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

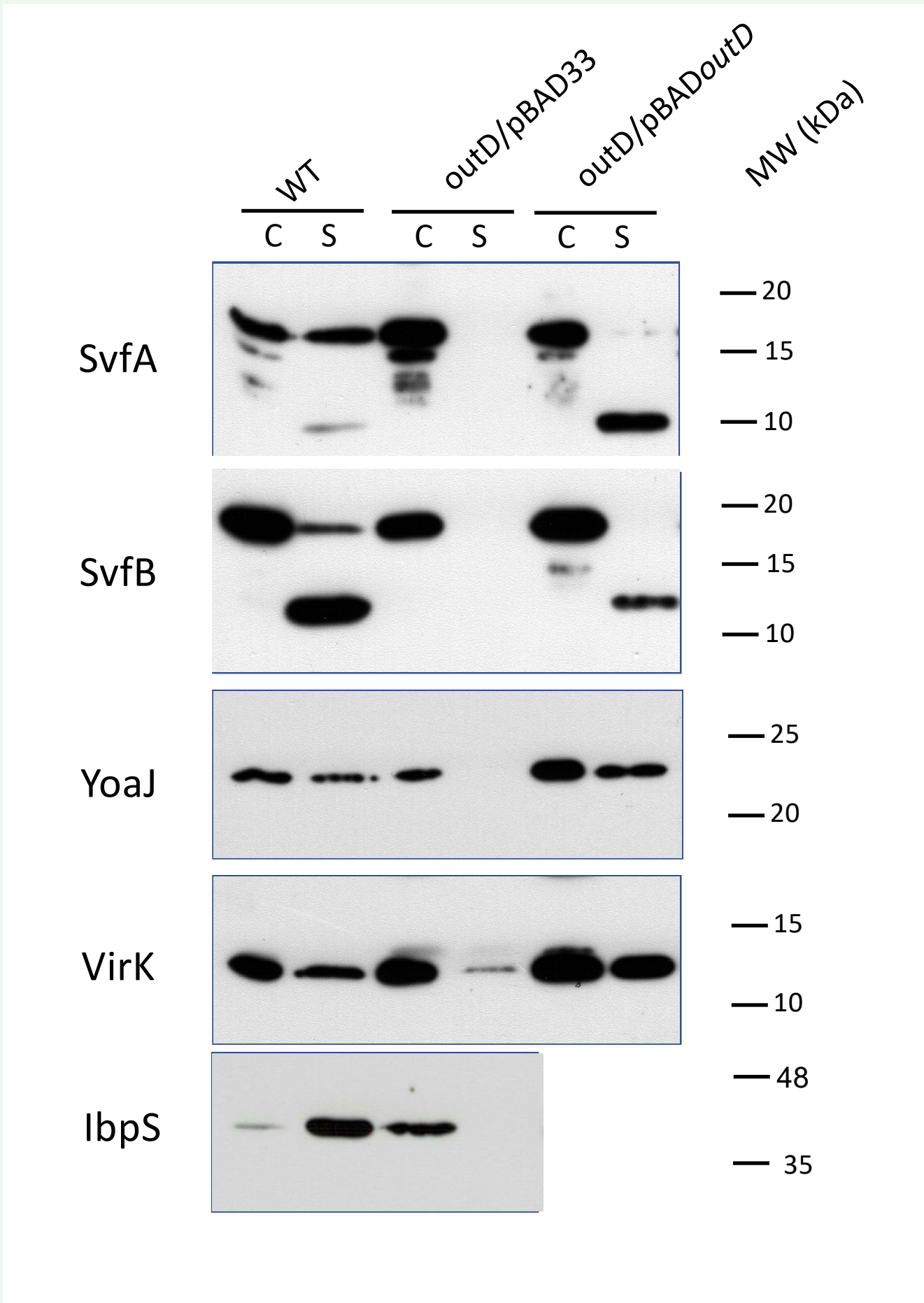
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Dickeya are plant pathogenic bacteria able to provoke disease on a wide range of plants. A type 2 secretion system (T2SS) named Out is necessary for bacterial virulence. Its study in *D. dadantii* showed that it secretes a wide range of plant cell 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 five new Out-secreted proteins: the iron-binding protein IbpS, the expansin YoaJ, the putative virulence factor VirK and two proteins of the DUF 4879 family, Svfa and Svfb.

