

## ***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 inoculums were tested in an empiric way to select for the 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*, *Diaphorina* insects. *Mamestra* and *Spodoptera* cell lines were not suitable for LAS growth. Two *Drosophila* and one *Aedes* cell lines showed to sustain *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 released *Ca. Liberibacter* genome sequences to define limiting factors and/or growth inhibitors and we complemented the primo-cultures with various additives (sugars, vitamins,...).

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 the axenization of this culture.

We also succeeded in getting high concentrations of LAS ( $>10^6$  cells/ml) in an *Aedes* cell culture + additives and are currently looking into best ways to maintain/increase those concentration levels and to stock those LAS cultures.

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