Alma Mater Studiorum Università di Bologna
Archivio istituzionale della ricerca

Study of the efficacy of bacterial antagonists against Cadophora luteo-olivacea of kiwifruit

(Article begins on next page)

19 December 2024

1   **Research article**

2

3   **Study of the efficacy of bacterial antagonists against *Cadophora luteo-olivacea* of kiwifruit**

4   A. Di Francesco[1†], F. Jabeen[1†], M. Di Foggia[2], C. Zanon[1], R. Cignola[1], A. Sadallah[1], V. Tugnoli[2], P.

5   Ermacora[1*], M. Martini[1]

6

7   [1] Department of Agriculture, Food, Environmental and Animal Sciences, University of Udine, Italy

8   [2] Department of Biomedical and Neuromotor Sciences, University of Bologna, Italy

9   [†] Contributed equally to this work

10   [*]Corresponding author: paolo.ermacora@uniud.it

11

12   **Abstract**

13   Skin pitting currently represents one of the major postharvest diseases of kiwifruit and one of the

14   most difficult to manage in packinghouses due to its latent behavior and the difficulty in predicting

15   its emergence. Our research demonstrates the potential to use different bacterial strains (*Pseudomonas*

16   *synxantha* and *Bacillus* spp.) instead of synthetic compounds to preserve kiwifruit from the

17   development of postharvest skin pitting symptoms, following the momentum towards sustainable

18   strategies. The antagonists tested with in vitro assays showed different efficacy rates against *C. luteo-*

19   *olivacea* (strain Cad21) mycelial growth by producing non-volatile metabolites. The biochemical

20   composition of the most active bacterial non-volatile secondary metabolites was described through

21   FT-IR (Fourier-Transform Infrared) spectroscopy. *Pseudomonas synxantha* strain 117-2b emerged as

22   the most active strain in in vivo experiments, both as a curative and preventive treatment (63% and

23   84.7% of inhibition, respectively). In addition to its ability to reduce disease incidence, the biological

24   antagonism exerted by *P. synxantha* strain 117-2b was further demonstrated by qPCR analysis as a

25   reduction in the pathogen's abundance. In view of these results, alternative solutions in the field and

26   during postharvest storage could be considered to control *C. luteo-olivacea* of kiwifruit.

1. Introduction

Kiwifruit is a recently domesticated plant belonging to the genus *Actinidia*, which contains ± 60 species (Zhang et al., 2020). However, two species, *A. deliciosa* and *A. chinensis*, dominate the kiwifruit industry (Huang et al., 2004). In particular, *Actinidia deliciosa* (A. Chev.) C.F. Liang & A.R. Ferguson) cv. "Hayward", which is planted worldwide in temperate-zone countries (Michaelidis and Elmer, 2000), remains one of the most commercially important cultivars (Choi et al., 2022). The consumption of kiwifruit has been increasing steadily; kiwifruit is one of the most popular and widely consumed fruits due to its flavor and remarkable nutrients (Wang et al., 2022a). Kiwifruit can be stored for about 4–5 months at 0 °C with relative humidity (R.H.) of 92–95% using standard refrigeration or for a longer time in a controlled atmosphere (CA) (Taş et al., 2022). Nevertheless, the fruit is susceptible to different fungal pathogens, among which the best known is *Botrytis cinerea* (Pers: Fr). It has always been considered one of the most important pathogens for stored kiwifruit (Costa et al., 1991), becoming a serious problem over the last 15 years in kiwifruit production (Michaelidis and Elmer, 2000, Di Francesco et al., 2018). During the last few years, the re-emerging pathogen *Cadophora luteo-olivacea*, which causes the skin-pitting disease of kiwifruit, has been detected in Italian and Chilean packaging houses (Di Francesco et al., 2022, Auger et al., 2018). Skin pitting symptoms (oval dark brown lesions) appear after several months of cold storage (Spadaro et al., 2010, Gilardi et al., 2007). *Cadophora luteo-olivacea* is a fungal pathogen that infects fruits during the developing period, remaining inactive for up to 4–5 months of cold storage (Di Francesco et al., 2022) and can appear immediately during the shelf-life period.

Usually, the risk of fungal postharvest diseases is mitigated with fungicide treatments before and after harvest (Palm and Kruse, 2012). However, issues related to chemical residues in the fruit have recently made these treatments challenging to justify, and this has stimulated the exploration of alternative strategies (Chowdhury et al., 2022). Microorganisms used as biocontrol agents (BCAs)

could represent one of the sustainable alternative methods (Wang et al., 2022b) to apply. Among

them, bacteria usually show interesting antagonistic mechanisms of action toward fungal pathogens,

making them attractive for biological control strategies. Sometimes, different mechanisms can act

simultaneously, and it is not easy to establish which individual mechanism contributes the most to

the inhibition of fungal pathogen growth (Di Francesco et al., 2016). Among the different species of

bacteria used as effective BCAs, *Bacillus*, and *Pseudomonas* spp. have been mainly exploited for

their synthesis of extracellular enzymes (Nihorimbere et al., 2011), production of volatile organic

compounds (VOCs) (Gotor-Vila et al., 2017, Ni et al., 2022), and induction of resistance responses

in the host (Carmona-Hernandez et al., 2019). All these characteristics could represent a useful

starting point for selecting active and effective BCAs.

The objective of the present work was to investigate the antifungal effect of four different bacterial

strains belonging to *Bacillus* and *Pseudomonas* spp. against *C. luteo-olivacea* by: i) in vitro assays

through a co-culturing method at different growth temperatures and using agar infusion of bacterial

cell filtrates from different growing times; ii) by their biochemical characterization (FT-IR); and iii)

via in vivo assays on kiwifruit to verify their effectiveness as a preventive or curative treatment.

2. Materials and methods

2.1 Fruit

Kiwifruit cv 'Hayward' (*Actinidia deliciosa* (A. Chev.) C.F. Liang & A.R. Ferguson) were obtained

from orchards cultivated under integrated pest management (IPM) production system located in

Zoppola (Pordenone province, Google: 45°56′56.91″ N, 12°47′15.83″ E) and Sedegliano (Udine

province: 46°02′11.02″ N, 12°57′46.51″ E). Fruits of uniform size and free from lesions were

collected and immediately stored at 4 °C with RH 92% until use.

2.2 Microorganisms

Cadophora luteo-olivacea strain Cad21 was isolated from the tissue of symptomatic kiwifruit; its fresh mycelium was used for genomic DNA extraction following a procedure by Lecellier and Silar (1994), modified by Martini et al. (2009). The extracted and quantified DNA was diluted in sterile water to obtain a concentration of about 2–5 ng/µl. The fungal strain was molecularly identified by using the specific primers Cad (KSDA)-f2 5′-GTG GCG GGG CTA CCC TAC-3′/Cad (KSDA)-r1 5′-GCT GGC AAG TAG ACC TAC CG-3′) (Martini et al., unpublished) to amplify a DNA fragment of 485 bp in 25 µL reactions containing 200 µM of each of the four dNTPs, 0.4 µM of each primer, 1.5 mM $MgCl_2$, 0.625 units of GoTaq Flexi DNA Polymerase (Promega, WI, USA) and 1 µL of diluted DNA. The amplification reactions were carried out in a T gradient thermal cycler (MiniAmp Plus, Thermo Fisher Scientific) using the following program: initial denaturation at 94 °C for 2 min; 35 cycles at 94 °C for 40 s, 58 °C for 30 s, 72 °C for 40 s; final extension at 72 °C for 7 min. PCR products were run on 1% agarose gels, stained with ethidium bromide, and visualized under UV light. The amplified DNA was purified with a Nucleospin Extracts II Kit (Macherey–Nagel, Germany) and, finally, Sanger sequenced by BMR Genomics (Padova, Italy). The sequences were compared to those of the GenBank database (https://www.ncbi.nlm.nih.gov/BLAST) using the BLASTN search. The fungal colony was maintained on potato dextrose agar (PDA, 39 g L^{-1}, Oxoid - UK) at 4 °C until use. Regarding the antagonists, the *Pseudomonas synxantha* 117-2b strain belonging to the microbiological collection of DI4A-University of Udine, was originally isolated from the surface of kiwifruits and subsequently molecularly characterized (Di Lenarda et al., 2010). The *Bacillus amyloliquefaciens* FZB24 strain was acquired from the DSM-Deutsche Sammlung von Mikroorganisms collection. The *Bacillus subtilis* QST713 and *Bacillus pumilus* QST2808 strains were purchased from NRRL (Northern Regional Research Laboratory, IL, USA). Two weeks before the experiments, *C. luteo-olivacea* strain Cad21 was grown on PDA (potato dextrose agar, 39 g L^{-1}; Oxoid - UK) at 25 °C. The conidial suspension was prepared by scraping and suspending conidia in sterile distilled water with 0.05% (v/v) Tween 80 and adjusted to the final required concentration (10^5 conidia mL^{-1}) with a hemocytometer. All the bacterial strains were maintained on nutrient agar (NA,

