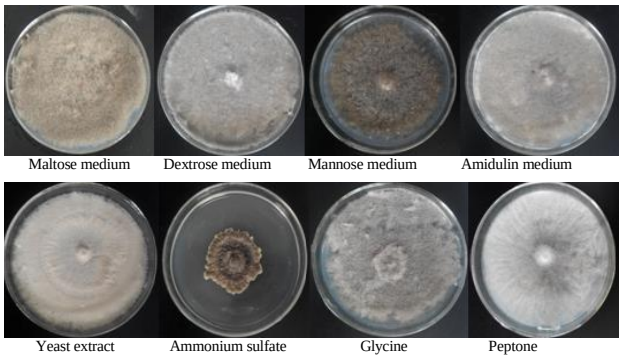

Fig. 5: Effect of carbon and nitrogen source medium on mycelial growth of *B. cinerea*

Influence of Different Nitrogen Source on the Mycelium Growth

When different nitrogen sources were used, the gray mold growth was fastest on the peptone, yeast extract, and glycine medium, but pathogenic fungal growth was slower on the $(NH_4)_2SO_4$ medium having 4.18 mm in diameter. After incubation for five days on peptone and yeast extract medium, the colony filled the whole dish. So we believe that the optimal nitrogen sources for gray mold growth were peptone and yeast extract. Colonies on $(NH_4)_2SO_4$ medium were most dense with dark and close to yellow-gray color. The colonies on peptone and yeast extract medium were relatively dense and color was relatively light while colonies on glycine medium were relatively sparse with gray color (Fig. 5).

Effect of Temperature on Mycelium Growth

Pathogenic fungal growth temperature ranged from 5 to 30°C to check the maximum growth. Results (Fig. 6) reveals that pathogenic fungal growth was slow at 30°C (4.67 mm in diameter), and after 30 days of incubation, there was no further expansion. The experiments showed that the pathogenic fungi at 10, 15 and 20°C (three degree variables in total) incubated and the colony filled the whole dish on the 7th day.

The color and morphology of gray mold colony on the four kinds of treatment showed some difference: colonies at 15°C and 20°C were most dense, and growth rate was fastest. So we believe that the optimal temperature ranges from 15 to 20°C for gray mold growth. Pathogenic fungal growth rate was fast, colonies were white and relatively sparse at 5°C and 10°C. The fungal growth was fast, the colony was gray and relatively sparse at 25°C and slow growth rate was observed at 30°C.

Influence of pH, Illumination Conditions and Culture Days on the Mycelium Growth

Various initial medium pH was used to check the growth of *B. cinerea* and results showed that the growth rate was relatively slowest at pH 4.0, 5.0, 9.0 and 11.0. The growth rate of the colony was relatively faster at pH 10.0. The growth of the colony was uniform speed at pH 6.0, 7.0 and 8.0. Mycelial growth reached a peak on the fifth day under the illumination condition and later mycelium almost stopped growing. The results prove that the effect of illumination on mycelium growth was not obvious. The mycelium growth rate was similar under the conditions of alternating light and dark periods. The growth rate was uniform for 5-15 days and then stagnated. The color of the colony was similar under three types of illumination procession: the colony was densest under the illumination condition, the color was dark and close to taupe, which prove that illumination stimulate the generation of melanin in the mycelium. The colony was denser under the condition of alternating light and dark, and the colony was sparsest under the dark condition. Colonies showed uniform speed for 10-15 days, and were white in the early stages and mycelium was thin and sparse. Colonies expanded rapidly and color became dark which appeared yellow-gray on the 10th day. With the increase in incubation time, the colony stopped expanding, mycelium became dense and color became dark (Fig. 7).

Determination of Germination Condition of *B. cinerea* Conidia

Influence of carbon source and nitrogen source: Different carbon sources incubated pathogenic fungi for 24 h, and the germination rate of conidia was different in the five kinds of carbon source broth. Conidia germination rate was highest (82.9%) in the glucose broth, respectively 35.9% and 34.6% in mannitol and soluble starch broth, and the germination rate was 52.9% in water for reference. The results prove that conidia germination needs some carbon source. Different nitrogen sources incubated pathogenic fungi after 24 h, germination rate of conidia was slow in the four kinds of nitrogen source broth. Conidia germination rate was relatively high (48.0%) in glycine broth, conidia almost didn't germinate and germination rate was only 9.4% in KNO_3 broth, and conidia germination rate was 52.9% in water for reference.

