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First report of fire blight on *Cotoneaster* in China

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In May 2023, characteristic symptoms of fire blight, suspected to be caused by *Erwinia amylovora*, were observed infected wild *Cotoneaster* plants in the secondary forest along the lower reaches of the Jilgilang River, Xinjiang, China. Symptomatic tissues were collected, and bacterial isolation and purification were performed. The bacterium was identified through morphological characterization of the colonies, pathogenicity assays, tobacco hypersensitivity tests, and specific polymerase chain reaction (PCR) amplification, followed by sequence analysis. The isolated bacterium exhibited morphological features consistent with *E. amylovora*, and pathogenicity tests conducted under greenhouse conditions confirmed its pathogenicity, as evidenced by bacterial dissemination from the main leaf veins to surrounding tissues and the presence of bacterial exudates at the petioles. Furthermore, a pronounced hypersensitive response was observed in tobacco. PCR followed by sequencing revealed over 99.6% similarity with *E. amylovora* plasmid pEa29 repeat region. These findings confirm the *E. amylovora* is the causal agent of the fire blight disease in wild *Cotoneaster* plants. This is the first documented case of the fire blight affecting *Cotoneaster* plants in China. The detection of this pathogen has significant implications for the conservation of wild Rosaceae germplasm in the Tianshan Mountains wild fruit forests in Xinjiang.

Keywords Xinjiang, China, Wild *Cotoneaster*, *Erwinia amylovora*, Rosaceae, PCR analysis

Cotoneaster (*Cotoneaster* spp.), a genus within the subfamily Amygdaloideae of the family Rosaceae, consists of over 90 species of deciduous, evergreen, or semi-evergreen shrubs, and small trees. These species are widely distributed across temperate regions in Asia (excluding Japan), Europe, and North Africa. China is recognized as the global center of *Cotoneaster* diversity, hosting 58 species, 37 of which are endemic[1–3]. Xinjiang, particularly the Yili Valley in the Western Tianshan Mountains, is home to seven wild species of *Cotoneaster*[4], many of which have notable horticultural and economic value[5].

Fire blight, a highly destructive disease caused by *Erwinia amylovora*, severely impacts numerous species in the Rosaceae family[6]. Due to its devastating effects, many countries have classified fire blight as a quarantine disease[7]. Since its first outbreak in the northeastern United States in 1780, the pathogen has rapidly spread to more than 60 countries across Europe, Australia, the Middle East, and Central Asia[8]. *Erwinia amylovora* infects over 200 species in 40 genera of the Rosaceae family, with major hosts including pear (*Pyrus* spp.), apple (*Malus pumila*), crabapple (*Malus* spp.), hawthorn (*Crataegus* spp.), *Cotoneaster*, loquat (*Eriobotrya japonica*), photinia (*Photinia* spp.), pyracantha (*Pyracantha* spp.), and rowan (*Sorbus* spp.)[9].

In China, the first confirmed outbreak of fire blight occurred in June 2015 in an apple orchard in Huocheng County, Yili, Xinjiang(which found was unreported, other researcher reported that the Fire blight spread to Korla Xinjiang at 2017[10]. The disease has since spread across 14 prefectures in Xinjiang, where it affects a range of important host plants, including pear (*Pyrus* spp.), apple, hawthorn, Chinese white pear, flowering crabapple (*Malus spectabilis*), quince (*Cydonia oblonga*), and apricot (*Armenica vulgaris*)[11]. According to reports from the Ministry of Agriculture and Rural Affairs of China, as of June 30, 2023, fire blight had been detected in 58 counties or cities across Xinjiang and Gansu provinces, making an increase of 10 counties since 2022. Of these, 22 counties in Xinjiang have successfully eradicated the disease[12].

