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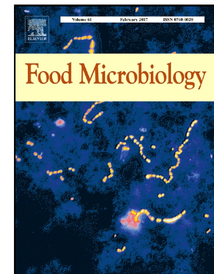

Potential of a new strain of Bacillus amyloliquefaciens BUZ-14 as a biocontrol agent of postharvest fruit diseases

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Highlights

- *Bacillus amyloliquefaciens* BUZ-14 inhibited major postharvest rots in fruits
- Preventive treatments were effective against *Penicillium* spp. in oranges and apples
- *B. amyloliquefaciens* exhibited a curative effect against brown rot in stone fruits
- BUZ-14 survived at cool temperatures making it suitable for postharvest treatment

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2 agent of postharvest fruit diseases**

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14 **ABSTRACT**

15 The biocontrol potential of the *Bacillus amyloliquefaciens* strain BUZ-14 was tested
16 against the main postharvest diseases of orange, apple, grape and stone fruit. After
17 characterizing the temperature and pH growth curves of strain BUZ-14, its *in vitro*
18 antifungal activity was determined against *Botrytis cinerea*, *Monilinia fructicola*, *M.*
19 *laxa, Penicillium digitatum, P. expansum* and *P. italicum*. Subsequently, *in vivo* activity
20 was tested against these pathogens by treating fruit with cells, endospores and cell-free
21 supernatants. The *in vitro* results showed that BUZ-14 inhibited the growth of all the
22 pathogens tested corresponding to the least susceptible species, *P. italicum*, and the
23 most susceptible, *M. laxa*. *In vivo* tests corroborated these results as most of the
24 treatments decreased the incidence of brown rot in stone fruit from 100 % to 0 %,
25 establishing 10^7 CFU mL^{-1} as the minimum inhibitory concentration. For the
26 *Penicillium* species a preventive treatment inhibited *P. digitatum* and *P. italicum* growth
27 in oranges and reduced *P. expansum* incidence in apples from 100 % to 20 %. Finally, it
28 has been demonstrated that BUZ-14 was able to survive and to control brown rot in
29 peaches stored at cool temperatures, making it a very suitable biocontrol agent for
30 application during the post-harvest storage and marketing of horticultural products.

31

32 *Keywords*: biocontrol, *Botrytis cinerea*, *Monilinia* spp., *Penicillium* spp.

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34 **1. Introduction**

35 It has been estimated that about 20-25 % of harvested fruits and vegetables
36 decay by filamentous fungi during postharvest handling even in developed countries, so
37 control of fungal infections at this stage is critical (Droby, 2006; Singh and Sharma,
38 2007). Currently control measures of phytopathogens during pre- and post-harvest
39 practices are primarily based on synthetic chemicals (El-Ghaouth et al., 2004; Ismail
40 and Zhang, 2004; Droby, 2006; Korsten, 2006; Zhu, 2006; Singh and Sharma, 2007).
41 However, the use of such chemicals is becoming increasingly problematic due to stricter
42 legislation (Dir. 91/414/CEE) and growing public pressure resulting from their
43 toxicological risks to human health (Dir. 2009/128/CE; U.S. National Research Council,
44 1987), especially children´s health (U.S. National Research Council, 1993) and possible
45 environmental pollution. A further limiting factor for chemical application is the
46 development of fungicide-resistant strains for the main phytopathogenic fungi (Kinay et
47 al., 2007; Zhao et al., 2010; Chen et al., 2013; Panebianco et al., 2015; Vitale et al.,
48 2016). Consequently, researchers are currently looking for alternative methods to
49 control postharvest diseases (Romero et al., 2007; Dimkic et al., 2013; Oro et al., 2014;
50 Jiang et al., 2015; Parafati et al., 2015).

51 Biological control, which includes the use of antagonist microorganisms (BCAs-
52 biological control agents) such as yeast and bacteria, has been a promising alternative to
53 synthetic fungicides since it is safer for both human health and the ecosystem (Wilson
54 and Wisniewski, 1994; Janisiewicz and Korsten, 2002; Korsten, 2006). During the last
55 30 years, over one thousand articles on postharvest biocontrol have been published and
56 several microorganisms have been tested against various postharvest fungal pathogens
57 on fruit. However, only few biologicals are commercially available for control of fruit
58 diseases during the postharvest phase. These include, Bio-Save® 10 LP (*Pseudomonas*

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59 *syringae*; Jet Harvest Solutions, USA) (Janisiewicz and Jeffers, 1997), registered in the
60 USA by the EPA (Environmental Protection Agency) and used mostly for the control of
61 sweet potato and potato diseases, BoniProtect® (*Aureobasidium pullulans*; Bio-Protect
62 GmbH, Germany) for the control of apple storage diseases and Candifruit (*Candida*
63 *sake* CPA-1, Sipcam Inagra S.A.) (Viñas et al., 1998), commercialized in Spain and
64 recommended against the major postharvest diseases of pome and citrus fruit.

65 *Bacillus* species are widely distributed in the rhizosphere. They have high
66 thermal tolerance, grow rapidly in liquid cultures, readily form spores and are not
67 phytopathogenic. Their spores are resistant to physical and chemical treatments such as
68 desiccation, heat, UV irradiation or organic solvents (Leelasuphakul et al., 2008), and
69 some species are able to produce biofilms. Several strains of the *Bacillus* genus have
70 been studied for their production of anti-fungal metabolites, which are potential BCAs
71 against a wide range of fungal pathogens (Touré et al., 2004; Ongena et al., 2005;
72 Chung et al., 2008; Arguelles-Arias et al., 2009; Torres et al., 2016). *B. subtilis* is the
73 species most extensively used against plant diseases and 4-5 % of its genome is
74 dedicated to antibiotics´ production (Stein 2005). Some strains have been employed to
75 develop commercial products such as Subtilex® (*B. subtilis* MBI 600; BASF), registered
76 in the USA by the EPA and used for the control of fruit, herb and vegetable diseases,
77 and Serenade® Max (*B. subtilis* QST 713, AgraQuest Inc., California, USA and BASF,
78 Ludwigshafen, Germany), currently registered for the use against *B. cinerea*,
79 *Pseudomonas* spp., *Sclerotinia* spp. *Venturia* spp. and *Monilinia* spp. in more than 20
80 countries in Europe, Africa, Middle East, Asia and Latin America. *B.*
81 *amyloliquefaciens*, closely related to *B. subtilis*, dedicates 8.5 % of its genome to
82 produce several bioactive compounds with high antifungal activity such as lipopeptides
83 including surfactin (Ahimou et al., 2000; Hsieh et al., 2004) iturin (Hsieh et al., 2008;

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84 Yu et al., 2002) or fengycin (Lin et al., 1999; Ongena et al., 2007) and several
85 polyketides such as macrolactin, bacillaene, bacilysin, bacillomycin and difficidin
86 (Chen et al., 2009). Furthermore, other antibiotics such as plantazolicin or chlorotetaine,
87 all of which have substantial antimicrobial and antifungal activity, are also secreted
88 (Scholz et al., 2011; Mudgal et al., 2013; Wang et al., 2016). Recently, Taegro® *(B.*
89 *amyloliquefaciens* FZB24); Novozymes (Copenhagen, Denmark) and Syngenta (Basel,
90 Switzerland) (Lecomte et al., 2016), recommended for suppressing selected soil-borne
91 and foliar diseases of fruits and leafy vegetables, cucurbits, and ornamentals, have been
92 registered in the USA by the EPA.

93 The main objective of this study is to evaluate the potential of the *B.*
94 *amyloliquefaciens* strain BUZ-14 for controlling postharvest diseases in orange, apple,
95 grape and stone fruit. Our study has four phases: (1) to characterize the strain growth at
96 different temperature and pH values; (2) to determine its *in vitro* antifungal activity
97 against several postharvest pathogens; (3) to establish the efficacy of cells, endospores
98 and cell-free supernatants against the main postharvest pathogens in fruit models; and
99 (4) to assess its survival rate and antifungal activity under cold storage temperatures.

100 **2. Materials and methods**

101 *2.1. Pathogens*

102 *Penicillium expansum* CECT 20140 and *Monilinia fructicola* ATCC 44557 were
103 obtained from the respective culture collections. *Botrytis cinerea* VG 1, *Penicillium*
104 *digitatum* VG 20*, Penicillium italicum* VG 101*,* and *Monilinia laxa* VG 105 were
105 obtained from the Plant Food Research Group culture collection at Zaragoza University
106 (Spain). All pathogens were incubated on potato dextrose agar (PDA) (Oxoid Ltd;
107 Basingstoke, Hampshire, England) Petri dishes supplemented with 1 % acetone in the

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108 case of *M. fructicola* and *M. laxa* to induce conidia production (Pascual et al., 1990).
109 Periodically, the strains were transferred to fresh fruit to induce infection and spore
110 production.

