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1 **From lichens to crops: Pathogenic potential of**
2 ***Pseudomonas syringae* from *Peltigera* lichens is similar to**
3 **world-wide epidemic strains**

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26 **Keywords:** non-agricultural habitat, pathogenicity test, plant pathogen, epidemic strains

27 **Ethics and integrity policies**

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31

32 **Abstract**

33 The presence of bacteria belonging to the *Pseudomonas syringae* complex (*P. syringae*) in
34 the natural vegetation of several Icelandic habitat types has been recently reported, raising
35 questions about the risk to Icelandic crops, particularly given the expected increase in
36 agricultural activity due to climate warming. This study takes advantage of Iceland's unique
37 characteristics and the discovery of *P. syringae* in *Peltigera* lichens to gain a better
38 understanding of the potential risk posed by this newly discovered ecological niche. The main
39 objective is to evaluate the pathogenic potential and fitness in crops of *P. syringae* strains
40 isolated from *Peltigera* lichen sampled in Iceland, focusing on strains that belong to
41 phylogroups 1 and 2, which commonly contain epidemic strains. The results indicate that *P.*
42 *syringae* isolated from Icelandic *Peltigera* lichen have a comparable fitness to epidemic strains
43 in eight out of ten tested plant species (rice, tomato, thale cress, annual mugwort, spinach,
44 garlic chives, tobacco, and kale). Furthermore, pathogenicity assessment on three plant
45 species highlighted that certain strains also caused similar symptoms and disease severity
46 compared to epidemic strains. These findings provide valuable insights into the potential risks
47 posed by *P. syringae* from Icelandic natural habitats and illustrate how strains from these
48 habitats have a wide pathogenic potential to crops without having encountered these crops in
49 the last several thousand years of their presence in Iceland.

50

51 **Introduction**

52 Bacterial strains within the *P. syringae* complex are present in connection with diverse biotic
53 and non-living substrates worldwide. The ability of *P. syringae* to adapt to a wide range of
54 habitats linked to the water cycle is thought to be a driver of its broad host range (Morris et al.
55 2013). This idea is reinforced by the observation that numerous strains from this group of
56 bacteria isolated from non-agricultural environments are phylogenetically closely related to
57 plant-associated strains and have also been shown to be pathogenic on plants such as
58 kiwifruit and tomato (Morris et al, 2019). For this reason, recent research has continued to
59 explore the ecology and pathogenicity of *P. syringae* outside the context of crops.

60 According to Ellis et al. (2010), agricultural land is becoming increasingly prevalent compared
61 to other vegetated areas on Earth. However, Iceland stands out as an atypical region with a
62 limited amount of cultivated land, thereby providing a unique opportunity to study the
63 adaptation of *P. syringae* without a predominant influence of local agriculture. Iceland's flora
64 is characterized by a relatively small number of native species of vascular plants, comprising
65 around 530 species (Wąsowicz 2020). Additionally, the country is home to a diverse range of
66 lichens (755 species) (Kristinsson and Heiðmarsson, 2009) and mosses (around 600 species)
67 (Jóhannsson 2003). However, the vegetation of Iceland evolved in the absence of large
68 herbivores and subsequently is vulnerable to grazing and human activities (Runólfsson 1987).
69 Furthermore, Iceland's position on the border between Arctic and Atlantic waters and air
70 masses, known as the polar front, makes for an interface (Jónsdóttir et al. 2005) which creates
71 favourable climate conditions for *P. syringae* with average temperatures between 2 to 14°C
72 throughout the year (Ogilvie and Jónsson, 2001).

73

74 A lichen is a composite organism resulting from a symbiotic association between a fungus,
75 known as the mycobiont, and one or more photosynthetic partners, termed photobionts. The
76 photosynthetic partners are typically green algae or cyanobacteria. This mutualistic

77 relationship forms a unique structure known as a thallus, which is the visible body of the lichen
78 However, they also harbor internal bacterial communities (Boustie and Grube, 2005; Feuerer
79 and Hawksworth, 2007; Cardinale et al, 2006; Leiva et al, 2021), as well as fungi, sometimes
80 pathogenic for the lichen (Bates et al, 2012; Spribille et al, 2016), archaea (Bjelland et al, 2011;
81 Garg et al, 2016), and viruses (Eymann et al, 2017). While lichens heavily depend on the
82 atmosphere for water intake, their remarkable resilience in challenging environments is partly
83 attributed to the microbiome associated with lichens, playing a crucial role in their survival.
84 (Leiva et al, 2021; Pisani et al, 2011; Bates et al, 2011; Bjelland et al, 2011; Cardinale et al,
85 2012, 2008, 2006; Grube and Berg, 2009; Hodkinson and Lutzoni, 2010; Mushegian et al,
86 2011; Selbmann et al, 2010; Sigurbjörnsdóttir et al, 2015).

