



2017

SYMPOSIUM

**A healthy food chain for a
balanced microbiome, Thanks
to algae!**



2017

Seaweed microbiome: Importance for cultivation, food & biotechnology

Philippe POTIN

Research director at CNRS / Roscoff Biologic station

A hot spot of biodiversity for understanding the seaweed microbiome



CNRS UPMC
Station Biologique
Roscoff

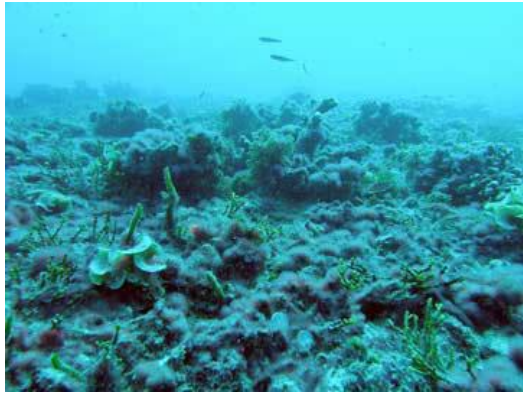


Philippe Potin, G. Michel, M. Czjzek, F. Thomas, R. Larocque, C. Leblanc, A. Gobet, J. Tapia, S. Dittami, JH Hehemann, G. Correc, C. Boyen, B. Kloareg



Why understanding the seaweed microbiome?

Impact on coral reefs



Green and golden tides



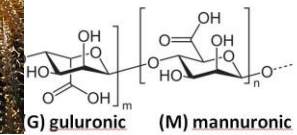
Seaweed Food and Feed



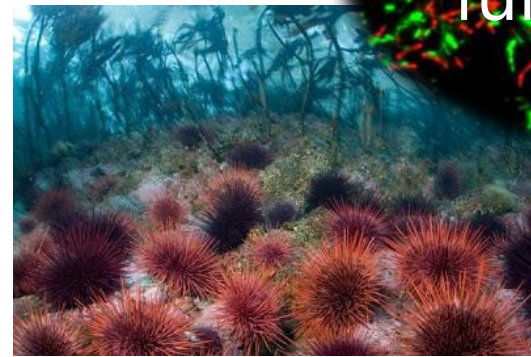
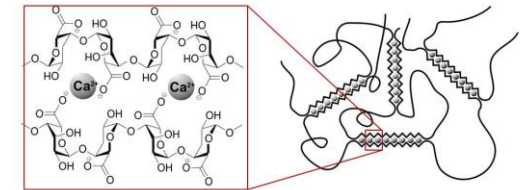
Applied

The microbiome functions?

Seaweed Cultivation



Ca²⁺



Decline of kelp forests worldwide



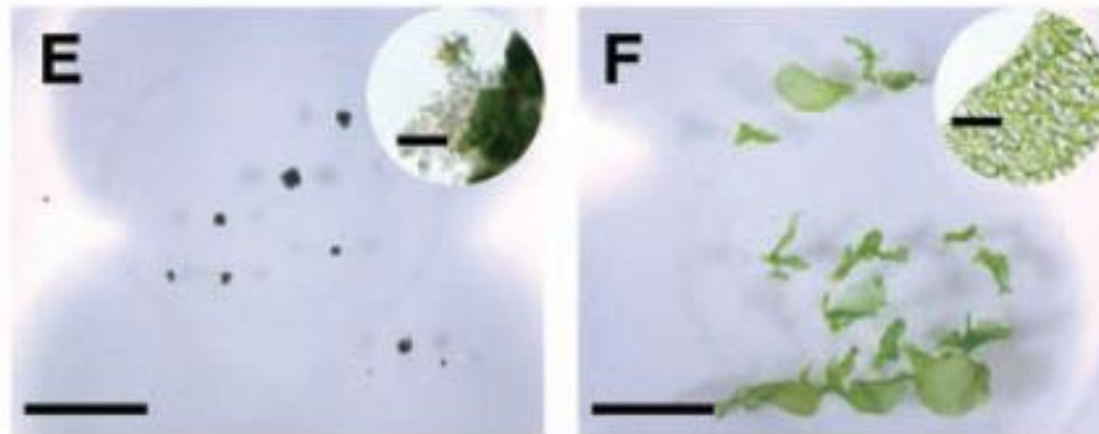
Alginate extraction/
Biotechnology

Fundamentals



As humans and all eukaryotes do, seaweeds interact with microbes

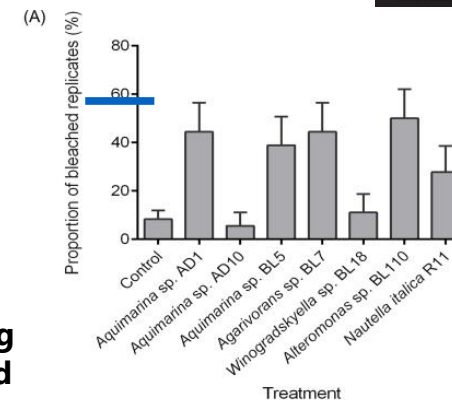
- Bacteria shape seaweed morphology



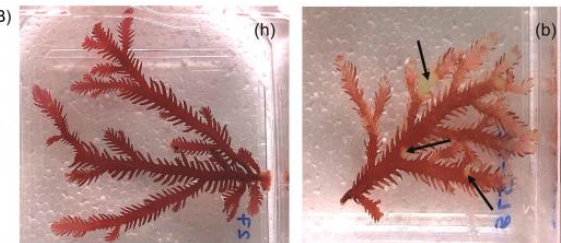
Matsuo et al. 2005 Science: Thallusin effect on *Ulva pertusa*

- Nitrogen assimilation
- Vitamins
- Defense/diseases...

Kumar et al. 2016
18, 3962–3975



Multiple opportunistic pathogens can cause a bleaching disease in the red seaweed *Delisea pulchra* (B)



MINIREVIEWS

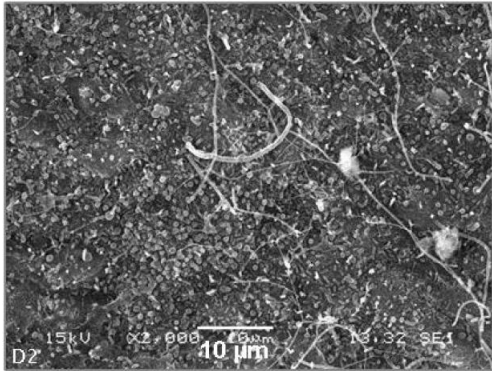
Algae Need Their Vitamins†

Martin T. Croft,^{1*} Martin J. Warren,² and Alison G. Smith¹



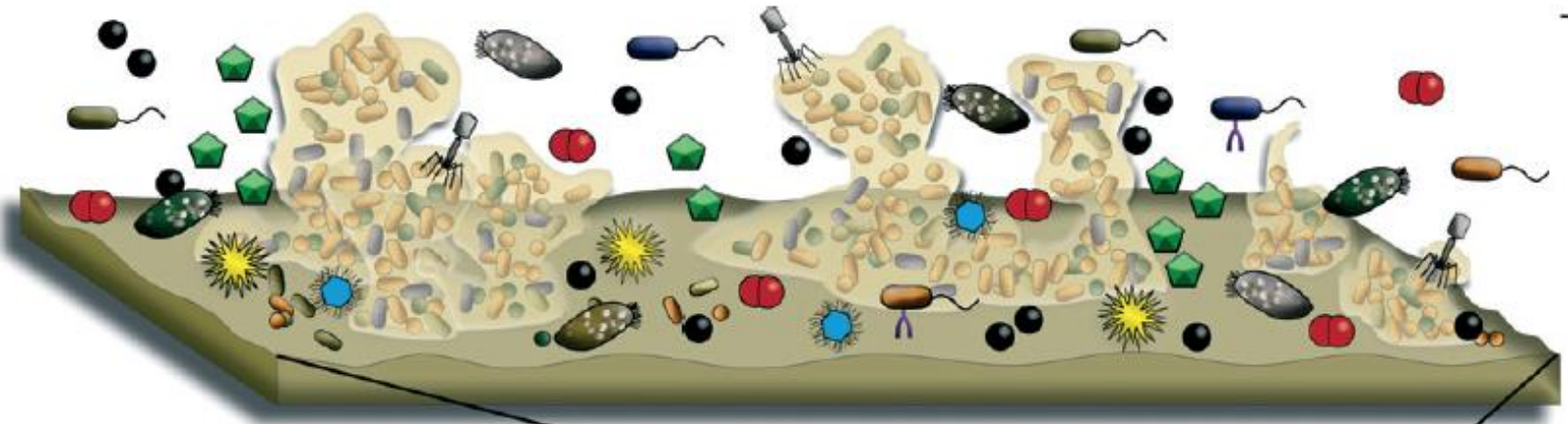


The seaweed microbiome: how it looks like?



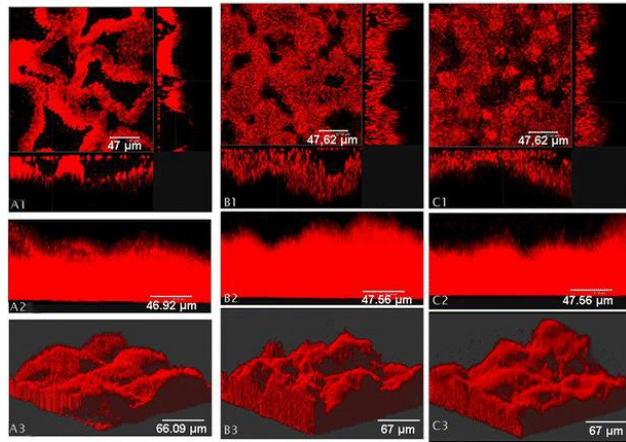
S. Salaün 2010.
L. digitata biofilm
by Scanning
Electron Microscopy

and by Fluorescence Confocal
Microscopy in flow cells.



Bacterial biofilm

Zobellia galactanivorans :



Control

CE

SE

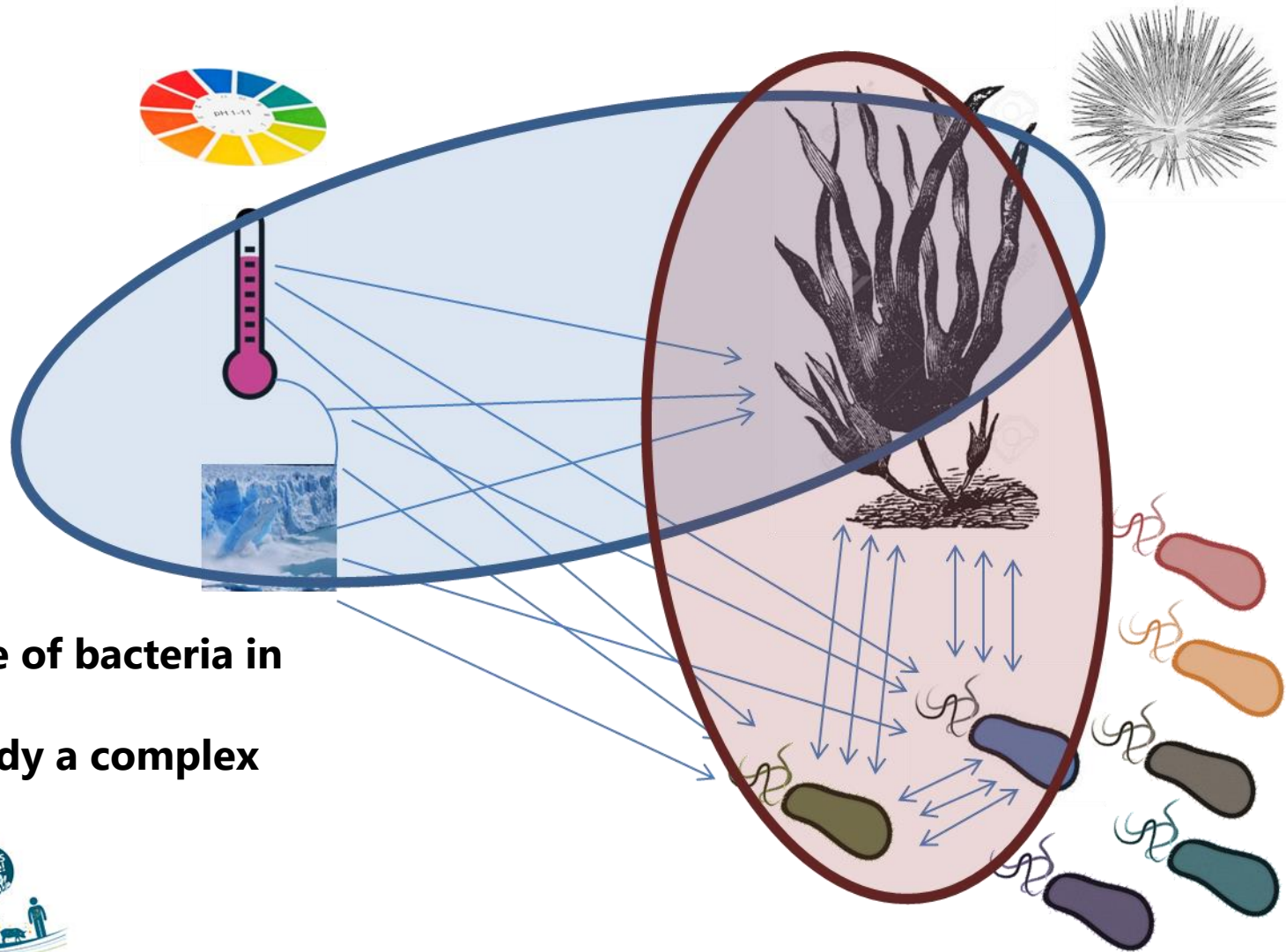


Seaweed

Egan et al. FEMS Microbiology reviews 37(3) ·2012



Seaweed and associated bacteria in a changing environment : a complex system = phycobiome



