

Supplementary Materials: Clarification of the Antagonistic Effect of the Lipopeptides Produced by *Bacillus amyloliquefaciens* BPD1 against *Pyricularia oryzae* via In Situ MALDI-TOF IMS Analysis

Jen-Hung Liao, Pi-Yu Chen, Yu-Liang Yang, Shu-Chen Kan, Feng-Chia Hsieh, and Yung-Chang Liu

1. Antagonistic Bioassay for Ba-BPD1

1.1. Results

Antifungal and Antibacterial Activities of Ba-BPD1

Based on the results of antagonistic plate bioassay, Ba-BPD1 can antagonize 21 phytopathogenic fungi (Table S1 and Figure S1) and 12 phytopathogenic bacteria (Table S2). The antifungal and antibacterial spectra of Ba-BPD1 were wide. This indicates that Ba-BPD1 is suitable to be developed as a widely-used biocontrol agent.

Table S1. Average inhibition distance between Ba-BPD1 disc and the fungus inoculum.

Species	Disease	Average Inhibition Distance (mm)
<i>Botrytis elliptica</i> (Be)	lily grey mold	9.2
<i>Botrytis cinerea</i> (Bc)	rose grey mold	8.8
<i>Glomerella cingulata</i> (Gc)	mango anthracnose	3.1
<i>Colletotrichum musae</i> (Cm)	banana anthracnose	9.8
<i>Rhizoctonia solani</i> (Rs)	sheath blight of rice	4.0
<i>Fusarium oxysporum</i> f. sp. <i>Pisi</i> (F307)	root rot of pea	10.5
<i>Fusarium oxysporum</i> f. sp. <i>Lycopersici</i> (F308)	fruit rot of tomato	5.2
<i>Fusarium oxysporum</i> f. sp. <i>Lycopersici</i> (Fol-33)	fruit rot of tomato	7.7
<i>Fusarium solani</i> (FSO)	Fusarium root rot of orchid	7.3
<i>Fusarium solani</i> (FSL)	Fusarium root rot of litchi	7.5
<i>Sclerotium rolfsii</i> Saccardo (Sr)	southern blight of lily	3.0
<i>Alternaria mali</i> (Am)	leaf spot of apple	8.0
<i>Phytophthora capsici</i> (PcS1)	Phytophthora blight of sweet pepper	5.0
<i>Aspergillus niger</i> (An12)	Aspergillus of onion	5.0
<i>Aspergillus niger</i> (An22)	Aspergillus of onion	4.0
<i>Penicillium italicum</i> (Pi13)	blue mould of citrus	13.5
<i>Penicillium italicum</i> (Pi28)	blue mould of citrus	12.3
<i>Colletotrichum gloeosporioides</i> (Cg-T4018)	anthracnose of sweet persimmon	7.8
<i>Colletotrichum gloeosporioides</i> (Cg-T4044)	anthracnose of sweet persimmon	9.4
<i>Pestalotiopsis eugeniae</i> (Pe)	fruit rot of wax apple	7.3
<i>Botryodiplodia theobromae</i> (Bot)	stem end rot of mango	9.3