104   13 g L^{-1}, Oxoid - UK) at 4 °C until use. For the assays, bacterial cells from two-day-old plates were

105   suspended in potassium phosphate buffer (PPB, 70 mL KH$_2$PO$_4$ 0.2 M; 30 mL K$_2$HPO$_4$ 0.2 M and

106   300 mL deionized water v/v/v pH 6·5) and adjusted to a final concentration of 106 cells mL^{-1} and

107   108 cells mL^{-1} according to the experiment.

108

109   **2.3 *In vitro* assays**

110   Two different experiments were performed to test the antagonism of the four bacterial strains. The

111   first, a co-culture assay, was conducted using bacterial cells grown in nutrient broth (NB, 13 g L^{-1},

112   Oxoid - UK) for 24 h. A mycelial plug (6 mm diameter) of the fungal strain Cad21 derived from 14-

113   day-old colonies was placed in the center of the PDA plate. A loopful (1 μL) of each bacterial culture

114   was deposited at four equidistant peripheral locations on the dish. Plates inoculated only with the

115   fungal pathogen were considered as control. Plates were incubated at 1 °C and 25 °C in dark

116   conditions, and the colony diameters were measured with a ruler after 8 and 4 weeks, respectively.

117   Five replicates for each condition were used, and the experiment was conducted twice.

118   The second assay tested the efficacy of bacterial cell filtrates collected at different growing times. A

119   loop of cells of each strain was grown in 100 mL of NB broth at 25 °C in a rotary shaker (250 rpm)

120   for 4 days. A flask was prepared for each sampling time (1 h, 24 h, 48 h, 72 h, 96 h). An aliquot of

121   50 mL of each sampling time was centrifuged (10,000 rpm at 4 °C for 30 min), and the supernatants

122   were filtered using 0.20 μm Millipore filters (Sigma Aldrich, USA). An aliquot of each collected cell

123   filtrate (50 mL) was infused into 50 mL of PDA and plated onto Petri dishes. Dishes were inoculated

124   with Cad21 strain plugs (6 mm diameter) and kept at 25 °C. After 2 weeks, the colony diameters were

125   measured using a digital caliper (Borletti, Italy). Plates inoculated with the fungal pathogen plug on

126   simple PDA were considered as control. Eight replicates were used for each condition, and the

127   experiment was conducted twice.

128

129   **2.4 FT-IR analysis of bacterial cell filtrates**

130   The most active bacterial cell filtrates were analyzed with FT-IR (Fourier-Transform Infrared)

131   spectroscopy to obtain a rapid and non-destructive characterization of their main antifungal

132   components. Filtrates were obtained as described above in paragraph 2.3. An aliquot (1.5 mL) of each

133   treated sample was collected in sterile tubes (2 mL), stored at −80 °C, and lyophilized. Infrared spectra

134   were recorded with a Bruker ALPHA series FT-IR spectrophotometer (Bruker, Ettlingen, Germany)

135   equipped with an attenuated total reflectance (ATR-Diamond crystal) apparatus at the standard

136   resolution of 4 cm^{-1}. Three spectra (averaged over 64 measures) were measured for each cell filtrate.

137   The spectra were collected as described by Di Francesco et al. (2021).

138

139   **2.5 *In vivo* assay**

140   For the in vivo assay, kiwifruits (6.5° Brix) were divided into two groups according to the applied

141   treatment: curative or preventive. For both treatments, fruits were surface sterilized with sodium

142   hypochlorite (0.1% v/v), rinsed with distilled water for one minute, and air dried at room temperature.

143   Fruits were artificially wounded once at the equatorial line by using a sterile steel nail (2 mm × 2 mm

144   × 2 mm). For curative treatment, 20 μL of pathogen conidial suspension (10^5 conidia mL^{-1}) were

145   pipetted into each wound. After 24 h, the same wounds were inoculated with 20 μL of *P. synxantha*

146   (117-2b), *B. amyloliquefaciens* (FZB24), *B. subtilis* (QST713), and *B. pumilus* (QST2808)

147   suspensions (10^8 cells mL^{-1}).

148   For preventive treatment, 20 μL of each bacterial cell suspension were inoculated first, and after 24

149   h, the same aliquot of the conidial suspension was added as described above. Treated fruits were kept

150   in plastic trays at 1 °C and 92% relative humidity (R.H.) for 4 months. After the cold storage, fruits

151   were maintained at 20 °C (shelf life) for 1 week. Kiwifruits inoculated with sterile water represented

152   the negative control, while fruits treated with Scholar® (active ingredient: Fludioxonil; Syngenta,

153   Basel, Switzerland) (1.3 mL L^{-1}) represented the positive one. The experiment consisted of three

154   replicates of 15 fruits for each treatment, and it was performed twice.

155

2.6 DNA Extraction and quantification of the amount of fungal DNA by qPCR

The total genomic DNA was extracted from artificially wounded-inoculated kiwifruits (as described above in paragraph 2.5) to quantify the amount of fungal pathogen DNA using a qPCR assay. DNA was extracted from 10 portions of kiwifruit tissue (150 mg) for each condition, collected at 1 cm from the inoculation wound following a Doyle and Doyle (1990) protocol modified by Martini et al. (2009). The quality and quantity of extracted DNA were assessed using a NanoDrop 1000 Spectrophotometer (Thermo Scientific, Wilmington, DE, USA), and each sample concentration was adjusted to 20 ng/µL by dilution in nuclease-free water. Qualitative qPCR was performed using ITS as a target using the *C. luteo-olivacea* specific primers Cad (KSDA)-f2 5′-GTG GCG GGG CTA CCC TAC-3′/Cad (KSDA)-r2 5′-CGC CAA AGC AAC AAA GGT AGT-3′ (fragment 105 bp long). qPCRs were performed in 15 µL per reaction in a 96-well Bio-Rad CFX96 RealTime PCR System (Bio-Rad Inc., Hercules, CA, USA). Reaction mixtures contained 0.3 µM of each primer, 1X SsoFast™ EvaGreen ® Supermix (Bio-Rad Inc., Hercules, CA, USA), molecular grade H2O; 2 µL of DNA solution containing 20 ng/µL of extracted DNA as a template. Cycling conditions were as follows: initial denaturation at 98 °C for 2 min; 50 cycles of 5 sec at 98 °C; 5 sec at 58 °C. A low-resolution melting curve (ramp from 65 °C to 95 °C with 0.5 °C increments and holding times of 5 s) was programmed at the end of the cycling reaction.

In all positive samples, the amount of pathogen DNA was assessed by qPCR as pg of *C. luteo-olivacea* DNA/mg of kiwifruit tissue to normalize the data. To quantify *C. luteo-olivacea* DNA, a standard curve was prepared with 10-fold serial dilutions of total DNA extracted from a pure culture of the Cad21 strain (quantified by using Qubit® 2.0 Fluorimeter), starting at 2 ng/µL and up to 2 pg/µL. PCR mixtures and cycling conditions were performed as described above.

The amount of fluorescence for each sample was measured at the end of each cycle and analyzed via CFX-Manager Software v. 2.0 (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The baseline was automatically determined, and the fluorescence threshold was set manually to maximize the standard curve efficiency. Each diluted sample and each standard were replicated three times in the

experiment. Fungal DNA quantity was expressed as pg of *C. luteo-olivacea* DNA/mg of kiwifruit tissue.

