Culture and genomic analysis of *Symbiopectobacterium purcellii*, gen.nov. sp. nov., isolated from the leafhopper *Empoasca decipiens*

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ABSTRACT

Bacterial endosymbionts are found in multiple arthropod species, where they play crucial roles as nutritional symbionts, defensive symbionts or reproductive parasites. Recent work has highlighted a new clade of heritable microbes within the gammaproteobacteria that enter into both obligate and facultative symbioses, with an obligately required unculturable symbiont recently given the name *Cand.* Symbiopectobacterium. In this study, we describe a culturable rod shaped non-flagellated bacterial symbiont from this clade isolated from the leafhopper *Empoasca decipiens*. The symbiont is related to the transovarially-transmitted 'BEV' bacterium that was first isolated from the leafhopper *Euscelidius variegatus* by Alexander Purcell, and we therefore name the symbiont *Symbiopectobacterium purcellii* sp. nov. gen. nov. We further report the closed genome sequence for *S. purcellii*. The genome is atypical for a heritable microbe, being large in size, without profound AT bias and with little evidence of pseudogenization. The genome is predicted to encode Type II, III and VI secretion systems and associated effectors and a non-ribosomal peptide synthase array likely to produce bioactive small molecules. Predicted metabolism is more complete than for other symbionts in the *Symbiopectobacterium* clade, and the microbe is predicted to synthesize a range of B vitamins. However, Biolog plate analysis indicate metabolism is depauperate compared to the sister clade, represented by *Pectobacterium carotovorum*. A quorum-sensing pathway related to that of *Pectobacterium* spp. (containing an overlapping *expI-expR1* pair in opposite directions and a "solo" *expR2*) is evidenced, and LC-MS/MS analysis reveals the presence of 3-hydroxy-C10-HSL as the sole *N*-acylhomoserine lactone (AHL) in our strain. This AHL profile is profoundly divergent from that of other *Erwinia* and *Pectobacterium* spp., which produce mostly 3-oxo-C6- and 3-oxo-C8-HSL and could aid group identification. Thus, this microbe denotes one that has lost certain pathways associated with a saprophytic lifestyle but represents an important baseline against which to compare other members of the genus *Symbiopectobacterium* that show more profound integration into host biology.

KEYWORDS

Symbiopectobacterium, *Empoasca*, symbiosis, leafhopper, quorum sensing

65 **INTRODUCTION**

66 It is now understood that microbes influence multiple aspects of animal
67 biology (1). Symbiont contributions extend from involvement in the process of
68 digestion in the gut, through anabolic activities and the supply of vitamins and amino
69 acids, to protection against natural enemies and defence against prey/hosts (2).
70 Conversely, other symbiotic microbes are pathogenic or parasitic, and many
71 symbioses combine both parasitic and beneficial aspects. Levels of symbiont
72 integration vary between symbioses (3). On the host axis, they vary from facultative
73 relationships where the host does not require a particular symbiont, to obligate
74 where the individual dies or becomes sterile in the absence of symbiosis. Likewise,
75 symbionts vary in the degree to which they rely on a host – some only replicating
76 within hosts with others having environmental replication. The process of symbiosis
77 formation also varies – from arising within the host lifecycle through acquisition by
78 the host or infection by the microbe, to being present through the host lifecycle,
79 with symbiont transfer/transmission from parent to offspring.

80

81 Whilst arthropod-microbe symbioses are diverse in terms of the microbial
82 partners, particular microbial taxa have established symbiosis with a number of host
83 species, commonly establishing in new host species through a host switch event.
84 Well-known 'heritable symbionts' found over a broad range of arthropods include
85 *Wolbachia*, *Rickettsia*, *Spiroplasma*, *Cardinium* and *Arsenophonus* (4). The
86 interactions found in these symbioses include obligate and facultative associations,
87 and ones which are beneficial, parasitic or have a combination of features.

88

89 Recent research has added a new clade of insect symbionts, *Cand.*
90 Symbiopectobacterium, to 'the big five' (5). The first member of this clade to be
91 described was the BEV strain – an acronym for bacterium from *Euscelidius*
92 *variegatus*, a planthopper host species. This strain was cultured (6), but never
93 formally named. The symbiosis was characterized as one with vertical transmission,
94 where the host's reproduction was negatively impacted by the microbe. In addition,
95 there was also transmission to other insects on the plant – thus establishing the
96 symbiosis as a pathogenic one maintained through mixed modes of transmission (7).

Experiments also suggested the symbiont facilitated the transmission of phytoplasma from its bug host to plant (8). Later, the pest species *Cimex lectularius* (common bedbug) was observed to carry a heritable symbiont related to BEV (9). This symbiont has not been established in cell-free culture, and symbiosis is facultative from the host perspective: the bedbug does not require the symbiont. Following this, a third hemipteran – the bulrush bug *Chilacis* was observed to carry a related vertically transmitted symbiont housed in a gut mycetome, in what appears to be an obligate association, in which the host requires the symbiont (10). More recently, symbioses involving members of this clade have extended beyond Hemiptera hosts to nematodes, with Martinson et al (5) characterizing symbionts related to BEV as obligate partners of *Howardula* nematodes. They named this microbe *Cand.* Symbiopectobacterium, reflecting its symbiotic lifestyle and its sister relationship to the well-characterized genus *Pectobacterium*.

Cand. Symbiopectobacterium has thus emerged as a potentially widespread and significant symbiotic associate of invertebrates. The original culturable BEV isolate, on which the genus could be formally described, was lost and genomic information for this strain is partial (11). Recovering a model culturable member of the genus is important, as it allows formal description of the microbe, completion of a closed genome sequence against which reductive evolution in symbiosis can be measured and presents a system in which gene function may be investigated. In this paper, we report the isolation to pure culture of a member of this clade from the planthopper *Empoasca decipiens*. We further present and analyse the complete genome sequence of this microbe, assess its growth requirements compared to *Pectobacterium carotovorum* and analyse its quorum sensing-signalling system.

124 **MATERIALS AND METHODS**

125 **Symbiont isolation, morphology *in vitro* and identification through 16S rRNA**
126 **sequence**

127 Initial Cicadellidae samples with light green coloration were collected in
128 Prince's Park, Liverpool in April 2018, scooping different plants with an insect net at a
129 maximum height of 2m. Fresh specimens were transported alive to the lab and
130 sacrificed by freezing at -20ºC for 15 min. The insect specimens were surface
131 sterilized by immersion in 70% ethanol and washed with sterile water to remove the
132 remaining alcohol. Insect legs were excised with a sterile surgical blade and stored at
133 -20ºC for *post hoc* host species determination through DNA barcoding.

