1.11 In vitro Culture of the Fastidious Bacteria Candidatus Liberibacter asiaticus in Association with Insect Feeder Cells

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Ca. Liberibacter asiaticus (LAS) is vectored by psyllids and is able to proliferate inside the insect. We therefore hypothesize that insect cells could act like feeder cells, providing nutrients in a continuous way and a favorable environment to the bacteria. Various insect cell lines and sources of LAS inoculum were tested in an empirical way to select for a suitable cell line and to establish a protocol for primo-cultures. LAS presence in the inoculated cell cultures was checked by direct PCR and confirmed by sequencing of the amplicons. Nine different cell lines were tested from Mamestra, Spodoptera, Drosophila, Aedes, and Diaphorina insects. Mamestra and Spodoptera cell lines were not suitable for LAS growth. One Aedes and two Drosophila cell lines sustained Liberibacter survival and growth. Diaphorina cell lines were recently received and are under investigation regarding their capacity to maintain the bacteria. To reach higher bacterial concentrations, we analyzed metabolic pathways potentially encoded by the Ca. Liberibacter genome to define limiting factors and/or growth inhibitors, and we complemented the primo-cultures with various additives (sugars, vitamins, etc.). A culture of LAS was obtained with Aedes cells as feeder cells. This culture has been continuously growing for 9 months and 16 successive transfers. We are currently working on axenization of this culture. By adding selected additives in our LAS/Aedes co-cultures, we increased the yield of LAS (~1.10^7 cells/ml), and we are currently looking into the best ways to maintain/increase this concentration and to stock those LAS cultures.