



**EUROPEAN COOPERATION
IN SCIENCE AND TECHNOLOGY**

Dr. Thomas Wichard

**Friedrich Schiller University Jena
Institute for Inorganic and Analytical Chemistry**

WP3: Final report - Towards macroalgal adult growth



PHYCOMORPH

**Advancing Knowledge On Seaweed
Growth And Development**

In this version of the report several images of the original presentation were replaced with the abstract of the cited publication.

Deliverable of task 3: “Towards adult growth”

DL3-1

Characterize whether cell-cell communication occurs within the embryo (apoplastic or symplastic communication). This will allow practice of excision of seaweed parts, which might be necessary in aquaculture processes.

DL3-2

Assess the impact of external and internal forces (mainly mechanical) on embryo fate. Most seaweed embryos develop directly in the sea, exposed to strong mechanical forces (such as sea current, waves and tides). In addition, growth and cell multiplication generate compression and shearing forces, which cells need to account for and respond to.

DL3-3

Identify bacterial chemicals used as external seaweed morphogens. This will lead to possible control of seaweed developmental steps by external application of morphogens present in natural compounds (in hatchery).

DL3-4

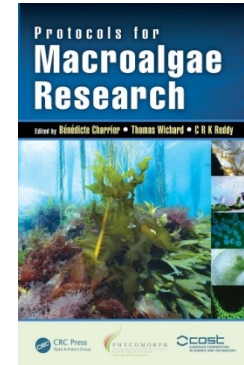
Characterize seaweed tissues or cellular structures enabling long-range transport through the seaweed body (differentiation of sieve elements and/or plasmodesmata, transport velocity). The intention would be to eventually manipulate the size of seaweed whole-body or specific organ to optimize yield should account for these knowledge.

DL3-5

Determine the existence of processes such as apical dominance, leading to the possibility of modifying overall seaweed morphology by promoting branching, thereby increasing seaweed biomass or production of reproductive organs, similarly to crop plants on land (e.g. “Green Revolution” breeds of wheat and rice).

COST Actions to achieve the goals

1. STSMs (# 25)
2. Workshops and training schools (#4)
3. Standardization of protocols
4. Shared projects and publications
5. Joint research proposals



TS 1: (2016) Macroalgal Cultivation joint with a **Training School** in Kavala, Greece

TS 2: (2016) Bioinformatics Workshop – Black Forest, Germany

TS 3: (2017) Crossing the kingdoms: Macroalgae – Bacteria interactions, a **Training School** in Analytical Chemistry, Jena, Germany

TS 4: (2018) State-of-the-art techniques for imaging cell and tissues of macroalgae, Roscoff, France

Deliverable of task 3: “Towards adult growth”

DL3-1

Characterize whether cell-cell communication occurs within the embryo (apoplastic or symplastic communication). This will allow practice of excision of seaweed parts, which might be necessary in aquaculture processes.

DL3-2

Assess the impact of external and internal forces (mainly mechanical) on embryo fate. Most seaweed embryos develop directly in the sea, exposed to strong mechanical forces (such as sea current, waves and tides). In addition, growth and cell multiplication generate compression and shearing forces, which cells need to account for and respond to.

DL3-3

Identify bacterial chemicals used as external seaweed morphogens. This will lead to possible control of seaweed developmental steps by external application of morphogens present in natural compounds (in hatchery).

DL3-4

Characterize seaweed tissues or cellular structures enabling long-range transport through the seaweed body (differentiation of sieve elements and/or plasmodesmata, transport velocity). The intention would be to eventually manipulate the size of seaweed whole-body or specific organ to optimize yield should account for these knowledge.

DL3-5

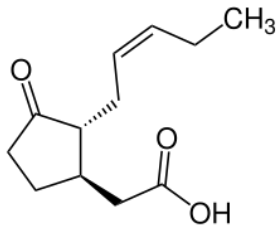
Determine the existence of processes such as apical dominance, leading to the possibility of modifying overall seaweed morphology by promoting branching, thereby increasing seaweed biomass or production of reproductive organs, similarly to crop plants on land (e.g. “Green Revolution” breeds of wheat and rice).

Example: "Towards adult growth"

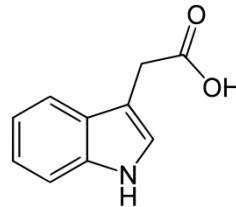
DL3-3

Identify **bacterial chemicals** used as external seaweed morphogens. This will lead to possible control of seaweed developmental steps by external application of morphogens present in natural compounds (in hatchery).

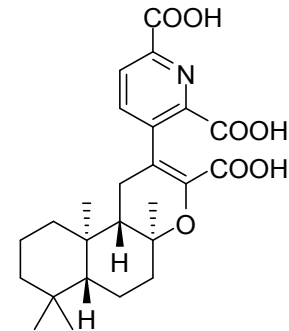
Three examples:



Jasmonic acid

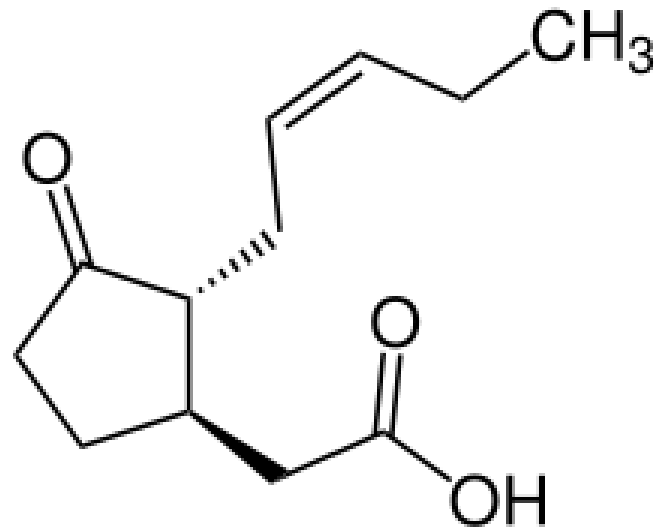


Auxin



Thallusin

Plant hormones: Jasmonic acid



Jasmonic acid

Plant hormones: Jasmonic acid

Molecular mechanisms underlying *Grateloupia imbricata* (Rhodophyta) carposporogenesis induced by methyl jasmonate