134

135 The remainder of the insect body was mechanically crushed and resuspended
136 in 100 μl of sterile water. An aliquot of 10 μl was plated on brain heart infusion (BHI,
137 Oxoid, UK) agar and grown at 30ºC for 6 d to allow the appearance of slow-growing
138 bacterial colonies. Morphology was examined through Gram staining and scanning
139 electron microscopy of overnight culture. To identify the bacterial species, we
140 performed colony PCR of the 16S rRNA gene colonies emerging on the agar plates
141 with primers 27F (AGAGTTTGATCMTGGCTCAG) (12) and 1492R(I)
142 (GGTTACCTTGTTACGACTT) (13), and sequenced by Eurofins genomics, Germany.
143 Sequences were manually curated and phylogenetic analysis performed based on
144 the 16S rRNA gene sequences which included a large assemblage of members from
145 the *Cand*. Symbiopectobacterium clade. To this end, 16S rRNA sequences were
146 aligned using the SSU-ALIGN software (14). A Bayesian phylogeny was estimated
147 with MrBayes v3.2.6 (15) by sampling across the GTR model space (nst=mixed,
148 rates=gamma). Two independent runs were performed for 5,000,000 generations
149 and sub-sampling every 500 generations using four Markov chains. The first 25% of
150 the samples were discarded as burn-in.

151

152 The leafhopper host was identified through DNA barcoding using the COI
153 sequence. To this end, insect legs were mechanically crushed and resuspended in 50
154 μl of sterile water and the genomic DNA (gDNA) was extracted using a Quick-DNA
155 Universal kit (Zymo research, USA). 2 μl of the gDNA were added to a GoTaq® Green

156 Master Mix (Promega, USA) and used to amplify part of the mitochondrial
157 cytochrome oxidase 1 (CO1) with primers C1-J-1718
158 (GGAGGATTTGGAAATTGATTAGTTCC) and C1-N-2191
159 (CCCGGTAAAATTAAAATATAAACTTC) (16). The PCR program consisted of an initial
160 denaturation step at 95ºC for 5 min, followed by 30 cycles of DNA denaturation at
161 94ºC for 15s, primer annealing at 55ºC for 45 seconds, and primer extension at 72ºC
162 for 1 min. A final extension was carried out at 72ºC for 5 min. A few microliters of
163 each PCR product were run on an agarose gel to assess the success of the PCR
164 reaction and the remains cleaned through an Isolate II PCR and Gel kit (Bioline, USA)
165 and sent for sequencing with primer C1-N-2191. Identity was checked through
166 analysis against the Barcode of Life Database, BOLD.
167
168 **In vitro Growth requirements**
169 BIOLOG GEN III plates (Cat. No. 1030) were used to ascertain the
170 physiological and biochemical characteristics of *S. purcellii* SyEd1 *in vitro*, and these
171 were conducted alongside *Pectobacterium carotovorum* subsp. *carotovorum* LMG
172 02404^{T} for comparison. Within this, we also performed the assay in the
173 presence/absence of 0.4 % polygalacturonic acid PGA (Sigma, P3850), which is
174 commonly used to induce the expression of plant cell wall-degrading enzymes (for
175 preparation of PGA, see (17). For the BIOLOG GEN III assays, we used IF-A inoculating
176 fluid (Biolog, Cat. No. 72401) with or without PGA supplementation to a final
177 concentration of 0.4% PGA. Both bacterial species were grown overnight, diluted to
178 an OD_{600}= 0.4 and 50 μl of this aliquot were added to a tube containing IF-A fluid.
179 The aliquot in the IF-A tube was homogeneously mixed using a vortex and 100 μl of
180 this suspension was added to each of the 96 wells of the BIOLOG GEN III plate. The
181 plate was subsequently incubated at 30ºC without shaking.
182
183 **Potato infection assays**
184 *Pectobacterium* spp. are well-known plant pathogens causing soft-rot disease
185 in several plants including potatoes, carrots and cabbages. This damage is caused by
186 a series of secreted enzymes (cellulases, proteases, pectate lyases (Pel), pectin
187 lyases, and polygalacturonases) commonly referred to as plant cell wall-degrading

188 enzymes (PCWDEs). The presence of 15 putative PCWDEs and two copies of the KdgR
189 regulator (associated to their expression) in *Symbiopectobacterium purcellii* led us to
190 assess whether this symbiont retains the plant pathogenic activity of its sister clade,
191 *Pectobacterium.* To this aim, virulence was tested in potatoes, using a method
192 previously described in Nadal-Jimenez et al (18) with minor modifications. Briefly,
193 potatoes were bought at local stores, washed with tap water, dried and surface
194 sterilized with 70% ethanol. Slices about 0.5 cm thick were placed in sterile Petri
195 dishes. Overnight cultures of *S. purcellii* SyEd1 and *Pectobacterium carotovorum* LMG
196 02404^{T} were diluted to an OD600=0.4, and 20 μl were placed at the centre of the
197 potato slice. The same amount of sterile BHI medium was added to the negative
198 controls. The plates were sealed with parafilm to avoid moisture loss and incubated
199 at 25ºC in dark conditions. Tissue maceration was assessed visually 24, 48 and 72 h
200 after incubation.
201
202
203 **Symbiont Genome sequencing, assembly and annotation**
204 The genome of the symbiont was completed using a combination of short
205 (Illumina) and long (nanopore) reads by MicrobesNG (Birmingham, UK) using their
206 enhanced genome service. Briefly, Illumina sequencing was performed using the
207 Nextera XT library prep protocol on a HiSeq platform (Illumina, San Diego, CA, USA)
208 using a 250bp paired end protocol. Reads were adapter trimmed using Trimmomatic
209 0.30, with a sliding window quality cutoff of Q15 (19). Long read genomic DNA
210 libraries are prepared with Oxford Nanopore SQK-RBK004 kit (ONT, UK) using 400-
211 500ng of HMW DNA and sequenced in a FLO-MIN106 (R.9.4.1) flow cell in a GridION
212 (ONT, UK). Hybrid genome assembly of both short and long reads was performed
213 using Unicycler version 0.4.0 under the normal mode (20). The final assembly was
214 manually inspected for potential misassemblies by mapping the raw reads back to it.
215 Genome annotation was performed with the NCBI Prokaryotic Genome Annotation
216 Pipeline (21). Metabolic and functional assessment of the symbiont genome was
217 conducted using the Kyoto Encyclopaedia of Genes and Genomes (KEGG) database
218 (22). Identification of secondary metabolite biosynthesis gene clusters was
219 performed using the antiSMASH server (23). Finally, prophage regions were

