1 **First report of Pseudomonas grapevine bunch rot caused by *Pseudomonas syringae* pv**
2 ***syringae***

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12 **Key words**
13 LOPAT test, GATTa tests, Red Globe, *Geotrichum candidum*, *Bacillus megaterium*
14 syringomycin, syringopeptin, *16S rDNA, gyrB*, *rpoB, rpoD* genes
15
16 **Abstract**
17 *Pseudomonas syringae* pv. *syringae* (*Pss*) a Gammaproteobacterium belonging to
18 genomospecies 2 within the *P. syringae* complex, is distributed worldwide and is responsible
19 for bacterial canker on more than 100 different hosts, including the grapevine. *Pseudomonas*
20 *syringae* pv. *syringae* induces necrotic lesions in the leaf blades, veins, petioles, shoots,
21 rachis and tendrils on grapevine cultivars in different areas. Recently, *Pss* has been associated
22 with severe economic losses in different grape cultivars in Australia where it causes
23 inflorescence rot. In mid- to late-summer 2017, symptoms of berry rots differing from those
24 caused by the common berry rots agents were observed in different 'Red Globe' vineyards of
25 Apulia (southern Italy). As proven by fulfilment of Koch's postulates, these symptoms were
26 caused by a bacterium which, according to the results of biochemical, physiological,
27 nutritional, antimicrobial activity, pathogenicity tests and sequencing of *16S rDNA, gyrB*,
28 *rpoB* and *rpoD* genes, was identified as *Pss.* This is the first report of Pseudomonas
29 grapevine bunch rot.
30
31 **Introduction**
32 *Pseudomonas syringae* (*Ps*) is probably the most extensively studied bacterial plant pathogen.
33 It occurs worldwide, comprises strains isolated from more than 200 cultivated and wild plants
34 (Mohr et al., 2008) and is responsible for symptoms ranging from foliar spots to blights,

35 stripes, and cankers. On the basis of visual symptoms (Bull et al., 2010), host range (Baltrus
36 et al., 2011), biochemical, physiological and nutritional tests (Lelliott et al., 1966), taxonomic
37 analysis (Young, 2010) and molecular typing, the *Ps* complex is nowadays subdivided into
38 57 pathovars (Bull et al., 2010). In addition, genome-wide comparative analysis between
39 pathovars has expanded the information on bacterial evolution and host specificity (Feil et al.,
40 2005, Marcelletti et al., 2011). Pyoverdin production is generally the first step in the
41 identification of plant pathogenic *Pseudomonas* spp. (Bultreys and Kaluzna, 2010; Gilbert et
42 al., 2009; Lelliott and Stead, 1987; Whitelaw-Weckert et al., 2011). Levan, oxidase, potato
43 soft rot, arginine dehydrolase, tobacco leaf hypersensitivity reaction (LOPAT tests) and the
44 assays based on gelatin liquefaction, aesculin hydrolase, tyrosinase activity and tartaric acid
45 utilization (GATTa tests) (Gašić et al., 2012; Jones, 1971; Latorre and Jones, 1979; Lelliott et
46 al., 1966) are currently used to discriminate *Ps* from other fluorescent *Pseudomonas* species
47 as well as the *P. syringae* pv. *syringae* (*Pss*) from the other pathovars.
48 *P. syringae* pv. *syringae* (*Pss*) is a Gammaproteobacterium belonging to genomospecies 2
49 within the *Ps* complex (Baltrus et al., 2011), has a worldwide distribution
50 (https://www.cabi.org/isc/datasheet/45014#B97A5089-5C15-4FA9-B3E4-B61E3DF9A535)
51 and is responsible for bacterial canker on a large number of horticultural and woody hosts
52 (Bultreys and Kaluzna, 2010; Golzar an Cother, 2008), including stone fruits (Abbasi et al.,
53 2013), apple (Mansvelt and Hattingh, 1989), pear (Moragrega et al., 2003) and olive
54 (Scortichini, 1997). The first report of *Pss* in grapevine dates to 1968 (Klingner et al., 1976),
55 in the province of Mendoza, Argentina, on cv. Cereza, causing symptoms such as necrotic
56 lesions in leaf blades, veins, petioles, shoots, rachis and tendrils. Long-considered a weak
57 pathogen, more recently *Pss* was associated with inflorescence rot causing severe economic
58 losses on cvs Sauvignon Blanc, Pinot Noir, Chardonnay and Riesling in Australia (Whitelaw-
59 Weckert et al., 2011; Hall et al., 2016) and in Kerch peninsula in Russia (Porotikova et al.,
60 2017).
61
62 During mid- to late-summer of 2017, previously unreported soft and watery spots on berries
63 were observed in different 'Red Globe' vineyards of Apulia (southern Italy). Similar
64 symptoms were observed also on VRG1, a new grapevine selection currently under trial in
65 two experimental vineyards located in the countryside of Noicattaro (province of Bari,
66 Apulia). This study was aimed to identifying and characterizing the pathogen/s responsible
67 for the symptoms observed by means of molecular, biochemical, pathological and
68 microbiological methods.

