SYMBIOTIC AGRICULTURE: INCREASING KNOWLEDGE ON THE MODE OF ACTION OF BENEFICIAL MICROORGANISMS

Università di Modena e Reggio Emilia, Dipartimento di Scienze della Vita, via Amendola 2, 42122 Reggio Emilia, Italy.

Student: *Vurukonda Sai Shivakrishnaprasad* **Tutor:** *Prof. Emilio Stefani*

E-mail: saishivakrishnaprasad.vurukonda@unimore.it

Ph.D. Annual Workshop, 1 December 2017, XXXII Cycle, Ist Year

INTRODUCTION:

Several microorganisms, part of the microbial communities present in soil and plant (as endophytes or epiphytes), play a significant role in agro-environments as plant growth promoters, control of plant diseases, facilitation of nutrients uptake by plants. Understanding the mode of action of such microbes (bacteria, yeasts, fungi) and their relationship with crop plants will help to focus on selected 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. The research focuses on searching microbial candidates in agricultural and natural environments suitable for crop management as biocontrol agents or plant growth promoters.

GOAL:

A set of already available microbes and new ones (bacteria and yeasts) will be thoroughly studied and used in field experiments on selected crop plants. This is 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 Microrganisms As Microbial Biocontrol Agents

A total of seven microorganisms belonging to different taxonomic groups (streptomycetes, pseudomonads, agrobacteria) available from the MICOSAT (commercial consortium) were screened for plant growth promoting and biocontrol activity (antibacterial & antifungal), The morphological and molecular characterization of such microbes was also performed. The most prospective antagonistic strains (they were: *Streptomyyces* spp. SA51 and *Agrobacterium* spp. AR39) singulary and as consortium were further evaluated in the field (2017) as bicontrol agents for the tomato spot disease organism, *Xanthomonas campestris* pv. *vesicatoria*.

PART II: Evaluation Of A Microbial Consortium For The Biocontrol Of Flavescence Dorée.

The standard microbial consortium (MICOSAT F) was sprayed (May-August, 2017, five treatments) in a large grapevine vineyard (*Lambrusco di Sorbara*) grown in Campagnola (RE), for the evaluation of possible biocontrol activity of grapevine yellows (Flavescence Dorée) and to study the elicitation of expression of several gene sequences *in planta*. The vineyard was severely affected by Flavescence Dorée the previous year. The experimental design was: four rows per treatment (replicates) and approx. 180 plants per row. Untreated rows were considered as negative control. Phytopathometrical analysis was performed at the end of August according a disease index scale, from 0 (healthy plant) to 3 (plant severely affected in all parts and unproductive).

RESULTS HIGHLIGHTS:

Plant growth promoting characteristics:

Test Bacteria	Ammonia Production	P-Solubilization Index	IAA production (µg/mL)	Siderophore activity
SB14	+++	150	19.76	-
SA51	+++	209	25.26	-
SL81	++	188	20.54	-
PT65	++	233	22.78	+
PN53	++	200	23.90	+
AR39	+++	271	18.70	-
PC50	++	0	-	-
DLS65	+++	200	21.65	-

Table 1 - Plant growth promoting traits

Fig. 1 - Ammonia Production

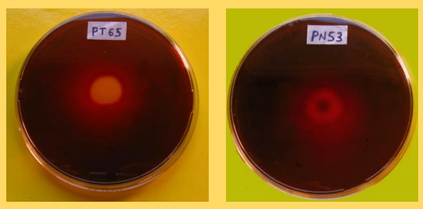


Fig. 2 - Siderophore activity: Orange halo

In vitro biocontrol activity:

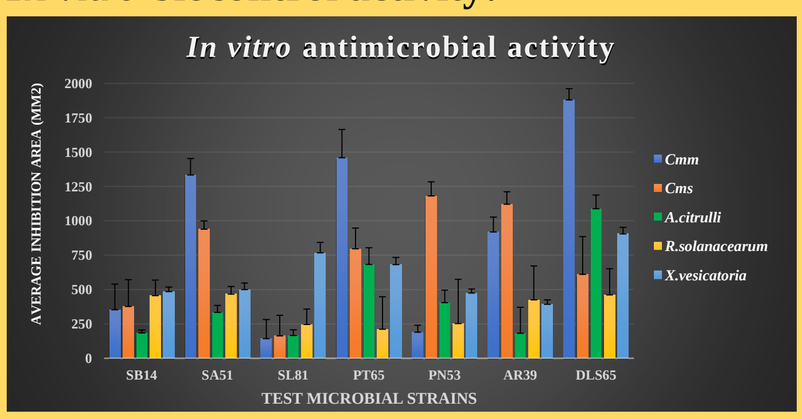


Fig. 3 – Antibacterial activity of a set of microorganisms. Error bars: ± SD, n=3

