POWER OF MICROBES – KRK – CROATIA – 2016

ANALYSIS OF THE EFFECT AND SURVIVAL OF PHYTOSTIMULATORY SOIL INOCULANTS

ZSUZSANNA NAGYMÁTÉ[1], ANDRÁS KARI[1], ZSUZSANNA POHNER[1], RITA KOVÁCS[2], ILDIKÓ PUSPÁN[2], ÉVA KÁRPÁTI[3], JÓZSEF KUTASI[2], CSABA ROMSICS[1], KÁROLY MÁRIALIGETI[1]

[1]EÖTVÖS LORÁND UNIVERSITY, INSTITUTE OF BIOLOGY, DEPARTMENT OF MICROBIOLOGY; [2]BIOFIL LTD.; [3]SANIPLANT LTD., BUDAPEST, HUNGARY

ANALYSIS OF THE EFFECT AND SURVIVAL OF PHYTOSTIMULATORY SOIL INOCULANTS

ZSUZSANNA NAGYMÁTÉ[1], ANDRÁS KARI[1], ZSUZSANNA POHNER[1], RITA KOVÁCS[2], ILDIKÓ PUSPÁN[2], ÉVA KÁRPÁTI[3], JÓZSEF KUTASI[2], CSABA ROMSICS[1], KÁROLY MÁRIALIGETI[1]

[1]EÖTVÖS LORÁND UNIVERSITY, INSTITUTE OF BIOLOGY, DEPARTMENT OF MICROBIOLOGY; [2]BIOFIL LTD.; [3]SANIPLANT LTD., BUDAPEST, HUNGARY

… A LITTLE HISTORY …

1886-88	Hellrieger, H. és Wilfarth, H.	Fixation of molecular N
1888	Beijerinck, M.	Pure culture of the nitrogen fixing *Rhizobium*
1896	Hellriegel, Wilfarth, Nobbe	Nitragin
1901	Ishiwata Shigetane	Silkworm disease
1911	Berliner, E.	*Bacillus thuringiensis*
1938		Sporein
1996		Bt cotton commercialised, Today: rice, broccoli, egg plant, apple, millet, sunflower, peanut, sugar cane, tobacco, potato, tomato, cajun (red) bean, chick pea…
1889	Trevisan, V.	*Description of Pseudomonas putida*
1972	Chakrabarty, A.	Patenting a „petroleum" degrading bacterium

