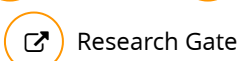


Citation index



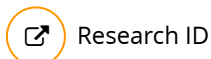
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Research Gate



ORCID



Research ID

EXPERIENCES

Founder & President

BlueCare discovery - Since September 2022



Post-doctoral researcher position (Marie Skłodowska-Curie program)

Station biologique de Roscoff - CNRS - June 2014 to June 2017 - Part-time - Roscoff - France



- ▶ OCEANCHARCoT program: OCEAN CHemodiversity Against Cell cycle Targets
- ▶ Development of a microalgae dereplication strategy assisted with metabolomics tools
- ▶ Exploration of signaling pathways hit by marine metabolites of interest
- ▶ Mode of action of marine metabolites using bioassays, flow cytometry, epifluorescence microscopy
- ▶ Marine bioresources valorization (cosmetoceuticals, nutraceuticals, feed/aquaculture)
- ▶ Involvement in OCEANOMICS Axis 7: Innovation platform for plankton screening for bioactive compounds and metabolites

Postdoctoral research position (Marie Skłodowska-Curie program)

Griffith Institute for Drug Discovery - Griffith University - July 2014 to August 2016 - Part-time - Brisbane - Australia - Queensland



- ▶ OCEANCHARCoT program: OCEAN CHemodiversity Against Cell cycle Targets
- ▶ Lead-Like Enhanced Fractions production and screening (HTS)
- ▶ Evaluation of marine fractions from Great Barrier Reef organisms against biological model
- ▶ NMR fingerprint dereplication

PhD student

IFREMER-Laboratory of Physiology and Biotechnology of Algae - March 2009 to December 2012 - Full-time - Nantes - France



- ▶ Research and production of metabolites of interest in photodynamic therapy from photosynthetic micro-organisms (Work under confidentiality clauses)
- ▶ Microalgae and cyanobacteria cultures
- ▶ Development of a biotest to assess a specific activity potentially interesting for high throughput screening
- ▶ Comparison of cell grinding methods by design of experiments methodology
- ▶ Optimization of the extraction of specific molecules of interest
- ▶ Development of a dereplication method for target molecules using hyphenated HPLC-UV DAD
- ▶ Purification by high performance liquid chromatography
- ▶ Image analysis
- ▶ Flow cytometry

Postgraduate student (EPHE)

Research group Sea Molecules Health - University of Nantes - September 2007 to July 2008 - Internship - Nantes - France



- ▶ Extraction, purification, characterization of marine phospholipids and sterols
- ▶ Scuba-diving expedition for collection of sponges, cnidarians, echinoderms
- ▶ Analysis and purification by analytical and preparative thin layer chromatography
- ▶ Purification by high performance liquid chromatography
- ▶ Analysis by hyphenated gas chromatography/mass spectrometry

Postgraduate student (EPHE)

Laboratory of Marine Biotechnology and Chemistry - University of South Brittany - January 2006 to August 2007 - Internship - Vannes - France



- ▶ Development of screening bioassays
- ▶ Cell culture (human cell lines, bacteria, virus)

- ▶ Anti-viral bioassays
- ▶ Anti-bacteria bioassays
- ▶ Anti-fouling bioassays
- ▶ Tests in cosmetology (anti-free radicals, anti-elastase, anti-oxidant, anti-inflammatory, cytotoxicity)

Undergraduate student (Intechmer)



IFREMER - Laboratory of Invertebrates Physiology - April 2004 to August 2004 - Internship - Brest - France

- ▶ Search for alternatives to the use of chloramphenicol in scallop larvae breeding (*Pecten maximus*)
- ▶ Larval breeding
- ▶ Microalgae culture (batch and continuous mode)
- ▶ Microbiology techniques
- ▶ Bioassays (cytotoxicity and growth inhibition)
- ▶ Image analysis

SKILLS

Scientific and technical expertise

- ▶ Pigment analysis by means of HPLC-UV DAD
- ▶ Optimized extraction of photosynthetic microorganisms
- ▶ Purification and culture of microalgae strains
- ▶ Purification of marine natural products
- ▶ Development testing of biological activities (anti-fouling, cosmetology, pharmacology)
- ▶ Development of a screening strategy for pharmacological activity
- ▶ Collection of marine organisms for studies in scuba-diving
- ▶ Light microscopy (cell counting and image analysis)
- ▶ Cell culture (human cell lines and animal cell lines, viruses)
- ▶ Basics of flow cytometry on Accuri C6
- ▶ Basic microbiology techniques (isolation, culture, bioassay)

Transversal skills

- ▶ Scientific and technological reporting (patent database)
- ▶ Studies in experimental designs (factors screening and optimization)
- ▶ Statistical analysis of data (Statgraphics, Xlstat, Matlab)
- ▶ Development and project management (Gantt, Mind Mapping on Mindmanager, quotations, inventory management)
- ▶ Drafting of specifications
- ▶ Results reporting
- ▶ Collaborations with external laboratories (LEMAR Brest, Nantes University Hospital, platform BIODIMAR Brest, Nantes Phycotoxins Laboratory, Veolia Environment)
- ▶ Publication in refereed journals
- ▶ Scientific networking
- ▶ Popular science (World Oceans Day, Square of Science, Science Fair, foreshore visit)

