Chemistry of the Deep: The first Screening of North-East Atlantic Deep-Sea Sponges and Initial Results

Sam Afoullouss[1,2], Shauna O'Brien[1,2], Kevin Calabro[1], Christine Morrow[2], Louise Allcock[2], Olivier P. Thomas[1]

[1] Marine Biodiscovery. School of Chemistry and Ryan Institute, National University of Ireland Galway University Road, H91TK33 Galway, Ireland.

[2] Zoology School of Natural Sciences and Ryan Institute, NUI Galway, University Road, Galway, Ireland.

S.AFOULLOUSS1@NUIGalway.ie

Project

Our research is part of a five year Science Foundation Ireland project **"Exploiting and conserving deep-sea genetic resources"**. This projects focus on Irish deep-sea biodiscovery with an emphasis on sponges and corals found between **700 m - 3,000 m** of the Celtic continental margin. There are three aims for this project:

1. Isolate and characterise **novel bioactive natural products** from the deep sea
2. Develop a **bioprospecting heat map** to increase the probability of finding bioactive natural products from the deep sea
3. **Inform conservation planning** to preserve biodiversity hotspots

Fig. 1: ROV *Holland 1* (left) and the RV *Celtic Explorer* (centre) used for sampling deep sea cold water reefs (right).

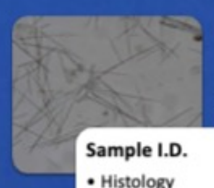
Research Focus

As part of the Irish deep-sea drug discovery project, we carried out chemical and biological screening on 126 deep-sea sponges. The results lead us to carry out a full chemical investigations on the sponge *Characella pachastrelloides* (Charter, 1876). Our work lead to the isolation and characterization of four novel glycolipopeptides named Characellides. These compounds consist of three moieties, a tripeptide (*O*-Me-Tyr-Asp-Thr), an unprecedented 3-amino-10-(5,6-dimethyltetrahydropyran-2)decanamide (nonamide for **C** and **D**) and a unique 2-amino-*α*-pyranuronamide.

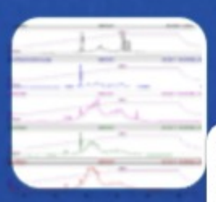
Fig.2: 'The Real Map of Ireland' showing Irish territorial waters with sampling locations marked with stars.

Screening

Sample Collection
- ROV *Holland 1*
- Sampled stored at -20°C
- Lyophilised before extraction



Sample I.D.
- Histology
- Genetic analysis
- Spicule characterisation



Screening
- 3 g of each sample
- Chemical Screening
  - UPLC-ELSD & MS
  - ^{1}H-NMR
- Bio-assays

Bio-assays
- Anti-viral
- Cytotoxicity
- Anti-parasitic
- Anti-bacterial

Structure Elucidation
- NMR (1D & 2D)
- UPLC-MS-MS
- Circular dichroism

Purification
- Fractionation
- RP-HPLC
- Isolation of pure compounds

Time	H_2O + 0.1%TFA	ACN + 0.1%TFA
-	90	10
5	90	10
25	0	100
30	0	100
31	90	10
35	90	10

