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1 **Running Head:** Pink-pigmented *Clavibacter michiganensis*

2

3 **Pink-pigmented variant of *Clavibacter michiganensis* expands phenotypic range of tomato**
4 **bacterial canker pathogen**

5

6 Malihe Haghverdi ^{1,2}, S. Mohsen Taghavi ¹, Sadegh Zarei ³, Hamzeh Mafakheri ¹, Hamid Abachi ²,
7 Martial Briand ⁴, Geraldine Taghouti ⁴, Perrine Portier ⁴, Marie-Agnes Jacques ⁴, Ebrahim
8 Osdaghi ^{2*}

9

10 1: Department of Plant Protection, School of Agriculture, Shiraz University, Shiraz 71441-65186,
11 Iran

12 2: Department of Plant Protection, College of Agriculture, University of Tehran, Karaj 31587-
13 77871, Iran

14 3: Department of Plant Protection, College of Agriculture, Isfahan University of Technology,
15 Isfahan 84156-83111, Iran

16 4: Univ Angers, Institut Agro, INRAE, IRHS, SFR QUASAV, CIRM-CFBP, F-49000 Angers, France

17

18 *: Corresponding author: eosdaghi@ut.ac.ir

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24 **Abstract**

25 Bacterial canker of tomato caused by the Gram-positive corynebacterial species *Clavibacter*
26 *michiganensis* is one of the most destructive seed-borne diseases in both open air and
27 greenhouse tomatoes. The pathogen is a regulated agent in all tomato-producing countries as
28 translocation of infected tomato materials transports the bacterium into new areas. *Clavibacter*
29 *michiganensis* is generally known to have yellow-pigmented colonies on culture media, which is
30 a key differentiative phenotypic feature in standard diagnostic guidelines. During 2020 and
31 2021, pink-pigmented corynebacterial strains were isolated from tomato seeds (cv. Sun
32 6189F1) and plants showing severe canker symptoms in Southern Iran. The six pink-pigmented
33 strains were pathogenic on tomato and pepper seedlings under greenhouse conditions, and
34 gave positive results with *C. michiganensis*-specific primers pairs described in the literature.
35 Phylogenomics and DNA similarity calculations showed that the pink-pigmented strains were
36 highly similar to the authentic yellow-pigmented members of the pathogen. Thus, they were
37 identified as a new phenotypic variant of tomato bacterial canker pathogen. Whole genome
38 screenings accomplished with PCR-based assays showed that the pink strains contain all
39 pathogenicity determinant genes described in *C. michiganensis*. Further, orthologous gene
40 clusters in the pink-pigmented strains were more similar to the pathogenic members of *C.*
41 *michiganensis* than to those of non-pathogenic tomato-associated *Clavibacter* species. Results
42 obtained in this study demonstrate the emergence of a new pink-pigmented variant of *C.*
43 *michiganensis* and highlight the importance of colony pigmentation/morphology in culture-
44 based detection of the bacterium. The need for updating diagnostic guidelines on the colony
45 variants of the pathogen is further discussed.

46

47

48 **Keywords:** Coryneform bacteria, Emerging pathogen, *Microbacteriaceae*, *Solanum lycopersicum*

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50

51 Introduction

52 Seed-borne plant pathogens are infectious microorganisms that are carried into the next
53 generation of their host plant or into new geographic areas via infected seeds (Gitaitis and
54 Walcott 2007). Most seed-borne plant pathogens are subjected to international quarantine
55 restrictions and inspections. Depending on their geographic distribution and economic impact,
56 the pathogens are included in various quarantine lists *e.g.*, A1 and A2 developed by the
57 European and Mediterranean Plant Protection Organization (EPPO). Development of universally
58 approved and constant methods of evaluating seed health would facilitate the global seed
59 trade and contribute to global food security (Madden et al 2007). Standard diagnostic
60 guidelines are available for all seed-borne quarantine plant pathogens under the authority of
61 International Seed Testing Association (ISTA), European Food Safety Authority (EFSA), and
62 EPPO. All the latter organizations are involved in the development of internationally approved
63 rules for sampling and testing seed health and quality.

64 According to the FAOSTAT (2024), tomato (*Solanum lycopersicum*) is the world's most popular
65 vegetable with annual production of >186 million tons in 2022. Tomatoes represent >17% of all
66 vegetables produced, followed by onions, cucumbers and cabbages. Tomato bacterial canker,
67 caused by the Gram-positive xylem-dwelling bacterium *Clavibacter michiganensis* is one of the
68 most destructive diseases of this crop in both open air fields and protected greenhouses (Jahr
69 et al. 1999; Kawaguchi et al. 2010; De Leon et al. 2011). Traditionally, tomato bacterial canker
70 pathogen was known as *Clavibacter michiganensis* subsp. *michiganensis* as one of the five
71 subspecies within the species (Davis et al., 1984). However, based on whole genome sequence
72 analyses, Li et al (2018) transferred all subspecies of *C. michiganensis sensu lato* into several
73 standalone species where *C. michiganensis* subsp. *michiganensis* was changed to *C.*
74 *michiganensis sensu stricto*. Thus otherwise mentioned, hereafter *C. michiganensis* will be used
75 to refer to the tomato pathogen in this study. The pathogen is seed-borne where infected seeds
76 and latently infected transplants are the main source of inoculum for long-distance
77 dissemination and the consequent disease outbreaks (Gleason et al. 1993; De Leon et al. 2011;
78 Tancos et al. 2013). Infected seedlings are often asymptomatic, leading to undetected spread of
79 the pathogen during cultural practices in nurseries (Chang et al., 1991; Gitaitis et al., 1991). As a

80 result of severe infection by this pathogen, yield losses of up to 84% have been reported in
81 tomato fields (Poysa, 1993; Hausbeck et al., 2000; Haghverdi 2022).

