SEABIOTECH

From sea-bed to test-bed: harvesting the potential of marine Microbes for industrial biotechnology
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SEABIOTECH FINAL REPORT

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1. FINAL PUBLISHABLE SUMMARY REPORT

1.1. Executive summary

The marine environment represents arguably the greatest source of environmental diversity available to mankind. However, despite that rich biodiversity relatively few products derived from the sea have made it through to industrial production compared to those arising from terrestrial environments. Although there are many factors involved, the major barriers to producing new products(e.g. antibiotics, anti-cancer agents, polymers, chemicals and cosmetics) include:

1) limited quality and quantity of marine resources available for biotechnological exploitation, (2)limitations in technical aspects of the bio-discovery pipeline which lengthen time to market, and increase cost of industrial production(3) limited sustainable modes of supply of raw materials for industry. The two last challenges centre on enabling activities to enhance the marine bio-discovery process: (4)the need for clarification and harmonization of legal aspects to facilitate access to marine resources, their sustainable use, & their secure exploitation; and(5) the need to improve the available framework(infrastructure) to improve access to marine biotechnology data & research materials.

SeaBioTech, a 48 month project partnership between companies (SME's) and research institutes, aims to overcome or significantly reduce these bottlenecks, and generate improved capability, knowledge and enhanced confidence to industry in each of these areas. The overall aim is to bring about a step-change in our ability to sustainably and economically exploit the biotechnological potential of the marine environment.

SeaBioTech has successfully addressed each hurdle. 1) Extensive sampling in a range of marine environments has augmented existing culture collections (SAMS, MATIS, SIPBS), while standardized sampling, processing and storage techniques developed within the project, have improved resource quality and availability in the long term. Likewise extensive use of advanced genomic, metagenomics and metabolomics has greatly increased the understanding of the metabolic potential of new samples and isolates in existing collections 2)Development and refinement of High Throughput Functional BioAssays for novel antibiotics, anticancer agents, antioxidants, enzyme activities and polymers, together with a systems biology approach, and advances in sample analysis, have accelerated the progress of promising lead candidates through the product pipeline, and should give confidence to industry that the use of marine microbes or their genes is a feasible route to novel industrial products 3)Metabolic engineering and metabolomics assisted fermentation both help ensure the product levels arising from initial marine isolates can be maintained across scales of culture. Traditionally, marine microbes might produce only tiny amounts of a desired product in culture. SeaBioTech has addressed this by engineering native strains of natural isolates with enhanced genetic potential to make a desire product. Finally, industrial workhorses (e.g *Escherichia coli)* have had gene sequences from marine isolates transferred in and the products have been produced at medium scale. Both routes increase the ready availability of marine products for further testing, analysis and validation by industry and give greater confidence to potential future investors in the marine bio sector. 4) Together with other KBBE Marine projects we have worked towards harmonisation of approaches to access and sustainable exploitation of marine resources and discussed and advised national, and international bodies 5) The creation of an accessible repository for all samples and extracts generated within the project and a database detailing marine resources with information on source, metabolic capabilities, bioassay results etc represents a significant new resource available to the marine biotechnology community. A Process Handbook has also been produced describing in detail all stages of the product pipeline and the methods, protocols and techniques used at each to ensure success. This is a valuable tool encapsulating the new knowledge generated within the project

Further, SeaBioTech partners have disseminated their project findings widely to the general public, legislators, and the wider scientific community. Partners have also extensively engaged with future scientists at Open Days, Workshops, and Science Centre activities. Public engagement and awareness activities have included numerous television, radio, newspaper and magazine interviews and articles.

1.2. Summary description of project context and objectives

The SeaBioTech project is designed and driven by SMEs to convert the huge potential from as yet underdeveloped marine biotechnology into novel bioactive industrial products. This project will be applied to the pharmaceutical (anti-cancer, anti-parasitic, antibiotic, antimalarial, anti-inflammatory, antioxidant and optic lenses), cosmetic (antioxidant), food (antioxidant) and industrial chemistry (biocatalysts, reagents) sectors. The project will make use of the biodiversity to be found in marine extreme environments. Such environments are characterized by geochemical and physical conditions at the edges of the compatibility with life, and they are colonized by highly adapted organisms called extremophiles. These can provide unique chemicals and novel enzymes that have enormous potential because they maintain their performance even in harsh industrial process conditions. However, there are significant bottlenecks that restrict the marine biodiscovery pipelines relating to:

- limited availability of collections of marine extremophiles and little knowledge of their potential use in biotechnology (lack of qualitative and quantitative data with respect to the application performance)
- limited transfer of knowledge from fundamental research into technically realizable and cost-effective products and technologies
- technical hurdles with methods and processes, including for cultivation and storage of organisms, and for extraction, isolation and characterization of bioactive components
- lack of industrial scale production techniques for marine substances

To develop efficiently marine biodiscovery pipelines and provide access to sustainable and economical production methods, SeaBioTech will tackle five key challenges with an integrated approach combining access to unique marine biodiversity, innovative culturing approaches, genomic and metagenomics analyses coupled with metabolomics, natural product chemistry, bioactivity evaluation and industrial bioprocessing along with legal aspects, market analysis and transfer of knowledge. SeaBioTech will not only drastically increase the number of new and potent marine-based products but also their success rate for future commercialization. SeaBioTech's research and technological progress will be completely in the framework provided by the participating SMEs relating to their definition of product opportunities and proof-of-concept demonstration activities.

- Challenge 1: The quality of marine resources: the approach to resource quality will begin by standardizing the sampling process from unique and previously untapped habitats, including geothermal intertidal biotopes in Iceland, hydrothermal vent fields and deep sea oligotrophic basins of the Eastern Mediterranean Sea, and unsampled areas of Scottish coasts that are likely to be highly productive sources of new bioactive compounds. The marine resources will also include the partners' existing biobanks (UK's Culture Collection of Algae and Protozoa, MATIS's Icelandic collection, Eastern Mediterranean Sea collections) as well as new in situ sampling. The SeaBioTech sampling process will guarantee the quality of marine resources for further industrial development, including identification of marine microorganisms and their variability based on genomics and metagenomics. This project will also integrate the critical aspect of the maintenance of the sampled species with their intrinsic quality and their secondary metabolites, by developing special cultivation media and storage conditions.
- Challenge 2: The improvement in technical aspects: to improve marine biodiscovery and reassure industries about its feasibility, SeaBioTech will perfectly combine metabolomics assisted by systems biology and functional bioassays to increase the ability to uncover positive hits with a cheaper and faster approach: an affordable, innovative and efficient method to separate, elucidate the structure, and identify the bioactive metabolites.
- Challenge 3: Sustainable modes of supply of raw materials for the industries: the last technical brick for industries is the sustainability of these newly discovered raw materials not only at lab scale but also at industrial scale. Thus, SeaBioTech will benefit from the power of well-controlled metabolic engineering of interesting organisms (bacteria, microalgae, cyanobacteria) to increase the yield of bioactive metabolites at lab scale and multiply this yield through fermentation technology at industrial scale to deliver promising enzymes, polymers and small molecules as industries need. The second level embraces the last two challenges as transversal activities: challenge 4, the legal framework necessary to secure the access to marine resources, their sustainable use and their exploitation process; and challenge 5, the access to a marine biotechnology database and biobank.

- Challenge 4: The whole biodiscovery process is completed by the clarification of all legal aspects to gain visibility and efficiency for industries. SeaBioTech will perfectly coordinate the legal procedures with national, European and international authorities/stakeholders to propose harmonization of the legal process related to marine bioprospecting, biodiscovery and marine biotechnology for commercial purposes.
- Challenge 5: To crystalize this innovative approach, SeaBiotech will create a centralized tool to describe the whole marine biodiscovery pipeline including available biobanks, the identified marine organisms, compounds and extracts, the cutting-edge methods in identification, elucidation, metabolic engineering to be further used for industrial purposes with all related procedures on legal process for companies, academia, and legal authorities.

The objectives of the project are to:

- Develop processes and methods at medium scale to ensure the production yield of active ingredients by the targeted microorganisms at least of 90% compared to original strains
- Develop standardised processes and methods according to market requirements (GMP…)
- Provide a pipeline of at least 10 commercially viable candidates of marine origin on the 6 targeted activities (anticancer, anti-inflammatories, antibiotics, fish antiparasitics, cosmetics, industrial sectors)
- Transfer bioprocessing methods at industrial scale suitable for commercial production of at least 10 marine-sourced materials with the same productivity as at medium scale
- Prepare a check list of documents required to ensure the compliance with the legal aspects in sampling and collection of marine microorganisms in the 5 explored extreme environments and 4 existing collections
- Draft a common template to fill in whatever the extreme environments involved in SeaBioTech project are or existing collections
- Create a central EU platform and biobank based on an integrated approach to biodiscovery pipelines for future use by other consortia, academia and companies

The following table summarises the main progress beyond the state of the art expected by SeaBioTech.

State of the Art	Progress beyond the SoA
Lack of marine-derived products on industrial applications: novel drugs, treatments, health and personal care products	Creation of a 'biodiscovery engine' focused on new ingredients for new medicines (including for cancer, infections, pain, inflammatory disease) for human health and antiparasitic for fish farming industry, cosmetics and functional foods (antioxidants) and industrial processing and life science research (enzymes as improved catalysts, oligosaccharides, biopolymers)
Declining pipelines of potential new medicines	Full assessment of the drug-like properties of bioactive small molecules to identify those that have potential to be clinical development candidates
Unadapted sampling process for novel microbes leading to limiting quality of marine resources to be used	Improved sampling process on novel microbes and microorganism consortia based on metagenomics - Better quality of novel marine microbes from extreme environments - Guidelines on metagenomics applied on micro-organism consortia
Limited genomics and metagenomics analysis of marine organisms	- Guidelines on genomics and metagenomics to be applied on marine microorganisms - Development of widely applicable sequence-driven metagenomics
Lack of technologies to culture and isolate marine microorganisms	Develop enabling technologies for culture and isolation of all types of microorganisms (cultivated and uncultivated species)
Lack of technologies to identify novel, industrially useful biocatalysts from marine derived organisms	Apply metagenomics approaches and high throughput solid phase screening to directly access novel, active biocatalysts targeting high value industrial compounds
Limiting yield of cultivated microorgnisms assisted by metabolomics	Bio-engineering of marine microorganisms to optimise yield of active compounds and provide sustainable compounds, through real-time monitoring with metabolomics

Limiting technologies on separation, structure elucidation and identification of the bioactive molecules	Combined technologies (high-throughput chromatography, NMR and MS analyses, and metabolomics) to lead to identification of the bioactive compounds
Lack of sustainability in discovered marine compounds	Provide sustainable modes of supply for marine bioactive compounds through application of industrial bioprocessing expertise
Lack of clarity in legal aspects	Simplify legal aspects related to the marine biodiscovery pipelines and keep policy makers informed of developments relating to sustainable marine bioprospecting
Lack of efficiency in time to market	Optimise the time to market through integration of collection processes, genetic analysis, bioactivity testing, metagenomic and metabolomics sampling, and industrial-scale production
Limited access to marine biotechnology data and marine derived molecules	- Develop and optimise biobank and marine infrastructure for all actors (industries, academia) - Implement a centralised repository of marine extracts and compounds for potential life science applications

In summary, the potential of marine biotechnology to generate novel drugs, polymers, enzymes and industrial compounds has been held back by the barriers described above. SeaBioTech had as its strategic aim the development of approaches which directly targeted these challenges with the aim of releasing more of the current latent potential of marine biotechnology. The outcome will be new drugs, polymers, and enzymes being delivered more quickly, to the benefit of human society. Additionally, through its work in harmonisation of the legal aspects of exploitation of marine resources, and its contribution to novel accessible infrastructure (database, repository, culture collections) SeaBioTech will also help enable future marine biotechnology ventures. Finally, by demonstrating the "manufacturability" of novel marine products, it will help to stimulate increased industrial interest in marine derived products.

SeaBioTech has made significant progress in tackling or reducing each of these barriers limiting marine biotechnology's potential. This has brought about a step change in our ability to sustainably exploit marine resources to the benefit of human society as a whole.

