***Pectobacterium colocasium* sp. nov. isolated from taro (*Colocasia esculenta*)**

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ABSTRACT

Pectobacterium, a genus comprising gram-negative, pectinolytic phytopathogens, is responsible for economic losses in a wide host range of plants. In this study, the bacterial strains PL152^T and PL155 were isolated from taro corms in Hawai'i in 2018, and characterized using genomic and biochemical assays. The Next Generation Sequencing technologies, Oxford Nanopore MinION and Illumina NovaSeq, were used for whole genome sequencing of the PL152^T strain. Short and long reads were assembled using the Unicycler tool accessible at the bioinformatic resource center, and PATRIC (PathoSystems Resource Integration Center) was used to generate a more accurate and reliable “hybrid” assembly. The 16S rRNA analysis of PL152^T with type strains of other known *Pectobacterium* species showed a close relationship with *P. fontis*. Multi-locus sequence analysis using nine housekeeping genes (*dnaA*, *gapA*, *gyrB*, *recA*, *dnaN*, *rpoS*, *mdh*, *rpoA* and *dnaK*) differentiated strain PL152^T from other species of *Pectobacterium* and formed a unique and well-defined clade. The concurrent results of average nucleotide identity (ANI) and digital DNA-DNA hybridization, with calculated values lower than 95 and 70%, respectively, supported the delineation of a novel bacterial species. Here, we proposed *Pectobacterium colocasium*, strain PL152^T (=ICMP 24362^T; LMG 32536^T) and PL155 as a novel species in the genus *Pectobacterium*.

INTRODUCTION

The genus *Pectobacterium* (formerly *Erwinia*) belonging to the family Proteobacteriaceae, is a gram-negative, fermentative, rod-shaped phytopathogen, with an ability to macerate plant cell walls by secreting multiple plant-cell wall degrading enzymes (PCWDEs)[[1, 2]. *Pectobacterium* ranked among the top ten scientifically and economically studied plant pathogenic bacteria due to its wide host range and the economic losses associated with soft rot diseases [3]. Species within *Pectobacterium* cause a broad spectrum of symptoms,

including soft rot, blackleg, and wilt on many crops [4]. In addition to the broad host range, species within this genus have also been isolated from soil, groundwater, and invertebrates [5].

The taxonomy of the genus *Pectobacterium* has been subjected to constant remodeling, re-evaluation, and reorganization, with the simultaneous development of new bacterial classification techniques (Figure 1). For instance, next generation sequencing (NGS), which provides the whole genome sequence, enables analysis of taxonomically complex genera and comparisons of phylogenetic/evolutionary relationships of different species and subspecies of plant pathogenic genera [6, 7]. Currently, *Pectobacterium* genus includes 20 recognized or proposed species till date. The recognized species includes *Pectobacterium actinidiae* [8, 9], *P. versatile* [8, 10], *P. brasiliense* [8, 11, 12], *P. odoriferum* [8, 13], *P. aquaticum* [14], *P. aroidearum* [15], *P. atrosepticum* [13, 16], *P. betavascularum* [13, 16], *P. wasabiae* [13, 16], *P. fontis* [17], *P. punjabense* [18], *P. cacticida* [13, 19, 20], *P. carotovorum* [13, 16, 21], *P. parmentieri* [22], *P. parvum* [23], *P. polaris* [24], *P. polonicum* [25], and *P. quasiquaticum* [26]. However, two additional species *P. peruviense* and *P. zantedeschiae* [27, 28], yet to be validated by the ad hoc committee [29]. Presently, not even the draft genome is available for *P. cacticida* in any databases. In this current study, two strains, PL152^T (ICMP 24362^T; LMG 32536^T) and PL155, were analyzed and proposed as a novel species, '*Pectobacterium colocasium*'.

ISOLATION AND BIONOMICS

The strains PL152^T and PL155, referred to hereafter as *P. colocasium*, were isolated from soft rot infected taro corm samples collected in Hawaii in 2018. Infected taro corms were surface sterilized with 0.6% sodium hypochlorite solution for 30s, followed by three consecutive rinses for 30s each in distilled water. The corm tissues were aseptically macerated in a 1.5 ml

Eppendorf tube with a sterile pestle, streaked onto Crystal Violet Pectate (CVP) medium [30] for isolation of the colonies within characteristic cavities caused by pectin degradation. Colonies that produced pits were purified by re-streaking the bacteria onto nutrient agar supplemented with 0.4% dextrose during two consecutive rounds, and confirmed with a PCR assay using *Pectobacterium* specific primers [31]. The purified cultures obtained from a single colony were stored at -80°C in 25% glycerol (25:75, Glycerol:LB liquid medium).

