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Preliminary study on the horizontal transfer and loss of the AM toxin gene of *Alternaria*

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The genus *Alternaria* has a global distribution and consists of a diverse group of pathogens. Plant-pathogenic *Alternaria* spp. can reduce the crop yield and pose serious threats to agricultural production. The pathogen *A. mali* is recognised as the key the pathogenic mechanism in the early defoliation of apples, which produces the host specific toxin (HST) that was named as an apple specific toxin (a specialised toxin of *A. alternata pv. mali*, AM toxin). The phenomenon of horizontal transfer of the AM toxin gene from different strains of *A. alternata* was found, and the relationship between the AM toxin and pathogenicity was confirmed. The representative strain *A. tuberculata* with the AM toxin gene was co-cultured with sixteen *Alternaria* strains without the AM toxin gene. As a result, four strains from different *Alternaria* species obtained the AM toxin gene, which indicated that the AM toxin gene can transfer among different *Alternaria* species. The AM toxin gene is easy to be lost after subculture, and high temperature and low nutrition can promote this loss. The symptoms of the *Alternaria* pathogen with or without the AM toxin gene are obviously different on the host. When infected by a pathogen with the AM toxin gene, green spots formed on the apple leaves, and rotten disease spots appeared in the fruit carpels. Contrary to this, when infected by a pathogen without this gene, only small epidermal spots without chlorosis formed on the apple leaves, and mildew-heart spots appeared in the fruit carpels.

Keywords: *Alternaria* spp., AM toxin, horizontal transfer, pathogenicity

The genus *Alternaria* is a compound fungus with multiple pathogens. It is widely distributed and poses a serious threat to the crop yield and agricultural production. Various *Alternaria* spp. cause a wide range of symptoms including leaf spot, blossom rot, fruit rot and blight (Andersen et al. 2005). During the pathogenic process of the *A. alternata* pathogen, the *A. alternata* toxin mainly destroys the cell wall of host cells through chemical or biochemical penetration and secretion of degrading enzymes, produces a mycotoxin to act on the plasma membrane, mitochondria, chloroplast and some metabolism-related enzymes of host cells, and mediates diseases through signal transduction pathways (Kang et al. 2013). Different sites and mechanisms of action of *Alternaria* toxin are different. The AC toxin (*A. alternata pv. citri* toxin), AB toxin (*A. alternata pv. brassicae* toxin) and AM toxin toxin (*A. alternata pv. mali* toxin) acting on chloroplast cell membranes can cause electrolyte leakage or loss of host cells such as Ca^{2+}, Mg^{2+}, K^{+}, sucrose and amino acids, leading to plasma membrane peroxidation, inhibiting the leaf photorespiration, destroying its defence mech-

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anism and leading to cell death (Liu & Li 2000). As for the pathogenic mechanism of the apple defoliation pathogen *A. mali*, previous research has shown that it was caused by the host specific toxin (HST) produced by the pathogen, which was named as the apple specific toxin (AM toxin). This toxin can not only assist the infection and colonisation of pathogenic bacteria on leaves and fruits, causing the diseases of leaves and fruits, but can also secrete into fruits, causing the fruits to rot and deteriorate at maturity, thus causing adverse effects on human body (Saha et al. 2012).

Researchers found that there were some small chromosomes less than 1.7 Mb in the pathogenic strains of *A. alternata*, but not in the non-pathogenic strains (Akamatsu et al. 1999). Among them, in the pathogenic type of the *A. alternata* apple, the peptide synthase gene (AM toxin gene) related to the biosynthesis of the host-specific toxin, the AM toxin is located on a small chromosome with a length of 1.1–1.7 Mb (Johnson et al. 2000; Johnson et al. 2001). Through physiological and pathological studies, it is found that these small chromosomes belong to extra chromosomes (supernumerary chromosomes) or unnecessary chromosomes (cridu-chat syndrome, CDCs) (Cover 1998; Johnson et al. 2001; Hatta et al. 2002). Non-essential chromosomes do not participate in the normal growth and physiological process of fungi, but participate in the infection process of host plants by occupying a specific niche (Kistler et al. 1996; VanEtten et al. 1998; Jain et al. 1999). It has been reported that the toxin genes of some fungi can be horizontally transferred among species. This gene transfer probably occurred just before 1941, creating a pathogen population with significantly enhanced virulence and leading to the emergence of a new damaging disease of wheat (Markham & Hille 2001; Friesen et al. 2006). The horizontal transfer of toxin makes the host-specific toxin of *A. alternata* no longer suitable for the classification of *A. alternata* in apple.

This study explored the horizontal transfer of the AM toxin gene of the *A. alternata* apple specialised toxin among different strains, and the relationship between the AM toxin and pathogenicity.

MATERIAL AND METHODS

Culture medium. PDA medium (potato dextrose agar medium): peeled potato 200 g, glucose 20 g, agar powder 15 g, distilled water to 1 L.

25% PDA medium: peeled potato 50 g, glucose 5 g, agar powder 15 g, distilled water to 1 L. PCA medium (potato carrot agar medium): 20 g peeled potatoes, 20 g peeled carrots, 15 g agar powder, distilled water to 1 L.

SNA medium (synthetic low nutrient agar medium): potassium nitrate (KNO_3) 1 g, potassium dihydrogen phosphate (KH_2PO_4) 1 g, magnesium sulfate heptahydrate ($MgSO_4$ $7H_2O$) 0.5 g, potassium chloride (KCl) 0.2 g, glucose 0.2 g, sucrose 0.5 g, agar powder 15 g, distilled water to 1 L.

