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

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

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14 Short title: *Dickeya dadantii* virulence factors

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17 **ABSTRACT**

18 *Dickeya* are plant pathogenic bacteria able to provoke disease on a wide range of plants. A type  
19 2 secretion system named Out is necessary for bacterial virulence. Its study in *D. dadantii*  
20 showed that it secretes a wide range of pectinolytic enzymes. However, the full repertoire of  
21 exoproteins it can secrete has not been identified. No secretion signal present on the protein  
22 allows the identification of substrates of a type 2 secretion system. To identify new Out  
23 substrates, we analyzed *D. dadantii* transcriptome data obtained in plant infection condition and  
24 searched for genes strongly induced encoding a protein with a signal sequence. We identified  
25 four new Out-secreted proteins: the expansin YoaJ, VirK and two proteins of the DUF 4879  
26 family, SvfA and SvfB. We showed that SvfA and SvfB are required for full virulence of *D.*  
27 *dadantii* and showed that Svf proteins are present with a variable number of copies, up to three  
28 in *D. fangzhongdai*, in other *Pectobacteriaceae*. This work opens the way to the study of the  
29 role in virulence of non-pectinolytic proteins secreted by the Out pathway in *Pectobacteriaceae*.

30

31 **IMPORTANCE**

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

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41

## 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 ornamentals 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 caryophyllus*, *D. zae* 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 or 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 acetylerase 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, YoaA,  
117 is among the most highly secreted protein of *Bacillus subtilis* (26). YoaA is also present in *B.*

118 *cereus* and in the insect pathogen *B. thuringiensis*. *Bacillus* Y01A 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 lyase 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 *svfA* 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 than  
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 (*pell*, *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 *D. dadantii*. 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. fangzhongdai* 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<sup>-1</sup>, kanamycin and chloramphenicol, 25  
279 mg l<sup>-1</sup>. 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 10,000g 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<sub>600</sub> 1 in M63  
306 medium. Potatoes were surface sterilized with 70% ethanol and dried. A hole was made with a  
307 pipette tip and 10 $\mu$ l 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  $\beta$ -glucuronidase assays were performed on toluenized extracts of cells grown to exponential  
313 phase using the method of Bardonnnet *et al* (42). with *p*-nitrophenyl- $\beta$ -D-glucuronate as the  
314 substrate.

315

316

317

318

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323

324

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335 nov. and *Dickeya paradisiaca* comb. nov. and delineation of four novel species, *Dickeya*  
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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. fangzhongdai* 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.  $\beta$ -glucuronidase activity was measured with *p*-  
485 nitrophenyl- $\beta$ -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  $\beta$ -  
490 glucuronidase activity was measured with *p*-nitrophenyl- $\beta$ -D-glucuronate. Activities are  
491 expressed in  $\mu$ moles of *p*-nitrophenol produced per minute and per milligram of bacterial dry  
492 weight  $\pm$  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  $p < 0.005=**$ ,  $p < 0.01=*$ .

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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.

501

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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
<i>D. dadantii</i>	1	1	0	1	1	1	1	1	2
<i>D. diffenbachiae</i>	1	1	0	1	1	1	1	0	2
<i>D. fangzhongdai</i>	1	1	1	1	1	1	1	1	2
<i>D. solani</i>	1	1	0	1	1	1	1	1	2
<i>D. zeaе</i>	0	1	0	1	1	1	1	1	1
<i>D. oryzae</i>	1	1	0	1	1	1	1	1	0
<i>D. dianthicola</i>	0	1	0	1	1	1	1	0	1
<i>D. undicola</i>	1	0	1	1	1	1	1	0	1
<i>D. lacustris</i>	1	0	0	0	0	1	1	0	2
<i>D. aquatica</i>	1	0	0	1	0	1	1	0	2
<i>D. chrysanthemi</i>	0	0	0	1	1	1	1	0	1
<i>D. paradisiaca</i>	0	0	0	0	1	0	0	0	0
<i>D. poaceiphila</i>	0	0	0	0	1	1	0	1	0
<i>P. atrosepticum</i>	0	0	0	1	0	1	1	0	1
<i>P. carotovorum</i>	1	1	0	1	0	1	1	0	1
<i>P. parmentieri</i>	0	0	0	1	0	1	1	0	1
<i>P. polaris</i>	0	0	0	1	0	1	1	0	1

