Localization and characterization of CYP76AE2 part of thapsigargin biosynthesis in Thapsia garganica

The Mediterranean plant *Thapsia garganica* (dicot, Apioaceae), also known as Deadly carrot, produces the highly toxic compound thapsigargin. This compound is a potent inhibitor of the SERCA calcium pump in mammals, and is of industrial importance as the active moiety of the anticancer drug Mipsagargin, currently in clinical trials. Knowledge of thapsigargin *in planta* storage and biosynthesis has so far been limited. Here we present the putative second step in thapsigargin biosynthesis, by showing that the cytochrome P450 *TgCYP76AE2*, transiently expressed in *Nicotiana benthamiana*, converts epikunzeaol into epidihydrocostunolide. Furthermore, we show that thapsigargin is likely to be stored in secretory ducts in the roots. Transcripts from *TgTPS2* (epikunzeaol synthase) and *TgCYP76AE2* in roots were only found in the epithelial cells lining these secretory ducts. This emphasizes the involvement of these cells in the biosynthesis of thapsigargin. This study paves the way for the further studies of thapsigargin biosynthesis.
Scopus rating (2015): SJR 3.575 SNIP 1.798 CiteScore 6.69
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 4.085 SNIP 2.096 CiteScore 7.16
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): SJR 4.162 SNIP 2.091 CiteScore 7.71
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): SJR 4.017 SNIP 1.945 CiteScore 6.78
ISI indexed (2012): ISI indexed yes
BFI (2011): BFI-level 2
Scopus rating (2011): SJR 3.864 SNIP 1.857 CiteScore 6.45
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 2
Scopus rating (2010): SJR 3.775 SNIP 1.715
BFI (2009): BFI-level 2
Scopus rating (2009): SJR 4.099 SNIP 1.796
Web of Science (2009): Indexed yes
BFI (2008): BFI-level 2
Scopus rating (2008): SJR 4.083 SNIP 1.653
Web of Science (2008): Indexed yes
Scopus rating (2007): SJR 4.405 SNIP 1.792
Web of Science (2007): Indexed yes
Scopus rating (2006): SJR 3.902 SNIP 1.622
Web of Science (2006): Indexed yes
Scopus rating (2005): SJR 3.459 SNIP 1.7
Web of Science (2005): Indexed yes
Scopus rating (2004): SJR 3.379 SNIP 1.693
Web of Science (2004): Indexed yes
Scopus rating (2003): SJR 3.207 SNIP 1.689
Web of Science (2003): Indexed yes
Scopus rating (2002): SJR 3.035 SNIP 1.761
Web of Science (2002): Indexed yes
Scopus rating (2001): SJR 2.879 SNIP 1.767
Web of Science (2001): Indexed yes
Scopus rating (2000): SJR 2.901 SNIP 1.653
Web of Science (2000): Indexed yes
Scopus rating (1999): SJR 2.993 SNIP 1.608

Original language: English
Publication: Research - peer-review › Journal article – Annual report year: 2018

On the biosynthetic origin of carminic acid
The chemical composition of the scale insect Dactylopius coccus was analyzed with the aim to discover new possible intermediates in the biosynthesis of carminic acid. UPLC-DAD/HRMS analyses of fresh and dried insects resulted in the identification of three novel carminic acid analogues and the verification of several previously described intermediates. Structural elucidation revealed that the three novel compounds were deoxyerythrolaccin-O-glucosyl (DE-O-Glcp), 5,6-didehydroxyerythrolaccin 3-O-β-D-glucopyranoside (DDE-3-O-Glcp), and flavokermesic acid anthrone (FKA). The finding of FKA in D. coccus provides solid evidence of a polyketide, rather than a shikimate, origin of coccid pigments. Based on the newly identified compounds, we present a detailed biosynthetic scheme that accounts for the formation of carminic acid (CA) in D. coccus and all described coccid pigments which share a flavokermesic acid (FK) core. Detection of coccid pigment intermediates in members of the Planococcus (mealybugs) and Pseudaulacaspis genera shows that the ability to form these pigments is taxonomically more widely spread than previously documented. The shared core-FK-biosynthetic pathway and wider taxonomic distribution suggests a common evolutionary origin for the trait in all coccid dye producing insect species.
Anthraquinones, Biosynthesis, Carmine, Carminic acid, Coccid pigment, Dactylopius coccus, Insects, Polyketide
Phylogenetic distribution of roseobacticides in the Roseobacter group and their effect on microalgae

