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First Report of *Epicoccum italicum* Causing Postharvest Rot on Pear Fruits in Italy

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Received: 8 December 2025 | **Accepted:** 9 February 2026

Keywords: emerging disease | fungal rot | *Pyrus communis*

In February 2025, diseased pear fruits (cvs. Max Red Bartlett and Williams) were collected during surveys in a storage facility located in Emilia-Romagna, Italy. The affected fruits had circular, brown and soft lesions covered with whitish mycelium (Figure 1). Diseased tissues from both cultivars were surface sterilised using 1% sodium hypochlorite (NaOCl) for 1 min and rinsed in sterile distilled water. The sterilised fragments were placed onto potato dextrose agar (PDA) and incubated at 27°C in the dark for 7

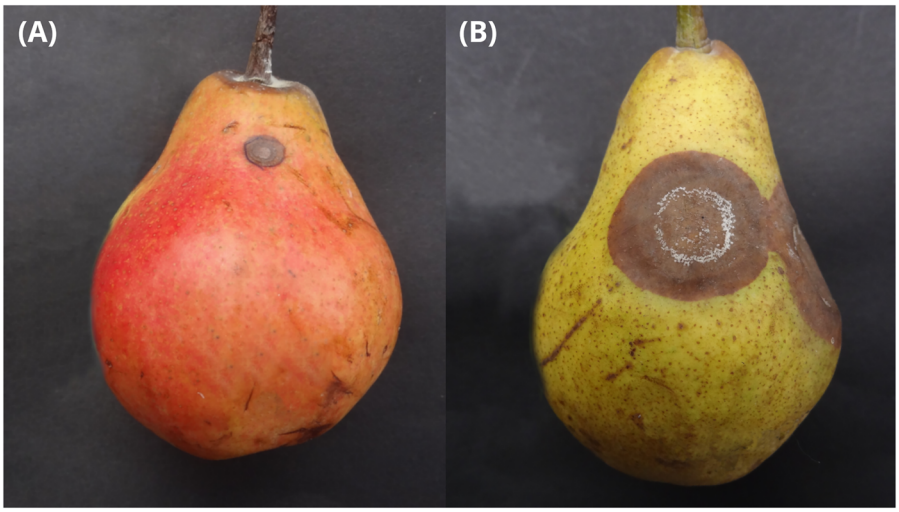

FIGURE 1 | Pear fruits of cvs. Max Red Bartlett (A) and Williams (B) showing brown circular lesions and whitish mycelium.

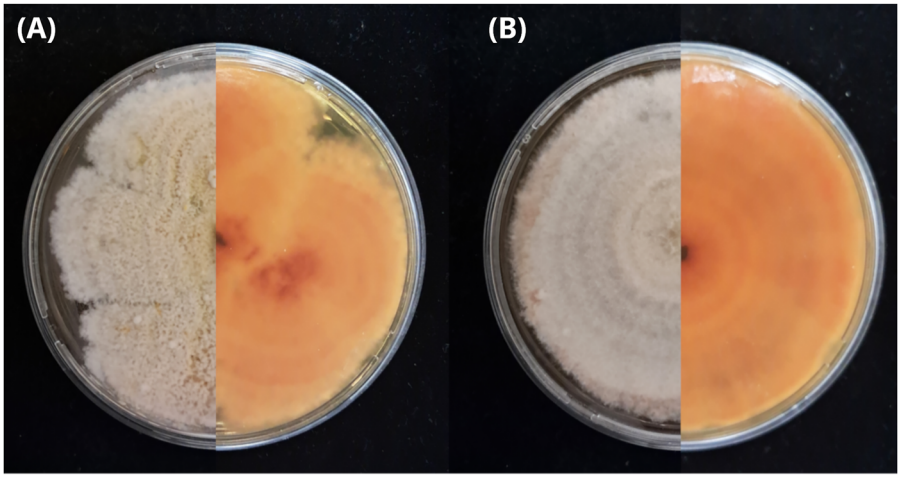

FIGURE 2 | Colony morphology of *Epicoccum italicum* isolates on potato dextrose agar: upper and reverse views of cultures of DLS2379 (A) and DLS2381 (B) 7 days after inoculation.

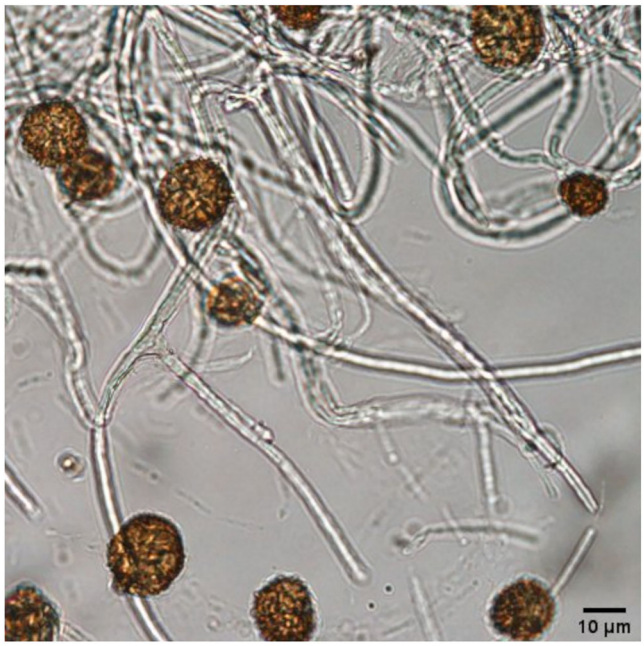

FIGURE 3 | Mycelium and spores of *Epicoccum italicum* isolates DLS2379. Bar = 10 µm.

