Identification of new *Dickeya dadantii* virulence factors secreted by the type 2 secretion system.

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ABSTRACT

Dickeya are plant pathogenic bacteria able to provoke disease on a wide range of plants. A type 2 secretion system named Out is necessary for bacterial virulence. Its study in *D. dadantii* showed that it secretes a wide range of pectinolytic enzymes. However, the full repertoire of exoproteins it can secrete has not been identified. No secretion signal present on the protein allows the identification of substrates of a type 2 secretion system. To identify new Out substrates, we analyzed *D. dadantii* transcriptome data obtained in plant infection condition and searched for genes strongly induced encoding a protein with a signal sequence. We identified four new Out-secreted proteins: the expansin YoaJ, VirK and two proteins of the DUF 4879 family, SvfA and SvfB. We showed that SvfA and SvfB are required for full virulence of *D. dadantii* and showed that Svf proteins are present with a variable number of copies, up to three in *D. fanghzongdai*, in other *Pectobacteriaceae*. This work opens the way to the study of the role in virulence of non-pectinolytic proteins secreted by the Out pathway in *Pectobacteriaceae*.

IMPORTANCE

The plant pathogen *Dickeya* rely on a type 2 secretion system named Out for their pathogenicity. Importance of plant cell wall degrading enzymes secreted by this system has been well studied. However, existence and role of other Out-secreted proteins has barely been investigated. By mining *D. dadantii* transcriptome data, we identified four new Out-secreted proteins. We showed that two of them, SvfA and SvfB, are necessary for the full virulence of the bacteria. These findings show that identification of all the proteins secreted by the *Dickeya* Out system is necessary for a better knowledge of the virulence of these bacteria.

42 **INTRODUCTION**

43 Soft rot *Pectobacteriaceae*, *Dickeya* and *Pectobacterium*, are plant pathogenic bacteria that can
44 provoke disease on a more than 35% of angiosperm plant orders, including both monocot and
45 dicot plants (1). Among those, there is a wide range of plants of agronomic interest such as
46 potato, rice, chicory, cabbage or ornementals on which they can cause severe losses. Symptoms
47 are usually soft rot but these bacteria can provoke blackleg or wilting on aerial parts of potato.
48 Recently, disease on woody plants caused by *Dickeya* have been reported (2). There is no
49 efficient way to fight the disease. There are actually twelve species of *Dickeya* described,
50 isolated either from infected plants (*D. chrysanthemi* from *Chrysanthemum morifolium*, *D.*
51 *dadantii* subsp. *dadantii* from *Pelargonium capitum*, *D. dadantii* subsp. *diffenbachiae* from
52 *Dieffenbachia* sp., *D. dianthicola* from *Dianthus caryophillus*, *D. zeae* from *Zea mays*, *D.*
53 *oryzae* from *Oryza sativa*, *D. paradisiaca* from *Musa paradisiaca*, *D. solani* from *Solanum*
54 *tuberosum*, *D. fangzhongdai* from *Pyrus pyrifolia*, *D. poaceiphila* from *Saccharum*
55 *officinarum*) (3)(4)(5)(6)(7) or from river of lake water (*D. aquatica*, *D. lacustris* and *D.*
56 *undicola*) (8)(9)(10). The role of protein secretion systems on the onset of the disease provoked
57 by these bacteria has been recognized long ago (11). In contrast to many plant pathogenic
58 bacteria, the type three Hrp secretion system is not the main determinant for *Pectobacteriaceae*
59 virulence (12). The main virulence factor for these bacteria is a type 2 secretion system (T2SS)
60 named Out. It allows the secretion of enzymes that degrade the components of the plant cell
61 wall, leading to the soft rot symptom distinctive of the disease. The first secreted proteins to be
62 identified were pectinases and a cellulase which are easily detectable by simple enzymatic tests
63 (13) (11). The pectinolytic secretome of the model strain *D. dadantii* 3937 has been studied in
64 detail by cloning the genes of these easily detectable enzymes. *D. dadantii* secretes nine pectate
65 lyases, one pectin methylesterase, one pectin acetylesterase and one rhamnogalacturonate lyase
66 (14). A proteomic analysis of the secreted proteins by 2D gel electrophoresis allowed the

67 identification of two other secreted proteins, the feruloyl esterase FaeD and a protein with
68 homology with a *Xanthomonas campestris* avirulence protein AvrL (15). A search in *D.*
69 *dadantii* of homologues of proteins secreted by *Pectobacterium atrosepticum* (16) led to the
70 characterization of the metal binding protein IbpS (17). Since all the pectinolytic enzymes
71 studied in *D. dadantii* are present in most of the *Dickeya* species they are probably not
72 responsible for the host preference observed for these bacteria (18). We hypothesized that
73 additional T2SS-secreted proteins specific for some species might exist and play a role in the
74 host preference. To identify such proteins, we analyzed *D. dadantii* transcriptome data, looking
75 for genes induced in plant infection conditions coding proteins with a signal sequence. We
76 identified several proteins secreted by the Out machinery and showed that two proteins of the
77 DUF4879 family, SvfA and SvfB are *D. dadantii* virulence factors.
78
79
80 **RESULTS**
81
82 **Identification of new Out-secreted proteins**
83 To have a more complete knowledge of the proteins secreted by the *D. dadantii* Out T2SS that
84 could be involved in the pathogenicity process, we searched for candidate genes in recently
85 published transcriptome data (19)(20). We selected genes strongly induced during plant
86 infection and coding for a protein possessing a signal sequence. We retained the genes
87 *Dda3937_01687*, *Dda3937_00585* (thereafter named SvfA and SvfB, respectively) and
88 *Dda3937_00081* (also named *yoaJ*). We also retained VirK, a protein with a signal sequence
89 identified among the genes controlled by PecS, a regulator of many virulence factors (21). The
90 proteins were tagged with a His-tag and their secretion analyzed in the *D. dadantii* wild type
91 strain and an OutD mutant. Proteins SvfA, SvfB, YoaJ and VirK were found in the supernatant
92 of the wild type strain but not of the mutant, proving their secretion by the Out machinery (Fig.

