COMBATTING BIOFILMS

An ESMB day at the LEAF conference

Gothenburg, 30.9.2015

The
European Society
for Marine
Biotechnology

Programme, abstracts, participants

Programme
ESMB DAY – 30th September 2015

Title	Starts
Welcome address – Torger Børresen	09:00
Introduction to the symposium – Grant Burgess	09:05
Invited Keynote Speaker – Johan Svenson ***Tuning Novel Natural Compounds from the Arctic to Target Unwanted Marine Colonizers***	09:10
Sergey Dobretsov ***Combatting biofilms using zinc oxide nanorod photocatalytic coatings***	09:40
Rozenn Trepos ***Anti-biofilms properties of sulphates polysaccharides from algae***	10:00
Coffee break and networking	10:20
Invited keynote speaker – Tim Sullivan ***The role of surface topography in combatting marine biofilms***	10:40
Maria Salta ***Marine biofilms: know thy "enemy"***	11:10
Francesco Secundo ***Immobilized hydrolytic enzymes for antibiofilm applications***	11:30
General discussion on morning presentations	11:50
Lunch and networking	12:00
Introduction to workshop topic: ***How to involve students in technology transfer?***	13:30
Breakout session in workshop groups	13:45
Presentation of workshop results	14:45
Tea and cake	15:00
Roundtable discussion on bridging the gap between research and industry	15:20
Presentation of an Erasmus placement programme – Claire Hellio	15:50
Concluding remarks – Grant Burgess	16:20
Adjourn	16:30

Abstracts

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Tuning Novel Natural Compounds from the Arctic to Target Unwanted Marine Colonizers

Presenting author: Johan Svenson
Co-authors:

***Affiliation of author(s)**: SP Technical Research Institute of Sweden
Chemistry, Materials and Surfaces, Box 857, SE-501 15 Borås, Sweden*

Presenting author present position: Senior Scientist

Research area: Antifouling, Medicinal Chemistry, Natural products

Example(s) of papers published: Hanssen, K.Ø.; Cervin, G.; Trepos, R.; Petitbois, J.; Haug, T.; Hansen, E.; Andersen, J. H.; Pavia, H.; Hellio, C.; **Svenson. J**. The bromotyrosine derivative Ianthelline isolated from the Arctic marine sponge *Stryphnus fortis* inhibits marine micro- and macro biofouling. *Mar. Biotechnol.* (2014) **16**, 684-694.

Trepos, R.; Cervin, G.; Hellio, C.; Pavia, H.; Stensen, W.; Stensvåg, K.; Svendsen, J.S.; Haug, T.; **Svenson. J.** Antifouling Compounds from the Sub-Arctic Ascidian *Synoicum pulmonaria*: Synoxazolidinone A and C, Pulmonarin A and B, and Synthetic Analogs. *J. Nat. Prod.* (2014) **77**, 2105-2113.

ABSTRACT

The uncontrolled and unwanted growth on marine surfaces leads to both economical and environmental costs. Marine micro and macroorganisms rapidly form biofilms on materials submerged in the ocean and the search for ways to control this fouling process in an environmentally benign fashion is intense. The new technologies should ideally be deterring and non-toxic.

The fouling process itself is natural and affects also the living organisms in the ocean. Slow and sessile marine organisms rely on both physical and chemical defences to evade predators and to avoid fouling overgrowth. Many novel antifouling compounds have been isolated from marine organisms and it has been heralded that natural products is the way forward towards new ”green” antifouling solutions. Not only marine natural products are under investigation as inhibitors of the marine biofilm but also terrestrial natural products. Of particular interest is the allelopathic class of compounds which prevent the growth of competing species by f.ex. acting as germination inhibitors.

The current presentation summarizes our efforts aimed at exploring new, naturally-inspired, chemistries to target unwanted marine growth. Specific focus lies on the development of Arctic natural products and compounds derived from the innate immune system to produce non-toxic inhibitors of the marine biofilm.