2.7 Population dynamics of *Pseudomonas synxantha* strain 117-2b on kiwifruit

To analyze the ability of *P. synxantha* (117-2b) to colonize and proliferate on kiwifruit wound sites, fruits were surface sterilized, as reported in section 2.5. Fruits were wounded at three equidistant points by using a sterile needle. Aliquots of 20 μL of *P. synxantha* strain 117-2b suspension (106 cells mL^{-1}) were pipetted into each wound. Fruits were kept at 0 °C for 4 months. BCA growth was determined at 0 (2 h from the inoculation), 1, 2, 3, and 4 months of storage. Plugs (6 mm, Ø) of fruit tissue from each wound site were removed with the help of a sterile cork borer. Fruit plugs were transferred into a sterile stomacher bag containing 5 mL of sterile distilled water and Tween 80 (0.05%). The bag was stomached for 20 min (Bag Mixer 400; Interscience, St Nom, France). The juice (100 μL), diluted in sterile distilled water, was surface-plated on NA, and the Petri dishes were incubated at 25 °C for 2 days. Three fruits representing three replicates were set up for each sampling time, and the experiment was performed twice.

2.8 Statistical analysis

All the experiments were analyzed by one-way analysis of variance (ANOVA). Statistical means were compared by using *Tukey's* test (α = 0.05). Data were reported as mean values ± SE of two independent experiments. All analyses were performed with the software MiniTab.16.

3. Results

3.1 *In vitro* assays

Different inhibition values of the fungal pathogen were observed with the in vitro assays after the direct application of the bacterial strain or their secondary metabolites. Furthermore, the colony diameter of the control observed at 1 °C showed values that support the ability of the pathogen to

grow at low temperatures. Regarding the results of the co-culture assay (Fig. 1), at 1 °C, *P. synxantha* (117-2b) showed the best antagonistic activity by reducing the growth of the fungal pathogen strain Cad21 by 74.7%. *Bacillus amyloliquefaciens* (FZB24) and *B. subtilis* (QST713) reduced the colony diameter of the *C. luteo-olivacea* strain by 35.9% and 42.1%, respectively. Conversely, *B. pumilus* (QST2808) was the least active strain against fungal pathogen growth, showing a reduction of only 15.1%. Compared to the lower temperature, the BCAs tested have shown a higher antagonism potential at 25 °C. Plates inoculated with *P. synxantha* (117-2b), *B. amyloliquefaciens* (FZB24), and *B. subtilis* (QST713) showed an inhibition of 73.3%, 72.1%, and 70.2%, respectively. *Bacillus pumilus* (QST2808) was the least active strain, with a 53.5% reduction in fungal mycelial growth. The culture filtrates of the tested bacterial strains were assayed against the growth of the fungal pathogen strain Cad21 and indicated a variable inhibition activity (Fig. 2). The effectiveness of *B. amyloliquefaciens* (FZB24) culture filtrates was significantly higher at growing times of 48 h, 72 h, and 96 h, inhibiting fungal growth by 43.6% on average, compared to the control. *Pseudomonas synxantha* (117-2b) filtrates showed a higher inhibition rate at 72 h and 96 h (9.2% and 24.2%, respectively). Culture filtrates of *B. subtilis* (QST713) had their highest activity at 48 h, 72 h, and 96 h, inhibiting the growth of the fungal mycelium by 31.7%, 41.7%, and 20%, respectively, compared to the control. The tested culture filtrates seemed to be, for all strains, most effective immediately after 48 h of growth. Nevertheless, the activity of *B. pumilus* (QST2808) culture filtrates was confirmed to be ineffective in inhibiting the growth of *C. luteo-olivacea* strain Cad21.

3.2 Biochemical analysis of bacterial cell filtrates

The spectroscopic measurements were carried out to determine the main non-volatile metabolites produced by the different bacterial antagonists. Fig. 3 shows the infrared difference spectra between cell-filtrate and the nutrient broth (the spectrum of nutrient broth is reported for comparison in Figure S1, supplementary material). Difference spectra were considered more valuable for discriminating the metabolites produced by bacteria (positive peaks) and the medium itself (negative peaks). The

234 bands of the nutrient broth medium were attributed thanks to previous data generated from cultures

235 grown on peptone-containing medium (Trivedi et al., 2015).

236 The prominent positive peaks were attributed to peptidic compounds: 1699, 1664, and 1625 cm^{-1}

237 bands are located in the amide I spectral region, arising from the C=O and N-H vibration of peptide

238 (and protein) backbones (Besson et al., 1996) and sensitive to their secondary structure. A positive

239 peak in the amide II spectral region at 1546 cm^{-1} (N-H and C-N vibrations) further confirms the

240 peptidic nature of the compounds in the cell filtrate. More specifically, the 1625 cm^{-1} band was

241 attributed to asparagine residues as observed in iturin A, a lipopeptide produced by *Bacillus subtilis*

242 (Besson et al., 1996), while the same authors attributed the 1699 and 1664 cm^{-1} bands to a type II β-

243 turn structure adopted by the lipopeptide. This secondary structure is characterized by the presence

244 of selected amino acids such as asparagine and proline (band at 1625 cm^{-1}), glycine (band at 698

245 cm^{-1}), aspartic acid (bands at 1218, 1069, 910, and 871 cm^{-1}) and serine (band at 988 cm^{-1}) (Barth,

246 2007).

247 1441, 1355, 1314, and 832 cm^{-1} bands were attributed to C-H vibrations; therefore, they may refer to

248 the hydrophobic side chains of amino acids (i.e., alanine, leucine, etc.) or a lipidic component of an

249 antimicrobial compound as observed in *Bacillus paralicheniformis* (Ahire et al., 2020). The bands at

250 1218 and 618 cm^{-1} have been previously observed in the IR spectra of linear lipopeptides produced

251 by *Paenibacillus polymixa* (Deng et al., 2011). The 1092 and 698 cm^{-1} peaks were associated with

252 the C-O and C-S bond vibrations in bacitracin, an antibiotic polypeptide produced by *B. subtilis* and

253 *B. licheniformis* (Li et al., 2017). Finally, the 1920 cm^{-1} band could be attributed to the O-N-O

254 vibration in an antibiotic peptide produced by *B. brevis* (Muhammad et al., 2016).

255 The presence of antibiotic peptides was further supported by the appearance of other bands already

256 reported for *Bacillus megaterius*: the O-N-O band at 1920 cm^{-1} and the C-S band at 698 cm^{-1} are

257 typical of bacitracin, a polypeptide rich in cysteine, glutamic acid, histidine, phenylalanine, lysine,

258 isoleucine, leucine, ornithine, and aspartic acid (Al-Thubiani et al., 2018).

259

260   IR spectra reported in Fig. 3 show many similarities. However, the differences between the different

261   cell filtrates may reflect a different chemical composition: 1625 (attributed to Asn and Pro amino

262   acids), and the 618 cm^{-1} bands were more intense in the QST2808 filtrate, which had a different

263   profile in the 1080–920 cm^{-1} spectral region. The *P. synxantha* strain 117-2b filtrate was

264   characterized by the most intense peaks, mainly in the 1400–800 cm^{-1} region, with a typical peak at

265   988 cm^{-1} attributed to Ser (Barth, 2007).

266

267   **3.3 *In vivo* assays**

268   The efficacy of all tested bacterial strains as curative and preventive applications was demonstrated

269   by *in vivo* assays.

270   The wounded fruits treated with *P. synxantha* (117-2b) showed the highest inhibition rate against

271   fungal pathogen strain Cad21 on kiwifruits (63% and 84.3%) in the case of both curative and

272   preventive application, respectively, compared to the other strains and relative to the control (Fig. 4).

273   However, *B. amyloliquefaciens* (FZB24) and *B. subtilis* (QST713) showed low curative activity

274   against the pathogen with reductions of only 13% and 18.5%, respectively, relative to the control.