CHALLENGES:

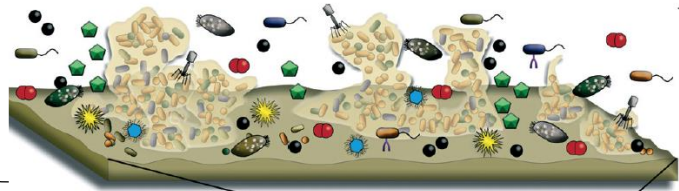
- Proofs of the role of bacteria in adaptation
- Learn how to study a complex system



Unraveling the Functions of the Macroalgal Microbiome?

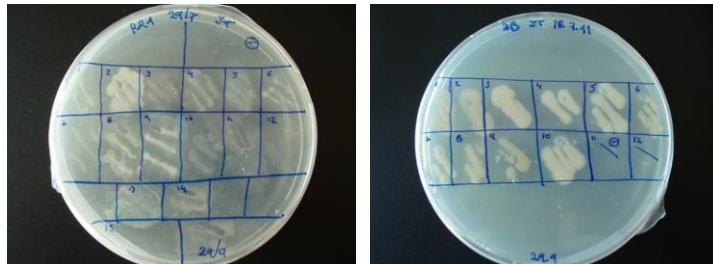
Bacterial biofilm

Uncultivable Surface Microbiota

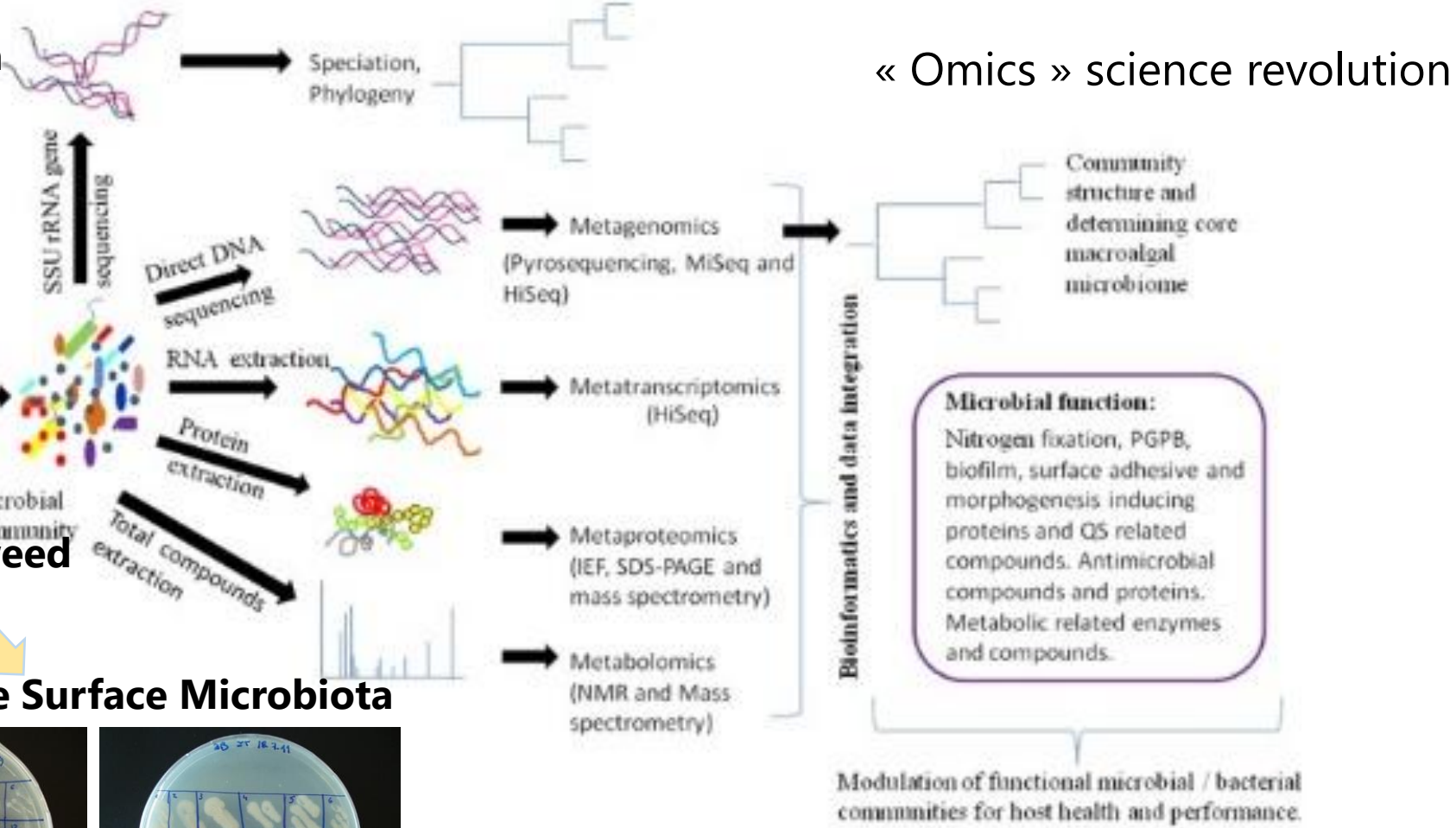


Seaweed

Cultivable Surface Microbiota

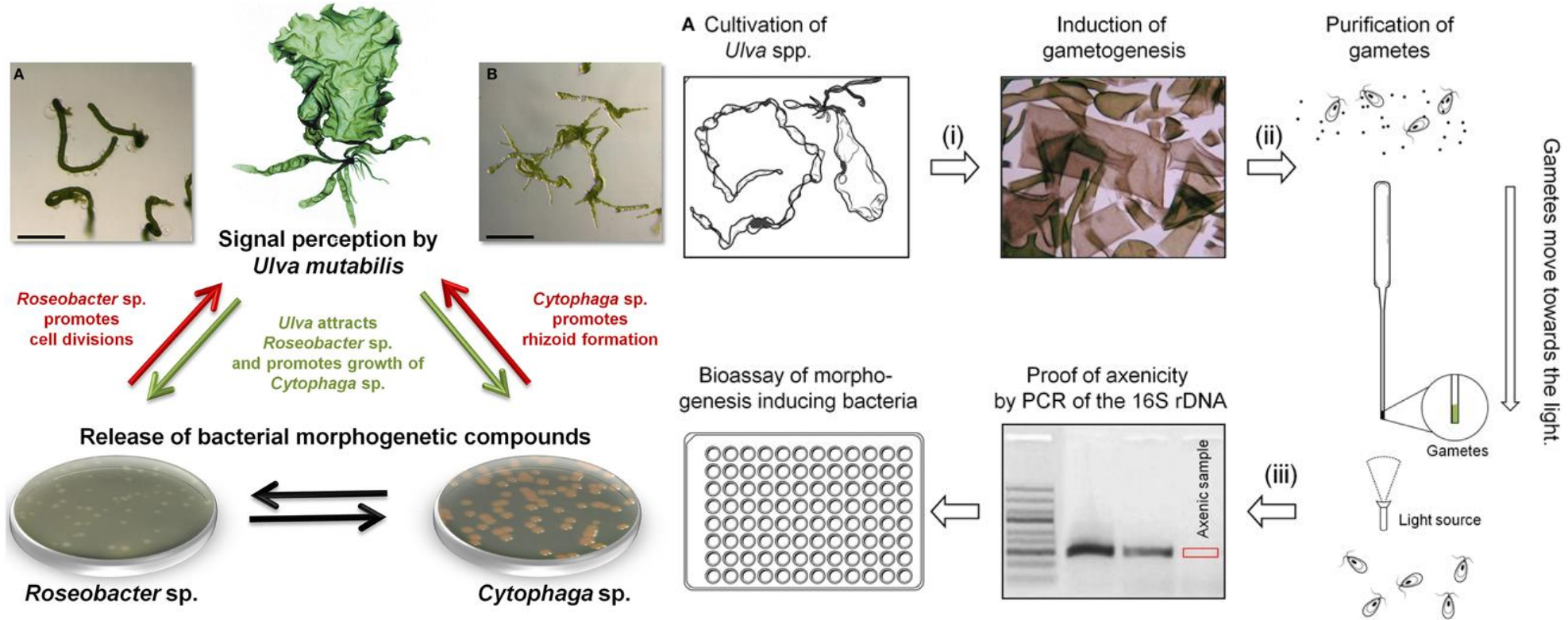


Egan et al. FEMS Microbiology re



Singh & Reddy, 2016
 Front. Microbiol., | <https://doi.org/10.3389/fmicb.2015.01488>