220 predicted using the PHAge Search Tool Enhanced Release (PHASTER) web server
221 (24).
222
223 **Phylogenomic analysis**
224 The phylogenetic position of the symbiont was assessed based on the
225 concatenated analysis of 527 single copy core proteins identified among 56 publicly
226 available genomes. These include members of the closely related genera
227 *Pectobacterium*, *Brenneria*, *Dickeya*, *Lonsdalea*, *Sodalis* and the recently
228 characterized *Cand*. Symbiopectobacterium (5). Single copy orthologue protein
229 sequences were identified using OrthoFinder v2.3.11 (25). A maximum likelihood
230 phylogeny was inferred with IQ-TREE 2.0.3 [25] using the JTT+F+R3 substitution
231 model selected using ModelFinder according to the Bayesian information criterion
232 [26]. Clade support was assessed based on 1000 ultrafast bootstrap replicates [27].
233
234 **Analysis of *N*-acyl homoserine lactone synthesis**
235
236 *S. purcellii* SyEd1 cultures were grown in 5 ml of BHI medium at 30ºC for 24h
237 and 200 rpm. After incubation the cultures were centrifuged and the supernatant
238 collected and filtered through a 0.2 mm filter (SLGP033RS, Millipore). 500 μl of
239 acidified ethyl acetate was added to 1 ml of supernatant sample and the mixture was
240 vortexed for 1-2 min. Subsequently. The mixture was centrifuged for 1 min to allow
241 the formation of a clear interface between the aqueous and organic layer. The
242 organic (upper) layer was transferred using a pipette (without disturbing the
243 aqueous layer) to a new 2ml Eppendorf. The extraction process was repeated twice
244 more, combining the extracts for each sample into one of approximately 1.5 ml
245 extract. Upon completion, the samples were dried under vacuum in a centrifugal
246 evaporator.
247 Dried extract samples were reconstituted in 50 μl of methanol (MeOH) prior
248 to analysis. LC-MS/MS analysis of 5 μl sample injections were conducted using a
249 Qtrap 6500 hybrid triple-quadrupole linear ion trap mass spectrometer in tandem
250 with an Exion LC system (Sciex). The overall method was a modification of that
251 described by Ortori et al (26). Chromatography was achieved using a Phenomenex

252 Gemini C18 column (3.0 um, 50 x 3.0 mm) with a constant flow rate of 450 μl/min of
253 mobile phase A (0.1 % (v/v) formic acid) and mobile phase B (0.1 % (v/v) formic acid
254 in methanol). The LC gradient began at 10% B for 1.0 min, increased linearly to 50% B
255 over 0.5 min, then to 99% B over 4.0 min. The composition remained at 99% B for
256 1.5 min, decreased to 10% B over 0.1 min, and stayed at this composition for 2.9
257 min. Analyte detection was conducted with the MS operating in MRM (multiple
258 reaction monitoring) mode, screening the LC eluent for specific AHLs (unsubstituted,
259 3-oxo and 3-OH AHLs with even chain lengths from C4-C14).
260
261 **Prevalence of symbiont in *Empoasca decipiens* leafhoppers**
262 In order to assess the prevalence of this bacterium in *E. decipiens*, we performed a
263 PCR screening on various specimens. Additional insect collections were completed at
264 the same location in August 2019 and tested for SyEd1 by PCR assay. Using the full-
265 genome sequence of our cultured strain, we developed two set of specific PCR
266 primers to amplify part of the DNA gyrase subunit (*gyrB*) gene of this bacterium:
267 BEV_gyrB_F1 (CCGTGGTGTCGGTGAAAGTA) + BEV_gyrB_R1
268 (TGGTCTTCTGTCAGCGTGTC) and BEV_gyrB_F2 (CTCGTGAAATGACACGACGC) +
269 BEV_gyrB_R2 (CAGCAGTTCCACTTGTTCGC). The gDNA was extracted in the same
270 manner as for the leg samples and used as a template for the PCR reactions.
271
272 **RESULTS**
273
274 **Symbiont isolation and identification**
275 The bacterium grows under standard aerobic conditions in Brain heart
276 infusion (BHI) medium (CM1032, Oxoid), forming circular white colonies approx. 2-3
277 mm in diameter on BHI agar, and cultures emitted a pronounced plant-like odour.
278 The bacterium is Gram negative, and SEM revealed it to be a non-flagellated rod
279 shape, of length 1-1.5 mm (Figure 1). The bacterium will also grow in LB (Miller)
280 (110285, Millipore/Merck KGaA) although at a slower rate, and growth is inhibited
281 by light.
282

283 Phylogenetic analysis based on the 16S rRNA gene (Accession number
284 OK044380) placed the isolated microbe well within the recently characterized clade
285 Cand. *Symbiopectobacterium* (Figure 2), a group of microbes commonly associated
286 with arthropods and nematodes. Sequence of the CO-1 amplicons from the insect
287 host revealed the leafhopper host to be *Empoasca decipiens* (Hemiptera,
288 Cicadellidae), a common species of leafhopper in Europe. *Empoasca decipiens* has
289 been implicated in the transmission of various plant pathogens (27), and is
290 considered a pest in various crops (28).
291
292 **In vitro Growth requirements**
293 *S. purcellii* SyEd1 and *P. carotovorum* LMG 2404^{T} were grown at 30ºC in
294 BIOLOG GEN III plates. For *P. carotovorum* LMG 2404^{T}, the presence of the purple
295 tetrazolium dye as a result of growth and respiration in the wells where the strains
296 had grown was visible after 24 h, while the wells that did not supported the growth
297 of this strain remained colourless. In the case of *S. purcelliii* SyEd1, the plates had to
298 be incubated for a total of 72h to allow bacterial growth. This is not surprising since,
299 in our hands, *S. purcellii* SyEd1 grows slowly in BHI media (requiring up to 48h), and
300 even slower in less rich media. Analyses indicated *S. purcellii* was considerably more
301 fastidious than the comparator outgroup strain *P. carotovorum* LMG 2404^{T} in terms
302 of metabolites that supported growth (Table 1) but had broader resistance to
303 xenobiotics than this strain (Table 2). Growth conditions for *S. purcellii* on Biolog
304 analysis was only modestly altered by addition of PGA.
305
306 **Potato infection assays**
307 Potato slices infected with *S. purcellii* SyEd1 exhibited a complete absence of
308 infection/ tissue maceration at the different time points tested (72h time point
309 shown in Figure 3) in contrast to *P. carotovorum* LMG 02404^{T}, used as positive
310 control for infection. The assay was maintained for a week without any sign of
311 infection being visible in *S. purcellii* SyEd1 infected potatoes.
312
313 **Genome sequence and assembly**

314 The genome of the symbiont presented as a single circular chromosome of
315 circa 4.9 MB with an average GC content of 52.5% (Table 3). No plasmids were
316 identified. The complete predicted gene set consists of 4,494 protein-coding genes
317 (including 312 predicted pseudogenes), 7 ribosomal RNA operons (5S, 16S, 23S) and
318 76 tRNAs. The average length of the protein-coding genes is 948 bp accounting for a
319 coding density of about 86.2%. Pseudogenization rates were estimated to be circa
320 7% (312 predicted pseudogenes). The main chromosome was predicted to contain
321 six intact prophage regions and three additional incomplete fragments. The
322 complete genome assembly and the raw reads have been submitted to the
323 DDBJ/EMBL/GenBank database under the BioProject accession number
324 PRJNA756769 (genome accession number CP081864).
325
326 **Phylogenomic and functional analysis**
327 To confirm the phylogenetic position of the *E. decipiens* symbiont we conducted a
328 phylogenomic analysis base on the concatenated set of 527 single copy orthologue
329 proteins across 56 related genomes (Figure 4). These results further support the
330 placement of the symbiont in the *Cand.* Symbiopectobacterium clade.
331
332 The genome is predicted to encode type II, III and VI secretion systems alongside a
333 wide array of predicted secreted toxins, compatible with its likely status as a
334 symbiont of its insect host. Anti-SMASH predicted five genomic regions associated
335 with small molecule production. Notable amongst these is a non-ribosomal peptide
336 synthase (NRPS) region. It is unclear if the NRPS system produces siderophore
337 molecules that permit growth in iron poor host environments, or antimicrobial
338 compounds. In addition, anti-SMASH predicted putative gene clusters for the
339 biosynthesis of thiopeptide, an aryl-polyene potentially providing defence against
340 ROS, and betalactone synthesis. There is also a predicted homoserine lactone
341 synthesis island *expI*/*expR1* that may be involved in sensing of microbial titre (see
342 below); the genome encodes additional conserved elements of the Quorum sensing
343 system, *expR2*, *gacA (expA)*, *gacS (expS)*, *rsmA* and *rsmB,* and *kdgR*. Finally, the
344 genome encodes complete biosynthetic pathways for several B vitamins including
345 thiamine (B1), riboflavin (B2), pantothenate (B5), biotin (B7), pyridoxine (B6) and