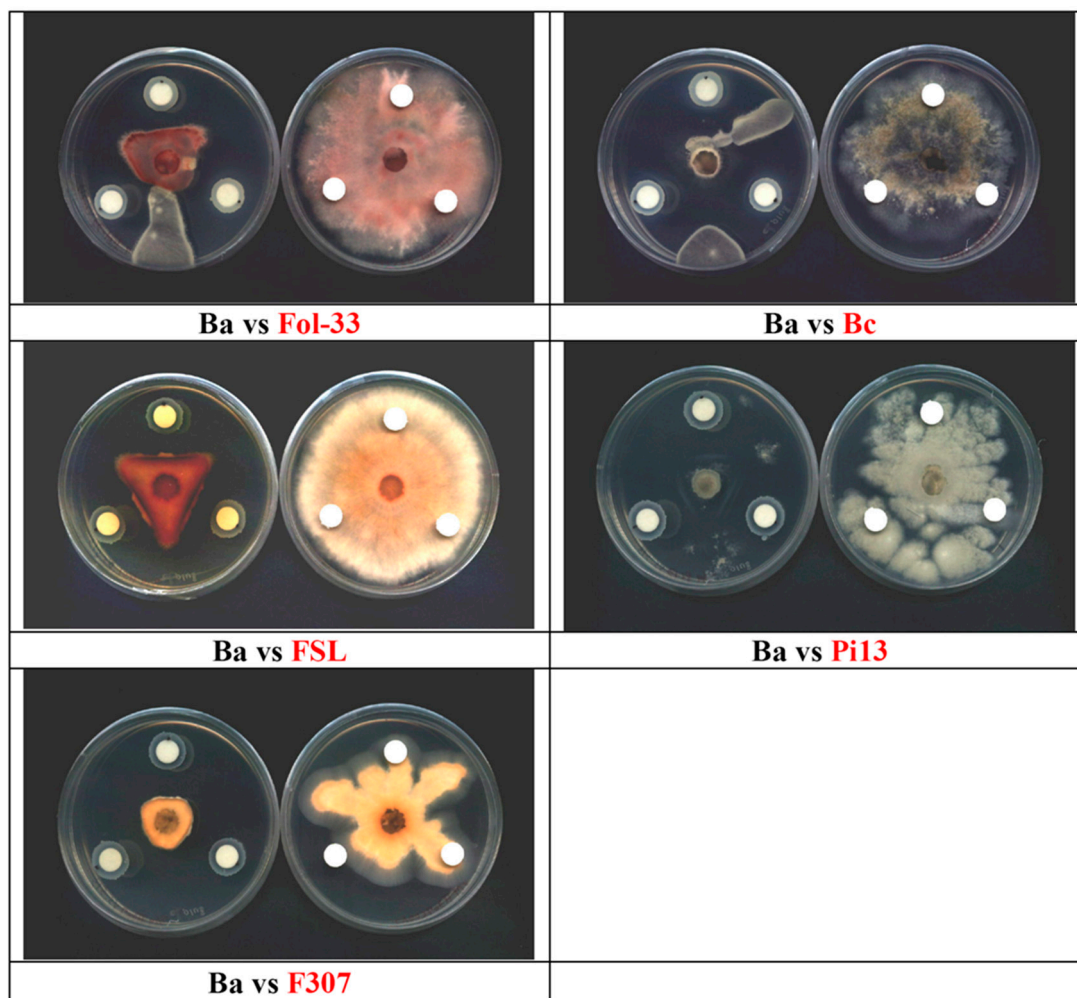


Figure S1. Cont.

