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 (T2SS) named Out is necessary for bacterial virulence. Its study in *D. dadantii* showed that it secretes a wide range of plant cell wall degrading enzymes, including pectinases and a cellulase. However, the full repertoire of exoproteins it can secrete has probably not yet been identified. Secreted proteins are first addressed to the periplasm before their secretion by Out. No secretion signal present on the protein allows the identification of substrates of a T2SS. 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, the putative virulence factor 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* genes are present in a variable number of copies in other *Pectobacteriaceae*, up to three in *D. fanghzongdai*. This work opens the way to the study of the role 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.

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43

44 **INTRODUCTION**

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

67 detectable enzymes. *D. dadantii* secretes by the Out machinery nine pectate lyases, one pectin
68 methylesterase, one pectin acetylesterase and one rhamnogalacturonate lyase (14). A proteomic
69 analysis of the secreted proteins by 2D gel electrophoresis allowed the identification of two
70 other secreted proteins, the feruloyl esterase FaeD and a protein with homology with a
71 *Xanthomonas campestris* avirulence protein AvrL (15). A search in *D. dadantii* of homologues
72 of proteins secreted by the Out T2SS of *Pectobacterium atrosepticum* (16) recently led to the
73 characterization of the metal binding protein IbpS (17). There is no strict host specificity for
74 Dickeya species, however some of them show a preference for some plant species. Since all the
75 pectinolytic enzymes studied in *D. dadantii* are present in most of other *Dickeya* species these
76 enzymes are probably not responsible for the host preference observed for these bacteria (18).
77 We hypothesized that additional T2SS-secreted proteins specific for some species might exist
78 and play a role in the host preference. To identify such proteins, we analyzed previously
79 published *D. dadantii* transcriptome data, looking for genes induced in plant infection
80 conditions and encoding proteins with a signal sequence. We identified several proteins
81 secreted by the Out machinery and showed that two proteins of the DUF4879 family, SvfA and
82 SvfB are *D. dadantii* virulence factors.
83

84 **MATERIAL AND METHODS**
85

86 **Bacterial strains and growth conditions**.

87 Bacterial strains, phages, plasmids and oligonucleotides used in this study are described in Table 2. *D.*
88 *dadantii* and *E. coli* cells were grown at 30 and 37°C respectively in LB medium or M63 minimal medium
89 supplemented with a carbon source (0.2%, w/v unless otherwise indicated). When required antibiotics
90 were added at the following concentrations: ampicillin, 100 mg l^{-1}, kanamycin and chloramphenicol, 25

91 mg l^{-1}. Media were solidified with 1.5% (w/v) agar. Transduction with phage ΦEC2 was performed
92 according to Résibois *et al.* (40)

93 **Mutant construction**.

94 To construct strain A6418 that contains a *svfA-uidA* fusion a 1.3 kb DNA fragment containing
95 *svfA* was amplified with primers 17176H+ and 17176A. The resulting fragment was inserted into
96 the pGEM-T plasmid (Promega). A XbaI site was created by site directed mutagenesis with the
97 primers 17176XbaF and 17176XbaR into the *svfA* coding sequence and a *uidA*-kanR cassette was
98 inserted into this XbaI site. To construct strain A6467 that contains a *svfB-uidA*-kanR fusion and
99 a CmR cassette a 2000bp DNA fragment containing *svfB* was amplified with the primers
100 15544L2+ and 15544L2-. The resulting fragment was inserted into the pGEM-T plasmid. A XmaI
101 site was created by site directed mutagenesis into *svfB* coding sequence with the primers
102 15544XmaF and 15544XmaR and a *uidA*-kanR cassette was inserted into this created unique XmaI
103 site. All the constructs were recombined into the *D. dadantii* chromosome according to Roeder
104 and Collmer (41). Recombinations were checked by PCR. His-tagged versions of the proteins
105 SvfA, SvfB, YoaJ and VirK were constructed by amplifying the corresponding genes with the
106 primers 17176H+ and 17176H-, 15544H+ and 15544H-, 14642H+ and 14642H-, VirKH+ and
107 VirKH-, respectively. The resulting DNA fragments were cloned into plasmid pGEMT.