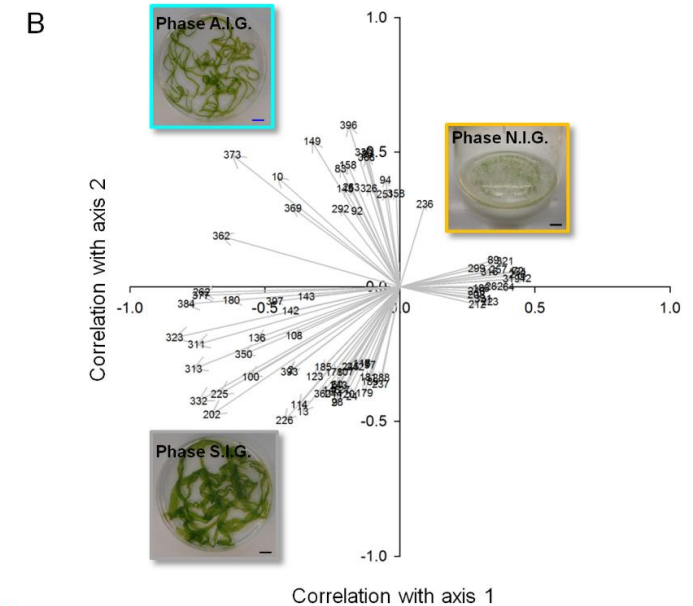
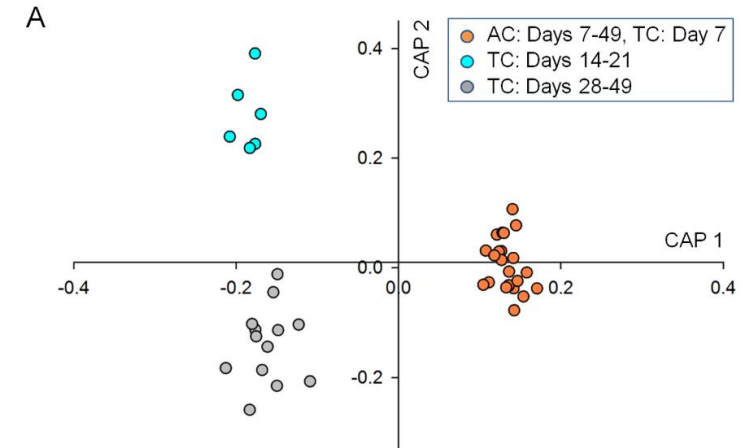
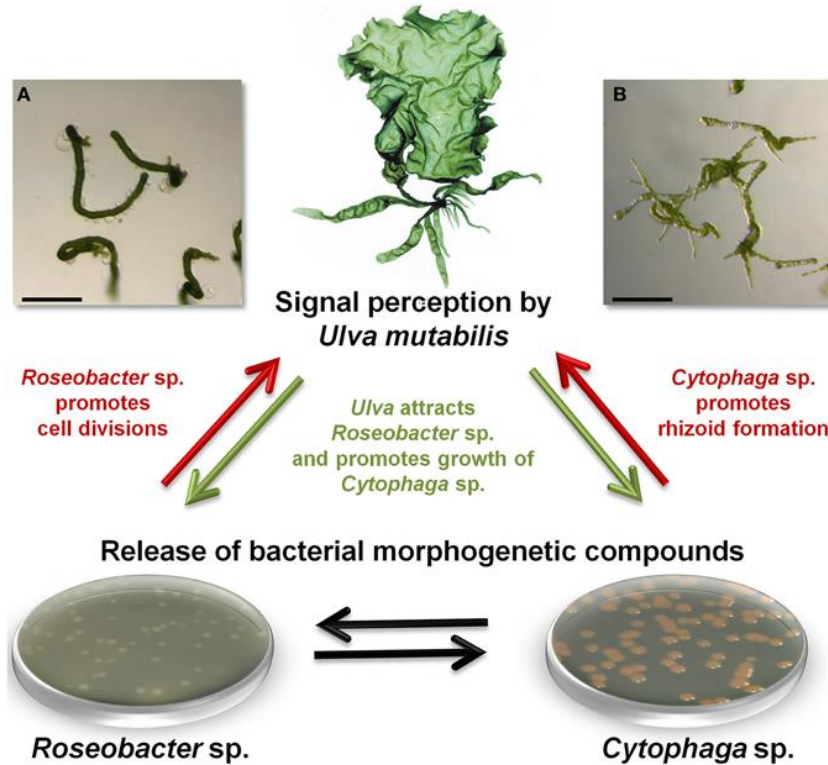
Bacteria-induced growth and morphogenesis in the green macroalga genus *Ulva*



Wichard T
Front. Plant Sci.,
03 March 2015 |



Metabolomics of bacteria-induced growth and morphogenesis in *Ulva mutabilis* (*compressa*)



Taghreed Alsufyani,
Anne Weiss
and Thomas Wichard
Mar Drugs 2017



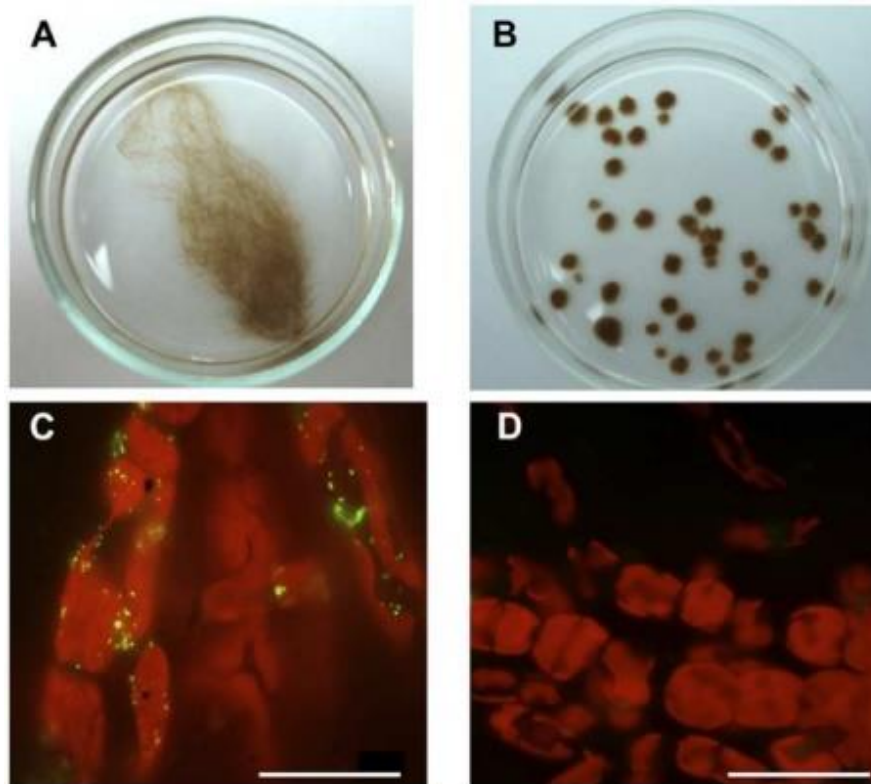


Bacteria-induced growth and morphogenesis in the brown alga *Ectocarpus sp.*

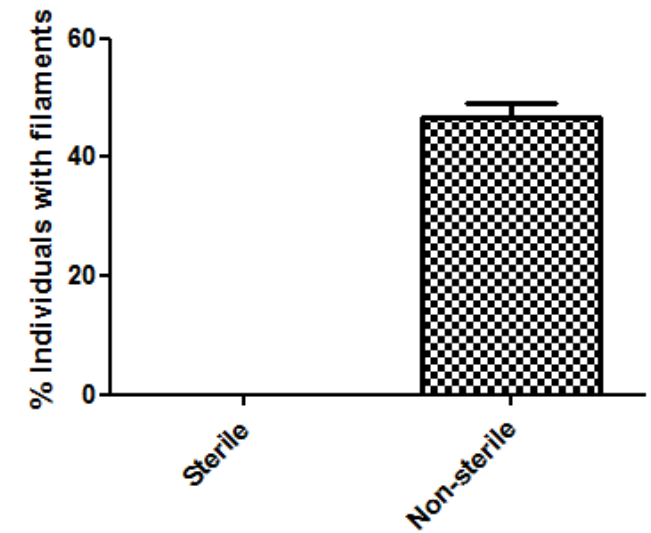
There is a clear **developmental difference** between the axenic and non-axenic *Ectocarpus sp.*

Non-axenic

Axenic



Under sterile conditions there is **not development of upright filament** in *Ectocarpus* axenic strain

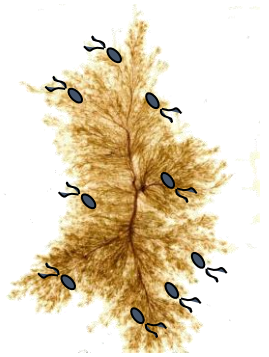


Tapia et al.,
2016
Front.
Microbiol.

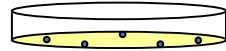


Bacteria-induced growth and morphogenesis in the brown alga *Ectocarpus sp.*

Culture-dependent technique

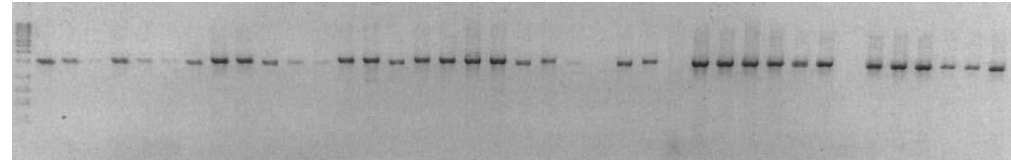


Grinding
Plating



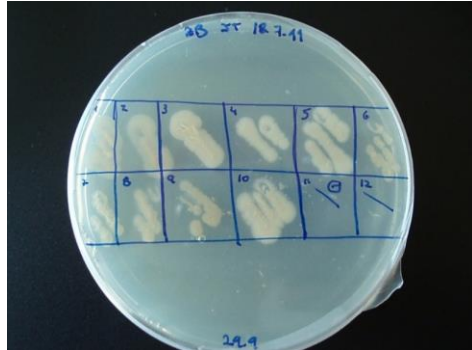
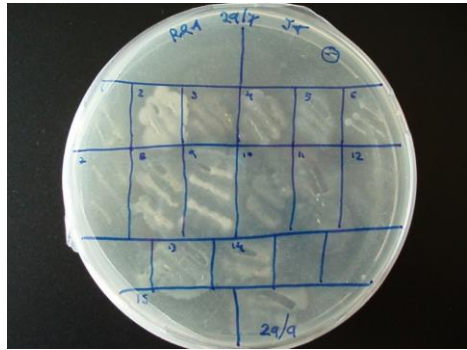
PCR

16S rRNA

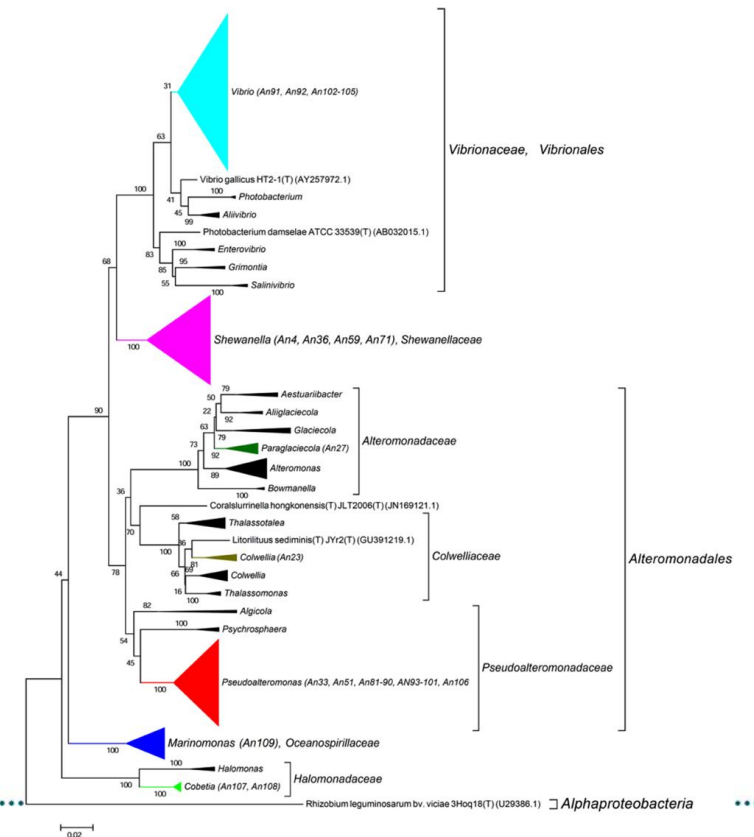


Sequencing

Sequence analysis



Tapia et al., 2016
Front. Microbiol.



Bacteria-induced growth and morphogenesis in the brown alga *Ectocarpus sp.*

Bacterial isolates identify by 16rRNA gene sequencing.

Isolate ID	Closest matching strain in BLAST	Class	% Sequence similarity
Z8_a	<i>Marinobacter sp.</i>	γ-Proteobacteria	100
R13	<i>Sulfitobacter sp.</i>	α-Proteobacteria	100
Es32869_2	<i>Marinobacter sp.</i>	γ-Proteobacteria	99
R15	<i>Sulfitobacter sp.</i>	α-Proteobacteria	99
Z3	<i>Halomonas sp.</i>	γ-Proteobacteria	99
Es32869_1	<i>Methylophaga sp.</i>	γ-Proteobacteria	99
R11_a	<i>Antarctobacter heliothermus</i>	α-Proteobacteria	99
R4_b	<i>Alteromonas sp.</i>	γ-Proteobacteria	99
Z1	<i>Alteromonas genovensis</i>	γ-Proteobacteria	99
Z9_b	<i>Bacterium DG940</i>	γ-Proteobacteria	98
R2_b	<i>Halomonas sp.</i>	γ-Proteobacteria	98
R6_a	<i>Bermanella marisubri</i>	γ-Proteobacteria	97
Es534869_2	<i>Halomonas sp.</i>	γ-Proteobacteria	96
R10_a	<i>Roseobacter sp.</i>	α-Proteobacteria	96
Z8_b	<i>Marinobacter sp.</i>	γ-Proteobacteria	90

Tapia et al., 2016
Front. Microbiol.



