A *gapA* PCR-sequencing Assay for Identifying the *Dickeya* and *Pectobacterium* Potato Pathogens

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Abstract

Several pectinolytic *Pectobacterium* and *Dickeya* species and subspecies are causative agents of blackleg and soft rot diseases on potato plants and tubers. Rapid and accurate identification of these taxa is a crucial issue for the production and international trade of potato seed-tubers. Here, we developed a PCR-sequencing tool to easily characterize the different *Pectobacterium* and *Dickeya* taxa. The *gapA* gene sequences from 53 published genomes were aligned and a phylogeny tree was constructed. A set of 35 signature nucleotides was

discovered to distinguish the *Pectobacterium* and *Dickeya* genera, species, and subspecies. Then, a PCR-primer couple was designed for amplifying the *gapA* gene in pectinolytic enterobacteria. The primers were tested on 22 isolates recovered from blackleg symptoms in several potato fields. Amplicons were sequenced and signature-nucleotides were analyzed. A phylogeny that includes *gapA* sequence specimens confirmed the taxonomical identification of these environmental isolates.

Dickeya and *Pectobacterium* are pectinolytic enterobacteria inducing damage on a wide range of plants (Ma et al. 2007; Samson et al. 2005; Waldee 1942). These pathogens produce extracellular plant cell-wall-degrading enzymes that cause blackleg and soft rot diseases (Barras et al. 1994). These diseases are a major problem for potato farmers. The disease dynamics are complex, as is the identification of the causative agent because several *Pectobacterium* and *Dickeya* species provoke similar symptoms on potato plants (Pérombelon 2002). In potato fields, the taxa isolated from symptomatic tissues are: *D. dianthicola* (Ddi); *D. solani* (Dsol), which recently emerged; and *D. dadantii* (Ddad); and *P. atrosepticum* (Pa); *P. parmentieri* (Pp), which results from the split of *P. wasabiae* (Pw); *P. carotovorum* subsp. *carotovorum* (Pcc); and *P. carotovorum* subsp. *brasiliense* (Pcb) (de Werra et al. 2015; Duarte et al. 2004; Gardan et al. 2003; Khayi et al. 2016; Samson et al. 2005; Toth et al. 2011). Remarkably, some *Pectobacterium* and *Dickeya* species isolated from nonpotato host plants cultivated in the same area should be considered as potential potato pathogens. As an example, *P. carotovorum* subsp. *odoriferum* (Pco), usually isolated from chicory, is able to induce soft rot symptoms when inoculated onto potato tubers (Waleron et al. 2014).

To limit the propagation of the pathogens, identification of blackleg fields and sorting of tuber-seed lots are regulated in several countries (Czajkowski et al. 2011). For 20 years, several PCR-based methods have been developed for detecting and identifying *Dickeya* and *Pectobacterium* pectinolytic enterobacteria. Among the first sets of PCR-primers designed and still used today, Y1-Y2 (Darrasse et al.

1994) and ADE1-ADE2 (Nassar et al. 1996) target *Pectobacterium* and *Dickeya* species, respectively. Some other PCR-primers are species-specific, targeting *P. atrosepticum* (Frechon et al. 1998), Pcb (Duarte et al. 2004), and *P. wasabiae* (Kim et al. 2012). Many other PCR-primer sets and methods are all available for the detection of pectinolytic bacteria. These include PCR-RFLP (Hélias et al. 1998), multiplex PCR (Potrykus et al. 2014), and real-time PCR (Laurila et al. 2010). These methods can be applied directly on environmental DNA and are therefore suitable for detecting pathogens in a complex matrix. Although rapid and easy to run, these taxon-specific PCR methods require one-by-one PCR-amplifications in numbers that are at least equal to—and often greater than—that of the searched taxon. To circumvent this constraint in the course of taxonomical characterization of pure isolates, some PCR-sequencing barcodes were developed, such as those targeting the *recA* and *fliC* genes in *Dickeya* (Parkinson et al. 2009; Van Vaerenbergh et al. 2012).