93 1). Production of these proteins from a plasmid may explain why secretion was not total in the
94 wild type strain.
95
96 YoaJ is a PecS-regulated gene (21) and it was found among the most induced genes during
97 *Arabidopsis* infection or culture in the presence of plant extracts (19). It encodes a protein with
98 homology to expansins. These proteins are able to non-enzymatically loosen cell wall cellulose.
99 They are found in all plants where they have a role in cell wall extension and also in many plant
100 pathogenic microorganisms (22). Their role in virulence has been shown in *Ralstonia*
101 *solanacearum*, in *P. atrosepticum*, *P. Brasiliense* and in the plant pathogen *Erwinia*
102 *tracheiphila* (23) (24). The role of *D. dadantii* expansin is probably the same. VirK is a protein
103 of unknown function that has homologues in several plant pathogenic bacteria such as *R.*
104 *solanacearum*, *Agrobacterium tumefaciens, Lonsdalea* and *Xanthomonas*. VirK is controlled
105 by PecS and induced during Arabidopsis infection or culture in the presence of plant extracts
106 (21) (20). No symptom for the *D. dadantii virK* mutant was observed whatever the plant tested
107 (21). The two proteins SvfA and SvfB are studied in the next paragraphs.
108
109 **SvfA and SvfB are virulence factors**
110 *svfA* and *svfB* are among the most induced genes during *D. dadantii* Arabidopsis infection or
111 culture of the bacteria in the presence of plant extracts (19). They are also strongly expressed
112 during maceration of potato by *D. dianthicola* and *D. solani* (25). The two *D. dadantii* proteins
113 share 43% identity and 58% similarity in amino acid composition (Fig. 2). SvfA is 187 amino
114 acid long (165 for the mature form, 17.5 kDa) and SvfB is 198 amino acid long (177 for the
115 mature form, 18.9 kDa). These proteins belong to the DUF 4879 family of proteins. These
116 proteins have no known function. A protein of this family with low homology with SvfB, YolA,
117 is among the most highly secreted protein of *Bacillus subtilis* (26). YolA is also present in *B.*

118 *cereus* and in the insect pathogen *B. thuringiensis*. *Bacillus* YolA is shorter than SvfA and SvfB,
119 missing the N-terminal more variable part (Fig. 2).
120
121 SvfA and SvfB mutants have been constructed and their pathogenicity has been tested on
122 potato. The *svfA* mutant was significantly less aggressive than the wild type while the *svfB*
123 mutant was not affected (Fig. 3A). Virulence of the *svfA* mutant could be restored by
124 introduction of a plasmid bearing the wild type *svfA* (Fig. 3B). Virulence of the double *svfA*
125 *svfB* mutant was further reduced showing that the role of SvfB is additive to that of SvfA (Fig.
126 3A). Thus, genes *Dda3937_01687*and *Dda3937_00585* were named *svfA* and *svfB* for secreted
127 virulence factor A and B.
128
129 All attempts to overproduce produce SvfA and SvfB to purify them and study more precisely
130 their function were unsuccessful because their production was toxic to the bacteria that
131 overproduce them.
132
133
134 **Expression of SvfA and SvfB**
135 To try to identify the function of SvfA and SvfB we analyzed the conditions in which their
136 genes are expressed. We tested the effect of galacturonate and polygalacturonate, two
137 compounds that are inducers of the expression of the main virulence factors, the pectate lyases,
138 and of glucose, which represses it. We also analyzed the effect of mutation in genes controlling
139 several aspects of *D. dadantii* virulence. KdgR represses the pectinase, pectin degradation and
140 the *out* genes (27). Its inducer is 2-keto-3-deoxygluconate, a polygalacturonate and
141 galacturonate catabolic derivative. PecS controls the pectinase, other secreted protein, the *out*
142 genes and those involved in resistance to oxidative stress (28). PecT is a regulator of the pectate