1.3. Description of the main S&T results, foregrounds, impacts and dissemination

WP1 – Definition of product opportunities, dissemination, exploitation plans and IPR	
Main objectives	- To identify precisely the industry bottlenecks to target their specific market - To manage IPR before, during and after the SeaBioTech project - To define an exploitation plan for each SMEs to optimise their target of the market - To ensure appropriate exploitation of the knowledge generated in the project, the industrial development of the promising compounds per target application - To disseminate on project outcomes to the relevant institutes and to the wider general public and promote results to the scientific community - To coordinate common activities with Bluegenics and PharmaSea consortia - To create a database of information and physical samples to facilitate the sustained exploitation of the SeaBioTech platform after the life of the FP7 project.
Contributing partners	**PKZ**, all partners
Main results	**PRKZ** : With the analysis of market opportunities and the generation of an initial exploitation plan, Prokazyme defined specific commercial goals and the implementation and strategy to reach these goals. The exploitation plan also further underlined the importance of collaboration between the company & RTD partner Matis as a key to the successful exploitation of the opportunities and potential of the SeaBioTech project. Through EU-funded projects Prokazyme has made agreements with academic institutions for such licensing of enzyme products with shared revenues according to the specific

agreements. Prokazyme has continued its strategy employed in this project to exploit potential collaborations with academic groups as a new business strategy for increased portfolio of products for the research laboratory market in EU and elsewhere.
Prokazyme has made strategic plans for future commercial production of enzymes on economical large scale in Ukraine. As part of this future strategy, it is the intention that production shall be transferred from Prokazyme to a subsidiary company in Ukraine where a sister company, Prokaria laboratories, has been established in cooperation with local parties. A feasibility study for such enzyme production in Ukraine is being conducted with a loan and grant from the Nordic Project Fund Nopef (Nopef.com).
Prokazyme has taken steps to further develop the successful strategy of alliance with its partners in the SeaBioTech project with continued collaboration that will extend well beyond the lifetime of the SeaBioTech project. Prokazyme and Matis have initiated a large research proposal with a consortium consisting of 15 partners in Europe. The research proposal, "Virus-X: Viral metagenomes for Innovation Value", has secured a EUR 8 million funding from the European Union under the Horizon2020 framework. Dr. Arnthor Ævarsson,Prokazyme will coordinate the project and within the project extend its collaboration with specific partners from SeaBioTech. A grant agreement was made during this period with the EU and the 4 year project started on April 1st 2016
The significant results of Prokazyme with respect to exploitation can be summarized as follows:

- Continued exploitation of viable targets for commercialization.
- Strengthen the Academic Alliance Program.
- Further development of strategic partnerships.
- Secured funding for new projects.
- Initiation and successful application for a EUR 8 Million grant under the Horizon 2020 program
- Strategic plan and feasibility study for economic future enzyme production on larger scale.
- Improved market strategy via internet marketing

HDL : HDL showed that several fractions containing single compounds had a marked ability for specifically killing cancer cells via inducing apoptosis. This is **proof-of-principle** that bioactive compounds isolated from these particular classes of marine organisms may have at least some of the required characteristics for exploitation in the oncology arena.
As a next step to support the potential commercialization of these bioactive compounds, further funding will be required to undertake the types of studies outlined in phase 2 above in order to better understand mechanism of action, develop a suitable patient stratification strategy and to assess tractability for conventional medicinal chemistry. Only once this information is in hand it will be possible to meaningfully engage potential partners.
AXXAM: supported the hit discovery programmes of SeaBioTech by performing 11 screening campaigns on a comprehensive number of 927 crude samples of marine origin received from SIPBS on an array of cell-based and enzymatic assays, which was refined based on the obtained results to seven assays suitable for high-throughput screening of complex extracts (TRPA1, TRPM8, TRPV1, PPARα, EL, HDAC6, HDAC2). These functional assays were developed to measure the activity of validated targets in three main disease indications: cancer (HDAC6 and HDAC2), metabolic syndrome (EL, PPARα) and pain (TRPA1, TRPM8, TRPV1). At the end of the primary screening activity, 287 crude extracts were confirmed as primary hits, distributed as follows: TRPA1 (12), TRPM8 (37), PPARα (36), HDAC6 (81), HDAC2 (3), EL (118). In collaboration with WP2-WP5, 31 crude extracts derived from 17 marine microorganisms were prioritized and included in the SeaBioTech pipeline. A subset of 15 crude extracts was fractionated by WP5 and 629 fractions were subjected to screening against the primary assays TRPM8, TRPA1, PPARα, HDAC6 and EL, respectively. The support to dereplication activities led to the identification of 148 fractions containing the sought bioactivity against the following primary targets: TRPA1 (9), TRPM8 (5), PPARα (5), HDAC6 (76), EL (53). Remarkably, one series of 27 fractions derived from the crude

extract SBT0541 (*Algoriphagus marincola*) was confirmed to containing negative modulators of the catalytic activity of Endothelial Lipase (EL). Among them, 8 fractions contained pure compounds (SBT2643, SBT2653, SBT2656, SBT2660, SBT2662, SBT2665, SBT2667, SBT2670), which were identified by WP5 as a series of structurally related fatty acids, which allowed the definition of a preliminary structure-activity relationship. This finding appears consistent with the targeted enzyme Endothelial Lipase (EL), which physiologically releases fatty acids from phospholipids in HDL particles. The compounds displayed a dose-dependent inhibition on EL, with partial inhibition at the highest compound concentrations tested. The negative modulation of the EL activity by fatty acids identically to those identified by AXXAM has never been reported in literature. However, a role of medium- and long-chain fatty acids in the regulation of EL activity has been already reported (Chen S, Subbaiah PV. Biochim Biophys Acta. 2007;1771:1319. Das UN. Prostaglandins Leukot Essent Fatty Acids. 2005;72:173), which indirectly supported AXXAM findings. The characterisation of this series of compounds is still ongoing to investigate their mechanism-of-action and their selectivity against a panel of assays developed by AXXAM on structurally related lipases.In addition, the collaboration between SIPBS, AXXAM and PHARMAQ has been enforced throughout the SeaBioTech project to promote an integrated hit discovery program for the identification of marine compounds with anti-parasitic activity directed against *Lepeophtheirus salmonis,* a major threat for aquaculture. Three high-throughput assays made available by AXXAM (TRPA1, TRPV1 and voltage-gated Na-channel) were applied as pre-selection tools for the prioritization of crude extracts and fractions to be tested by PHARMAQ with the low-throughput phenotypic assay on living parasites. In total, AXXAM screened over 750 crude extracts for this purpose, which generated a list of 135 hits prioritized for testing at PHARMAQ. A number of these hits were confirmed for their parasiticidal activity on *L. salmonis*, and further characterization is ongoing at PHARMAQ on a subset of fractions to identify the pure compounds responsible for the sought bioactivity.

MBL: The first periodic activity report focussed on defining MBL's interests in terms of polysaccharide compounds and their growing market demand. As the project progressed through its second period, it was clear to MBL that in addition to the polysaccharides, the sampling events (both macroepiphytic and microepiphytic) have presented interesting and new chemistry and bioactivity across a range of compounds. MBL put considerable effort into advancing the analysis and fractionation of the priority samples selected internally and by the projects partners. Period 3 then focussed on the upscale of the bacterial samples and further fractionation and elucidation work on pure compounds. MBL continued sampling some of the key macroalgal species to develop seasonal metabolomic data. Alongside MBLs research effort, initial market analysis defining the potential market size/demand and market areas the compounds could feed into, whether that be as a stand-alone product or as an ingredient in a current or new formulation has been explored. Due to impracticality at commercial scale or inconsistency in results, some of the compounds were discarded. MBL has also communicated with various potential end users and current market producers to develop collaborations for the future development of the priority compounds. MBL has not released any publications on the priority compounds allowing there to be an element of commercial sensitivity especially if patents are to be filed in the coming years.

The final exploitation plan (June 2016) detailed the period 3 focus for the samples in the table below. It also highlighted difficulties in defining market information in some samples where limited research or market data is available. Although MBL initially had a strong focus on polysaccharides, it can clearly be observed that there are additional compounds that MBL now plan to commercialise over the coming years subject to the availability and success of appropriate funding mechanisms.

Development Bioactive	Application	Development Route	Estimated Commercial Potential (5 years)	Timing to start next progress stage	Comments

Polysiphonia extract (brominated compounds)	Pharmaceutical, Animal Health (Aquaculture)	BBI-JU call Submitted proposal September 2016	€ 1m	Q2 2017 (for 4 years)	Promising performance on Sea Lice LeSa assay and cancer therapeutics
Seaweed Bark Extract (complex compound mix)	Natural biostat/biocide	PhD, James Hutton Institute,	€ 1m	Under way – early 2016 (for 3 years)	More detailed fractionation is 1st step
Exo-polysaccharide / small bioactives from Bacillus	Cosmetics/Personal Care	UK funded – IBioIC Process Demonstrator	€ 1-5 m	End 2016/First half 2017 (6 months)	Key step now is scale-up feasibility and elucidation
Polysaccharide / small bioactives from Celeribacter	Cosmetics/Personal Care, Biomedical Plastics	UK funded – IBioIC /Innovate grant	€ 1 m	First half 2017 (6 months)	Key step now is scale-up feasibility and elucidation
Small bioactives from Ulva Lactuca	Pharmaceutical	PhD, Strathclyde University	€ 1 m	Q1/2 2017 (3 years)	PhD to be confirmed, definition of bioactives
High Value Saccharides – Fucoidan, Laminarin	Consumer Healthcare, Cosmetics	UK funded – SMART project-proposal underway	€ 10 – 25 m	Q1 2017 (1 year)	Topical skin treatment, anti-obesity
Polysaccharide and small bioactives-Ruegeria	Cosmetics/Personal Care, Biomedical Plastics	MRS 8 week studentship followed by UK grant funding	€ 1-5 m	Q2 2016 first half 2017	Key step now is scale-up feasibility and elucidation

PHARMAQ: The SeaBioTech project gave goods result for PHARMAQ. Very good collaboration with both academia and SME's that will continue after the end of the project is one of the main and high impact results for PHARMAQ. In addition, a HTS assay directed against a target special for salmon lice has been developed. This assay will be very valuable in screening of large libraries in the search for new actives against one of the most devastating parasites in aquaculture. Another main result is the increase in the capacity of the phenotypic screening assay, this will be very helpful in the future. Some compounds with effect against salmon lice have been identified. Although the effect has so far only been identified at a relative high concentration, the compounds are worth to further explore.

IGZ: Further development and implementation of inABLE® which is Ingenza's combinatorial genetics technology for the efficient and selective assembly of DNA expression vectors. This development included work on nested inABLE®. These technologies were key tools for improvement of strain construction and screening, and have been used and developed through SeaBioTech and the technology is of core importance to all of Ingenza's commercial interests.