… GOALS …

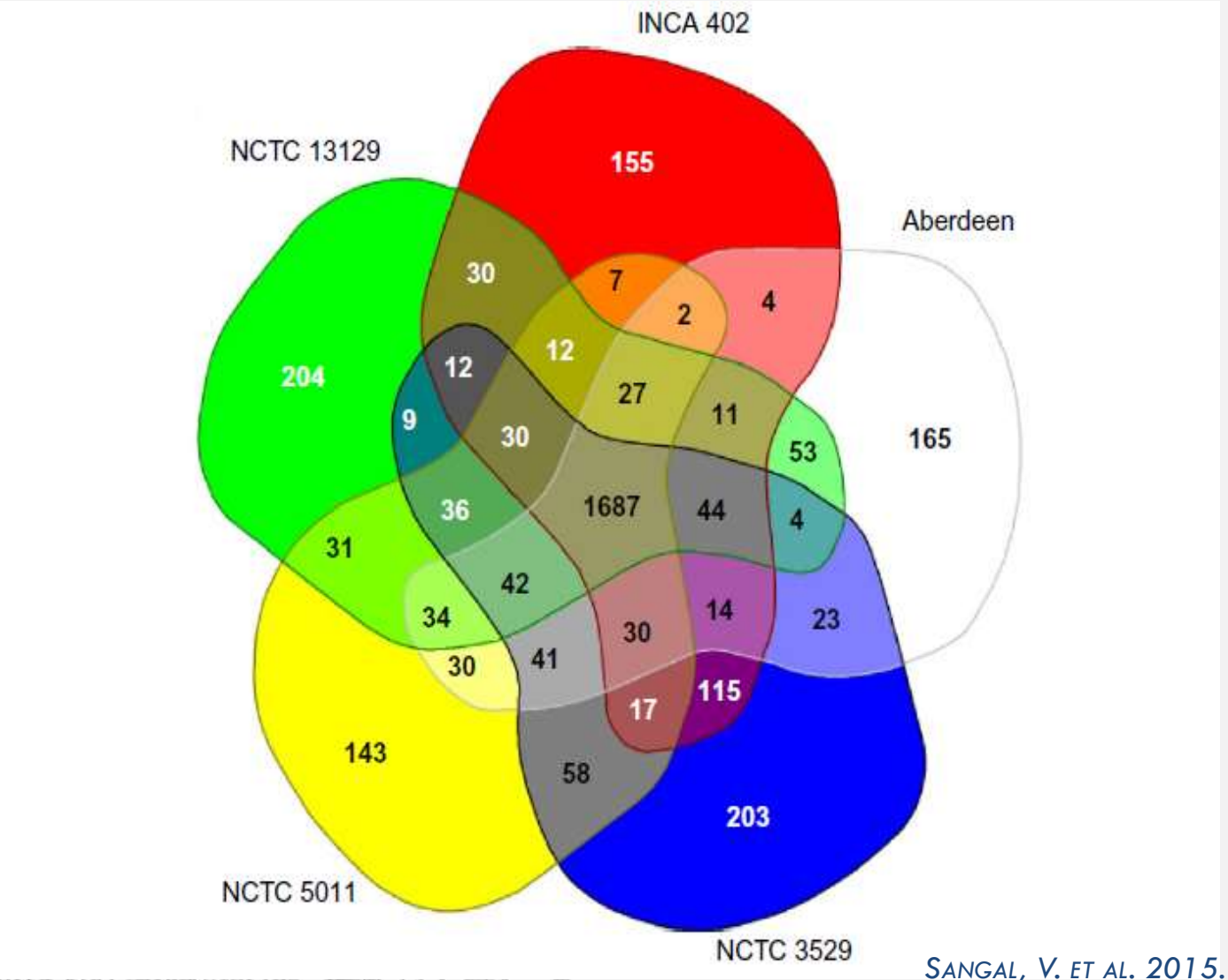
PLANT GROWTH PROMOTON	
	N FIXATION; SUPPLY OF AVAILABLE P; PROVISION OF OTHER NUTRIENTS; PRODUCTION OF PLANT HORMONES, INFLUENCING SIGNAL MOLECULES/PATHWAYS; …
PLANT PROTECTION	
	TOXINS, METAL CHELATING COMPOUNDS, PRODUCTION OF ANTIMICROBIAL COMPOUNDS; CHITIN DEGRADATION; QUORUM QUENCHING; …
SOIL STRUCTURE MODIFICATION	
	PRODUCTION OF EXTRACELLULAR MATRIX; FILAMENTOUS STRUCTURE; …
STALK RESIDUE MULCHING	
	LIGNOCELLULOSE, PECTINE DEGRADATION; COLD TOLERANCE; …
BIOREMEDIATION	
	SURFACTANT PRODUCTION; ROS SYNTHESIS; GENE CASCADES FOR BIODEGRADATION; ANAEROBITY TOLERANCE; …

… SPECIES …

Agrobacterium spp., *Agrobacterium tumefaciens*, *Alcaligenes* spp., *Azospirillum brazilense*, *Azospirillum* spp., *Azotobacter vinelandii*, *Bacillus alcalophilus*, *Bacillus licheniformis*, *Bacillus* spp., *Bacillus subtilis*, *Bacillus thuringiensis* var. *berliner*, *Bacillus thuringiensis* var. *israelensis*, *Bacillus thuringiensis* var. *tenebrionis*, *Bradyrhizobium japonicum*, *Bradyrhizobium lupini*, *Bradyrhizobium* spp., *Brevibacterium flavum*, *Chromobacterium violaceum*, *Corynebacterium glutamicum*, *Corynebacterium* spp., *Dehalococcoides mccarthyi*, *Enterobacter* spp., *Erwinia herbicola*, *Erwinia* spp., *Frankia* spp., *Geobacillus* spp., *Herbaspirillum* spp., *Klebsiella* spp., *Kocuria palustris*, *Kocuria rhizophila*, *Kocuria* spp., *Leifsonia xyli*, *Mesorhizobium ciceri*, *Mesorhizobium loti*, *Methylococcus* spp., *Photorhabdus* spp., *Pseudomonas fluorescens*, *Pseudomonas* spp., *Ralstonia eutropha*, *Rhizobium etli*, *Rhizobium leguminosarum*, *Rhizobium* spp., *Rhodococcus* spp., *Serratia* spp., *Sinorhizobium meliloti*, *Sinorhizobium saheli*, *Sinorhizobium tarangae*, *Streptomyces rubiginosus*, *Streptomyces* spp., *Xenorhabdus* spp.

