SIMBA: Design, formulation and optimization of plant growth-promoting microbes for their use as microbial consortia inoculants

SUSTAINABLE INNOVATION OF MICROBIOME APPLICATIONS IN THE FOOD SYSTEM

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INTRODUCTION

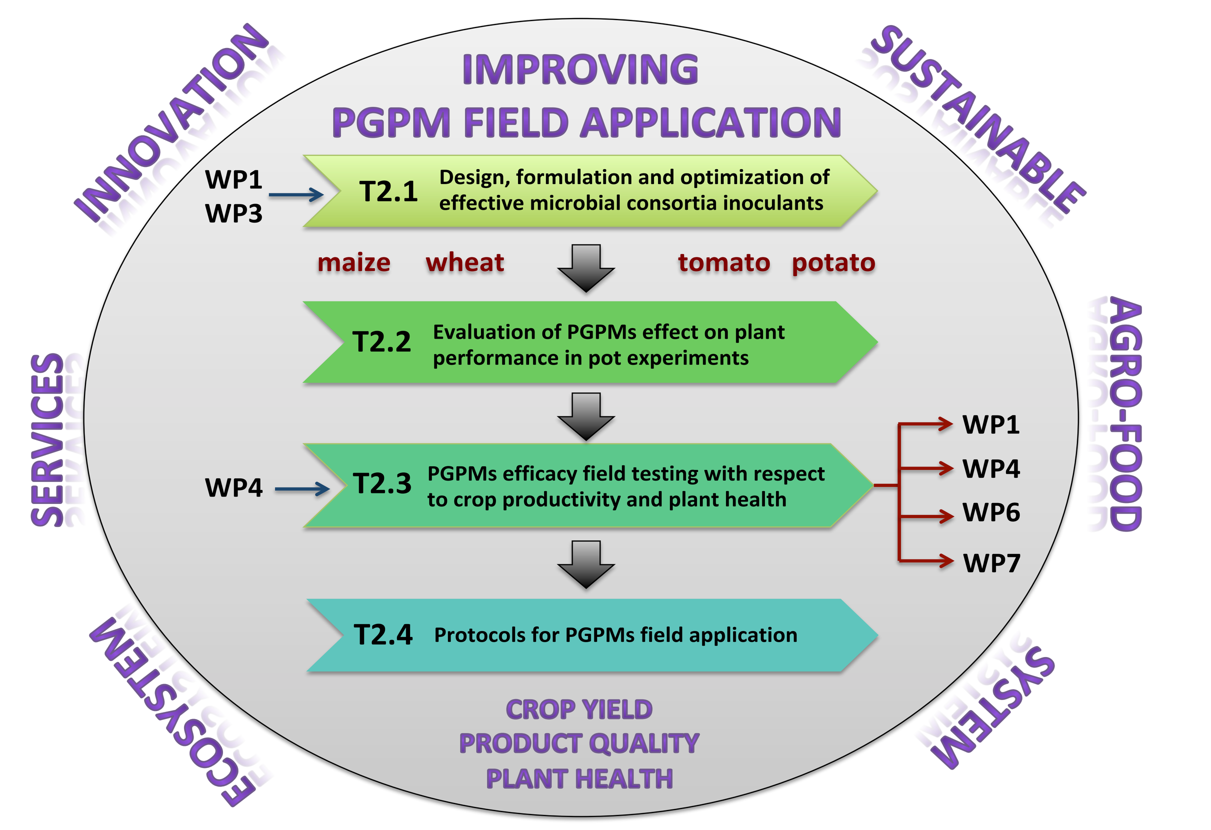
The use of efficient inoculants is considered an important strategy for sustainable management and for reducing chemical inputs in agriculture. Plant Growth-Promoting Microbes (PGPMs) are important determinants of soil fertility and plant health for their potential to improve crop productivity and nutritional quality, as well as resistance to plant pathogens and environmental stresses and plant tolerance to abiotic stresses. Nevertheless, in open field numerous biotic and abiotic factors may hinder their plant growth-promoting efficacy and reproducibility, limiting their successful use in agriculture.