275   *Bacillus pumilus* (QST2808) had the lowest efficacy against skin pitting disease, with no reduction

276   as a curative treatment and only a 16.3% reduction as a preventive treatment. The synthetic product

277   (Scholar®) was confirmed in both experiments as the most effective treatment. However, the

278   effectiveness of *P. synxantha* (117-2b) was very close to the performance of the chemical treatment,

279   particularly in the preventive application.

280

281   **3.4 qPCR analysis**

282   qPCR analysis quantified the amount of fungal pathogen DNA in each portion of the kiwifruit tissue

283   analyzed. The standards were used to construct a standard curve, which presented an optimal

284   efficiency of 94.8%, with y = −3.453x + 16.578 and R^2 = 0.999 (Fig. 5a).

285   The real-time amplification reaction gave positive results, especially with all DNA samples obtained

286   by processing the portions of untreated fruits (negative controls) and with the majority of DNA

287   samples obtained from *P. synxantha* (117-2b) treated fruits, thus allowing their quantification. The

288   samples' melting temperatures (Figure 3.2.4) corresponded to 82.5 °C, which was the same as the

289   standards (Fig. 5b) and indicated that the amplification was specific.

290   The quantification of *C. luteo-olivacea* in infected samples, measured after 4 months after the

291   artificial inoculation of fruits, was obtained by extrapolating from the standard curve the pg of *C.*

292   *luteo-olivacea* DNA and normalizing these data with mg of kiwifruit tissue sampled at 1 cm from the

293   inoculation wound. The results of the quantification are summarized in Table 1. The amount of fungal

294   DNA in the negative control (fungus and water) was estimated at around 30.02 pg/mg. In kiwifruits

295   treated with *P. synxantha* strain 117-2b, selected as the best bacterial strain for controlling the

296   development of skin pitting symptoms, and Scholar®, the amounts of DNA were drastically reduced

297   to 0.13 pg/mg and 0.03 pg/mg, respectively (Table 1).

298

299   **3.5 *Pseudomonas synxantha* strain 117-2b population dynamics**

300   The *Pseudomonas synxantha* (117-2b) population dynamics on artificially wounded-inoculated

301   kiwifruit were determined during the 4-month cold storage period (Fig. 6). During the first three

302   months of storage, a slight but significant increase in the CFU of the strain was registered, reaching

303   almost 4.9×10^3 CFU wound^{-1}. After the third month of storage, the strain showed a slight but

304   significant decrease with respect to the previous months.

305

306   **4. Discussion**

307   *Cadophora luteo*-olivacea has presented a critical problem in recent years in Italian packaging houses,

308   and the connection between its incidence during the postharvest phase and field conditions at the time

309   of fruit production has made it difficult to manage (Di Francesco et al., 2021). The possible

310   appearance of skin pitting during storage is a challenge for kiwifruit marketing operators and often

311 prevents them from waiting for the best market combination of demand and more profitable prices.

312 This study aimed to find a sustainable solution to preserve kiwifruit from skin pitting losses by using

313 BCAs as an alternative o fungicides. The most intensively studied bacteria for use as BCAs belong

314 to the genera of *Pseudomonas, Bacillus,* and *Streptomyces* spp. (Bonaterra et al., 2022). Moreover,

315 many of these have already been registered and marketed as biopesticides.

316 Among the most interesting, Pseudomonas spp. have been studied as potential antagonists against a

317 wide range of fungal pathogens, mainly for their ability to produce active antifungal compounds

318 (Rojas-Solis et al., 2020, Sang and Kim, 2014, Aiello et al., 2019). For example, *Pseudomonas*

319 *synxantha* produces bioactive compounds such as a biosurfactant effective against several

320 microorganisms (Mukherjee et al., 2014). For this reason, the strain *P. synxantha* 117-2b belonging

321 to our microbiological collection has been studied and tested in the present work as an alternative

322 method to control kiwifruit skin pitting, together with other known bacterial strains.

323 Among the tested BCAs, *B. amyloliquefaciens* (FZB24), *B. pumilus* (QST2808), and *B. subtilis*

324 (QST713) were assayed for their effectiveness against a broad range of plant pathogens. In fact, these

325 strains are the active components of the bio-products Taegro®, Sonata®, and Serenade®,

326 respectively, whose effectiveness we also wanted to test against *C. luteo-olivacea* for a possible and

327 valuable field-ready treatment.

328 It is known that bacterial antagonists can produce bioactive compounds such as hydrolytic enzymes,

329 phytohormones, and volatile and non-volatile metabolites or induce systemic resistance in plants and

330 promote plant growth (Calderón et al., 2015, De Vleesschauwer et al., 2008, Lo Cantore et al., 2015,

331 Raza et al., 2016, Rojas-Solis et al., 2020). In this regard, non-volatile metabolites, selected as the

332 most active against *C. luteo-olivacea* by in vitro assay, were analyzed after different growing times.

333 As noted, all the tested strains, except for *B. pumilus* QST2808, produced active metabolites against

334 *C. luteo-olivacea*, starting from the stationary phase.

335 The biochemical analysis conducted using infrared spectroscopy on bacterial cell filtrate revealed the

336 presence of peptidic compounds, mainly from the appearance of amide I and amide II bands in 1699–

337   1625 cm^{-1} and 1546 cm^{-1} spectral regions (Fig. 3). These compounds could be related to the well-

338   known production of antibiotic peptides and lipopeptides by several bacteria, such as *B. subtilis,*

339   which produces iturin A, a heptapeptide closed in a ring with an amino fatty acid, whose antifungal

340   activity is related to its ability to induce a massive leakage of K$^+$ and other cell components from the

341   cytosol (Besson et al., 1996). The different profiles observed in the IR spectra could also explain the

342   behavior of the antagonists in the in vivo assays. The corresponding in vivo effects of the QST713

343   and FZB24 strains could be related to their similar chemical composition due to the analogous

344   positions and intensities of their peaks (Fig. 3). At the same time, the different spectral profile of the

345   QST2808 strain may reflect the production of antifungal compounds that are less effective against

346   the infection. However, the IR data should be interpreted carefully because it is well known that some

347   bacteria, such as various *Pseudomonas* spp., can produce several antagonistic antifungal compounds

348   in the form of peptides, including proteolytic enzymes (i.e., proteases, chitinases, cellulases,

349   pectinases or amylases) or even siderophores (non-proteogenic peptides) (Karmegham et al., 2020).

350   The similar chemical composition of these antagonistic compounds is reflected in their IR spectra, as

351   evidenced in previous papers on non-volatile metabolites in *Aureobasidium pullulans* in which all the

352   previous compounds were detected (Di Francesco et al., 2020, Di Francesco et al., 2021).

353   Nevertheless, none of the BCA cultures filtrates totally inhibited *C. luteo-olivacea* mycelial growth,

354   even though they were active against *B. cinerea* mycelium (data not reported). This fact showed a

355   broad spectrum of antagonism against different plant pathogens based on antimicrobial metabolites

356   (Roselló et al., 2013).

357   Despite this, the curative and preventive in vivo experiments on fruits showed that *P. synxantha* strain

358   117-2b had higher effectiveness against *C. luteo-olivacea* (strain Cad21) than the other tested BCAs

359   (Fig. 4). This finding was further confirmed by the IR analysis composition of culture filtrates (Fig.

360   3). *Pseudomonas synxantha*'s filtrates showed the most intense peaks (at 988 cm^{-1}), which can be

361   related to the highest concentration of antifungal compounds. Also, according to the co-culturing

362   assay, the strain had the best adaptation to low temperatures (1 °C), permitting long persistence during

14

363 the storage phase, although, as seen in population dynamics, with a significant cellular decrease.

364 Nevertheless, different studies suggested that high spore concentrations in inoculum were not always

365 connected to the best performance of a BCA (Verma et al., 2007). However, a higher initial cellular

366 concentration will be required for further applications such as in the field.

367 Indeed, this fact could be connected to the endophytic behavior of *P. synxantha* (Aiello et al., 2019),

368 which can move, colonize, and persist inside its host's vascular tissues. This characteristic should be

369 interesting to develop in the future for managing *C. luteo-olivacea,* whose epidemiology is not still

370 well understood but whose long latency is known and poorly manageable.

371 The management of kiwifruit diseases by employing BCAs has been successfully evaluated with

372 many pathogens, but to our knowledge, *C. luteo-olivacea* had not been explored previously.