111 *2.2.* Bacillus *strain and inoculum production*

112 The strain BUZ-14 used in this study was obtained from the Plant Food
113 Research Group Collection at Zaragoza University. It was isolated from the surface of
114 peach fruit from an orchard in Zaragoza and tested for its preliminary antifungal
115 potential against *M. fructicola* (data not shown). A partial 16s rRNA sequence from the
116 BUZ-14 strain was conducted by the Spanish Type Culture Collection (CECT). It was
117 identified as a member of the *B. amyloliquefaciens* species complex. Cultures were
118 stored at 5 ºC and subcultured on tryptose soy agar (TSA, Oxoid Ltd) supplemented
119 with 0.6% yeast extract at 30 ºC for 24 h when required. Criobilles (Deltalab,
120 Barcelona, Spain) were used for long-term storage at -80 ºC. To prepare the initial fresh
121 cell suspension of BUZ-14, the 24 h-old culture on TSA was transferred to 7 mL of
122 tryptose soy broth (TSB, Oxoid Ltd). This initial suspension was incubated for 24 h at
123 30 ºC and adjusted to 40 ± 5 % transmittance at 700 nm with a spectrophotometer,
124 corresponding to 2 x 10^8 colony forming units (CFU) per milliliter. 100 µL of this
125 suspension was transferred to 250 mL conical flasks containing 50 mL of 863 medium
126 (10 g L^{-1} peptone, 10 g L^{-1} yeast extract and 20 g L^{-1} glucose; pH 7) (Yánez-Mendizábal
127 et al., 2010) to obtain the initial cultures. Cultures were always incubated on a rotary
128 shaker at 150 rpm.

129 *2.3. BUZ-14 growth temperature, pH curves and endospore production*

130 Three Erlenmeyer flasks containing 50 mL of 863 medium inoculated with 100
131 µL of the initial suspension were disposed per time, temperature and pH on a rotary

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132 shaker at 150 rpm.

133 BUZ-14 strain growth (log_{10} CFU mL^{-1}) at 4, 10, 20, 30 and 37 ºC at 150 rpm
134 was determined by subsampling bacterial cultures every 2 h from 0 to 24 h, and at 36,
135 48, 72, 96 and 120 h. Besides, its growth at different pH values at 30 ºC was studied
136 (3.0, 4.0, 4.5, 5.0 and 7.0) using citric acid (Merck) to adjust the medium. In this case
137 BUZ-14 growth was determined by subsampling bacterial cultures at 0, 10, 24, 48 and
138 120 h. Endospore production at 30 ºC was determined from 24, 48, 72, 96 and 120 h old
139 BUZ-14 strain cultures. Bacterial cells (vegetative cells + endospores) were separated
140 from the supernatant (antifungal metabolites) by centrifugation for 10 min at 9000 x *g* at
141 10 ºC (Beckman Coulter™). The pellet obtained was resuspended in buffered peptone
142 water and vegetative cells were killed by heat treatment at 80 ºC for 12 min. The entire
143 experiment was repeated three times. Bacteria and endospores counting (CFU mL^{-1})
144 were done by dilution and plated on TSA.

145 *2.4. In vitro antifungal activity*

146 Cultures (vegetative cells + endospores + supernatant), endospores and cell-free
147 supernatant from the *B. amyloliquefaciens* strain BUZ-14 were tested against *B.*
148 *cinerea, M. fructicola, M. laxa, P. digitatum, P. expansum,* and *P. italicum*. An agar
149 plug (5 mm diameter) from actively-growing margins of fungal colonies (7 day-old
150 culture on PDA) was placed at the center of PDA dishes for each *M. fructicola* and *M.*
151 *laxa*. For *B. cinerea*, *P. digitatum*, *P. expansum* and *P. italicum*, the inoculum was
152 obtained from a 7-day old culture in potato dextrose broth (PDB) (Oxoid Ltd). The
153 concentrations of CFU units were determined using a haemocytometer and a Leica
154 microscope (Leica Microsystems, Germany) and the suspensions were adjusted to 10^5
155 CFU mL^{-1} and inoculated at the center of PDA dishes using a sterile needle. Then,

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156 BUZ-14 strain obtained from a 24 h-old culture on 863 broth (10^9 CFU mL^{-1}) was
157 inoculated using a sterile needle in three equidistant parts from the center of the plate (3
158 cm) where the fungal pathogens was previously placed. The fungal pathogens with slow
159 growth (*M. fructicola* and *M. laxa*) were inoculated 24 h prior to the bacteria. To test the
160 efficacy of the endospores 30 µL aliquots of endospore suspension from 72, 96 and 120
161 h-old cultures in 863 medium, adjusted to 10^7 endospores mL^{-1} by decimal dilution,
162 were dispensed in 3 wells (6 mm diameter) made in the gel medium with a sterile
163 scalpel before placing the fungal inoculum. Supernatant samples obtained by
164 centrifugation at 9000 x *g* from 72, 96 and 120 h-old BUZ-14 cultures in 863 medium at
165 30 ºC were mixed with PDA in proportions of 1:1, 1:4 and 1:10. Each mixture was then
166 sterilized at 121 ºC for 15 min and poured into 90 mm diameter Petri plates. After
167 solidification, single agar plugs of *M. fructicola* and *M. laxa* 7 day-old cultures were
168 placed on each plate, whereas *B. cinerea*, *P. digitatum, P. expansum* and *P. italicum*
169 inocula from PDB liquid culture (10^5 conidia mL^{-1}) were punctured at three equidistant
170 places from each other and at 3 cm from the center of the plate. The control samples
171 consisted of PDA Petri dishes with only the fungal inoculum. The PDA Petri dishes
172 were incubated for 7 days at 25 ºC under aerobic conditions. Fungal growth inhibition
173 was evaluated by measuring the diameter of the fungal colony and expressed as the
174 percentage of fungal growth inhibition compared to that of the control plates. Mean
175 values and standard errors of the mean were calculated from five PDA dishes for each
176 pathogen and treatment.

177 *2.5. Antifungal activity of* B. amyloliquefaciens *BUZ-14 strain on fruit*

178 *2.5.1 Fruit*

179 The fruit used in this study were obtained from local packinghouses and were

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180 grown in different areas of Spain (La Almunia de Doña Godina, Tarragona, and Teruel).
181 Fruit free of visible wounds and rot and homogeneous in size and maturity were stored
182 at 1 ºC and used for experiments within 3 days of collection. Prior to the experiments,
183 all fruit were surface-disinfected by immersion for 2 min in 1 % sodium hypochlorite,
184 rinsed with tap water, and allowed to air-dry at room temperature (20 ºC).

185 *2.5.2 Phytopathogenic fungal and bacteria inocula preparation*

186 The pathogenic fungal inocula consisted of aqueous conidial suspensions
187 prepared from 7 day-old cultures of *B. cinerea*, *P. digitatum, P. expansum* and *P.*
188 *italicum* grown on PDA at 25 ºC and 80 % RH. *M. fructicola* and *M. laxa* isolates were
189 inoculated onto peaches or nectarines in order to obtain a high conidia production
190 (Casals et al., 2010). Fruit were wounded with a sterilized steel rod (1 x 2 mm) and
191 conidia and mycelium were transferred to the wound site with a sterile pipette tip. Fruit
192 were then incubated at 25 °C and 80 % RH for 7 days. Conidia were loop-washed from
193 the PDA plates or from the surface of infected fruits, filtrated through four layers of
194 sterile cheesecloth and transferred to a test tube with 9 mL of sterile distilled water with
195 0.01 % Tween 80. The suspensions were adjusted at 10^5 conidia (CFU) mL $^{-1}$ for *B.*
196 *cinerea*, *P. digitatum, P. expansum* and *P. italicum* and at 10^4 CFU mL $^{-1}$ for *Monilinia*
197 spp.

198 Cultures (vegetative cells + endospores + supernatant), cells (vegetative cells +
199 endospores), at different concentrations (10^8,10^7, 10^6 CFU mL^{-1}), endospores (10^7 CFU
200 mL^{-1}) and cell-free supernatant undiluted and 1:10 diluted were prepared from cultures
201 at different incubation times (24, 28, 72, 96 and 120 h). To obtain the cell suspensions,
202 the supernatant was separated by centrifugation at 9000 x *g* for 10 min at 10 ºC and the
203 pellet obtained was resuspended in buffered peptone water. Endospores and cell-free

204 supernatants were obtained as described in sections 2.3. and 2.4, respectively. Cultures
205 and cells were adjusted by spectrophotometry to 10^8 CFU mL^{-1} and subsequent
206 concentrations were obtained by dilution and verified via viable counts on TSA.