MULTI-LOCUS SEQUENCE ANALYSIS

The bacterial DNA was isolated from purified colonies using DNeasy Blood and Tissue kit following the manufacturer's protocol (Qiagen, Valencia, CA). For identification and initial taxonomic classification of novel bacterial strains PL152^T and PL155, the isolated DNA was amplified using three housekeeping genes – chromosomal replication initiator protein DnaA (*dnaA*), glyceraldehyde-3-phosphate dehydrogenase (*gapA*), and DNA topoisomerase ATP-hydrolyzing subunit B (*gyrB*) primers [32, 33]. The amplified PCR product was enzymatically cleaned using ExoSAP-IT (Affymetrix, Santa Clara, CA) sequenced at Genewiz facility (La Jolla, CA) using both forward and reverse primers, and further aligned and manually edited to remove errors using Geneious R10 [34]. The consensus sequences of *dnaA*, *gapA* and *gyrB* gene sequences for PL152^T and PL155 strains were submitted to the NCBI GenBank database under the accession numbers MZ542535 - MZ542540. Taxonomic classification and analyses were performed using 51 different bacterial strains, including novel strains of *P. colocasium* (PL152^T and PL155), 48 other species of *Pectobacterium* and *D. zeae* EC1^T as an outgroup. The sequences of all *Pectobacterium* and *Dickeya* strains used in the analysis were retrieved from the NCBI GenBank genome database in July 2020, with an exception of newly added *P. quasiquaticum* (retrieved in January 2022) (Supplemental Table 1). The sequences were aligned with ClustalW, trimmed and concatenated using

Geneious R10 [34]. MEGA11 software [35] was used to generate the phylogenetic tree using the Maximum Likelihood method with a bootstrap test of 1,000 replicates (Supplemental Figure 1). Based on phylogenetic tree analysis, the novel strains (PL152^T and PL155) clustered together, indicating clonality, and formed a novel clade which was well differentiated from other *Pectobacterium* species. To refine the taxonomic classification and delineation of a novel species, Multi-Locus Sequence Analysis (MLSA) [36] was performed with nine housekeeping genes - *dnaA*, *gapA*, *gyrB*, recominase RecA (*recA*), DNA polymerase III subunit beta (*dnaN*), RNA polymerase sigma factor RpoS (*rpoS*), malate dehydrogenase (*mdh*), DNA-directed RNA polymerase subunit alpha (*rpoA*), and molecular chaperone DnaK (*dnaK*) using 54 bacterial species including novel strain PL152^T and other *Pectobacterium* and *Dickeya* species retrieved from NCBI GenBank database (Supplemental Table 1). The sequences were aligned using ClustalW, trimmed and concatenated using Geneious R10 [34]. Phylogenetic analysis was performed with MEGA11 software using the Maximum Likelihood method and Tamura- Nei model with a 1,000 bootstrap replicates (Figure 2). Based on MLSA analysis, a novel strain of *P. colocasium* branched at the roots of other *Pectobacterium* species, forming a distinctively separate and a novel branch delineating it as a novel *Pectobacterium* species. *Pectobacterium colocasium* rooted close to *P. aroidearum* CFBP8737, KC20 and L6, shown as its closest neighbor species.

16S rRNA GENE ANALYSIS

To confirm and validate the generic level identification of novel bacterial strain PL152^T, 16S rRNA analysis was performed with all 19 type strains of *Pectobacterium* species, and *D. zeae* EC1^T, as an outgroup, available in NCBI database. The 16S rRNA sequences of *P. actinidiae* KKH3^T, *P. carotovorum* NCPPB312^T, *P. aroidearum* SCRI109^T, *P. brasiliense* LMG21371^T, *P. polaris* NIBIO1006^T, *P. parvum* s0421^T, *P. odoriferum* LMG17566^T, *P. versatile*

CFBP6051^T, *P. aquaticum* A212-S19-A16^T, *P. zantedeschiae* 9M^T, *P. fontis* M022^T, *P. polonicum* DPMP315^T, *P. punjabense* SS95^T, *P. parmentieri* RNS08-42-1A^T, *P. wasabiae* CFBP3304^T, *P. atrosepticum* LMG2386^T, *P. peruviana* IFB5232^T, *P. betavascularum* CFBP2122^T, *P. quasipolonicum* A477-S1-J17^T, and *D. zeae* EC1^T were retrieved from NCBI GenBank database (Supplemental Table 2). The 16S rRNA gene sequence of PL152^T was extracted from the whole genome sequence of PL152^T and was submitted to NCBI GenBank with accession number OM457660. Based on 16S rRNA analysis, PL152^T clustered with other *Pectobacterium* species, confirming its identity within *Pectobacterium* genus (Figure 3). The PL152^T was closely rooted to *P. fontis* M002^T strain in this analysis.

