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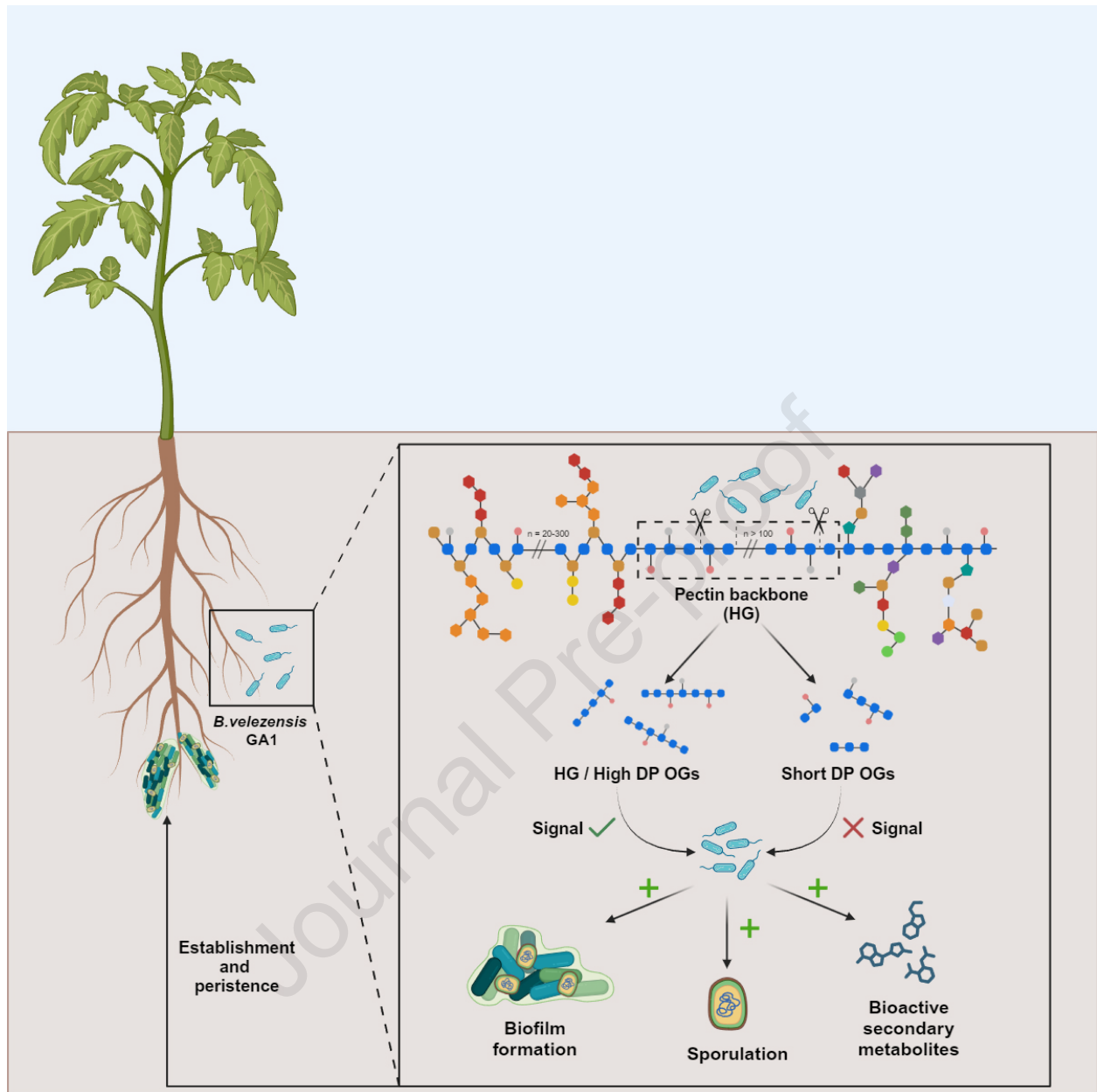
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1 **Pectic homogalacturonan sensed by *Bacillus* acts as host associated cue to**
2 **promote establishment and persistence in the rhizosphere**

3

4 Farah Boubsi^{1*}, Grégory Hoff^{1,2}, Anthony Arguelles Arias¹, Sébastien Steels¹, Sofija Andrić¹, Adrien
5 Anckaert¹, Romain Roulard³, Augustin Rigolet¹, Olivier van Wuytswinkel³, and Marc Ongena^{1,4*}

6

7 ¹ Microbial Processes and Interactions, TERRA Teaching and Research Center, University of Liège - Gembloux Agro-Bio Tech,
8 Gembloux, 5030, Belgium

9 ² Present address : MYCOPHYTO, Grasse, 06130, France

10 ³UMRT INRAE 1158 Plant Biology and Innovation, University of Picardie Jules Verne, UFR des Sciences, Amiens, 80039,
11 France

12

13 ⁴Lead contact

14 *Correspondence : farah.boubsi@uliege.be, marc.ongena@uliege.be

15

16 **Abstract**

17

18 *Bacillus velezensis* isolates are among the most promising plant-associated beneficial bacteria used as
19 biocontrol agents. However, various aspects of the chemical communication between the plant and
20 these beneficials, determining root colonization ability, remain poorly described. Here we investigated
21 the molecular basis of such interkingdom interaction occurring upon contact between *Bacillus*
22 *velezensis* and its host via the sensing of pectin backbone homogalacturonan (HG). We showed that *B.*
23 *velezensis* stimulates key developmental traits via a dynamic process involving two conserved
24 pectinolytic enzymes. This response integrates transcriptional changes leading to the switch from
25 planktonic to sessile cells, a strong increase in biofilm formation and an accelerated sporulation
26 dynamics while conserving the potential to efficiently produce specialized secondary metabolites. As
27 a whole, we anticipate that this response of *Bacillus* to cell wall-derived host cues contributes to its
28 establishment and persistence in the competitive rhizosphere niche and *ipso facto* to its activity as
29 biocontrol agent.

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33 **Key Words:** *Bacillus velezensis*, pectin, homogalacturonan, biofilm, sporulation, bioactive
34 secondary metabolites, plant-bacteria interactions, root colonization

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42 Introduction

43 The rhizosphere is viewed as the narrow interface between plant roots and bulk soil
44 directly influenced by root exudates¹. These root-secreted compounds represent the major
45 nutrient source for carbon-starved soil dwelling microbes and contribute to create a densely
46 populated but spatially limited niche characterized by intense interspecies interactions being
47 neutral, cooperative or antagonistic²⁻⁴. Root exudates thus shape the assembly of microbial
48 communities associated with roots according to the potential of individuals to adapt to the
49 host and to face such highly competitive context. This results in dynamic and complex
50 rhizosphere microbiomes that comprise commensal, pathogenic but also beneficial
51 microorganisms including bacterial species that have evolved as true mutualists establishing
52 intricate interactions with the plant. These bacteria use root exuded chemicals to fuel their
53 catabolism and sustain growth while in turn, they provide beneficial services to their host by
54 promoting growth and/or by augmenting resistance to biotic and abiotic stresses⁵⁻⁷. These
55 beneficial rhizobacteria are usually characterized by efficient root colonization ability and
56 stress tolerance^{8,9}. Moreover, they possess a higher number of genes involved in chemotaxis,
57 motility and biofilm formation compared with bacteria found in the bulk soil^{10,11}.

58 Species of the Bacillaceae belonging to the *Bacillus subtilis* complex are common
59 inhabitants of the rhizosphere and are among the most studied plant-beneficial bacteria
60 (PBB), especially for their positive effects on plant health and growth^{12,13}. It includes *Bacillus*
61 *velezensis*, one of the most promising rhizobacterial species to be used as biological control
62 agent¹⁴⁻¹⁷. This biocontrol activity mainly relies on i) the ability to compete for space and
63 nutrients with the other inhabitants of the rhizosphere, ii) to develop strong direct antagonism
64 towards pathogenic (micro)organisms and iii) to stimulate host immunity leading to induced
65 systemic resistance (ISR)^{12,18}. The expression of these traits correlates with the potential of *B.*
66 *velezensis* to produce a wide range of chemically diverse and functionally specialized
67 secondary metabolites (SMs) acting as signals, siderophores, antimicrobials or immunity
68 elicitors^{19,20}. From an ecological perspective, this arsenal is also considered as an adaptative
69 trait to improve establishment and persistence in the competitive rhizosphere niche.

70 A few recent reports have described how soil bacilli and other plant beneficial species
71 may modulate the expression of developmental traits and adapt the production of SMs upon
72 facing microbial competitors in interspecies interactions²¹⁻²⁵. However, the molecular
73 dialogue occurring with the host plant at the root level also represents a key component of
74 the multitrophic rhizospheric interactions that may influence the behaviour of these
75 beneficials. Certain root-exuded compounds, essentially amino acids, organic acids and to a
76 lesser extent sugars are perceived as host-derived signals by rhizobacteria which in response,
77 move towards the host roots *via* chemotaxis²⁶⁻²⁹. This promotes bacterial cell attachment to
78 the root surface to initiate root colonization by forming biofilms, which are highly-organized
79 bacterial community assembly comprising vegetative cells and spores enclosed in a self-
80 produced matrix^{25,30}. Root exudates have also been shown to modulate SMs production,
81 among which surfactin^{31,32}, and the expression of genes involved in bacillibactin, bacilysin,
82 bacilaene, difficidin, macrolactin, surfactin and fengycin synthesis^{33,34} in diverse *B. velezensis*
83 strains.

84 Another facet of the plant-rhizobacteria interaction, occurring upon direct contact with
85 the host plant, involves the perception of plant cell wall polymers (CWPs). We and others
86 previously showed that the production of the multifunctional lipopeptide surfactin is
87 specifically stimulated upon sensing pectin^{35,36}, and more precisely pectin backbone³², playing
88 a key role in the first steps of root colonization. Moreover, pectin but also arabinogalactan
89 and xylan were shown to promote biofilm formation in several bacilli species^{36–38} through the
90 induction of the expression of biofilm matrix-related genes^{36,37}. In *B. subtilis*, the galactose
91 contained in these polymers is used as a substrate to synthesize matrix
92 exopolysaccharides^{37,39}. However, the phenotypic and metabolite responses of beneficial
93 bacilli to CWPs perception still remain largely uncharacterized.

94 In this study, we investigated the mechanistic underlying the impact of pectin, as first
95 CWP potentially sensed by *B. velezensis* upon contact with root tissues⁴⁰, on the expression of
96 key developmental traits sustaining efficient colonization and on SMs production. We showed
97 that the pectin backbone homogalacturonan (HG) acts as host-associated cue that triggers
98 early biofilm formation, fast sporulation and enhanced production of some specialized
99 secondary metabolites. Our data indicated that such HG sensing contributes to the
100 establishment and persistence of *B. velezensis* cell populations in the rhizosphere *via* a
101 dynamic process involving bacterial pectinolytic enzymes readily active *in planta*.

102

103 Results

104

105 Conserved polygalacturonate lyases contribute to root colonization by *B. velezensis*

106 In this work, the genetically amenable plant-associated strain GA1 (GenBank: CP046386) was
107 used as representative of the *B. velezensis* species⁴¹. We previously showed that GA1
108 possesses two genes encoding polygalacturonate lyases (*pelA*, GL331_08735; *pelB*,
109 GL331_04125) that are conserved with a high nucleotide identity in most strains of the *B.*
110 *velezensis* species which belong to the functional group *amyloliquefaciens* within the *B. subtilis*
111 complex³². These lyases act as pectin degrading enzymes by generating oligogalacturonides
112 from pectin backbone (homogalacturonan), constituting up to 65% of total pectin
113 encountered in the plant primary cell wall^{40,42}. Comparative genomic analysis of the enzymatic
114 content of pectin remodelling and degrading enzymes in other soil-dwelling bacilli reveals that
115 PelA and PelB are the only enzymes potentially involved in pectin degradation/remodelling
116 that are produced by *B. velezensis* (**Table1, TableS1**). On the one hand, it highlights a highly
117 reduced pectinase arsenal in GA1 compared to other saprophytic soil species such as *B.*
118 *subtilis*, *B. paralicheniformis* or *B. licheniformis* (**Table1, TableS1**). On the other hand, none of
119 these enzymes are synthesized by ubiquitous species such as *B. mycoides*, *B. simplex*, *B.*
120 *thuringiensis* or *B. weihenstephanensis* (**Table1**). We assume that such a limited potential to
121 synthesize pectin-degrading enzymes in *B. velezensis* might be linked to its typical plant-
122 associated lifestyle⁴³. In terms of phylogenetic relationship between pectinases of the PL1 and
123 PL3 families found in these soil-dwelling bacilli, the maximum likelihood tree shows low
124 similarity between PelA and PelB of GA1 with pectinases of the other *Bacillus* species (**Fig1A,**

125 **TableS2**). Moreover, PelA and PelB of *B. velezensis* are separated in two different clusters,
126 suggesting notable differences in their structure and function.

