Scientific Report – Short Term Scientific Mission (STSM)

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COST Action F1103

Host institution: **AIT GmbH** in Tulln (Austria)

Period: 14/06/2015 to 27/06/2015

Reference code: COST-STSM-FA1103-27038

STSM Topic:

Identification and characterization of plant beneficial activities in micro-organisms

(i) Abstract

The aim of the proposed STSM is to learn how to select bacterial strains, with the potential to enhance plant performance by rigorous testing in the lab addressing a multitude of biochemical characteristics. The tests will include assessment of ACC deaminase activity, qualitative and quantitative activity in phytohormone production and P-solubilization, plant polymer hydrolyzing enzyme activity, growth parameters and optional tests for antagonistic activities against plant pathogenic bacteria, fungi and oomycetes.

(ii) Purpose of the STSM

The goal of this STSM was to learn classical microbiological methods for characterization of endophytic bacteria in their biochemistry and physiology. Hereby different plate assays and spectrophotometric measurements were used. In particular the handling of various materials and tools were practiced.

(iii) Description of the work carried out during the STSM

<u>Material used for experiments:</u>

8 endophytic bacterial isolates from *Setaria viridis*:

TP-R1	TP-R8b
TP-R9b	TP-R3y
TP-W4	TP-R6a
TP-W10	TP-W1

Positive control:

Burkholderia phytofirmans PsJN

Assay for siderophores production:

Bacterial isolates, including *B. phytofirmans,* were assayed for siderophores production on the Chrome azurol S (CAS) agar medium described by Schwyn and Neilands (1987).

Characterization of Gram negative bacteria via BIOLOG plates (GN2 MicroPlate):

5 ml 50% (v/v) TSB-Medium was filled in sterile tubes and inoculated with each bacterial isolate. The inoculated tubes were agitated at 200 rpm, 28°C overnight.
Afterthat the tubes were centrifugated at 2000 rpm for 5 min. The supernatant was discarded and the pellet was washed 3 times with VL 55 medium, centrifugated at 2000 rpm for 2 min, supernatant was discarded again.
Tube with washed pellet was filled with 15 ml medium VL55 and vortexed. 1,5 µl bacterial suspension was filled in each well of "N2 Microplate". The inoculated microplates were incubated at 28°C overnight.

Used media:

TSB-Medium:

Tryptic Soy Broth	15 g
Agar Agar	15 g
Aqua dest.	1000 ml

Medium was autoclaved for 15 min at 121°C.

VL55 Medium:

2-(N-morpholino)ethanesulfonic acid (MES)	1,95 g
Magnesiumsulfate heptahydrate	48,2 mg
Calciumchloride dihydrate	88,2 mg
Diammoniumhydrogenphosphate	53,8 mg
Selenit/Tungstat-solution	1 ml
Trace element solution SL 10	1 ml
Aqua dest.	900ml
Base mix	100 ml

pH 5,5

autoclavate (15 min at 121°C)

Selenit/Tungstat-solution:

NaOH	0,5 g
Na_2SeO_3 x 5 H_2O	3 mg
Na_2WO_4 x 2 H_2O	4 mg
Aqua dest.	1000 ml

Trace element solution SL 10:

H_3BO_3	6 mg
$CuCl_2$ x 2 H_2O	2 mg
Aqua dest.	1000ml

Base mix:

NaOH (200 mM)	0,8 g
KOH (100 mM)	1,1 g
Aqua dest.	100 ml

Every well of “N2 Microplate” contains a specific test substance where the bacteria can grow or not. This plate performs 95 discrete tests simultaneously and gives a characteristic reaction pattern called a “metabolic fingerprint”.

After 1 and 4 days every well of microtiter plates was checked for bacterial growth.

Assay for availability of ACC deaminase:

Cultivation of bacterial isolates on 10% TSA-Medium.

10 % TSA-Medium:

Tryptic Soy Broth	3 g
Agar Agar	15 g
Aqua dest.	1000 ml

Production of ACC medium:

Basal medium:

Aqua dest.	950 ml
Agar Agar	12 g

Medium was autoclaved at 121°c for 15 min.

Afterthat following solutions were putted filter sterile in lukewarm basal medium:

10 ml of solution 1:

KH_2PO_4	10,8 g
K_2HPO_4	42 g
Aqua dest.	300 ml

10 ml of solution 2:

$MgSO_4$ x 7 H_2O	7,5 g
CaCl x 2 H_2O	0,6 g
NaCl	6 g
Aqua dest.	300 ml

1 ml of solution 3:

$FeCl_3$ x 6 H_2O	1,1 g
EDTA	0,015 g
Aqua dest.	300 ml

1 ml of solution 4:

Thiamine HCl (Vit. B_1)	0,1 g
Ca-Phanthotenate (Vit. B_5)	0,2 g
0,01g Biotin/ 100ml Biotin	20 ml
Aqua dest.	80 ml

20 ml of Glucose solution:

Glucose	5 g
Aqua dest.	50 ml

Each 300 ml of the basal medium (including 5 above called solutions) was filled in each 1 sterile bottle:

Bottle A: 300 ml basal medium as negative control

Bottle B: 300 ml basal medium and 2,1 ml NH_4Cl-solution as positive control

Bottle C: 300 ml basal medium and 2,1 ml ACC solution

The medium from each bottle was filled in sterile petri dishes.