Isolates. The seventeen putative *Alternaria* isolates, came from five different *Alternaria* species (YLBD_8 was clustered into *A. tuberculata*; YLBD_19, YLBD_14, and HNBD_F6 were clustered into *A. alternata*; YLMX15, QYBD, BSBD_123, and YLBD_2 were clustered into *A. alternatoide*; BSBD_39, TGBD_19, LBBD03, WQBD_10, ZQ111521, ZQT, and HNBD_F1 were clustered into *A. constrictum*; XJ78T_4 and BSBD_X2 were clustered into *A. tuberculata*) (Dang 2018), used in this study were obtained from the margins of black dot lesions on the pericarp and cultured on potato dextrose agar (PDA) slants at 25 °C in darkness for a week. Purified colonies were preserved in glycerol (15%) at −80 °C in the Fungal Herbarium of Northwest A&F University (HMUABO), Yangling, Shaanxi Province, China.

DNA extraction, PCR, and sequencing. The protocol of Pryor and Gilbertson (2000) was used to extract the genomic DNA from the mycelium of single-conidium-origin colonies grown on PDA plates at 25 °C in darkness for 7 days. The AM genes (AM 1-F: ATATCGTTTTCGGCCGCAC; AM 1-R: AACGGGTCCATGTAACCTAG) were amplified. The amplification reaction mixtures consisting of 2.5 μL 10× PCR buffer, 2 Mm $MgCl_2$, 0.2 mM of each dNTP, 0.4 μM of each primer, and 0.5 U AmpliTaq polymerase, 1 μL of the template DNA, were made up to a total volume of 25 μL with sterile water. The polymerase chain reactions (PCRs) were performed on a PTC-200TM Bio-Rad PCR System. The conditions for the PCR amplification consisted of an initial denaturation step of 5 min at 94 °C followed by 40 cycles of 30 s at 94 °C, 1 min at 53 °C, 45 s at 72 °C, followed by a final elongation step of 7 min at 72 °C.

The PCR amplification products were detected by 1.0% agarose electrophoresis (1× TAE electrophoresis buffer, 5V/cm voltage), stained with fluorescent dye EZVISION One or ethidium bromide (EB), and

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the loading amount was 2–3 μL. The bands were observed and photographed by a BIO-RAD gel imaging system, and the PCR amplification results and product fragment length were recorded.

Factors affecting the inheritance of the AM toxin. The Secondary Culture: the wild strain YLBD_8 containing the AM toxin gene was activated by PDA (pH = 7.0) medium. A 0.5 cm diameter plug containing both the hyphae and conidia was inoculated into the PDA culture medium plate, marked as YLBD_8 (F1), and was cultured in the dark for 7 days at 25 °C. An appropriate amount of mycelium was collected from the edge of the colony to extract the DNA, and whether the AM toxin gene existed or not was detected by the AM1-F and AM1-R primers. At the same time, the mycelium plug from the edge of the colony was transferred into a new PDA medium labelled YLBD_8 (F2), the above-mentioned culture steps were repeated, until the AM toxin gene could not be detected. According to the above method, the mutant strain HNBD_F6-2, which was cultured in confrontation with the wild strain YLBD_8, was cultured and labelled as HNBD_F6-2 (F1).

Nutrition: the wild strain YLBD_8 containing the AM toxin gene was cultured with PDA, 25% PDA, PCA, SNA and MM media. The mycelium plug was inoculated into different culture medium plates, labelled as YLBD_8 (F1), and was cultured in the dark at 25 °C for 7 days, and the appropriate hyphae were collected from the edge of the colony to extract the DNA. The existence of the AM toxin gene was detected by AM1-F and AM1-R primers. At the same time, the mycelium plug from the edge of the colony was transferred into a new PDA medium labelled YLBD_8 (F2), the above-mentioned culture steps were repeated, until the AM toxin gene could not be detected. According to the above method, the mutant strain HNBD_F6-2, which was cultured in confrontation with the wild strain YLBD_8, was cultured and labelled as HNBD_F6-2.

Temperature: the wild strain YLBD_8 containing the AM toxin gene was cultured at 0 °C, 35 °C, 30 °C, 25 °C, 20 °C, 15 °C, 10 °C and 5 °C. The mycelium plug was inoculated into different culture medium plates, marked as YLBD_8 (F1), was cultured in the dark for 7 days at different temperatures, and a proper amount of mycelium was collected from the edge of the colony to extract the DNA. The existence of the AM toxin gene was detected by the AM1-F and AM1-R primers. At the same time, the mycelium plug was taken from the edge of the colony, transferred to a new PDA medium labelled YLBD_8 (F2), and the above-mentioned culture steps were repeated, and so on until the AM toxin gene could not be detected. According to the above method, the mutant strain HNBD_F6-2, which was cultured in confrontation with the wild strain YLBD_8, was cultured and labelled as HNBD_F6-2 (F1).

Pathogenicity tests on apples. Four isolates [YLBD_8 (with AM toxin gene), YLBD_8 (2) (lost AM toxin gene), HNBD_F6 (without the AM toxin gene) and HNBD_F6 (2), the obtained AM toxin gene], were used for the pathogenicity tests on the pericarps of cultivars Golden Delicious, Red Delicious, Gala, and Fuji, respectively. Five fruits of each cultivar were used for each inoculation method. Mature asymptomatic fruit harvested from a commercial orchard located near Yangling were cleaned with pure water and wiped gently with cotton wool dipped in 75% ethanol for 30–40 s, then air dried before inoculation. Inoculations were performed on wounded and non-wounded leaves. Insect mounting pins (size #2; length = 40 mm, width = 0.38 mm, Beijing Zhecheng Technology Co. Ltd., Beijing, China) were used to make 8–10 wounds (epidermal punctures) located arbitrarily on the fruit. After the culture was incubated for a week in darkness at 25 °C, 0.5 cm diameter plugs, containing hyphae, were excised from the actively growing margin of the colony and placed on the calyx dimple of the fruit (two plugs per fruit), with the culture side facing the fruit. Sterile water and agar plugs without the fungus were used as the controls. The inoculated and control fruit were sealed in individual plastic bags with wet cotton wool (soaked with sterile water) to provide continuous high relative humidity (> 90%) and were incubated at 25 °C in darkness.