127 In the plant cell wall, pectin backbone is mainly low methylesterified, but it can also be found
128 in its highly methylesterified form⁴⁰. Therefore, we wanted to assess whether PelA and PelB
129 may display some substrate specificity regarding the level of methylation of the HG polymer,
130 allowing to distinguish between pectin and pectate lyase activities⁴². To that end, we
131 generated and cultivated the two GA1 mutants $\Delta pelA$ and $\Delta pelB$ in a medium containing the
132 specific sugars, organic acids and amino acids found in root exudates of Solanaceae³¹ (root
133 exudates mimicking medium). Pel enzymes are readily produced in this medium and we
134 measured the activity of the corresponding cell-free supernatants on commercially available
135 homogalacturonan polymer of low ($\leq 5\%$, HG) and high ($\geq 85\%$, HGHM) methylesterification
136 degree. Our data reveal that PelB is moderately but exclusively active on HGHM (**Fig1B**) while
137 PelA is highly and preferentially active on HG (**Fig1C**), suggesting that PelA is a pectate lyase
138 and PelB is a pectin lyase. *In silico* analysis of structural features in the protein sequences of
139 PelA and PelB strongly suggests that the low activity of PelB compared to PelA might be due
140 to the absence of disordered domain(s) (**TableS3**), responsible of structural plasticity and
141 proper exposition of the catalytic site for optimal functioning of the enzyme^{44,45}. Therefore,
142 PelA and PelB thus display complementary lyase activities on HG. In addition, the double
143 mutant $\Delta pelA\Delta pelB$ is fully impaired in its potential to breakdown HG, providing functional
144 evidence in support to genomic data that these two Pels represent the only enzymes involved
145 in HG degradation in GA1 (**Fig1D**).

146 As mutualistic rhizobacteria, one of the key traits of *B. velezensis* isolates relies on their
147 potential to efficiently colonize host roots. We previously showed that GA1 tends to form
148 biofilm structures when colonizing tomato plantlets roots⁴¹. As they represent unique
149 functional pectin-degrading enzymes that have been conserved in the species, we postulated
150 that PelA/B lyases may play some role in the rhizosphere fitness of the bacterium. Monitoring
151 of bacterial population dynamics on tomato roots on solidified Hoagland medium reveals that
152 the mutant $\Delta pelA\Delta pelB$ impaired in lyases production is also significantly affected in its
153 colonization potential compared to the wild type strain (**Fig2A**). In addition, $\Delta pelA\Delta pelB$ is
154 strongly outcompeted by GA1 upon co-inoculation at the same initial cell concentration,
155 further indicating some reduced root fitness related to the loss of PelA/B synthesis (**Fig2B**). In
156 support to the role of these enzymes in the *Bacillus*-plant interaction *in vivo*, we detected
157 substantial amounts of oligogalacturonides released from native pectin extracted from
158 tomato roots (PEC) after treatment with a PelA/B-enriched extract (**Fig2C**). This indicates that
159 these enzymes may effectively alter the pectin polymer naturally found in the plant cell wall.

160 Based on all these data, we assumed that PelA/B are readily produced by the bacterium quite
161 early upon root colonization. In liquid cultures, pectin degrading activity is detected as soon
162 as GA1 cells enter into the exponential growth phase (**Fig2D**), suggesting that quorum sensing
163 might regulate their expression. This population-dependent process plays a key role in various
164 developmental traits driving the fate of cell communities including biofilm formation and *in*

165 *extenso* root colonization^{46,47}. RT-qPCR measurements of the expression levels of *pelA* and
166 *pelB* in a $\Delta comA$ mutant unable to synthesize the major regulator of quorum sensing in bacilli
167 show that *pelA* is not expressed anymore while the expression of *pelB* is markedly repressed
168 compared to GA1 (**Fig2E**). This indicates that these enzymes also differ in their regulation in
169 addition to differences in their activities.

170 Therefore, GA1 may somehow degrade to a limited extent pectin backbone during root
171 colonization via its Pel enzymes, without impairing cell wall integrity since we never observed
172 any adverse effect on plant health and growth caused by this beneficial bacterium. We thus
173 hypothesized that besides root exudates, the bacterium may use oligomers of galacturonic
174 acid (GA) generated from HG degradation as an additional carbon source to sustain growth
175 and thereby, favour root colonization. However, according to KEGG database⁴⁸, the pentose
176 and glucuronate interconversion pathway necessary for the assimilation of oligomers of GA is
177 incomplete in *B. velezensis* (**Fig2F**). Intriguingly, GA1 possesses the complete pathway to
178 metabolize and use GA monomers as carbon source but is unable to generate GA monomers
179 from HG since it does not synthesize polygalacturonases (EC.3.2.1.15) and the other required
180 isomerases. We confirmed this experimentally since GA1 and the mutant $\Delta pelA\Delta pelB$ display
181 a reduced but similar growth rate in minimal medium supplemented with HG while it is
182 significantly higher in presence of GA (**Fig2G**). Taking all these results together, the ability to
183 degrade HG confers to GA1 an advantage for its establishment on roots at the early stages of
184 colonization but without using HG degradation products as an additional carbon source.

185 **Homogalacturonan sensing stimulates early biofilm formation in *B. velezensis***

186 The root colonization potential of *B. velezensis* and related species such as *B. subtilis* correlates
187 positively with their ability to form robust biofilm *in vitro* and *in vivo*^{49–52}. Therefore, we next
188 hypothesized that enhanced fitness of GA1 during root colonization may be due to an
189 improved potential to form biofilm in response to HG or HG degradation products. Based on
190 measurements of biofilm pellicles at the air-liquid interface, we observed an enhanced biofilm
191 formation in presence of HG for both GA1 and the mutant $\Delta pelA\Delta pelB$ compared to
192 unsupplemented control conditions (**Fig3A**). Furthermore, $\Delta pelA\Delta pelB$ responds optimally to
193 HG of high degree of polymerization (DP) by increasing biofilm formation but only moderately
194 to oligogalacturonides with intermediate DP (OG_A). However, it does not respond to low-DP
195 oligomers (OG_B) or GA monomer (**Fig3B, FigS1A** for DP distributions of OG_A and OG_B). This
196 indicates that HG of high DP is responsible for triggering biofilm formation in GA1. To further
197 investigate the dynamics of this phenomenon, we observed the biofilm pellicle formation by
198 microscopy at early stages. In presence of HG, bacterial cells start to agglomerate within a few
199 hours and form long chains reducing their motility (**VideoS1A**), while individual cells remain
200 highly motile in control conditions 7h post inoculation (**VideoS1B**). This earlier shift from
201 motile to sessile behaviour upon HG supplementation correlates with a global repression of
202 genes related to flagellar protein synthesis as revealed by genome-wide RNAseq analysis of
203 the transcriptional reprogramming induced in GA1 within the first 8 hours in presence of
204 pectin backbone (**Fig3C**).

205 In bacilli, biofilm initiation and maturation is related to the production of an extracellular
206 matrix mainly composed of exopolysaccharides (EPS), cohesion proteins (TasA) and
207 extracellular DNA (eDNA), which is essential to hold the bacterial community together within
208 the biofilm structure²⁵. Measurements of the expression level of *epsB* and *tasA* by RT-qPCR on
209 GA1 cells harvested from biofilm at the early stages of formation reveal an overexpression of
210 both genes in the medium supplemented with HG compared to control conditions (**Fig3D**).
211 Our RNAseq data also confirmed that all the genes constituting the operons responsible of
212 EPS and cohesion protein synthesis are up-regulated in presence of HG (**FigS2A**). Interestingly,
213 among genes of the *epsA-O* operon, the protein encoded by *epsE*, in addition of being a key
214 protein involved in EPS synthesis, has been shown to be involved in motility cessation by acting
215 like a clutch on the flagellar motor switch protein FliG^{53,54}.

216 On the other hand, it has been shown that the lipopeptide surfactin acts as signal triggering
217 the expression of *eps* and *tasA* genes^{55,56} and in a previous study, we showed that the
218 production of this compound by GA1 is stimulated in response to HG in the early exponential
219 growth phase³². Therefore, we wanted to evaluate whether this early surfactin production in
220 presence of HG could have an impact on the enhanced biofilm formation observed here.
221 Colorimetric quantification of biofilm formation by crystal violet staining shows that the
222 mutant Δ *srfAA* impaired in surfactin synthesis is strongly affected in its potential to produce
223 biofilm compared with GA1, but still displays a high responsiveness to HG regarding both
224 pellicle formation (**FigS2B**) and biofilm gene expression (**FigS2C**). This indicates that surfactin
225 synthesis stimulation by HG does not markedly contribute to the strongly enhanced biofilm
226 phenotype triggered by the polymer in GA1.

227 Besides EPS and cohesion proteins, eDNA is also required at early stage of biofilm formation
228 and contributes to its 3D architecture⁵⁷. Relative eDNA quantification assay shows a
229 significantly higher eDNA content in pellicles formed in presence of HG compared with the
230 control medium after 24h (**Fig3E**), which is concomitant with a higher number of cells
231 embedded in the biofilm pellicle (**Inset Fig3E**). However, no significant differences were
232 observed in terms of total number of cells in the wells (i.e. planktonic cells and cells embedded
233 in the biofilm) in both conditions (approximately 4.5×10^8 CFU/ml at 24h after inoculation),
234 indicating that the accumulation of eDNA is not due to cell lysis but rather to an active
235 secretion as reported for *B. subtilis*⁵⁸.

236 Accordingly, both macroscopic observations and colorimetric quantification of biofilm show
237 that GA1 pellicle starts to form earlier in presence of HG and gives rise to a thicker biofilm
238 after 24h compared with control conditions (**Fig3F-G**). A similar increase in biofilm formation
239 was also measured upon addition of natural pectin extracted from tomato plantlets roots
240 (PEC) at the same concentration (**Fig3G**). In addition, we observed a significant stimulation of
241 biofilm formation upon addition of HG at concentrations as low as 0.03 g/l (**Fig3H**), suggesting
242 the involvement of a quite sensitive perception system of the polymer in GA1. This would also
243 discard the possibility that HG acts indirectly by creating some osmotic stress if added at
244 sufficient concentration to the medium, which is supported by the fact that the chemical

245 polymer PEG, added at the same concentration, does not trigger any response in GA1 (**FigS1B**).
246 That said, HG of high polymerization degree with low level of methylesterification can form
247 egg-box structures stabilized by calcium bridges⁵⁹ that might favour cell cohesion and
248 contribute to biofilm architecture via a biologically-unrelated process as it has been shown for
249 cellulose in *E. coli* biofilms⁶⁰. Nevertheless, involvement of such a physico-chemical structuring
250 effect can be ruled out since a similar increase in biofilm pellicle formation is observed upon
251 addition of highly-methylated HG, unable to form such supra-molecular conformation (**Fig3I**).
252 Collectively, these data strongly suggest that pectin perception underpins a signalling process
253 through which bacterial cells actively detect homogalacturonan fragments with a minimal
254 length for triggering biofilm establishment.

255 **Homogalacturonan sensing enhances the sporulation dynamics in *B. velezensis***

256 It is commonly assumed that during biofilm formation, *Bacillus* enters into a process of cell
257 differentiation, leading to the coexistence of vegetative cells, endospores and spores into the
258 same multicellular structure⁶¹. Within the population colonizing tomato roots, we observed
259 that a majority of cells tends to rapidly differentiate into spores, representing more than half
260 of the total population after 3 days and around 90% after 6 days (**Fig4A**). This prompted us to
261 also investigate the possible impact of HG recognition on sporulation as key developmental
262 trait. We set up a method to monitor the sporulation dynamics of GA1 cells by flow cytometry
263 using Redox Sensor Green dye (RSG) as a marker of cellular activity and cell size as proxies (See
264 STAR method and **FigS3A**). A strong increase in spore population is observed upon
265 supplementation of liquid cultures of GA1 with both PEC or HG compared with the control
266 medium after 48h (**Fig4B**). We next investigated the dynamics of spore formation in GA1
267 population between 24h and 48h, this time frame corresponding to the end of the exponential
268 growth phase/beginning of the stationary phase in our culture conditions. We observed that
269 sporulation is triggered earlier and faster in GA1 cells upon HG sensing, allowing the biomass
270 to remain constant while it sharply decreases in control conditions (**Fig4C**). As observed for
271 biofilms, the response of the bacterium regarding sporulation in presence of HG is DP-
272 dependant. Undegraded HG and OG_A induce a strong increase in spore population in the
273 $\Delta peIA\Delta peIB$ mutant while OG_B and GA monomer display a limited or no effect respectively
274 compared with the control conditions (**Fig4D**).

275 The initiation of sporulation in bacilli is regulated by the master transcription factor Spo0A,
276 depending on its abundance and phosphorylation state⁶². The active form Spo0A-P is indeed
277 responsible of the direct regulation of hundreds of genes involved in the sporulation
278 mechanism. It includes repression of the global transition state regulator AbrB, known to
279 influence the expression of a large number of genes at late growth phase^{63,64}. We therefore
280 anticipated that differences in expression of both *spo0A* and *abrB* genes should be induced in
281 response to HG before the release of the first mature spores observed after 30h. Accordingly,
282 RT-qPCR measurements show a significant increase in *spo0A* expression and a concomitant
283 repression of *abrB* in presence of HG in cells collected after 24h (**Fig4E**). The strong repression

284 of *abrB* in presence of HG thus reflects a higher cellular concentration of Spo0A-P, necessary
285 to initiate the sporulation mechanism.

