

Diatom-associated bacteria?

Crashing phenomenon and the late 70's paper.

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Apr. 1978, p.791-796
0099-2240/78/0035-0791\$02.00/0
Copyright © 1978 American Society for Microbiology

Vol. 35, No. 4

Printed in U.S.A.

Interactions Between the Diatom *Thalassiosira pseudonana* and an Associated Pseudomonad in a Mariculture System

KATHERINE H. BAKER* AND DIANE S. HERSON

Cell and Molecular Biology Section, School of Life and Health Sciences, University of Delaware, Newark, Delaware 19711

Received for publication 12 October 1977



Pseudomonas T827/2B

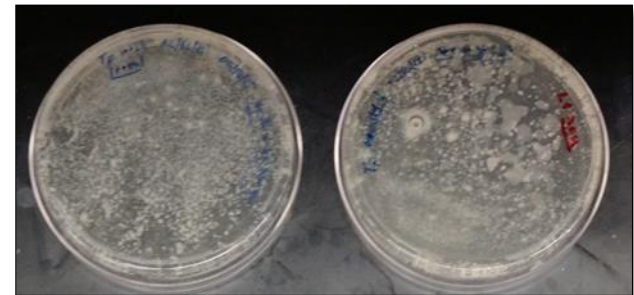
- competition and indirect parasitism
- "harmful proteinaceous excrete"

Glassy, shiny morphology.

Plating *T. pseudonana* at high densities.



L1, 1.5 % agar

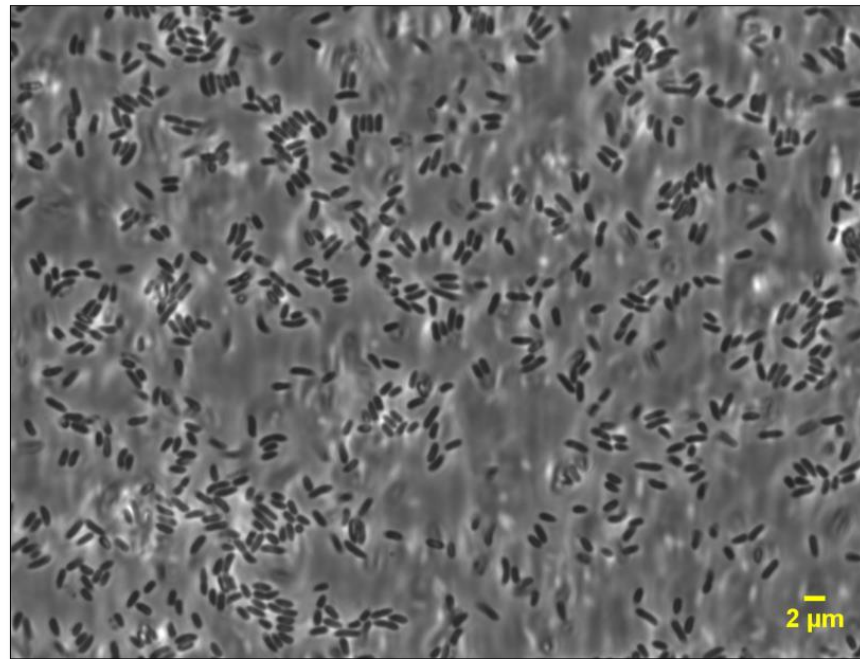


Gene gun experiment last summer.

Karas *et al.* (2015)