EDUCATION

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Laureate of the vocation award
2006
(Bleustein Blanchet Foundation)

Laureate 2013 - Marie Curie
Outgoing Fellowship
OCEANCHARCoT programm

Member of the French
Speaking Network of
Metabolomics and Fluxomics

Member of the French
Speaking Society of
Pharmacognosy

Member of the American
Society of Pharmacognosy

Member of the Association for
the Sciences of Limnology and
Oceanography

Reviewer for scientific peer-
reviewed journals :
Marine Drugs, Algal Research

Invited Speaker at the 5th
World Congress on
Biotechnology (Valencia, Spain,
June 2014)

In brief :

Voluntary, passionate and
sensitive to human values, I
want to use my skills to the
search for molecules with high
added value from marine
biodiversity.

Golden prize of the most
scholar, caring, and honest
supervisor awarded to:

Prof. Ronald J. QUINN
(Eskitis Institute for Drug
Discovery, Brisbane, Australia)

Mentor :

Emeritus Prof. Jean-Michel
KORNPROBST
(University of Nantes, France)

PhD of Science speciality in biomolecules, pharmacology and therapy

PHD SCHOOL VENAM - UNIVERSITY OF NANTES

March 2009 to December 2012

MSc in medical and biological science research specialising in Mechanisms and optimisation of marine bioproduction

UNIVERSITY OF NANTES - FACULTY OF SCIENCES AND TECHNIQUES

September 2007 to June 2008

Marine pharmacognosy, aquaculture, maritime Law, marine biology

MSc in Cell signalling and integrated systems in biology

PRACTICAL SCHOOL OF HIGH STUDIES (EPHE)

September 2006 to July 2008

Cellular and molecular biology, cancerology, flow cytometry, virology

BSc in Biochemistry-Molecular biology

UNIVERSITY OF NANTES - FACULTY OF SCIENCES AND TECHNIQUES

September 2004 to March 2006

Enzymology, biochemistry, molecular biology, bio-organic chemistry, integration of metabolic pathways

DTSM degree : Higher standard technician in marine sciences and techniques specialising in bioengineering and marine productions

INTECHMER CHERBOURG

September 2002 to September 2004

Physical and biological oceanography, marine zoology, algology, aquariology, marine geology, fisheries, aquaculture

Intensive course in science subjects

UCO - INSTITUTE OF APPLIED MATHEMATICS

September 2001 to June 2002

National diploma in aquaculture

MFR LES PLANTES

September 1999 to July 2001

Aquaculture technologies, economy, accounting

First diploma in marine cultures

MFR LES PLANTES

September 1997 to July 1999

Study of breeding and culture techniques of marine species, marine biology

INTERESTS

Selection of publications

- ▶ Study on the microalgal pigments extraction process: performance of microwave-assisted extraction (2011) *Process Biochemistry* 46: 59-67
- ▶ Antiproliferative activity of violaxanthin isolated from bioguided fractionation of *Dunaliella tertiolecta* extracts (2011) *Marine Drugs* 9: 819-831
- ▶ Selection and optimisation of a method for efficient metabolites extraction from microalgae (2012) *Bioresource Technology* 124: 311-320
- ▶ Screening marine resources to find novel chemical inhibitors of disease-relevant protein kinases (2015) *Medecine/Sciences* 31(5): 538-545
- ▶ Community analysis of pigment patterns from 37 microalgae strains reveals new carotenoids and porphyrins characteristic of distinct strains and taxonomic groups (2017) *PLoS One* 12(2): 1-35

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- ▶ Constituents of *Acacia nilotica* (L.) Delile with novel kinase inhibitory activity (2017) *Planta Medica International Open* 4:108-113
- ▶ Marine pigment diversity: applications and potential (2018, in process) In: *Blue Biotechnology: production and use of marine molecules*
- ▶ Microalgal biomass of industrial interest: methods of characterization (2018, in process) In: *Biomass, waste and related by-products characterization*

Sport

- ▶ Scuba-diving
- ▶ Surfing
- ▶ Swimming
- ▶ Kayaking
- ▶ Tai-chi-chuan
- ▶ Apnea

Travel


- ▶ Senegal
- ▶ Germany
- ▶ Scotland
- ▶ USA
- ▶ Australia
- ▶ Portugal
- ▶ Fiji
- ▶ Norway

Hobbies


- ▶ Aquariophilly
- ▶ Oenology

PORTFOLIOS


OCEANCHARCoT: bioprospection of the Great Barrier Reef chemodiversity




Griffith UNIVERSITY
Griffith Institute for Drug Discovery
Creating Knowledge that Transforms Lives



Station Biologique Roscoff



CNRS



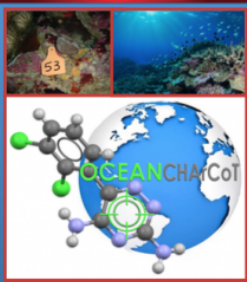
BIOPROSP_17
The 8th International Conference on Marine Bioprospecting

OCEANCHARCoT: bioprospection of the Great Barrier Reef chemodiversity

Serive B.^{1,2}, Quinn R.J.¹, Bach S.²

¹ Griffith Institute for Drug Discovery, Griffith University, Brisbane, QLD 4111, Australia.
² Kinase Inhibitor Specialized Screening facility, KISSf, USR3151 CNRS/UPMC, Station Biologique de Roscoff, 29688 Roscoff cedex, France.