82 According to the EPPO and EFSA diagnostic guidelines (EFSA 2014; EPPO 2016), *C.*
83 *michiganensis* possesses slow-growing, shining yellow-pigmented smooth round colonies with
84 entire margins on basic or semi-selective culture media *i.e.*, yeast-extract peptone glucose agar
85 (YPGA), yeast extract-dextrose-calcium carbonate (YDC) agar, and bacterial canker of tomato
86 (BCT) (Ftayeh et al 2011). Thus, in the culture-based detection assays, after streaking the cell
87 suspension of suspected bacterial strains on one of the above media, the culture plates should
88 be examined for growth of yellow mucoid colonies 72-96 h post incubation at 25-27 °C. Until
89 the beginning of the genomics era, it has been thought that non-yellow variants (*i.e.*, white,
90 pink, red, and orange) of *C. michiganensis* do also occur on tomato (Hayward and Waterson,
91 1964). However, DNA sequence-based phylogenetic analyses showed that non-yellow-
92 pigmented strains could not be considered authentic members of tomato bacterial canker
93 pathogen *C. michiganensis* (Jacques et al 2012). Rather, they were generally non-pathogenic on
94 tomatoes and belong to several hypothetical novel species within the genus (Thapa et al 2017;
95 Osdaghi et al., 2020). Further comparative genomics and phylogenomics analyses have also
96 confirmed that tomato-pathogenic clade of *Clavibacter* sp. includes only yellow-pigmented
97 strains. Members of the latter clade are known for carrying two plasmids pCM1 (≈27 kb) and
98 pCM2 (≈70 kb) and a 129-kb *chp/tomA* pathogenicity island in their chromosome (Eichenlaub
99 and Gartemann 2011). For instance, the non-pathogenic *Clavibacter* strain LMG 26808 isolated
100 from tomato seed did not contain plasmids pCM1 and pCM2, or the majority of important
101 virulence factors described in pathogenic *C. michiganensis* strains (Załuga et al 2014). In some
102 cases, tomato-associated non-pathogenic *Clavibacter* spp. strains were designated as
103 standalone species *e.g.*, *Clavibacter californiensis* (Yasuhara-Bell and Alvarez 2015), *Clavibacter*
104 *phaseoli* (formerly *Clavibacter michiganensis* subsp. *chilensis*; González and Trapiello 2014;
105 Arizala et al. 2022), and *Clavibacter lycopersici* (Osdaghi et al. 2023). Almost all records of the *C.*
106 *michiganensis* epidemics in the past two decades reported only yellow-pigmented strains while
107 tomato-pathogenic non-yellow variants of the bacterium were almost entirely missing in the
108 literature (Basim et al., 2018; Osdaghi et al., 2018; Ansari et al., 2019).

109 Since 2015, local plant protection agencies in Southern Iran have been witnessing rapidly
110 spreading occurrence of bacterial canker disease across major tomato growing areas leading to
111 severe yield losses (Osdaghi et al 2018). *Clavibacter michiganensis* has originally been reported
112 in Northwestern Iran in 1993 (Mazarei et al 1993), while no serious epidemics of the disease
113 were recorded in the country within the subsequent two decades. Until 2016, geographic
114 distribution of the pathogen was mostly restricted to the northern and northwestern provinces
115 of the country that are characterized with cooler temperature and lower extent of tomato
116 cultivation compared to the southern provinces (Nazari et al 2007). A nationwide
117 comprehensive field surveys and samplings in 2016 and 2017 revealed the occurrence of the
118 disease in the hub of tomato production in Southern Iran *i.e.*, Fars and Bushehr provinces
119 (Ansari et al 2019). In 2020 and 2021, the disease was established in all southern provinces of
120 the country with such a destructive severity where dozens of tomato fields were either
121 abandoned or eradicated before fruiting due to the substantial crop losses and mortality. In
122 most cases, tomato seeds and transplants have previously been inspected for infection with *C.*
123 *michiganensis* in local plant health laboratories using culture-based detection methods
124 according to the EFSA, EPPO and ISTA protocols (EPPO 2016). However, the pathogen could
125 escape the standard detection methods leading to the occurrence of the disease in the areas
126 with no history of the disease. Hence, we have initiated a comprehensive survey and sampling
127 to shed light on the dark behind the recent outbreaks of the disease in the area. As a result, we
128 have unexpectedly isolated several pink-pigmented Gram-positive coryneform bacterial strains
129 from both tomato seeds and symptomatic plants. The pink strains were preliminarily identified
130 as members of *Clavibacter* spp. and shown to be pathogenic on tomato plants. This is contrary
131 to established diagnostic EFSA, EPPO and ISTA guidelines, in which pathogenic *Clavibacter* spp.
132 are described as only having a yellow pigmentation. The international seed health initiative
133 (ISHI; International Seed Federation) also published a protocol for detection of *C. michiganensis*
134 in tomato seeds where yellow-pigmentation was specified as differentiative phenotypic feature
135 of the pathogen.

136 These observations raised a question whether the current criteria for detection of *C.*
137 *michiganensis* are broad enough to cover all phenotypic range of the pathogen. On the other

138 hand, biological characteristics, virulence repertoires, and phylogenomic features of the new
139 pink-pigmented variant of the pathogen remain undetermined. Thus, the main objectives of
140 this study were: i) to evaluate phenotypic and genetic features of the new pink-pigmented
141 strains of *Clavibacter* sp. isolated from tomato; ii) to assess the pathogenicity and host range of
142 the pink-pigmented strains on different hosts in comparison to the previously-reported yellow
143 strains of the pathogen, and iii) to determine genetic features and phylogenetic position of the
144 pink-pigmented strains via whole genome sequence-based comparative genomics and
145 phylogenomics.

146

147 **Materials and methods**

148 **Field surveys, sampling and bacterial isolation**

149 Upon the widespread occurrence of tomato bacterial canker in Southern Iran (Ansari et al
150 2019), comprehensive field surveys and inspections were carried out during two consecutive
151 years (May 2020 to September 2021) across tomato-growing areas and greenhouses in Central,
152 Southern, Southwestern, and Western Iran. The surveyed areas are described in Table S1. Dates
153 of sampling within a given year varied across the areas due to different climatic conditions and
154 agronomic practices *e.g.*, planting dates. Tomato seeds lots and transplants/plants with
155 suspected bacterial canker symptoms showing cracks (canker) and wilt on stems, shoots, and
156 petioles were collected from all surveyed areas and brought to the laboratory. Isolation of the
157 putative bacterial pathogens from seed lots and plant specimens was carried out on YPGA
158 medium using the procedure described in EPPO, EFSA, and ISTA guidelines (EFSA 2014; EPPO
159 2016). In brief, symptomatic pieces of each sample were cut using a sterile scalpel and surface
160 sterilized by dipping into 0.5% sodium hypochlorite for 20 s followed by two to three rinses in
161 sterile distilled water (SDW), and macerated in 10-20 mL of SDW. A loopful of the resulting
162 suspension was streaked onto YPGA medium, the plates were incubated at 25-27 °C for 72-96
163 h, and the resulting bacterial colonies were purified by repetitive streaking on fresh YPGA
164 plates. For seed samples, approximately 50-100 seeds were randomly tested from each seed
165 lot. To remove the protective chemicals or antimicrobial agents *i.e.*, Thiram, Mefenoxam, and

166 Fludioxonil, the seeds were dipped into SDW for 30 s, rinsed three times, transferred into a
167 sterile mortar containing 5mL SDW, and ground with a sterile pestle. The resulting suspensions
168 were used for bacterial isolation as described above. Purified strains were re-suspended in
169 SDW, and stored at 4 °C for further use. Sterile 15% glycerol was used for their long-term
170 storage at -70 °C.