286 Next, we wanted to verify if enhanced sporulation was also triggered by HG in biofilm pellicles
287 of GA1. We analysed by flow cytometry the population diversity of cells embedded in biofilm
288 pellicles of GA1 and we observed that cells also sporulate faster upon HG supplementation,
289 with a 8.5 fold increase of mature spore population in the 48h-pellicle compared with the
290 control medium. As a consequence, the bacterial population in the mature biofilm in presence
291 of HG is almost exclusively constituted of spores after 120h (**Fig4F**). Still, such a higher
292 sporulation dynamics could be an indirect effect of HG rather than a direct effect of its
293 perception since it has been shown that the sporulation rate of *B. subtilis* NCIB 3610 increases
294 when the bacterium produces exopolysaccharides⁶⁵. However, sporulation is also significantly
295 increased in the non-EPS producing mutant $\Delta epsA-O$ upon HG supplementation (**Fig4G**),
296 confirming that HG perception has a direct effect on the sporulation mechanism.

297 **Homogalacturonan sensing impacts the production of bioactive secondary metabolites in *B.*** 298 ***velezensis***

299 We also investigated the possible modulation of SMs production by *B.velezensis* in response
300 to HG since these small-size chemicals contribute to the rhizosphere fitness of the bacterium
301 and play key roles in its biocontrol activity^{14,15,66}. We first performed an untargeted profiling
302 of metabolites secreted by planktonic GA1 cells in liquid cultures supplemented or not with
303 HG. Cell-free extracts collected at stationary growth phase were analysed by UPLC-qTOF-MS
304 and data processing with MZmine2⁶⁷ allowed us to detect features and the corresponding
305 compounds differentially produced upon HG sensing. Beside unknown hits, we could identify
306 some non-ribosomal lipopeptides (LPs) and polyketide (PKs)-type metabolites with production
307 modulated in response to HG but in a specific way depending on the family (**Fig5A**). The
308 production of the surfactin- and iturin-type LPs and to a lower extent, the PKs bacillaene and
309 difficidin, are significantly increased in presence of HG while fengycins and macrolactins were
310 not or negatively impacted respectively (**Fig5A-FigS4A**). However, due to specific
311 transcriptional regulations, the kinetic of synthesis of these metabolites differs depending on
312 the growth phase, which means that the amplitude and timing of the stimulating effect of HG
313 can vary according to the molecule and sampling time. Thus, we decided to refine the time
314 course analysis focusing on the LPs as key compounds widely produced by several plant-
315 beneficial *Bacillus* species⁶⁸. We observed a pronounced early stimulation of surfactins
316 production (**Fig5B left**) as previously described³², which correlates with an enhanced
317 expression of the *urfAA* gene, one of the genes encoding for the surfactins synthetase (**Inset**
318 **Fig5B left**). However, our data also reveal a clear stimulation of iturins and fengycins
319 production upon HG supplementation (**Fig5B middle and right**), which is concomitant with a
320 higher expression of the related biosynthesis genes *ituC* and *fenC* (**Inset Fig5B middle and**
321 **right**). This phenomenon is independent of pectin degradation products since the stimulation

322 is also observed in cultures of $\Delta peIA\Delta peIB$ cells upon HG supplementation as shown for iturins
323 production (**FigS4B**).

324 We also wondered whether the acceleration of sporulation dynamics in GA1 upon HG
325 perception has an effect on the potential of the bacterium to efficiently synthesize these
326 bioactive SMs when it forms thick spore-enriched biofilms. UPLC-qTOF-MS profiling of LPs
327 produced by mature biofilm of GA1 with and without HG added to the medium reveal similar
328 amounts of iturins, fengycins and surfactins in both conditions (**Fig5C**). Therefore, the faster
329 increase in spore population in biofilm triggered upon HG perception does not impair the
330 ability of the bacterial community to efficiently synthesize these SMs. This can be explained
331 considering the higher production rate of iturins, surfactins and fengycins by active cells in the
332 population even if the relative proportion of these vegetative cells is lower in presence of HG.

333

334 Discussion

335 According to our current knowledge, the molecular dialogue between beneficial rhi-
336 zobacteria and their host plant is mainly mediated by small-size diffusible compounds^{32,69,70}.
337 However, signaling that may occur upon direct contact remains relatively unknown. In this
338 work, we demonstrate that pectin backbone homogalacturonan (HG) is perceived as a host
339 cue by the beneficial species *B. velezensis*, which in turn, readily stimulates key developmental
340 traits such as biofilm formation, sporulation, but also production of various bioactive second-
341 ary metabolites with specific functions. Our data come in support to the few studies mainly
342 performed on *B. subtilis* reporting that some plant cell wall polysaccharides may act as trigger
343 of biofilm formation^{32,36-39}. However, we provide a more comprehensive picture of the multi-
344 faceted response mounted by the typical plant-associated *B. velezensis* aiming at increasing
345 its fitness and ensuring its persistence in its natural competitive ecological niche.

346 We found that the potential of *B. velezensis* to perceive and respond to HG is asso-
347 ciated with its ability to secrete two pectin/pectate lyases, constituting its very limited arsenal
348 of pectin remodeling and degrading enzymes (**Table1-Tables1**). These Pels contribute to bac-
349 terial fitness since depleted mutants are significantly affected in root colonization (**Fig2A-B**).
350 *B. velezensis* can feed on galacturonic acid, the constitutive monomer of HG, only if it is already
351 available in the rhizosphere due to its uncomplete pathway to assimilate HG degradation
352 products (**Fig2F-G**), in contrast with microbial plant pathogens generating and consuming
353 monomers from HG directly encountered in the plant cell wall^{71,72}. From an ecological point
354 of view, the presence of such enzymes in the genome of beneficials raises questions. Indeed,
355 the ability to synthesize active plant cell wall degrading enzymes, which are very abundant in
356 phytopathogenic microorganisms, is considered as one of the main traits responsible of their
357 virulence⁷²⁻⁷⁴. Therefore, it is tempting to speculate that such mutualistic bacteria living in
358 intricate association with roots might have co-evolved with their host, leading to a decrease
359 of their arsenal of pectin degrading enzymes to be tolerated by the plant. We assume that the
360 role of Pels in colonization of plant roots by *B. velezensis* relies on the fact that their activity

361 may either facilitate accessibility to the HG backbone for the bacterium without being detri-
362 mental to the host plant and release some high-DP OGs from the complex polymer, to which
363 the bacterium is able to respond. A reduced arsenal of pectin degrading enzymes, with mod-
364 erate activity, could also be used to weaken cell-to-cell adhesion by gently degrading pectin
365 of the middle lamella to colonize intercellular spaces in root tissues as it has been shown for
366 *Enterobacter sp.* SA187⁷⁵.

367 The ability of bacteria to form robust biofilms in their natural ecological niche is con-
368 sidered as an essential adaptive trait. In the case of rhizobacteria, it facilitates attachment to
369 the root and efficient colonization, which is required to exert their beneficial effects on the
370 host plant^{25,30,51,76,77}. Here we showed that, in *B. velezensis*, this process is favoured by the
371 ability of the bacterium to sense HG and high-DP OGs (**Fig3B**). Early biofilm pellicle formation
372 triggered by HG (**Fig3F**) correlates with transcriptional activation of genes playing key roles in
373 biofilm initiation (i.e. EPS and cohesion proteins synthesis) and concomitant repression of
374 genes involved in motility (**Fig3C-D**), further illustrating the trade-off and incompatibility of
375 both processes as reported in *B. subtilis*⁷⁸. Moreover, we detected a higher eDNA content in
376 the biofilm pellicle upon HG sensing (**Fig3E**), which can contribute to biofilm robustness since
377 eDNA is known to interact with EPS to promote cell adhesion⁵⁷. Besides ensuring cell cohesion
378 and adhesion to surfaces, which are two traits governing biofilm stability⁷⁹, EPS and the hy-
379 drophobin layer of the biofilm also act as a shield to protect the cell community within the
380 biofilm from external stresses and against toxins and/or infiltration by competitors^{25,80,81}. This
381 confers an advantage to *B. velezensis* in terms of ecological fitness in the highly competitive
382 rhizosphere niche where microbial warfare dominates.

383 Cell differentiation is inherent to biofilm formation and guarantees the survival of
384 the community and proliferation of the species in response to adverse abiotic conditions or
385 microbial competitors⁸²⁻⁸⁵. Biofilm formation and sporulation can be seen as interlinked pro-
386 cesses since both are controlled by the same global regulators^{62,86,87}. Interestingly, our data
387 show that the sporulation dynamics of GA1 cells is highly accelerated upon HG sensing both
388 in biofilms and in cells in planktonic cultures (**Fig4B, Fig4F**), independently of EPS synthesis
389 (**Fig4G**) indicating that it results directly from HG perception inducing transcriptional changes
390 in key regulators genes of sporulation such as *spo0A* and *abrB* (**Fig4E**). Via an enhanced spor-
391 ulation, GA1 also avoids the strong decrease in population observed at the end of the expo-
392 nential growth phase in liquid cultures (**Fig4C**). Bacilli are known to exhibit a social behaviour
393 called cannibalism when they face nutrient starvation during which a portion of the popula-
394 tion feed itself on the other to survive⁸⁸⁻⁹¹. Therefore, by enhancing sporulation dynamics
395 upon HG perception, GA1 might hijack this phenomenon and allow a greater persistence of
396 cells on plant roots. A prompt formation of spore-enriched biofilms should globally favour the
397 establishment of robust populations on roots.

398 The secretion of SMs is also one of the factors contributing to the modulation of the
399 fitness of the bacteria in the highly competitive rhizosphere and *B. velezensis* is one of the
400 most prolific species regarding this aspect¹⁴⁻¹⁷. In this work, we observed that HG sensing also
401 results in modulation of the secondary metabolome of GA1 (**Fig5A-B, FigS4A**). As recently

402 reported, it includes an increased synthesis of the cyclic LPs surfactins, favouring biofilm
403 formation and motility and priming the plant immune system leading to systemic
404 reinforcement of the host against phytopathogens^{32,92}. Iturins and fengycins are other
405 lipopeptides boosted upon HG perception, mainly described for their strong antifungal activity
406 against a wide range of phytopathogens^{15,19} but also reported as elicitors of plant systemic
407 resistance^{93–96}. PKs synthesis of the bacillaene and difficidin families, with broad-spectrum
408 antibiotic activities against gram-negative bacteria^{97–101}, is also enhanced in response to HG.
409 Enhanced production of these SMs is thus of clear benefit for plant health and biocontrol.
410 However, in the rhizosphere as in any other populated environment, microbes dwell in a
411 context of competitive interactions where antibiotics are raised as weapons for chemical
412 warfare^{12,68}. The changes occurring in the secondary metabolome described here may result
413 in an enhanced global antimicrobial potential of *B. velezensis* as we reported recently upon
414 sensing bacterial competitors in interspecies interactions²¹. Importantly, our data also show
415 that efficient production of these bioactive metabolites by *B. velezensis* is still occurring in
416 spore-enriched biofilms (**Fig5C**) and therefore in multicellular communities developing on
417 roots of its host on which the bacteria tend to rapidly differentiate into spores (**Fig4A**).

418 That said, the molecular basis underpinning HG perception by bacilli cells is still
419 unclear. Our data show that the bacterial response is not indirectly triggered by some physico-
420 chemical cues such as structuring effect or osmotic stress that may have been caused by the
421 addition of the polymer (**Fig3I, FigS1B**). Moreover, *B. velezensis* responds to relatively low
422 concentrations of HG (**Fig3H**) but also does not react to short-DP OGs and GA monomer,
423 suggesting some sensitive recognition system triggered selectively by high DP HG of a minimal
424 length. Therefore, we assume that polymer sensing may rely on a specific receptor-based
425 recognition process as it has been reported for *Sphingomonas spp*¹⁰², but further investigation
426 is required to identify such putative receptor. The membrane-anchored histidine kinases
427 (KinA-E) are good candidates since in *B. subtilis*, they are activated in response to a range of
428 environmental cues including potassium leakage induced by surfactin²², plant
429 polysaccharides³⁷ and potentially oxygen, light and redox potential^{103,104} which results in
430 phosphorylation of the master regulator Spo0A acting on both biofilm formation and
431 sporulation, according to the level of its phosphorylation state^{62,85}. However, whether such
432 Kin system is involved or not in the perception of HG by *B. velezensis* remains to be
433 established.

434 In conclusion, perception of HG as host-associated cue underpins a new aspect of
435 interkingdom molecular interactions between plants and their bacterial associates such as *B.*
436 *velezensis*. Using HG as signal allows *Bacillus* to eavesdrop its host, establish and proliferate in
437 the rhizosphere. Improved fitness in terms of root colonization and persistence means a
438 higher niche competition and establishment of threshold populations necessary to produce,
439 in biologically relevant amounts, its unique arsenal of antimicrobials and plant immunity elic-
440 itors, all of them contributing to a better protection of the plant against pathogens and *in fine*
441 to biocontrol. So beyond the interest in microbial chemical ecology in the broad sense, this

442 knowledge is essential for optimizing the use of *B. velezensis* as environmentally robust inoc-
443 ulant in sustainable crop production.