NH_4Cl-solution:

NH_4Cl	1 g
Aqua dest.	10 ml

The solution was sterile filtrated.

ACC-solution:

ACC(1-aminocyclopane-1-carboxylate)	1 g
Aqua dest.	10 ml

The solution was sterile filtrated.

The plates were inoculated with different bacterial isolates and incubated at 28°C in darkness for 10 days.

Analysis:

The growing bacterial isolates on the plates were characterized:
0= no bacterial growth, 1= weak bacterial growth, 2= middle bacterial growth, 3= strong bacterial growth.

The total value was calculated following: The value of negative control was substracted from ACC value.
A high total value means a high production of ACC deaminase.

Quantification of auxin production of bacterial isolates:

Auxin production of bacterial isolates in presence and absence of L-Tryptophan was determined colorimetrically after the method of Sarwar et al. 1992.

5 ml 50% (v/v) TSB-Medium was inoculated with each bacterial isolate and incubated at 28°C overnight.

50% TSB-Medium:

Tryptic Soy Broth	15 g
Agar Agar	15 g
Aqua dest.	1000 ml

Medium was autoclaved at 121°C for 15 min.

Afterthat each 5 ml basal medium with and without tryptophan was inoculated with bacterial suspension of 50% TSB-overnight culture. The inoculated media were agitated at 180 rpm for 72h. The next step was that the solutions were centrifugated at 2000 rpm for 10 min at 4°C.

Basal medium with tryptophan:

Glucose	5 g
Yeast extract	0,025 g
L-Tryptophan	0,204 g
Aqua dest.	1000 ml

Medium was autoclaved at 121°C for 15 min.

Basal medium without tryptophan:

Glucose	5 g
Yeast extract	0,025 g
Aqua dest.	1000 ml

Medium was autoclaved at 121°C for 15 min.

For determination of IAA-concentration of bacterial isolates:
Each well of a 96-microtiter plate was filled with:
200 µl supernatant and 80 µl Salkowski-reagent

The microtiter plate was covered with aluminium foil and incubated at 28°C for 30 min. After incubation the concentration of IAA in media in the wells of microtiterplate was measured with spectrophotometer “Gen 5” at 560 nm.

Salkowski-reagent:

0,5 M FeCl3	1 ml
35% (v/v) Perchloric acid	49 ml

Auxin concentration produced by bacterial isolates was determined using standard curves for IAA prepared from serial dilutions of 5 – 200 µg/ml (Fig. 1).

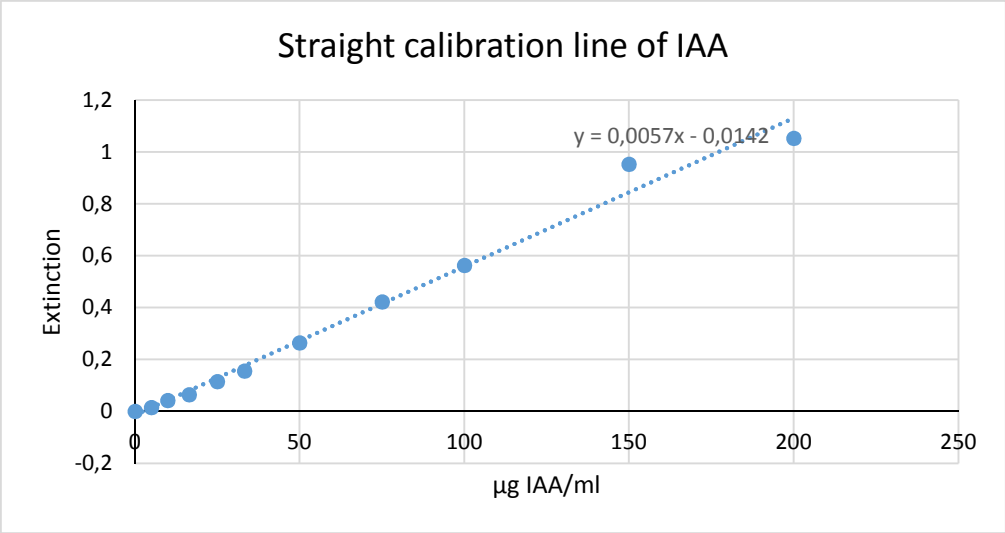

Fig. 1: Straight calibration line of IAA from a spectrophomotric measurement of different IAA concentrations

<u>Characterization of growth of different bacterial isolates via growth curves:</u>

Tubes with sterile 5 ml 10% TSB-Medium was inoculated with each bacterial isolate. The inoculated tubes was incubated at 28°C overnight and agitated at 200 rpm.

TSB-Medium 10 %:

Tryptic Soy Broth	3 g
Aqua dest.	1000 ml

Liquid medium was autoclaved at 121°C for 15 min.

Each well from 3 wells of a 96-Microtiter plate was filled with:

16 µl Bacterial suspension

+ 144 µl TSB-Medium 10 %

= 160 µl total of 1:10 dilution

16 µl Bacterial suspension

+ 144 µl TSB-Medium 10 %

= 160 µl total of 1:100 dilution

The optical density of bacterial suspension was measured at 600 nm in spectrophotometer “Gen 5” for 20 h.