171

172 **Phenotypic characterization of the strains**

173 Besides yellow-pigmented *C. michiganensis* strains which have frequently been isolated from
174 symptomatic tomato tissues and seeds, pink-pigmented Gram-positive, domed circular shining
175 colonies were also isolated from plant tissues making the speculation that an atypical variant of
176 the bacterial canker pathogen is responsible for infection of the plant specimens. Thus, all
177 purified bacterial strains (Table 1; S1) were subjected to standard phenotypic tests according to
178 Schaad et al (2001). Gram reaction, aerobic/anaerobic growth (O/F), oxidase, catalase, and
179 urease activity, levan production, starch, aesculin, and tween 80 hydrolysis, as well as colony
180 characteristics on YDC medium were determined (Schaad et al. 2001). Type strains of
181 *C. michiganensis* LMG 7333^T and *C. lycopersici* CFBP 8615^T were used as controls. All phenotypic
182 tests were repeated twice.

183

184 **Pathogenicity and host range assays**

185 The bacterial strains *i.e.*, yellow- and pink-pigments variants (Table 1) were evaluated for their
186 pathogenicity on the host of isolation (tomato cv. Sun 6189F1). Further, to determine the host
187 range of the pink-pigment strains isolated from tomato, their pathogenicity was evaluated on a
188 set of selected solanaceous plants *i.e.*, pepper (cv. Mert F1), eggplant (cv. Shadow F1),
189 nightshade (*Solanum nigrum*), and potato (cv. Agria). Inoculum preparation, plant inoculation,
190 and incubation conditions were the same as described in EPPO, EFSA, and ISTA guidelines (EFSA
191 2014; EPPO 2016). In brief, plants were grown in 20-cm diameter pots (3 plants/pot and 3
192 pots/species). The plants were inoculated at 10-15 days post emergence, when they had at

193 least three fully-expanded leaves (approximately 15 cm of height). Inoculation was made by
194 inserting a sterile dissecting needle dipped into a freshly prepared bacterial suspension on
195 YPGA medium (1×10^8 CFU/ml in SDW) through the first internode of each plant. All inoculated
196 plants were maintained under greenhouse conditions ($27 \pm 3^\circ\text{C}$ and 14 h natural lights) up to 30
197 days post-inoculation (dpi), and periodically monitored for the appearance of disease
198 symptoms. The positive control plants were inoculated using the type strain of *C. michiganensis*
199 LMG 7333^T, while type strain of the non-pathogenic peach-pigmented species *C.*
200 *lycopersici* isolated from tomato phyllosphere and SDW were used in the same manner as
201 negative controls. Koch's postulates were accomplished by re-isolating the inoculated strains on
202 YPGA medium from plants showing symptoms. Confirmation of the re-isolated bacteria was
203 made using colony morphology on YDC medium, Gram reaction, as well as *C. michiganensis*-
204 specific PCR primers CMM5/CMM6 (Dreier et al., 1995; Table S2).

205

206 **Molecular-phylogenetic analyses**

207 **Genus- and species-specific PCRs**

208 All bacterial strains were tested using the generic primers for *Clavibacter* spp., and species-
209 specific primers for *C. michiganensis* (Lee et al. 1997; Pastrik & Rainey, 1999; Dreier et al., 1995;
210 Sousa-Santos et al., 1997). Culture preparation and bacterial DNA extraction were carried out
211 using the procedure described previously (EPPO 2016). For PCR reactions, Universal PCR Kit,
212 Ampliqon® Taq DNA Polymerase Master Mix Red (Ampliqon A/S, Odense, Denmark) was
213 applied according to the manufacturer's recommendations. For each strain, a 20 μl PCR
214 including 50 ng total DNA and 1 μl of each primer ($10 \text{ pmol} \times \mu\text{l}^{-1}$) were used. The annealing
215 temperatures and the corresponding primer sequences are described in Table S2.

216

217 **Phylogenetic analysis**

218 To provide a precise and reliable insight into taxonomic status and phylogenetic position of the
219 pink-pigment strains isolated in this study they were subjected to MLSA along with

220 representative yellow-pigmented strains isolated in the same area/plant using the sequences of
221 five housekeeping genes *i.e.*, *atpD*, *gyrB*, *ppk*, *recA* and *rpoB* (Jacques et al. 2012). These
222 selected genes were shown to be reliable enough to resolve phylogenetic relationships among
223 the pathogenic and non-pathogenic tomato-associated *Clavibacter* strains (Jacques et al. 2012).
224 The sequences and annealing temperature of primer pairs are shown in Table S2, while the PCR
225 procedure was the same as described above. The PCR products were sent to Bioneer
226 Corporation (<http://www.Bioneer.com>) to be sequenced via Sanger sequencing technology. The
227 resulted sequences were analyzed with BLASTn (<http://blast.ncbi.nih.gov/>), and
228 phylogenetically compared to those of *Clavibacter* spp. strains retrieved from the GenBank
229 database (Ansari et al 2019). The sequences were aligned with ClustalW (Larkin *et al.*, 2007)
230 using MEGA 7.0 software (Kumar *et al.*, 2016), and concatenated following the alphabetic order
231 of the genes. Phylogenetic trees were constructed using both individual gene sequences and
232 sequences of the concatenated dataset of the five genes via Maximum Likelihood method with
233 MEGA 7.0. The model of evolution for Maximum Likelihood analysis was determined using
234 Modeltest tab in MEGA 7.0 with 1000 bootstrap replicates (Hall, 2011).