This work proposes a PCR-sequencing barcode method for the identification, in a single assay, of all *Dickeya* and *Pectobacterium* pectinolytic species or subspecies causing blackleg and soft rot diseases on a potato plant host. The most recent description of the *Dickeya* and *Pectobacterium* genera and classification (Zhang et al. 2016) was retained to develop this “all-in-one” molecular tool that exploits the housekeeping gene *gapA* (coding for the glyceraldehyde-3-phosphate dehydrogenase A) as a molecular barcode.

Materials and Methods

Genome resource and *gapA* gene alignment. All 53 *Dickeya* and *Pectobacterium* genomes were retrieved from the National Center for Biotechnology Information (NCBI). These genomes are representative of the known diversity of the two genera (Zhang et al. 2016). Genome accession numbers and additional characteristics are provided in Table 1. The *gapA* genes were retrieved from genomes and aligned using CLC Genomics Workbench 9.0.1 (<https://www.qiagenbioinformatics.com>). Taxon-specific nucleotides were defined as those distinctive as compared with the other taxons. Phylogeny reconstruction was performed with the neighbor-joining statistical method (Tamura et al. 2004) using MEGA7 (Kumar et al. 2016).

Isolation of *Dickeya* and *Pectobacterium* strains from potato field. Plants with blackleg symptoms were collected from 10 French potato fields. Symptomatic tissues were cut into 3- to 5-mm pieces and incubated during 1 h at room temperature in a sterile solution buffer (Na₂HPO₄ 12 H₂O 1.07%, KH₂PO₄ 0.27%, pH 7.0). Bacterial suspensions were diluted and plated 48 h at 28°C on CVP

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gapA sequences from strains isolated in potato field appear in the GenBank nucleotide sequence databases with the accessions number KY047578 to KY047599.

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Table 1. *Dickeya* and *Pectobacterium* genomes used in this study

