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Research Article

Isolation of culturable bacteria from Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hem., Liviidae): Toward potential applications in symbiont-based pest management

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Abstract. The Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hem.: Liviidae), is a key pest in many citrus-growing regions and transmits the pathogen *Candidatus* Liberibacter asiaticus (CLas), the causal agent of citrus Huanglongbing (HLB) or citrus greening disease, in a propagative manner. This disease, which spreads rapidly, has been reported in citrus-growing areas of southern Iran and is considered a serious threat to citrus production in Iran and globally. Despite extensive research on various control strategies for this pest, information about the interactions between the pathogen, endosymbiotic bacteria, and the vector remains unavailable. In the present study, some bacterial endosymbionts of *D. citri* were identified using culture-dependent methods from adult insects collected from Jahrom and Rudan cities in Iran. Cultivation on different growth media led to the identification of four bacterial genera, including *Burkholderia* sp., *Bacillus* sp., *Enterococcus* sp., and *Staphylococcus* sp.Their potential use in managing this vector aimed at reducing or eliminating pathogen acquisition and transmission efficiency are discussed and warrants further investigation.

Keywords: *Diaphorina citri*, endosymbionts, huanglongbing, vector control

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Introduction

The Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), not only causes direct damage by feeding on host plants but also serves as a vector for the bacterium *Candidatus Liberibacter asiaticus* (CLas), which is a phloem-limited bacterium and the causative agent of Huanglongbing (HLB), a devastating citrus disease (Wang *et al.*, 2017). The spread of HLB poses a significant threat to global citrus production (Gottwald, 2010). Despite extensive efforts to control this vector and mitigate HLB, these measures have failed to prevent the disease's proliferation. Chemical control methods, though effective in the short term, face increasing challenges due to environmental hazards, disruption of natural enemy populations (Bové, 2006), and the development of pesticide resistance in psyllid populations (Tiwari *et al.*, 2011; Hall *et al.*, 2013; Chen *et al.*, 2017; Pardo *et al.*, 2018). Furthermore, the high cost and non-specificity of chemical treatments have diminished their efficacy. Consequently, the development of alternative pest management strategies is imperative. Among these, biological control methods present a sustainable and environmentally benign approach that could reduce reliance on chemical interventions.

Microbial symbionts in insects play pivotal roles in reproduction, survival, fecundity, oviposition, nutrient provisioning, and pathogen defense (Kikuchi *et al.*, 2012; Gosalbes *et al.*, 2010; Ayoubi *et al.*, 2025; Kashkouli *et al.*, 2019; Karamipour *et al.*, 2016). In recent years, the biological control of insect vectors through the manipulation of their endosymbionts has emerged as a promising strategy for managing both the vectors and their associated pathogens (Nouri *et al.*, 2018; Heck, 2018; Britt *et al.*, 2020; Wu *et al.*, 2025). Recent studies highlight

that identifying bacterial endosymbionts in insects, including the *D. citri*, is critical for understanding host biology, pathogen resistance, and even pathogen transmission dynamics (Ramsey *et al.*, 2015). Endosymbiotic bacteria can significantly influence the growth, reproduction, dispersal, and transmission efficiency of *D. citri*; thus, characterizing these microbial communities may provide novel avenues for sustainable pest management (Guidolin & Consoli, 2013; Fagen *et al.*, 2012).

Insect endosymbionts have emerged as innovative tools for sustainable pest management strategies (Engel & Moran, 2013; Kashkouli *et al.*, 2019). Among the most promising approaches are heterologous associations, microbial symbiont disruption, the Incompatible Insect Technique (IIT), and paratransgenesis. The IIT method, which leverages *Wolbachia*-induced cytoplasmic incompatibility (CI), functions by releasing *Wolbachia*-infected males into target populations. These males mate with wild females, resulting in reproductive failure and population suppression (Zheng *et al.*, 2019; Lim *et al.*, 2024). Paratransgenesis offers another sophisticated approach, involving genetic modification of insect symbionts to alter host traits (Beard *et al.*, 1998). First developed in the 1990s for managing disease vectors and agricultural pests (Beard *et al.*, 1993; Arora & Douglas, 2017), this technique requires symbionts with specific characteristics: laboratory cultivability, genetic transformability, efficient vertical/horizontal transmission to hosts and either high host specificity or non-target safety (Qadri *et al.*, 2020). By exploiting these intimate host-microbe relationships, these methods provide targeted, environmentally sound alternatives to conventional pest control, representing a paradigm shift toward precision agriculture and vector management.

Although paratransgenesis research has primarily targeted medically important insect vectors, its application to insects that transmit phytopathogens remains largely unexplored. A critical first step in developing paratransgenic strategies is the identification and cultivation of suitable vector-associated endosymbionts. In this study, we employed culture-dependent methods to isolate and characterize potential bacterial candidates for such an approach. The ecological significance and biotechnological potential of these isolates are also discussed.

Materials and methods

1. ACP Collection and Rearing

The ACP specimens were collected from field populations in Rudan County (Hormozgan Province) and Jahrom County (Fars Province), Iran, and subsequently transferred to the research greenhouse at the Faculty of Agriculture, Tarbiat Modares University for colony establishment. The insects were maintained on two-year-old key lime (*Citrus aurantifolia*) seedlings housed in double-layered mesh cages under strict conditions. Environmental parameters were regulated throughout the rearing period, maintaining a temperature of 25±3°C, relative humidity of 60-70%, and a photoperiod of 14:10 hours (light: dark). To optimize host plant quality and promote adult oviposition, regular pruning was conducted biweekly. Plant nutrition was maintained through the application of balanced NPK fertilizer (20-20-20 formulation) supplemented with iron chelate, ensuring adequate micronutrient availability for both plant health and insect development.

2. Insect Dissection and Bacterial Cultivation

For bacterial isolation, adult ACP specimens were collected from established colonies and immobilized on ice for 1 hour. Following surface sterilization with 70% ethanol and sterile distilled water washes, males and females were separated, and intestinal tissues were aseptically dissected from 20 individuals. The dissected tissues were homogenized in sterile phosphate-buffered saline and transferred to 15 mL conical tubes containing liquid culture medium, Luria-Bertani (LB). These suspensions were incubated at 37°C for 12-13 hours with constant agitation (150 rpm) in an orbital shaker to facilitate bacterial growth. Two distinct culture media were employed for bacterial isolation: LB agar (per liter: 10g tryptone, 5g yeast extract, 10g NaCl; pH 7.0 ± 0.2) and Acetic acid bacteria-specific medium (per liter: 20g D-sorbitol, 5g peptone, 3g yeast extract, 100mg cycloheximide). Inoculation loops were sterilized by immersion in 70% ethanol followed by flaming, with adequate cooling time before use. Quadrant streaking was performed at 45° angles to achieve isolated colonies, with particular attention to maintaining aseptic conditions throughout the process.