Combatting biofilms using zinc oxide nanorod photocatalytic coatings

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Presenting author: Sergey Dobretsov[1]
Co-authors: Priyanka Sathe[1, 3], Jutta Richter[2], Myo Tay Zar Myint[1], Joydeep Dutta[3]

Affiliation of author(s):[1]Sultan Qaboos University, [2] Hochschule Bremerhaven, Bremen, Germany, [3] KTH Royal Institute of Technology, Sweden

Presenting author present position: Head of Department of Marine Science and Fisheries

Research area: Biofouling and antifouling, chemical microbial ecology

Example(s) of papers published:

Dobretsov S., Al-Wahaibi A.S.M., Lai D., Al-Sabahi J., Claereboudt M., Proksch P., Soussi B. 2015. Inhibition of bacterial fouling by soft coral natural products. International Biodeterioration and Biodegradation. 98: 53-58

Muthukrishnan T., Abed, R., Dobretsov, S., Kidd, B., and Finnie, A. 2014. Long-term microbial fouling on commercial biocidal fouling control coatings. Biofouling 30: 1155-1164

Dobretsov S., Abed R M. M., Voolstra C.R. 2013. The effect of surface colour on the formation of marine micro- and macro-fouling communities. Biofouling 29: 617-627

ABSTRACT

Previously it has been shown that zinc oxide (ZnO) nanostructures can photocatalytically inhibit growth of bacterial and fungal strains under solar irradiation. The objective of this study was to investigate the prevention of formation of marine biofilms by ZnO nanorod coatings in the out-door mesocosm experiment. ZnO nanorod coatings were fabricated on microscope glass substrates by a simple hydrothermal technique using equimolar solutions of 10 mM zinc nitrate and hexamethyltetramine. In the continuous 5 days out-door experiment conducted in aquaria (volume = 70 L) with sea water collected from the Sea of Oman (23°34′55″ N, 58°36′27″ E), the nanorod coatings significantly reduced density of bacteria in comparison to the control (no coatings) under the sunlight conditions. In the absence of sunlight (auqauria covered with a non-trasparent box), test and control slides were equally colonized by bacteria. Most of bacteria on the coatings under sunlight were dead, while bacteria in the absence of sunglight were alive, as shown using live and dead stating. *De novo* sequencing of bacterial biofilms on MiSeq system® demonstrated that different communities were formed in the presence and absence of sunlight on the coatings. Our study suggests that ZnO nanorod coatings effectively prevent biofilm formation and can be used as a novel green antifouling technology.

Work financed by: TRC chair in Nanotechnology and SQU Internal Grant IG/AGR/FISH/15/02, Oman

ANTI-BIOFILMS PROPERTIES OF SULPHATED POLYSACCHARIDES FROM ALGAE

Presenting author: Rozenn Trepos
Co-authors: Claire Hellio
Affiliation of author(s): University of Portsmouth, School of Biological Sciences , King Henry Building, King Henry Street I, PO1 2DY , Portsmouth , UK.
IUEM-LEMAR UMR 6539 , Rue Dumont D'Urville,29280 Plouzane,France

Presenting author present position: Research Associate at University of Portsmouth,UK

Research area: bioactive marine natural products and biofouling

Example(s) of papers published:

Trepos R., Cervin G., Pile C., Pavia H., Hellio C., Svenson J. (2015) Evaluation of cationic micropeptides derived from the innate immune system as inhibitors of marine biofouling. Biofouling, 2015,Vol 31(4): 393-403

Trepos R., Cervin G., Hellio C., Pavia H., Stensen W., Stensvåg K., Svendsen J., Haug T., Svenson J. (2014) Antifouling Compounds from the Sub-Arctic Ascidian *Synoicum pulmonaria*: Synoxazolidinones A and C, Pulmonarins A and B, and Synthetic Analogs, Journal of Natural Products , 77(9): 2105-13.