In May 2023, symptoms consistent with fire blight were observed for the first time on wild *Cotoneaster* in the secondary forests along the lower reaches of the Jilgilang River, Gongliu County, Yili, Xinjiang. These symptoms included blackened leaf veins, bacterial exudates at the petioles, wilting, necrosis, and inward curling of infected leaves. To confirm the presence of *E. amylovora* on wild *Cotoneaster*, we conducted pathogen isolation and identification as well as pathogenicity tests on the collected samples. Our findings are crucial for understanding the potential of the pathogen to spread within the Tianshan wild fruit forests and for preventing other wild

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Rosaceae species from infection. The study is particularly significant for the conserving wild Rosaceae germplasm in this region, which serves as a vital reservoir of biodiversity.

Materials and methods

Materials

Symptomatic branches and leaf tissues exhibiting fire blight-like symptoms were collected from wild *Cotoneaster* plants in the secondary forests along the lower reaches of the Jilgilang River, Gongliu County, Xinjiang. Samples were immediately transported to the laboratory and stored at 4 °C for further analysis.

Healthy wild *Cotoneaster* seedling was collected and transplanted to Plant Protect Lab of Yili Vocational and Technical College, Yining city from the reaches of the Jilgilang River which were use in pathogenicity test.

A positive control strain of *E. amylovora* (strain E.a YZ003), previously isolated from apple was prepared for pathogenicity investigation, was provided by Professor Hu Baishi of Nanjing Agricultural University.

Isolation of the bacterial pathogen

Infected stem segments (0.5–1 cm) and leaf tissues (1 × 1 cm) were surface-sterilized using 75% ethanol for 30 s, followed by 1% sodium hypochlorite for 1 min. The tissues were rinsed three times in sterile water to remove residual disinfectants and then dried with sterile absorbent paper. The sterilized tissues were homogenized in phosphate-buffered saline (PBS) which was 5.0 mL 0.01 M with pH 7.4 using a sterile mortar. The homogenate was streaked onto nutrient agar (NA) and selective Zeller medium plates using a three-step dilution streaking technique. The plates were incubated at 28 °C under a 12-hour light/dark cycle, with a relative humidity of 75%, for 24–48 h in an inverted position. Emerging bacterial colonies were examined for characteristic morphology. On NA medium, colonies displaying a milky-white color with smooth, raised, and glossy surfaces were selected. On Zeller medium, colonies appearing orange-red, highly raised, sticky, and circular, with well-defined edges and a dark central ring, were selected for further analysis. The colonies were purified through three rounds of streaking, and isolated strains which were named ZGXJXZ-1 and ZGXJXZ-2 were chosen for subsequent characterization.

Hypersensitivity reaction in tobacco

In order to recognizing the pathogenicity of the two purified bacterium speedy, the hypersensitivity response on tobacco were investigated followed Dong Xiuzhu's method[13]. Isolates ZGXJXZ-1, ZGXJXZ-2 and positive control were cultured on NA plates at 28 °C for 48 h. A single colony from each strain was then transferred to nutrient broth (NB) and shaken at 150 rpm at 28 °C for 12 h. The bacterial suspension was adjusted to an optical density at 600 nm (OD_{600}) of 0.4 to serve as the inoculum.

The bacterial suspension was injected into the mesophyll of *Nicotiana benthamiana*(tobacco) leaves using a disposable syringe, three replicates were finished through each strains and with sterile distilled water as the negative control. After 24–48 h, the development of white necrotic lesions at the injection sites was measured with positive control.

Pathogenicity testing

Pathogenicity tests were conducted by spray-inoculating three-year-old potted *Cotoneaster* plants with bacterial suspensions of isolates ZGXJXZ-1 and ZGXJXZ-2, adjusted to a concentration of 1×10^8 CFU/mL. Plants were maintained under controlled greenhouse conditions, and three replicates were used for each isolate. Sterile distilled water was applied as a negative control. Disease symptoms were monitored, and bacterial re-isolation from symptomatic tissues was performed to confirm the presence of the pathogen.

Specific primer detection and sequence analysis

16 S rRNA gene was partially amplified of the strains' genome followed by Jeng R.S[14] and identical with *E. amylovora* (GenBank Accession: KM597069.1, HF546214.1, MF780734.1, AM980507.1 and AF141892.1), the similarity was up to 99.6%.