Work Package 2 of SIMBA project is aimed to exploit the full potential of PGPMs for sustainable crop production by optimizing the efficacy and reproducibility of field applications.

AIM OF THE PRESENT WORK:

Identify efficient microbial formulations to be applied as bioinoculants in arable crops in Italy and Germany i.e. **WHEAT, MAIZE, POTATO and TOMATO**

WP2: Improvement of PGPMs field application efficiency and reproducibility



EXPERIMENTAL PROCEDURE

comprensive literature survey → selection of PGPMs → *in vitro* compatibility tests → set up of microbial consortia → pot experiments

RESULTS

Table 1. Microorganisms selected by literature survey

Microorganism	Strain	Origin	Country of isolation	Properties
Acaulospora morrowiae	CL290	Rhizosphere	STATI UNITI	PGP
Agrobacterium radiobacter	AR 39	soil near peach tree	Ascoli Piceno, IT	biocontrol / PGP
Azospirillum brasiliense	CD/ATTC 29710	*Cynodon dactylon* rhizosphere	USA	N-fixation
Azospirillum brasiliense	NCCB 78036	soil under soy field	India	N-fixation
Azospirillum lipoferum	CRT1	field grown maize	FR	N-fixation
Azotobacter chroococcum	76A	soil	South IT	Nitrogen fixation
Azotobacter chroococcum	DSM 2286	unknown	unknown	Nitrogen fixation
Azotobacter chroococcum	LS132	Rhizosphere	South IT	N-fixation
Azotobacter chroococcum	LS163	Rhizosphere	South IT	N-fixation
Azotobacter chroococcum	S-5	unknown	Iran	N-fixation
Azotobacter vinelandii	DSM 2289	unknown	unknown	N-fixation
Bacillus sp.	BV84	Grape leafs	Ascoli Piceno, IT	biocontrol/PGP
Bacillus amyloquefaciens	BA41	Wheat rhizosphere	Ascoli Piceno, IT	biocontrol/PGP
Bacillus amyloquefaciens	FZB42	plant pathogen infested soil	DE	biocontrol/PGP
Bacillus amyloquefaciens	LMG 9814	soil	UK	alpha-amylase , alpha-glucosidase, iso-amylase production
Bacillus atrophaeus	ABI02A		Berlin, DE	PGP
Bacillus licheniformis	PS141	Rhizosphere	South IT	Indole acetic acid (IAA) production
Bacillus megaterium	M3	rice	unknown	P-solubilisation
Bacillus megaterium	PMC 1855	unknown	unknown	P-solubilisation
Bacillus pumilus	LMG 24415	soil	Ecuador	PGP
Bacillus simplex	R49538	unknown	Ecuador	PGP/IAA production
Bacillus subtilis	FZB24 WG		Berlin, DE	PGP
Bacillus subtilis	LMG 23370	Forest soil	India	PGP/ biocontrol against *Rhizoctonia solani*
Bacillus subtilis	LMG 24418	soil	Ecuador	PGP
Bacillus subtilis	OSU-142	pepper	unknown	N-fixation, biocontrol
Burkholderia ambifaria	MCI7	Maize rhizosphere	Lazio, IT	PGP
Burkholderia ambifaria	PHP7/LMG 11351	Maize rhizosphere	FR	PGP
Gigaspora gigantea	PA125	Rhizosphere	STATI UNITI	PGP
Gigaspora rosea	NY328A	Rhizosphere	STATI UNITI	PGP
Komagataella pastoris	PP59	Grape rhizosphere	Ascoli Piceno, IT	PGP
Paenibacillus sp	R47065	unknown	Ecuador	PGP/IAA production
Paraburkholderia tropica	MDIIIAzo225	Maize rhizosphere	Caserta, IT	Nitrogen fixation
Pseudomonas granadensis	A23/T3c	soil	Lazio, IT	PGP
Pseudomonas fluorescens	DR54	Sugar beet rhizosphere	Holeby, DK	biocontrol
Pseudomonas putida	P1-20/08	soil	Ecuador	PGP
Pseudomonas sp.	PN53	Grass rhizosphere	Ascoli Piceno, IT	PGP
Rahnella aquatilis	BB23/T4d	soil	Lazio, IT	PGP
Raoultella terrigena	FS152	Rhizosphere	South, IT	Phytase activity, siderophore production
Septoglomus constrictum	FL328	Rhizosphere	STATI UNITI	PGP
Streptomyces sp.	SA 51	Rhizosphere	Liguria, IT	biocontrol
Trichoderma gamsii	6085	uncultivated soil	Crimea, UA	biocontrol
Trichoderma harzianum	OmG-08	Orchid roots	Bernburg, DE	P- solubilisation
Trichoderma harzianum	OmG-16		Bernburg, DE	P-solubilisation
Trichoderma harzianum	T6776	soil	Pisa, IT	biocontrol/PGP
Trichoderma harzianum	TH01	Grass soil and rhizosphere	Ascoli Piceno, IT	PGP
Trichoderma harzianum	CBS 354.33/ATCC 48131	soil	USA	chitinase production, biocontrol