108

109 **Secretion assays and Western blots.**

110 *D. dadantii* strains containing the plasmid to test were grown overnight in LB medium in the
111 presence of the appropriate antibiotic. Culture supernatant containing secreted proteins was
112 separated from cells by centrifugation at 10,000 g for 3 min, and both fractions were loaded onto
113 12% polyacrylamide gel electrophoresis (SDS-PAGE). The proteins were next transferred onto
114 Immobilon P membrane (Merck) and probed with Ni-NTA-HRP.

115

116 **Pathogenicity tests**.

117 Bacteria were grown overnight in LB medium, centrifuged and resuspended at OD_{600} 1 in M63
118 medium. Potatoes were surface sterilized with 70% ethanol and dried. A hole was made with a
119 pipette tip and 10 μl of bacteria were deposited in the hole which was covered with mineral oil.
120 Potatoes were placed over a wet paper in a tray contained in a plastic bag to maintain moisture.
121 After 48 h at 30°C, the weight of rotten tissue was measured.

122

123 **Enzymatic assays**.

124 β-glucuronidase assays were performed on toluenized extracts of cells grown to exponential
125 phase using the method of Bardonnet *et al* (42) with *p*-nitrophenyl-β-D-glucuronate as the
126 substrate.

127

128

129 # RESULTS
130

131 **Identification of new Out-secreted proteins**

132 To have a more complete knowledge of the proteins secreted by the *D. dadantii* Out T2SS that
133 could be involved in the pathogenicity process, we searched for candidate genes in recently
134 published transcriptome data (19)(20). We selected genes strongly induced during plant
135 infection and coding for a protein possessing a signal sequence. We retained the genes
136 *Dda3937_01687*, *Dda3937_00585* (thereafter named SvfA and SvfB, respectively) and
137 *Dda3937_00081* (also named *yoaJ*). We also retained VirK, a protein of unknown function
138 with a signal sequence identified among the genes controlled by the transcriptional regulator
139 PecS of many virulence factors (21). Each protein was tagged with a His-tag and its secretion
140 was analyzed in the *D. dadantii* wild type strain and an *outD* mutant in which the Out machinery
141 is not functional. The proteins SvfA, SvfB, YoaJ and VirK were detected in the supernatant of

142 the wild type strain but not of the mutant, demonstrating their secretion by the Out machinery
143 (Fig. 1). Production of these proteins from a gene cloned on a multicopy plasmid may explain
144 why secretion was not total in the wild type strain.
145
146 YoaJ is a PecS-regulated gene (21) and it was found among the most induced genes during
147 *Arabidopsis* infection or culture in the presence of plant extracts (19). It encodes a protein with
148 homology to expansins. These proteins are able to non-enzymatically loosen cell wall cellulose.
149 They are found in all plants where they have a role in cell wall extension and also in many plant
150 pathogenic microorganisms (22). Their role in virulence has been shown in *Ralstonia*
151 *solanacearum*, in *P. atrosepticum*, *P. Brasiliense* and in the plant pathogen *Erwinia*
152 *tracheiphila* (23) (24). The role of *D. dadantii* expansin is probably the same. VirK is a protein
153 of unknown function that has homologues in several plant pathogenic bacteria such as *R.*
154 *solanacearum*, *Agrobacterium tumefaciens, Lonsdalea* and *Xanthomonas*. VirK is controlled
155 by PecS and induced during Arabidopsis infection or culture in the presence of plant extracts
156 (21) (20). No symptom for the *D. dadantii virK* mutant was observed whatever the plant tested
157 (21). The two proteins SvfA and SvfB are studied in the next paragraphs.
158
159 **SvfA and SvfB are virulence factors**
160 *svfA* and *svfB* are among the most induced *D. dadantii* genes during Arabidopsis infection or
161 during culture of the bacteria in the presence of plant extracts (19). They are also strongly
162 expressed during maceration of potato tubers by *D. dianthicola* and *D. solani* (25). The two *D.*
163 *dadantii* proteins share 43% identity and 58% similarity in amino acid composition (Fig. 2).
164 SvfA is 187 amino acid long (165 for the mature form, 17.5 kDa) and SvfB is 198 amino acid
165 long (177 for the mature form, 18.9 kDa). These proteins belong to the DUF 4879 family of
166 proteins. Proteins of this family have no known function. YolA,a protein of the DUF 4879