Genomic DNA was extracted from the isolates ZGXJXZ-1 and ZGXJXZ-2 for molecular identification. A nested polymerase chain reaction (PCR) was performed using two pairs of primers, P29A/P29B and PEANT1/PEANT2, targeting the pEa29 plasmid of *E. amylovora*[15]. For the first round of PCR, a reaction mixture containing 10 µL of 2× *Taq* PCR mix, 0.3 µL of each primer (P29A/P29B, 10 µM), 1 µL of template DNA, and ddH_2O to a final volume of 20 µL. The second-round reaction contained 25 µL of 2× *Taq* PCR mix, 1 µL of each primer (PEANT1/PEANT2, 10 µM), 1 µL of template DNA, and ddH_2O to a final volume of 50 µL. The PCR protocol for the first round consisted of an initial denaturation at 94 °C for 2 min, followed by 30 cycles of 94 °C for 1 min, 52 °C for 30 s, and 72 °C for 55 s, with a final extension at 72 °C for 45 s. The second-round conditions were 94 °C for 2 min, followed by 35 cycles of 95 °C for 1 min, 56 °C for 30 s, and 72 °C for 25 s, with a final extension at 72 °C for 10 min. The PCR products were analyzed *via* 1% agarose gel electrophoresis. PCR products were sequenced bidirectionally, and sequence data were analyzed using BLAST in the NCBI database (blast genome No: CP117555.1). High-similarity sequences were employed to construct a phylogenetic tree using the neighbor-joining method in MEGA 7.0.26.

Results and analysis

Disease symptoms

In the field, *E. amylovora* initially infected the inflorescences of wild *Cotoneaster*, causing wilting and necrosis, which led the flowers to discolor from brown to dark brown, characteristic of fire blight (Fig. 1a). The infection subsequently spread along the leaf veins, causing progressive necrosis that resulted in leaves discoloring from

Fig. 1. Field symptoms of fire blight on wild *Cotoneaster*. (**a**) Blossom blight; (**b**, **c**) Leaf blight; (**d**) Shoot blight; (**e**,**f**) Fruit blight with bacterial exudates.

light reddish-brown to dark brown, followed by curling, wilting, and complete necrosis, manifesting as leaf blight (Fig. 1b,c). In early stem infections, light brown lesions appeared. As the disease progressed, the stems developed a reddish-brown to dark brown coloration, eventually taking on a characteristic hooked shape before desiccation and death, indicative of shoot blight (Fig. 1d). Infected young fruits displayed water-soaked lesions, with bacterial exudates observed on the surface. The fruits then darkened and necrotized, ultimately mummification (Fig. 1e,f).

Colony morphology characteristics

On NA medium, bacterial colonies were circular, with smooth, well-defined edges, appearing creamy-white to off-white, and exhibiting a raised, glossy, and viscous texture (Fig. 2a). On Zeller medium, colonies were orange-red, circular, and highly raised, with a stick consistency, darker central regions, and a distinct yolk-like central ring, along with well-defined edges (Fig. 2b).

Hypersensitive response in tobacco

Three days after inoculation, white necrotic lesions developed on tobacco leaves, indicating a positive hypersensitive response (Fig. 3a). This response was absent in the control inoculated with sterile distilled water (Fig. 3b).

Pathogenicity testing

Seven days after inoculation under greenhouse conditions, the infected *Cotoneaster* plants exhibited wilting and necrosis in both leaves and shoots (Fig. 4a). In contrast, no symptoms were observed in the control plants (Fig. 4b). Bacteria were re-isolated from the symptomatic tissues, and the re-isolated colonies exhibited morphological characteristics identical to the original strains. PCR and sequence analysis using specific primers further confirmed the presence of *E. amylovora* in the re-isolated samples.