ABSTRACT

The specific activity of surfaces by biocidal molecules, active against biofilms, is a great challenge, leading to the development of materials with antimicrobial barriers. Applications are of interest to packaging and marine antifouling surfaces. The project focused on preventing the growth of micro-organisms by using natural biocides or anti-biofilm molecules. Algae have the ability to produce a large variety of chemical defences which prevent over-predation, defence against competition from foreign microorganisms or changes in environmental conditions. Tests were performed on the antifouling and antimicrobial activities of sulphated polysaccharides extracted from algae against 12 strains of bacteria and 4 strains of marine microalgae involved in surface colonisation process. Nine extracts have shown promising results by inhibiting the growth of bacteria with a Minimum Inhibition Concentration (MIC) value ≤0.01µg/ml. The most promising compounds were subsequently combined and both antifouling and antibacterial activities were re-assessed. One of the mixture of compounds displayed remarkable results by inhibiting the growth of five bacteria and two microalgae strains with MIC value ≤ 0.01 µg/ml. Our research has led to the discovery of new compounds with antimicrobial and antifouling properties.

The role of surface topography in combatting marine biofilms

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Presenting author: Timothy Sullivan
Co-authors Prof. Fiona Regan

Affiliation of author(s):
***Timothy Sullivan**:*
Dept. of Chemical Engineering and Chemistry
Functional Organic Materials & Devices (SFD)
Building 14 - HELIX STO 0.33
De Rondom 70 - 5612 AP
P.O. Box 513 - 5600 MB
Eindhoven - The Netherlands

Professor Fiona Regan
School of Chemical Sciences,
Marine Environment Sensing Technology Hub (MESTECH)
Director, DCU Water Institute
Dublin City University, Glasnevin, Dublin 9

Presenting author present position: Postdoctoral Researcher

Research area: Antifouling Materials

Example(s) of papers published:

1. **Sullivan T.**, and F. Regan, *The characterization, replication and testing of dermal denticles of Scyliorhinus canicula for physical mechanisms of biofouling prevention*. Bioinspiration and Biomimetics, 2011, **6**, 1-11.
2. **Sullivan T.**, and F Regan, *Biomimetic design for the development of novel sustainable antifouling methods for ocean monitoring technology*. Journal of Ocean Technology, 2011, **6**, 41-54.
3. Chapman J., Hellio, C., **Sullivan, T.**, Brown, R., Russel, S., Kiterringham, E., Le Nor, L., and Regan, F. *Bioinspired synthetic macroalgae: examples from nature for antifouling applications*, International Biodeterioration & Biodegradation Volume 86, Part A, January 2014, Pages 6–13

ABSTRACT:
Marine biofouling is often considered as unwanted growth of marine organisms such as bacteria, invertebrates and algae on immersed surfaces in aquatic environments (e.g. surfaces of aquaculture cages or ships). Control of biofouling is important for many marine industries due to increased drag or other factors and subsequent financial losses associated with fouling, and much effort is expended annually in reducing, removing or attempting to prevent biofouling. Controlling bioadhesion and growth of fouling organisms using either static or dynamically modified surface chemistry and topography is an attractive option for developing novel antifouling systems. However, the fact that there are many adhesion strategies and different organisms competing for space on exposed surfaces means that developing a surface that is an effective non-biocidal antifouling material or coating against all or even most organisms remains challenging. Careful control of surface chemistry or release/delivery of biocidal compounds or materials are approaches commonly used for control of biofouling, however appropriate scaling and engineered control of surface topography at nano and microscale can also effect initial cell settlement, attachment, adhesion strength and the required shear forces for removal of organisms. Determining the topographic parameters that influence settlement is however difficult, especially where different competing organisms of different length scales are involved. This talk discusses the results from both laboratory and field-testing of surface topography and structures for effects on biofouling, with particular focus on diatom adhesion and growth and the implications for developing materials with enhanced antifouling capability.

