

Application of Ecoplates/GenI II plates in the studies with bacteria

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EXCALIBUR

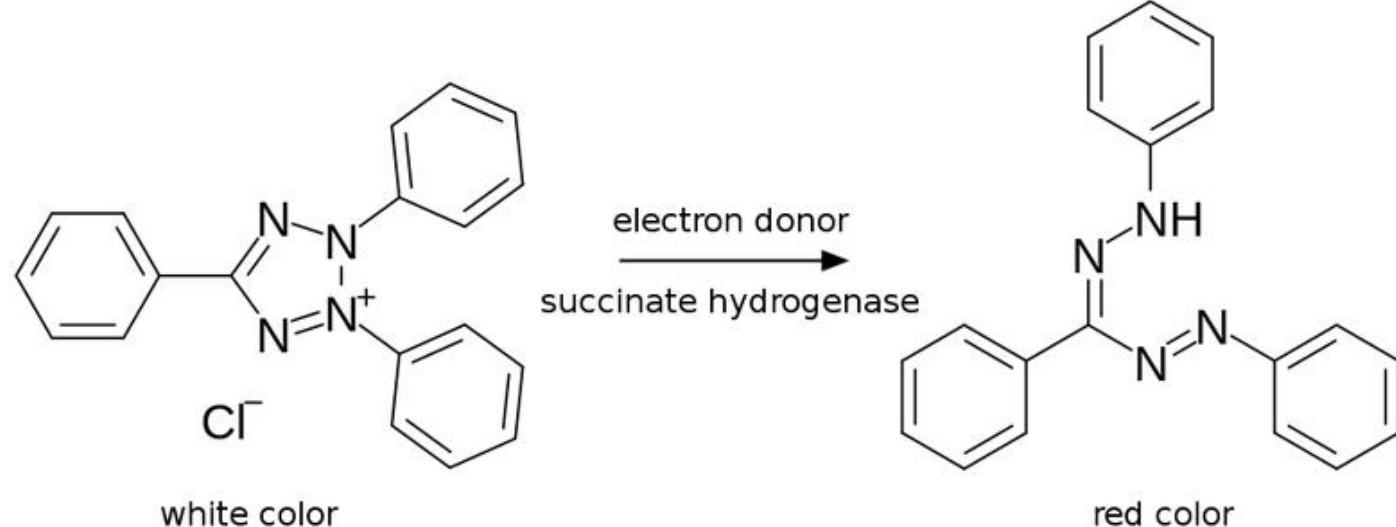


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817946



What is the Biolog system

The Biolog is a system based on the measurement of microbial dehydrogenase activity in the presence of different carbon sources or chemical compounds. The obtained phenotype profile could be used for the identification of microorganisms.



Biolog - Microbial Identification - MicroStation



<https://www.exportersindia.com/product-detail/microbial-identification-system-3518166.htm>

Biolog - OmniLog



<https://www.biomaxima.com/193-systemy-do-identyfikacji-drobnoustrojow-firmy-biolog.html>

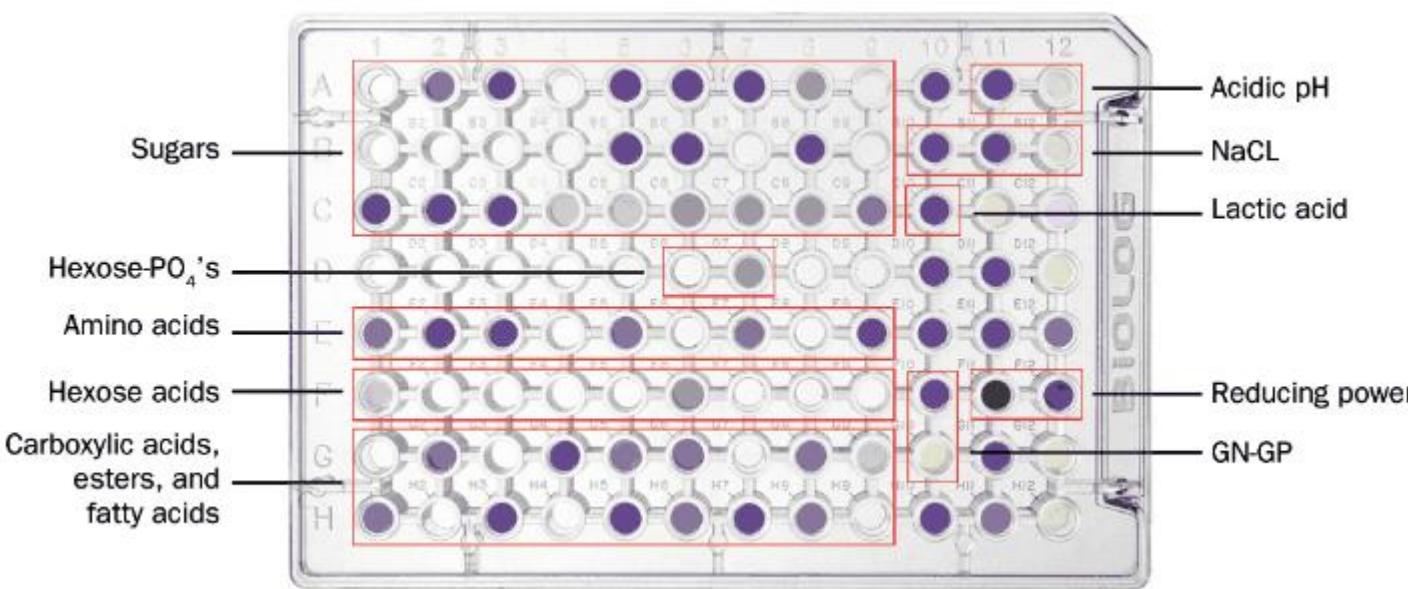
Microbial identification & characterisation

There are currently four main types of Biolog tests designed for microbial identification:

- GENIII - for aerobic bacteria identification
- AN - for anaerobic bacteria identification
- FF - for fungi & yeast identification
- YT - for yeast identification



Reagents used in GENIII plates



GEN III MicroPlate

A1 Negative Control	A2 Dextrin	A3 D-Maltose	A4 D-Trehalose	A5 D-Cellobiose	A6 Gentibiose	A7 Sucrose	A8 D-Turanose	A9 Stachyose	A10 Positive Control	A11 pH 6	A12 pH 5
B1 D-Raffinose	B2 α -D-Lactose	B3 D-Mellibiose	B4 β -Methyl-D-Glucoside	B5 D-Salicin	B6 N-Acetyl-D-Glucosamine	B7 N-Acetyl- β -D-Mannosamine	B8 N-Acetyl-D-Galactosamine	B9 N-Acetyl Neuraminc Acid	B10 1% NaCl	B11 4% NaCl	B12 8% NaCl
C1 α -D-Glucose	C2 D-Mannose	C3 D-Fructose	C4 D-Galactose	C5 3-Methyl Glucose	C6 D-Fucose	C7 L-Fucose	C8 L-Rhamnose	C9 Inosine	C10 1% Sodium Lactate	C11 Fusidic Acid	C12 D-Serine
D1 D-Sorbitol	D2 D-Mannitol	D3 D-Arabitol	D4 myo-Inositol	D5 Glycerol	D6 D-Glucose-6-PO4	D7 D-Fructose-6-PO4	D8 D-Aspartic Acid	D9 D-Serine	D10 Troleandomycin	D11 Rifamycin SV	D12 Minocycline
E1 Gelatin	E2 Glycyl-L-Proline	E3 L-Alanine	E4 L-Arginine	E5 L-Aspartic Acid	E6 L-Glutamic Acid	E7 L-Histidine	E8 L-Pyroglutamic Acid	E9 L-Serine	E10 Lincomycin	E11 Guanidine HCl	E12 Nlaprof 4
F1 Pectin	F2 D-Galacturonic Acid	F3 L-Galactonic Acid Lactone	F4 D-Gluconic Acid	F5 D-Glucuronic Acid	F6 Glucuronamide	F7 Mucoic Acid	F8 Quinic Acid	F9 D-Saccharic Acid	F10 Vancomycin	F11 Tetrazolium Violet	F12 Tetrazolium Blue
G1 p-Hydroxy-Phenylacetic Acid	G2 Methyl Pyruvate	G3 D-Lactic Acid Methyl Ester	G4 L-Lactic Acid	G5 Citric Acid	G6 α -Keto-Glutamic Acid	G7 D-Malic Acid	G8 L-Malic Acid	G9 Bromo-Succinic Acid	G10 Nalidixic Acid	G11 Lithium Chloride	G12 Potassium Tellurite
H1 Tween 40	H2 γ -Amino-Butyric Acid	H3 α -Hydroxy-Butyric Acid	H4 β -Hydroxy-D,L-Butyric Acid	H5 α -Keto-Butyric Acid	H6 Acetoacetic Acid	H7 Propionic Acid	H8 Acetic Acid	H9 Formic Acid	H10 Aztreonam	H11 Sodium Butyrate	H12 Sodium Bromate

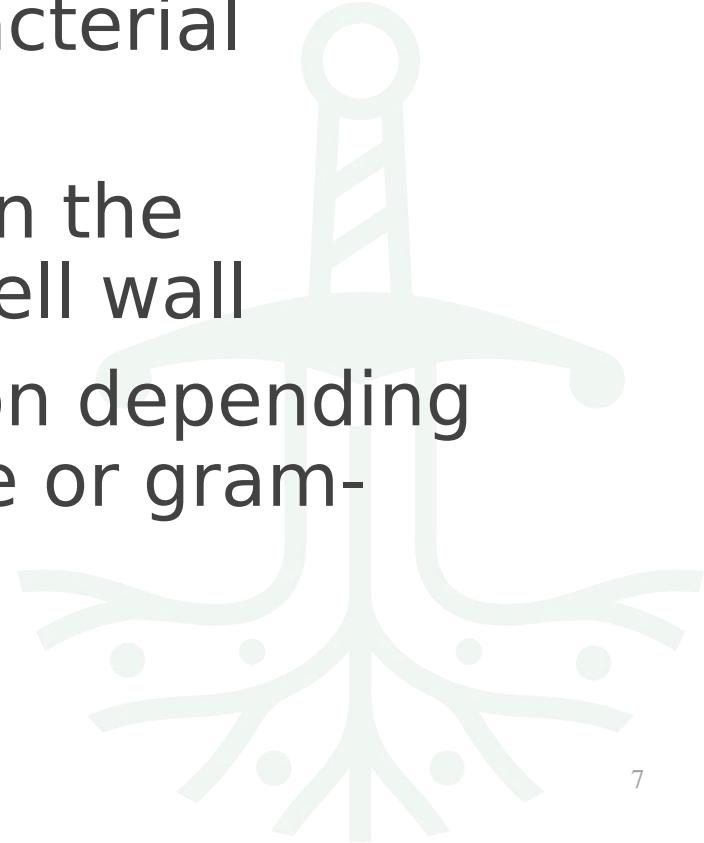
https://www.biolog.com/wp-content/uploads/2020/04/Universal_GEN_III_Protocol_07172013.pdf

https://www.biolog.com/wp-content/uploads/2020/04/00P_185_GEN_III_MicroPlate_IFU.pdf

Aerobic bacteria identification & characterization (GENIII plates)

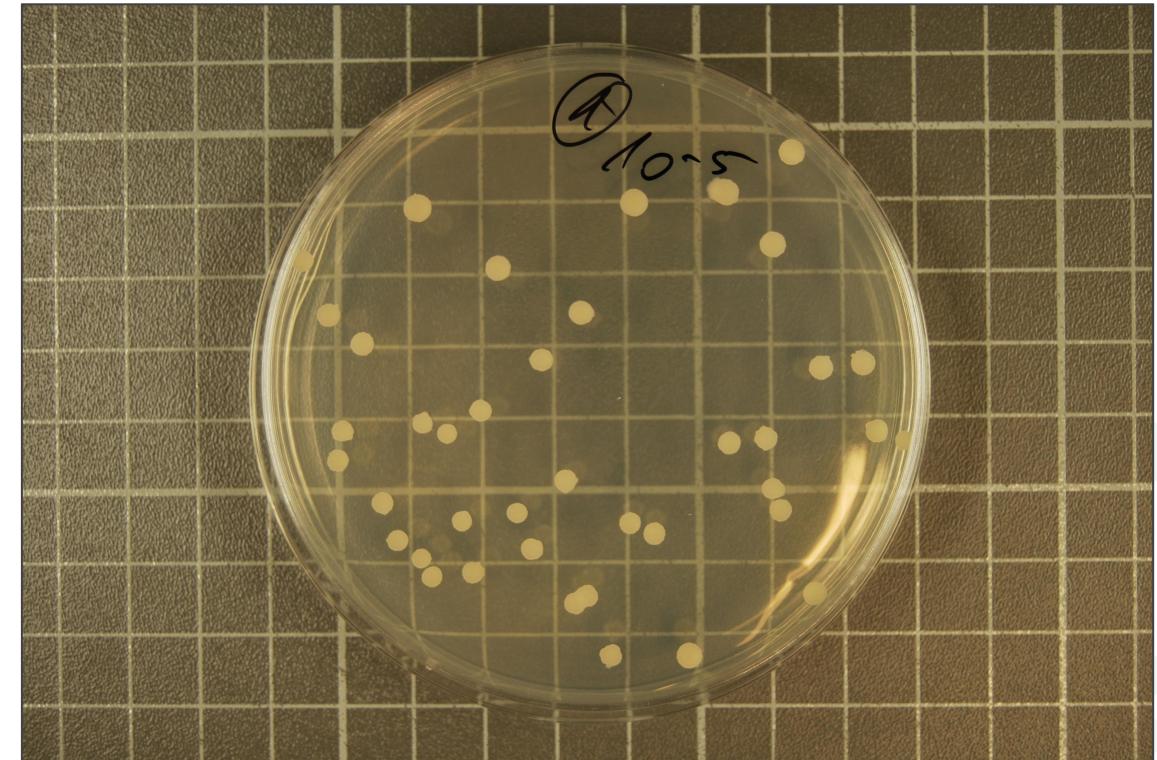
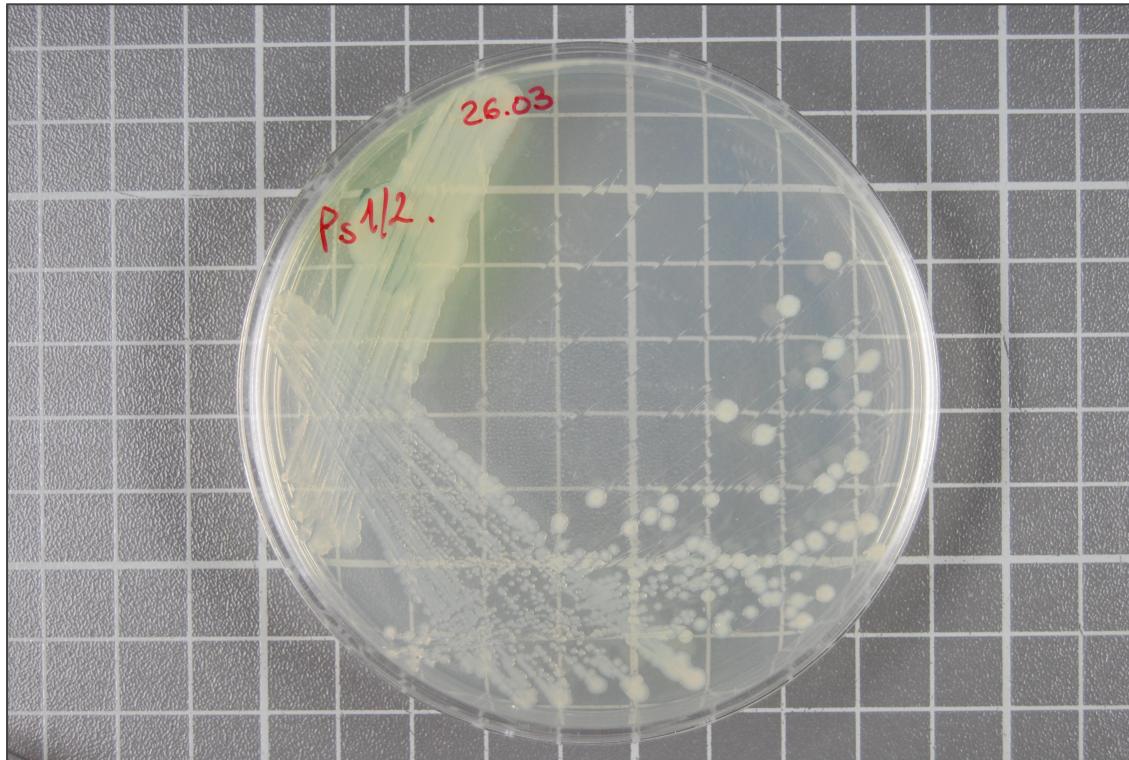
Sample preparation:

1. Obtaining the pure culture of bacteria
2. Choosing the agar media for obtaining bacterial biomass for the test
3. Differentiation of tested bacteria based on the chemical and physical properties of the cell wall
4. Choosing the protocol for the identification depending on the cell wall properties (gram-negative or gram-positive) and metabolism
5. Plates inoculation & incubation



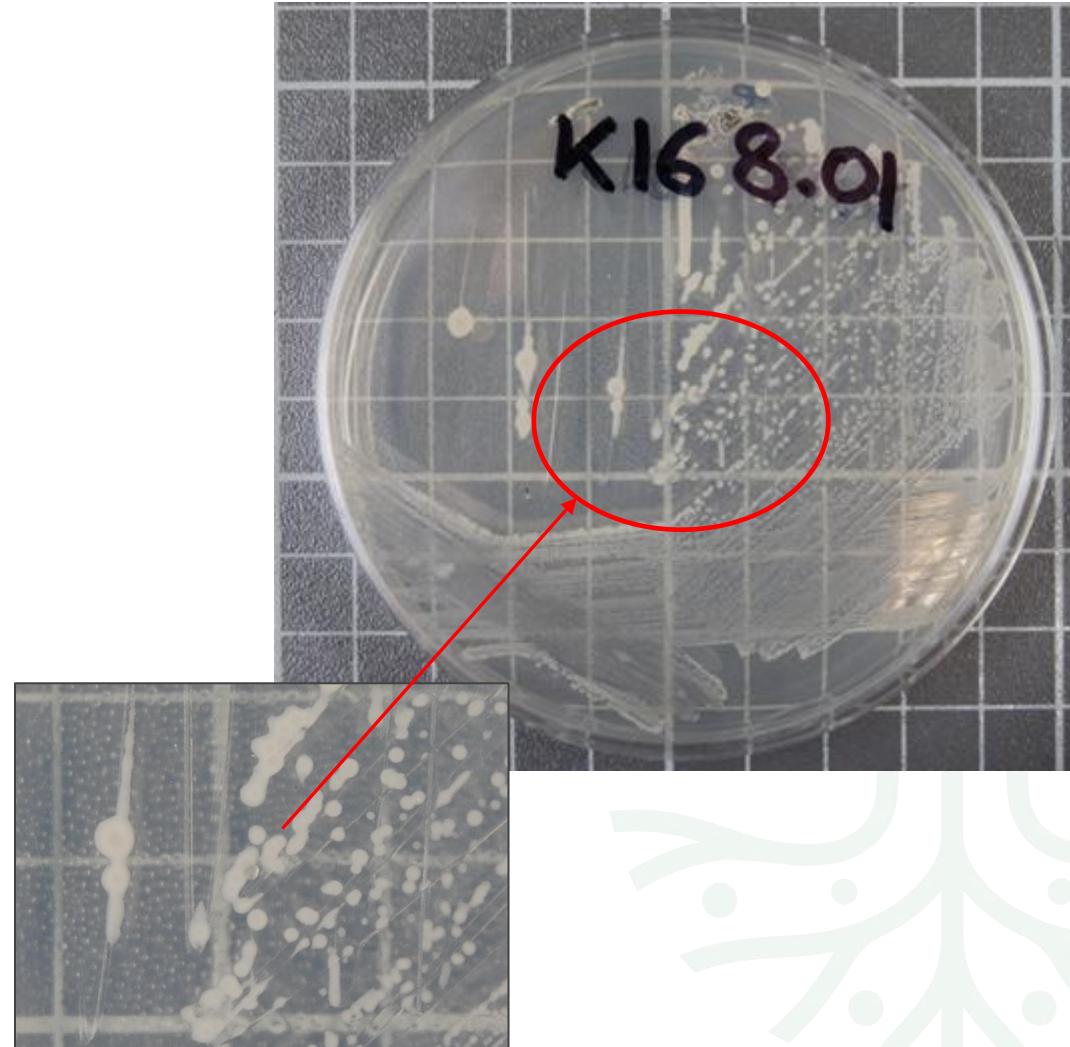
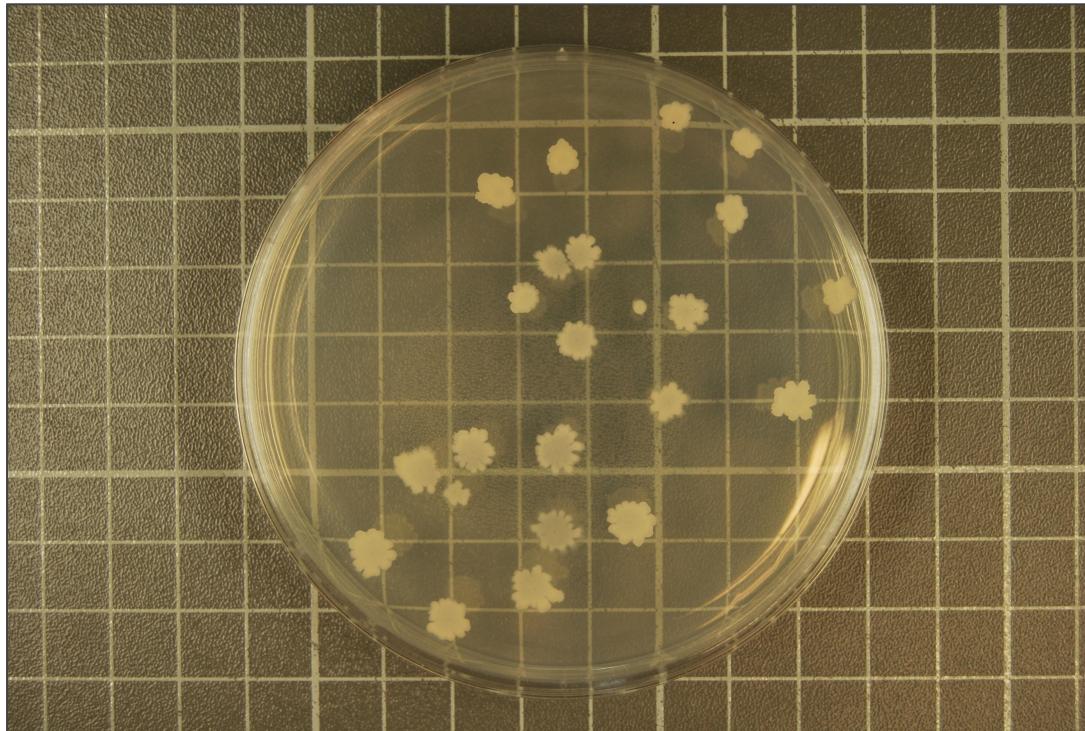
Aerobic bacteria identification & characterization (GENIII plates)

Obtaining the pure culture of bacteria



Aerobic bacteria identification & characterization (GENIII plates)

Obtaining the pure culture of bacteria

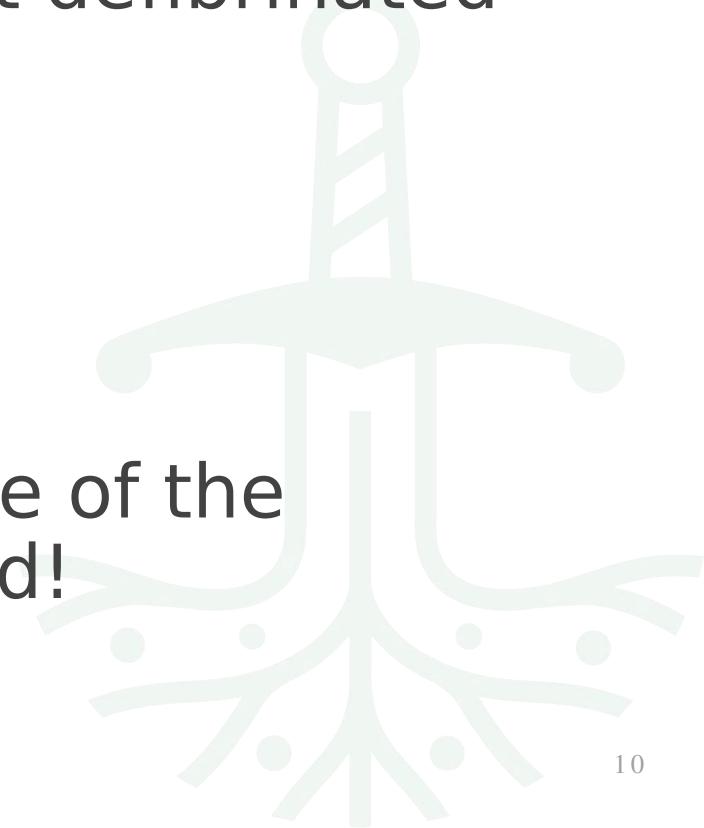


Aerobic bacteria identification & characterization (GENIII plates)

Choosing the agar media for obtaining bacterial biomass for the test

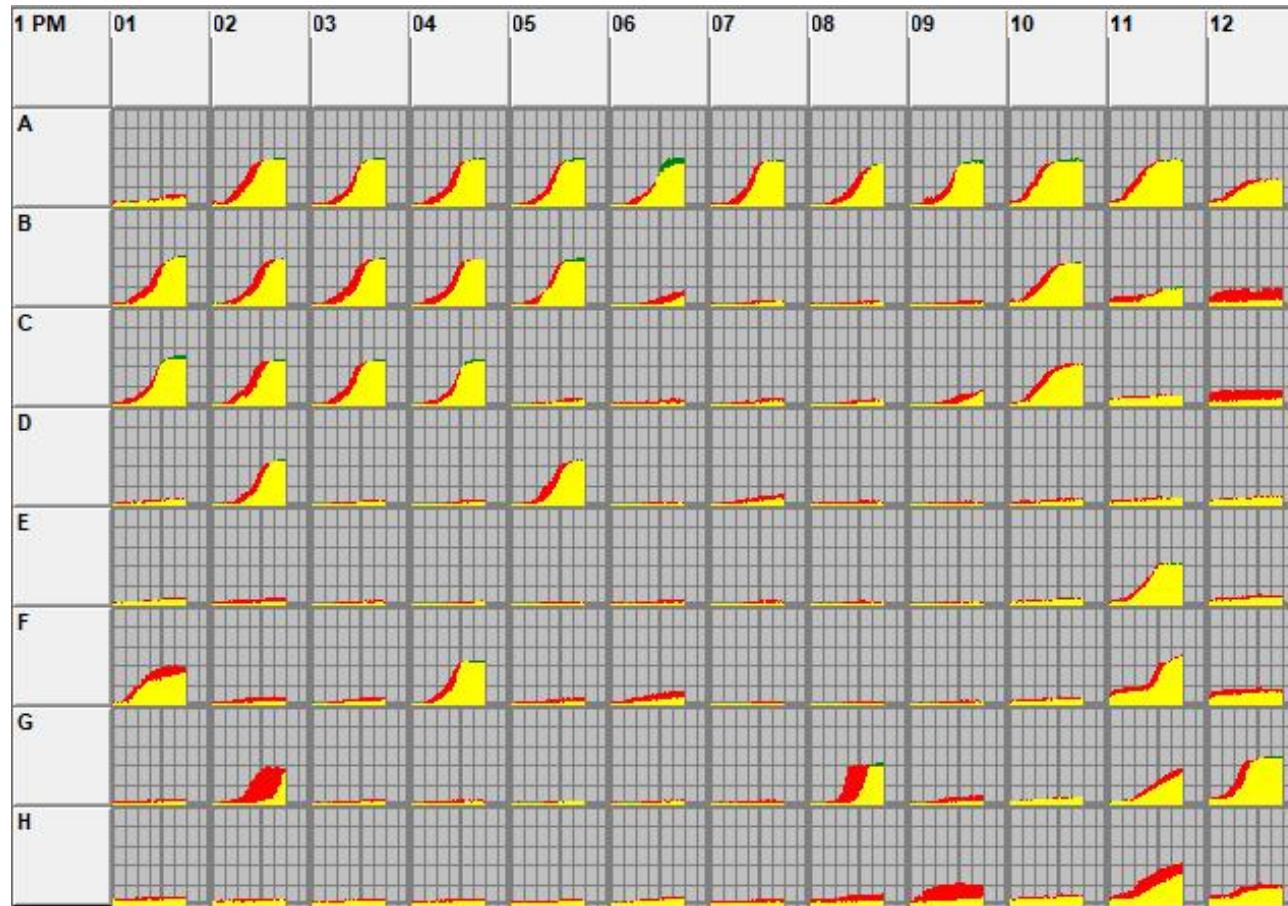
- Biolog Universal Growth Agar with/without defibrinated blood (Biolog Inc)
- Trypticase Soy Agar (Any Brand)
- Chocolate agar (Any Brand)
- R2A agar (Any Brand)

For the purposes of the test, the fresh culture of the bacteria (typically 24-48h old) has to be used!