GENOME CHARACTERIZATION

Bacteria from stored pure culture of PL152^T were cultured on nutrient agar supplemented with 0.4% dextrose for total genomic DNA isolation. A half loopful of pure bacterial culture was scrapped for DNA isolation using QIAGEN Genomic-tip 100/G (Qiagen, Valencia, CA) and quantified using the Qubit dsDNA HS kit and Qubit 4 (Thermo Fisher Scientific, Waltham, MA), and analyzed on 1.5% agarose gel. The library was prepared using Rapid Barcoding kit (SQB-RBK004) (Oxford Nanopore Technologies, Oxford Science Park, UK) with 400 ng of high-quality genomic DNA, and sequencing was performed using a MinION vR9.4 flow-cell following the manufacturer instructions (Oxford Nanopore Technologies, Oxford, UK). Sequencing data generated in the flow-cell was monitored in real time using the MinKNOW software (version 4.0.20). The obtained FAST5 sequences from MinION were base-called using MinKNOW (version 4.0.20). Later, the obtained long reads were corrected using the tool “Correct Long Reads (beta)” of the CLC Genomics Workbench version 20.0. (Qiagen), with default parameters. Additionally, Illumina DNA library was prepared using Seqwell plexWell LP384 Library Preparation kit (seqWell, Beverly, MA) using 10 ng gDNA.

The prepared library was amplified with 8 PCR cycles, analyzed using Bioanalyzer 2100 (Agilent, Santa Clara, CA), quantified with Qubit, and combined into two pools at equimolar ratios. The library pool was quantified by qPCR with a Kapa Library-Quant kit (Kapa Biosystems/Roche, Basel, Switzerland) and sequenced on an Illumina NovaSeq system (Illumina, San Diego, CA) with paired-end 150-bp reads. The Illumina library preparation and sequencing was performed at Novogene facility (Sacramento, CA). The Illumina paired-end short reads along with the corrected long reads from Oxford Nanopore were used to create an accurate and complete hybrid assembly using the Unicycler assembly pipeline version 0.4.8 [37] with default parameters, as plugged in the web-server PathoSystems Resource Integration Center (PATRIC) [38]. The generated complete genome sequence was annotated using the Prokaryotic Genome Annotation Pipeline (PGAP) version 4.10 and Rapid Annotation using Subsystem Technology (RAST) toolkit [39]. The genome of PL152^T has a total of 5,019,495 bp and DNA G+C content of 54.6 mol%. The assembled genome was submitted to the NCBI GenBank genome database under the accession number CP091064.

To assign the *P. colocasium* strain PL152^T as a novel species, the average nucleotide identity (ANI) and digital DNA-DNA hybridization (dDDH) was calculated using Qiagen CLC Genomics workbench and Type (Strain) Genome Server (<https://tygs.dsmz.de/>), respectively. However, the ANI value for *P. peruvienne* A97-S13-F16 strain was calculated against *P. quasiaquaticum* A477-S1-J17^T and *P. parvum* Y1 using EzBiocloud ANI calculator [40], and dDDH for *P. quasiaquaticum* against all other bacterial strains including in the analysis was calculated using Genome-to-Genome Distance Calculator 3.0 (GGDC) [41]. ANI, defining the nucleotide-level genomic similarity of PL152^T strain genome against other *Pectobacterium* species genomes and *D. zeae* strain EC1, was below the suggested cut-off value of 95-96% to delineate bacterial species [42]. The ANIm values for *P. colocasium* strain PL152^T for analysed *Pectobacterium* species ranged between 88.26 - 91.46%: *P.*