The excised leaves (fifth to sixth leaves, counting proximally from the shoot tip) of the Golden Delicious, Red Delicious, Gala, and Fuji apples were inoculated. Agar discs with mycelium were placed on each side of the midrib of the blade of the excised leaves. Five samples of each tissue type were inoculated by each method. The inoculated leaves were placed in a sealed plastic bag with wet cotton wool to provide continuous high relative humidity (> 90%); and were incubated at 25 °C in darkness. The symptom development on the leaves was assessed after 10 days after the inoculation. The trial was performed three times.

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RESULTS

The wild strains containing the AM toxin gene were confronted cultured with different *Alternaria* sp. The wild strain YLBD_8 containing the AM toxin gene and strains from the different species, including YLBD_19, YLBD_14, and HNBD_F6, were cultured in confrontation. As well as the strains YLMX15, QYBD, BSBD_123, YLBD_2, BSBD_39, TGBD_19, LBBD03, WQBD_10, ZQ111521, ZQT, HNBD_F1, XJ78T_4, BSBD_X2. It was found that YLBD_8 could grow in affinity with YLBD_14, HNBD_F6, BSBD_123, and BSBD_X2, and the hyphae of the two strains cultured in confrontation could be crossed or covered. Moreover, the colonies of YLBD_14, HNBD_F6, BSBD_123, and BSBD_X2, which are amiable and growing, all cover the colonies of YLBD_8. However, the strain incompatible with YLBD_8 will form an obvious dividing line at the edge of two colonies (Figure 1).

The AM 1-F and AM 1-R primers were used to detect the change of the AM toxin in the strain before and after confronting with YLBD_8, YLBD_14, HNBD_F6, BSBD_123 and BSBD_X2 could not detect the AM toxin gene before confronting with

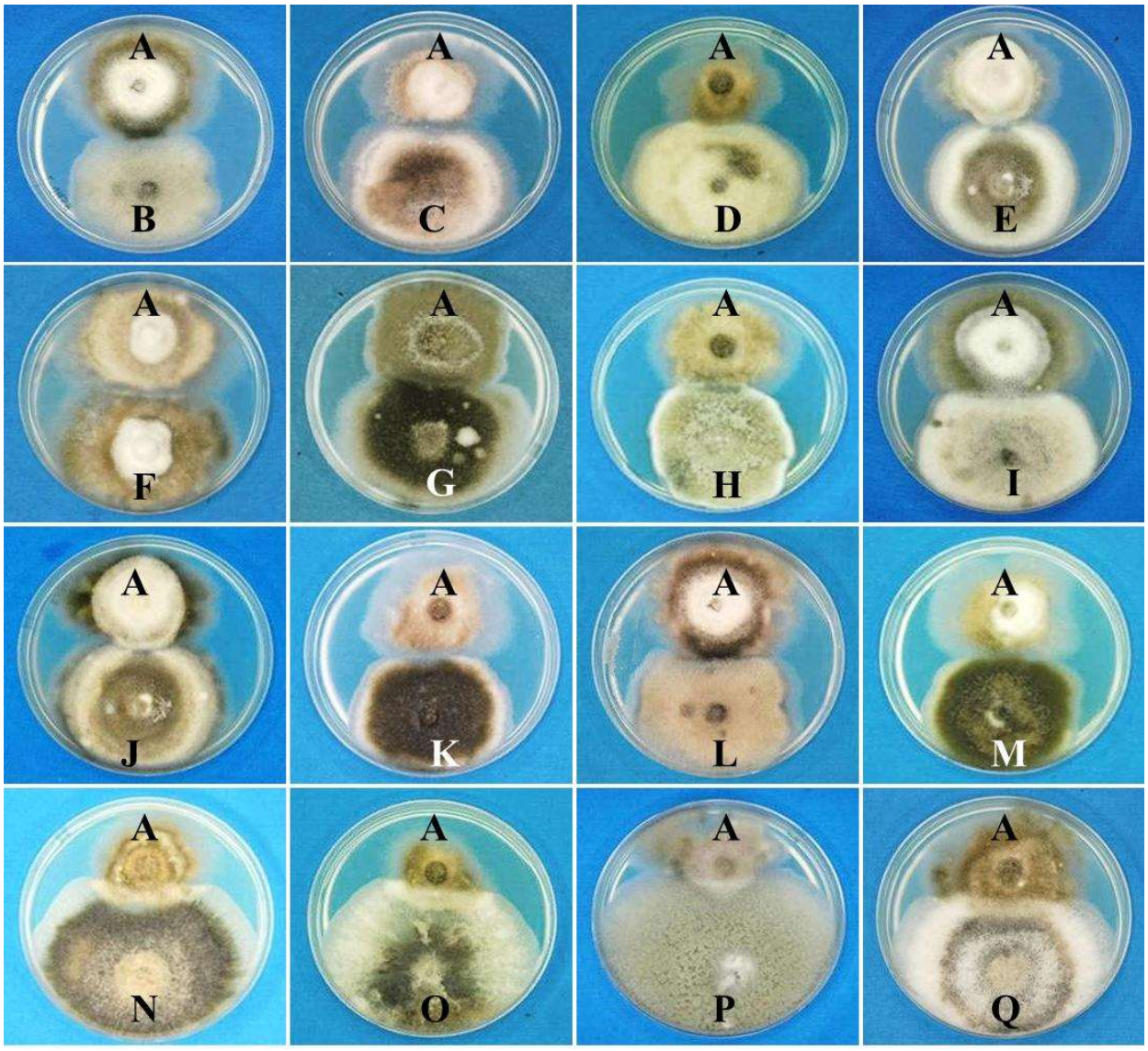

Figure 1. The co-culture of the *A. tuberculata* representative strains YLBD_8 and different *Alternaria* strains
A-Q: YLBD_8, YLBD_19, YLMX15, QYBD, YLBD_2, BSBD_39, TGBD_19, LBBD03, WQBD_10, ZQ111521, ZQT, HNBD_F1, XJ78T_4, BSBD_123, YLBD_14, HNBD_F6, and BSBD_X2, respectively