87 Previous research has shown that *P. syringae* is prevalent in Iceland on wild vascular plants
88 and moss (Morris et al. 2022), confirmed by the observations of *P. syringae* genes in the lichen
89 metagenome of *Peltigera membranacea* (Sigurbjörnsdóttir et al. 2015; Sigurbjörnsdóttir,
90 2016). This prompted researchers to investigate its ubiquity across several types of plants and
91 lichens in Iceland, hypothesizing that lichens may serve as non-host reservoirs for *P. syringae*
92 (Vilhelmsson et al. 2016). Morris and colleagues (Morris et al. 2022) unveiled how the genetic
93 lines of *P. syringae* in the Icelandic region are monophyletic, indicating that they may have
94 evolved separately from the *P. syringae* populations elsewhere in the world during the
95 relatively short geological history of Iceland. However, these monophyletic haplotypes
96 represent different phylogroups (PGs) (Morris et al. 2022). This illustrates the extraordinary
97 adaptive properties throughout the *P. syringae* complex.

98 *P. syringae* was found in lichens in Iceland, specifically in species of the genus *Peltigera*
99 (Ramírez et al. 2023). To delve deeper into this discovery, a phylogenetic analysis of *P.*
100 *syringae* strains collected from various sources, including lichens, tracheophytes, and moss
101 was conducted. The analyses revealed significant differences among strains between
102 geographical locations, showing a greater similarity of *P. syringae* within a site across all
103 vegetation types rather than within vegetation types across sites. Moreover, *Peltigera* thalli

104 harbored a consistent population density of *P. syringae* although it was lower than that on
105 moss and tracheophyte samples (Ramírez et al. 2023). This finding underscores the
106 adaptability of *P. syringae* to inhabit a diverse range of vegetation beyond higher plants,
107 offering novel insights into its evolutionary dynamics.

108 Assigning *P. syringae* strains to phylogroups based on their citrate synthase gene sequences
109 (Berge et al. 2014) is a useful tool for understanding their phenotypic variations. Many of the
110 strains isolated from *Peltigera* lichens can be assigned to phylogroups PG01 and PG02. These
111 phylogroups contain a wide range of epidemic strains, as documented by Berge and
112 colleagues (Berge et al. 2014). Roughly fifty percent of the lichen thalli were found to host
113 PG01 and/or PG02 strains. Among the *P. syringae* isolates, PG02 included approximately
114 14% of strains and PG01 accounted for a mere 4% of the overall *P. syringae* population
115 derived from *Peltigera*, while the remaining 82% were assigned to the environmental habitat-
116 associated phylogroups PG10 and PG13 (Ramírez et al. 2023).

117 In light of the ubiquity of bacteria in the *P. syringae* complex on vegetation in Iceland and, in
118 particular, the presence of the PG01 and PG02 phylogroups showing high frequencies of
119 strains displaying a functional T3SS (Berge et al. 2014), our goal was to assess the fitness
120 and pathogenic potential – mainly on crops - of strains from PG01 and PG02 from Icelandic
121 *Peltigera* compared to strains in the same phylogroup isolated from epidemics on crops
122 elsewhere in the world.

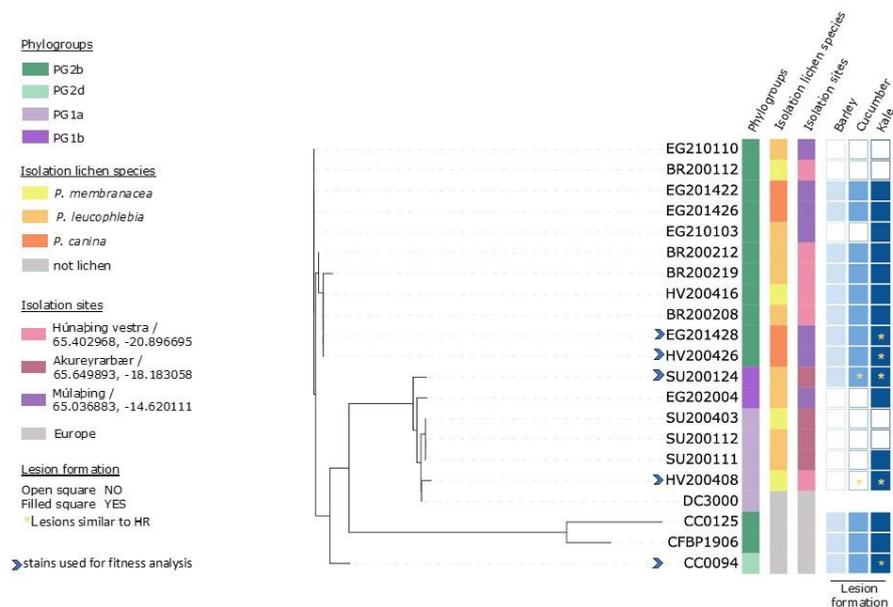
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124 **Materials and methods**

125 **Bacterial strains**

126 *P. syringae* strains belonging to PG01 and PG02 were randomly selected from a collection of
127 strains isolated from *Peltigera* lichen that previously tested positive in a HR test, conducted
128 according to Morris et al, (2007), to represent the range of diversity of these phylogroups
129 associated with this group of lichens (Fig. 1). As positive controls, strains belonging to PG01
130 and PG02 from epidemic occurrences were chosen due to their well-established pathogenic

131 potential and consistent behavior, as revealed by previous work (Morris et al. 2019). For
 132 fitness assessment, the reference strains included CC0094 (PG02d) and Pto DC3000
 133 (PG01a). For the evaluation of pathogenicity, CC0125 and CFBP1906 (PG02b), and CC0094
 134 (PG02d), were employed as reference controls, aligning with the findings outlined previously
 135 (Morris et al. 2000).