373 According to the results obtained with qPCR analysis, *C. luteo-olivacea* showed great persistence in

374 kiwifruit despite being inoculated with an antagonist. In fact, the pathogen DNA was detected in

375 inoculated but asymptomatic kiwifruit, although in much smaller quantities than the control.

376 Conversely, in the positive control (fungicide application), almost no traces of the fungal pathogen

377 biomass were detected, confirming the different mechanisms of action between a BCA and a

378 fungicide.

379 Therefore, further studies such as genomic analysis are needed to deeply evaluate the mechanisms of

380 action of *P. synxantha* strain 117-2b. It will be useful to characterize it as a good BCA and for its

381 eventual consideration as an active ingredient in a bioformulation.

382

383 **CRediT authorship contribution statement**

384 A. Di Francesco: Conceptualization, Methodology, Investigation, Formal analysis, Writing – original

385 draft, Writing – review & editing. F. Jabeen: Investigation, Writing – original draft. M. Di Foggia:

386 Methodology, Investigation, Writing – original draft, Writing – review & editing. C. Zanon:

387 Investigation. R. Cignola: Methodology, Investigation. A. Sadallah: Methodology, Investigation. V.

388 Tugnoli: Methodology, Writing – review & editing. P. Ermacora: Conceptualization, Writing –

389   original draft, Writing – review & editing, Supervision, Funding acquisition. M. Martini:

390   Conceptualization, Methodology, Writing – original draft, Writing – review & editing, Supervision.

391

392   **Acknowledgements**

396

397   **Conflict of Interest Statement**

398   The authors declare that they have no known competing financial interests or personal relationships

399   that could have appeared to influence the work reported in this paper.

400

401   **References**

402   Ahire, J.J., Kashikar, M.S., Lakshimi, S.G., Madempudi, R., 2020. Identification and characterization

403   of antimicrobial peptide produced by indigenously isolated *Bacillus paralicheniformis* UBBLi30

404   strain. 3 Biotech 10, 112.

405

406   Aiello, D., Restuccia, C., Stefani, E., Vitale, A., Cirvilleri, G., 2019. Postharvest biocontrol ability of

407   *Pseudomonas synxantha* against *Monilinia fructicola* and *Monilinia fructigena* on stone fruit.

408   Postharvest Biol. Technol. 149, 83-89.

409

410   Al-Thubiani, A.S.A., Maher, Y.A., Fathi, A., Abourehab, M.A.S., Alarijah, M., Jhan, M.S.A., Al-

411   Ghamsi, S.B., 2018. Identification and characterization of a novel antimicrobial peptide compound

412   produced by *Bacillus megaterium* strain isolated from oral microflora. Saudi Pharm. J. 26, 1089-

413   1097.

414

415 Auger, J., Pérez, I., Osorio-Navarro, C., Esterio, M., 2018. First report of *Cadophora luteo-olivacea*

416 causing side rot on kiwifruit in Chile. Plant Dis. 102, 680. https://doi.org/10.1094/PDIS-09-17-1349-

417 PDN

418

419 Barth, A. Infrared spectroscopy of proteins. (2007). Biochimica et Biophysica Acta1767, 1073-1101.

420

421 Besson, F., Raimbault, C, Hourdou, M.L., Buchet, R., 1996. Solvent-induced conformational

422 modifications of iturin A: an infrared and circular dichroic study of a D,L-lipopeptide of *Bacillus*

423 *subtilis.* Spectrochimica Acta A 52, 793-803.

424

425 Bonaterra, A., Badosa, E., Daranas, N., Francés, J., Roselló, G., Montesinos, E., 2022. Bacteria as

426 biological   control   agents   of   plant   diseases.   Microorganisms   10,   1759.

427 https://doi.org/10.3390/microorganisms10091759

428

429 Calderón, C.E., Ramos, C., de Vicente, A., Cazorla, F.M., 2015. Comparative genomic analysis of

430 *Pseudomonas chlororaphis* PCL1606 reveals new insight into antifungal compounds involved in

431 biocontrol Mol. Plant Microbe Interact. 28, 249-260.

432

433 Carmona-Hernandez, S.; Reyes-Pérez, J.J.; Chiquito-Contreras, R.G.; Rincon-Enriquez, G.; Cerdan-

434 Cabrera, C.R.; Hernandez-Montiel, L.G., 2019. Biocontrol of postharvest fruit fungal diseases by

435 bacterial antagonists: A review. Agronomy 9, 121. https://doi.org/10.3390/agronomy9030121

436

437 Choi, H.R., Baek, M.W., Tilahun, S., Jeong, C.S., 2022. Long-term cold storage affects metabolites,

438 antioxidant activities, and ripening and stress-related genes of kiwifruit cultivars. Postharvest Biol.

439 Technol. 189, 111912.

440

441   Chowdhury, N.N., Islam, M.N., Jafrin, R., Rauf, A., Khalil, A.A., Emran, T.B., Simal-Gandara, J.,

442   2022. Natural plant products as effective alternatives to synthetic chemicals for post-harvest fruit

443   storage management. Crit. Rev. Food Sci. Nut. 1-19.

444

445   Costa, G., Kukuriannis, B., Monet, R., 1991. "Kiwifruit production in Europe." II International

446   Symposium on Kiwifruit, 297.

447

448   Deng, Y., Lu, Z., Bi, H., Lu, F., Zhang, C., Bie, X., 2011. Isolation and characterization of peptide

449   antibiotics LI-F04 and polymyxin B6 produced by *Paenibacillus polymyxa* strain JSa-9. Peptides 32,

450   1917-1923.

451

452   De Vleesschauwer, D., Djavaheri, M., Bakker, P. A. H. M., Hoefte, M., 2008. *Pseudomonas*

453   *fluorescens* WCS374r-induced systemic resistance in rice against *Magnaporthe oryzae* is based on

454   pseudobactin-mediated priming for a salicylic acid-repressible multifaceted defence response. Plant

455   Physiol. 148, 1996-2012.

456

457   Di Francesco, A., Martini, C., Mari, M., 2016. Biological control of postharvest diseases by microbial

458   antagonists: how many mechanisms of action? Eur. J. Plant Pathol. 145, 711–717.

459   https://doi.org/10.1007/s10658-016-0867-0

460

461   Di Francesco, A., Mari, M., Ugolini, L., Baraldi, E., 2018. Effect of *Aureobasidium pullulans* strains

462   against *Botrytis cinerea* on kiwifruit during storage and on fruit nutritional composition. Food

463   Microbiol. 72, 67–72.

464

465 Di Francesco, A., Di Foggia, M., Zajc, J., Gunde-Cimerman, N., Baraldi, E., 2020. Study of the

466 efficacy of *Aureobasidium* strains belonging to three different species: A. *pullulans, A. subglaciale*

467 and *A. melanogenum* against *Botrytis cinerea* of tomato. Ann. Appl. Biol. 177, 266-275.

468

469 Di Francesco, A., Di Foggia, M., Corbetta, M., Baldo, D., Ratti, C., Baraldi, E., 2021. Biocontrol

470 activity and plant growth promotion exerted by *Aureobasidium pullulans* strains. J. Plant Growth

471 Regul 40, 1233-1244.

472

473 Di Francesco, A., Di Foggia, M., Baldo, D., Ratti, C., Baraldi, E., 2022. Preliminary results on

474 *Cadophora luteo-olivacea* pathogenicity aspects on kiwifruit. Eur. J. Plant Pathol. 163, 997–100.

475 https://doi.org/10.1007/s10658-022-02518-6

476

477 Doyle, J.J., Doyle, J.L., 1990. Isolation of plant DNA from fresh tissue. Focus 12, 13-15.

478

479 Gilardi, G., Galliano, A., Vittone, F., Bevilacqua, A., Spadaro, D., Garibaldi, A., 2007. Difesa in post-

480 raccolta dell'actinidiadagli attacchi di *Phialophora sp.* e di *Botrytis cinerea* agenti di marciume dei

481 frutti. In: Atti degli Incontri Fitoiatrici 2007, 28 Febbraio–1 Marzo 2007, Torino, Italy, 78 (abstract).