3. PCR Amplification

For detection of cultured endosymbiotic bacteria from ACP, single colonies were isolated from solid culture media. 16S rRNA gene amplification was performed using universal primers (F: 5'-AGAGTTTGATCMTGGCTCAG-3' and R: 5'-TACGGYTACCTTGTTACGACTT-3') in 20 µL reaction volumes containing: 10 µL 2× Master Mix Red (Ampliqon), 1 µL of each primer (10 µM), 2 µL template DNA, and 6 µL nuclease-free water. Following brief centrifugation to pellet reaction components, thermal cycling was performed with initial denaturation at 95°C for 10 min, followed by 40 cycles of denaturation (95°C, 5 sec), annealing (57°C, 45 sec), and extension (72°C, 45 sec), with a final extension at 72°C for 2 min (Applied Biosystems 2720 Thermal Cycler). Amplified products were verified by 1.5% agarose gel electrophoresis before Sanger sequencing (Pishgam Biotech, Iran).

4. Sequencing and Phylogenetic Analysis

The PCR products from the extracted DNA of the cultured endosymbiotic bacteria were submitted for Sanger sequencing. The obtained sequences were analyzed using the NCBI's (National Center for Biotechnology Information) BLAST tool for taxonomic identification. For phylogenetic reconstruction, reference sequences of related bacterial species were retrieved from the NCBI database. Sequence alignment was performed using the PRATT software, followed by phylogenetic tree construction in MEGA X software. When necessary, manual sequence adjustments were made to optimize alignments. The neighbor-joining method was employed for phylogenetic analysis with 1000 bootstrap replicates to assess nodal support, using the Kimura 2-parameter substitution model.

Results

1. Identification and Characterization of Cultured Bacterial Isolates from ACP

Bacterial isolation was successfully performed using both LB agar and acetic acid bacteria-specific media, with numerous distinct colonies observed (Fig. 1). Single colonies were selected based on morphological characteristics (colony color, size, and morphology) and subjected to DNA extraction. A portion of the 16S rRNA gene was amplified by PCR and the products were sequenced. After quality trimming, the obtained sequences were analyzed using the nucleotide BLAST, leading to the identification of four bacterial genera from the psyllid populations. *Bacillus* sp. and *Enterococcus* sp. were detected in populations from the colonies collected from Jahrom and Rudan populations, while *Staphylococcus* sp. was exclusively found in the Jahrom colony and *Burkholderia* sp. only in the Rudan colony. The 16S rRNA sequences were deposited in the NCBI GenBank under accession numbers PV875931 (*Burkholderia*), PV875933 (*Enterococcus*), and PV875935 (*Bacillus*).

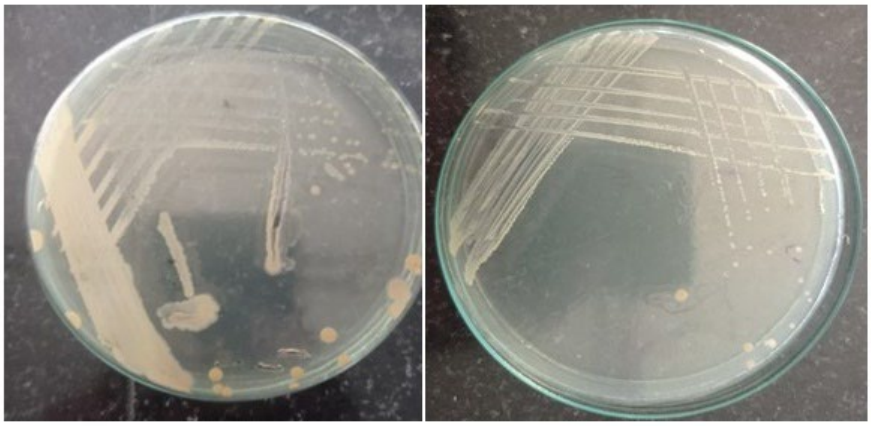

Fig. 1. Culture and isolation of *Diaphorina citri* gut bacteria. (A) Primary streak plate on nutrient agar for isolation of bacterial colonies from the dissected gut of *D. citri*. (B) Purified single colonies obtained after subsequent streaking were used for further molecular identification.

The BLAST analysis revealed the following closest matches: *Bacillus* sp. showed 98% query coverage and 87% identity to *Bacillus safensis*, *Enterococcus* sp. showed 100% query coverage and 98% identity to *Enterococcus casseliflavus*, *Staphylococcus* sp. showed 100% query coverage and 98% identity to *Staphylococcus saprophyticus*, *Burkholderia* sp. showed 92% query coverage and 95% identity to *Burkholderia cepacia*.

2. Phylogenetic Analysis of Bacterial Isolates

The phylogenetic relationships of the isolated bacteria (*Bacillus* sp., *Enterococcus* sp., *Staphylococcus* sp., and *Burkholderia* sp.) were analyzed using MEGA10 software with reference sequences from NCBI. For *Bacillus* species, the phylogenetic tree revealed two sister clades, with isolates from both Jahrom and Rudan counties clustering together in a distinct clade (Fig. 2). *Enterococcus* sp. isolates formed a single cluster regardless of their geographic origin (Fig. 3). The *Staphylococcus* sp. isolate from Jahrom showed relationship to the phylogenetic affinity to *S. saprophyticus*, forming a well-defined branch adjacent to reference strains (Fig. 4). Similarly, the *Burkholderia* sp. isolate from Rudan demonstrated the highest similarity to *B. diffusa* (Fig. 5). All phylogenetic analyses were performed using the Neighbor-Joining with 1000 bootstrap replicates. The Tamura-Nei evolutionary model was applied for *Bacillus* and *Burkholderia* sp., while all sequences were aligned using the MUSCLE algorithm. Branch support values exceeded 85% bootstrap for all significant nodes, confirming robust phylogenetic relationships. These results demonstrate clear species-level classification for *Staphylococcus* sp. and *Burkholderia* sp., while revealing interesting patterns of geographic distribution among the *Bacillus* populations and consistent evolutionary relationships for *Enterococcus* isolates across different locations.