444

445 Limitations of the study

446

447 In this study, we highlight the importance of HG as major constituent of pectin in the context
448 of plant-bacteria interactions. However, the mechanism underlying the perception of this
449 polymer as molecular pattern by *B. velezensis* remains to be determined, including the
450 identification of potential specific receptor at the bacterial cell surface. Moreover, it could be
451 relevant to study if this ability to perceive and respond to such plant cell wall polymer is also
452 found in other bacterial species and genera commonly used as PGPR. Biocontrol assays would
453 also be interesting to assess if the stimulation of lipopeptide production by *B. velezensis* upon
454 HG supplementation may lead to higher protection of the host plant against the attack of
455 phytopathogens. Finally, it would be relevant to extend the scope of this study and further
456 investigate the impact of the application of exogenous HG at the plant microbiome level.

457

458 Author contributions

459

460 F.B and M.O conceived, designed and coordinated the project. F.B, G.H, A.A.A, S.S, S.A, A.A,
461 R.R, A.R and O.VW generated materials, performed experiments, and/or analysed data. F.B
462 and M.O wrote the manuscript. O.VW, A.A and G.H revised the manuscript. O.VW and G.H
463 were involved in the discussion of the work.

464

465 Main figure titles and legends

466

467 **Figure 1. General characterization of *B. velezensis* pectin degrading enzymes PelA and PelB.** (A) Phylogenetic relationship of
468 HG degrading enzymes between PelA and PelB of *B. velezensis* GA1 and archetype strains of other soil-dwelling bacilli. (B and
469 C) Time-course measurement of pectin degradation activity of cell-free culture supernatants of (B) *B. velezensis* GA1 Δ pelA
470 and (C) Δ pelB mutants on HG (light gray bars) and HGHM (dark grey bars) (Mean \pm SD, n=3). (D) Kinetics of oligogalacturonides
471 released over time from HG by 24h-cell-free culture supernatants of GA1 (black line) and the mutant Δ pelA Δ pelB (light grey
472 line). See also Table S2 and S3.

473 **Figure 2. Conserved polygalacturonate lyases contribute to root colonization by *B. velezensis*.** (A and B) Time-course
474 measurement of (A) GA1 (white bars) and Δ pelA Δ pelB (grey bars) populations on tomato plantlets roots and (B) upon co-
475 inoculation (50:50) on tomato plantlets roots (Mean \pm SD, n=3 for (A) and n=7 for (B), t-test; ns, non-significative; *, P<0.05;
476 ****, P<0.0001). (C) Schematic representation of pectin degradation products generated by Pels enzymes from pectin
477 extracted from tomato plant roots (PEC, Created with biorender.com). Column chart represents simple sugar composition
478 (expressed as molar ratio percentages) of PEC fraction. Line graph represents the kinetics of oligogalacturonides released over
479 time from PEC fraction by cell-free culture supernatants of GA1. (D) Time-course measurement of pectin degrading activity of
480 cell-free culture supernatants of GA1 (bars, left axis) regarding bacterial growth (line, right axis) (Mean \pm SD, n=3). (E)
481 Expression pattern of pelA (up) and pelB (down) in GA1 (white bars) and Δ comA mutant (grey bars) at early growth stages
482 (Mean \pm SD, n=3, t-test; *, P<0.05; **, P<0.01; ***, P<0.001; ****, P<0.0001). (F) Simplified pathway of HG degradation and
483 assimilation (pentose and glucuronate interconversion, adapted from KEGG pathway database^{A8}). Enzymes encoded and not
484 encoded in GA1 genome are marked in green and red respectively. (G) Growth profile of GA1 and Δ pelA Δ pelB mutant on
485 minimal medium supplemented with 0.2% (w/v) HG (black and grey line respectively) or GA (green and red lines respectively)
486 (mean \pm SD, n=3). Growth rate in each condition is indicated next to the corresponding curve and was determined by calculating
487 the slope of the linear part of $\ln(OD_{600})$.

488 **Figure 3. Homogalacturonan sensing stimulates early biofilm formation in *B. velezensis*.** (A and B) Assessment of biofilm
489 formation at the air-liquid interface by (A) GA1 and Δ pelA Δ pelB in the control medium (white bars) and upon supplementation

490 with 0.1% (m/v) HG (grey bars) in a 96-well microplate by crystal violet staining and (B) by GA1 upon supplementation with
 491 0.1% (m/v) HG of different DP (HG, mean DP \approx 170³²; OG_A, mean DP=13; OG_B, mean DP=4; GA, DP=1) (Mean \pm SD, n=8, Tukey's
 492 multiple comparisons, α =0.05). (C) Heatmap representing differential expression of genes involved in motility in GA1 upon
 493 supplementation with 0.1% (m/v) HG normalized to the control medium after 2.5, 5 and 8h of culture. Green and red shadings
 494 represent respectively higher and lower relative expression level compared with the control medium. (D) Expression pattern
 495 of *epsB* (left) and *tasA* (right) genes in GA1 in the control medium (white bars) and upon supplementation with 0.1% (m/v) HG
 496 (grey bars) at early biofilm formation stages (Mean \pm SD, n=3, t-test; **, P<0.01; ***, P<0.001). (E) Time-course measurement
 497 of eDNA content in biofilm pellicles of GA1 formed at the air-liquid interface in the control medium (black line) and upon
 498 supplementation with 0.1% (m/v) HG (grey line) (Mean \pm SD, n=8, t-test; ****, P<0.0001). Inset: Time-course measurement of
 499 the number of cells embedded into the biofilm pellicle in the control medium (white bars) and upon supplementation with
 500 0.1% (m/v) HG (Mean \pm SD, n=3, t-test; *, P<0.05; **, P<0.01). (F) Macroscopic observation of the kinetic of biofilm formation
 501 at the air-liquid interface by GA1 in the control medium and upon supplementation with 0.1% (m/v) HG. (G) Assessment of
 502 biofilm formation (as described in panel A and B) by GA1 in the control medium and upon supplementation with 0.1% (m/v)
 503 pectin fraction extracted from tomato plant roots (PEC) and HG (Mean \pm SD, n=8, Tukey's multiple comparisons, α =0.05). (H)
 504 Assessment of biofilm formation (as described in panel A and B) by GA1 upon supplementation of HG at different
 505 concentrations (Mean \pm SD, n=8, Tukey's multiple comparisons, α =0.05). (I) Assessment of biofilm formation (as described in
 506 panel A and B) by GA1 in the control medium and upon supplementation with 0.1% (m/v) HG and HGHM (Mean \pm SD, n=8,
 507 Tukey's multiple comparisons, α =0.05). See also Figure S1 and S2.

508 **Figure 4. Homogalacturonan sensing enhances the sporulation dynamics in *B. velezensis*.** (A) Time-course measurement of
 509 GA1 population (vegetative cells and spores) by CFU counting upon root colonization of tomato plantlets roots (Mean \pm SD,
 510 n=6). (B) Assessment of spores percentages by flow cytometry (see STAR method) in planktonic cultures of GA1 after 48h upon
 511 supplementation with 0.1% (m/v) PEC or HG (Mean \pm SD, n=3, Tukey's multiple comparisons, α =0.05). (C) Time-course
 512 measurement of sporulation dynamics over time of GA1 (left axis) in control medium (light grey bars) and upon
 513 supplementation with 0.1% (m/v) HG (dark grey bars) regarding bacterial OD₆₀₀ (right axis) in the control medium (red dots)
 514 and upon supplementation with 0.1% (m/v) HG (green dots) (Mean \pm SD, n=3). (D) Assessment of spores percentages (as
 515 described in Panel B) in planktonic cultures of GA1 after 48h upon supplementation with 0.1% (m/v) HG of different DP (HG,
 516 mean DP \approx 170³²; OG_A, mean DP=13; OG_B, mean DP=4; GA, DP=1) (Mean \pm SD, n=3, Tukey's multiple comparisons, α =0.05). (E)
 517 Relative gene expression of *spo0A* and *abrB* in GA1 cells in the control medium (white bars) and upon supplementation with
 518 0.1% (m/v) HG (grey bars) in planktonic cultures of 24h (Mean \pm SD, n=3, t-test; *, P<0.05; **, P<0.01). (F) Population diversity
 519 in biofilm pellicles formed at the air-liquid interface determined by flow cytometry after RSG staining (see STAR method) in
 520 the control medium and upon supplementation with 0.1% (m/v) HG. Gate P1, P2, P3, P4 stand for highly metabolically active
 521 vegetative cells, low metabolically active vegetative cells, spore-forming cells and mature spores respectively. (G) Assessment
 522 of spores percentages (as described in panel B) in planktonic cultures of GA1 and the mutant Δ epsA-O after 48h upon
 523 supplementation with 0.1% (m/v) HG (Mean \pm SD, n=3, t-test; ns, ****, P<0.0001). See also Figure S3.

524 **Figure 5. Homogalacturonan sensing impacts the production of bioactive secondary metabolites in *B. velezensis*.** (A) Impact
 525 of HG supplementation on the secondary metabolome of GA1 at late exponential/stationary phase. Each dot represents a
 526 feature detected corresponding to a specific compound (molecular ion with exact mass in UPLC-MSMS). The variety of dots
 527 in each family of metabolites reflects the natural co-production of structural variants differing in the length and isomery of
 528 the fatty acid chain and in the position of some amino acid residues in the peptide moiety. Data are expressed as peak area
 529 fold change per OD₆₀₀, compared to control culture (Mean \pm SD, n=3). (B) Relative production of surfactins, iturins and fengycins
 530 (from left to right) in planktonic cultures of GA1 in the control medium (white bars) and upon supplementation with 0.1%
 531 (m/v) HG (grey bars) (Mean \pm SD, n=3, t-test; ns, non-significative; *, P<0.05; **, P<0.01; ***, P<0.001). Inset: Expression
 532 pattern of *srfAA*, *ituC* and *fenC* genes (from left to right) in planktonic cultures of GA1 in the control medium (white bars) and
 533 upon supplementation with 0.1% (m/v) HG (grey bars) (Mean \pm SD, n=3, t-test; ns, non-significative; **, P<0.01; ***, P<0.001).
 534 (C) UPLC-MS total ion chromatogram (TIC) illustrating the relative abundance of lipopeptides surfactins, iturins and fengycins
 535 and their corresponding structure synthesized by GA1 cells forming biofilm at the air liquid interface in the control medium
 536 (red) and upon supplementation with 0.1% (m/v) HG (black) after 72h. The different peaks eluted for each lipopeptide family
 537 correspond to structural variants and homologs as described in panel A. See also Figure S4.

538

539 Main tables and legends

540 **Table1.** Content of enzymes involved in pectin degradation (polygalacturonases, (PG); rhamnogalacturonan hydrolases,
 541 (RGH); polygalacturonate lyases, (PL)) and pectin remodelling (pectin methylsterases, (PME) and pectin acetylsterases,
 542 (PAE)) in archetype strains of soil-dwelling bacilli. Enzymes are grouped by family following the classification established in
 543 the CAZy database¹⁰⁵. * means that one or several protein sequences are referred as domain of unknown function (DUF),
 544 unknown protein, hypothetical protein or putative protein. See also TableS1.

545

| | Pectin degradation | | | Pectin remodelling | | |
|---|--------------------|-----|----|--------------------|-----|-------|
| | PG | RGH | PL | PME | PAE | TOTAL |
| <i>B. altitudinis</i> CHB19 | 1 | 1 | 2* | 1 | 1 | 6 |
| <i>B. atrophaeus</i> GQJK17 | 0 | 2 | 6* | 0 | 3 | 11 |
| <i>B. clausii</i> DSM8716 | 0 | 2 | 4* | 0 | 0 | 6 |
| <i>B. licheniformis</i> ATCC-14180 | 1 | 2 | 8* | 1 | 4 | 16 |
| <i>B. mycooides</i> KBAB4 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>B. paralicheniformis</i> CBMAI1303 | 1 | 2 | 8* | 1 | 4 | 16 |
| <i>B. pumilus</i> SAFR032 | 1 | 1 | 2* | 1 | 1 | 6 |
| <i>B. simplex</i> SH-B26 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>B. subtilis</i> 168 | 0 | 2 | 7* | 0 | 3 | 12 |
| <i>B. thuringiensis</i> CT-43 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>B. velezensis</i> GA1/FZB42 | 0 | 0 | 3* | 0 | 0 | 3 |
| <i>B. weihenstephanensis</i> WSBC 10204 | 0 | 0 | 0 | 0 | 0 | 0 |

563 STAR methods

564 Resource availability

565 Lead contact

566 Further information and requests for resources and reagents should be directed to and will
567 be fulfilled by the Lead Contact, Marc Ongena (marc.ongena@uliege.be).

568 Materials availability

571 Bacterial strains and mutants generated in this study are available upon request from Marc
572 Ongena (marc.ongena@uliege.be). This study did not generate new unique plasmids or rea-
573 gents.

574 Data and code availability

- 576 • Data

577 The RNA-seq data sets (generated by Hoff *et al.*³²) exploited for this study are deposited
578 at <https://www.ebi.ac.uk/ena/> under the project reference [PRJEB39762](https://www.ebi.ac.uk/ena/).

579

580 • *Code*

581 This paper does not report original code.

582 • Any other item

583

584 Any additional information regarding the data reported in this study is available from
585 the Lead contact upon request.