The Roseobacter-group species Phaeobacter inhibens produces the antibacterial tropodithietic acid (TDA) and the algaecidal roseobacticides with both compound classes sharing part of the same biosynthetic pathway. The purpose of this study was to investigate the production of roseobacticides more broadly in TDA-producing roseobacters and to compare the effect of producers and non-producers on microalgae. Of 33 roseobacters analyzed, roseobacticide production was a unique feature of TDA-producing P. inhibens, P. gallaeciensis and P. piscinae strains. One TDA-producing Phaeobacter, 27-4, did not produce roseobacticides, possibly due to a transposable element. TDA-producing Ruegeria and Pseudovibrio did not produce roseobacticides. Addition of roseobacticide-containing bacterial extracts affected the growth of the microalgae Rhodomonas salina, Thalassiosira pseudonana and Emiliania huxleyi, while growth of Tetraselmis suecica was unaffected. During co-cultivation, growth of E. huxleyi was initially stimulated by the roseobacticide producer DSM 17395, while the subsequent decline in algal cell numbers during senescence was enhanced. Strain 27-4 that does not produce roseobacticides had no effect on algal growth. Both bacterial strains, DSM 17395 and 27-4, grew during co-cultivation presumably utilizing algal exudates. Furthermore, TDA-producing roseobacters have potential as probiotics in marine larviculture and it is promising that the live feed Tetraselmis was unaffected by roseobacticides-containing extracts.

General information
State: Published
Organisations: Department of Biotechnology and Biomedicine, Bacterial Ecophysiology and Biotechnology, DTU Metabolomics Core, Natural Product Discovery
Pages: 383-393
Publication date: 2018
Main Research Area: Technical/natural sciences

Publication information
Journal: Environmental Microbiology Reports
Volume: 10
Issue number: 3
ISSN (Print): 1758-2229
Ratings:
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 3.47 SJR 1.475 SNIP 0.952
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 1.599 SNIP 0.959 CiteScore 3.39
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 1.524 SNIP 0.861 CiteScore 3.14
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 1.465 SNIP 0.941 CiteScore 3.24
ISI indexed (2013): ISI indexed yes
Scopus rating (2012): SJR 1.472 SNIP 0.907 CiteScore 2.99
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
Scopus rating (2011): SJR 1.631 SNIP 1.247 CiteScore 2.77
ISI indexed (2011): ISI indexed no
Scopus rating (2010): SJR 1.251 SNIP 0.935
Localization and in-vivo characterization of thapsia garganica CYP76AE2 indicates a role in thapsigargin biosynthesis

The Mediterranean plant Thapsia garganica (dicot, Apiaceae), also known as deadly carrot, produces the highly toxic compound thapsigargin. This compound is a potent inhibitor of the sarcoplasmic-endoplasmic reticulum Ca\(^{2+}\) -ATPase calcium pump in mammals and is of industrial importance as the active moiety of the anticancer drug mipsagargin, currently in clinical trials. Knowledge of thapsigargin in planta storage and biosynthesis has been limited. Here, we present the putative second step in thapsigargin biosynthesis, by showing that the cytochrome P450 TgCYP76AE2, transiently expressed in Nicotiana benthamiana, converts epikunzeaol into epidihydrocostunolide. Furthermore, we show that thapsigargin is likely to be stored in secretory ducts in the roots. Transcripts from TgTPS2 (epikunzeaol synthase) and TgCYP76AE2 in roots were found only in the epithelial cells lining these secretory ducts. This emphasizes the involvement of these cells in the biosynthesis of thapsigargin. This study paves the way for further studies of thapsigargin biosynthesis.