Bacteria-induced growth, morphogenesis and reproduction in the brown alga *Ectocarpus* sp.

Non-axenic



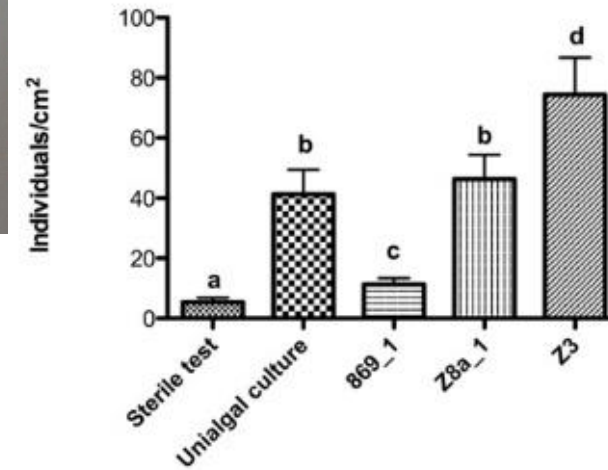
Axenic



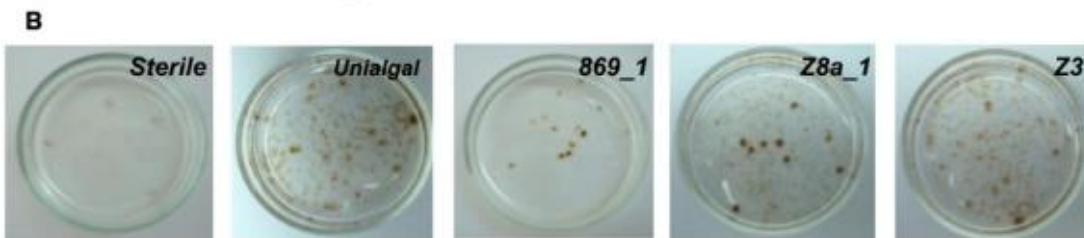
Z8a



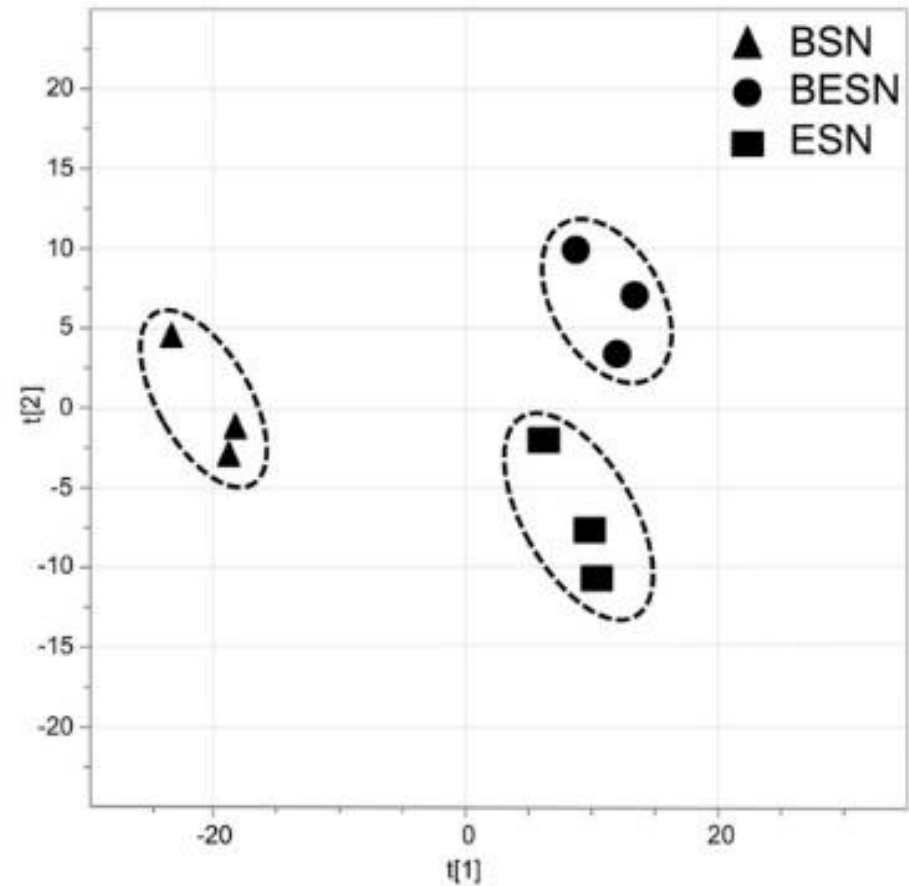
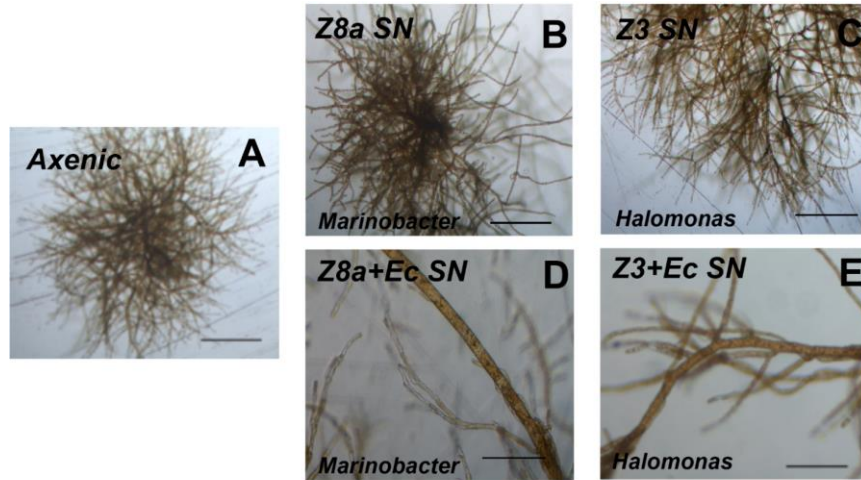
Bacterial effect on sporulation



Tapia et al., 2016
Front. Microbiol.



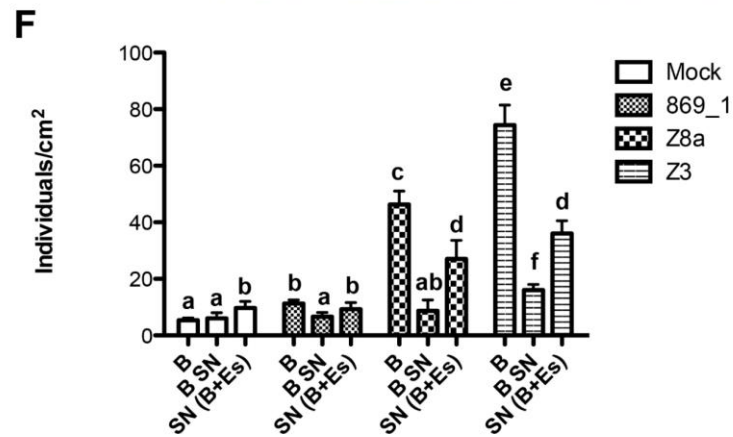
Waterborne cues in bacteria-induced growth and morphogenesis in the brown alga *Ectocarpus sp.*



R2X[1] = 0.746

R2X[2] = 0.142

Ellipse: Hotelling's T² (95%)



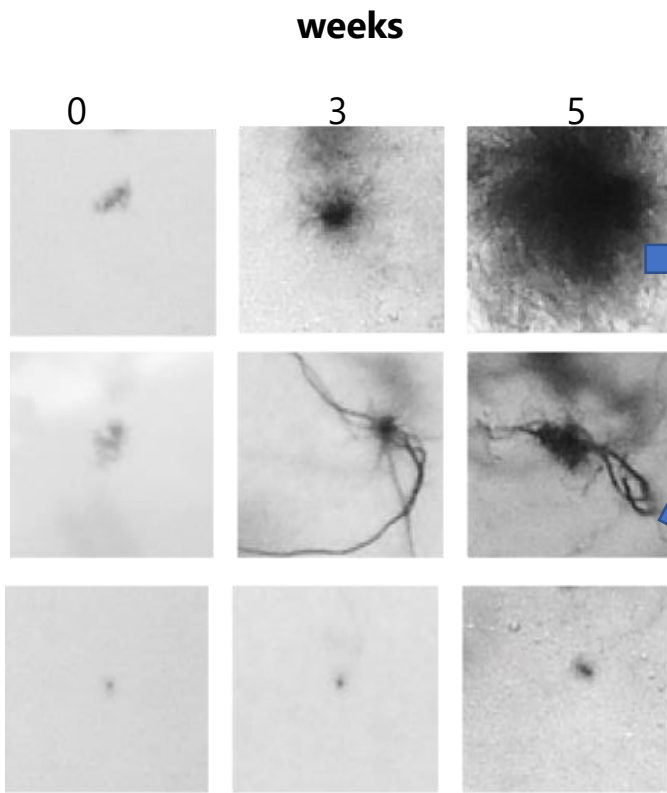
Tapia et al., 2016
Front. Microbiol.



Host–microbe interactions as a driver of acclimation to salinity gradients in *Ectocarpus subulatus*

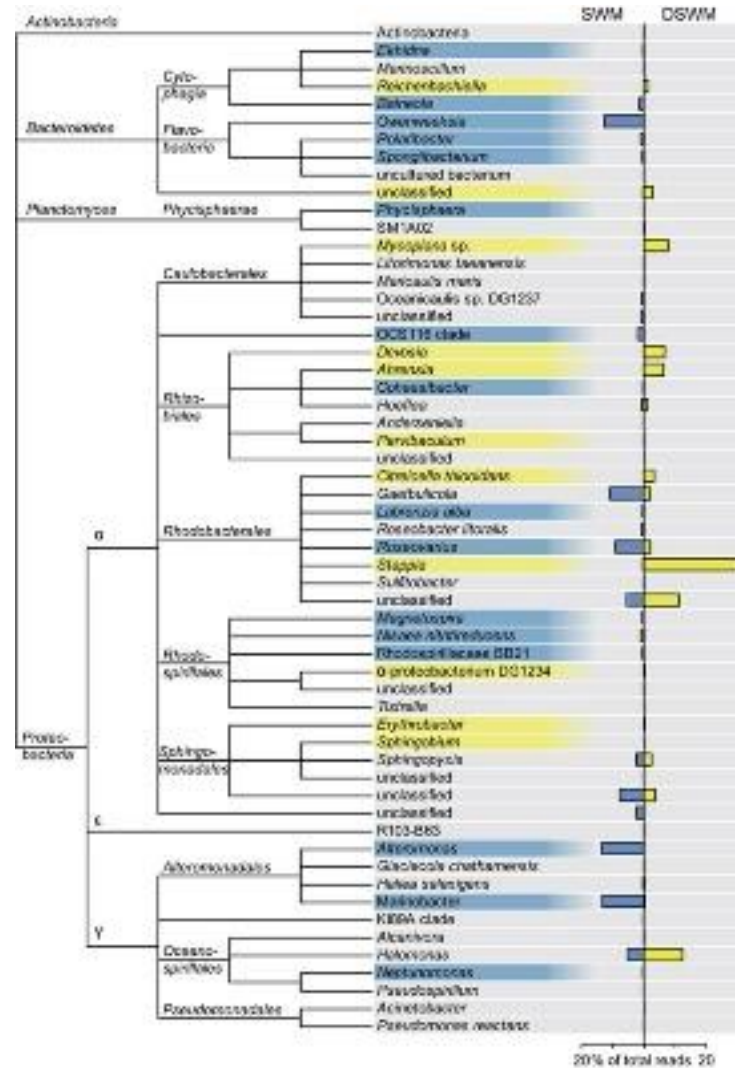
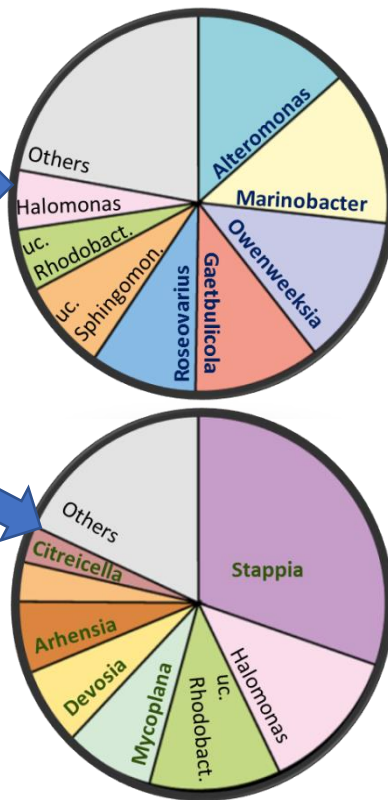