586

587 **Experimental model and subject details**

588

589 *Bacterial strains and construction of B. velezensis GA1 knockout mutants*

590 All the bacterial strains used in this study are listed in **TableS4**. Knockout mutant strains of *B.*
591 *velezensis* GA1 were constructed by gene replacement by homologous recombination. Briefly,
592 a cassette containing a chloramphenicol or phleomycin resistance gene flanked by 1 kb of the
593 upstream and downstream region of the targeted gene (see **TableS5** for primers) was
594 constructed by PCR overlap following the method developed by Bryksin and Matsumura¹⁰⁶.
595 This recombinant cassette was introduced into *B. velezensis* GA1 following a slightly modified
596 protocol developed by Jarmer *et al.*¹⁰⁷ to induce natural competence *via* nitrogen limitation.
597 Briefly, one colony of GA1 was inoculated in LB medium during 6h at 37°C under shaking
598 (160rpm). Then, 1mg of the recombinant cassette was added to the GA1 washed cell
599 suspension adjusted to an OD₆₀₀ of 0.01 in MMG medium at pH 7 (19g/l K₂HPO₄, 6g/l KH₂PO₄,
600 1g/l Na₃citrate, 0.2g/l MgSO₄·7H₂O, 2g/l Na₂SO₄, 50mM FeCl₃ [sterilized by filtration at
601 0.22µm], 2mM MnSO₄ [sterilized by filtration at 0.22 µm], 8g/l glucose, and 2g/l L-glutamic
602 acid). Incubation was performed at 37°C under shaking (160rpm) during 24h. Cells were plated
603 on LB medium solidified with agar (14g/l) supplemented with 5µg/ml chloramphenicol or
604 4µg/ml phleomycin to only select cells having integrated the recombinant cassette by double
605 crossing over. Gene deletions were confirmed by PCR analysis with the corresponding specific
606 upstream forward and downstream reverse primers.

607 *Culture media and growth conditions*

608 Bacterial cultures were performed at 26°C under shaking (160rpm) in root exudates mimicking
609 medium diluted to the half (REM)³¹ at pH 7: 1g/l glucose, 1.7g/l fructose, 0.2g/l maltose, 0.3g/l
610 ribose, 2g/l citrate, 2g/l oxalate, 1.5g/l succinate, 0.5g/l malate, 0.5g/l fumarate, 0.34g/l
611 KH₂PO₄, 10.5g/l MOPS, 0.25g/l MgSO₄·7H₂O, 0.25g/l KCl, 0.5g/l yeast extract, 0.5g/l Casamino
612 acids, 1g/l (NH₄)₂SO₄, 600µg/l Fe₂(SO₄)₃, 200µg/l MnSO₄, 800µg/l CuSO₄, 2mg/l Na₂MoO₄.
613 Following the experiment, PEC, HG(HM) (Elicityl), oligogalacturonides (OGs) or galacturonic
614 acid (GA, Sigma) were normalized in terms of weight and were added at a final concentration
615 of 0.1% (m/v) in the REM.

616 To assess the use of pectin backbone as a carbon source, cultures were performed in modified
617 M9 medium (M9 stock solution adjusted to pH7: 42.5g/l Na₂HPO₄·2H₂O, 15g/l KH₂PO₄, 5g/l
618 NH₄Cl, 2.5g/l NaCl; trace elements solution: 0.1g/l MnCl₂·4H₂O, 0.17g/l ZnCl₂, 0.043g/l
619 CuCl₂·2H₂O, 0.06g/l CoCl₂·6H₂O, 0.06g/l Na₂MoO₄·2H₂O; Calcium chloride solution: 14.7g/l

620 CaCl₂.2H₂O; Magnesium sulfate solution: 246g/l MgSO₄.7H₂O; Iron chloride solution: 13.5g/l
621 FeCl₃.6H₂O, 21g/l citric acid.H₂O). All the solutions were filter sterilized (0.22µm CA
622 membrane). M9 medium was reconstituted by mixing 200ml M9 stock solution, 1ml calcium
623 chloride solution, 10ml trace elements solution, 1ml magnesium sulfate solution, 1ml iron
624 chloride solution, 0.1% (m/v) glycerol and the volume was adjusted to 1l with ultrapure water.
625 The carbon sources tested were normalized in terms of weight and were added to the medium
626 at a final concentration of 0.2% (w/v).

627 For planktonic and biofilm cultures, the cells of an overnight preculture of the strain of interest
628 were washed twice with sterile phosphate-buffered saline (PBS, 8g/l NaCl, 0.2g/l KCl, 1.44g/l
629 Na₂HPO₄, 0.24g/l KH₂PO₄; pH 7.0), before being inoculated in the liquid medium at a final
630 OD₆₀₀ of 0.02 (planktonic cultures) or 0.1 (biofilm cultures).

631 *Plant growth conditions*

632 For plant root colonization assays, tomato seeds were sterilized in a 70% (v/v) ethanol solution
633 for 2min, transferred in a 20% (v/v) bleach solution under shaking during 20min, and rinsed
634 three times with sterile water. Sterilized tomato seeds were pre-germinated during 4 days in
635 square petri dishes containing Hoagland medium solidified with agar (14g/l) at 22°C under a
636 16 h/8 h day/night cycle.

637 To extract pectin from tomato plant roots, tomato seeds were sterilized as described above.
638 Then, seeds were transferred into seedholders filled with Hoagland medium with 0.7% (m/v)
639 agar and grown during 4 weeks under a 16h/8h day/night cycle in hydroponic systems
640 (Araponics, Belgium) filled with a nutritive solution (500µl/l FloraBloom, FloraMicro and
641 FloraGro (General Hydroponics Europe)).

642

643 **Method details**

644 *Phylogenetic and sequence identity analysis*

645 All the analysis were carried out on MEGA11 software¹⁰⁸. Phylogenetic analysis was performed
646 on a total of 20 pectin/pectate lyase sequences (classified in PL1 and PL3 family following the
647 CAZy Database¹⁰⁵) of soil-dwelling bacilli species retrieved from NCBI protein database. To
648 construct the maximum likelihood tree, protein sequences were aligned by MUSCLE alignment
649 and phylogenetic evolution was inferred using the “Maximum likelihood” method and
650 “Whelan And Goldman” model (WAG) with a discrete Gamma distribution (+G). The best-fit
651 substitution model was selected among all the substitution models implemented in MEGA11
652 by choosing the one yielding to the lowest BIC score for the dataset analyzed.

653 *Pectin degradation activity measurement*

654 Pectin degradation activity of cell-free culture supernatants (CFCS) of *B. velezensis* GA1 or GA1
655 knockout mutants was measured by spectrophotometry. Accumulation of unsaturated
656 oligogalacturonates generated by the enzymes via the mechanism of β-elimination was
657 evaluated by measuring the increase of absorbance at 232nm. Briefly, CFCS of the different
658 strains were obtained by centrifugation (6000rpm, 10min) of the samples from the planktonic

659 bacterial cultures in REM at different time points before being sterilized on a 0.22 μ m PTFE
660 filter. Then, 25 μ l of the CFCS were added to 100 μ l of HG or HGHM (2.5g/l) dissolved in 50mM
661 Tris-HCl buffer at pH 8. The reaction mixture was then incubated at 30°C under shaking and
662 the increase of absorbance was measured at 232nm in a multiplate reader (Tecan SPARK,
663 Männedorf, Switzerland). The molar extinction coefficient for unsaturated oligogalacturonates
664 at 232nm is 4600 M⁻¹ cm⁻¹ 10⁹.

665 *root colonization assay*

666 Root colonization assays were performed on tomato plantlets under gnotobiotic conditions.
667 4 days-old tomato plantlets roots were inoculated with the bacterial strain of interest in the
668 square petri dishes of solidified Hoagland medium in which the seeds were pre-germinated.
669 To do so, cells from an overnight preculture in REM were washed twice with sterile PBS and
670 OD₆₀₀ was adjusted to 0.5. For the co-inoculation assay, 500 μ l of GA1 and Δ pelA Δ pelB cell
671 suspensions, both at OD₆₀₀ adjusted to 0.5, were mixed together in an Eppendorf and
672 vortexed. Then, 5 μ l of the bacterial suspensions were deposited on the root top. To quantify
673 the bacterial colonization *in-planta* overtime, the roots were separated from the aerial part of
674 the plant after 1, 3 and 7 days of colonization. The roots were placed in tubes containing a
675 solution of PBS supplemented with 0.1% (m/v) Tween20 and vortexed vigorously to tear off
676 the bacterial cells from the roots. The total colony-forming units (CFUs) were determined by
677 plating serial dilutions of the bacterial solution on solid LB medium (for GA1 cells) or solid LB
678 medium supplemented with 5 μ g/ml chloramphenicol (for Δ pelA Δ pelB mutant cells) and
679 incubated overnight at 30°C before proceeding to plate counting. For spores counting, the
680 same solution containing the bacteria was heated at 85°C during 20min to kill vegetative cells
681 before proceeding to the plating.

682 *Extraction of pectin from tomato roots*

683 Roots of 4 weeks-old tomato plants were harvested and lyophilized before being grinded with
684 a Retsh MM400 mixer mill. The extraction of root cell wall was performed following an
685 adapted protocol from Carpita¹¹⁰ and Silva et al¹¹¹. Briefly, 200mg of cell wall powder were
686 resuspended into 40ml of ethanol 80% (v/v) and incubated at 90°C during 20min. After
687 centrifugation (10,000g, 5min), supernatant was discarded and the same procedure described
688 above was repeated 3 times on the pellet. Then, the pellet was washed with 20ml of ultrapure
689 water and after centrifugation (10,000g, 5min), the pellet was resuspended in 10ml of
690 acetone. The solution was centrifuged (10,000g, 5min) and the pellet was lyophilized to obtain
691 the alcoholic residue (AR) for fractionation. The AR was resuspended in 20ml of ultrapure
692 water and incubated at 80°C during 1h. After centrifugation (10,000g, 5min), the pellet was
693 resuspended in 100ml of ammonium oxalate 1% (v/v) and incubated at 80°C during 2h. After
694 centrifugation (10,000g, 5min), the supernatant was recovered, dialyzed on a 3.5kD MWCO
695 Spectra/Por membrane (Spectrum Laboratories Inc.) and lyophilized to obtain the PEC
696 fraction.

697 *Simple sugar analysis of pectin fraction*

698 To hydrolyse simple sugars from PEC fraction, 1ml of 2M trifluoroacetic acid (TFA) were added
699 to 2mg of lyophilized pectin fraction and incubated at 121°C during 90min. Then, TFA was

700 evaporated under N₂ flow and the residue was resuspended in 1ml ultrapure water and
701 filtered on a 0.2µm filter. Before analysis, the solution was diluted to 1mg/ml in ultrapure
702 water. Neutral and acidic monosaccharide composition was determined by high-performance
703 anion exchange chromatography (ICS3000 system) coupled with a pulsed amperometric
704 detector (HPAEC-PAD) (Dionex, Thermo Scientific) equipped with a CarboPac PA-1 column
705 (4mm × 250mm) and a CarboPac PA1 guard column (ID 4mm × 50mm). The injection volume
706 was 25µl and elution was performed at 30°C with a constant flow rate of 1ml/min in gradient
707 mode. For neutral sugar quantification, the mobile phases were ultrapure H₂O (A), 160 mM
708 NaOH (B) and 200 mM NaOH (C). The elution profiles were as follows : 0–25 min 90% A and
709 10% B, 25–26 min 0–100% C, 26–35 min 100% C, 25-36 min 100–0% C, 36–50 min 90% A and
710 10% B. For uronic acids quantification, the mobile phases were 0.16 M NaOH (A) and 0.6 M
711 NaOAc in 0.16 M NaOH (B). The elution profiles were as follows: 0–5 min 100% A, 5–35 min
712 0–100% B, 35–40 min 100% B, 40–42 min 100–0% B and finally column re-equilibration by
713 100% A from 42 to 50 min. Monosaccharides arabinose, fucose, galactose, glucose, rhamnose,
714 xylose, galacturonic acid and glucuronic acid (Sigma–Aldrich) were used as standards for
715 quantification.

716 *RNA extraction and RT-qPCR analysis*

717 RNA extractions were performed with the NucleoSpin RNA Kit (Macherey Nagel, Germany),
718 following the manufacturer's protocol for Gram positive bacteria. The amount of RNAs
719 extracted and their purity were assessed with the UV-vis spectrophotometer NanoDrop 2000
720 (Thermo scientific). RT-qPCR was performed in a ABI StepOne™ qPCR apparatus (Applied
721 Biosystems) using the kit Luna® Universal One-Step RT-qPCR Kit (New England Biolabs,
722 Ipswich, MA, United States). Reactions were carried out in a 20µl volume containing 10µL of
723 Luna Universal One Step Reaction Mix, 1µL of Luna WarmStart® RT Enzyme Mix, 0.8µl of each
724 primer (10µM) and 100ng of RNA under the following thermal cycling conditions: a 10min
725 reverse transcription step at 55°C, a 1min initial denaturation step at 95°C and 40 cycles of 10s
726 at 95°C and 30s at 60°C. Melting curves were also realized from 65 to 95°C with an increase
727 rate of 0.5°C/5s to evaluate the specificity of the amplified products. The transcription level of
728 genes of interest was analysed using the mathematical model proposed by Pfaffl¹¹². The *gyrA*
729 gene encoding the DNA gyrase subunit A was used as housekeeping gene to normalise the
730 expression level of the gene of interest.