136
 137 **Fig.1** Phylogenetic tree illustrating the strains included in the study, along with details on lichen of isolation,
 138 phylogroup classification, site of isolation, and lesion formation in kale, cucumber, and barley. The color code
 139 represents different categories, and an arrow indicates the strains selected for fitness analyses.

141 **Inoculum preparation**

142 Bacterial inoculum was prepared from 24-72 hr growth on King's B (KB) medium (King et al.
 143 1954). A loopful of growth was resuspended in phosphate buffer (TP1: 8,75g of K₂HPO₄ and
 144 6,75g of KH₂PO₄ diluted in 1L of distilled water) and adjusted with a spectrophotometer to 10⁸
 145 CFU mL⁻¹ (OD_{600 nm} = 0.1). The inoculum was further diluted in phosphate buffer, resulting in
 146 a density of 10⁷ CFU mL⁻¹ for the pathogenicity tests and 10⁶ CFU mL⁻¹ for the in planta in the
 147 fitness tests. Inoculum concentration was verified by dilution plating.

148 **Plant material**

149 For fitness tests, plant species belonging to 10 families were used: rice (*Oryza sativa*), tomato
150 (*Solanum lycopersicum*), thale cress (*Arabidopsis thaliana*), annual mugwort (*Artemisia*
151 *annua*), spinach (*Spinacia oleracea*), garlic chives (*Allium tuberosum*), tobacco (*Nicotiana*
152 *tabacum*), kale (*Brassica oleracea*), cucumber (*Cucumis sativus*) and barley (*Hordeum*
153 *vulgare*). Pathogenic potential was evaluated on the last three plant species, which are
154 cultivated in Iceland. Cultivar details, growing conditions, and cultivation dates can be found
155 in Table S2. The duration of cultivation in the greenhouse was tailored to the specific plant
156 species. In the greenhouse environment of Montfavet, France (43.946678, 4.864758) the
157 plants were cultivated during a period spanning from September 2022 to January 2023.
158 Adequate watering was provided in accordance with the plants' needs. Plants were grown in
159 TS3 substrate mixture from Klasmann-Deilmann (Geeste, Germany).

160 **Evaluation of fitness *in planta***

161 Fitness of strains in plants was determined in terms of population growth after inoculation
162 using a modified protocol based on methodologies outlined in Clarke et al.(2010), Donati et
163 al.(2020), and Kim et al. (2022). Leaf tissue was wounded and strains were inoculated into
164 plants by placing a 5 μ L-drop of inoculum (10^6 CFU mL⁻¹), letting the inoculum be absorbed by
165 the plant. An emery board/emery paper was used gently to create a fresh wound on the
166 surface of the leaf. Each plant received a single inoculation, and there were 10 replicate plants
167 per strain.

168 Three to five plants were inoculated with phosphate buffer as negative control for each plant
169 species. For a given plant species, all strains were inoculated simultaneously to facilitate
170 between-strain comparisons. Via a randomized complete block design, the plants were set up
171 in a growth chamber and incubated at 24 °C (14 h light, 10 h dark), under a light intensity
172 between 151- 165 μ mol/s/cm² and 75% humidity.

173 At 1 and 8 days post inoculation (dpi), five leaves were collected from each plant species for
174 each strain, except in the case of *A. thaliana*, where six leaves were collected. Prior to
175 maceration, leaves bearing soil residues were gently cleaned using dry paper. Each leaf was
176 stomached for 2–3 min in sterile phosphate buffer (10 μ L of 0.1 M buffer per mg of fresh plant

177 weight amounting to between 1-30 ml depending on the leaf size) in sterile stomacher bags.
178 Aliquots of serial dilutions of the macerate were plated on KB medium. The plates were placed
179 in a dark environment and incubated at room temperature for a period of 48-72 hours. *P.*
180 *syringae* colonies were identified based on their shape, size, color, texture, elevation, and
181 margin (Morris et al. 2022). The abundance of *P. syringae* in the inoculated leaves was
182 expressed per leaf.

183 **Inoculation, incubation, and disease assessment for pathogenicity assays**

184 We employ a modified protocol inspired by Bartoli et al,(2015) and Cazorla et al, (1998). One
185 leaf per plant was infiltrated with a needleless syringe with 5-10 μL of inoculum (10^7 CFU
186 mL^{-1}). Six to eight replicate plants were utilized per plant species per strain. The negative
187 control consisted of phosphate buffer. Plants were arranged in a growth chamber in a
188 randomized complete block design and incubated at 24 °C (14 h light, 10 h dark), under a light
189 intensity between 151- 165 $\mu\text{mol/s/cm}^2$ and 75% humidity for 14 days.

190 Symptoms were scored at 2, 5, 9 and 14 dpi in terms of the maximum length of the necrosis
191 that developed at the inoculated site except for cucumber that was observed only until 9 dpi.
192 Symptoms were also photographed and described at each scoring date. Strains were
193 classified as pathogenic if the necrotic length was significantly different than the negative
194 control.