235

236 **Whole genome sequencing and comparative genomics**

237 Specific PCRs and phylogenetic analyses revealed that the pink-pigmented bacterial strains
238 isolated from symptomatic tomato plant were members of *C. michiganensis*. Hence, the
239 representative pink-pigmented strains M34 and M98 as well as the yellow-pigmented strain
240 M26 were subjected to whole genome sequencing to shed light on the genomic features and
241 repertoires of virulence factors of these two variants. Culture preparation, DNA extraction, and
242 genome sequencing were the same as described previously (Osdaghi et al 2018). DNAs were
243 sequenced via DNBseq and assembled using Unicycler v0.4.8. Genome annotation was
244 performed using the GeneMarkS+ (v 4.6) suite implemented in the NCBI Prokaryotic Genome
245 Annotation Pipeline with default settings. Total number of protein-coding genes, RNA genes,
246 and pseudogenes were determined for all the genomes. Comparative genomics tools were used
247 to analyze whole genome sequences of the pink-pigmented strains M34 and M98 as detailed in

248 Osdaghi et al (2020). BLASTn-based average nucleotide identity (ANi) and digital DNA-DNA
249 hybridization (dDDH) indexes were calculated among the strains sequenced in this study and all
250 validly described *Clavibacter* species. ANI values were calculated using three different
251 algorithms i.e., JSpeciesWS, ANI calculator, and OrthoANIu (Richter et al 2016; Rodriguez-R et al
252 2016; Yoon et al 2017). Additionally, Genome-to-Genome Distance Calculator online service
253 (<http://ggdc.dsmz.de/distcalc2.php>) was used to calculate digital DNA-DNA hybridization
254 (dDDH) value which infers to the genome-to-genome distances between pairs of genomes
255 based on the Genome Blast Distance Phylogeny (Meier-Kolthoff et al 2013). For whole-genome
256 sequence-based classification of the bacterial strains, the genome assemblies were used as
257 input for the pyANI v0.2.11 Python pipeline (Pritchard et al. 2016), with the ANIm parameter to
258 calculate pairwise distances using MUMmer (nucmer). The pipeline generates a distance matrix
259 and a double hierarchical clustered heatmap, in which a red color indicates ANI percent
260 identities above 95%. Genome wide comparisons and visualization of orthologous gene clusters
261 were performed via online pipeline OrthoVenn3 using the procedure described by the service
262 provider (Sun et al 2023).

263

264 **PCR-based and *in silico* detection of pathogenicity determinant genes**

265 In order to determine if genomic elements with significant contribution to pathogenicity of *C.*
266 *michiganensis* were present in the genomes of pink-pigmented strains, sequences of these
267 virulence genes - as summarized by Peritore-Galve et al (2021) - were retrieved from the
268 complete genome sequence of *C. michiganensis* NCPPB 382 (GenBank: AM711867.1). One-vs.-
269 one BLASTn/BLASTp search was implemented using the sequences of these genes (*i.e.*, a 129-kb
270 low G+C pathogenicity island which contains *chp* and *tomA* clusters; Peritore-Galve et al 2021)
271 against the whole genome sequences obtained in this study. Proteins with amino acid sequence
272 similarities higher than 50% and query coverage higher than 70% were considered homologs.
273 Complete genome sequence of *C. michiganensis* NCPPB 382 was used as control.

274 PCR-based detection of pathogenicity determinant genes was also performed to validate *in*
275 *silico* data. PCR tests were performed on both pink-pigmented strains and representatives of

276 yellow-pigmented strains. The primer pairs cel-578up/cel-2752low, pCRcel-593/pCRcel-1860
277 and PFC3/PFC5 specific for different domains of *ceIA* gene, primer pair Cmm-5/Cmm-6 specific
278 for the *pat-1* gene (Dreier et al., 1995; Jahr et al., 2000; Kleitman et al., 2008), and primer pairs
279 tomA-F/tomA-R, ppaA-F/ppaA-R, chpC-F/chpC-R, and chpG-F/chpG-R specific for *tomA*, *ppaA*,
280 *chpC* and *chpG* genes, respectively were used for detection of pathogenicity genes (Kleitman et
281 al., 2008). PCR conditions were the same as described above. Type strains of *C. michiganensis*
282 (LMG 7333^T) and non-pathogenic *C. lycopersici* CFBP 8615^T were used as positive and negative
283 controls, respectively. The annealing temperatures and the corresponding primer sequences
284 are described in Table S2.

285

286 **Data availability**

287 The sequenced nucleotides were deposited into the GenBank database under the following
288 accession numbers for partial gene sequences: *atpD*: OP271522 to OP271530; *gyrB*: OP271531
289 to OP271545; *ppk*: OP271546 to OP271554; *recA*: OP271555 to OP271567; *rpoB*: OP271568 to
290 OP271576. Furthermore, pure culture of the representative strains isolated in this study was
291 deposited in CIRM-CFBP culture collection (https://www6.inra.fr/cirm_eng/CFBP-Plant-Associated-Bacteria) and assigned accession numbers as follow: M26 = CFBP 9079, M34 = CFBP
292 9078, M98 = CFBP 9080, M93 = CFBP 9114, ML10 = CFBP 9115 and M126 = CFBP 9116. Whole
293 genome sequences of the three strains have also been deposited in the NCBI GenBank as
294 follows: JBBEFE0000000000 for M34 = CFBP 9078; JBBEFF0000000000 for M98 = CFBP 9080; and
295 JBBEFG0000000000 M26 = CFBP 9079.

297

298 **Results**

299 **Disease incidence and bacterial strains**

300 During 2020 and 2021, severe outbreaks of bacterial canker were observed in Southern Iran,
301 negatively affecting tomato industry in the area. In some cases, 100% crop destruction was
302 recorded in Fars Province where tomato plantings were abandoned in the mid-season due to

303 the bacterial canker epidemics. The most destructive epidemics were observed in Marvdasht
304 County (Fars Province) that is characterized by dry and warm environmental conditions during
305 transplanting season (mid-April to mid-May). Typical disease symptoms were observed on all
306 aerial plant parts including leaves, stems, and sepals in Fars, Hamedan, Isfahan, Hormozgan,
307 Kurdistan, Tehran, and Yazd provinces (Figure S1). With the exception of Fars Province, tomato
308 bacterial canker symptoms were recorded for the first time in the other mentioned provinces.

309 Bacterial canker symptoms were observed on the main stems and lateral branches of young
310 tomato plants 15-25 days post transplanting (Figure 1A). The canker symptoms increased in size
311 over time forming extended cracks (canker) especially in the areas characterized with high
312 temperature and water stress. With the increase in disease severity, interveinal chlorosis were
313 observed on the leaf surface leading to the necrotic areas towards leaf margins. Then, the
314 leaves wrinkled, the whole branch was wilted, and the phyllosphere of the plant was desiccated
315 (data not shown). Following systemic infections, the vascular tissues and pith of the infected
316 stems became dark yellow to brown. Disease symptoms were observed also on tomato sepals,
317 which consisted of deep cankers, leading to chlorotic tissue and drying of the sepals. On
318 severely infected plants, fruits remained small and ripened unevenly, or remained unripe
319 (Figure 1B). In the interior part of the ripening fruit, discoloration of the placenta from white to
320 yellow was observed (data not shown).