BIOFECTOR and VALORAM strains highlighted, respectively, in green and yellow.

Table 2. Compatibility among selected bacterial strains

BACTERIAL STRAINS WITH HIGH COMPATIBILITY	BACTERIAL STRAINS WITH MODERATE COMPATIBILITY	BACTERIAL STRAINS WITH LOW COMPATIBILITY
A. chrococcum LS132	*A. chrococcum* DSM2286	*Bacillus* spp. BV84
A. chrococcum LS163	*A. brasilense* CD	*B. amyloliquefaciens* LMG 9814
A. radiobacter AR39	*A. brasilense* NCCB 78036	*B. amyloliquefaciens* BA41
A. vinelandii DSM2289	*B. ambifaria* MCI7	*B. pumilus* LMG24415
Enterobacter spp. BB23t3/d	*B. ambifaria* PHP7	*B. subtilis* LMG 23370
R. terrigena FS152	*B. licheniformis* PS141	*B. subtilis* LMG24418
	K. pastoris PP59	*P. fluorescens* DR54
	P. granadensis A23/T3c	
	P. fluorescens PN53	
	P. tropica MDIIIAzo225	

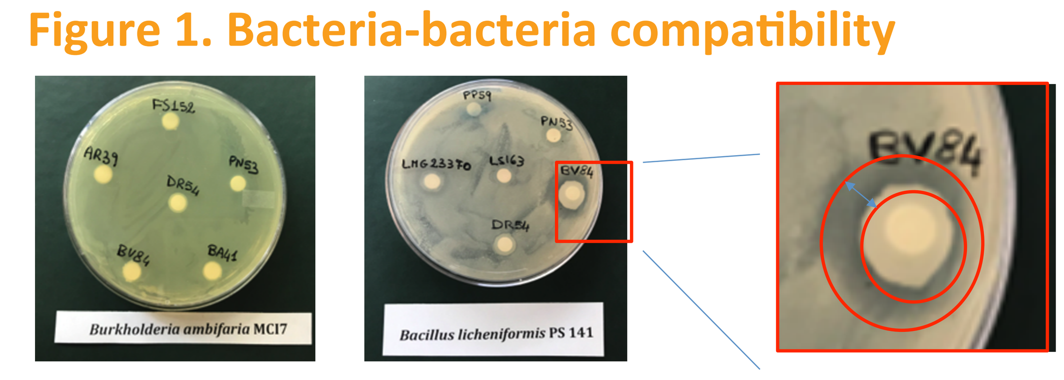
Twenty-three strains were cross-checked through *in vitro* assays and ranked by compatibility: High : microorganisms compatible with > 20 strains; Moderate: microorganisms compatible with 16 to 19 strains; Low: micr-organisms compatible with 12 to 15 strains

Table 3. Compatibility between bacterial strains and *T. harzianum* ATCC48131 and TH01