Aerobic bacteria identification & characterization (GENIII plates)

Choosing the agar media for obtaining bacterial biomass for the test



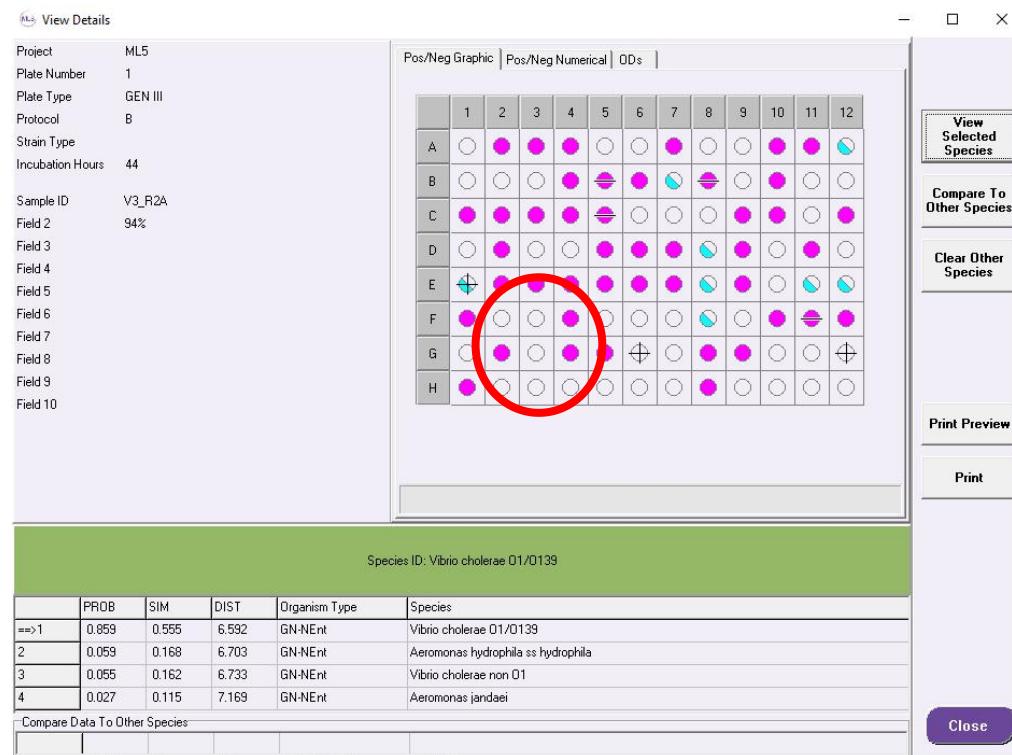
Green - *Paenibacillus polymyxa* (K16)
cultivated on BUG agar medium

Red - *Paenibacillus polymyxa* (K16)
cultivated on R2A agar medium

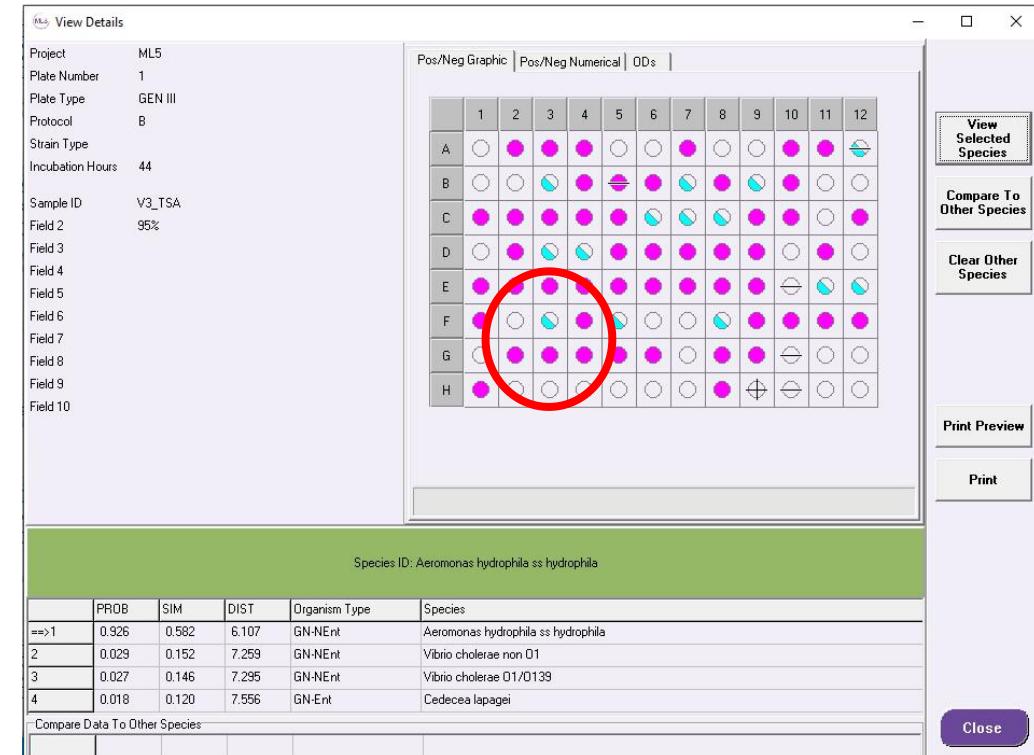
Yellow - common part
of the graph

Aerobic bacteria identification & characterization (GENIII plates)

Differences between bacteria metabolism (strain V3) depending on the cultivation agar medium



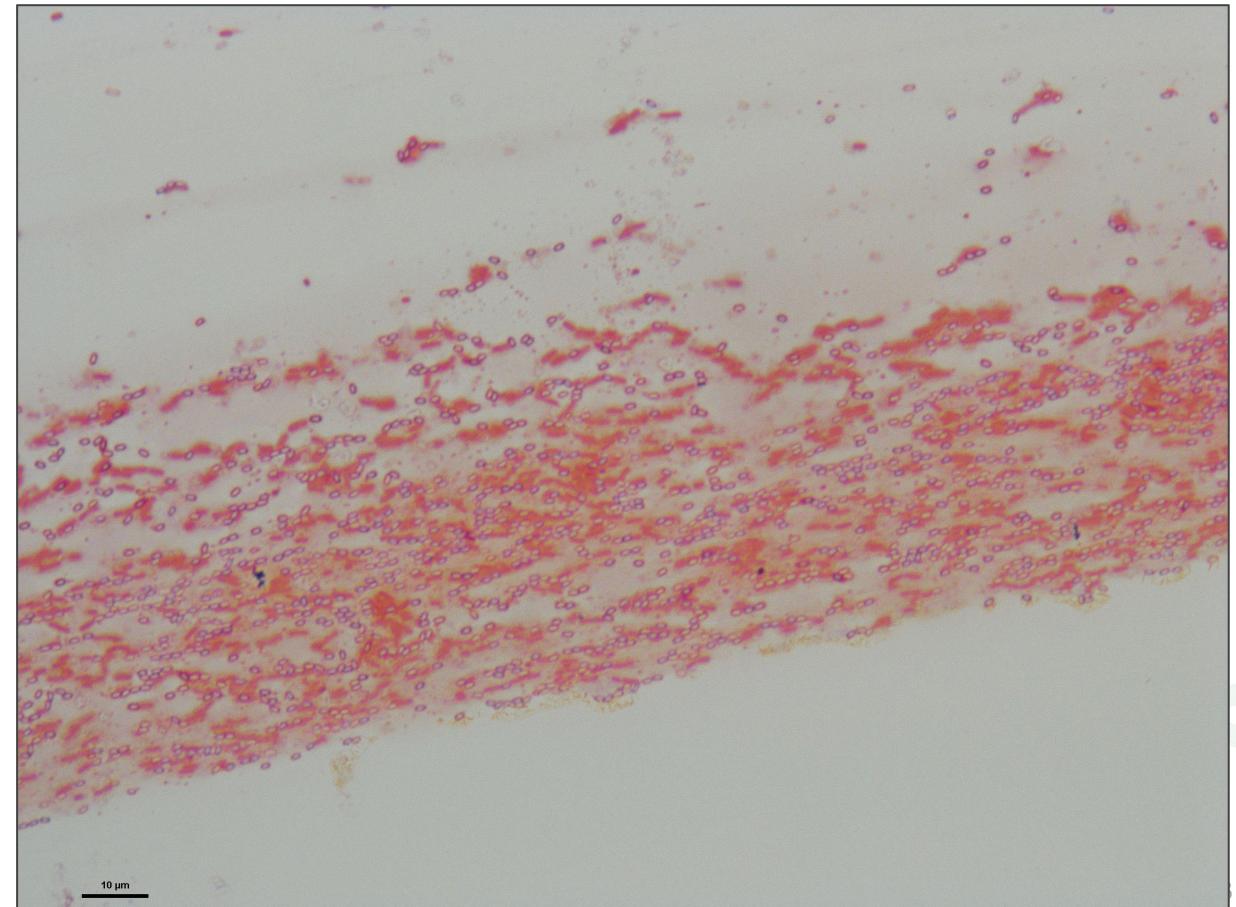
V3 strain cultivated on R2A agar



V3 strain cultivated on TSA agar

Aerobic bacteria identification & characterization (GENIII plates)

Differentiation of the chemical and physical properties of the bacteria cell wall via gram staining or similar tests like KOH or susceptibility to antibiotics like vancomycin or polymyxin.



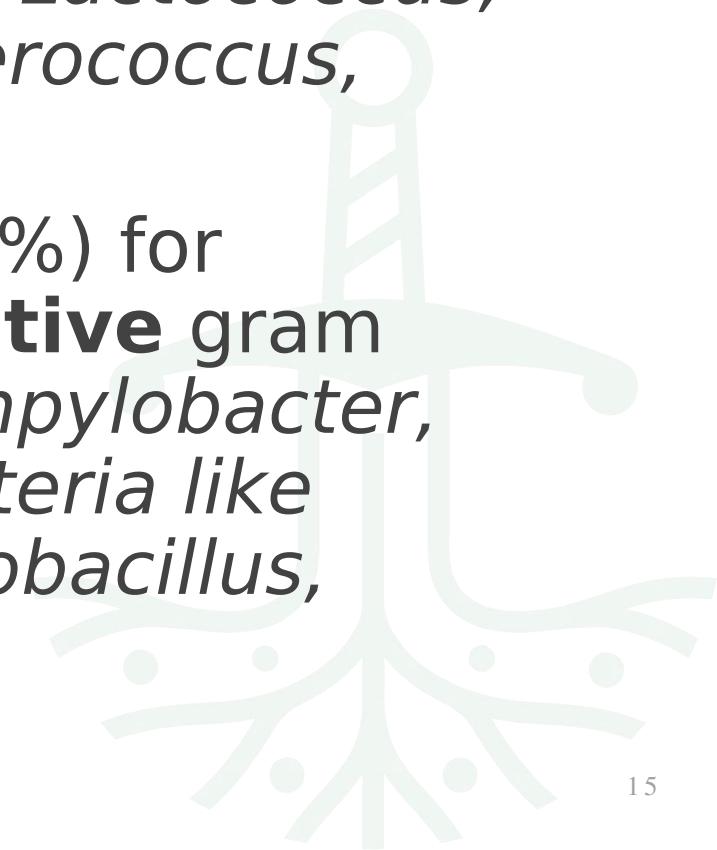
Aerobic bacteria identification & characterization (GENIII plates)

Choosing the protocol for the identification depending on the cell wall properties (gram-negative or gram-positive) and metabolism:

- Protocol A (Inoculation fluid A, T – 90-98%) universal protocol, mainly used for **gram-negative bacteria**
- Protocol B (Inoculation fluid B, T – 90-98%) mainly for **gram positive spore-forming bacteria** and for **strongly reducing and capsule producing gram-negative** bacteria like *Aeromonas* or *Vibrio*

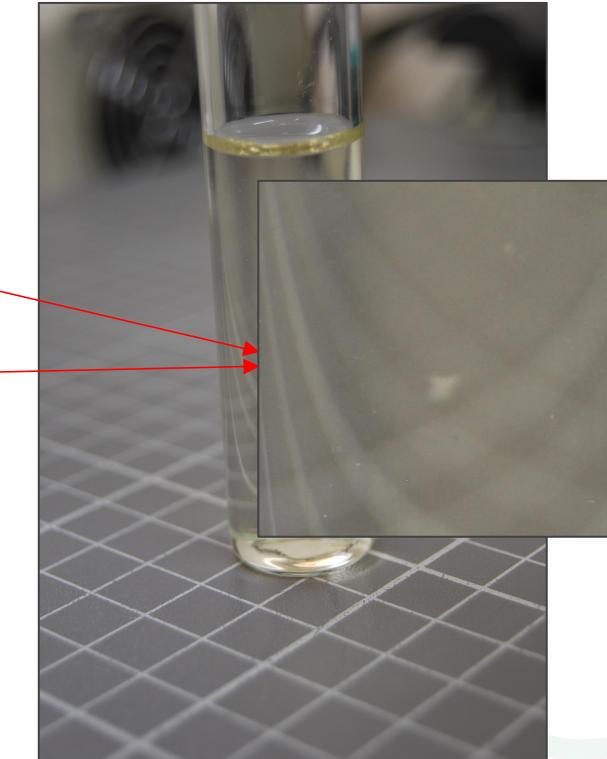
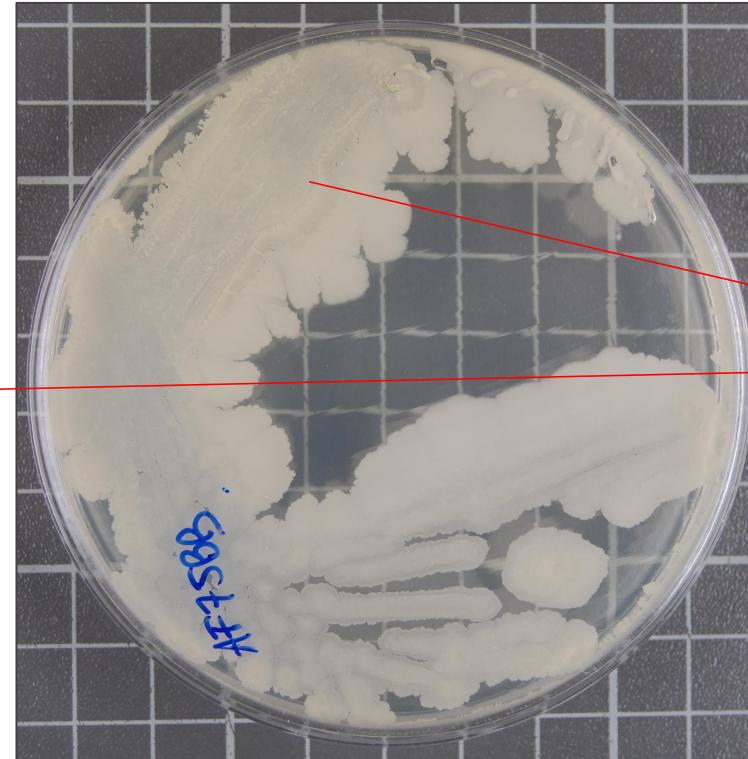
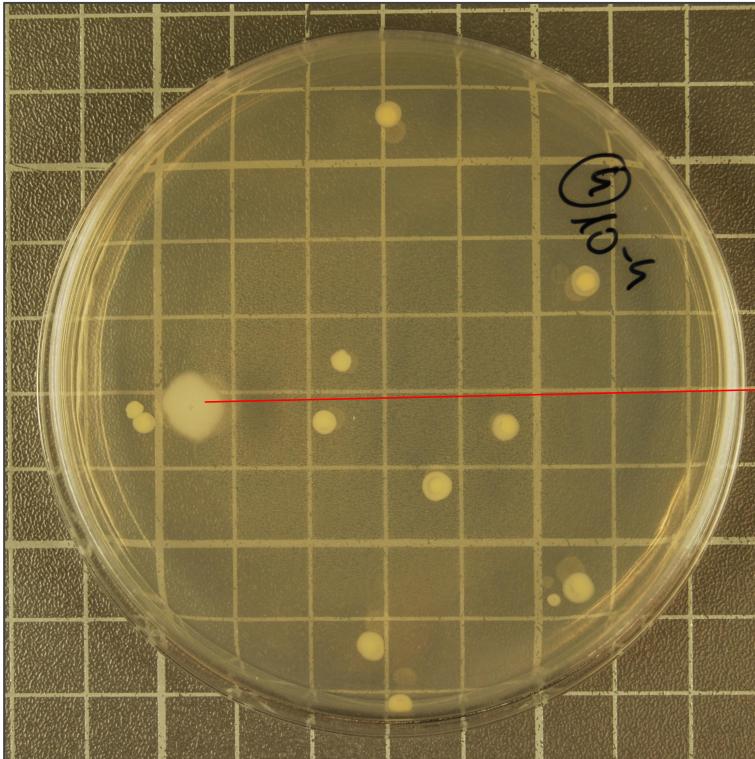
Aerobic bacteria identification & characterization (GENIII plates)