321 Gram-positive bacterial strains possessing domed round and shiny mucoid colonies were
322 isolated from the stems, leaves, and fruits of symptomatic tomato plants as well as tomato
323 seed lots provided from the marketplaces. Besides yellow-pigmented coryneform bacterial
324 strains with characteristic features of *C. michiganensis* on YPGA medium (Figure 2A), six strains
325 possessing pink-pigmented colonies were also isolated from symptomatic tomato tissues and
326 seeds 72-96 h post incubation on YPGA medium (Figure 2B). In some cases, yellow- and pink-
327 pigmented coryneform bacterial strains were simultaneously isolated from the same plant
328 specimen as shown in Figure 2B. Interestingly, both yellow- and pink-pigmented strains isolated
329 from the same plant sample found to be *C. michiganensis*. Both yellow- and pink-pigmented
330 strains had mucoid, domed, round colonies, with entire margins reaching 2-3 mm in diameter
331 after 72-96 days on YPGA (Figure 2C, D). Colony pigmentation was more noticeable on YDC

332 medium 48-72 hpi (Figure 2E, F). Regardless of colony pigmentation, no phenotypic differences
333 were observed between the yellow- and pink-pigmented bacterial strains in terms of
334 morphological and biochemical characteristics. Both yellow- and pink-pigmented strains were
335 positive for hydrolysis of aesculin and catalase, while they were negative for anaerobic growth,
336 oxidase, and Tween 80 hydrolysis. The list of bacterial strains isolated in this study, their host,
337 and the source of isolation are reported in Table S1.

338

339 **Pathogenicity and host range assay**

340 All yellow- and pink-pigmented strains induced wilting symptoms on the host of isolation where
341 inoculated tomato plants showed leaf wilting 12-15 dpi and were close to collapse at 21-28 dpi
342 (Figure 1C; Table S1). Both yellow- and pink-pigmented stains produced canker on stems of
343 pepper plants (Figure 1D), only yellow strains induced stem canker on eggplant 20 dpi, while
344 none of the strains showed wilting symptoms on pepper and eggplantm (Table S1). The
345 nightshade and potato plants inoculated with yellow- and pink-pigmented strains did not show
346 any symptoms. All plants inoculated with type strain of the non-pathogenic peach-pigmented
347 species *C. lycopersici* CFBP 8615^T remained healthy. Yellow- and pink-pigmented strains, similar
348 to those originally inoculated, were consistently re-isolated from symptomatic plants on YPGA
349 medium and their identity was confirmed using specific PCR primers PSA-4/PSA-R (Table S2).

350

351 **Molecular-phylogenetic analyses**

352 All yellow-/pink-pigmented bacterial strains were subjected to generic and specific PCRs for
353 precise identification. The primer pair CMR16F1/CMR16R1 specific for *Clavibacter* spp. directed
354 the amplification of the expected 1,425 bp fragment in all bacterial strains (Table S1). Further,
355 the *C. michiganensis*-specific primer pairs PSA-4/PSA-R (271 bp), CMM5/CMM6 (614 bp), and
356 CM3/CM4 (639 bp) amplified the expected DNA fragments in all yellow- and pink-pigmented
357 strains. The latter DNA fragments were not amplified using the corresponding species-specific
358 primer pairs in the non-pathogenic species *C. lycopersici* CFBP 8615^T (Table S1). Thus, the six

359 pink-pigmented strains isolated in this study were confirmed as a phenotypic variant of *C.*
360 *michiganensis*. In order to shed light on the phylogenetic position of the pink-pigmented strains
361 within *C. michiganensis* populations, all pink-pigmented strains (M30, M34, M93, M94, M98,
362 and M126) as well as three representative yellow-pigmented strains (M26, Ms65, and ML10)
363 isolated in the vicinity of the pink strains were subjected to phylogenetic analyses using the
364 sequences of five housekeeping genes *i.e.*, *atpD*, *gyrB*, *ppk*, *recA*, and *rpoB*. BLASTn search on
365 the GenBank database using the sequences of individual genes showed that all pink- and
366 yellow-pigmented strains had the highest sequence identity (99-100%) with the reference
367 strains of *C. michiganensis e.g.*, NCPPB 382 and LMG 7333^T. Maximum likelihood-based
368 phylogenetic tree constructed using the concatenated sequences of five housekeeping genes
369 confirmed the results obtained from the BLASTn analyses. All pink-pigmented strains were
370 clustered among *C. michiganensis* strains in a monophyletic clade separated from the other
371 nine previously described *Clavibacter* species (Figure 3A). Similar results were obtained when
372 the sequences of single housekeeping genes were subjected to phylogenetic analyses (data not
373 shown).

374 Based on the isolation source, pathogenicity features, and MLSA results, two pink-pigmented
375 strains M34 and M98 as well as the yellow-pigmented strain M26 were subjected to whole
376 genome sequencing. All three strains had the genome size of \approx 3,447 kb which was in the same
377 range with that of the reference strain of *C. michiganensis* NCPPB 382 (3,297 kb; NC_009480.1).
378 In all three strains sequenced in this study, DNA G+C contents were 72.50% which was similar
379 to that of the strain NCPPB 382 (72.66%). Average nucleotide identity (ANI) calculations using
380 pyANI and JSpeciesWS showed that the pink-pigmented strains M34 and M98 had 99.90 to
381 100.00% sequence identity with the type strain of *C. michiganensis* LMG 7333^T. In addition, ANI
382 value between the two pink-pigmented strains and type strains of other validly-described
383 *Clavibacter* species were <94.10% indicating that the strains M34 and M98 authentically belong
384 to *C. michiganensis*. Digital DNA-DNA hybridization indexes were also in congruence with ANI
385 where the pink-pigmented strains had 92.9% dDDH with the type strain of *C. michiganensis*
386 (Table S3). ANI-based pyANI matrix has also confirmed the inclusion of pink-pigmented strains
387 in *C. michiganensis* as shown in Figure 3B.

388

389 **Comparative genomics**

390 As for virulence-related gene repertoires of the pink-pigmented strains, all tested primer pairs
391 for detection of pathogenicity determinant genes directed the amplification of the expected
392 DNA fragments in all yellow- and pink-pigmented bacterial strains (Table 2). Furthermore, one-
393 vs.-one BLASTn-/BLASTp-based explorations using the complete genome sequence of *C.*
394 *michiganensis* NCPPB 382 as the reference genome vs. the individual strains sequenced in this
395 study revealed the presence of pathogenicity determinant genes/clusters in both pink-
396 pigmented strains M34 and M98 and the yellow-pigmented strain M26 evaluated in this study
397 (Table 2). All this evidence showed that the pink-pigmented bacterial strains isolated in this
398 study were close relatives of the authentic *C. michiganensis* strains while colony pigmentation is
399 the only differentiative feature between these two variants (Table 2).