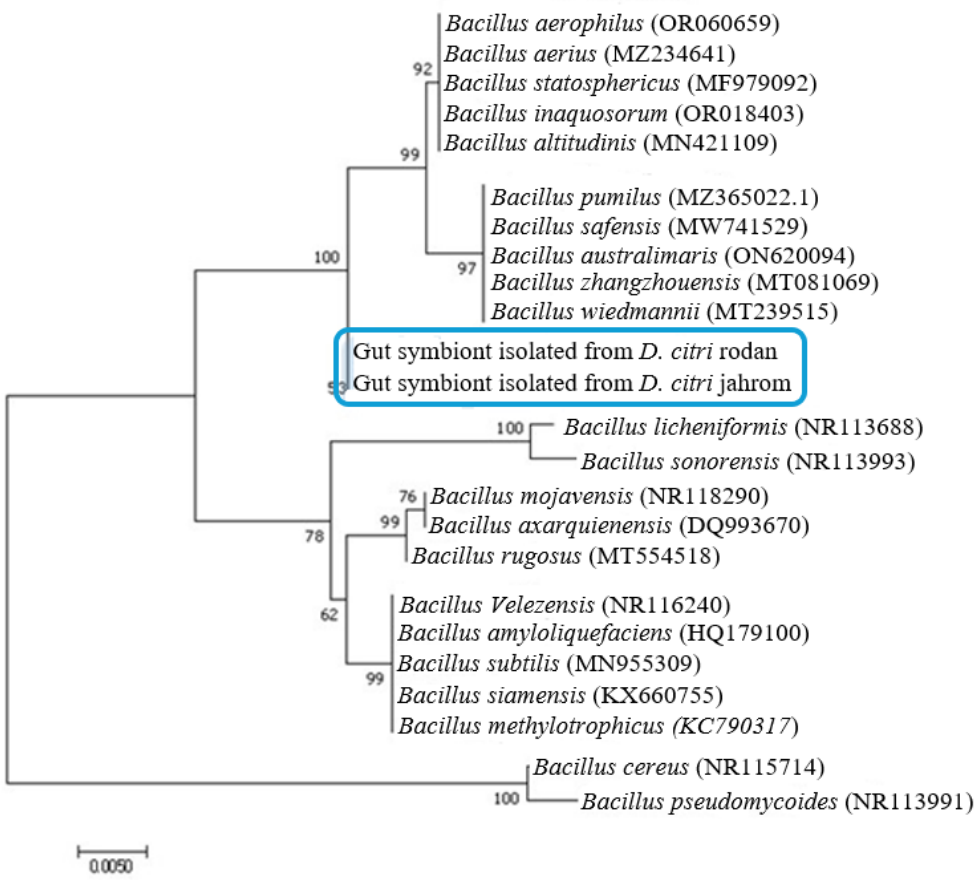

Fig. 2. Phylogenetic analysis of bacterial isolate *Bacillus* sp. from *D. citri* gut. The evolutionary history was inferred using the Neighbor-Joining method based on the 16S rRNA gene sequence. The bootstrap consensus tree (from 1000 replicates) shows the position of the isolate (highlighted in blue) relative to closely related type strains. The scale bar indicates the number of base substitutions per site.

Discussion

The ACP is recognized as the primary vector of *Candidatus* Liberibacter asiaticus, the causal agent of citrus greening disease (huanglongbing). In addition to this pathogen, recent studies have reported the presence of other associated bacteria within the insect's body, which may play a role in host biology, disease transmission, or microbial-host interactions. In this study, as part of efforts to exploit symbiotic bacteria for the biological control of this pest, we aimed to identify culturable bacteria present in the gut of *Diaphorinacitri*. Four bacterial isolates belonging to the genera *Bacillus* sp.*, Burkholderia* sp.*, Enterococcus* sp.*,* and *Staphylococcus* sp. were successfully isolated and identified. Studies indicate that the bacteria associated with *D. citri* are predominantly citrus-related endophytes (Araújo *et al.*, 2002; Azevedo *et al.*, 2000).

Each genus holds distinct biological significance: certain *Bacillus* species produce antimicrobial metabolites with demonstrated biocontrol potential against plant pests and pathogens (Fira *et al.*, 2018); *Burkholderia* comprises both beneficial symbionts and phytopathogens that may influence insect-plant interactions (Compant *et al.*, 2008); while *Enterococcus* and *Staphylococcus*, frequently reported as gut symbionts, may contribute to antibiotic resistance or host immune modulation (Dillon & Dillon, 2004). Although previous studies have characterized some *D. citri* endosymbionts such as *Bacillus sp.*, *Staphylococcus sp.*, *Cardinium sp.*, *Hamiltonella sp* (Kolora *et al.*, 2015; Hosseinzadeh *et al.*, 2019; Rahimpour *et al.*, 2025; Zanganeh *et al.*, 2025), the full diversity and functional roles of its bacterial associates remain incompletely understood. These findings advance our understanding of the tripartite psyllid-symbiont-pathogen interplay and provide a foundation for developing novel management strategies, including biocontrol approaches or genetic manipulation of endosymbionts. Based on the obtained results, the bacterium *Burkholderia* sp. was exclusively identified in the *Diaphorina citri* population from Rudan County. To our knowledge, this bacterium has not been previously reported in the Asian citrus psyllid. Members of the *Burkholderia* sp. genus (β-proteobacteria) are primarily soil-dwelling bacteria commonly found in the rhizosphere of plants, surrounding environments, and other moist habitats (Woods *et al.*, 2006). Certain Burkholderia species are established as specialized, beneficial symbionts in various hemipteran insects.

