

# First Report of Postharvest Fruit Rot in *Solanum muricatum* Aiton Caused by *Alternaria alternata* in southwest China

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*Solanum muricatum* is native to South America and well known for its sweet,  
attractive, nutritious fruits. *S. muricatum* has been cultivated in China since the 1980s  
and increasingly popular (Li et al. 2015). In November 2021, an unknown fruit rot  
was observed in Shilin County of Yunnan Province (24.77 °N, 103.28 °E). The  
incidence of this disease was about 16% of 500 postharvest *S. muricatum* fruits after 7  
d in storage room (25°C, 90% relative humidity). The initial symptoms were small  
brown spots on the fruit surface, which gradually expanded into irregular brown or  
black lesions, and gray-white mold developed in the center of the lesions, eventually  
the fruit turned rot. To isolate the pathogen, ten fruits with typical symptoms were  
collected and surface-sterilized with 75% ethanol for 45 s. Small fragments (5 × 5  
mm) from the margin of lesions on fruit were disinfected with 1% sodium  
hypochlorite for 60 s, washed three times with sterile water then transferred to potato  
dextrose agar (PDA), and incubated at 28 ± 1 °C for 3 days (Li et al. 2022). Two  
fungal isolates with the same morphology were obtained and purified by single-spore  
isolation method. The colony was covered with thick fluffy aerial mycelia and the  
center was dark brown or black with white margins. Conidia were brown, pyriform or  
ellipsoid, with 1 to 3 longitudinal and 2 to 6 transverse septa, 15.12 to 34.01 × 6.90 to  
12.73 μm (21.22 × 9.69 μm on average, n=50) in size. These morphological  
characteristics were consistent with *Alternaria alternata* (Li et al. 2015; Xiang et al.  
2021; Alberto. 1992). For molecular identification, genomic DNA was extracted from  
a representative isolate, and primers ITS1/ITS4 (Gardes et al. 1993), TEF-F/TEF-R

(Lawrence et al. 2013), Alt-F/Alt-R (Hong et al. 2005), GPD-F/GPD-R (Berbee et al. 1999) and EPG-F/EPG-R (Peever et al. 2004) were used to amplify the internal transcribed spacer (*ITS*), translation elongation factor 1-alpha (*TEF*), *Alternaria* major allergen (*Alt a1*), glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) and endo-polygalacturonase (*endoPG*), respectively. The obtained DNA sequences (*ITS*: OM049821; *TEF*: OM069656; *Alt a1*: OM069655; *GAPDH*: OM069654 and *endoPG*: OM069653) showed over 99% homology with that of *A. alternata* (GenBank Accession No. MN856355.1 (565/573 bp); MN258023.1 (267/267 bp); KY923227.1 (491/501 bp); LC131645.1 (608/609 bp) and MN698284.1 (452/454 bp)). A phylogenetic tree based on the combined *ITS*, *TEF*, *Alt a1*, *GAPDH*, and *endoPG* sequences using the maximum likelihood methods with Kimura 2-parameter model, bootstrap nodal support for 1000 replicates in MEGA7.0 (Li et al. 2019) revealed that the isolate was assigned to *A. alternata*. To confirm pathogenicity, 10  $\mu$ L spore suspension ( $1.0 \times 10^6$  conidia/ml) obtained from 7-day-old PDA cultures of each isolate were inoculated on 15 needle-wounded and 15 non-wounded surface-disinfected fruits, respectively. Healthy fruits were inoculated with sterile water as controls and the experiment was repeated 3 times. All fruit were incubated at  $25 \pm 1^\circ\text{C}$ , 90% relative humidity. After 7 days, all the wounded and non-wounded fruit inoculated with *A. alternata* showed similar symptoms to those observed on the previously fruits, while the control fruits remained healthy. The same pathogen was again isolated from the inoculated fruits, thus Koch's postulates were fulfilled. *A. alternata* causing fruit rot of *Prunus avium* and *Mangifera indica* in China were reported in previous studies (Ahmad et al. 2020; Liu et al. 2019). As far as we know, this is the first report of postharvest fruit rot on *S. muricatum* caused by *A. alternata* in southwest China. This work provides a basis for the development of control strategies of the disease in the future.