400 Orthologous gene clusters were visualized using OrthoVenn3 online service through five-vs.-five
401 designations of the representative strains from different tomato-associated *Clavibacter*
402 lineages using whole genome protein sequences (Figure 4A). In taxonomically related species,
403 orthologous genes are clusters of genes that vertically descended from a single gene in their
404 last common ancestor. Comparative analysis of the organization of orthologous clusters in
405 phenotypic variants of plant pathogenic bacteria is important for understanding the rules of
406 genome structure and gene function driving those variations (Wang et al 2015). When the three
407 strains sequenced in this study were compared to the type strain (LMG 7333^T) and reference
408 strain of *C. michiganensis* NCPPB 382, the five strains shared 2,835 orthologous genes while the
409 two pink-pigmented strains shared only two unique genes. When type strains of the non-
410 pathogenic species *C. lycopersici* CFBP 8615^T and *C. californiensis* CFBP 8216^T were subjected to
411 the analyses, three pathogenic strains isolated in this study shared 476 orthologous genes most
412 of which possessing hypothetical role in the pathogenicity of the bacterium. Interestingly, no
413 unique orthologous gene was observed among the two pink-pigmented strains and the peach-
414 pigmented strain CFBP 8615^T. The orange-pigmented pepper-pathogenic strain of *C. capsici*
415 PF008^T showed four unique genes in comparison with the three strains sequenced in this study
416 and tomato-associated non-pathogenic strain of *C. phaseoli* CFBP 8217. However, no unique

417 gene was found between the pink-pigmented strains of *C. michiganensis* and the orange-
418 pigmented *C. capsici* PF008^T. On the other hand, whole genome nucleotide sequence-based
419 comparative genomics revealed that genome size of the three strains sequenced in this study
420 were in range (3,447 kbp) with the other tomato-associated *Clavibacter* strains (3,044 - 3,447
421 kbp). Further, the number of pseudo-genes was significantly higher in the non-pathogenic
422 strains compared to the pathogenic strains of *Clavibacter* regardless of the host of isolation and
423 colony pigmentation (Figure 4B).

424

425

426 **Discussion**

427 In this study, we describe biological characteristics, pathogenicity features, and genomic
428 repertoires of the emerging pink-pigmented variant of *C. michiganensis* the causal agent of
429 tomato bacterial canker in Southern Iran. The results indicate virulence of the pink-pigmented
430 strains on their host of isolation and probability of simultaneous occurrence of pink- and
431 yellow-pigmented variants not only in the same area but also on the same plant. Furthermore,
432 MLSA and whole genome sequence-based analyses showed that the pink- and yellow-
433 pigmented strains are phylogenetically closely related possessing the same set of pathogenicity-
434 determinant genes. Emergence of the pink-pigmented variant of *C. michiganensis* highlights a
435 concern from both epidemiological and phytosanitary perspectives, underlining the need for
436 updating diagnostic guidelines and seed test standards.

437 Corynebacterial plant pathogens *i.e.*, members of *Clavibacter* spp. and *Curtobacterium* spp. are
438 well known for their multicolored colony morphology. Until the beginning of the genomics era,
439 colony pigmentation was considered minor phenotypic features in corynebacteria where the
440 latter characteristics had insignificant impact on taxonomy and classification of these organisms
441 (Dye and Kemp 1977). For instance, *Curtobacterium* strains infecting dry beans were
442 characterized by their variability in colony pigmentation possessing orange, pink, purple, red,
443 and yellow phenotypes in either fluidal, mucoid, or dry form on culture media (Harveson and
444 Vidaver 2008; Osdaghi et al. 2016). However, all these colony variants were classified within a

445 single pathovar as *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* (Carlson and Vidaver
446 1982). On the other hand, the complex species *C. michiganensis sensu lato* has had five validly-
447 described subspecies with different colony pigmentation. Within the latter framework, dozens
448 of non-pathogenic multicolored strains originated from tomato plants were preliminarily
449 designated as *C. michiganensis* subsp. *michiganensis* in the absence of precise molecular-
450 phylogenetic analyses (Jacques et al. 2012).

451 During the past decade, DNA sequence-based investigations *i.e.*, MLSA, whole genome
452 sequence comparisons, and ANI-based similarity calculations resulted in a profound refinement
453 in the classification of multicolored corynebacterial plant pathogens (Osdaghi et al 2022, 2023).
454 Interestingly, recent changes in the classification of coryneform bacteria were in congruence
455 with their categorization on the basis of colony pigmentation. For instance, Jacques et al (2012)
456 showed that *C. michiganensis* subsp. *michiganensis* was a monophyletic clade consisting of
457 mostly yellow-pigmented strains being distinct from the other subspecies of *C. michiganensis*.
458 Furthermore, it has been shown that non-pathogenic non-yellow-pigmented *Clavibacter* strains
459 isolated from tomato were phylogenetically distinct from the pathogenic strains but belonged
460 to the *C. michiganensis sensu lato*. In 2018, Li and his colleagues reclassified the genus
461 *Clavibacter* where all former subspecies were elevated at the species level. Since then, the
462 tomato canker pathogen has been known as *C. michiganensis sensu stricto*, which included only
463 yellow-pigmented tomato-pathogenic strains. Later, Osdaghi et al (2020) showed that, in most
464 of the cases, non-pathogenic *Clavibacter* strains isolated from tomato were non-yellow-
465 pigmented, and could be designated as several standalone species. The non-pathogenic species
466 *C. californiensis* (orange-yellow) and *C. lycopersici* (peach-pigmented) both isolated from
467 tomato are indicative of the role of colony pigmentation in differentiation of *Clavibacter*
468 lineages (Yasuhara-Bell and Alvarez 2015; Osdaghi et al 2023). All these observations led to a
469 conviction that among *Clavibacter* strains originated from tomato, colony pigmentation is
470 correlated with their pathogenicity on tomato where only yellow-pigmented strains are
471 considered serious pathogens (Osdaghi et al 2023; Yasuhara-Bell and Alvarez 2015; Arizala et al
472 2022). Due to the latter presumption, in all quarantine guidelines issued by EFSA, EPPO and
473 ISTA it is recommended to pick up yellow pigmented colonies from the culture media after 72-

474 96 h incubation of plates (EFSA 2014; EPPO 2016). Our results indicate that pink-pigmented
475 strains of the species could play a significant role in tomato infection and should be considered
476 as a new virulent variant of the pathogen.