aquaticum A212-S19-A16^T (90.42%), *P. polonicum* DPMP315^T (88.92%), *P. actinidae* KKH3^T (89.95%), *P. fontis* M022^T (88.26%), *P. betavascularum* NCPPB2795^T (88.85%), *P. polaris* NIBIO1006^T (90.76%), *P. brasiliense* LMG21371^T (91.02%), *P. carotovorum* NCPPB312^T (90.65%), *P. aroidearum* L6 (93.87%), *P. versatile* CFBP6051^T (90.44%), *P. wasabiae* CFBP3304^T (88.52%), *P. odoriferum* BC S7 (90.24%), *P. parmentieri* RNS08-42-1A^T (88.44%), *P. punjabense* SS95^T (88.78%), *P. parvum* Y1 (91.46%), *P. zantedeschiae* PC2 (88.55%), *P. atrorepticum* NCPPB549^T (88.9%), *P. peruviense* A97-S13-F16 (88.28%), and *P. quasiaquaticum* A477-S1-J17^T (91.12%) (Table 1). The predicted dDDH values for PL152^T as a query against other strains of *Pectobacterium* species ranged between 21.2-44.7%; A212-S19-A16^T (41.3%), DPMP315^T (36.4%), KKH3^T (39.2%), M022^T (34.8%), NCPPB2795^T (36.6%), NIBIO1006^T (41.8%), LMG21371^T (42.5%), NCPPB312^T (41.4%), L6 (54.2%), CFBP6051^T (40.8%), CFBP3304^T (35.6%), BC S7 (40.4%), RNS08-42-1A^T (35.4%), SS95^T (36%), Y1 (44.7%), PC2 (35.3%), NCPPB549^T (36.4%), A97-S13-F16 (34.6%), and A477-S1-J17^T (43.1%) and *D. zeae* EC1^T (21.2%) were lower than the threshold value of 70% (Wayne et al. 1987) (Table 1). Therefore, based on the concurrence results of comparative genomic, whole genome analysis and clustering of strains in MLSA analysis, authors clearly propose *Pectobacterium colocasium* sp. nov. PL152^T and PL155 strains as a novel *Pectobacterium* species.

Table 1. Average nucleotide identity (ANI) and digital DNA-DNA hybridization (dDDH; in bold type) estimations of *Pectobacterium colocasium* strain PL152^T (=ICMP 24362^T; LMG 32536^T) against other species of *Pectobacterium* genus and *Dickeya zeae* EC1^T (an outgroup). The upper right triangle and lower left triangle represent ANIm and dDDH values, respectively.