The screening of both alternative metagenomics libraries and those of the work package partners for new and novel enzymes of commercial interest to Ingenza was carried out. This allowed expression constructs to be made and screens to be developed (WP6) which led to subsequent production processes (WP7). These generic fermentation protocols which had been developed previously were then implemented to test the growth and expression of

	positive hits which were highlighted in the subsequent screening of the work package partner's databases. These novel marine enzymes were cloned into an industrially relevant *E.coli* strain using inABLE® compatible parts. Further optimisation of the expression of these strains has been carried out in shake flasks followed by activity assays of the successfully expressed enzymes. Based on these results, fermentation development has been implemented, linking into the deliverables required for WP7. A production process of the most successful enzymes was implemented and scaled up during the course of WP7. Ingenza is continually conducting new business development activities and establishing new and expanded relationships with end users of industrial biotechnology in a broad range of industry sectors. On a quarterly basis the company re-evaluates opportunities and re-prioritises work as necessary to establish exploitation priorities which have been enabled by the work carried out during the course of the SeaBiotech project.
Potential impacts	**PRKZ**: Prokazyme as a small SME puts immense value on the opportunity to collaborate with front-line scientists in Europe through funding from the EU in projects like SeaBioTech. This is at the core of the company's strategy for competitiveness and has further strengthen the alliance with key partners and led to new opportunities for future discoveries and innovations. Prokazyme has made strategic plans for future commercial production of enzymes on economical large scale that will have great economic impact and help to bring innovations to the market for the benefits of the society and the European industry. **HDL**: Horizon has close links with the Cambridge Science Centre, an educational charity which hosts hands-on exhibitions, workshops, shows and talks to get the public excited about science and technology. Horizon employees volunteering at events hosted by the Cambridge Science Centre are able to engage with members of the public and talk about the work that Horizon does including our contribution to grants, such as SeaBioTech. Horizon submitted material to Strathclyde University intended for a poster presentation at the Marine Microbiome event hosted by MaCuMBA. Furthermore, Horizon has created valuable links with innovative scientists from around Europe and has a strong client base within the European pharmaceutical and Biotech industry. Horizon has entered into a number of strategic partnerships and is involved in several EU grant consortia. These alignments allow Horizon to access additional research fields. Participation in the SeaBioTech programme has opened access to many more natural products than currently available, for profiling in cell-based screens to directly find candidate new therapies for cancer. Using Horizon's existing client base and contacts in biotech and pharmaceutical sectors, candidate drugs can be quickly commercialised. **AXXAM**: Novel and underexplored species of marine microorganisms were demonstrated to be effective sources of novel therapeutics to be progressed to address unmet medical needs and threatening parasitic infections for aquaculture. Thus, the availability of novel therapeutics for human health and aquaculture will directly contribute towards improving quality of life, health, employment and economic strength. In addition, the knowledge gained through SeaBioTech concerning the assay development and screening of complex marine extracts may directly or indirectly translate into new opportunities for the CROs to expand their potential market and for pharmaceutical and life science companies to undertake novel R&D projects. **MBL**: Pending the success of grant funding MBL would envisage that their Fucoidan product would be the first to market which would be competitive to existing fucoidans produced in FMC (Norway), several manufacturers in China and Marinova (Australia). With an absence of grant funding, MBL would still commercialise fucoidan, albeit with a longer time to market. The expected economic impact of the fucoidan and associated saccharides laminarin and mannitol is expected to reach a commercial potential of €10-25 M. The successful grant funding of the polysiphonia based compound would have a significant impact on the salmon market but a commercial potential can only be estimated at this point in time (observed in table). Referring to the above table, MBL plan to seek grant and governmental support for the compounds/ bioactives which if successful, would lead to increased revenue and industrial output and employment within MBL but also potentially

	with partner organisations and end users. **PHARMAQ :** With the recently increased capacity of the phenotypic screening assay (Lesa) it will be possible increase the search for a new compound against salmon lice. The single compound identified at the end of the project period with effect against salmon lice will be further explored (in a new EU-project) and may result in a patent or also evaluated as a new potential product candidate. A new product will be very helpful for PHARMAQ as a company and competitiveness but also for the salmon farming industry. **IGZ** : Ingenza's involvement within EU projects such as SeaBioTech allows us to explore new commercial opportunities, as well as, improve on current production processes. Enzymes are key to the success of Ingenza, now, and in the future. Allowing collaboration within a grant such as this makes Ingenza, as a company, more competitive but the combined collaboration also makes our EU grant partners more competitive as a whole which benefits Europe's place within other competing world economies. Europe benefits from access to a diverse set of marine ecosystems and to the corresponding biodiversity partly due to its proximity to four seas and two oceans. These marine ecosystems are largely unexplored, understudied and underexploited in comparison with terrestrial ecosystems and organisms. Marine Biotechnology will become even more, central to delivering these benefits from the sea, and projects like these go some way to Europe realising its potential in the marine Biotechnology sector. Biotechnology, involves the application of biological knowledge and cutting-edge techniques to develop products and other benefits for humans, is of growing importance for Europe and will increasingly contribute to shape the future of our societies. Marine Biotechnology is fast becoming an important component of the global biotechnology sector. The global market for Marine Biotechnology products and processes is currently estimated at € 2.8 billion (2010) with a cumulative annual growth rate of 4-5%. Less conservative estimates predict an annual growth in the sector of up to 10-12% in the coming years, revealing the huge potential and high expectations for further development of the Marine Biotechnology sector at a global scale. Ingenza and other EU partners, both in the academic and commercial sectors being part of this, are essential if we want to remain competitive with the rest of the world.
Main dissemi-nation / exploitation	See ref. **80 and 98** in section 2

WP2 – Sampling process	
Main objectives	- Define sampling process according to the explored marine ecosystems and sampling in situ - Characterise the microorganisms - Optimise the storage of marine organisms - ACentralise the knowledge about the existing biobanks
Contributing partners	**HCMR**, SAMS, MBL, MATIS, UWUERZ, SIPBS
Main results	***SAMPLING*** ***MATIS:*** From coastal geothermal sites in Iceland, a total of 49 samples were collected, primarily from photosynthetic microbial mats and also from polysaccharide enrichments in situ and a total of 194 strains were isolated: 122 from Laugarvík, 47 from Yngingarlindir and 25 from Reykhólar. Numerous strains representing novel species and genera were isolated, especially from Yngingarlindir. Alginate degrading anaerobic isolates from Reykhólar were close to the genus of *Clostridium* and five of them were selected for whole genome sequencing and genome annotation analyses in WP4. A preliminary study of the species composition of Cyanobacteria from the clone sequences from the YL samples was performed and the largest taxon contained several species representing

distant (88-95% 16S rDNA similarity) relatives of *Geitlerinema sp*. within the *Oscillatoriales*. A similar study on the composition of Cyanobacteria in four of the Laugarvík biomat samples revealed the majority of sequences belonged to a filamentous *Leptolyngbya sp* highly related to a Leptolyngbya sp. found in arctic hot springs in Greenland. Results from culture independent biodiversity studies in Yngingarlindir and Laugarvík indicated novel species of Cyanobacteria. Seven Cyanobacteria strains were (M24-M36) isolated from mat samples and identified. Strains of interest (32) were selected for extractions in WP3. The extracts (62) and relevant control samples (6) were labelled and sent to the relevant partners for bioactivity screening. Based on novelty, 39 strains were selected for whole genome sequencing and annotations in WP4 & WP6. From the total of 39 strains, 38 strains were sequenced and their genomes annotated.

HCMR:

Santorini volcanic complex (Santorini caldera and Kolumbo submarine volcano) is a part of the Hellenic Volcanic Arc characterized by a unique convergent setting and by a unique enrichment of polymetallic spires in As, Sb, Zn etc. Two major sampling events were organized by HCMR in September 2013 and in May 2014 in this volcanic complex with the Research Vessel Aegaeo and the remote operated vehicle of HCMR from which a large number of water samples (>100), pollymetalic active and inactive gas chimneys (>30 samples and subsamples) from the submarine Kolumbo volcano and microbial mat samples from Santorini caldera and Kolumbo volcano (>30) were collected and used for microbial strain isolation, community characterization and metagenomic libraries construction. In total, 280 microbial strains were finally isolated from the Kolumbo/Santorini samples for the other tasks and WPs, belonging to different species mainly within the *Bacillales* of *Firmicutes* phulym and within the *Pseudomonadales* of *Gammaproteobacteria*. Several novel species were also identified whereas additional strains isolated from the Milos sampling event of May 2013 are available also in MATIS strain collection. In addition a series of physicochemical parameters (e.g. gas analysis of the active vents, nutrients, organic carbon, metals, chloropigments etc) were also estimated in order to explain microbiological results and further evaluate the potential risks of the active submarine volcanoes of the Hellenic arc (Paper published in Nature Scientific Reports Rizzo et al., 2016). A detailed description of the collected samples has been presented in deliverable D2.5.

SAMS:

In addition to samples from the CCAP, Isolates have been generated from the following sources: Milos sponges (120, of which 57 have been processed); Scottish sponge isolates (~150); Scottish & Antarctic sediment cores (~100 of which 54 have been processed); Polar Antarctic & Arctic sediment cores (~150).

MBL:

Throughout the SeaBioTech project, MBL have submitted 654 samples onto the internal database which can be broken into:

- Bacterial samples/extracts: 241 samples
- Macroepiphytes/algae samples: 144 samples
- Fractions: 193 samples
- Washes/MBL process residues: 75 samples

UWUERZ:

2 collection efforts to the Greek islands yielded the following biomaterial:

- 64 unique actinomycetes were isolated from 12 different marine sponge species, which were affiliated to 23 genera representing 8 different suborders based on nearly full-length 16S rRNA gene sequencing.
- 4 putatively novel species belonging to the genera *Geodermatophilus*, *Microlunatus*, *Rhodococcus*, and *Actinomycetospora* were identified based on a sequence similarity <98.5% to validly described 16S rRNA gene sequences.
- 13 isolates showed antioxidant, antimicrobial, and antitrypanosomal activities.
- *Streptomyces* sp. SBT345 and SBT348 were prioritized for compounds isolation

	based on metabolomics analyses and bioassay screening in follow-up work with WP3 and WP5. ***METAGENOMES (all partners):*** Samples for metagenome libraries were already available from period 1 of the project (i.e. from Yngingarlindir in Iceland (water sample), microbial mats and sponges from Milos Island in Greece and Santorini volcanic complex in Greece i.e. Santorini microbial mats, water samples from Kallisti lakes, Kolumbo microbial mats covering the ocean floor and the polymetallic chimneys, water samples from the active area). During period 2, from Santorini volcanic complex, a total of 5 samples were successfully processed for metagenomic library construction and have been sequenced in Illumina MiSeq. These libraries include two microbial mat samples from the newly discovered Kallisti lakes in Santorini caldera, and three different microbial mat layers from a gas chimney in Kolumbo volcano, Greece. A mat sample from Yngingarlindir in Iceland was processed for metagenomics analysis described in WP4. In period 3 no more libraries were constructed. ***STRAIN COLLECTIONS:*** ***HCMR strain collection:*** HCMR has created a collection of 280 strains from the extreme environments of the Hellenic Volcanic arc. ***MATIS strain collection:*** MATIS has created a collection of 194 strains from the three intertidal sampling sites in Iceland, 122 from Laugarvík, 25 from Reykhólar and 47 from Yngingarlindir. Additionally 9 strains from Milos during the 2013 expedition and 34 strains from Santorini volcanic complex 2013 expedition. Selected strains from Milos 2013 and Santorini 2013 expeditions have been shipped to HCMR. ***UWUERZ strain collection:*** UWUERZ has created a collection of 64 actinomycete strains from Milos and Crete sponge sampling of 2013. ***SAMS strain collection:*** SAMS has created a unique collection of strains encompassing of a wide range of taxa including: a range of heterotrophic eubacteria, cyanobacteria and eukaryotic micro-algae. Full details of the procedures employed were reported in deliverable D2.6. In total 480 biological isolates have been identified in the project and processed down the biodiscovery pipeline by SAMS, with 116 of these being identified by 18S rRNA gene sequence NCBI blast results in Period 3. Of these 310 biological isolates; were processed down the biodiscovery pipeline. Of the 310 samples processed, 246 were of bacterial isolated identified in this project by molecular barcoding (16S rRNA gene) and 64 were algal, with identity confirmed by 18S rRNA gene sequence NCBI blast results. All the live microorganisms that have been identified are held in the bacterial and protistan collections at SAMS. All bacterial isolates are held as frozen/ cryopreserved master stock-cultures at -80^{o}C, with glycerol (5% in medium) as cryoprotectant. The algal isolates are maintained by serial transfer and where practicable they are also held as cryopreserved master-cultures and stored at -196^{o}C in the CCAP Cryostore. ***MBL strain collection:*** MBL has created a collection of 165 strains over 4 sampling sessions; two in 2013 in Culzean bay and Oban and two in 2014 at Culzean bay. Of those strains which were isolated, the dominant members were affiliated within the class of *Gammaproteobacteria* and the phylum of *Firmicutes*. Period 3 has furthered the depth of analysis on some of these sampled strains such as SBT111 (*Celeribacter*), SBT148(*Ruegeria*) and SBT153 (*Bacillus licheniformis*).
Potential impacts	***Scientific breakthrough:*** WP2 gave the opportunity to investigate some of the most unique environments/habitats on earth, isolate/characterize microbial species living there and create large strain collections for biotechnological exploitation. Some of the isolated strains were characterized by high novelty and biotechnological potential as they showed very low similarity with any other previously characterized bacteria. We gained new knowledge about gene diversity in extreme environments, as well as