207 *2.5.3. Efficacy of cultures, endospores and cell-free supernatant curative treatments*
208 *against the main postharvest pathogens*

209 The efficacy of cultures (10^8 CFU mL^{-1}), endospores (10^7 CFU mL^{-1}), and cell-
210 free supernatant (undiluted and 1:10 diluted) from 72, 96 and 120 h-old cultures were
211 determined on fruit. For this purpose, oranges (cv. Valencia) were inoculated with *P.*
212 *digitatum* and *P. italicum,* apples (cv. Golden Delicious) with *P. expansum*, grapes (cv.
213 Sultanina) with *B. cinerea* and cherries (cv. Lapins) with *M. fructicola* and *M. laxa*.
214 Apples and oranges were inoculated by making two wounds (3 x 3 mm width and
215 depth) on the fruit surface with a sterile micropipette tip, but only one wound was made
216 in the case of cherries and grapes. Each wound was inoculated with 10 μL of 10^5
217 conidia mL^{-1} for *B. cinerea, P. digitatum, P. expansum* and *P. italicum* and 10 μL of 10^4
218 conidia mL^{-1} for *M. fructicola* and *M. laxa* prior to bacterial treatment. The fruits were
219 stored for 1 hour at room temperature to permit the absorption of the conidia
220 suspension. After that, 10 μL of bacterial treatment was inoculated in each wound. Fruit
221 with only fungal inocula served as control treatments. All the samples were stored at 20
222 ºC and 80 % RH for 7 days. Incidence (percentage of rotted wounds) and severity were
223 measured to compare the treatments. In the case of small fruits (grapes and cherries) the
224 severity rating scale of infected wounds was: 0 = no symptoms; 1 = 1-25 % of the fruit
225 infected; 2 = 25-50% of the area infected; 3 = sporulation cover 50-75 %; 4 > 75% of
226 the fruit infected. In addition, the lesion diameter (mm) around the wound was
227 measured for the rest of the fruits to determine the severity. Ten fruits were used for

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228 each treatment and pathogen. The entire experiment was repeated three times.

229 *2.5.4. Efficacy of BUZ-14 curative and preventive treatments against* B. cinerea *and*
230 Penicillium *species*

231 In the case of *B. cinerea* and *Penicillium* species, the effects of curative and
232 preventive treatments using 24 h-old cultures were tested. Oranges (cv. Valencia) were
233 inoculated with *P. digitatum* and *P. italicum,* apples (cv. Golden Delicious) with *P.*
234 *expansum* and grapes (cv. Sultanina) with *B. cinerea* as previously described. For the
235 preventive treatments, the strain BUZ-14 was first inoculated. Thus, 10 µL from a 24 h-
236 old culture in 863 medium (10^8 CFU mL^{-1}) were transferred to each wound and fruits
237 were placed at 20 ºC and 80 % RH for 24 h. Afterwards, 10 µL from 10^5 conidia mL^{-1}
238 suspension of the pathogen was inoculated to the fruits and stored at 20 ºC and 80 %
239 RH for 7 days. For the curative treatment, fruits were inoculated with 10 µL of conidia
240 suspensions (10^5 conidia mL^{-1}) 1 h prior to the bacterial treatment (10 µL from a 24 h-
241 culture at 10^8 CFU mL^{-1}) and incubated at 20 ºC and 80 % RH for 7 days. In both cases,
242 a control treatment with only pathogen conidia (controls) was included. Incidence and
243 severity were measured to compare the treatments as described above. Ten fruits were
244 used for each treatment and pathogen. The entire experiment was repeated three times.

245 *2.5.5. Efficacy of different concentrations of culture, cell and cell-free supernatant*
246 *treatments against* M. fructicola *and* M. laxa *on wounded stone fruit*

247 Cultures (vegetative cells + endospores + supernatant in 863 medium) adjusted
248 to 10^8, 10^7 and 10^6 CFU mL^{-1}, cells (vegetative cells + endospores resuspended in
249 buffered peptone water) adjusted to 10^8, 10^7 and 10^6 CFU mL^{-1} and cell-free supernatant
250 treatments (undiluted and 1:10 diluted) from 24, 48 and 72 h-old culture of the *B.*
251 *amyloliquefaciens* strain BUZ-14 were tested against both *Monilinia* species on stone

252 fruit (peaches cv. Calante). Treatment with Serenade® Max at the commercial dose of
253 2.5 g L^{-1} (approximately 1.8 x 10^7 CFU mL^{-1}), with the fungicide Scholar at the
254 recommended dose of 2 mL L^{-1}, and a control treatment with only distilled water were
255 also included. Bacterial concentrations were verified after the application by plate count
256 on TSA. Peaches were inoculated by making two wounds (3 x 3 mm width and depth)
257 on the fruit surface with a sterile micropipette tip. Each wound was inoculated with 10
258 µL of *M. fructicola* and *M. laxa* (10^4 conidia mL^{-1}) 2 h prior to bacterial treatment. After
259 that, 10 µL of bacterial treatments was inoculated in each wound. Fruits with only
260 fungal inocula served as control treatments. Treated peaches were stored at 20 ºC, 80 %
261 RH for 7 days. Incidence (percentage of rotted wounds) and severity (lesion diameter
262 (mm)) were measured to compare the treatments. Ten peaches were used for each
263 treatment and pathogen. The entire experiment was repeated three times.

264 *2.5.6. Survival and efficacy against brown rot of* B. amyloliquefaciens *in wounded fruit*
265 *at cool and room temperatures*

266 Peaches cv. Calante inoculated with *M. fructicola* or *M. laxa* and treated with *B.*
267 *amyloliquefaciens* BUZ-14 were used to determine bacterial survival and efficacy
268 against brown rot at cool and room temperatures.

269 For the bacterial survival experiment, peaches were inoculated by making two
270 wounds (3 x 3 mm width and depth) on the fruit surface with a sterile micropipette tip.
271 Each wound was inoculated with 10 µL of 10^4 conidia mL^{-1} of *M. fructicola* and *M. laxa*
272 prior to bacterial treatment. The fruits were stored for 2 hours at room temperature to
273 favour the absorption of the conidia suspension. After that, 10 µL (10^7 CFU mL^{-1})
274 obtained from a 24 h-old culture (vegetative cells + endospores + supernatant) was
275 inoculated in each wound. Then, one batch of fruits were stored for 10 days at 1 ºC

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276 followed by a shelf-life period of 3 days at 20 ºC and another batch was disposed at
277 room temperature (4 days at 20 ºC). BUZ-14 counts were determined on days 0, 5 and
278 10 at cool temperature and daily during the shelf-life period. At room temperature the
279 counts were conducted daily during the four days of the experiment. A plug sample (10
280 x 5 mm, diameter and depth) of the wound previously inoculated with bacterial
281 inoculum was removed with a sterile scalpel. The peach plugs were placed in filter
282 blender bags with 0.1 % sterile peptone water. The mixture was homogenized in a
283 laboratory blender Stomacher 400 Circulator (Seward Laboratory, London, England) for
284 120 s at 260 rpm and the resulting suspension was diluted, plated on TSA plates and
285 counted after 24 h at 30 ºC. Three peaches (2 wounds per peach) were analyzed at each
286 sampling point and the results were expressed as CFU *Bacillus* per wound (CFU $wound^{-1}$
287). The entire experiment was repeated three times.

288 The antifungal activity was studied in parallel to the BCA survival experiments.
289 For that purpose, ten peaches for each *Monilinia* species and storage conditions were
290 wounded and inoculated retracing the steps described above, and stored at cool and
291 room temperatures. The entire experiment was repeated three times.

292 *2.6. Statistical analysis*

293 The data were statistically analyzed using an SPSS software package for
294 Windows version 19.0 (SPSS Inc., Chicago, IL, USA). Differences in the mean values
295 of parameters were tested by one-way analysis of variance and separated by Tukey's
296 honestly significant difference test ($P < 0.05$).

297 **3. Results and discussion**

298 *3.1. Characterization of* B. amyloliquefaciens *strain BUZ-14 growth*

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299 Characterization of the growth of the *B. amyloliquefaciens* strain BUZ-14 at
300 different temperatures and pH values is necessary to ascertain whether this strain will be
301 able to survive in the intrinsically acidic conditions of the fruit and at the cool
302 temperatures employed during postharvest storage, distribution and commercialization.
303 These tests were conducted previously on laboratory media and subsequently on fruit as
304 described in section 2.5.6.

305 *3.1.1. Growth temperature and pH curves and endospore production*

306 The growth temperature curve of *B. amyloliquefaciens* (Figure 1A) showed that
307 the population increased from 4.1 to 5.0 log_{10} CFU mL $^{-1}$ in only 2 h at 37 ºC. At this
308 temperature, the lag phase was almost inexistent and the maximum population of 9.1
309 log_{10} CFU mL $^{-1}$ was reached after 16 h. At 20 ºC the lag phase was prolonged during 8
310 h of incubation, after which logarithmic growth was observed to reach the stationary
311 phase after 48 h. This means that the BUZ-14 strain at 20 ºC needs around 40 hours to
312 attain the stationary phase, keeping large differences with higher temperatures. It can
313 also be observed that the population decreased 1-1.5 log units after 120 h of incubation
314 at cool temperatures (4 and 10 ºC).