- Protocol C1 (Inoculation fluid C, T – 90-98%) for **microaerophilic capnophilic gram positive bacteria** like *Helcococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Streptococcus* and some *Aerococcus*, *Mycobacterium*, *Nocardia**
- Protocol C2 (Inoculation fluid C, T – 62-68%) for **fastidious, capnophilic, oxygen sensitive** gram negative bacteria like *Actinobacillus*, *Campylobacter*, *Methylobacterium* and gram positive bacteria like *Actinomyces*, *Aerococcus* and some *Lactobacillus*, *Pediococcus*



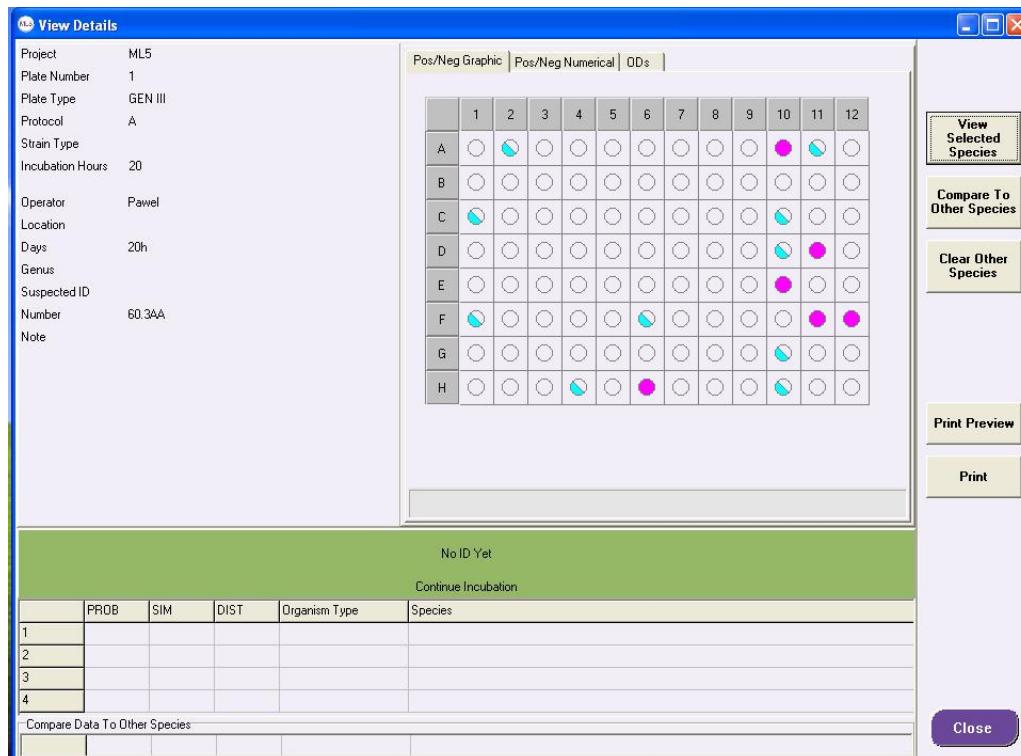
Aerobic bacteria identification & characterization (GENIII plates)

Some common problems with suspending the bacterial biomass

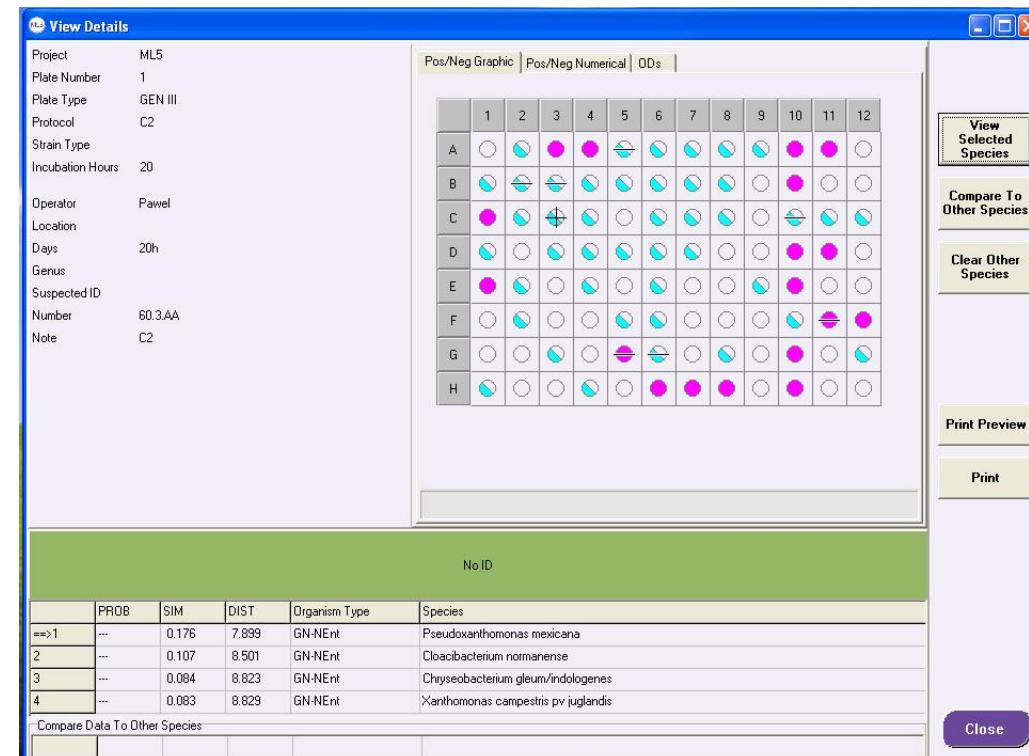


Aerobic bacteria identification & characterization (GENIII plates)

Differences between bacteria metabolism (Lysobacter sp - 60.3AA) depending on the Inoculation fluid



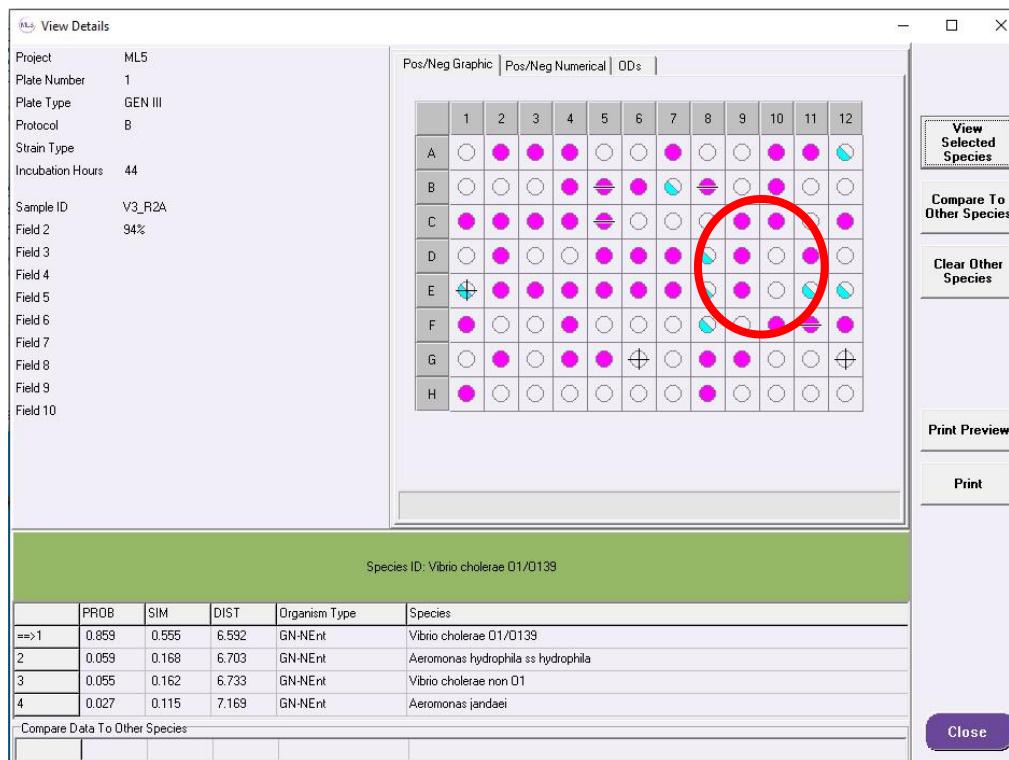
60.3AA strain protocol A



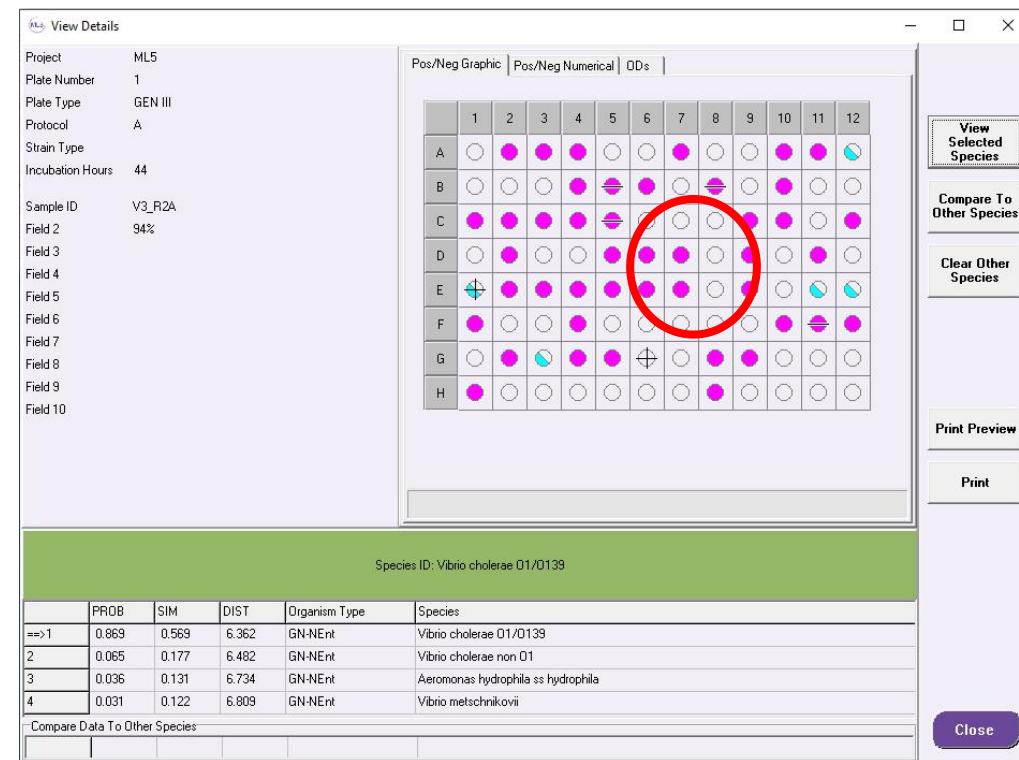
60.3AA strain protocol C2

Aerobic bacteria identification & characterization (GENIII plates)

Differences between bacteria metabolism (strain V3) depending on the Inoculation fluid



V3 strain protocol B



V3 strain protocol A

Aerobic bacteria identification & characterization (GENIII plates)

Plates incubation

- Temperature: general incubation temperature is 33 °C, but it could be from 26 to 37 °C or depending on your studies