346 folate (B9). A broad array of complete amino acid biosynthesis pathways was also
347 observed, including serine, threonine, cysteine, methionine, valine, leucine,
348 isoleucine, arginine, ornithine, arginine, proline, histidine, tryptophan, phenylalanine
349 and tyrosine. Vitamin and amino acid provision are common means through which
350 symbionts contribute to host function. There are also 5 *pel* genes predicted to
351 encoded pectate lyase enzymes. The failure of the strain to utilize pectin on Biolog
352 plates or on potato tubers may thus be context dependent.
353
354 **Analysis of homoserine lactones**
355
356 *S. purcellii* SyEd1 analysis using LC-MS/MS revealed the presence of a single
357 AHL that was characterised as *N*-(3-hydroxydecanoyl)-L-homoserine lactone (3-OH-
358 C10-HSL). Figure 5 shows the LC-MS/MS chromatogram obtained from the *S. purcellii*
359 SyEd1 sample compared to the 3-OH-C10-HSL standard and the uncultured BHI
360 medium. Members of the genus *Pectobacterium* have been reported to produce 3-
361 oxo-C6-HSL, 3-oxo-C8-HSL, C10-HSL (29), but, to the best of our knowledge, the
362 presence of 3-OH-C10-HSL as the sole AHL in *S. purcellii* is unreported in related
363 genera. This trait may help to identify novel members of this genus that we presume
364 may have been previously misidentified as *Pectobacterium* spp. associated to the
365 plants where the leafhoppers feed.
366
367 **Prevalence of *S. purcellii* in *E. decipiens* samples.**
368 Seven new *E. decipiens* specimens were collected and their identity
369 confirmed by CO-1 amplification and sequencing. All samples were confirmed to be
370 *E. decipiens* with >99.6% identity with previously deposited sequences in NCBI. The
371 same gDNA extract was used to screen for the presence of the bacterial symbiont by
372 PCR using our BEV_gyrB_F2 and BEV_gyrB_R2 primers. All seven samples produced
373 an amplicon for *S. purcellii* (10), and the identity of the amplicon was confirmed
374 through sequencing accounting for a 100% prevalence in the population tested
375 (95%CI: 64% - 100%).
376
377 **Description of *Symbiopectobacterium purcelli gen. nov., sp. nov*.**

378 *Symbiopectobacterium purcellii*. *Symbiopectobacterium* (L. n. *sym bio pecto*
379 *bacterium*) references the related *Cand.* Symbiopectobacterium that is an obligate symbiont
380 of nematode worms, this name reflecting the symbiotic habit of the microbe, and the
381 relationship of the genus as sister to *Pectobacterium*. The species name *purcellii* [pur.cell ii.
382 L. m. gen.] is given in reference to Alexander Purcell, who isolated the first member of this
383 clade, which he named the BEV symbiont (bacterium from *Euscelidius variegatus*).

384 Gram-negative rod-shaped bacterium. Grows optimally at 30 °C in BHI medium in
385 the dark forming colonies within 24-48 h. Using Biolog GENIII plates, *S. purcelli* responded
386 positively to the following carbon sources: D-glucose, D-mannose, D-fructose, glycerol, N-
387 acetyl glucosamine, L-rhamnose, and weaker to methyl pyruvate and acetic acid. Growth
388 was inhibited at pH5, by 4% and 8% NaCl, by D-serine, minocycline and sodium bromate.
389 Growth was not impaired by 1% sodium lactate, fusidic acid, Troleandomycin, Rifamycin S,
390 Lincomycin, Guanidine HCl, Vancomycin, Tetrazolium Violet, Tetrazolium blue, Potassium
391 tellurite, Nalidixic Acid, Lithium Chloride, Aztreonam, Sodium Butyrate. The microbe does
392 not cause macerations on potato slices.

393 *Symbiopectobacterium purcellii* gen. nov. sp. nov. form a cluster with a variety of
394 uncultured symbionts of insects and nematodes, as well as the previously cultured strain
395 BEV.

396 The type strain is SyEd1 (LMG 32449, CECT 30436) and was isolated from *Empoasca*
397 *decipiens* (Hexapoda: Hemiptera: Cicadellidae) from Liverpool UK (53.3868° N, 2.9565° W).
398 The genome consists of a single circular chromosome of size 4.9MB and DNA G+C content is
399 52.5 mol%. The 16S rRNA sequence of the type strain is available at accession OK044380.
400 The complete genome assembly and the raw reads have been submitted to the
401 DDBJ/EMBL/GenBank database under the BioProject accession number PRJNA756769
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403

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Conflicts of interest

The authors declare that there are no conflicts of interest.

REFERENCES

1. McFall-Ngai M, Hadfield MG, Bosch TCG, Carey H V., Domazet-Lošo T, Douglas AE, et al. Animals in a bacterial world, a new imperative for the life sciences. Vol. 110, Proceedings of the National Academy of Sciences of the United States of America. 2013. p. 3229–36.
2. Hurst GDD. Extended genomes: Symbiosis and evolution. Interface Focus [Internet]. 2017 [cited 2021 Sep 22];7(5). Available from: https://royalsocietypublishing.org/doi/abs/10.1098/rsfs.2017.0001
3. Gerardo N, Hurst G. Q&A: Friends (but sometimes foes) within: The complex evolutionary ecology of symbioses between host and microbes. BMC Biol [Internet]. 2017 Dec 27 [cited 2021 Sep 22];15(1):1–6. Available from: https://bmcbiol.biomedcentral.com/articles/10.1186/s12915-017-0455-6
4. Duron O, Bouchon D, Boutin S, Bellamy L, Zhou L, Engelstädter J, et al. The diversity of reproductive parasites among arthropods: Wolbachia do not walk alone. BMC Biol. 2008;6.
5. Martinson VG, Gawryluk RMR, Gowen BE, Curtis CI, Jaenike J, Perlman SJ. Multiple origins of obligate nematode and insect symbionts by a clade of bacteria closely related to plant pathogens. Proc Natl Acad Sci U S A [Internet]. 2020 Dec 15 [cited 2021 Sep 22];117(50):31979–86. Available from: https://www.pnas.org/content/117/50/31979
6. Purcell AH, Steiner T, Mégraud F, Bové J. In vitro isolation of a transovarially transmitted bacterium from the leafhopper Euscelidius variegatus (Hemiptera: Cicadellidae). J Invertebr Pathol. 1986;48(1):66–73.
7. Purcell AH, Suslow KG, Klein M. Transmission via plants of an insect pathogenic bacterium that does not multiply or move in plants. Microb Ecol [Internet]. 1994 Jan [cited 2021 Sep 22];27(1):19–26. Available from: https://link.springer.com/article/10.1007/BF00170111
8. Purcell AH, Suslow KG. Pathogenicity and effects on transmission of a mycoplasmalike organism of a transovarially infective bacterium on the leafhopper Euscelidius variegatus (Homoptera: Cicadellidae). J Invertebr Pathol. 1987;50(3):285–90.
9. Hypša V, Aksoy S. Phylogenetic characterization of two transovarially transmitted endosymbionts of the bedbug Cimex lectularius (Heteroptera:

452 Cimicidae). Insect Mol Biol [Internet]. 1997 [cited 2021 Sep 22];6(3):301–4.
453 Available from: https://pubmed.ncbi.nlm.nih.gov/9272448/
454 10. Kuechler SM, Dettner K, Kehl S. Characterization of an obligate intracellular
455 bacterium in the midgut epithelium of the bulrush bug Chilacis typhae
456 (Heteroptera, Lygaeidae, Artheneinae). Appl Environ Microbiol.
457 2011;77(9):2869–76.
458 11. Degnan PH, Bittleston LS, Hansen AK, Sabree ZL, Moran NA, Almeida RPP.
459 Origin and examination of a leafhopper facultative endosymbiont. Curr
460 Microbiol [Internet]. 2011 May [cited 2021 Sep 22];62(5):1565–72. Available
461 from: /pmc/articles/PMC3069327/
462 12. Lane, D. J. (1991). 16S/23S rRNA sequencing, p. 115-175. *In* E. Stackebrandt
463 and M. Goodfellow (ed.), Nucleic acid techniques in bacterial systematics.
464 John Wiley & Sons, New York. [Internet]. [cited 2021 Sep 22]. Available from:
465 http://www.sciepub.com/reference/56892
466 13. Turner S, Pryer KM, Miao VPW, Palmer JD. Investigating deep phylogenetic
467 relationships among cyanobacteria and plastids by small subunit rRNA
468 sequence analysis. J Eukaryot Microbiol [Internet]. 1999 [cited 2021 Sep
469 22];46(4):327–38. Available from:
470 https://pubmed.ncbi.nlm.nih.gov/10461381/
471 14. Nawrocki EP. Structural RNA Homology Search and Alignment using
472 Covariance Models. PhD thesis [Internet]. 2009 Jan 1 [cited 2021 Sep 22];282.
473 Available from: http://openscholarship.wustl.edu/etd/256
474 15. Ronquist F, Teslenko M, Van Der Mark P, Ayres DL, Darling A, Höhna S, et al.
475 Mrbayes 3.2: Efficient bayesian phylogenetic inference and model choice
476 across a large model space. Syst Biol [Internet]. 2012 May [cited 2021 Sep
477 22];61(3):539–42. Available from:
478 https://pubmed.ncbi.nlm.nih.gov/22357727/
479 16. Simon C, Frati F, Beckenbach A, Crespi B, Liu H, Flook P. Evolution, weighting,
480 and phylogenetic utility of mitochondrial gene sequences and a compilation of
481 conserved polymerase chain reaction primers. Ann Entomol Soc Am [Internet].
482 1994 Nov 1 [cited 2021 Sep 22];87(6):651–701. Available from:
483 https://academic.oup.com/aesa/article/87/6/651/19291
484 17. Nadal-Jimenez P, Valente RS. Preparation of polygalacturonic acid (PGA) to
485 study virulence in Erwinia/Pectobacterium [Internet]. protocols.io. 2021.
486 Available from: https://doi.org/10.17504/protocols.io.bydyps7w
487 18. Jimenez PN, Koch G, Papaioannou E, Wahjudi M, Krzeslak J, Coenye T, et al.
488 Role of PvdQ in Pseudomonas aeruginosa virulence under iron-limiting
489 conditions. Microbiology. 2010;156(1):49–59.
490 19. Bolger AM, Lohse M, Usadel B. Trimmomatic: A flexible trimmer for Illumina
491 sequence data. Bioinformatics [Internet]. 2014 Aug 1 [cited 2021 Sep
492 22];30(15):2114–20. Available from:
493 https://academic.oup.com/bioinformatics/article/30/15/2114/2390096
494 20. Wick RR, Judd LM, Gorrie CL, Holt KE. Unicycler: Resolving bacterial genome
495 assemblies from short and long sequencing reads. PLoS Comput Biol
496 [Internet]. 2017 Jun 1 [cited 2021 Sep 22];13(6):e1005595. Available from:
497 https://journals.plos.org/ploscompbiol/article?id=10.1371/journal.pcbi.10055
498 95

21. Tatusova T, Dicuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, et al. NCBI prokaryotic genome annotation pipeline. Nucleic Acids Res. 2016 Aug 19;44(14):6614–24.
22. Kanehisa M, Goto S. KEGG: Kyoto Encyclopedia of Genes and Genomes. Nucleic Acids Res [Internet]. 2000 Jan 1 [cited 2021 Sep 22];28(1):27–30. Available from: /pmc/articles/PMC102409/
23. Blin K, Shaw S, Kloosterman AM, Charlop-Powers Z, Van Wezel GP, Medema MH, et al. AntiSMASH 6.0: Improving cluster detection and comparison capabilities. Nucleic Acids Res. 2021 Jul 2;49(W1):W29–35.
24. Arndt D, Grant JR, Marcu A, Sajed T, Pon A, Liang Y, et al. PHASTER: a better, faster version of the PHAST phage search tool. Nucleic Acids Res [Internet]. 2016 Jul 8 [cited 2021 Sep 22];44(W1):W16–21. Available from: https://www.readcube.com/articles/10.1093%2Fnar%2Fgkw387
25. Emms DM, Kelly S. OrthoFinder: solving fundamental biases in whole genome comparisons dramatically improves orthogroup inference accuracy. Genome Biol [Internet]. 2015 Aug 6 [cited 2021 Sep 22];16(1):1–14. Available from: https://genomebiology.biomedcentral.com/articles/10.1186/s13059-015-0721-2
26. Ortori CA, Halliday N, Cámara M, Williams P, Barrett DA. LC-MS/MS quantitative analysis of quorum sensing signal molecules. Methods Mol Biol [Internet]. 2014 [cited 2021 Sep 22];1149:255–70. Available from: https://pubmed.ncbi.nlm.nih.gov/24818911/
27. Galetto L, Marzachì C, Demichelis S, Bosco D. Host Plant Determines the Phytoplasma Transmission Competence of Empoasca decipiens (Hemiptera: Cicadellidae). J Econ Entomol. 2011;104(2):360–6.
28. Fathi SAA, Nouri-Ganbalani G, Rafiee-Dastjerdi H. Life cycle parameters of Empoasca decipiens Paoli (Hom.: Cicadellidae) on four potato cultivars (Solarium tuberosum L.) in Iran. J Entomol. 2009;6(2):96–101.
29. Barnard AML, Salmond GPC. Quorum sensing in Erwinia species. Anal Bioanal Chem. 2007;387(2):415–23.