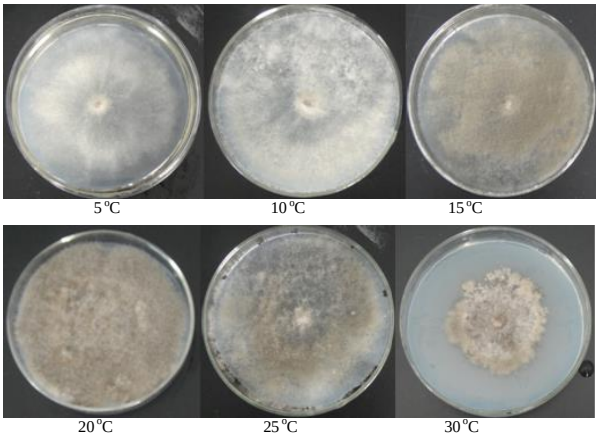

Fig. 6: Effect of temperature on mycelial growth of *B. cinerea*

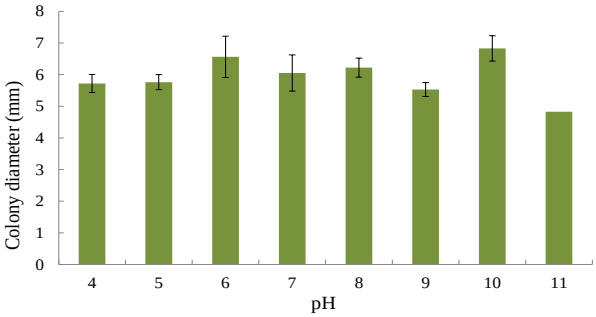

Fig. 7: Effect of pH on mycelial growth of *B. cinerea*

The results prove that conidia germination does not need a nitrogen source, on the contrary, the nitrogen source could, to a certain extent, inhibit conidia germination (Fig. 8).

Influence of Temperature, pH and Illumination Conditions

The conidia of fungi was incubated at different temperatures for 24 h. The results showed that conidia almost did not germinate at 5°C having germination rate of 8.9% only while germination was fastest at 25°C with germination rate of 52.9% (Fig. 9). So we believe that the optimal temperature for conidia germination was 25°C (Fig. 9). Conidia can germinate at pH between 2.0 and 12.0 while fastest germination rate was observed at pH 4.0 (60.1%) and 5.0 (63.2%) as shown in Fig. 9. Extreme acidic conditions inhibit the spore germination. The influence of different light conditions for conidia germination were limited, the difference in spore germination rate was not significant, so we believe that light did not significantly affect the spore germination.

Observation of Germination and Speed of Spore and Determination of the Lethal Temperature of Conidia

B. cinerea conidia have three main means of germination:

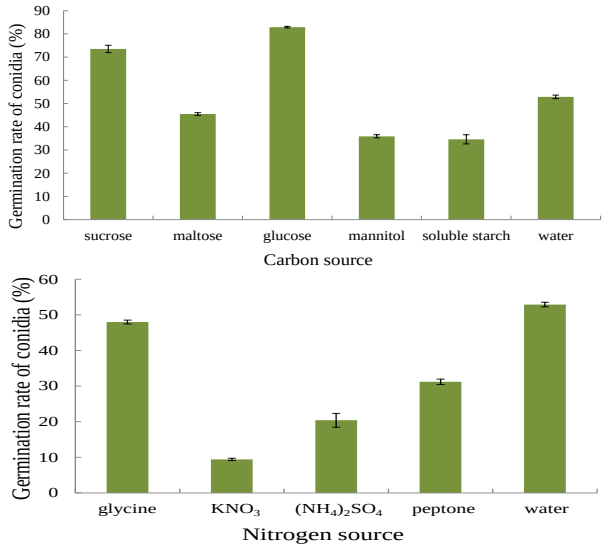

Fig. 8: Effect of carbon and nitrogen source on conidia germination of *B. cinerea*