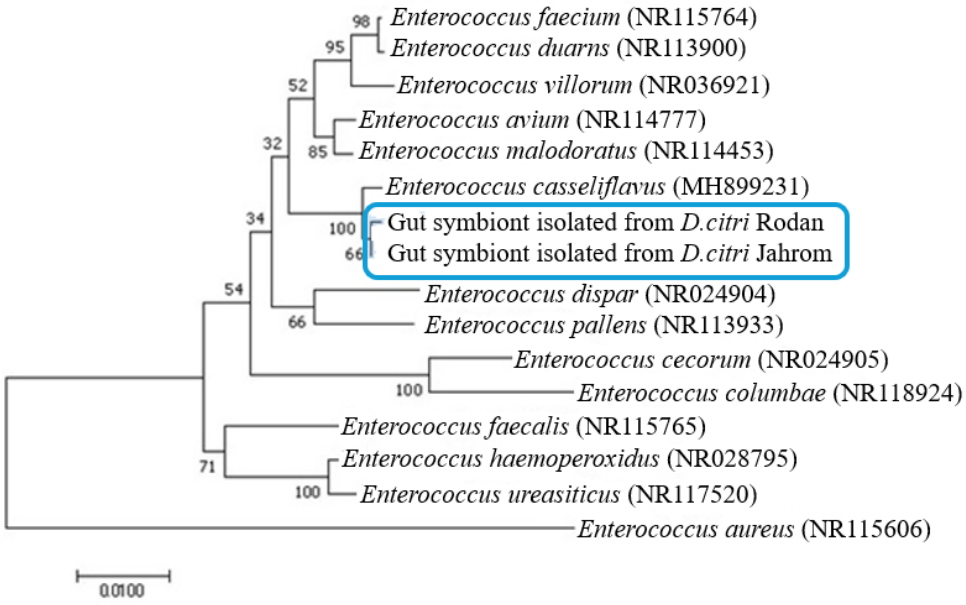

Fig. 3. Phylogenetic analysis of bacterial isolate *Enterococcus* sp. from *D. citri* gut. The evolutionary history was inferred using the Neighbor-Joining method based on the 16S rRNA gene sequence. The bootstrap consensus tree (from 1000 replicates) shows the position of the isolate (highlighted in blue) relative to closely related type strains. The scale bar indicates the number of base substitutions per site.

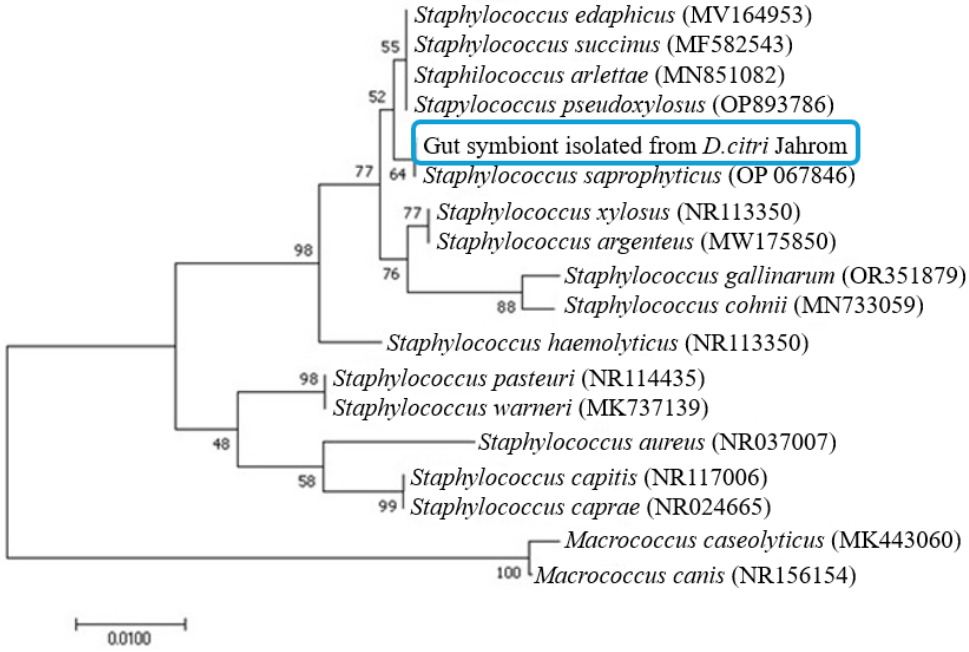

Fig. 4. Phylogenetic analysis of bacterial isolate *Staphylococcus* sp. from *D. citri* gut. The evolutionary history was inferred using the Neighbor-Joining method based on the 16S rRNA gene sequence. The bootstrap consensus tree (from 1000 replicates) shows the position of the isolate (highlighted in blue) relative to closely related type strains. The scale bar indicates the number of base substitutions per site.

They are typically acquired environmentally by nymphs and colonize the midgut to facilitate host growth (Kikuchi *et al.*, 2005, 2011). Evidence also suggests potential endosymbiotic and vertical transmission, as Burkholderia has been detected in insect bacteriomes and ovaries (Kikuchi *et al.*, 2005, 2011). Its presence across diverse insect hosts, including stinkbugs, leafhoppers, and scale insects, indicates a broader symbiotic role, potentially through nutrient provisioning or metabolic contributions (Takeshita & Kikuchi, 2020). The detection of *Burkholderia* sp. in *D. citri* is particularly noteworthy, as this genus encompasses species with diverse ecological roles, ranging from plant pathogens to insect symbionts. Certain *Burkholderia* sp. strains are known to aid insects in nutrient acquisition or detoxification (Kikuchi *et al.*, 2012).

Bacillus sp. was also isolated from both Jahrom and Rudan Counties psyllid populations. This bacterium has been previously reported from ACP (Kolora *et al.*, 2015). Beyond the ACP, *Bacillus* sp. has been reported in other insect species, including the kissing bug, *Meccus pallidipennis* (Jiménez *et al.*, 2021), and the tobacco hornworm *Manduca sexta* (Van der Hoeven *et al.*, 2008). Members of the *Bacillus* sp. genus are Gram-positive, rod-shaped, spore-forming bacteria that are widely distributed in soil, plant surfaces, and insect digestive systems, often establishing symbiotic or antagonistic relationships with their hosts. Certain *Bacillus* species (e.g., *B. thuringiensis*) are known to function as biological pesticides (Zhao *et al.*, 2016; Guo *et al.*, 2017). Given that some *Bacillus* strains can produce antimicrobial compounds, their presence in citrus psyllids may influence the insect's susceptibility to *Candidatus* Liberibacter asiaticus (CLas) or other gut pathogens. *Enterococcus* sp. was also identified in the psyllid populations collected from both Jahrom and Rudan. *Enterococcus* species are ubiquitous in nature, commonly found in water, soil, food products, and the gastrointestinal tracts of various hosts (Ran *et al.*, 2015). While these bacteria are typically associated with vertebrate microbiomes, several studies have documented their presence in different insects (Vilanova *et al.*, 2016; Yun *et al.*, 2014; Li *et al.*, 2020). Enterococci may play roles in food digestion and immune regulation (Johnston & Rolff, 2015). *Staphylococcus* sp. was the other bacterial genus exclusively cultured and isolated from the psyllid population collected from Jahrom. These bacteria are frequently reported as components of the normal surface or gut flora of insects (El Shazely *et al.*, 2019; Oliveira *et al.*, 2014), including *Manduca sexta* (Van der Hoeven *et al.*, 2008), *Brithys crini* and *Hyles euphorbiae* (Vilanova *et al.*, 2016).