Species, strain	Isolation source	Geographical origin, year of isolation	GenBank Accession number
<i>Dickeya dadantii</i>			
NCPPB 898 ^T	<i>Pelargonium capitatum</i>	Comoros, 1960	CM001976.1
3937	<i>Saintpaulia ionantha</i>	France, 1977	CP002038.1
NCPPB 3537	<i>Solanum tuberosum</i>	Peru	CM001982.1
<i>Dickeya dianthicola</i>			
NCPPB 453 ^T	<i>Dianthus caryophyllus</i>	UK, 1956	CM001841.1
IPO 980	<i>Solanum tuberosum</i>	Netherlands	CM002023.1
RNS04.9	<i>Solanum tuberosum</i>	France, 2004	APVF00000000.1
GBBC 2039	<i>Solanum tuberosum</i>	Belgium	CM001838.1
NCPPB 3534	<i>Solanum tuberosum</i>	Netherlands	CM001840.1
<i>Dickeya paradisiaca</i>			
NCPPB 2511 ^T	<i>Musa paradisiaca</i>	Colombia, 1970	CM001857.1
<i>Dickeya solani</i>			
IPO 2222 ^T	<i>Solanum tuberosum</i>	Netherlands, 2007	CM001859.1
05.1.2A	<i>Solanum tuberosum</i>	France, 2005	JWMJ00000000.1
MK10	<i>Solanum tuberosum</i>	Israel	CM001839.1
MK16	River water	UK	CM001842.1
GBBC 2040	<i>Solanum tuberosum</i>	Belgium	CM001860.1
RNS08.23.3.1.A	<i>Solanum tuberosum</i>	France, 2008	AMYI00000000.1
07.7.3B	<i>Solanum tuberosum</i>	France, 2007	JWLR00000000.1
<i>Pectobacterium carotovorum</i> subsp. <i>actinidiae</i>			
KKH3 ^T	<i>Actinidia chinensis</i>	South Korea, 2006	JRMH00000000.1
<i>Pectobacterium carotovorum</i> subsp. <i>brasiliense</i>			
LMG 21371 ^T	<i>Solanum tuberosum</i>	Brazil, 1999	JQOE00000000.1
LMG 21372	<i>Solanum tuberosum</i>	Brazil, 1999	JQOD00000000.1
CFIA1009	<i>Solanum tuberosum</i>	Canada, 2008	JPSN00000000.1
CFIA1033	<i>Solanum tuberosum</i>	Canada, 2009	JPSO00000000.1
YC D49	<i>Brassica rapa</i> subsp. <i>chinensis</i>	China, 2013	JUJB00000000.1
YC D62	<i>Brassica rapa</i> subsp. <i>chinensis</i>	China, 2013	JUJD00000000.1
YC D64	<i>Brassica rapa</i> subsp. <i>chinensis</i>	China, 2013	JUJE00000000.1
YC D21	<i>Brassica rapa</i> subsp. <i>chinensis</i>	China, 2013	JUJJ00000000.1
YC D29	<i>Brassica rapa</i> subsp. <i>chinensis</i>	China, 2013	JUJK00000000.1
YC T31	<i>Brassica rapa</i> subsp. <i>chinensis</i>	China, 2013	JUJL00000000.1
YC D65	<i>Brassica rapa</i> subsp. <i>chinensis</i>	China, 2013	JUJN00000000.1
YC D52	<i>Brassica rapa</i> subsp. <i>chinensis</i>	China, 2013	JUJC00000000.1
<i>Pectobacterium carotovorum</i> subsp. <i>carotovorum</i>			
ICMP 5702 ^T	<i>Solanum tuberosum</i>	Denmark, 1952	AODT00000000.1
WPP14	<i>Solanum tuberosum</i>	USA, 2001	ABVY00000000.1
NCPPB 3395	<i>Solanum tuberosum</i>	Netherlands, 1985	JQHN00000000.1
YC D57	<i>Brassica rapa</i> subsp. <i>chinensis</i>	China, 2013	JUJG00000000.1
YC T1	<i>Brassica rapa</i> subsp. <i>chinensis</i>	China, 2013	JUJH00000000.1
YC D16	<i>Brassica rapa</i> subsp. <i>chinensis</i>	China, 2013	JUJO00000000.1
YC T39	<i>Brassica rapa</i> subsp. <i>chinensis</i>	China, 2013	JUJQ00000000.1
BC T2	<i>Brassica rapa</i> subsp. <i>pekinensis</i>	China, 2013	JUJR00000000.1
BC T5	<i>Brassica rapa</i> subsp. <i>pekinensis</i>	China, 2013	JUJS00000000.1
<i>Pectobacterium aroidearum</i>			
PC1	<i>Ornithogalum dubium</i>	Israel, 2004	CP001657.1
<i>Pectobacterium carotovorum</i> subsp. <i>odoriferum</i>			
NCPPB 3839 ^T	<i>Cichorium intybus</i>	France, 1978	JQOG00000000.1
NCPPB 3841	<i>Cichorium intybus</i>	France, 1979	JQOF00000000.1
BC S7	<i>Brassica rapa</i> subsp. <i>pekinensis</i>	China, 2007	CP009678.1
<i>Pectobacterium atrosepticum</i>			
ICMP 1526 ^T	<i>Solanum tuberosum</i>	UK, 1957	ALIV00000000.1
SCRI1043	<i>Solanum tuberosum</i>	UK, 1985	BX950851.1
JG10-08	<i>Solanum tuberosum</i>	China, 2010	CP007744.1
21A	<i>Solanum tuberosum</i>	Belarus, 1978	CP009125.1, CP009126.1
CFBP 6276	<i>Solanum tuberosum</i>	France, 1999	ASAB00000000.1
<i>Pectobacterium parmentieri</i>			
RNS08.42.1A ^T	<i>Solanum tuberosum</i>	France, 2008	CP015749.1
WPP163	<i>Solanum tuberosum</i>	USA, 2004	CP001790.1
SCC3193	<i>Solanum tuberosum</i>	Finland, 1980s	CP003415.1
CFIA1002	<i>Solanum tuberosum</i>	Canada, 2007	JENG00000000.1
<i>Pectobacterium wasabiae</i>			
CFBP 3304 ^T	<i>Eutrema wasabi</i>	Japan, 1985	CP015750.1
NCPPB3702	<i>Eutrema wasabi</i>	Japan, 1985	JQOH00000000.1