Light microscopy of the bacteria

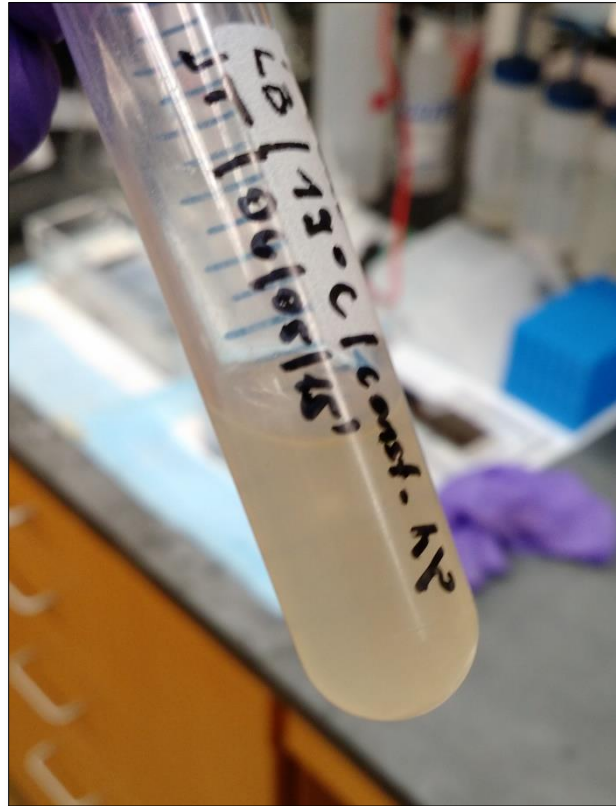
Behavior of the cells on a glass slide (I have a movie as well).



This activity might indicate they're in search of a certain (diatom-derived?) chemical signal → chemotaxis assays with a library of potential infochemicals?

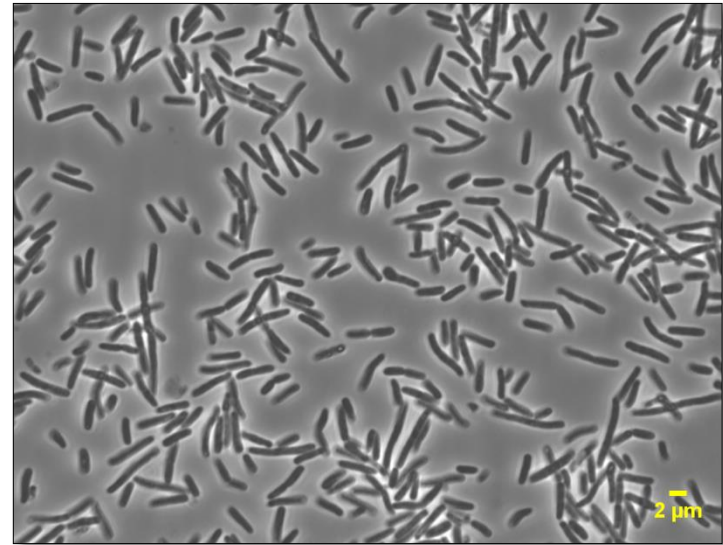
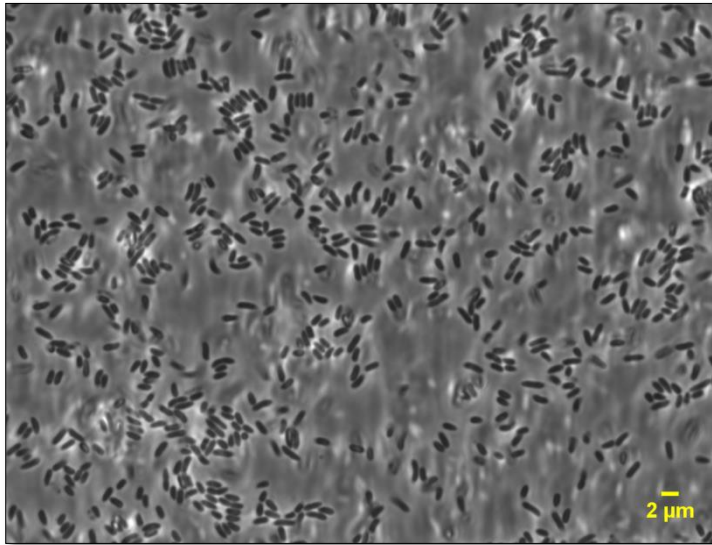
The bacteria swims and doesn't settle down

The culture turbid after being unperturbed for ~ 7 days at 4° C.