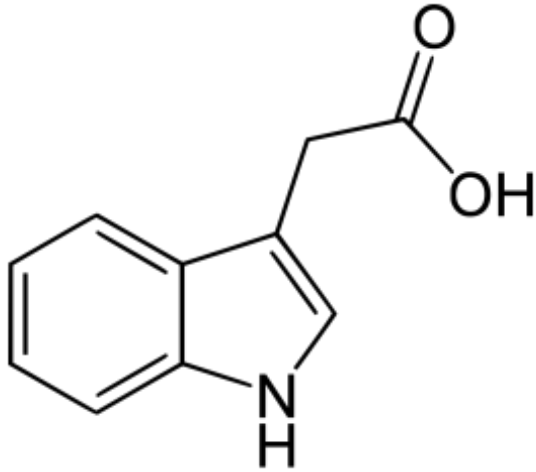
Grateloupia imbricata



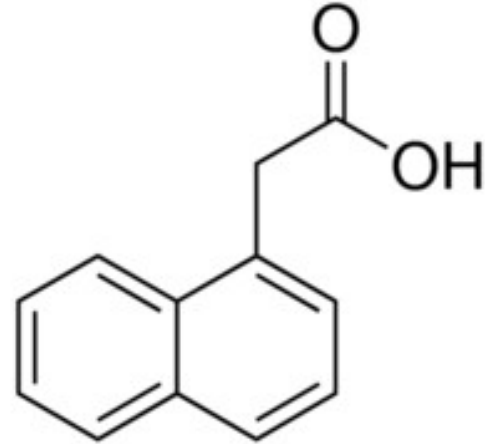
Abstract (Garcia-Jimenez et al. (2017) Journal of Phycology)

When applied in vitro, methyl jasmonate is sensed by the red seaweed *Grateloupia imbricata*, substantially and visually affecting its carposporogenesis. However, although there is some understanding of the morphological changes induced by **methyl jasmonate** in vitro, little is known about the genes that are involved in red seaweed carposporogenesis and how their protein products act. For the work reported herein, the expression of genes in red seaweed that encode enzymes involved in the synthesis of methyl jasmonate (jasmonic acid carboxyl methyl transferase and a putative methyl transferase) was monitored. Additionally the genes involved in oxidation (cytochrome P450 and WD40), jasmonate synthesis, signal transduction, and regulation of reactive oxygen species (MYB), and reproduction (ornithine decarboxylase) were monitored. To determine when or if the aforementioned genes were expressed during cystocarp development, fertilized and fertile thalli were exposed to methyl jasmonate and gene expression was measured after 24 and 48 h. The results showed that methyl jasmonate promoted differential gene expression in fertilized thalli by 24 h and upregulated expression of the ornithine decarboxylase gene only by 48 h in fertile thalli (0.75 ± 0.03 copies $\cdot \mu\text{L}^{-1}$ at 24 h vs. 1.11 ± 0.04 copies $\cdot \mu\text{L}^{-1}$ at 48 h). We conclude that Ornithine decarboxylase expression involves methyl jasmonate signaling as well as development and maturation of cystocarps.