731 *Biofilm formation assay*

732 Quantification of total biofilm was performed by crystal violet staining. Briefly, the strain of
733 interest was inoculated in a 96-wells microplate containing 200µl of REM supplemented or
734 not with 0.1% (m/v) HG, HGHM, OGs or GA. The plate was incubated at 30°C during 24h
735 without shaking. Medium and planktonic cells were discarded, and wells were washed with
736 PBS. The biofilm pellicle was stained with 0.1% (v/v) crystal violet during 10min and then wells
737 were washed three times with PBS. The stained biofilm was dissolved with 30% (v/v) acetic
738 acid. Absorbance was measured at 595nm.

739

740

741 *Generation of short DP OGs*

742 OGs of short polymerization degree (OG_B) were generated from the reaction of concentrated
743 24h-CFCS of *B. velezensis* GA1 Δ *sfp* unable to synthesize lipopeptides, on HG as substrate.
744 Briefly, the CFCS was obtained as described in the section "Pectin degradation activity
745 measurement". The CFCS was then concentrated 50x on a Vivaspin20 centrifugal concentrator
746 with a polyethylene sulfone membrane of 10,000Da MWCO (Sartorius). The enzymatic
747 reaction was performed at 30°C by mixing 25% (v/v) of concentrated supernatant with a
748 solution of 0.5% (m/v) HG resuspended in 50mM Tris-HCl buffer at pH 8. Absorbance at 232nm
749 was followed until the enzymatic reaction reached a plateau. Then, short DP OGs were
750 separated from larger molecules according to the protocol of Voxeur *et al*¹¹³ via ethanol
751 precipitation. The supernatant was then recovered and lyophilized.

752 *Analysis of OGs profile*

753 Relative quantification and polymerization degree of OGs were evaluated by UPLC-qTOF MS.
754 For each sample, a volume of 10µl was injected in an Agilent 1290 Infinity II apparatus coupled
755 with a diode array detector and a mass detector (Jet Stream ESI-Q-TOF 6530) with the
756 parameters set up as follows: capillary voltage: 3.5kV; nebulizer pressure: 35psi; drying gas:
757 8l/min; drying gas temperature: 300°C; flow rate of sheath gas: 11l/min; sheath gas
758 temperature: 350°C; fragmentor voltage: 175V; skimmer voltage: 65V; octopole RF: 750V.
759 Accurate mass spectra were recorded in negative mode in the range of $m/z = 50-1700$. OGs
760 were separated on a Luna HILIC column (3mm x 150mm x 5µm, Phenomenex, Torrance,
761 California, USA) using a gradient of water (solvent A) and 90% acetonitrile (solvent B) that
762 were both acidified with 0.1% (v/v) formic acid and supplemented with 15mM ammonium
763 formate as mobile phase, with a constant flow rate of 0.5ml/min and a temperature of 40°C.
764 For the separation of OGs, initial gradient was decreased from 100% B to 85% B in 4min before
765 decreasing to 0% B in 16min. Solvent B was kept at 0% during 10min before going back to
766 100% B. Polymerization degree of the detected OGs was determined based on accurate mass
767 measurement and relative quantification was based on peak area of the extracted ion
768 chromatogram generated by MassHunter workstation v10.0.

769 *Extracellular DNA (eDNA) detection in biofilm pellicles*

770 Biofilms of GA1 were grown in a 96-wells black microplate containing 200µl of REM
771 supplemented or not with 0.1%(m/v) HG and incubated at 30°C without shaking. eDNA
772 present in the biofilm pellicles formed at the air-liquid interface was measured using the kit
773 QuantiFluor dsDNA system (Promega, Madison, WI, USA) according to manufacturer's
774 protocol. Briefly, medium and planktonic cells were discarded, and wells were washed with
775 PBS in order to only keep the biofilm pellicles. Then, 200µl of QuantiFluor® dsDNA Dye working
776 solution (1:400 QuantiFluor® dsDNA Dye in 1X TE buffer) were dispensed into each well and
777 homogenized with the pellicles by pipetting up and down. Microplate was incubated in the
778 dark at room temperature during 5min and the fluorescence intensity (504nm_{Ex}/531nm_{Em})
779 was measured using a microplate reader (Tecan SPARK, Männedorf, Switzerland).

780

781 *Cell counting in biofilm pellicles*

782 To evaluate the bacterial population embedded in the biofilm pellicles, GA1 cells were
783 inoculated in a 24-wells microplate containing 2ml of REM supplemented or not with 0.1%
784 (w/v) HG and incubated at 30°C without shaking. The biofilm pellicle formed at the air-liquid
785 interface was then collected, resuspended into 2ml PBS and mildly sonicated (Power 60%, 2
786 cycles, 1min) on a Sonoplus apparatus HD 2070 (Bandelin). Several dilutions were plated on
787 solid LB medium and incubated at 30°C overnight before counting.

788 *Stereomicroscopy acquisitions*

789 Pictures of biofilm pellicles of GA1 formed at the air-liquid interface were taken with a Nikon
790 SMZ1270 stereomicroscope (Nikon, Japan) equipped with a Nikon DS-Qi2 monochrome
791 microscope camera and a DS-F 1× F-mount adapter. Pictures were captured in the bright field
792 channel with an ED Plan Apo 1×/WF objective and a 0.63x magnification of with an exposure
793 time of 40ms. Additionally, pictures of biofilm pellicles at 6h were captured with a 3x
794 magnification and an exposure time of 10ms to get a better view of the structures in
795 formation. Acquisitions were then processed with NIS-Element AR software (Nikon, Japan).

796 *Microscopy acquisitions*

797 Microscopy acquisitions were performed using a Nikon Ti2-E inverted microscope (Nikon,
798 Japan) equipped with a ×20/0.45 NA S Plan Fluor objective lens (Nikon, Switzerland) and a
799 Nikon DS-Qi2 monochrome microscope camera. Images and videos taken in the bright field
800 channel were acquired using a Ti2 Illuminator-DIA and an exposure time of 20ms. GA1 cells
801 strained with RedoxSenor Green (RSG) (*BaC*Light vitality kit, Thermofisher) were visualized by
802 conventional epifluorescence microscopy with a ×100/1 oil NA S Plan Fluor objective lens
803 (Nikon, Switzerland). A lumencor sola illuminator (Lumencor, USA) was used as the source of
804 excitation with an exposure time of 500ms and the GFP-B HC Bright-Line Basic Filter was used.
805 Acquisitions were then processed with NIS-Element AR software (Nikon, Japan).

806 *Flow cytometry analysis*

807 Cell activity of GA1 cells was evaluated by RSG staining. For the experiments conducted under
808 biofilm conditions, the pellicles formed at the air-liquid interface were collected, resuspended
809 into PBS and mildly sonicated as described in the section “Cell counting in biofilm pellicles”,
810 before staining. To stain GA1 cells, 1µl of RSG 10x diluted were added to 1ml of cell culture
811 appropriately diluted in filtered PBS and incubated in the dark during 10min. RSG fluorescence
812 (FL1-490nm_{ex}/520nm_{em}) and cell size (FSC-H) were measured by flow cytometry using a BD
813 Accuri™ C6 apparatus (BD Biosciences) under the following conditions: Medium fluidics
814 (35µl/min); 20,000 events recorded; FSC-H threshold 20,000.

815

816 *Analysis of BSMs*

817 The detection of *B. velezensis* metabolites was performed by UPLC-qTOF MS. Before analysis,
818 samples from planktonic cell cultures were filtered on a 0.22µm PTFE filter. For the analysis of
819 metabolites of biofilm-forming cells, the content of each well of the microplate was mildly

820 sonicated as described in the section “Cell counting in biofilm pellicles”, before filtration. For
821 each sample, a volume of 5µl was injected in an Agilent 1290 Infinity II apparatus coupled with
822 a diode array detector and mass detector (Jet Stream ESI-Q-TOF 6530) with the parameters
823 set up as described in the section “Analysis of OGs profile”. Accurate mass spectra were
824 recorded in positive mode in the range of $m/z = 100-1700$. Metabolites were separated on a
825 C18 Acquity UPLC BEH column (2.1 × 50mm × 1.7µm; Waters, milford, MA, USA) using a
826 gradient of water (solvent A) and acetonitrile (solvent B) both acidified with 0.1% formic acid
827 as a mobile phase with a constant flow rate 0.6ml/min and a temperature of 40°C. The
828 gradient was set up to start at 10% B and kept in this condition during 1min before raising to
829 100% B in 10 min. Solvent B was kept at 100% for 3.5 min before going back to the initial ratio.
830 MassHunter v10.0 workstation and the open-source software MzMine2⁶⁷ were used for data
831 collection and analysis.

832 *RNA-seq data analysis*

833
834 The analysis of RNAseq data used in this study has been described in details previously³². In
835 the present study, we focused especially on genes involved in motility and EPS and cohesion
836 protein synthesis. Briefly, raw RNA-seq reads were trimmed with Trimmomatic v0.39¹¹⁴ and
837 quality control was assessed using FastQC v0.11.8 (Babraham Bioinformatics). The trimmed
838 reads were then aligned to the genome of *B. velezensis* GA1 (GenBank: CP046386) using BWA-
839 MEM v0.7.17¹¹⁵. Read counts were calculated using the python-based tool HTSeq v0.9¹¹⁶ and
840 the tables of fragments per kilobase of transcript per million mapped reads (FPKM) were
841 generated using the Cufflinks function cuffnorm¹¹⁷. Genes with read counts <25 were
842 excluded. Differential expression analysis was conducted using DESeq2 pipeline¹¹⁸,
843 considering the following parameters: P-value<0.05 and fold change ≥1.5. Heatmaps were
844 performed on GraphPad Prism 9.

845

846 **Quantification and Statistical Analyses**

847 All statistical analyses were performed on GraphPad Prism 9. Results are expressed as mean
848 ± SD (standard deviation). Analysis of variance (ANOVA) was performed on each data set and
849 statistical differences between means were evaluated by two-tailed Student’s t-test or Tukey’s
850 multiple comparisons ($\alpha=0.05$). The number of biological replicates used for each experiment
851 and P-values are indicated in the legends of the corresponding figures.

852

853 **Video files**

854 **VideoS1A.** *Microscopic observation of early biofilm formation (at 7 hours post-inoculation) of B. velezensis GA1 in microplate*
855 *in the control medium. Related to Figure 3.*

856 **VideoS1B.** *Microscopic observation of early biofilm formation (at 7 hours post-inoculation) of B. velezensis GA1 in microplate*
857 *upon supplementation with 0.1% (m/v) HG. Related to Figure 3.*