(iv) Description of the main results obtained

Assay for siderophores production:

Unfortunately the CAS-plates were inoculated wrong with bacterial isolates. So the bacteria overgrown the plates and the visual analyse of a possibly color change of medium was not seen.

Characterization of Gram negative bacteria via BIOLOG plates (GN2 MicroPlate):

The bacterial isolates TP-W4, TP-W1 and B. phytofirmans PsJN showed bacterial growth on the most test substances in Biolog plates (Tab. 1 and 2).

Analysis of Biolog plates after 1 day:

- = no bacterial growth, + = weak bacterial growth, ++ = middle bacterial growth, +++ = strong bacterial growth

Des-cription of well	Test substance	Isolate TP-R1	Isolate TP-R9b	Isolate TP-W4	Isolate TP-W10	Isolate TP-R8b	Isolate TP-R3y	Isolate TP-R6a	Isolate TP-W1	Isolate B. PsJN
A 1	Water	-	-	-	+	-	-	-	+	-
B 1	i-Erythritol	-	-	-	+	-	-	-	++	-
C 1	D-Melibiose	-	-	+++	+	-	-	-	++	-
D 1	Acetic Acid	-	-	-	+	-	-	-	+++	-
E 1	p-Hydroxy Phenylacetic Acid	-	+	-	+	-	-	-	++	-
F 1	Bromosuc-cinic Acid	++	++	+++	+	-	+	-	++	++
G 1	L-Histidine	-	-	++	+	-	-	-	++	++
H 1	Urocanic Acid	+	+	-	++	-	+	-	+++	-
A 2	a-Cyclodextrin	-	-	-	+	-	-	-	+	-
B 2	D-Fructose	-	-	++	+	-	-	-	+	-
C 2	ß-Methyl-D-Glucoside	-	-	+++	+	-	-	-	+	-
D 2	Cis-Aconitic Acid	+++	+++	+++	+	-	+++	-	++	+++
E 2	Itaconic Acid	++	+	-	+	-	+	-	++	+
F 2	Succinamic Acid	-	-	-	+	-	-	-	++	-
G 2	Hydroxy-L-Proline	+++	+	-	+	-	++	-	++	-

H 2	**Inosine**	**-**	**-**	**-**	**+**	**-**	**-**	**-**	**++**	**-**
A 3	**Dextrin**	**-**	**-**	**+**	**+**	**+**	**-**	**+**	**++**	**-**
B 3	**L-Fucose**	**-**	**-**	**-**	**+**	**-**	**-**	**-**	**++**	**-**
C 3	**D-Psicose**	**-**	**-**	**++**	**+**	**-**	**-**	**-**	**+**	**-**
D 3	**Citric Acid**	**+++**	**+++**	**++**	**+**	**-**	**+++**	**-**	**+**	**++**
E 3	**a-Keto Butyric Acid**	**-**	**-**	**-**	**+**	**-**	**-**	**-**	**+**	**+**
F 3	**Glucuron-amide**	**+**	**+++**	**-**	**+**	**-**	**+**	**-**	**++**	**+**
G 3	**L-Leucine**	**-**	**-**	**-**	**+**	**-**	**-**	**-**	**++**	**-**
H 3	**Uridine**	**-**	**-**	**-**	**+**	**-**	**-**	**-**	**++**	**-**
A 4	**Glycogen**	**-**	**-**	**-**	**+**	**-**	**-**	**-**	**+**	**-**
B 4	**D-Galactose**	**+**	**-**	**+++**	**+**	**-**	**++**	**-**	**+**	**++**
C 4	**D-Raffinose**	**-**	**-**	**++**	**+**	**-**	**-**	**-**	**+**	**-**
D 4	**Formic Acid**	**-**	**-**	**-**	**+**	**-**	**-**	**-**	**+**	**-**
E 4	**a-Keto Glutaric Acid**	**+++**	**+**	**-**	**+**	**-**	**++**	**-**	**++**	**-**
F 4	**L-Alaminamide**	**-**	**-**	**-**	**+**	**-**	**-**	**-**	**++**	**-**
G 4	**L-Ornithine**	**-**	**-**	**-**	**+**	**-**	**-**	**-**	**++**	**-**
H 4	**Thymidine**	**-**	**-**	**-**	**+**	**-**	**-**	**-**	**++**	**-**
A 5	**Tween 40**	**-**	**-**	**+**	**++**	**-**	**-**	**-**	**+++**	**+**
B 5	**Gentiobiose**	**-**	**-**	**+++**	**+**	**-**	**-**	**-**	**+**	**-**
C 5	**L-Rhamnose**	**-**	**-**	**+++**	**+**	**-**	**-**	**-**	**+**	**++**
D 5	**D-Galactonic Acid Lactone**	**-**	**-**	**-**	**+**	**-**	**-**	**-**	**+**	**+**
E 5	**a-Keto Valeric**	**-**	**+**	**-**	**+**	**-**	**-**	**-**	**++**	**-**
F 5	**D-Alanine**	**+**	**-**	**+**	**+**	**-**	**-**	**-**	**++**	**-**
G 5	**L-Phenyl-alanine**	**-**	**-**	**-**	**+**	**-**	**-**	**-**	**++**	**++**
H 5	**Phenyethyl-amine**	**-**	**-**	**-**	**+**	**-**	**-**	**-**	**+**	**-**
A 6	**Tween 80**	**-**	**-**	**+**	**++**	**-**	**-**	**-**	**+++**	**+**
B 6	**α-D-Glucose**	**+**	**-**	**+++**	**+**	**-**	**-**	**-**	**++**	**++**
C 6	**D-Sorbiol**	**-**	**-**	**+++**	**+**	**-**	**-**	**-**	**+**	**++**
D 6	**D-Galactur-onic Acid**	**+++**	**+++**	**+++**	**+**	**-**	**+++**	**-**	**+**	**+++**
E 6	**D,L-Lactic Acid**	**+++**	**++**	**+++**	**+**	**-**	**++**	**-**	**+++**	**++**
F 6	**L-Alanine**	**++**	**+**	**++**	**+**	**-**	**+**	**-**	**++**	**+**