GEN III Database Species and Their Characteristics

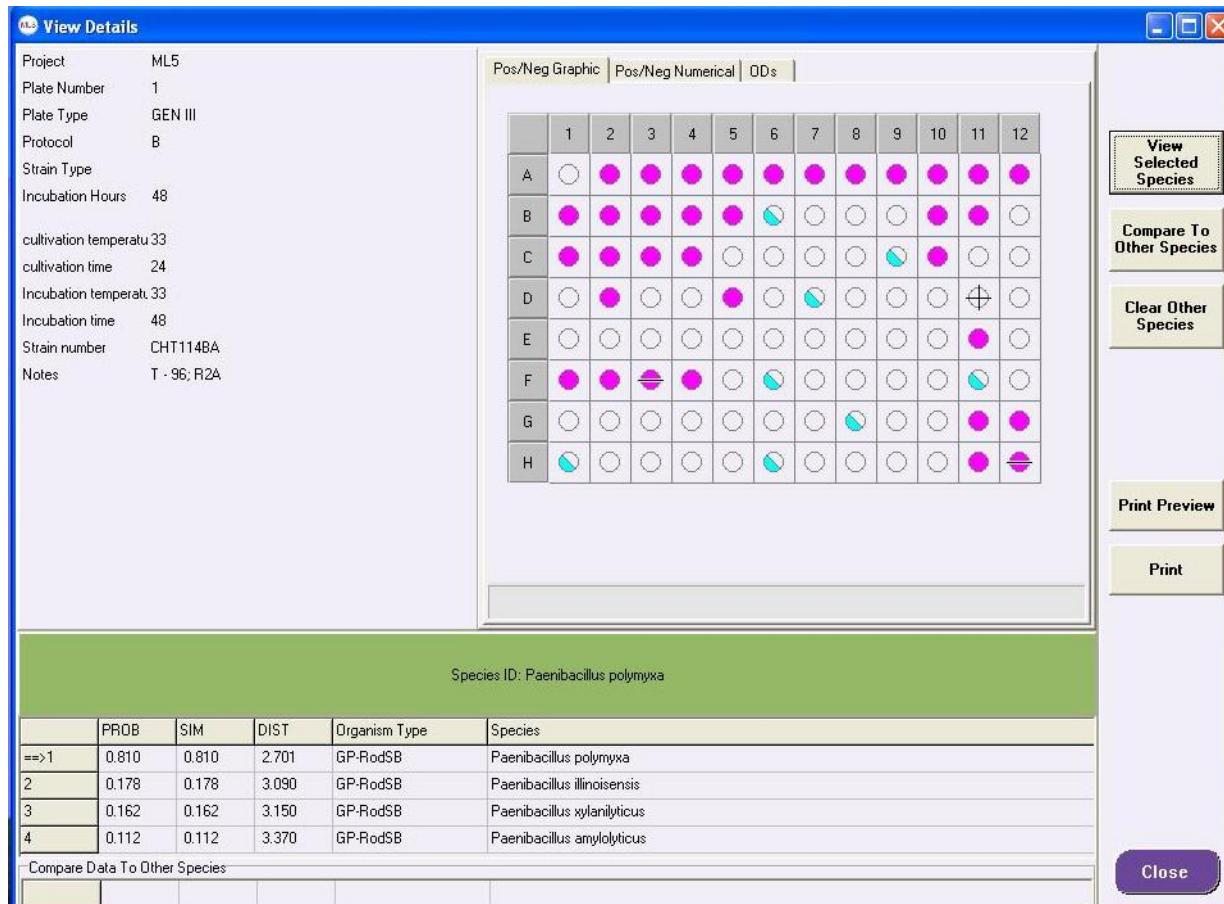
Species Name	Organism		Medium		
	Type	Test	Medium	Atm	Temp
142 <i>Corynebacterium simulans</i>	GP-ROD	A	BUG+B	Air	33
143 <i>Corynebacterium singulare</i>	GP-ROD	A	BUG+B	Air	33
144 <i>Corynebacterium spheniscorum</i>	GP-ROD	A	BUG+B	Air	33
145 <i>Corynebacterium</i> spp. (CDC.G)	GP-ROD	A	BUG+B	Air	33
146 <i>Corynebacterium striatum</i> (CDC.I-1)	GP-ROD	A	BUG+B	Air	33
147 <i>Corynebacterium terpenotabidum</i>	GP-ROD	A	BUG+B	Air	33
148 <i>Corynebacterium testudinoris</i>	GP-ROD	A	BUG+B	Air	33
149 <i>Corynebacterium thomssenii</i>	GP-ROD	C1	BUG+B	Air	33
150 <i>Corynebacterium ulcerans</i>	GP-ROD	A	BUG+B	Air	33
151 <i>Corynebacterium urealyticum</i>	GP-ROD	A	BUG+B	Air	33
152 <i>Corynebacterium variable</i>	GP-ROD	A	BUG+B	Air	33
153 <i>Corynebacterium vitaeruminis</i>	GP-ROD	A	BUG+B	Air	33
154 <i>Corynebacterium xerosis</i> (GPC)	GP-COCCUS	A	BUG+B	Air	33
155 <i>Curtobacterium albidum</i>	GP-ROD	A	BUG	Air	30
156 <i>Curtobacterium citreum</i>	GP-ROD	A	BUG	Air	30
157 <i>Curtobacterium flaccumfaciens</i>	GP-ROD	A	BUG	Air	30
158 <i>Curtobacterium luteum</i>	GP-ROD	A	BUG	Air	30
159 <i>Curtobacterium pusillum</i>	GP-ROD	A	BUG	Air	30
160 <i>Deinococcus proteolyticus</i>	GP-COCCUS	B	BUG+B	Air	30
161 <i>Deinococcus radiodurans</i>	GP-COCCUS	A	BUG+B	Air	30
162 <i>Deinococcus radiopugnans</i>	GP-COCCUS	A	BUG+B	Air	30
163 <i>Dermabacter hominis</i>	GP-ROD	A	BUG+B	Air	33
164 <i>Dermacoccus nishinomiyaensis</i>	GP-COCCUS	A	BUG+B	Air	33
165 <i>Dietzia maris</i>	GP-ROD	A	BUG+B	Air	33
166 <i>Dolosicoccus paucivorans</i>	GP-COCCUS	C1	BUG+B	6.5%CO ₂	33-37
167 <i>Dolosigranulum pigrum</i>	GP-COCCUS	C1	BUG+B	6.5%CO ₂	33-37
168 <i>Enterococcus asini</i>	GP-COCCUS	A	BUG+B	Air	33

Aerobic bacteria identification & characterization (GENIII plates)



Aerobic bacteria identification & characterization (GENIII plates)

Interpretation of the results:



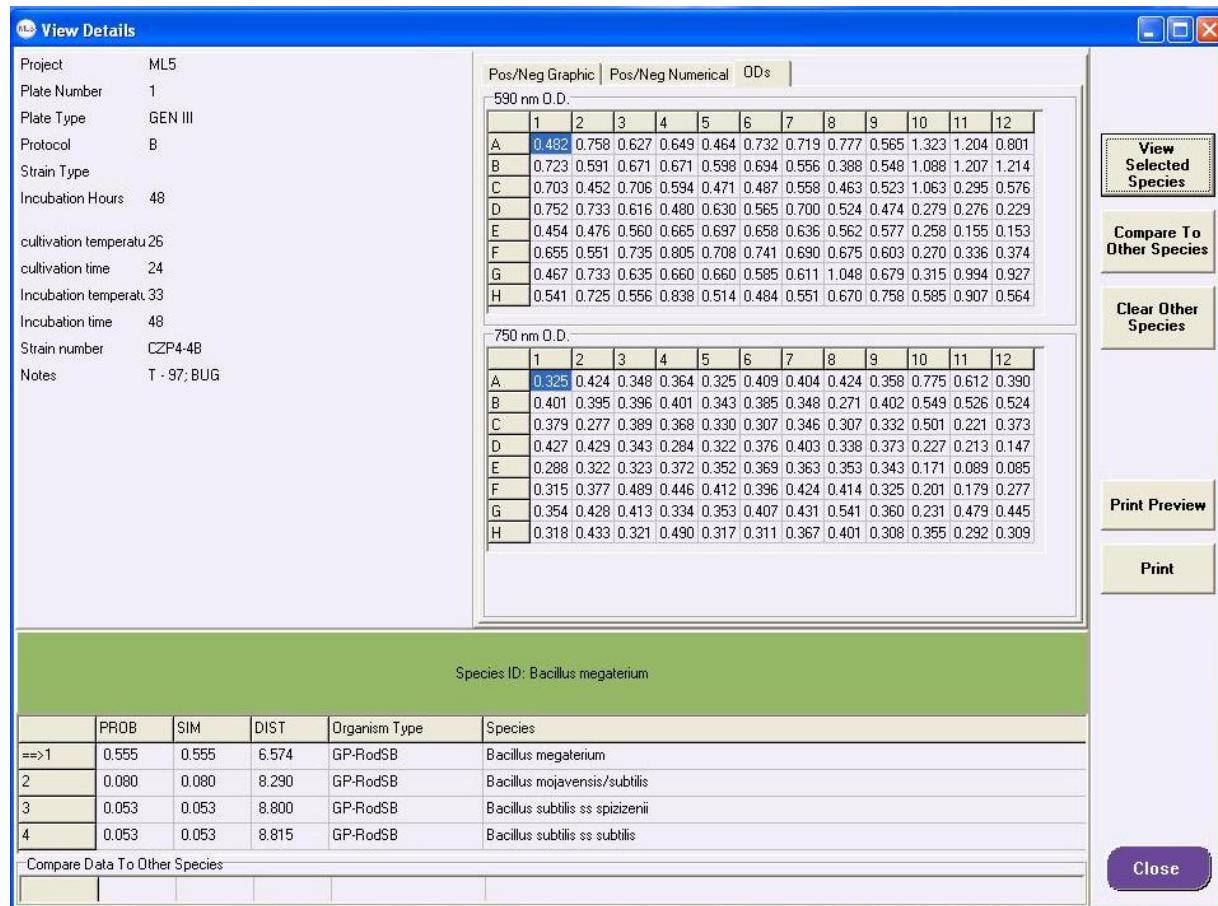
PROB index – the probability of successful identification. The value ranged from 0 to 1, where 1 is 100% of the probability of correct identification

SIM index – indicates how 'well' a sample is identified. The value ranged from 0 to 1, where 1 is perfect identification. For successful identification, the SIM index of tested bacteria has to exceed the threshold value. The threshold value is changing over time. For identification, the SIM value has to exceed 0.5 threshold

DIST index – indicates the approx number of mismatch reactions between tested bacteria and database

Aerobic bacteria identityfication & characterization (GENIII plates)

Interpretation of the results:



The results are based on measurements of the absorbance:

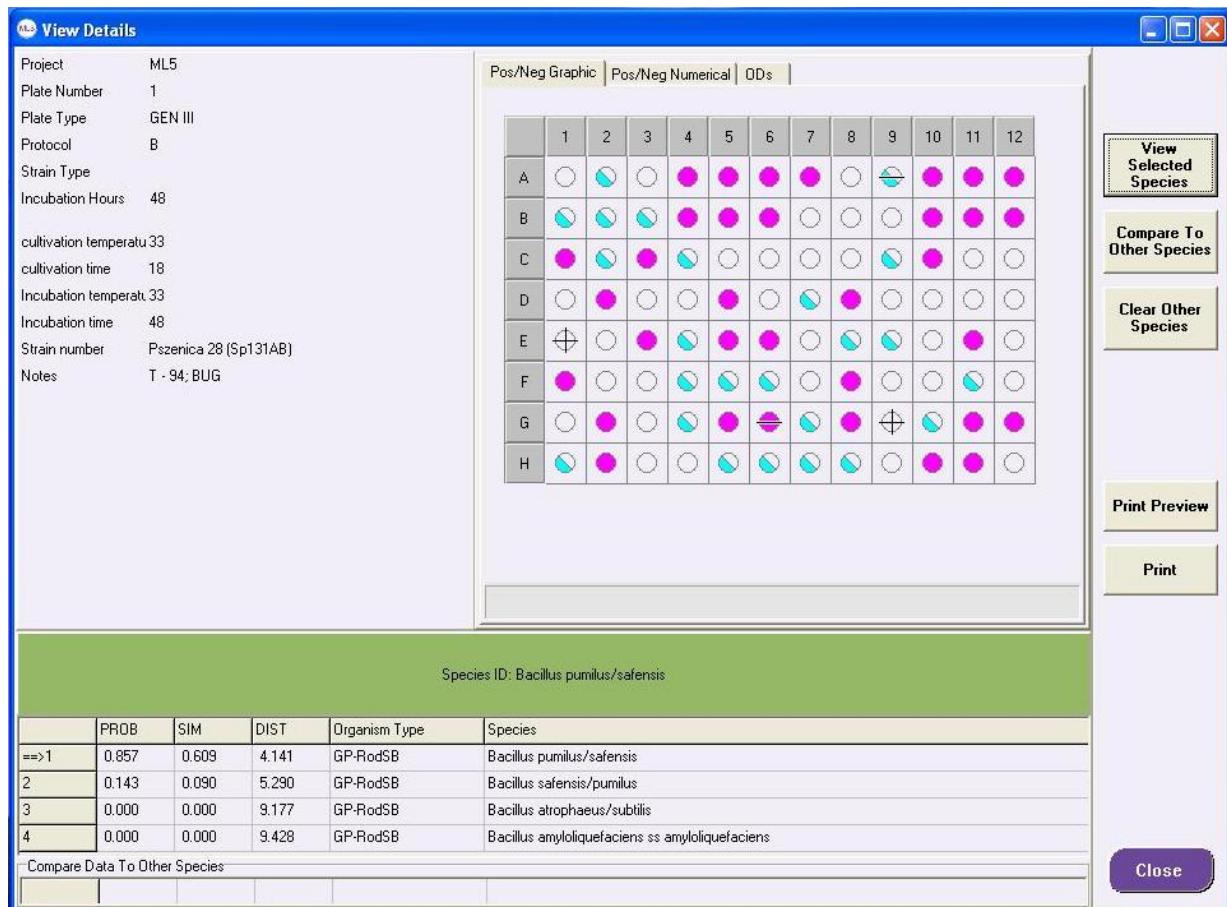
The absorbance of 590 nm wavelength is corelated with the color development

The absorbance of 750 nm wavelength is corelated with the biomass development

Aerobic bacteria identification & characterization (GENIII plates)