gene	séquence signal	secretion	function
pelI	+	2	pectate lyase I
prtA	-	1	protease A
svfA	+	?	unknown
rhiE	+	2	rhamnogalacturonate lyase E
paeY	+	2	pectin acetyltransferase Y
rhaD	-	-	rhamnulose-1-phosphate aldolase
ibpS	+	?	secreted iron binding protein
prtB	-	1	protease B
pelZ	+	2	pectate lyase Z
pemA	+	2	pectin methylesterase A
rhiT	-	-	rhamnogalacturonide transporter
DUF1471	+	?	unknown
rhiN	-	-	rhamnogalacturonase
svfB	+	?	unknown
yoaJ	+	?	expansin
17674	-	-	MCP
16286	-	-	unknown
46940	-	-	hydrolase
pelD	+	2	pectate lyase D
cytB	-	-	toxin cytB
avrM	+	2	avirulence protein M

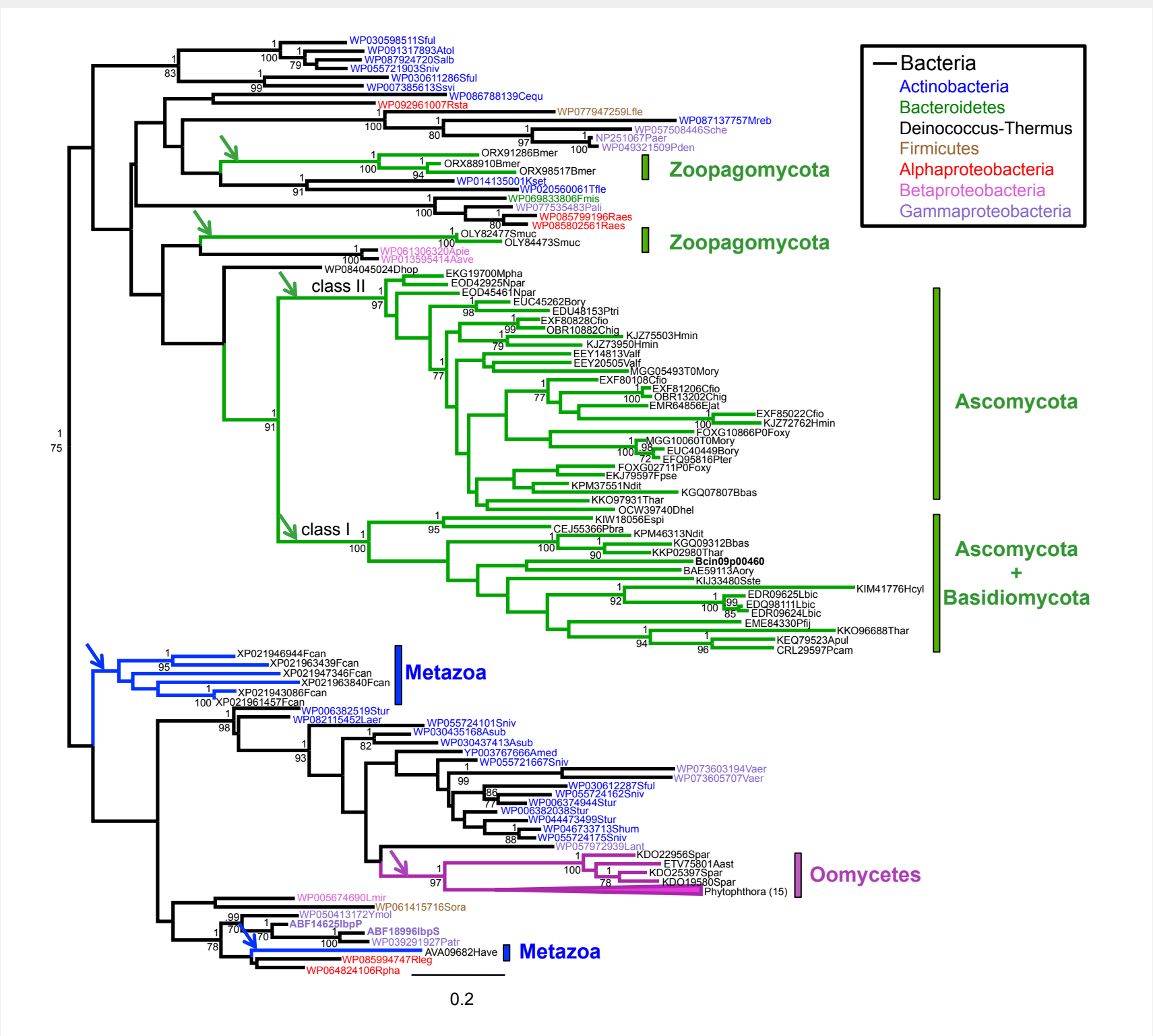
Most induced *D. dadantii* genes during *A. thaliana* infection (Pedron et al., 2018). Many genes encode PCWDE secreted by the Out T2SS. Four genes encode proteins with a sequence signal that are candidate T2SS substrates (in blue).