315 The growth curves of the *B. amyloliquefaciens* strain BUZ-14 at different pH
316 values are shown in Figure 1B. At pH 7, used as control, the population increased from
317 4.8 to 9.0 log_{10} CFU mL $^{-1}$ in the first 24 h whereas at pH 5 the same maximum
318 concentration was achieved after 48 h of incubation following a lag phase of 24 h. At
319 lower pH values (4.5, 4.0 and 3.5) the counts decreased by 2 log units in just 12 h of
320 incubation, the final counts after 120 h being about 2-2.5 log_{10} CFU mL^{-1}. It is clear that
321 a pH below 5 has an inhibitory effect on BUZ-14 growth, decreasing the initial
322 populations by almost 3 log units. Although it would be expected that the concentration

323 of BUZ-14 in acid fruits such as strawberry or oranges should be significantly reduced,
324 its survival was observed instead. However, its growth can be assured in fruits with
325 higher pH such as such as ripe peaches used in this study (section 3.4).

326 Endospore production (Figure 1A) reached 4.3 log units after 24 h and increased
327 by more than 1 log unit per day until the fourth day of incubation, achieving 8.5 log
328 units after 120 h of incubation. The BUZ-14 spore production (3.2 x 10^8 spores mL^{-1})
329 and sporulation efficiency (33 %) on 683 culture media were in the range reported for
330 other *Bacillus* strains, with densities from 1.0 x 10^5 spores mL^{-1} (Cayuela et al., 1993) to
331 3.0 x 10^9 spores mL^{-1} (Warriner and Aites, 1999) and typical efficiencies of 30-100%
332 (Nicholson and Setlow, 1990). Industrial exploitation of spores requires high cell
333 density bioreaction and good sporulation efficiency. For that purpose the cultivation
334 parameters (pH, dissolved oxygen concentration, and media composition) have to be
335 optimized. Monterio et al. (2005) for *B. subtilis* strain MB24 established an optimal pH
336 value of 7.5, no significative influence of the dissolved oxygen concentration within the
337 studied range (10-50% of the oxygen saturation concentration) and that as nutrient
338 depletion is the main stimulus for sporulation, it is very important to achieve glucose
339 depletion at the end of the exponential growth phase.

340 *3.2. In vitro antifungal activity of* B. amyloliquefaciens *strain BUZ-14*

341 The strain BUZ-14 was able to inhibit mycelium growth of all the tested
342 postharvest fungal pathogens *in vitro* (Table 1). Vegetative cells (24 h-old cultures)
343 reduced fungal growth between 39 %, for *P. italicum*, and 73 %, for *M. laxa*, in
344 comparison with the untreated pathogen control. Endospores also showed strong
345 antifungal activity, those obtained from 96 and 120 h-old cultures being the most
346 effective. BUZ-14 cell-free supernatants obtained after 72, 96 and 120 h of incubation
347 completely inhibited the growth of all tested pathogens, except for *P. digitatum* and *P.*

348 *italicum*. Total inhibition of *P. digitatum* was obtained with metabolites 1:1 and 1:4
349 diluted obtained after 72 h, and at any concentration in the case of 96 and 120 h of
350 incubation. The worst results were observed for *P. italicum* as total reduction was only
351 achieved with 1:1 and 1:4 supernatant dilutions obtained from 96 and 120 h cultures.

352 Vegetative cells, endospores and cell-free supernatants of BUZ-14 have shown
353 strong *in vitro* antifungal activity against *B. cinerea, M. fructicola, M. laxa, P.*
354 *digitatum, P. expansum,* and *P. italicum.* The activity of cells and endospores was
355 associated with white precipitates surrounding the bacterial colonies inwards from the
356 zone of mycelium inhibition. Touré et al. (2004) observed these white precipitates when
357 testing the *in vitro* activity of *B. subtilis* GA1 endospores against *B. cinerea* and
358 suggested that they were related with the excretion of fungitoxic compounds which
359 precipitate in contact with the acidified medium induced by the mold growth. This
360 hypothesis was exemplified by testing filter-sterilized crude supernatants and verifying
361 their high antifungal activity. Several studies with different strains of *B. subtilis*
362 suggested that antibiosis could be the principal mode of action in postharvest disease
363 suppression and some of them have identified the presence of powerful antifungal
364 metabolites, in special lipopeptides of surfactin, iturin and fengycin families (Touré at
365 al., 2004; Ongena et al. 2005; Stein 2005; Chung et al., 2008; Joshi et al., 2008;
366 Waewthomgrak et al., 2015; Torres et al., 2016). The ability to produce a wide range of
367 antifungal compounds has also been reported for *B. amyloliquefaciens* strains (Yoshida
368 et al., 2001; Arguelles-Arras et al., 2009; Arrebola et al., 2010; Hao et al., 2011; Ben
369 Ayed et al., 2015; Torres et al., 2016). Chen et al. (2006) revealed the capacity of *B.*
370 *amyloliquefaciens* FZB42 to produce several lipopeptides such as surfactin,
371 bacillomycin, fengycin and bacillibactin with antifungal, antibacterial and even
372 nematocidal activity. Subsequently, Schneider et al. (2007) detected that this strain was

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373 also able to produce some polyketides with high antifungal activity such as difficidin
374 and bacylisin. The *in vitro* antifungal activity of BUZ-14 cell-free supernatants, that
375 equal or better that of cells and endospores, supported the role of these antifungal
376 compounds in the biocontrol activity of the strain. However, there are some bacteria that
377 produce great amounts of antibiotics *in vitro*, but cannot always do so in fruit (Droby et
378 al. 1992, Bull et al., 1997; Touré et al. 2004; Kim et al., 2007; Lai et al., 2012).
379 Consequently, *in vitro* assays should be followed by *in vivo* assays to check that the
380 mechanism of action does not change.

381 *3.3 In vivo assays of* B. amyloliquefaciens *strain BUZ-14 to control postharvest*
382 *incidence and severity of representative postharvest diseases*

383 *3.3.1 Efficacy of cells, endospores and cell-free supernatant curative treatments against*
384 *postharvest pathogen molds*

385 The efficacy of cultures (vegetative cells + endospores + cell-free supernatant),
386 endospores and cell-free supernatant treatments at 72, 96 and 120 h of incubation
387 against postharvest pathogenic fungi is shown in Figure 2. *B. amyloliquefaciens*
388 treatments did not significantly reduce the incidence (% of rotted wounds) or severity
389 (lesion diameter) of the diseases caused by *Penicillium* species, neither by *P. digitatum*
390 or *P. italicum* in oranges neither by *P. expansum* in apples. Nevertheless, *P. expansum*
391 decreased its growth from 23 mm to 17 mm with undiluted cell-free supernatant
392 obtained after 120 h of incubation. Similar results were obtained against gray mold on
393 grapes in terms of incidence, although the severity reduction was higher. The best
394 reduction was provided by undiluted cell-free supernatants, since the severity decreased
395 from 2.5 (untreated samples) to 1.2. These data show that BUZ-14 treatments were not
396 effective in controlling previous Penicilia and Botrytis fruit infections. Our results are
397 consistent with those found by Yánez-Mendizábal et al. (2011) that testing the activity

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398 of *B. subtilis* CPA-8 against *P. digitatum* and *P. italicum* found minimal or no efficient
399 control of green and blue mold decay on orange, despite the good results achieved in the
400 *in vitro* assays. In this study the inoculation of the pathogens was prior to that of the
401 BCA. It seems that in the case of *Bacillus* species the preventive application of the BCA
402 is crucial to achieving a good control of Penicillia and Botrytis infections.
403 Leelasuphakul et al. (2008) with *B. subtilis* 155 endospores applied prior to *P. digitatum*
404 spores in citrus fruit obtained better results in terms of decay incidence and severity than
405 when the two microorganisms were inoculated together. Hang et al. (2005) detected that
406 a pre-application of *Bacillus subtilis* S1-0210 before *B. cinerea* inoculation was more
407 effective in controlling gray mold in strawberry than a post-application, concluding that
408 the pre-colonization of antagonistic agents on host plants can be a critical factor in
409 protecting the host from infection of fungal pathogens. Lai et al. (2012) also detected
410 that the control of postharvest green mold on citrus fruit improved when the period
411 between the treatment with *Paenibacillus polymyxa* strain SG-6 and the inoculation
412 with the fungal pathogen was increased. In view of these results, the effect of preventive
413 treatments with BUZ-14 cultures, 10^8 CFU mL^{-1} applied 24 h prior to *Penicillium*
414 species and *B. cinerea,* was investigated (section 3.3.2).