	valuable information about environmental microbial functioning through the application of modern metagenomic deep-sequencing techniques. Genomic sequence data by UWUERZ have revealed the presence of a large fraction of putatively silent biosynthetic gene clusters in the genomes of actinomycetes that encode for secondary metabolites that remain silent under standard fermentation conditions. Our work has provided here novel insights into actinomycete biodiversity as well as into the effects and consequences of elicitation of secondary metabolism in actinomycetes. Huge metagenomics data were created and used as a source for bioprospecting. ***Economic impact (health costs, market,…):*** WP2 did not have a direct economic impact, as it did not produce any new product/service of economic significance. WP2 served as the foundation of SeaBioTech discovery pipeline. By focusing on previously unexplored environments, WP2 attempted to increase the odds of discovering novel bacterial species that would contain novel bioactive compounds of potential economic interest. Indeed, WP2 supplied the other workpackages with novel cultivable strains holding a great potential for the discovery of novel natural products of high-added value ***Societal impact (quality of life, health, education, employment, citizen awareness,…):*** WP2 gave the opportunity to: - recruit young researchers, PhD students and technical staff - >raising citizen awareness by diverse outreach activities to pupils, students and the interested public - >exchange young scientists between SeaBioTech partners, provide them hands-on training for different aspects of the biodiscovery pipeline and enhance their research skills - >collaborate in order to collect samples that would better serve the purposes of SeaBioTech and the discovery of novel natural products (e.g. HCMR-UWUERZ collaborative sampling campaigns in Milos and Chania, Greece) In addition through Seabiotech sampling campaigns we produced knowledge on the activity of the extreme environments of the Hellenic Volcanic Arc. A relevant paper was recently published in Nature Scientific Reports (Rizzo et al., 2016) demonstrating the need of a monitoring program for this dangerous environment. ***European competitiveness / standards and policies:*** **HCMR:** SeaBioTech became a source of inspiration for HCMR and increased the biodiscovery expertise/knowledge of a critical mass of scientists working at the *Institute of Marine Biology, **Biotechnology** and Aquaculture (IMBBC)* Biotechnology and discovery of novel natural products turned into a high priority and a strategic goal for our institute which has recently acquired a BIOFLO-320 10 L fermenter and an Agilent 6460 Triple quadrupole LC-MS/MS. In addition, HCMR coordinates a National Platform targeted to the exploitation of Marine Biological Resources (preparatory phase starts Nov 2016). **SAMS:** SeaBioTech has helped inform the ongoing development of the BSC Marine Biotechnology module of the University of the Highlands and Islands (UHI) BSc in Marine Science. In addition, the Bioprospecting pipeline developed in SeaBiotech has helped facilitate the development of a focussed study being undertaken in the EU funded EMBRIC project http://www.embric.eu/ on exploring options to streamline future marine microbial biodiscovery pipelines. It has also helped inform the Culture Collection of Algae and Protozoa www.ccap.ac.uk of the needs of the rapidly developing algal biotechnology sector, so that services can be better targeted to help the development of the sector. **MBL:** SeaBioTech has encouraged and grown MBL's knowledge and product research in development of algal and marine derived bacterial compounds. The project has been strategic in developing knowledge and partnerships with academia and other industry allowing MBL to move closer towards commercialisation on a range of algae derived

	products while generating meaningful metabolomics and chemical data and an understanding of seasonal yield. MBL will continue to pursue several research threads that have arisen due to the involvement in the project and will continue to collaborate with several of SeaBioTech partners on future projects. Referring to WP1, MBLs further work on the SeaBioTech pipeline will lead to a 5 year commercial potential in excess of 10M GBP. **MATIS:** The SeaBioTech project has and will encourage further research on microbial life at the unique habitats at the intertidal geothermal sites in Iceland. The sites explored within the project have revealed numerous novel microbial species and genera, providing material for bioprospecting for novel bioactive compounds, and robust enzymes and microbes for bioconversion of sustainable marine biomass to value added products. Culture independent studies revealed unknown taxa, yet to be isolated and investigated. Collaboration and student exchange has been important for transferring knowledge and skills.
Main dissemination / exploitation	see ref. **3, 6, 11, 15, 16, 17** in section 2

WP3 – Detection and characterisation of commercially relevant bioactivities	
Main objectives	- To assemble a repository of extract and compounds of marine origin - To identify novel bioactive molecules of marine origin from extract and compound collections - To contribute to the isolation of the compounds responsible for the desired pharmacologic effects present in complex bioactive extracts and fractions - To characterize the pharmacological properties of the most promising lead compounds progressed from the screening process
Contributing partners	**AXXAM**, HDL, PHARMAQ, MATIS, PKZ, MBL, UWUERZ
Main results	**1. Repository of extracts and compounds of marine origin.** The first objective of WP3 within the SeaBioTech integrated project was the assembly of a repository of extract and compounds of marine origin. This goal was achieved by WP3 in collaboration with WP2 and WP5 members through the implementation of centralized repository of marine samples housed at SIPBS. The centralized repository contains at the end of the project 3209 samples of marine origin, including 1140 crude samples and 606 fractions plated in ready-to-screen format and 63 pure compounds. In addition, the repository contains samples which were received in a too small amount for general screening. Thus, they were stored and annotated in case further sample is obtained to ensure sufficient material is available for assaying. The annotation of samples, fractions and pure compounds stored in the centralized repository was managed through a database implemented by SIPBS and accessible in a secure manner through the SeaBioTech Portal to all partners involved in sampling, screening and dereplication activities (http://spider.science.strath.ac.uk/seabiotech/index.php). Each sample was assigned a unique SeaBioTech code in the format "SBTXXXX" (where "X" is a number from 0 to 9) and all information associated to each sample related to parental microorganism, genomics, LCMS, NMR data, bioactivity results and pharmacological profiling generated during the SeaBioTech collaboration was entered into the database. In addition, each sample was connected to its relevant negative control sample (e.g., culture media) to enable validation and correct analysis of potentially active entities during bioactivity screening. The database played an essential role on the prioritization of samples, fractions and compounds along the SeaBioTech collaboration and represents a valuable asset for the prospective exploitation of the results obtained by SeaBioTech. **2. Assay development and screening** WP3 members in charge of the screening activities improved along the project the performance and throughput of their assays, to comply with the requirement to process

a remarkably high number of extracts, fractions and compounds of marine origin. Major improvements were obtained for the development of automated, high-throughput screening platform to provide cell-based assays for the detection of hits with anti-cancer activities, in particular for cell proliferation (HDL). Moreover, assay systems were modified to achieve a suitable robustness to screen complex marine extracts and subsequently to produce more accurate and reliable results (SIPBS, AXXAM). In addition, the phenotypic assay performed on the fish parasite of aquaculture plants *Lepeophtheirus salmonis* was optimized to increase its capacity and processivity, thereby expanding the possibility to screen extracts and fractions of marine source (PHARMAQ).

3. Isolation and characterization of bioactive molecules of marine origin.

The central goal of WP3 (and of the entire SeaBioTech consortium) was the isolation and pharmacological characterization of novel lead candidates of marine origin. This goal was achieved through an integrated effort between WP2-WP5 with the six members of WP3 (SIPBS, AXXAM, HDL, PHARMAQ, UWUERZ, MATIS), who have made available comprehensively an array of 41 functional assays with relevance to 12 therapeutic and life science indications. The screening process and the bioactivity-assisted dereplication of crude extracts and fractions have led to the isolation and characterization of 35 pure compounds with promising therapeutic properties. Notable examples are the followings: (1) SBT0345 from *Streptomyces sp.* was fractionated by UWUERZ to yield three novel natural products, namely strepthonium A, ageloline A and strepoxazine A. Strepthonium A inhibited the production of Shiga toxin produced by enterohemorrhagic *E. coli* at a concentration of 80 µM, without interfering with the bacterial growth. Ageloline A exhibited antioxidant activity and inhibited the inclusion of *Chlamydia trachomatis* with an IC_{50} value of 9.54 ± 0.36 µM without cytotoxicity towards human kidney 2 cells. Strepoxazine A displayed antiproliferative property towards human promyelocytic HL-60 cells with an IC_{50} value of 16µg/mL. Moreover, SBT0345 from *Streptomyces sp.* was yielded also the known compound phencomycin, which displayed cytotoxicity against colon cancer cell line SW48 at 30 µg/mL, and tubermycin B, which showed cytotoxicity against colon cancer cell lines DLD-1 and HCT116 at 30µg/mL. (2) SBT0348 from *Streptomyces sp.* was fractionated by UWUERZ to yield one novel compound, petrocidin A, exhibiting significant cytotoxicity towards the human promyelocytic HL-60 and the human colon adenocarcinoma HT-29 cell lines, with IC_{50} values of 3.9 and 5.3 µg/mL, respectively. (3) SBT0961 from *Polysiphonia lanosa* yielded three fractions, which were identified by HDL as active and selective for rapidly dividing cancer cells, with anti-proliferative properties strongly correlated with the induction of cell death via apoptosis. (4) MATIS identified from microorganisms collected from the Icelandic coastline 11 hits were identified by displaying high anti-oxidant activity, 9 hits that inhibited cell viability of breast cancer cell line and 13 hits that inhibited viability of intestine cancer cell line. (5) SIPBS isolated 13-methyltetradecanoic acid (SBT2309) from *Muricauda ruestringensis,* a compound with activity against PTP1B, a target to treat diabetes and metabolic syndrome. Remarkably, SIPBS isolated the same compound showing comparable activity against PTP1B at the end of an independent bioactivity-assisted screening campaign from extracts of another microorganism, *Algoriphagus marincola.* (6) SIPBS isolated a series of structurally related fatty acids from extracts of *Algoriphagus marincola*, which showed activity against PTP1B (SBT2656, SBT2660, SBT2662, SBT2665 and SBT2667) and allowed the definition of a preliminary structure-activity relationship on the basis of the relative potency. This finding corroborated previous studies, which indicated that length of carbon chain backbones of fatty acids was correlated with greater inhibition of PTP1B (Planta Med 2012;78:219; Cell Physiol Biochem 2013;32:871). Remarkably, AXXAM isolated with an independent screening campaign for inhibitors of endothelial lipase, a validated target for atherosclerosis, a series of fatty acids derived from *Algoriphagus marincola* partially overlapping with the hits showing activity against PTP1B at SIPBS (SBT2643, SBT2653, SBT2656, SBT2660, SBT2662, SBT2665, SBT2667, SBT2670). This finding appears consistent with the targeted enzyme EL, which physiologically releases

	fatty acids from phospholipids in HDL particles. (7) SBT1997, a pure compound isolated by SIPBS from *Polysiphonia lanosa* as active against α-glucosidase, was identified as a known compound termed lanosol. Lanosol was documented in literature as an α-glucosidase inhibitor (J Nat Prod, 1999;62:882; Mar Drugs. 2011;9:1273). A related bromide compound, termed SBT1998 (2,3-dibromo-4,5-dihydroxybenzaldehyde), was shown to inhibit α-glucosidase as well. (8) A series of homologous compounds have been identified by PHARMAQ from *Polysiphonia lanosa* extracts and fractions having a potent parasiticidal activity against *Lepeophtheirus salmonis*, a major threat for farmed salmon in aquaculture.
Potential impacts	**1. Scientific breakthroughs** The repository of extracts, fractions and pure compounds derived from underexplored marine microorganisms and the related information managed by the centralized database represents a valuable infrastructure for future R&D projects in diverse life science areas. Novel and underexplored species of marine microorganisms were investigated for the first time as potential sources of novel therapeutics and they provide positive indications that lead compounds can be isolated and progressed to address significant unmet medical needs (e.g., cancer, infections against, metabolic syndrome and inflammation) and threatening parasitic infections for aquaculture. **2. Economic impact** The personalised medicine market worldwide is estimated to be over 400 billion Euro and the core diagnostic and therapeutic segment of the market is estimated at over 40 billion Euro. The need to address this market and the benefit of doing so is supported by many facts, including a 75% increase in personalised medicine investment over the last 5 years and 30% of all pharma companies now require compounds in R&D to have patient-relevant treatments. The potential novel marine products identified through the SeaBioTech collaboration may enable such novel therapeutics to be progressed through the R&D process. In particular, potential lead compounds have been isolated with a potential to address therapeutic indications for human health such as cancer, bacterial infections and metabolic syndrome, and to develop an effective treatment against the fish parasite *L. salmonis*, which represent a major threat for aquaculture. In addition, the knowledge gained through SeaBioTech concerning the assay development and screening of complex marine extracts may directly or indirectly translate into new opportunities for the CROs to expand their potential market and for pharmaceutical and life science companies to undertake novel R&D projects. **3. Societal impact** The lead compounds isolated at the end of the SeaBioTech collaboration have the potential to be evolved into novel therapeutics, which will be further pursued, e.g., as in the grant application AlgaChem (H2020-BBI-JTI-2016). The availability of novel therapeutics for human health and aquaculture will directly contribute towards improving quality of life, health, employment and economic strength. The positive societal impact of results achieved by WP3 have been disseminated to public through interviews on TV programmes, talks and exhibits to school children and adults (e.g., Glasgow Science festival and Explorathon), talks and publications delivered to the scientific community and general audiences, and cooperation with charities, such as Cambridge Science Centre, which hosts hands-on exhibitions, workshops, shows and talks to get the public excited about science and technology. To end, WP3 gave the opportunity for education of PhD and masters students, who contributed to the activities of SeaBioTech. **4. European competitiveness** WP3 has strongly participated in fostering European research excellence in the field of drug discovery project based on natural compounds of marine origin. Technological and scientific progresses made by WP3 partners along the SeaBioTech collaboration have substantially increased the European competitiveness by producing, organizing and

	making available a repository of extracts, fractions and pure compounds of marine origin, which may represent the foreground for future drug discovery programmes. Moreover, the lead candidates isolated by WP3-WP5 may disclose novel chemical scaffolds to treat unmet biomedical and agrochemical needs. Consistently, key partners of WP3 are now engaged in the H2020 grant application AlgaChem (H2020-BBI-JTI-2016), aimed at developing and bringing to the market compounds with potential anti-parasitic activity for aquaculture isolated during the SeaBioTech collaboration.
Main dissemination / exploitation	See ref. **3, 15, 18, 21, 22** in section 2 **2 further papers in revision**