Introduction

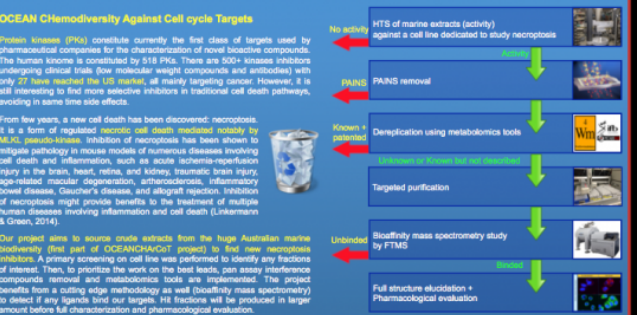


OCEAN Chemodiversity Against Cell cycle Targets

Protein kinases (PKs) constitute currently the first class of targets used by pharmaceutical companies for the characterization of novel bioactive compounds. The human genome is constituted by 518 PKs. There are 500+ kinases inhibitors undergoing clinical trials (low molecular weight compounds and antibodies) with only 27 have reached the US market, all mainly targeting cancer. However, it is still interesting to find more selective inhibitors in traditional cell death pathways, avoiding in same time side effects.

From few years, a new cell death has been discovered: necroptosis. It is a form of regulated **apoptotic cell death mediated notably by MLKL, pseudokinase**. Inhibition of necroptosis has been shown to mitigate pathology in mouse models of numerous diseases involving cell death and inflammation, such as acute ischaemia-reperfusion injury in the brain, heart, retina, and kidney, traumatic brain injury, age-related muscular degeneration, atherosclerosis, inflammatory bowel disease, Gaucher's disease, and allograft rejection. Inhibition of necroptosis might provide benefits to the treatment of multiple human diseases involving inflammation and cell death (Linkermann & Green, 2014).

Our project aims to **assess crude extracts from the huge Australian marine biodiversity** (first part of OCEANCHARCoT project) to find new necroptosis inhibitors. A primary screening on cell line was performed to identify any fractions of interest. Then, to prioritize the work on the best leads, an assay interference compounds removal and metabolomics tools are implemented. The project benefits from a cutting edge methodology as well (bioaffinity mass spectrometry) to detect if any ligands bind our targets. **Hit fractions** will be produced in larger amount before full characterization and pharmacological evaluation.



HTS of marine extracts (activity) against a cell line dedicated to study necroptosis

PAINS removal

Derivatization using metabolomics tools

Targeted purification

Bioaffinity mass spectrometry study by FTMS

Full structure elucidation + Pharmacological evaluation

Chemodiversity high-throughput screening

Material and methods

Extraction step: 300 mg of beds are loaded in a C18 SPE cartridge and then, different solvents are eluted on it following a standardized protocol (Fig. A). 125 µL of the LLE extract obtained are injected in HPLC according to a standardized elution method. Five Lead-Like Enhanced Fractions (LLEFs) are obtained. Nature Bank manages this step and Compounds Australia is in charge to store fractions and plating for assays.

This entire protocol allows to remove very polar and very apolar parts of the raw extracts to meet the Lipinski's rule of five (RO5). The latter evaluates the druglikeness or determines if a chemical compound with pharmacological or biological activity has properties that would make it a likely orally active drug in humans. To follow the RO5, a compound has no more than one violation of these criteria (Lipinski, 2004):

- No more than 5 hydrogen bond donors.
- No more than 10 hydrogen bond acceptors.
- A molecular mass less than 500 daltons.
- An octanol-water partition coefficient log P not greater than 5.


Cell based bioassay: Protection against induced cell death (Jurkat cells FADD deficient)

High Throughput Screening performed on BioCoat500 Agent, Technologies robot platform.

Results

35 965 LLEFs screened in the following phyla: Porifera, Chordata, Arthropoda, Annelida, Anthophyta, Bryozoa, Cnidaria, Echinodermata, Ectoprocta, Mollusca, Chlorophyta, Rhodophyta, Heterokontophyta, Cyanophyta.