533 **Table 1: Utilization of carbon sources for Growth by *S. purcellii* in the presence and**
534 **absence of PGA, with comparison to *Pectobacterium carotovorum* LMG 2404^T. +++:**
535 **strong growth; ++: medium growth; +: weak growth; and -: no growth.**
536

	S. purcelli	***S. purcelli* + PGA**	***P. carotovorum.* LMG 2404**	***P. carotovorum* LMG 2404 + PGA**
Dextrin	-	-	+++	+++
D-maltose	-	-	+++	+++
D-Trehalose	-	-	+++	+++
D-Cellobiose	-	-	+++	+++
Gentiobiose	-	-	+++	+++
Sucrose	-	-	+++	+++
D-Turanose	-	-	+++	+++
Stachyose	-	-	+++	+++
D-raffinose	-	-	+++	+++
α-D-lactose	-	-	+++	+++
D-melibiose	-	-	+++	+++
B-Methyl-D-Glucoside	-	-	+++	+++
D-Salicin	-	-	+++	+++
N-Acetyl-D-Glucosamine	+++	+++	+++	+++
N-Acetyl-B-D-Mannosamine	-	-	+++	+++
N-Acetyl-D-Galactosamine	-	-	+++	+++
N-Acetyl Neuraminic Acid	-	-	+++	+++
α-D-Glucose	+++	+++	+++	+++
D-mannose	+++	+++	+++	+++
D-Fructose	+++	+++	+++	+++
D-Galactose	-	-	+++	+++
3-Methyl Glucose	-	-	+++	+++
D-Fucose	-	-	+++	+++
L-Fucose	-	-	+++	+++
L-Rhamnose	+++	+	+++	+++
Inosine	-	-	+++	+++
D-sorbitol	-	-	+++	+++
D-mannitol	-	-	+++	+++
D-Arabitol	-	-	+++	+++
myo-inositol	-	-	+++	+++
Glycerol	+++	+++	+++	+++
D-Glucose-6-PO4	-	-	+++	+++
D-Fructose-6-PO4	-	-	+++	+++
D-Aspartic Acid	-	-	+++	+++
D-Serine	-	-	+++	+++
Gelatin	-	-	+++	+++
Glycyl-L-Proline	-	-	+++	+++

L-Alanine	-	-	+++	+++
L-Arginine	-	-	+++	+++
L-Aspartic Acid	-	-	+++	+++
L-Glutamic Acid	-	-	+++	+++
L-Histidine	-	-	-	-
L-Pyroglutamic Acid	-	-	-	-
L-Serine	-	-	+++	+++
Pectin	-	-	+++	+++
D-Galacturonic acid	-	-	+++	+++
L-Galactonic Acid Lactone	-	-	+++	+++
D-Gluconic Acid	-	-	+++	+++
D-Glucuronic Acid	-	-	+++	+++
Glucuronamide	-	-	+++	+++
Mucic Acid	-	-	+++	+++
Quinic Acid	-	-	-	-
D-Saccharic Acid	-	-	+++	+++
ρ-hydroxy phenyl acetic acid	-	-	-	-
Methyl Pyruvate	+	+	+++	+++
D-Lactic Acid Methyl Ester	-	-	-	-
L-Lactic acid	-	-	-	-
Citric Acid	-	-	+++	+++
α-Keto-Glutaric-Acid	-	-	-	-
D-Malic Acid	-	-	-	-
L-Malic Acid	-	-	+++	+++
Bromo-Succinic Acid	-	-	+++	+++
Tween-40	-	-	-	+++
Gamma-Amino-Butyric acid	-	-	-	+++
α-Hydroxy-Butyric Acid	-	-	-	+++
B-Hydroxy-D-L-Butyric Acid	-	-	-	+++
α-Keto-Butyric-Acid	-	++	-	+++
Acetoacetic Acid	-	-	+	+++
Propionic Acid	-	-	-	-
Acetic Acid	+	+	+++	+++
Formic Acid	-	-	+++	+++

539 Table 2: Impact of environmental and xenobiotic stress conditions on *S. purcellii*
540 growth on Biolog III plates compared to *P. carotovorum,* in the presence/absence of
541 PGA. +++: maintains full growth under condition stated, ++: medium growth; +: weak
542 growth; and - :no growth under condition stated.
543

	S. purcelli	***S. purcelli* + PGA**	***P. carotovorum* LMG 2404^T**	***P. carotovorum* LMG 2404^T + PGA**
pH 6	+++	+++	+++	+++
pH 5	-	-	-	-
1% NaCl	+++	+++	+++	+++
4% NaCl	+	+	+++	+++
8% NaCl	-	-	-	-
1% Sodium lactate	+++	+++	+++	+++
Fusidic acid	+++	+++	+++	+++
D-Serine	-	+++	-	-
Troleandomycin	+++	+++	+++	+++
Rifamycin SV	+++	+++	+++	+++
Minocycline	-	+++	-	-
Lincomycin	++	++	+++	+++
Guanidine HCl	++	++	+++	+++
Niaproof 4	++	++	+++	+++
Vancomycin	+++	+++	+++	+++
Tetrazolium Violet	++	++	+++	+++
Tetrazolium Blue	+++	+++	+++	+++
Nalidixic Acid	++	++	-	-
Lithium Chloride	+++	+++	+++	+++
Potassium Tellurite	+++	+++	-	-
Aztreonam	+++	+++	-	-
Sodium Butyrate	++	++	+++	+++
Sodium Bromate	-	-	-	-

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547 **Table 3.** Genome features of the *Symbiopectobacterium purcellii* strain SyEd1
548 isolated from the leafhopper *Empoasca decipiens*.
549

Chromosome size (bp)	4,942,431
Plasmids	no
GC contents (%)	52.5
Number of predicted CDS	4,494
Number of pseudogenes	312
Average CDS length (bp)	948
Coding density (%)	86.2
Number of rRNA operons	7 (5S, 16S, 23S)
Number of tRNAs	76