days. The mycelium growing from the diseased tissues was aerial and floccose, with yellow pigmentation and a white margin on the upper side, while the reverse side was orange with a yellow margin (Figure 2). Microscopic observation of conidia showed their multicellular-phragmosporous, subglobose-pyriform structure, with a brown colouration (Figure 3). Colony morphology and single spores of two representative isolates, DLS2379 (from cv. Max Red Bartlett) and DLS2381 (from cv. Williams), showed characteristics consistent with the description of the ascomycete *Epicoccum* sp. (Chen et al. 2017).

Single-spore cultures of DLS2379 and DLS2381 were used for DNA extraction. The internal transcribed spacer (ITS) region, β-tubulin (*TUB*), and second largest subunit of nuclear RNA polymerase II (*RPB2*) genes were amplified and sequenced with the primer pairs ITS1/ITS4 (White et al. 1990), Bt2a-Bt2b (Glass and Donaldson 1995) and 5F2/7cR (Liu et al. 1999), respectively. Sequences were deposited in GenBank under Accession Nos. PX470109 and PX470110 (ITS), PX552162 and PX552163 (*TUB*), and PX505262 and PX505263 (*RPB2*) for DLS2379 and DLS2381 isolates, respectively. BLAST analysis showed that both strains DLS2379 and DLS2381 were identical to each other, and they exhibited 100% sequence identity with *E. italicum* ex-type strain LC 8150^{T} for ITS (NR_158264.1), *TUB* (KY742341.1) and *RPB2* (KY742172.1) regions. The phylogenetic tree constructed from concatenated ITS, *TUB* and *RPB2* gene sequences of both isolates DLS2379 and DLS2381, using the maximum-likelihood method, placed isolates within the *E. italicum* clade (Figure 4), confirming the BLAST results.

To fulfil Koch's postulates, 10 detached pear fruits (cv. Williams) per isolate were surface disinfected with 1% NaOCl for 1 min and rinsed with sterile distilled water. The fruits were wounded and inoculated with a conidial suspension (10^5 conidia mL^{-1}) of DLS2379 and DLS2381. Fruits were then placed in plastic boxes to maintain high humidity (RH > 90%) and incubated in the dark under controlled conditions (22 ±2°C). After 7 days' incubation, inoculated pear fruits showed rot symptoms, similar to those observed initially. No symptoms occurred on the control fruits inoculated with sterile water (Figure 5). *Epicoccum italicum* was reisolated from symptomatic fruits and identified through sequencing of the ITS region.

To our knowledge, this is the first report of *E. italicum* as a postharvest pathogen on pear fruits worldwide. The limited current understanding of the infection biology of *E. italicum* during long-term pear storage, combined with the lack of commercially approved fungicides or biocontrol products for its management in Italy, highlights the significant challenge faced by growers and postharvest operators in controlling this pathogen.

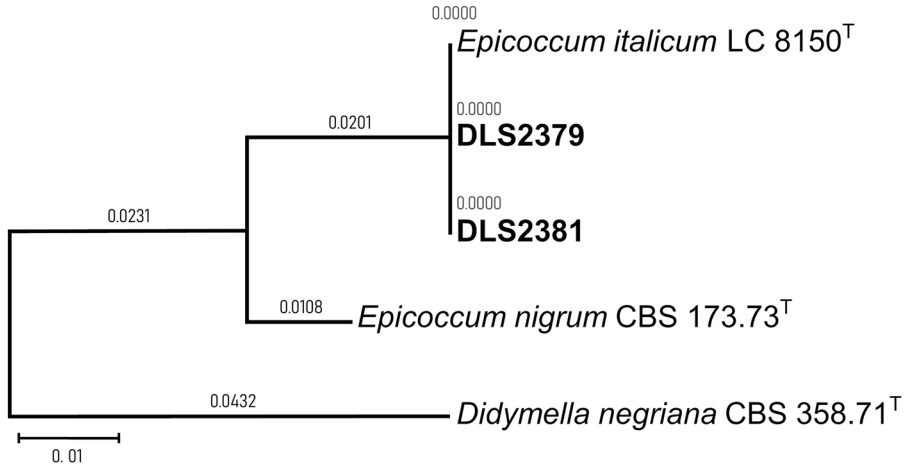

FIGURE 4 | Maximum likelihood phylogenetic tree generated with MEGA 12 by applying 1000 bootstrap replications based on analyses of a concatenated alignment of ITS, *TUB* and *RPB2* sequences. The values (from 1000 replicates) are indicated at the branch nodes as the percentages supported by bootstrap. The phylogenetic tree is rooted to the *Epicoccum italicum* ex-type strain LC 8150^{T}, *E. nigrum* ex-type strain CBS 173.73^{T} and the outgroup *Didymella negriana* representative strain CBS 358.71^{R}. Sequences used in these trees were obtained from GenBank. The *E. italicum* isolates obtained in this study are highlighted in bold.

FIGURE 5 | Pathogenicity of *Epicoccum italicum* isolates on pear fruits (cv. Williams); photos were taken 7 days post-inoculation.

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