Marine biofilms: know thy "enemy"

Presenting author: Maria Salta, Lecturer in Environmental Microbiology

Affiliation of author(s): School of Biological Sciences, University of Portsmouth, United Kingdom

Presenting author present position: Lecturer

Research area: Biofilms/Biofouling/Antifouling
Example(s) of papers published:
Salta M. (2014) Biomimetic strategies in antifouling coatings. *Journal of Ocean Technology* Vol.9, No. 4
Salta, M., Wharton, J.A., Blache, Y., Stokes, K.R., and Briand, J.-F. (2013) Marine biofilms on artificial surfaces: structure and dynamics. *Environmental Microbiology* 15: 2879-2893
Salta, M., Capretto, L., Carugo, D., Wharton, J.A., and Stokes, K.R. (2013) Life under flow: A novel microfluidic device for the assessment of anti-biofilm technologies. *Biomicrofluidics* 7: 064118
Salta, M., Wharton, J., Stoodley, P., Dennington, S., Goodes, L., Werwinski, S. et al. (2010) Designing biomimetic antifouling surfaces. *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences* 368: 4729-4754

ABSTRACT

Marine biofilms, mainly comprised of bacteria and diatoms, are now recognized to be a significant issue for a wide range of engineered structures such as ship hulls, energy systems, sensors, oil/gas pipe lines, heat exchange systems, and aquaculture. Despite their importance, there is a significant gap in mechanistic understanding of "how" marine micro-organisms remain adhered on moving vessels and "why" they preferentially attach on substrates with certain properties.
The aim of this talk is to present an overview on recent developments in experimental methods for the assessment of biofilm processes on inert and antifouling surfaces. Dr Salta's expertise is focused on understanding and tackling biofilm formation by utilising a wide range of approaches, such as natural products and biomimetics, flow cells, microfluidics, and molecular biology. Additionally, newly designed experiments that assess biofilm diversity on artificial surfaces using metagenomics will be briefly presented.

Immobilized hydrolytic enzymes for antibiofilm applications

Presenting author: Francesco Secundo[1]
Co-authors: Piotr Biniarz[2], Francesca Cappitelli[3], Anna Krasowska[2], Marcin Łukaszewicz[2], Eugenio Spadoni Andreani[1], Federica Villa[3]

Affiliation of author(s):
[1]Institute of Chemistry of Molecular Recognition, National Council of Research, via Mario Bianco 9, 20131, Milan, Italy. [2]University of Wrocław, Faculty of Biotechnology, Joilot-Curie 14a, 50-383 Wrocław, Poland. [3]Department of Food Environmental and Nutritional Sciences, Università degli Studi di Milano, via Celoria 2, 20133 Milan, Italy.

Presenting author present position: Research Scientist

Research area: Biocatalysis, enzymology

Example(s) of papers published: F. Villa, F. Secundo, A. Polo, F. Cappitelli
Immobilized Hydrolytic Enzymes Exhibit Antibiofilm Activity Against *Escherichia coli* at Sub-Lethal Concentrations. Curr Microbiol (2015) 71:106–114. DOI10.1007/s00284-015-0834-6

ABSTRACT
Microbial colonization on a surface leads to the development of a biofilm, which is a surface-associated multicellular community embedded in a self-produced polymeric substance (EPS). This latter one consists of polysaccharides, proteins, glycopeptides, nucleic acids and lipids.
Numerous strategies have been adopted to prevent or inhibit undesired and (often) patohogenic biofilm formation on surfaces. These can be summarized in
i) chemical treatments of the surface aiming at minimizing van der Waals interactions or to form non-homogeneous surface (e.g., charged and hydrophobic);
ii) use of coatings as hydrophilic polymeric materials that form highly hydrated surfaces (e.g. PEG);
iii) structuring micro- and nanoscale surface architecture for developing superhydrophobic properties (e.g., mimicking the Lotus leaf);
iv) use of coatings or bulk materials designed to release biocidal compounds or able to prevent microbial adhesion by interfering with the quorum sensing or affecting the EPS integrity.

Concerning this latter approach, enzyme based coatings have also been developed by different research groups. Different kind of enzymes belonging to the class of hydrolases can be employed to degrade EPS, thus the biofilm structure. Indeed, proteases and glycosidases can hydrolyze the two main components of the EPS, that is, proteins and polysaccharides, respectively.
In this communication a brief review of the state of the art of hydrolytic enzymes mostly used as anitibiofilm agents and some example of enzyme immobilization on plastic surface carried out in our laboratories will be presented. Finally, conclusions for addressing the use of hydrolases as antibiofilm coating will also be discussed.

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