Identification of *Candidatus* Liberobacter-induced Small RNAs for early diagnosis of HLB Citrus Greening

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Citrus greening or "Huanglongbing" (HLB), caused by bacteria *Candidatus* Liberobacter, is one of the most devastating diseases of citrus. The serious spread of HLB in Florida is of great concern to the citrus industry as well as scientists. Recent detection of Asian citrus psyllids in several additional states, including California, makes it an urgent need for scientist to develop effective measures to prevent its further spread.

To prevent its further spread, early diagnosis before the appearance of the dreadful symptoms is particularly important. However, the unculturable nature of the bacteria and their low concentration and uneven distribution in the hosts make it extremely difficult to detect HLB infection.

Instead of focusing on the bacteria, we took advantage of host rapid defense responses and propose to identify unique host biomarkers for early diagnosis of HLB. Some host small RNAs are rapidly and specifically induced by pathogens, which makes them one of the most attractive markers for early diagnosis.

Our goal of this project is to identify HLB specific small RNAs that can be used as early diagnosis markers. The objectives for the second year include: a). Small RNA isolation and small RNA library construction on plant tissue collected from control and HLB-infected plants at 5 weeks and 9 weeks post infection; and b). Small RNA library sequencing by high- throughput deep sequencing.

We have completely accomplished what we proposed in the second year, and we even initiated the data analysis and small RNA validation at the end of the second year. The detailed progress is listed below:

1. We have isolated and purified small RNAs from HLB- infected plants and HLB-free control plants at 5 weeks and 9 weeks post inoculation.
2. The small RNAs were ligated with 5'- and 3'- RNA adaptors and reverse transcripted into cDNA libraries.
3. Small RNA libraries were deep-sequenced and total of over 4 million reads were obtained. After removing the sequences that match non-sRNAs, such as tRNA, rRNA, snoRNA and snRNA and removing the sequences longer or shorter than expected sizes, we obtained total of 2,656,558 reads.
4. We then assembled all the 483,092 citrus ESTs that are publicly available (included the large dataset of the newly released citrus ESTs) and got 26,153 contigs and 255,108 singlets.
5. We mapped all the small RNA reads onto the citrus contigs and singlets. Excitingly, total of 704,773 small RNAs (excluded the tRNA and rRNA matching reads) can match citrus sequences.
6. We discovered 28 citrus miRNAs that are conserved within Arabidopsis and citrus, which confirmed the success of our small RNA cloning and data analysis. We also found some of the small RNAs are differentially expressed in different libraries. Small RNA validation and cross-hybridization analysis are underway.

We expect to identify several HLB-induced small RNAs, which will have the potential to be developed into early diagnosis markers.

Deep Sequencing Results

	Control 5wpi	HLB 5wpi	Control 9wpi	HLB 9wpi	All libraries
total reads of raw sequences	968,921	1,008,918	1,097,886	999,685	**4,075,410**
number of unique sequences	521,086	468,986	481,568	445,792	1,662,694
> 18nt & <28nt unique sequences	418,130	370,818	367,322	351,301	1,286,097
number of unique sequences hit Rfam	205,763	162,076	124,003	137,329	547,752
total reads hit Rfam	394,771	348,075	346,463	329,543	1,418,852
nunber of unique sequencs after remove Rfam	212,367	208,742	243,319	213,972	718,345
total reads after remove Rfam	574,150	660,843	751,423	670,142	2,656,558
number of unique sequences hit citrus Uniset (assembled)	54,492	53,911	53,092	55,227	**176,059**
total reads hit citrus Uniset (assembled new EST)	136,905	165,735	184,938	217,195	**704,773**