G 6	L-Proline	++	++	+++	+	-	+	-	++	++
H 6	Putrescine	++	-	-	+	-	-	-	++	-
A 7	N-Acetyl-D-Galactosamine	-	-	-	+	-	-	-	+	-
B 7	m-Inositol	-	-	+++	+	-	-	-	+	+
C 7	Sucrose	-	-	+++	+	-	-	-	+++	-
D 7	D-Gluconic Acid	-	-	+++	+	-	+	-	+	++
E 7	Malonic Acid	+	-	-	+	-	-	-	+	++
F 7	L-Alanyl-glycerin	-	-	+	+	-	-	-	++	-
G 7	L-Pyroglu-tamic Acid	++	+	-	+	-	+	-	+++	++
H 7	2-Amino-ethanol	-	-	-	+	-	-	-	++	++
A 8	N-Acethyl-D-Glucosamine	-	-	+++	+	-	-	-	+	++
B 8	a-D-Lactose	-	-	+++	+	-	-	-	+	-
C 8	D-Trehalose	-	+	+++	+	-	+	-	++	-
D 8	D-Glucos-aminic Acid	-	-	+++	+	-	-	-	++	++
E 8	Propionic Acid	-	-	-	+	-	-	-	+	-
F 8	L-Asparagine	+++	++	+++	+	-	++	-	++	++
G 8	D-Serine	-	-	-	+	-	-	-	++	-
H 8	2,3-Butanediol	-	-	-	+	-	-	-	++	-
A 9	Adonitol	-	-	-	+	-	-	-	+	++
B 9	Lactulose	-	-	-	+	-	-	-	+	-
C 9	Turanose	-	-	-	+	-	-	-	++	-
D 9	D-Glucuronic Acid	+++	++	+++	+	-	+++	-	++	++
E 9	Quinic Acid	+++	+	++	+	-	+	-	+	++
F 9	L-Aspartic Acid	+++	+	+++	+	-	++	-	++	++
G 9	L-Serine	++	+	+	+	-	+	-	++	+
H 9	Glycerol	+	-	+++	+	-	-	+	++	+
A 10	L-Arabinose	+++	+++	+++	++	++	++	-	++	++
B 10	Maltrose	-	-	+++	+	-	-	-	+++	-
C 10	Xylitol	-	-	-	+	-	-	-	+	-

D 10	**α-Hydroxy-butyric Acid**	**-**	**-**	**-**	**+**	**-**	**-**	**-**	**++**	**-**
E 10	**D-Saccharic Acid**	**+++**	**-**	**+++**	**+**	**-**	**-**	**-**	**+**	**+++**
F 10	**L-Glutamic Acid**	**+++**	**++**	**+++**	**+**	**-**	**++**	**-**	**+++**	**+++**
G 10	**L-Threonine**	**-**	**-**	**-**	**+**	**-**	**-**	**-**	**++**	**+**
H 10	**D,L-α-Glycerol Phosphate**	**+**	**-**	**+**	**+**	**-**	**-**	**-**	**++**	**-**
A 11	**D-Arabitol**	**-**	**-**	**+++**	**+**	**-**	**-**	**-**	**+**	**++**
B 11	**D-Mannitol**	**+**	**-**	**+++**	**+**	**-**	**+**	**-**	**++**	**++**
C 11	**Pyruvic Acid Methyl Ester**	**++**	**-**	**+**	**+**	**-**	**+**	**-**	**+++**	**++**
D 11	**ß-Hydroxy-butyric Acid**	**-**	**-**	**-**	**+**	**-**	**+**	**-**	**++**	**++**
E 11	**Sebacic Acid**	**-**	**-**	**++**	**+**	**-**	**-**	**-**	**+**	**+**
F 11	**Glycyl-L-Aspartic Acid**	**-**	**-**	**-**	**+**	**-**	**-**	**-**	**++**	**-**
G 11	**D,L-Carnitine**	**+**	**-**	**-**	**+**	**-**	**-**	**-**	**++**	**+**
H 11	**α-D-Glucose-1-Phosphate**	**-**	**-**	**+++**	**+**	**-**	**-**	**-**	**++**	**-**
A 12	**D-Cellobiose**	**-**	**-**	**+++**	**+**	**-**	**-**	**-**	**+**	**-**
B 12	**D-Mannose**	**-**	**-**	**+++**	**+**	**-**	**-**	**-**	**++**	**+**
C 12	**Succinic Acid Mono-Methyl-Ester**	**-**	**-**	**-**	**+**	**-**	**-**	**-**	**++**	**-**
D 12	**y-Hydroxy-butyric Acid**	**-**	**-**	**-**	**+**	**-**	**-**	**-**	**+**	**-**
E 12	**Succinic Acid**	**+++**	**++**	**+++**	**++**	**-**	**++**	**-**	**++**	**+++**
F 12	**Glycyl-L-Glutamic Acid**	**-**	**-**	**-**	**+**	**-**	**-**	**-**	**++**	**-**
G 12	**y-Amino-Butyric Acid**	**+++**	**+**	**-**	**+**	**-**	**+**	**-**	**++**	**+**
H 12	**D-Glucose-6-Phosphate**	**-**	**-**	**++**	**++**	**-**	**-**	**-**	**++**	**-**