195 To verify that symptoms were caused by *P. syringae*, isolations were made from at least three
196 infected tissue replicates per strain. Sections of tissue (at the interface of necrotic and the
197 green surrounding tissue) were aseptically collected and placed on KB plates and incubated
198 at 24°C in darkness. After 2-3 days, colony morphology, colour and fluorescence under 366
199 nm were assessed.

200 **Statistical analyses**

201 Utilizing Microsoft Excel and R Studio, statistical analyses were conducted. This included t-
202 tests, graph plotting with the ggplot2 package, and one-way ANOVA. The t-test was applied
203 to determine the differences between strains inoculated into the same plant species at various

204 time points. Moreover, one-way ANOVA was employed, followed by post hoc Tukey-Kramer
205 analysis to unravel strain statistical differences in necrotic tissue length..

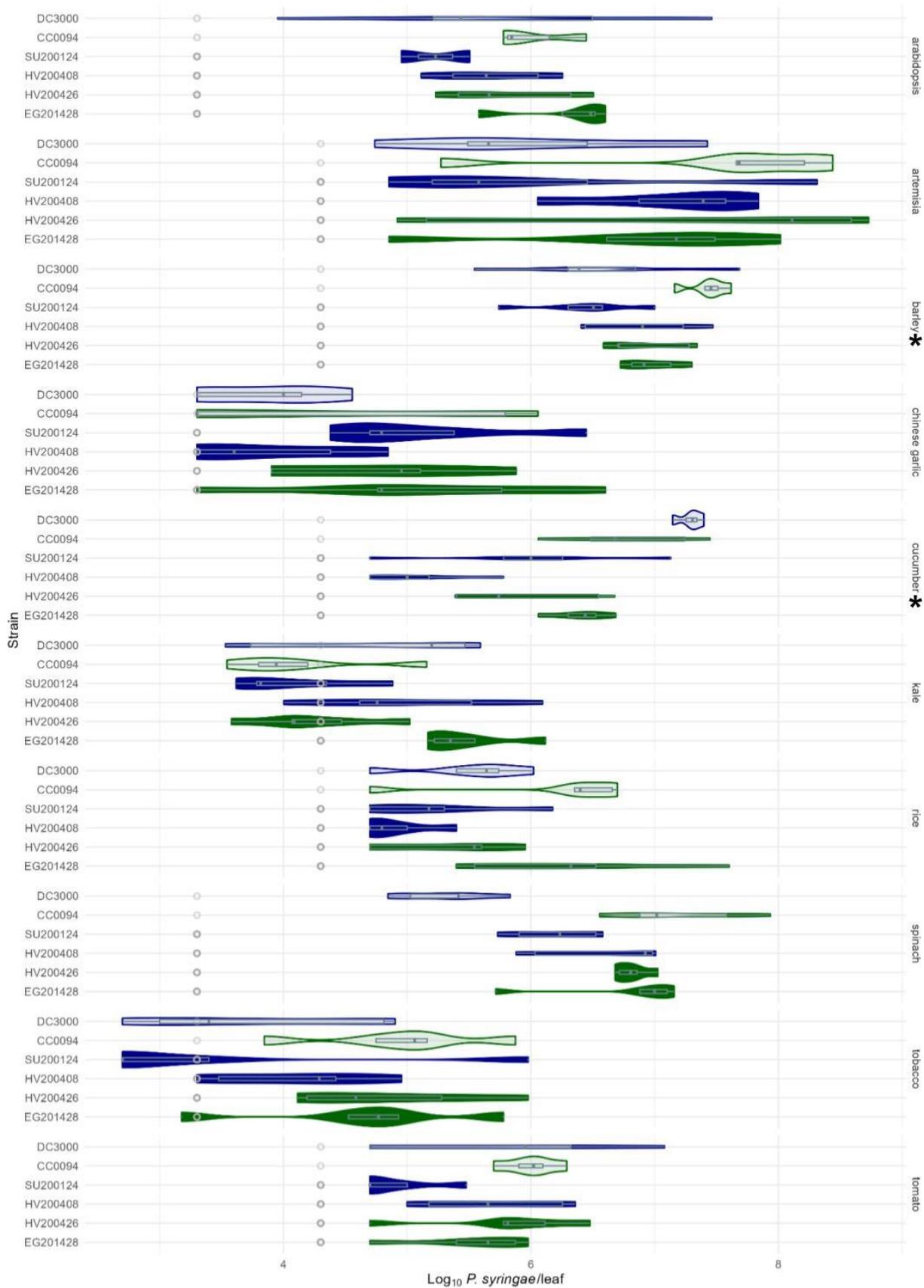
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207 **Results**

208 ***P. syringae* isolated from Icelandic *Peltigera* lichens and epidemic strains have similar** 209 **fitness in plants**

210 The fitness analysis revealed that *P. syringae* strains SU200124, HV200408, EG201426 and
211 EG201428 isolated from Icelandic *Peltigera* lichen (hereafter referred as lichen strains)
212 exhibited overall population growth levels similar to the epidemic strains CC0094 and DC3000
213 at both 1 and 8 dpi under the specified culture conditions across the ten plant species tested,
214 except for cucumber, barley and tomato. Indeed, differences emerged in tomato at 1 dpi, with
215 the population size of *Peltigera* lichen *P. syringae* strains reaching higher densities compared
216 to epidemic ones (Figure S1), although no difference was further observed at 8 dpi.
217 Conversely, despite a similar population density at 1 dpi, the *Peltigera* strains showed a lower
218 population level at 8 dpi compared with epidemic strains in cucumber and barley (Fig. 2). For
219 the latter, however, considering both time points, the comparison of the total population of
220 lichen strains was significantly higher than the population density of epidemic strains (p value
221 < 0.05). This indicates that *Peltigera* strains display, as whole, the same *in-planta* fitness than
222 epidemic strains, with some variability among plant species.