508 The strains used in this study are *D. dadantii* 3937, *D. aquatica* 174/2, *D. chrysanthemi*  
 509 ATCC 11663, *D. dadantii* subsp *dieffenbachiae* NCPPB 2976, *D. dianthicola* NCPPB 453, *D.*  
 510 *fangzhongdai* DSM 101947, *D. lacustris* S29, *D. oryzae* ZYY5, *D. paradisiaca* ATCC 33242,  
 511 *D. poaceiphila* NCPPB 569, *D. solani* IPO 2222, *D. undicola* 2B12, *D. zeaе* NCPPB 2538, *P.*  
 512 *atrosepticum* ATCC 33260, *P. carotovorum* subsp. *carotovorum* ATCC 15713, *P.*  
 513 *parmentieri* RNS08.42.1A and *P. polaris* NIBIO 1006. The presence and number of proteins  
 514 detected in each strain is indicated.  
 515

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	CACGGTAACCCGAGACAGCCCCGGGAGCGGGGGCTCGCAAGGC
530	15544H-	GGTTAGTGATGGTGATGGTGATGTTTAATATAGGTCTGTGTGAAC
531	14642H+	GGTGAGGAATAATTCTGGCC
532	14642H-	GGTTAGTGATGGTGATGGTGATGAGGCAGTTGTACTTTTCCAG
533	VirKH+	TGCCGTATGTGATAGTCACG
534	VirKH-	CCTTAGTGATGGTGATGGTGATGTTGCTTGAAGAAGCGGATATC

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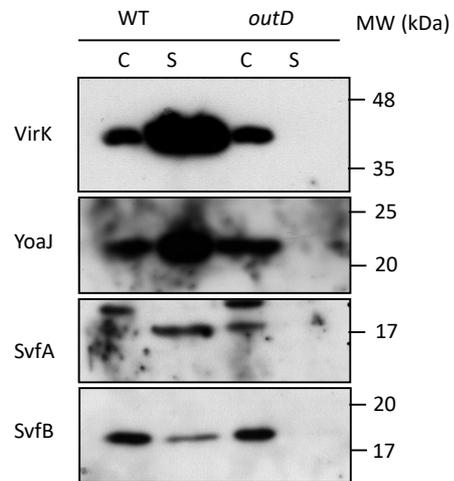
537 *Dickeya dadantii* strains

538

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

552	A6437	<i>gacA::CmR svfA::uidA-kanR</i>	This work
553	A6467	<i>svfB::uidA-kanR</i>	This work
554	A6469	<i>kdgR::Mu-CmR svfB::uidA-kanR</i>	This work
555	A6470	<i>pecS::Mu-CmR svfB::uidA-kanR</i>	This work
556	A6471	<i>pecT::CmR svfB::uidA-kanR</i>	This work
557	A6472	<i>pir::CmR svfB::uidA-kanR</i>	This work
558	A6473	<i>gacA::CmR svfB::uidA-kanR</i>	This work
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Fig. 1

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

SvfA      SSVQVYAVYSSSLKGGWQAVPT-NTLSL-SGYAGGTLRIAVLEVYGGNRIGWLNGGQTS- 104
SvfC      SGLRIKVLSGVYGGTWQYAPV-NAVSI GPGYAGGTLQIAVVE TGYGGNRIGWINGEQKK- 64
Bacillus  TSLNVVKVESQL-GGVEFIGA--NNLSTVKDHGGSYLYIYT NEMGYGRNPIAQMSGQK LKK 64
SvfB      SRVTVYAVGSSN-CGWEYMTSIGQLSTTC DHGGAQLRVAVQE IGYGNPVAWMNGGVLPR 113
Photorhabdus TNMWVYAVGSTN-CGWEYTSNL--FATTC DHGQQLRAAVLE IGYGYSSPAMMNGGLLPN 107
          : : : : . : .: .:* * . * *** . . :.*

SvfA      --PYQVNPVCVVSGRYTESCPAGSIVSGWMAYFNADNMSSVTFRYQSTSTNFPNRTLSTS 162
SvfC      --PDSVKLACLVKGEMTDNCPRGATGAGWIA YFSANYQTSVTFRYQSTSANFPYKTLSTS 122
Bacillus  VDS---KMI-----DINGDRTVDGW EYKWDASGQQNGQFKYQNTSTNAPWN TLFTS 112
SvfB      SANYQTDGICIVGNQYTFPCPAGYTVV GWMYYNLDGTDNGQFKFQDTSNAPRN TLFQ 173
Photorhabdus SAMYSSKTVCITNGYYTWPCTAGQTVV GYLHEYNL DGNQNGTFRYQNTSTNSPWN TMSVQ 167
          . . * : . . . * : * . * * * * * : . .