Tab. 1: Overview about growth of different bacterial isolates on various test substances in Biolog plates

Analysis after 4 days:

Des-cription of well	**Test substance**	**Isolate TP-R1**	**Isolate TP-R9b**	**Isolate TP-W4**	**Isolate TP-W10**	**Isolate TP-R8b**	**Isolate TP-R3y**	**Isolate TP-R6a**	**Isolate TP-W1**	**Isolate B. PsJN**
A 1	**Water**	**-**	**-**	**-**	**+**	**-**	**-**	**-**	**+**	**-**

B 1	i-Erythritol	-	-	-	+	-	-	-	++	-
C 1	D-Melibiose	-	-	+++	+	-	-	-	++	-
D 1	Acetic Acid	-	-	-	+	-	-	-	+++	-
E 1	p-Hydroxy Phenylacetic Acid	-	+	-	+	-	-	-	+++	+
F 1	Bromosuc-cinic Acid	++	++	+++	+	-	+	-	++	++
G 1	L-Histidine	+++	+	+++	+	-	+	+	++	++
H 1	Urocanic Acid	+++	++	-	++	-	+++	-	+++	+++
A 2	a-Cyclodextrin	-	-	-	+	-	-	-	+	-
B 2	D-Fructose	-	-	++	+	-	-	-	++	++
C 2	ß-Methyl-D-Glucoside	-	-	+++	+	-	-	-	+	-
D 2	Cis-Aconitic Acid	+++	+++	+++	+	-	+++	-	++	+++
E 2	Itaconic Acid	+++	+	-	+	-	+	-	++	++
F 2	Succinamic Acid	+	-	-	+	-	-	-	++	++
G 2	Hydroxy-L-Proline	+++	++	-	+	-	+++	-	++	++
H 2	Inosine	-	-	-	+	-	-	-	++	++
A 3	Dextrin	-	-	+	+	+	-	+	+++	-
B 3	L-Fucose	-	-	-	+	-	-	-	+	++
C 3	D-Psicose	-	+	+++	+	-	-	-	+	-
D 3	Citric Acid	+++	+++	+++	+	-	+++	-	+	+++
E 3	a-Keto Butyric Acid	-	+	-	+	-	-	-	+++	++
F 3	Glucuron-amide	+	+++	-	+	-	+	-	++	+
G 3	L-Leucine	+	-	-	+	-	-	-	++	-
H 3	Uridine	-	-	-	+	-	-	-	++	-
A 4	Glycogen	-	-	-	+	-	-	-	+	-
B 4	D-Galactose	+++	++	+++	+	-	+++	-	+	+++
C 4	D-Raffinose	-	-	+++	+	-	-	-	+	-
D 4	Formic Acid	-	-	+	+	-	-	-	+	++
E 4	a-Keto Glutaric Acid	+++	+	-	+	-	++	-	+++	+++

F 4	L-Alaminamide	-	-	-	+	-	-	-	++	++
G 4	L-Ornithine	-	-	-	+	-	-	-	++	++
H 4	Thymidine	-	-	-	+	-	-	-	++	-
A 5	Tween 40	-	+	+++	+	-	++	-	+++	++
B 5	Gentiobiose	-	-	+++	+	-	-	-	+	++
C 5	L-Rhamnose	-	+	+++	+	-	-	-	+	+++
D 5	D-Galactonic Acid Lactone	-	++	-	+	-	++	-	++	++
E 5	a-Keto Valeric	-	+	-	+	-	-	-	++	-
F 5	D-Alanine	+++	+	+++	+	-	++	-	++	+
G 5	L-Phenyl-alanine	-	-	-	+	-	-	-	++	++
H 5	Phenyethyl-amine	-	-	-	+	-	-	-	+	++
A 6	Tween 80	-	+	+++	++	++	++	-	+++	++
B 6	α-D-Glucose	+++	++	+++	+	-	+++	-	++	+++
C 6	D-Sorbiol	-	-	+++	+	-	-	-	+	++
D 6	D-Galactur-onic Acid	+++	+++	+++	+	-	+++	-	+	+++
E 6	D,L-Lactic Acid	+++	++	+++	+	-	++	-	+++	+++
F 6	L-Alanine	+++	++	+++	+	-	++	-	+++	++
G 6	L-Proline	+++	+++	+++	+	-	++	-	++	++
H 6	Putrescine	+++	+	-	+	-	++	-	++	+
A 7	N-Acetyl-D-Galactosamin e	-	-	-	+	-	-	-	+	-
B 7	m-Inositol	-	-	+++	+	-	-	-	+	++
C 7	Sucrose	-	-	+++	+	-	-	-	+++	-
D 7	D-Gluconic Acid	-	++	+++	+	-	++	-	+	+++
E 7	Malonic Acid	+	-	-	+	-	-	-	+	+++
F 7	L-Alanyl-glycerin	-	-	+++	+	-	-	-	+++	+
G 7	L-Pyroglu-tamic Acid	+++	+++	-	+	+++	++	+	+++	++
H 7	2-Amino-ethanol	+++	++	-	+	-	++	-	++	++
A 8	N-Acethyl-D-Glucosamine	-	-	+++	+	-	-	-	+	++