medium (Hélias et al. 2012). Single colonies on CVP medium were purified on TY medium (tryptone 5 g/liter, yeast extract 3 g/liter, and agar 1.5%) and then incubated 48 h at 28°C. Before *gapA* sequencing, all the isolates were characterized using the taxon-specific PCR

methods (Darrasse et al. 1994; Duarte et al. 2004; Frechon et al. 1998; Kang et al. 2003; Kim et al. 2012; Nassar et al. 1996).

***gapA*-primers and PCR conditions.** The *gapA* primers *gapA*-7-F 5'-ATCAAAGTAGGTATCAACGG and *gapA*-938-R 5'-TCRTACCARGAAACCAGTT were designed using Primer3 (Untergasser et al. 2012). The size of the amplicon is 932 bp in length. Primers were synthesized by Eurofins Genomics (Ebersberg, Germany).

The *gapA*-PCR assay was performed directly on liquid bacterial cultures. One single colony of each strain was cultured in 5 ml liquid TY medium (tryptone 5 g/liter, yeast extract 3 g/liter) overnight. Two microliters of the culture were diluted with 18 µl of TE buffer, heated 10 min at 99°C, and immediately frozen at -20°C. PCR assays were performed in 25 µl reaction mixture (final volume) containing 0.1 µl of EurobioTaq (5 u/µl); 2.5 µl of 10× buffer; 2 µl of MgCl₂ 25 mM; 2.5 µl of Eurobio mix dNTP 1 mM; 1 µl of each primer 1 µM; 13.9 µl of sterile Milli-Q H₂O; and 2 µl of lysed culture in TE buffer. PCRs were carried out with a 2720 Thermal Cycler (Applied Biosystems, Foster City, CA). Cycling parameters were 94°C for 5 min, 40 cycles of 94°C for 30 s, 54°C for 30 s, and 72°C for 30 s, with a final extension at 72°C for 5 min. PCR products were separated in 2% agarose gels in TBE buffer, at 150 V for 30 min and bands were revealed with ethidium bromide staining.

Sequencing and analyses of PCR products. PCR products were sequenced using Sanger technology by the GATC NightXpress service (GATC Biotech AG, Cologne, Germany). After alignment and trimming of all PCR products, 812 bp of *gapA* sequences were analyzed for the presence of signature-nucleotides and a phylogeny was established, all as described above.

Results

The *gapA* phylogeny in *Dickeya* and *Pectobacterium*. A phylogeny of 53 *gapA* genes was constructed with neighbor-joining statistical method. Subtrees were compressed and grouped by species or subspecies (Fig. 1). In the case of *P. carotovorum* subspecies, the classification G1 to G5 was added in reference to the data from Zhang et al. 2016. The *gapA* phylogeny permitted a clear separation of the investigated species and subspecies, supporting the proposition of this gene as an appropriate genetic marker. Noticeably, the new species *P. parmentieri* (Khayri et al. 2016) was clearly separated from *P. wasabiae* species. Furthermore, strain PC1, which was isolated in Israel from Star of Bethlehem plants (*Ornithogalum dubium*; Luzzatto et al. 2007) and previously identified as a Pcc, was

Fig. 1. The *gapA* phylogeny in *Dickeya* and *Pectobacterium*. The *gapA* genes were recovered from 53 genomes, aligned, and used for building a phylogenetic tree with the neighbor-joining statistical method using MEGA7. One thousand bootstrap replicates were computed. The subtree was compressed and grouped by species or subspecies. The number of genomes used is indicated next to each taxon.