482

483 Gotor-Vila, A., Teixidó, N., Di Francesco, A., Usall, J., Ugolini, L., Torres, R., Mari, M., 2017.

484 Antifungal effect of volatile organic compounds produced by *Bacillus amyloliquefaciens* CPA-8

485 against fruit pathogen decays of cherry. Food Microbiol. 64, 219–225.

486

487 Huang, H.; Wang, Y.; Zhang, Z.; Jiang, Z.; Wang, S. 2004. Actinidia germplasm resources and

488 kiwifruit industry in China. Hort. Sci. 39, 1165-1172.

489

490 Karmegham, N., Vellasamy, S., Natesan, B., Sharma, M. P., Al Farraj, D. A., Elshikh, M.S., 2020.

491 Characterization of antifungal metabolite phenazine from rice rhizosphere fluorescent pseudomonads

492 (FPs) and their effect on sheath blight of rice. Saudi J. Biol. Sci. 27, 3313-3326.

493

494 Lecellier, G., and Silar, P. 1994. Rapid method for nucleic acid extraction from Petri dishgrown

495 mycelia. Curr. Genet. 25, 122-123.

496

497 Li, Y., Wang, Z., Li, X., Yin, T., Bian, K., Gao, D., 2017. Facile synthesis of bacitracin-templated

498 palladium nanoparticles with superior electrocatalytic activity. J. Power Sources 341, 183-191.

499

500 Liu, Q., Li, Y., Liao, G., Xu, X., Jia, D., Zhong, M., Ye, B., 2022. Transcriptome and metabolome

501 reveal AsA regulatory network between metabolites and genes after fruit shading by bagging in

502 kiwifruit (*Actinidia eriantha*). Sci. Hort. 302, 111184.

503

504 Lo Cantore, P., Giorgio, A., Iacobellis, N., 2015. Bioactivity of volatile organic compounds produced

505 by *Pseudomonas tolaasii*. Front. Microbiol. https://doi.org/10.3389/fmicb.2015.01082

506

507 Manning, M.A., Mundy, D.C., 2009. Fungi associated with grapevine trunk disease in established

508 vineyards in New Zealand. Phytopathol. Mediterr. 48, 160–161.

509

510 Martini, M., Musetti, R., Grisan, S., Polizzotto, R., Borselli, S., Pavan, F., Osler, R., 2009. DNA-

511 dependent detection of the grapevine fungal endophytes *Aureobasidium pullulans* and *Epicoccum*

512 *nigrum.* Plant Dis. 93, 993-998.

513

514 Michaelidis, T.J., Elmer, P., 2000. *Botrytis* gray mold of kiwifruit caused by *Botrytis cinerea* in the

515 United States and New Zealand. Plant Dis. 84, 208–223.

516

517 Mitchell, F.G., 1994. Composition, maturity and quality. kiwifruit growing and handling. Eds: JK

518 Hasey et al. Publication 3344, 94-98.

519

520 Muhammad, S.A., Ali, A., Naz, A., Hassan, A., Riaz, N.,Saeed-ul-Hassan, S., Andleeb, S., Barh, D.,

521 2016. A new broad-spectrum peptide antibiotic produced by *Bacillus brevis* strain MH9 isolated from

522 Margalla Hills of Islamabad., Pakistan. Int. J. Pept. Res. Ther. 22, 271-279.

523

524 Mukherjee, K., Mandal, S., Mukhopadhyay, B., Mandal, N.C., Sil, A.K., 2014. Bioactive compound

525 from *Pseudomonas synxantha* inhibits the growth of Mycobacteria. Microbiol Res. 169, 794-802.

526 doi: 10.1016/j.micres.2013.12.005.

527

528 Ni, H., Kong, W.L., Zhang, Y., Wu, X.Q., 2022. Effects of volatile organic compounds produced by

529 *Pseudomonas aurantiaca* ST-TJ4 against *Verticillium dahliae*. J. Fungi 2022; 8, 697.

530 https://doi.org/10.3390/jof8070697

531

532 Nihorimbere, V., Ongena, M., Smargiassi, M., Thonart, P., 2011. Beneficial effect of the rhizosphere

533 microbial community for plant growth and health. Biotechnol. Agron. Soc. Environ. 15, 327-337.

534

535 Palm, G., Kruse, P., 2012. Wie ist in der Zukunft Lagerfäulnis zu verhindern? Mitteilungen des

536 Obstbauversuchsringes des Alten Landes, 67, 306–311.

537

538 Raza, W., Yousaf, S., and Rajer, F.U., 2016. Plant growth promoting activity of volatile organic

539 compounds produced by biocontrol strains. Sci. Lett. 4, 40–43.

540

541   Rojas-Solis, D., Vences-Guzmán, M.A., Sohlenkamp, C., 2020. *Bacillus toyonensis* COPE52

542   modifies lipid and fatty acid composition, exhibits antifungal activity, and stimulates growth of

543   tomato   plants   under   saline   conditions.   Curr   Microbiol   77,   2735–2744.

544   https://doi.org/10.1007/s00284-020-02069-1

545

546   Roselló, G., Bonaterra, A., Francés, J., Montesinos, L., Badosa, E., Montesinos, E., 2013. Biological

547   control of fire blight of apple and pear with antagonistic *Lactobacillus plantarum*. Eur. J. Plant.

548   Pathol. 137, 621–633.

549

550   Sang, M.K, Kim, K.D., 2014. Biocontrol activity and root colonization by *Pseudomonas corrugata*

551   strains CCR04 and CCR80 against *Phytophthora* blight of pepper. Biocontrol 59, 437-448.

552   10.1007/s10526-014-9584-9

553

554   Spadaro, D., Galliano, A., Pellegrino, C., Gilardi, G., Garibaldi, A., Gullino, M.L., 2010. Dry matter

555   and mineral composition, together with commercial storage practices, influence the development of

556   skin pitting caused by *Cadophora luteo-olivacea* on kiwifruit 'Hayward'. J. Plant Pathol. 92, 339–

557   346.

558

559   Taş, A., Berk, S. K., Kibar, H., Gündoğdu, M., 2022. An in-depth study on post-harvest storage

560   conditions depending on putrescine treatments of kiwifruit. J. Food Compos. Anal, 111, 104605.

561

562   Trivedi, M. K., Branton, A., Trivedi, D., Nayak, G., Mishra, R. K., Jana, S., 2015. Physicochemical

563   evaluation of biofield treated peptone and malmgren modified terrestrial orchid medium. Americ J.

564   Biosci. Bioeng. 3, 169-177.

565

566 Wang, H., Li, X., Wang, J., Vidyarthi, S.K., Wang, H., Zhang, X. G., Xiao, H.W., 2022a. Effects of

567 post-harvest ripening on water status and distribution, drying characteristics, volatile profiles,

568 phytochemical contents, antioxidant capacity and microstructure of kiwifruit (*Actinidia*

569 *deliciosa*). Food Control 139, 109062.

570

571 Wang, S.Y., Herrera-Balandrano, D.D., Wang, Y.X., Shi, X.C., Chen, X., Jin, Y., Laborda, P., 2022b.

572 Biocontrol Ability of the *Bacillus amyloliquefaciens* Group, B. J Agric Food Chem. 70, 6591-6616.

573 doi: 10.1021/acs.jafc.2c01745.

574

575 Zhang, X., Bao, W., Zhang, A., Pathirana, R., Wang, Q.C., Liu, Z.E., 2020. Cryopreservation of shoot

576 tips, evaluations of vegetative growth, and assessments of genetic and epigenetic changes in cryo-

577 derived   plants   of   Actinidia   spp.   Cryobiology,   94,   18-25.