143 lyase, motility and exopolysaccharide synthesis genes (29). Pir regulates hyperinduction of
144 pectate lyses in response to plant signal (30). Finally, GacA, the regulator of the two-component
145 regulation system GacA-GacS, is a global regulator required for disease expression in response
146 to the metabolic status of the bacteria (31). Expression of *svfA* was slightly induced by
147 polygalacturonate but not by galacturonate (Fig. 4A). However, expression of the gene was not
148 modified in a *kdgR* background indicating that induction by polygalacturonate is not mediated
149 by *kdgR*. Growth in the presence of chicory chunks strongly induced s*vfA* expression as
150 expected from transcriptomic data showing induction in the presence of plant extract. A high
151 concentration of glucose led to a strong induction of the gene expression (Fig. 4A). This
152 regulation is mediated by the catabolite repressor protein CRP since a mutation in the *crp* gene
153 derepressed *svfA* expression. Thus, Crp is a repressor of *svfA*. Although it had not been detected
154 as a PecS-regulated gene (21), its expression is elevated in a *pecS* background. A *pir* mutation
155 derepressed also a little *svfA* expression. Neither PecT nor GacA significantly regulate *svfA*
156 expression (Fig. 4A).
157
158 Regulation of *svfB* is quite similar to that of *svfA*: it was not induced by galacturonate,
159 polygalacturonate or regulated by KdgR, it was induced by glucose and repressed by Crp, and
160 it was repressed by PecS (Fig. 4B). However, few differences can be noted: in contrast to what
161 is observed with *svfA*, no induction by plant pieces was observed and PecT was a repressor of
162 *svfB* expression while pir did not seem to control it (Fig. 4B).
163
164
165 **Occurrence of new secreted proteins in other Dickeya species**
166 Presence of *svfA*, *svfB*, *virK* and *yoaJ* was searched in all the *Dickeya* type strains, and in a few
167 *Pectobacterium* strains (Table 1). Presence and number of proteins of the DUF 4879 family is

168 variable among *Dickeya* species. *svfA* is present in all strains except *D. zeae*, *D. chrysanthemi*,
169 *D. poaceiphila* and *D. paradisiaca*. *svfB* is absent in *D. chrysanthemi*, *D. poaceiphila* and *D.*
170 *paradisiaca*, *D. undicola* and *D. aquatica*. A third gene located next to *svfA* and resulting
171 probably from a duplication is found in *D. fangzhongdai* and *D. undicola*. It has 53% homology
172 with *D. dadantii* SvfA and 34% with *D. dadantii* SvfB. It was named *svfC*. SvfC is shorter that
173 SvfA and SvfB (126 amino acid for the mature protein, 13.2 kDa) and has the same size as *B.*
174 *cereus* YolA (Fig. 2). It possesses a signal sequence, indicating that it could also be secreted by
175 the Out system. Thus, the number of genes of this family in *Dickeya* strains varies from 0 to 3.
176 Homologues of the *svf* genes can be found in some *Pectobacterium* strains (Table 1). For
177 example, two copies are present in *P. carotovorum* subsp *carotovorum*. However, even in a
178 given species the gene may be present or not (presence of a homologue of *svfB* in 10 out of the
179 23 *P. brasiliense* strains present in the ASAP data bank
180 (https://asap.ahabs.wisc.edu/asap/home.php). Outside *Pectobacteriaceae*, homologues of *svfB*
181 can be found in a few gammaproteobacteriaceae, i.d. in some *Photorhabdus*, *Luteibacter* and
182 *Pseudoalteromonas* strains. *yoaJ* is present in all *Dickeya* and *Pectobacterium* strains except
183 *D. poaceiphila* and *D. paradisiaca*. *virK* is absent in *D. aquatica* and in all *Pectobacterium*
184 strains.
185
186 We examined also the presence or absence of genes of other non-pectinolytic proteins known
187 to be secreted by a T2SS in *Dickeya* or *Pectobacterium*: *ibpS*, *nipE*, *xynA*, *avrL*/*avrM* (Table
188 1). IbpS is a metal binding protein that prevents ROS-induced killing of bacteria (17). NipE is
189 a toxin that provoke plant cell death (32). XynA is a xylanase that was identified in a corn strain
190 of *Erwinia chrysanthemi* (33). AvrL is a protein with homology with a *Xanthomonas campestris*
191 avirulence protein AvrL (15). Two very similar proteins, AvrL and AvrM, are encoded by *D.*
192 *dadantii* 3937. AvrL was named Svx in *P. atrosepticum* where a role in virulence has been

193 proven (34). However, its function in *Dickeya* has not been studied. While IbpS and NipE are
194 present in almost all the species, except *D. paradisiaca* and *D. poaceiphila* for NipE, a variation
195 in the presence and number of XynA and AvrL was observed (Table 1). Thus, the repertoire of
196 T2SS-secreted protein known to be important for virulence is very variable from species to
197 species.
198
199
200 **DISCUSSION**

