**Supplementary Table 1.** MICs of CFCFs of JCK-1618 and JCK-1696 collected at various incubation times against *Mycosphaerella cerasella* (Mc), *Epicoccum tobaicum* (Et), and *Xanthomonas arboricola* pv. *pruni* (Xap)

| Strain | Incubation time (h) | MIC (%) | | |
|---|---|---|---|---|
| | | Mc | Et | Xap |
| JCK-1618 | 12 | 10 | 10 | 5 |
| | 24 | 5 | 5 | 0.625 |
| | 36 | 2.5 | 1.25 | 0.3125 |
| | 48 | 1.25 | 1.25 | 0.3125 |
| | 60 | 1.25 | 1.25 | 0.625 |
| | 72 | 2.5 | 1.25 | 1.25 |
| | 84 | 2.5 | 2.5 | 1.25 |
| | 96 | 5 | 5 | 5 |
| JCK-1696 | 12 | 10 | 10 | 5 |
| | 24 | 5 | 5 | 0.3125 |
| | 36 | 2.5 | 2.5 | 0.3125 |
| | 48 | 1.25 | 1.25 | 0.3125 |
| | 60 | 5 | 2.5 | 0.3125 |
| | 72 | 5 | 2.5 | 0.625 |
| | 84 | 5 | 5 | 2.5 |
| | 96 | 10 | 10 | 2.5 |

MIC, minimum inhibitory concentrations; CFCF, cell-free culture filtrate.

**Supplementary Table 2.** Typical antimicrobial VOCs of JCK-1618 and JCK-1696 detected by GC-MS analysis

| JCK-1618 | | | JCK-1696 | | | Note | Reference |
|---|---|---|---|---|---|---|---|
| Rt (min) | Area (%) | Possible compound | Rt (min) | Area (%) | Possible Compound | | |
| 2.785 | 11.45 | 3-Methyl-1-butanol | 2.797 | 15.86 | 3-Methyl-1-butanol | AF | Chaves-López et al. (2015) |
| 2.932 | 1.65 | Dimethyl disulfide | 2.945 | 1.89 | Dimethyl disulfide | AF | Li et al. (2010) |
| 3.168 | 2.19 | Toluene | 3.18 | 3.03 | Toluene | AF | Yuan et al. (2012) |
| 5.469 | 1.29 | Isopropyl benzene | | | | AF | Yuan et al. (2012) |
| 6.103 | 4.35 | 5-Methyl-2 heptanone | 6.107 | 3.69 | 5-Methyl-2-heptanone | AF | Morita et al. (2019) |
| 8.337 | 12.37 | 2-Nonanone | 8.34 | 12.81 | 2-Nonanone | AB, AF | Fernando et al. (2005), Raza et al. (2016), Yuan et al. (2012) |
| 9.532 | 5.12 | 2-Decanone | 9.533 | 3.88 | 2-Decanone | AF | Fernando et al. (2005), Yuan et al. (2012), Zhang et al. (2013) |
| 11.624 | 6.61 | 2-Undecanone | 11.625 | 7.80 | 2-Undecanone | AB | Raza et al. (2016) |
| | | | 11.765 | 5.72 | 1-Hexadecene | AF | Zhang et al. (2013) |
| 12.588 | 3.17 | 2-Dodecanone | 12.589 | 4.86 | 2-Dodecanone | AF | Yuan et al. (2012) |
| | | | 14.532 | 2.06 | 2-Tridecanone | AB | López-Lara et al. (2018) |

VOC, volatile organic compound; GC-MS, gas chromatography-mass spectrometry; Rt, retention time; AF, antifungal activity; AB, antibacterial activity.

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**Supplementary Table 3.** Typical VOCs produced by JCK-1618 detected by GC-MS analysis