… GENES …

Gene	Protein	Effect
phlACB, phlD	2,4-diacetyl-phloroglucinol synthesis enzyme	Antimicrobial compound
hcnABC	Hydrogen-cyanide synthesis enzyme	antimicrobial compound
pqqG, pggF, pqqBCDE	Pyrroloquinoline quinone synthesis enzyme	Phosphate solubilization
nifDHK	Nitrogenase enzyme	Nitrogen fixation
budAB, budC	2,3-butanediol synthesis enzyme	Induced systemic resistance
ipdC, ppdC, hisC1, hisC2	Indole-3-pyruvate decarboxilase, phenylpyruvate decarboxilase, histidinol phosphate aminotransferase 1 and 2 enzymes	Indole-acetic acid synthesis
alkB	E.g. alcane hydroxylase enzyme	Degradation of saturated hydrocarbons
vcrA	Vinyl-chloride reductase enzyme	Cleavage of vinyl chloride to ethene and hydrochloric acid



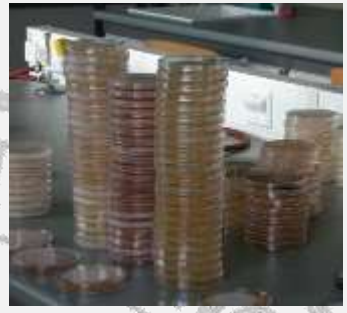
Sangal, V. et al. 2015.

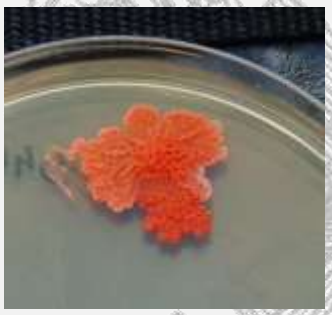
Venn diagram:

Pangenome: 3264 genes; Core genome: 1687 genes; Indispensable genome: 1577 genes

Horisontal gene transfer; Genetic variability

… MICROBIOLOGICAL METHODS … LABORATORY BENCH …





SAMPLE

↓

ENRICHMENT

ISOLATION → PURE CULTURE → STRAIN

MIXED INOCULUM CULTURE → ? → „STRAIN" MIXED INOCULUM

… MICROBIOLOGICAL METHODS … LABORATORY BENCH …

CLOSED CULTURE

CLOSED MICRO- AND MESOCOSMS

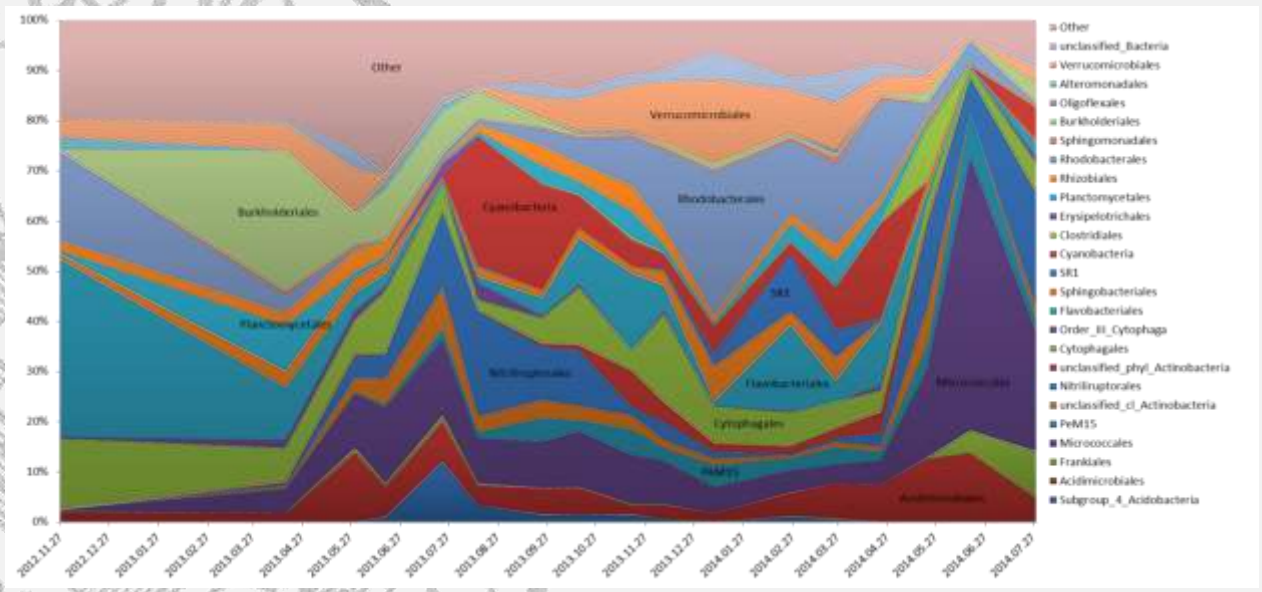
OPEN SYSTEM

LIQUID CULTURE AND POT EXPERIMENT

… MICROBIOLOGICAL METHODS … LABORATORY BENCH …

~~CELL / MPN DETERMINATION~~

MOLECULAR FINGERPRINTING TECHNIQUES (E.G. T- RFLP, ARDRA, SNUPE, HOPE, BRENDA, ERIC…) /
FISH /
METAGENOME STUDIES



… EXAMPLE …


… CROP SAFETY AND INCREASED CROP PRODUCTION IN CORN USING INOCULANTS ADAPTED TO SOIL TYPE

Strain	Nearest neighbour type strain	Similarity	
		%	Alignment (bp)
242_9	*Azospirillum brasilense*	98,16	26/1415
NF_10	*Azospirillum brasilense*	98,23	25/1416
NF_11	*Azospirillum brasilense*	98,89	30/1420
NF_6	*Azospirillum irakense*	99,42	8/1381
Nf_7	*Azospirillum brasilense*	98,36	12/1420
B_41	*Azospirillum largimobile*	98,5	20/1387
S_28	*Bacillus simplex*	100,00	0/1476
S_153	*Arthrobacter crystallopoietes*	98,83	17/1454
S_225	*Kocuria rhizophila*	99,73	4/1456
S_33	*Pseudomonas frederiksbergensis*	98,85	17/1472
S_125	*Pseudomonas jessenii*	99,45	8/1462
S_284	*Paenibacillus peoriae*	99,73	4/1476
S_47	*Agreia pratensis*	99,59	6/1449
LU_44	*Bacillus arybbhattai*	99,93	1/1491
12_4	*Pseudomonas chlororaphis*	99,76	3/1239

Tested features

- Nitrogen fixation
- Phosphorus mobilization
- IAA production
- Siderophore production
- EPS production
- Acid tolerance
- Alkali tolerance
- Desiccation tolerance
- Strain to strain tolerance
- Antibiosis to selected plant pathogens

Strain combinations were made for different soil types, and mass cultivation was optimised