477 Morphological variation and colony pigmentation is a predominant characteristic in *Clavibacter*
478 species. Schaad et al (2001) summarized the colony variants of *C. michiganensis sensu lato*
479 subspecies. For instance, *Clavibacter insidiosus* causing bacterial wilt of alfalfa produces yellow
480 colonies on general culture media while blue pigmentation is also seen in some strains.
481 *Clavibacter nebraskensis* causing Goss's wilt of maize is known for orange colonies but yellow
482 variant of the bacterium is infrequently observed. Various atypical strains of *C. michiganensis*
483 subsp. *michiganensis* with phenotypic and genotypic variations *i.e.*, dry, sticky, less mucoid,
484 pink, red, orange, white, or colorless colonies have been reported in the pre-2010s literature
485 (Hayward and Waterston 1964; Davis and Vidaver 2001). While Schaad et al (2001) referred to
486 occasional pink, red, orange, white or even colorless variants of tomato canker pathogen, it
487 should be noted that assignment of all those non-yellow strains to *C. michiganensis* subsp.
488 *michiganensis* was made mostly on the basis of the host of isolation in the absence of
489 molecular-phylogenetic analyses. Subsequent studies have excluded most of those non-yellow
490 strains from the *C. michiganensis sensu stricto*. For instance, orange-pigmented *Clavibacter*
491 strains causing bacterial canker on pepper were once referred to as a variant of *C.*
492 *michiganensis* subsp. *michiganensis* (Yim et al 2012), then transferred to a new subspecies as
493 *Clavibacter michiganensis* subsp. *capsici* (Oh et al 2016), and finally elevated to the species level
494 as *Clavibacter capsici* (Li et al 2018). Thus, non-yellow variants of tomato canker pathogen
495 described in the pre-genomics era need to be re-confirmed using current phylotaxonomic
496 criteria which is the case in the present study.

497 *Curtobacterium* is another multicolored sibling of *Clavibacter* known for variation in colony
498 morphology and pigmentation. Until recently, all plant pathogenic members of *Curtobacterium*
499 were classified within the complex species *C. flaccumfaciens* and divided into five pathovars
500 based on their host of isolation and pathogenicity scheme (Collins and Jones 1983). Among
501 these pathovars, the poinsettia pathogen included yellow and orange colonies while the bean
502 pathogen possessed yellow, orange, pink, purple and red colonies on culture media (Osdaghi et

2016). Interestingly, in both latter pathogens variations in colony pigmentation were in congruence with DNA sequence-based clustering. It has been shown that yellow-pigmented strains of the bean pathogen *C. flaccumfaciens* pv. *flaccumfaciens* were genetically and phylogenetically distinct from the red- and orange-pigmented strains of the pathovar (Osdaghi et al. 2018). MLSA-based results showed that the *C. flaccumfaciens* pv. *poinsettiae* strains causing bacterial canker of *Euphorbia pulcherrima* in the USA, and the orange-/red-pigmented strains of *C. flaccumfaciens* pv. *flaccumfaciens* pathogenic on bean were too distinct from each other and from type strain of the species to be considered members of *C. flaccumfaciens*. This led to the reclassification of *C. flaccumfaciens* where orange-pigmented poinsettia strains and red-/orange-pigmented bean strains were elevated at the species level as *C. poinsettiae* and *C. aurantiacum*, respectively (Osdaghi et al 2024). Although colony pigmentation divides plant pathogenic *Curtobacterium* strains into different taxa even when they cause similar diseases on the same host plants, pink-pigmented variant of *C. michiganensis* could not be differentiated from the yellow-pigmented strains using pathogenicity scheme, phylogenetic criteria, and comparative genomics. Data provided in this study is insufficient to hypothesize origin of the pink-pigmented variant of bacterial canker pathogen. Indeed, the yellow- and pink-pigmented strains of the bacterial canker pathogen share almost 100% DNA similarity, and contain a similar set of pathogenicity determinant genes. This suggest that a few (if not one) number of genetic variations drive colony pigmentation in *Clavibacter*. Jacobs et al (2005) evaluated the role of pigmentation in population dynamics, leaf colonization strategies, and field survival of *C. michiganensis*. Pigment-deficient *C. michiganensis* mutants were significantly reduced in UVA (320 to 400 nm) radiation survival in vitro and showed reduced field populations on peanut when compared to the wild-type strain. Further analyses using functional genomics analyses would shed light on the origin and biological impact of phenotypic variations in *C. michiganensis*. Furthermore, phylogeographic investigations using a worldwide population of the pathogen could delineate if the pink-pigmented variant was originated in Iran and overlooked for years due to guidelines referring only to yellow colonies or introduced from the outside of the country.

531 Considering all this evidence, one could generalize that colony pigmentation is a fundamental
532 phenotypic feature of corynebacterial plant pathogens in their current taxonomic designation.
533 This overgeneralization is the case for tomato canker pathogen *C. michiganensis sensu stricto*
534 where all diagnostic guidelines refer to its colony pigment as yellow (EFSA 2014; EPPO 2016).
535 Such a restricted range in targeting the enemy in detection and diagnosis processes could lead
536 to the situation where non-yellow colony variants of the pathogen become unintentionally
537 ignored. The impact of phenotypic variations on phylogenetic relationships of microbial
538 lineages could indicate the length of divergence of those variants from each other. In the case
539 of *Curtobacterium* species, it could be hypothesized that the colony pigmentation is a historical
540 phenotype being mirrored in the phylogenetic features of the yellow and orange lineages
541 (Osdaghi et al 2024). However, pigment variation in *C. michiganensis* seems to be a recent
542 phenotypic disparity since it has not yet been imitated in the phylogenetic position and
543 biological features of the bacterium.

544 Since the mid-20th century, international tomato seed trades have spread *C. michiganensis* in
545 intra- and intercontinental scales due to the seed-borne nature of the pathogen (EPPO, 2016).
546 It has been shown that even a few fractions of contaminated seeds, as low as 0.01 - 0.05% (one
547 to five seeds per 10,000) could be sufficient to start a bacterial canker outbreak in production
548 fields (Chang et al., 1991; Gitaitis et al., 1991). The severe bacterial canker outbreaks in
549 Southern Iran could be attributed directly to the cultivation of infected seeds. With the
550 emergence of new pink-pigmented variant of the pathogen, it will be even more likely for the
551 pathogen to be escaped from standard culture-based detection methods (De Leon et al., 2006).
552 We have isolated all six pink-pigmented *C. michiganensis* strains from either seeds or plant
553 tissues of tomato cultivar Sun 6189F1 (Table S1). While several other tomato cultivars were
554 positive for the *C. michiganensis* infection, only yellow-pigmented strains were isolated from
555 those cultivars. This might indicate that pink-pigmented variant of the pathogen was originated
556 from a restricted source somewhere in the production site of the Sun 6189F1 cultivar. More
557 interestingly, our results revealed simultaneous infection of Sun 6189F1 cultivar with both
558 yellow- and pink-pigmented strains of *C. michiganensis* which would complicate the detection
559 procedure (Figure 2B).