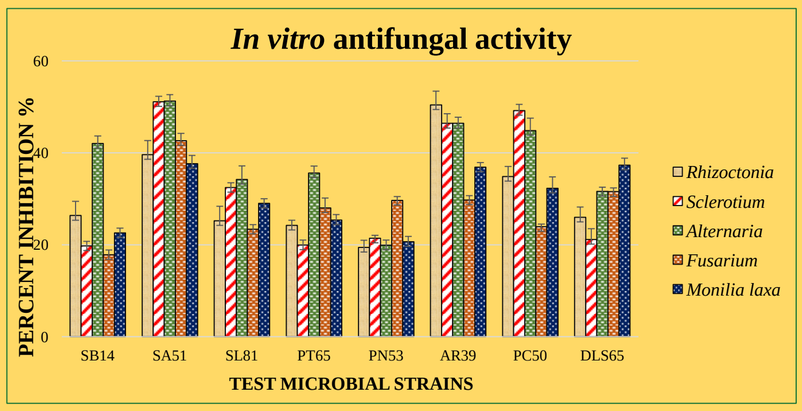
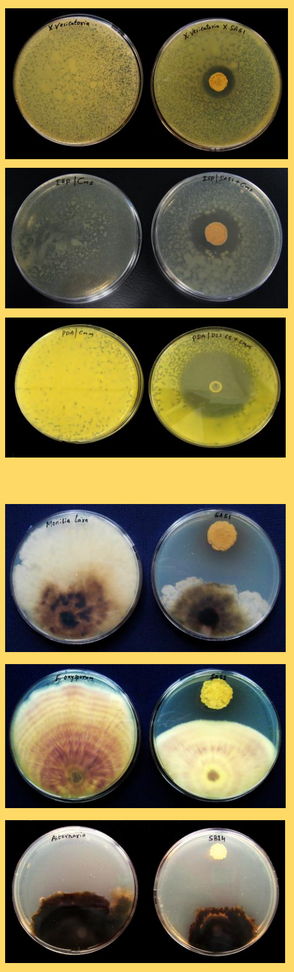


Fig. 4 – Antifungal activity of a set of microorganisms. Error bars ± SD, n=3.



Field Experiments:

Thesis	Marketable Production	
	Kg's / 40plants	Tons / Hectare
NC	37.94±1.92	18.97
PC	35.42±1.41	17.71
SA51	40.33±2.13	20.16
AR39	24.31+3.44	12.15
MICOSAT	43.37+0.61	21.68
DLS65	47.75±9.15	23.87

Table 2. Average of marketable fruit production after different treatments during the growing season 2017

Fig. 5 - Flavescence dorée of grapevine at different stages during the growing season 2017

1. The eight microorganisms studied confirmed their potential as plant growth promoting agents (Fig. 1 & 2).
2. Their antimicrobial activity was excellent against most of the phytopathogens taken into consideration (fungi and bacteria) (Fig. 3 & 4).
3. The growth promoting activity was confirmed for strains SA51, DLS65 and the commercial MICOSAT (Table 2).
4. From the phytometrical analysis it was noted that control plants were more affected than treated plants.
5. Grape samples from each control and treatment rows (4 x 5 bunches) were collected just before vintage and evaluated for different qualitative parameters (in progress).

CONCLUSION:

The present research will improve the understanding on the use of microbial biocontrol agents and will implement innovative biocontrol strategies to bacterial diseases. In particular, *Streptomyces* sp. SA51 and *Agrobacterium* sp. AR39 were found to be most active for *in vitro* biocontrol activity. *In vitro* biocontrol activity has shown very significant results also related to possible growth promoting activity. Field experiments highlighted the growth promoting activity of MICOSAT, as for strains DLS65 and SA51. No significant control of bacterial spot was noted during field experiments, since the disease occurred at very low incidence and severity, possibly due to the exceptionally hot and dry season 2017.

REFERENCES:

1. **Vurukonda S.S.K.P., Vardharajula S., Shrivastava M., Shaik Zulfikar A. (2016).** Multifunctional *Pseudomonas putida* strain FBV-2 from arid rhizosphere soil and its growth promotional effects on Maize under drought stress. *Rhizosphere* 1, 4-13.
2. **Tontou R., Gaggia F., Baffoni L., Devescovi G., Venturi V., Giovanardi D., Stefani E. (2016).** Molecular characterisation of an endophyte showing a strong antagonistic activity against *Pseudomonas syringae* pv. *actinidiae*. Plant Soil 405: 97-106.

ACKNOWLEDGEMENT:

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