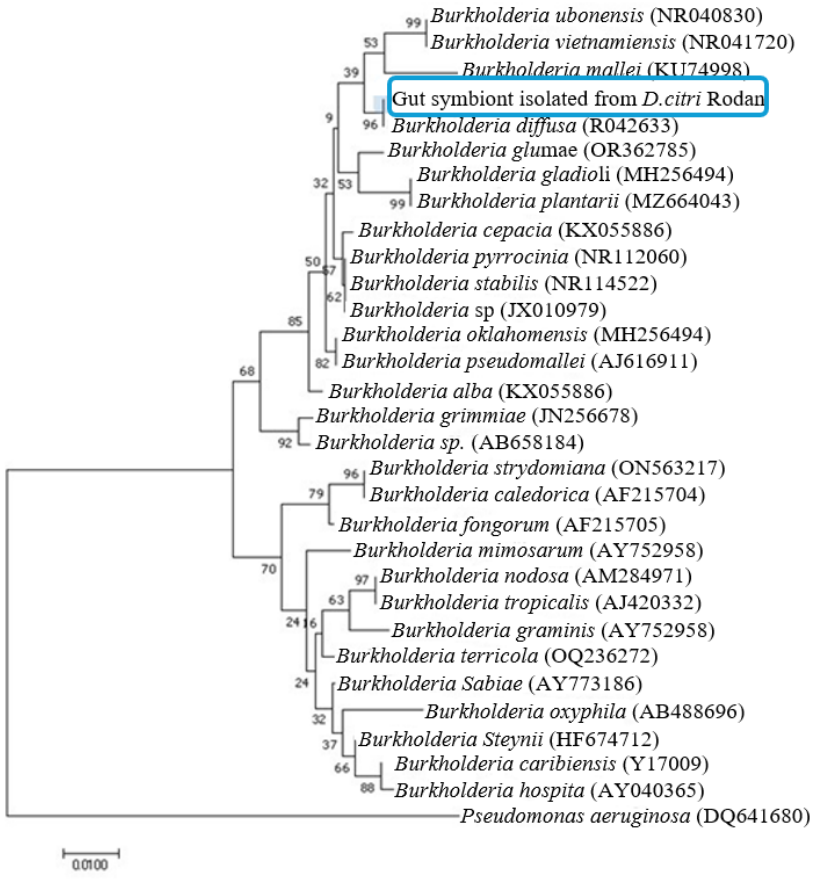

Fig 5. Phylogenetic analysis of bacterial isolate *Burkholderia* sp. from *D. citri* gut. The evolutionary history was inferred using the Neighbor-Joining method based on the 16S rRNA gene sequence. The bootstrap consensus tree (from 1000 replicates) shows the position of the isolate (highlighted in blue) relative to closely related type strains. The scale bar indicates the number of base substitutions per site.

Staphylococci sp. are Gram-positive, coccoid bacteria, with certain species such as *S. aureus* being human pathogenic (Oliveira *et al.*, 2014). In *D. citri*, their presence may result from environmental exposure or contact with plants. Although *Staphylococcus* sp. is not recognized as a primary insect pathogen, its detection could indicate secondary contamination or alterations in the insect microbiome under stressful conditions. The isolation of *Bacillus*, *Burkholderia* sp., *Enterococcus* sp., and *Staphylococcus* sp. from ACP carrying the HLB pathogen expands current knowledge about the microbial communities associated with citrus psyllids. The widespread occurrence of these bacteria across diverse insect orders suggests they may not form specialized associations with specific hosts. Instead, they likely confer general beneficial traits when symbiotic, or may represent transient environmental acquisitions. The results raise important questions about potential interactions between these bacteria and psyllid biology, including their possible influence on pathogen transmission dynamics and potential applications in biological control strategies. The presence of these bacterial genera, particularly those with known plant-growth-promoting or antimicrobial properties, warrants deeper exploration of their functional roles in the HLB pathosystem.

While this study successfully isolated and identified several culturable bacterial associates with *D. citri*, it is important to acknowledge the limitations inherent in the culture-dependent approach employed. Our methodology, while optimal for obtaining live isolates for downstream biotechnological applications, is inherently selective. The use of specific growth media and incubation conditions inevitably favors fast-growing bacteria and may overlook a significant portion of the microbial community, including unculturable, fastidious, or slow-growing symbionts. For instance, well-known primary and secondary symbionts of *D. citri*, such as CLasand *Wolbachia*, which are typically detected via molecular methods, were not recovered in our cultures, as reported in

previous studies. Consequently, the findings presented here should not be interpreted as a complete census of the *D. citri* microbiome but rather as a targeted isolation of cultivable components with potential for manipulation. Further studies investigating the microbial composition of field-collected psyllids from different regions and seasons would help elucidate the influence of environmental factors. Finally, functional experiments, including the gnotobiotic rearing of psyllids with the isolated bacteria, are essential to unequivocally determine their roles in host fitness, immune response, and, most critically, their interaction with and impact on CLas acquisition and transmission.

Author's Contributions

Hamed Rahimpour: methodology; formal analysis; investigation; draft preparation; final review and edit. **Mohammad Mehrabadi:** Conceptualization, supervision; project administration and funding acquisition. **Abbasali Raz:** final review and edit; project administration. **Aliasghar Talebi:** final review and edit; project administration.

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Data Availability Statement

All data used in this study are available to the authors and can be provided upon request.

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Ethics Approval and Consent to Participate

All national and international guidelines related to the care and use of insects were strictly followed. This study did not involve any experiments or participation of humen subjects.

Conflict of Interest

The authors declare that they have no conflict of interest.