WP4 – Genomic and metagenomic bioprospecting	
Main objectives	- To establish extensive genome / metagenome / single cell genome databases for bioprospecting of genes/enzymes of classes of interest. - To identify genes of interest in the databases and whole biosynthetic pathways of target compounds - To advise WP1 on the potential commercialisation of target molecules/enzymes identified by genomic/metagenomic bioprospecting
Contributing partners	**UWUERZ**, MATIS, SAMS, HCMR, PKZ, SIPBS
Main results	The main results within WP4 are summarized as follows: ➢ Large sequence databases (totalling >350 Gb) generated ➢ Newest NGS technologies integrated ➢ Bioinformatic pipelines established ➢ Novel bacteria discovered ➢ Thousands of gene clusters, among them many novel ones, identified (in particular: secondary metabolism, Cazy) ➢ Student teaching at BA and Masters level ➢ Publications in open access journals on-going **UWUERZ** **Genome mining of bacterial isolates** UWUERZ provided draft genomes of 3 selected actinomycetes (Horn et al., Mar Genomics 2015). Metabolomic analysis in WP5 has shown the chemical richness of the sponge-associated actinomycetes *Streptomyces* sp. SBT349, *Nonomureae* sp. SBT364, and *Nocardiopsis* sp. SBT366 that had been isolated from sponges during a SBT sampling expedition. The genomes of these three actinomycetes were subsequently sequenced and draft genomes were mined using antiSMASH and NapDos. Streptomyces sp. SBT349 displayed the most diverse read-out. A total of 108 potential secondary metabolite gene clusters were predicted, encoding for 23 type I polyketide synthases (PKS), 11 non-ribosomal peptide synthetases (NRPSs), 2 terpenes, 21 saccharides, 3 siderophores, 3 lantipeptides, 1 butyrolacetone, 1 bacteriocin, 1 phenazine, 1 ladderane, and 1 linaridin, as well as 26 unidentified putative clusters. Furthermore, NaPDoS predicted the presence of natural products such as nystatin, rapamycin, rifamycin, epothilone, and tetronomycin. For Nonomureae sp. SBT364, NaPDoS predicted the presence gene clusters encoding for rifamycin, avermectin, avilamycin, concanamycin, and tetronomycin. Thirdly, for Nocardiopsis sp. SBT366, gene clusters encoding for pikromycin, alnumycin, amphotericin, and mycinamicin were predicted. In summary, UWUERZ efforts provided new insights into the genomic underpinnings of actinomycete secondary metabolism, which may deliver novel chemical scaffolds with interesting biological activities for the drug discovery pipeline. **Metagenomic bioprospecting** UWUERZ employed a metagenomic bioprospecting approach to unravel the differences in the functional gene repertoire between three Mediterranean sponge species, *Petrosia ficiformis*, *Sarcotragus foetidus*, *Aplysina aerophoba* and seawater, collected during a SBT

sampling expedition (WP2). Microbial diversities were compared to those of other sponges within an EMP global sponge microbiome effort and contributed to the largest microbiology survey in sponges so far conducted (Thomas et al., Nature Comm 2016).

With respect to gene function, different signatures were observed between sponge and seawater metagenomes with regard to microbial community composition, GC content, and estimated bacterial genome size. Our analysis showed further a pronounced repertoire for defense systems in sponge metagenomes. Specifically, Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR), restriction modification, DNA phosphorothioation and phage growth limitation systems were enriched in sponge metagenomes (Horn et al., Frontiers in Microbiol, in review). These data suggest that the "defensosome" is an important functional trait for an existence within sponges that requires mechanisms to defend against foreign DNA from microorganisms and viruses.

With respect to secondary metabolism, the most abundant marker genes in the microbial metagenomes belonged to the groups of saccharides, bacteriocins, terpenes and fatty acids. Other indicator genes of secondary metabolism – linaridin, lantipeptides, ectoines, phosphonates, proteusin, polyketide synthases, nucleosides, microcins, siderophore or homoserine lactones - were found only in low copy numbers. Interestingly, while siderophores and homoserine lactone hits were only identified in seawater, lantipeptides, linaridines, and Type I Polyketide synthases were exclusively found in the sponge metagenomes.

We further identified a total of 120 Type I PKS genes in the three sponge metagenomes. Phylogenetic analysis assigned the majority (109/120) to the symbiont ubiquitous *supA*-type PKS group. Most similar sequences from the sponge metagenomes were derived from bacterial symbionts of other sponge species. Most of the polyketide synthases in the *supA* clade of the tree resulted in a hit to epothilone with low to moderate sequence identities. Despite the variance of possible products in the FAS-like PKS clade, the order of the genes surrounding the polyketide synthase was highly conserved.

MATIS

Matis sequenced 34 novel bacterial strains from geothermal intertidal areas in Iceland, assembled and annotated for bioprospecting. An additional 4 strains that had been sequenced before SeaBioTech were also annotated at the beginning of SeaBioTech to allow bioprospecting to start.

Of the 38 sequenced strains, 13 (34%) belong to the α-*Proteobacteria*, 10 (26%) to *Bacteroidetes*, 7 (18%) to Firmicutes, 6 (16%) to γ-*Proteobacteria* and one strain each to *Actinobacteria* and *Chloroflexi*. All strains are thermophiles or moderate thermophiles.

An extremely high level of novelty was presented by this panel of novel strains. Based on 16S rRNA gene sequencing of the 38 genomes, 19 strains (50%) shared less than 94% similarity with their closest relative and are therefore considered novel species and novel genera. 10 (26%) shared between 94% and 97% similarity and are considered novel species and the remaining 9 strains (24%) shared more than 97% similarity with their closest relative.

Strain MAT4553 which has 90% similarity with its closest relative *Rhodothermus marinus* (16S rRNA gene) was selected for further characterisation. It has been assigned the species name *Rubrimicrobum thermolitorum* and characterisation is ongoing with the aim for publication in the International Journal of Systematic and Evolutionary Microbiology.

All 38 strains were annotated using subsystem annotation servers (RAST and MG-RAST), the genomes mined for novel genes of interest and analysed by antiSMASH for putative secondary metabolite gene clusters. A total of 2432 putative gene clusters were predicted, including 20 Non-Ribosomal Peptide Synthetase clusters and a total of 30 Polyketide Synthase clusters of Types I, II or III.

A total of 64 genes encoding novel enzymes for applications in marine macroalgal biorefineries were identified and delivered for cloning, expression and functional analysis in WP6 including, 51 carbohydrate active enzymes (CAE) 3 enzymes (oxidases) putatively active on polyphenols, 5 alcohol dehydrogenases, a sulfatase and 4 proteases

A total of 58 genes encoding novel enzymes including thioesterase, cyclic peptide related

genes, and (3) lysine exporters , for application in synthesis of added value chemical and pharmaceutical were identified and delivered to IGZ for, cloning , expression in their proprietary inABLE® system and for further analysis and selection in WP6.

SAMS

SAMS undertook whole genome sequencing of five bacterial strains and delivered a total of four draft whole bacterial genomes. The fifth bacterial genome was to be of the filamentous cyanobacterium, *Nodularia harveyana* CCAP 1452/2. This was advanced to the point of achieving an axenic culture (WP2) and development of a useable DNA extraction protocol based on mechanical tissue disruption without pre-digestion of the cell walls using the lysozyme, and purification using the quarternary ammonium detergent cetyl trimethyl ammonium bromide. However, significant quantities of polysaccharide were found to contaminate the DNA preparations, and refinements to the protocols were not successful in removing this. This meant the genome sequencing centre were unable to prepare the DNA library required for PacBio RSII genome sequencing.

All genome data was mined for enzymatic and secondary metabolite potential. In terms of carbohydrate active enzymes and xenobiotic degradation potential, *Colwellia* and *Rhodococcus*, respectively, had the greatest potential of the four organisms. The *Colwellia* genome data will serve as an important resource for the scaling up and commercialisation of the gel-forming biopolymer this organism produces (WP7) during a PhD studentship working in conjunction with the multinational company, Unilever. The *Rhodococcus* genome is undergoing further analysis to link the secondary metabolite clusters identified with the metabolome of this organism fermented under different conditions (WP5 and WP7).

The Acidobacteria (Holophagales) genome showed an especially high number of novel secondary metabolite gene clusters belonging to the non-ribosomal peptide synthetase (NRPS) and polyketide synthase (PKS) classes. Metabolomic analysis (WP5) did not identify production any secondary metabolites putatively linked with these cluster, nor was any bioactivity identified (WP3). The lack of novel secondary metabolite production by the Acidobacteria is hypothesized to be a failure to induce the many cryptic secondary metabolite operons. This hypothesis is given some support by the observation that many signal transduction systems were found within or immediately adjacent to these clusters. This suggests that these clusters are tightly regulated and are part of a signal transduction relay activated by specific signalling molecules or environmental stressors. In conclusion, this organism holds significant potential for secondary metabolite production. To achieve this though, further funding is required try to activate the cryptic secondary metabolite clusters, & continue to isolate & genome sequence new marine Acidobacteria from the environment.

Vibrio splendidus SBT0000027 was shown to produce a range of bisindoles, including the compound Turbomycin. Several putative genes were identified that may be linked with Turbomycin production. First, the biosynthetic pathway for the assumed precursor, L-tryptopan, was identified. Second, the enzyme 4-hydroxyphenylpyruvate dioxygenase had previously been identified as a part of Turbomycin production, and this was identified in this genome. Third, inosine-5'-monophosphate dehydrogenase has been shown to be important in bisindole production previously, and this gene was also identified. However, as these genes are not organized in an apparent gene cluster, it is uncertain how these genes are involved in Turbomycin production by this *Vibrio*. Moreover, the above genes are all highly conserved and syntenic in all other *Vibrio splendidus* genome sequenced isolates. This suggests either, that all *V. splendidus* are capable of Turbomycin production, or that the main pathway for bisindole and/or Turbomycin production in *V. splendidus* SBT0000027 has not been correctly identified. Clearly, further work is required to identify this pathway.

