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Clues into the metagenome of Huanglongbing infected Citrus by analysis of ancillary sequences from Ion Torrent whole genome *Candidatus Liberibacter asiaticus* sequencing

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**Abstract:**

Huanglongbing (HLB) is a globally devastating disease of citrus. Presently, three etiological agents are associated with HLB and include; *Candidatus Liberibacter asiaticus* (CLas), *Candidatus Liberibacter americanus*; and *Candidatus Liberibacter africanus*. Attempts to determine alternate (non-Liberibacter) associated etiological agents of HLB have been performed, namely by metagenomic analyses with HLB phenotypic citrus of phloem tissue isolated from bark [ ] and whole leaf midribs [ ]. These reports indicated a strong correlation for Liberibacter species associated with HLB etiology, but they do not indicate the presence of other significant associated etiological agents. Utilizing both PCR and non-PCR based metagenomic strategies; these previous reports present an undersized view (relating to inherent technique limits, restricted sample scope, and/or bacterial bias) into the etiology of HLB. Here we report ancillary contiguous metagenomic sequences contained amongst whole CLas genome amplification contiguous sequences that were sequenced on the next generation Ion Torrent PGM sequencing system.

Within these ancillary sequences, a diverse metagenomic community is present, indicating a rich diversity of both prokaryotic and eukaryotic organisms that are unique and in common within these globally isolated HLB citrus samples. Although this report does not represent a complete metagenomic study of HLB diseased citrus, it does suggest that the use of Ion Torrent PGM sequencing system can be employed for metagenomic analysis. These findings justify a more

complete analysis of the metagenome of HLB etiology, which may help further elucidate the HLB disease complex.

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### Clues into the metagenome of Huanglongbing infected *Citrus* by analysis of ancillary sequences from Ion Torrent whole genome *Candidatus Liberibacter asiaticus* sequencing

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Huanglongbing (HLB) is a globally devastating disease of citrus. Presently, three etiological agents are associated with HLB and include; *Candidatus Liberibacter asiaticus* (CLAs), *Candidatus Liberibacter americanus*; and *Candidatus Liberibacter africanus*. Attempts to determine alternate (non-Liberibacter) associated etiological agents of HLB have been performed, namely by metagenomic analyses with HLB phenotypic citrus of phloem tissue isolated from bark [1] and whole leaf midribs [2]. These reports indicated a strong correlation for Liberibacter species associated with HLB etiology, but they do not indicate the presence of other significant associated etiological agents. Utilizing both PCR and non-PCR based metagenomic strategies; these previous reports present an undersized view (relating to inherent technique limits, restricted sample scope, and/or bacterial bias) into the etiology of HLB. Here we report ancillary contiguous metagenomic sequences contained amongst whole CLAs genome amplification contiguous sequences that were sequenced on the next generation Ion Torrent PGM sequencing system. Within these ancillary sequences, a diverse metagenomic community is present, indicating a rich diversity of both prokaryotic and eukaryotic organisms that are unique and in common within these globally isolated HLB citrus samples. Although this report does not represent a complete metagenomic study of HLB diseased citrus, it does suggest that the use of Ion Torrent PGM sequencing system can be employed for metagenomic analysis. These findings justify a more complete analysis of the metagenome of HLB etiology, which may help further elucidate the HLB disease complex.

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