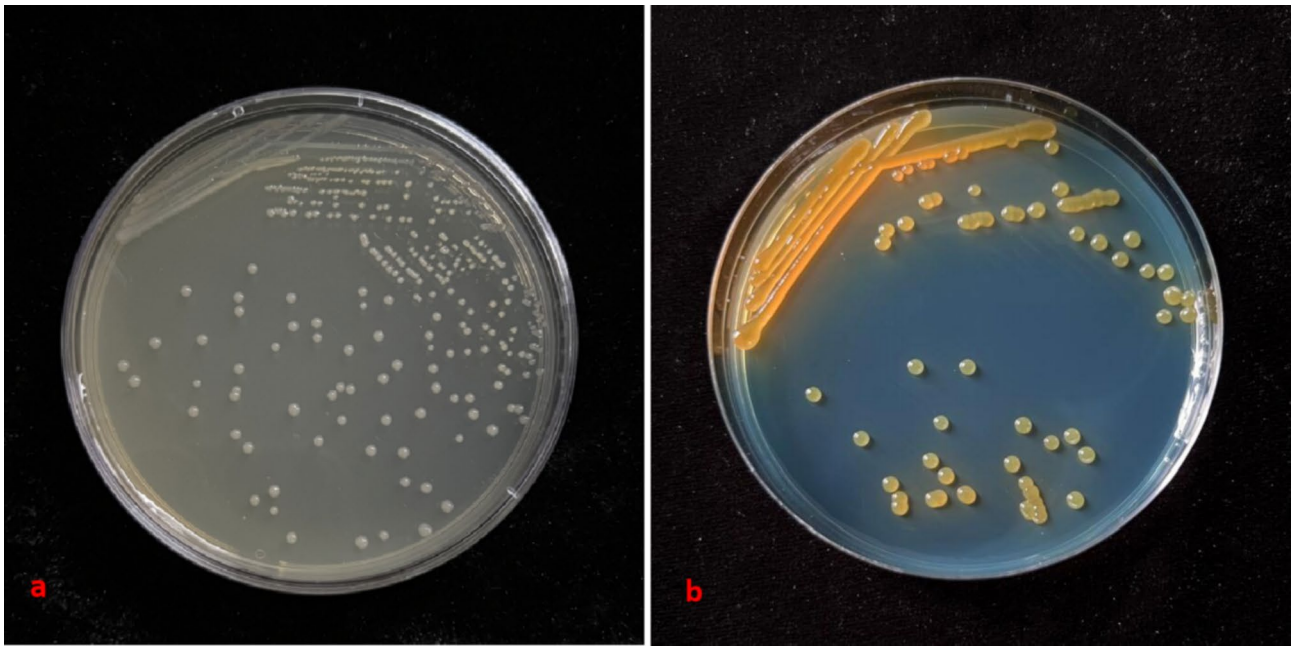

Fig. 2. Colony morphology of *E. amylovora* isolated from wild *Cotoneaster.* (**a**) Colonies on NA medium; (**b**) Colonies on Zeller medium.

