Interactions of microalgae and microbial communities present in microalgae cultures and larval rearing systems

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Bacteria in microalgae cultures?

Greenwater = microalgae + bacteria

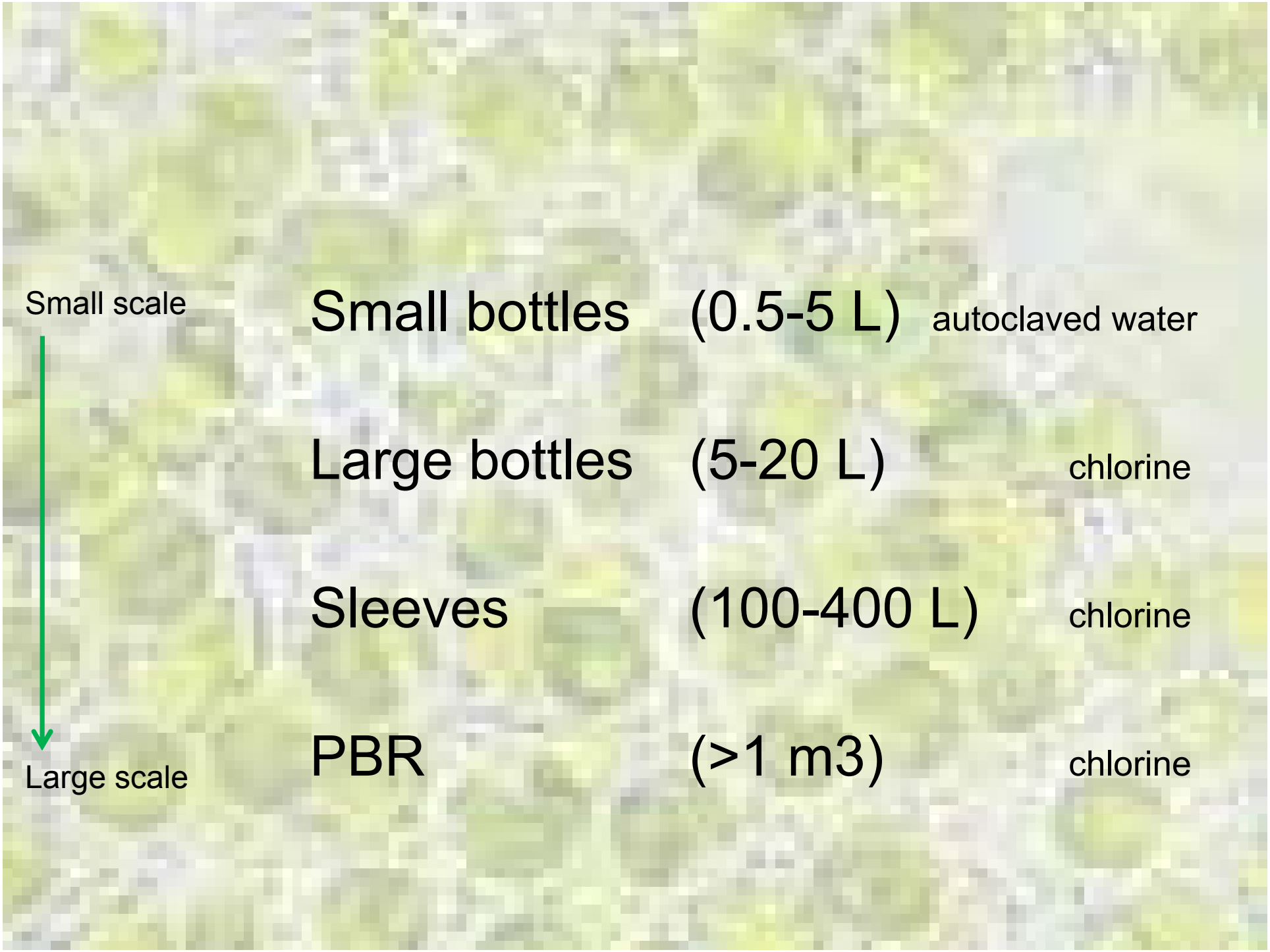
Bacteria in microalgae cultures may have a positive influence for the larvae

Three series of experiments:

1. bacterial communities in cultures of *Chlorella minutissima*

Bacterial communities influenced by:

- algal species cultured
- cultivation system
- phase of the culture (lag, log, stationary, death)
- state of culture
- other factors (use of antibiotics, contaminations etc)

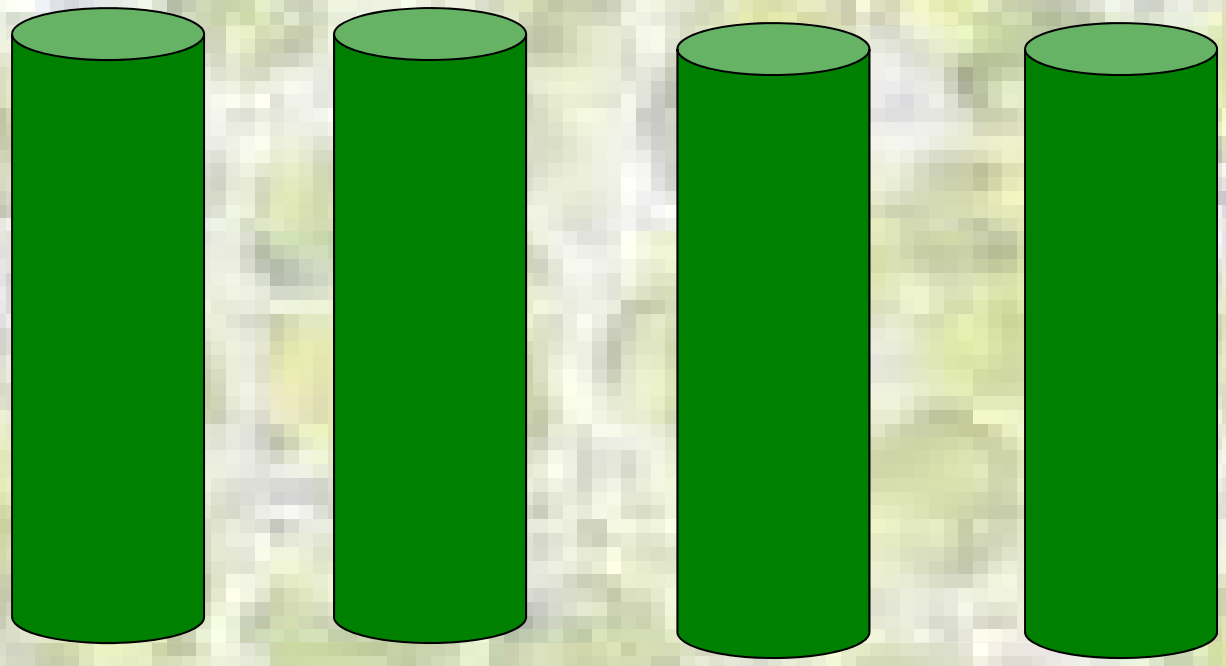

Small scale
Large scale
Small bottles (0.5-5 L) autoclaved water
Large bottles (5-20 L) chlorine
Sleeves (100-400 L) chlorine
PBR (>1 m3) chlorine