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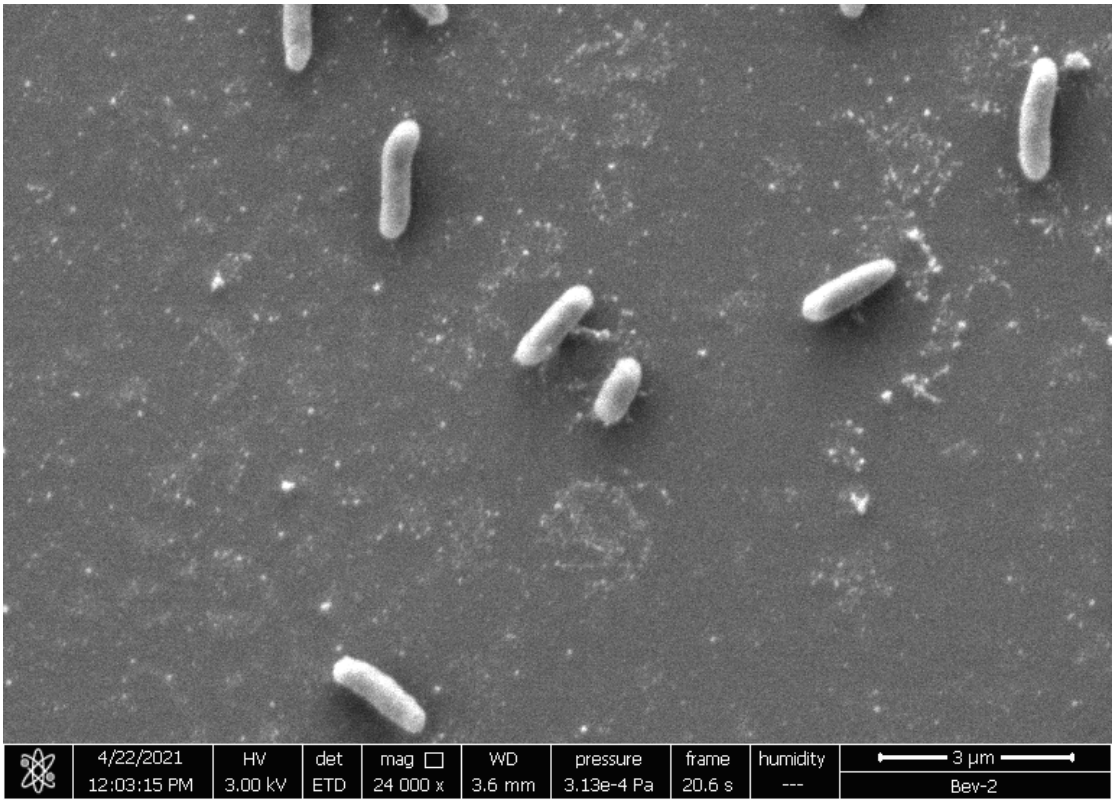
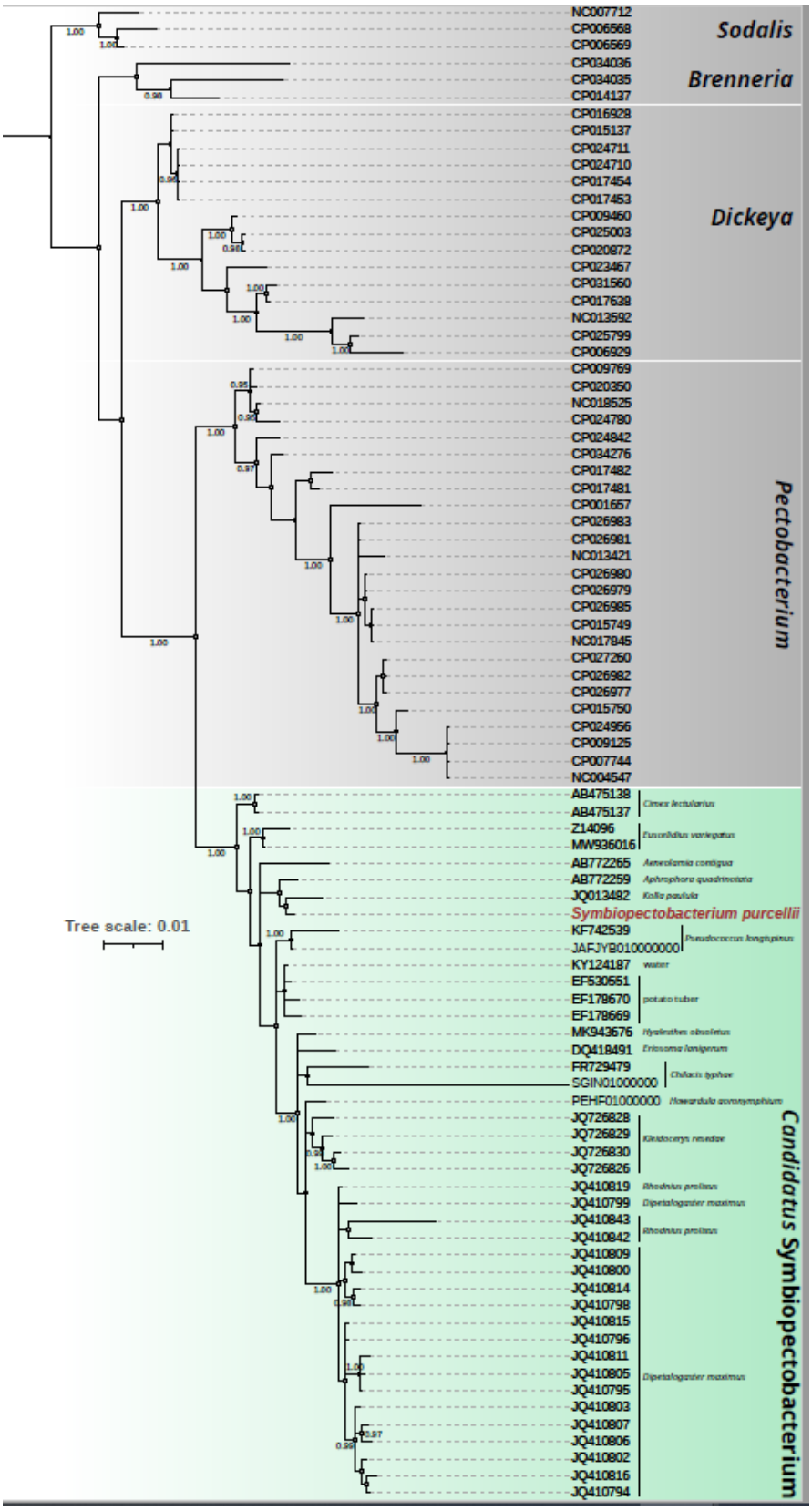
551

Figure 1: SEM of *S. purcellii* SyEd1.



556 Figure 2: Phylogenetic affiliation of the 16S rRNA of *S. purcellii* compared to other
557 strains, as estimated with Mr Bayes. Numbers on nodes represent posterior
558 probability.



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560 Figure 3: Virulence assay in potatoes.