Plant hormones: Auxin

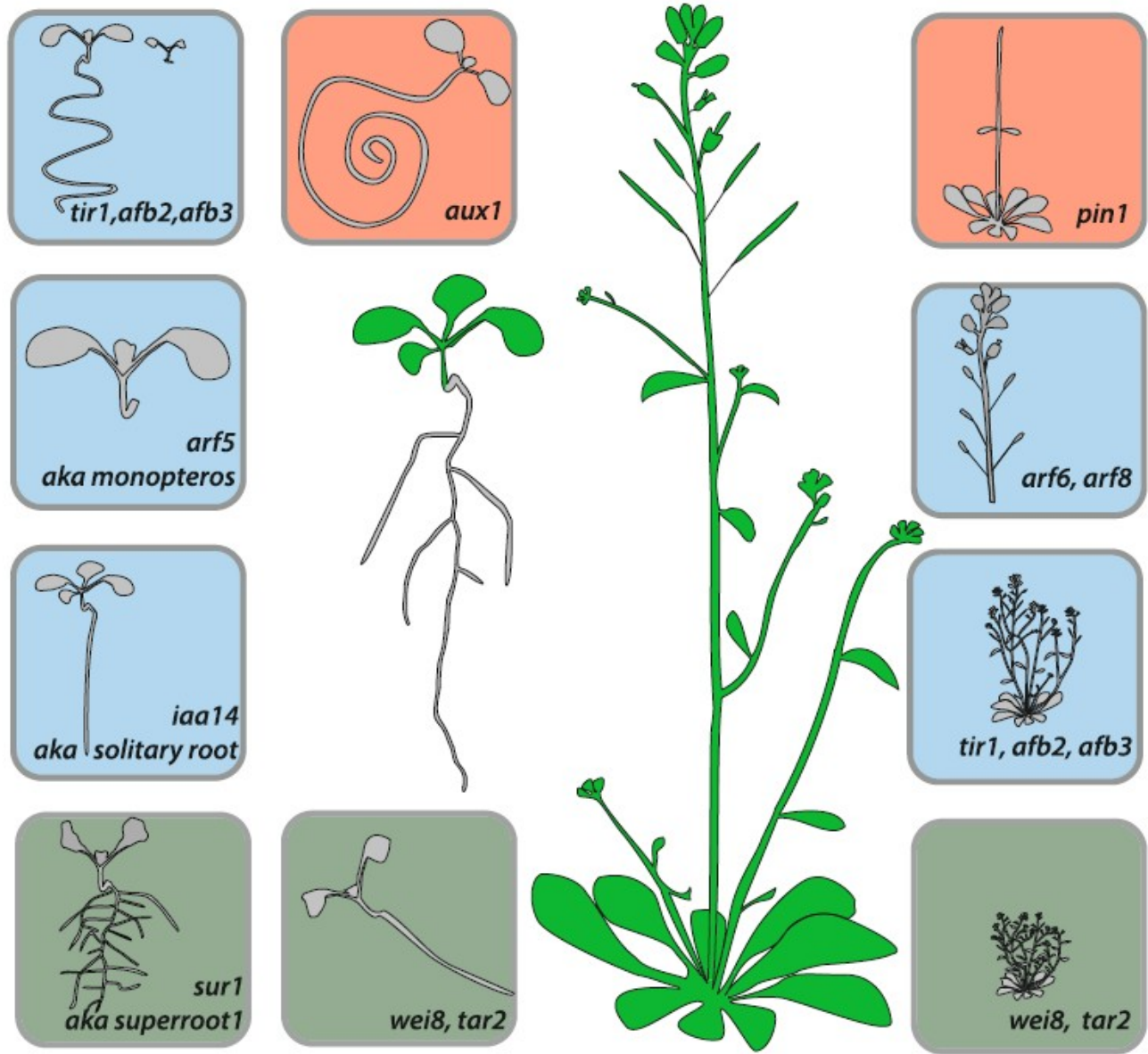


Indole-3-acetic acid



1-Naphthaleneacetic acid (NAA)