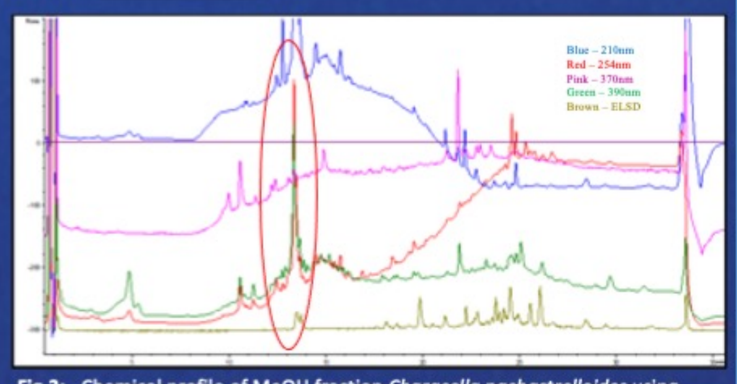
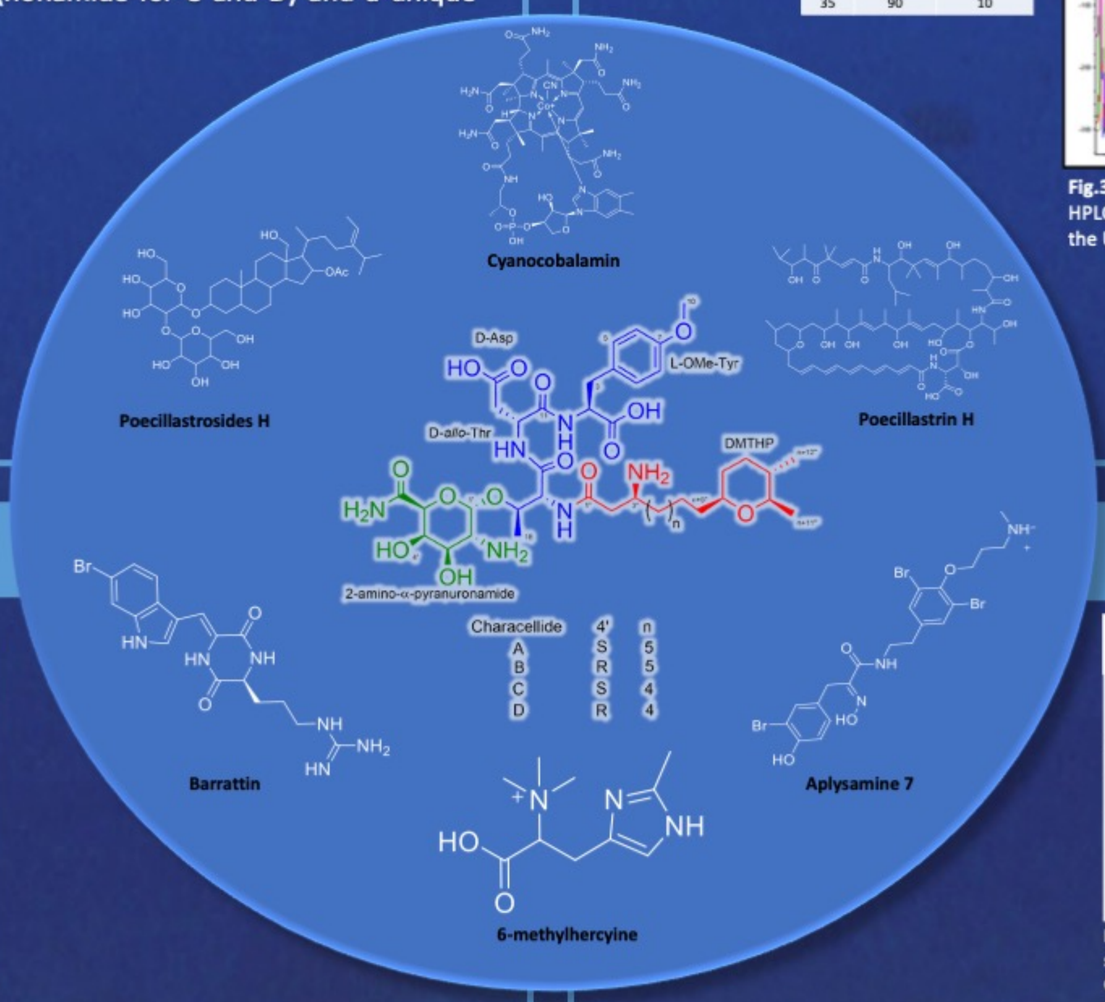


Fig.3: –Chemical profile of MeOH fraction *Characella pachastrelloides* using HPLC-ELSD profile using Waters Xselect HSS C18 5µm . The red ring incorporates the UV and ELSD signals that corresponding to the Characellides.