HCMR

HCMR generated 2 metagenomic libraries from the Kallisti lakes in Santorini caldera characterized by high concentrations of metals and differences in pH, temperature and nutrient concentrations.HCMR generated another 3 metagenomic libraries from a polymetallic spire located within the submarine Kolumbo volcano of the Hellenic Volcanic Arc. Each library has been constructed from different microbial mat layers of the spire

	characterized by differences in metal concentrations. Elevated amounts of As, Pb, Sb have been also measured.
Potential impacts	**Scientific breakthrough**: g**eneration of novelty** Novel bacterial isolates within the following phyla and genera were discovered: Acidobacteria, Actinobacteria, Bacteroidetes, Proteobacteria, Rhodothermus sp. Novel gene clusters encoding for enzymes were identified: Carbohydrate-active enzymes, ADHs and proteases, aminotransferases, methyltransferases, hydratases, phosphatases, EPS production, bioindole production, viral/phage defenses, etc Novel gene clusters encoding for secondary metabolism were discovered: PKS, NRPS, nystatin, rapamycin, rifamycin, epothilone, tetronomycin, avermectin, avilamycin, concanamycin, pikromycin, alnumycin, amphotericin, saccharides, bacteriocins, terpenes, and fatty acids and many, many novel ones The Acidobacteria genome is the first marine acidobacterial representative to have been sequenced. The number and size of the NRPS and PKS gene clusters account for ca. 15 % of the whole genome. This secondary metabolite genome specialism is similar to that observed in the most commercially important bacterial secondary metabolite producers, the Streptomyces. Metagenomic data have revealed the presence of complex and diverse functions in the investigated extreme environments of the Hellenic Volcanic Arc related to metal resistance and key processes e.g. nitrification and CO2 fixation. The microbial diversity of the Mediterranean sponges was included in the largest effort known to date in sponge microbiology, contributing to a high impact publication in Nature Communications. **Economic impact (health costs, market,…):** PhD funding IBioIC (Scotland) to develop *Colwellia* sp. DG1864 Exopolysaccharide production. Supervisors: Green, Harvey, McNeil & Unilever; Large sequence databases totaling 350 Gb were generated;Novel enzymes of commercial relevance were identified **Societal impact** (quality of life, health, education, employment, citizen awareness,…): **UWUERZ selected examples** Darwin-Day, public speech to about 1000 high school students at Kiel (http://www.uni-kiel.de/pressemeldungen/index.php?pmid=2015-417-darwintag; Nov. 13, 2015) Boys Day 2016, (http://www.boys-day.de/; April 28, 2016) MyOSD, citizen science project at Kiel (http://www.my-osd.org/index.html; June 21 2016) **SAMS selected examples** Duncan, K.R, *et al* . 2014.Identifying secondary metabolites and their biosynthetic pathways using GnPS molecular networking. Natural Products Biotechnology, Inverness, Scotland. UK Green, DH. Marine biotechnology lectures to Marine Science BSc 3rd year students, (University of the Highlands and Islands). Feb/Mar. 2015, 2016 Green, DH. Marine enzymes and bioinformatics tutorial and laboratory to Industrial Biotechnolgy Master by Science students (University of Strathclyde). Mar., 2015, 2016. **European competitiveness / standards and policies** : A strong, unique combination of skills, tools and experiences was achieved within WP4 and in close interaction with other WPs of SeaBioTech HCMR participation to WP4 of SeaBioTech became a source of inspiration by increasing the biodiscovery expertise/knowledge of a critical mass of scientists working at the *Institute of Marine Biology, Biotechnology and Aquaculture (IMBBC).* Indeed, biotechnology and discovery of novel natural products turned into a high priority and a strategic goal for our institute which has recently acquired a BIOFLO-320 10 L fermenter and an Agilent 6460 Triple quadrupole LC-MS/MS.
Main dissemination / exploitation	See ref. **9, 12, 13, 14** in section 2 **4 further Manuscripts under preparation**

WP5 – Natural Products - Metabolomics

Main objectives	- To determine the metabolomes of priority strains by dereplication study to confirm the results of biosynthetic gene-based screening. - To optimise the much needed computational tools to analyse all data sets - To evaluate the extracts, fractions, and purified secondary metabolites for their applicability as sources of potential novel drugs. - To isolate, purify and elucidate novel natural products for structure-activity relationship (SAR) studies - To manipulate fermentation systems by metabolomics-assisted studies to enhance the titre of the desired product within the metabolome. - To correlate metabolomic data to the effect of variable changes in the bioprocessing parameters on the biosynthesis of secondary metabolites and hence, aid in optimizing their production at an already early stage
Contributing partners	**SIPBS**, VTT, MBL, Wuerzburg, SAMS
Main results	• MZmine was modified and Excel Macros were developed to automate data processing and dereplication. MZmine 2 data processing programme was optimised by adding the chemical formula prediction tool as a module for the framework. This computational tool provided the core functionality for MS data processing: raw data import, peak detection, MS/MS scan recognition, and isotope pattern detection and comparison. VTT was collaborating with SIPBS in implementing the tool for benefit of EU SeaBioTech project. • For metabolite profiling studies during scale up and analysis of fractions, higher column efficiency can improve the chromatographic output and then the number of detected metabolites. We tested two HPLC columns with the same C18 reverse phase stationary phase but different column dimensions: 150 x 3mm with 3µm particle size and 75 x 4.6 mm with 5µm particle size which we used in all previous analyses. TICs (Total Ion Current) of the same bacterial extract (SBT 0000328) were measured with the different column dimensions; A) longer, narrower column with small particle size material and B) the shorter, wider column with larger particle size material column. As observed with the same eluting time scale, using the long-narrow-small column enhanced the retention and the separation performance and improved the peak shape. • Dereplication work was finalized for samples originating from Milos, Crete, and the geothermal vents of Iceland as well as those covering Scottish coastline and additional sample strains from the Antarctica region. Seventy-seven (77) bacterial samples were dereplicated from the NPMG-Orkney archive. A total A total of 34 bacterial extracts from Milos and Crete were analyzed, yielding SBT348 and SBT687 as the candidate strains for further compounds isolation and purification. While based on mass spectrometry profiles of strains from the Scottish coastline and the Antarctica region, three isolates revealed distinct patterns, KP130 (an unidentified bacteria isolated from Maud Rise, Antarctica), KP044 (a Streptomyces strain isolated from St. Andrews sediment) and KP121 (a *Bacillus* strain from Bransfield Strait, Antarctica). The metabolites responsible for these unique profiles were identified using principle component analysis (PCA) and found to be a series of polymers m/z 363-1911 with spacing of 86 Da (KP130), a series of piscicides and antimycins known to be produced by *Streptomyces* spp. (KP044). These PCA outliers were also identified in the molecular network, demonstrating their complementary nature of metabolomic tools for secondary metabolite discovery. Metabolomic profiles have been documented into the SeaBioTech database. • Metabolomes were dereplicated for priority strains while biosynthetic gene-based screening explored the presence of the genes for the respective secondary metabolite (w/WP4). However, bioactivity was used to prioritise strains for the WP7 pipeline (w/WP3). **Chemical dereplication study & biological activities: strains were prioritized for the SBT pipeline as requested by WP3. (Highlighted isolates selected for the WP7 pipeline).**

			Bioactivity					
SBT Code	**Organism name**	**In charge of its analysis**	**SIPBS**	**AXXAM**	**HDL**	**WUE**	**PHARMAQ**	
017	*Rhodococcus sp. ZS402*	SIPBS/Axxam		PPARa/ TRPM8		E. faecalis		
027	*Vibrio splendidus Isolate 28*	SIPBS	*M. marin-um*	HDAC6-backup			Sea Lice	
111	*Celeribacter sp. SK032*	MBL/SIPBS/ Axxam		PPARa/ HDAC6				
144	*Bacillus licheniformis BAB-1836*	MBL/SIPBS/ Axxam			Cancer			
148	*Ruegeria sp. QDHT-13*	MBL/SIPBS/ Axxam		PPARa/ TRPM8/ HDAC6			Sea Lice	
167	*Polysiphonia lanosa*	MBL/SIPBS/ Axxam/HDL		HDAC6/ EL-high	Cancer		Sea Lice	
169	*Laminaria hyperborea*	MBL/SIPBS/ Axxam		HDAC6				
345	*Streptomyces* sp.	Wuerzburg			DLD-1 HCT116	Anti-oxid.		
531/ 587	*Muricauda ruestringensis*	SIPBS/Axxam/ Matis	Metab-olic	EL-high	Cancer			
541	*Algoriphagus marincola*	SIPBS/Matis	Metab-olic					
687	*Micromonospora sp. N17*	SIPBS/ Wuerzburg				Trypa-noso-ma	Sea Lice	
692	*Micromonospora sp. N74*	SIPBS/ Wuerzburg					Sea Lice	

- At VTT, axenic *Euglena gracilis* microalgae was introduced as a model organism for metabolic profiling. It was cultivated in 2 L stirred glass tank bioreactors in the presence of glucose under constant light or in the dark. The analyses showed that in light the glucose intake was delayed while the culture generated more biomass suggesting the contribution of photosynthesis. Lipidomic profiling by UPLC-QTof-MS in ESI+ mode (VTT) indicated that phosphatidylcholines were the prior lipid species, but in light cells accumulated large amounts of galactosyldiacylglycerols and ether-bonded lipids, while in dark medium-chain wax-esters were typically formed. LTQ-Orbitrap based metabolomic profiling (SIPBS), on the other hand, showed the richness of metabolites formed in dark especially, and numerous spectral library suggestions for terpenoids of marine origin were obtained. Bioactivity testing (AXXAM) was also indicating some HDAC6 and PPARα activities for the ethylacetate extract of cells cultivated in dark.
- Extracts of priority strains were prepared from scale-up for further fractionation and isolation of bioactive secondary metabolites. Metabolomic-guided targeted isolation work was done in parallel to and in support of the bioassay resulting to a quick identification of the active metabolites.
- 65 natural products have been elucidated and have been documented in the SeaBioTechdatabase (http://spider.science.strath.ac.uk/seabiotech/pure_compounds_show.php), which has been linked to Chemspider and PubChem databases.
- Fermentation systems were manipulated by metabolomics-assisted studies to enhance the titre of the desired product within the metabolome (w/WP7). SBT017 was active in target-based functional assays, TRPM8 and PPARα as well as against

Enteroccoccus faecium. SBT017 was also interesting as it is also positive for the presence of non-ribosomal peptide synthetase. Fractions were sent to WP3 screening for screening in TRPM8 and PPARα screening. However, the agonistic activity reported from original extract, SBT017, was not found in the fractions. Many fractions were found to have antagonistic activity which is not a desired bioactivity. However, one suggestion that has been discussed is to retest the most chemically interesting fractions in PTP1B and TRPA1 assay systems. Co-culturing *Rhodococcus* (SBT017) with Streptomyces (SBT 1625 and 681) for instance has increased the potency of the extracts against PPARa and TRPM8. The production of the bioactive metabolites were optimised by co-culturing with three species of Strepyomyces (two obtained from the Mediterrenean and one obtained from the West Coast of Scotland.

- Metabolomic data was correlated to the effect of variable changes in the bioprocessing parameters on the biosynthesis of secondary metabolites to aid in optimizing their production (w/ WP7).

⊡ Metabolomics assisted comparison of the fractions from shake flask bacterial culture versus bioreactor culture of *Vibrio splendidus* (SBT27). The bisindole turbomycin B was one of the most significant bioactive metabolite produced from the fractionation of the shake flask bacterial culture of *Vibrio splendidus*. It has a remarkable activity against *Mycobacterium marinum* as has been reported in deliverable 5.4. Turbomycin B was afforded from the non-polar fractions of the culture extract. Fermentatiion of *Vibrio splendidus* in the bioreactors was also producing turbomycin B. However, the amount was less than in the shake flask although tryptophan and phenylalanine were added to the fermentation culture as precusors to increase the amount of turbomycin naturally biosynthesised by the *Vibrio*. Multivariate data analysis of extracts from the bioreactors and those from the shake cultures was accomplished in order to highlight similarities and differences in the produced metabolites between the two groups. PCA analysis demonstrated the clustering of the fractions from the fermentation culture against the shake culture's fractions. This indicated a variation in the produced metabolites on the multivariate level between the two groups of fractions. A supervised OPLS-DA was employed to disclose the unique metabolites contributed in the variation between the two groups. S-plot revealed the unique and significant metabolites in each group. Most of the significant metabolites contributed to the variation of both group were found undescribed in the antiMarine and DNP databases. Two metabolites at *m/z* 144.0455 [M-H]- and 176.0706 [M+H]+ eluting at 6.21 min and 10.55 min, respectively were highlighted among the significant unique metabolites produced by the fermentation culture in the bioreactor. These metabolites were respectively identified as indole-3-carboxaldehyde and methyl-indole-3-carboxylate. Presence of those metabolites in fermentation culture's fractions can be deduced to the addition of the tryptophan, which is converted by the bacterium into indole-3-carboxaldehyde. This finding may explains the low yield of turbomycins as tryptophan was consumed by *Vibrio* to give indole-3-carboxaldehyde rather than turbomycin. The biosynthesis of turbomycin maybe using a different carbon source.