69

70 **Materials and methods**

71 *Sample collection and isolation*

72 Isolations were made from 20 symptomatic and 20 asymptomatic berries sampled from at
73 least 10 different bunches in each of three different vineyards of table grape 'Red Globe' and
74 one stand of ‘VRG1’, a new grape selection under study. Berries were rinsed with tap water,
75 surface-sterilized with a sodium hypochlorite solution (1% available chloride) for 3 min and
76 rinsed four times with sterile distilled water. Sterilized tissues from the rot area were
77 aseptically cut into 5x5 mm pieces, finely minced in a few drops of sterile distilled water,
78 streaked on 5% sucrose-nutrient-agar (SNA) medium and incubated in the dark at 25°C for
79 up to 48 h. The “levan type” colonies, the only ones isolated exclusively from symptomatic
80 berries, were subcultured onto SNA to obtain pure colonies These were streaked on King’s
81 medium B (King et al., 1954) and fluorescence was observed under UV light (254 nm) after
82 48 h at 25°C. Pure colonies were stored in nutrient agar (NA) slant tubes at 4°C and in 20%
83 glycerol at −80°C for further use. Colonies grown for 48 h onto NA slant tubes were used for
84 biochemical and pathogenic assays.

85

86 *Biochemical and nutritional tests*

87 The selected bacterial isolates DiSSPA_Pss_20, DiSSPA_Pss_21, DiSSPA_Pss_22 and
88 DiSSPA_Pss_23, showing “levan type” colonies, representative of the populations present in
89 the four sampled vineyards, and the type strain of *Pss* NCPPB 281 were characterized by
90 LOPAT test for levan production from sucrose (L); oxidase activity (O); pectolytic activity
91 on potato tubers; arginine dihydrolase (A) and tobacco hypersensitivity (T) (Lelliott and
92 Stead, 1987). The same isolates were also subjected to GATTa tests consisting of gelatine
93 hydrolysis (G); aesculin hydrolysis (A); tyrosinase activity (T) and tartrate utilization (Ta)
94 (Latorre and Jones, 1979). Their capability in 2-keto gluconate production, nitrate reduction,
95 acid production from sucrose, arbutin hydrolysis and utilization of inositol, sorbitol,
96 erythritol, L(-)lactate and D(-)tartrate as carbon sources was ascertained according to Lelliott
97 and Stead (1987) and Schaad et al. (2001).

98

99 *Molecular assay*

100 Genomic DNA was extracted from a 24-h LB culture according to the protocol described by
101 Marmur (1961) and analysed for the partial sequences of four core genes (*16S rDNA*, *gyrB*,

102 *rpoB* and *rpoD*). The primer pairs for the target sequences are listed in Table 1. The isolates
103 DiSSPA_Pss_20 DiSSPA_Pss_21, DiSSPA_Pss_22, DiSSPA_Pss_23 and NCPPB 281 were
104 used in the experiment.
105
106 PCR reaction mixture contained 5 µL of 10x LA Taq buffer, 5 µL of 25 mM $MgCl_2$, 8 µL of
107 10 mM dNTPs mixture, 200 nM of each primer (Table 1), 0.5 µL of Takara LA Taq, 3 µL of
108 template DNA (50 ng μL^{-1}) and ultrapure water up to 50 µL. Amplifications were carried out
109 in a MyCycler Thermocycler (Bio-Rad Laboratories, Hercules, CA) using the following PCR
110 conditions: 94°C for 3 min, followed by 35 cycles of 94°C for 1 min, 58°C for 1 min, and
111 72°C for 1 min, followed by final extension to 72°C for 10 min. All PCR-products were
112 purified by using PCR Kleen Purification Spin Columns (Bio-Rad, CA) and custom-
113 sequenced (Genewiz, Takeley, UK), in both directions (*16S rDNA*, 900 bp) or in single
114 direction for the others (*gyrB*: 500 bp; *rpoB*: 844 bp and *rpoD*: 431 bp).
115
116 Amplicons of the *16S rDNA* sequences of all the isolates used (DiSSPA_Pss_20-23)
117 (GeneBank Accession No. MK156155, MK156156, MK156157and MK156158, in the order)
118 were previously aligned in both directions and self-compared using the SeqMan Pro software
119 (DNASTAR Madison, USA), before their comparison with sequences deposited in GenBank
120 (www.ncbi.nlm.nih.gov/). Ientification was completed by alignment and self-comparison of
121 the *gyrB* (GeneBank Accession No. MK165100, DiSSPA_Pss_20; MK165101,
122 DiSSPA_Pss_21; MK165102 DiSSPA_Pss_22; and MK165103, DiSSPA_Pss_23), *rpoB*
123 (MK165104, DiSSPA_Pss_20; MK165105, DiSSPA_Pss_21; MK165106, DiSSPA_Pss_22;
124 MK165107, DiSSPA_PSS_23) and *rpoD* (MK165108, DiSSPA_PSS_20; MK165109,
125 DiSSPA_Pss_21; MK165110, DiSSPA_Pss_22, MK165111_ DiSSPA_Pss23) sequenced
126 genes with those from GeneBank (www.ncbi.nlm.nih.gov/). The *16S rDNA*, *gyrB*, *rpoB* and
127 *rpoD* gene sequences of *Pseudomonas* sp. (Table 2), directly downloaded from "The
128 Pseudomonas Genome Databas" (www.pseudomonas.com), were aligned and trimmed with
129 SeqMan Pro Software (DNASTAR, Inc., Madison, WI, USA) in order to obtain concatenated
130 sequences. Phylogenetic analyses using *16S rDNA*-*gyrB*-*rpoB*-*rpoD* concatenated sequences
131 and rpoD sequences were conducted in MEGA6 (Tamura et al., 2013) using the UPGMA
132 method. Similarly, the *Pss* isolated from grape were compared using *ropB* sequences,
133 because the only ones available in GenBank.
134
135 *Antimicrobial activity*