201 The T2SS of *Dickeya* and *Pectobacterium* is the main virulence factor of these bacteria. The
202 knowledge of the repertoire of secreted proteins is necessary to better understand the
203 mechanisms of virulence of these bacteria. This analysis has been undertaken with the model
204 strain *D. dadantii* 3937 and partially with *Pectobacterium atrosepticum* (35)(16). *Dickeya* and
205 *Pectobacterium* are characterized by their ability to degrade pectin and new species are
206 identified by this characteristic on the semi selective Crystal Violet Pectate medium. They all
207 secrete enzymes capable of degrading this polysaccharide (pectate lyases, polygalacturonases,
208 pectin methylesterases) which are produced by all these bacteria. However, recent work shows
209 that other proteins are secreted by the Out T2SS (15)(16). In this work we used published
210 transcriptome data to identify new substrates of the *Dickeya dadantii* T2SS. The most induced
211 genes in a transcriptome experiment of *D. dadantii* infecting *A. thaliana* are known virulence
212 genes (*pelI, prtA*, *rhiE*, *paeY*, *rhaD*, *ibpS*, etc...) (19). However, in this top list some genes have
213 no known function. The presence of a signal sequence in their product suggested that these
214 proteins could be substrates of the T2SS necessary for the infection process. We showed here
215 that the proteins SvfA, SvfB and YoaJ present in the top list are substrates of the Out T2SS.
216 YoaJ belongs to the family of expansins, proteins that loosen cellulose fibers. Their role as a
217 virulence factor has been shown in *P. brasiliense* and *P. atrosepticum* (23). It probably has the
218 same function in *D. dadantii*. No function could be predicted for SvfA and SvfB which belong

219 to the DUF 4879 family of proteins. However, a reduction of virulence of a *svfA* mutant and a
220 *svfA svfB* double mutant on potato could be observed proving a role of the proteins in the
221 bacterial pathogenicity. Although an additive effect of the mutations was observed, they could
222 not have exactly the same function. The mutants should be tested on various hosts to detect
223 possible differences. It can be supposed that each protein would be more active on one type or
224 one family of plant. Presence of three proteins of this family in *D. fanghzongdai* could explain
225 its wide host range. Presence of homologues of SvfA and SvfB in *Photorhabdus* and in *B.*
226 *thuringiensis* strains, two insect pathogens, indicates that the role of these proteins is not
227 restricted to plant virulence but may participate to a common process of bacterial pathogeny
228 such as cell lysis. We showed also here that the PecS-regulated protein VirK is also secreted by
229 Out. No role on virulence had been found for this protein with the chicory leaf model of
230 infection (21). Other models should be tested to find the role of this protein.
231
232 Regulation of expression of the *svfA* and *svfB* genes is atypical for a *D. dadantii* gene involved
233 in pathogeny. While expression of most of the virulence factors is induced in the presence of
234 pectin or its derivatives through the repressor KdgR and repressed by glucose, that of *svfA* and
235 *svfB* is opposite: it is activated by glucose and not controlled by KdgR. Expression of *svfA* is
236 induced in the presence of plant tissue. This pattern of regulation has been described for *ibpS*,
237 which is also strongly induced in *A. thaliana* (17). This could correspond to conditions
238 encountered during the early phases of infection: pectin has not yet been degraded and glucose
239 and saccharose are plentiful in plant tissues. *svfA* and *ibpS* could be among the earliest gene to
240 be induced at the onset of infection, before the genes involved in pectin degradation. However,
241 regulation of these genes by PecS and PecT shows that *svfA* and *svfB* are fully integrated in the
242 network of regulators that controls *D. dadantii* virulence.
243

244 This work has extended our knowledge of the Out-dependent secretome of *D. dadantii*, showing
245 that besides pectinases several other proteins are secreted. If the number of pectinolytic
246 enzymes secreted is almost identical in the various *Dickeya* species, the number of additional
247 secreted proteins varies markedly. Among the proteins we analyzed (Table 1), *D. paradisiaca*
248 has only one (VirK) while *D. fanghzongdai* has ten. All the intermediate combinations can be
249 found in the various species. There seems to be less variations in the *Pectobacterium* strains
250 that we surveyed. It is tempting to speculate that the presence/absence of these proteins could
251 be one of the origin of the host preference of the *Dickeya* strains, providing additional virulence
252 factors necessary to infect certain hosts. Articles that compare *Dickeya* strains to understand
253 what makes their difference in their host range or aggressivity often focus only on the presence
254 of the six known types of secretion systems without analyzing what proteins could be secreted
255 (18)(36)(37). An exhaustive analysis of the secreted proteins would be more informative.
256
257 Is there other T2SS-secreted proteins to be identified in *Dickeya* strains? No specific signal is
258 present on T2SS-secreted proteins that would allow their identification. 2D gels which were
259 used in previous studies performed on *D. dadantii* and *P. atrosepticum* to identify these proteins
260 have a limited sensitivity (15)(34). More sensitive methods such as liquid chromatography-
261 tandem mass spectrometry (LC-MS/MS) can now be used (38). However, they give many false
262 positive results since periplasmic and cytoplasmic proteins are often found in the culture
263 supernatant. The approach we used here allowed the identification of four new secreted
264 proteins. However, all these methods have a drawback. They can only detect proteins that are
265 produced. The rhamnogalacturonate lyase RhiE could only be detected when the bacteria were
266 cultivated in the presence of rhamnose (39). YoaJ and VirK were not induced in *D. dianthicola*
267 grown on potato (25). Another problem is that a protein may not exist in the strain tested. An
268 analysis of the secretome of several *Dickeya* strains grown in several conditions will be

269 necessary to have a global view of all the virulence factors that can be secreted by *Dickeya*
270 species and evaluate their potential role in pathogenicity.
271

272 **MATERIAL AND METHODS**
273

274 **Bacterial strains and growth conditions**.