B 8	a-D-Lactose	-	-	+++	+	-	-	-	+	-
C 8	D-Trehalose	-	++	+++	+	-	++	-	+++	-
D 8	D-Glucos-aminic Acid	-	-	+++	+	-	-	-	+	+++
E 8	Propionic Acid	+	-	-	+	-	-	-	+	+
F 8	L-Asparagine	+++	+++	+++	+	-	+++	+	++	++
G 8	D-Serine	-	-	-	+	-	-	-	++	-
H 8	2,3-Butanediol	-	-	-	+	-	-	-	++	-
A 9	Adonitol	-	-	-	+	-	-	-	+	++
B 9	Lactulose	-	-	++	+	-	-	-	+	-
C 9	Turanose	-	-	-	+	-	-	-	+++	-
D 9	D-Glucuronic Acid	+++	+++	+++	+	+++	+++	-	++	+++
E 9	Quinic Acid	+++	+++	+++	+	-	+++	-	+	++
F 9	L-Aspartic Acid	+++	+++	+++	+	-	+++	-	++	++
G 9	L-Serine	+++	++	+++	+	-	++	-	+++	++
H 9	Glycerol	+++	+	+++	+	-	++	+	++	++
A 10	L-Arabinose	+++	+++	+++	++	++	+++	++	++	++
B 10	Maltrose	-	-	+++	+	-	-	-	+++	
C 10	Xylitol	-	-	-	+	-	-	-	+	+
D 10	α-Hydroxy-butyric Acid	-	-	-	+	-	-	-	++	+++
E 10	D-Saccharic Acid	+++	++	+++	+	-	++	-	+	+++
F 10	L-Glutamic Acid	+++	+++	+++	+	-	+++	-	+++	+++
G 10	L-Threonine	-	-	-	+	-	-	-	++	++
H 10	D,L-α-Glycerol Phosphate	+	-	+	+	-	-	-	++	-
A 11	D-Arabitol	-	-	+++	+	-	-	-	+	+++
B 11	D-Mannitol	+++	++	+++	+	-	+++	-	+++	+++
C 11	Pyruvic Acid Methyl Ester	+++	-	++	+	-	++	-	+++	+++
D 11	ß-Hydroxy-butyric Acid	+++	++	-	+	-	++	-	++	+++

E 11	**Sebacic Acid**	-	-	++	+	-	-	-	+	++
F 11	**Glycyl-L-Aspartic Acid**	-	-	-	+	-	-	-	+++	-
G 11	**D,L-Carnitine**	+	+	-	+	-	+	-	+++	+
H 11	**α-D-Glucose-1-Phosphate**	-	-	+++	+	-	-	-	+++	-
A 12	**D-Cellobiose**			+++	+				+	
B 12	**D-Mannose**	+	+++	+++	+	-	+++	-	++	++
C 12	**Succinic Acid Mono-Methyl-Ester**	-	++	-	+	-	-	-	++	-
D 12	**y-Hydroxy-butyric Acid**	-	-	-	+	-	-	-	+	-
E 12	**Succinic Acid**	+++	++	+++	++	-	++		++	+++
F 12	**Glycyl-L-Glutamic Acid**	-	-	-	+	-	-	-	++	
G 12	**y-Amino-Butyric Acid**	+++	++	-	+	-	++	-	++	++
H 12	**D-Glucose-6-Phosphate**	-	-	+++	++	-	-	-	++	+

Tab. 2: Overview about growth of different bacterial isolates on various test substances in Biolog plates

Assay for availability of ACC deaminase:

Only the bacterial isolate *Burkholderia phytofirmans* PsJN showed ACC deaminase activity on plate where this bacterium grown alone (Tab. 3).

Bacterial isolate	Positive control	ACC	Negative control	Total
1 PsJN	3	3	1	2
2 PsJN	0	0	2	-2
2 TP-W4	3	1	1	0
2 TP-R6a	3	0	1	-1
3 TP-W4	3	1	1	0
3 TP-W10	0	0	0	0
3 TP-R8b	1	0	0	0
4 TP-W10	1	0	1	-1
4 TP-R9b	1	0	1	-1

4 TP-R6a	0	0	1	-1
5 TP-R8b	1	0	1	-1
5 TP-W1	2	0	1	-1
5 PsJN	0	0	0	0
6 TP-R3y	2	0	1	-1
6 TP-R9b	2	0	1	-1
6 TP-R8b	2	0	0	0
7 TP-R1	2	0	0	0
7 TP-W10	1	0	1	-1
7 TP-W1	1	0	0	0
8 TP-W4	2	0	1	-1
8 TP-R1	2	0	0	0
8 TP-R3y	2	0	1	-1
9 TP-W1	1	0	1	-1
9 TP-R3y	3	0	1	-1
9 TP-R6a	1	0	1	-1
10 PsJN	0	0	0	0
10 TP-R1	2	0	0	0
10 TP-R9b	2	0	1	-1

Tab. 3 Growth of different bacterial isolates on Media with and without ACC

The isolates TP-R3y, TP-W4 and TP-R1 could grow only positive control medium and not on ACC and negative control medium (fig. 2).