Nucleotide position		81	141	147	153	156	168	180	207	216	231	233	241	279	315	355	435	507	513	537	543	546	561	618	627	636	662	672	720	789	840	876	900	911	972	978													
Genus,species or subspecies		<i>P.wasabiae</i> and <i>P.parmentieri</i> *	<i>Pectobacterium</i> or <i>Dickeya</i>		<i>P.atrosepticum</i>			<i>D.dadantii</i>	<i>D.dadantii</i>	<i>D.solani</i>	<i>Pectobacterium</i> or <i>Dickeya</i>			<i>P.atrosepticum</i>	<i>Pectobacterium</i> or <i>Dickeya</i>		<i>P.c. odoriferum</i>	<i>D.dadantii</i>	<i>P.atrosepticum</i>	<i>D.solani</i> **	<i>D.dianthicola</i>	<i>D.dianthicola</i>	<i>D.dianthicola</i>	<i>D.dianthicola</i>	<i>D.dadantii</i>	<i>P.atrosepticum</i>	<i>Pectobacterium</i> or <i>Dickeya</i>		<i>P.c. carotovorum</i> G2	<i>Pectobacterium</i> or <i>Dickeya</i>		<i>Pectobacterium</i> or <i>Dickeya</i>		<i>P.wasabiae</i> and <i>P.parmentieri</i>	<i>D.dianthicola</i>	<i>Pectobacterium</i> or <i>Dickeya</i>		<i>P.parmentieri</i>	<i>Pectobacterium</i> or <i>Dickeya</i>		<i>D.dianthicola</i>	<i>D.solani</i>	<i>P.carotovorum</i>	<i>P.c. odoriferum</i> and <i>P.c. brasiliense</i>		<i>Pectobacterium</i> or <i>Dickeya</i>		<i>D.dadantii</i>	<i>P.wasabiae</i> and <i>P.parmentieri</i>
Signature nucleotide	<i>P.atrosepticum</i>	C	C	T	T	T	C	T	T	T	G	G	T	T	T	C	T	T	A	G	A	G	T	A	C	T	A	C	T	T	T	G	A	A	T	C													
	<i>P. wasabiae</i>	T	C	A	T	T	C	T	C	T	G	G	G	T	T	C	T	T	A	C	A	G	T	A	T	T	A	C	T	T	T	G	A	A	T	T													
	<i>P. parmentieri</i>	T	C	A	T	T	C	T	C	T	G	G	G	T	T	C	T	T	A	C	A	G	T	A	T	T	A	T	T	T	T	G	A	A	T	T													
	<i>P.c. subsp. brasiliense</i> (G1)	C	C	A	T	T	C	T	C	T	G	G	G	T	T	C	T	T	A	Y	A	G	T	A	C	T	A	C	T	T	T	A	A	T	A	T	C												
	<i>P.c. subsp. carotovorum</i> (G2)	C	C	A	T	T	C	T	C	T	G	G	G	T	T	C	T	T	A	C	A	A	T	A	C	T	A	C	T	T	T	A	A	A	T	C													
	<i>P.c. subsp. carotovorum</i> (G3)	C	C	A	T	T	C	T	C	T	G	G	G	T	T	C	T	T	A	C	A	G	T	A	C	T	A	C	T	T	T	A	A	A	A	T	C												
	<i>P.c. subsp. odoriferum</i> (G4)	C	C	A	T	T	C	T	C	T	A	G	G	T	T	C	T	T	A	Y	A	G	T	A	C	T	A	C	T	T	T	A	G	A	A	T	C												
	<i>D. dianthicola</i>	C	T	C	T	T	C	G	C	C	G	G	G	T	C	T	G	C	A	T	T	G	C	G	C	C	C	C	G	G	T	G	A	C	T	C													
	<i>D. solani</i>	C	T	C	T	T	T	G	C	C	G	G	G	C	T	C	T	T	A	T	T	G	C	G	C	T	C	C	G	T	A	G	A	C	T	C													
	<i>D. dadantii</i>	C	T	C	C	C	C	G	C	C	G	A	G	T	T	C	T	T	G	T	T	G	C	G	C	T	C	C	G	T	T	G	A	C	C	C													