Light microscopy of the bacteria

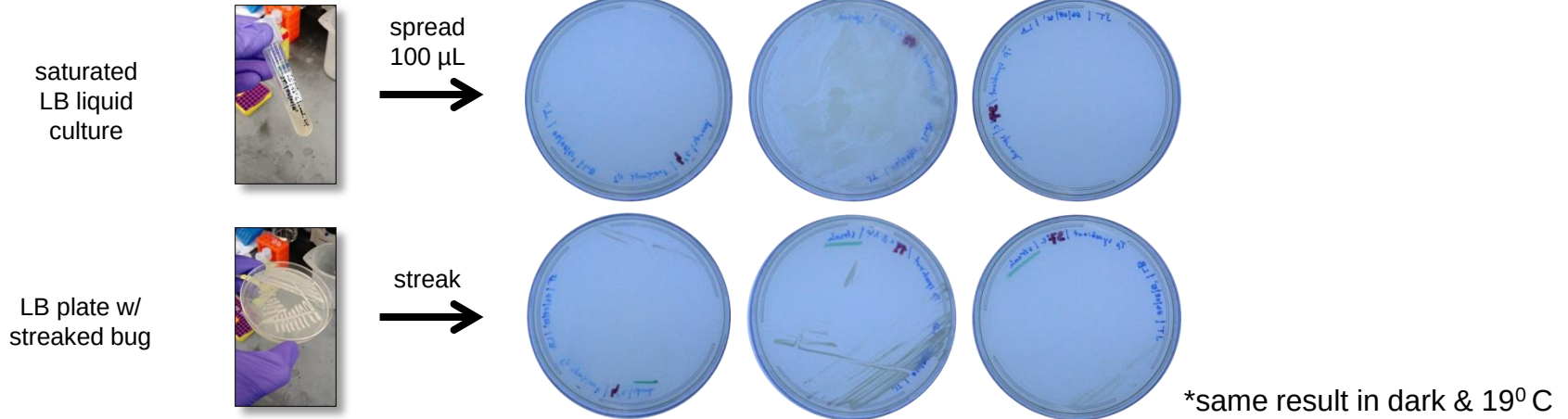
Side by side comparison with *E. coli*.