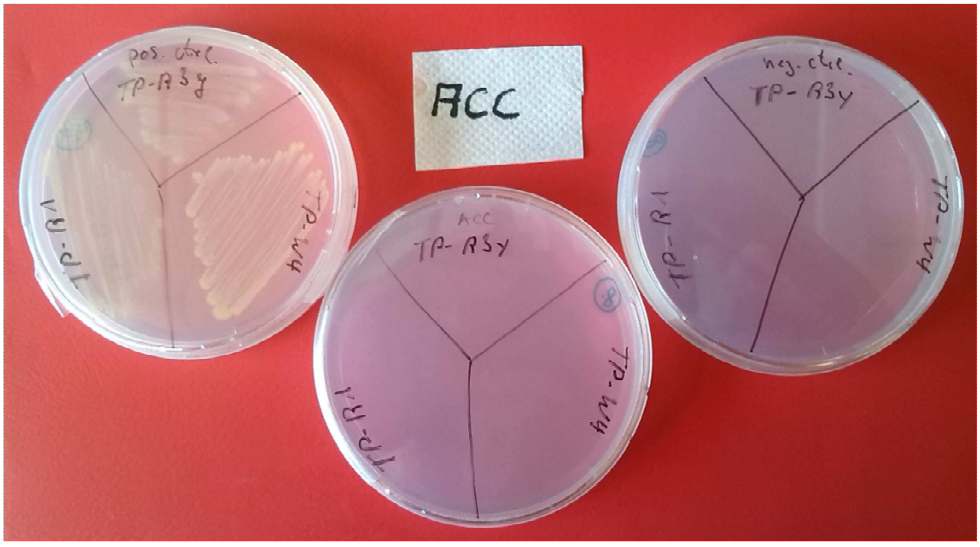

Fig. 2: Medium of positive control (left), ACC-medium (middle) and medium of negative control with streaked out bacterial isolates TP-R3y, TP-W4 and TP-R1

Quantification of auxin production of bacterial isolates:

The bacterial isolate *TP-W4* was characterized by the highest concentration of IAA (~15 µg IAA/ml) in comparison to the other isolates. The other isolates produced 2 – 6 µg/ml of IAA (Fig. 3).

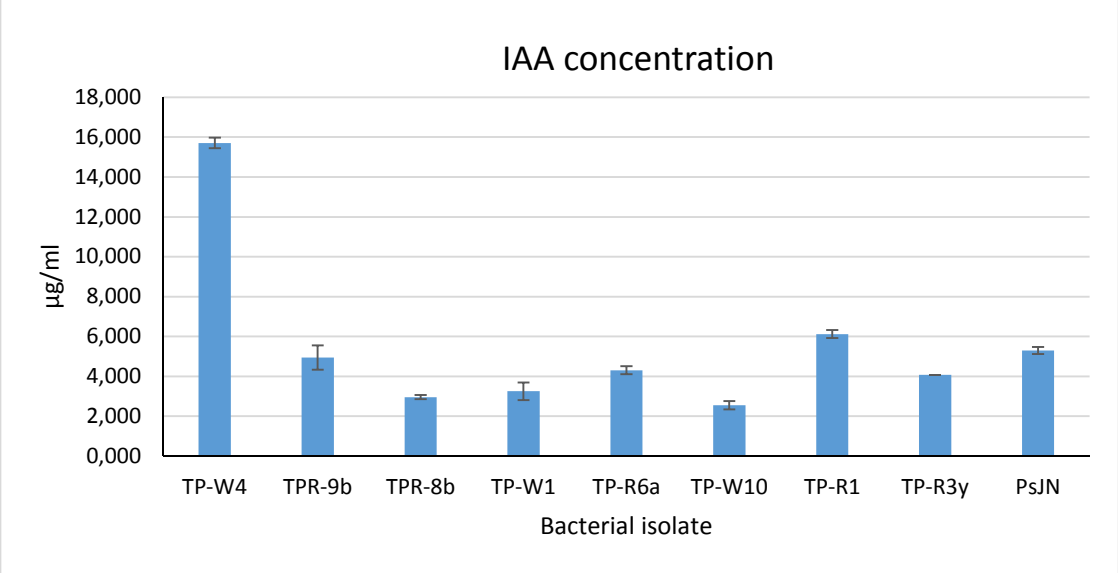

Fig. 3: IAA concentrations of different bacterial isolates measured via spectrophotometer

Characterization of growth of different bacterial isolates via growth curves:

The bacterial isolates TP-W10, TP-R9b, TP-R3y and TP-W4 showed similar growth phases in liquid medium for 20 h. Their lag-phase was from 0 – 2 h, the exponential phase from 2 – 5 h, the stationary phase from 5 – 8 h. Afterthat the die off phase began. The bacterial isolates TP-R6a and TP-R8b showed a longer lag-phase from 0 – 8 h. The exponential phase was from 8 – 13 h, following of a short stationary phase and a quick die off phase. The isolate TP-W1 showed no stationary and die off phase after 20 h, but a lag –phase from 0 – 8 h, following an exponential phase. The bacterial isolate TP-R1 was characterized by a more linear cell growth which is not typically for microbial cultures (Fig. 4).