167 family showing low homology with SvfB, is among the most highly secreted protein of *Bacillus*
168 *subtilis* (26). YolA is also present in *B. cereus* and in the insect pathogen *B. thuringiensis*. *B.*
169 *subtilis* YolA is shorter than SvfA and SvfB, missing the N-terminal more variable part (Fig.
170 2).
171
172 *svfA* and *svfB* mutants have been constructed and their pathogenicity has been tested on potato.
173 The *svfA* mutant was significantly less aggressive than the wild type strain while the *svfB* mutant
174 was not significantly affected (Fig. 3A). Virulence of the *svfA* mutant could be restored by
175 introduction of a plasmid bearing the wild type *svfA* gene (Fig. 3B). Virulence of the double
176 *svfA svfB* mutant was further reduced showing that the role of SvfB is additive to that of SvfA
177 (Fig. 3A). Thus, genes *Dda3937_01687*and *Dda3937_00585* were named *svfA* and *svfB* for
178 secreted virulence factor A and B.
179
180 All our attempts to overproduce the proteins SvfA and SvfB in order to purify them and to study
181 more precisely their function were unsuccessful because their production was toxic to the
182 bacterial cells engineered to overproduce them.
183
184
185 **Expression of *svfA* and *svfB***
186 To try to identify the function of SvfA and SvfB, we analyzed the conditions in which their
187 genes are expressed. We tested the effect of galacturonate and polygalacturonate, two
188 compounds that are inducers of the expression of the main virulence factors, the pectate lyases,
189 and of glucose, which represses it. We also analyzed the effect of mutations in genes controlling
190 several aspects of *D. dadantii* virulence. KdgR represses the pectinase, pectin degradation and
191 *out* genes (27). Its inducer is 2-keto-3-deoxygluconate, a polygalacturonate and galacturonate

192 catabolic derivative. PecS controls genes encoding the pectinases, diverse secreted protein, the
193 Out machinery and proteins involved in resistance to oxidative stress (28). PecT is a regulator
194 of the pectate lyase, motility and exopolysaccharide synthesis genes (29). Pir regulates
195 hyperinduction of pectate lyses in response to plant extracts (30). GacA, the regulator of the
196 two-component regulatory system GacA-GacS, is a global regulator required for disease
197 expression in response to the metabolic status of the bacteria (31). Expression of *svfA* was
198 slightly induced by polygalacturonate but not by galacturonate (Fig. 4A). However, expression
199 of this gene was not modified in a *kdgR* background indicating that induction by
200 polygalacturonate is not mediated by KdgR. Growth in the presence of chicory chunks strongly
201 induced s*vfA* expression as expected from transcriptomic data showing induction in the
202 presence of plant extract. A high concentration of glucose led to a strong induction of *svfA*
203 expression (Fig. 4A). This regulation is mediated by the catabolite repressor protein CRP since
204 a mutation in the *crp* gene derepressed *svfA* expression. Thus, Crp is a repressor of *svfA*.
205 Although it had not been previously identified as a PecS-regulated gene (21), *svf*A expression
206 is increased in a *pecS* background. A *pir* mutation provoked a weak derepression of *svfA*
207 expression. Neither PecT nor GacA significantly regulate *svfA* expression (Fig. 4A).
208
209 Regulation of *svfB* shows some similarity to that of *svfA*: it was not induced by galacturonate,
210 polygalacturonate or regulated by KdgR, it was induced by glucose and repressed by Crp, and
211 it was repressed by PecS (Fig. 4B). However, a few differences can be noted: in contrast to
212 what is observed with *svfA*, no induction by plant pieces was observed for *svfB* and PecT was
213 a repressor of *svfB* expression while Pir did not seem to control it (Fig. 4B).
214
215
216 **Occurrence of the new secreted proteins in other Dickeya species**