⊡ Metabolomics assisted comparison of the fractions from shake flask bacterial culture versus bioreator culture of *Muricauda ruestringensis* (SBT 531). SBT531_Bioreactor versus SBT531_ShakeFlasks were compared in terms of their metabolomics profile. The shake flasks yielded aseanostatin (AP-6) as the major bioactive metabolite against TRPB1 while the bioreactor afforded other lipids and hydroxylated compounds as well. In the medium scale, AP-6 was a major component as shown by the NMR analysis. AP6 does not ionize well and is more difficult to detect by mass spectrometry. AP-6 was produced at its maximum concentration within 22 hrs.The Bioreactor at 120 hours, still shows the present of AP-6 (Figure 3.3.2b), however together with the production of other components which includes other cholic acid (CA) derivatives and BHAs. The quick production AP-6 in the bioreactor is a great advantage. However, there is still a need to optimize the parameters to

	increase the bacterial biomass and hence, the yield of the target metabolite.
Potential impacts	**Scientific breakthrough:** - Automated dereplication and chemical profiling aid screening for diversity and novelty were established. Marine invertebrate-associated symbiotic bacteria produce a plethora of novel secondary metabolites, which may be structurally unique with interesting pharmacological properties. Selection of strains usually relies on literature searching, genetic screening and bioactivity results, often without considering the chemical novelty and abundance of secondary metabolites being produced by the microorganism until the time-consuming bioassay-guided isolation stages. To fast track the selection process, metabolomic tools were used to aid strain selection by investigating differences in the chemical profiles of bacterial extracts from diverse extreme environments using liquid chromatography-high resolution mass spectrometry (LC-HRMS) and nuclear magnetic resonance (NMR) spectroscopy. Following mass spectrometric analysis and dereplication using an Excel macro developed in-house, principal component analysis (PCA) was employed to differentiate the bacterial strains based on their chemical profiles. NMR 1H and correlation spectroscopy (COSY) were also employed to obtain a chemical fingerprint of each bacterial strain and to confirm the presence of functional groups and spin systems. These results were combined with taxonomic identification & bioassay screening data to identify bacterial strains to be prioritized for scale-up based on their chemically interesting secondary metabolomes, established through dereplication and interesting bioactivities, determined from bioassay screening. Additionally, that strains with nearly identical 16S rRNA sequences do not necessarily produce the same secondary metabolites. - Metabolomic-assisted isolation of target compounds efficiently improved the purification of the bioactive secondary metabolites. High resolution Fourier transform mass spectrometry (HRFTMS) and nuclear magnetic resonance (NMR) spectroscopy were employed as complementary metabolomic tools to dereplicate the chemical profile of bacterial extracts. Principal Component (PCA), hierarchical clustering (HCA), and orthogonal partial least square-discriminant analysis (OPLS-DA) were used to evaluate the HRFTMS and NMR data of crude extracts from different fermentation approaches. Statistical analysis identified the best culture one-strain-many-compounds (OSMAC) condition and extraction procedure, which was used for the isolation of novel bioactive metabolites. As a result, new natural products can be isolated from cultivated broth cultures. - New natural products with novel mechanisms of actions were isolated. Biologically active compounds were isolated and purified from prioritized strains. SBT345 (Streptomyces sp.) showed anti-oxidant, anti-cancer cell lines (DLD-1, HCT116) activities, and some activities in the enzymatic reactions. Four compounds have been isolated from SBT345. Compounds SBT1620 (phencomycin), SBT1621 (tubermycin B), SBT1186 (benzethonium), and SBT1187 (ageloline A, new compound) have been structurally elucidated while SBT1877 showed anti-oxidant and anti-Chlamydia trachomatis activities. SBT0017 (Rhodococcus sp.) yielded 16 pure compounds after scale-up, one of which was elucidated as isohalobacillin B. SBT0027 (Vibrio splendidus) yielded 27 pure compounds, 7 of which are bis-indole analogues with strong to medium potency against Mycobacterium marinum. Three analogues are new. Other pure compounds from SBT0027 consisted of diketopiperazines, long chain amines, and hydroxylated fatty acids, the activities of which still need to be determined. SBT167 (Polysiphonia lanosa), an algal macro-epiphyte yielded the di-bromo-dihydroxylated-benzaldehyde as its major component. SBT167 was found to be active against parasitic sea lice and in several enzymatic assays against metabolic diseases. From the Icelandic collection, new BHA congeners bioactive against metabolic diseases were isolated. **Economic impact (health costs, market,…):** - Defining target compounds by metabolomic-assisted isolation has proved to be more

	economical and more efficient in designing high-throughput purification protocols for bioactive natural products. Multivariate analyses of LC-HRMS and NMR data by principal component analysis (PCA) were used to successfully compare the secondary metabolite profiles of crude extracts. PCA was shown to be an effective tool to differentiate bacterial strains based on their chemical diversity and novelty of metabolites, providing a means to select bacterial isolates with diverse chemistry **without having to carry out full isolation work on each extract**. PCA was used to reveal bacterial species producing similar chemical groups of metabolites grouped together whilst those producing distinct secondary metabolomes were observed as outliers. By using an Exactive mass spectrometer, which enabled fast-polarity switching, it was possible to obtain efficient and greater metabolite coverage in a single experiment, **greatly speeding up analysis times**. The development of a comprehensive metabolomics workflow pathway including **an in-house developed Excel macro embedded with a database made it possible to rapidly dereplicate higher number of strains**, providing putative identities of known metabolites in each extract. It is also shown that the dereplication results can also be correlated with bioassay screening results to support drug discovery efforts with the objective of both finding a bacterial isolate that has a unique diverse chemistry and is biologically active. Overall, this shows that metabolomics approaches are worthwhile for the selection of strains for the isolation of novel natural products and **that this methodology reduces redundancy in drug discovery programs.** - New aquaculture applications of the use of seaweed micro and macro-epiphytes maybe further explored in the future to manage waste management in the seaweed industry. SeaBioTech was able to open new challenges in the development of high-value marine algal biomolecules as antiparasitics and antifungals for fish pharmaceutical applications. This can be achieved by increasing the awareness of the biotechnological potential of the marine epiphytic macroalgae Polisiphonia (Vertebrata lanosa) as a valuable chemical resource, which is currently considered as a waste product from Ascophyllum harvest for alginate production. SeaBiotech has opened new possibilities to achieve sustainability, efficiency, and development processes for novel marine agrochemicals particularly for fish pharmaceuticals in aquaculture. SeaBiotech will seed to accelerate the development of novel, sustainable, high-value chemicals from waste products of macroalgae processing, to advance the application of bio-based fish pharmaceutical products for future end markets. **Societal impact (quality of life, health, education, employment, citizen awareness,…):** WP5 has Involved 4 posdocs, 3 research assistants 2 PhD, 2 MSc/MRes students, Master and undergraduate students through the ERASMUS and Science without border Programmes. Several undergraduates contributed to the project over the 4 years. WP5 has organised community outreach activities through the annual Science Festivals and Explorathon events sponsored by the EU WP5 has led and participated in collaborative global workshops (i.e. With PharmaSea in India; with Macumba in Malaysia. **European competitiveness / standards and policies**: - Utilisation of waste from the seaweed industry for potential sources of natural products for new applications in aquaculture - Database and repository of marine extracts, fractions, and purified compounds with well-documented chemical profile and bioactive type from several screening programmes can be offered for future exploration.
Main dissemination / exploitation	See ref. **4, 5, 6, 7, 8** in section 2 **2 further papers and 4 further Manuscripts under preparation**

WP6 – Genetic and metabolic engineering of production organisms	
Main	- Collection of –omics data from metabolic engineering of secondary metabolite and

objectives	polysaccharide synthesis in selected production organisms: E. coli, microalgae and marine platform thermophiles - Construction of microalgal expression system - Metabolic engineering of proven production organism (E. coli) for synthesis of secondary metabolites from cyanobacteria - Construction and metabolic engineering of a polysaccharide production organism, expressing genes and synthetic pathways from marine extremophiles
Contributing partners	**MATIS**, LUND, IGZ, SAMS, VTT, PKZ, SIPBS
Main results	**MATIS**, LUND **Enzyme bioprospecting. (MATIS)** A variety of efficient and robust enzymes, with a range of specificities that can be used for complete or selective degradation or modification of marine polymers, polysaccharides and proteins were selected from genomes of novel bacteria isolated and annotated in WP2 and WP4, respectively. A total of 34 enzymes were expressed and evaluated, including *Short chain dehydrogenases.* One of these enzymes was an uronic reductase capable of reducing unsaturated uronic acids to 2-keto-3-deoxy gluconate (KDG), a potential valuable platform chemical. The enzyme can also be used in synthetic pathways enabling heterologous hosts Entner-Douderoff utilization of uronic acids generated by alginate lyases. *Alginate lyases.* A number of thermophilic alginate lyases were cloned, expressed, characterized and compared. Thermostable (stable at 80°C) alginate lyases have not been described before. High temperature decreases viscosity and facilitates enzymatic degradation of alginate. They enzymes were capable of both selective degradation of alginate to oligosaccharides and complete degradation of alginate to mono-uronates. The best enzymes are expressed in high yields in *E. coli*. Production protocols were developed (WP7) & production in pilot scale demonstrated (WP10). *Chondroitin lyase:* A chondroitin lyase from a novel marine microbe was isolated and fully characterized. This enzyme was capable of degrading the complex polysaccharide, chondroitin sulfate found in cartilage and cell walls of sea-cucumbers and generating bioactive oligosaccharides. *Transglucosidases:* Novel thermostable transglucosidases were discovered in novel thermophilic *Bacteroidetes* species. These enzymes were expressed in high yields and can be used to modify 1,3-beta glucans for increased complexity and enhanced bioactivity. This discovery supports a patent application pending from MATIS on homologous enzymes produced and assessed in WP7 by LU. *Proteases*. Novel robust thermophilic glutamate specific endopeptidases were discovered, expressed & characterized. These enzymes cleave proteins into glutamate ending peptides that confer the umami taste perception in food products. **Enzyme development (LU)** *Carbohydrate active enzymes.* In collaboration with Prokazyme & Matis, LU selected robust beta-glucanases belonging to families GH3 and GH17 for in depth study, development, production, characterization and assessment in WP6/WP7. The five GH3 enzymes were exo-glycosidases from *R. marinus* with a range of activities of potential application in biorefineries, including chitinase, laminarinase, xylosidase and glucosidase. The work involved defining their genetic context, and functional and structural studies in order to determine the molecular determinants for the observed variety in activity in this particular protein family. (manuscriptin preparation).The GH17 enzymes were transglucosidases from *Proteobacteria* able to modify the marine polysaccharide lamainarin. Similarily, to the GH3 enzymes, the GH17 showed a range of activities forming 1,3, 1,6 and to some extent 1,4 linkages. The enzymes constitute together a potential highly valuable tool box for generating complex mixed linkage, linear and branched beta glucans of various industrial interest. The work involved computational

modelling of these enzymes in order to determine the molecular determinants of specificity and the potential influence of specific residues for the observed variety of these enzymes. And, how it could be effected, enhanced or focused by protein engineering. Valuable information was gained regarding these aspects. (Manuscripts in preparation)

Metabolic reconstruction and engineering of *R. marinus* (MATIS & LU)

The metabolic engineering task involving the marine thermophile *Rhodothermus marinus* was a highly integrated work of LU and MATIS aiming at on exploring and establishing the biorefinery potential of the organism. The short term emphasis was on studying the production of extracellular polysaccharide (EPS) and glycosylated carotenoid in the species. The work included:

Genomic scale metabolic network reconstruction. Rhodothermus marinus is potentially a very valuable biorefinery strain on carbohydrate rich feedstocks. It grows on all the lignocellulose sugars as well as on the constituent sugars of seaweed polysaccharides such the uronic acids in alginate. It produces also great variety of polysaccharide degrading enzymes such as alginate lyases, xylanases, and various beta-glucanases. The biorefinery potential of *R. marinus* is being optimized in collaborative work of LU and MATIS and the synthetic potential of the organism explored. Matis has constructed a genome scale metabolic network model of *R. marinus* that will be used for model based enhancement of its biorefinery possibilities.