Generative AI statement

The authors declare that no generative AI tools were used in the writing, analysis, or preparation of this manuscript.

REFERENCES

Araújo, W. L., Marcon, J., Maccheroni Jr, W., Van Elsas, J. D., Van Vuurde, J. W. & Azevedo, J. L. (2002) Diversity of endophytic bacterial populations and their interaction with *Xylella fastidiosa* in citrus plants. *Applied and Environmental Microbiology*, *68*(10), 4906-4914. https://doi.org\10.1128\AEM.68.10.4906-4914.2002

Arora, A. K. & Douglas, A. E. (2017) Hype or opportunity? Using microbial symbionts in novel strategies for insect pest control. *Journal of Insect Physiology*, *103*, 10-17. https://doi:%2010.1016/j.jinsphys.2017.09.011

Ayoubi, A., Talebi, A. A., Fathipour, Y., Hoffmann, A. A. & Mehrabadi, M. (2025) Symbiont-mediated insect host defense against parasitism: insights from the endosymbiont, *Hamiltonella defensa* and the insect host, *Myzus persicae*. *Pest*

Management Science. https://doi.org\10.1002\ps.8844

Azevedo, J. L., Maccheroni Jr, W., Pereira, J. O. & De Araújo, W. L. (2000) Endophytic microorganisms: a review on insect control and recent advances on tropical plants. *Electronic Journal of Biotechnology*, *3*(1), 15-16. https://doi:%2010.2225/vol3-issue1-fulltext-4

Beard, C. B., O'Neill, S. L., Tesh, R. B., Richards, F. F. & Aksoy, S. (1993) Modification of arthropod vector competence via symbiotic bacteria. *Parasitology Today*, *9*(5), 179-183. https://doi.org/10.1016/0169-4758(93)90142-3

Beard, C. B., Durvasula, R. V. & Richards, F. F. (1998) Bacterial symbiosis in arthropods and the control of disease transmission. *Emerging Infectious Diseases*, *4*(4), 581. https://doi.org/10.3201/eid0404.980408

Bové, J. M. (2006) Huanglongbing: a destructive, newly-emerging, century-old disease of citrus. *Journal of Plant Pathology*, 7-37. https://doi.org/10.4454/JPP.V88I1.828

Britt, K., Gebben, S., Levy, A., Al Rwahnih, M. & Batuman, O. (2020) The detection and surveillance of Asian citrus psyllid (*Diaphorina citri*) associated viruses in Florida citrus groves. *Frontiers in Plant Science*, *10*, 1687. https://doi.org/10.3389/fpls.2019.01687

Chen, X. D., Gill, T. A., Pelz-Stelinski, K. S. & Stelinski, L. L. (2017) Risk assessment of various insecticides used for management of Asian citrus psyllid, *Diaphorina citri* in Florida citrus, against honey bee, *Apis mellifera*. *Ecotoxicology*, *26*(3), 351-359. https://doi.org\10.1007\s10646-017-1768-5

Compant, S., Nowak, J., Coenye, T., Clement, C. & Ait Barka, E. (2008) Diversity and occurrence of *Burkholderia* spp. in the natural environment. *FEMS Microbiology Reviews*, *32*(4), 607-626. https://doi.org/10.1111/j.1574-6976.2008.00113.x

Dillon, R. J. & Dillon, V. M. (2004) The gut bacteria of insects: nonpathogenic interactions. *Annual Reviews in Entomology*, *49*(1), 71-92. https://doi.org/10.1146/annurev.ento.49.061802.123416

El Shazely, B., Urbański, A., Johnston, P. R. & Rolff, J. (2019) In vivo exposure of insect AMP resistant *Staphylococcus aureus* to an insect immune system. *Insect Biochemistry and Molecular Biology*, *110*, 60-68. https://doi.org/10.1016/j.ibmb.2019.04.017

Engel, P. & Moran, N. A. (2013) The gut microbiota of insects–diversity in structure and function. *FEMS Microbiology Reviews*, *37*(5), 699-735. https://doi.org/10.1111/1574-6976.12025

Gosalbes, M. J., Latorre, A., Lamelas, A. & Moya, A. (2010) Genomics of intracellular symbionts in insects. *International Journal of Medical Microbiology*, *300*(5), 271-278. https://doi.org/10.1016/j.ijmm.2009.12.001

Fagen, J. R., Giongo, A., Brown, C. T., Davis-Richardson, A. G., Gano, K. A. & Triplett, E. W. (2012) Characterization of the relative abundance of the citrus pathogen Ca. Liberibacter asiaticus in the microbiome of its insect vector, *Diaphorina citri*, using high throughput 16S rRNA sequencing. *The Open Microbiology Journal*, *6*, 29. https://doi.org/10.2174/1874285801206010029

Fira, D., Dimkić, I., Berić, T., Lozo, J. & Stanković, S. (2018) Biological control of plant pathogens by *Bacillus* species. *Journal of Biotechnology*, *285*, 44-55. https://doi.org/10.1016/j.jbiotec.2018.07.044

Gottwald, T. R. (2010) Current epidemiological understanding of citrus huanglongbing. *Annual Review of Phytopathology*, *48*(1), 119-139. https://doi.org/10.1146/annurev-phyto-073009-114418

Guo, M., Wu, F., Hao, G., Qi, Q., Li, R., Li, N., Wei, L. & Chai, T. (2017) *Bacillus subtilis* improves immunity and disease resistance in rabbits. *Frontiers in Immunology*, *8*, 354. https://doi.org/10.3389/fimmu.2017.00354

Guidolin, A. S. & Cônsoli, F. L. (2013) Molecular characterization of *Wolbachia* strains associated with the invasive Asian citrus psyllid *Diaphorina citri* in Brazil. *Microbial Ecology*, *65*(2), 475-486. https://doi.org\10.1007\s00248-012-0150-7

Hall, D. G., Richardson, M. L., Ammar, E. D. & Halbert, S. E. (2013) Asian citrus psyllid, *Diaphorina citri*, vector of citrus huanglongbing disease. *Entomologia Experimentalis et Applicata*, *146*(2), 207-223. https://doi.org/10.1111/eea.12025

Heck, M. (2018) Insect transmission of plant pathogens: a systems biology perspective. *MSystems*, *3*(2), 10-1128. https://doi.org/10.1128/msystems.00168-17