136 The syringomycin and syringopeptin production of DiSSPA_Pss_20, DiSSPA_Pss_21,
137 DiSSPA_Pss_22 and DiSSPA_Pss_23 and NCPPB 281 was assayed according to Gross and
138 DeVay (1977). Aliquots (5 µl) of bacterial suspension (about 10^8 CFU ml^{-1}) were spotted onto
139 potato dextrose agar (PDA) and maintained at25°C. After four days, plates were oversprayed
140 either with a dense arthrospore suspension of the fungus *Geotrichum candidum*, known for its
141 sensitivity to syringomycin and insensitivity to syringopeptin (Grgurina et al., 1996;
142 Lavermicocca et al., 1997), or with a suspension of *Bacillus megaterium*, a bacterium highly
143 sensitive to syringopeptin and less sensitive to syringomycin (Lavermicocca et al. 1997). For
144 each test three replicated plates were used. The suspension of *G. candidum* and *B.*
145 *megaterium* were obtained from colonies grown on PDA for four days at 25°C. Inhibition
146 was recorded after 48 h at 25°C in the dark.
147
148 *Pathogenicity and host range tests*
149 All *Pss* isolates were tested for their ability to elicit a hypersensitivity reaction by infiltration
150 in Samsun tobacco leaves. The pathogenicity of isolates DiSSPA_Pss_20, DiSSPA_Pss_21,
151 DiSSPA_Pss_22 and DiSSPA_Pss_23 was proven by inoculating detached table grape
152 berries, unripe green lemon fruits, and lilac leaves (Lelliott and Stead, 1987; Klement, 1990).
153 Two-day-old bacterial cells grown on SNA at 25°C were suspended in sterile distilled water
154 and adjusted to an appropriate final concentration as detailed below. Detached berries, fresh
155 lemons and lilac leaves were also inoculated with the type strain NCPPB 281 and sterile
156 distilled water as positive and negative controls, respectively.
157
158 Ten surface-sterilized (NaClO solution 1% for 3 min) berries of four different table grape
159 cultivars ('Red Globe', 'Sweet Globe', 'VRG1' and 'VRG2', two new selections currently under
160 evaluation) were prick-inoculated with a hypodermic needle, placing a droplet of bacterial
161 suspension (10^8 CFU ml^{-1}) at the inoculation site. Inoculated berries were maintained at high
162 humidity on moist filter paper in a sterile sealed plastic container at room temperature till to
163 20 days.
164
165 Lemons were surface-sterilized by dipping into 2% NaClO solution for 2 min, then rinsed
166 with sterile distilled water. Four punctures were made on the fruits (1 mm wide, 3 mm deep),
167 with a sterile hypodermic needle, then 20 µl of bacterial suspension (10^6 CFU ml^{-1}) were
168 placed on each lesion (Lelliot and Stead, 1987). Four lemons for each condition were used.

Inoculated fruits were placed on moist filter paper in a sterile sealed plastic box and incubated at 25°C for a week.

Detached lilac leaves were wounded, according to Ivanović et al. (2017), with a sterile hypodermic needle by pricking at 3-4 points the leaf midrib and areas on the wing of the petiole of fully expanded leaves and placing a 10 µl drop of bacterial suspension (10^6 CFU ml^{-1}). Inoculated leaves and petioles were placed in sterile plastic bags and maintained ten days at room temperature (never exceeding 21°C) and 70–80% humidity. Reactions were assessed 7 and 10 days post inoculation (dpi). All the experiments were repeated twice.