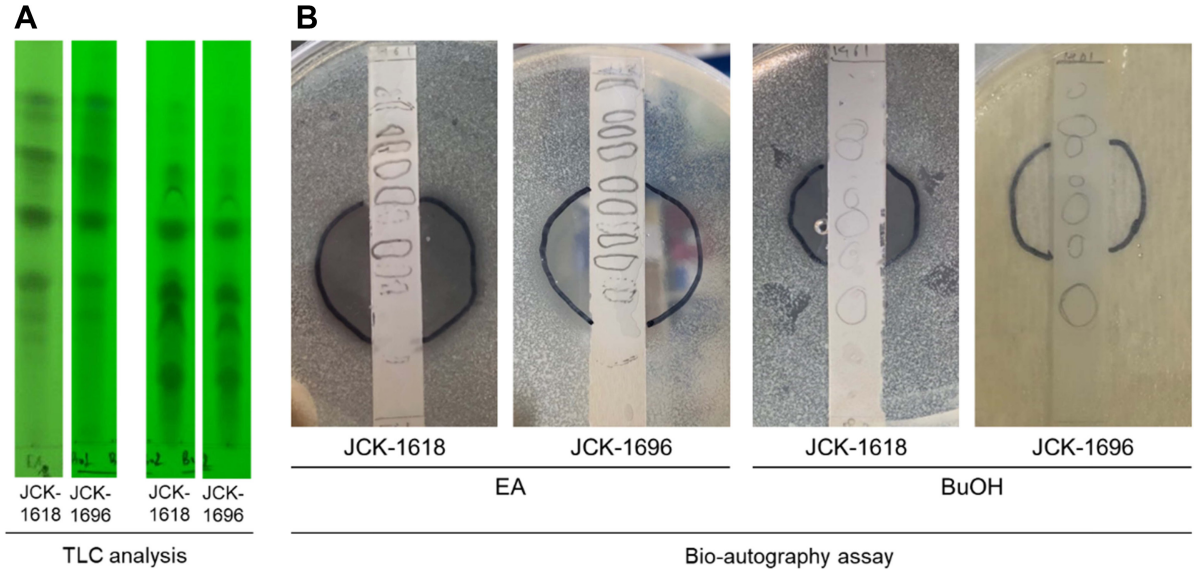
| Rt (min) | Total area (%) | Possible compound |
|---|---|---|
| 2.293 | 5.20 | Benzene |
| 4.576 | 2.62 | 1,4-Dimethyl benzene |
| 5.932 | 3.81 | 6-Methyl-2-heptanone |
| 6.484 | 3.60 | 1-Methylethenyl-benzene |
| 6.729 | 3.72 | 1,1'-(1-Ethenyl-1,3-propanediyl) bis-benzene |
| 6.761 | 3.75 | Butyl octyl carbonate |
| 8.301 | 6.97 | 1,1-Dimethylbutyl-oxirane |
| 8.517 | 3.96 | 2-Propyl-1-heptanol |
| 9.61 | 1.25 | 6-Methyl-1-heptanol |
| 9.732 | 2.37 | Cyclohexyl-tricyclo [6.2.2.0 2,7] dodeca-2(7),3,5-trien-9-ylidene-amine |
| 10.521 | 5.76 | Docosanoic acid, docosyl ester |
| 10.632 | 4.62 | 1-Naphthol |
| 11.763 | 2.82 | 1-Nonadecene |
| 11.863 | 1.61 | 2-Methyl-naphthalene |
| 12.694 | 5 | 6-Dodecanone |
| 14.151 | 2.19 | 1,1'-Biphenylene |

VOC, volatile organic compound; GC-MS, gas chromatography-mass spectrometry; Rt, retention time.

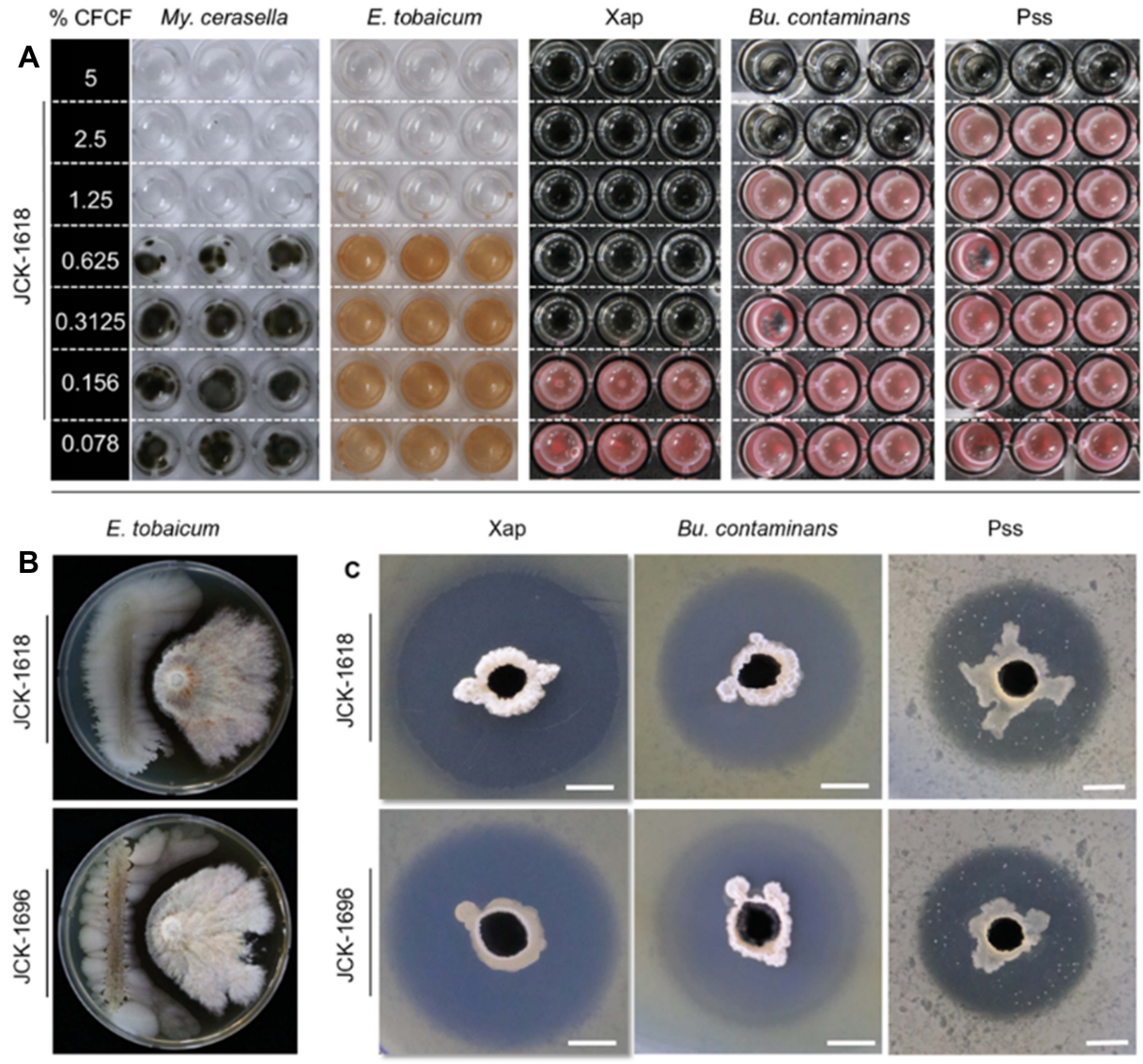
**Supplementary Table 4.** Typical VOCs produced by JCK-1696 detected by GC-MS analysis