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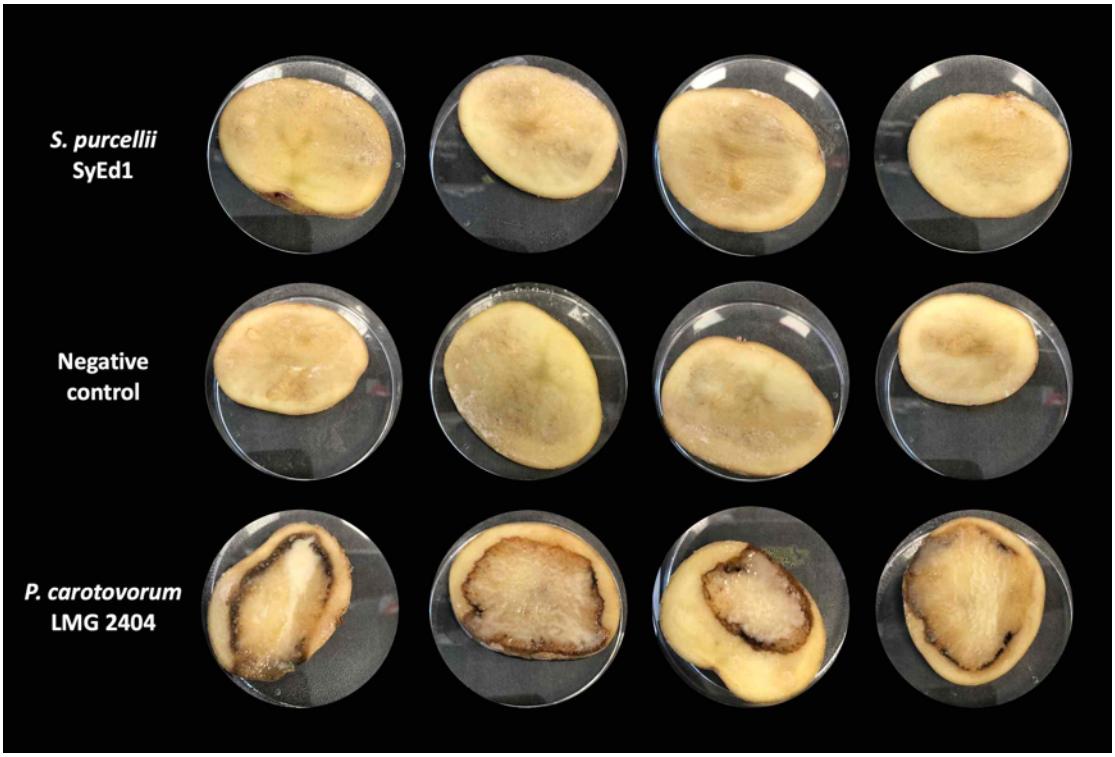

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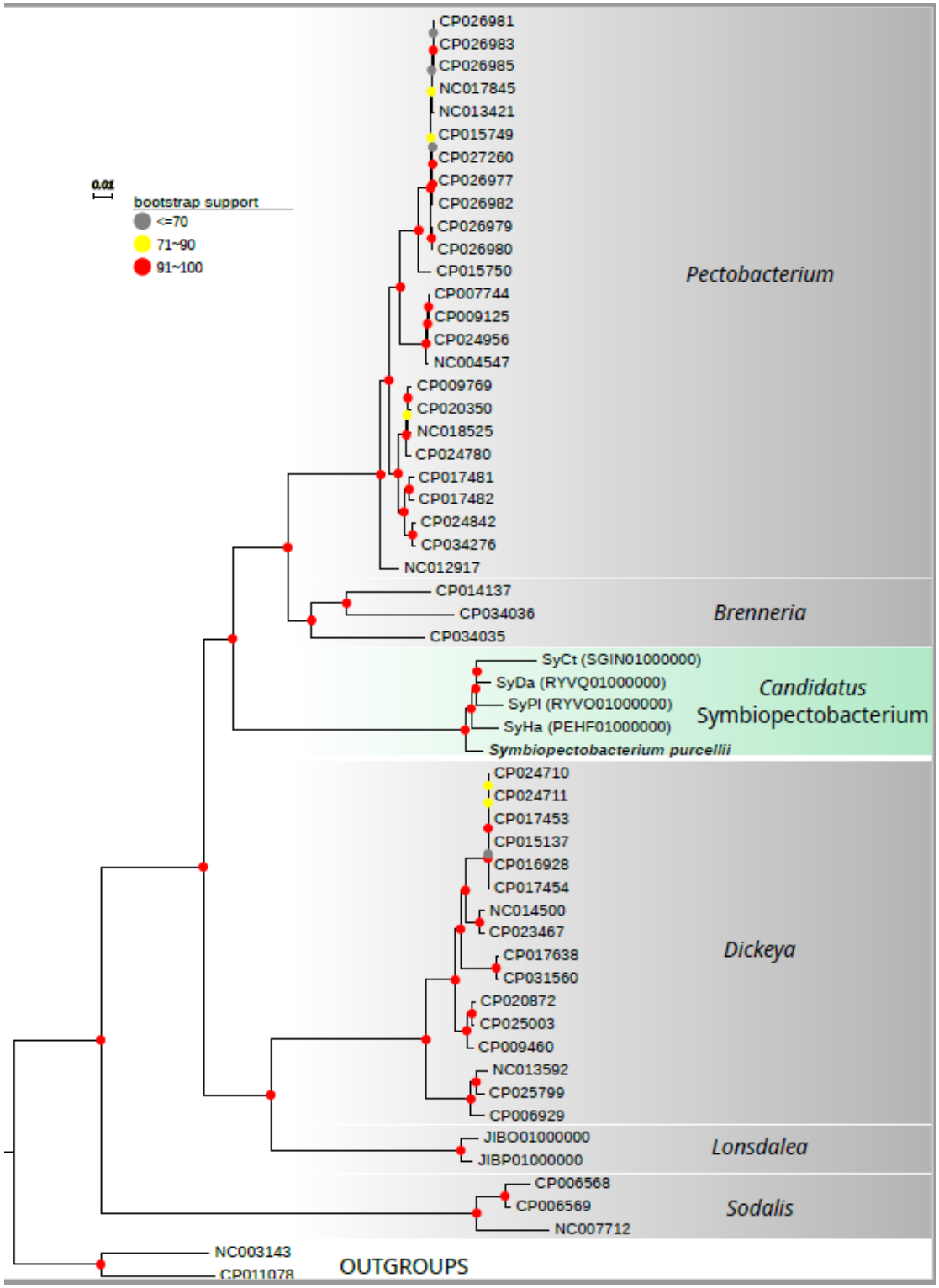
Figure 4: Affiliation of *S. purcellii* with other bacteria as estimated using IQTREE based on 527 shared single copy orthologues. Coloured dots on nodes represent bootstrap support.



570 Figure 5: LC-MS/MS of an extraction of *S. purcellii* SyEd1 grown in BHI medium (A), 3-
571 OH-C10-HSL standard (B) and negative control using a sterile BHI medium extract (C).
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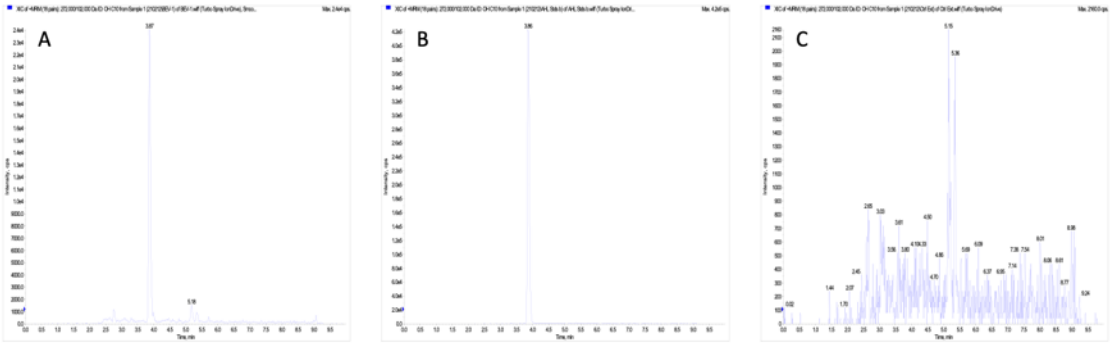

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