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Effects of rhizobacteria *Paenibacillus polymyxa* APEC136 and *Bacillus subtilis* APEC170 on biocontrol of postharvest pathogens of apple fruits

Key words: Anthracnose, Antagonistic activity, Biological control, White rot, Apple

Summary

This study evaluates the plant growth-promoting rhizobacteria (PGPR) as potential biocontrol agents against postharvest pathogens of apple fruits.

Among 30 isolates screened, isolates *Paenibacillus polymyxa* APEC136 and *Bacillus subtilis* APEC170 were selected based on the inhibitory effects against the mycelial growth of fungal plant pathogens.

Treatment of harvested apples with suspensions of either strain reduced the symptoms of anthracnose disease caused by two fungal pathogens, *Colletotrichum gloeosporioides* and *C. acutatum*, and white rot disease caused by *Botryosphaeria dothidea*.

Increased production of amylase and protease by APEC136, and increased production of chitinase, amylase, and protease by APEC170 might have been responsible for inhibiting mycelial growth.

The isolates caused a greater reduction in the growth of white rot than of anthracnose.

These results indicate that both isolates APEC136 and APEC170 are promising agents for the biocontrol of anthracnose and white rot diseases in apples after harvest, and suggest that they may be useful in controlling these diseases under field conditions.

Methods

Antagonistic rhizosphere bacteria were isolated from apple orchards at various provinces and cultured in nutrient agar (NA).

All the bacterial isolates were tested for *in vitro* for antagonistic activity against *C. gloeosporioides, C. acutatum* and *B. dothidea* on potato dextrose agar (PDA; Difco, USA) with 0.5% peptone (PDAP) using a disc diffusion method (Park *et al*., 2013).

Fungal pathogen inocula was prepared from the symptomatic tissue after surface sterilization. Conidia suspensions were prepared by suspending mycelia scraped from 7-d-old cultures of the pathogenic fungi on PDA plates.

The selected isolates APEC136 and APEC170 were subjected to the molecular identification based on sequence homology of their 16S rRNA genes (Weisburg *et al*., 1991). Sequence alignment and phylogenetic tree construction were performed using the MEGA 4.0 program (Tamura *et al*., 2007).

Optimization of growth media, temperature, and pH for culturing APEC136 and APEC170

The growth of isolates APEC136 and APEC170 in different culture media, such as brain-heart infusion (BHI), potato dextrose broth (PDB), nutrient broth (NB), tryptic soy broth (TSB), and Luria-Bertani (LB) broth was determined. For optimization of pH, BHI broth was adjusted to different pH levels (3 - 9) using 1.0 N HCl or 1.0 N NaOH before autoclaving. For optimization of temperature, the isolates were cultured in BHI broth at different incubation temperatures (15°C - 45°C) for 48 h under shaking conditions.

Chitinase, amylase, cellulase, and protease activities of APEC136 and APEC170

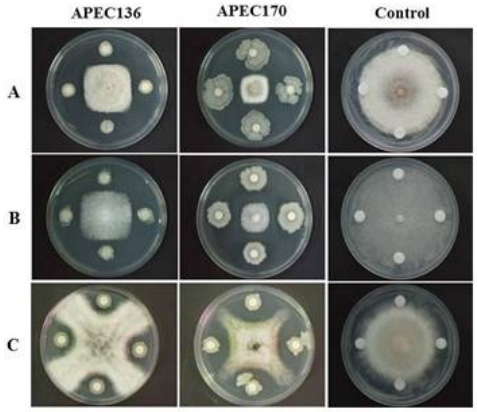
Chitinase assays were conducted according to the method developed by Roberts and Selitrennikoff (1988). Amylolytic activity was also assessed according to a previously described method (Shaw *et al*., 1995). Cellulase activity assays were carried out qualitatively, using carboxymethylcellulose (CMC) as the sole carbon and energy source, as described by Hankin and Anagnostakis (1977). Proteolytic assays were performed according to the method described by Fleming *et al*. (1975).

***In vivo* reduction of anthracnose and white rot diseases by APEC136 and APEC170 on harvested apples**

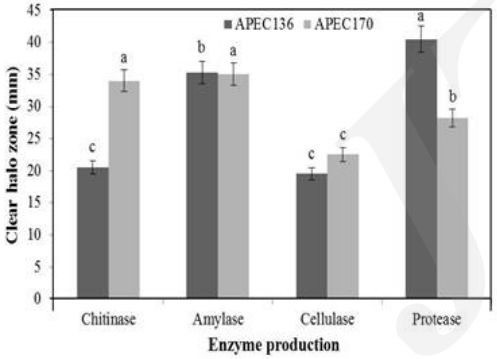
The surface sterilized apple fruits were treated with antagonistic suspension by piercing them 1 to 2 mm deep. The wounds of the apple fruits were then inoculated with spore suspensions (10 µl) of the pathogenic fungi. Disease symptoms were observed 10 d after incubation at 25°C and compared with non-treated controls.

Results

Antagonistic activity



Enzyme activity



Anthracnose suppression on harvested apples

Conclusion

This study demonstrates that APEC136 and APEC170 isolates exhibit stronger *in vitro* antifungal activity against *C. gloeosporioides* and *C. acutatum* than against *B. dothidea*.

In vivo, only APEC136 reduces the white rot disease caused by *B. dothidea*.

Postharvest treatment with bacterial suspensions resulted in significant reductions in disease development during storage at room temperature.

Our results indicate that both isolates are promising agents for the biocontrol of apple diseases after harvest and under field conditions.