200 L sleeves

Natural light conditions

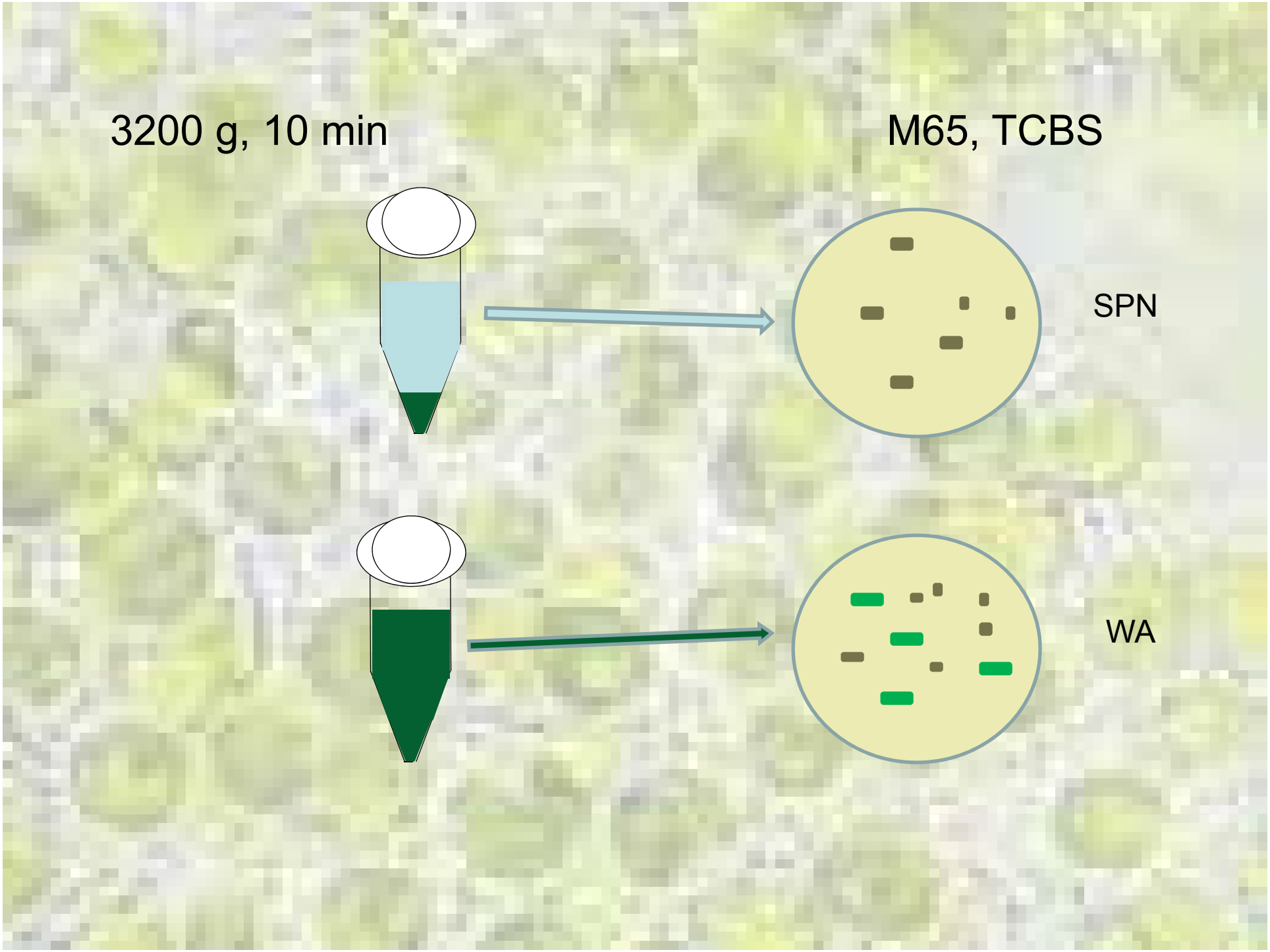
Sampling for microbiological analysis

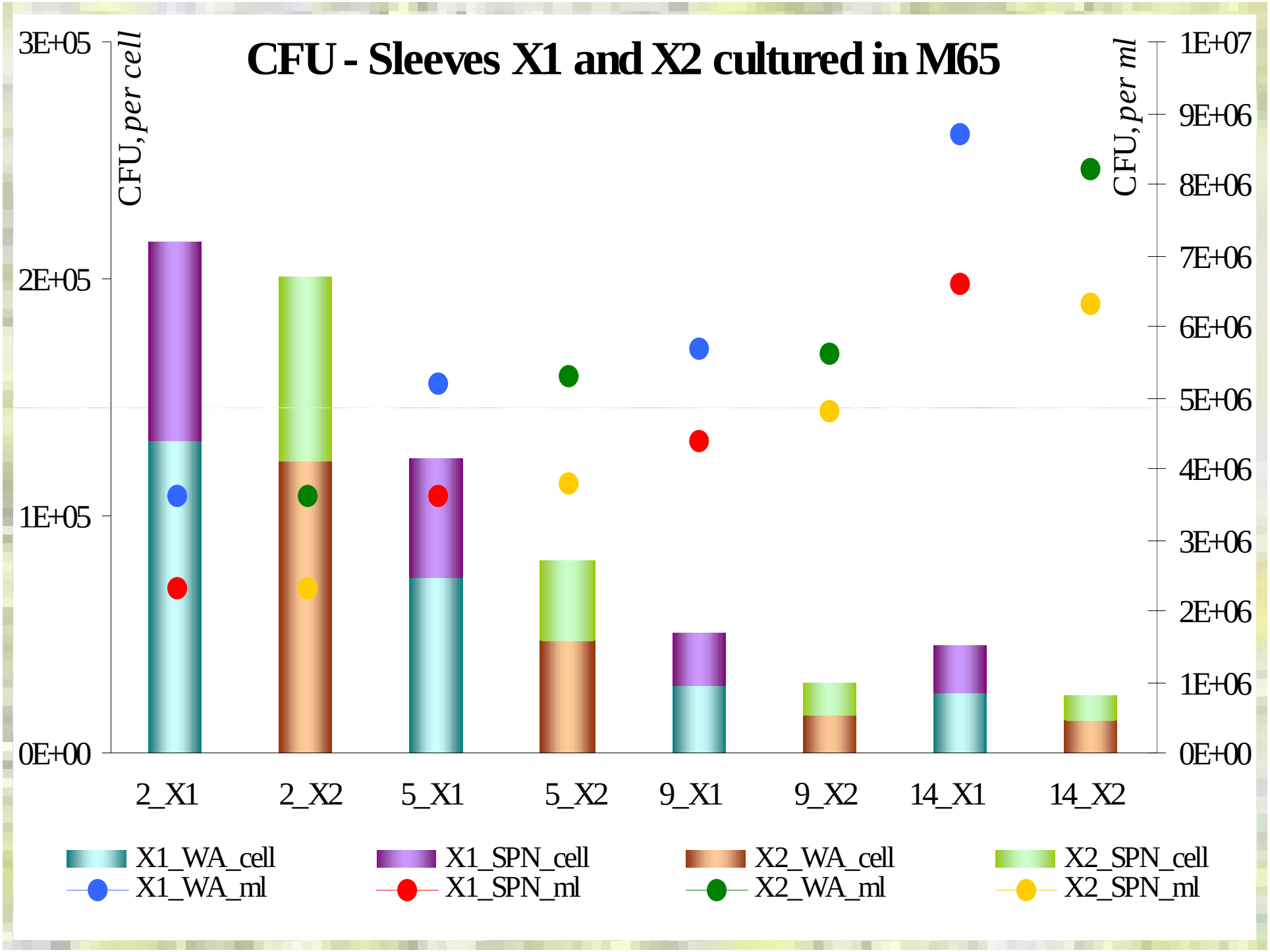
2, 5, 9, 14 days after inoculation

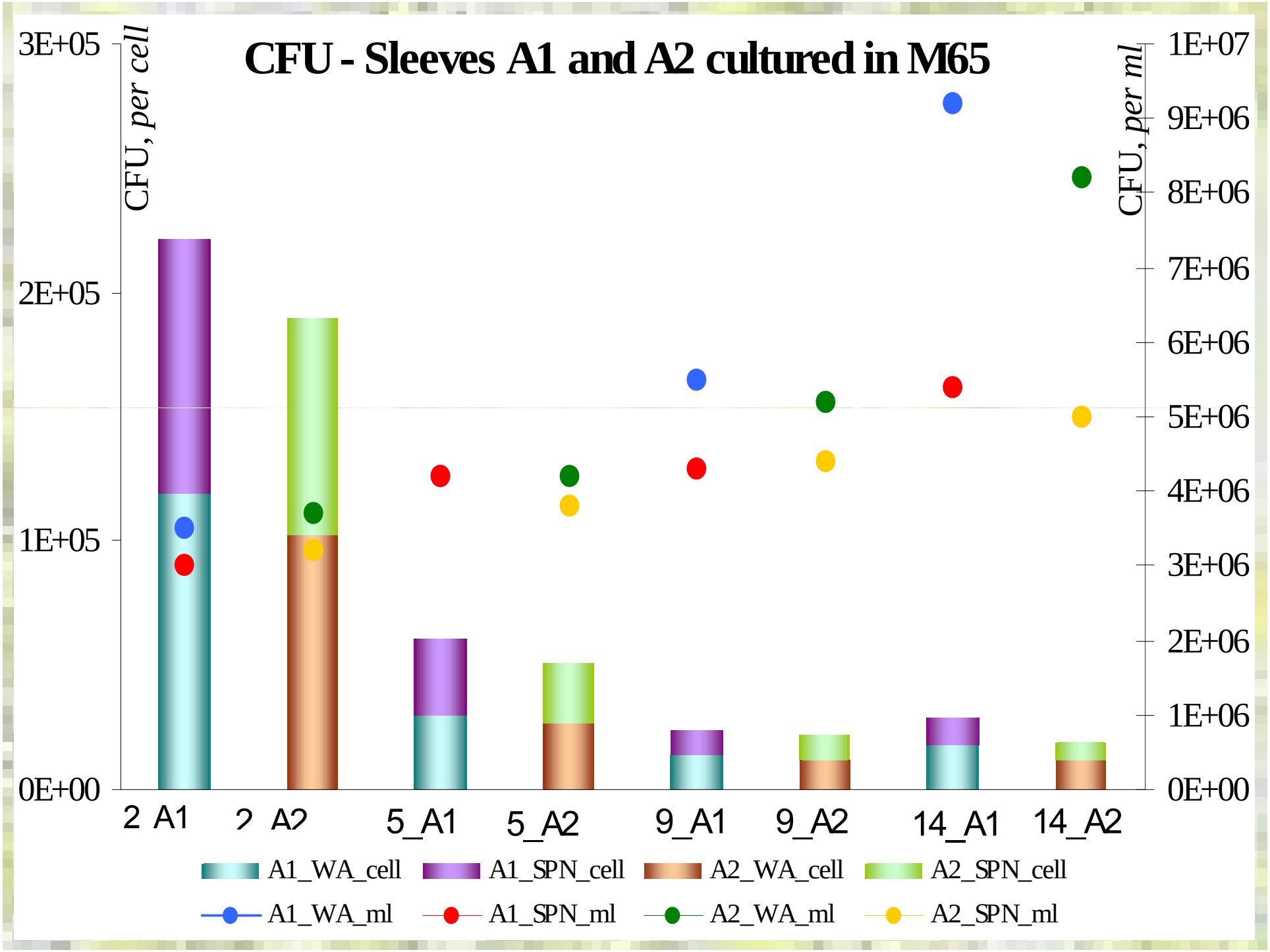


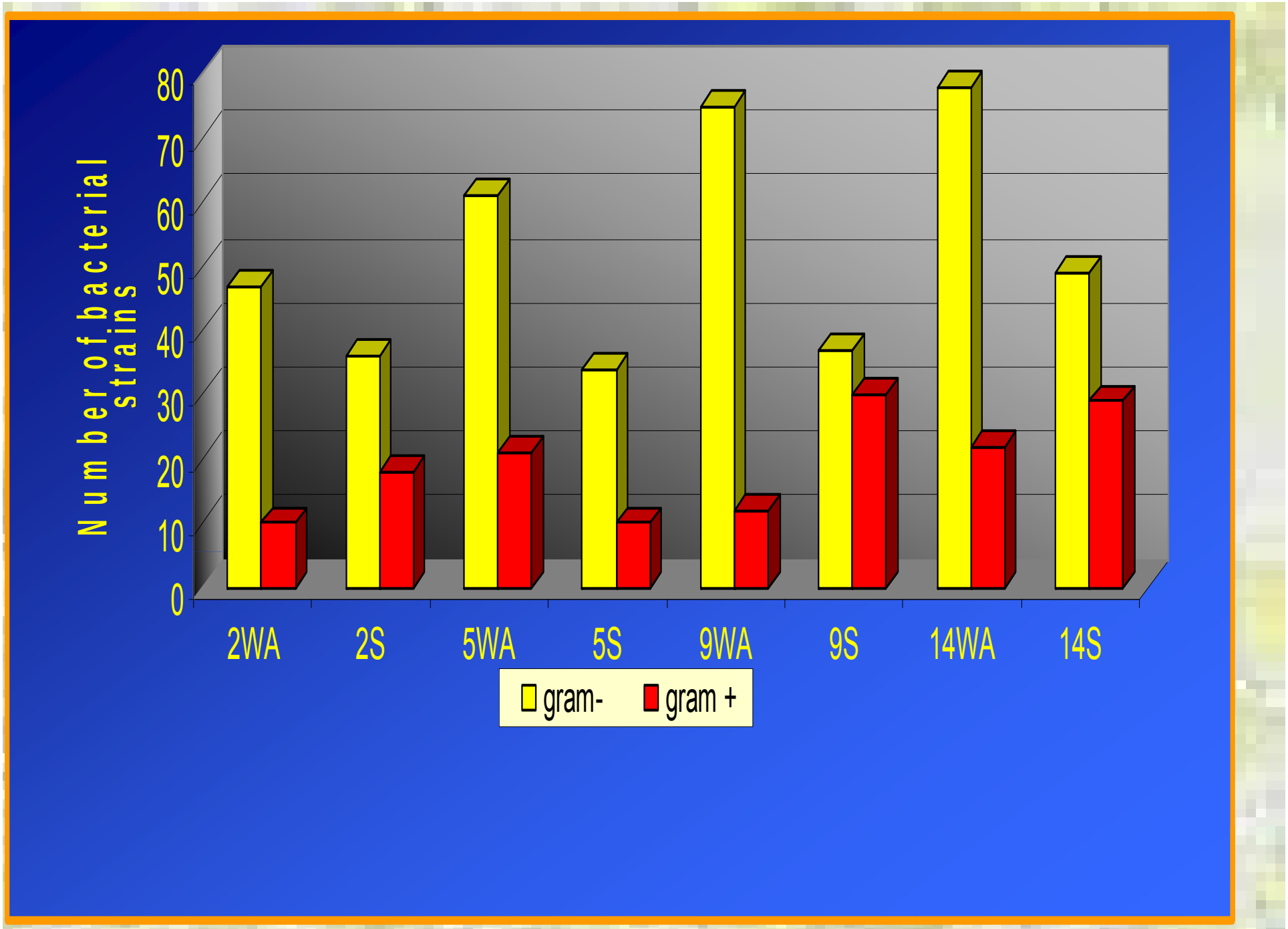
nitrofurantoin (10 mg L^{-1})

control


3200 g, 10 min
M65, TCBS
SPN
WA


CFU - Sleeves X1 and X2 cultured in M65
CFU, per cell
CFU, per ml
3E+05
2E+05
1E+05
0E+00
1E+07
9E+06
8E+06
7E+06
6E+06
5E+06
4E+06
3E+06
2E+06
1E+06
0E+00
2_X1
2_X2
5_X1
5_X2
9_X1
9_X2
14_X1
14_X2
X1_WA_cell
X1_SPN_cell
X2_WA_cell
X2_SPN_cell
X1_WA_ml
X1_SPN_ml
X2_WA_ml
X2_SPN_ml


