

Title Growth inhibitory properties of *Bacillus subtilis* strains and their metabolites against the green mold pathogen (*Penicillium digitatum* Sacc.) of citrus fruit

Author Wichitra Leelasuphakul, Punpen Hemmane and Samerchai Chuenchitt

Citation Postharvest Biology and Technology, Volume 48, Issue 1, April 2008, Pages 113-121

Keywords *Bacillus subtilis*; *Penicillium digitatum*; Antifungal; Antibiotic; Citrus; Postharvest disease

Abstract

Twenty three strains of *Bacillus* spp. screened from 205 *Bacillus* spp. isolated from soil, showed antagonistic activities *in vitro* towards the *Penicillium digitatum* pathogen, a cause of citrus fruit rot disease. Culture supernatants from nine strains caused >80% inhibition of *P. digitatum* growth when they were serially diluted to 1:32. Volatile compounds produced by these strains also caused 30–70% inhibition of fungal growth. An ethanol extract from a *Bacillus subtilis* 155 cell-free supernatant referred to as secondary metabolites (SMs) produced the best inhibitory effect on mycelial growth and spore germination of the fungus with EC₅₀ values of 77.26 and 82.10 µgm L⁻¹, respectively. Inhibitory compounds, separated from the SMs by preparative thin-layer chromatography (CHCl₃/MeOH/H₂O: 65/25/4, v/v/v), had R_f values of 0.14, 0.28, 0.31, 0.49, and 0.64 with EC₅₀ values of 95.73, 14.07, 15.19, 108.59, and 99.98 µg mL⁻¹, respectively. Protein precipitated with 80% saturated ammonium sulphate, from the culture supernatant, had an EC₅₀ of 288 µg mL⁻¹. After native polyacrylamide gel electrophoresis of this protein the antifungal protein activity was detected only in the lowest band. Inoculation of a suspension of *P. digitatum* conidia (10⁴ conidia mL⁻¹) onto wounded citrus fruit induced disease symptoms at day 3 and decay at day 5. Inoculation with 20 µL of a 10⁸ CFU mL⁻¹ *B. subtilis* endospore suspension 24 h prior to fungal spore inoculation decreased disease incidences by 86.7%, and disease symptoms were delayed by 6 days and decay symptoms to day 9. Addition of the SMs (10 mg mL⁻¹), simultaneously with the fungus decreased disease incidence by 72.5%, delayed disease symptoms up to 5 days after inoculation, and no sign of decay was observed up to 9 days. The average lesion diameters observed from treatments with bacterial endospores, SMs and a control fungicide, imazalil were significantly different from the size of the wounds in the control set treated only with fungal conidia. *B. subtilis* 155 and its antibiotics are considered to be potent biological control agents to suppress growth of *P. digitatum* in the postharvest protection of citrus.