| Rt (min) | Total area (%) | Possible compound |
|---|---|---|
| 2.308 | 1.75 | Benzene |
| 5.703 | 1.27 | 1-(Dimethoxymethyl)-4-(1-methoxy-1-methylethyl) benzene |
| 5.75 | 0.83 | Methy-2-oxohexanoate |
| 5.938 | 1.83 | 6-Methyl-2-heptanone |
| 6.26 | 1.15 | 4,4-Dimethyl-1,3-diphenyl-1-(trimethylsilyloxy)-1-pentene |
| 6.492 | 1.37 | 1-Ethenyl-2-methyl-benzene |
| 6.73 | 1.71 | 1,1'-(1-Ethenyl-1,3-propanediyl) bis-benzene |
| 6.769 | 1.61 | 1-Fluoro-dodecane |
| 8.305 | 5.53 | 4-Methyl-2-hexanol |
| 8.742 | 0.99 | 1-(4-Methyl-3-cyclohexen-1-yl)- ethanone |
| 9.728 | 1.65 | 3-Tetradecene |
| 10.523 | 5.50 | Docosanoic acid, docosyl ester |
| 10.636 | 4.01 | 1-Naphthol |
| 12.694 | 5.32 | 6-Dodecanone |
| 12.826 | 1.24 | 9-Octadecene |

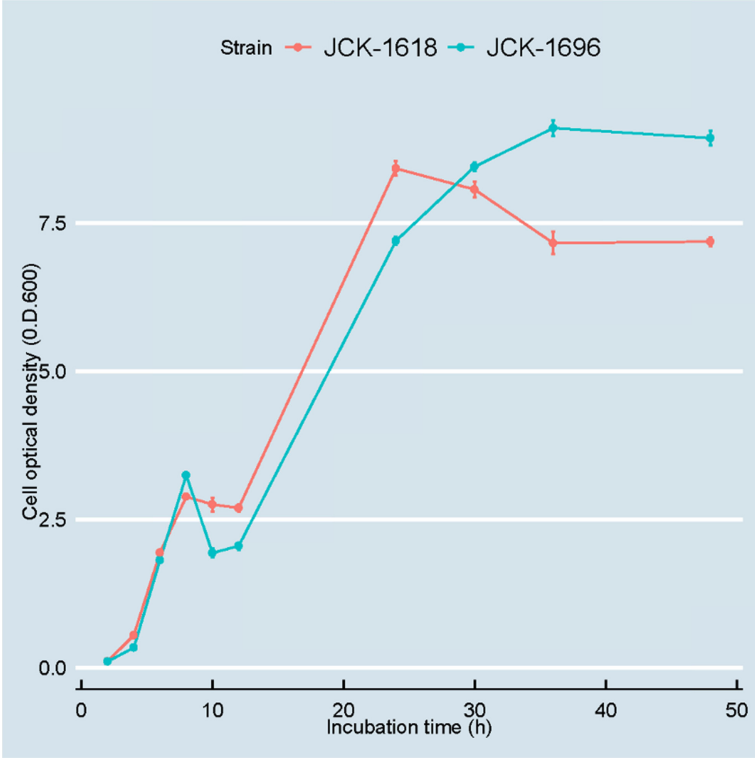
VOC, volatile organic compound; GC-MS, gas chromatography-mass spectrometry; Rt, retention time.