415 *M. fructicola* and *M. laxa* were the most susceptible species as the incidence
416 obtained with undiluted cell-free supernatant at any incubation times and 72 and 96 h-
417 old cultures treatments was reduced to 0 % in comparison with 100 % for the untreated
418 sample. For endospores, the best result was achieved with those collected after 96 h of
419 incubation as *M. fructicola* growth was totally inhibited and the incidence and severity
420 of *M. laxa* was reduced to 10 % and 1.2, respectively. These results confirm the
421 potential of cultures, endospores and cell-free supernatants of BUZ-14 to control decay
422 by *M. fructicola* and *M. laxa* already reported in previous studies for other *Bacillus*

423 strains (Pusey and Wilson, 1984; Altindag et al., 2006; Yañez-Mendizabal et al., 2010;
424 Liu et al., 2011; Rungjindamai et al., 2013; Gao et al., 2016). The high efficacy against
425 brown rot of BUZ-14 endospores is very interesting as they are more stable than
426 vegetative cells maintaining their viability for years and far more resistant to drying
427 processes for powder formulations (Brannen and Kenney, 1997), all of them being
428 crucial aspects to develop a commercial formulation. In addition, the cell free
429 supernatant, composed by bioactive compounds, such as lipopeptides and polyketides,
430 with low toxicity, high biodegradability and environmentally friendly characteristics,
431 show great potential for future applications, being an effective alternative to the
432 chemical pesticides usually applied to control brown rot. In section 3.3.3. the
433 relationship between the number of cells and the protection level is elucidated and
434 compared to those of BUZ-14 cell-free supernatants, fungicide Scholar® and Serenade®
435 Max.

436 *3.3.2 Efficacy of curative and preventive treatments against* B. cinerea and Penicillium
437 *species*

438 The results of preventive and curative treatments from 24 h-old cultures against
439 *B. cinerea* and *Penicillium* species are shown in Figure 3. Preventive inoculation
440 avoided *P. digitatum* and *P. italicum* growth in oranges and reduced *P. expansum*
441 incidence in apples from 100 % to 20 %. For *B. cinerea*, only a 20 % of incidence
442 reduction was observed, although the severity decreased by 40 %. Curative treatments
443 using 24 h-old cultures had no effect on the incidence and slighty reduced the severity,
444 showing similar results to those obtained with 72, 96 and 120 h-old cultures, except in
445 the gray mold severity that was greater (Fig. 2). These data confirmed that BUZ-14
446 cultures have a limited curative effect on *P. expansum*, *P. digitatum* and *P. italicum*
447 infections and, as has been discussed in the previous section, preventive inoculation of

448 the BCA is crucial to achieve a good disease control. For these species, the bacterial
449 tissue colonization and a certain level of antifungal compounds production that protect
450 the fruit against the pathogen infection could be necessary (Arrebola et al., 2010, Hang
451 et al., 2005).

452 However, Arrebola et al. (2010) demonstrated that the effectiveness of the
453 treatments with the antagonist *B. amyloliquefaciens* PPCB004 applied 1 day after or 1
454 day before pathogen application depends on the pathogen tested. For example, to
455 control *Alternaria citri* or *Penicillium crustosum* the antagonist treatment 1 day before
456 showed promise; however, for *Colletotrichum gloeosporioides* the antagonist
457 application 1 day after was more effective so the efficacy of curative and preventive
458 treatments should be determined for each antagonism and pathogen. Hao et al. (2011)
459 and Hong et al. (2014) obtained good control of citrus green and blue mold and sour rot
460 inoculating the pathogens almost immediately after the BCA (*B. amyloliquefaciens* HF-
461 01) and three hours later, respectively. So, another aspect to study is the determination
462 of the optimal interval of time between the BCA and the pathogen inoculation (also in
463 reverse order of inoculation) to obtain the maximum postharvest disease control.

464 *3.3.3 Efficacy of different concentrations of culture, cell and cell-free supernatant*
465 *treatments against* M. fructicola *and* M. laxa *on wounded stone fruit*

466 Since our previous data show that *M. fructicola* and *M. laxa* are most susceptible
467 to BUZ-14 applications, these fungi were chosen for subsequent bioassays in Calante
468 peaches. The activity of 24, 48 and 72 h-old cultures were tested in this assay to shorten
469 incubation times, which are always preferred when obtaining BCAs. The results of the
470 culture, cells and cell-free supernatant treatments are shown in Figure 4. Cultures and
471 cells at 10^8 and 10^7 CFU mL^{-1} and cell free supernatant obtained from 24 h-old cultures

472 reduced the incidence of brown rot to 0 %, compared to 100 % in the untreated control.
473 However, 10^6 CFU mL^{-1} treatments were ineffective since the disease incidence was
474 similar to that of untreated samples although the lesion diameter was reduced. A notable
475 decrease of efficacy was detected with treatments obtained from 48 h-old cultures since
476 only cultures and cells at 10^8 CFU mL^{-1} and undiluted cell free supernatant reduced
477 disease incidence to 0% for both species. Cultures and cells obtained from a 72 h-
478 culture were also unable to control the disease and only the cell free supernatant
479 obtained a total inhibition of *M. laxa* growth. The BUZ-14 treatments were compared
480 with two commercial standards, Serenade® Max, a biocontrol product formulated with
481 *Bacillus subtilis* QST-713, and Scholar®, a postharvest treatment based on fludioxonil.
482 No evidence of disease was detected with Scholar®. Serenade® Max (1.7 x 10^7 CFU
483 mL^{-1}) significantly reduced the incidence and severity compared to untreated control
484 samples but did not fully control the disease.

485 For both species the experimental data show that concentrations of 10^8 and 10^7
486 CFU mL^{-1} of cultures and cells obtained after 24 h of incubation of the BCA provided a
487 total control of brown rot in peaches. Data reported by Zhang and Dou (2002), Touré et
488 al. (2004) and Yánez-Mendizábal et al. (2010) using *B. subtilis* to green mold, brown
489 rot and gray mold control indicated that $>10^7$ CFU mL^{-1} was the appropriate
490 concentration for bacterial treatments. An evident decrease in efficacy was detected
491 with the reduction of the number of cells in the treatment and with the increasing of
492 previous incubation times of the BUZ-14 cultures. Touré at al. (2004) suggested that
493 antibiosis could play a major role in the inhibition of the disease since the protection
494 level by the bacterium increased proportionally with the number of bacterial cells used
495 for treatment although competition for nutrients cannot obviously be ruled out.
496 Treatment of fruits with BUZ-14 cell free supernatants also provided a strong protective

497 effect against brown rot that was similar to, or higher than the one observed with live
498 cells. This indicated the important role of the antifungal compounds in the biocontrol
499 activity of BUZ-14. However, the maximum inhibition of supernatants (that is, no
500 evidence of disease detected with undiluted and 1:10 diluted treatments) was obtained
501 with extracts from different times of incubation, 48 h-old cultures for *M. fructicola* and
502 72 h-old cultures for *M. laxa*. This could be due to the different amounts and types of
503 antifungal compounds in the supernatant and the different susceptibility of each species.
504 Although several authors have revealed the major role of fengycnin-like lipopeptides in
505 the biological control of *Bacillus* species against fungal pathogens (Ongena et al., 2005;
506 Romero et al., 2006; Hu et al., 2007; Alvarez et al., 2012) including *M. fructicola* (Lui
507 et al., 2011; Yánez-Mendizábal et al., 2012), the activity of other lipopeptides such as
508 iturins (Yu et al., 2002; Arrebola et al., 2010) or other antimicrobial compounds such as
509 plantazolicin (Scholz et al., 2010) or chlorotetaine (Wang et al., 2016) and the presence
510 of synergistic effects (Maget-Dana et al., 1992; Lui et al., 2011; Tao et al., 2011) could
511 not be ruled out. Thus, the characterization of the bioactive compounds produced by
512 BUZ-14 in terms of identity, antifungal activity using non-producing mutants, total and
513 individual amounts, relative proportions and progressive accumulation both in culture
514 media and in fruit are key aspects to understand the mechanism of action of this BCA
515 against both fungal pathogens.