Seaweed in seawater
 Seaweed in freshwater
 Algue traitée aux antibiotiques en eau douce



Controls that exclude direct effect of antibiotics

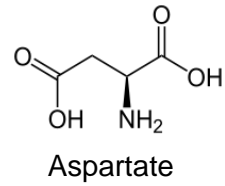
86 bacterial taxa



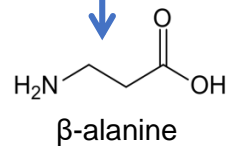
Dittami et al. ISME J. 2015

An example of complementarity: Biosynthesis of vitamin B5

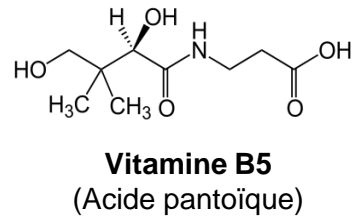
β-alanine biosynthesis III



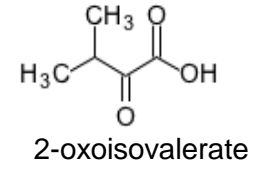
Phect502
EC 4.1.1.11



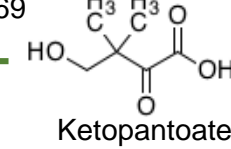
Esi0070_0043
Phect3248
EC 6.3.2.1



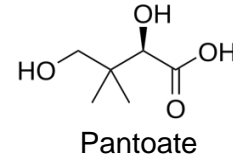
Phosphopantothenate biosynthesis I



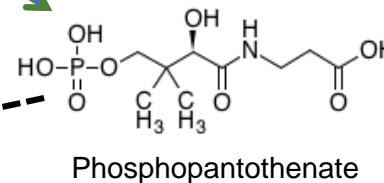
Phect2640
Esi0112_0042
EC 2.1.2.11



Esi0133_0041
EC 1.1.1.169



Esi0031_0150
Phect2770
EC 2.7.1.33



Synthèse de Coenzyme A

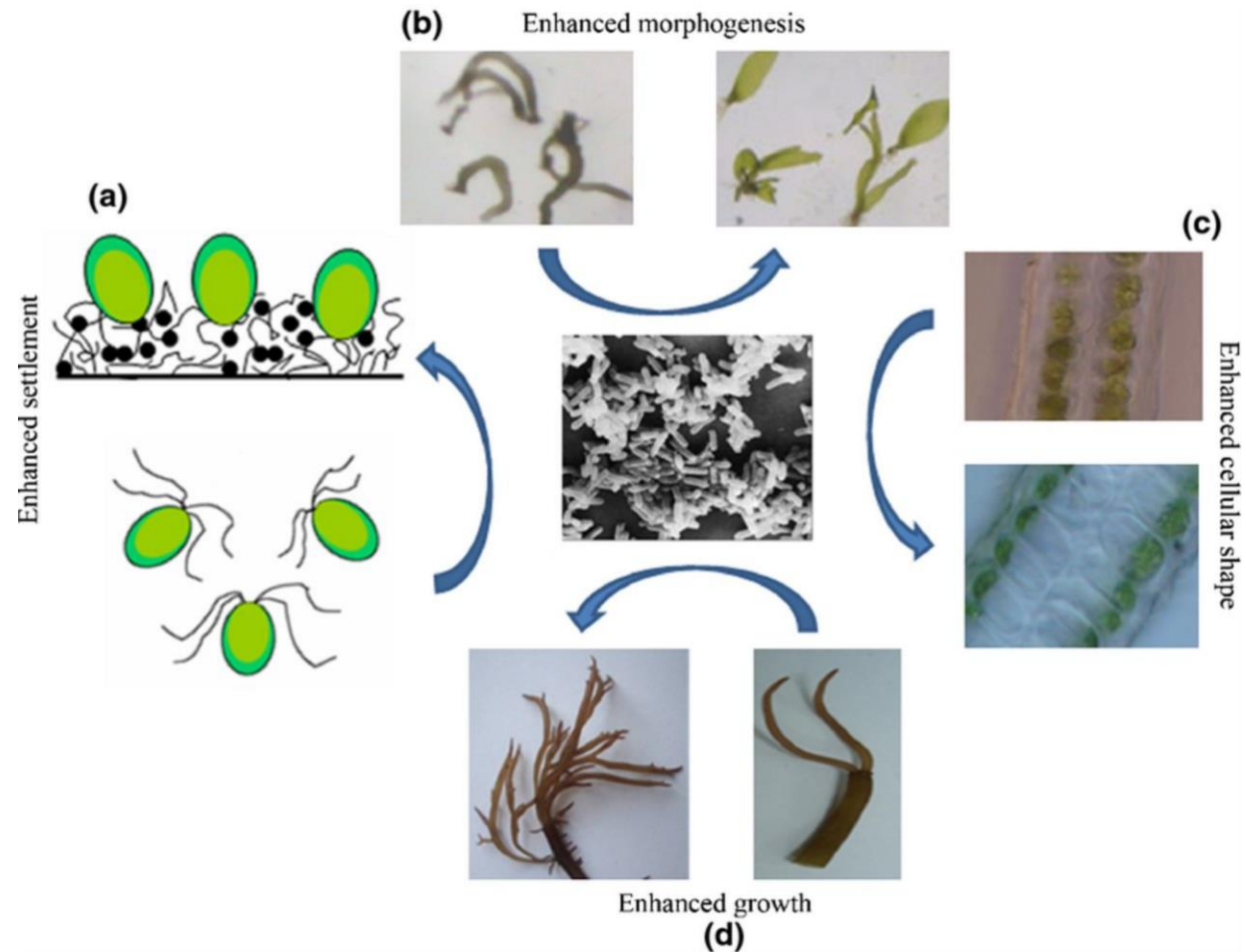
Ectocarpus



*Pyruvatibacter
ectocarpi*

Dittami et al.

Key functions of the seaweed microbiome



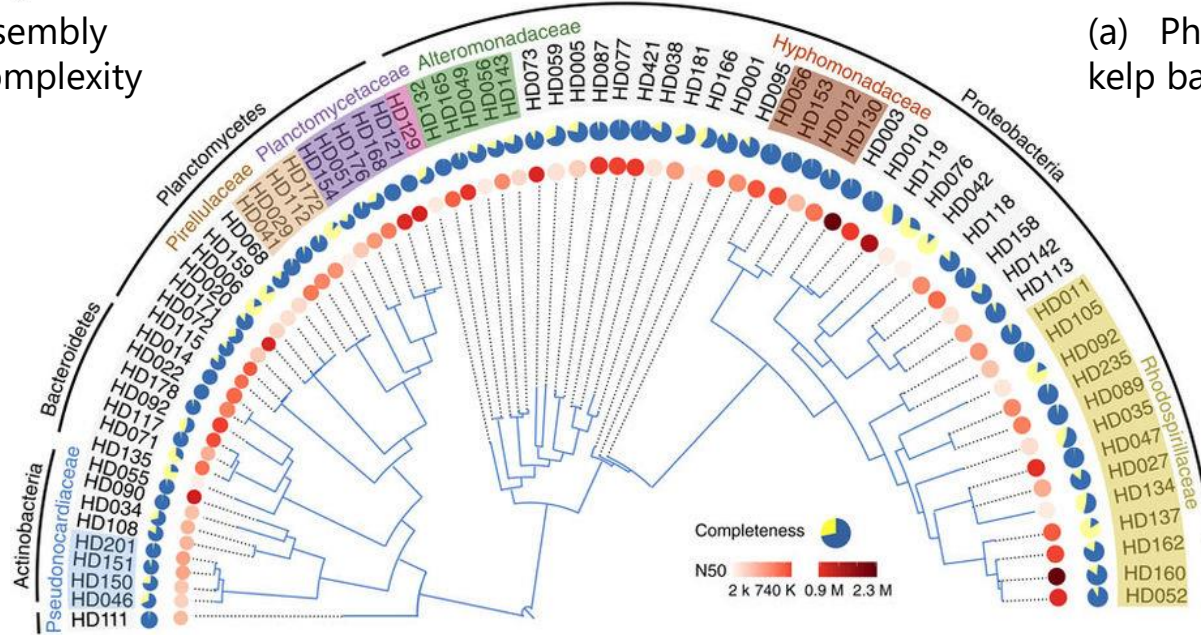
From: Seaweed–microbial interactions: key functions of seaweed-associated bacteria
FEMS Microbiol Ecol. 2014;88(2):213-230. doi:10.1111/1574-6941.12297
FEMS Microbiol Ecol | © 2014 Federation of European Microbiological Societies



The kelp microbiome: a first metagenomics approach

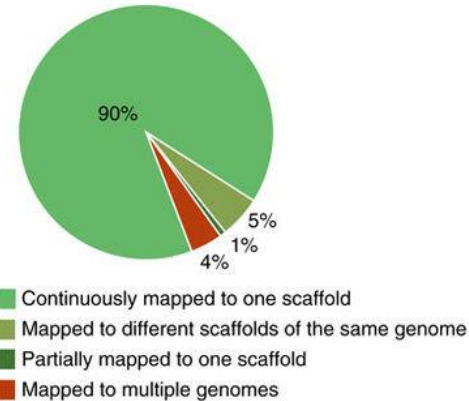
a

MetaSort untangles metagenome assembly by reducing microbial community complexity

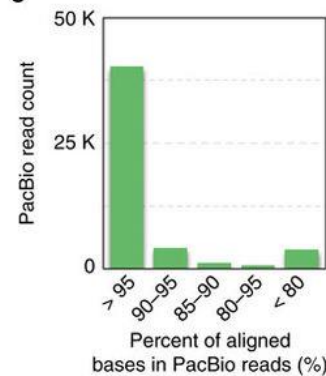


(a) Phylogenetic tree of 75 assembled kelp bacterial genomes belonging to five phyla.