217 Presence of *svfA*, *svfB*, *virK* and *yoaJ* was searched in the genome of all the *Dickeya* type strains,
218 and in a few *Pectobacterium* strains (Table 1). Presence and number of proteins of the DUF
219 4879 family is variable among *Dickeya* species. The gene *svfA* is present in all strains except
220 *D. zeae*, *D. chrysanthemi*, *D. poaceiphila* and *D. paradisiaca*. The gene *svfB* is present in most
221 species but is absent in *D. chrysanthemi*, *D. poaceiphila* and *D. paradisiaca*, *D. undicola* and
222 *D. aquatica*. A third gene located next to *svfA* and probably resulting from a duplication is
223 found in *D. fangzhongdai* and *D. undicola*. It was named *svfC*. It has 53% homology with *D.*
224 *dadantii* SvfA and 34% with *D. dadantii* SvfB. SvfC is shorter that SvfA and SvfB (126 amino
225 acid for the mature protein, 13.2 kDa) and has the same size as *B. cereus* YolA (Fig. 2). It
226 possesses a signal sequence, indicating that it could also be secreted by the Out system. Thus,
227 the number of genes of the DUF 4879 family in *Dickeya* strains varies from 0 to 3. Homologues
228 of the *svf* genes can also be found in some *Pectobacterium* strains (Table 1). For example, two
229 copies are present in *P. carotovorum* subsp *carotovorum*. However, even in a given species, the
230 gene may be present or not (presence of a homologue of *svfB* in 10 out of the 23 *P. brasiliense*
231 strains present in the ASAP data bank (https://asap.ahabs.wisc.edu/asap/home.php). Outside
232 *Pectobacteriaceae*, homologues of *svfB* can be found in a few Gammaproteobacteriaceae, i.d.
233 in some *Photorhabdus*, *Luteibacter* and *Pseudoalteromonas* strains. *yoaJ* is present in all
234 *Dickeya* and *Pectobacterium* strains except *D. poaceiphila* and *D. paradisiaca*. *virK* is present
235 in all Dickeya strains except in *D. aquatica* and absent in all *Pectobacterium* strains tested.
236
237 We also examined the presence or absence of genes of other non-pectinolytic proteins known
238 to be secreted by a T2SS in *Dickeya* or *Pectobacterium*: *ibpS*, *nipE*, *xynA*, *avrL*/*avrM* (Table
239 1). IbpS is a metal binding protein that prevents ROS-induced killing of bacteria (17). NipE is
240 a toxin that provoke plant cell death (32). XynA is a xylanase that was identified in a corn strain
241 of *Dickeya zeae* previously named *Erwinia chrysanthemi* (33). AvrL is homologous to the