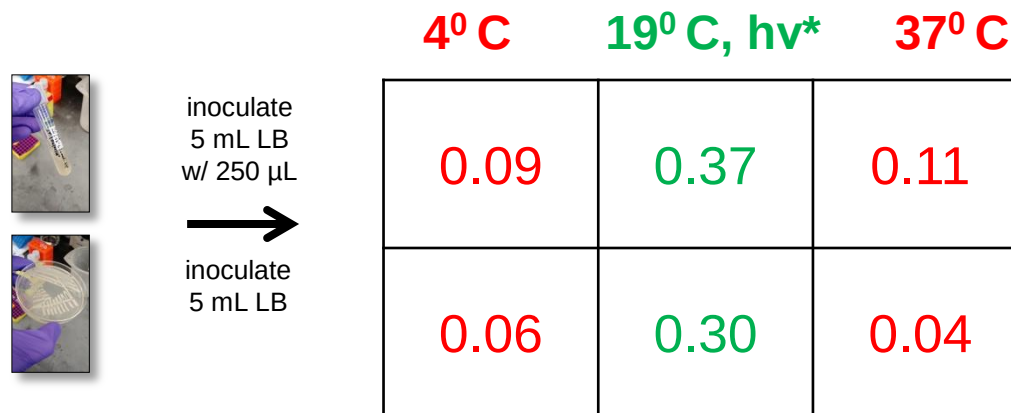
E. coli EPI300 w/ pTA-MOB & pTpPuc3

The bacteria is heterotrophic and mesophilic

Plating assay

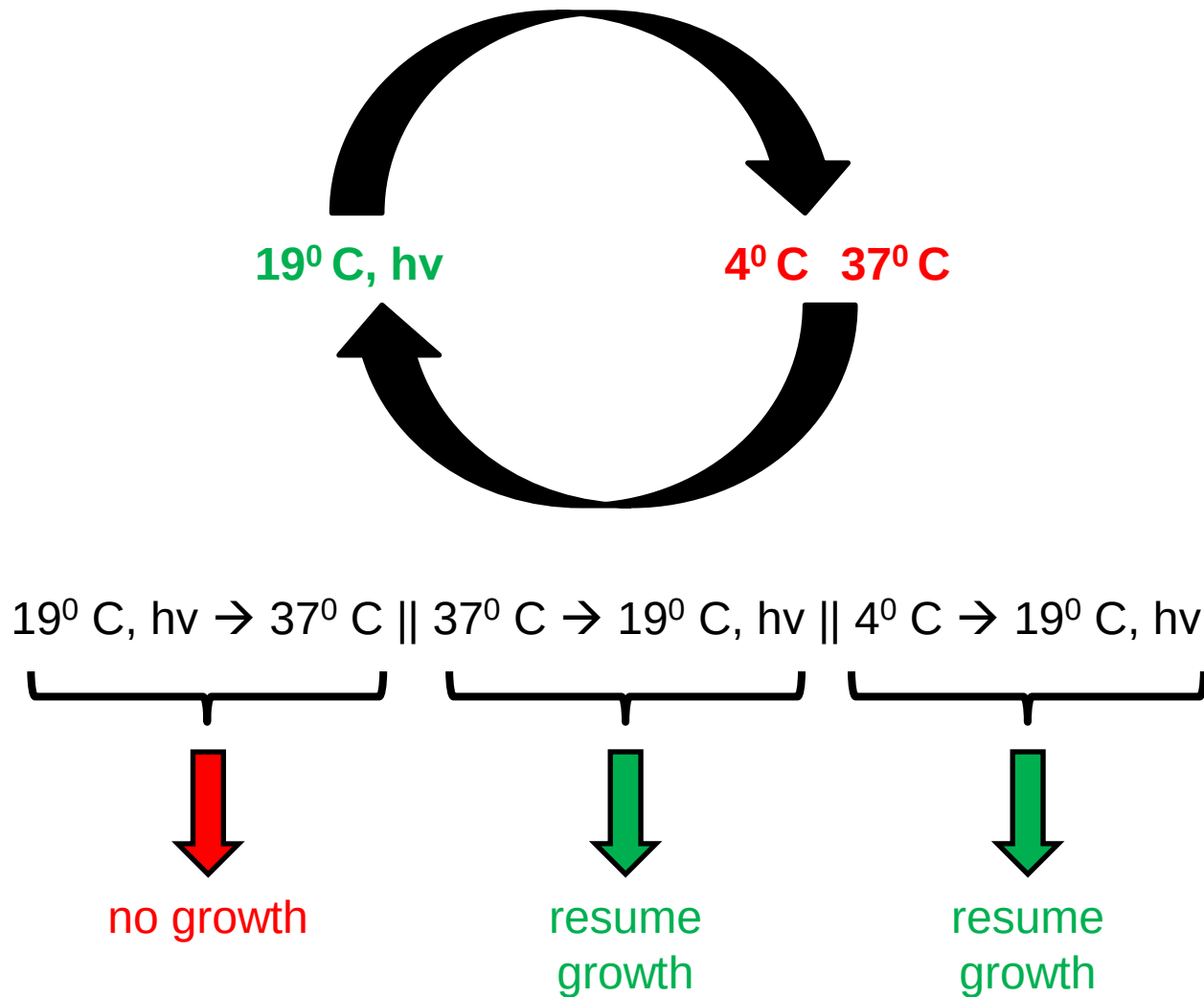


Liquid culture assay (OD₆₀₀)



after ~14 h incubation

Switch conditions for ~24 hours

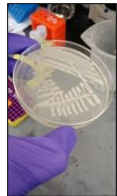


Definitely heterotrophic and mesophilic

Plating assay



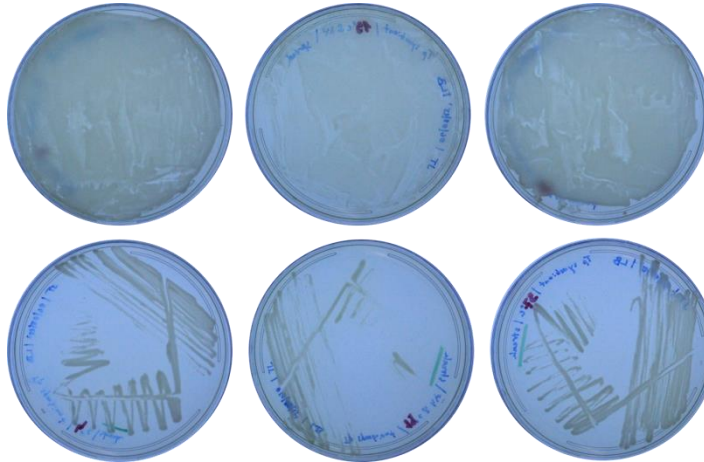
spread
100 μ L



streak



4^0C_{19} $19^0 \text{C}_{37}, \text{hv}^*$ 37^0C_{19}



Liquid culture assay (OD_{600})