#### References:

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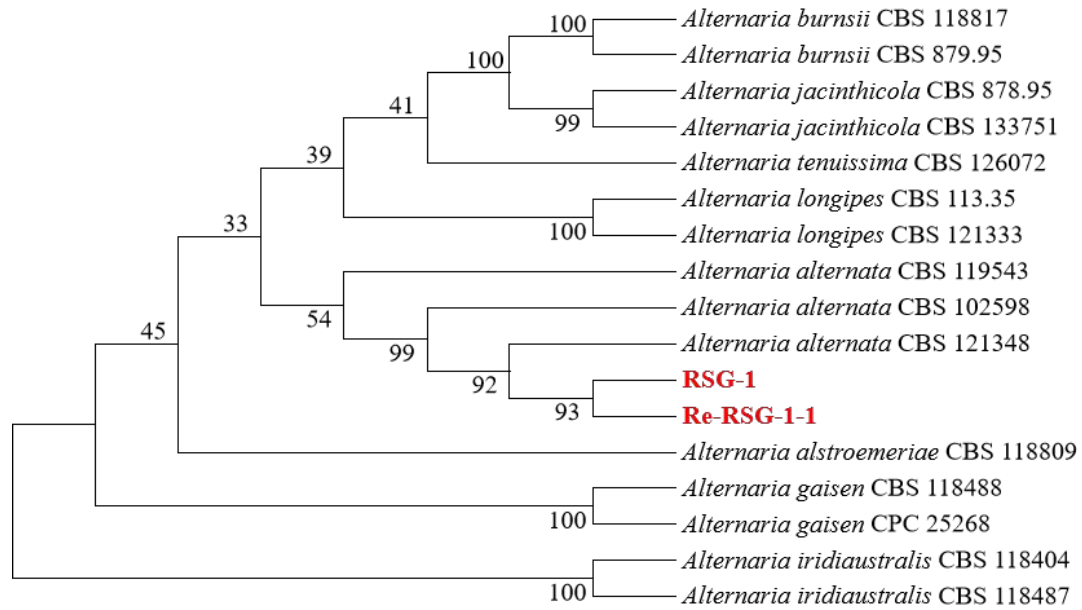


Fig 1 A multilocus phylogenetic analysis based on *ITS*, *TEF*, *Alt a1*, *GAPDH* and *endoPG* genes with Kimura 2-parameter model using MEGA7.0. The bootstrap analysis was conducted using maximum likelihood with 1,000 replicates. RSG-1 was isolated strain; Re-RSG-1-1 was re-isolated strain.