CFU - Sleeves A1 and A2 cultured in M65
CFU, per cell
CFU, per ml
3E+05
2E+05
1E+05
0E+00
1E+07
9E+06
8E+06
7E+06
6E+06
5E+06
4E+06
3E+06
2E+06
1E+06
0E+00
2_A1
2_A2
5_A1
5_A2
9_A1
9_A2
14_A1
14_A2
A1_WA_cell
A1_SPN_cell
A2_WA_cell
A2_SPN_cell
A1_WA_ml
A1_SPN_ml
A2_WA_ml
A2_SPN_ml


Number of bacterial strains
80
70
60
50
40
30
20
10
0
2WA
2S
5WA
5S
9WA
9S
14WA
14S
gram-
gram +

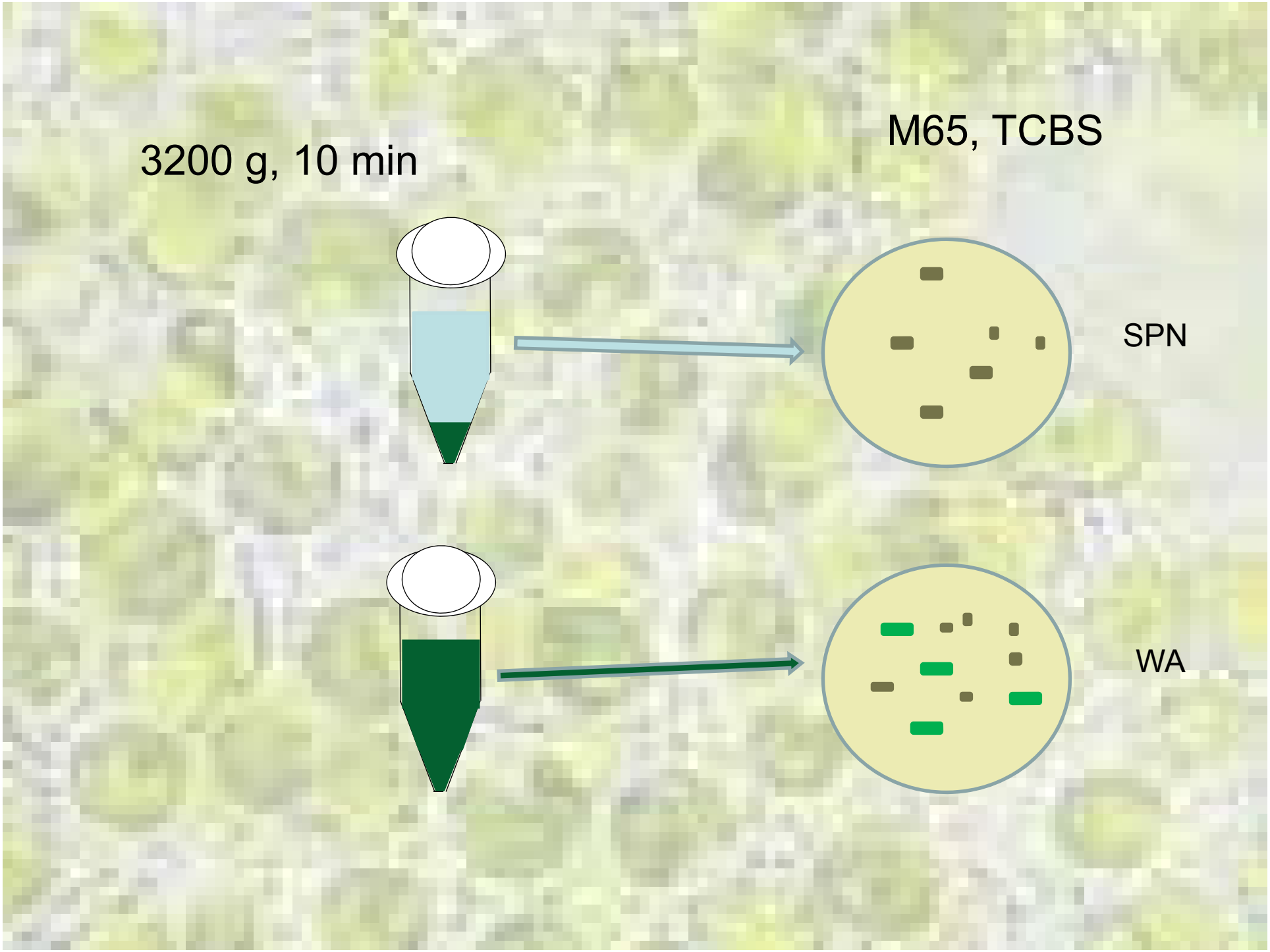
Strain	BLAST result	Homology %
2SA01	Pseudomonas sp. D6-9	99
2SA02	Stenotrophomonas maltophilia isolate SMJR2	100
2SA03	Vibrio sp. SMB7	99
2AX04	Gamma proteobacterium 12IX/A01/168	99
2AX05	Muricauda aquimarina strain SW-72	98
2SX06	Loktanella rosea	99
5AA07	Roseobacter sp. YS-57	99
5AX08	Halomonas sp. B12	99
5SX09	Pseudoalteromonas sp es32	99
5SX10	Maribacter goseongensis strain IS14	99
5SX11	Paracoccus marcusii	100
9SX12	Arenibacter sp. CC10	99
14AA13	Erythrobacter sp. SMB19	99
14AA14	Maribacter goseongensis strain IS14	99
14AA15	Halomonas sp. B12	99
14AA16	Gamma proteobacterium 12IX/A01/168	99
14AA17	Marinobacter sp. NT N31	99
14AA18	Loktanella rosea	99
14SA19	Flexibacter aggregans strain BSs20185	99
14AX20	Roseobacter sp. YS-57	99

counts in TCBS

Densities		**CFU *per ml***				**CFU *per cell***			
Extract	**DAI**	**2**	**5**	**9**	**14**	**2**	**5**	**9**	**14**
WA	**A1**	10	250	190	100	0	2	1	0
	A2	0	0	100	120	0	0	0	0
	X1	0	0	30	0	0	0	0	0
	X2	0	20	20	0	0	0	1	0
SPN	**A1**	300	100	270	630	10	1	1	1
	A2	270	0	430	440	7	0	1	1
	X1	180	40	50	250	7	1	0	1
	X2	230	20	70	0	8	0	0	0

Summary of findings

1. Low numbers of presumptive *Vibrio*
2. Counts in M65 higher in whole algae samples than in SPN sampes
3. The numbers of presumptive Vibrios and opportunistists in whole algae was lower than in SPN
4. The numbers of presumptive *Vibrio* in cultures added antibiotic was higher than in the control cultures