578 https://doi.org/10.1016/j.cryobiol.2020.05.004

579

580 **Figures**

581 Figure 1. *In vitro* co-colture assay. Efficacy of bacterial antagonists at two different temperatures (1

582 °C and 25 °C) on the colony growth of *Cadophora luteo-olivacea* (Cad 21). The data were the average

583 of two experiments. Data reporting the same letters are not statistically significant according to
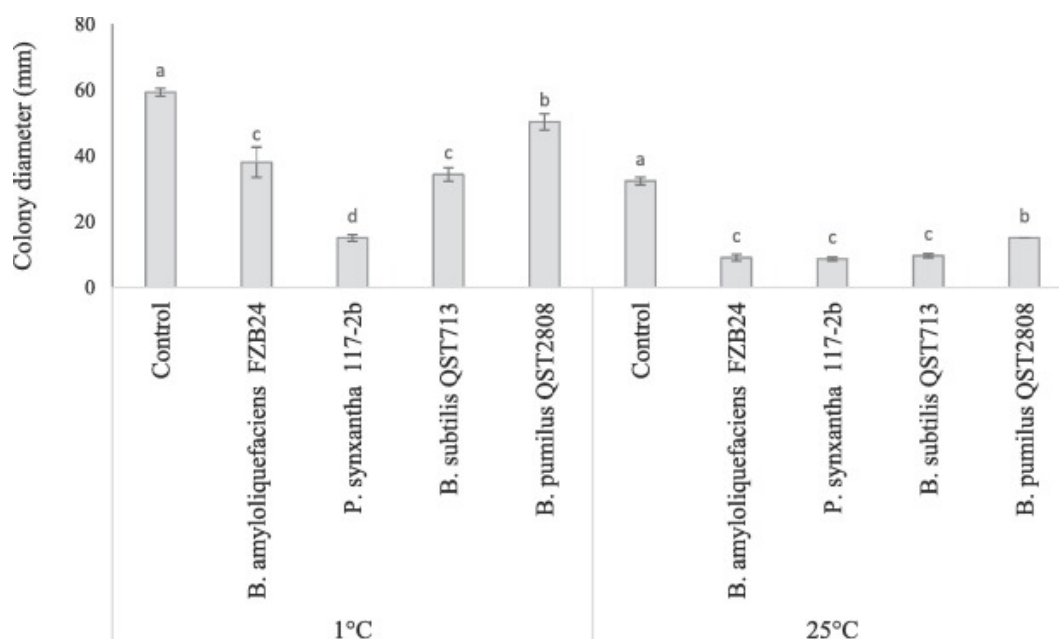
584 Tukey's test (a = 0.05).

585

Figure 2. Effect of bacterial strain cell-filtrates infused onto PDA plates on the colony growth of *Cadophora luteo-olivacea* (Cad21). Biocontrol agents were grown in nutrient broth at 20 °C for one week and filtrates were sampled at 1 h and then every 24 h for up to 96 h. Data are the means of *Cadophora luteo-olivacea* (Cad21) colony growth observed after 14 days at 20 °C. For each antagonist different letters indicate significant differences according to Tukey's test ($\alpha = 0.05$).
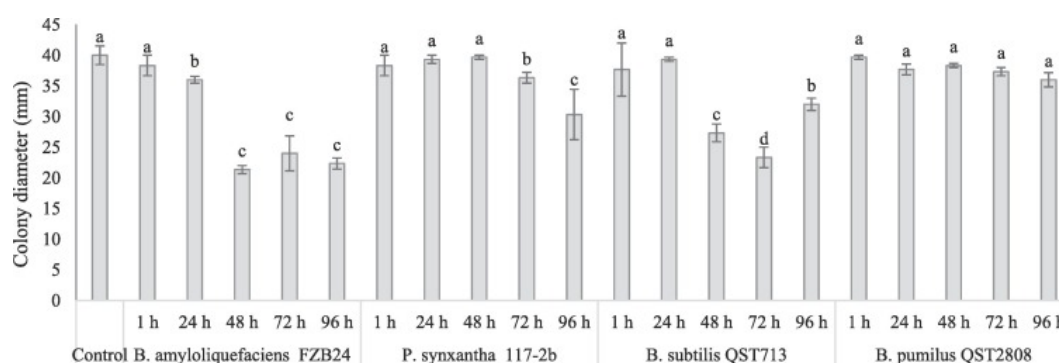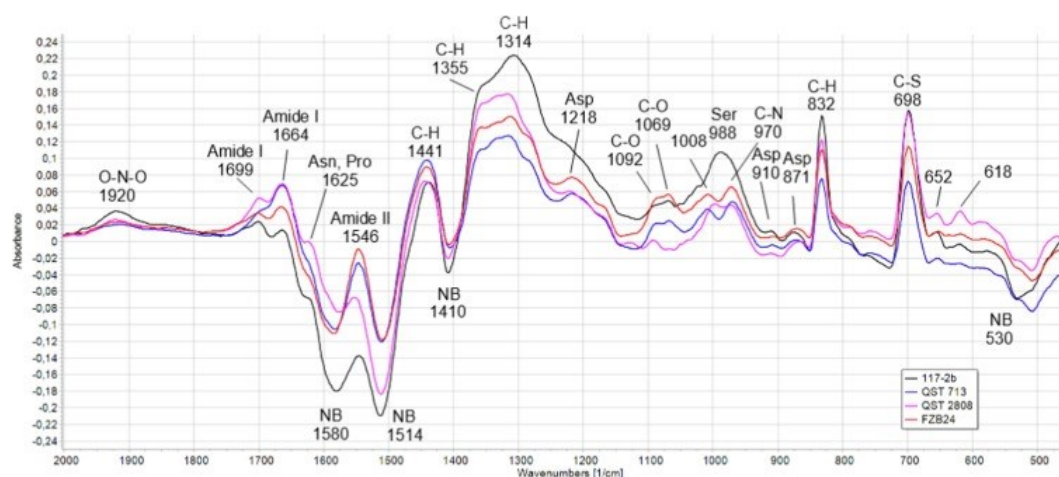

Figure 3. Difference FT-IR spectra of bacterial cell filtrates with the main positive bands related to the production of non-volatile compounds discussed in the text. Negative peaks are related to the consumption of the nutrient broth (NB).

24

598

599

600 Figure 4. Curative and preventive effect of bacterial strains (108 cells mL^{-1}) and Scholar® (1.3 mL

601 L^{-1}) on disease incidence on artificially inoculated kiwifruit. Fruits were kept at 1 °C for four months.

602 For the curative effect fruit wounds were first treated with 20 μL of pathogen suspension and after air

603 drying they were inoculated with 20 μL of each antagonist. For the preventive effect antagonists were

604 inoculated first. Data are the means of fifteen fruits for each antagonist and treatment effect. For each

605 treatment effect different letters indicate significant differences according to Tukey's test (α = 0.05).
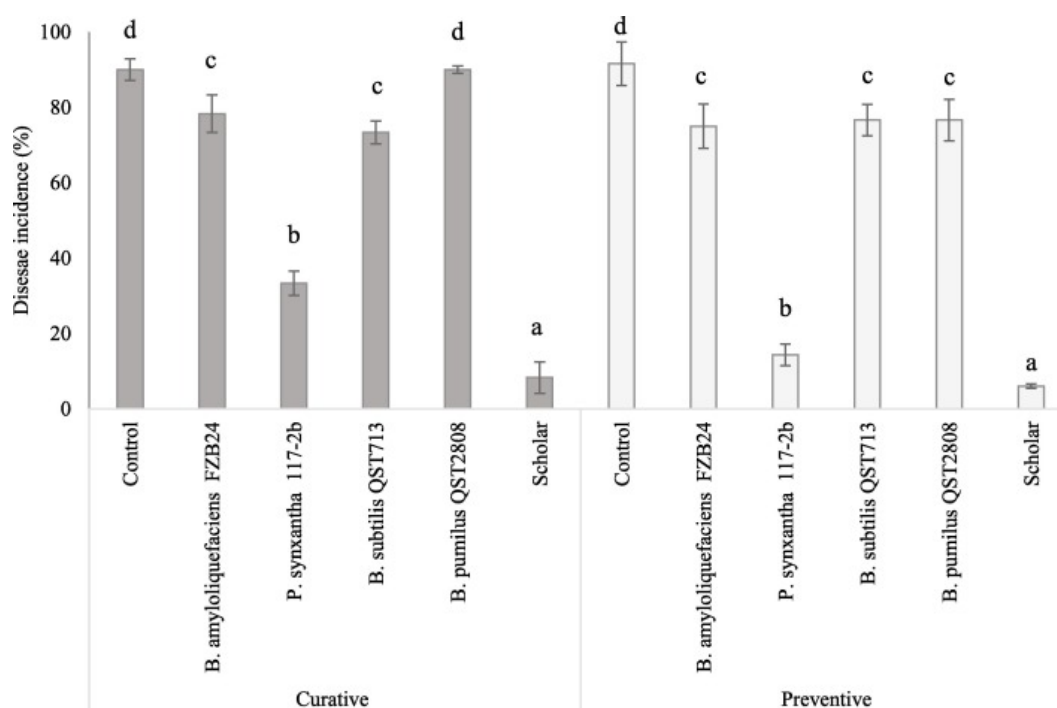