ANI value																						
Species		1 <i>P. colocasium</i> PL152 [†]	2 <i>P. aquaticum</i> A212-S19-A16 [†]	3 <i>P. polonicum</i> DPMP315 [†]	4 <i>P. actinidae</i> KKH3 [†]	5 <i>P. fontis</i> M0222 [†]	6 <i>P. betavascularum</i> NCPBP2795 [†]	7 <i>P. polaris</i> NIBIO1006 [†]	8 <i>P. brasiliense</i> LMG21371 [†]	9 <i>P. carotovorum</i> NCPBP312 [†]	10 <i>P. aroidearum</i> L6	11 <i>P. versatile</i> CFBP6051 [†]	12 <i>P. wasabiae</i> CFBP3304 [†]	13 <i>P. odoriferum</i> BC S7	14 <i>P. parmentieri</i> RNS08-42-1A [†]	15 <i>P. punjabense</i> SS95 [†]	16 <i>P. parvum</i> Y1	17 <i>P. zantedeschiae</i> PC2	18 <i>P. atrosepticum</i> NCPBP549 [†]	19 <i>P. peruviansae</i> A97-S13-F16	20 <i>P. quasiaquaticum</i> A477-S1-J17 [†]	21 <i>Dickeya zeae</i> EC1
1	<i>Pectobacterium colocasium</i> PL152 [†]		90.42	88.92	89.95	88.26	88.85	90.76	91.02	90.65	93.87	90.44	88.52	90.24	88.44	88.78	91.46	88.55	88.90	88.28	91.12	83.02
2	<i>P. aquaticum</i> A212-S19-A16 [†]	41.3		89.64	91.99	88.42	89.25	93.50	93.2	94.05	90.08	94.45	88.76	93.43	88.73	88.91	93.82	88.89	89.53	88.75	95.62	82.87
3	<i>P. polonicum</i> DPMP315 [†]	36.4	38.8		89.34	87.71	89.16	89.6	89.44	90.26	88.84	90.13	90.98	89.89	91.07	93.96	90.41	88.92	89.53	88.78	89.63	82.99
4	<i>P. actinidae</i> KKH3 [†]	39.2	46.3	37.7		82.90	88.94	91.79	91.48	93.25	89.71	93.02	88.53	93	88.47	88.54	92.09	88.57	89.22	88.31	91.12	82.90
5	<i>P. fontis</i> M0222 [†]	34.8	35.2	33.6	35.4		87.59	88.40	88.31	88.63	88.22	88.50	87.36	88.42	87.33	87.37	89.05	87.37	87.85	86.97	88.69	82.89
6	<i>P. betavascularum</i> NCPBP2795 [†]	36.6	37.7	37.5	36.8	33.4		89.51	89.53	89.47	89.01	89.44	89.47	89.25	89.3	89.15	90.15	91.35	92.15	91.25	89.57	82.98
7	<i>P. polaris</i> NIBIO1006 [†]	41.8	53.3	38.3	45.2	35.2	38.3		93.97	93.47	90.36	93.81	88.98	92.77	88.92	89.06	96.58	89.04	89.73	88.84	93.47	82.93
8	<i>P. brasiliense</i> LMG21371 [†]	42.5	51.7	37.5	43.8	34.9	38.1	54.4		93.23	90.64	92.90	88.88	92.36	88.83	89.14	94	89	89.59	88.78	93.94	83.05
9	<i>P. carotovorum</i> NCPBP312 [†]	41.4	55.6	40.2	51	35.7	38.1	52.4	51		90.29	95.60	89.02	95.19	88.94	89.08	93.81	89.10	89.81	89.05	92.99	83
10	<i>P. aroidearum</i> L6	54.2	40.1	36.3	38.8	34.6	37.1	40.4	41.3	40.2		90.05	88.59	90.00	88.53	88.81	91	88.97	88.98	88.21	90.35	82.97
11	<i>P. versatile</i> CFBP6051 [†]	40.8	57.8	39.6	50	35.4	38	53.5	49.2	64.2	39.5		88.92	95.01	88.86	88.98	94.33	89.06	89.71	89.08	93	82.87
12	<i>P. wasabiae</i> CFBP3304 [†]	35.6	36.6	42.8	35.8	32.9	38.5	36.8	36.3	36.8	36	36.5		88.88	93.98	91.17	89.72	89.13	90.09	89.14	88.67	83.05
13	<i>P. odoriferum</i> BC S7	40.4	52.6	39.2	50.4	35.6	37.8	49.2	47.4	61.4	39.5	59.8	36.7		88.79	88.81	93.15	88.82	89.6	88.94	92.56	82.93
14	<i>P. parmentieri</i> RNS08-42-1A [†]	35.4	36.4	43.1	35.6	32.6	37.8	36.6	36.2	36.7	35.7	36.4	54.7	36.5		91.40	89.74	89.01	89.88	88.85	88.97	82.98
15	<i>P. punjabense</i> SS95 [†]	36	36.9	54.6	35.5	32.8	37.3	36.9	36.8	36.8	36.3	36.4	43.3	36.3	44.1		89.58	88.82	89.55	88.81	89.26	83.10
16	<i>P. parvum</i> Y1	44.7	55.1	41.3	47.1	37.2	40.3	71.3	55.2	54	43	56.7	39.5	51.5	39.1	39.2		89.69	90.44	*73.9	93.85	82.62
17	<i>P. zantedeschiae</i> PC2	35.3	36.1	36.3	35.4	32.9	43.8	36.5	36.3	36.4	36.5	36.2	37.2	35.7	36.7	36.1	38.6		91.18	90.83	88.43	82.73
18	<i>P. atrosepticum</i> NCPBP549 [†]	36.4	38.2	38.2	37.4	33.8	46.8	38.5	38	38.8	36.6	38.3	40.0	38.4	39.4	38.4	41.1	43.5		92.96	89.49	83
19	<i>P. peruviansae</i> A97-S13-F16	34.6	35.9	35.8	34.9	31.6	43.6	36.2	35.7	36.5	34.3	36.1	36.8	36.2	36	35.5	60.1	42	50.3		*68.7	83.18
20	<i>P. quasiaquaticum</i> A477-S1-J17 [†]	43.1	64.2	39.1	42.8	36.1	38.3	53.5	55.1	50.8	41.2	50.8	36.7	48.3	36.5	37.8	55.5	35.5	38.7	50.4		82.25
21	<i>Dickeya zeae</i> EC1	21.2	20.8	20.8	21.2	21.1	20.8	20.9	21.1	20.9	21.2	20.7	21.2	21.2	20.8	20.6	20.4	20.5	20.8	20.3		
dDDH value																						

*ANI calculated using EzBiocloud software ANI calculator incorporating OrthoANLu algorithm.