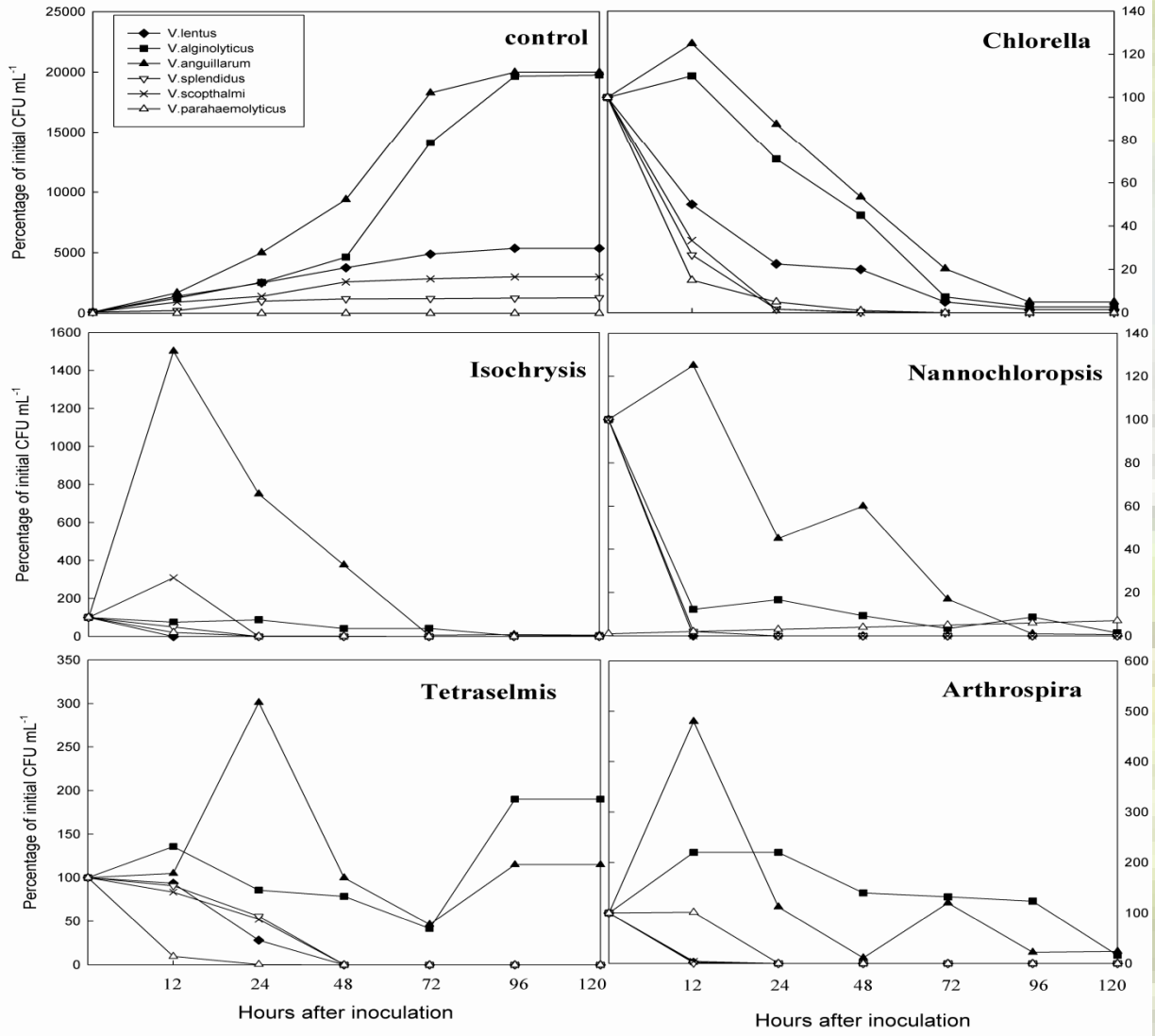

3200 g, 10 min
M65, TCBS
SPN
WA

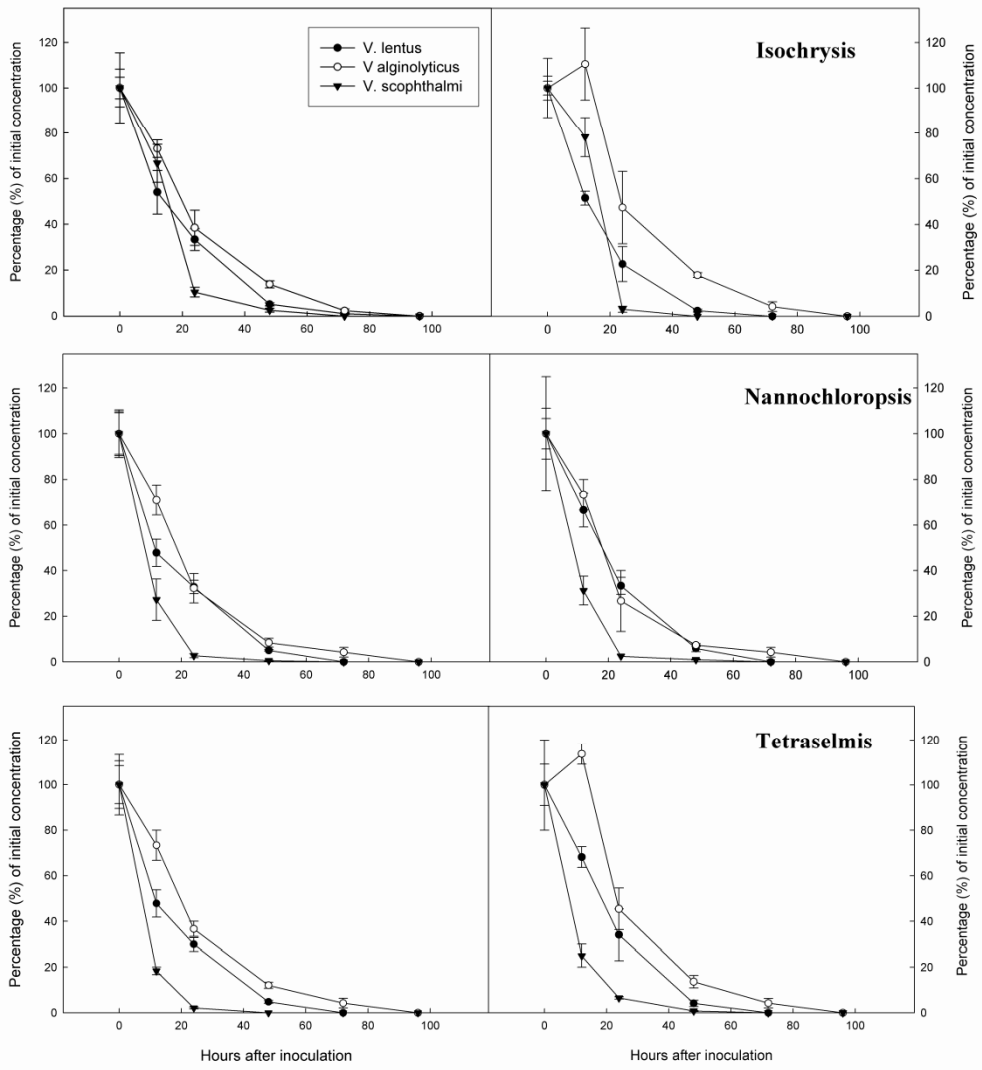
Three series of experiments:

1. bacterial communities in cultures of *Chlorella minutissima*

2. Effect of five microalgae species on *Vibrio* bacteria

- Incubation of each bacterial strain separately in axenic microalgae culture
- A control for each trial with bacteria inoculated in sterile medium
- Natural light conditions
- Initial bacterial concentration 10^4 cells per mL


control
Chlorella
Isochrysis
Nannochloropsis
Tetraselmis
Arthrospira
V.lentus
V.alginolyticus
V.anguillarum
V.splendidus
V.scopthalmi
V.parahaemolyticus
Percentage of initial CFU mL-1
Hours after inoculation
12
24
48
72
96
120


V. lentus
V alginolyticus
V. scophthalmi
Isochrysis
Nannochloropsis
Tetraselmis
Percentage (%) of initial concentration
Hours after inoculation
0
20
40
60
80
100
120