General information
State: Published
Organisations: Natural Product Discovery, Department of Biotechnology and Biomedicine, Photosynthetic Cell Factories, University of Copenhagen, University of Melbourne, University of California at Berkeley, Bruker Biospin Scandinavia AB, Technical University of Denmark
Authors: Andersen, T. B. (Ekstern), Martinez-Swatson, K. A. (Ekstern), Rasmussen, S. A. (Intern), Boughton, B. A. (Ekstern), Jørgensen, K. (Ekstern), Andersen-Ranberg, J. (Ekstern), Nyberg, N. (Ekstern), Christensen, S. B. (Ekstern), Simonsen, H. T. (Intern)
Number of pages: 17
Pages: 56-72
Publication date: 1 May 2017
Main Research Area: Technical/natural sciences

Publication information
Journal: Plant Physiology
Volume: 174
Issue number: 1
ISSN (Print): 0032-0889
Ratings:
BFI (2018): BFI-level 2
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 2
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 6.58 SJR 3.735 SNIP 1.75
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 3.575 SNIP 1.798 CiteScore 6.69
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 4.085 SNIP 2.096 CiteScore 7.16
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): SJR 4.162 SNIP 2.091 CiteScore 7.71
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): SJR 4.017 SNIP 1.945 CiteScore 6.78
ISI indexed (2012): ISI indexed yes
BFI (2011): BFI-level 2
Scopus rating (2011): SJR 3.864 SNIP 1.857 CiteScore 6.45
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
Characterization of a membrane-bound C-glucosyltransferase responsible for carminic acid biosynthesis in Dactylopius coccus Costa

Carminic acid, a glucosylated anthraquinone found in scale insects like Dactylopius coccus, has since ancient times been used as a red colorant in various applications. Here we show that a membrane-bound C-glucosyltransferase, isolated from D. coccus and designated DcUGT2, catalyzes the glucosylation of flavokermesic acid and kermesic acid into their respective C-glucosides dcII and carminic acid. DcUGT2 is predicted to be a type I integral endoplasmic reticulum (ER) membrane protein, containing a cleavable N-terminal signal peptide and a C-terminal transmembrane helix that anchors the protein to the ER, followed by a short cytoplasmic tail. DcUGT2 is found to be heavily glycosylated. Truncated DcUGT2 proteins synthesized in yeast indicate the presence of an internal ER-targeting signal. The cleavable N-terminal signal peptide is shown to be essential for the activity of DcUGT2, whereas the transmembrane helix/cytoplasmic domains, although important, are not crucial for its catalytic function.

General information
State: Published
Organisations: Department of Biotechnology and Biomedicine, Natural Product Discovery, Biosynthetic Pathway Engineering, University of Copenhagen, University of Southern Denmark, Chr. Hansen A/S
Authors: Kannangara, R. (Ekstern), Siukstaite, L. (Ekstern), Borch-Jensen, J. (Ekstern), Madsen, B. (Ekstern), Kongstad, K. T. (Ekstern), Stærk, D. (Ekstern), Bennedsen, M. (Ekstern), Okkels, F. T. (Ekstern), Rasmussen, S. A. (Intern), Larsen, T. O. (Intern), Frandsen, R. J. N. (Intern), Møller, B. L. (Ekstern)
Number of pages: 12
Publication date: 2017
Main Research Area: Technical/natural sciences

Publication information
Journal: Nature Communications
Volume: 8
Issue number: 1
HPLC-HRMS Quantification of the Ichthyotoxin Karmitoxin from Karlodinium armiger

