Bioagro 37(2): 245-252. 2025 doi: http://www.doi.org/10.51372/bioagro3721.10

Technical Note

MICROORGANISMS IN THE RHIZOSPHERE OF SYMPTOMATIC AND ASYMPTOMATIC PLANTS EXPOSED TO HLB IN A COMMERCIAL *Citrus* ORCHARD IN TAMAULIPAS, MEXICO

Jesús G. García Olivares[1], Homar R. Gill Langarica[1] and José A. Narváez Zapata[1]

ABSTRACT

Huanglongbing (HLB) is a devastating disease of citrus characterized by yellow spots on the foliage, leaf chlorosis, small and deformed fruits, premature leaf drop, and tree decline which is caused by Candidatus *Liberibacter asiaticus*. The state of Tamaulipas in Mexico is one of the main producers of *Citrus* in the country. Given the arrival of HLB in the region, this work focuses on analyzing the individual rhizosphere from symptomatic and asymptomatic orange, lemon, and grapefruit plants cultivated in an orchard in the state. Microbial profiles were analyzed by using a metagenomic approach and bioinformatic tools. The results show that microbial diversity is different between symptomatic and asymptomatic lemon plants. Particularly, the asymptomatic grapefruit plants show the lowest microbial diversity. In general, all *Citrus* species show differences in their bacterial population structure, identifying groups related to beneficial bacteria, and differences in microbial diversity, according to the presence of HLB symptoms.

Additional keywords: Citrus, diversity, HLB, metagenomic

RESUMEN

Microorganismos en la rizosfera de plantas sintomáticas y asintomáticas expuestas a HLB en un huerto comercial de cítricos en Tamaulipas, México

Huanglongbing (HLB) es una enfermedad devastadora de los cítricos que se caracteriza por manchas amarillas en el follaje, clorosis de las hojas, frutos pequeños y deformados, caída prematura de las hojas y deterioro de los árboles, causada por Candidatus *Liberibacter asiaticus*. El estado de Tamaulipas en México es uno de los principales productores de cítricos en el país. Ante la llegada del HLB a la región, este trabajo se enfoca en analizar las muestras de rizosfera de plantas sintomáticas y asintomáticas de naranja, limón y toronja cultivadas en un huerto del estado. Las diferencias en el perfil microbiano se analizaron utilizando un enfoque metagenómico y herramientas bioinformáticas. Los resultados muestran que la diversidad microbiana es diferente entre plantas de limón sintomáticas y asintomáticas. En particular, las plantas de toronja asintomáticas muestran la menor diversidad microbiana. En general, todas las especies de cítricos muestran cambios en su estructura poblacional bacteriana, identificándose grupos de bacterias benéficas y diferencias en la diversidad microbiana, de acuerdo con la presencia de síntomas de HLB. Los resultados podrían ayudar a desarrollar estrategias de manejo para aumentar la tolerancia al HLB en plantas de cítricos.

Palabras clave adicionales: Cítricos, diversidad, HLB, metagenómica

INTRODUCTION

Huanglongbing (HLB) is a disease that affects the phloem of plants, including the roots, and causes symptoms such as leaf spots, fruit drop and deformation, and reduced production, which can lead to plant death, depending on the species, between 5 and 8 years after infection (Márquez *et al*., 2018). In Mexico, HLB was first detected in July 2009 in the Yucatan Peninsula. By November 2015, the disease had spread to 347 municipalities in 18 citrus-producing states (Garza *et al.*, 2017). In the northeast of Mexico in the state of Tamaulipas, HLB is present in all *Citrus* orchards. *Citrus* production is a key agricultural activity in Mexico, especially for export. Tamaulipas, Veracruz and San Luis Potosí are the main producing states (Biblioteca de Publicaciones Oficiales, 2022). At the end of 2021, Mexico had 625 thousand hectares of citrus crops, of which

---

Received: October 19, 2024 Accepted: February 7, 2025

Instituto Politécnico Nacional- CBG. Blvd. del Maestro esq. Elías Piña, Col. Narciso Mendoza, Reynosa, Tamaulipas, México. e-mail: jggarcia@ipn.mx; hgill@ipn.mx; jnarvaez@ipn.mx (corresponding author).