inoculate
5 mL LB
w/ 250 μ L



inoculate
5 mL LB

4^0C_{19} $19^0 \text{C}_{37}, \text{hv}^*$ 37^0C_{19}

0.69	0.37	0.67
0.67	0.28	0.54

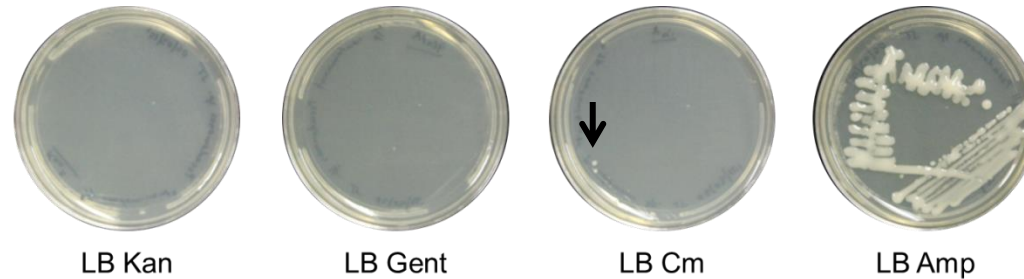
$$\overline{\text{green}} = 0.64 \pm 0.07$$

$$\overline{\text{red}} = 0.33 \pm 0.06$$

after additional ~28 h incubation (compare to slide 5)

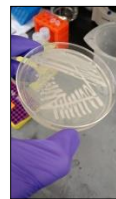
The bacteria sensitive to Gent, Tet (and Km)

Plating assay*

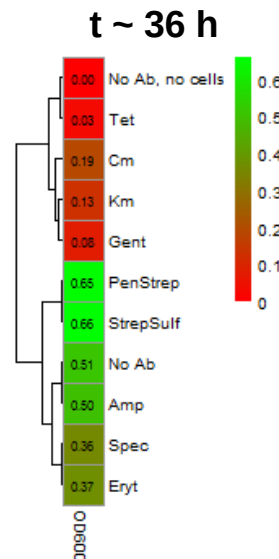


Liquid culture assay (OD₆₀₀)

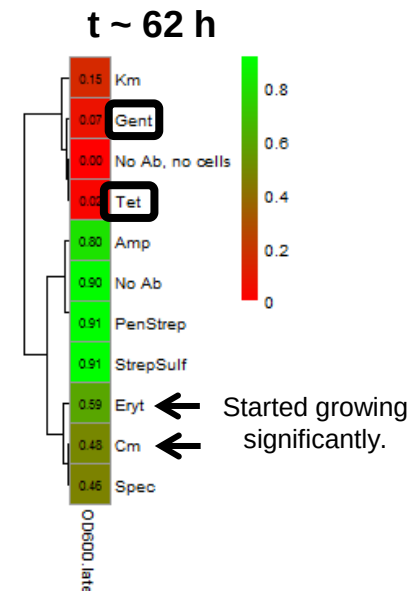
Ab	c [ug/mL]
No Ab, no cells	0
No Ab	0
Amp	50 or 100
Km	50?
Gent	20
Cm	25
PenStrep	1x
Spec	50
Eryt	50
Tet	15
Strep	100



~18 °C
inoculate
5 mL LB



RT

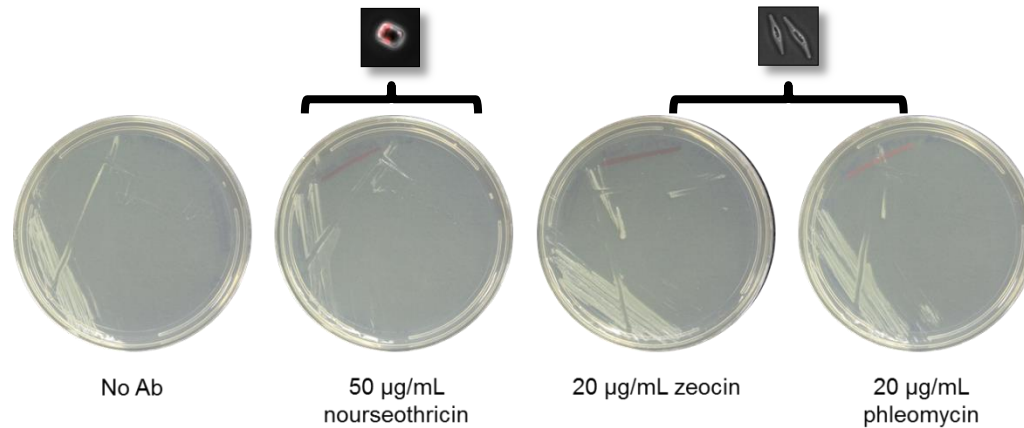


Clusters don't really make a lot of sense.