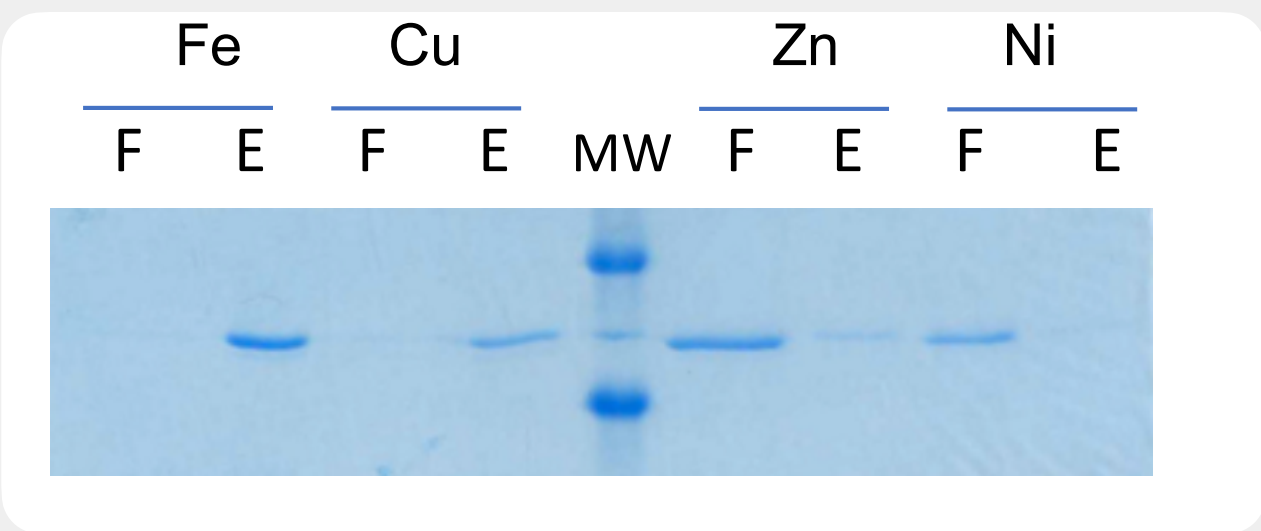
Five new substrates of the Out T2SS are not PCWDE:

- Expansin YoaJ
- Iron binding protein IbpS
- Secreted virulent factors A and B
- Virulence protein homologue VirK

Detection of His-tagged versions of the proteins in the WT and an OutD mutant complemented or not



Homologues of IbpS are found in Gram- and Gram+ bacteria, oomycetes, fungi and even insects. Most of these organisms are necrotrophic plant pathogens. Bacterial genes seem to have been transferred several times to these eukaryotes .