55.1% are dedicated to oranges, 33.4% to lemons and 10.5% to other varieties of citrus (SENACICA, 2022). Currently, there are no commercial varieties, resistant grafts or curative methods for HLB. Prevention is therefore essential. Control measures include the use of certified plant material, eradication of infected trees and chemical control of the insect vector (Qureshi *et al*., 2014). Also, efforts to control HLB have focused on correcting nutritional deficiencies through foliar and soil nutrition to maintain the productivity of affected trees (Shahzad *et al*., 2020).

Chemical control has been one of the most widely used tools, but it offers only limited protection and is harmful to beneficial entomofauna and public health (Qureshi *et al*., 2014). In addition, these chemical control methods can change the microbial communities of the rhizosphere and reduce the populations of microorganisms beneficial for HLB tolerance in citrus plants (Ginnan *et al*., 2020). Therefore, it is crucial to develop sustainable alternatives to control HLB (Blacutt *et al*., 2020). Since microorganisms in the rhizosphere, phyllosphere and endosphere of citrus affect plant health and growth, it is important to study how HLB influences the diversity and structure of bacterial communities. Using the traditional and culture-independent microbiological approach in combination with bioassays against *Liberibacter crescens*, a cultivable relative of "Candidatus *Liberibacter asiaticus*", it was possible to identify in the roots, stems and leaves of different citrus plantations some bacteria and fungi as potential biocontrol agents against HLB (Blacutt *et al*., 2020). A restructuring of the bacterial community has also been demonstrated in the rhizosphere of *C. sinenesis* infected by HLB (Trivedi *et al*., 2012).

In general, it has been proposed that in *Citrus* roots during HLB infection some specific microbial communities are reduced while some beneficial microorganisms are induced, although only in tolerant plants they accumulate mostly (Ginnan *et al*., 2020). In Tamaulipas, Mexico, no studies have been conducted on the microbial communities associated with the rhizospheres of citrus infected by HLB. Therefore, this study focuses on understanding the structure and microbial diversity of rhizospheres of different species of symptomatic and asymptomatic citrus with HLB in the zone, which could be useful to identify bacterial groups with beneficial potential against HLB infection in this important group of plants.

MATERIALS AND METHODS

Sampling. For the evaluation of HLB, samples were taken from 5 points (200 g) of rhizosphere in representative trees of each species; orange (*Citrus × sinensis*), lemon (*Citrus latifolia*) and grapefruit (*Citrus paradisi*) from a production orchard located in Padilla, Tamaulipas (24°05'07.2"N 99°05'10.8"W) during the 2024 production season. This location was selected because it was reported as one of the *Citrus* producing regions with the highest incidence of HLB in the state (SIAP, 2018). The symptomatology of the plants was evaluated by visual inspection considering the symptoms associated with HLB such as yellow spots on the leaves, chlorosis, small and deformed fruits, premature leaf fall, and tree deterioration in each one of these species. Asymptomatic plants were those plants that did not exhibit any visible symptoms of the disease and that were associated by their proximity to those plants with severe and evident symptoms. This study focused on analyzing the rhizosphere of plants and no determinations were made on plant tissues. This limitation makes it impossible to confirm if the symptom-free plants are entirely free of the HLB pathogen. However, their seemingly healthy conditions enable a comparison of their rhizospheres with those of severely affected plants.

DNA extraction and sequencing. Metagenomic DNA was extracted from rhizosphere samples (1 g) using the Quick-DNA Fecal/Soil Microbe Miniprep Kit from Zymo Research. Sequencing was performed at the Laboratorio Nacional de Nutrigenómicos y Microbiomática, Instituto Politécnico Nacional campus (LAMNDA-IPN) with an Illumina Miniseq sequencer using the Illumina reference guide (Illumina document no. 1000000025416 v09). The V4 region (~250 bp) of the bacterial 16S rRNA gene of each amplified DNA sample was specifically sequenced with the universal primers 27F (5' AGAGTTTGATCMTGGCTCAG

3') and 1492R (5' GGTTACCTTGTTACGACTT 3'). Detailed review of the reads was performed using the dada2 package v1.30.0 (Callahan *et al*., 2016). Reads were filtered and preprocessed, including quality filtering, identification of unique sequences, calculation of error matrices, and correction of unique sequences. Sequence pairs were then assembled, chimeras were corrected, and a table of sequences and their abundance in each sample was created. Taxonomy assignment was done using the "rdp_train_set_16.fa.gz" database.