516 *3.4.* B. amyloliquefaciens *survival and efficacy against brown rot on wounded stone*
517 *fruit stored at room and cool temperatures*

518 Our experimental data show that BUZ-14 was more effective than Serenade®
519 Max in reducing brown rot caused by *M. laxa* and *M. fructicola* in peaches, providing
520 opportunities for the use of a new *Bacillus* strain to develop commercial formulations.
521 However, given that fruits are usually stored and distributed at cool temperatures (1-10

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522 ºC), the BCA strain must be able to grow in this temperature range or at least not
523 decrease during the postharvest storage period. Thus, it is important to establish the
524 survival and antifungal activity of BUZ-14 in fruit stored at cool temperatures. These
525 data were compared with those obtained at room temperature. Bacterial populations of
526 *B. amyloliquefaciens* BUZ-14 on wound inoculated peaches (pH 5.1) at 20 ºC was
527 characterized by a 24 h lag-phase followed by a constant increase of cell population
528 from 3.2 x 10^5 CFU $wound^{-1}$ to values up to 10^8 CFU $wound^{-1}$ from the 48 h and
529 onwards (Figure 5) indicating a good bacterial survival and growth. BUZ-14 bacterial
530 concentration after 10 days of storage at 1 ºC and 80 % RH decreased from 1.3 x 10^5 to
531 2.5 x 10^4 CFU $wound^{-1}$. When the fruits were transferred to room temperature
532 conditions (20 ºC) in order to simulate a period of commercialization, the populations
533 recovered after 48 h reached 4 x 10^6 CFU $wound^{-1}$ in 72 h (Figure 5). Compared with
534 the initial concentration (10^5 CFU $wound^{-1}$), the bacterial population only decreased by
535 0.7 log units after 10 days at cool temperature. These data coincide with those obtained
536 previously in 863 medium at 4 ºC, where bacterial growth decreased by around 0.8 log
537 units. No symptoms of decay were detected in any of the inoculated and treated peaches
538 so we can be sure that BUZ-14 survived and colonized the injured fruit tissue and
539 maintained its antifungal activity even after a cool storage period of 10 days.

540 **4. Conclusions**

541 The results obtained in this study have demonstrated that *B. amyloliquefaciens*
542 BUZ-14 is a promising BCA for major postharvest fruit diseases. Its relevance in
543 curative treatments against *M. fructicola* and *M. laxa* and preventive treatments against
544 *P. expansum, P. digitatum* and *P. italicum* should be highlighted. In addition, the BUZ-
545 14 strain survives at cool temperatures, as low as 1 ºC, which make it suitable for
546 postharvest application. Its mode of action, antibiotics´ production and activity, and

547 biotechnological issues of production and formulation are the subjects of ongoing
548 research.

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798

799 **Figure Captions**

800

801 **Fig. 1. Growth of *B. amyloliquefaciens* BUZ-14 in 863 medium.** Effect of the
802 temperature: 4 (▲), 10 (△), 20 (■), 30 (□) and 37 (●) ºC and endospore production at
803 30 ºC (✕) (**A**). Effect of the pH: 3.0 (▲), 4.0 (△), 4.5 (■), 5.0 (□) and 7.0 (●) (**B**). Each
804 value is the mean of three separate replicates of three Erlenmeyer flasks each and
805 vertical bars represent the standard error of the mean.

806 **Fig. 2. Effect of *B. amyloliquefaciens* BUZ-14 curative treatments from 72, 96 and**
807 **120 h-old cultures against fruit postharvest rots caused by several mold pathogens**.
808 Incidence as % of rotted wounds (□) and severity as lesion diameter (mm) for apples
809 and oranges and % of the single fruit rotted referred to 0-to-4 scale for grapes and
810 cherries (■). Golden delicious apples infected with *P. expansum* (A); Sultanina
811 seedless grapes infected with *B. cinerea* (B); Valencia oranges infected with *P.*
812 *digitatum* (C) and *P. italicum* (D); Lapins cherries infected with *M. fructicola* (E) and
813 *M. laxa* (F). Fruit were stored at 20 ºC for 7 d. Cont: untreated pathogen control; Cul:
814 vegetative cells + endospores + supernatant (10^8 CFU mL^{-1}); End: endospores at 10^7
815 CFU mL^{-1}; CF Sup.: cell-free supernatant undiluted and 1:10 diluted. Each value is the

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816 mean of three replicates of ten fruits each, 30 fruits per treatment. For each pathogen,
817 different letters above the columns (capital letters for rot incidences and lower-case
818 letters for lesion severities) indicate statistical difference at $P < 0.05$. Incidence columns
819 for A, B, C and D are omitted since their values are similar (about 100%) without
820 statistical differences.

821 **Fig. 3: Curative and preventive effect of *B. amyloliquefaciens* BUZ-14 treatments**
822 **from 24 h-old cultures on fruit.** Golden delicious apples infected with *P. expansum*
823 (A); Sultanina seedless grapes infected with *B. cinerea* (B) and Valencia oranges
824 infected with *P. digitatum* (C) and *P. italicum* (D). Incidence as % of rotted wounds (□)
825 and severity as lesion diameter (mm) for apples and oranges and % of the single fruit
826 rotted referred to 0-to-4 scale for grapes (■). Fruit were stored at 20 ºC for 7 d. Control:
827 untreated pathogen control; Cur 24 h: 24 h-old culture (vegetative cells + endospores +
828 supernatant (10^8 CFU mL^{-1})) inoculated 1 h after pathogen infection; Prev 24 h: 24 h-
829 old culture inoculated 1 day prior to pathogen infection. Each value is the mean of three
830 replicates of ten fruits each, 30 fruits per treatment. For each pathogen, different letters
831 above the columns (capital letters for rot incidences and lower-case letters for lesion
832 severities) indicate statistical difference at $P < 0.05$.

833 **Fig. 4: Efficacy of several concentrations of culture, cell and cell-free supernatant**
834 **curative treatments of *B. amyloliquefaciens* BUZ-14 from 24, 48 and 72 h-old**
835 **cultures against *Monilinia* species in Calante peaches.** Incidence (% of rotted
836 wounds): □ and severity (lesion diameter (mm)): ■. *M. fructicola* (A) and *M. laxa* (B).
837 Peaches were stored at 20 ºC for 7 d. Cont: untreated pathogen control (10^4 conidia mL^-
838 ^{1}); Cul: vegetative cells + endospores + supernatant at 10^8, 10^7 and 10^6 CFU mL^{-1}; Cel:
839 Cell suspensions at 10^8, 10^7 and 10^6 CFU mL^{-1}; CF sup: Cell-free supernatant; CF sup
840 1:10: CF sup 1:10 diluted; Com. Stand.: Commercial standards (Ser: Serenade® Max

841 (1.8 x 10^7 CFU mL^{-1}), Scho: Scholar® (2 mL L^{-1})). Each value is the mean of three
842 replicates of ten fruits each, 30 fruits per treatment. For each pathogen, different letters
843 above the columns (capital letters for rot incidences and lower-case letters for lesion
844 diameters) indicate statistical difference at $P < 0.05$.

845 **Fig. 5: *B. amyloliquefaciens* BUZ-14 growth in wounded Calante peaches during**
846 **cold storage and at ambient temperature.** 10 days at 1 ºC followed by a shelf life
847 period of 3 days at 20 ºC (●) and 4 days at 20 ºC (○). Each value is the mean of three
848 replicates (3 fruits with 2 wounds per peach per replicate) and vertical bars correspond
849 to standard deviation.