242 *Xanthomonas campestris* avirulence protein AvrL (15). Two very similar proteins, AvrL and
243 AvrM, are encoded by *D. dadantii* 3937. AvrL was named Svx in *P. atrosepticum* where a role
244 in virulence has been proven (34). However, its function in *Dickeya* has not been studied. IbpS
245 is present in almost all the species, except *D. paradisiaca*. NipE is absent in *D. paradisiaca* and
246 *D. poaceiphila*. Presence of XynA is variable in *Dickeya* strains and it is absent in
247 *Pectobacterium*. A variation in the presence and number of AvrL can be observed in Dickeya
248 strains (Table 1). Thus, the repertoire of T2SS-secreted protein known to be important for
249 virulence is very variable from species to species.
250
251
252 **DISCUSSION**
253 The T2SS of *Dickeya* and *Pectobacterium* is a major virulence factor of these bacteria. The
254 knowledge of the repertoire of secreted proteins is necessary to better understand the precise
255 mechanisms of virulence of these bacteria. These analyses have been undertaken with the model
256 strain *D. dadantii* 3937 and partially with *Pectobacterium atrosepticum* (35)(16). *Dickeya* and
257 *Pectobacterium* are characterized by their ability to degrade pectin and they are identified by
258 this characteristic on the semi selective Crystal Violet Pectate medium. They all secrete
259 enzymes capable of degrading pectin (pectate lyases, polygalacturonases, pectin
260 methylesterases). However, recent works show that other proteins are secreted by the Out T2SS
261 (15)(16). In the present work we used published transcriptome data to identify new potential
262 substrates of the *D. dadantii* T2SS. The most highly induced genes in a transcriptome
263 experiment of *D. dadantii* infecting *A. thaliana* are known virulence genes (*pelI, prtA*, *rhiE*,
264 *paeY*, *rhaD*, *ibpS*, etc...) (19). However, in this top list some genes have no known function.
265 The presence of a signal sequence in their product suggested that these proteins could be
266 substrates of the T2SS necessary for the infection process. We showed here that the proteins
267 SvfA, SvfB and YoaJ produced by genes present in the top list of those induced in Arabidopsis

268 are substrates of the Out T2SS. YoaJ belongs to the family of expansins, proteins that loosen
269 cellulose fibers. Their role as a virulence factor has been shown in *P. brasiliense* and *P.*
270 *atrosepticum* (23) and it probably has the same function in *D. dadantii* and other *Dickeya*
271 species. No function could be predicted for SvfA and SvfB which belong to the DUF 4879
272 family of proteins. However, a reduction of virulence of a *svfA* mutant and a *svfA svfB* double
273 mutant on potato could be observed, proving a role of the proteins in the bacterial pathogenicity.
274 Although an additive effect of the mutations was observed, they could not have exactly the
275 same function. The mutants should be tested on various hosts to detect potential differences. It
276 can be supposed that each protein would be more active on one type or one family of plant.
277 Presence of three DUF 4879 proteins in *D. fanghzongdai* could explain its wide host range,
278 from orchid to pear trees. Presence of homologues of SvfA and SvfB in *Photorhabdus* and in
279 *B. thuringiensis* strains, two insect pathogens, indicates that the role of these proteins is not
280 restricted to plant virulence but may participate to a common process of bacterial pathogeny.
281 We also showed here that the PecS-regulated protein VirK is secreted by Out. No role on
282 virulence had been observed for this protein with the chicory leaf model of infection (21). Other
283 models should be tested to find the role of this protein.
284
285 Regulation of expression of the *svfA* and *svfB* genes is atypical for a *D. dadantii* gene involved
286 in pathogeny. While expression of most of the virulence factors is induced in the presence of
287 pectin or its derivatives through the repressor KdgR and repressed by glucose, that of *svfA* and
288 *svfB* is opposite: it is activated by glucose and not controlled by KdgR. Expression of *svfA* is
289 induced in the presence of plant tissue. This pattern of regulation has been described for *ibpS*,
290 which is also strongly induced in *A. thaliana* (17). This could correspond to conditions
291 encountered during the early phases of infection: pectin has not yet been degraded and glucose
292 and saccharose are plentiful in plant tissues. *svfA* and *ibpS* could be among the earliest gene to