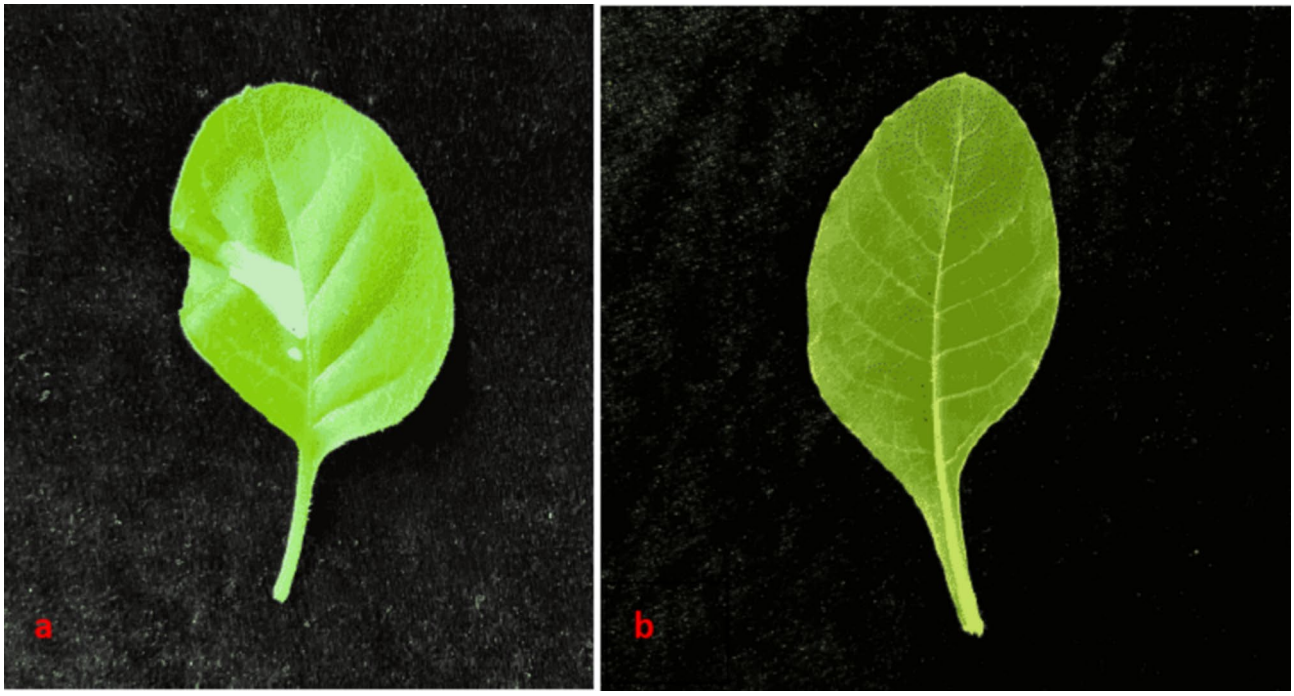

Fig. 3. Hypersensitive response in tobacco leaves. (**a**) Inoculated leaves with necrotic lesions; (**b**) Control leaves.

Specific PCR amplification and sequence analysis results

PCR amplification using *E. amylovora*-specific primers resulted in the expected 391 bp amplicon, identical to the positive control, while no amplification was observed in the negative control (Fig. 5).

The sequenced PCR products were submitted to GenBank (accession number: CP117555.1) and analyzed using BLAST in the NCBI database. Phylogenetic analysis conducted with MEGA software confirmed the isolates ZGXJXZ-1 and ZGXJXZ-2 clustered closely with the plasmid pEa29 repeat region of 11 *E. amylovora* reference strains(KJ457294.1, EU725790.1, LN794190.1, LN794191.1, LN794192.1, LN794197.1, LN794198.1,

Fig. 4. Pathogenicity symptoms in greenhouse-inoculated *Cotoneaster* plants. (**a**) Wilting and necrosis of leaves and shoots seven days post-inoculation; (**b**) Control plants with no symptoms.