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863

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870

871 **Declaration of interests**

872

873 The authors declare no competing interests.

874

875 **References**

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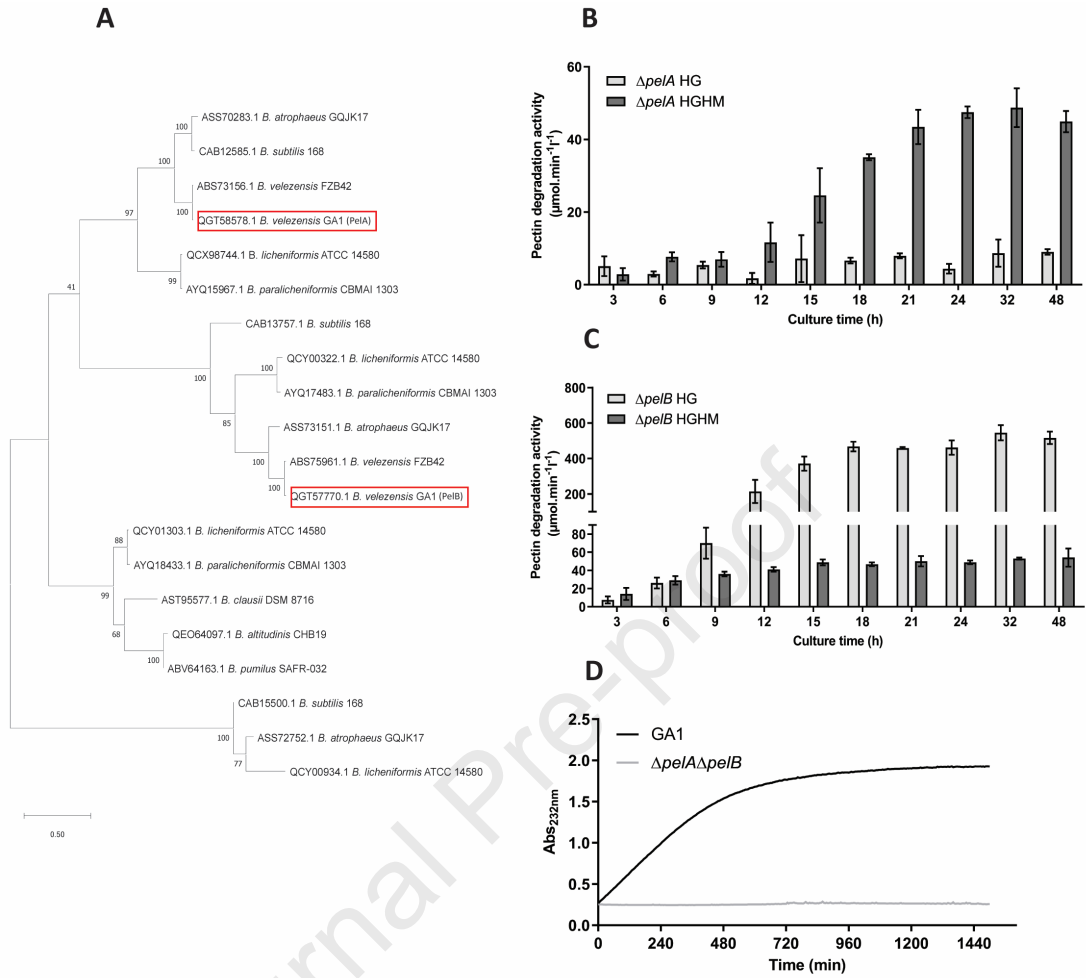
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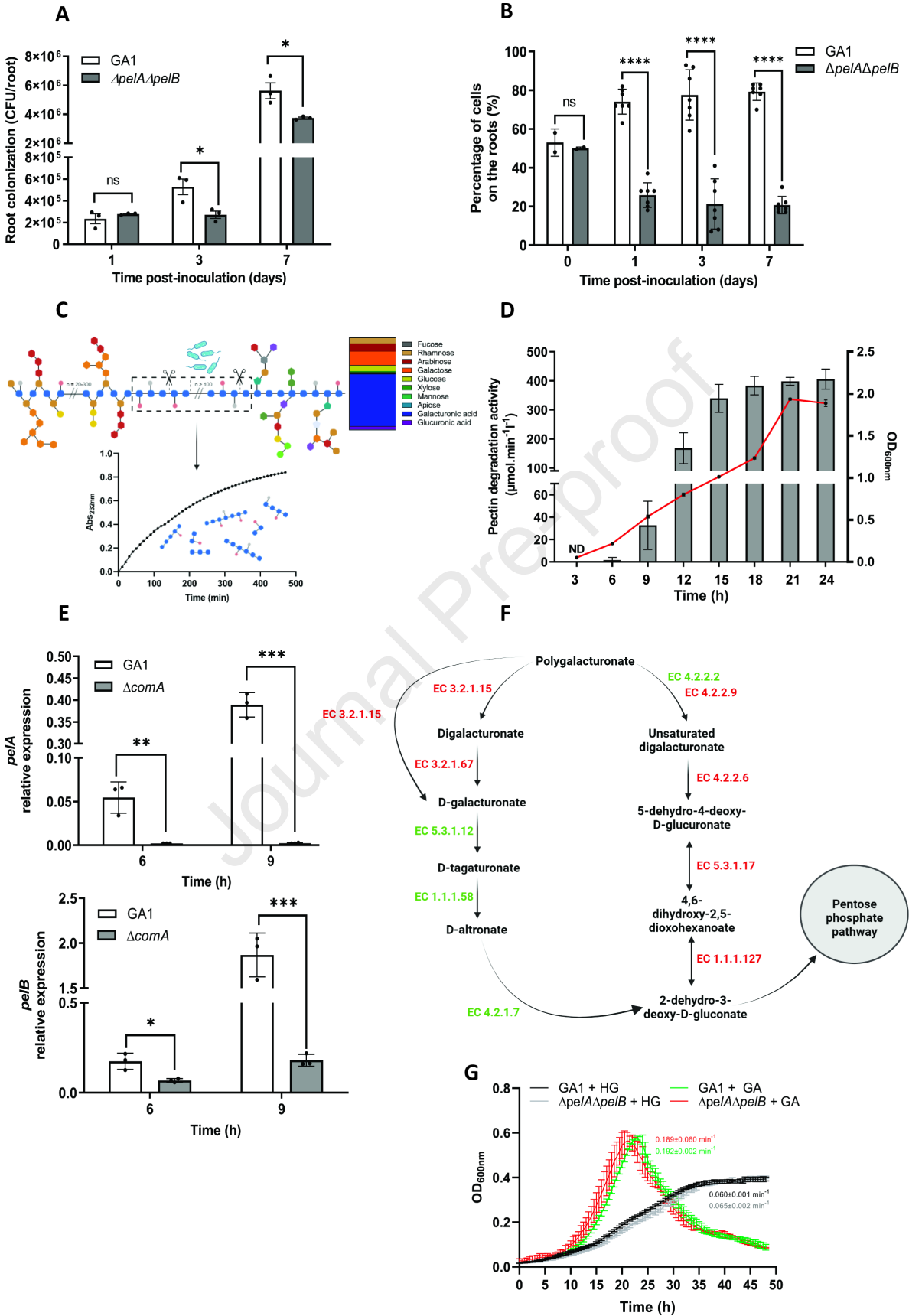
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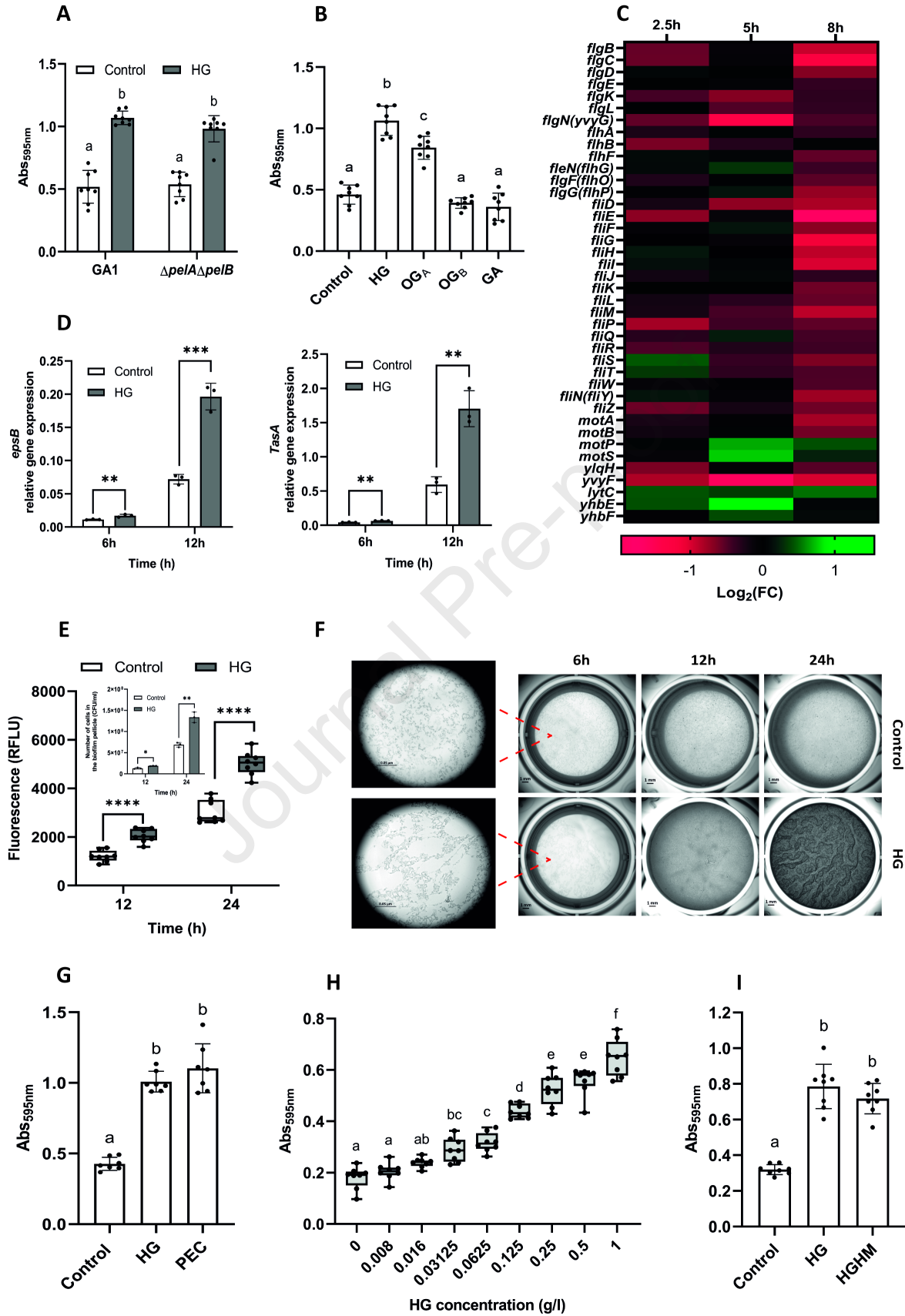
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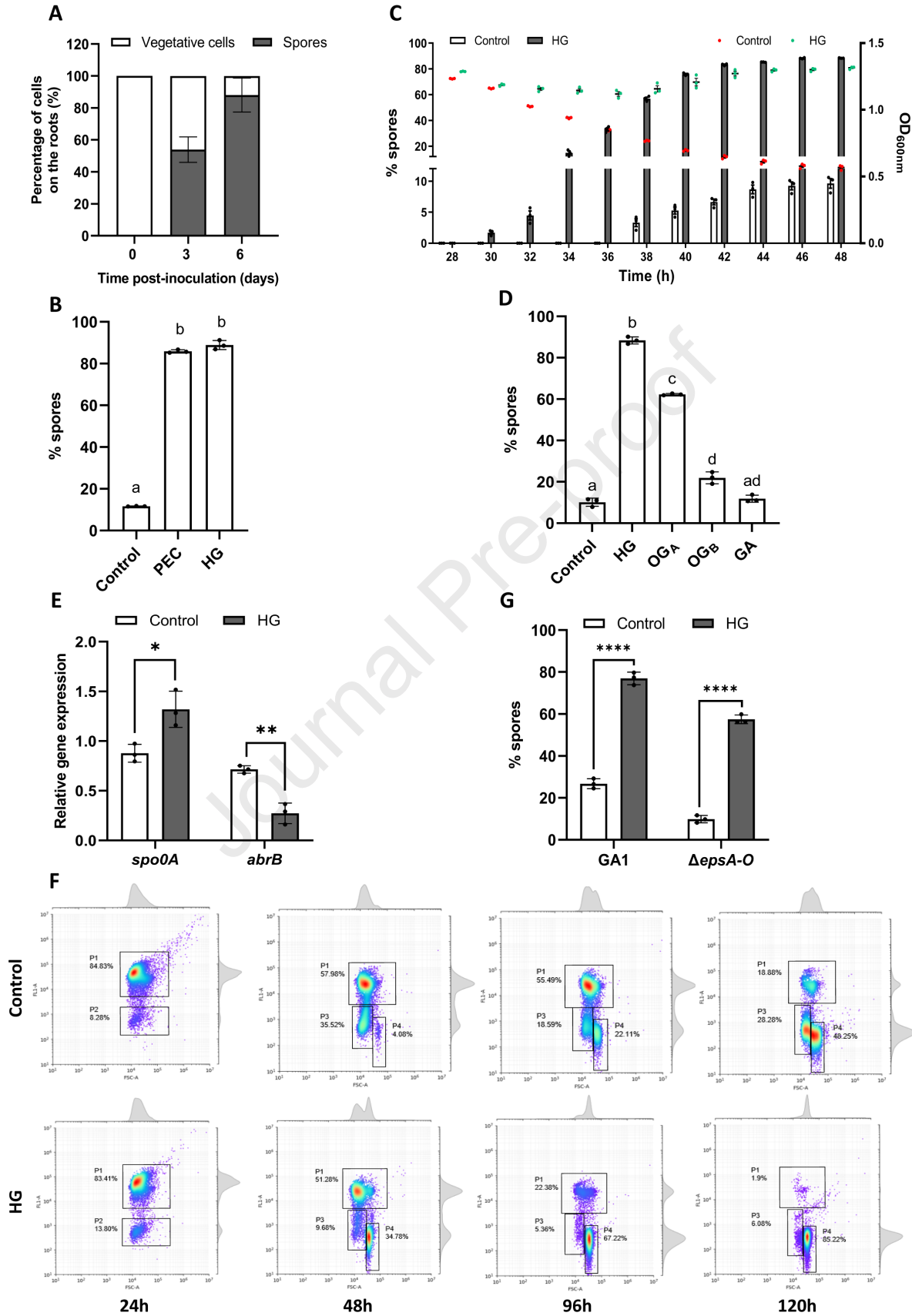