293 be induced at the onset of infection, before the genes involved in pectin degradation. However,
294 regulation of these genes by PecS and PecT shows that *svfA* and *svfB* are fully integrated in the
295 network of regulators that controls *D. dadantii* virulence.
296
297 This work has extended our knowledge of the Out-dependent secretome of *D. dadantii*, showing
298 that besides pectinases several other proteins are secreted. If the number of pectinolytic
299 enzymes secreted is almost identical in the various *Dickeya* species, the number of additional
300 secreted proteins varies markedly. Among the proteins analyzed (Table 1), *D. paradisiaca* has
301 only one (VirK) while *D. fanghzongdai* has ten. All the intermediate combinations can be found
302 in the various species. There seems to be less variations in the *Pectobacterium* strains surveyed.
303 It is tempting to speculate that the presence/absence of these proteins could influence the host
304 preference of some *Dickeya* species, providing additional virulence factors favorable to infect
305 certain hosts. Works that compare *Dickeya* strains to understand what makes difference in their
306 host range or aggressivity often focus only on the presence of the six known types of secretion
307 systems without analyzing what proteins could be secreted (18)(36)(37). An exhaustive analysis
308 of the secreted proteins would be more informative.
309
310 Is there other T2SS-secreted proteins to be identified in *Dickeya* strains? No specific signal is
311 present on T2SS-secreted proteins that would allow their identification. 2D gels which were
312 used in previous studies performed on *D. dadantii* and *P. atrosepticum* to identify their
313 secretome have a limited sensitivity (15)(34). More sensitive methods such as liquid
314 chromatography-tandem mass spectrometry (LC-MS/MS) can now be used (38). However,
315 they give many false positive results since periplasmic and cytoplasmic proteins are often found
316 in the culture supernatant. The approach we used here allowed the identification of four new
317 secreted proteins. However, all these methods have a drawback. They can only detect proteins

318 in conditions where they are produced. For instance, the rhamnogalacturonate lyase RhiE could
319 only be detected when the bacteria were cultivated in the presence of rhamnose (39). The genes
320 encoding YoaJ and VirK were not induced in *D. dianthicola* grown on potato (25). Another
321 problem is that a protein may not exist in the strain tested. An analysis of the secretome of
322 several *Dickeya* strains grown in several conditions will be necessary to have a global view of
323 all the additional virulence factors that can be secreted by *Dickeya* species and evaluate their
324 potential role in pathogenicity.
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478

479 Fig 1: Identification of new secreted proteins by *D. dadantii*. Wild-type and *outD* mutant strains
480 containing plasmid bearing the gene of the selected protein were grown overnight in LB
481 medium. The supernatant (S) and cellular (C) fractions were separated by SDS-PAGE. After
482 blotting, the proteins were detected with Ni-NTA-HRP.

483

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

489

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

497

498

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

503 Strains with additional mutations were grown with glycerol as a carbon source except the *crp*
504 mutant that was grown with 0.2% glucose. ß-glucuronidase activity was measured with *p*-
505 nitrophenyl-ß-D-glucuronate. B. Similar experiment for the *D. dadantii* strain A6467
506 containing the *svfB-uidA* fusion and its derivative strains containing an additional regulatory
507 mutation. Activities are expressed in µmoles of *p*-nitrophenol produced per minute and per
508 milligram of bacterial dry weight ± standard deviation. Data are expressed as the mean (n = 6)
509 from six independent experiments. Statistical tests were performed using the Wilcoxon- Mann-
510 Whitney test. The *p*-value were compared with an alpha risk of 4%. $p < 0.001$=***, $p <$
511 0.005=**, $p < 0.01$=*.
512
513
514 Table 1: Presence of Out-secreted proteins in various *Dickeya* and *Pectobacterium* strains.
515
516 Table 2: Oligonucleotides and strains used in this study.
517
518
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520 .
521

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

523

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

524

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

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

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

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

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

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

531 detected by search of the corresponding gene in the genome in each strain is indicated.