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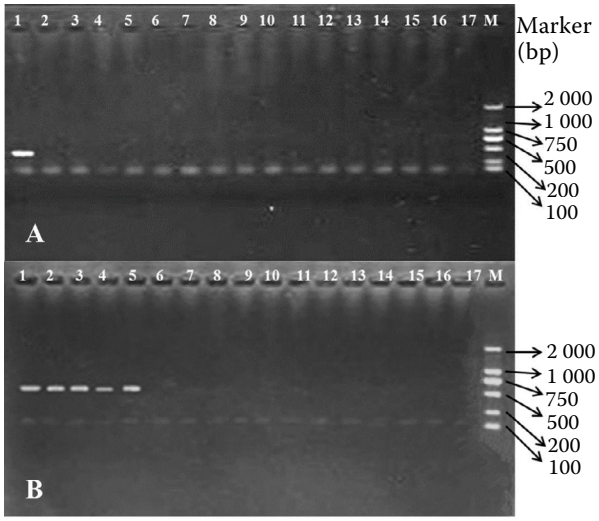

Figure 2. Amplified band of 17 *Alternaria* stains obtained with the AM specific primer, and the strain containing the AM toxin gene can be detected

A (1–17): YLBD_8, YLBD_14, HNBD_F6, BSBD_123, BSBD_X2, YLBD_19, YLMX15, QYBD, YLBD_2, BSBD_39, TGBD_19, LBBD03, WQBD_10, ZQ11152, ZQT, HNBD_F1, and XJ78T_4, respectively; B (1–17): YLBD_8 (2), YLBD_14 (2), HNBD_F6 (2), BSBD_123 (2), BSBD_X2 (2), YLBD_19 (2), YLMX15 (2), QYBD (2), YLBD_2 (2), BSBD_39 (2), TGBD_19 (2), LBBD03 (2), WQBD_10 (2), ZQ111521 (2), ZQT (2), HNBD_F1 (2), and XJ78T_4 (2), respectively; M: DNA marker (DL 2000)

YLBD_8. However, the AM toxin gene can be detected after confronting with YLBD_8, and becomes the mutant strains YLBD_14 (2), HNBD_F6 (2), BSBD_123 (2) and BSBD_X2 (2). No AM toxin gene was detected in the other incompatible strains before and after the confrontation culture (Figure 2).

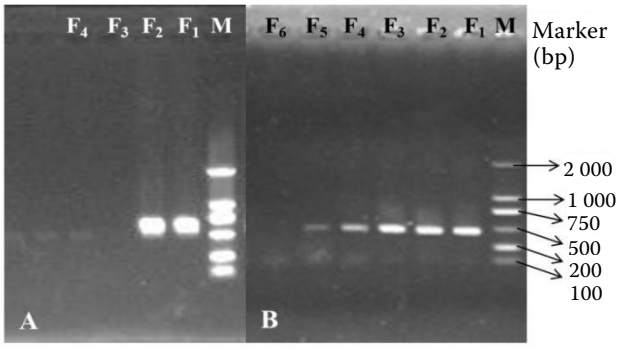

Figure 3. The difference of the AM-toxin gene in the wild and mutant strains after being sub-cultured

A – mutant strain HNBD_F6-2; B – wild strain YLBD_8; F1–F6: first–sixth generation; M – DNA marker (DL 2000)

Effect of the culture conditions on the retention of the AM toxin gene. *(i)* Influence of the subculture: YLBD_8, a wild strain with the AM toxin gene, was sub-cultured with the PDA (pH = 7.0) medium at 25 °C. It was found that the AM toxin gene could not be detected in YLBD8 by the 6th generation [YLBD_8 (F6)]. The mutant strain HNBD_F6-2 which obtained the AM toxin after the confrontation culture found that the AM toxin could not be detected by the third generation [HNBD_F6-2 (F3)] (Figure 3).

(ii) Effect of the temperature: YLBD_8, a wild strain containing the AM toxin gene, was cultured at 5–40 °C. It was found that the mycelium stopped growing at 40 °C, and the DNA could not be extracted to detect whether the AM toxin gene changed. The AM toxin gene can still be detected after 6 generations of culture [YLBD_8 (F6)] at 30 °C and 35 °C. At the temperatures of 25 °C, 20 °C, 15 °C, 10 °C and 5 °C, the AM toxin gene could not be detected until the 6th generation [YLBD_8 (F6)] (Figure 4). The effect of the temperature on the mutant strain HNBD_F6-2 is similar to that of the wild strain. The AM toxin gene can still be detected after three generations of culture [HNBD_F6-2 (F3)] at 30 and 35 °C, while it can be cultured to the third generation at 5 °C, 20 °C, 15 °C, 10 °C and 5 °C.

(iii) Effects of the nutritional conditions: The PDA medium, 25% PDA medium, PCA medium, SNA medium (low nutrient agar medium) and MM medium (inorganic salt medium) were used to culture the wild strain YLBD_8 containing the AM toxin gene. At room temperature (25 °C), the AM toxin gene can still be detected after 6 generations of culture YLBD_8 (F6) in the SNA and MM medium. In the PDA, 25% PDA and PCA medium, the AM toxin gene could not be detected until the 6th generation YLBD_8 (F6) (Figure 5). The effect of the temperature on the mutant strain HNBD_F6-2 is similar to that of the wild strain. In the SNA and MM medium, the AM toxin gene can still be detected after three generations of culture HNBD_F6-2 (F3). In the PDA, 25% PDA and PCA medium, AM toxin gene could not be detected until the third generation HNBD_F6-2 (F3) (Figure 5).