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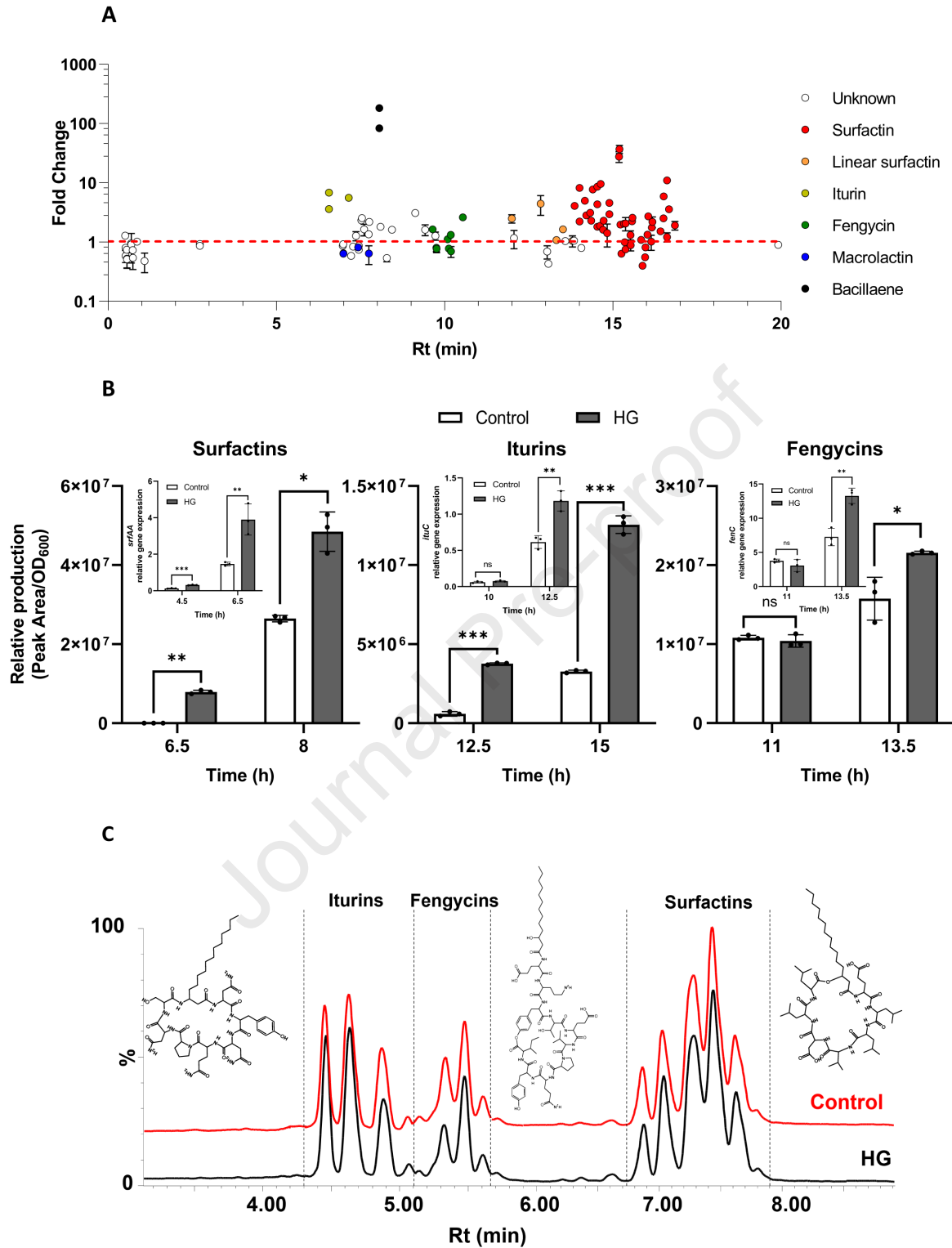


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Highlights

- *B. velezensis* (*B.v*) secretes two unique enzymes degrading homogalacturonan (HG)
- These pectinases contribute to bacterial fitness and root colonization
- HG stimulates biofilm, sporulation and secondary metabolite production in *B.v*
- These traits contribute to establishment and persistence of *B.v* in the rhizosphere

KEY RESOURCES TABLE

The table highlights the reagents, genetically modified organisms and strains, cell lines, software, instrumentation, and source data **essential** to reproduce results presented in the manuscript. Depending on the nature of the study, this may include standard laboratory materials (i.e., food chow for metabolism studies, support material for catalysis studies), but the table is **not** meant to be a comprehensive list of all materials and resources used (e.g., essential chemicals such as standard solvents, SDS, sucrose, or standard culture media do not need to be listed in the table). **Items in the table must also be reported in the method details section within the context of their use.** To maximize readability, the number of **oligonucleotides and RNA sequences** that may be listed in the table is restricted to no more than 10 each. If there are more than 10 oligonucleotides or RNA sequences to report, please provide this information as a supplementary document and reference the file (e.g., See Table S1 for XX) in the key resources table.

Please note that ALL references cited in the key resources table must be included in the main references list. Please report the information as follows:

- **REAGENT or RESOURCE:** Provide the full descriptive name of the item so that it can be identified and linked with its description in the manuscript (e.g., provide version number for software, host source for antibody, strain name). In the experimental models section (applicable only to experimental life science studies), please include all models used in the paper and describe each line/strain as: model organism: name used for strain/line in paper: genotype. (i.e., Mouse: OXTR^{fl/fl}; B6.129(SJL)-Oxtr^{tm1.1Wsy/J}). In the biological samples section (applicable only to experimental life science studies), please list all samples obtained from commercial sources or biological repositories. Please note that software mentioned in the methods details or data and code availability section needs to also be included in the table. See the sample tables at the end of this document for examples of how to report reagents.
- **SOURCE:** Report the company, manufacturer, or individual that provided the item or where the item can be obtained (e.g., stock center or repository). For materials distributed by Addgene, please cite the article describing the plasmid and include “Addgene” as part of the identifier. If an item is from another lab, please include the name of the principal investigator and a citation if it has been previously published. If the material is being reported for the first time in the current paper, please indicate as “this paper.” For software, please provide the company name if it is commercially available or cite the paper in which it has been initially described.
- **IDENTIFIER:** Include catalog numbers (entered in the column as “Cat#” followed by the number, e.g., Cat#3879S). Where available, please include unique entities such as RRIDs, Model Organism Database numbers, accession numbers, and PDB, CAS, or CCDC IDs. For antibodies, if applicable and available, please also include the lot number or clone identity. For software or data resources, please include the URL where the resource can be downloaded. Please ensure accuracy of the identifiers, as they are essential for generation of hyperlinks to external sources when available. Please see the Elsevier [list of data repositories](#) with automated bidirectional linking for details. When listing more than one identifier for the same item, use semicolons to separate them (e.g., Cat#3879S; RRID: AB_2255011). If an identifier is not available, please enter “N/A” in the column.
 - **A NOTE ABOUT RRIDs:** We highly recommend using RRIDs as the identifier (in particular for antibodies and organisms but also for software tools and databases). For more details on how to obtain or generate an RRID for existing or newly generated resources, please [visit the RII or search for RRIDs](#).

Please use the empty table that follows to organize the information in the sections defined by the subheading, skipping sections not relevant to your study. Please do not add subheadings. To add a row,

place the cursor at the end of the row above where you would like to add the row, just outside the right border of the table. Then press the ENTER key to add the row. Please delete empty rows. Each entry must be on a separate row; do not list multiple items in a single table cell. Please see the sample tables at the end of this document for relevant examples in the life and physical sciences of how reagents and instrumentation should be cited.

TABLE FOR AUTHOR TO COMPLETE

Please upload the completed table as a separate document. **Please do not add subheadings to the key resources table.** If you wish to make an entry that does not fall into one of the subheadings below, please contact your handling editor. **Any subheadings not relevant to your study can be skipped.** (NOTE: References within the KRT should be in numbered style rather than Harvard.)

Key resources table

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|--|-----------------------------------|--------------|
| Antibodies | | |
| N/A | | |
| Bacterial and virus strains | | |
| N/A | | |
| Biological samples | | |
| N/A | | |
| Chemicals, peptides, and recombinant proteins | | |
| MOPS | Sigma | Cat#M1254 |
| Bacto™ Casamino acids | ThermoFisher Scientific | Cat#223050 |
| Bacto™ Yeast extract | ThermoFisher Scientific | Cat#212750 |
| HG: Galacturonan polysaccharides LM | Elicityl | Cat#GAT100 |
| HGHM: Galacturonan polysaccharides HM | Elicityl | Cat#GAT101 |
| GA: Galacturonic acid | Sigma | Cat#93478 |
| OG _A | Pr. Emmanuel Petit (UPJV, France) | N/A |
| OG _B | This study | N/A |
| PEC | This study | N/A |
| Chloramphenicol | AppliChem | Cat#A1806 |
| Phleomycin | InvivoGen | Cat#ant-ph-1 |
| Tris-HCl | Sigma | Cat#T3253 |
| Tween20 | Sigma | Cat#P9416 |
| TFA: Trifluoroacetic acid | Sigma | Cat#302031 |
| Crystal Violet solution | Sigma | Cat#V5265 |
| FloraBloom | General Hydroponics Europe (GHE) | N/A |
| FloraMicro | General Hydroponics Europe (GHE) | N/A |
| FloraGro | General Hydroponics Europe (GHE) | N/A |

| Critical commercial assays | | |
|---|------------------------------|---|
| NucleoSpin RNA Kit | Macherey Nagel | Cat#740955.250 |
| Luna® Universal One-Step RT-qPCR Kit | New England Biolabs | Cat#E3005L |
| QuantiFluor dsDNA system | Promega | Cat#E2670 |
| BacLight™ RedoxSensor™ Green vitality kit | ThermoFisher Scientific | Cat#B34954 |
| Deposited data | | |
| RNAseq data exploited in this paper | Hoff <i>et al.</i> , 2021 | https://www.ebi.ac.uk/ena/ Project reference: PRJEB39762 |
| Experimental models: Cell lines | | |
| N/A | | |
| Experimental models: Organisms/strains | | |
| Bacterial strains (see TableS4) | | |
| Tomato: <i>Solanum Lycopersicum</i> L. | Sluis Garden | Cat#SL0765 |
| Oligonucleotides | | |
| Oligonucleotides (see TableS5) | This study | N/A |
| Recombinant DNA | | |
| N/A | | |
| Software and algorithms | | |
| MEGA11 | Tamura <i>et al.</i> , 2021 | https://www.megasoftware.net/ |
| MassHunter v10.0 | Agilent | https://www.agilent.com/en/product/software-informatics/mass-spectrometry-software |
| MzMine2 | Pluskal <i>et al.</i> , 2010 | http://mzmine.github.io/features.html |
| Trimmomatic v0.39 | Bolger <i>et al.</i> , 2014 | http://www.usadellab.org/cms/?page=trimmomaticv |
| FastQC v0.11.8 | Babraham Bioinformatics | https://www.bioinformatics.babraham.ac.uk/projects/fastqc/ |
| BWA-MEM v0.7.17 | Li and Durbin., (2009) | https://maq.sourceforge.net/ |
| HTSeq v0.9 | Anders <i>et al.</i> , 2015 | https://htseq.readthedocs.io/en/release_0.9.1/history.html#version-0-9-0 |
| DESeq2 | Love <i>et al.</i> , 2014 | https://bioconductor.org/packages/release/bioc/html/DESeq2.html |

| | | |
|--|-------------------------|---|
| NIS-Element AR software | Nikon | https://www.microscope.healthcare.nikon.com/products/software/nis-elements/nis-elements-advanced-research |
| GraphPad Prism 9 | GraphPad Software | https://www.graphpad.com/features |
| Other | | |
| SPARK multiplate reader | Tecan | https://lifesciences.tecan.com/multimode-plate-reader |
| Mixer Mill MM400 | Retsch | https://www.retsch.com/products/milling/ball-mills/mixer-mill-mm-400/ |
| Hydroponic systems | Araponics | Cat#K72L |
| Dionex™ ICS-3000 Ion Chromatography System | Conquer scientific | https://conquerscientific.com/product/dionex-ics-3000-ion-chromatography-system/ |
| Dionex™CarboPac™ PA-1 column (4mm x 250mm) | ThermoFisher Scientific | Cat#035391 |
| NanoDrop 2000 | ThermoFisher Scientific | Cat#ND-2000 |
| StepOne™ Real-Time PCR system | ThermoFisher Scientific | Cat#4376357 |
| 3.5kD MWCO Spectra/Por dialysis membrane | VWR | Cat#25219-085 |
| Vivaspin20 centrifugal concentrator polyethylene sulfone 10,000Da MWCO | Sartorius | Cat#VS2001 |
| UHPLC Agilent 1290 Infinity II | Agilent | https://www.agilent.com/en/product/liquid-chromatography/hplc-systems/analytical-hplc-systems/1290-infinity-ii-lc-system |
| Luna® HILIC 200 Å column (150 x 3 mm x 5 µm) | Phenomenex | Cat#00F-4450-Y0 |
| Acquity UPLC BEH C18 column (2.1 x 50mm x 1.7µm) | Waters | Cat#186002350 |
| 6530 Q-TOF mass spectrometer | Agilent | Cat#G6530AA |
| Sonoplus HD 2070 ultrasonic homogenizer | Bandelin | https://profilab24.com/en/laboratory/ultrasonic/bandelin-sonopuls-hd-2070-homogeniser |

| | | |
|-----------------------------------|----------------|---|
| BD Accuri™ C6 Plus Flow cytometer | BD Biosciences | https://www.bdbiosciences.com/en-us/products/instruments/flow-cytometers/research-cell-analyzers/bd-accuri-c6-plus |
| Nikon SMZ1270 stereomicroscope | Nikon | https://www.microscope.healthcare.nikon.com/products/stereomicroscopes-microscopes-smz1270-smz1270i |
| Nikon Ti2-E inverted microscope | Nikon | https://www.microscope.healthcare.nikon.com/products/inverted-microscopes/eclipse-ti2-series |