Hit rate: 0.3% (102 hits) (Fig. B)



Phylogenic tree of screened beds for OCEANCHARCoT project

Hit rate: 0.3% (102 hits)

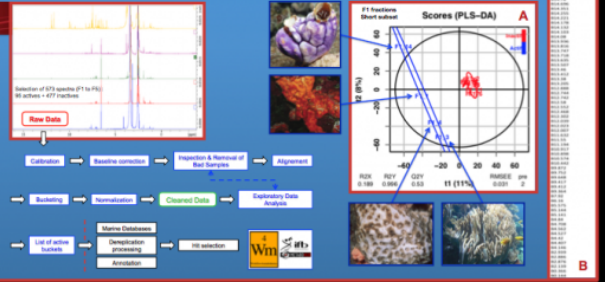
NATURE BANK

Hit dereplication

In drug discovery and particularly in screening projects, the goal is to identify as early as possible known molecules to focus on new molecules or known molecules but not described for the activity that we work on. Traditionally, bioguided purification was the gold strategy but it is often time-consuming and expensive especially for minor compounds. Moreover, there is a risk to hunt a patented or already described molecule.

Dereplication aims to identify active molecules in presence of raw extracts. With the advent of NMR1 techniques and bioinformatic, new tools are currently available at the interface between biologists and chemists. These metabolomics workflows allow to merge the big data collected (herein, NMR data acquired from a Bruker 800 MHz spectrometer), process the various technical steps required to make a proper exploratory data analysis (A). Then, the goal is to **discriminate active buckets** from non-active buckets (B) and curate them to (i) make targeted purification (C) to compare the acquired NMR fingerprints to in-house acquired NMR spectra and/or predicted NMR spectra databases.

This last step is still a challenge to overcome since most natural products databases are (i) not open source (ii) are not structured for metabolomics workflow (iii) are not fully available.



Raw Data

Acquisition

Acquisition & Removal of Bad Samples

Alignment

Reference

Dereplication

Curated Data

Exploratory Data Analysis

Hit selection

Purification

Scores (PLS-DA)

PLS-DA Scores (PLS-DA)

Hit selection

This research was supported by a Marie Curie International Outgoing Fellowship within the 7th European Community Framework Programme. The authors thank Nature Bank, Queensland Museum and Compounds Australia for growing and plating samples. We thank also Toi-Wei for his assistance on the high throughput screening robot. Photos Copyrights: Aqualand Pty Ltd, Rick Stuart-Smith (Petal Life Survey)

Bibliography: Linkermann A., Green D.R. (2014) The New England Journal of Medicine 370(5). Lipinski C.A. (2004) Drug Discovery Today: Technologies 1(4).

Follow the project on: www.facebook.com/Oceancharcot @oceancharcot

Contact: serive.bach@roscoff.fr

Poster displayed at the 8th International Conference on Marine Bioprospecting

Creation date

07 mars 2017

5 / 17



► Les scientifiques de la station biologique de Roscoff (SBR) travaillent depuis plusieurs dizaines d'années sur la régulation de la division cellulaire en utilisant, comme modèles, des organismes marins. Ceci a notamment conduit à l'étude de kinases dépendantes des cyclines (CDK) qui contrôlent le déroulement du cycle cellulaire. Ces cibles ont ensuite été utilisées afin de caractériser des inhibiteurs pharmacologiques, ou « touches » (*hits*), en mettant en place un criblage automatisé. Le mécanisme d'action des meilleures touches sélectionnées a également été étudié en les dérivant, afin de procéder à des approches de criblages inverses par chromatographie d'affinité. À l'interface entre biologie et chimie, le travail de cette plate-forme place au centre des recherches le composé chimique, qui est à la fois (1) une molécule d'intérêt thérapeutique et (2) un outil moléculaire permettant d'analyser la fonction cellulaire des kinases ciblées. À partir d'organismes marins, huit familles structurales d'inhibiteurs ont été caractérisées sur la plate-forme, et l'espoir est grand de voir la mer nous en apporter de nouveaux, encore plus puissants. ►

La plate-forme de criblage KISSf de Roscoff, un site dédié à la recherche sur les inhibiteurs de kinases

En 1872, Henri de Lacaze-Duthiers, professeur à la Sorbonne, fonde à Roscoff le « laboratoire de zoologie expérimentale ». Ses objectifs sont la recherche, l'enseignement et l'accueil scientifique. Le choix de Roscoff s'explique, notamment, par la grande biodiversité végétale et animale, l'accessibilité au matériel biologique, rendue aisée grâce au phénomène de marée, mais aussi par la proximité avec Paris par le train. Plus de 140 ans

Cet article fait partie de la série « Chémobiologie » qui a débuté dans le n° 12, vol. 30, décembre 2014 (www.medicinesciences.org).
Vignette (éponge *Axinella verrucosa*, © Océanopolis, Brest, France). Organisme marin duquel a été extrait un puissant inhibiteur de kinase (l'hymenialdisine, représenté sur la Figure 2).