Microbial structure and diversity. Species diversity (Shannon index) found in the rhizospheres of plants with severe symptoms and those free of symptoms of HLB was obtained from the Lumina 16S Metagenomics report. Heat maps and population structure histograms were created from the taxa assignment tables generated in the Lumina report using the gplots and ggplot2 packages, respectively, in RStudio ver. 4.3.2.

RESULTS AND DISCUSSION

In the present study, the microbial communities of the rhizospheres of symptomatic and asymptomatic orange (*Citrus* × *sinensis*), lemon (*Citrus* × *latifolia*), and asymptomatic grapefruit (*Citrus paradisi*) plants showed an average diversity index around 3 in all lemon and orange samples, but a lower diversity (2.289) in the asymptomatic grapefruit sample, which is a *Citrus* species more tolerant to HLB, since it displays genes related to the SA defense mechanism (Table 1; Gao *et al*., 2023). Changes in microbial diversity were also observed in HLB-infected leaves of mandarin (*C. reticulata* Blanco), where specifically a reduction in the abundance of OTUs related to the endophytic fungal fraction was also observed (Yan *et al*., 2021). The analysis of microbial diversity in citrus rhizospheres is important since this is the compartment with the greatest biodiversity in relation to the associated soil and other plant tissues. In addition, the bacterial community in general is more diverse than the fungal community (Xi *et al*., 2023). Using the metagenomic approach in rhizospheres of a *Citrus* plantation (*Citrus sinensis* Osbeck cv. Newhall and *Citrus sinensis* L. Osb × *Poncirus trifoliate* L.) infected with HLB from acidified soils, it was shown that soil amendments enrich the diversity and structure of the endophytic microbial community in the roots, represented by the enrichment of beneficial microorganisms (Li *et al*., 2022).

Table 1. Diversity index (Shannon) and number of identified species in the rhizosphere of citrus (2024 production season)

Sample	Specie	Shannon index	Number of species
NI	Orange	3.163	584
NS	Orange	3.148	567
LS	Lemon	2.805	231
LI	Lemon	3.314	529
PS	Grapefruit	2.289	786

Regarding population structure, the current study shows that only a few specific taxa switched between asymptomatic and symptomatic plants (Figure 1). Changes in the microbial population structure have been previously documented in rhizospheres of *Citrus sinensis* Osbeck cv. Newhall and *Citrus sinensis* L. Osb × *Poncirus trifoliate* L infected with HLB when acidified soil amendments are applied, which improves tolerance and enriches functional categories involved in host-microbial interactions as well as nitrogen and sulfur metabolism (Li *et al*., 2022). A restructuring of the bacterial community in the rhizosphere has also been observed in *C. sinenesis* where Proteobacteria counts are higher in healthy *Citrus* rhizospheres, while Acidobacteria, Actinobacteria and Firmicutes were more abundant in HLB-infected citrus rhizospheres (Trivedi *et al*., 2012).

In the present study the main phyla on average for lemon were Actinobacteria (18 %), Protobacteria (21 %), Acidobacteria (17 %) and

Firmicutes (7 %), in orange; Actinobacteria (43 %), Protobacteria (21 %), Acidobacteria (16 %) and Firmicutes (5 %), and in the asymptomatic sample of grapefruit; Actinobacteria (1 %), Protobacteria (90 %), Acidobacteria (13 %), and Firmicutes (6 %). Using the metagenomic approach another study has also shown that the microbial core of *Citrus* rhizospheres is composed of four bacterial phyla (Proteobacteria, Actinobacteria, Acidobacteria, and Bacteroidetes), one archaea (*Thaumarchaeota*), and two fungal phyla (Ascomycota and Basidiomycota) (Lombardo *et al.*, 2024). In the present study, significant differences were observed between symptomatic and asymptomatic orange plants for the taxa Planctomycetaceae and Rubrobacteraceae. The asymptomatic grapefruit plant is abundant in OTUs related to the taxa Solirubrobacteraceae (Figure 2). The taxa Rubrobacteraceae also showed strong differences in abundance between rhizospheres of the different species (i.e. orange and lemon). Streptomycetaceae taxa also shows an increase in the asymptomatic lemon regarding its symptomatic counterpart. A related study using bacterial co-occurrence network shows that the taxa Streptomycetaceae may act as a functional unit against HLB (Park *et al.*, 2024).