Hosseinzadeh, S., Shams-Bakhsh, M., Mann, M., Fattah-Hosseini, S., Bagheri, A., Mehrabadi, M. & Heck, M. (2019) Distribution and variation of bacterial endosymbiont and "Candidatus Liberibacter asiaticus" titer in the Huanglongbing insect vector, *Diaphorina citri* Kuwayama. *Microbial Ecology*, *78*(1), 206-222. https://doi.org\10.1007\s00248-018-1290-1

Jiménez-Cortés, J. G., García-Contreras, R., Bucio-Torres, M. I., Cabrera-Bravo, M., López-Jácome, L. E., Franco-Cendejas, R., Vences-Blanco, M. O. & Salazar-Schettino, P. M. (2021) Bacteria cultured from the gut of *Meccus pallidipennis* (Hemiptera: Reduviidae), a triatomine species endemic to Mexico. *Medical and Veterinary Entomology*, *35*(3), 478-

483. https://doi.org/10.1111/mve.12496

Johnston, P. R. & Rolff, J. (2015). Host and symbiont jointly control gut microbiota during complete metamorphosis. *PLoS Pathogens*, *11*(11), e1005246. https://doi.org/10.1371/journal.ppat.1005246

Karamipour, N., Fathipour, Y. & Mehrabadi, M. (2016). Gammaproteobacteria as essential primary symbionts in the striped shield bug, *Graphosoma lineatum* (Hemiptera: Pentatomidae). *Scientific Reports*, *6*(1), 33168. https://doi.org\10.1038\srep33168

Kashkouli, M., Fathipour, Y. & Mehrabadi, M. (2019). Heritable gammaproteobacterial symbiont improves the fitness of *Brachynema germari* Kolenati (Hemiptera: Pentatomidae). *Environmental Entomology*, *48*(5), 1079-1087. https://doi.org/10.1093/ee/nvz089

Kikuchi, Y., Meng, X. Y. & Fukatsu, T. (2005) Gut symbiotic bacteria of the genus *Burkholderia* in the broad-headed bugs *Riptortus clavatus* and *Leptocorisa chinensis* (Heteroptera: Alydidae). *Applied and Environmental Microbiology*, *71*(7), 4035-4043. https://doi.org/10.1128/AEM.71.7.4035-4043.2005

Kikuchi, Y., Hosokawa, T. & Fukatsu, T. (2011) Specific developmental window for establishment of an insect-microbe gut symbiosis. *Applied and Environmental Microbiology*, *77*(12), 4075-4081. https://doi.org/10.1128/AEM.71.7.4035-4043.2005

Kikuchi, Y., Hayatsu, M., Hosokawa, T., Nagayama, A., Tago, K. & Fukatsu, T. (2012) Symbiont-mediated insecticide resistance. *Proceedings of the National Academy of Sciences*, *109*(22), 8618-8622. https://doi.org/10.1073/pnas.1200231109

Kolora, L. D., Powell, C. M., Hunter, W., Bextine, B. & Lauzon, C. R. (2015) Internal extracellular bacteria of *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae), the Asian citrus psyllid. *Current Microbiology*, *70*(5), 710-715. https://doi.org\10.1007\s00284-015-0774-1

Li, F., Li, M., Mao, T., Wang, H., Chen, J., Lu, Z., Qu, J., Fang, Y., Gu, Z. & Li, B. (2020) Effects of phoxim exposure on gut microbial composition in the silkworm, *Bombyx mori*. *Ecotoxicology and Environmental Safety*, *189*, 110011. https://doi.org/10.1016/j.ecoenv.2019.110011

Lim, J. T., Bansal, S., Chong, C. S., Dickens, B., Ng, Y., Deng, L., Lee, C., Tan, L. Y., Chain, G., Ma, P., Sim, S., Tan, C. H., Cook, A. & Ng, L. C. (2024) Efficacy of *Wolbachia*-mediated sterility to reduce the incidence of dengue: a synthetic control study in Singapore. *The Lancet Microbe*, *5*(5), e422-e432. https://doi.org/10.1056/nejmoa2030243

Nouri, S., Matsumura, E. E., Kuo, Y. W. & Falk, B. W. (2018) Insect-specific viruses: from discovery to potential translational applications. *Current Opinion in Virology*, *33*, 33-41. https://doi:%2010.1016/j.coviro.2018.07.006

Oliveira, P. S., Souza, S. G., Campos, G. B., Silva, D. C. D., Sousa, D. S., Araújo, S. P., Ferreira, L., Santos, V., Timenetsky, J., Cruz, M. P., Yatsuda, R. & Marques, L. M. (2014) Isolation, pathogenicity and disinfection of *Staphylococcus aureus* carried by insects in two public hospitals of Vitória da Conquista, Bahia, Brazil. *Brazilian Journal of Infectious Diseases*, *18*(02), 129-136. https://doi.org/10.1016/j.bjid.2013.06.008

Pardo, S., Martínez, A. M., Figueroa, J. I., Chavarrieta, J. M., Viñuela, E., Rebollar-Alviter, Á., Miranda, M. A., Valle, J. & Pineda, S. (2018) Insecticide resistance of adults and nymphs of Asian citrus psyllid populations from Apatzingán Valley, Mexico. *Pest Management Science*, *74*(1), 135-140. https://doi.org/10.1002/ps.4669

Qadri, M., Short, S., Gast, K., Hernandez, J. & Wong, A. C. N. (2020) Microbiome innovation in agriculture: development of microbial based tools for insect pest management. *Frontiers in Sustainable Food Systems*, *4*, 547751. https://doi.org/10.3389/fsufs.2020.547751

Rahimpour, H., Talebi, A. A., Raz, A., Azarbad, H. & Mehrabadi, M. (2025) Geographic variation and diversity of bacterial endosymbionts in Asian citrus psyllid, *Diaphorina citri*, from Iran. *Pest Management Science*. https://doi.org/10.1002/ps.70100

Ramsey, J. S., Johnson, R. S., Hoki, J. S., Kruse, A., Mahoney, J., Hilf, M. E., Hunter, W. B., Hall, D., Schroeder, F., MacCoss, M. & Cilia, M. (2015) Metabolic interplay between the Asian citrus psyllid and its *Profftella* symbiont: an Achilles' heel of the citrus greening insect vector. *PLoS One*, *10*(11), e0140826. https://doi.org/10.1371/journal.pone.0140826