Characellide	4'	n
A	S	5
B	R	5
C	S	4
D	R	4

Characellides

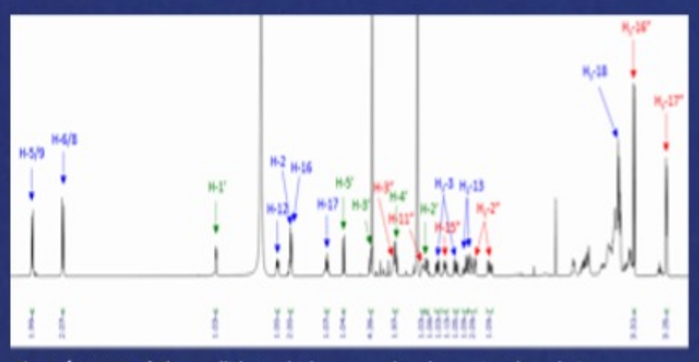
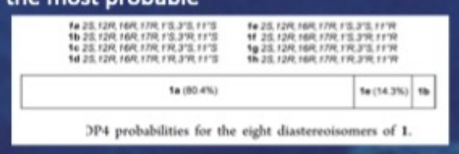


Fig. 4: ^{1}H-NMR of Characellide A which was used to determine the relative configuration of sugar motif by studying J-coupling values

DP4 NMR based computational methods were used to determine which of the eight possible diastereoisomers were the most probable



Computational Vibrational Circular Dichroism (**VCD**) spectra of the two most probable configurations were compared to the experimental VCD spectra to confirm the absolute configuration (**Fig. 5**) DP4 and VCD calculation were carried out by Dr. Gregor Geta-Jouve, University Paris Descartes.

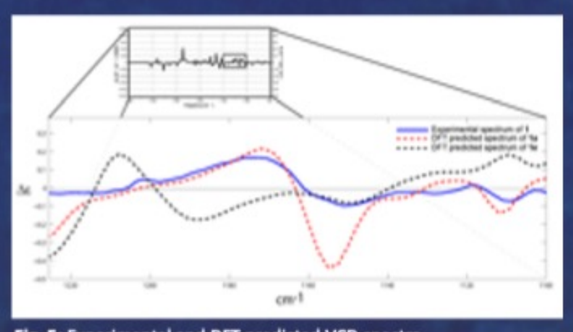


Fig. 5: Experimental and DFT predicted VCD spectra

Discussion

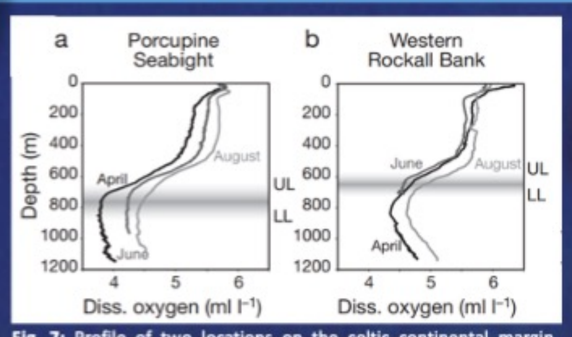
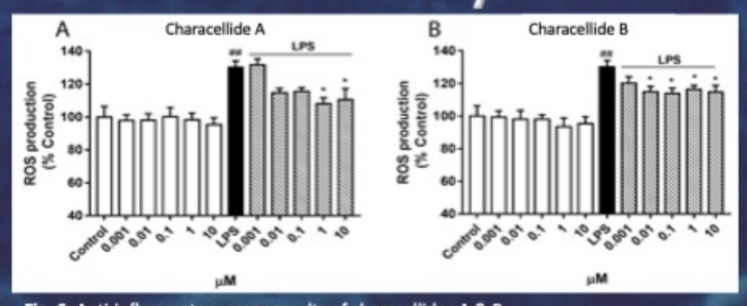


Fig. 7: Profile of two locations on the celtic continental margin showing dissolved oxygen content variations with depth for three different times of the year[7]. The blurred, grey bar represents the depth habitat of cold water reefs. UL upper limit. LL lower limit.

