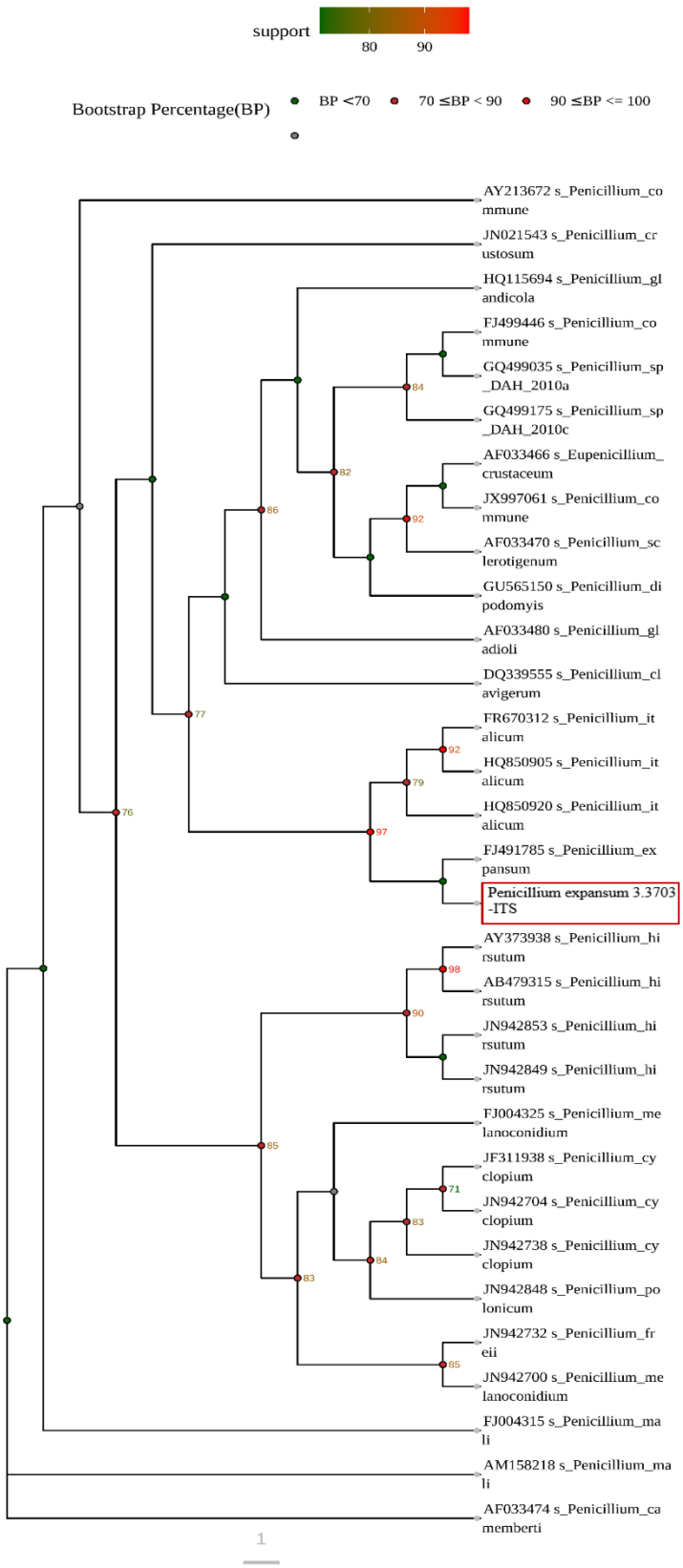



**Figure S1.** Phylogenetic tree constructed from ITS sequences, showing evolutionary relationships between *P. expansum* strain 3.3703 and related species. Bootstrap values (>70%) are indicated at nodes.