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850 **Tables**

851 **Table 1**

852 Antifungal activity of *B. amyloliquefaciens* strain BUZ14 on PDA plates against several postharvest mold pathogens.

Treatment	Percentage of inhibition[a] against:					
	Botrytis cinerea	*Monilinia fructicola*	*Monilinia laxa*	*Penicillium digitatum*	*Penicillium expansum*	*Penicillium italicum*
Veg. cells[b] 24 h	52.0 ± 10.9ab,AB[e]	66.6 ± 0.7a,AB	73.1 ± 4.2a,B	54.5 ± 2.5a,AB	56.8 ±6.3a,AB	39.3 ± 7.5a,A
End[c] 72 h	65.4 ± 7.2b,A	66.4 ± 2.2a,AB	81.5 ± 2.1b,C	76.5 ± 1.8bcd,BC	59.5 ± 1.3a,A	36.3 ± 1.2a,D
End 96 h	97.0 ± 2.4c,A	60.0 ± 3.9b,B	89.6 ± 1.2c,AC	81.9 ± 0.7d,CD	79.2 ± 2.0b,D	68.5 ± 1.2bc,B
End 120 h	92.1 ± 4.7c,A	57.0 ± 1.2b,BC	88.2 ± 0.9c,AD	80.9 ± 4.4cd,D	51.6 ± 1.7a,B	63.8 ± 2.8b,C
CF Sup[d] 1:1 24 h	100.0 ± 0.0c,A	100.0 ± 0.0c,A	100.0 ± 0.0d,A	77.4 ± 6.6bcd,B	32.11 ± 0.82c,C	84.8 ± 7.6de,D
CF Sup 1:4 24 h	62.0 ± 6.1ab,A	80.9 ± 1.7d,B	100.0 ± 0.0d,C	73.2 ± 7.7bc,B	8.65 ± 0.78d,D	79.7 ± 10.2cd,B
CF Sup 1:10 24 h	50.3 ± 6.1a,A	76.0 ± 2.5e,B	45.5 ± 6.7e,A	70.9 ± 8.4b,B	1.29 ± 0.46d,C	2.8 ± 2.4f,C
CF Sup 1:1 72 h	100.0 ± 0.0c,A	100.0 ± 0.0c,A	100.0 ± 0.0d,A	100.0 ± 0.0e,A	100.0 ± 0.0e,A	96.6 ± 0.8eg,B
CF Sup 1:4 72 h	100.0 ± 0.0c,A	100.0 ± 0.0c,A	100.0 ± 0.0d,A	100.0 ± 0.0e,A	100.0 ± 0.0e,A	88.4 ± 1.9de,B
CF Sup 1:10 72 h	100.0 ± 0.0c,A	100.0 ± 0.0c,A	100.0 ± 0.0d,A	92.4 ± 0.8e,B	100.0 ± 0.0e,A	84.3 ± 1.4de,C
CF Sup 1:1 96 h	100.0 ± 0.0c,A	100.0 ± 0.0c,A	100.0 ± 0.0d,A	100.0 ± 0.0e,A	100.0 ± 0.0e,A	100.0 ± 0.0g,A
CF Sup 1:4 96 h	100.0 ± 0.0c,A	100.0 ± 0.0c,A	100.0 ± 0.0d,A	100.0 ± 0.0e,A	100.0 ± 0.0e,A	100.0 ± 0.0g,A
CF Sup 1:10 96 h	100.0 ± 0.0c,A	100.0 ± 0.0c,A	100.0 ± 0.0d,A	100.0 ± 0.0e,A	100.0 ± 0.0e,A	81.7 ± 0.8de,B
CF Sup 1:1 120 h	100.0 ± 0.0c,A	100.0 ± 0.0c,A	100.0 ± 0.0d,A	100.0 ± 0.0e,A	100.0 ± 0.0e,A	100.0 ± 0.0g,A
CF Sup 1:4 120 h	100.0 ± 0.0c,A	100.0 ± 0.0c,A	100.0 ± 0.0d,A	100.0 ± 0.0e,A	100.0 ± 0.0e,A	100.0 ± 0.0g,A
CF Sup 1:10 120 h	100.0 ± 0.0c,A	100.0 ± 0.0c,A	100.0 ± 0.0d,A	100.0 ± 0.0e,A	100.0 ± 0.0e,A	92.6 ± 1.6eg,B

853

854 [a] Data are expressed as percentage of fungal growth inhibition compared with control plates without bacteria or supernatant and represent mean values of five plates

855 (replicates) ± standard errors.

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856 [b] Cells at 10^9 CFU mL^{-1}.

857 [c] Endospores at 10^7 CFU mL^{-1}.

858 [d] Cell-free supernatant.

859 [e] Values in the same colum followed by different lower-case letters show significant differences between treatments for the same fungal pathogen ($P < 0.05$). Values in the

860 same line followed by different capital letters show significant differences between fungal pathogens for the same treatment ($P < 0.05$).

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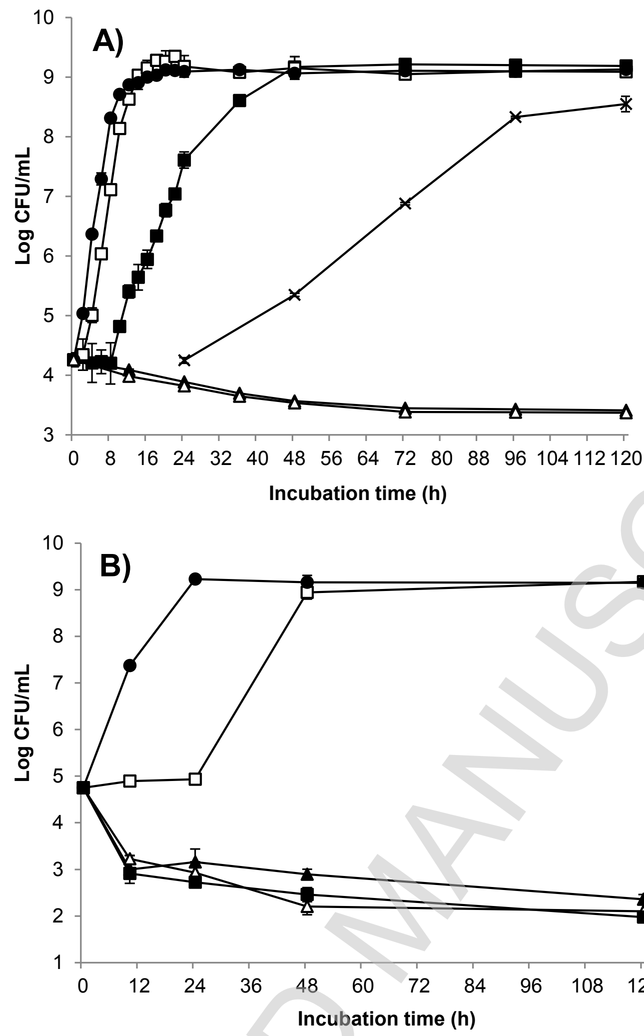