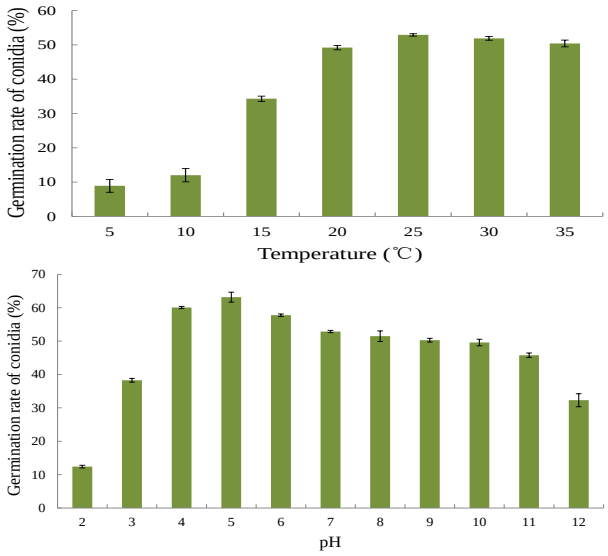

Fig. 9: Effect of temperature and pH on conidia germination of *B. cinerea*

two ends germination, center germination and both ends and center germination at the same time. Two ends germination accounted for the largest proportion (67.1%), the second was the center germination at 20.3%. Spore germination in both ends and center germination at the same time accounted for the smallest proportion (12.6%). When we observed conidia germination, we found that *B. cinerea* conidia germination rate was not high, ranging from 50 to 70% compared with fungal conidia germination. The results prove that conidia can normally germinate at 45-71°C for 10 min; conidia did not germinate at more than 72°C for 10 min. So we believe that the lethal temperature of *B. cinerea* conidia was 72°C for 10 min.

Discussion

Grey mould is a kind of facultative parasitism fungi. The hosts of grey mould are very extensive, and can grow on an artificial medium. Some mycelium aggregates can be globular and produce sclerotia at a later growth stage. Sclerotium is black with irregular shapes, which is a dormant structure. When the temperature rises or there is high humidity, sclerotia will germinate to produce conidia, and through air flow it can be released. Grey mould sclerotia can germinate in 5~30°C. The optimum temperature for generation of lily grey mould sclerotia is 15~20 °C (Tang *et al.*, 1998). Sclerotia need 48~80 h to generate at 15~20°C. A low temperature treatment on sclerotia at 15~20°C needs 5~7 d to germinate. However, with heat temperature treatment at 35°C, sclerotia cannot germinate (Xu *et al.*, 2006). There are large differences among different strains for the time and size of producing that are affected by the ambient temperature. The mycelium of grey mould can be grown at 5~30°C, and the mycelium of Lily grey mould was most suitable for the growth at 20~25°C. When the temperature exceeds 30°C, mycelium will brown, making the growth of the mycelium inhibited or even cease (Bai *et al.*, 2013). When the temperature is 5~30°C, the humidity is 100% and there is a wet environment, conidia will germinate. The optimum temperature for spore germination is 15~20°C. When the humidity is less than 93%, the spores do not germinate (Tang *et al.*, 1998). In this study, we have found that the grey mould pathogen can grow in the temperature range of 5~30°C. The pathogen grows slowly at 30°C, and the lethal temperature is 75°C for 10 min. The preliminary study on the biological characteristics of Lily grey mould provides the experimental basis for illuminating the relationship between the production of the lily grey mould and the various environmental factors.

During the early stages of disease, the leaf surface initially showed pinpoint tawny spots, which enlarged and developed into reddish water-soaked shape lesions, distinguishing the ill health junction. During the middle stage of the disease, the spots at the margin were brown, the center of the lesions was thin, fragile, white and transparent when the climate was dry, visible white mycelium were produced on both sides of the leaves, and black sclerotia was produced on the mold layer of the lesions area. There can be connections between the spots which develop into pieces, then large irregular spots in the late stage, with the leaves and stem appearing fire-like, and the flower showing soft rot.