532

533 Table 2: Oligonucleotides and strains used in this study

534

535 Oligonucleotides

536 17176H+	CCTCCTGAGATTAGAGAGAG
537 17176A	CGCGCCGGTGTTTTTCTTGCG
538 17176H-	GGTTAGTGATGGTGATGGTGATGCTGAATATTGAGCGACGTGC
539 17176XbaF	GAACAGGATGTGTCCTCTTCTAGACACAAAGCGCTGCGTGCG
540 17176XbaR	CGCACGCAGCGCTTTGTGTCTAGAAGAGGACACATCCTGTTC
541 15544L2+	CTAAGAATCAGTCAGTTTGCG
542 15544L2-	AGGCAGATAACGCTACTCGCC
543 15544H+	ATTTATACTCGCCACCGATGC
544 15544XmaF	GCCTTGCGAGCCCCCGCTCCCGGGCTGTCTCGGGTTACCGTG
545 15544XmaR	CACGGTAACCCGAGACAGCCCGGGAGCGGGGGCTCGCAAGGC
546 15544H-	GGTTAGTGATGGTGATGGTGATGTTTAATATAGGTCTGTGTGAAC
547 14642H+	GGTGAGGAATAATTCTGGCC
548 14642H-	GGTTAGTGATGGTGATGGTGATGAGGCAGTTGTACTTTTCCAG
549 VirKH+	TGCCGTATGTGATAGTCACG
550 VirKH-	CCTTAGTGATGGTGATGGTGATGTTGCTTGAAGAAGCGGATATC

551

552

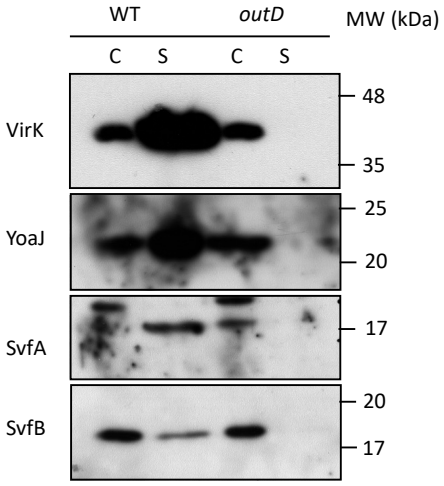
553 *Dickeya dadantii* strains

554

555 3937	Wild type	Laboratory collection
556 A5653	*outD* ::CmR	Laboratory collection
557 A6417	*svfB* ::CmR	This work
558 A6418	*svfA* ::*uidA*-kanR	This work
559 A3838	*kdgR* ::Mu-CmR	(19)
560 A3845	*pecS*::Mu-CmR	(28)
561 A3846	*pecT*::CmR	(29)
562 A3849	*pir*::CmR	Laboratory collection
563 A4237	*gacA*::CmR	(31)
564 A6434	*kdgR*::Mu-CmR *svfA*::*uidA*-kanR	This work
565 A6435	*pecS*::Mu-CmR *svfA* :*uidA*-kanR	This work
566 A6436	*pecT*::CmR *svfA*::*uidA*-kanR	This work
567 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