PHENOTYPIC CHARACTERIZATION

Biochemical analysis of novel strain PL152^T was performed with Biolog GEN III MicroPlate (Biolog, Inc., Hayward, CA), utilizing 71 carbon source utilization and 23 chemical sensitivity assays. The pure bacterial culture was streaked on BUG agar (Biolog, Inc.) and incubated for 24 hours at $\pm 28^{\circ}\text{C}$. Colonies were picked using the sterile loops and inoculated into inoculating fluid B to prepare the inoculum. Inoculum (100 μl) was dispensed into each well of a 96-well GEN III microplate. Plates were incubated for 24 hours at 28°C and read using the BIOLOG microplate reader; *P. colocasium* utilized α -D-Glucose, Pectin, α -D-Lactose, D-Mannose, D-Mannitol, D-Galacturonic Acid, Methyl Pyruvate, D-Fructose, L-Galactonic Acid Lactone, β -Methyl-D-Glucoside, D-Galactose, myo-Inositol, D-Gluconic Acid, D-Cellobiose, D-Salicin, Glycerol, L-Aspartic Acid, Citric Acid, Gentiobiose, N-Acetyl-D-Glucosamine, D-Glucose-6-PO₄, Sucrose, D-Fructose-6-PO₄, Mucic Acid, L-Rhamnose, D-Aspartic Acid, L-Malic Acid, Acetic Acid, D-Serine, L-Serine, D-Saccharic Acid, Bromo-Succinic Acid; and weak utilization of Dextrin, D-Melibiose, D-Trehalose, L-Glutamic Acid, Glucuronamide, Acetoacetic Acid and Formic Acid (Table 2). In this test, PL152^T was not able to utilize D-Raffinose, D-Sorbitol, Gelatin, p-Hydroxy-Phenylacetic Acid, Tween 40, Glycyl-L-Proline, γ -Amino-Butyric Acid, D-Maltose, D-Arabitol, L-Alanine, D-Lactic Acid Methyl Ester, α -Hydroxy-Butyric Acid, L-Arginine, L-Lactic Acid, β -Hydroxy-D,L-Butyric Acid, 3-Methyl Glucose, D-Glucuronic Acid, α -Keto-Butyric Acid, D-Fucose, α -Keto-Glutaric Acid, N-Acetyl- β -D-Mannosamine, L-Fucose, L-Histidine, D-Malic Acid, Propionic Acid, D-Turanose, N-Acetyl-D-Galactosamine, L-Pyroglutamic Acid, Quinic Acid, Stachyose, N-AcetylNeuraminic Acid and Inosine (**Table 2**). In addition, PL152^T was sensitive to 1% NaCl, 1% Sodium Lactate, Troleandomycin, Vancomycin, pH 6, 4% NaCl, Rifamycin SV, Guanidine HCl, Tetrazolium Violet, Lithium Chloride, Niaproof 4 and Tetrazolium Blue; while insensitive to Nalidixic Acid, Aztreonam, pH 5, Minocycline,

Potassium Tellurite and Sodium Bromate, as per results of chemical sensitivity assay (Table 2).

The proposed novel strain PL152^T was further tested for antibiotic sensitivity using the disc diffusion method. The bacterial inoculum of 100 µl (0.5 OD value) of novel strain PL152^T was uniformly spread onto Nutrient Agar medium supplemented with 0.4% dextrose. A commercial disc was impregnated with 20 µl of Penicillin (50 mg/ml), Kanamycin (50 mg/ml), Tetracycline (40 mg/ml), Chloramphenicol (50 mg/ml), Carbenicillin (100 mg/ml), Gentamicin (50 mg/ml) and Bacitracin (50 mg/ml) and placed at the center of the plates containing inoculated media.. After an incubation period of 24 hours at ±28°C, a “zone of inhibition” was observed determining the sensitivity of the novel strain PL152^T to the antibiotics (Figure 4).

Table 2. Phenotypic characterization of *Pectobacterium colocasium* PL152^T (=ICMP 24362^T; LMG 32536^T) using Biolog GEN III MicroPlate analyzing 71 carbon source utilization assays and 23 chemical sensitivity assays. Microplate incubated at 28°C was read using Biolog’s Microbial Identification System software after 24 hours.