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Fig. 1

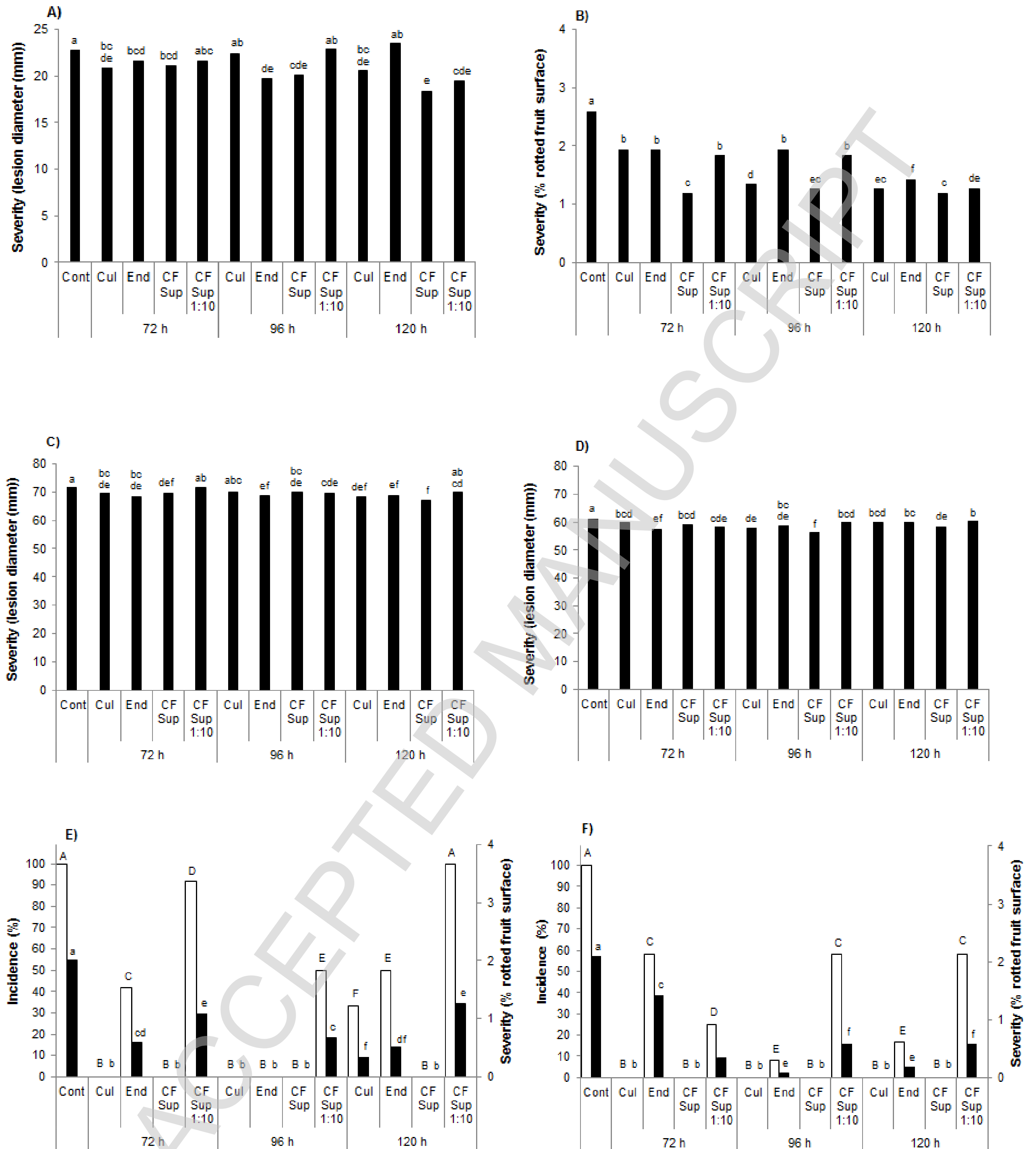

Fig. 2

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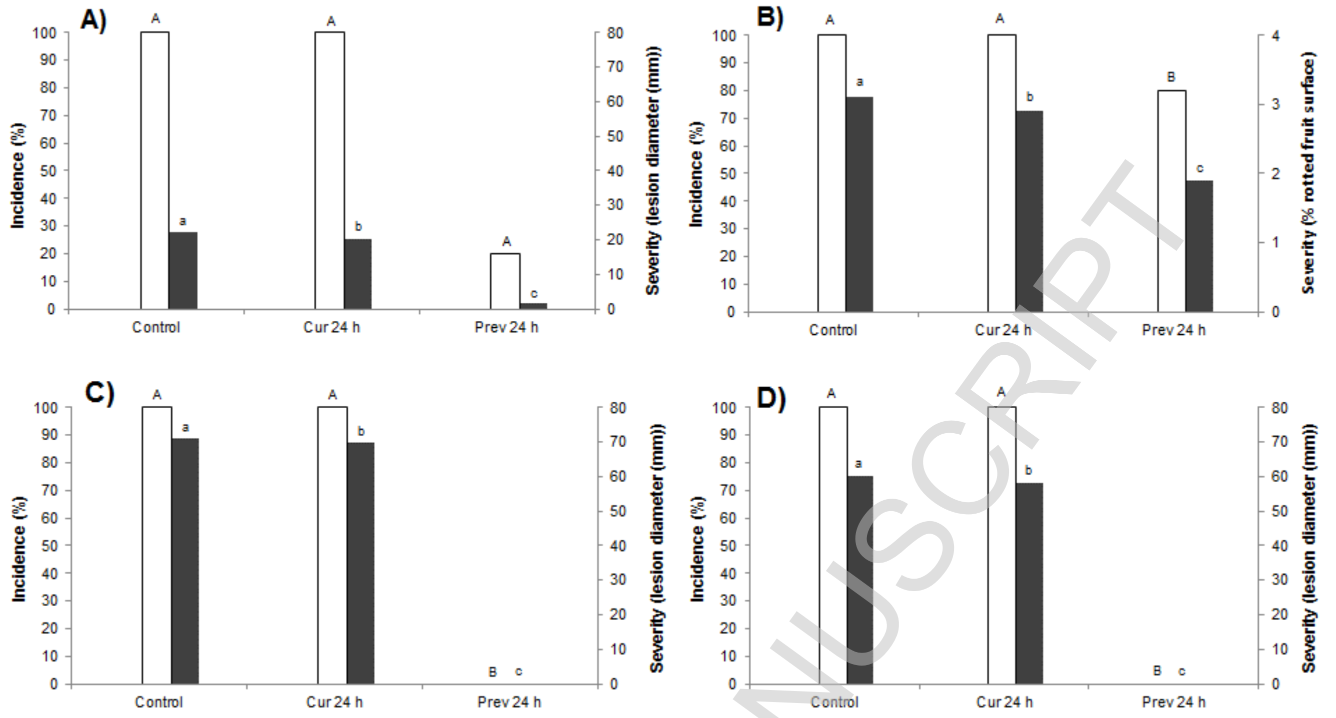

Fig. 3

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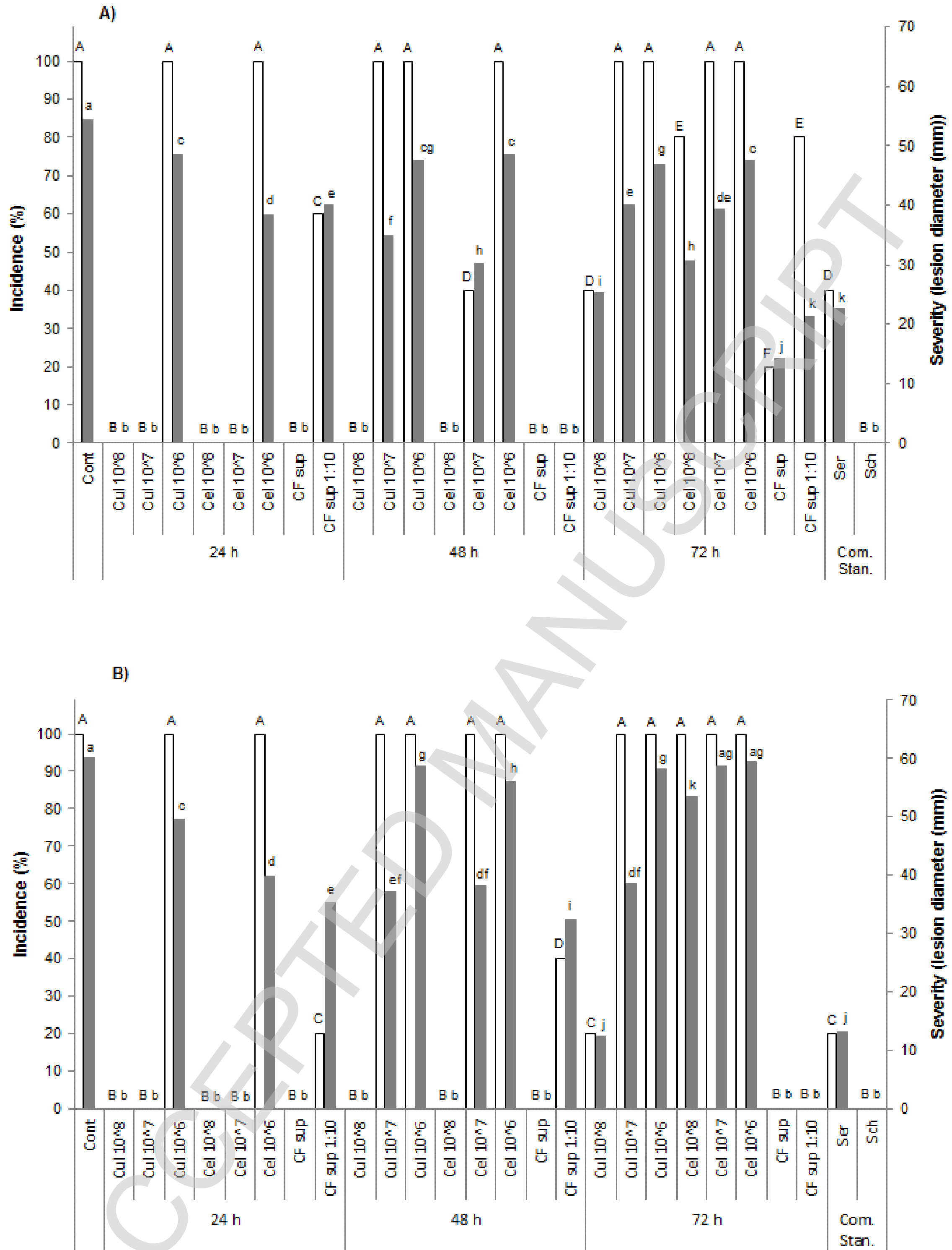

Fig. 4

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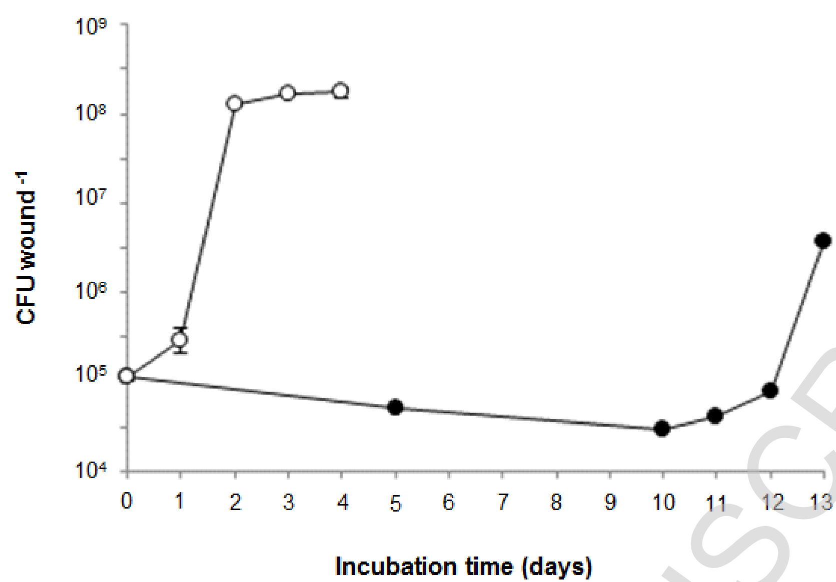

Fig. 5

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