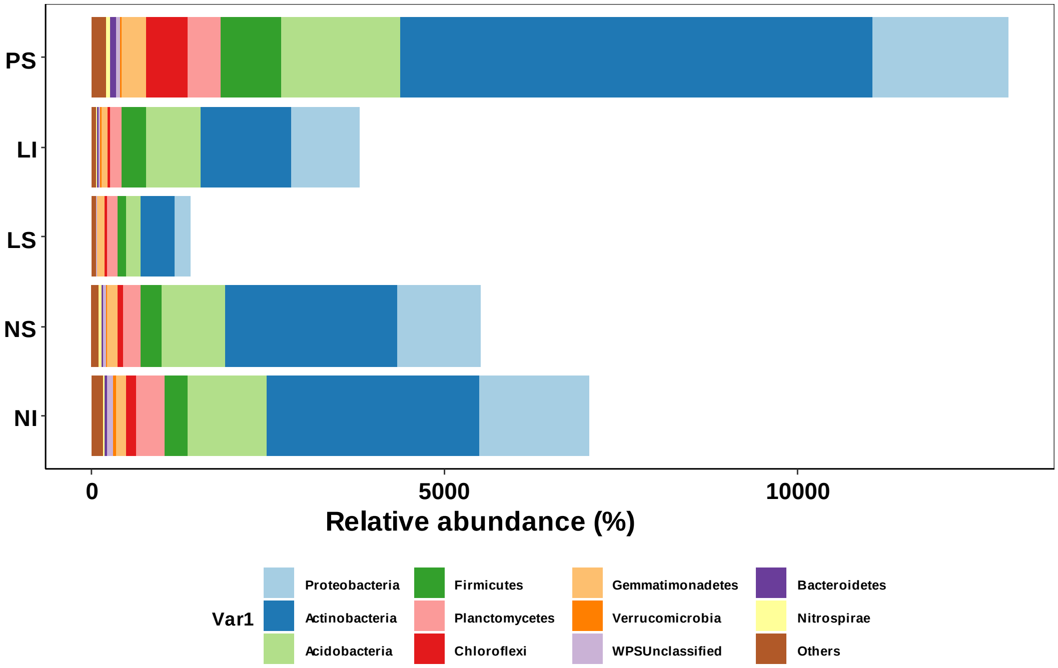

Figure 1. Relative abundance of *Citrus* rhizosphere, collected from representative soils of symptomatic and asymptomatic citrus of HLB, from Padilla, Tamaulipas, production season, 2024. NS: asymptomatic orange. NI: symptomatic orange. LS: asymptomatic lemon. LI: symptomatic lemon. PS: Asymptomatic grapefruit.

Finally, knowledge about the abundance of Solirubrobacteraceae in the rhizosphere of the most tolerant grapefruit species (*C. paradisi*) and the differences between the taxa Planctomycetaceae and Rubrobacteraceae in asymptomatic orange plants (*Citrus* × *sinensis*) can contribute to the knowledge about management strategies and specific stimulation of some bacterial groups in rhizospheres of *Citrus* plants to increase HLB tolerance traits in this important group of plants for Mexico.