223



225 **Fig. 2** *P. syringae* population in inoculated leaves of ten plant species on the 8th day post-inoculation. Violin and
226 boxplot graphs resume the values of *P. syringae* per leaf for 5 or 6 replicates per strain inoculated in each plant.
227 The Icelandic strains isolated from lichens are represented by a solid color while the epidemic strains used as
228 control are plotted as transparent. PG1 strains are represented by the color blue, while PG2 strains are depicted
229 in green. Grey dots indicate the minimum detection level. Asterisk denotes a plant species where statistically
230 significant differences in *P. syringae* population sizes between epidemic and *Peltigera* strains were observed.

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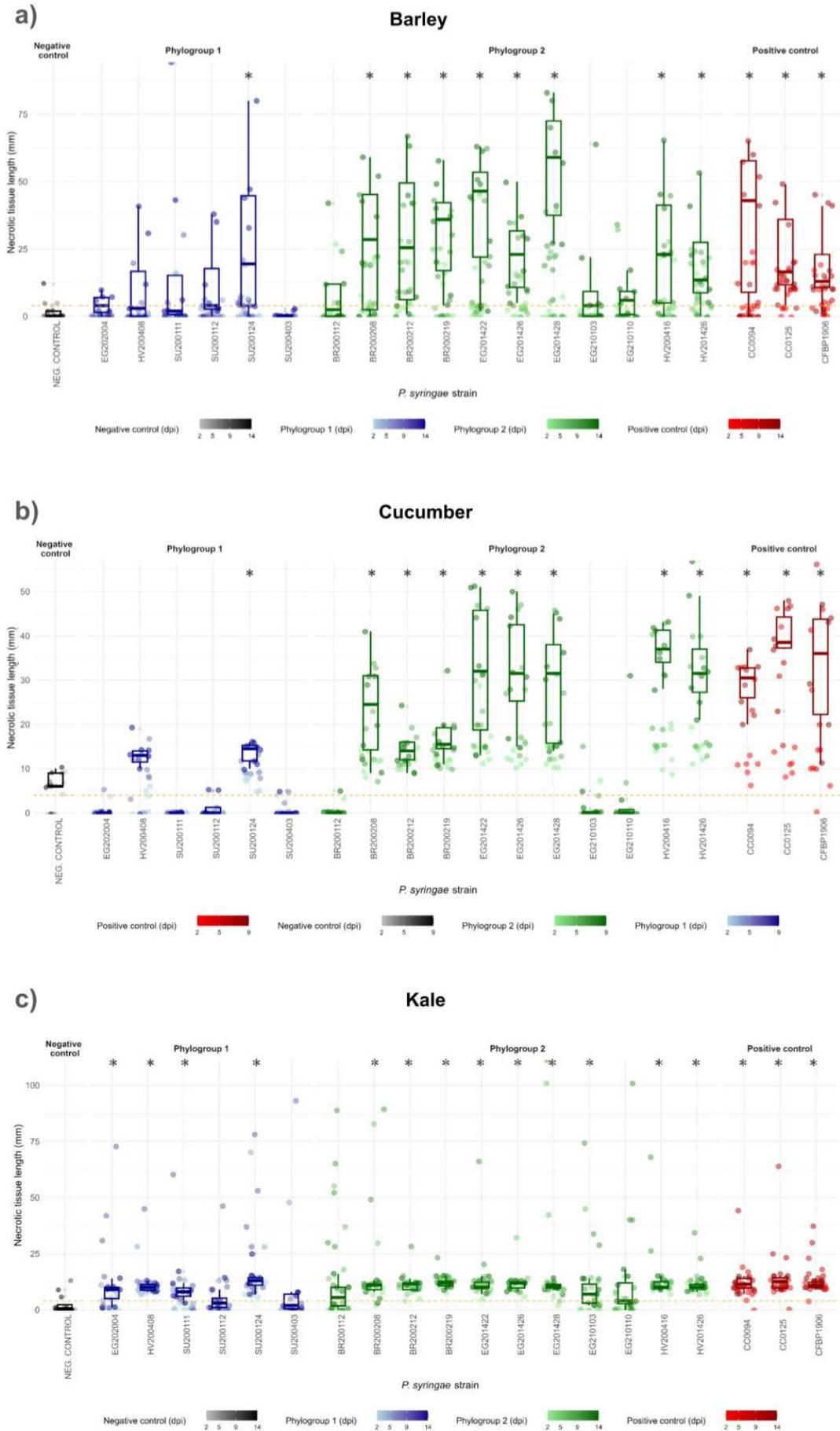
232 Interestingly, each lichen strain behave like the epidemic one belonging to the same
233 phylogroup. Indeed, the main differences were observed more at phylogroup level, with PG02
234 (including CC0094, HV201426 and EG201428) showing higher bacterial densities compared
235 to PG01 (DC3000, SU200124 and HV200408), at 1 dpi only, in 7 out of the 10 analyzed plant
236 species. Such discrepancy, that was not noticed anymore at 8 dpi, suggests a better capacity
237 of PG02 strains to adapt and start growing in plants independently of their origin.