Being able to quantify ichthyotoxic metabolites from microalgae allows for the determination of ecologically-relevant concentrations that can be simulated in laboratory experiments, as well as to investigate bioaccumulation and degradation. Here, the ichthyotoxin karmitoxin, produced by Karlodinium armiger, was quantified in laboratory-grown cultures using high-performance liquid chromatography (HPLC) coupled to electrospray ionisation high-resolution time-of-flight mass spectrometry (HRMS). Prior to the quantification of karmitoxin, a standard of karmitoxin was purified from K. armiger cultures (80 L). The standard was quantified by fluorescent derivatisation using Waters AccQ-Fluor reagent and derivatised fumonisin B₁ and fumonisin B₂ as standards, as each contain a primary amine. Various sample preparation methods for whole culture samples were assessed, including six different solid phase extraction substrates. During analysis of culture samples, MS source conditions were monitored with chloramphenicol and valinomycin as external standards over prolonged injection sequences (>12 h) and karmitoxin concentrations were determined using the response factor of a closely eluting iturin A2 internal standard. Using this method the limit of quantification was 0.11 μg·mL⁻¹, and the limit of detection was found to be 0.03 μg·mL⁻¹. Matrix effects were determined with the use of K. armiger cultures grown with 13C-labelled bicarbonate as the primary carbon source.

General information
State: Published
Organisations: National Food Institute, Department of Biotechnology and Biomedicine, Natural Product Discovery, DTU Metabolomics Core, Research Group for Analytical Food Chemistry, Universidade Federal de Sao Paulo, University of Copenhagen
Authors: Andersen, A. J. C. (Intern), Soman De Medeiros, L. (Ekstern), Binzer, S. B. (Ekstern), Rasmussen, S. A. (Intern), Hansen, P. J. (Ekstern), Nielsen, K. F. (Intern), Jørgensen, K. (Intern), Larsen, T. O. (Intern)
Karmitoxin: An amine containing polyhydroxy-polyene toxin from the marine dinoflagellate Karlodinium armiger

Marine algae from the genus Karlodinium are known to be involved in fish-kill events worldwide. Here we report for the first time the chemistry and bioactivity of a natural product from the newly described mixotrophic dinoflagellate Karlodinium armiger. Our work describes the isolation and structural characterization of a new polyhydroxy-polyene named karmitoxin. The structure elucidation work was facilitated by use of 13C enrichment and high-field 2D NMR spectroscopy, where 1H–13C long-range correlations turned out to be very informative. Karmitoxin is structurally related to amphidinols and karlotoxins; however it differs by containing the longest carbon–carbon backbone discovered for this class of compounds, as well as a primary amino group. Karmitoxin showed potent nanomolar cytotoxic activity in an RTgill-W1 cell assay as well as rapid immobilization and eventual mortality of the copepod Acartia tonsa, a natural grazer of K. armiger.
Black perithecial pigmentation in *Fusarium* species is due to the accumulation of 5-deoxybostrycoidin-based melanin