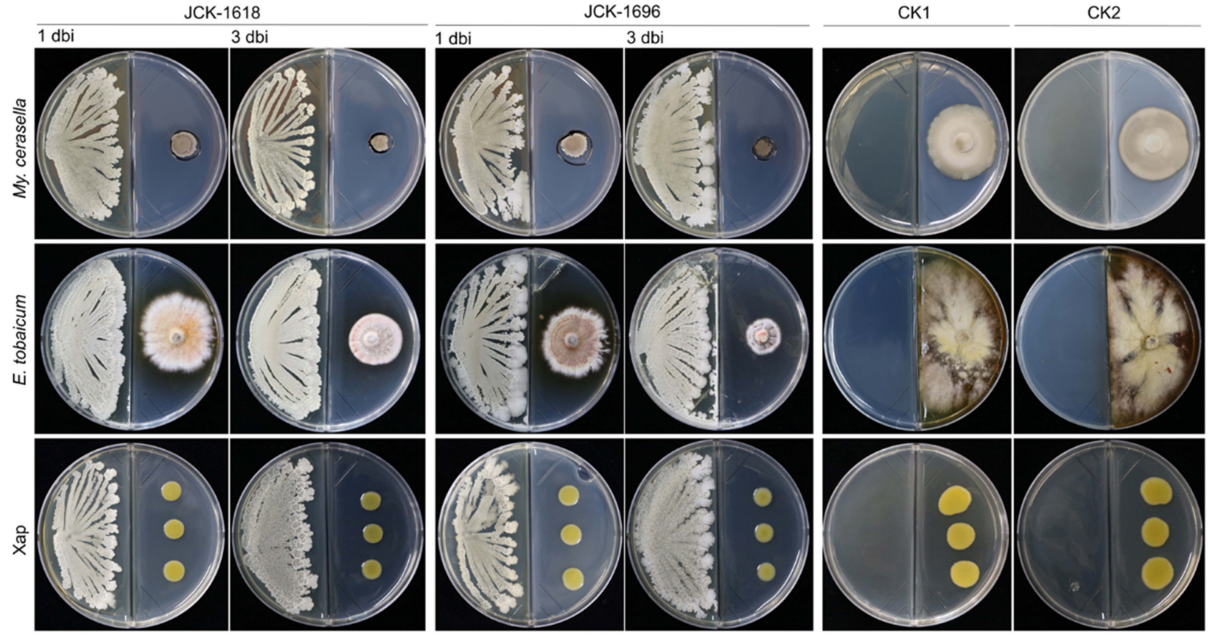

**Supplementary Fig. 1.** Crude solvent extracts of JCK-1618 and JCK-1696 exhibiting a highly similar profile of general and bioactive compounds. (A) Thin-layer chromatography (TLC) analysis. (B) Bio-autography assay against *Xanthomonas arboricola* pv. *pruni*. Ea, ethyl acetate; BuOH, *n*-butanol.