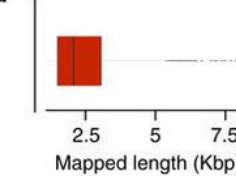
b



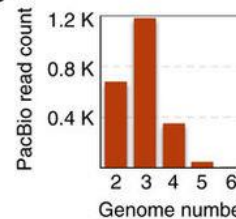
c



d



e



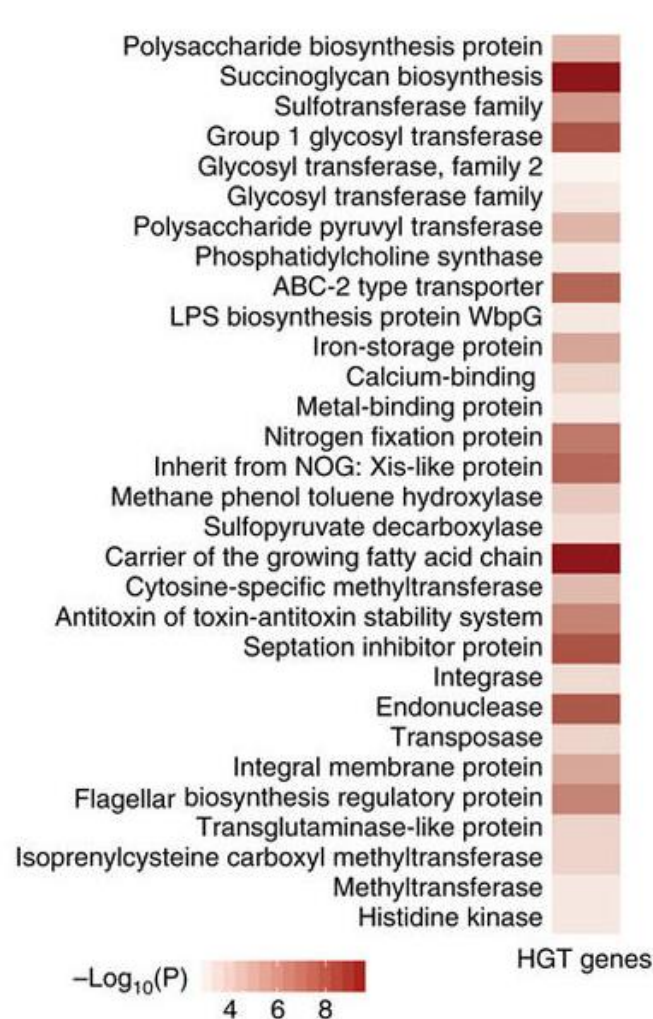
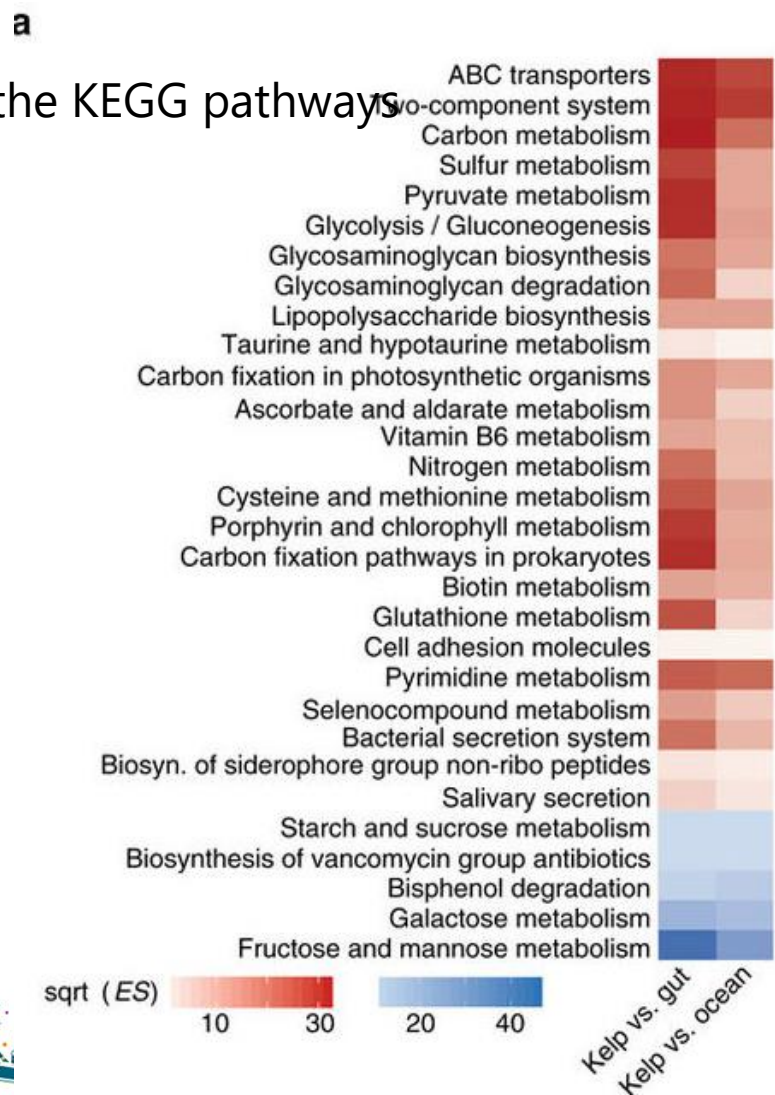
Peifeng Ji, Yanming Zhang,
Jinfeng Wang & Fangqing Zhao

Nature Communications **8**:
14306 (2017)
•doi:10.1038/ncomms14306



The kelp microbiome is very distinct than the surrounding ocean and human gut microbiomes

Comparison of the KEGG pathways



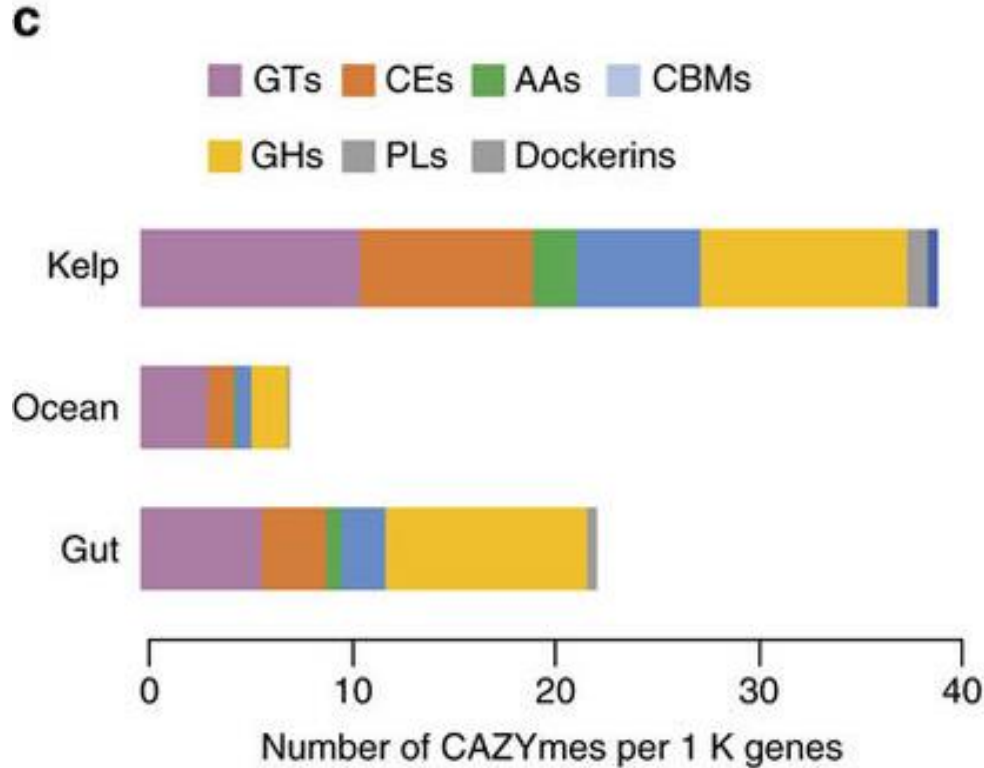
(b) The enriched EGGNOG functions of horizontally transferred genes.

Ji et al. (2017)
Nature Communications **8**,
 14306

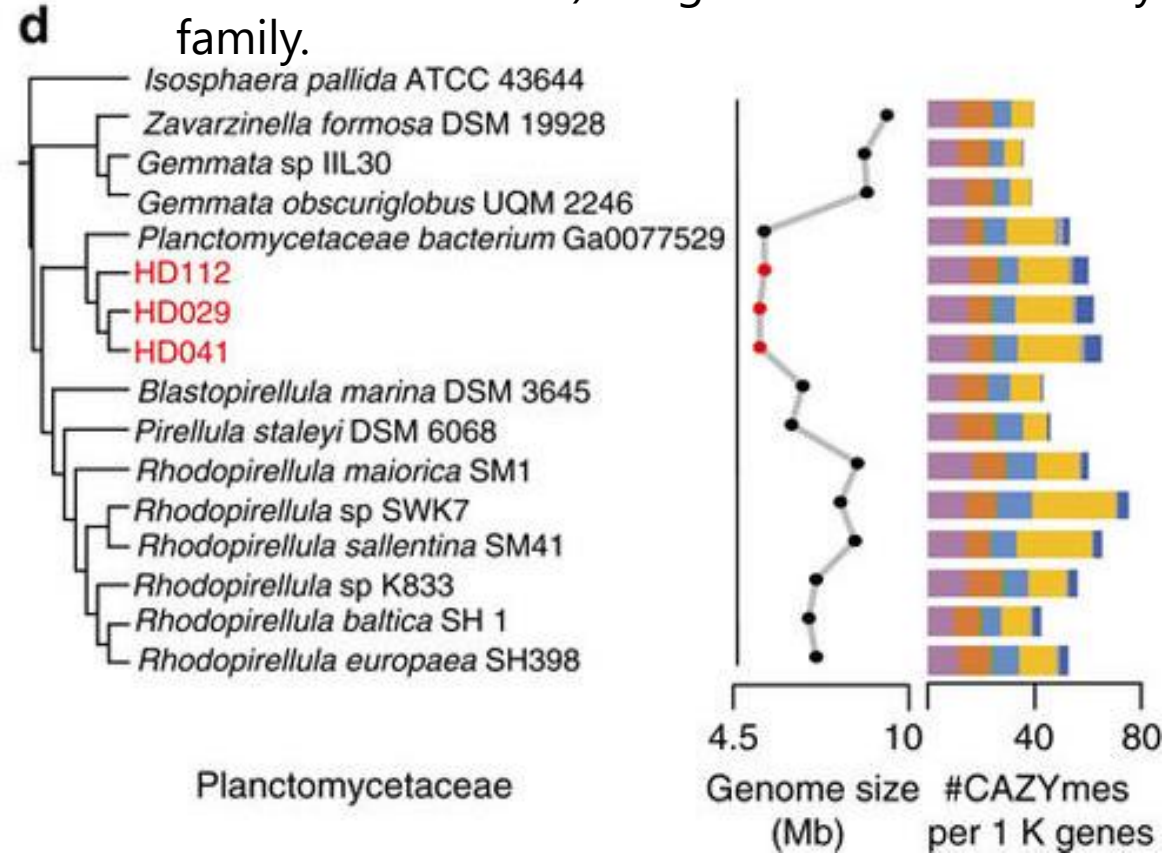


The kelp microbiome is very distinct than the surrounding ocean and human gut microbiomes

(c) Comparison of the number of CAZyme genes among the three microbiomes.



(d) Features of the three kelp bacterial genomes (HD112, HD029 and HD041) assigned to the Planctomycetaceae family.

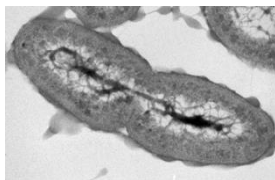
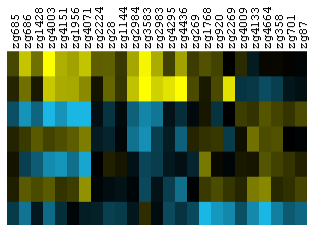


Ji et al. (2017)
Nature Communications **8**,
 14306

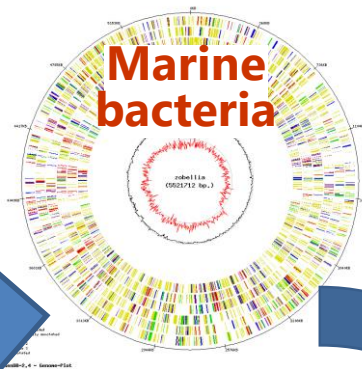
Genome size is shown in curved line, and the number of CAZyme genes in each genome is shown in bar plot.



