Purification, characterization, and heterologous expression of an antifungal protein from the endophytic *Bacillus subtilis* strain Em7 and its activity against *Sclerotinia sclerotiorum*

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ABSTRACT. An antifungal protein exhibiting a high activity against *Sclerotinia sclerotiorum in vivo* was purified by ammonium sulfate precipitation, hydrophobic chromatography, and gel filtration chromatography from the culture filtrate of the endophytic *Bacillus subtilis* strain Em7. The protein was characterized as a β-1,3-1,4-glucanase according to amino acid analysis, and showed excellent properties in thermal stability and acid resistance. At the same time, the antifungal protein was cloned and heterologously expressed in *Escherichia coli* BL21. The recombinant protein was purified and showed similar enzymatic properties to the native protein, exhibiting strong inhibitory activity against *S. sclerotiorum.* This shows that the β-1,3-1,4-glucanase may play a very important role in *B. subtilis* Em7 biocontrol function. In addition, many physiochemical properties of the native and purified recombinant protein were compared, including the effect of pH, temperature, metal cations, substrate specificity, and kinetic parameters. All parameters were similar between the native and recombinant pu-

rified protein, indicating that the purified recombinant protein has potential for industrial applications.

Key words: Antifungal activity; Heterologous expression; *Bacillus subtilis*; β-1,3-1,4-glucanase; *Sclerotinia sclerotiorum*