Auxin homeostasis



Auxin
influx/efflux
transporter

Mutants in
In auxin TRF

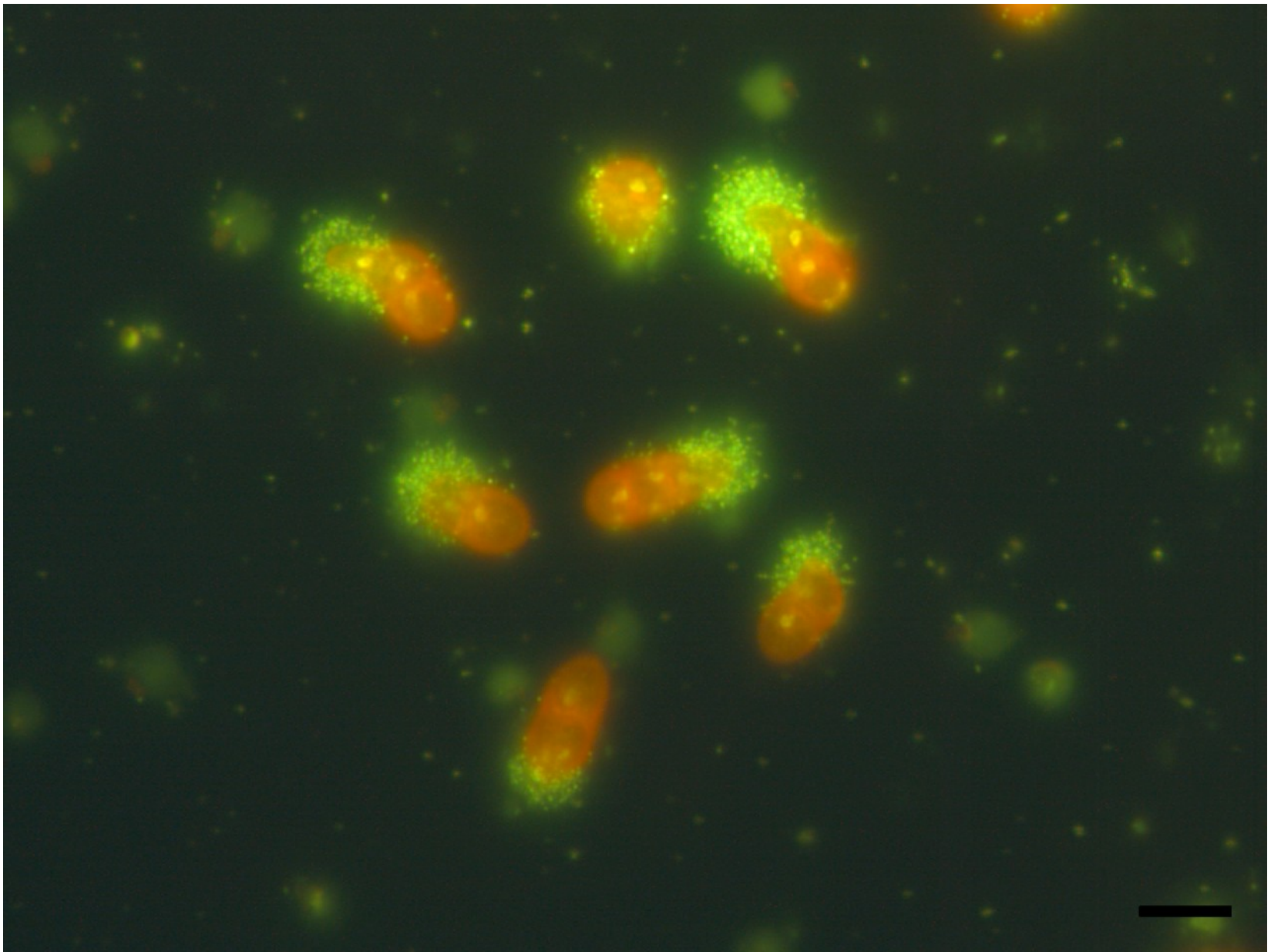
Auxin
biosynthesis

Auxin function in the brown alga *Dictyota dichotoma*

Auxin Function in the Brown Alga *Dictyota dichotoma*

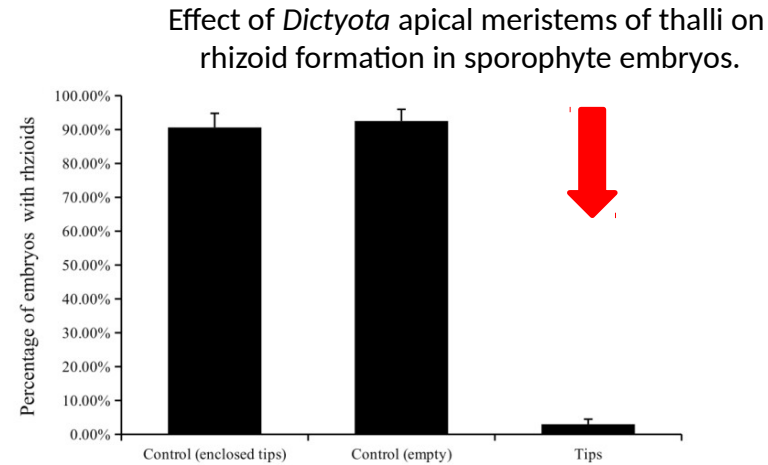
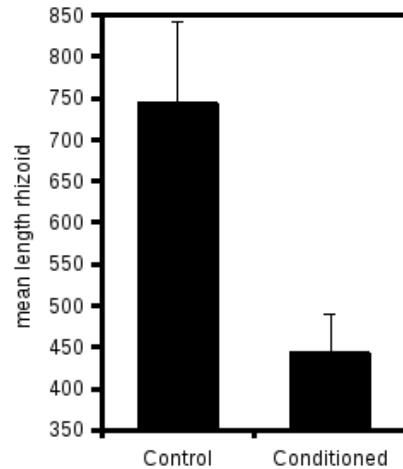
Abstract (Bogaert et al. 2019)

Auxin controls body plan patterning in land plants and has been proposed to play a similar role in the development of brown algae (Phaeophyta) despite their distant evolutionary relationship with land plants. The mechanism of auxin action in brown algae remains controversial because of contradicting conclusions derived from pharmacological studies on *Fucus*. In this study, we used *Dictyota dichotoma* as a model system to show that auxin plays a role during the apical-basal patterning of the embryo of brown algae. Indole-3-acetic acid was detectable in *D. dichotoma* germlings and mature tissue. Although two-celled *D. dichotoma* zygotes normally develop a rhizoid from one pole and a thallus meristem from the other, addition of exogenous auxins to one-celled embryos affected polarization, and both poles of the spheroidal embryo developed into rhizoids instead. The effect was strongest at lower pH and when variable extrinsic informational cues were applied. 2-[4-(diethylamino)-2-hydroxybenzoyl]benzoic acid, an inhibitor of the ABC-B/multidrug resistance/P-glycoprotein subfamily of transporters in land plants, affected rhizoid formation by increasing rhizoid branching and inducing ectopic rhizoids. An in silico survey of auxin genes suggested that a diverse range of biosynthesis genes and transport genes, such as PIN-LIKES, and the ATP-binding cassette subfamily (ABC-B/multidrug resistance/P-glycoprotein) transporters from land plants have homologs in *D. dichotoma* and *Ectocarpus siliculosus*. Together with reports on auxin function in basal lineages of green algae, these results suggest that auxin function predates the divergence between the green and brown lineage and the transition toward land plants.



Wichard 2015 *Frontiers in Plant Science* Exploring bacteria-induced growth and morphogenesis in the green macroalga order Ulvales (Chlorophyta) 6: 86.

STSM: Ongoing research within a STSM



Developmental plasticity in polarisation process of *Dictyota* zygotes. (A) Representative embryos **that divide asymmetrically** resulting in a unirhizoidal embryo. (B) zygote development via an **intermediate situation**. (C) symmetrically dividing embryo resulting in an **dithallic** embryo.

Bogaert, Kenny. 2015. "Cell Polarisation and Asymmetric Cell Division Using *Dictyota Dichotoma* as a New Experimental Model". Ghent, Belgium: Ghent University. Faculty of Sciences.



Saccharina laminaria

Phytohormones in red seaweeds: a technical review of methods for analysis and a consideration of genomic data

Abstract (Izumi et al. 2017)

Emerging studies suggest that seaweeds contain phytohormones; however, their chemical entities, biosynthetic pathways, signal transduction mechanisms, and physiological roles are poorly understood. Until recently, it was difficult to conduct comprehensive analysis of phytohormones in seaweeds because of the interfering effects of cellular constituents on fine quantification. In this review, we discuss the details of the latest method allowing simultaneous profiling of multiple phytohormones in red seaweeds, while avoiding the effects of cellular factors. Recent studies have confirmed the presence of indole-3-acetic acid (IAA), N^6 -(Δ^2 -isopentenyl)adenine (iP), (+)-abscisic acid (ABA), and salicylic acid, but not of gibberellins and jasmonate, in *Pyropia yezoensis* and *Bangia fuscopurpurea*. In addition, an *in silico* genome-wide homology search indicated that red seaweeds synthesize iP and ABA *via* pathways similar to those in terrestrial plants, although genes homologous to those involved in IAA biosynthesis in terrestrial plants were not found, suggesting the epiphytic origin of IAA. It is noteworthy that these seaweeds also lack homologues of known factors involved in the perception and signal transduction of IAA, iP, and ABA. Thus, the modes of action of these phytohormones in red seaweeds are unexpectedly dissimilar to those in terrestrial plants.