Pathogenicity tests. Small dark brown spots with a brown ring and black mildew layers around the lesion (Figure 6) appeared on both the wounded and unwound pericarps 7 days after being inoculated by YLBD_8 and YLBD_8 (2) (Figure 6).

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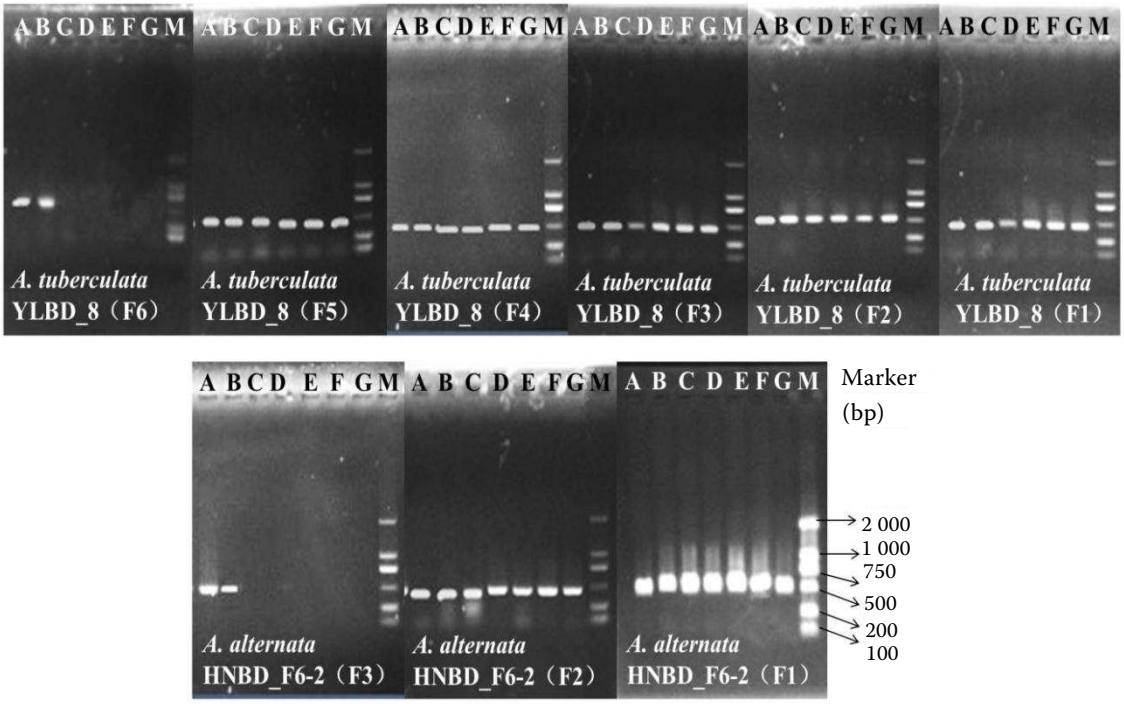

Figure 4. The difference of the AM-toxin gene in the wild and mutant strains under different temperatures
A: 35 °C; B: 30 °C; C: 25 °C; D: 20 °C; E: 15 °C; F: 10 °C; G: 5 °C. YLBD_8 (F1–F6) were the first–sixth generation of the wild strain YLBD_8; HNBD_F6 (2) (F1–F3) were the first–third generation of the mutant strain HNBD_F6-2. M – DNA marker (DL 2000)