Fig. 2. The *gapA* signature nucleotides. This figure shows signature nucleotides of the *Dickeya* and *Pectobacterium* species and subspecies. Genera signature nucleotides are represented in dark gray, *P. atrorepticum* signature nucleotides are colored in light gray, *P. parmentieri* and *P. wasabiae* in dark blue, *Pectobacterium carotovorum* in purple, *D. dadantii* in yellow, *D. dianthicola* for light red, and *D. solani* in salmon pink. Nucleotide numbering starts at the first nucleotide of the codon ATG. Asterisks indicate uncommon variations: * = T at position 81 in the strain Pcb YC D65; ** = C at position 279 in the strain Pcc YC T1. Y represents C or T nucleotide.

Fig. 3. The *gapA* phylogeny for taxonomic assignment of field isolates. The *gapA* phylogeny combines 22 isolates from potato symptomatic tissues (green) listed in **Table 2** and 53 genomes of *Pectobacterium* and *Dickeya* (black) listed in **Table 1**. Tree was performed with the neighbor-joining statistical method using MEGA7. One thousand bootstrap replicates were computed.

recently reclassified in *P. aroidearum* (Nabhan et al. 2013). This strain clearly emerged in a separated branch that was distant from the Pcc clade.

Identification of signature-nucleotides. The *gapA* alignment also revealed 35 signature nucleotides that distinguished the *Pectobacterium* and *Dickeya* taxa (Fig. 2). Nine nucleotides distinguished the genera *Pectobacterium* and *Dickeya*. In addition to these genus signature nucleotides, additional positions discriminated the different species: three nucleotides were specific for *D. solani*, five for *D. dadantii*, six for *D. dianthicola*, four for *P. atrosepticum*, one for *Pectobacterium carotovorum*, three for the sister species *P. wasabiae* and *P. parmentieri*, and one for distinguishing *P. parmentieri* from *P. wasabiae*. At the subspecies level, three additional nucleotides separated Pcc, Pco, and Pcb, as well as G2 and G3 phyla.

Identification of pectinolytic strains isolated from blackleg symptoms in potato fields. Twenty-two strains isolated on blackleg symptoms from 10 French potato fields have been collected since 2013. Isolates were assigned to *Dickeya* and *Pectobacterium* taxa using the already available PCR methods (Darrasse et al. 1994; Duarte et al. 2004; Frechon et al. 1998; Kang et al. 2003; Kim et al. 2012; Nassar et al. 1996). Then, after PCR with *gapA* primers and amplicon sequencing, *gapA* sequences were trimmed and aligned and a phylogeny tree was constructed (Fig. 3). All the pectinolytic strains isolated from potato fields branched with known *Dickeya* and *Pectobacterium* clades. In addition, the signature nucleotides were examined. For each of the 22 field strains, the signature nucleotides allowed unambiguous assignment to a species, or subspecies in the case of *P. carotovorum* (Table 2). The taxonomic assignments performed with the *gapA* phylogeny and the *gapA* signature-nucleotide analysis were congruent. Using the *gapA* PCR-sequencing tool, three Pcb, three Pcc, one Pco, three *P. atrosepticum*, four *P. parmentieri*, four *D. dianthicola*, and four *D. solani* were identified.