580



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586

587 Fig. 1

588

```
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

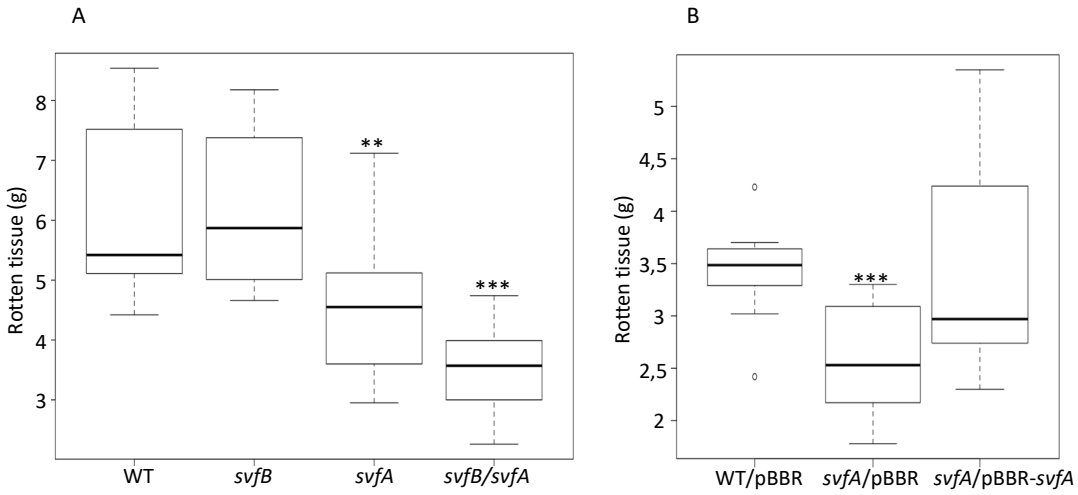
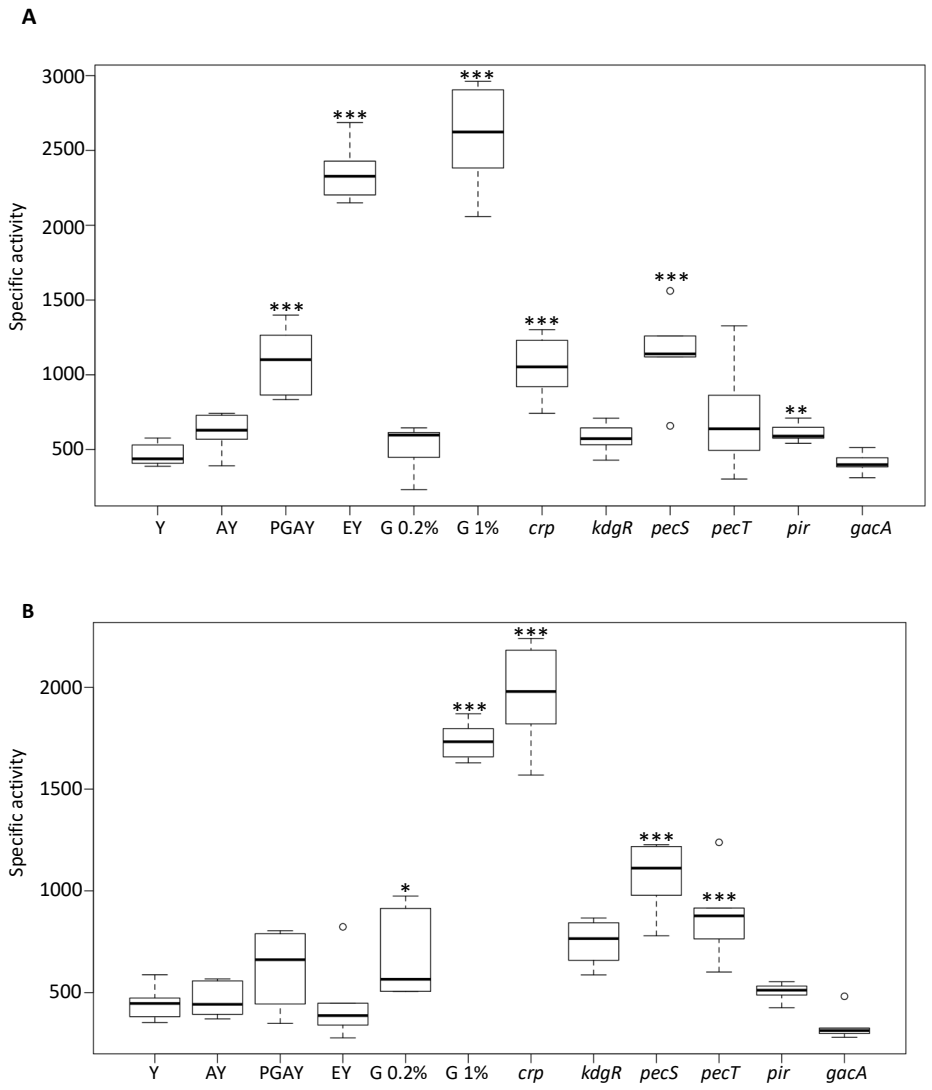

A
B
Rotten tissue (g)
8
7
6
5
4
3
**
***
WT
svfB
svfA
svfB/svfA
Rotten tissue (g)
5
4,5
4
3,5
3
2,5
2
***
WT/pBBR
svfA/pBBR
svfA/pBBR-svfA

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