Light

Dark

Conclusions

Vibrio bacteria grew well in microalgae medium in the absence of microalgae

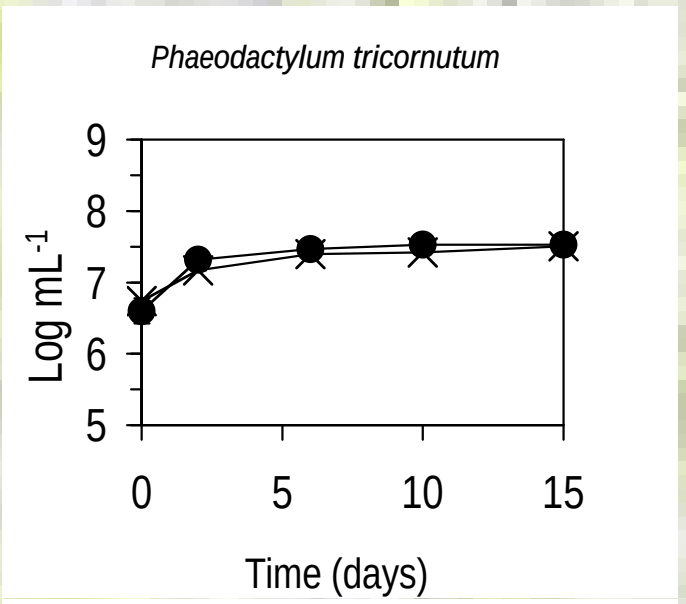
All five microalgae species tested showed antibacterial activity against six *Vibrio* bacteria
Both in the darkness and in light conditions

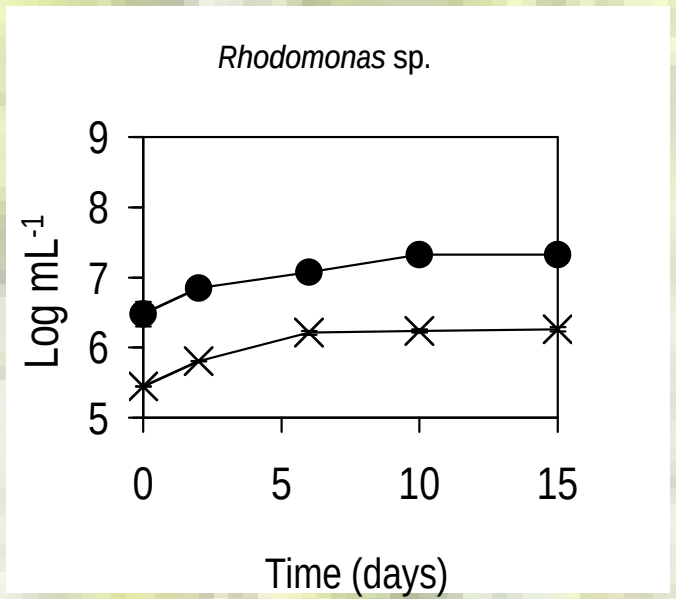
Three series of experiments:

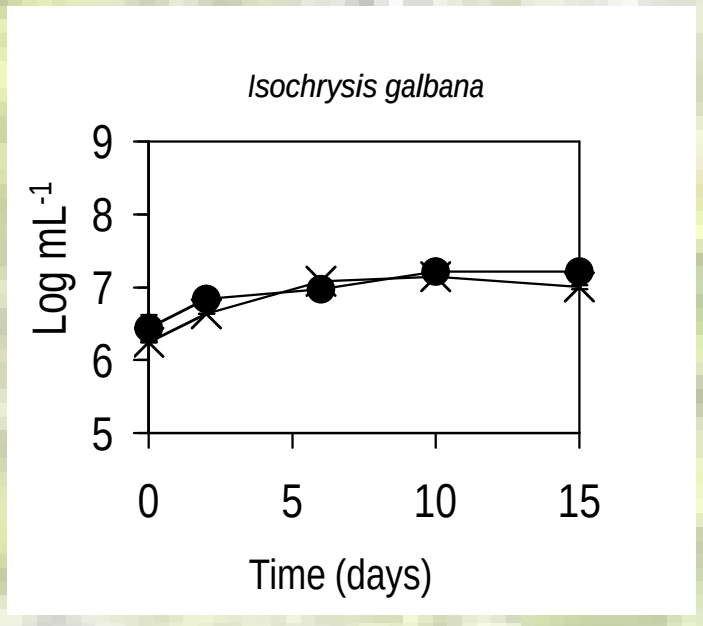
1. bacterial communities in cultures of *Chlorella minutissima*

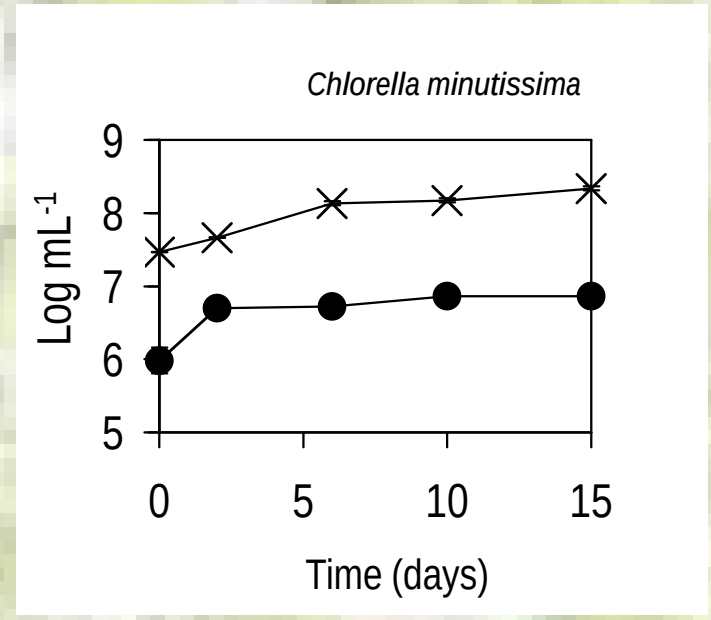
2. Effect of five microalgae species on *Vibrio* bacteria

3. Bacterial communities in four species of microalgae



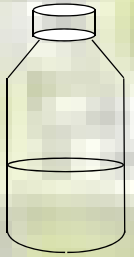






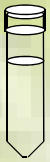
Microalgae cells (X) and total CFU grown in MA (●).

PROTOCOL



Microalgae culture
In Flask or bags

- take 50 ml
- shake vigorously

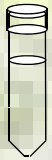


- plate in MA dilutions 10^3, 10^4, 10^5
- plate in TCBS dilutions 10^1

- incubate
7 days at 20°C
in obscurity

- count CFU

- count CFU
- incubate
1 day at 20°C
in obscurity

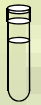


in the rest of the volume, measure:
- pH
- Oxygen
- Chlorophyll fluorescence (PEA)
- Absorbance 750 nm
- Microalgae cell counts (Malassez cell)

- centrifuge 3.200 x g, 10 min

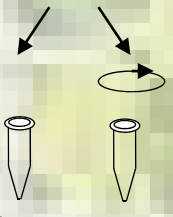
- purify the isolates by 3 passes

- keep bacterial biomass for DNA extraction at - 20ºC



- keep the isolates in cryotubes at -80ºC in TSA-glicerol

- resuspend the pellet in 1ml
-centrifuge 10.000 x g, 10 min
- keep the microalgae pellet at -20ºC



- centrifuge the supernatant at 10.000 x g, 10 min
-resuspend the pellet in 1ml
-centrifuge 10.000 x g, 10 min
- keep the bacteria pellet at -20ºC