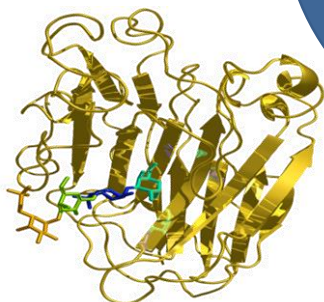
Our current strategy for the discovery of enzymes that degrade seaweed polysaccharides



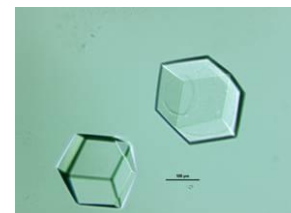
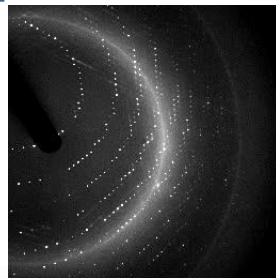
functional characterization



From genome to enzymatic tools



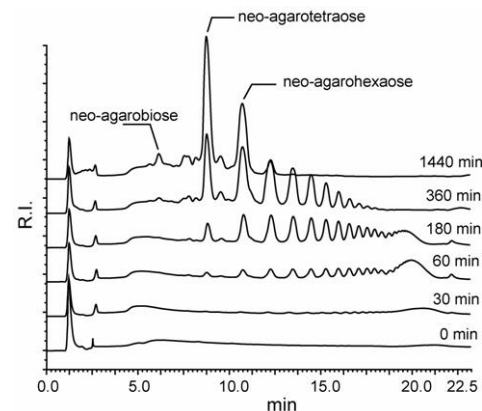
3D structure



crystallization

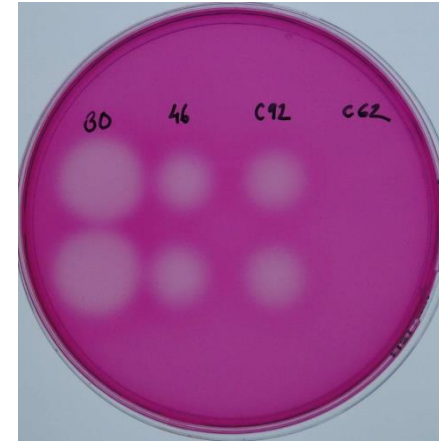
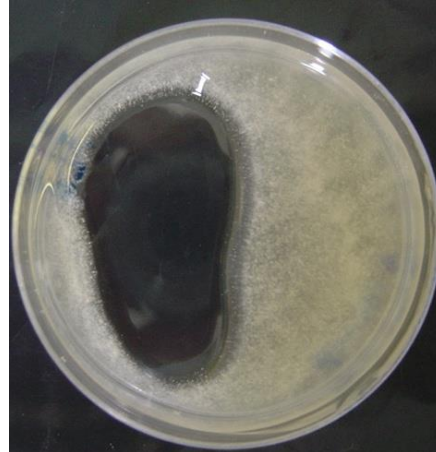
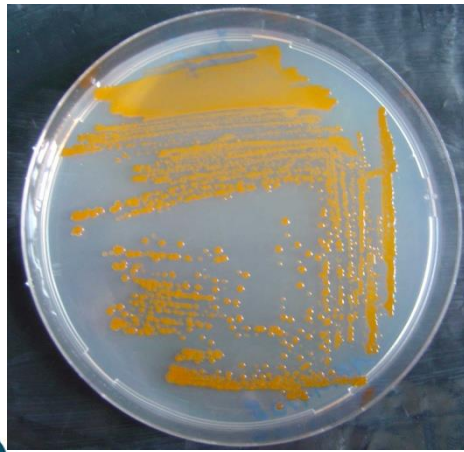
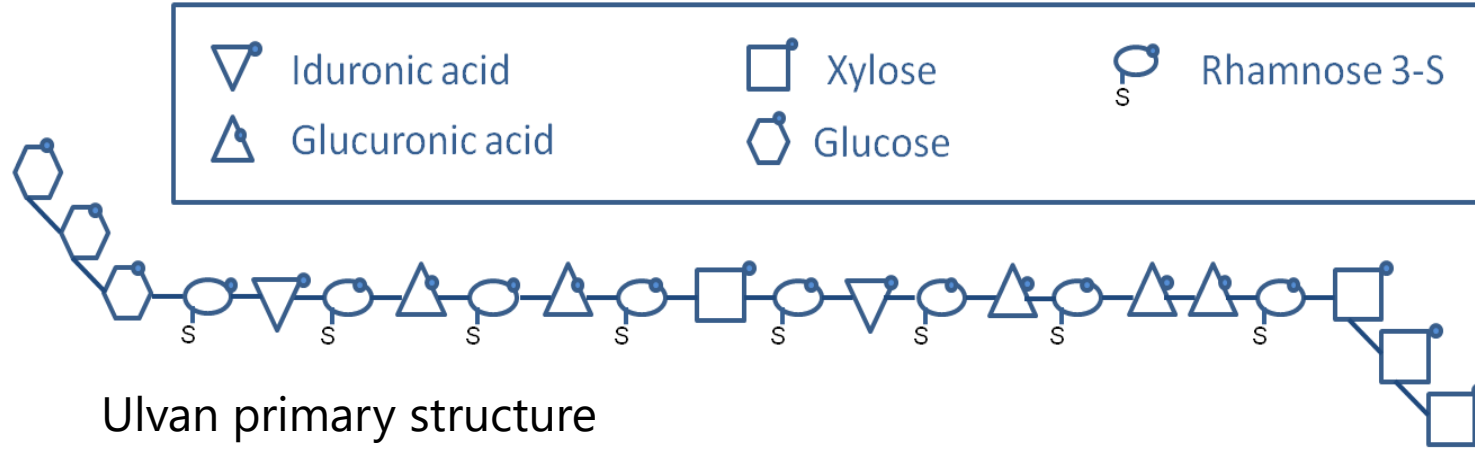


medium throughput expression



biochemical characterization

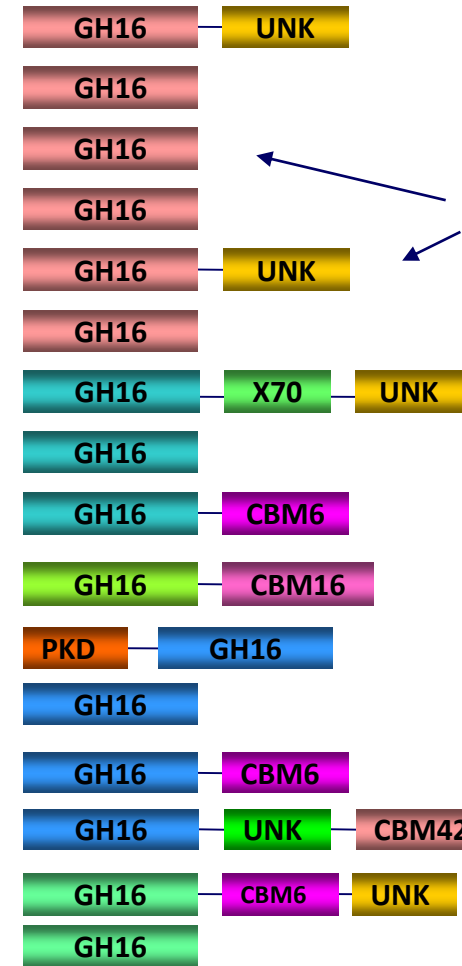
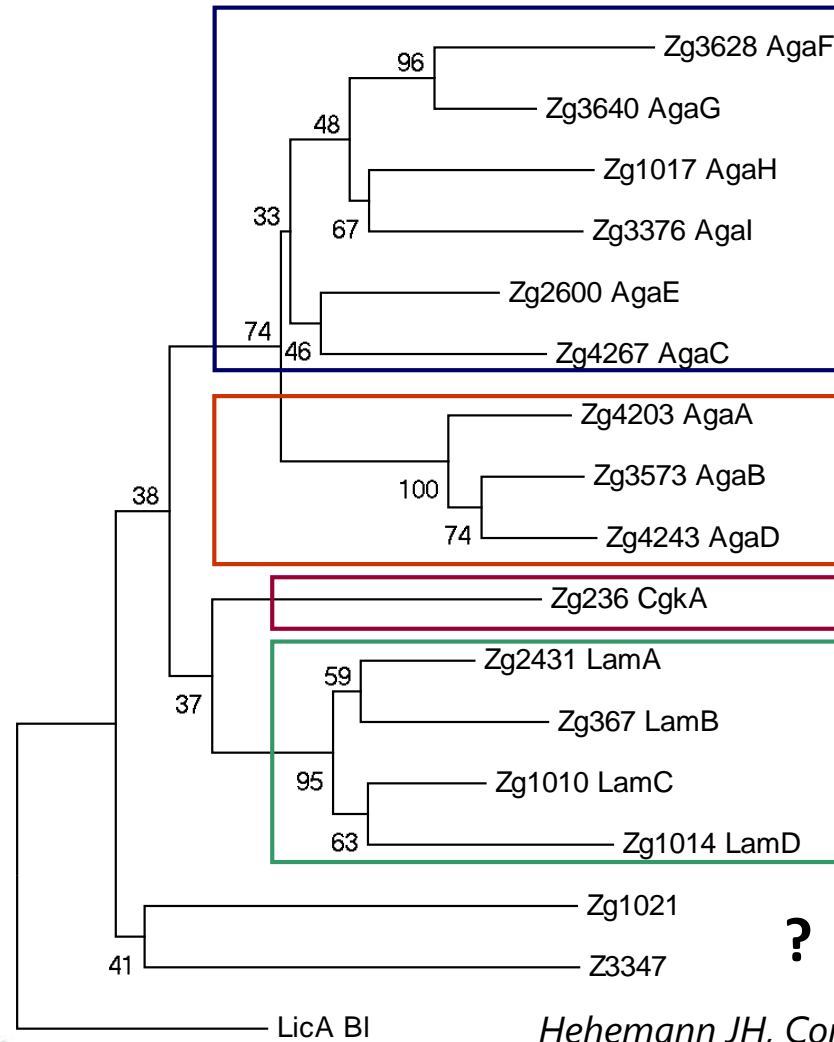
Expression and characterisation of a ulvan lyase from the marine bacterium *Persicivirga ulvanivorans*



P. ulvanivorans colonies on agar plate; Degradation of a 4% ulvan plate; Activity of recombinant ulvan lyase.

Pi Nyvall Collén et al., *J. Biol. Chem.*, 2011

Phylogenetic analysis of Zobellia enzymes from family GH16



heterologous over expression of Zg1017 & Zg2600

β -agarases

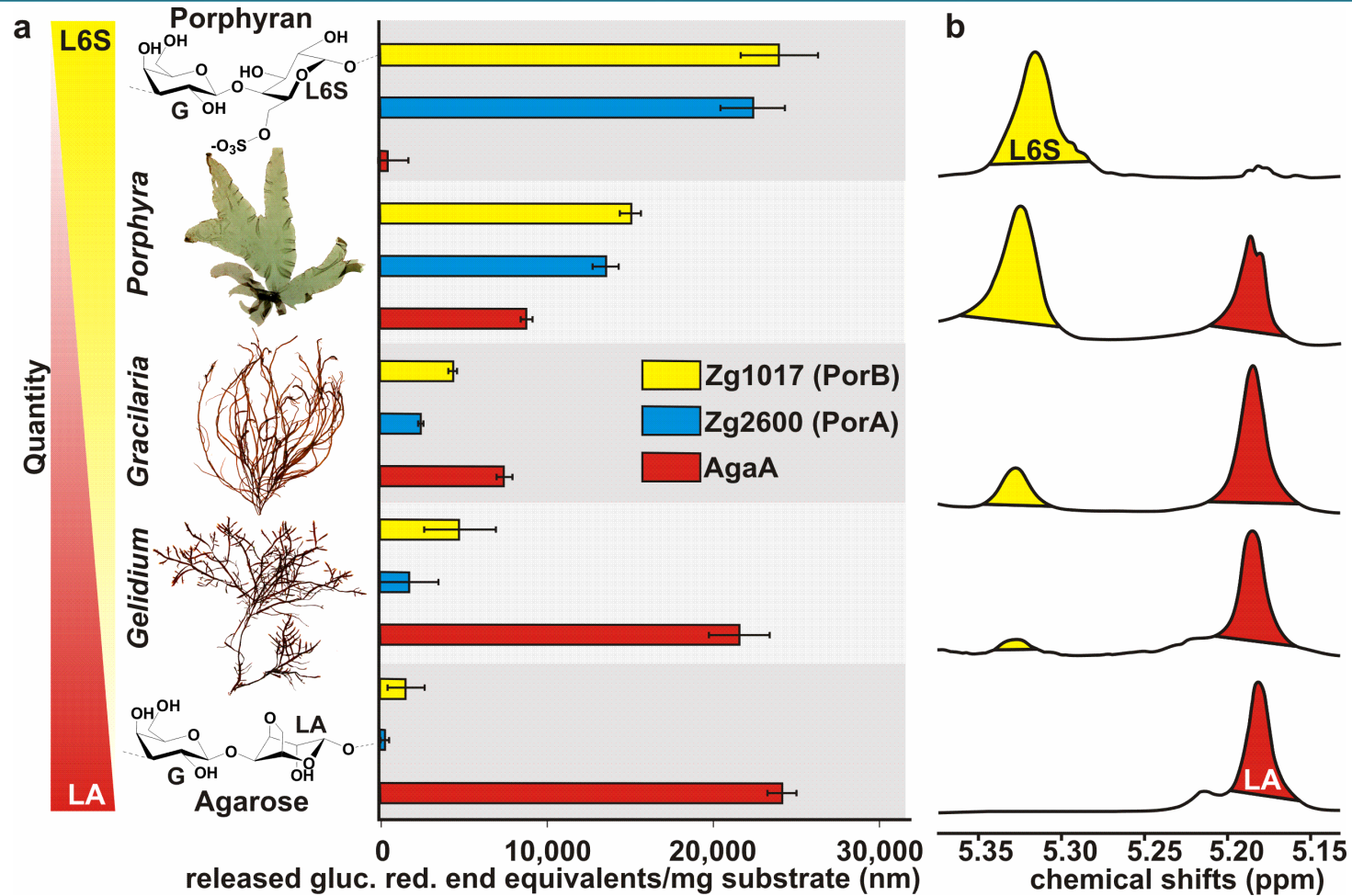
κ -carrageenases

laminarinases

Hehemann JH, Correc G, Barbeyron T, Helbert W, Czjzek M, Michel G. Nature. 2010 464:908-12.