RESULTS

Unexpected and new soft and watery spots on berries (Fig. 1 a) evolving in necrotic spots were observed on bunches of cv Red Globe and VCG1. The season in which these symptoms appeared was particularly humid during blossoming and then hot with broad night/day thermal excursions. Only "levan type" bacterial colonies were isolated from all symptomatic grape berries. All isolates were Gram-negative and on King-B medium produced a green to blue diffusible pigment fluorescent under UV light, as the NCPPB 281 type strain. Data on biochemical, physiological and nutritional characterization as well as data on antimicrobial activity and pathogenicity are summarized in Table 3 and Fig. 1. The four representative grape berry isolates were positive for levan, negative for oxidase, potato soft rot, and arginine dihydrolase and elicited a hypersensitive reaction on tobacco leaves. They, furthermore, produced gelatin and aesculin hydrolysis but not tyrosinase, and, unlike the NCPPB281 type strain, were able to utilize L(+)-tartrate ($GATTa^{\pm}$). Unlike NCPPB 281type strain, the grape isolates did not utilize L-lactic acid. All isolates, however, hydrolyzed arbutin, produced acidity from sucrose, did not reduce nitrates or produce 2-ketogluconate, and were able to use sorbitol, inositol and erythritol, but not D-tartrate.

The antimicrobial activity was assayed *vs B. megaterium* and *G. candidum* on PDA. The grape *Pseudomonas* isolates strongly inhibited *B. megaterium* but were ineffective against *G. candidum,* which indicated the ability to produce syringopeptin but not syringomycin, unlike the NCPPB 281 type strain, which inhibited both microorganisms.

203 To fulfill identification of the four *Pss* isolates (DiSSPA_Pss_20-23) the partial gene
204 sequences of *16S rDNA*, *gyrB*, *rpoB*, and *rpoD* were analyzed and the self-identity between
205 *Pss* strains was 100%. (e_value 0.0, coverage 100%). For all of them, the nucleotide blast
206 analysis of the *16SrDNA*, *gyrB*, *rpoB*, and *rpoD* gene sequences showed, in the order, an
207 identity of 99-100% (e-value 0.0, coverage 100%), with different *P. syringae* pathovars, 99-
208 100% (e-value 0.0, coverage 100%) with *P. syringae* pv. *syringae* and *P. syringae* pv. *aptata*
209 99% (e-value 0.0, coverage 98%) with *P. syringae* pv. *syringae*, *P. syringae* pv. *lapsa* and *P.*
210 *syringae* pv. *atrofaciens* and 98% (e-value 0.0, coverage 99-100%) with *P. syringae* pv.
211 *syringae*, *P. syringae* pv. *aptata*, *P. syringae* pv. *lapsa* and *P. syringae* pv. *atrofaciens*.
212 Generally, lower identities values were observed for other *P. syringae* pathovars as well as
213 other *Pseudomonas* species.

214 Similarly, the phylogenetic analysis confirmed that all the grape berry isolates
215 DiSSPA_Pss_20-23 clad with *Pss* strains isolated by grape (*rpoD*) in Australia and different
216 hosts (*16SrDNA-gyrB-rpoB-rpoD* concatenated sequences) (Fig. 2).

217 All tested isolates were pathogenic to grape. On all VRG1 grape berries, water-soaked lesions
218 and necrosis of tissues were observed around the inoculation site 20 dpi with all the grape
219 isolates DiSSPA_Pss_20-23, and NCPPB type strain, even though symptoms were generally
220 less severe than those observed in the field. Instead, on 'VRG2' and 'Sweet Globe', which
221 were symptomless in the field, the symptoms appeared only on 10-20% of the inoculated
222 berries. Re-isolated colonies exhibited the same morphological, biochemical and molecular
223 traits as those used for inoculation.
224

225 Lemon fruits inoculated with the grape *Pseudomonas* isolates showed within one week black
226 pit lesions, less extensive, but comparable to those observed on fruits inoculated with NCPPB
227 281 type strain. Lilac leaves reacted to the grape *Pseudomonas* isolates and NCPPB 281 type
228 strain with the production of a water-soaked or darkened area, expanding along the mid vein.
229 No symptoms were observed in lemons and lilac leaves used as controls.
230

231 **Discussion**

232 A large number of studies on *P. syringae* have been conducted as this bacterium is used as a
233 model for studying plant–microbe interactions (Alfano and Collmer, 2004; Xin and He, 2013;
234 Hulin et al., 2018). *Ps* is known to be responsible for a large number of disease emergencies