Biosynthesis of the black perithecial pigment in the filamentous fungus *Fusarium graminearum* is dependent on the polyketide synthase PGL1 (oPKS3). A seven-membered PGL1 gene cluster was identified by over-expression of the cluster specific transcription factor pglR. Targeted gene replacement showed that PGL1, pglJ, pglM and pglV were essential for the production of the perithecial pigment. Over-expression of PGL1 resulted in the production of 6-O-demethyl-5-deoxybostrycoidin (1), 5-deoxybostrycoidin (2), and three novel compounds 5-deoxybostrycoidin anthrone (3), 6-O-demethyl-5-deoxybostrycoidin anthrone (4) and purpurfusarin (5). The novel dimeric bostrycoidin purpurfusarin (5) was found to inhibit the growth of *Candida albicans* with an IC50 of 8.0 +/-1.9 μM. The results show that *Fusarium* species with black perithecia have a previously undescribed form of 5-deoxybostrycoidin based melanin in their fruiting bodies.
Microalgae, particularly those from the lineage Dinoflagellata, are very well-known for their ability to produce phycotoxins that may accumulate in the marine food chain and eventually cause poisoning in humans. This includes toxins accumulating in shellfish, such as saxitoxin, okadaic acid, yessotoxins, azaspiracids, brevetoxins, and pinnatoxins. Other toxins, such as ciguatoxins and maitotoxins, accumulate in fish, where, as is the case for the latter compounds, they can be metabolized to even more toxic metabolites. On the other hand, much less is known about the chemical nature of compounds that are toxic to fish, the so-called ichthyotoxins. Despite numerous reports of algal blooms causing massive fish kills worldwide, only a few types of compounds, such as the karlotoxins, have been proven to be true ichthyotoxins. This review will highlight marine microalgae as the source of some of the most complex natural compounds known to mankind, with chemical structures that show no resemblance to what has been characterized from plants, fungi, or bacteria. In addition, it will summarize algal species known to be related to fish-killing blooms, but from which ichthyotoxins are yet to be characterized.
Blooms of the microalga <i>Prymnesium parvum</i> cause devastating fish kills worldwide, which are suspected to be caused by the supersized ladder-frame polyether toxins prymnesin-1 and -2. These toxins have, however, only been detected from <i>P. parvum</i> in rare cases since they were originally described two decades ago. Here, we report the isolation and characterization of a novel B-type prymnesin, based on extensive analysis of 2D- and 3D-NMR data of natural as well as 90% 13C enriched material. B-type prymnesins lack a complete 1,6-dioxadecalin core unit, which is replaced by a short acyclic C2 linkage compared to the structure of the original prymnesins. Comparison of the bioactivity of prymnesin-2 with
prymnesin-B1 in an RTgill-W1 cell line assay identified both compounds as toxic in the low nanomolar range. Chemical investigations by liquid chromatography high-resolution mass spectrometry (LC-HRMS) of 10 strains of *P. parvum* collected worldwide showed that only one strain produced the original prymnesin-1 and -2, whereas four strains produced the novel B-type prymnesin. In total 13 further prymnesin analogues differing in their core backbone and chlorination and glycosylation patterns could be tentatively detected by LC-MS/HRMS, including a likely C-type prymnesin in five strains. Altogether, our work indicates that evolution of prymnesins has yielded a diverse family of fish-killing toxins that occurs around the globe and has significant ecological and economic impact.

**General information**
State: Published
Organisations: Department of Systems Biology, Department of Chemistry, Organic Chemistry, Metabolomics Platform, University of Copenhagen
Authors: Rasmussen, S. A. (Intern), Meier, S. (Intern), Andersen, N. G. (Ekstern), Blossom, H. E. (Ekstern), Duus, J. Ø. (Intern), Nielsen, K. F. (Intern), Hansen, P. J. (Ekstern), Larsen, T. O. (Intern)
Number of pages: 7
Pages: 2250-2256
Publication date: 2016
Main Research Area: Technical/natural sciences

**Publication information**
Journal: Journal of Natural Products
Volume: 79
Issue number: 9
ISSN (Print): 0163-3864
Ratings:
BFI (2018): BFI-level 2
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 2
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 3.41 SJR 1.22 SNIP 1.408
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 1.395 SNIP 1.758 CiteScore 4.14
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 1.333 SNIP 1.827 CiteScore 3.68
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): SJR 1.516 SNIP 1.716 CiteScore 3.75
ISI indexed (2013): ISI indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): SJR 1.428 SNIP 1.538 CiteScore 3.23
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 2
Scopus rating (2011): SJR 1.39 SNIP 1.508 CiteScore 3.11
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 2
Scopus rating (2010): SJR 1.368 SNIP 1.601
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 2
Scopus rating (2009): SJR 1.461 SNIP 1.5
Web of Science (2009): Indexed yes
BFI (2008): BFI-level 2
Scopus rating (2008): SJR 1.328 SNIP 1.423
Web of Science (2008): Indexed yes
Chemodiversity of the ladder-frame prymnesin polyethers of the fish-killing microalgal Prymnesium parvum