275 Bacterial strains, phages, plasmids and oligonucleotides used in this study are described in Table 2. *D.*
276 *dadantii* and *E. coli* cells were grown at 30 and 37°C respectively in LB medium or M63 minimal medium
277 supplemented with a carbon source (0.2%, w/v unless otherwise indicated). When required antibiotics
278 were added at the following concentrations: ampicillin, 100 mg l^{-1}, kanamycin and chloramphenicol, 25
279 mg l^{-1}. Media were solidified with 1.5% (w/v) agar. Transduction with phage ΦEC2 was performed
280 according to Résibois *et al.* (40)

281 **Mutant construction**.

282 To construct strain A6418 that contains a *svfA-uidA* fusion a 1.3 kb DNA fragment containing
283 *svfA* was amplified with primers 17176H+ and 17176A. The resulting fragment was inserted into
284 the pGEM-T plasmid (Promega). A XbaI site was created by site directed mutagenesis with the
285 primers 17176XbaF and 17176XbaR into the *svfA* coding sequence and a *uidA*-kanR cassette was
286 inserted into this XbaI site. To construct strain A6467 that contains a *svfB-uidA*-kanR fusion and
287 a CmR cassette a 2000bp DNA fragment containing *svfB* was amplified with the primers
288 15544L2+ and 15544L2-. The resulting fragment was inserted into the pGEM-T plasmid. A XmaI
289 site was created by site directed mutagenesis into *svfB* coding sequence with the primers
290 15544XmaF and 15544XmaR and a *uidA*-kanR cassette was inserted into this created XmaI site
291 unique. All the constructs were recombined into the *D. dadantii* chromosome according to Roeder
292 and Collmer (41). Recombinations were checked by PCR. His-tagged versions of the proteins
293 SvfA, SvfB, YolA, VirK were constructed by amplifying the corresponding genes with the primers

294 17176H+ and 17176H-, 15544H+ and 15544H-, 14642H+ and 14642H-, VirKH+ and VirKH-,
295 respectively. The resulting DNA fragments were cloned into plasmid pGEMT.
296
297 **Secretion assays and Western blots.**
298 *D. dadantii* strains containing the plasmid to test were grown overnight in LB medium in the
299 presence of the appropriate antibiotic. Culture supernatant containing secreted proteins was
300 separated from cells by centrifugation at 10,000*g* for 3 min, and both fractions were loaded onto
301 12% polyacrylamide gel electrophoresis (SDS-PAGE). The proteins were next transferred onto
302 Immobilon P membrane (Merck) and probed with Ni-NTA-HRP.
303
304 **Pathogenicity tests**.
305 Bacteria were grown overnight in LB medium, centrifuged and resuspended at OD_{600} 1 in M63
306 medium. Potatoes were surface sterilized with 70% ethanol and dried. A hole was made with a
307 pipette tip and 10μl of bacteria were deposited in the hole which was covered with mineral oil.
308 Potatoes were placed over a wet paper in a tray contained in a plastic bag to maintain moisture.
309 After 48h at 30°C, the weight of rotten tissue was measured.
310
311 **Enzymatic assays**.
312 β-glucuronidase assays were performed on toluenized extracts of cells grown to exponential
313 phase using the method of Bardonnet *et al* (42). with *p*-nitrophenyl-β-D-glucuronate as the
314 substrate.
315
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457

458

459 Fig 1: Identification of new secreted proteins. Wild-type and *outD* mutant strains containing
460 plasmid bearing the gene of the protein to test were grown overnight in LB medium. The
461 supernatant (S) and cellular (C) fractions were separated by SDS-PAGE. After blotting, the
462 proteins were detected with Ni-NTA-HRP.

463

464 Fig. 2: Alignment of Svf proteins. The sequences of *D. dadantii* SvfA (Dda3937_01687), SvfB
465 (Dda3937_00585) and *D. fanghzongdai* SvfC (CVE23_15565), *B. cereus* WP-193674364.1
466 and *Photorhabdus asymbiotica* CAQ86327.1 without their signal sequence were aligned with
467 Clustal omega. Identical residues are indicated by a star and chemically equivalent residues by
468 a double dot

469

470 Fig. 3. Virulence of *svfA* and *svfB* mutants. A. Potatoes were infected with the wild type, and
471 the *svfA*, the *svfB* and the *svfA svfB* mutants. Rotten tissue was weighed after 48 h. B.
472 Complementation of the *svfA* mutation. Potatoes were infected with the wild type strain and the
473 *svfA* mutant containing the empty plasmid pBBRGm and the *svfA* mutant containing the pBBR
474 plasmid bearing *svfA*. Rotten tissue was weighed after 48h. Statistical tests were performed
475 using the Wilcoxon- Mann-Whitney test. The *p*-value were compared with an alpha risk of 4%.
476 $p < 0.001$=***, $p < 0.005$=**, $p < 0.01$=*.

477

478

479 Fig 4 : Expression of *svfA and svfB* in various growth conditions. A. The *D. dadantii* strain
480 A6418 containing the *svfA-uidA* fusion and derivative strains containing an additional
481 regulatory mutation were grown in M63 medium in the presence of the indicated compounds
482 (Y = glycerol, G = glucose, A = galacturonate, PGA = polygalacturonate, E = chicory chunks).