235 (Mohr et al., 2008; Yan et al., 2008; Cai et al., 2011; Diallo et al., 2012; Morris et al., 2013;
236 Bartoli et al., 2015a, b). In fact, considering only this century, new diseases caused by *Ps*
237 have been reported on at least 20 different species of woody plants (Elena et al., 2011; Santini
238 et al., 2013, Lamichhane et al., 2014). In the EPPO region, bacterial canker of kiwifruit
239 caused by *P. syringae* pv. *actinidiae* was first detected in Central Italy in 1992 (Scortichini,
240 1994) where it remained sporadic and with a low incidence for 15 years, becoming very
241 severe in 2007/2008, particularly in the Lazio region, where it induced heavy economic
242 losses, then moved to other kiwifruit-producing regions in the Mediterranean area.
243 On grapevine, *Ps* has been reported as the cause of extensive damage, including necrosis of
244 leaf tissues, shoots, tendrils and rachises (Klingner et al., 1976; Hall et al., 2002), bark
245 necrosis (Cugusi et al., 1986), generically bacteriosis (Samedov et al., 1988), and recently *Pss*
246 has been ascertained to be responsible for bacterial inflorescence rot in Australia (Whitelaw-
247 Weckert et al., 2011). It was proved to be a motile inhabitant of plant surfaces, soil and water,
248 overwintering in woodnd following grapevine dormancy, it can spread across wet surfaces of
249 emerging shoots, leaves and inflorescences (Whitelaw-Weckert et al., 2011).
250 As proven by the fulfilment of Koch's postulates, this is the first report of Pseudomonas
251 bunch rot of grapevine, caused by *P. syringae* pv *syringae*, according to the results of
252 biochemical, physiological, nutritional, antimicrobial activity, pathogenicity tests, and
253 sequencing of *16S rDNA, gyrB*, *rpoB* and *rpoD* genes. Likely, Pseudomonas bunch rot was
254 favored by seasonal weather that was particularly humid during blossoming and then hot with broad
255 night/day thermal excursions.
256 According to the LOPAT test, the four representative grape berry isolates proved to belong to
257 the LOPAT group Ia, *sensu* Lelliott et al. (1966), which includes *P. syringae* pathovars. They,
258 unlike the NCPPB281 type strain and the Pss strains, were able to utilize L(+)-tartrate
259 (GATTa±) but did not utilize L-lactic acid. The LOPAT and GATTa testing schemes were
260 developed to separate *Pss* from other *Pseudomonas* species and *Ps* pathovars, but it can occur
261 that these did not match for all (Scheck et al., 1997).
262 The differences of *16S rDNA* sequences are always very small, and although are effective for
263 the identification of genera, they may be not enough to distinguish strains of the same genus
264 (Yamamoto and Harayama, 1995). Thus, three additional gene sequences for the isolate DNA
265 were examined according to previous works on *Pseudomonas* pathovars identification
266 (Sawada et al., 2009; Whitelaw-Weckert et al., 2011). Recently genome-wide analyses of the
267 effector- and toxin-encoding genes were used to examine the phylogenetics and evolution of
268 pathogenicity amongst diverse strains of *Ps* causing bacterial canker of cherry (*Prunus*

269 *avium*), including pathovars *P. syringae* pv. *morsprunorum* races 1 and 2, *Pss* and *P.*
270 *syringae* pv. *avii*. Phylogenetic analyses of the Italian grape berry *Pss* strains revealed that all
271 were claded and interspersed amongst strains from other host species, providing an example
272 of convergent evolution of pathogenicity (Hulin et al 2018).
273 In pathogenicity tests, 'Red globe' and the new grapevine selection 'VRG1' proved to be
274 much more susceptible than 'Sweet Globe' and 'VRG2'. Likewise, the grapevine bacterial
275 strains differed slightly from the NCPPB 281 type strain. *Pss*_20 was a high syringopeptin
276 producer but in the experimental condition adopted it was unable to produce syringomycin,
277 result in accordance with Lotorre and Jones (1979). Syringomycins and syringopeptins are
278 two classes of necrosis-inducing lipodepsipeptide toxins characterizing *Pss* (Scholz-
279 Schroeder et al., 2001), are produced in infected plant tissues (Fogliano et al., 1999, Grgurina
280 et al., 1997), and they play roles as virulence factors in plant diseases (Scholz-Schroeder et
281 al., 2001). Although both elicit necrotic symptoms in host tissues and are highly phytotoxic,
282 syringomycin was 30 times more active in antifungal activity assays and was also shown to
283 reduce stomata apertures in leaves of *Vicia faba* (Mott and Takemoto, 1989). However, it has
284 been shown that not all strains produce such compounds (Gross and De Vay, 1977; Zeller et
285 al., 1997; Scortichini et al., 2003). Different authors, instead, showed that syringopeptin is
286 more phytotoxic than syringomycin (Iacobellis et al. 1992a, Scholz-Schroeder et al., 2001;
287 Dalla Serra et al. 1999; Hutchison and Gross, 1997), and *Ps* pathovars are known to produce
288 a wide spectrum of secondary metabolites exhibiting phytotoxic capabilities (Bender et al.
289 1999; Gross 1991). Thus, the pathogenic contribution of a phytotoxin depends on the specific
290 host–pathogen interaction. In addition to their phytotoxic effects, these lipopeptides have
291 prominent antibiotic properties exhibiting differential antifungal and antibacterial activity
292 against a variety of microorganisms (Iacobellis et al. 1992a, b; Lavermicocca et al. 1997).
293 They are strongly inhibitory to gram-positive bacteria, particularly *Bacillus* spp.
294 (Lavermicocca et al. 1997). The use of biocontrol agents in the management of bunch rots is
295 increasing (Rotolo et al., 2017). As many of them are lipopetide producers, it can be
296 supposed that, in some way, they can increase the virulence and/or the fitness of *Pss.* This
297 concern is heightened by changes in climate and commercial networks that could intensify
298 emerging epidemics of plant disease in areas well known for their crop vocation. The
299 characterization of the metabolites associated with the bunch rot PSS type will be improved,
300 and studies on the relationship between grapevine cultivars and *Ps* pathovars are under study.
301