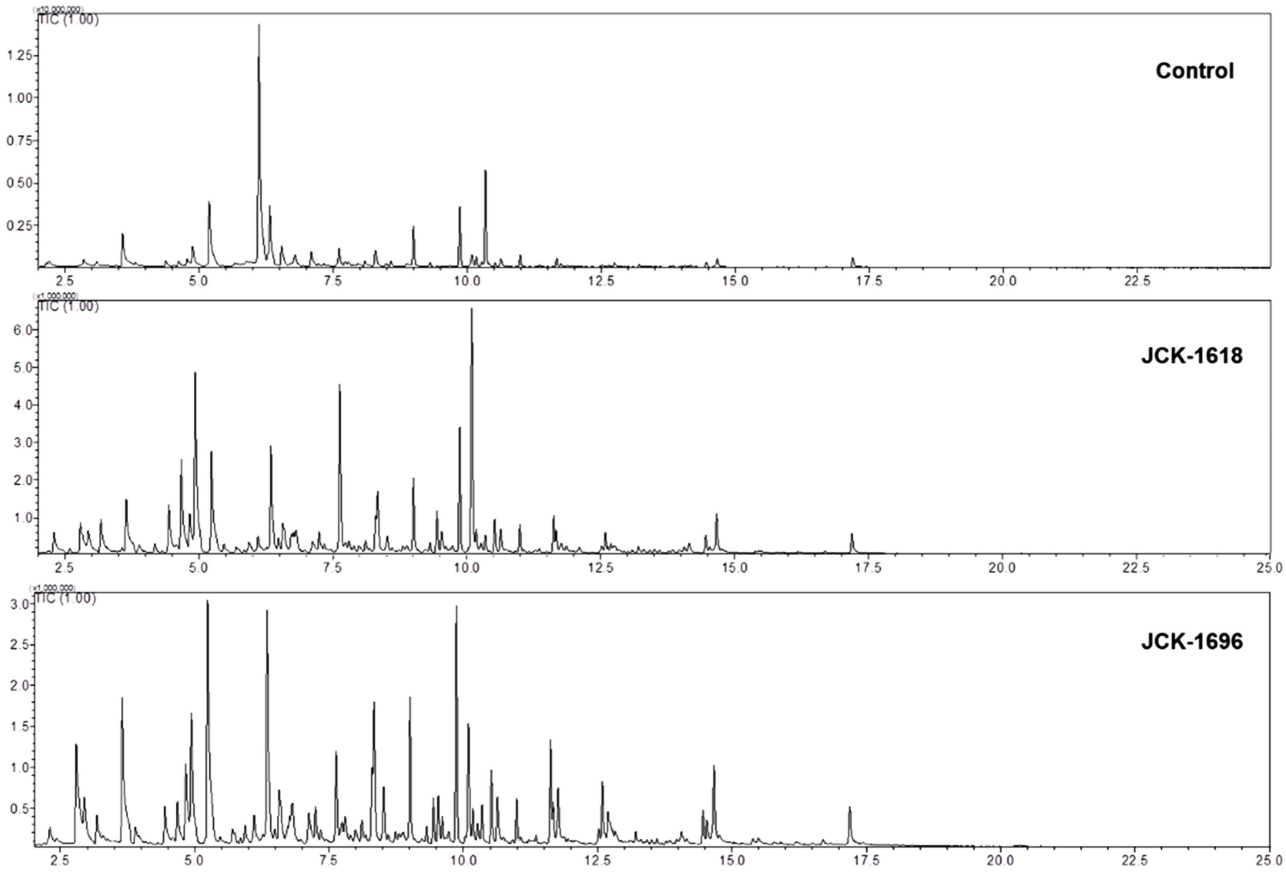

**Supplementary Fig. 2.** Growth inhibition of flowering cherry shot-hole pathogens. (A) Microtiter broth dilution assay of JCK-1618. Values represent the percentage of cell-free culture filtrates (CFCFs) present. The minimum inhibitory concentrations of CFCFs were determined as the lowest concentrations at which the growth of the pathogens was completely inhibited (empty wells). Each concentration was repeated in triplicate. Pink wells indicate the activity of bacterial cells after administration of 10 µl TTC (0.01%) as a stain for 2 h. (B, C) Dual-culture and spot-on-lawn assays, respectively, of JCK-1618 and JCK-1696. Scale bars = 5 mm.