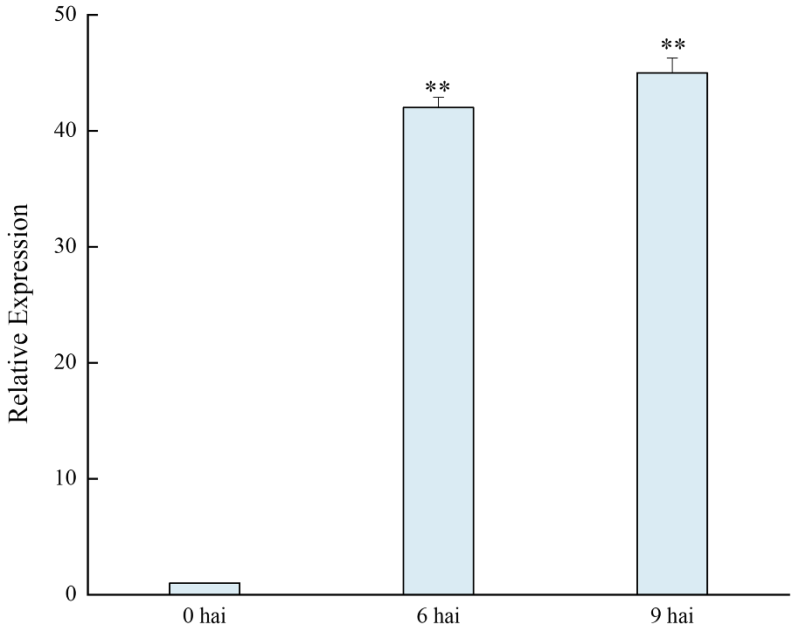



**Figure S2.** qRT-PCR analysis of *PeMep* relative expression levels in apple fruit tissues at different time points after inoculation (hai). Asterisks indicate significant differences compared to 0 hai (**$P < 0.01$). The 18S rDNA gene was used as an endogenous control. Data represent mean ± SD from three independent biological replicates.

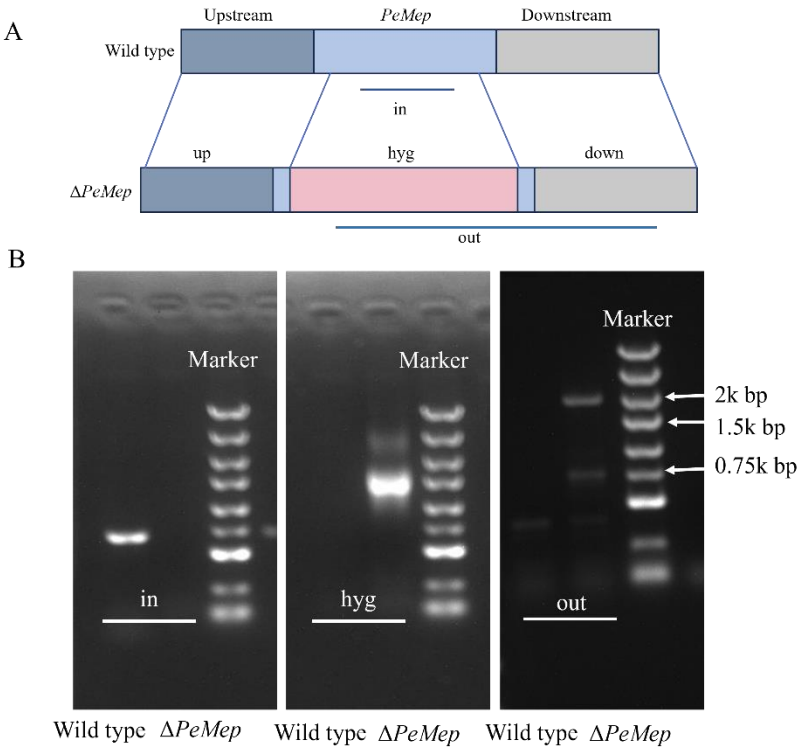



**Figure S3.** Diagrammatic representation of Deletion of *PeM35* in *P. expansum* 3.3703. (A) Schematic diagram illustrating the construction principle of the *PeMep* gene knockout mutant. The knockout strategy was based on homologous recombination, where left and right homologous arms were designed to replace the *PeMep* gene sequence. The fusion fragment carries the hygromycin resistance gene (*hyg*) as a selection marker. Upon transformation, the target gene was disrupted via a double-crossover recombination event. (B) Verification of Δ*PeMep* deletion strains with PCR. Primers hyg-F/R and PeMep-out-F/R amplified the expected bands exclusively in mutant strains, with no product detected in wild-type controls. The absence of amplification using PeMep-in-F/R primers in mutants demonstrates that the PeMep gene was precisely replaced by the hygromycin cassette without non-specific insertions.