# COST STSM Scientific Report – Elena Gonella

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**COST Office science Officer:** Mr. Albino MAGGIO, amaggio@cost.esf.org

**COST STSM Manager:** Dr. Matthew DISKINSON, matthew.dickinson@notthnham.ac.uk

**STSM Title:** Molecular characterization of the microbial communities affiliated to different species and populations of psyllid and cixiid vectors of phytoplasmas

**Applicant:** Dr. Elena GONELLA; DIVAPRA - Entomologia e Zoologia Applicate all'Ambiente "Carlo Vidano", Facoltà di Agraria, University of Torino; e-mail: elena.gonella@unito.it

**Host:** Dr. Maria KOLBER; Fitolab Plant Pest Diagnostic and Advisory Ltd.; e-mail kolber.maria@fitolab.hu

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# Molecular characterization of the microbial communities affiliated to different species and populations of leafhopper and cixiid vectors of phytoplasmas

## Background

Sap-feeding insects are known to host several bacterial symbionts providing nutrients missing in their unbalanced diets. Among these insects, different Auchenorrhyncha and Sternorrhyncha species, including several phytoplasma vectors, have been recently described to host huge microbial communities co-occurring with the pathogenic agents. The study of interactions between the insect microbiota and phytoplasmas within the host's body is of great interest, also with the aim of exploiting symbionts to control the transmission of the plant pathogens. Indeed, an innovative control approach would be promising, as currently there is no direct cure for the phytoplasmosis in the plant, and the only technique applied in the practice of crop management is the control of the vectors with insecticides.

## Purpose of the visit

The aim of this STSM was to understand the differences of the microbial communities affiliated to leafhopper and planthopper vectors of Stolbur (Group 16Sr XII) and Group 16Sr V phytoplasmas to potato and grapevine residing in different geographical areas and processed with different DNA extraction methods. Several populations (from Hungary, Romania and Russia) of the cixiids *Reptalus panzeri*, *R. quinquecostatus*, *R. cuspidatus*, *R. melanochetus*, and *Hyalesthes obsoletus* were taken into consideration to assess the variability of microbiotas; furthermore Hungarian specimens of the cicadellids *Scaphoideus titanus* and *Oncopsis alni* were analyzed. Different sample manipulation methods were compared, namely DNA extraction with the LC Magna Pure DNA isolation robot and with a modified CTAB method, to evaluate the possibility of comparing samples processed in different ways to characterize their microbial communities. Moreover, molecular analyses were carried out on the samples for determination of phytoplasma infection.

## Description of the work carried out during the visit

Insect sampling

Joint samplings were organized to collect insects in Hungarian vineyards and potato fields, in different sites of southern, central and western Hungary. Morphological identification of the collected cixiids and cicadellids from Hungary and of previously collected insects from Romania was performed on a total of 40 insect individuals, which result to belong to the following species: *R. quinquecostatus, H. obsoletus, S. titanus,* and *O. alni*. (Table 1).

DNA extraction

The collected samples were submitted to nucleic acid isolation with a modified CTAB method (Raddadi *et al*., 2011). Further insect samples (belonging to the following species: *R. panzeri*, *R. quinquecostatus, R. cuspidatus, R. melanochetus,* and *H. obsoletus*, and previously collected in Romania and Russia) were submitted to DNA extraction with the LC Magna Pure, Tissue kit II (Roche).

PCR screenings for detection of microbial symbionts of cixiids and cicadellids

The total DNA isolated from single cixiid individuals was used as template in PCR amplifications on the 16S rRNA gene with primers specific for the two primary symbionts "*Candidatus* Vidania fulgoroideae" and "*Candidatus* Sulcia muelleri", previously found with high infection rates in Italian population of *H. obsoletus* (Gonella *et al*., 2011). Reaction and cycling conditions were as in the original paper. None of the tested samples were positive for *Vidania*, while a total of 9 samples out of 38 (23.7% of individuals) gave a positive amplification signal for *Sulcia* (Table 1). This symbiont was found in specimens of two species: *H. obsoletus* and *R. quinquecostatus*, while it was not detected in other *Reptalus* species.

*Sulcia* was found with high infection rates (between 50 and 100%) in all the *H. obsoletus* samples collected in different geographical areas, confirming the strict symbiotic relation with this host. The differences in detected infection percentages are due to the low number of tested samples, and do not seem to be linked to different DNA extraction methods.

*Sulcia* was also observed in Romanian *R. quinquescostatus* individuals with similar infection rates (around 10%) among samples submitted to the two different DNA extraction methods, suggesting they are both suitable for the study of the symbiotic microbiota of planthoppers. The symbiont was not detected in Hungarian samples, which could have a different microbial community, even if a higher number of individuals should be tested. Moreover, the low overall infection rate of *Sulcia* in *R. quinquecostatus* suggests that in this species *Sulcia* is not a primary symbiont; alternatively the *Sulcia* strain affiliated to *R. quinquecostatus* could be rather different from the strain associated to *H. obsoletus* (on which the primer pair was designed), and could be only difficultly amplified by this PCR essay.