STRAINS

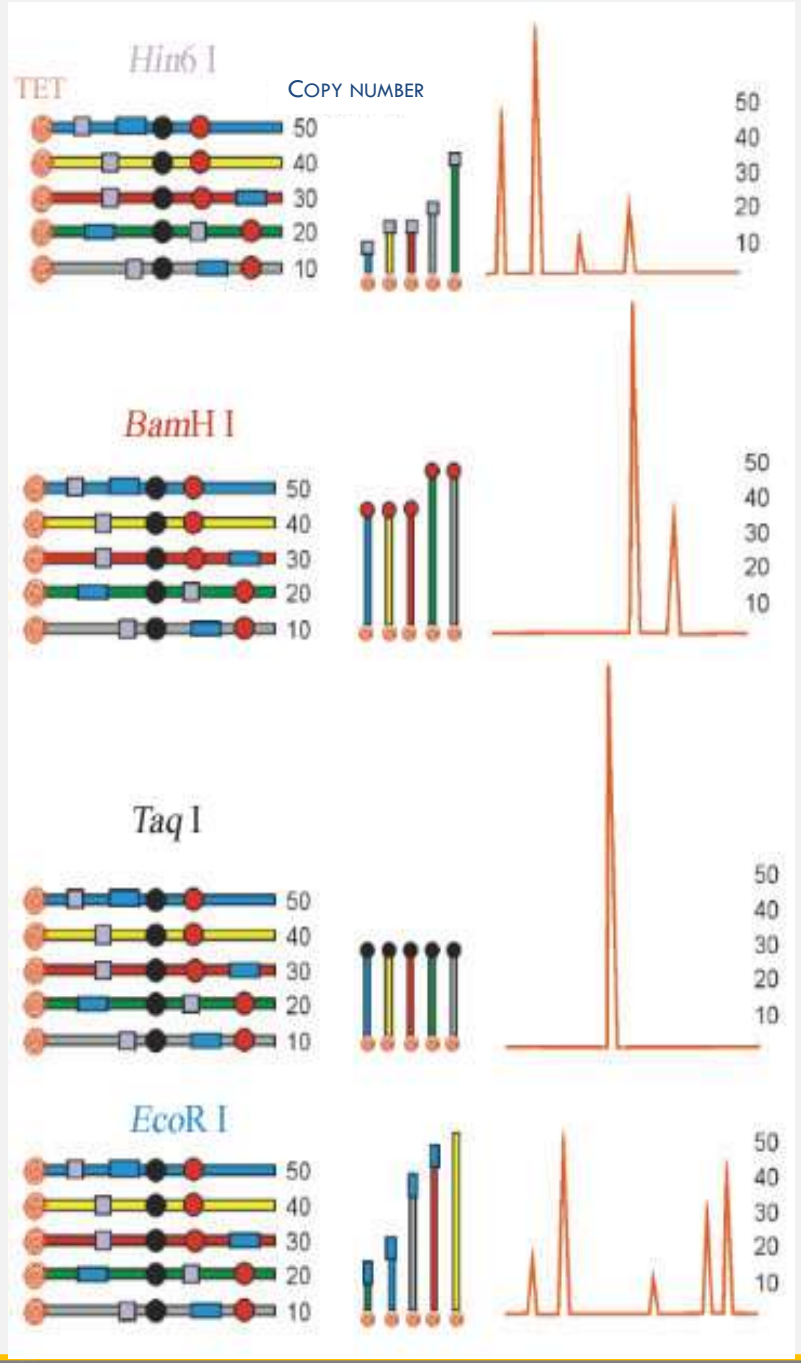
SHAKEN CULTURES

THREE PHASE SCALE UP PROCESS

THREE PHASE FERMENTATION

T-RFLP

- NA extraction
- NA multiplication with labelled primers
- Restriction digestion
- (Capillary) electrophoresis
- Analysis of electropherograms
- Qualitative and quantitative detection of strains



Törzs kód	Enzim neve / Hasítóhely	*Alu* I AG/CT	*Bsh* 12361 CG/CG	*Bsp* 143I /GATC	*BsuR* I GG/CC	*Csp* 6I G/TAC	*Fsp* BI C/TAG	*Hha* I GCG/C	*Hin* 1II CATG/	*Hin* 6I G/CGC	*Mbo* I /GATC	*Msp* I C/CGG	*Rsa* I GT/AC	*Tai* I ACGT/	*Taq* T/CGA	*Tas* I /AATT	*Tru* 1I T/TAA
242_9	**27F-519R** primerek által közre fogott 16S rDNS szakaszok T-RF-jeinek bázispár hossza	238	185	229	61	n.a.	64	72	174	70	229	140	n.a.	90	n.a	482	n.a.
NF_10		238	185	229	61	n.a.	n.a.	72	174	70	229	140	n.a.	90	n.a	482	n.a.
NF_11		238	185	229	61	n.a.	n.a.	72	174	70	229	140	n.a.	90	n.a	482	n.a.
NF_6		238	185	229	215	n.a.	469	70	348	68	229	138	n.a.	88	n.a	482	n.a.
S_28		63	225	293	221	445	237	230	178	228	293	143	446	104	54	547	n.a.
S_153		226	215	53	218	444	n.a.	359	176	357	53	57	445	102	n.a	517	n.a.
S_225		62	383	n.a.	220	446	n.a.	463	176	461	n.a	271	447	102	n.a	519	n.a.
S_33		226	381	259	192	61	103	199	378	197	259	482	62	163	n.a	537	417
S_125		62	379	257	190	n.a.	101	197	376	195	257	481	n.a.	132	134	72	415
13_4		62	379	257	190	432	101	197	376	195	257	133	433	132	n.a	536	415
S_284		65	396	298	226	479	82	235	183	233	298	133	480	108	n.a	552	n.a.
LU_44		64	227	164	223	447	239	n.a.	180	n.a.	164	133	448	80	n.a	549	n.a.
S_47		60	213	n.a.	167	442	57	n.a.	174	n.a.	n.a.	139	443	100	n.a	190	n.a.
B_41		236	183	227	59	n.a.	467	70	n.a	68	227	138	n.a.	88	n.a.	480	n.a.