238 On the other hand, when examining strains individually, at 1 dpi, CC0094 consistently showed
239 a greater population size compared with DC3000 in all plant species but *Arabidopsis*, and
240 higher than all strains but HV200426 in tobacco (Fig. 2). Conversely, at 8 dpi, distinctions were
241 observed only in cucumber, with DC3000 having greater population sizes than all lichen
242 strains, and in barley, where CC0094 had a significantly higher population size than SU200124
243 (p-value <0.05) (Fig. 2). Finally, looking at the dynamic of population growth, we observed
244 that, in general, all strains (epidemic and lichen) display a population increase between 1 dpi
245 and 8 dpi in all plant species, except for HV200408 and SU200124 that decreased over time
246 in cucumber (Fig.S2), while no strain but EG210103 did grow in kale between 1 and 8 dpi
247 (Fig.S3).

248 ***P. syringae* from *Peltigera* and from crop epidemics have similar pathogenic potential**

249 For this analysis the set of lichen strains was enlarged to a total of 17, including the 4 strains
250 used for *in-planta* fitness investigation, together with 4 and 9 additional strains belonging to
251 PG01 and PG02, respectively. To account for non-specific reactions of the plants due to
252 wounding, the size of the necroses on plants inoculated with selected strains was compared
253 to those observed on control plants. Nine out of the 17 strains of *P. syringae* isolated from

254 lichen produced necrotic symptoms that were significantly longer than any of the necroses
255 observed on the negative controls in barley (Fig. 3.a) and cucumber (Fig. 3.b), and comparable
256 to the lesions caused by epidemic strains. These same nine *Peltigera* strains and four
257 additional strains, for a total of 13 out of 17, also caused lesions that were significantly longer
258 on kale when compared to damage on control plants, and in the same size range than those
259 observed with epidemic strains (Fig. 3.c).

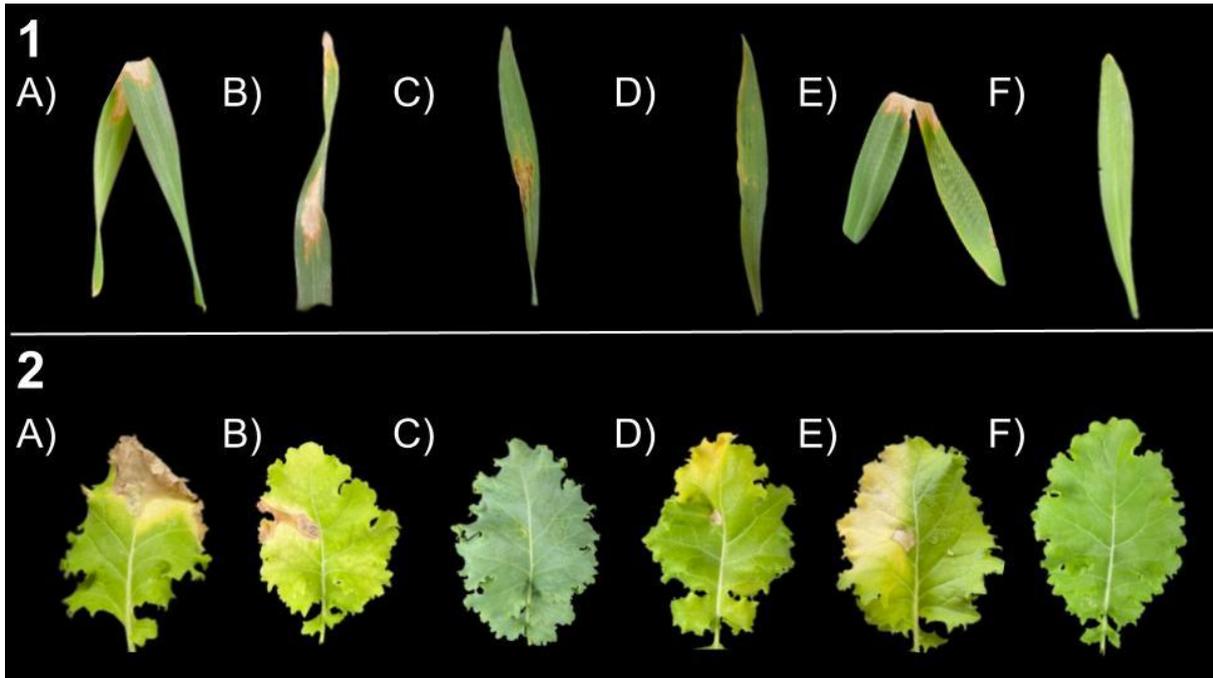


261 **Fig.3** Necrotic tissue length in mm on leaves infiltrated with *P. syringae*. Dots represent all measurements at
262 different time points distinguished by the darkness of the color and the green color correspond to PG1 strains and
263 PG2 was colored in blue while the boxplots summarize the necrotic length on: a) Barley at 14 dpi; b) Cucumber
264 on 9 dpi and; c) Kale on 14 dpi. All plants were incubated at 24 °C (14 h light, 10 h dark) and 75% humidity for up
265 to 14 days. The *P. syringae* strains that exhibit statistical significance from the negative control (p value < 0.05)
266 are marked with an asterisk on this graph.