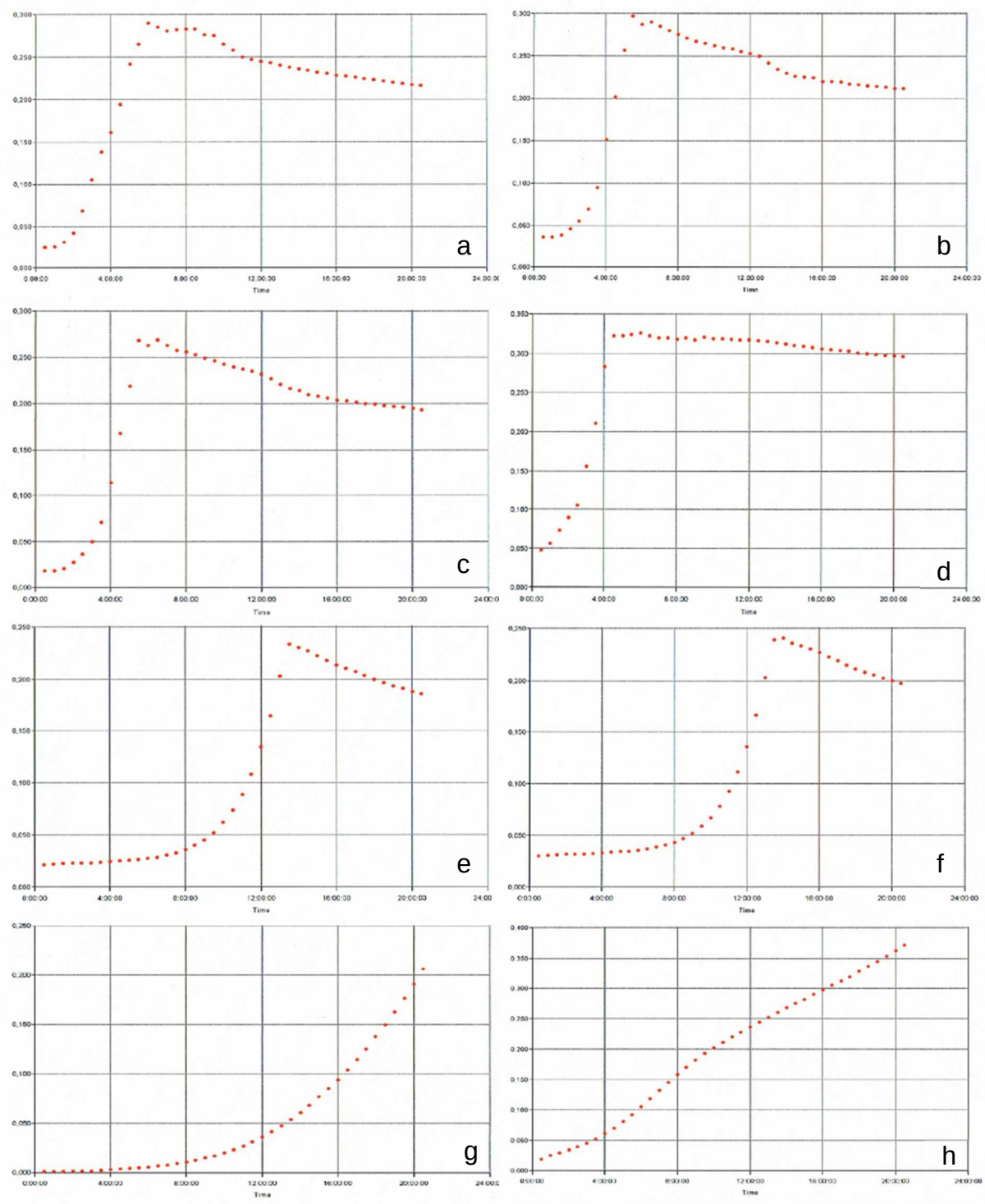

Fig. 4: Growth phases of batch cultures of different bacterial isolates: TP-W10 (a), TP-R9b (b), TP-R3y (c), TP-W4 (d), TP-R6a (e), TP-R8b (f), TP-W1 (g), TP-R1 (h)

(v) Future collaboration with host institution

Actually there is no plan for that.

(vi) Foreseen publications/articles resulting or to result from the STSM (if applicable)

The skills which I learned during this STSM will help me by my further lab work.

(vii) References

Sarwar, M., Arshad, M., Martens, D.A. and Frankenberger, W.T. Jr. 1992. Trypto-phan dependent biosynthesis of auxins in soil. Plant Soil 147:207-215.

Schwyn, B. and Neilands, J.B. 1987. Universal chemical assay for the detection and determination of siderophores. Anal. Biochem. 160:47-56.