*Growth conditions: ~19° C | light.

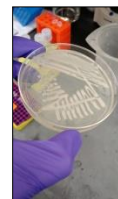
The bacteria resistant to all Abs for work with diatoms

Plating assay*

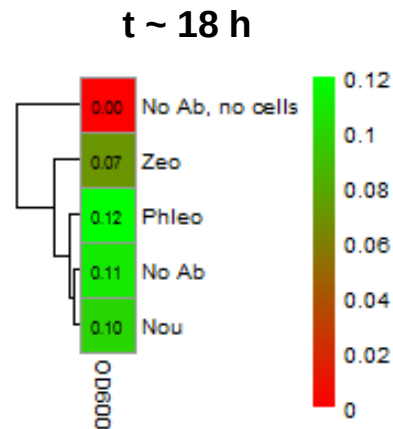


Liquid culture assay (OD₆₀₀)

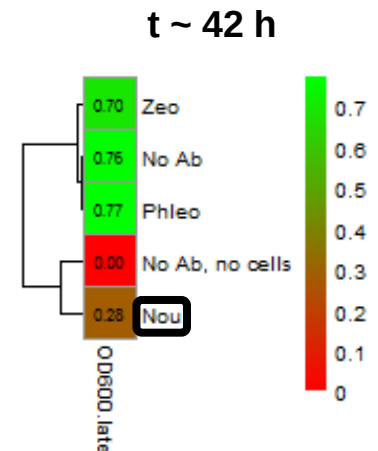
Ab	c [µg/mL]
No Ab, no cells	0
No Ab	0
Nou	50
Zeo	20
Phleo	20



~15 °C
inoculate
5 mL LB



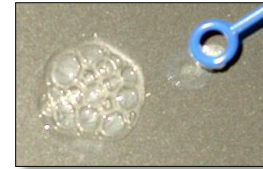
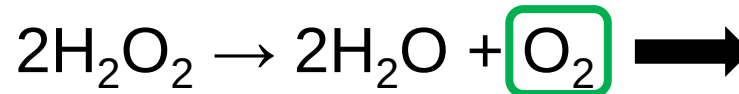
RT



*Growth conditions: ~15° C | light → lower T because of *T. pseudonana*. All antibiotics in this experiment are used to select for diatom exconjugants.

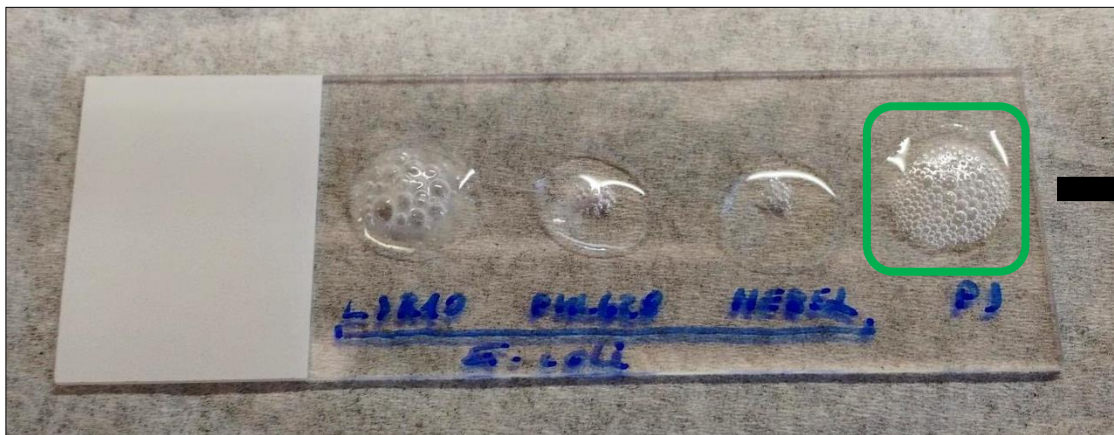
Catalase assay

Catalase: a common enzyme found in nearly all living organisms exposed to oxygen. It catalyses the decomposition of hydrogen peroxide to water and oxygen.