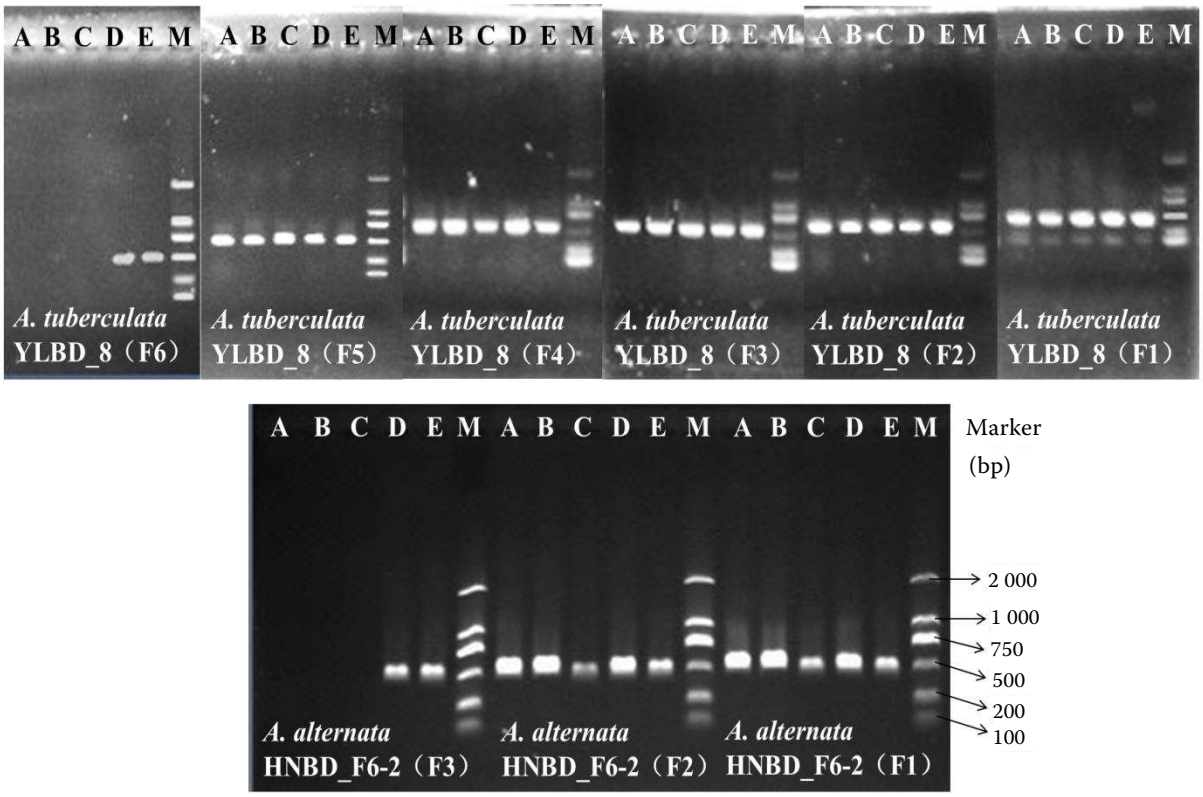

Figure 5. The difference of the AM-toxin gene in the wild and mutant strains cultured with different nutritional ingredients
A – PDA; B – 25% PDA; C – PCA; D – SNA; E – MM. YLBD_8 (F1–F6) were the first–sixth generation of the wild strain YLBD_8; HNBD_F6 (2) (F1–F3) were the first–third generation of the mutant strain HNBD_F6 (2). M – DNA marker (DL 2000)

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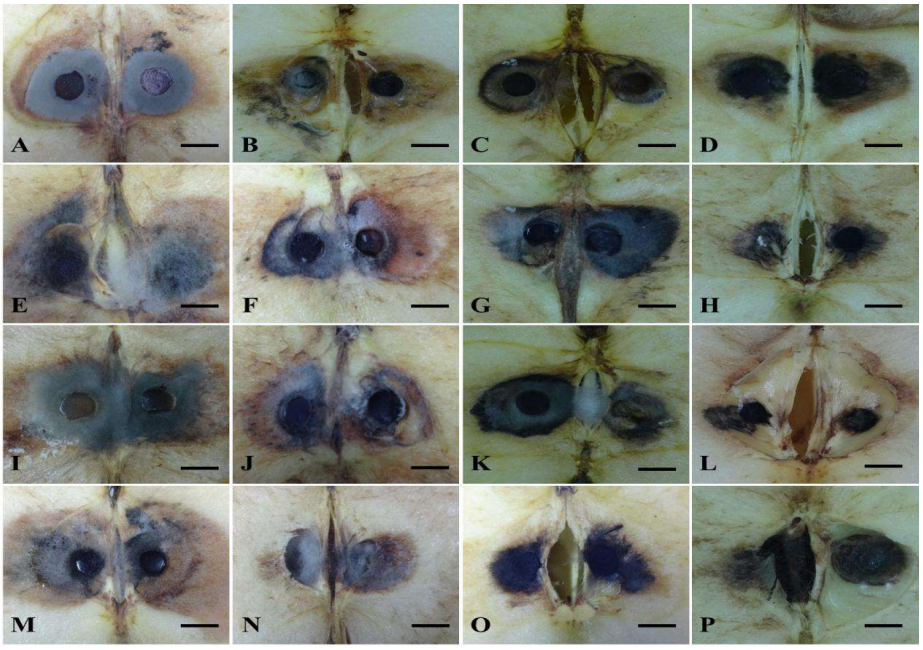
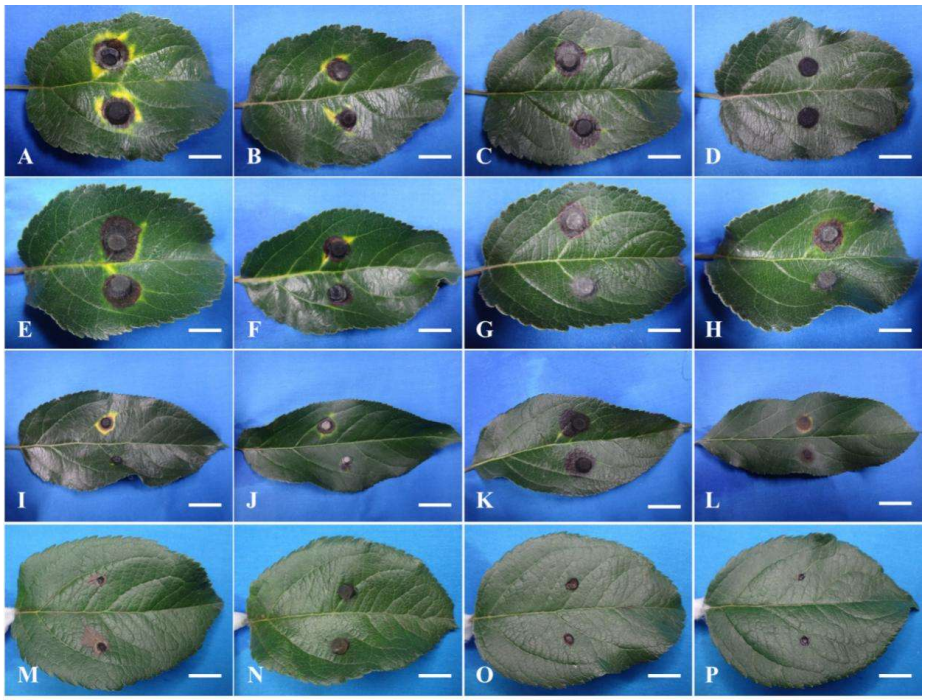


A–D – Golden Delicious; E–H – Red Delicious; I–L – Gala; M–P – Fuji. A, C, E, G, I, K, M, and O were wound inoculated; B, D, F, H, J, L, N, and P were inoculated without wounding. A, B, E, F, I, J, M, and N were inoculated by YLBD_8; C, D, G, H, K, L, O, and P were inoculated by YLBD_8 (2). YLBD_8 – the strain with the AM gene, YLBD_8 (2) – the strain with the lost AM gene. All the symptoms were photographed 5 days after inoculation. Scale bars = 1.0 cm

Figure 6. Symptoms on the fruit carpels of the cvs. Golden Delicious, Red Delicious, Gala and Fuji inoculated by YLBD_8 and YLBD_8 (2)

Symptoms of leaf blotch developed more slowly. Ten days after the leaves were inoculated with hyphae, slight chlorosis appeared around the inoculation sites in the detached leaf assay of the Golden Delicious, Red Delicious and Gala apples (Figures 7A–7N), whereas no lesions were observed after inoculation on the Fuji apples (Figures 7M–7P).