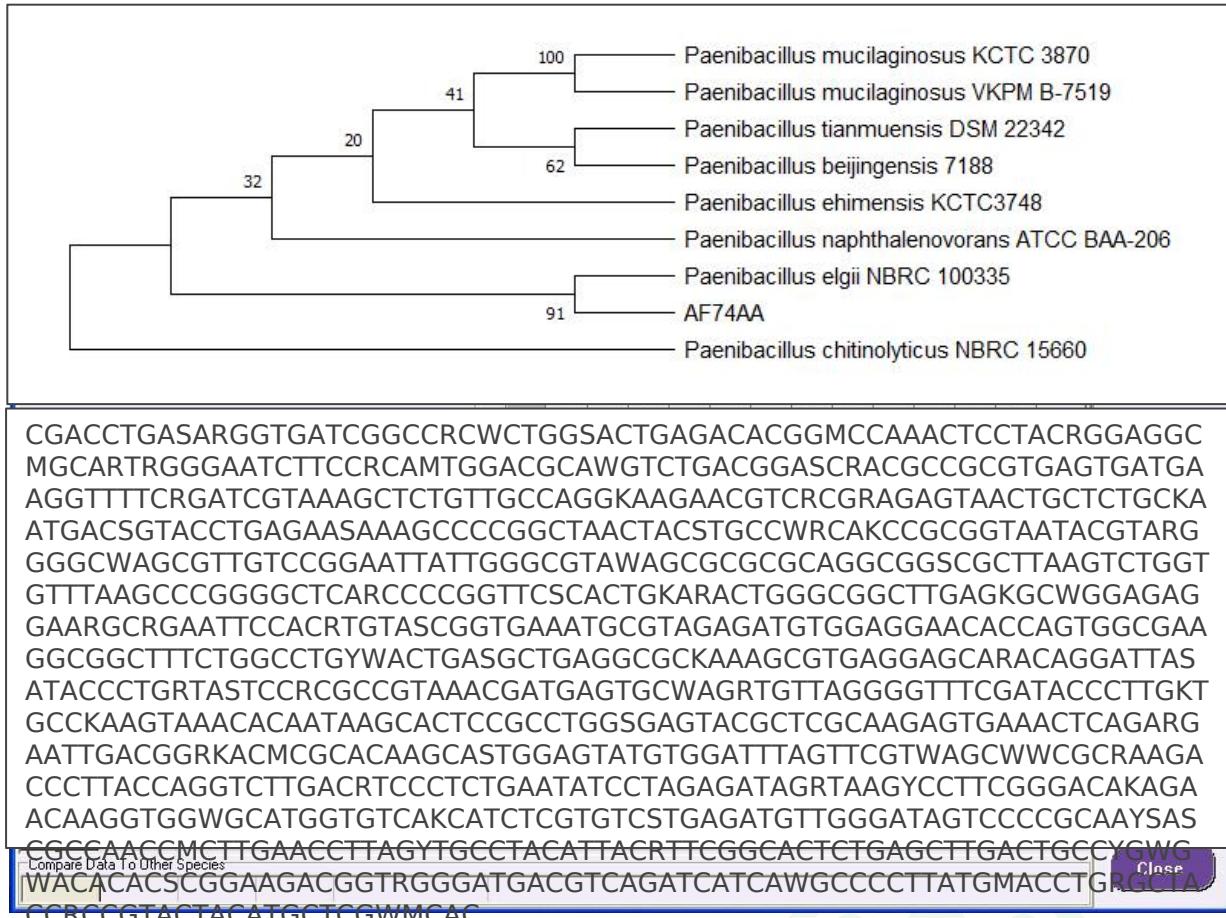
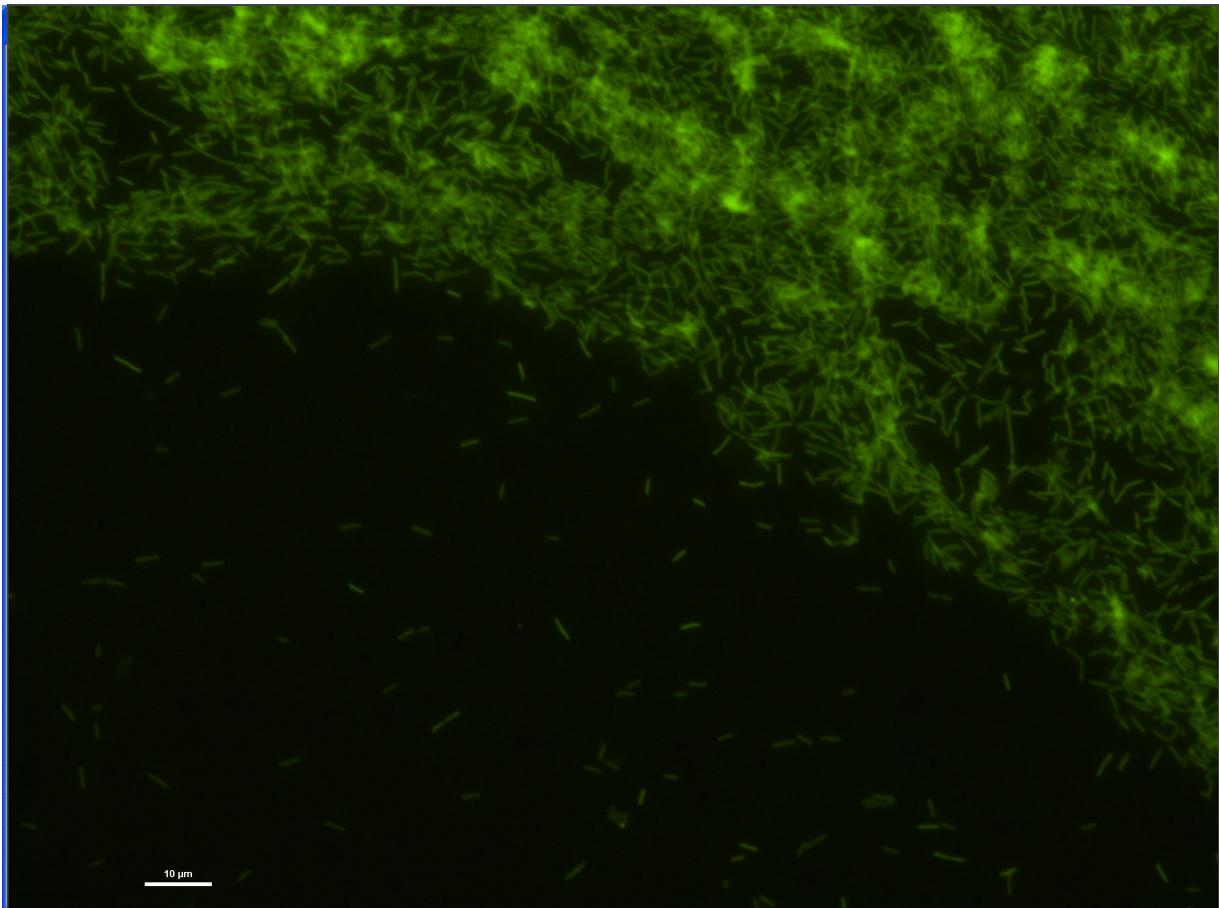
Interpretation of the results:

Sometimes the identification 'gives' two species. This is due to the similar phenotype of these species. For differentiation other biochemical tests are needed or sequencing of the specific genes.



Aerobic bacteria identification & characterization (GENIII plates)

Interpretation of the results:



Aerobic bacteria identification & characterization (GENIII plates)

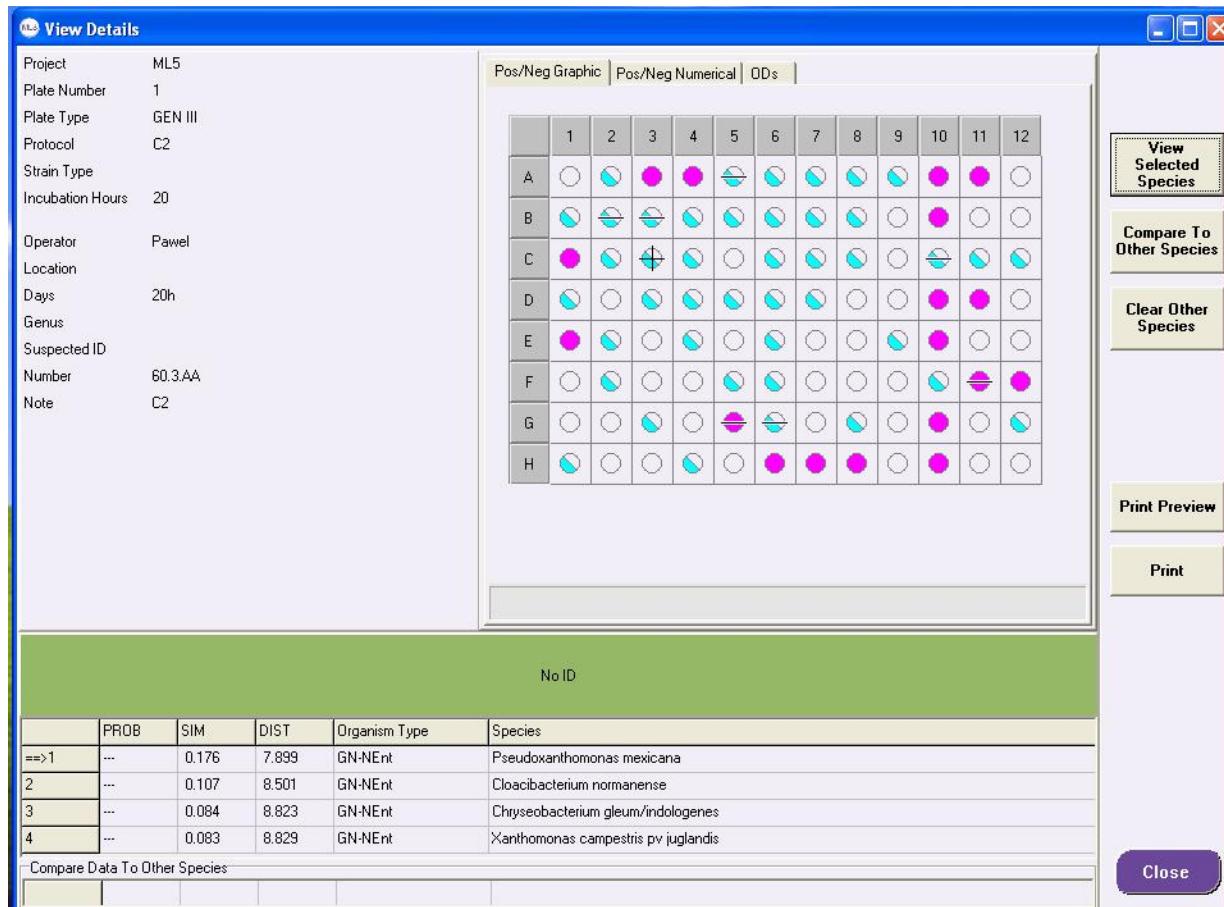
Common problem:



False positive reaction

Aerobic bacteria identity & characterization (GENIII plates)

Common problem:

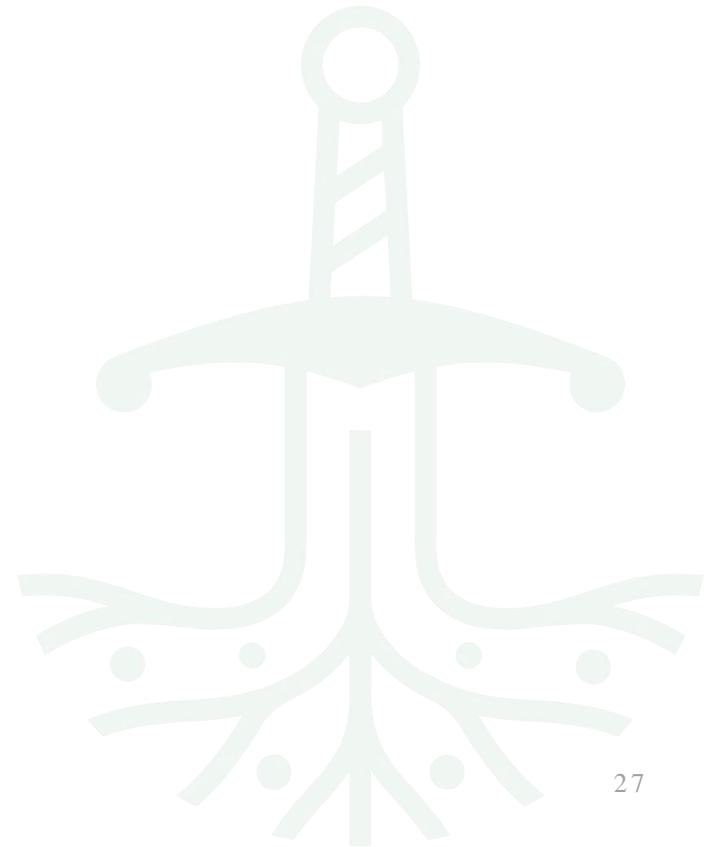


No identification



Aerobic bacteria identification & characterization (GENIII plates)

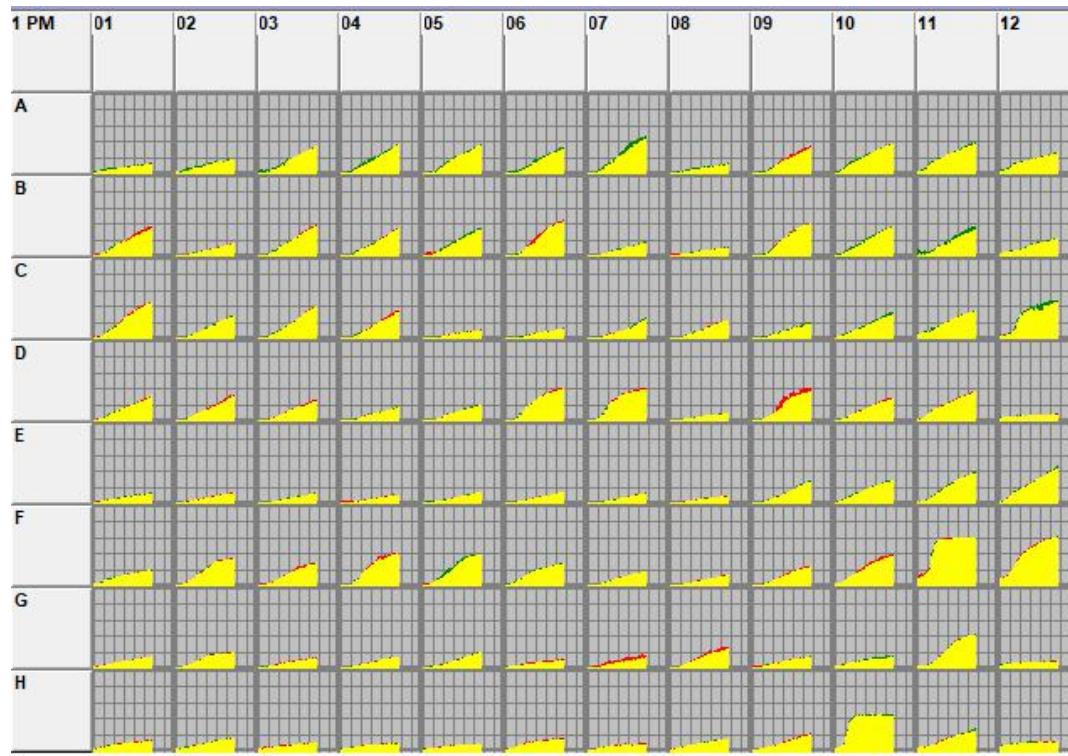
Other application of the Biolog/Omnilog system



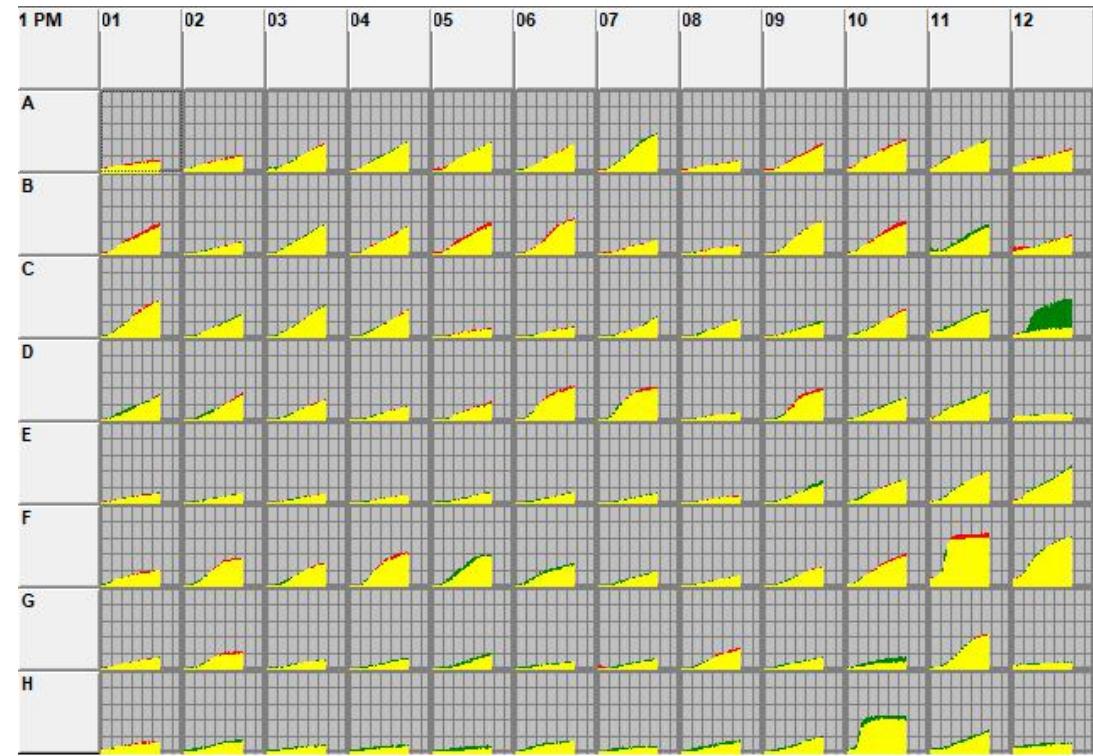
Aerobic bacteria identification & characterization (GENIII plates)