SvfA      LNIQ 166
SvfC      LTIK 126
Bacillus  LNIK 116
SvfB      TYIK 177
Photorhabdus INIL 171
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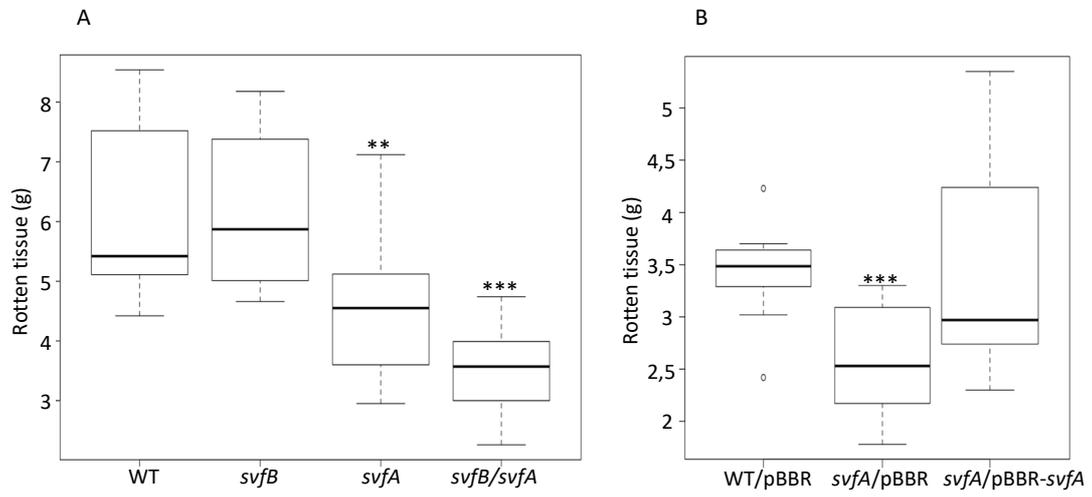
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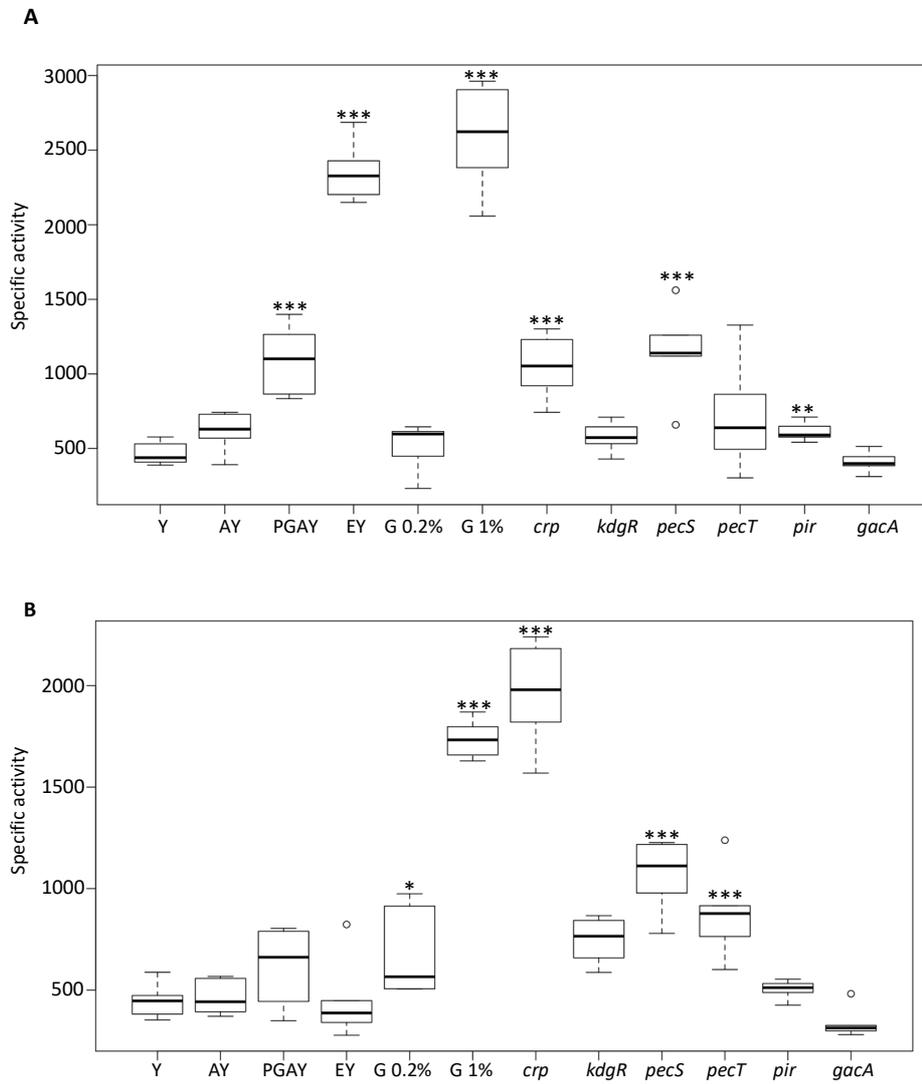
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Fig. 2

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Fig. 4