General information
State: Published
Organisations: Department of Systems Biology, Department of Chemistry, Organic Chemistry, Metabolomics Platform, University of Copenhagen
Authors: Rasmussen, S. A. (Intern), Meier, S. (Intern), Gedsted Andersen, N. (Ekstern), Blossom, H. (Ekstern), Duus, J. Ø. (Intern), Nielsen, K. F. (Intern), Larsen, T. O. (Intern)
Number of pages: 1
Publication date: 2016
Conference: 9th Joint Meeting of AFERP, ASP, GA, JSP, PSE & SIF, Copenhagen, Denmark, 24/07/2016 - 24/07/2016
Main Research Area: Technical/natural sciences

Publication information
Journal: Planta Medica
Volume: 81
Issue number: S 01
ISSN (Print): 0032-0943
Ratings:
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 1.97 SJR 0.654 SNIP 0.94
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 0.637 SNIP 0.991 CiteScore 2.1
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 0.762 SNIP 1.135 CiteScore 2.15
Web of Science (2014): Indexed yes

Bibliographical note
This is an open access article published under an ACS AuthorChoice License, which permits copying and redistribution of the article or any adaptations for non-commercial purposes.
Source: FindIt
Source-ID: 2342049522
Publication: Research - peer-review › Journal article – Annual report year: 2016
Prymnesium parvum revisited: relationship between allelopathy, ichthyotoxicity, and chemical profiles in 5 strains

Bioassay-guided discovery of ichthyotoxic algal compounds using in vivo fish assays is labor intensive, costly, and highly regulated. Since the mode of action of most known algal-mediated fish-killing toxins is damage to the cell membranes in the gills, various types of cell-based bioassays are often used for bioassay-guided purification of new ichthyotoxins. Here we tested the hypothesis that allelopathy is related to ichthyotoxicity and thus that a microalgal bioassay can be used as a proxy for ichthyotoxicity by comparing the toxicity of five strains of Prymnesium parvum toward rainbow trout (Oncorhynchus mykiss, 10 g) and the microalga Teleaulax acuta. No relationship between median effective concentrations (EC₅₀) on fish and median lethal concentrations (LC₅₀) on algae was observed in the 5 strains showing that a microalgal bioassay cannot be used as a proxy for ichthyotoxicity. Fish were more sensitive to P. parvum with EC₅₀s ranging from 6 × 10⁻³ to 40 × 10⁻³ cells ml⁻¹, compared to the test alga where LC₅₀s ranged from 30 × 10⁻³ to nearly non-toxic at 500 × 10⁻³ cells ml⁻¹. In addition, the cellular concentrations of two recently suggested ichthyotoxins produced by P. parvum, the “golden algae toxins”, GAT 512 and a novel GAT 510, did not show any relationship to either ichthyotoxicity or allelopathy, and are not the biologically relevant toxins, but are simply lipids found in algal chloroplasts. Finally, we demonstrated that the recently suggested ichthyotoxin, oleamide, could not be detected in any of the five P. parvum strains above the limit of detection, nor was it found in a ¹³C-labeled strain. Instead we document that oleamide can easily be extracted from plastic materials, which may have been the source of oleamide reported previously.