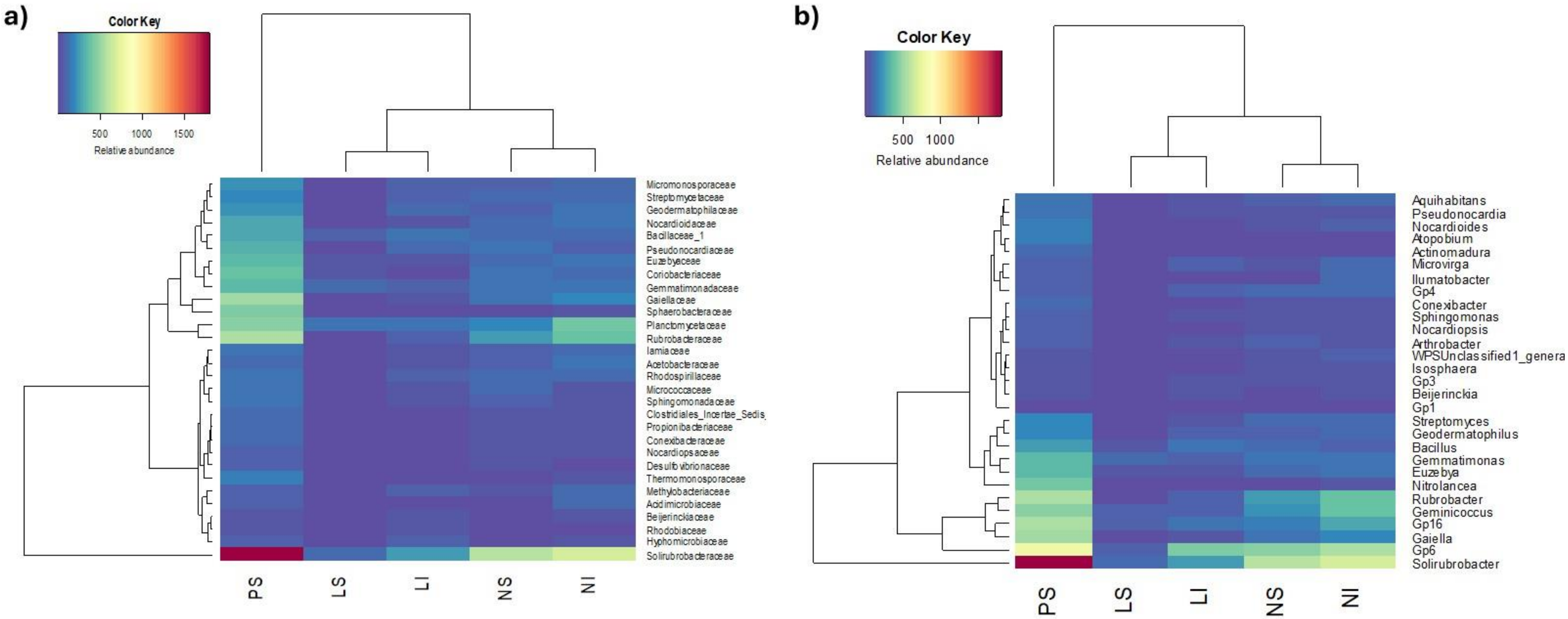

Figure 2. Heat map analysis of the main family (a) and genera taxa (b) of rhizosphere in different citrus genera with symptomatic and asymptomatic HLB from Padilla, Tamaulipas, production season, 2024. NS: asymptomatic orange. NI: symptomatic orange. LS: asymptomatic lemon. LI: symptomatic lemon. PS: Asymptomatic grapefruit.

CONCLUSION

Understanding the abundance of Solirubrobacteraceae in the rhizosphere of tolerant grapefruit (*C. paradisi*) and differences in Planctomycetaceae and Rubrobacteraceae in asymptomatic orange (*Citrus* × *sinensis*) can help develop management strategies to stimulate specific bacterial groups, potentially increasing HLB tolerance in citrus plants in Mexico.

ACKNOWLEDGMENTS

This research was supported by the projects SIP-20241414 and SIP-20241679 from Instituto Politécnico Nacional (IPN).