These novel embellished glycolipopeptides exhibit a unique 3-amino-10-(5,6-DMTHP-2)decanamide (or nonamide). Such a ring is not common in fatty acids, nor is an amine group in position 3. The incorporation of D-Asp and D-*allo*-Thr into the peptide moiety may indicate bacterial biosynthesis[3]. The presence of cyanocobalamin and poecillastrins C-H confirms that a microbial community thrives within *Characella pachastrelloides*.

Of the 126 specimens (approximately 40 species) of deep-sea sponges we screened, only six of the sponges showed interesting chemical profiles with compounds of medium polarity. This lower than expected chemical diversity could be explained by the extreme and highly stable environmental conditions[6], limiting the resources available and competition required to produce specialized metabolites.

Bioassays



Bioassays carried out by Prof. Luis M. Botana, Universidade de Santiago de Compostela

Fig. 6: Anti-inflammatory assay results of characellides A & B

- MTT assay to test cell viability
  - Microglia BV-2 cells
  - **Non-toxic effects**
- Anti-inflammatory assay
  - ROS production
  - Characellide A active at 1 µM
  - Characellide B active at **10 nM**

Conclusions

While the characellides posses a novel structure, there is a high level of similarity in terms of biosynthetic pathways, to primary metabolites. This can also be said for other metabolites we isolated including poecillastroides[2], 6-methylhercyine and aplysamine 7 [5]. Although we have observes a lower level of chemical complexity, these specialized metabolites tend to possess strong bioactivity such as characellide B which is active in anti-inflammatory bio-assay to 10 nM [1] and poecillastrin H has an IC_{50} value of showed potent 4.1 nM against 3Y1 cells[4]. Deep-sea sponges and associated microbes may have evolved to produce extremely potent specialized metabolites to aid the conserve of valuable nutrients which are in low supply.

Acknowledgements

This research was carried out with the support of the Marine Institute under the Marine Research Programme and the support of the Irish Government. This publication has emanated from research supported in part by a research grant from Science Foundation Ireland (SFI) and the Marine Institute under the Investigators Programme Grant Number SFI/15/1A/3100, co-funded under the European Regional Development Fund 2014-2020. Experimental VCD spectra was carried out by Biotools, Florida. Ryan Young and Bill J. Baker for aiding sample collection and the organisation of biological screening.

References

1. S. Afoullouss and K. Calabro, G. Genta-Jouve, S. Gegunde, A. Alfonso, R. Nesbitt, C. Morrow, E. Alonso, L. M. Botana, A. L. Allcock, and O. P. Thomas *Org. Lett.* **2019** *21* (1), 246-251
2. K. Calabro, E. Lotfi Kalahroodi, D. Rodrigues, C. Diaz, M. de la Cruz, B. Cautain, R. Laville, F. Reyes, T. Perez, B. Soussi and, O. P. Thomas. *Mar. Drugs* **2017**, *15*(7), 199-211.
3. T. Kubota, T. Kobayashi, T. Nunoura , F. Maruyama and S. Deguchi. *Front. Microbiol. 2016,* 7, 511-514
4. R. Suo, K. Takada, R. Irie, R. Watanabe, T. Suzuki, Y.I Ise, S. Ohtsuka, S. Okada, and S. Matsunaga. *J. Nat. Prod.* **2018**, *81* (5), 1295-1299.
5. T. D. Tran, N. B. Pham, G. Fechner, J. N. A. Hooper, and R. J. Quinn. *J. Nat. Prod.* **2013**, *76, 4, 516-523*
6. R. Danovaro, C. Corinaldesi, A Dell'Anno and P. V.R. Snelgrove. *Curr. Biol.* **2017**, 27, 11, 461-465.
7. W.C. Dullo, S. Flogel, A.Ruggeberg. *Mar. Ecol. Prog. Ser.* **2008**. 371: 165-176

Ryan Institute | European Union, European Regional Development Fund | Marine Institute, Foras na Mara | NUI Galway, OÉ Gaillimh | SFI Science Foundation Ireland