Current Biology

Insights into the Evolution of Multicellularity from the Sea Lettuce Genome

Highlights

- The *Ulva* genome is the first whole-genome sequence of a green seaweed
- Gene families associated with multicellularity are distinct from freshwater algae
- Cell-cycle S-phase entry does not depend on the RB/E2F pathway or D-type cyclins
- *Ulva*, a renowned DMS-producer, uses homologs of the Alma protein to cleave DMSP

Contributions from seven COST Action countries

Authors

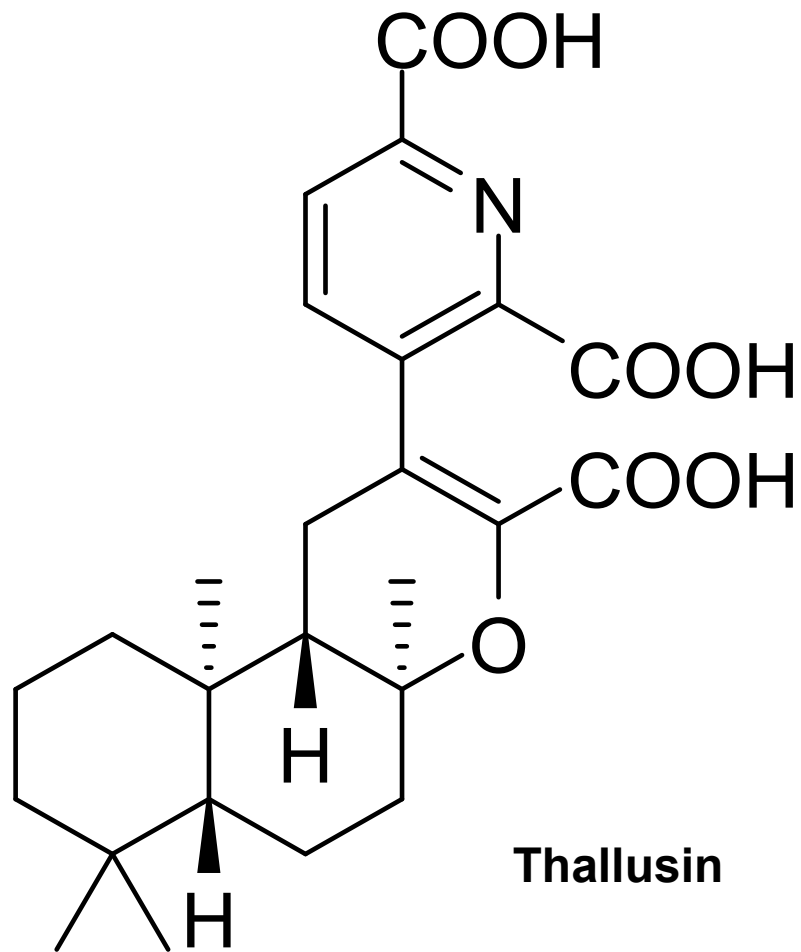
Olivier De Clerck, Shu-Min Kao, Kenny A. Bogaert, ..., Yves Van de Peer, Thomas Wichard, John H. Bothwell

Correspondence

olivier.declerck@ugent.be (O.D.C.),
j.h.bothwell@durham.ac.uk (J.H.B.)

In Brief

De Clerck et al. present the first genome sequence of a green seaweed, a dominant group of primary producers in coastal environments. The *Ulva* genome informs on an independent acquisition of multicellularity, sheds light on adaptations to life in intertidal habitats, and identifies candidate genes involved in DMSP biosynthesis and conversion to DMS.





Ulva mutabilis Føyn (slender)

Collaboration with
Aschwin Engelen
CCMAR, Portugal

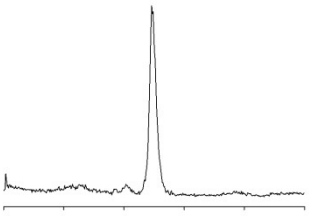


Solid phase extraction of growth medium

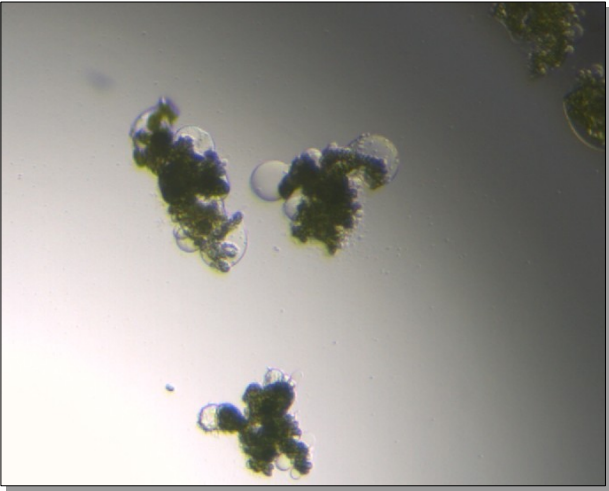
Workflow:



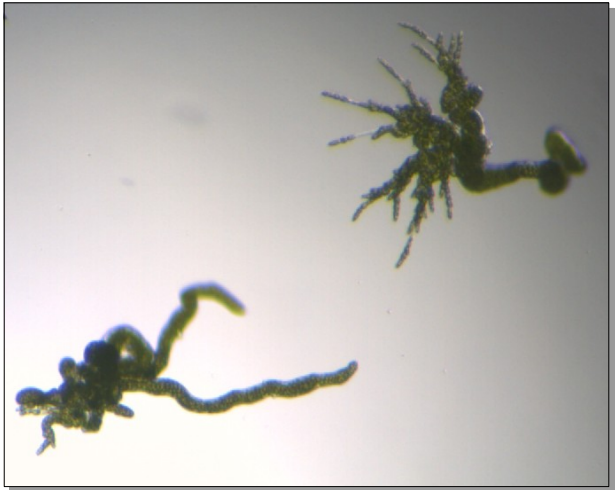
Bioassays



Mass spectrometry

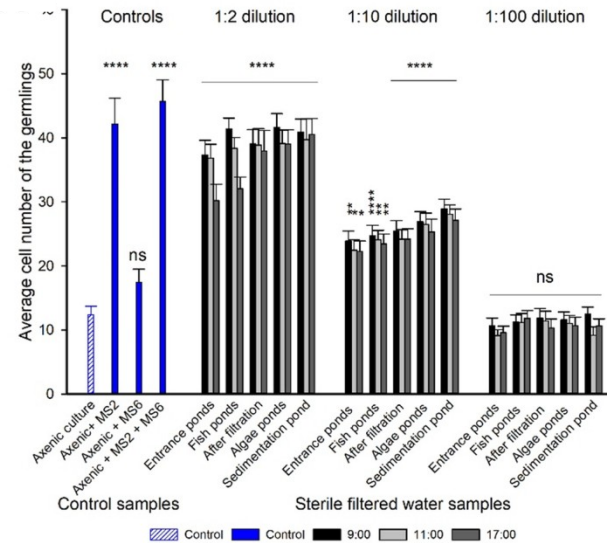
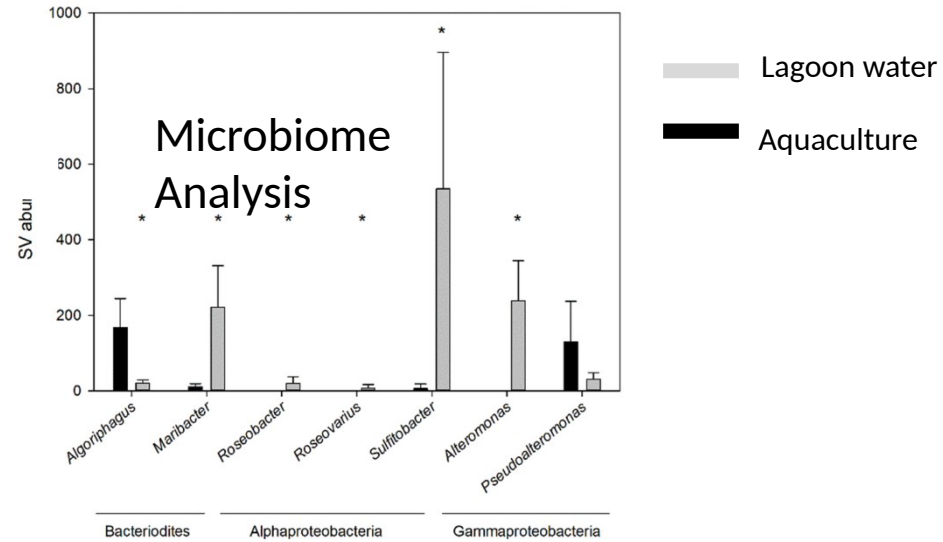
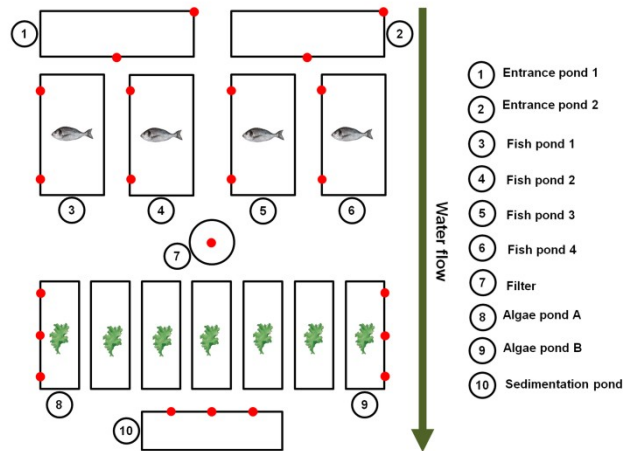
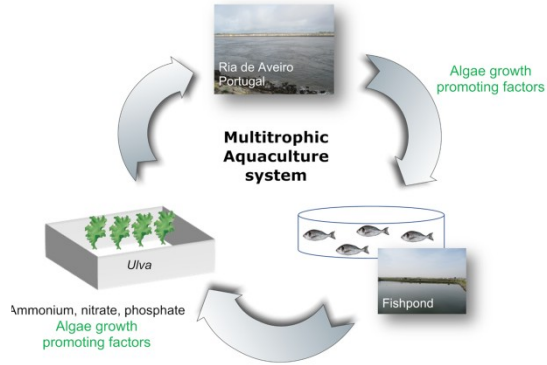


Non active fraction



Active fraction

Mutualistic interactions in aquatic systems



Deliverable of task 3: “Towards adult growth”

DL3-1

Characterize whether cell-cell communication occurs within the embryo (apoplastic or symplastic communication). This will allow practice of excision of seaweed parts, which might be necessary in aquaculture processes.

DL3-2

Assess the impact of external and internal forces (mainly mechanical) on embryo fate. Most seaweed embryos develop directly in the sea, exposed to strong mechanical forces (such as sea current, waves and tides). In addition, growth and cell multiplication generate compression and shearing forces, which cells need to account for and respond to.

DL3-3

Identify bacterial chemicals used as external seaweed morphogens. This will lead to possible control of seaweed developmental steps by external application of morphogens present in natural compounds (in hatchery).

DL3-4

Characterize seaweed tissues or cellular structures enabling long-range transport through the seaweed body (differentiation of sieve elements and/or plasmodesmata, transport velocity). The intention would be to eventually manipulate the size of seaweed whole-body or specific organ to optimize yield should account for these knowledge.