BACTERIAL STRAINS	FUNGAL STRAINS	
	Trichoderma harzianum ATCC48131	*Trichoderma harzianum* TH 01
A. brasilense CD	nc	–
A. brasilense NCCB78036	+	–
A. chrococcum DSM2286	+	nc
A. chrococcum LS132	–	–
A. chrococcum LS163	+	–
A. radiobacter AR39	+	+
A. vinelandii DSM2289	–	–
Bacillus sp. BV84	+	–
B. amyloliquefaciens BA41	+	+
B. amyloliquefaciens LMG9814	+	+
B. licheniformis PS141	–	–
B. pumilus LMG24415	+	ND
B. subtilis LMG23370	+	+
B. subtilis LMG24418	+	ND
B. ambifaria MCI7	+	+
B. ambifaria PHP7	+	+
Rahnella aquatilis BB23T3/d	+	–
K. pastoris PP59	–	–
P. tropica MDIIIAzo225	–	nc
P. granadensis A23/T3c	–	–
Pseudomonas sp. PN53	+	–
P. fluorescens DR54	–	nc
R. terrigena FS152	+	–

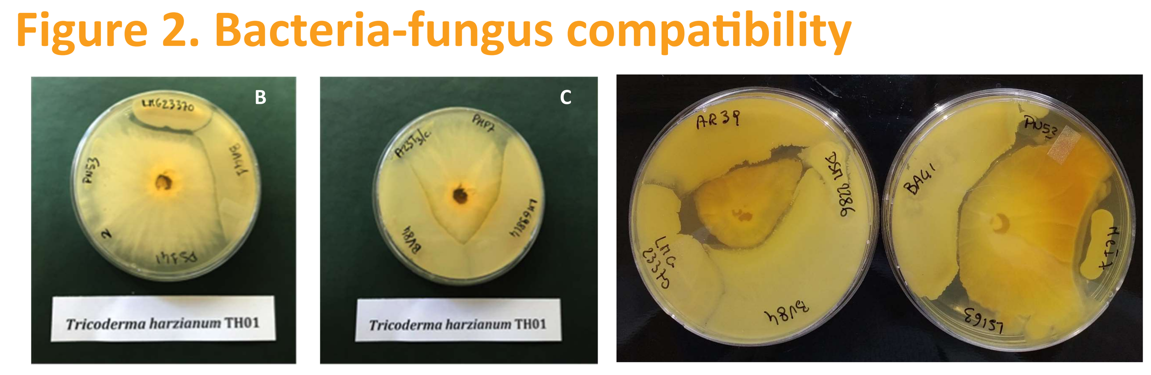
+ = compatible combination; - = incompatible combination; nc= unclear interaction; nd = not determined

Figure 1. Bacteria-bacteria compatibility



On the left, examples of compatibility between *B. ambifaria* MCI7 and tested bacteria. In the middle and on the right, example of incompatibiltiy between *B. licheniformis* PS141 and *Bacillus* sp. BV84.

Figure 2. Bacteria-fungus compatibility



On the left (B) and in the middle (C): examples of incompatibility/compatibility between *T. harzianum* TH01 and four bacterial strains. On the right: examples of incompatibility/compatibility between *T. harzianum* ATCC48131 and four bacterial strains.

Figure 3. Prebiotic test of biostimulant compounds

Prebiotic effects of BS1 (Plant-derived protein hydrolysate) on germination and growth of our beneficial consortia in starvation conditions (WA, water agar) to be used to rapidly increase the number of microorganisms when applied in the soil.