Ran, S., Liu, B., Jiang, W., Sun, Z. & Liang, J. (2015) Transcriptome analysis of *Enterococcus faecalis* in response to alkaline stress. *Frontiers in Microbiology*, *6*, 795. https://doi.org/10.3389/fmicb.2015.00795

Takeshita, K. & Kikuchi, Y. (2020) Genomic comparison of insect gut symbionts from divergent *Burkholderia* subclades. *Genes*, *11*(7), 744. https://doi.org/10.3390/genes11070744

Tiwari, S., Mann, R. S., Rogers, M. E. & Stelinski, L. L. (2011) Insecticide resistance in field populations of Asian citrus psyllid in Florida. *Pest Management Science*, *67*(10), 1258-1268. https://doi.org/10.1002/ps.2181

Van Der Hoeven, R., Betrabet, G. & Forst, S. (2008) Characterization of the gut bacterial community in *Manduca sexta* and effect of antibiotics on bacterial diversity and nematode reproduction. *FEMS Microbiology Letters*, *286*(2), 249-256. https://doi.org/10.1111/j.1574-6968.2008.01277.x

Vilanova, C., Baixeras, J., Latorre, A. & Porcar, M. (2016) The generalist inside the specialist: Gut bacterial communities of two insect species feeding on toxic plants are dominated by *Enterococcus sp*. *Frontiers in Microbiology*, *7*, 1005. https://doi.org/10.3389/fmicb.2016.01005

Wang, N., Pierson, E. A., Setubal, J. C., Xu, J., Levy, J. G., Zhang, Y., Li, J., Rangel, L. & Martins Jr, J. (2017) The Candidatus Liberibacter–host interface: insights into pathogenesis mechanisms and disease control. *Annual Review of Phytopathology*, *55*(1), 451-482. https://doi.org/10.1146/annurev-phyto-080516-035513

Woods, D. E. & Sokol, P. A. (2006) The genus *Burkholderia*. In *The prokaryotes* (pp. 848-860). Springer, New York, NY. https://doi.org/10.1007/0-387-30745-1_40

Wu, K., Vu, E. D., Ghosh, S., Mishra, R. & Bonning, B. C. (2025) Continuous cell lines derived from the Asian citrus psyllid, *Diaphorina citri*, harbor viruses and *Wolbachia*. *Scientific Reports*, *15*(1), 124. https://doi.org/10.1038/s41598-024-83671-2

Zhao, Y., Zhang, S., Luo, J. Y., Wang, C. Y., Lv, L. M. & Cui, J. J. (2016) Bacterial communities of the cotton aphid *Aphis gossypii* associated with Bt cotton in northern China. *Scientific Report. 6: 22958*. https://doi.org/10.1038/srep22958

Yun, J. H., Roh, S. W., Whon, T. W., Jung, M. J., Kim, M. S., Park, D. S., Yoon, C., Nam, Y. D., Kim, Y. D., Choi, J. H., Kim, J. Y., Shin, N. R., Kim, S. H., Lee, W. J. & Bae, J. W. (2014) Insect gut bacterial diversity determined by environmental habitat, diet, developmental stage, and phylogeny of host. *Applied and Environmental Microbiology*, *80*(17), 5254-5264. https://doi.org/10.1128/AEM.01226-14

Zanganeh, M., Fathipour, Y., Hoffmann, A. & Mehrabadi, M. (2025) Population incompatibility associated with *Wolbachia* in the Asian citrus psyllids, *Diaphorina citri*: insights for pest management. *Pest Management Science*. https://doi.org/10.1002/ps.8914

Zheng, X., Zhang, D., Li, Y., Yang, C., Wu, Y., Liang, X., Liang, Y., Pan, X., Hu, L., Sun, Q., Wang, X., Wei, Y., Zhu, J., Qian, W., Yan, Z., Parker, A. G., Gilles, J. RL., Bourtzis, K., Bouyer, J., Tang, M., Zheng, B., Yu, J., Liu, J., Zhuang, J., Hu, Z., Zhang, M., Gong, J. T., Hong, X. Y., Zhang,Z., Lin, L., Liu, Q., Hu, Z., Wu, Z., Baton, L. A., Hoffmann, A. A. & Xi, Z. (2019) Incompatible and sterile insect techniques combined eliminate mosquitoes. *Nature*, *572*(7767), 56-61. https://doi.org/10.1038/s41586-019-1407-9

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جداسازی باکتری‌های قابل کشت از پسیل آسیایی مرکبات *Diaphorina citri* Kuwayama (Hem., Liviidae) به‌سوی کاربردهای بالقوه در مدیریت آفات بر پایه همزیست‌ها

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چکیده: پسیل آسیایی مرکبات، *Diaphorina citri*، یک آفت کلیدی در بسیاری از مناطق اصلی کشت مرکبات بوده و باکتری *Candidatus* Liberibacter asiaticus (CLas) را به‌صورت تولیدمثلی منتقل می‌کند. این بیمارگر، عامل بیماری Haunglungbing (HLB) یا سبز شدن مرکبات است. این بیماری که به سرعت گسترش می‌یابد، در مناطق مرکبات‌خیز جنوب ایران گزارش شده و تهدیدی جدی برای تولید مرکبات در ایران و جهان محسوب می‌شود. علیرغم تحقیقات گسترده بر روی راهبردهای مختلف کنترل این آفت، اطلاعاتی درباره تعاملات میان بیمارگر، باکتری‌های همزیست درون‌سلولی و ناقل بیماری هنوز در دسترس نیست. در مطالعه حاضر، برخی از همزیست‌های باکتریایی درون‌سلولی *D. citri* با استفاده از روش‌های وابسته به کشت، از حشرات بالغ جمع‌آوری‌شده از شهرهای جهرم و رودان ایران شناسایی شدند. کشت روی محیط‌های رشد مختلف منجر به شناسایی چهار جنس باکتریایی شامل *Burkholderia* sp.، *Bacillus* sp.، *Enterococcus* sp. و *Staphylococcus sp.* گردید. کاربرد بالقوه این باکتری‌ها در مدیریت این ناقل با هدف کاهش یا حذف کارایی اکتساب و انتقال پاتوژن مورد بحث قرار گرفته و نیازمند بررسی‌های بیشتر است.

کلمات کلیدی: پسیل آسیایی مرکبات، درون همزیست، ناقل بیماری، بیماری HLB

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