**Chémobiologie (11)
Le criblage à Roscoff**

Une recherche d'inhibiteurs de kinases tournée vers la mer




Blandine Baratte, Benoît Serive, Stéphane Bach



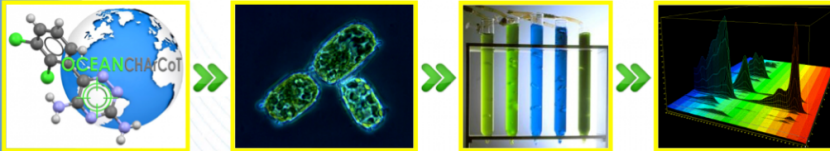
CNRS/UPMC USR3151, plate-forme de criblage KISSf (*kinase inhibitor specialized screening facility*), station biologique de Roscoff, place Georges Teissier, CS 90074, 29688 Roscoff Cedex, France. bach@sb-roscoff.fr

plus tard, le site roscovite, dont l'effectif dépasse les 300 personnes, est un des centres de biologie marine parmi les plus importants en Europe¹. Au sein de cet institut se trouve une plate-forme de criblage spécialisée dans l'identification d'inhibiteurs chimiques de protéine kinases, la plate-forme KISSf (*kinase inhibitor specialized screening facility*) (Figure 1). Cette spécialisation prend racine dans les années 1970, une vingtaine d'années après la première observation d'une activité kinase assignée à une enzyme hépatique, la caséine kinase, par Eugene Kennedy [1]. En effet, les travaux pionniers de Pierre Guerrier et Marcel Dorée sur l'implication de la phosphorylation dans la reprise du processus de la méiose, notamment chez les ovocytes d'invertébrés marins, ont été réalisés à Roscoff [2]. Ces études ont conduit à la mise en évidence de l'inhibition d'activité de phosphorylation par de petits composés chimiques, tels que le 6-diméthylaminopurine (6-DMAP) [3]. Par la suite, Laurent Meijer poursuivit ce travail sur différents modèles marins (*Arenicola marina*, *Urechis caupo*, *Marthasterias glacialis*, etc.). Il participa ainsi à la caractérisation des acteurs kinasiques contrôlant le cycle de division cellulaire, et notamment à la découverte de la kinase dépendante de cycline, CDK1/cycline B [4]. La conservation des acteurs régulant la division cellulaire a permis l'utilisation d'une grande variété de modèles cellulaires. Le choix des modèles marins a été judicieux pour les études biochimiques du fait de la division synchronisée des cellules, qui donne ainsi accès à des quantités importantes de protéines. Le laboratoire a alors développé des stratégies permettant de purifier, à partir d'ovocytes d'étoile de mer, des quantités


¹ <http://www.embric.eu>

Optimized extraction of microalgae's metabolites: a crucial step in High-Throughput Screening programs dedicated to phytoplankton chemodiversity



5th World Congress on **Biotechnology** Biotechnology-2014
 June 25-27, 2014, Valencia, Spain Track 12-1 Marine natural products and biomolecules

 Serive B., Kaas R., La Barre S., Bach S., Cadoret J-P.

With the recent development of state-of-the-art technologies (e.g. hyphenated MS techniques) and methodologies (e.g. dereplication), the scientific community is interested in the exploration of poorly chemically studied bioresources. The high diversity of interacting phytoplankton species suggests an important and highly diverse chemical repertoire (e.g. isoprenoids, toxins, polysaccharides, PUFAs, oxylipins, phycobiliproteins) which may inspire applications in health, nutrition and biotechnology. Biosynthesis of these metabolites is strongly dependent upon their environment/culture conditions which may be investigated using OMICS approaches. In microalgae, a major bottleneck is the difficulty in extracting deeply inaccessible molecules, an important issue that demands adapted solutions prior to considering High-Throughput Screening (HTS). Bioactive minority metabolites may pass unnoticed on spectra and thus require special attention. The extraction of metabolites may prove difficult due to the presence of highly resistant cell walls (*Phaeodactylum tricorneratum*), or of exopolysaccharidic secretions surrounding the cell membrane (*Porphyridium purpureum*). The Mix Mill process (vibrating microbeads) which gave excellent extraction yields without chemical alteration of the analytes and is fully compatible with HPLC and LC-MS analysis was optimised. Being accurate, simple to operate, rapid, safe and preserving sensitive molecules, makes the Mix Mill process suitable for the screening of microalgal chemodiversity. This methodology was applied in the Photomer, and currently in OCEANOMICS and OCEANCHARCoT programs, all being dedicated to the identification of new marine metabolites with high added value. Finally, this methodology represents a significant improvement in the field of OMICS studies from microalgae, as it provides the most representative estimate of their exploitable chemical diversity.

Marie Curie International Outgoing Fellowship – Laureate 2013

OCEANCHARCoT
OCEAN CHemodiversity Against Cell cycle Targets



CNRS UPMC
Station Biologique
Roscoff



Griffith UNIVERSITY
Eskitis Institute



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Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Selection and optimisation of a method for efficient metabolites extraction from microalgae

Benoît Serive^a, Raymond Kaas^{a,*}, Jean-Baptiste Bérard^a, Virginie Pasquet^b, Laurent Picot^c, Jean-Paul Cadoret^a

^aIFREMER, Laboratoire de Physiologie et Biotechnologie des Algues, 44311 Nantes, France

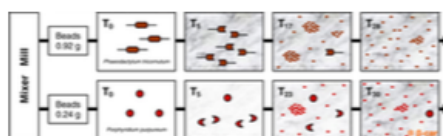
^bMer, Molécules, Santé, Institut Universitaire Mer et Littoral, FR 3473 CNRS, LUNAM Université, Université du Maine, EA 2160, IUT de Laval, 53020 Laval, Cedex 9, France

^cUniversity of La Rochelle, UMR CNRS 7266 LIENSs, F-17042 La Rochelle, France

HIGHLIGHTS

- ▶ Nine disruption techniques were tested on two microalgae models.
- ▶ Image analysis was used to evaluate the efficiency of disruption techniques.
- ▶ The best grinding method was the mixer mill with polypropylen grinding jars.
- ▶ The disruption method was optimised in the objective of high throughput screening.
- ▶ Pigments were good candidates to follow extraction of fragile metabolites.