606

607

608 Figure 5. (a) Standard curve established for the quantification of *Cadophora luteo-olivacea* by

609 plotting the log of the starting quantity (ng) of 1:10 serial dilutions of fungal genomic DNA versus

610 the cycle number (Cq); (b) melting peaks of amplicons generated from 1:10 serial dilutions and

611 genomic DNA samples extracted from artificially wounded-inoculated kiwifruit.

a)

b)

612
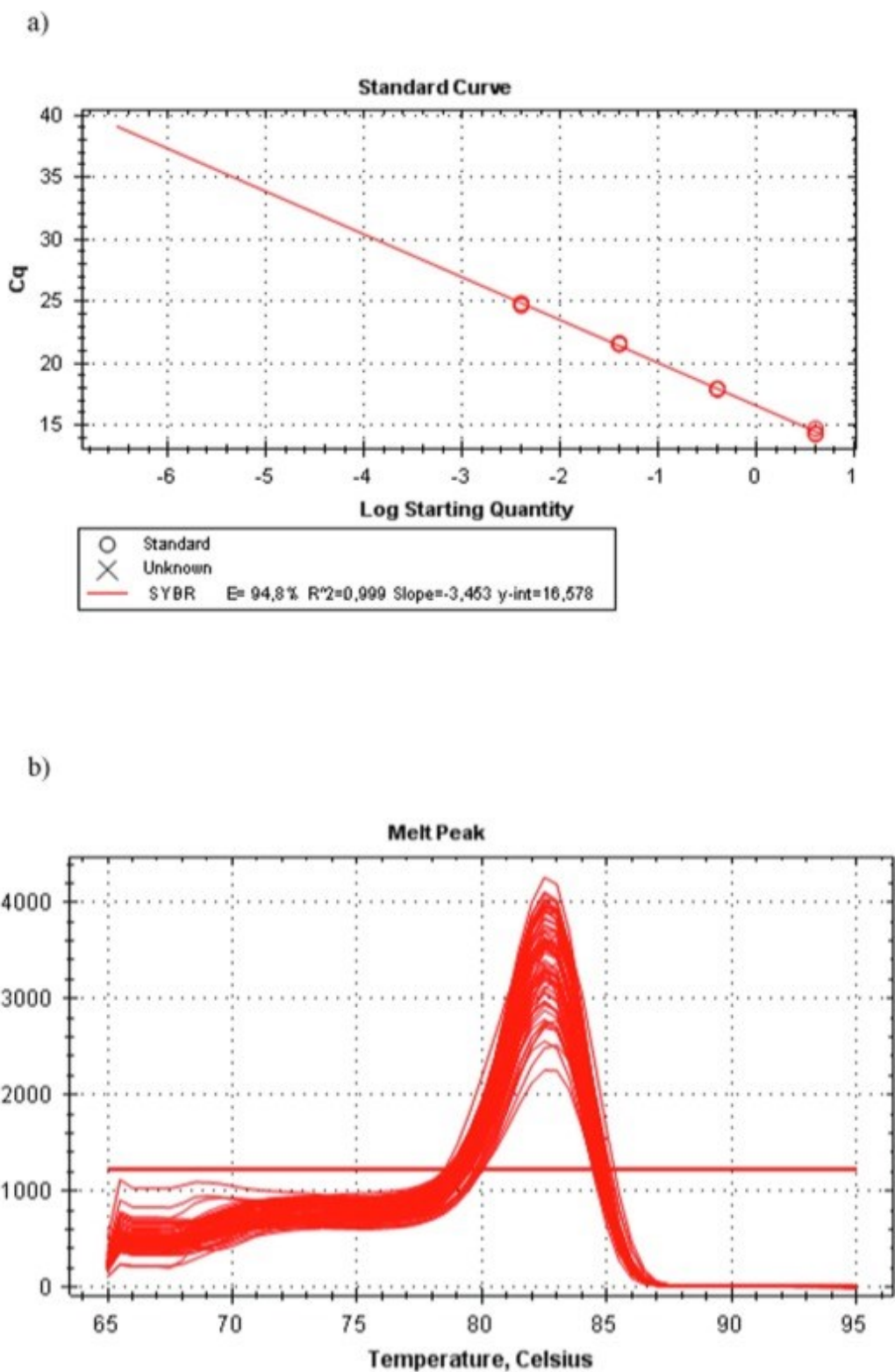
613 Figure 6. Population dynamics of *Pseudomonas synxantha* (118-2b) in previously wounded-
614 inoculated kiwifruit. Fruits were stored at 0 °C in normal refrigeration for 4 months. Each point
615 represents the mean of the number of colony forming units (CFUs) from four replicates (kiwifruit)
616 for each sampling time. For each sampling time different letters indicate significant differences
617 according to Tukey's test ($\alpha = 0.05$).

618

619
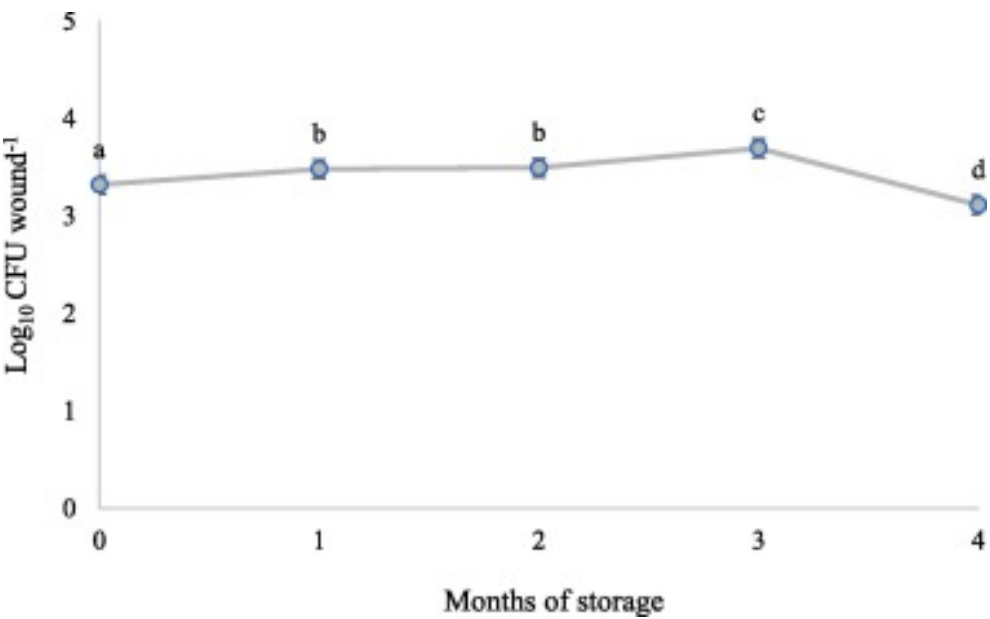
620 **Tables**

621 Table 1. EC_{50} values ($\mu L\ mL^{-1}$) of synthetic volatile organic compounds (VOCs) emitted by two
622 strains of Aureobasidium pullulans (L1 and L8) evaluated on mycelium growth (Ø) and conidia
623 germination (CFU) of the four Monilinia species. The evaluations were carried out after 2 days and
624 5 days of incubation at 25 °C respectively for CFU and mycelium growth.

625

Treatments	Cad21 DNA pg/mg
Control	30.02 ± 9.05 a
P. synxantha 117-2b	0.13 ± 0.02 b
Scholar®	0.03 ± 0.00 c