Table 5. Selected microbial consortia

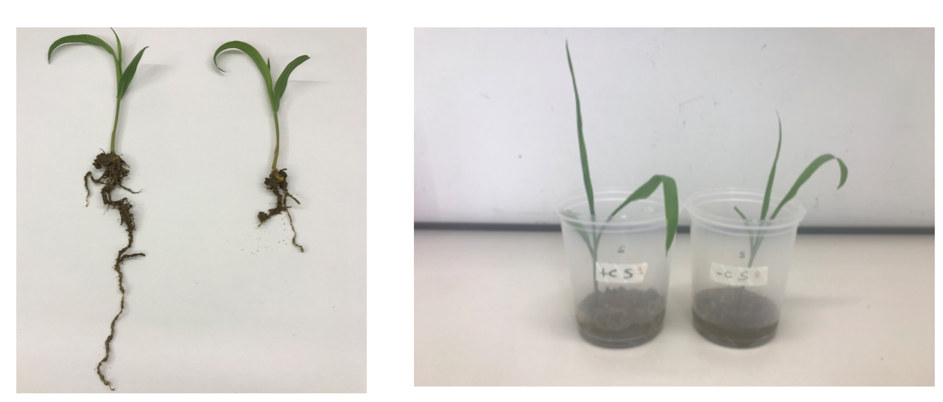
MICROBIAL CONSORTIA (MC)	MICROORGANISMS
A	*Trichoderma harzianum* TH01 *Pseudomonas granadensis* A23/T3c *Paraburkholderia tropica* MDIII Azo225 *Bacillus licheniformis* PS141 *Azotobacter chrococcum* LS132 *Pichia pastoris* PP59
B	*Bacillus amyloliquefaciens* LMG9814 *Pseudomonas fluorescens* DR54 *Bacillus* sp. BV 84 *Rahnella aquatilis* BB23T3/d *Azotobacter vinelandii* DSM2289

Table 6. Microbial combination

Combination	MC	MC_AMF	BS
C-1	X		
C-2	X	X	
C-3	X		X
C-4	X	X	X

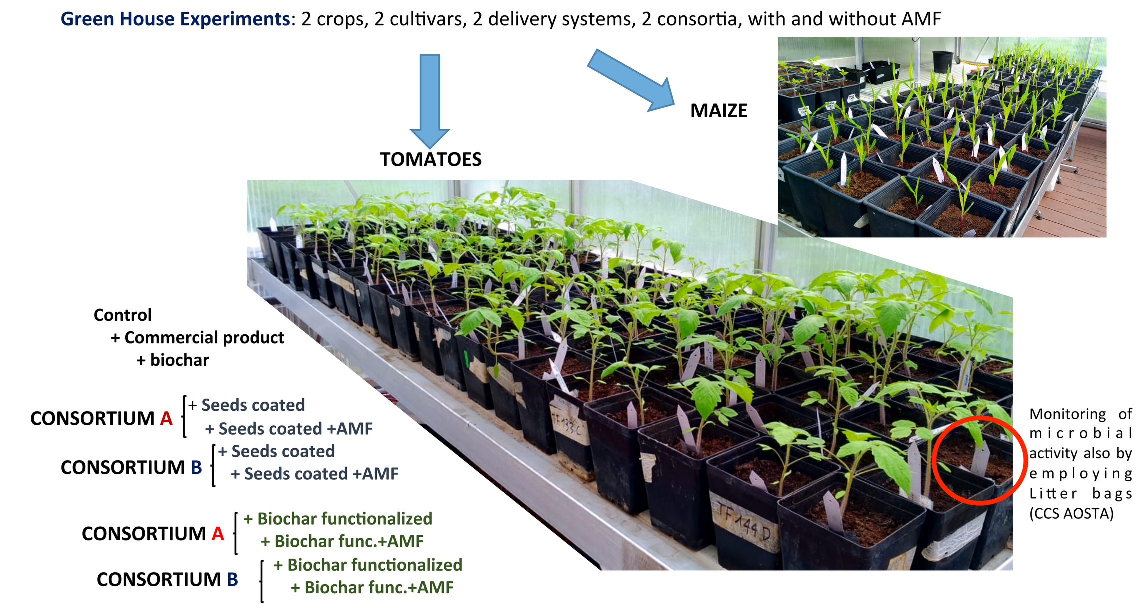
MC: Microbial Consortium (A or B); MC_AMF (consortium of arbuscolar mychorrizal fungi) : *Acaulospora morrowiae* CL290, *Septoglomus constrictum* FL328, *Gigaspora gigantea* PA125; BS (Biostimulant compounds): a combination of seaweed plant and compost extracts, humic substances, plant-microbial signal compounds.

Figure 5. PGP effect of consortium B on maize plants



Greenhouse experiments were carried out in sterile sandy loam/loess from organic farm Wiesengut (Germany). Maize seeds (cv. Benedictio) were coated with microbial consortium B. On the left, the effect on root growth (at 8 days); on the right , the effect on shoot growth (at 13 days).

Figure 4. Ongoing pot-experiments



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