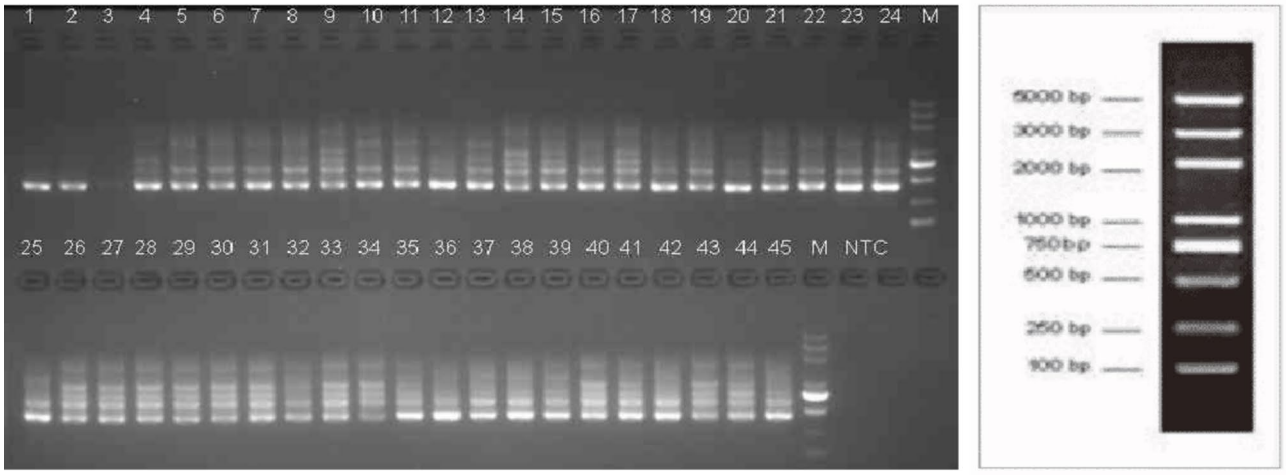

Fig. 5. PCR detection results. M: Marker 5000; 44: ZGXJXZ-1; 45: ZGXJXZ-2; 25: Positive control; NTC: Negative control.

LN794195.1, LN794196.1, FN668623.1, and U19254.1), sharing over 99% sequence identity (Fig. 6). These molecular and morphological findings conclusively identify the isolates as *E. amylovora*.

Discussion and conclusion

Discussion

Fire blight infections on *Cotoneaster* species have been widely reported in several regions of the world. Severe outbreaks were documented in the Netherlands in 1966, affecting long-leaved *Cotoneaster* in urban parks and gardens. After 1972, the disease had spread to hawthorn hedges and *Cotoneaster* shrubs in France. In 1990, a notable outbreak occurred near Stavanger on the West coast of Norway[16]. Additionally, in July 2007, fire blight was detected at a commercial nursery in Vukovarsko-Srijemska County, Eastern Croatia, affecting dwarf *Cotoneaster*. The symptoms included necrotic branches and petioles, and this infection was confirmed through

