Unraveling metabolically active fungal-bacterial diversity in commercial organic vineyard soils

Carmen Biel1, Miriam Guivernau2, Marc Viñas2, Xavier Aranda3, and Felicidad de Herralde3

1IRTA, Sustainable Plant Protection Program, Cabrils, Spain (carmen.biel@irta.cat)
2IRTA, Integral Management of Organic Waste Program, Torre Marimon, Caldes de Montbui, Spain
3IRTA, Fruit Production Program, Torre Marimon, Caldes de Montbui (Spain)

This study aims to assess the impact of the pre-bloom and post-harvest periods on the diversity of metabolically active soil-rhizosphere microbiota in a commercial vineyard in Sant Sadurní d’Anoia, a typical wine producing region (Penedès DO, Catalonia, Spain). Thereby, total genomic DNA and RNA was simultaneously monitored to distinguish total from active bacterial-fungal microbiota, by molecular tools in both periods. The studied organic vineyard had 20 years old plants of the white grape variety of Macabeu and 41B as a rootstock. Soil had last been amended (14 tm/ha of composted cow manure) 5 years before.

The soil was monitored in April 2018 in the pre-bloom period (stages 09 to 12 Eichhorn and Lorenz 1977) and the post-harvest period (October 2018) in 2 different plots of the vineyard: Zone1 (loam texture with permanent cover crop) and Zone 4 (sandy-loam without vegetal cover). Samples soils were obtained at a soil depth of30 cm and 20 cm of distance from a plant (n=4 for each plot and sampling event). Each soil sample was submerged in a DNA/RNA preservative solution at 4⁰C and afterward stored at -20⁰C until the further analysis. In order to quantify and to assess bacterial and fungal diversity (total and active), (RT)qPCR and MiSeq-Illumina analysis (16SrRNA/ITS1rRNA region) were performed.

Results showed that in post-harvest period the bacterial populations were more active in both zones (2 and 5 orders of magnitude in Zone1 and Zone4, respectively) vs. pre-bloom period. Metabolically active fungal population was increased in both plots by 4 orders of magnitude. It is noteworthy to mention that fungal population was present but not active in pre-harvest period. This fact could be explained for the mutualistic microbe interaction and the environmental conditions (soil temperature and soil water content), including grape drop in harvest linked to rainy conditions.

High-throughput sequencing analysis revealed that the microbial diversity was specific for each plot, vine and sampling period. Bacterial population in post-harvest was more diversified but still dominated by Actinobacteria (mainly by Actinomycetales order), Proteobacteria (mainly by Rhizobiales and Pseudomonadales orders). Interestingly, during post-harvest Clostridiales (Firmicutes phylum), present in the pre-bloom period, completely disappeared. Alpha bacterial diversity was higher than fungal one in both plots. Interestingly, the bacterial diversity (H Shannon
index) of metabolically active bacteria (cDNA) was higher during post-harvest season compared to April, suggesting more activity and diversity in the former. On the contrary, fungal diversity was smaller and less uniform in both periods. They were predominated by Ascomycota, Basidiomycota and Zygomycota phyla. Noticeably, the relative abundance (RA) of existing fungal population (DNA) in the soil were highly different compared to the RA of active fungal community (cDNA).

In conclusion, simultaneous RNA/DNA-based molecular biology tools could improve the knowledge of metabolically active microbial populations in vineyard soils under different seasons.

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