Törzs kód	Enzim neve / Hasítóhely	*Bcn* I CC/SGG	*Bme* 135 CC/NGG	*Cfr* 13I GG/NCC	*Hin* fI G/ANTC	*Hpy* F3I C/TNAG	*Mva*I CC/WGG	*Nmu* CI /GTSAC	*Pfe* I G/AWTC	*Sat* I GC/NGC	*Taa*I ACN/GT	*Tau*I GCSG/C	*Bta* CI GGATG/	*Bse* NI ACTGG/	*Bse* XI GCAGC/	*Bsp* LI GGN/NC(	*Bve* I ACCTGC	*Cfr* 13I G/GNCC	*Faq* I GGGAC/
242_9	**27F-519R** primerek által közre fogott 16S rDNS szakaszok T-RF-jeinek bázispár hossza	428	427	61	286	271	548	n.a.	n.a.	301	408	351	146	268	304	94	100	60	271
NF_10		428	427	180	286	63	548	n.a.	n.a.	301	408	351	146	268	304	94	100	179	271
NF_11		428	427	180	286	63	548	n.a.	n.a.	301	408	351	146	268	304	94	100	179	271
NF_6		428	427	281	286	271	548	393	n.a.	179	408	351	144	268	182	n.a	n.a	280	271
S_28		n.a	n.a.	220	51	72	n.a	205	51	82	196	84	n.a	127	344	221	115	219	311
S_153		70	70	311	118	274	132	281	n.a.	216	443	218	209	298	334	445	113	310	301
S_225		n.a	133	313	118	276	132	283	n.a.	333	445	383	211	144	336	447	113	312	303
S_33		n.a	71	183	107	274	71	288	107	50	310	52	284	272	56	183	n.a	182	185
S_125		n.a	113	181	105	272	113	286	105	80	308	82	55	270	332	181	n.a	180	127
13_4		n.a	113	181	105	27	113	286	105	80	308	82	282	270	332	181	n.a	180	183
S_284		497	497	225	331	289	437	210	356	86	478	88	224	313	349	480	119	224	316
LU_44		n.a	437	222	328	286	n.a	n.a.	353	84	475	86	n.a	129	346	223	117	221	313
S_47		n.a	n.a	311	118	274	n.a	281	n.a.	170	142	172	209	137	334	445	113	310	301
B_41		425	425	178	284	242	546	n.a.	n.a.	229	406	349	252	266	302	92	98	177	269

Pot experiments with sweet pepper and tomato were used to optimize strain combinations, doses, etc.

Different soil types

Adequate controls

Different doses

Different inoculum formulations

Small plot field experiment with corn

Two different characteristic Hungarian soil types

Adequate controls

Different doses

Different inoculum formulations

Detection of phenophasic characters

Continuous measurement of environmental parameters

Rizosphere soil sampling through the vegetation period

Corn variety: Szeged 346

„Acidic” soil:

Ramann type brown forest soil; Physical soil type: clayey loam

pH_{KCL}: 5,75; CaCO3: <0,1 m/m%; P_2O_5: 284 mg/kg (good); K_2O: 146 mg/kg (medium); NO_2^- + NO_3^- -N: 44 mg/kg (poor);
Organic matter (humus): 1,32 m/m%

„Alkaline-neutral” soil:

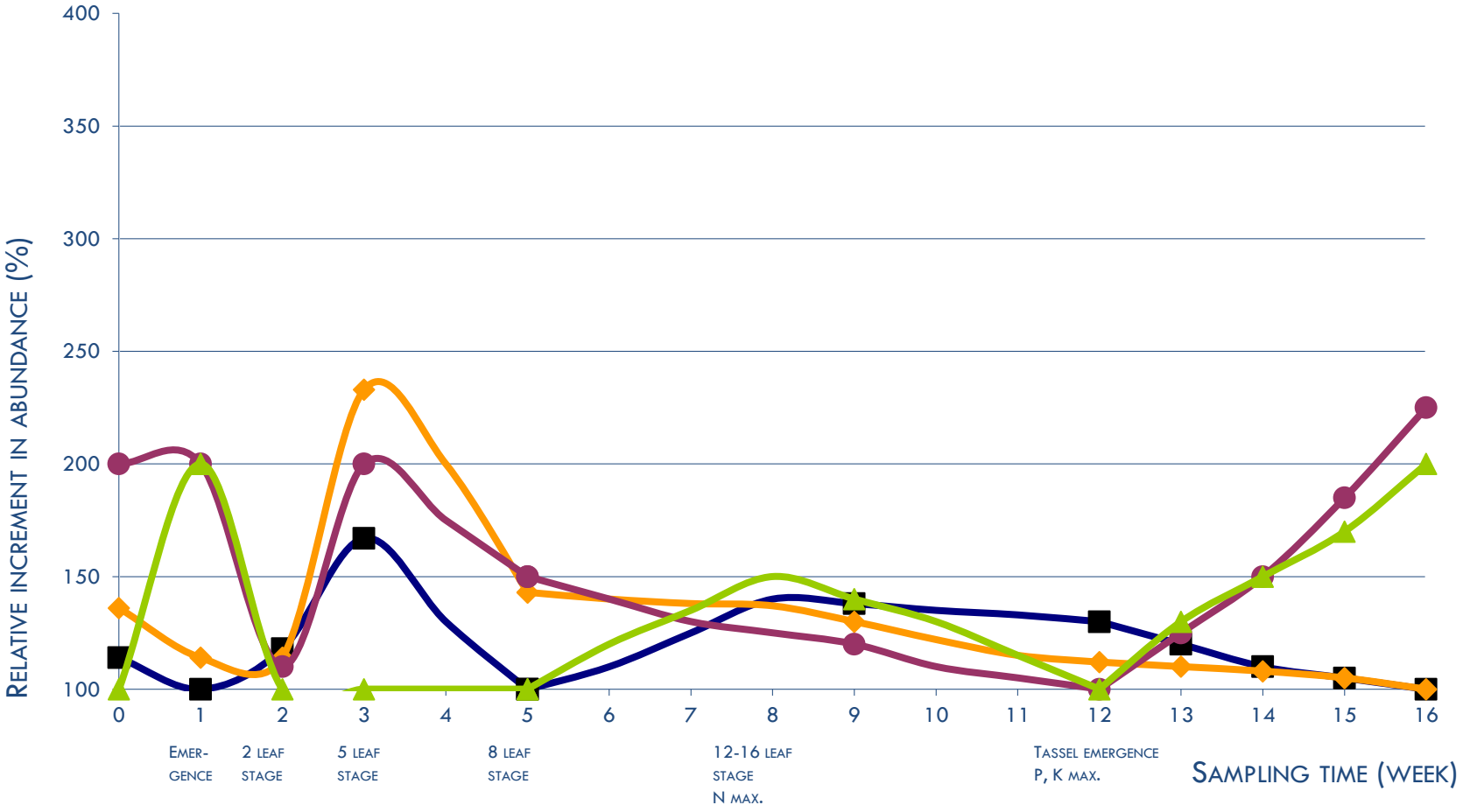
Clay infiltered brown forest soil; Physical soil type: clayey loam

pH_{KCL}: 7,35; CaCO3: 10 m/m%; P_2O_5: 470 mg/kg (good); K_2O: 298 mg/kg (good); NO_2^- + NO_3^- -N: 106 mg/kg (good);
Organic matter (humus): 3,87 m/m%