DL3-5

Determine the existence of processes such as apical dominance, leading to the possibility of modifying overall seaweed morphology by promoting branching, thereby increasing seaweed biomass or production of reproductive organs, similarly to crop plants on land (e.g. “Green Revolution” breeds of wheat and rice).

Mutualistic interactions in aquatic systems

The growing interest in commercialization of seaweeds for human food has stimulated research into the physical properties of seaweed tissue.

Texture analysis of *Laminaria digitata* (Phaeophyceae) thallus reveals trade-off between tissue tensile strength and toughness along lamina

Abstract (Lubsch, A., & Timmermans, K. 2017)

Texture analysis is a method to test the physical properties of a material by tension and compression. The growing interest in commercialisation of seaweeds for human food has stimulated research into the physical properties of seaweed tissue. These are important parameters for the survival of sessile organisms consistently exposed to turbulent flow and varying drag-forces. These tactile properties also affect consumer perception and acceptance of materials. Here, we present a standardised method to determine these physical properties using, as an example, the brown seaweed *Laminaria digitata* (Hudson) J.V. Lamouroux, which is prevalent on coastlines along the northern Atlantic Ocean. Morphological features of a healthy *L. digitata* thallus (lamina) seem modified to withstand physical distress from hydrodynamic forces in its wave-swept habitat. The trade-off in tissue responses to tensile and compression forces along the lamina, linked to an age gradient, indicates a twinned alignment of its cellular microstructure, similar to those of modern nanotechnology, to optimise the toughness and flexibility of constituent tissue. Tensile strength increased from young to old tissue along a positive toughness gradient of 75%. Based on our results, a short interpretation is given of the heterogeneity in *L. digitata* lamina from morphological, ecological and physiological perspectives.

Deliverable of task 3: “Towards adult growth”

DL3-1

Characterize whether cell-cell communication occurs within the embryo (apoplastic or symplastic communication). This will allow practice of excision of seaweed parts, which might be necessary in aquaculture processes.

DL3-2

Assess the impact of external and internal forces (mainly mechanical) on embryo fate. Most seaweed embryos develop directly in the sea, exposed to strong mechanical forces (such as sea current, waves and tides). In addition, growth and cell multiplication generate compression and shearing forces, which cells need to account for and respond to.

DL3-3

Identify bacterial chemicals used as external seaweed morphogens. This will lead to possible control of seaweed developmental steps by external application of morphogens present in natural compounds (in hatchery).

DL3-4

Characterize seaweed tissues or cellular structures enabling long-range transport through the seaweed body (differentiation of sieve elements and/or plasmodesmata, transport velocity). The intention would be to eventually manipulate the size of seaweed whole-body or specific organ to optimize yield should account for these knowledge.

DL3-5

Determine the existence of processes such as apical dominance, leading to the possibility of modifying overall seaweed morphology by promoting branching, thereby increasing seaweed biomass or production of reproductive organs, similarly to crop plants on land (e.g. “Green Revolution” breeds of wheat and rice).

Immunolocalization of cell wall carbohydrate epitopes

Immunolocalization of cell wall carbohydrate epitopes in seaweeds: presence of land plant epitopes in *Fucus vesiculosus* L. (Phaeophyceae)

Abstract (Raimundo et al. 2016)

Land plant cell wall glycan epitopes are present in *Fucus vesiculosus*. RG-I/AG mAbs recognize distinct glycan epitopes in structurally different galactans, and 3-linked glucans are also present in the cell walls.

Cell wall-directed monoclonal antibodies (mAbs) have given increased knowledge of fundamental land plant processes but are not extensively used to study seaweeds. We profiled the brown seaweed *Fucus vesiculosus* glycome employing 155 mAbs that recognize predominantly vascular plant cell wall glycan components. The resulting profile was used to inform in situ labeling studies. Several of the mAbs recognized and bound to epitopes present in different thallus parts of *Fucus vesiculosus*. Antibodies recognizing arabinogalactan epitopes were divided into four groups based on their immunolocalization patterns. Group 1 bound to the stipe, blade, and receptacles. Group 2 bound to the antheridia, oogonia and paraphyses. Group 3 recognized antheridia cell walls and Group 4 localized on the antheridia inner wall and oogonia mesochite. This study reveals that epitopes present in vascular plant cell walls are also present in brown seaweeds. Furthermore, the diverse in situ localization patterns of the RG-I/AG clade mAbs suggest that these mAbs likely detect distinct epitopes present in structurally different galactans. In addition, 3-linked glucans were also detected throughout the cell walls of the algal tissues, using the β -glucan-directed LAMP mAb. Our results give insights into cell wall evolution, and diversify the available tools for the study of brown seaweed cell walls.

Deliverable of task 3: “Towards adult growth”

DL3-1

Characterize whether cell-cell communication occurs within the embryo (apoplastic or symplastic communication). This will allow practice of excision of seaweed parts, which might be necessary in aquaculture processes.

DL3-2

Assess the impact of external and internal forces (mainly mechanical) on embryo fate. Most seaweed embryos develop directly in the sea, exposed to strong mechanical forces (such as sea current, waves and tides). In addition, growth and cell multiplication generate compression and shearing forces, which cells need to account for and respond to.

DL3-3

Identify bacterial chemicals used as external seaweed morphogens. This will lead to possible control of seaweed developmental steps by external application of morphogens present in natural compounds (in hatchery).

DL3-4

Characterize seaweed tissues or cellular structures enabling long-range transport through the seaweed body (differentiation of sieve elements and/or plasmodesmata, transport velocity). The intention would be to eventually manipulate the size of seaweed whole-body or specific organ to optimize yield should account for these knowledge.