Characteristics (Carbon Source/Chemical Sensitivity)	PL152 ^T	Characteristics (Carbon Source/Chemical Sensitivity)	PL152 ^T
D-Raffinose	-	Sucrose	+
α-D-Glucose	+	N-Acetyl-β-D-Mannosamine	-
D-Sorbitol	-	L-Fucose	-
Gelatin	-	D-Fructose-6-PO4	+
Pectin	+	L-Histidine	-
p-Hydroxy-Phenylacetic Acid	-	Mucic Acid	+
Tween 40	-	D-Malic Acid	-
Dextrin	(+/-)	Propionic Acid	-
α-D-Lactose	+	D-Turanose	-
D-Mannose	+	N-Acetyl-D-Galactosamine	-
D-Mannitol	+	L-Rhamnose	+
Glycyl-L-Proline	-	D-Aspartic Acid	+
D-Galacturonic Acid	+	L-Pyrogutamic Acid	-
Methyl Pyruvate	+	Quinic Acid	-
γ-Amino-Butyric Acid	-	L-Malic Acid	+
D-Maltose	-	Acetic Acid	+
D-Melibiose	(+/-)	Stachyose	-
D-Fructose	+	N-Acetyl Neuraminic Acid	-

D-Arabitol	-	Inosine	-
L-Alanine	-	D-Serine	+
L-Galactonic Acid Lactone	+	L-Serine	+
D-Lactic Acid Methyl Ester	-	D-Saccharic Acid	+
α -Hydroxy-Butyric Acid	-	Bromo-Succinic Acid	+
D-Trehalose	(+/-)	Formic Acid	(+/-)
β -Methyl-D-Glucoside	+	1% NaCl	+
D-Galactose	+	1% Sodium Lactate	+
myo-Inositol	+	Troleandomycin	+
L-Arginine	-	Linomycin	(+/-)
D-Gluconic Acid	+	Vancomycin	+
L-Lactic Acid	-	Nalidixic Acid	-
β -Hydroxy-D,L-Butyric Acid	-	Aztreonam	-
D-Cellobiose	+	pH 6	+
D-Salicin	+	4% NaCl	+
3-Methyl Glucose	-	Fusidic Acid	(+/-)
Glycerol	+	Rifamycin SV	+
L-Aspartic Acid	+	Guanidine HCl	+
D-Glucuronic Acid	-	Tetrazolium Violet	+
Citric Acid	+	Lithium Chloride	+
α -Keto-Butyric Acid	-	Sodium Butyrate	(+/-)
Gentiobiose	+	pH 5	-
N-Acetyl-D-Glucosamine	+	8% NaCl	(+/-)
D-Fucose	-	D-Serine	(+/-)
D-Glucose-6-PO ₄	+	Minocycline	-
L-Glutamic Acid	(+/-)	Niaproof 4	+
Glucuronamide	(+/-)	Tetrazolium Blue	+
α -Keto-Glutaric Acid	-	Potassium Tellurite	-
Acetoacetic Acid	(+/-)	Sodium Bromate	-

DESCRIPTION OF *PECTOBACTERIUM COLOCASII* SP. NOV.

Pectobacterium colocasium (co.lo.ca'si.um L. neut. gen. n. *colocasiae*, pertaining to taro from which strains PL152^T and PL155 were isolated).

Gram negative pectinolytic bacteria forms cavities on CVP medium and grows optimally within 24 hours on Nutrient Agar supplemented with 0.4% dextrose at 28°C. The colonies were smooth, moist consistency and ceramic white on solid SOB media (2% tryptone, 0.5% yeast extract, 10 mM NaCl, 2.5 mM KCl, 10 mM MgSO₄, 1.5% agar supplemented with 2% glycerol) after 2 days at 28°C. Sensitive to antibiotics: Kanamycin, Tetracycline, Cephalosporin, Carbenicillin, Gentamicin and Bacitracin. Able to utilize α D-Glucose, Pectin, α D-Lactose, D-Mannose, D-Mannitol, D-Galacturonic Acid, Methyl Pyruvate, D-Fructose, L-Galactonic Acid Lactone, β -Methyl-D-Glucoside, D-Galactose, myo-Inositol, D-Gluconic

Acid, D-Cellobiose, D-Salicin, Glycerol, L-Aspartic Acid, Citric Acid, Gentiobiose, N-Acetyl-D-Glucosamine, D-Glucose-6-PO₄, Sucrose, D-Fructose-6-PO₄, Mucic Acid, L-Rhamnose, D-Aspartic Acid, L-Malic Acid, Acetic Acid, D-Serine, L-Serine, D-Saccharic Acid and Bromo-Succinic Acid. Sensitive to 1% NaCl, 1% Sodium Lactate, Troleandomycin, Vancomycin, pH 6, 4% NaCl, Rifamycin SV, Guanidine HCl, Tetrazolium Violet, Lithium Chloride, Niaproof 4 and Tetrazolium Blue.