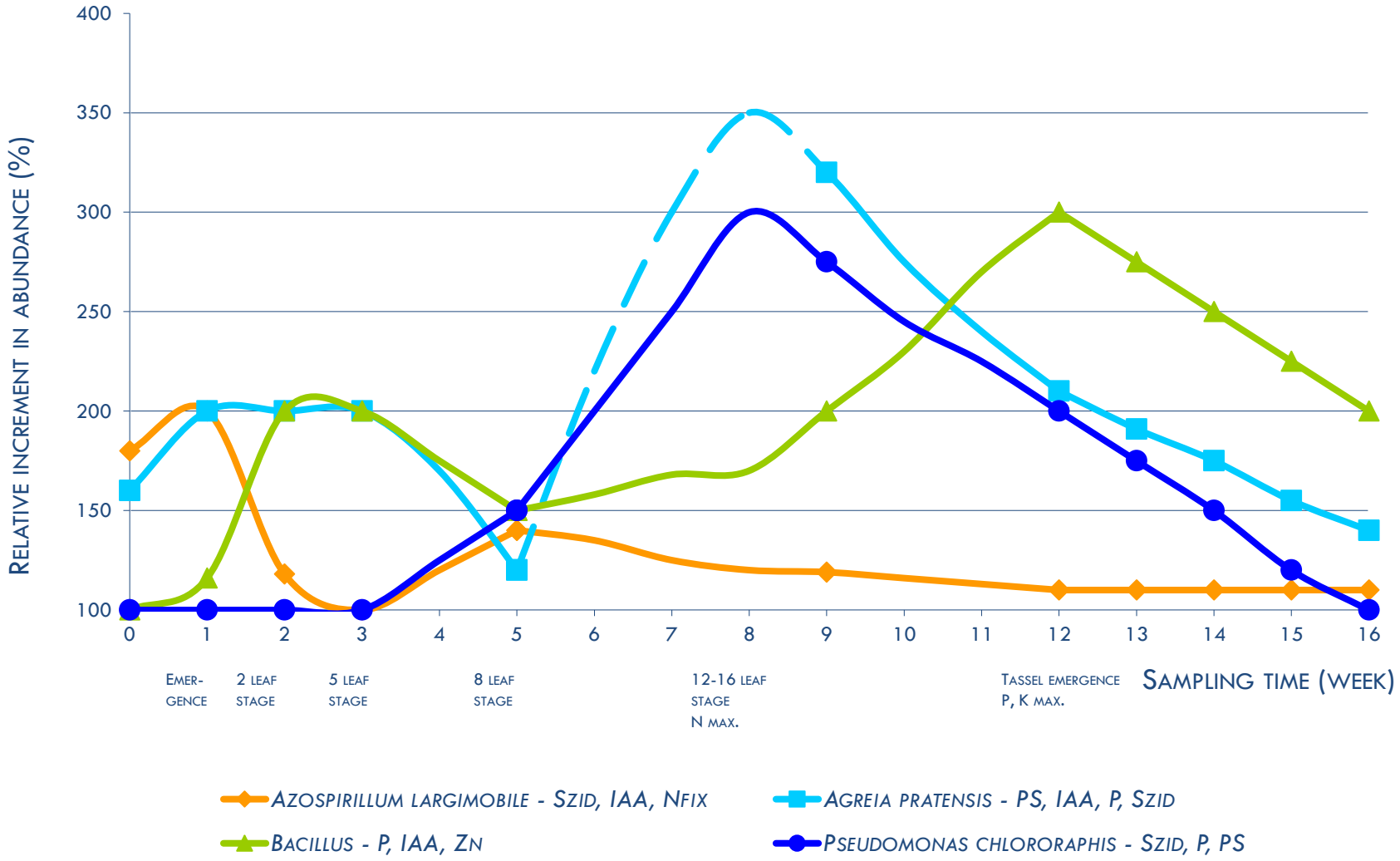
SURPLUS CROP
(GIVEN YEAR, GIVEN CULTIVATION PRACTICES)

„ALKALINE-NEUTRAL SOIL”:	4,6 - 8,8 %
„ACIDIC SOIL”:	16,3 -28,2 %

ANP CORN


RELATIVE INCREMENT IN ABUNDANCE (%)
400
350
300
250
200
150
100
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
EMER-GENCE
2 LEAF STAGE
5 LEAF STAGE
8 LEAF STAGE
12-16 LEAF STAGE N MAX.
TASSEL EMERGENCE P, K MAX.
SAMPLING TIME (WEEK)
AZOSPIRILLUM BRASILENSE - IAA, NFIX
AZOSPIRILLUM LARGIMOBILE - SZID, IAA, NFIX
ARTHROBACTER - IAA, SZID, P, PS
BACILLUS - P, IAA, ZN

ACP CORN


RELATIVE INCREMENT IN ABUNDANCE (%)
400
350
300
250
200
150
100
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
EMER-GENCE
2 LEAF STAGE
5 LEAF STAGE
8 LEAF STAGE
12-16 LEAF STAGE N MAX.
TASSEL EMERGENCE P, K MAX.
SAMPLING TIME (WEEK)
AZOSPIRILLUM LARGIMOBILE - SZID, IAA, NFIX
AGREIA PRATENSIS - PS, IAA, P, SZID
BACILLUS - P, IAA, ZN
PSEUDOMONAS CHLORORAPHIS - SZID, P, PS

And what is still missing:

Transcription analysis

Post-harvest Survival

Rhizosphere/rhizoplane composition changes …

… AND THE PARTNERS …

INFALLIBLE

BIOFIL®

FERMENTIA
fermentation company

SZÉCHENYI 2020
NEMZETI KUTATÁSI, FEJLESZTÉSI ÉS INNOVÁCIÓS ALAP
MAGYARORSZÁG KORMÁNYA
BEFEKTETÉS A JÖVŐBE

FALLIBLE

norway grants
SZENT ISTVÁN EGYETEM
www.norwaygrants.org


THANK YOU FOR YOUR ATTENTION!