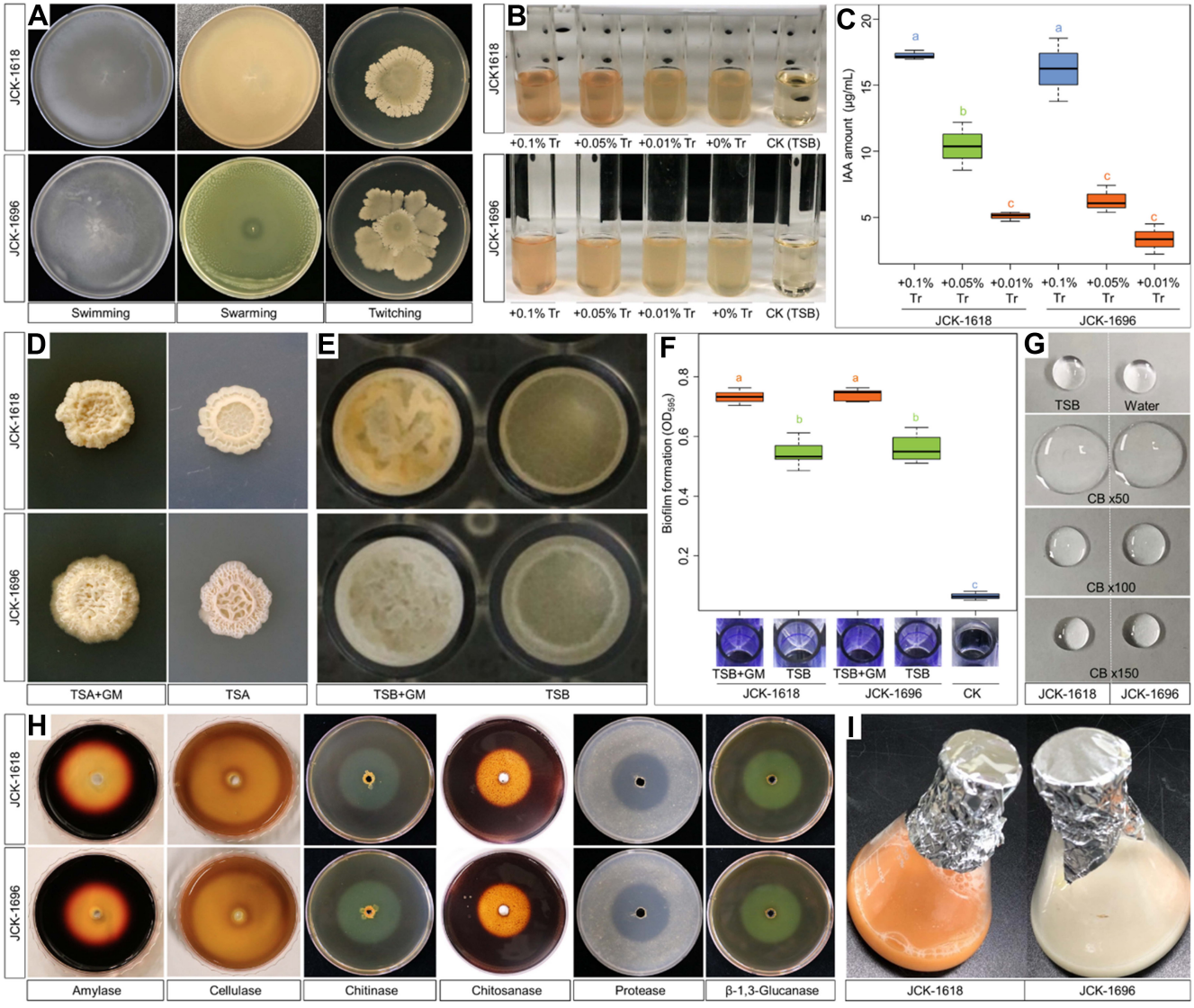

**Supplementary Fig. 3.** The growth curves of JCK-1618 and JCK-1696 constructed from the cell optical density (O.D. 600) of the culture broth media collected at various incubation times.



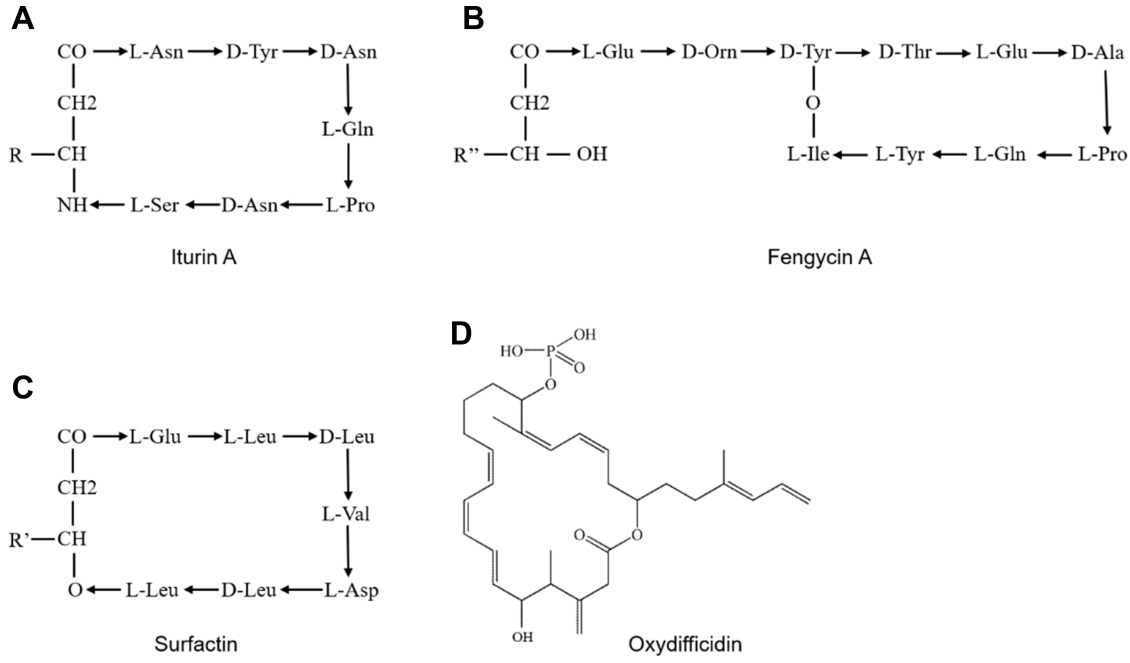

**Supplementary Fig. 4.** Antimicrobial volatile organic compound (VOC) production of JCK-1618 and JCK-1696 against *Mycosphaerella cerasella*, *Epicoccum tobaicum*, and *Xanthomonas arboricola* pv. *pruni* (Xap). CK1, pathogens inoculated only, plates sealed with Parafilm to expose pathogens with VOCs; CK2, pathogens inoculated only, plates not sealed to assess the normal growth of pathogens; dbi, days before pathogen inoculation.