483 Strains with additional mutations were grown with glycerol as a carbon source except the *crp*
484 mutant that was grown with 0.2% glucose. ß-glucuronidase activity was measured with *p*-
485 nitrophenyl-ß-D-glucuronate. B. The *D. dadantii* strain A6467 containing the *svfB-uidA* fusion
486 and derivative strains containing an additional regulatory mutation were grown in M63 medium
487 in the presence of the indicated compounds (Y = glycerol, G = glucose, A = galacturonate, PGA
488 = polygalacturonate, E = chicory chunks). Strains with additional mutations were grown with
489 glycerol as a carbon source except the *crp* mutant that was grown with 0.2% glucose ß-
490 glucuronidase activity was measured with *p*-nitrophenyl-ß-D-glucuronate. Activities are
491 expressed in µmoles of *p*-nitrophenol produced per minute and per milligram of bacterial dry
492 weight ± standard deviation. Data are expressed as the mean (n = 6) from six independent
493 experiments. Statistical tests were performed using the Wilcoxon- Mann-Whitney test. The *p*-
494 value were compared with an alpha risk of 4%. $p < 0.001$=***,
495 $p < 0.005$=**, $p < 0.01$=*.
496
497
498 Table 1: Presence of Out-secreted proteins in various *Dickeya* and *Pectobacterium* strains.
499
500 Table 2: Oligonucleotides and strains used in this study.
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505

506 Table 1 : Presence of Out-secreted proteins in various *Dickeya* and *Pectobacterium* strains.

507

Strain	SvfA	SvfB	SvfC	YoaJ	VirK	IbpS	NipE	XynA	AvrL
D. dadantii	1	1	0	1	1	1	1	1	2
D. diffenbachiae	1	1	0	1	1	1	1	0	2
D. fangzhongdai	1	1	1	1	1	1	1	1	2
D. solani	1	1	0	1	1	1	1	1	2
D. zeae	0	1	0	1	1	1	1	1	1
D. oryzae	1	1	0	1	1	1	1	1	0
D. dianthicola	0	1	0	1	1	1	1	0	1
D. undicola	1	0	1	1	1	1	1	0	1
D. lacustris	1	0	0	0	0	1	1	0	2
D. aquatica	1	0	0	1	0	1	1	0	2
D. chrysanthemi	0	0	0	1	1	1	1	0	1
D. paradisiaca	0	0	0	0	1	0	0	0	0
D. poaceiphila	0	0	0	0	1	1	0	1	0
P. atrosepticum	0	0	0	1	0	1	1	0	1
P. carotovorum	1	1	0	1	0	1	1	0	1
P. parmentieri	0	0	0	1	0	1	1	0	1
P. polaris	0	0	0	1	0	1	1	0	1

508

509 The strains used in this study are *D. dadantii* 3937, *D. aquatica* 174/2, *D. chrysanthemi*

510 ATCC 11663, *D. dadantii* subsp *dieffenbachiae* NCPPB 2976, *D. dianthicola* NCPPB 453, *D.*

511 *fanghzongdai* DSM 101947, *D. lacustris* S29, *D. oryzae* ZYY5, *D. paradisiaca* ATCC 33242,

512 *D. poaceiphila* NCPPB 569, *D. solani* IPO 2222, *D. undicola* 2B12, *D. zeae* NCPPB 2538, *P.*

513 *atrosepticum* ATCC 33260, *P. carotovorum* subsp. *carotovorum* ATCC 15713, *P.*

514 *parmentieri* RNS08.42.1A and *P. polaris* NIBIO 1006. The presence and number of proteins

515 detected in each strain is indicated.

516

517 Table 2: Oligonucleotides and strains used in this study

518

519 Oligonucleotides

520 17176H+	CCTCCTGAGATTAGAGAGAG
521 17176A	CGCGCCGGTGTTTTTCTTGCG
522 17176H-	GGTTAGTGATGGTGATGGTGATGCTGAATATTGAGCGACGTGC
523 17176XbaF	GAACAGGATGTGTCCTCTTCTAGACACAAAGCGCTGCGTGCG
524 17176XbaR	CGCACGCAGCGCTTTGTGTCTAGAAGAGGACACATCCTGTTC
525 15544L2+	CTAAGAATCAGTCAGTTTGCG
526 15544L2-	AGGCAGATAACGCTACTCGCC
527 15544H+	ATTTATACTCGCCACCGATGC
528 15544XmaF	GCCTTGCGAGCCCCCGCTCCCGGGCTGTCTCGGGTTACCGTG
529 15544XmaR	CACGGTAACCCGAGACAGCCCGGGAGCGGGGGCTCGCAAGGC
530 15544H-	GGTTAGTGATGGTGATGGTGATGTTTAATATAGGTCTGTGTGAAC
531 14642H+	GGTGAGGAATAATTCTGGCC
532 14642H-	GGTTAGTGATGGTGATGGTGATGAGGCAGTTGTACTTTTCCAG
533 VirKH+	TGCCGTATGTGATAGTCACG
534 VirKH-	CCTTAGTGATGGTGATGGTGATGTTGCTTGAAGAAGCGGATATC