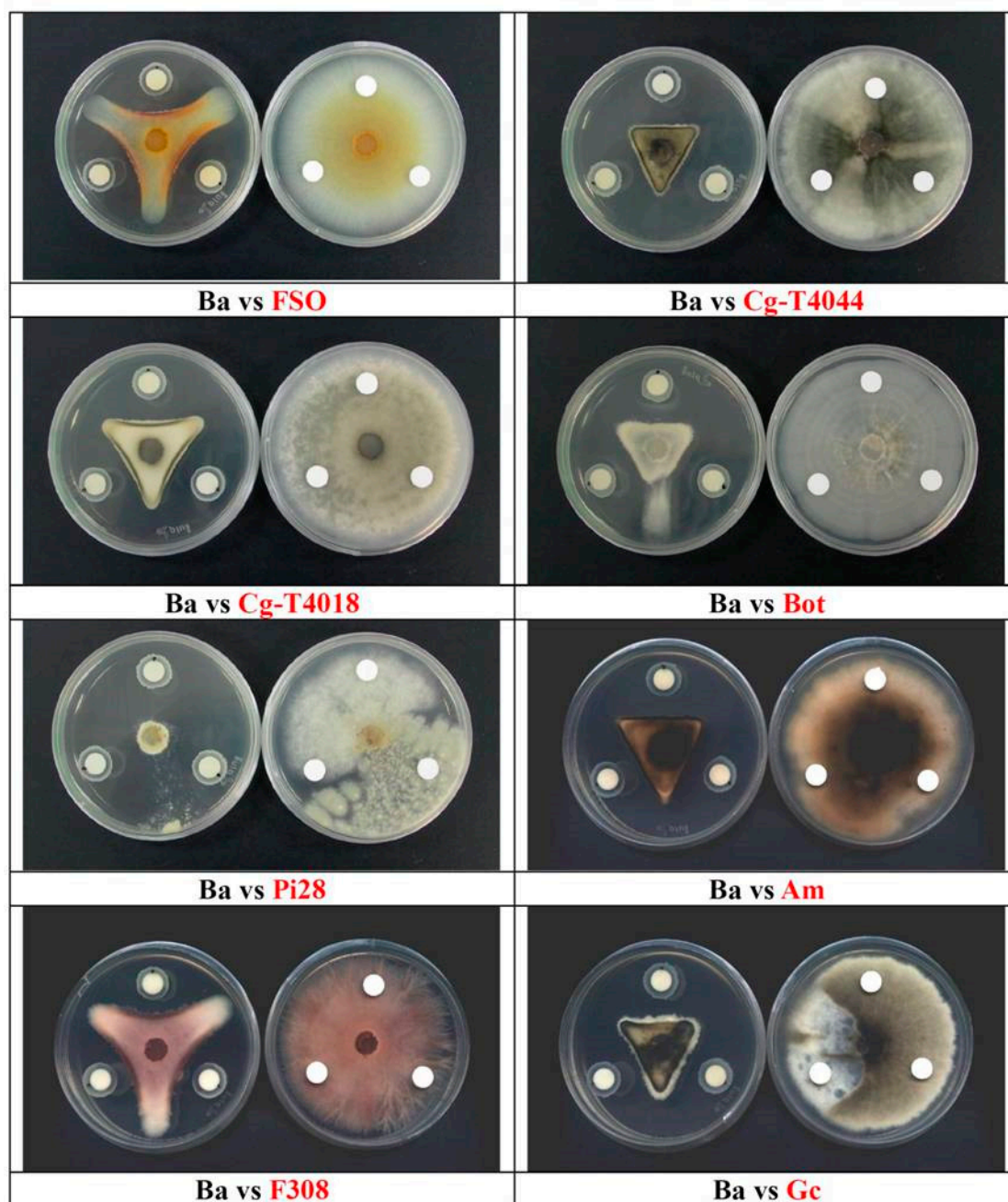


Figure S1. Cont.