**Supplementary Fig. 5.** Typical gas chromatography-mass spectrometry chromatograms of tryptic soy agar (negative control), JCK-1618, and JCK-1696.




**Supplementary Fig. 6.** Biochemical and phenotypic characteristics of *Bacillus velezensis* strains JCK-1618 and JCK-1696. (A) Motility. (B, C) Indole-3-acetic acid (IAA) production. (D-F) Biofilm formation. (G) Drop collapse assay. (H) Enzyme production. (I) Bacterial colonies grown in tryptic soy broth medium. Tr, L-tryptophan; CB, culture broth; TSA, tryptic soy agar; TSB, tryptic soy broth.




**Supplementary Fig. 7.** Chemical structures of bioactive compounds. (A) Iturin A. (B) Fengycin A. (C) Surfactin. (D) Oxydifficidin. A, B, C. R, R', and R'' denote an aliphatic chain.

## Supplementary Methods

**Bacterial isolation.** Soil samples for bacterial isolation were collected from soil at a depth of 5 to 10 cm from various crop and tree fields in South Korea. The bacteria were isolated by the plate dilution method. One gam of each soil sample was suspended in 9 ml sterile distilled water (SDW) and mixed thoroughly by shaking the tubes on a rotatory shaker for 10 min. Each suspension was serially diluted up to $10^{-4}$, and then 50 µl diluted suspensions were spread onto sterilized and coagulated TSA Petri plates (diameter: 15 cm). Inoculated plates were placed at 28°C in an incubator for 48 h. The bacterial isolates produced abundant colonies, with a rough surface. The isolates were purified and maintained on TSA plates at 4°C until further use. For long-term preservation, the isolates were kept in 20% glycerol at –80°C.

To isolate bacterial endophytes from FC leaves, healthy and un-damaged leaf samples were collected at Chonnam National University (Gwangju campus, Gwangju, Korea). The leaves were then surface-disinfected with 2% sodium hypochlorite for 2 min and 70% ethanol for 1 min before being washed with SDW twice. The last rinse (100 µl) was plated on a TSA plate to assess the efficacy of surface disinfection. The plates were then incubated at 28°C for 72 h and checked for the presence or absence of any bacterial colonies. Leaf samples exhibiting no microorganisms from the last rinse were used for isolation. Approximately 3-mm-long leaf pieces were macerated into a suspension with 10 mM phosphate-buffered saline (PBS) using Tissue Lyser II (Qiagen, Seoul, Korea). Next, 20 µl of the original suspension (10-fold and 100-fold dilutions when required) was spread over the surface of a TSA plate. Bacterial isolates were then picked and maintained following the aforementioned procedure.

**Screening of antagonistic bacteria.** For the initial screening of the antagonistic bacteria, 403 selected bacterial isolates were challenged for their antimicrobial activities against Mc, Et, and Xap by microdilution broth assays on 96-well plates (Nguyen et al., 2019). Each well received 90 µl of PDB supplemented with 1% (w/v) fungal mycelia suspension (50 mg/ml). Then, 10 µl of CFCFs were added to each well, followed by a 2-fold dilution to reach the two final concentrations of 10 and 5%, respectively. Negative and positive controls were established by adding 10 µl TSB or difenoconazole (Merck KGaA, Darmstadt, Germany), respectively, to wells instead of CFCFs. After 3 to 5 days at 25C, wells that not displaying any mycelial growth were recorded as "inhibited mycelial growth."

For the antibacterial test, 10 µl of CFCF was mixed with 90 µl of pathogenic bacterial cell suspension ($10^5$ cfu/ml in TSB) in a well, followed by a 2-fold dilution to reach the two final concentrations of 10 and 5%, respectively. Wells filled 10 µl TSB or streptomycin sulfate (Merck KGaA) instead of CFCFs served as negative and positive controls, respectively. After 36 h at 28°C, wells without visual growth of bacteria were recorded as "inhibited bacterial growth."

***In vitro* antimicrobial activity and minimum inhibitory concentration (MIC).** The microtiter broth dilution method, as previously described, was employed to determine the MIC value of antagonistic bacteria against the SH pathogens. The lowest concentration of CFCFs which completely inhibited the growth of bacteria or fungi by visual observation was defined as the MIC value. To determine bacterial cell viability, 10 µl of 0.01% TTC (2,3,5-triphenyltetrazolium chloride; Merck KGaA) was added into each well. After 2 h, wells without the appearance of pink or red colors indicated completely inhibited bacterial growth.