535

536

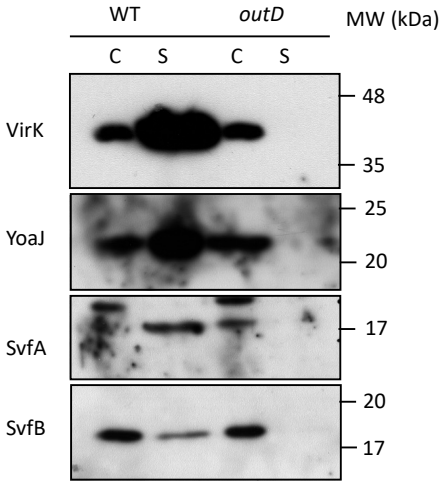
537 *Dickeya dadantii* strains

538

539 3937	Wild type	Laboratory collection
540 A5653	*outD* ::CmR	Laboratory collection
541 A6417	*svfB* ::CmR	This work
542 A6418	*svfA* ::*uidA*-kanR	This work
543 A3838	*kdgR* ::Mu-CmR	(19)
544 A3845	*pecS*::Mu-CmR	(28)
545 A3846	*pecT*::CmR	(29)
546 A3849	*pir*::CmR	Laboratory collection
547 A4237	*gacA*::CmR	(31)
548 A6434	*kdgR*::Mu-CmR *svfA*::*uidA*-kanR	This work
549 A6435	*pecS*::Mu-CmR *svfA* :*uidA*-kanR	This work
550 A6436	*pecT*::CmR *svfA*::*uidA*-kanR	This work
551 A6436	*pir*::CmR *svfA*::*uidA*-kanR	This work

A6437	*gac*A::CmR *svfA*::*uidA*-kanR	This work
A6467	*svfB*::*uidA*-kanR	This work
A6469	*kdgR*::Mu-CmR *svfB*::*uidA*-kanR	This work
A6470	*pecS*::Mu-CmR *svfB*::*uidA*-kanR	This work
A6471	*pecT*::CmR *svfB*::*uidA*-kanR	This work
A6472	*pir*::CmR *svfB*::*uidA*-kanR	This work
A6473	*gacA*::CmR *svfB*::*uidA*-kanR	This work

564



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571 Fig. 1

572

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SvfA            -------AQTS----DEPVQTVITALD--SPFVDYPLSAGSEQDVSSSEHKALRAPAPAL 47
SvfC            -------------------------------------------------------APAPAL 6
Bacillus        -------------------------------------------------------APAPPL 6
SvfB            GYSQTINNTDEAGPPIELG---LTAFD-SL-ESNSPLSAARDTS-RASTSGALRAPAPAL 54
Photorhabdus    --------QTEQLPKVPAAQKVLIEQSQLLPNVKSPIR-AEERD-LKIEDGSVHAPAPGL 50
                                                                  **** *

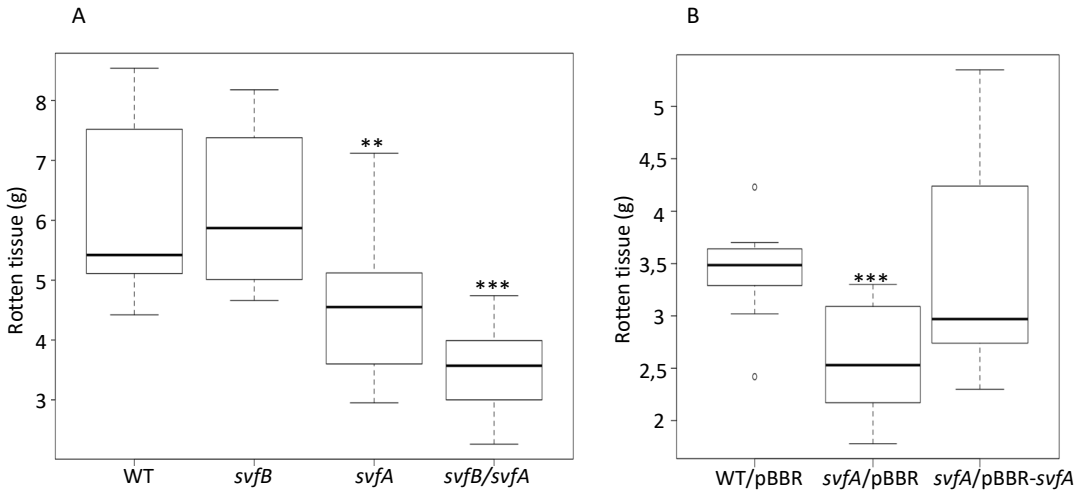
SvfA            SSVQVYAVYSSLKGGWQAVPT-NTLSL-SGYAGGTLRIAVLEVGYGGNRIGWLNGGQTS- 104
SvfC            SGLRIKVLSGVYGGTWQYAPV-NAVSIGPGYAGGTLQIAVVETGYGGNRIGWINGEQKK- 64
Bacillus        TSLNVVKVESQL-GGVEFIGA-NNLSTVKDHGGSYLYIYTNEMGYGRNPIAQMSGQKLKK 64
SvfB            SRVTVYAVGSSN-CGWEYMTSIGQLSTTCDHGGAQLRVAVQEIGYGNNPVAWMNGGVLPR 113
Photorhabdus    TNMWVYAVGSTN-CGWEYTSNL--FATTCDHGGQQLRAAVLEIGYGYSSFAWMNGGLLPN 107
                : : :  : .      :       .:   .:.*  *   . * *** . .. :.*