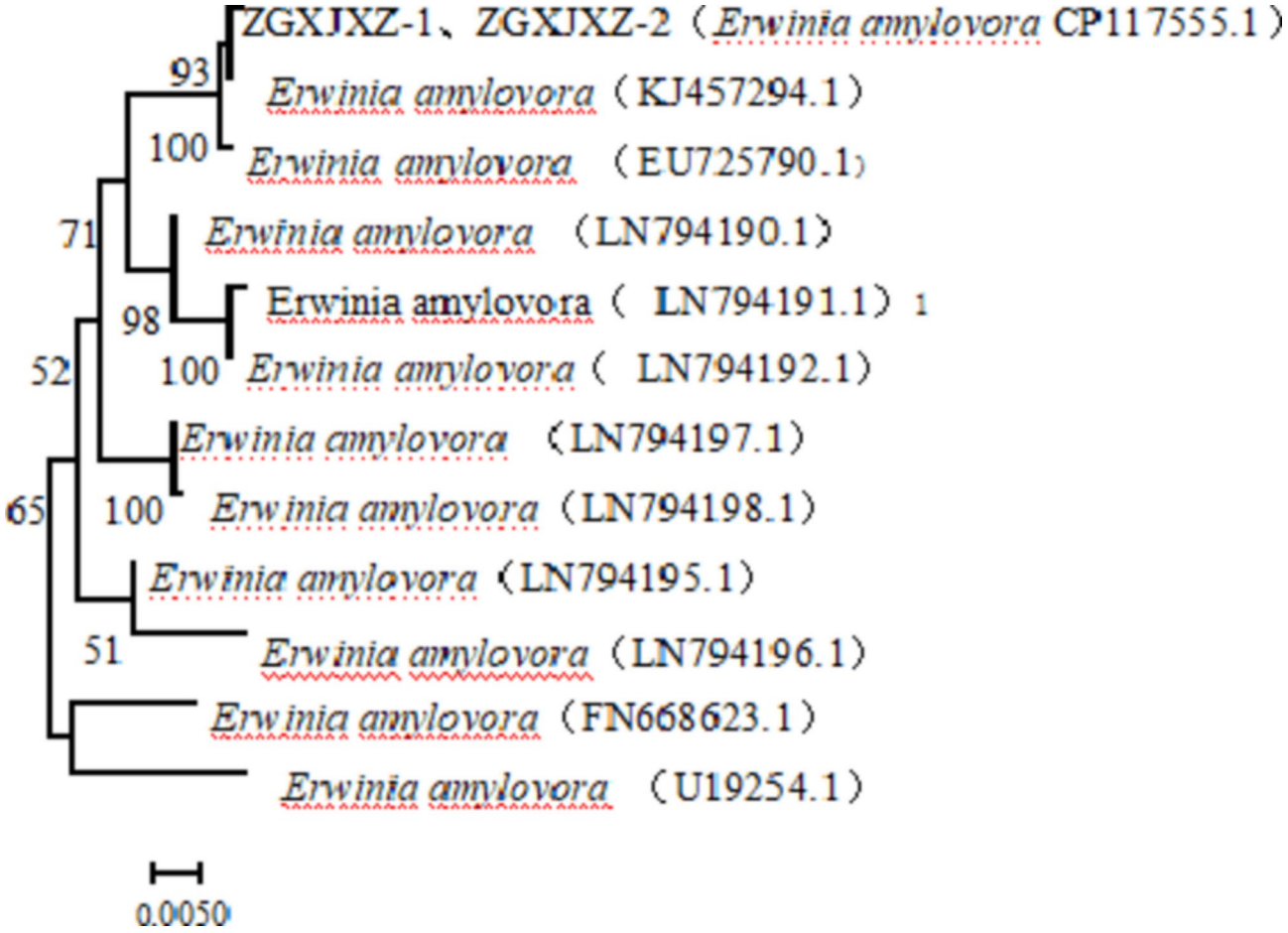

Fig. 6. A phylogenetic tree.

immunofluorescence assays, Koch's postulates, and PCR detection, marking the first report of fire blight on *Cotoneaster* in Croatia[17].

Since the first identification of fire blight in China in June 2015, *E. amylovora* has primarily affected pome fruit species, such as pear (*Pyrus* spp.), apple (*Malus pumila*), Chinese white pear (*Pyrus betulifolia*), crabapple (*Malus spectabilis*), hawthorn (*Crataegus pinnatifida*), quince (*Cydonia oblonga*), and apricot (*Armeniaca vulgaris*). Prior to this study, no fire blight infections had been recorded on *Cotoneaster* or other non-pome Rosaceae species in China. This new finding demonstrates an expansion in the host range of this pathogen in China. Xinjiang is recognized as a key center of origin for several important fruit tree species, hosting a rich diversity of wild fruit trees, particularly in the mountainous regions of Yili, Tacheng, and Altay in Northern Xinjiang. These areas are home to a variety of wild fruit species, including wild apple, apricot, almond (*Amygdalus communis*), hawthorn, plum (*Prunus salicina*), cherry (*Cerasus pseudocerasus*), rowan, raspberry (*Rubus corchorifolius*), *Cotoneaster*, and bird cherry (*Prunus padus*). This region is critical for conserving wild fruit tree germplasm in China[18,19]. The recent discovery of fire blight on wild *Cotoneaster* in May 2023 raises serious ecological concerns. If *E. amylovora* continues to spread within the Tianshan wild fruit forests, other wild Rosaceae species may become infected, resulting in potentially losing valuable genetic resources.

Conclusion

Through integrated assays—field observations, colony morphology, tobacco hypersensitivity tests, pathogenicity tests, PCR amplification, and sequence analysis—isolates ZGXJXZ-1 and ZGXJXZ-2 from wild *Cotoneaster* in Gongliu County, Yili, Xinjiang, were identified as *E. amylovora*, the causative agent of fire blight. This represents the first documented instance of fire blight on *Cotoneaster* in China.

Data availability

Sequence data that support the findings of this study have been deposited in NCBI with the primary accession No. OR754584 and OR754583. The other datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

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Author contributions

Conceptualization, C.W.M., C.X.L.and H.L.L.; formal analysis: C.W.M.and C.X.L.; investigation: H.L.L and W.J.H.; methodology, C.W.M. and C.X.L.; writing of (original draft): C.W.M.and H.L.L.; writing (review, and editing): C.W.M., C.X.L.,W.J.H.and H.L.L. All authors have read and agreed to the published version of the manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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