**Incubation time optimization and bacterial growth curve construction.** A single bacterial colony was sub-cultured in 5 ml TSB in a glass tube. Overnight-grown bacterial cultures were inoculated into 120-ml TSB in a 500-ml flask adjusted to the final optical density of bacterial cells ($OD_{600}$, measured at 600 nm) of 0.02. JCK-1618 and JCK-1696 were then incubated at 30°C on a rotating shaker (150 rpm/min) for up to 96 h. The number of bacterial cells were estimated using a spectrophotometer ($OD_{600}$) (SmartSpec 3000, BioRad Laboratories, Inc., CA, USA) every 2 h for the first 12 h, and subsequently at 12-h intervals for up to 48 h. The data obtained from the OD measurements were then used for the construction of bacterial growth curves. The culture broths were also collected every 12 h and prepared for the production of CFCFs before being tested for their antimicrobial activities as described above. An appropriate incubation time was determined at which CFCFs exhibited the best effects against the test pathogens. The CFCFs obtained from an appropriate time were devoted to further experiments, including an *in vivo* assay and the extraction of bioactive compounds.

**IAA production.** In a tube, 2 ml of CFCFs, obtained from the culture broth, were mixed with two drops of O-phosphoric acid and 4 ml of Salkowski reagent (50 ml of 35% perchloric acid, 1 ml 0.5 M $FeCl_3$ solution). After incubation at 28C for 20 min in darkness, the appearance of

a pink or red color confirmed the production of IAA. Next, mixtures with a pink or red coloration were analyzed using a UV-Vis spectrophotometer (UV-1650PC, Shimadzu, Kyoto, Japan) at 530 nm. The amount of bacterial IAA was quantified based on the standard curve, which was constructed using various concentrations (µg/ml) of pure IAA (Merck KGaA).

**Biofilm formation.** Biofilm production was carried out on flat-bottom 96-well plates (Gudiña et al., 2010; O'Toole et al., 1999). Bacteria adhering to the wells were fixed in 200 µl methanol (99%) and visualized using 1% crystal violet solution. After 15 min, wells were washed thoroughly and vigorously with SDW to remove unattached cells and residual dye. The appearance of a violet color at the bottom of the wells or at the air–medium interface indicated biofilm production. Biofilm quantification was performed as described by Gudiña et al. (2010).

**Preparation of crude enzyme.** The 48-h CFCFs of JCK-1618 and JCK-1696 were used for the preparation of the crude enzyme following the methods described by Wang and Chang (1997) with minor modifications. CFCFs (200 ml) were first prepared in a glass cup and kept on ice at 4°C in a fridge. Ammonium sulfate was slowly added to the cup until a saturation of 40% was reached while gently stirring at 4°C. After 5 h, the precipitate was collected by centrifugation at 4°C at 10,000 rpm for 15 min and then dissolved in a minimal amount of 10 mM Tris-HCl buffer (pH = 8), before being transferred into a dialysis bag (10 kDa, Spectra/Por Dialysis Membrane, Fisher Scientific, Göteborg, Sweden). The dialysis bag was then immersed into the same buffer at 4°C for the dialysis process, with the buffer being replaced every 2 h for the first 12 h and subsequently at 6-h intervals. After 24 h, the resultant dialysate inside the bag was collected as the crude enzyme and then sterilized by filtration using a 0.22-µm pore size membrane before being examined for antimicrobial activity on 96-well plates.

**Biosurfactant production and isolation.** The 48-h culture broth (240 ml) was centrifuged at 4,500 rpm for 20 min at 4°C. Cells were collected and washed twice with demineralized water before being resuspended in 40 ml of phosphate-buffered saline (PBS, 0.01 M $KH_2PO_4/K_2HPO_4$ and 0.15 M NaCl, pH = 7.0). The bacterial suspensions were kept at 25°C for 3 h with gentle shaking (60 rpm) to induce the secretion of biosurfactants (Gudiña et al., 2010). Bacterial cells were then removed by centrifugation and the supernatant was filtered through a 0.2-µm pore-size membrane filter. The CFCFs were acidified to pH 2.0 with 1 M HCl and kept on ice for 4 h. The precipitate was obtained by centrifugation and washed twice with sterile acidic (pH 2.0) demineralized water (SDeW) (de Souza et al., 2003). The precipitate was resuspended in SDeW, adjusted to pH 7.0 with 0.2 M NaOH, and lyophilized. The lyophilized biosurfactant extracts were checked for their surfactant activity using a drop-collapsing assay along with the assessment of their antimicrobial activity using the methods previously described and then kept at –20°C for further studies.

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