267

268 According to lesion size, symptoms were most severe on barley and cucumber compared to
269 kale, although we cannot rule out the possibility that such differences could be attributable to
270 technical aspects (e.g, plant growth conditions, plant age). Moreover, not only the number of
271 PG02 strains (7 in barley and cucumber, 9 in kale) causing significant lesions was higher than
272 the number of PG01 strains (1 in barley and cucumber, 4 in kale) but, considering only
273 necrosis-inducing strains, symptoms caused by PG02 strains were overall more severe than
274 those caused by PG01 strains. Indeed, symptom severity (lesion length) in most cases was
275 within the same range as those caused by the strains from crop epidemics (Fig. 3).

276 In barley, in addition to necrosis, other symptoms such as chlorosis, leaf collapse, wilting,
277 water-soaked areas or a slightly pale appearance were also recorded, mainly following
278 infection with necrosis-inducing strains (Fig. 4.1). Similarly, the infiltration of the *Peltigera*
279 strains in kale caused symptoms such as chlorosis, water-soaked areas sometimes
280 surrounded by a yellow halo and pale appearance, besides necrotic lesions (Fig. 4.2). The
281 observed symptoms in cucumber included necrosis, chlorosis, and lesions with a yellow halo
282 (images not available).



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Fig.4 Leaf symptoms at 14 dpi. The leaves represent some of the most common symptoms observed on each plant species. 1. Barley infected with strains a) HV201426; b) EG201422; c) EG201428; d) HV201426 e) Positive control CFBP1906; and f) Negative control. 2. Kale infected with strains a) HV201426; b) EG210110; c) SU200403; d) EG201426; e) Positive control CFBP1906; and f) Negative control.

In some cases, however, necroses resembled hypersensitive-like cell death, *i.e.* occurring shortly after infiltration and apparently localized to the site of infection. In particular, such a phenotype was observed in cucumber inoculated with the *Peltigera* strains belonging to PG01, namely HV200408 and SU200124, which induced smaller lesions, reaching a maximum length of around 15 mm, compared to the lesions induced by PG02 strains with a lesion size comprised between 30 and 40 mm (Fig.S2). Interestingly, the two above-mentioned strains showed a decrease in population size in cucumber 8 dpi compared to 1 dpi, likely related to the induction of HR leading to the restriction of bacterial growth.

The induction of HR-like cell death was also common to numerous strains tested in kale, including the lichen strains HV200408 and SU200124 from PG01, and HV201426 and EG201408 belonging to PG02, together with the epidemic strain CC0094 (Fig.S3). Interestingly, the lesions induced by these strains were already clearly visible after only 2dpi, but did not increase further up to 14 dpi. Considering the absence of bacterial population

302 increase between 1 and 8 dpi in fitness experiments (Fig.2), these results indicate the capacity
303 of these *Peltigera* strains to induce a rapid HR in kale that in turn stop bacterial growth.
304 Conversely, the PG02 lichen strain EG210103 induced significant lesions in kale only after 5
305 dpi and these necroses were characterized by the presence of chlorosis (Fig.S3.b). Moreover,
306 this strain was the only one capable of growing between 1 and 8 dpi in fitness experiments,
307 thus supporting the absence of HR in this case.

308

309 **Discussion**

310 Here we have revealed that *P. syringae* isolated from *Peltigera* lichen in Iceland has fitness
311 and pathogenicity levels comparable to strains isolated from epidemic crops worldwide. As a
312 component of pathogenicity, some *Peltigera* strains were capable of inducing HR. Hence,
313 even though we do not know what the host range would be in a cropping system, the strains
314 from lichens possess a functional system to deliver effectors and to be recognized by a plant
315 as something more than a saprophyte.

316 Numerous studies have demonstrated that *P. syringae* retains its pathogenic capability even
317 in the absence of significant agricultural pressures (Morris et al. 2008; Morris et al. 2013;
318 Morris and Moury, 2019), especially in those strains that have a wider range of habitat. The
319 hypothesis suggests that the pathogenicity of *P. syringae* could be more pronounced and
320 evident in agricultural contexts due to the lack of genetic diversity of the host and some
321 practices for cultivation that might favor a rapid growth of *P. syringae*, even though it can also
322 grow in non-agricultural plants as *Arabidopsis thaliana*, grass and ornamental plants (Jones
323 et al, 1986; Katagiri et al, 2002; Sato et al, 2001). However, the unique context of the Icelandic
324 *P. syringae* strains that have evolved in Iceland for thousands of years makes them an
325 interesting case study (Morris et al. 2022) as it illustrates the ancestral nature of the traits that
326 confer pathogenicity and their maintenance in populations in natural habitats (Xin et al. 2018).

