

INTRODUCTION:

Microbial communities present in soil and plant play a significant role as growth promoters, control of plant diseases, facilitation of nutrients uptake by plants. Understanding the mode of action of such microbes and their relationship with plants will help the selection of microorganisms, with the scope to develop innovative products (Microbials) to be used in sustainable crop production, gaining a positive impact on agricultural environments in terms of microbial diversity and stability.

GOAL:

A set of microbes will be studied and used in field experiments on selected crop plants. This to evaluate their potential use as Microbials in symbiotic agriculture. Additionally, their mode of action in plants will be unraveled through biochemical analyzes and a transcriptomic approach, coupled with RNA-seq analysis.

EXPERIMENTAL APPROACH:

PART I: Characterisation Microorganisms As Microbial Biocontrol Agents

A set of microbial strains were characterized for various activities and the most prospective strain SA51 *Streptomyces* sp. was selected to evaluate its efficacy under *in vivo* conditions (tomato). Field experiments confirmed the ability of strain SA51 to act as plant growth promoting agent: such promoting activity was also reflected into an increase of fruit production by approximately 7%. We also performed whole genome sequence (WGS) of strain SA51 to further evaluate its novel characteristics at genome level. Based on Sequence analysis with reference genome present on NCBI and 16s rRNA gene BLAST results, strain SA51 was found as *Streptomyces avermitilis*. The draft genome sequences were deposited at NCBI GenBank under BioProject no. [PRJNA545025](#) with an accession no. VEXM000000000.1.

PART II: Evaluation Of A Microbial Consortium For The Biocontrol Of Esca and Flavesence Dorée (FD).

MICOSAT F, a consortium containing strain SA51, was sprayed in two vineyards (*Lambrusco di Sorbara*) grown in Campagnola (RE), for the evaluation of possible biocontrol activity. Results confirmed the antimicrobial activity of MICOSAT F against Esca, but not much against FD. Biochemical aspects of grapes were evaluated and the positive effect of MICOSAT F reflected on the higher stability of most and pigments content. Gene expression of grape following MICOSAT F sprays was studied in controlled conditions. We observed that the microbial consortium profusely induced the upregulation of grapevine genes involved in maintenance of biocontrol and plant growth promotion activity. Almost all the genes were downregulated initially after 0 hpi and 2 hpi, but later from 4 hpi genes like *ACC*, *CHS*, *PAL* & *PER* were significantly upregulated. Particularly, *PR11* and *PR12* genes were significantly upregulated after 4 hpi.

RESULTS

WGS Analysis:

Category	Subcategory	Subsystem	Role
Cofactors, Vitamins, Prosthetic Groups, Pigments	Folate and pterines	Folate Biosynthesis	Dihydrofolate reductase
		Dihydrofolate reductase	
Iron acquisition and metabolism	Siderophores	Siderophore assembly kit	Putative ABC iron siderophore transporter, fused permease and ATPase domains
Iron acquisition and metabolism	no subcategory	Encapsulating protein for DyP-type peroxidase and ferritin-like protein oligomers	Predicted dye decolorizing peroxidase (DyP), YfeX like subgroup
Iron acquisition and metabolism	no subcategory	Heme, hemin uptake and utilization systems in Gram Positives	Heme ABC transporter, cell surface heme and hemoprotein receptor HmuT
Membrane Transport	Cation transporters	Copper Transport System	Repressor CsoR of the copZA operon
Membrane Transport	Cation transporters	Copper transport and blue copper proteins	Copper binding protein, plastocyanin/azurin family
Stress Response	Detoxification	Uptake of selenate and selenite	Various polyols ABC transporter, ATP-binding component
Stress Response	Osmotic stress	Choline and Betaine Uptake and Betaine Biosynthesis	HTH-type transcriptional regulator BetI
Stress Response	Osmotic stress	Choline and Betaine Uptake and Betaine Biosynthesis	High-affinity choline uptake protein BetT
Virulence, Disease and Defense	Resistance to antibiotics and toxic compounds	Beta-lactamase	Beta-lactamase

Table 1: Important pathways and their roles which exclusively present in the strain SA51

- ❖ Strain SA51 was identified as *Streptomyces avermitilis*. Genome data was submitted to NCBI under the accession No. [VEXM000000000.1](#).
- ❖ Genome analysis was done using RAST server and KEGG annotation.
- ❖ Strain SA51 evidenced some intriguing differences related to the unique presence of genes related to folate biosynthesis, to the beta-lactamase production, to the uptake of selenate and selenite and to the iron, copper and niche transport/metabolism.