LITERATURE CITED

1. Biblioteca de Publicaciones Oficiales. 2022. Producción de cítricos en México. Gobierno de México: https://www.gob.mx/publicaciones/articulos/produccion-de-citricos-en-mexico
2. Blacutt, A., Ginnan, N., Dang, T., Bodaghi, S., Vidalakis, G., Ruegger, P *et al*. (2020). An *in vitro* pipeline for screening and selection of citrus-associated Microbiota with potential anti-“Candidatus Liberibacter asiaticus” properties. Applied and Environmental Microbiology 86(8) 1-18.
3. Callahan, B.J., P.J. McMurdie, M.J Rosen, A.W. Han, A.J.A. Johnson and S.P. Holmes. 2016. DADA2: High-resolution sample inference from Illumina amplicon data. Nature Method 13: 581-583.
4. Gao, C., C. Li, Z. Li, Y. Liu, J. Li, J. Guo *et al*. 2023. Comparative transcriptome profiling of susceptible and tolerant citrus species at early and late stage of infection by “Candidatus Liberibacter asiaticus”. Frontiers in Plant Science 14:1-13.
5. Garza-Saldaña, J.J., S. Varela-Fuentes and W. Gómez-Flores. 2017. Métodos para la detección presuntiva de Huanglongbing (HLB) en cítricos CienciaUAT 11(2): 93-104
6. Ginnan, N.A., T. Dang, S. Bodaghi, P.M. Ruegger, G. McCollum, G. England. *et al*. 2020. Disease-induced microbial shifts in citrus indicate microbiome-derived responses to huanglongbing across the disease severity spectrum. Phytobiomes Journal 4(4): 375-387.
7. Li, B., Y. Wang, T. Hu, D. Qiu, F. Francis, S. Wang and S. Wang. 2022. Root-associated Microbiota response to ecological factors: Role of soil acidity in enhancing citrus tolerance to Huanglongbing. Frontiers in Plant Science 13.
8. Lombardo, M.F., Y. Zhang, J. Xu, P. Trivedi, P. Zhang, N. Riera *et al*. 2024. Global citrus root microbiota unravels assembly cues and core members. Frontiers in Microbiology 15: 1-18.
9. Márquez-Pérez, F.J., J.L. Flores-Sánchez, L. Rodríguez Mejía, J. Márquez Gómez, S.J. Michereff, V. Ancona *et al*. 2018. Progress and spatial pattern of huanglongbing in Persian lime in Nayarit, Mexico. Revista Bio Ciencias 5(nesp1), e351.
10. Park, J.W., W.E. Braswell and M. Kunta. 2023. Co-occurrence analysis of citrus root bacterial Microbiota under citrus greening disease. Plants 13(1): 80.
11. Qureshi, J.A., B.C. Kostyk and P.A. Stansly. 2014. Insecticidal suppression of Asian citrus psyllid Diaphorina citri (Hemiptera: Liviidae) vector of huanglongbing pathogens. PloS One 9(12): e112331.
12. SENACICA. 2022. Análisis de impacto económico ante un posible establecimiento y dispersión del cancro de los cítricos en México en áreas comerciales. https://n9.cl/1f71p
13. Shahzad, F., C. Chun, A. Schumann and T. Vashisth. 2020. Nutrient uptake in Huanglongbing-affected sweet orange: Transcriptomic and physiological analysis. Journal of the American Society for Horticultural Science145(6): 349-362.
14. SIAP. (Servicio de Información Agroalimentaria y Pesquera) 2018. Infografía: Huanglongbing, Análisis Epidomiológico Tamaulipas. https://prod.senasica.gob.mx/SIRVEF/ContenidoPublico/HLB/Infografias/Tamaulipas.pdf
15. Trivedi, P., Z. He, J.D. Van Nostrand, G. Albrigo, J. Zhou and N. Wang. 2012.

Huanglongbing alters the structure and functional diversity of microbial communities associated with citrus rhizosphere. The ISME Journal 6(2): 363-383.

16. Xi, M., E. Deyett, J.E. Stajich, A. El-Kereamy, M.C. Roper and P.E. Rolshausen, 2023. Microbiome diversity, composition and assembly in a California citrus orchard. Frontiers in Microbiology 14: 1-11.
17. Yan, H., B. Zhou, B. Jiang, Y. Lv, M.D. Moniruzzaman, G. Zhong and Y. Zhong 2021. Comparative analysis of bacterial and fungal endophytes responses to *Candidatus Liberibacter asiaticus* infection in leaf midribs of *Citrus reticulata* cv. Shatangju. Physiological and Molecular Plant Pathology 113(101590): 1-10.