Algae Water

Isolates

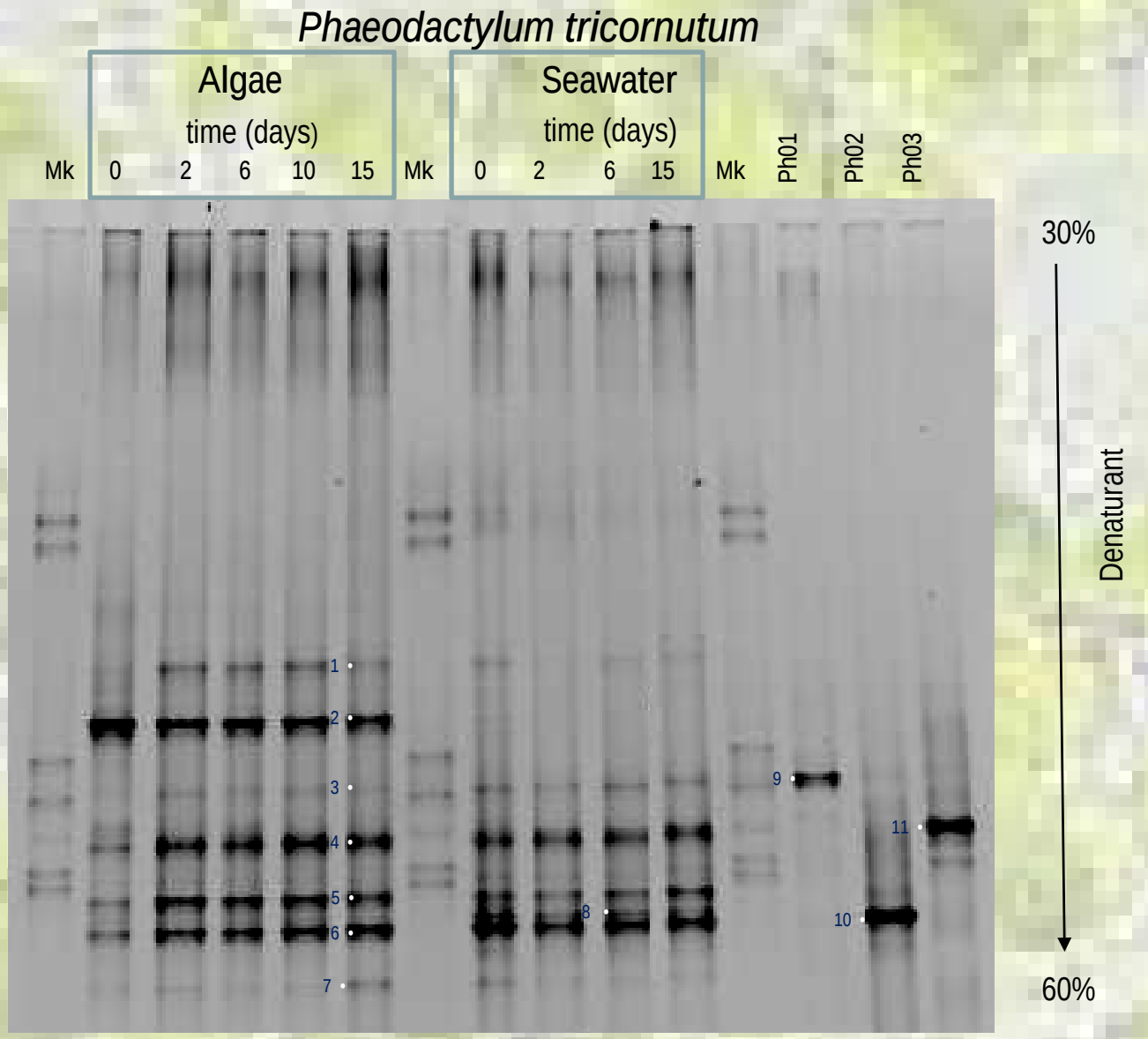
DNA extraction

DNA extraction

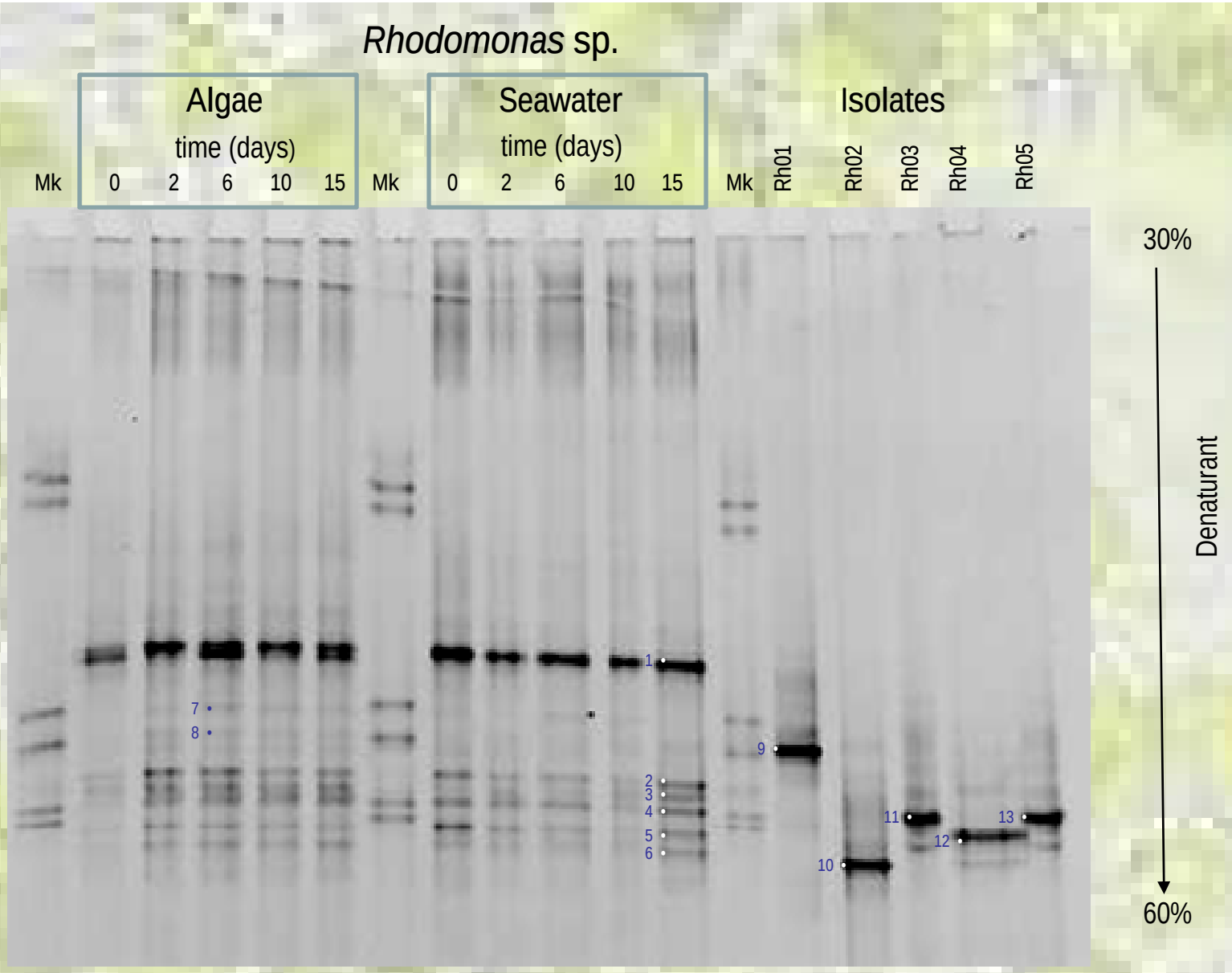
DGGE

Gel analysis and band identification

José Pintado HCMR, Aprril 2009



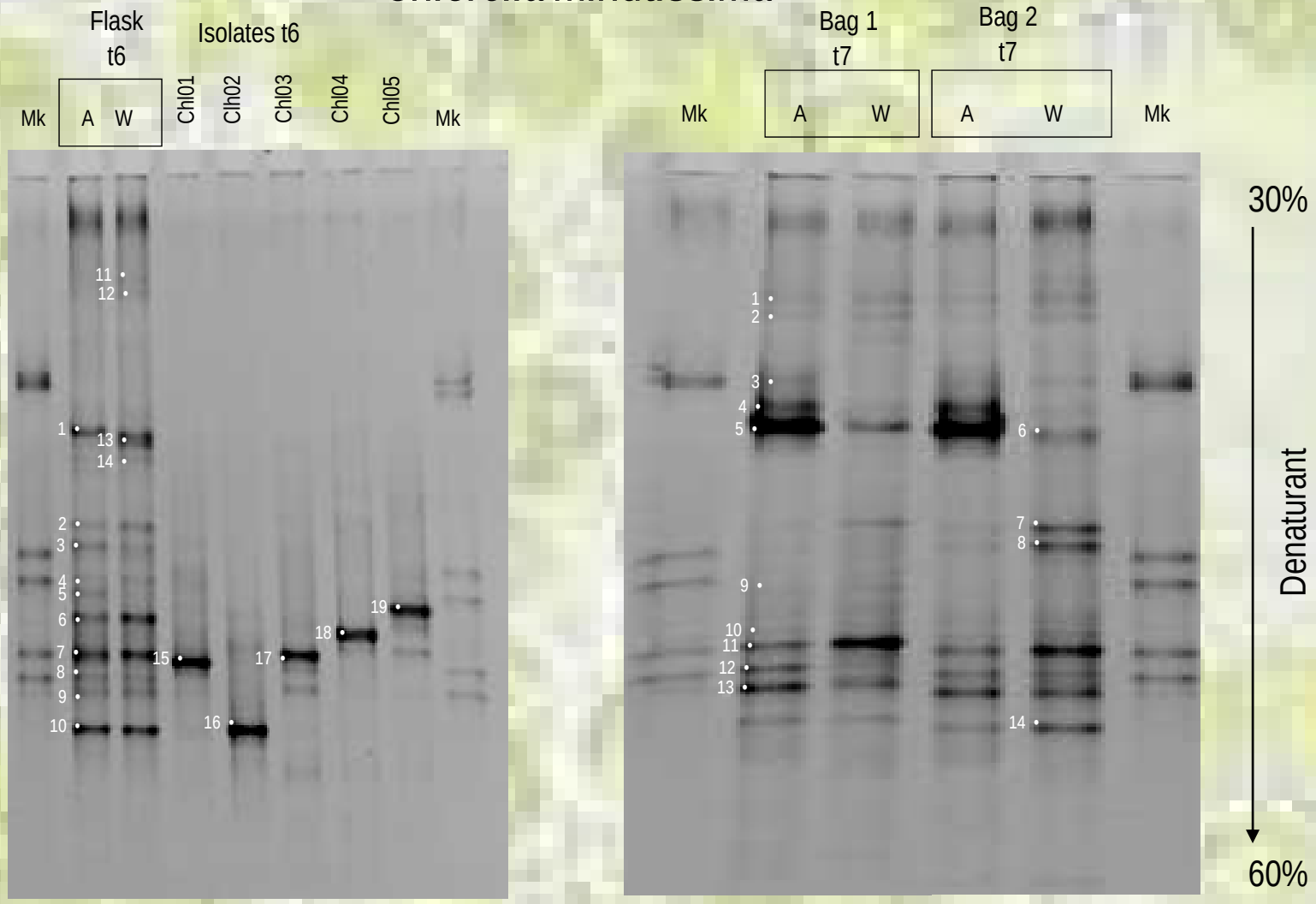
DGGE profiles of the bacterial communities present in the algae and in the seawater, in a culture of Phaeodactylum tricornutum



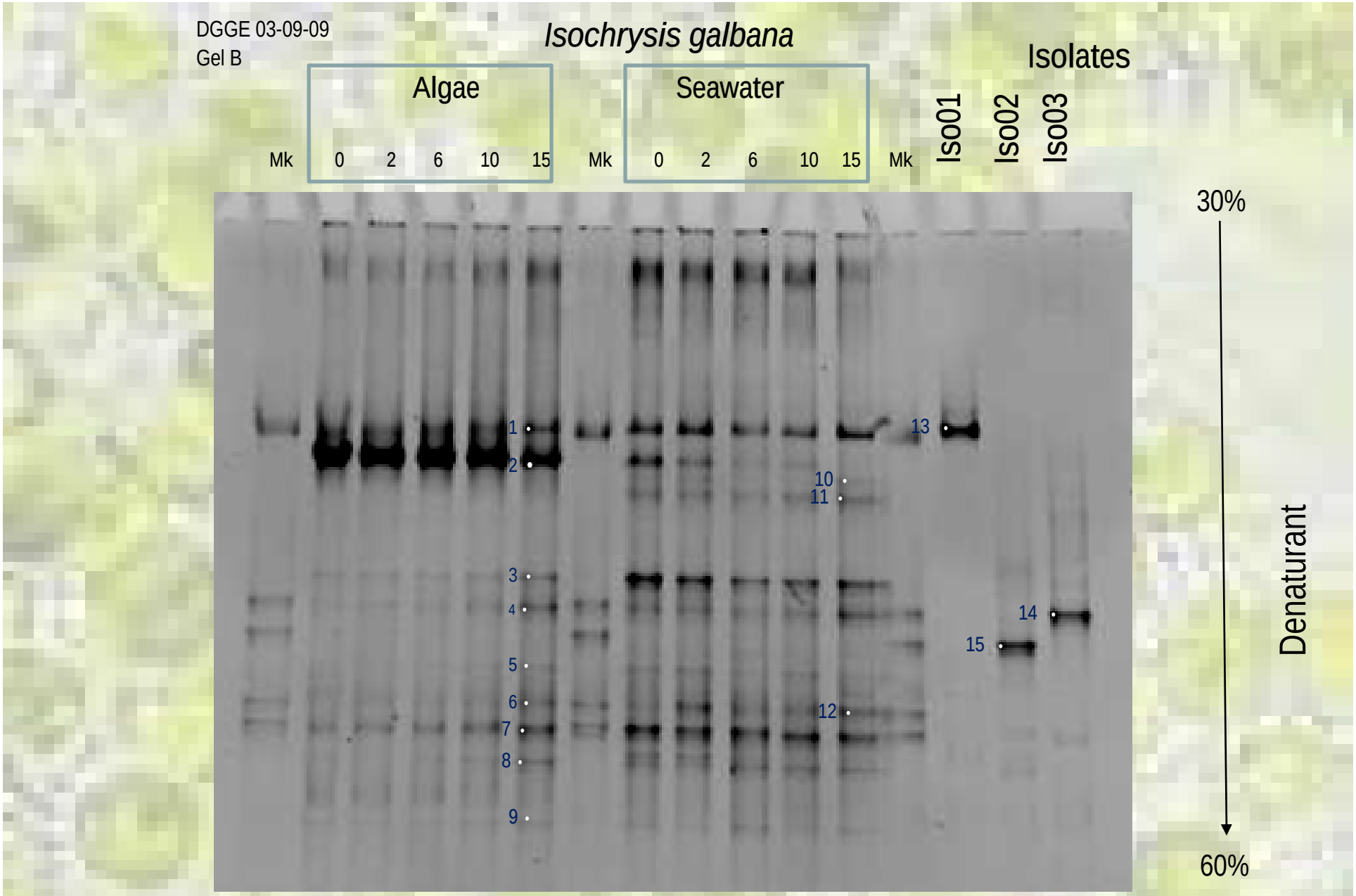
DGGE profiles of the bacterial communities present in the algae and in the seawater, in a culture of *Rhodomonas* sp.

Chlorella minutissima

DGGE 03-09-09
Gel A



DGGE profiles of the bacterial communities present in the algae (A) and in the seawater (W), in a culture of *Chlorella minutissima* in a preculture in Flasks (6 days) in sterile seawater and in a scale-up culture in bags with non sterile seawater.



DGGE profiles of the bacterial communities present in the algae and in the seawater, in a culture of *Isochrysis galbana* at different times, and the predominant isolates from Marine Agar plates at t6. Numbered bands were excised for re-amplification and sequencing. Mk: marker.

Conclusions

1. The DGGE pattern was different for each microalgae species
2. No changes were observed throughout the trials for each algae
3. In three out of four cases culturable strains were representative for the bacteria present in the cultures


Thank you for your attention