DNA samples isolated from leafhoppers were tested for the secondary symbiont *Asaia* sp., which was previously observed in Italian *S. titanus* individuals, and which has a great potential for the development of symbiotic control strategies (Crotti *et a*l., 2009). PCR assays were carried out as reported in the original paper. Nor the *S. titanus* specimens or the *O. alni* individuals were positive for the symbiont (Table 1), suggesting that a higher number of samples should be employed to properly assess the association between Asaia and the Hungarian insect populations.

PCR/RFLP for identification of phytoplasma

Cixiid and cicadellid samples were tested for phytoplasma infection using P1/P7 and F2/R2 primers. The RFLP analysis, using *Tru*I restriction enzyme, of the positive samples showed an infection by Stolbur (16SrXII) phytoplasmas in 13 cixiids (1 *R. panzeri* and 10 *R. quinquecostatus* from Romania, and 2 *H. obsoletus* from Russia) and in 1 *O. alni*, while an infection by group 16SrV phytoplasmas was observed in 4 *O. alni* (Table 1). No phytoplasma infection was found in *S. titanus* individuals, confirming the absence of Flavescence dorée in Hungary.

**References**

Crotti E., Damiani C., Pajoro M., Gonella E., Rizzi A., Ricci I., Negri I., Scuppa P., Rossi P., Ballarini P., Raddadi N., Marzorati M., Sacchi L., Clementi E., Genchi M., Mandrioli M., Bandi C., Favia G., Alma A., Daffonchio D. 2009 *Asaia,* a versatile acetic acid bacterial symbiont, capable of cross-colonizing insect of phylogenetically distant genera and orders. Environmental Microbiology 11 (12):3252-3264.

Gonella E., Negri I., Marzorati M., Mandrioli M., Sacchi L., Pajoro M., Crotti E., Rizzi A., Clementi E., Tedeschi R., Bandi C., Alma A., Daffonchio D. 2011 Bacterial endosymbiont localization in *Hyalesthes obsoletus*, the insect vector of Bois Noir in *Vitis vinifera.* Applied and Environmental Microbiology 77(4):1423-1435.

Raddadi N., Gonella E., Camerota C., Pizzinat A., Tedechi R., Crotti E., Mandrioli M., Bianco P.A., Daffonchio D and Alma A. 2010. '*Candidatus* Liberibacter europaeus' sp. nov. that is associated with and transmitted by the psyllid *Cacopsylla pyri* apparently behaves as en endophyte rather than a pathogen. Environmental Microbiology 13(2):414–426.

**Table 1:** List of insect samples with their geographical origin and the used DNA extraction method. For each group the percentage of infected specimens is indicated for every tested symbiont and for the phytoplasmas. The symbiotic bacteria *Vidania* and *Sulcia* were searched in planthoppers, while *Asaia* was sought in leafhoppers. The occurrence of phytoplasmas was tested in all the samples.

| Insect species | Origin | DNA extraction method | No. samples | % infected by *Vidania* | % infected by *Sulcia* | % infected by *Asaia* | % infected by Stolbur phytoplasmas | % infected by 16SrV phytoplasmas |
|---|---|---|---|---|---|---|---|---|
| *R. panzeri* | Romania | LC Magna Pure | 4 | 0 | 0 | - | 25 | - |
| *R. quinquecostatus* | Romania | LC Magna Pure | 11 | 0 | 9.1 | - | 54.5 | - |
| *R. quinquecostatus* | Romania | CTAB method mod. | 9 | 0 | 11.1 | - | 44.4 | - |
| *R. quinquecostatus* | Hungary | CTAB method mod. | 2 | 0 | 0 | - | 0 | - |
| *R.cuspidatus* | Russia | LC Magna Pure | 2 | 0 | 0 | - | 0 | - |
| *R. melanochetus* | Russia | LC Magna Pure | 1 | 0 | 0 | - | 0 | - |
| *H.obsoletus* | Russia | LC Magna Pure | 3 | 0 | 100 | - | 66.7 | - |
| *H.obsoletus* | Romania | LC Magna Pure | 2 | 0 | 50 | - | 0 | - |
| *H.obsoletus* | Romania | CTAB method mod. | 3 | 0 | 66.7 | - | 0 | - |
| *H.obsoletus* | Hungary | CTAB method mod. | 1 | 0 | 100 | - | 0 | - |
| *S. titanus* | Hungary | CTAB method mod. | 15 | - | - | 0 | 0 | - |
| *O.alni* | Hungary | CTAB method mod. | 10 | - | - | 0 | 10 | 40 |