DL3-5

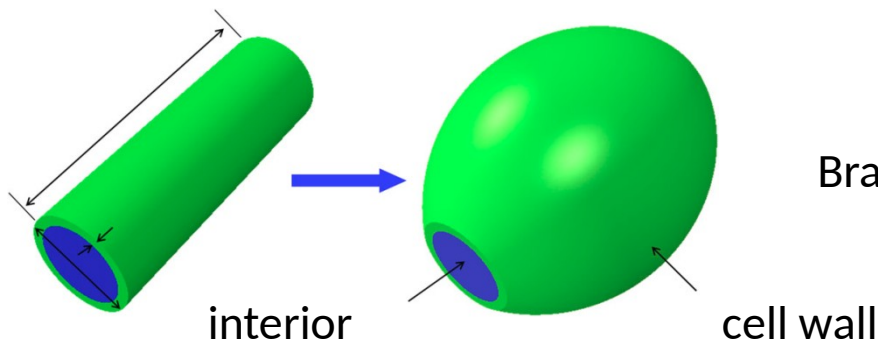
Determine the existence of processes such as apical dominance, leading to the possibility of modifying overall seaweed morphology by promoting branching, thereby increasing seaweed biomass or production of reproductive organs, similarly to crop plants on land (e.g. “Green Revolution” breeds of wheat and rice).

Biophysics of cell differentiation in *Ectocarpus*

Branching mainly occurs when a cylindrical cell becomes rounded and swells, forming a spherical cell.

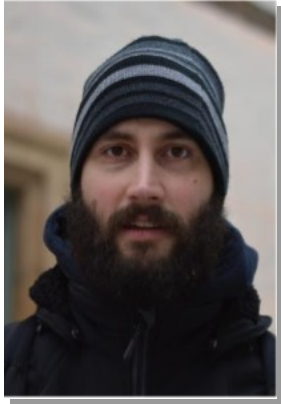


Transformation of a cylindrical shape into spherical shape with a volumetric increase, and then lateral branching.



Branching occurs mainly on round cells.

COST Action and Master thesis



Ioannis Theodorou finished his undergraduate studies in Biology (University of Athens) and is interested in the evolution and development of photosynthetic organisms. He continued his postgraduate studies in the Master's programme at the FSU Jena, before he has recently started his PhD programme at the Marine Station in Roscoff, France

First contact with the COST Action: Workshop and Training School participation “Seaweed Development and Cultivation” in Kavala, Greece, 2016

Bachelor thesis: **Study of the morphogenesis of thallus in Brown Algae representatives of particular phylogenetic interest.** (National and Kapodistrian University of Athens, Greece)

Publication: Katsaros, C., Weiss, A., Llangos, I., Theodorou, I., Wichard, T.(2017). Cell structure and microtubule organisation during gametogenesis of *Ulva mutabilis* Føyn (Chlorophyta). *Botanica Marina*

Master Programme: Molecular Life Sciences
(FSU Jena, Germany)

Master thesis: **Identification and characterisation of homeobox genes in *Ulva mutabilis***
(FSU Jena, Germany)

PhD project: **Cell fate and blade patterning during the early growth of *Saccharina latissima* sporophytes**
(Roscoff, France)

COST Action and PhD thesis



Fatemeh Ghaderiardakani has studied microbiology at the Alzahra and Islamic Azad University (Iran). She is currently interested in bacteria-macroalgae interactions and aquacultures and finished her PhD at the University of Birmingham.

COST Action supported her research through STSMs and Training schools during the Phd programme at the University of Birmingham (UK)

1st Short-Term Scientific Mission (STSM) of the COST ACTION FA1407

Extending cultivation protocols that will enable and support *Ulva* sp. (*Ulva mutabilis* and *Ulva linza*) cultivation under laboratory conditions

2nd Short-Term Scientific Mission (STSM) of the COST ACTION FA1407

ALGAPLUS, Lda, Aveiro Area, Portuga

Participation on COST ACTION workshops and training schools.

Her research was further supported by DAAD and FEMS.

Ghaderiardakani et al. Bacteria-induced morphogenesis of *Ulva intestinalis* and *Ulva mutabilis* (Chlorophyta): a contribution to the lottery theory. *FEMS Microbiology Ecology* 93(8):fix094

Green Talents award



Mark Polikovsky works on developing a biorefinery concept. He aims at using seaweeds as feedstock instead of polluted oil refinery. His research topic is the influence of seaweed associated bacteria on seaweed sugar and protein content and composition.

First contact with the COST ACTION via STSM: Preparing axenic *Ulva* sp. and defined bacterial impact on proteins and sugars changes in *Ulva* sp

2017, The Rieger Foundation-Jewish National Fund Program for Environmental Studies. (USD 5,000 /per year).

2017, The national found for engineering and applied science (Mia). Ministry of science, technology, and space. State of Israel (250,000 NIS/ per 3 years). For a project called: New bioprocess for *Ulva* sp. biomass conversion into bioethanol.

2017, Travel grant for EUALGAE Training school, Microalgae processes: from fundamentals to industrial scale, Almería (Spain) (700 Euro).

2018, Green Talents award. German Federal Ministry of Education and Research (BMBF).
25 World High Potentials in Sustainable Development Award. <https://www.greentaleants.de>

Polikovsky et al. (2019) Engineering bacteria-seaweed symbioses for modulating *Ulva* sp. monosaccharides content important for bioethanol production, in preparation



**EUROPEAN COOPERATION
IN SCIENCE AND TECHNOLOGY**

Dr. Thomas Wichard

**Friedrich Schiller University Jena
Institute for Inorganic and Analytical Chemistry**

WP3: Final report - Towards macroalgal adult growth



PHYCOMORPH

**Advancing Knowledge On Seaweed
Growth And Development**