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600 **Captation**

601 Fig. 1. (A) Symptoms on Pseudomonas grape bunch rot on 'VRG1' in the field; (B) symtoms on
602 'VRG1'berries 20 day after inoculation (DAI) with Pss_20 (up) and water as negative control
603 (down); (C) patogenicity assay on lilac leaves 10 DAI with Pss_20 (1), NCPPB type strain (2),
604 and water as negative control (3); (D) black pit lesions on lemon fruits 7 DAI with NCPPB type
605 strain (1), DiSSPA_Pss_20 (2), and water as negative controls (3, 4).

606

607 Fig. 2. Phylogeneny of isolates DiSSPA_Pss_20-23 with others *Pss* strains and *Ps* pathovars by
608 using concatenated *16S rDNA*, *gyrB*, *rpoB* and *rpoD* gene sequences (a) and only *rpoD* gene
609 sequences (b). Dendrograms was generated by the UPGMA method. *P. marginalis*
610 (ICMP11289), *P. cichorii* (JBC1), *P. tolaasii* (2192T), *P. fluorescens* (L228), *P. corrugata*
611 (RM1-1-4) and *P. brassicacearum* (DF41) were used as outgroup. Percentage bootstrap values of
612 more than 50% (from 1000 replicates) are indicated at the nodes. *Probably synonyms of *Pss*
613 (Whitelaw-Weckert et al., 2011).

614

615

616

1 **Table 1. Primer used.**

Primers		Target	References
Name	Sequence (5'-3')		
FD1	CCGAATTCGTCGACAACAGAGTTTGATCCTGGCTCAG	16S ribosomal DNA	Weisburg et al., 1991
RD1	CCCGGGATCCAAGCTTAAGGAGGTGATCCAGCC		
Pss_gyrBF	AAGTATCCGGTGGTTTGCAC	*gyrB*	Whitelaw-Weckert et al., 2011
Pss_gyrBR	CAGACCTTCCTGCTCGATGT		
LAPS	TGGCCGAGAACCAGTTCCGCGT	*rpoB*	
LAPS27	CGGCTTCGTCCAGCTTGTTCAG		
Pss_rpoDF	AGAGGCATCCGTGAAGTGAT	*rpoD*	
Pss_rpoDR	CATGATGGCGCGTTCCTG		

2

1 **Table 2. Description of *Pseudomonas* species used in the phylogenetic analyses.**