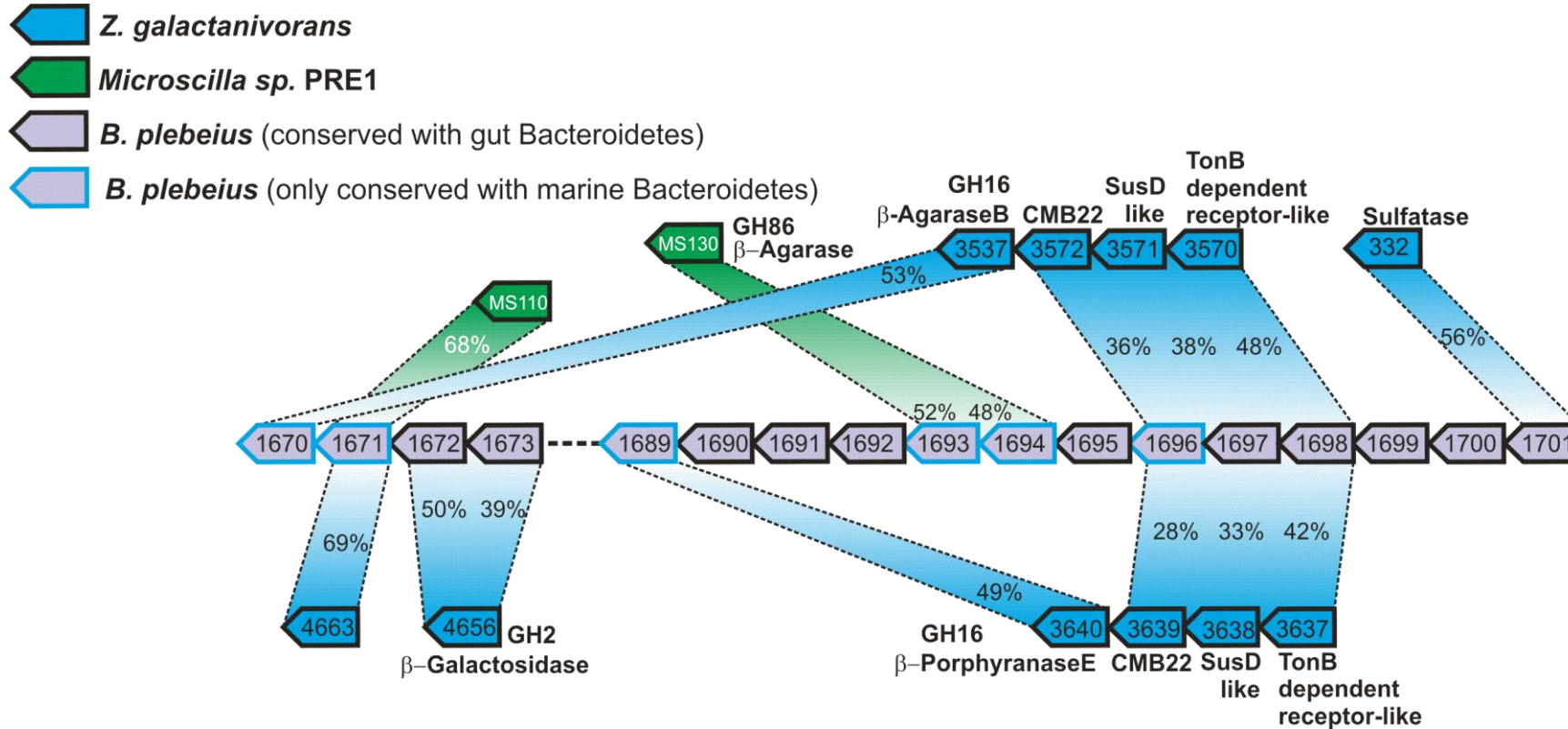
Activity screening on algal cell wall extracts



Hehemann JH,
Correc G,
Barbeyron T,
Helbert W,
Czjzek M,
Michel G.
Nature. 2010
464:908-12.

- correlation with increasing L-galactose-6-sulfate units
- maximum activity on red algae *Porphyra* sp.

The intestinal bacteria of Japanese people received a complete porphyranolytic system from a marine Bacteroidetes



6 of the genes in *B. plebeius* are conserved with marine bacteria only

Hehemann JH, Correc G, Barbeyron T, Helbert W, Czjzek M, Michel G. Nature. 2010 464:908-12.

A genetic gift for sushi eaters

Gut-metagenome analysis shows that porphyranases and agarases:

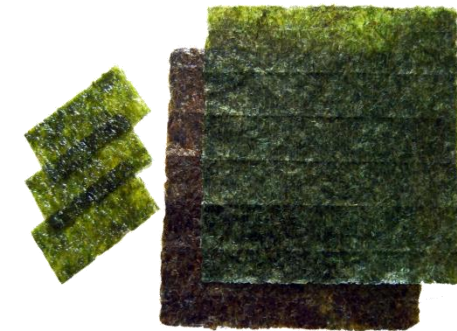
- are **frequent** in **Japanese** gut microbiota
- are **absent** in **American** gut microbiota

Dietary seaweed is the most probable **vector** for contact with marine microbes that led to the HGT

Porphyra/Pyropia



Nori



Sushi



Transfer of carbohydrate-active enzymes from marine bacteria to Japanese gut microbiota. *Hehemann JH, Correc G, Barbeyron T, Helbert W, Czjzek M, Michel G. Nature. 2010 464:908-12.*

The intestinal bacteria of Japanese and Japanese-Hawaiian people received a complete alginolytic system from the marine *Bacteroidetes Zobellia*

Characterization of the first alginolytic operons in a marine bacterium: from their emergence in marine *Flavobacteria* to their independent transfers to marine *Proteobacteria* and human gut *Bacteroides*

Thomas et al 2012 *Env Microbiol*



BACTEROIDETES

Flavobacteriia (13)
12 genera, 13 species

Cytophagia (1)
1 genera, 1 species

Sphingobacteriia (1)
1 genera, 1 species

Bacteroidia (5)
1 genus, 5 species

PROTEOBACTERIA

Alphaproteobacteria (3)
3 genera, 3 species

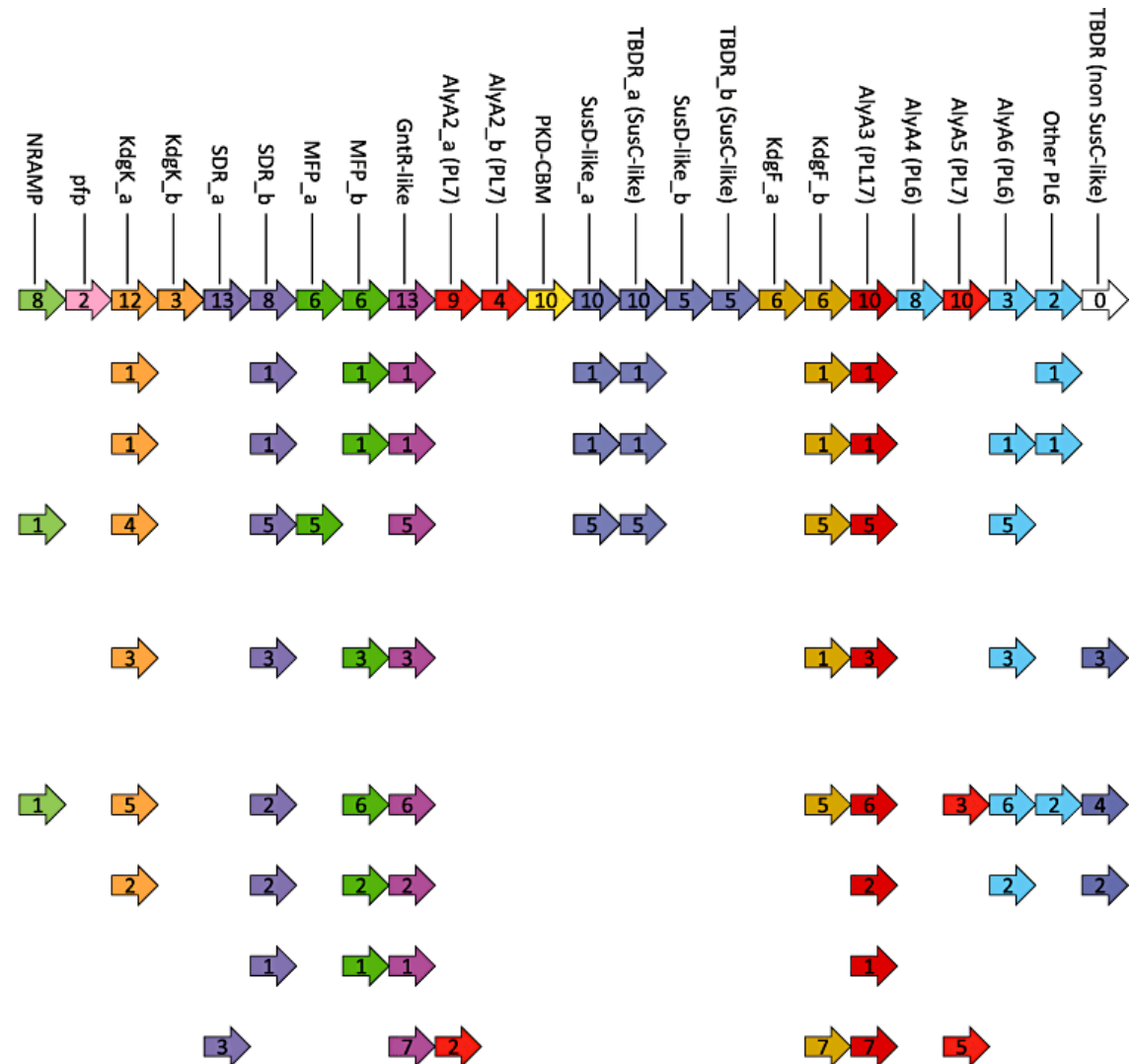
Gammaproteobacteria

Alteromonadales (6)
5 genera, 6 species

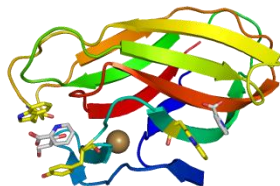
Xanthomonadales (2)
1 genus, 1 species, 2 strains

Enterobacteriales (1)
1 genus, 1 species

Vibrionales (7)
1 genus, 6 species, 7 strains



From the seaweed microbiome toward biotechnology and biorefineries



- Enzyme/protein engineering

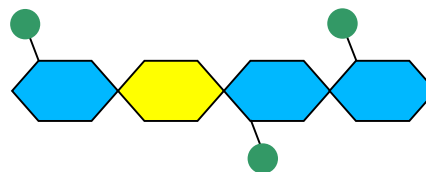
Probes, markers

optimisation/thermostability

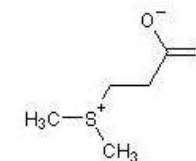
" glycosynthases "



- Enzyme-assisted hemisynthesis

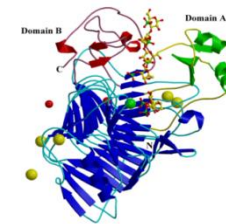


- Controlled production of chemicals and bioactive molecules



Dimethylsulfoniopropionate (DMSP)

- Bioprocessing of the biomass using enzymes



Algolife

ISI bpi**france**

 GENIALG



2017

**Thank you
for your attention!**



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