GRAPHICAL ABSTRACT



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ABSTRACT

Over the last decade, the use of microalgae for biofuel production and carbon dioxide sequestration has become a challenge worldwide. Processing costs are still too high for these methods to be profitable though, leading to a need to find high value by-products to optimise the added value of this biomass. For high-throughput screening of such metabolites, it is essential to reach the inner content of the cell. This paper presents research and development of a technique enabling a high extraction yield of any metabolite, taking into account the difficulty of extracting bound and/or inaccessible molecules with a wide variety of polarities. To this end, several disruption techniques were tested at laboratory scale on two biological models: *Porphyridium purpureum* and *Phaeodactylum tricornutum*. A mixer mill gave the best results, offering access to a broad diversity of metabolites from microalgae for high-throughput screening.

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1. Introduction

Microalgae are nowadays considered to be the best source for biofuel production due to their ability to produce large amounts of triglycerides or to be converted into biogas. These photosynthetic micro-organisms are capable of converting carbon dioxide

into lipids representing up to the half of their dry weight (Chisti, 2007). While primary metabolites are the result of the unity of life on earth, secondary metabolites are the expression of its biodiversity (Kornprobst, 2005). Many of them have a high added value (Harun et al., 2010), such as isoprenoids, alkaloids, toxins, polysaccharides, polyunsaturated fatty acids, oxylipins, enzymes, phycobiliproteins and non-ribosomal peptides, which find applications in health, pharmacology, nutrition and biotechnology. Among all new marine molecules identified, the proportion produced by

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E-mail address: Raymond.Kaas@ifremer.fr (R. Kaas).

Article

Antiproliferative Activity of Violaxanthin Isolated from Bioguided Fractionation of *Dunaliella tertiolecta* Extracts

Virginie Pasquet¹, Perrine Morisset¹, Said Ihammouine¹, Amandine Chepied¹, Lucie Aumailley¹, Jean-Baptiste Berard², Benoit Serive², Raymond Kaas², Isabelle Lanneluc¹, Valerie Thiery¹, Mathieu Lafferriere¹, Jean-Marie Piot¹, Thierry Patrice³, Jean-Paul Cadoret² and Laurent Picot^{1*}

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³ Department LASER, CHU Nantes, F-44093 Nantes, France; E-Mail: thierry.patrice@chu-nantes.fr

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Abstract: *Dunaliella tertiolecta* (DT) was chemically investigated to isolate molecules inhibiting cancer cell proliferation and inducing apoptosis *in vitro*. The potency to inhibit cell growth was used for the bio-guided fractionation and isolation of active compounds using chromatographic techniques. The DT dichloromethane extract exhibited a strong anti-proliferative activity on MCF-7 and LNCaP cells, and was further fractionated and sub-fractionated by RP-HPLC. High resolution mass spectrometry and spectrophotometric analysis unequivocally identified violaxanthin as the most antiproliferative molecule present in DT DCM extract. Violaxanthin purified from DT induced MCF-7 dose-dependent growth inhibition in continuous and discontinuous treatments, at concentrations as low as 0.1 $\mu\text{g}\cdot\text{mL}^{-1}$ (0.17 μM). Phosphatidylserine exposure, typical of early apoptosis, was observed after 48 h treatment at 8 $\mu\text{g}\cdot\text{mL}^{-1}$ (13.3 μM) but no DNA fragmentation, characteristic of late apoptosis steps, could be detected even after 72 h treatment at



Study on the microalgal pigments extraction process: Performance of microwave assisted extraction

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ABSTRACT

The performance of microwaves irradiation (MAE and VMAE) to extract pigments from two marine microalgae was compared to conventional processes (cold and hot soaking and ultrasound-assisted extraction). Pigments were quantified by RP-HPLC and extraction performance was assessed regarding rapidity, reproducibility and extraction yields. Scanning electron microscopy was used at all extraction steps to assess the impact of the process on microalgal cell integrity. Freeze-drying and pigments extraction preserved microalgal cell integrity (except sonication) and evoked agglutination in superposed cells layers. All processes performed on *Dunaliella tertiolecta* (chlorophyte) lead to rapid pigments extraction, and equivalent pigments extraction yields, the absence of frustule allowing immediate solvent penetration in microalgal cells. In contrast, presence of the frustule in the diatom *Cylindrotheca closterium* (bacillariophyte) constituted a mechanical barrier to pigment extraction. MAE was identified as the best extraction process for CC pigments as it combined rapidity, reproducibility, homogeneous heating and high extraction yields.