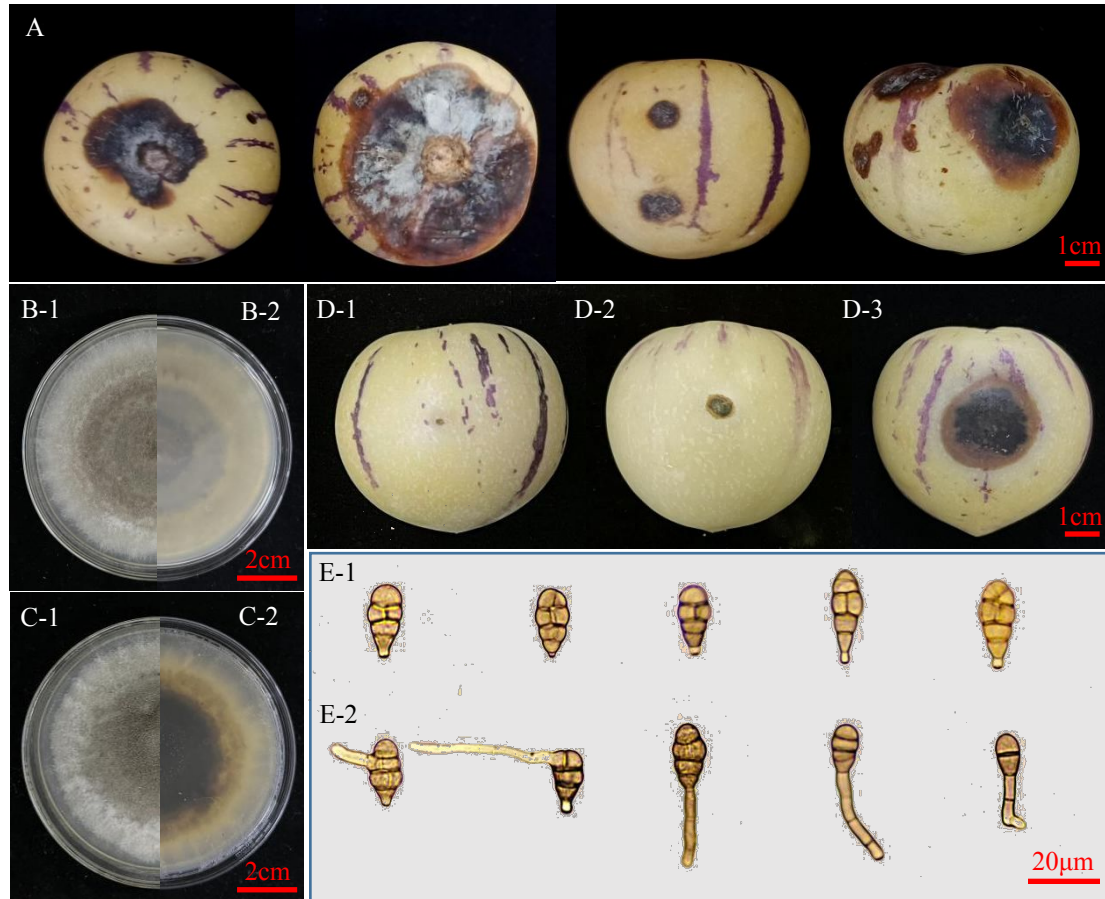


Fig 2 Fruit rot on *Solanum muricatum* Aiton caused by *Alternaria alternata*  
 A: Symptoms of *S. muricatum* fruit rot (Bar=1cm); B-1, B-2: The front and reverse sides of colonies of 7-day-old isolated strain on PDA medium; C-1, C-2: The front and reverse sides of colonies of 7-day-old re-isolated strain on PDA medium (Bar=2cm); D: Pathogenicity tests (D-1 is control group, D-2 is non-wounded inoculation group and D-3 is wounded inoculation group)(Bar=1cm); E-1, E-2: Conidia (Bar=20µm).

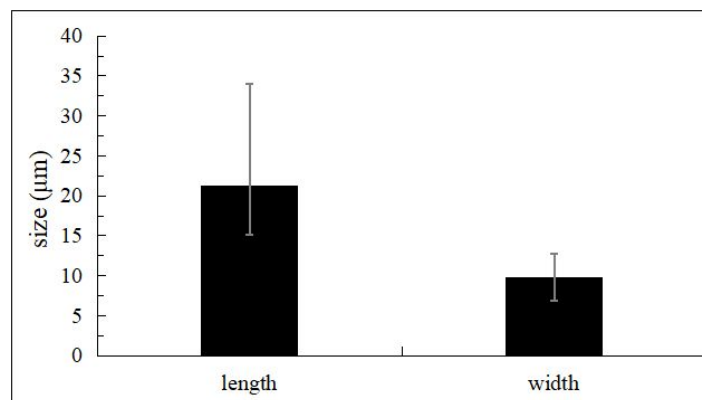


Fig 3 The bar graph of conidia size

Table 1 Comparison of morphological characteristics of pathogen

| Morphological characteristics | Described in manuscript                                                               | Described in reference     |                                              |
|-------------------------------|---------------------------------------------------------------------------------------|----------------------------|----------------------------------------------|
|                               |                                                                                       | Li et al. 2015             | Xiang et al. 2021                            |
| Colony morphology             | thick fluffy aerial mycelia and the center was dark brown or black with white margins | round, dark brown          | brown to black with abundant whitish margins |
| Conidia color                 | brown                                                                                 | dark brown                 | brown                                        |
| Conidia morphology            | pyriform or ellipsoid                                                                 | ellipsoid to ovoid         | obclavate or ovoid                           |
| Conidia transverse septa      | 2 to 6                                                                                | 3 to 6                     | 2 to 5                                       |
| Conidia longitudinal septa    | 1 to 3                                                                                | 0 to 3                     | 0 to 3                                       |
| Size (μm)                     | 15.12 to 34.01 × 6.90 to 12.73                                                        | 10.5 to 32.5 × 7.5 to 10.9 | 9.90 to 32.80 × 6.50 to 14.50                |