Comparison of the kinetics of the metabolism

A



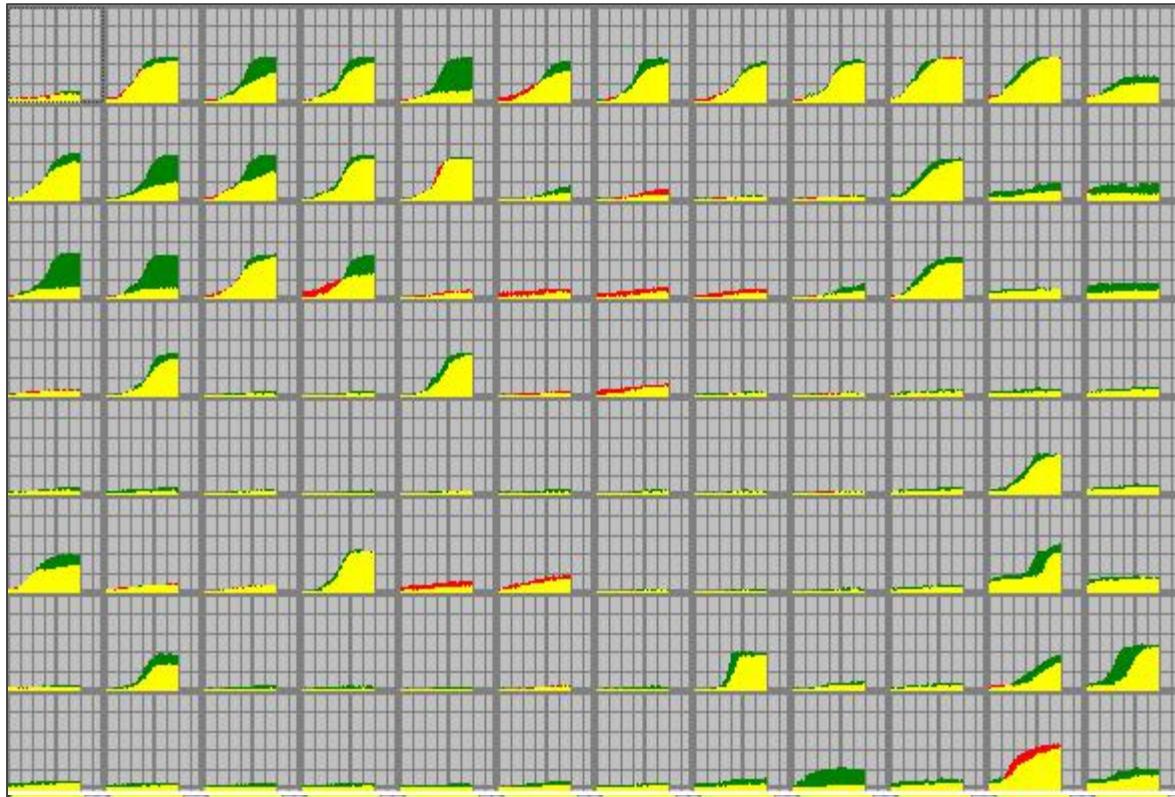
B



Green - Klebsiella oxytoca (RXAAC) reference strain; Red - Klebsiella oxytoca (isolate A or isolate B) collected from the cultivation tank 2 weeks after inoculation ; Yellow - common part of the graph

Aerobic bacteria identification & characterization (GENIII plates)

Comparison of the kinetics of the metabolism



Comparison of biochemical profile of applied *P. polymyxa*, K16 strain, with the isolate from the growing substrate

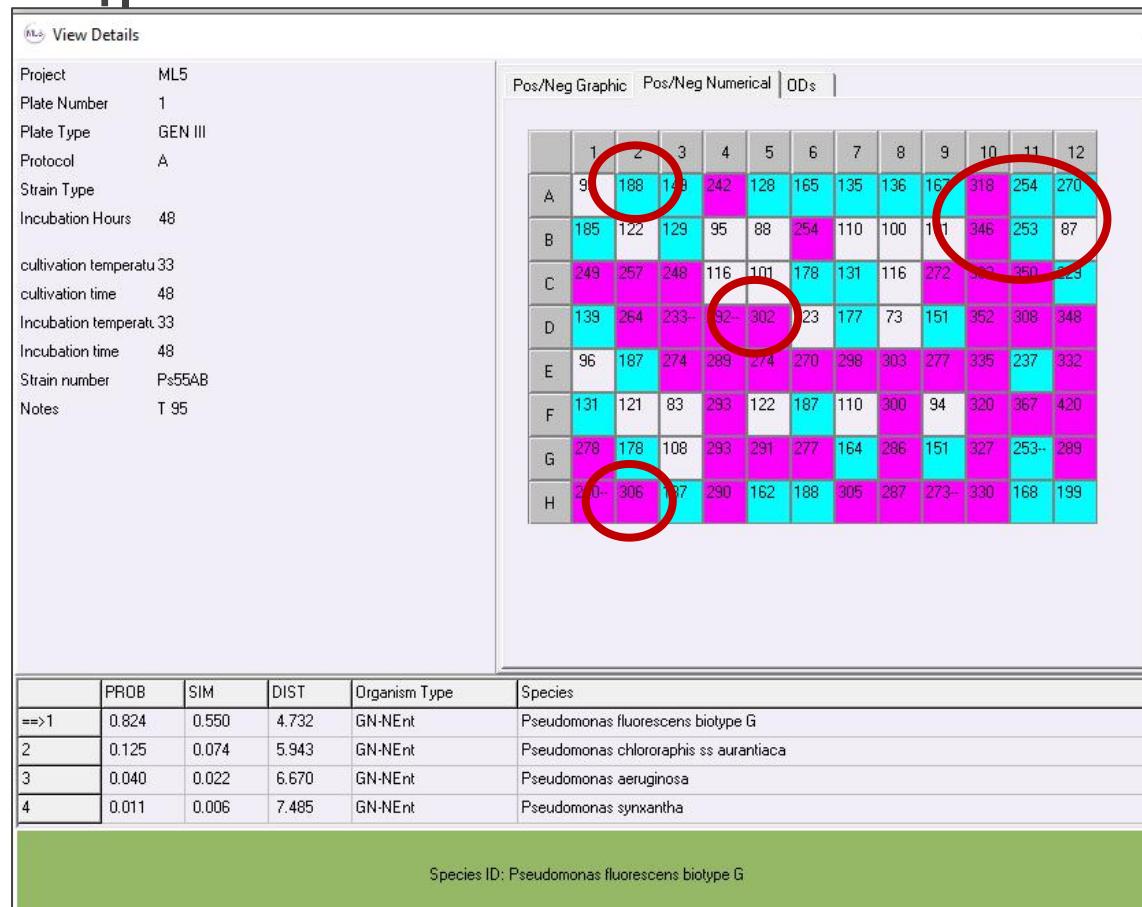
Green color – Biochemical profile (GENIII plate) of K16 strain

Red color – Biochemical profile (GENIII plate) of the strain isolated from growing substrate

Yellow color – Overlapping parts of both profiles

Aerobic bacteria identification & characterization (GENIII plates)

Development the medium composition for bacteria multiplication, medium for recovery of the bacteria from the

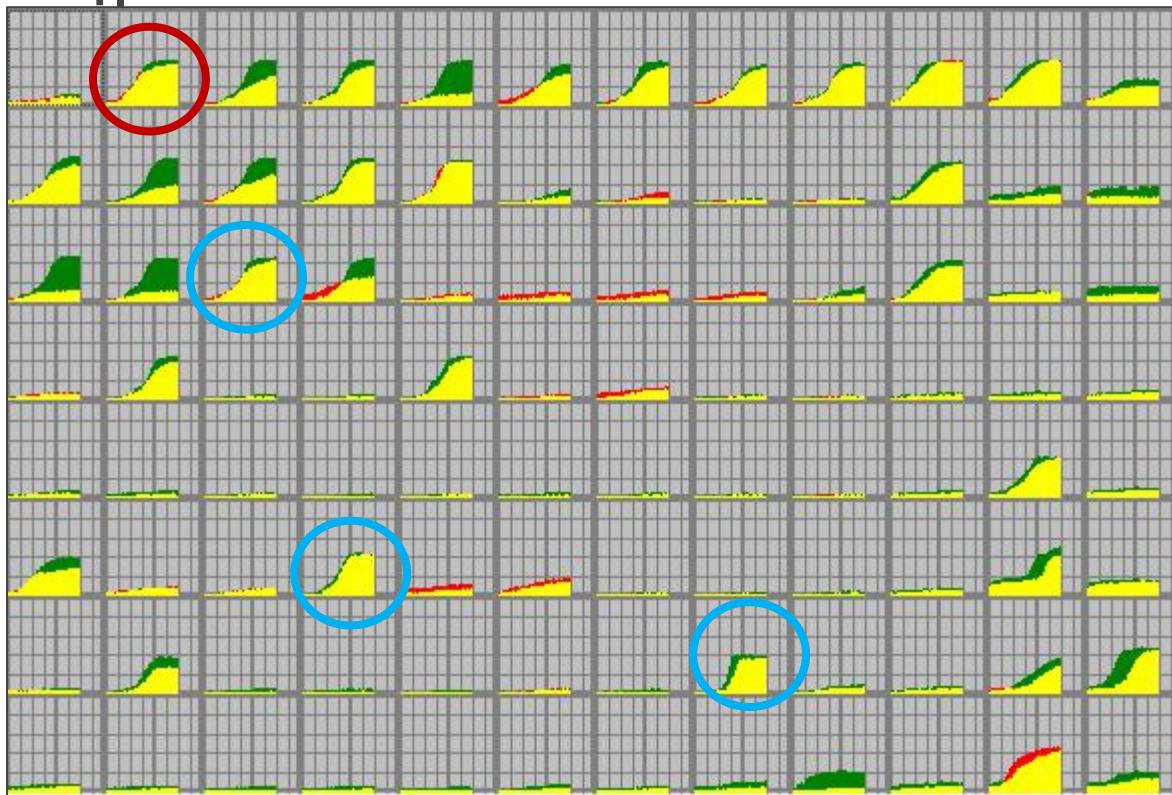


For cultivation of *Pseudomonas fluorescens* (Ps55AB) the growing medium should contain glycerol or γ -Aminobutyric acid as a carbon source than dextrose with is commonly used in growing mediums.

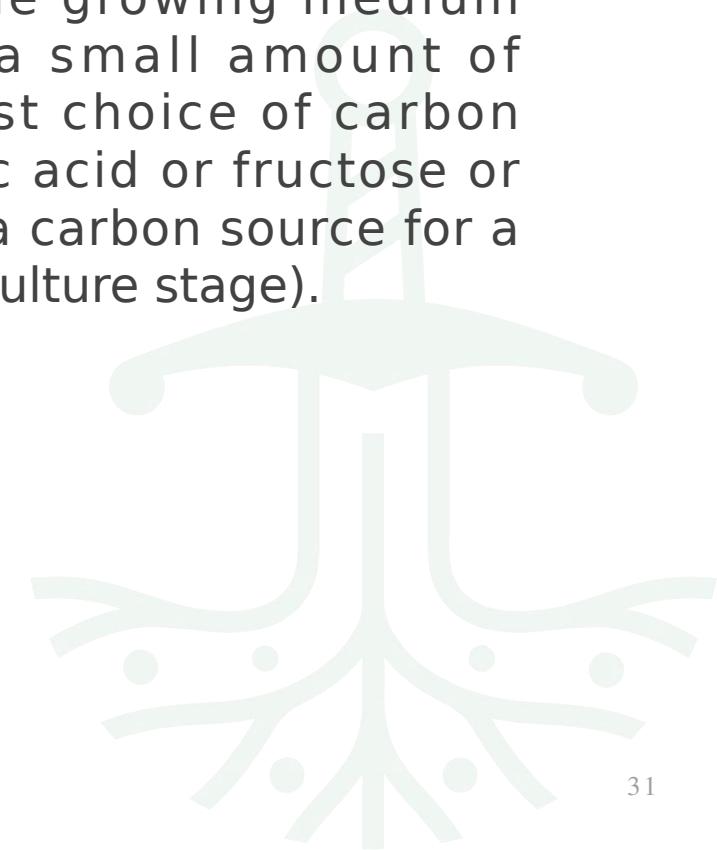
The medium should be buffered at pH 7 due to the PS55AB lower activity in pH 6 or 5.

Aerobic bacteria identification & characterization (GENIII plates)

Development the medium composition for bacteria multiplication, medium for recovery of the bacteria from the



For the cultivation of *P. polymyxa* (isolate DN-1) the growing medium should contain a small amount of glucose (as a first choice of carbon source) and malic acid or fructose or gluconic acid (as a carbon source for a later stage of the culture stage).