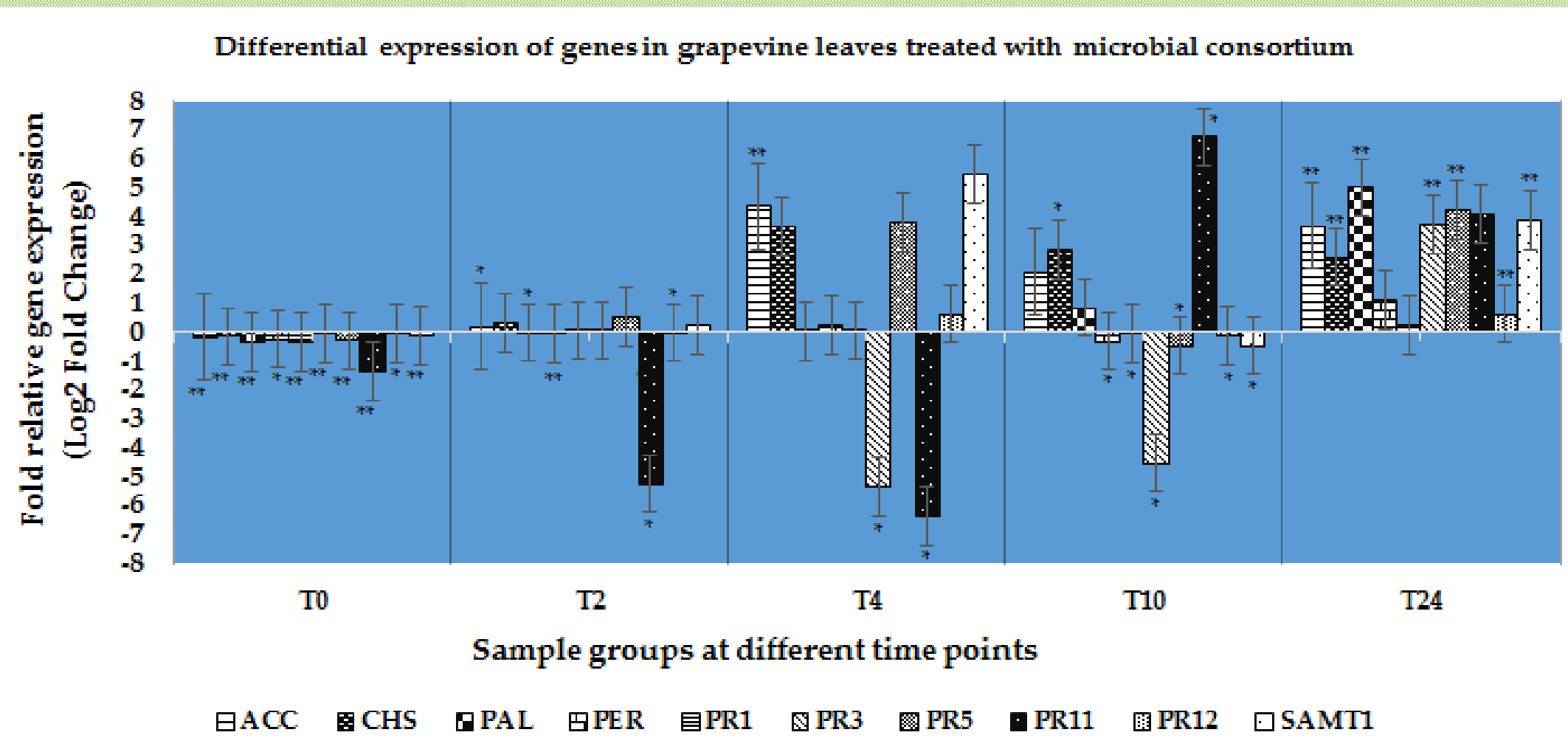


Figure 1: Relative gene expression in treated leaf samples compared with control leaf samples at different time points after inoculation with microbial consortium. Data were normalized on the most stable housekeeping genes Elongation factor (*EF1*) and α -Tubulin (*TA*). Fold change and statistical analysis according to REST. Data are the means $\pm$ SEM from three independent assays. One star (*): significance difference at $p < 0.05$; two stars (**): highly significance difference at $p < 0.01$; no star: no significance difference observed. Three independent biological replicates were used for each sample for the analysis. The threshold fold change difference considered as 1 for Log2 scale.

CONCLUSION:

Results showed that beneficial microbes either singularly or as consortium increase the plant health in terms of growth and protection. WGS analysis of strain SA51 gave us evidences why this strain is so prospective due to its unique nature of producing various secondary metabolites/pathways. As a consortium its effect on grapevine significantly increased grape quality. Microbial inoculants has upregulated growth promoting genes which terms changed the regulation of various *PR* protein gene pattern. Initially inoculation does not show its effect but from 4 hpi microbes were effectively showing its characteristics.

REFERENCES:

1. Vurukonda Sai Shiva Krishna Prasad, Mauro Mandrioli, Greta D'Apice and Emilio Stefani. (2019). Draft genome sequence of a plant growth promoting *Streptomyces* sp., strainSA51, isolated from olive trees. *Microbiological resource announcement*, (ASM) in press.

ACKNOWLEDGEMENT:

The authors are thankful to the CCS Aosta Srl, Quart (AO), for funding the project.