Species (strain)	Host	Location
Pseudomonas amygdali pv. *morsprunorum* (2341)	*Prunus cerasus*	Hungary
Pseudomonas brassicacearum (DF41)	*Brassica napus*	Canada, Manitoba
Pseudomonas cichorii (JBC1)	*Glycine max*	South Korea
Pseudomonas corrugata (RM1-1-4)	*Brassica napus*	Austria, Graz
Pseudomonas fluorescens (L228)	*Miscanthus giganteus*	Ireland, Carlow, Oakpark
Pseudomonas marginalis (ICMP 11289)	*Actinidia deliciosa*	New Zealand
Pseudomonas savastanoi pv. *phaseolicola* (1448A)	*Phaseolus vulgaris*	Ethiopia
Pseudomonas syringae pv. *actinidiae* (ICMP 9617)	*Actinidia deliciosa*	New Zealand
Pseudomonas syringae pv. *aptata* (ICMP459)	*Beta vulgaris*	USA
Pseudomonas syringae pv. *atrofaciens* (ICMP4394)	*Triticum aestivum*	New Zealand, Auckland
Pseudomonas syringae pv. *cerasicola* (ICMP17524)	*Prunus x yedoensis*	Japan
Pseudomonas syringae pv. *lapsa* (ATCC 10859)	*Triticum aestivum*	Unknown
Pseudomonas syringae pv. *persicae* (NCPPB 2254)	*Prunus persica*	France
Pseudomonas syringae pv. *pisi* (PP1)	*Pisum sativum*	Japan
Pseudomonas syringae pv. *syringae* (642)	*Plantago rugelii*	USA, Blacksburg, VA
Pseudomonas syringae pv. *syringae* (1212)	*Pisum sativum*	United Kingdom
Pseudomonas syringae pv. *syringae* (2339)	*Prunus avium*	Hungary
Pseudomonas syringae pv. *syringae* (A2)	*Pyrus calleryana*	Unknown
Pseudomonas syringae pv. *syringae* (Alf3)	*Medicago sativa*	USA, Laramie, Wyoming
Pseudomonas syringae pv. *syringae* (B64)	*Triticum aestivum*	Unknown
Pseudomonas syringae pv. *syringae* (B301D)	*Pyrus communis*	United Kingdom
Pseudomonas syringae pv. *syringae* (B728a)	*Phaseolus vulgaris*	USA, Wisconsin
Pseudomonas syringae pv. *syringae* (CRAFRU11)	*Corylus avellana*	Italy
Pseudomonas syringae pv. *syringae* (DAR 73915)	*Vitis vinifera* (leaves)	Australia
Pseudomonas syringae pv. *syringae* (DAR 77819)	*Vitis vinifera* (berry)	Australia Tumbarumba
Pseudomonas syringae pv. *syringae* (DAR 77820)	*Vitis vinifera* (rachis)	Australia Tumbarumba
Pseudomonas syringae pv. *syringae* (HR-IW 7924)	*Prunus cerasus*	United Kingdom
Pseudomonas syringae pv. *syringae* (HS191)	*Panicum miliaceum*	Australia
Pseudomonas syringae pv. *syringae* (MW844)	*Vitis vinifera (*rachis)	Australia Tumbarumba
Pseudomonas syringae pv. *syringae* (MW953)	*Vitis vinifera* (shoot)	Australia Tumbarumba

Pseudomonas syringae pv. *syringae* (ICMP3023)	*Syringa vulgaris*	United Kingdom
Pseudomonas syringae pv. *syringae* (SM)	*Triticum aestivum*	USA
Pseudomonas syringae pv. *theae* (NCPPB 2598)	*Camellia sinensis*	Japan
Pseudomonas syringae pv. *tomato* (DC3000)	*Lycopersicon esculentum*	USA
Pseudomonas tolaasii (2192T)	*Agaricus bisporus*	United Kingdom, Reading

2

1 **Table 3: Biochemical, pathological, and antimicrobial characters of the bacterial grape**
2 **berry isolates and *Pss* type strain used.**

Tests	**Grape berry isolates**				***Pss* type strain NCPPB 281***
	DiSSPA _Pss_20	**DiSSPA _Pss_21**	**DiSSPA _Pss_22**	**DiSSPA _Pss_23**	
Gram	-	-	-	-	-
Fluorscence	+	+	+	+	+
Levan (L)	+	+	+	+	+
Oxidase (O)	-	-	-	-	-
Potato soft rot (P)	-	-	-	-	-
Arginine dehydrolase (A)	-	-	-	-	-
Tobacco hypersensitivity (T)	+	+	+	+	+
Gelatin hydrolysis (G)	+	+	+	+	+
Aesculin hydrolysis (A)	+	+	+	+	+
Tyrosinase activity (T)	-	-	-	-	-
L (+)-tartrate (Ta)	+	+	+	+	-
Utilization of					
L-lactate	-	-	-	-	+
D-tartrate	-	-	-	-	-
Sorbitol	+	+	+	+	+
Inositol	+	+	+	+	+
Erythritol	+	+	+	+	+
Arbutin hydrolysis	+	+	+	+	+
Nitrate reduction	-	-	-	-	-
2-ketogluconate production	-	-	-	-	-
Pathogenicity on					
lemon fruit	+	+	+	+	+
lilac leaves	+	+	+	+	+
Inhibition of					
Bacillus megaterium	+	+	+	+	+
Geotrichum candidum	-	-	-	-	+

3 *DiSSPA: Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Università degli
4 Studi di Bari Aldo Moro, Bari, IT; NCPPB: National Collection of Plant Pathogenic Bacteria,
5 Harpenden, U.K.