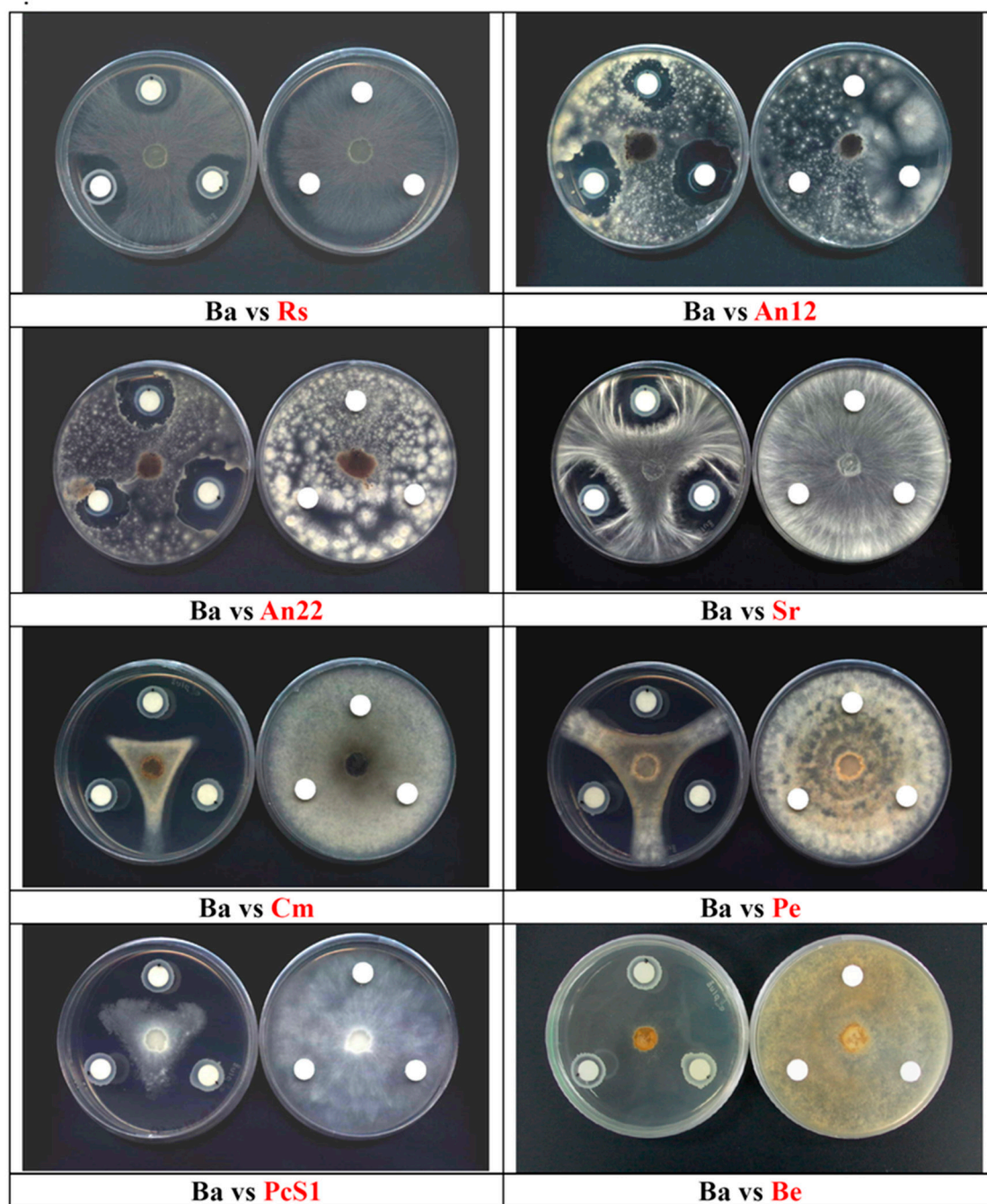


Figure S1. Antagonistic bioassay of Ba-BPD1 against phytopathogenic fungi. The short names of the fungi are listed in Table S1.

Table S2. The inhibition zone of Ba-BPD1 to the pathogenic bacteria.

Bacterium	Disease	Inhibition Zone in Diameter (cm)
<i>Acidovorax avenae</i> subsp. <i>citrulli</i>	Bacterial fruit blotch of melon	3.4
<i>Agrobacterium tumefaciens</i>	Crown gall	2.3
<i>Burholderia caryophylli</i>	Bacterial wilting	3.5
<i>Enterobacter cloacae</i>	Bacterial basal rot	2.5
<i>Erwinia carotovora</i> subsp. <i>carotovora</i>	Soft rot disease	2.3
<i>Erwinia chrysanthemi</i>	Soft rot disease	3.1
<i>Pseudomonas syringae</i>	Bacterial leaf spots	3.1
<i>Ralstonia solanacearum</i>	Bacterial wilting	2.9
<i>Xanthomonas axonopodis</i> pv. <i>cirti</i>	Citrus canker	4.5
<i>Xanthomonas axonopodis</i> pv. <i>vesicatoria</i>	Bacterial spot of tomato	4.5
<i>Xanthomonas compestris</i> pv. <i>compestris</i>	Black rot of brassica	4.5
<i>Xanthomonas oryzae</i> pv. <i>Oryzae</i>	Bacterial leaf blight	3.2

1.2. Materials and Methods

1.2.1. Antagonistic Bioassay with Phytopathogenic Fungi

The antifungal activity of Ba-BPD1 towards 21 phytopathogenic fungi was tested on PDA plates. A plug (about 1 cm in diameter) of each phytopathogenic fungus cut from the leading edge of the cultured plate (grown on PDA at 25 °C for five days) was placed in the center of the antagonistic plate. Simultaneously, three paper discs with 9 mm diameter were placed at a distance of 1.8 cm from the fungi inoculum. Then 30 µL of Ba-BPD1 cultured broth were loaded on each paper disc. The plates were incubated at 25 °C until the fungi in untreated contrast covered the plate. The distance of the inhibition zone was measured.

1.2.2. Antagonistic Bioassay with Phytopathogenic Bacteria

The antibacterial activity of Ba-BPD1 towards 12 phytopathogenic bacteria was tested on LA plates. A paper disc with 9 mm diameter was placed at the center of plate, and 30 µL of Ba-BPD1 cultured broth (grown in LB at 30 °C overnight) was loaded on paper disc. Then the phytopathogenic bacteria-cultured broth (grown in LB at 30 °C overnight) was sprayed evenly on the LA plate. The plates were incubated at 30 °C till the inhibition zone appeared. The diameter of the inhibition zone was measured.