

Nematocidal activity and biocontrol efficacy of endophytic *Bacillus velezensis* Pt-RP9 from *Pinus tabuliformis* against pine wilt disease caused by *Bursaphelenchus xylophilus*

Mengjiao Sun ^a, Chaoqiong Liang ^b, Xiao Fu ^a, Guixiang Liu ^c, Yanru Zhong ^d, Ting Wang ^a,
Guanghui Tang ^a, Peiqin Li ^a

(^a Key Laboratory of National Forestry and Grassland Administration on Management of Western Forest
Bio-Disaster, College of Forestry, Northwest A&F University, Yangling, Shaanxi 712100, China

^b Shaanxi Academy of Forestry, Xi'an, Shaanxi 710082, China

^c Ningshan Forestry Technology Promotion Station, Ankang, Shaanxi 711600, China

^d Huangguan Management Station of Changqing Management Sub-bureau, Panda National Park, Ankang, Shaanxi
711600, China)

^e Interdisciplinary Innovation Center in Agricultural Nematodes, Institute of Future Agriculture, Northwest A&F
University, Yangling, Shaanxi 712100, China

Abstract: Pine wilt disease (PWD) is a globally significant quarantine forest disease caused by *Bursaphelenchus xylophilus* (PWN), resulting in substantial ecological and economic losses. Traditional nematode management practices are neither cost-effective nor environmentally friendly, prompting the exploration of biocontrol as a promising alternative for managing this devastating forest disease. Obtaining novel and specific biocontrol agents is extremely crucial for the effective and precise control of PWD. In the present study, a total of 136 endophytic isolates were obtained from various tissues of *P. tabuliformis* trees in the Qinling Mountains of China, comprising 65 isolates from roots, 41 from stems, and 11 from needles, which were then subjected to nematocidal activity assay against PWN in vitro. Among which, nine endophytic bacterial isolates exhibited exceptionally strong nematocidal capacity, with a corrected mortality rate exceeding 90%, which were then identified as the genus of *Bacillus* through morphological features, endospore staining, and 16S rDNA sequencing, with one strain as *B. mycoides*, two as *B. cereus*, and six as *B. velezensis*. Additionally, the inhibition effects of the three *Bacillus* species on the reproduction of PWN in vitro was assessed using an original detection model. Overall, the bacterial fermentation suspension exhibited stronger inhibition compared to the bacteria-free fermentation filtrate. Among the three *Bacillus* isolates, *B. velezensis* Pt-RP9 displayed the highest level of inhibition, with significantly lower values of reproduction rate represented a 244.4-fold reduction, the egg amounts represented a 1069.7-fold reduction. Subsequently, the biocontrol efficacy of *B. velezensis* Pt-RP9 against PWD was evaluated in greenhouse experiments. Pt-RP9 demonstrated significant biocontrol effectiveness against PWD, with control efficiencies ranging from 31.25 % to 68.89 % across all treatments, particularly showing improved efficacy when pine seedlings were pretreated with Pt-RP9 before PWN inoculation. Besides, the density of PWN in the seedlings treated with Pt-RP9 was significantly lower than that of PWN group, indicating

that *B. velezensis* Pt-RP9 can effectively inhibit *PWN* reproduction in vivo. Furthermore, to delve deeper into the protective mechanisms of *B. velezensis* Pt-RP9 against PWD, the MDA content and activities of defensive enzymes in *P. tabuliformis* seedlings were assayed. The pine seedlings treated with Pt-RP9 exhibited significantly reduced lipid peroxidation levels in cell membranes compared to the control groups, along with increased activities of peroxidase, catalase, and polyphenol oxidase, but superoxide dismutase, Chitinase, and Phenylalanine ammonia-lyase showed no significant differences among all treatment groups. To our knowledge, this study is the first to showcase the nematocidal activity of endophytes from *P. tabuliformis* against *PWN* and their biocontrol efficacy against PWD, marking a significant advancement in the field. The findings highlight the potential of *B. velezensis* Pt-RP9 as a crucial biological control agent against PWD, presenting a novel and sustainable disease management approach for pine forests.