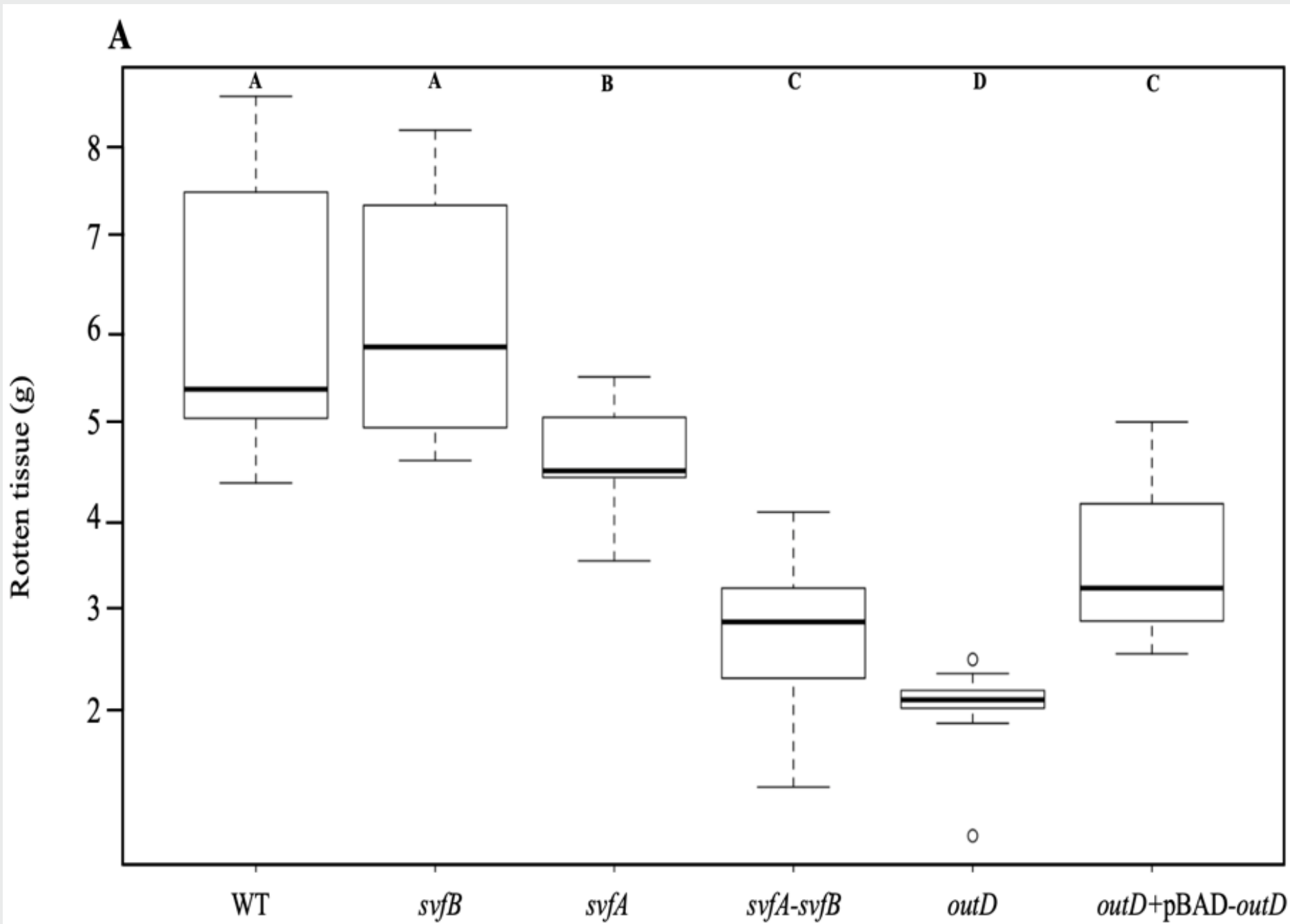
IbpS is an iron/copper binding protein.

IbpS was loaded on metal-NTA resin. Flowthrough (F) or eluted by EDTA fractions (E) were analyzed. IbpS binds iron and copper.

By binding these metals IbpS prevents formation of ROS by the Fenton reaction and protects the bacteria

Virulence of ibp mutants

D. dadantii *ibpS* mutant is less virulent to *A thaliana* (a and b). *Botrytis cinerea* *Bcibp* mutant is less virulent to cucumber and bean (c and d)



SvfA and Svfb mutants are less virulent on potato

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

Non PCWDE type 2 secreted proteins necessary for virulence are present in variable number in the different *Dickeya*. Their presence should be considered when analyzing the presence of virulence factors in new species.