Microbial Community Analysis (EcoPlates)

Phenotype profiling could be used for the characterization of bacterial communities. For the purposes of this kind of analysis, the EcoPlates were developed. The EcoPlate contains 31 carbon sources repeated 3 times to obtain replicates of the data. Communities of microorganisms will give a characteristic reaction pattern **EcoPlate™**

A1 Water	A2 β -Methyl-D-Glucoside	A3 D-Galactonic Acid γ -Lactone	A4 L-Arginine	A5 Water	A6 β -Methyl-D-Glucoside	A7 D-Galactonic Acid γ -Lactone	A8 L-Arginine	A9 Water	A10 β -Methyl-D-Glucoside	A11 D-Galactonic Acid γ -Lactone	A12 L-Arginine
B1 Pyruvic Acid Methyl Ester	B2 D-Xylose	B3 D-Galacturonic Acid	B4 L-Asparagine	B5 Pyruvic Acid Methyl Ester	B6 D-Xylose	B7 D-Galacturonic Acid	B8 L-Asparagine	B9 Pyruvic Acid Methyl Ester	B10 D-Xylose	B11 D-Galacturonic Acid	B12 L-Asparagine
C1 Tween 40	C2 i-Erythritol	C3 2-Hydroxy Benzoic Acid	C4 L-Phenylalanine	C5 Tween 40	C6 i-Erythritol	C7 2-Hydroxy Benzoic Acid	C8 L-Phenylalanine	C9 Tween 40	C10 i-Erythritol	C11 2-Hydroxy Benzoic Acid	C12 L-Phenylalanine
D1 Tween 80	D2 D-Mannitol	D3 4-Hydroxy Benzoic Acid	D4 L-Serine	D5 Tween 80	D6 D-Mannitol	D7 4-Hydroxy Benzoic Acid	D8 L-Serine	D9 Tween 80	D10 D-Mannitol	D11 4-Hydroxy Benzoic Acid	D12 L-Serine
E1 α -Cyclodextrin	E2 N-Acetyl-D-Glucosamine	E3 γ -Amino Butyric Acid	E4 L-Threonine	E5 α -Cyclodextrin	E6 N-Acetyl-D-Glucosamine	E7 γ -Amino Butyric Acid	E8 L-Threonine	E9 α -Cyclodextrin	E10 N-Acetyl-D-Glucosamine	E11 γ -Amino Butyric Acid	E12 L-Threonine
F1 Glycogen	F2 D-Glucosaminic Acid	F3 Itaconic Acid	F4 Glycyl-L-Glutamic Acid	F5 Glycogen	F6 D-Glucosaminic Acid	F7 Itaconic Acid	F8 Glycyl-L-Glutamic Acid	F9 Glycogen	F10 D-Glucosaminic Acid	F11 Itaconic Acid	F12 Glycyl-L-Glutamic Acid
G1 D-Cellobiose	G2 Glucose-1-Phosphate	G3 α -Keto Butyric Acid	G4 Phenylethylamine	G5 D-Cellobiose	G6 Glucose-1-Phosphate	G7 α -Keto Butyric Acid	G8 Phenylethylamine	G9 D-Cellobiose	G10 Glucose-1-Phosphate	G11 α -Keto Butyric Acid	G12 Phenylethylamine
H1 α -D-Lactose	H2 D,L- α -Glycerol Phosphate	H3 D-Malic Acid	H4 Putrescine	H5 α -D-Lactose	H6 D,L- α -Glycerol Phosphate	H7 D-Malic Acid	H8 Putrescine	H9 α -D-Lactose	H10 D,L- α -Glycerol Phosphate	H11 D-Malic Acid	H12 Putrescine

FIGURE 1. Carbon Sources in EcoPlate

Microbial Community Analysis (EcoPlates)

The procedure used in the Excalibur project (for soil):

Step 1: Estimation of soil dry mass (drying the samples in 105°C for 1 day)

Step 2: Preparation of soil solution: each sample must have the same amount of soil dry mass. Addition of sterile distilled water to obtain 10% soil solution for example:

12 g of moisture soil (with dry mass of 83.33%) and 88 g of sterile distilled water

10.363 g of moisture soil (with dry mass of 96.51%) and 89.637 g of sterile distilled water

Step 3: Dilution of soil suspension (dilution of soil suspension to 10^{-3})

Step 4: Inoculation of test plates (per 100 µl per well)

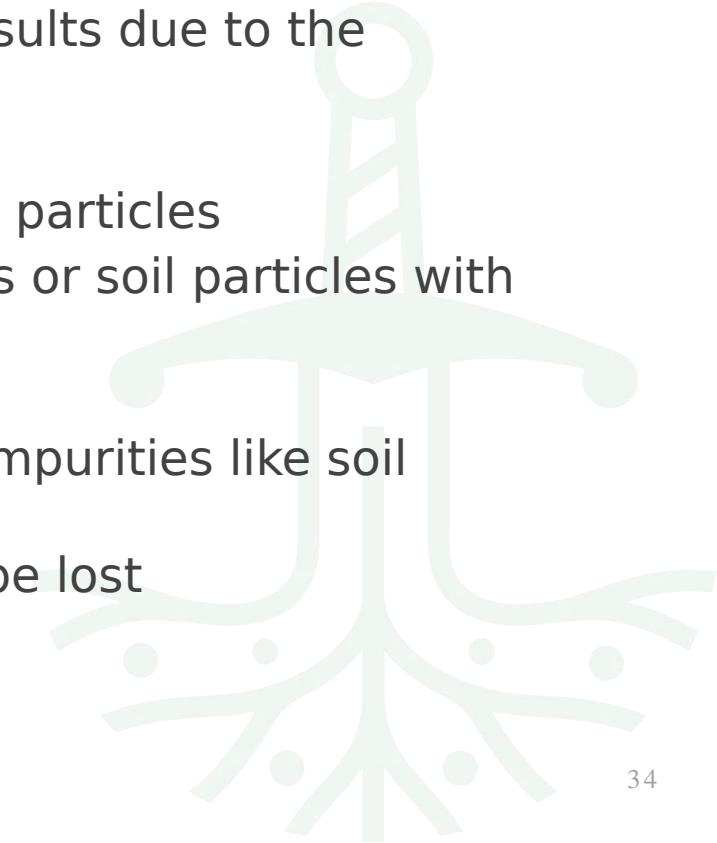
Step 5: Incubation: 26°C, 72 hours

Step 6: Estimation of biodiversity and activity

Microbial Community Analysis (EcoPlates)

Sample preparation modification:

- Dilution of the soil suspension
 - Pros: Easy to prepare, the soil solution contains relatively high amount of microorganisms
 - Cons: some false positive reactions or scattering of the results due to the impurities like soil particles
- Centrifugation of the soil suspension
 - Pros: obtained soil suspension is free of impurities like soil particles
 - Cons: during the centrifugation, some of the bacterial cells or soil particles with bacteria attached will be lost
- Decantation of the soil suspension
 - Pros: easy to prepare, obtained soil suspension is free of impurities like soil particles
 - Cons: during decantation, some of the bacterial cells will be lost
- Other suspending agents like saline or buffer
- Other sample dilution



Microbial Community Analysis (EcoPlates)

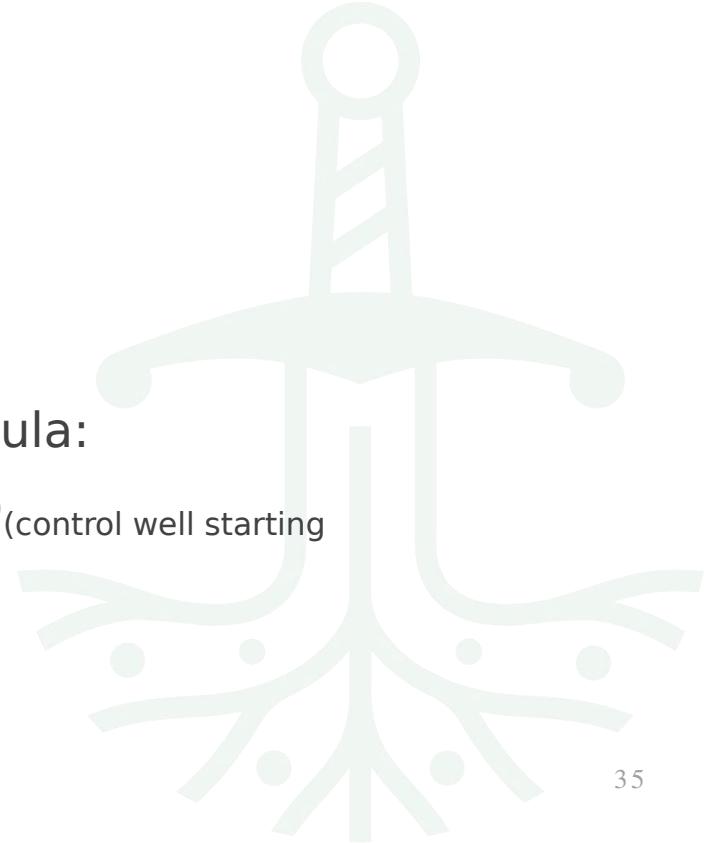
Calculation of activity of bacteria:

Microbial activity, is a activity of dehydrogenase enzymes, estimated on the basis of the Average Well Color Development (AWCD). The value of this parameter is calculated using the following formula:

$$AWCD = \Sigma OD_i/31,$$

where: OD_i is the optical density of each well calculated using the formula:

$$OD_i = (OD_{i(\text{absorbance after 72h})} - OD_{i(\text{starting absorbance})}) - (OD_{(\text{control well after 72h})} - OD_{(\text{control well starting absorbance})})$$



Microbial Community Analysis (EcoPlates)

Calculation of biodiversity of bacteria:

Microbial diversity - Shannon-Weaver index (H) is estimated on the basis of dehydrogenase activity. The value of this parameter is calculated using the following formula:

$$H = -\sum p_i (\ln p_i)$$

where: p_i is the level of microbial activity in individual wells (OD_i) divided by the sum of the activity in all the wells ($\sum OD_i$).



Microbial Community Analysis (EcoPlates)

Interpretation of the results and data processing

	1	2	3	4	5	6	7	8	9	10	11	12
A	0,104	0,131	1,198	0,432	0,12	0,257	1,773	2,02	0,071	0,904	1,154	2,121
B	1,42	-0,012	0,012	2,41	1,41	-0,027	0,037	2,791	1,029	-0,028	0,195	2,8
C	1,65	0,219	0,029	0,647	1,538	0,252	-0,19	0,516	1,862	0,08	0,074	0,264
D	1,341	0,849	0,604	1,674	0,883	0,875	0,59	2,393	0,741	0,873	0,777	2,033
E	0,196	0,92	0,183	0,658	0,3	0,928	0,161	0,299	0,145	1,099	0,275	0,248
F	0,26	1,449	0,224	0,153	0,308	1,365	0,15	0,123	0,148	1,713	0,215	0,136
G	0,263	0,353	0,055	0,291	0,152	0,032	0,105	0,718	0,172	0,028	0,047	0,016
H	0,013	0,333	0,447	1,992	0,174	0,289	0,598	2,323	0,018	0,231	0,659	1,538

For data validation use the median of the repetition and compare individual well absorbance with

Microbial Community Analysis (EcoPlates)

Common problems:

Soil dilution	AWCD	Index H
1:9	0,28 ± 0,11	3,02 ± 0,24
1:99	0,93 ± 0,06	3,07 ± 0,06
1:999	0,61 ± 0,04	2,74 ± 0,07
1:9999	0,19 ± 0,11	1,88 ± 0,66

Bacteria activity and biodiversity of soil stored over one year in the refrigerator

There is no 'golden standard' for the soil dilution used for the test. Some samples required higher density inoculum (like degraded or polluted soil) others need a lower density of inoculum due to the high bacteria concentration (compost, fertile soil).

For the tests with unknown types of samples, we should do some screening tests to estimate the dilution to be used in the studies.

Microbial Community Analysis (EcoPlates)

Common problems:

A

Pos/Neg Graphic Pos/Neg Numerical ODs												
590 nm O.D.												
	1	2	3	4	5	6	7	8	9	10	11	12
A	2.800	3.062	3.084	3.389	2.738	3.100	3.123	3.402	2.936	3.091	3.212	3.306
B	3.027	3.327	3.263	3.601	3.348	3.426	3.380	3.611	3.287	3.244	3.341	3.525
C	3.097	3.017	2.861	3.294	3.395	3.314	3.274	2.913	3.264	3.213	2.863	2.909
D	2.944	3.336	3.217	3.563	3.233	3.446	3.242	3.529	3.165	2.911	3.272	3.286
E	3.086	3.108	3.010	2.475	2.708	3.333	2.982	2.698	2.636	2.957	2.842	2.865
F	2.964	3.293	2.986	2.979	3.275	3.517	3.085	2.907	3.074	2.997	2.855	2.735
G	2.612	2.574	2.612	3.205	2.583	2.790	2.729	3.046	2.752	2.415	2.588	2.878
H	3.045	2.726	2.612	2.977	2.934	2.753	2.317	2.879	2.992	2.667	2.867	2.743
750 nm O.D.												
	1	2	3	4	5	6	7	8	9	10	11	12
A	2.371	2.347	2.375	2.388	2.309	2.443	2.406	2.403	2.472	2.425	2.397	2.318
B	2.417	2.512	2.472	2.549	2.474	2.600	2.495	2.530	2.318	2.460	2.410	2.303
C	2.372	2.540	2.450	2.644	2.568	2.768	2.474	2.379	2.415	2.665	2.445	2.250
D	2.345	2.619	2.467	2.564	2.463	2.715	2.518	2.539	2.403	2.235	2.258	2.284
E	2.464	2.405	2.335	2.090	2.206	2.581	2.300	2.259	2.139	2.208	2.296	2.254
F	2.281	2.398	2.478	2.528	2.643	2.830	2.524	2.385	2.365	2.223	2.284	2.251
G	2.035	2.091	2.174	2.234	1.985	2.251	2.281	2.311	2.149	1.975	2.139	2.125
H	2.432	2.287	2.077	2.322	2.215	2.318	1.856	2.280	2.261	2.244	2.196	2.136

B

Soil dilution problems:

A - Soil diluted to the ratio 1:9 – problems with high amounts of impurities

Pos/Neg Graphic Pos/Neg Numerical ODs												
590 nm O.D.												
	1	2	3	4	5	6	7	8	9	10	11	12
A	0.102	0.109	0.101	1.519	0.187	0.150	0.098	0.173	0.459	0.115	0.650	0.582
B	1.020	0.357	0.196	2.304	1.260	0.359	0.179	1.746	1.244	0.359	0.133	0.097
C	0.553	0.227	0.140	0.139	1.146	0.143	0.144	0.434	0.220	0.224	0.107	0.324
D	0.262	0.099	0.127	1.207	0.294	1.678	0.248	0.134	0.254	1.184	0.100	0.107
E	0.122	0.558	0.139	0.213	0.182	0.149	0.142	0.141	0.197	0.178	0.110	0.106
F	0.236	0.113	0.160	0.183	0.277	0.444	0.140	0.137	0.265	0.136	0.156	0.109
G	0.196	0.170	0.277	0.183	0.209	0.215	0.187	0.161	0.231	0.219	0.251	0.560
H	0.307	0.157	0.217	0.137	0.102	0.169	0.210	0.840	0.132	0.102	0.156	0.115
750 nm O.D.												
	1	2	3	4	5	6	7	8	9	10	11	12
A	0.099	0.103	0.096	0.574	0.152	0.129	0.095	0.142	0.446	0.107	0.303	0.363
B	0.491	0.151	0.146	0.864	0.553	0.143	0.137	0.620	0.676	0.155	0.098	0.094
C	0.369	0.181	0.139	0.135	0.764	0.135	0.142	0.292	0.195	0.191	0.104	0.223
D	0.188	0.096	0.126	0.541	0.219	0.724	0.207	0.132	0.199	0.752	0.097	0.100
E	0.116	0.353	0.137	0.185	0.168	0.146	0.140	0.139	0.179	0.167	0.108	0.103
F	0.184	0.111	0.147	0.169	0.223	0.312	0.135	0.134	0.216	0.133	0.133	0.103
G	0.160	0.149	0.221	0.173	0.177	0.186	0.160	0.152	0.194	0.209	0.174	0.281
H	0.251	0.135	0.188	0.121	0.098	0.142	0.197	0.395	0.118	0.099	0.148	0.110

B - Soil diluted to the ratio 1:9999 – problem low repeatability

Conclusions

- The phenotyping with the use of GENIII/EcoPlates (and other types) is a tool for environmental studies (ie. comparing the impact of different stressing factors etc), biotechnology (ie. multiplication of microbes), or for studies on microorganism's metabolism (ie. tracking changes caused by mutations or environmental pressure)
- It could be successfully used for identification, but other tests will be needed for confirmation of identification or for more accurate analysis



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A close-up, low-angle photograph of two hands reaching down towards the ground. One hand is wearing a plaid shirt cuff, and the other is bare. They are both holding a small amount of dark, moist soil between their fingers. The scene is bathed in warm, golden sunlight, suggesting either sunrise or sunset. The background is blurred, showing more of the earthy terrain.

Thanks for you attention