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1. Introduction

Marine microalgae constitute one of the most diverse group of oceanic microorganisms, with an estimated 2×10^5 to several millions species [1], from which only 35,000 are described. Extensive screening of cultivated species has led to the isolation and chemical determination of over 15,000 compounds, including fatty acids, sterols, phenolic compounds, terpenes, enzymes, polysaccharides, alkaloids, toxins and pigments [2]. Because of their high biodiversity and huge productivity, microalgae represent an untapped resource offering great possibilities for the isolation of original natural substances of interest for food, health or biotechnological applications [3,4]. Their interest also lies in their convenient use as a biotechnological biomass, as they can easily be grown in controlled conditions, handled as conventional lab

microorganisms, and genetically modified without any risk of dissemination.

Lipids and pigments extraction processes applied to microalgae are mainly derived from phytochemical techniques developed on superior plants and macroalgae. The main parameters driving selection of an extraction technology are biochemical characteristics of extracted molecules, rapidity, limitation of solvent use, reproducibility, extraction yield, selectivity, protection of extracted molecules against chemical transformation, dimension, cost and easiness [5,6]. Classical organic solvent extraction techniques, including maceration (soaking), percolation, counter-current extraction, pressurized liquid extraction, and soxhlet are widespread technologies described to extract lipids and pigments. These processes are reproducible, allow the rapid extraction of chemicals, but usually imply the use of large amounts of solvents, and the risk of thermal denaturation or transformation of molecules of interest [5]. Coupling steam distillation or hot water extraction with maceration in solvent increases extraction yields for plant essential oils and bioactive compounds [7], but thermolabile molecules are damaged using this technology. The use of enzymes, such as xylanases, pectinases or cellulases, to enhance pigments extractability rates was proposed and validated for superior plants tissues [8,9] and macroalgae [10], and

Abbreviations: CC, *Cylindrotheca closterium*; Chl, chlorophyll; DT, *Dunaliella tertiolecta*; MAE, microwave-assisted extraction; PVDF, poly(vinylidene difluoride); RT, room temperature; SCF, supercritical fluid; UAE, ultrasound assisted extraction; VMAE, vacuum-microwave assisted extraction.

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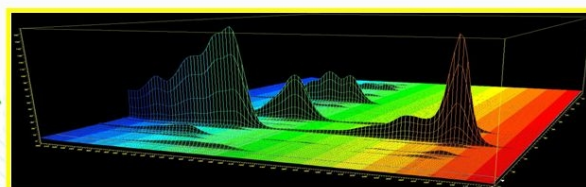
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Microalgae pigment study for oceanography and biotechnologies : potential, uses and limits.



Étude des pigments de microalgues en océanographie et en biotechnologies : potentiel, applications, limites



Assemblée générale BIOCHIMAR – Lorient, 7 et 8 novembre 2013

Serive B., Kaas R., Nicolau E., Bérard J-B., Cadoret J-P.

Annual general meeting of BIOCHIMAR group

Website

<http://biochimar.icsn.cnrs-gif.fr/spip.php?article29>

Creation date

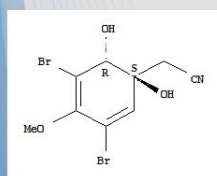
08 nov. 2013

Conference Uses from sponges

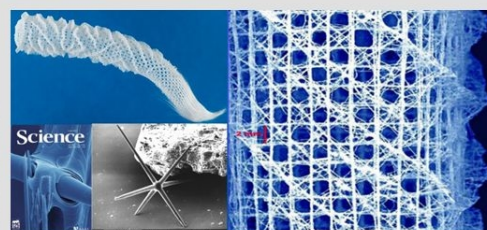


Les spongiaires :

des organismes primitifs au potentiel industriel et biotechnologique conséquent




Aeropylsinin-1



Lézardrieux, le 18/05/2013 – Stage de validation Bio N1

Benoit.Serive@ifremer.fr

Conference 01/03/2012



Les algues

Conférence de la
Commission
Environnement
et Biologie
Subaquatique
FFESSM CODEP 44

**Un réservoir d'applications incroyables
à l'image de leur diversité biologique**

Les algues en quelques mots...

Le : 1^{er} mars 2012
de 20h à 22h


Lieu : Salle La Mano
3, rue Eugène Thomas
44300 NANTES

Les océans couvrent 71% de la superficie de notre planète, un des écosystèmes privilégiés des algues. Qu'elles soient microscopiques ou visibles à l'œil nu, elles représentent 80% des végétaux présents sur Terre et constituent donc un des poumons de celle-ci. Elles nous fournissent par conséquent la majeure partie de l'oxygène que nous respirons. En dehors de ces quelques considérations non négligeables, que savons-nous vraiment d'elles ?

A travers cette conférence, nous découvrirons quel immense patrimoine biologique se cache derrière ce petit mot si banal, quelles sont les nombreuses applications que nous pouvons tirer de leurs spécificités dans le quotidien, et le potentiel des applications qui restent à inventer.