Discussion

We reported here a simple (a single PCR primer pair for amplifying *Dickeya* and *Pectobacterium gapA* barcodes) and rapid (one day for external Sanger sequencing) methodology for identifying species and subspecies of pectinolytic potato pathogens belonging to the *Dickeya* and *Pectobacterium* genera. PCR assays were performed on a bacterial cell suspension without DNA extraction. Previous work has already considered partial sequence of the *gapA* gene in

Table 2. Pectinolytic strains isolated from potato fields

Isolate	Field	Year of isolation	<i>gapA</i> identification	GenBank <i>gapA</i> sequence
10C1	Field A	2013	Pcc	KY047578
40C2	Field B	2013	<i>D. dianthicola</i>	KY047579
MO30a	Field C	2014	Pco	KY047580
16Ka9	Field D	2016	<i>D. dianthicola</i>	KY047581
16Ka18a	Field D	2016	<i>P. parmentieri</i>	KY047582
ME1	Field E	2016	<i>D. solani</i>	KY047583
AI1	Field F	2016	<i>D. dianthicola</i>	KY047584
AI28	Field F	2016	<i>P. parmentieri</i>	KY047585
AM7	Field G	2016	Pcb	KY047586
AM9	Field G	2016	<i>D. solani</i>	KY047587
M2	Field H	2016	<i>D. solani</i>	KY047588
M16	Field H	2016	<i>P. atrosepticum</i>	KY047589
M22	Field H	2016	<i>P. atrosepticum</i>	KY047590
M28	Field H	2016	Pcb	KY047591
M30	Field H	2016	Pcb	KY047592
M32	Field H	2016	<i>P. atrosepticum</i>	KY047593
M34	Field H	2016	Pcc	KY047594
KD19	Field I	2016	<i>P. parmentieri</i>	KY047595
KD21	Field I	2016	Pcc	KY047596
KD26	Field I	2016	<i>D. dianthicola</i>	KY047597
CH1	Field J	2016	<i>D. solani</i>	KY047598
CH3	Field J	2016	<i>P. parmentieri</i>	KY047599

MLSA (Ma et al. 2007; Nabhan et al. 2012). In this study, special attention was paid to the selection of primers in order to generate large-sized *gapA* amplicons, and hence to maximize the associated taxonomic information. The *gapA* phylogeny was congruent with the topology of the phylogeny based on the concatenation of 895 nucleotide sequences (Zhang et al. 2016.). Noticeably, in silico analysis showed that these *gapA* primers potentially amplify the *gapA* gene in some other enterobacteria, so the *gapA* PCR-product needs to be sequenced for taxonomical assignation. This is expected for a DNA-barcode.

For an accurate taxonomic assignation, we recommended performing an alignment of the tested *gapA* sequence with *gapA* genes belonging to the different species and subspecies listed in Table 1. Then, this alignment may be used to construct a phylogenetic tree (e.g., Fig. 3) and to identify the signature nucleotides described in the Figure 2. These two complementary analyses reinforced the taxonomic assignation based on *gapA* sequencing. Unlike species-specific, PCR identification methods based on the presence or absence of an amplicon, the *gapA* method authorizes the archiving of sequences that can be stored and reused for further analyses. Another value of the *gapA* tool lies in its adaptability to the evolution of *Dickeya* and *Pectobacterium* taxonomy. As an example, the phyla G2 and G3 that are potential *P. carotovorum* subspecies may be distinguished using *gapA* sequences.

By testing the *gapA* tool on 22 pectinolytic strains isolated from potato fields, several expected taxa (Pcb, Pcc, *P. atrosepticum*, *P. parmentieri*, *D. dianthicola*, and *D. solani*) were identified, including Pco. To our knowledge, this is the first published report of such an identification of this subspecies on a potato host. Pco were recurrently isolated from chicory or others plants. Waleron et al. 2014 showed that Pco strains isolated from chicory could induce severe maceration symptoms when inoculated directly on potato tuber. Our work suggests that pathogen transmission may occur between plants cultivated in the same area.

Another potential interest of the *gapA* tool involves investigations on the natural diversity of pectinolytic *Dickeya* and *Pectobacterium* collected from different host and nonhost plants, and soil and surface water samples. A broader knowledge of this diversity will help understanding the genetic resource and reservoirs of potential forthcoming disease outbreaks.

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