327 Although the pathogenicity observed in controlled conditions do not precisely reflect the
328 pathogenicity of bacterial strains in the field, our results clearly illustrate the pathogenic
329 potential of Icelandic strains, under favorable conditions. Thus, while this suggests the
330 potential threat posed by *P. syringae* in Iceland, it is crucial to keep in mind that *P. syringae* is
331 ubiquitous in the environment. In this light, it is notable that there have been no reported
332 instances of *P. syringae* causing diseases in local crops in Iceland nor to the native vegetation.
333 This highlights the role that environmental factors, such as cold temperatures that reduce the
334 cultivation season or average temperatures under the optimal for *P. syringae* to develop
335 aggressive traits (Bender et al. 1999), likely play in constraining bacterial proliferation and
336 safeguarding plant health. The interplay of environmental conditions significantly contributes
337 to the assessment of disease potential (Morris et al. 2023).

338 *P. syringae* in Iceland has evolved over the past 10000 years, at least, separate from contact
339 with plants that are common to agricultural landscapes such as rice and tomato (Morris et al.
340 2022). Nevertheless, Icelandic strains display some level of fitness in these plants, which could
341 be then considered as novel potential hosts for such stains. This raises intriguing questions
342 about the establishment of host specificity. However, the aggressiveness detected for
343 Icelandic *P. syringae* might be related to the adaptation to environmental habitats, which have
344 been linked to the evolution of their pathogenicity (Morris et al. 2010).

345 As previously demonstrated for *P. syringae* overall (Morris et al, 2019), it has not been possible
346 to make any inference of pathogenic capacity/host range based on the substrate of isolation
347 (habitat or lichen species) or according to the phylogroup of the Icelandic strains (Fig.1). This
348 is also consistent with the observation of the mixing of populations of *P. syringae* between
349 crop and environmental habitats, whereby they are not genetically distinguishable as different
350 populations (Monteil et al, 2016; Morris et al, 2010). Moreover, the lichen strains resemble more
351 the strains of *P. syringae* at a same site than other strains from lichens at distal sites (Ramírez
352 et al, 2023). Thus, overall, these observations demonstrate that the habitat from which a strain

353 is isolated is not necessarily the habitat on which it has spent the most time and via which
354 selection pressures might have dominated.

355 The finding of *P. syringae* raised concerns about a possible threat to Icelandic crops in case
356 the strains isolated showed pathogenic properties. Icelandic cool climate can play an important
357 role in pathogenicity of *P. syringae*, due to tissue damage that intensified by frost episodes
358 and humid environments, as it has been shown that the presence of a layer of free water is
359 essential for infection (Lamichhane et al. 2015). Furthermore, certain studies have indicated
360 that lower temperatures enhance the functionality of T3SS (Type III Secretion System) and
361 T6SS (Type VI Secretion System), which are typically associated with increased
362 aggressiveness (Tribelli and López, 2022; Puttilli et al, 2022). Despite the limited agricultural
363 land in Iceland (Denk et al. 2011), certain crops are cultivated on an industrial scale in
364 greenhouses, with yields exceeding 1000 tones annually. These include tomatoes and
365 cucumbers, while outdoor cultivation involves crops like barley and potatoes. Nevertheless,
366 the anticipation is that the range of plant species grown outdoors and their yields in Iceland
367 will rise over the coming decades due to the predicted rise in temperatures (Parry 1991). The
368 expansion of cultivable land, coupled with the anticipated milder, yet still relatively cold
369 temperatures in Iceland, may pose a potential threat to Icelandic crops.

370 The results of this study are one more example of *P. syringae* strains isolated from
371 environmental habitats, where they are not causing any obvious damage, or where they might
372 even be beneficial (Morris et al. 2013). However, these strains can affect some plant species
373 under specific conditions – conditions that might be increasingly probable with the changing
374 climate and land use in Iceland.

375

376 **Disclosure statement**

377 The authors affirm that the research was conducted without any commercial or financial
378 affiliations that could be construed as potential conflicts of interest.

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513

514 **Supplementary material**

515 **Table S1.** Description of the plant species used in fitness and pathogenicity test with
516 information of the cultivation conditions and time periods.

517 **Figure S1** *P. syringae* population in inoculated leaves of ten plant species on the 1st day post-
518 inoculation.. Violin and boxplot graphs resume the values of *P. syringae* per leaf for 5 or 6

519 replicates per strain inoculated in each plant. The Icelandic strains isolated from lichens are
520 depicted by a solid color while the epidemic strains used as control are represented with
521 transparent filling. PG1 strains are represented by the color blue, while PG2 strains are
522 depicted in green. Grey dots point the minimum detection level. Asterisk denotes statistically
523 significant differences in *P. syringae* abundance between epidemic and *Peltigera* strains.

524 **Figure S2.** Necrotic tissue length in cucumber a) across all strains at 2 dpi. b) in strains
525 displaying HR lesions at 2, 5, and 9 dpi.

526 **Figure S3.** Necrotic tissue length in kale a) across all strains at 2 dpi. b) in strains displaying
527 HR lesions at 2, 5, 9, 14 dpi.