Pour les plus téméraires, la soirée se terminera par une petite dégustation d'algues.

Présenté par Benoit SERIVE - Entrée libre



Algae : an incredible reservoir of applications as their biodiversity

Creation date

01 mars 2012

Conference 20/10/2011



Étude des pigments de microalgues en océanographie et en biotechnologies au moyen d'un procédé de déréplication CLHP-UV DAD



Laboratoire de Physiologie et Biotechnologie des Algues
Directeur de thèse : Raymond Kaas

Journées scientifiques de l'École Doctorale VENAM – 20 et 21 octobre 2011

par Benoit.Serive@ifremer.fr

Study of microalgal pigments in oceanography and biotechnology through a process of dereplication HPLC-UV DAD

Creation date

20 oct. 2011

MMS
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molécules
santé

Laboratoire EA2180
Faculté de Pharmacie
Université de Nantes

Collecte d'organismes marins tropicaux en plongée



De l'étude de la biodiversité à l'émergence
de nouveaux médicaments

Conférence du 18/03/2011 - de 19h30 à 21h30
Présentée par B. Serive - Amphithéâtre A
IUT - 2, avenue du Professeur Jean Rouxel
44 475 Carquefou

Entrée libre



Collection of marine tropical organisms in scuba-diving - From the study of biodiversity to the emergence of new drugs

Creation date

18 mars 2011

adebioTech **IFREMER** **ANR** **CNRS**

Composition pigmentaire d'espèces phytoplanctoniques originales au moyen d'un procédé de déréplication CLHP-UV DAD

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Introduction
 Depuis les années 1990, les pigments sont reconnus comme un moyen d'étudier la diversité ainsi que la répartition géographique et bathytropique du phytoplancton en océanographie. Le phytoplancton présente une très grande diversité génétique qui se traduit par une diversité tout aussi importante de pigments. Les espèces peuvent être ainsi regroupées en divisions ou classes d'algues selon l'abondance ou non de certains pigments. Le programme CHIBITAX a été développé afin d'intégrer les concentrations molaires détectées lors de l'extraction en CLHP d'échantillons naturels. Cependant, ce système est encore limité de par le manque de données pigmentaires sur les espèces phytoplanctoniques.

Objectifs
 Le rôle en place de l'étude de déréplication des pigments a mené à l'identification de certains plus représentés présents dans 3 espèces. L'une appartient au phylum des Charophytes (Chlorococum peruvianum), le second appartient au phylum des Charophytes (Chlorococum adhaerens) et le troisième au phylum des Coccoliphytes (Pheobrobacteres salinis) (Fig. 1). Chacun de ces 3 phyla est peu connu sur le plan pigmentaire et les résultats corréleront ainsi un apport de connaissances non biologiques qui élucidera les positions de ces espèces dans les écosystèmes aquatiques.

Démarche de déréplication
 Le processus de déréplication consiste à identifier au sein de mélanges complexes toutes les molécules connues grâce à leurs propriétés physico-chimiques. Cette technique a été appliquée à l'étude des pigments organo-solubles par CLHP couplée à un détecteur UV-DAD. La méthode d'élution standardisée et reproductible retenue est celle de Van Heulekom & Thunnis, 2001. Celle-ci a été modifiée pour optimiser l'élution des molécules d'élites par plus d'expériences. Pour l'obtention d'une discrimination efficace des pigments, le choix de la résolution constitue l'étape la plus importante par conséquent des différentes méthodes testées. Elle reflète la capacité d'un système chromatographique à retrouver la ligne de base entre 2 molécules consécutives.

Optimisation de la méthode d'élution | **Algorithme d'identification** | **Résultats**

Perspectives
 Le couplage CLHP - détecteur UV-DAD est un outil qui s'avère rapide et puissant si l'on possède une bibliothèque de standards suffisante pour couvrir la diversité des pigments naturels que l'on traite chez les microalgues. La présence de la déréplication sera délicate en ce qui concerne le couplage d'un spectromètre de masse. Celui-ci permettra de confirmer l'identification des pigments et sera un outil très intéressant à développer en océanographie. La quantification de masses permettrait également une quantification précise des cultures. En plus, leur caractère d'élution moléculaire différents ne permet pas de les quantifier en utilisant UV-DAD et un standard de chaque pigment n'est pas adapté en ce qui concerne la calibration, quantification. D'autre part, le spectromètre de masse permet une grande caractérisation des pigments moléculaires car connus et de leurs dérivés (polymères et formes de dégradation). Les études quantitatives peuvent évaluer des échantillons par l'utilisation d'un détecteur ESI-D (réponse Light Scattering Detector) ou d'un détecteur cationique.

Yves René Servin@nantes.fr - Tel : 02 40 37 42 20 2018 - Colloque Algues - Nantes de 2018

A pigment composition of original phytoplankton species by means of a method of HPLC-UV DAD dereplication

Collection of marine organisms in diving



Mission in Senegal mangrove