[Source.](#)

3 wt. % H_2O_2 in H_2O



catalase positive*

E. coli controls

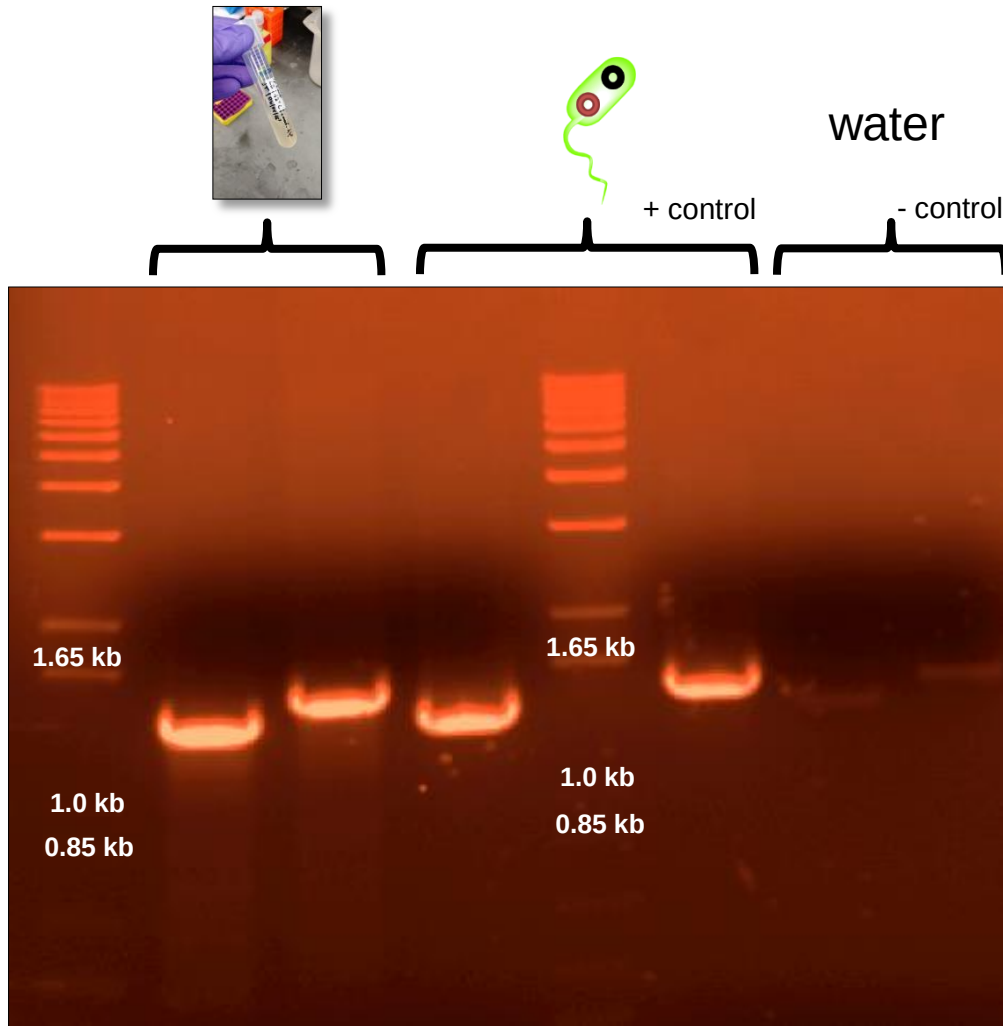
?

*Characteristic of *Pseudomonas* sp. → [link](#).

16S sequencing

green
genes*

16S rRNA gene database and
workbench compatible with ARB
greengenes.lbl.gov



2 16S primer pairs

Control

- *Escherichia* and *Shigella* species

Experiment

- *Pseudomonas* sp.