560 In conclusion, description of the pink-pigmented variant of the bacterial canker pathogen
561 expands phenotypic range of *C. michiganensis* highlighting a higher level of global population
562 diversity than that has previously been documented. More information can only be obtained
563 through future studies based on the population structure of the pathogen on a large number of
564 strains isolated from different geographical regions. The main question raises from the findings
565 of this research is whether the current diagnostic guidelines for culture-based detection of the
566 pathogen are comprehensive enough to cover all phenotypic range of the pathogen. In all EFSA,
567 EPPO and ISTA guidelines it is clearly stated that one should look for yellow-pigmented mucoid
568 colonies on culture media after a reasonable time of incubation. Our results highlight the need
569 for revision of the diagnostic guidelines for bacterial canker. *Clavibacter michiganensis* is a
570 seed-borne pathogen possessing high probability of being distributed into the areas with no
571 history of the disease via infected seeds. The situation would be more complicated when
572 quarantine authorities and diagnostician are cheated with an undetectable enemy masked with
573 an unfamiliar colony pigment. This escape of the bacterium could lead to the introduction of
574 the pathogen into new areas. With a growing scale of tomato demand, production, and trade,
575 the economic consequences of these outbreaks are severe, resulting in a loss of trust among
576 seed providers, plant protection agencies, and growers. Thus, updating the diagnostic standards
577 and adjustment of the methods for early detection and identification of the pathogen are the
578 major prerequisites for successful management of the disease.

579

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583

584 **Conflict of Interest Statement**

585 The authors declare that the research was conducted in the absence of any commercial or
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587

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783

784 **Legends for Figures and Tables**

785 **Figure 1:** Disease symptoms of bacterial canker caused by *Clavibacter michiganensis* on young
786 plants (A) and tomato fruit (B) under natural field conditions. Tomato plant artificially
787 inoculated with the pink-pigmented variant of *C. michiganensis* (M126) showed wilt (C),
788 while pepper plant inoculated with the same strain showed stem canker (D) under
789 greenhouse conditions 15-20 days post-inoculation.

790

791 **Figure 2:** Colonies of yellow- (A) and pink-pigmented (B) strains of *Clavibacter michiganensis* on
792 YPGA medium resulted from streaking of the water suspension of tomato leaves 96 h post
793 incubation. Pure culture and single colonies of yellow- (C) and pink-pigmented (D)
794 variants of *C. michiganensis* streaked on YPGA and YDC (E, F) media. Bacterial cultures in
795 section “F” include *C. michiganensis* strains M26 (I) and M34 (II), *C. tessellarius* strain
796 sh2113 (III), *Rathayibacter* sp. strain sh3093 (IV), *C. lycopersici* strain CFBP 8615^T (V), and
797 *C. zhangzhongii* strain sh3003 (VI).

798

799 **Figure 3:** Maximum likelihood phylogenetic tree constructed by MEGA7 software using the
800 concatenated sequences of *atpD*, *gyrB*, *ppk*, *recA*, and *rpoB* genes (1,414 bp) obtained
801 from the strains isolated in this study and those of validly described *Clavibacter* species.
802 Bootstrap scores (1000 replicates) are displayed at each node. Pink-pigmented strains of
803 *Clavibacter michiganensis* were phylogenetically closely related to the yellow-pigmented
804 strains of the species. Color circles indicate the strains isolated in this study. Color shade
805 on each phylogenetic branch indicates colony pigmentation of the corresponding species.
806 Average nucleotide identity-based pyANI matrix also confirmed the inclusion of pink-
807 pigmented strains in *C. michiganensis* (B).

808

809 **Figure 4:** Venn diagrams constructed using the OrthoVenn3 online service showing the
810 distribution of shared gene families (orthologous clusters) among different sets of
811 *Clavibacter* spp. strains (A). Genomic features of three *Clavibacter* strains sequenced in
812 this study in comparison to representative members of tomato-associated *Clavibacter*
813 species (B).

814

815 **Table 1:** Bacterial strains investigated in this study, their origin, host, and source of isolation.
816 For a full list of the strains isolated in this study see Table S1.

817

818

819 **Table 2:** Results of one-vs.-one BLASTn/tBLASTn/tBLASTx searches using the sequences of
820 pathogenicity determinant genes/regions in the genome of *Clavibacter michiganensis*
821 NCPPB 382 (GenBank: AM711867.1) against pink-pigmented (M34 and M98) and yellow
822 pigmented (M26) strains sequenced in this study. Results of the PCR tests using the
823 primer pairs specific for pathogenicity determinants genes are a confirmation for the *in*
824 *silico* analyses. List of the pathogenicity determinants genes/regions were adopted from

825 Peritore-Galve et al (2021). In the BLAST analyses, the first number shows percentage
826 identity while the second number in the parenthesis shows query coverage.

827

828 **Figure S1:** Distribution map of *Clavibacter michiganensis* in Iran from 1993 to 2021. Each of the
829 dashed color circles corresponds to first reports of bacterial wilt and canker of tomato in
830 each area (dark red for 1993, orange for 2004, purple for 2005, blue for 2015, black for
831 2016, red for 2017, gray for 2020, and green for 2021). The yellow and pink circles inside
832 the dashed circles indicate the presence of yellow and new pink-pigmented strains of *C.*
833 *michiganensis* in Iran.

834

835 **Table S1:** Bacterial strains isolated in this study, their origin, host and source of isolation, and
836 the results of pathogenicity tests on different solanaceous plants.

837

838 **Table S2:** Primer pairs used in this study.

839

840 **Table S3:** Average nucleotide identity (ANI; lower diagonal) and digital DNA–DNA hybridization
841 (dDDH; upper diagonal) values among the type strains of *Clavibacter* species and three
842 strains CFBP 9078, CFBP 9079, and CFBP 9080 sequenced in this study. ANI values were
843 calculated using three different algorithms *i.e.*, JSpeciesWS, ANI calculator, and
844 OrthoANIu (Richter et al 2016; Rodriguez-R et al 2016; Yoon et al 2017).