The type strain PL152^T (ICMP 24362^T; LMG 32536^T) and clonal strain PL155 were isolated in 2018 from infected taro corms in Hawai'i. The DNA G+C content of type strain is 54.6 mol%.

AUTHOR STATEMENTS

Conflicts of interest

The author(s) declare that there are no conflicts of interest.

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Ethical approval

N/A

Consent for publication

N/A

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FIGURES AND TABLES

Figure 1. Timeline of major taxonomic events based on taxonomic description of *Pectobacterium*.

Figure 2. Multi-Locus Sequence Analysis (MLSA) of 54 strains, comprising *Pectobacterium colocasium* (PL152^T; ICMP 24362^T; LMG 32536^T) (brown color) and other known *Pectobacterium* sp. and *Dickeya zeae* EC1 (as an outgroup), was performed using nine housekeeping genes (*dnaA*, *gapA*, *gyrB*, *recA*, *dnaN*, *rpoS*, *mdh*, *rpoA* and *dnaK*). MEGA11 (Maximum Likelihood method) with a bootstrap test of 1,000 replicates was used for analysis. Bootstrap values are indicated at nodes. *Pectobacterium colocasium* formed a novel clade.

Figure 3. The 16S rRNA tree was generated using MEGA 11 (Maximum Likelihood method) for all type strains within *Pectobacterium*: *P. actinidiae* KKH3^T, *P. carotovorum* NCPPB312^T, *P. aroidearum* SCRI109^T, *P. brasiliense* LMG21371^T, *P. polaris* NIBIO1006^T, *P. parvum* s0421^T, *P. odoriferum* LMG17566^T, *P. versatile* CFBP6051^T, *P. aquaticum* A212-S19-A16^T, *P. zantedeschiae* 9M^T, *P. fontis* M022^T, *P. polonicum* DPMP315^T, *P. punjabense* SS95^T, *P. parmentieri* RNS08-42-1A^T, *P. wasabiae* CFBP3304^T, *P. atrosepticum* LMG2386^T, *P. peruviense* IFB5232^T, *P. betavascularum* CFBP2122^T, *P. colocasium* PL152^T and *Dickeya zeae* EC1^T (as an outgroup). *Pectobacterium colocasium* strain PL152^T (ICMP 24362^T; LMG 32536^T) labelled with red solid circle, rooted among *Pectobacterium* species.

Figure 4. Antibiotic assay determining the sensitivity of the novel strain PL152^T (ICMP 24362^T; LMG 32536^T) to seven different antibiotics- Penicillin (50 mg/ml), Kanamycin (50 mg/ml), Tetracycline (40 mg/ml), Chloramphenicol (50 mg/ml), Carbenicillin (100 mg/ml),

Gentamicin (50 mg/ml) and Bacitracin (50 mg/ml) (A-G) by forming a “zone of inhibition”.

Distilled water was used as control (H).

SUPPLEMENTAL MATERIALS

Supplemental Figure 1. Initial identification and phylogenetic analysis of *Pectobacterium colocasium* strains (PL152^T and PL155), 48 different species of *Pectobacterium* genus and *Dickeya zeae* EC1^T as an outgroup, using MEGA11 (Maximum likelihood method) with 1,000 bootstrap replication. Partial sequences of three housekeeping genes, *dnaA*, *gapA* and *gyrB*, were concatenated and used to generate phylogenetic tree. PL152^T and PL155 are labelled with a red solid circle on the phylogenetic tree, depicting two strains as clonal, rooted among other *Pectobacterium* species in a novel clade.

Supplemental Table 1. List of 53 bacterial strains including 52 known strains of *Pectobacterium* species and *Dickeya zeae* EC1 (as an outgroup) retrieved from NCBI GenBank database for multi-locus sequence analysis.

Supplemental Table 2. List of type strains of known *Pectobacterium* species and *Dickeya zeae* EC1 (as an outgroup), retrieved from the NCBI GenBank database and used for the 16S rRNA analysis.

A. Penicillin (50 mg/ml)

B. Gentamicin (50 mg/ml)

C. Kanamycin (50 mg/ml)

D. Tetracycline (40 mg/ml)

E. Carbenicillin (100 mg/ml)

F. Bacitracin (50 mg/ml)

G. Chloramphenicol (50 mg/ml)

H. Control (Water)