Similar results were found in the HNBD_F6 and HNBD_F6 (2) tests. The development of symp-



A–D – Golden Delicious; E–H – Red Delicious; I–L – Gala; M–P – Fuji. A, C, E, G, I, K, M, and O were wound inoculated; B, D, F, H, J, L, N, and P were inoculated without wounding. A, B, E, F, I, J, M, and N were inoculated by YLBD_8; C, D, G, H, K, L, O, and P were inoculated by YLBD_8 (2). YLBD_8 – the strain with the AM gene, YLBD_8 (2) – the strain with the lost AM gene. All the symptoms were photographed 7 days after inoculation. Scale bars = 1.0 cm

Figure 7. Symptoms on the leaves of the cvs. Golden Delicious, Red Delicious, Gala and Fuji inoculated by YLBD_8 and YLBD_8 (2)

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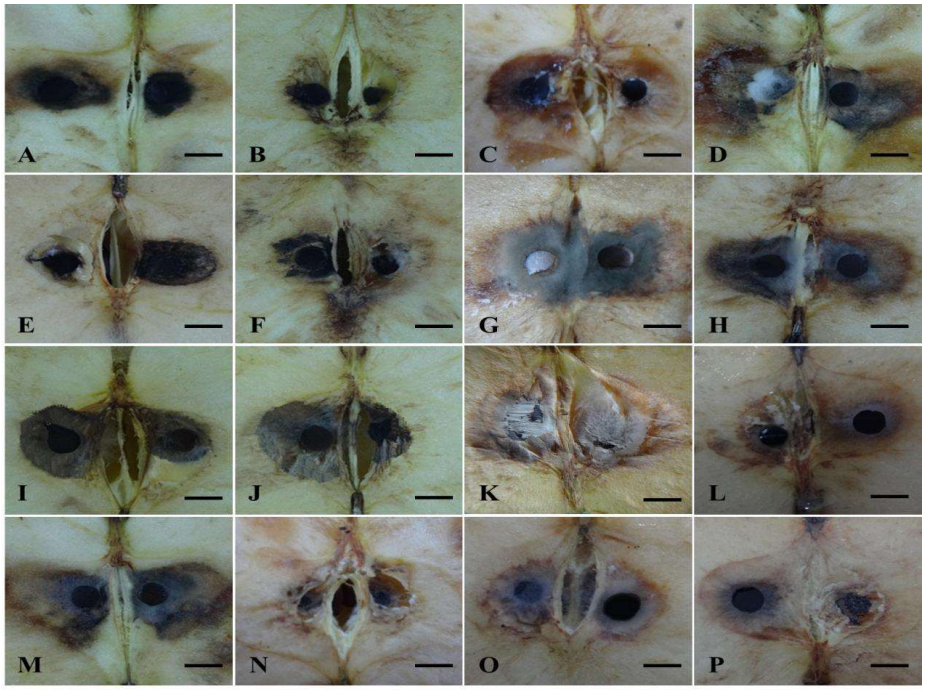
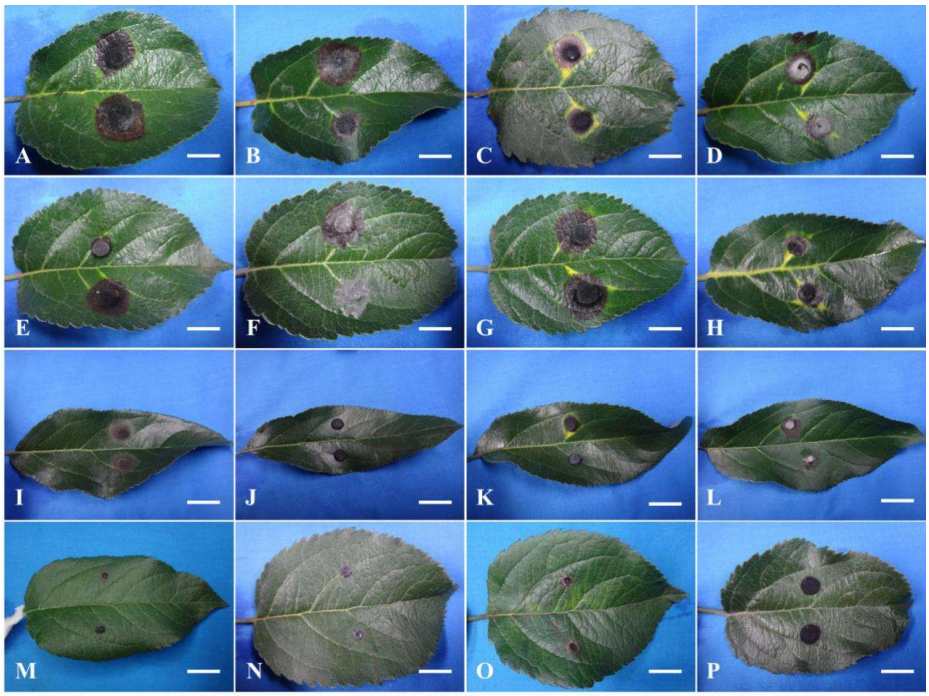