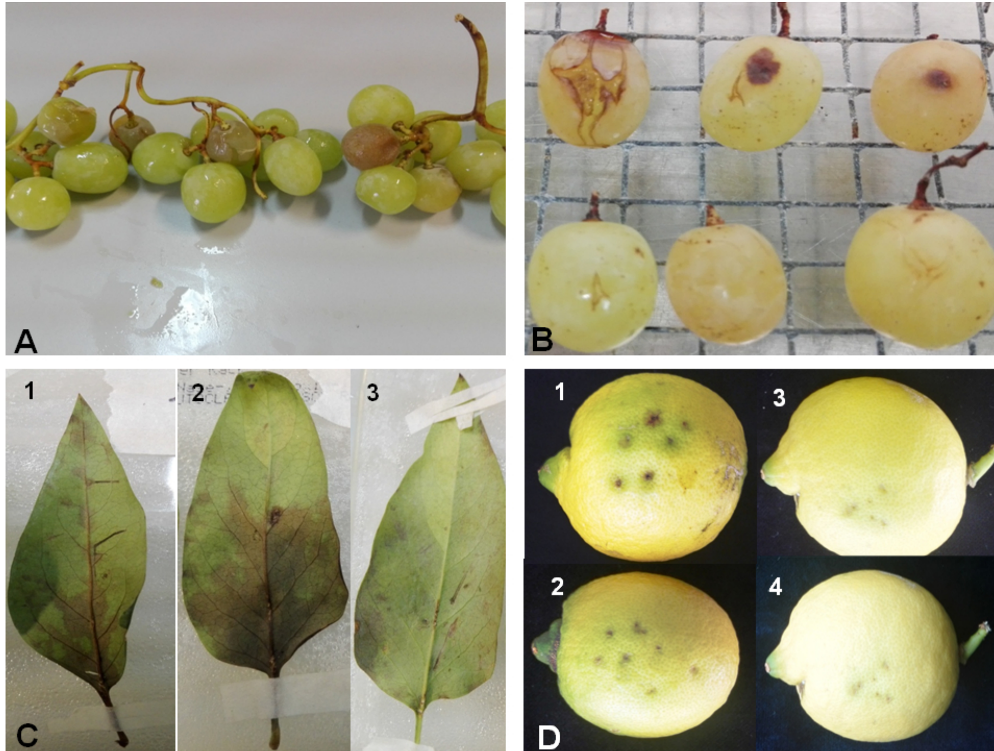

Fig. 1. (A) Symptoms on Pseudomonas grape bunch rot on 'VRG1' in the field; (B) symtoms on 'VRG1'berries 20 day after inoculation (DAI) with Pss_20 (up) and water as negative control (down); (C) patogenicity assay on lilac leaves 10 DAI with Pss_20 (1), NCPPB type strain (2), and water as negative control (3); (D) black pit lesions on lemon fruits 7 DAI with NCPPB type strain (1), DiSSPA_Pss_20 (2), and water as negative controls (3, 4).

254x190mm (96 x 96 DPI)

a

Ps pv. aptata ICMP 459 *
Pss CRAFRU11
Pss Alf3
Ps pv. atrofaciens ICMP 4394 *
Ps pv. pisi PP1 *
Pss SM
Pss NCPPB 281
Pss ICMP3023
Ps pv. lapsa ATCC 10859 *
Pss B64
DiSSPA Pss 20
DiSSPA Pss 23
DiSSPA Pss 21
DiSSPA Pss 22
Pss HS191
Pss B301D
Pss B728a
Pss 1212
Pss 2339
Pss HRIW 7924
Pss 642
Pss A2
Pseudomonas amygdali pv. morsprunorum 2341
Pseudomonas savastanoi pv. phaseolicola 1448A
Ps pv. cerasicola ICMP 17524
Ps pv. actinidiae ICMP 9617
Ps pv. theae NCPPB 2598
Ps pv. persicae NCPPB 2254
Ps pv. tomato DC3000
Pseudomonas marginalis ICMP 11289
Pseudomonas cichorii JBC1
Pseudomonas brassicacearum DF41
Pseudomonas corrugata RM1-1-4
Pseudomonas fluorescens L228
Pseudomonas tolaasii 2192T

0.08 0.06 0.04 0.02 0.00

b

Pss DAR 73915
Pss MW844
DiSSPA Pss 21
DiSSPA Pss 22
DiSSPA Pss 20
DiSSPA Pss 23
Pss SM
Pss DAR 77820
Pss DAR 77819
Pss MW953
Pss NCPPB 281
Ps pv. aptata ICMP 459 *
Pss CRAFRU11
Ps pv. lapsa ATCC 10859 *
Ps pv. pisi PP1 *
Pss ICMP3023
Pss Alf3
Pss B64
Ps pv. atrofaciens ICMP 4394 *
Pss 642
Pss A2
Pss HS191
Pss 2339
Pss 1212
Pss B301D
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Pseudomonas savastanoi pv. phaseolicola 1448A
Ps pv. actinidiae ICMP 9617
Pss NCPPB 2598
Ps pv. persicae NCPPB 2254
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0.06 0.04 0.02 0.00