- *rpoD*-based PCR (Mulet *et al.*, 2009)

rpoD sequencing

An *rpoD*-based PCR procedure for the identification of *Pseudomonas* species and for their detection in environmental samples

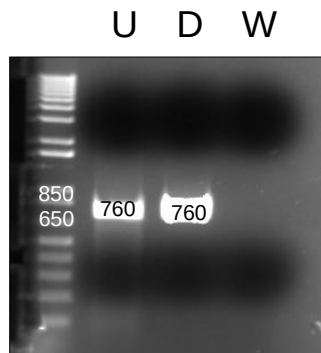
Magdalena Mulet^a, Antonio Bennasar^a, Jorge Lalucat^{a,b}, Elena García-Valdés^{a,b,*}

^a Microbiologia, Departament de Biologia, Universitat de les Illes Balears, Campus UIB, 07122 Palma de Mallorca, Spain

^b Institut Mediterrani d'Estudis Avançats (CSIC-UIB), Campus UIB, 07122 Palma de Mallorca, Spain

Molecular and Cellular Probes (2009)

760 bp long amplicon based on the internal conserved sequences of 33 selected *rpoD* gene sequences (the sigma 70 factor subunit of the DNA polymerase) of *Pseudomonas* type strains.



U: undiluted culture
D: 20x diluted culture
W: water control

seq
→
NCBI
BLAST
nr

2 99 % hits:

☐ [Aphanomyces euteiches cDNA](#) → water mold; notorious plant pathogen

☒ [Pseudomonas sp. 8H1 RNA polymerase sigma factor 70 \(rpoD\) gene, partial cds](#)

Max score	Total score	Query cover	E value	Ident	Accession
996	1991	84%	0.0	99%	CU367837.1
1168	2336	100%	0.0	99%	JX987895.1

Paper associated with the *Pseudomonas* hit

Pseudomonas yamanorum sp. nov., a
psychrotolerant bacterium isolated from a
subantarctic environment

Víctor Gonzalo Arnau,¹ Leandro Arturo Sánchez¹
and Osvaldo Daniel Delgado²

International Journal of Systematic and Evolutionary Microbiology (2015)

Facts

- 1] *Pseudomonas* known to be involved in diatom-bacteria interactions (Baker *et al.*, 1978; Amin *et al.*, 2012).
- 2] Half of the algal kingdom are vitamin B₁₂ auxotrophs, including *T. pseudonana* (Croft *et al.*, 2005).
- 3] Methionine synthase (MetH) in *T. pseudonana* requires vitamin B₁₂ (Croft *et al.*, 2005).
- 4] *Pseudomonas denitrificans* used for industrial-scale vitamin B₁₂ production (Xia *et al.*, 2015).

Hypothesis: The bacteria produces vitamin
B₁₂ and feeds it to *T. pseudonana*

At least 2 recent publication are strengthening my hypothesis

Cryptic carbon and sulfur cycling between surface ocean plankton

Bryndan P. Durham^a, Shalabh Sharma^b, Haiwei Luo^b, Christa B. Smith^b, Shady A. Amin^c, Sara J. Bender^d, Stephen P. Dearth^e, Benjamin A. S. Van Mooy^d, Shawn R. Campagna^e, Elizabeth B. Kujawinski^d, E. Virginia Armbrust^c, and Mary Ann Moran^{b,1}

^aDepartment of Microbiology, University of Georgia, Athens, GA 30602; ^bDepartment of Marine Sciences, University of Georgia, Athens, GA 30602; ^cSchool of Oceanography, University of Washington, Seattle, WA 98195; ^dDepartment of Marine Chemistry and Geochemistry, Woods Hole Oceanographic Institution, Woods Hole, MA 02543; and ^eDepartment of Chemistry, University of Tennessee, Knoxville, TN 37996

PNAS (2015)

LETTER

doi:10.1038/nature14488

Interaction and signalling between a cosmopolitan phytoplankton and associated bacteria

S. A. Amin^{1,2}, L. R. Hmelo³, H. M. van Tol¹, B. P. Durham⁴, L. T. Carlson¹, K. R. Heal¹, R. L. Morales¹, C. T. Berthiaume¹, M. S. Parker¹, B. Djunaedi¹, A. E. Ingalls¹, M. R. Parsek³, M. A. Moran⁵ & E. V. Armbrust¹

Nature (2015)