A–D – Golden Delicious; E–H – Red Delicious; I–L – Gala; M–P – Fuji. A, C, E, G, I, K, M, and O were wound inoculated; B, D, F, H, J, L, N, and P were inoculated without wounding. A, B, E, F, I, J, M, and N were inoculated by HNBD_F6; C, D, G, H, K, L, O, and P were inoculated by HNBD_F6 (2). HNBD_F6 – the strain without the AM gene, HNBD_F6 (2) – the strain with the obtained AM gene. All the symptoms were photographed 5 days after inoculation. Scale bars = 1.0 cm

Figure 8. Symptoms on the fruit carpels of the cvs. Golden Delicious, Red Delicious, Gala and Fuji inoculated by HNBD_F6 and HNBD_F6 (2)

toms of both pericarps and leaves are consistent with the YLBD_8 and YLBD_8 (2) tests (Figure 8) (Figure 9).

After inoculation with YLBD_8(2) and HNBD_F6 strains, the leaves were normal, with no diseased spots and no yellowing around the veins.



A–D – Golden Delicious; E–H – Red Delicious; I–L – Gala; M–P – Fuji. A, C, E, G, I, K, M, and O were wound inoculated; B, D, F, H, J, L, N, and P were inoculated without wounding. A, B, E, F, I, J, M, and N were inoculated by HNBD_F6; C, D, G, H, K, L, O, and P were inoculated by HNBD_F6 (2). HNBD_F6 – the strain without the AM gene, HNBD_F6 (2) – the strain with the obtained AM gene. All the symptoms were photographed 5 days after inoculation. Scale bars = 1.0 cm

Figure 9. Symptoms on the leaves of the cvs. Golden Delicious, Red Delicious, Gala and Fuji inoculated by HNBD_F6 and HNBD_F6 (2)

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DISCUSSION

The genus *Alternaria* consists of a diverse group of pathogens and has a global distribution. The AM toxin plays a very important role in the Alternaria apple disease.

The wild strain YLBD_8 was clustered into *A. tuberculata* with the AM toxin gene, which was co-cultured with 16 strains without the AM toxin gene. Four strains from different *Alternata* spaces (YLBD_14, HNBD_F6, and BSBD_123 were clustered into *A. alternata*; BSBD_X2 was clustered into *A. tuberculata*) obtained the AM toxin gene. It shows that the AM toxin gene can be transferred horizontally among different *Alternaria* sp. This result is apparently consistent with the report that toxin genes can be horizontally transferred among species (Markham & Hille 2001; Friesen et al. 2006). The influence of environmental factors, the temperature, pH, type of culture media, light and dark on the growth of *Alternaria* sp. was significant, and the colony morphology, colour and overall character showed variability under these circumstances (Sagarika & Perumal 2014). The influence of the microenvironment may disturb the same strains to different colours and colony morphology.

The AM toxin gene can easily be lost among the subculture of the strain. The AM toxin gene from the wild strain YLBD_8 was lost when the strain was cultured to the 6th generation. The strain HNBD_F6 obtained the AM toxin gene from YLBD_8 when co-cultured together, but lost it in the 3rd generation.

Different temperature conditions affect the inheritance of the AM toxin gene. A high temperature (30 °C and 35 °C) can promote the AM toxin to be passed on to the offspring. While a normal temperature and low temperature (25 °C, 20 °C, 15 °C, 10 °C and 5 °C) are unfavourable for its inheritance. The AM toxin gene can be detected after three generations of culture in the SNA medium (containing inorganic salts and a small amount of sugar) and the MM medium (containing only inorganic salts), but not in the PDA, 25% PDA and PCA medium, which indicates that the AM toxin gene can be passed on to the offspring under low nutritional stress. To sum up, the existence and inheritance of the AM toxin gene are related to the temperature and nutrition, and the high temperature and low nutrition are beneficial to its inheritance, which may be related to the self-protection mechanism of pathogenic bacteria under unhealthy growth conditions. The specific mechanism of temperature and nutrition affecting the AM toxin gene needs further study.

More future studies are needed for the reaction mechanism of how the AM gene transfer and loss functions. The peptide synthetase gene (AM toxin) related to the biosynthesis of the host-specific toxin AM toxin is located on a small chromosome with a length of 1.1–1.7 Mb in the *A. alternata* apple pathogenic type (Johnson et al. 2000; Johnson et al. 2001). We concluded preliminarily that the transfer and loss may be related to the hyphal cell fusion and the minichromosome. During the process of inheritance and evolution of this pathogen, the minichromosome containing genes related to the AM toxin biosynthesis may be lost or mutated, resulting in the abnormal synthesis of the AM toxin.

The symptoms were obviously different in the strains with or without the AM toxin gene [YLBD_8 and YLBD_8 (2), HNBD_F6, and HNBD_F6 (2)] based on the pathogenic test results. The symptoms of the lesion inoculated by the strains with the AM toxin gene are more serious and obvious than that without the gene. When inoculated by *A. alternata*, the symptoms of leaf blotch developed. Besides, the concentration of the cytoplasm and chloroplast matrix, and lipid globules at the junction of diseased and healthy tissues were changed. The chloroplast and plasma membrane in the host leaf cells changed significantly, most organelles were degraded, which indicated that the pathogen had secreted the AM toxin to act on the host (Zhang et al. 2012).

The present paper explores the construction of the transfer and loss of the AM toxin gene, and the pathogenicity on apples. The study preliminary proved that the AM toxin gene can transfer among different *Alternata* stains, and can form special symptoms on the host. The result of the study has particular guiding significance over the pathogenic mechanism, the occurrence and expansion, disease-resistant and control of the *Alternata* disease.

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