Observation of the morphology of *B. cinerea* under an optical microscope found gray mold mycelium was dark gray, straight and thick, gathered into a mass or cluster and had a few branches. The mycelium had a diaphragm approximately 20 to 25 μm in length. Conidia were colorless, fell off from the conidiophores after the conidia matured, were an oval or round shape and from 10 to 15 × 5

to 10 μm. Conidia had three main means of germination: two ends germination, center germination and both ends and the middle same time germination.

The biological research data on *B. cinerea* mycelium showed that its growth rate was relatively fast in different media and differences were not obvious. Fungi on Oats, Lily leaf, Lily flower, Rechard and Czapek medium had the fastest growth rate, the fungal growth rate was faster on PDA and Agar medium, fungi on PSA had the slowest growth rate and the colony color was relatively special and appeared tawny. Mycelium in different carbon sources and nitrogen sources had a fast growth rate, so we believe the growth of mycelium needs carbon sources and nitrogen sources, but selectivity was not high. Mycelium in mannitol as a carbon source had a relatively slow growth rate and the colony became dark, so we believe mannitol as a carbon source was conducive to the mycelium pigmentation. Mycelium in $(NH_4)_2SO_4$ as a nitrogen source had the slowest growth rate and the colony stopped growing on the seventh day, so $(NH_4)_2SO_4$ as a nitrogen source was not conducive to gray mold growth. Gray mold can grow in temperatures ranging from 5 to 30°C, the growth rate of *B. cinerea* was slow at 30°C, and *B. cinerea* growth rate was relatively fast in temperatures ranging from 5 to 25°C. On the basis of observation and analysis of colony morphology and density, mycelium were gray and most dense at 15°C and 20°C, so we believe that the optimal temperature was 15°C and 20°C for mycelium growth.

Mycelium could grow and had roughly similar growth rate under different pH values. Light had a certain influence on mycelium growth and mycelium growth rate was fast in the 24 h dark and 12 h dark/light cycle. Mycelium growth rate was slow and color became dark in the 24 h light cycle. Upon analysis, the illumination condition was not conducive to mycelium growth but generated more pigmentation. The lethal temperature for *B. cinerea* conidia was 75°C for 10 min. From the point of culture days, the growth rate was uniform for 5-15 days and peaked on the fifteenth day and later mycelium almost stopped growing, colony morphology became dense and color became dark. From the point of mycelium culture algebra, the first generation of fungal colony was yellow-gray and mycelium was relatively dense; the second generation of fungal colony became dark, mycelium dense and thick and produced lots of quasi-sclerotia; the third generation of fungal colony was gray and mycelium was relatively dense; the fourth generation of fungal colony became yellow, color was lightest and mycelium was thick but sparse.

Based on the above analysis, results showed that gray mold mycelium grew doggedly, the growth of the required environment was not critical, and it was able to survive in a relatively wide temperature range. The lethal temperature for mycelium was as high as 75°C for 10 min, the results prove that the thermostability of mycelium was high. *B. cinerea* was prone to degeneration and colony between different algebra had obvious difference.

The optimal temperature for conidia germination was 25°C and germination temperature ranged from 5 to 35°C; conidia can normally germinate after high-temperature processing and finally the lethal temperature was determined as 72°C for 10 min. Conidia germination rate was highest when the pH was 5, obviously the optimal condition for conidia germination was a weak acid environment. Conidia germination rate was highest (82.9%) and germination speed fastest in glucose broth; spore germination rate in different nitrogen source broth was lower than in water, so we believe that conidia germination does not need a nitrogen source. The influence of different light conditions on conidia germination was not significant. The spore has three main means of germination: two ends germination, center germination, both ends and middle same time germination, and two ends germination accounted for the largest proportion.

Conclusion

Based on the above analysis, results showed that up to certain extent environmental conditions decided germination of spores. The lethal temperature for spore was high as 72°C for 10 min, and obviously conidia thermostability was very high. We found that *B. cinerea* conidia germination rate was not high, ranging from 50 to 70%, and was far lower than the other plants' separated gray mold conidia germination rate. But on the whole, gray mold has a strong anti-adversity and has a direct relationship with temperature.

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