General information
State: Published
Organisations: Department of Systems Biology, Metabolomics Platform, University of Copenhagen
Authors: Blossom, H. E. (Ekstern), Rasmussen, S. A. (Intern), Andersen, N. G. (Intern), Larsen, T. O. (Intern), Nielsen, K. F. (Intern), Hansen, P. J. (Ekstern)
Number of pages: 8
Pages: 159-166
Publication date: 2014
Stability of the intra- and extracellular toxins of Prymnesium parvum using a microalgal bioassay

Prymnesium parvum produces a variety of toxic compounds, which affect other algae, grazers and organisms at higher trophic levels. Here we provide the method for development of a sensitive algal bioassay using a microalgal target, Teleaulax acuta, to measure strain variability in P. parvum toxicity, as well as the temporal stability of both the intracellular and the extracellular lytic compounds of P. parvum. We show high strain variation in toxicities after 3h incubation with LC50s ranging from 24 to 223×10³cellsml⁻¹. Most importantly we prove the necessity of testing physico-chemical properties of P. parvum toxins before attempting to isolate and characterize them. The extracellular toxin in the supernatant is highly unstable, and it loses significant lytic effects after 3 days despite storage at −20°C and after only 24h stored at 4°C. However, when stored at −80°C, lytic activity is more easily maintained. Reducing oxidation by storing the supernatant with no headspace in the vials significantly slowed loss of activity when stored at 4°C. We show that the lytic activity of the intracellular toxins, when released by sonication, is not as high as the extracellular toxins, however the stability of the intracellular toxins when kept as a cell pellet at −20°C is excellent, which proves this is a sufficient storage method for less than 3 months. Our results provide an ecologically appropriate algal bioassay to quantify lytic activity of P. parvum toxins and we have advanced our knowledge of how to handle and store the toxins from P. parvum so as to maintain biologically relevant toxicity.
Integrated analytical approaches towards toxic algal natural products discovery

Microalgae are known to produce toxins which affect the marine ecosystems. This include compounds active against competitors, grazers and in many cases also fish (1,2). Many strategies can be followed for discovery of novel bioactive secondary metabolites from marine sources. We have previously demonstrated that phenotypic based chemotaxonomy can be successfully used as the initial step in selection of talented strains for testing in various bioassays, using multivariate methods for clustering of whole profiles of metabolites. The second and very important step in the discovery process is dereplication, where we use explorative solid-phase extraction (E-SPE), and UHPLC-state-of-the-art high resolution mass spectrometry (<1 ppm mass accuracy and accurate isotope pattern) in combination with comprehensive compound databases in order to ensure not to waste time isolating and elucidating the structures of already known compounds (3). When likely unknown compounds have been identified, we use E-SPE results (4) to predict a fast and optimal purification strategy towards the pure novel compounds for NMR characterization. This presentation will highlight our integrated analytical approaches and present some of the initial results that we have gained looking into the chemistry of Alexandrium and Prymnesium in the new larger Danish strategic project: “Harmful algal blooms and fish kills”(5).

General information
State: Published
Organisations: Department of Systems Biology, Center for Microbial Biotechnology, University of Copenhagen
Authors: Larsen, T. O. (Intern), Rasmussen, S. A. (Intern), Gedsted Andersen, M. (Ekstern), Blossom, H. E. (Forskerdatabase), Christophersen, C. (Intern), Hansen, P. J. (Forskerdatabase), Nielsen, K. F. (Intern)
Publication date: 2012
Event: Abstract from 15th International Conference in Harmful Algae, Gyeongnam, Korea, Republic of.
Main Research Area: Technical/natural sciences
Electronic versions:
Larsen_TO_doc.docx
Publication: Research - peer-review › Conference abstract for conference – Annual report year: 2012

Projects:

Algal analytical natural product chemistry
Department of Systems Biology
Period: 01/06/2012 → 02/12/2015
Number of participants: 6
Phd Student:
Rasmussen, Silas Anselm (Intern)
Supervisor:
Nielsen, Kristian Fog (Intern)
Main Supervisor:
Larsen, Thomas Ostenfeld (Intern)
Examiner:
Gotfredsen, Charlotte Held (Intern)
Hess, Philipp (Ekstern)
Sterner, Olov (Ekstern)

Financing sources
Source: Internal funding (public)
Name of research programme: 1/3 FUU, 1/3 inst 1/3 Andet
Project: PhD