SvfA            --PYQVNPVCVVSGRYTESCPAGSIVSGWMAYFNADNMSSVTFRYQSTSTNFPNRTLSTS 162
SvfC            --PDSVKLACLVKGEMTDNCPRGATGAGWIAYFSANYQTSVTFRYQSTSANFPYKTLSTS 122
Bacillus        VDS---KMI---------DINGDRTVDGWEYKWDASGQQNGQFKYQNTSTNAPWNTLFTS 112
SvfB            SANYQTDGICIVGNQYTFPCPAGYTVVGWMYYYNLDGTDNGQFKFQDTSTNAPRNTLFTQ 173
Photorhabdus    SAMYSSKTVCITNGYYTWPCTAGQTVVGYLHEYNLDGNQNGTFRYQNTSTNSPWNTMSVQ 167
                      .              .    *:   :. .   .  *::*.**:* * .*: ..

SvfA            LNIQ 166
SvfC            LTIK 126
Bacillus        LNIK 116
SvfB            TYIK 177
Photorhabdus    INIL 171
                  *
```

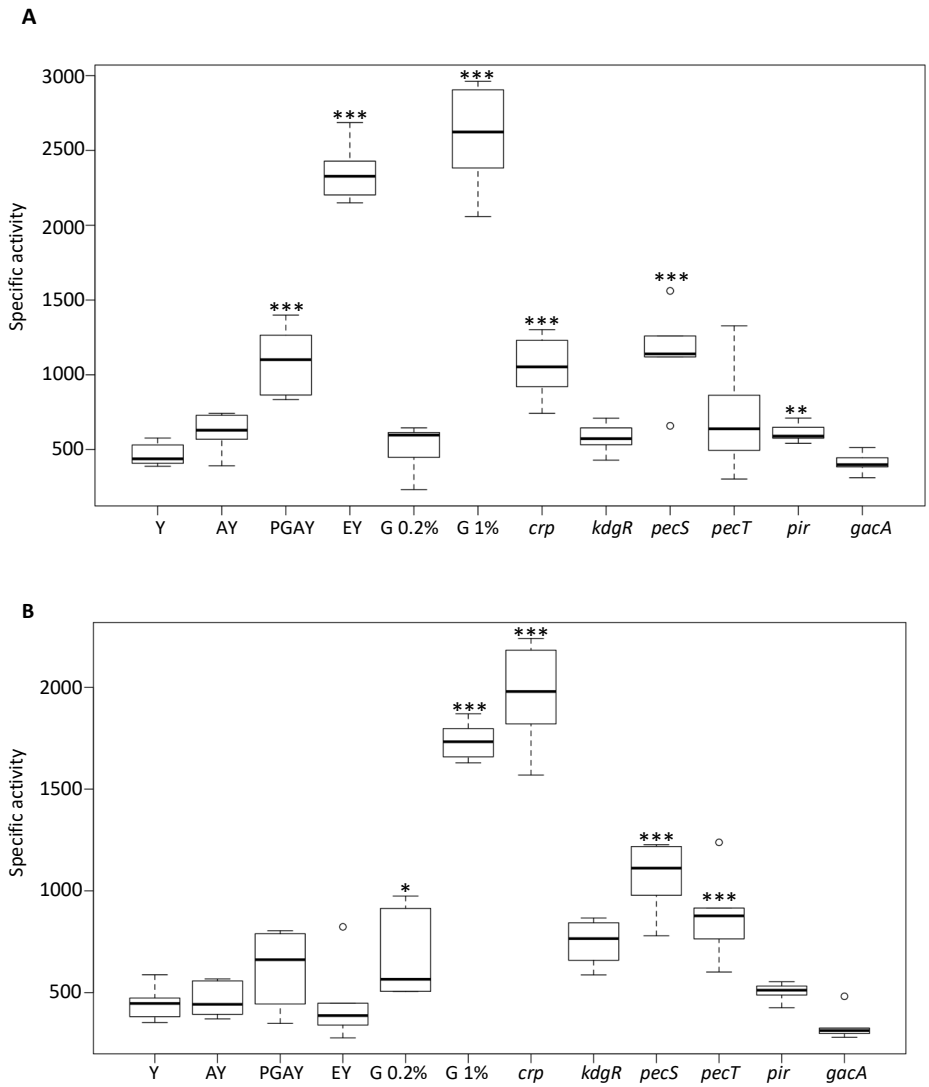
Fig . 2

579



580

581 Fig. 3


A
Specific activity
3000
2500
2000
1500
1000
500
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Y
AY
PGAY
EY
G 0.2%
G 1%
crp
kdgR
pecS
pecT
pir
gacA
B
Specific activity
2000
1500
1000
500
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Y
AY
PGAY
EY
G 0.2%
G 1%
crp
kdgR
pecS
pecT
pir
gacA

Fig. 4