Physiology studies. Cultivation conditions effecting production of EPS and carotenoids were carried and evaluated out by LU and MATIS and developed further and assessed in WP7 by LU.

Structural characterization of EPS. The complex structure of *R. marinus* EPS was elucidated . The EPS was sulfated and had unusually high content of xylose and arabinose. Sulfated exopolysaccharide derivatives are known to have advantageous properties, in particular as therapeutic substances. E.g. heparin commercially extracted from porcine intestinal mucosa as anticoagulant & antithrombotic agent in the prevention and treatment of venous thrombosis and fucoidan from. Novel polysaccharides from bacterial origin offer an alternative and may also expand the potential range of activities and potency of EPS derived health promoting agents.

Structural characterisation of carotenoids. Detailed studies on carotenoids in different strains of *R. marinus* were carried out and bioactivity was established.

Pathway analysis. Synthetic pathways were resolved for EPS and carotenoid and supported by reverse genetics studies by MATIS involving gene knock-outs.

INGENZA

Demonstration of Identification of novel biocatalytic activities of industrial relevance

Ingenza has focused devloping improved inABLE for screening for novel commercial enzymes that were provided to the work by package partners.

Engineered Strains of *E.coli* for Production

inABLE® Technology. Ingenza optimised and adapted an in-house developed combinatorial genetics technology (known commercially as inABLE®) for the efficient and selective assembly of DNA expression vectors. Traditional digestion/ligation cloning methods allow for the efficient ligation of at most 3 fragments of DNA, however, inABLE® permits up to 10 DNA fragments to be mixed together in a single reaction and then correctly & efficiently assembled to generate cloning & expression vectors, thus, highlighting the phenomenal selectivity it offers. inABLE® has allowed Ingenza to accelerate the systematic combination of DNA fragments ("parts") allowing, e.g. the construction of libraries of regulatory elements responsible for control of gene expression. Screening such libraries then allows us to rapidly identify the most effective combination of gene(s) & regulatory elements.

Nested inABLE®: The inABLE® technology in its original format permits construction of DNA vectors from up to 10 fragments of DNA. However, as the complexity of the assembled constructs increases the need to further develop the technology became

apparent. As the number of fragments increases the efficiency of the assembly reaction decreases and the technique becomes less suitable to construct libraries where there is an extensive requirement to mix and match multiple genes and regulatory elements. Ingenza identified an opportunity to develop a 'nested' approach which assists in the construction of these much more complex vectors. The nested approach involves the addition of a junction in the linker sequences, resulting in the formation of constructs from an initial assembly which can be used in a second round of inABLE® assembly. In the first stage of assembly the combination of a single gene with multiple regulatory regions will be performed prior to a second round of assembly to construct a vector containing multiple genes. i.e. convergent rather than a linear synthesis. In WP6 the screening of the metagenomic libraries for novel enzymes of industrial relevance is a key part. Therefore, as previously reported using the nested inABLE® technology, libraries were created to allow for the screening of P450s. P450s provide a particularly useful test for nested inABLE® due to the size and complexity of the genes which encode P450 enzymes. This allowed Ingenza to develop a better understanding of nested inABLE® .These technologies are key tools for improvement of strain construction and screening, and have been used and developed through the course of this project.

The work package partners screened their in-house data bases for new and novel enzymes related to those listed in D6.3. While Ingenza waited on feedback from the work package partners Ingenza carried out its own phylogenetic analysis of these enzymes and screened alternative metagenomics libraries in order to progress with the construction of expression vectors and the screening of these enzymes. Ingenza used inABLE® to construct host/vector systems for these targets. The ultimate aim was to substitute and test novel enzymes using these adaptable production systems as they become available. Ingenza has been systematically working with the enzymes of interest and developing the tools for their construction, expression, screening and production. One particular example which was described in more detail was carboxylic acid reductase (CAR) which was cloned and expressed in E.coli.

Engineered Strains of E.coli expressing marine enzymes

Sequences of enzymes useful to Ingenza were sent to WP6 partner Matís, who screened in-house databases for putative genes with homology to those sequences. Positive hits were then sent back to Ingenza, and Ingenza performed a phylogenetic analysis on these hits. Two putative marine enzymes, IGZ.ENZ.SBT001 and IGZ.ENZ.SBT002, with sequence homology to thioesterases, were shortlisted so that they could be screened for activity against a range of substrates of commercial interest to Ingenza. These novel marine enzymes were cloned into an industrially relevant *E.coli* strain using inABLE® compatible parts. Master cell banks were made and sterility checked before carrying out initial shake flask expression studies. Further optimisation of the expression of these strains has been carried out in shake flasks followed by activity assays of the successfully expressed enzymes. Based on these results, fermentation development has been implemented, linking into the deliverables required for WP7. A production process of the most successful enzymes was implemented and scaled up during the course of work package 7.

Results of Production Trials

Ingenza developed a 5 L generic fermentation process based on commercial enzymes of interest from alternative metagenomic and strain repository sources which were used to prepare expression constructs, develop screens (WP6) and subsequent production processes (WP7). The development of such generic systems allowed flexibility in terms of the enzymes to be produced, in a variety of divergent microorganisms depending on their growth requirement e.g. oxygen demands. This was key to successfully express enzymes which are sourced from marine stocks or carrying out fermentation on sea-borne microorganisms. These generic fermentation protocols which had been developed previously were then implemented to test the growth and expression of two thioesterase enzymes (noted above) in the constructed strains ING.STR.SBT001 and

	IGZ.STR.SBT002 from WP6. At the moment the processes have been scaled up to 5 L but the simple generic system is fully expected to render further scale-up as very straightforward. From Ingenza's experience, in-house 5 L protocols have provided high predictability of success at both 30 000 L (yeast) and 50 000 L (*E.coli*) using the host organisms of choice for any of the commercial fermentation scale-ups from this project. **SAMS** *Transformation protocols developed and optimized for the marine microalgal species Nannochloropsis based on site-specific homologous recombination to facilitate the heterologous expression of eukaryotic proteins*. The focus within this particular task was to improve the transformation efficiency creating gene knockouts from the VCP1, a light harvesting protein, promoter gene from N. oculata fused to a zeocin resistance marker. This allowed positive gene-knockouts to be screened through antibiotic resistance pressure of the zeocin, in other words only those cells that had been positively transformed to express the zeocin would then grow on agar plated containing the antibiotic. An electroporation method was adapted from Vieler et al., 2012, but the recombinant cells produced were not stable in the long-term. This particular promoter is primarily active during log phase growth and less active during stationary pphase growth. The result is that Zeocin resistance (or whatever gene is downstream) may be 'turned -off' and the recombinant colonies are then killed by the Zeocin. *Transformation vectors constructed in conjunction with the company Algenuity based on promoter trap studies.* This resulted in a stable transformation protocol and promoter trapping again utilising electroporation with both N. oculata and N. oceanica and Hygromycin as the resistance marker instead of zeocin. Hygromycin at concentrations of between 200-400 µg ml-1 were shown to be effective for selection of the promoter trap transformants. Using these proprietary promoter trap vectors, the transformation frequency of N. oceanica was 10- 1000 fold more effective than of N. oculata. The reason for the differences between the two species is not clear at this point. From this sixty promoter trap-mutants were characterized and the promoter suitability is being further assessed by Algenuity. *Comparative metabolomics analysis of nine N. oceanica transformants against the wild type.* From molecular network analysis of the ions present 12 ions, 23% of network, are gene-knockout or transformant specific ions as a result of genetic manipulation of the strain. While variation between gene knockout strains at the chemical level was very minimal (in comparison to total chemistry) the technique demonstrated that even minimal changes can generate strains that synthesize potential interesting novel secondary metabolites. In conclusion the use of metabolomics and molecular network analysis has the capability of identifying mutations even when no specific phenotypic difference is observed. **VTT** *Strain improvement of Chlamydomonas reinhardtii for heterologous protein production.* The GFP-HFBI fusion was successfully expressed in *Chlamydomonas reinhardtii.* Accumulation level of ca. 190 µg/mg cells (FW) of GFP or GFP-HFBI in was recorded and production yield of 1 mg/l could be expected if the best harvesting point approximately after five days of growth would be applied. Unfortunately, the HFB-fusion did not improve the GFP accumulation level and did not facilitate the aqueous two phase separation (ATPS) purification of the target protein. It is speculated that the fusion protein was not correctly folded in *Chlamydomonas*. Thus, the protein of interest would need to be targeted and retained in ER in order to fold HFB properly & thus facilitate ATPS purification.
Potential impacts	**MATIS & LU** Novel thermostable counterparts of high industrial interest were discovered and evaluated, including, alginate lyases, glutamate specific endopeptidases, novel robust transglucosidases, uronic reductases, sulfatases, GH3# glycosidses, glucosaminidases and fucosidases Major progress was made in the development of *R. marinus* as a versatile biorefinery

	organism for utilizing carbohydrate rich biomass of terrestrial and marine origin. This involved detailed analysis of synthesis pathways in production of the bioactive secondary metabolites, EPS and carotenoids, in *R. marinus* and genetic confirmation of roles of specific genes. The work was furthermore supported by important structural and bioactivity studies of these compounds and physiological/cultivation studies on the conditions effecting their production, yield and the composition of the EPS. **VTT & SAMS** Novel microalgal autotrophic host-vector systems for production of proteins and small molecules VTT: Improved *Chlamydomonas reinhardti:* for protein expression. Thresholds defined and overcome. SAMS : Novel Nannochloropsis production hosts, *N. occulata* and *N. oceanica* and associated new genetic tools and protocols **IGZ** Novel robust enzymes for synthesis of biochemicals of industrial enzymes were expressed, analysed and developed by IGZ. This included thioesterases from novel strains of MATIS useful in the development of synthetic biology routes to polymer intermediate, Reductases: exploitable in novel biosynthetic pathways to polymer intermediates, with particular focus upon carboxylic acid reductase; aminotransferases: exploitable in several multi step pathways to large volume chemical building block intermediates; Decarboxylases: exploitable in native form and potentially following adaptation to key industrial targets; Hydrolases: exploitable in the short term for bioenergy applications; Post translation modifying enzymes such as cyclases, relevant to the synthesis and diversification of cyanobactin and other peptides of potential pharmaceutical relevance Improved inABLE vectors were engineered for expression, activity screening and modification of heterologous enzymes of high industrial interest.
Main dissemination / exploitation	See ref. **10, 23, 24 , 65, 85** in section 2 **3 further manuscripts under preparation** **Patent :** THERMOSTABLE ALGINATE DEGRADING ENZYMES AND THEIR METHODS OF USE. 2016. European Patent Application No. 15703635 & and USA Patent Application No. 15/110,132 **(MATIS)**

WP7 – Industrial bioprocessing	
Main objectives	- To understand and develop methods to overcome the hurdles to successful industrial exploitation of promising activities in natural isolates from marine environments. - Accelerate the process development for source microorganisms - Investigate factors contributing to changes in metabolite production during scale and location transfer - Accelerate process development for novel constructs at small production scale and formulate scale-up predictions. - Process targeted compounds using selected enzymes.
Contributing partners	**SIPBS**, IGZ, LUND, MATIS
Main results	All the main objectives and deliverables of the program were completed during the program of work. The program has developed standard operating protocols for the growth and exploitation of resources from both natural isolates from the marine environment and from construct microorganisms, developed by identifying, isolating and genes of interest from marine species and inserting them into organisms which are regarded as industry work horses e.g. *Escherichia coli.* In this work package we have concentrated on laboratory or small production scale protocols which allowed us to formulate scale up predictions for processes developed WP10. Accelerated process development has been achieved either by utilizing powerful gene

technologies to create construct organisms or by utilizing bioprocessing techniques with metabolomics with source microorganisms to identify bottlenecks in the relevant catabolic pathways. Both of these techniques resulted in successful bioprocess intensification of the relevant target compounds or enzymes.

Industrial partners identified appropriate target compounds which allowed us to selectively mine the gene pool of the marine organisms for useful enzymes. Suitable protocols were then generated for the bioprocess and these are reported in the Process Manual.

Scaled industrial fermentation protocols for *source* microorganisms

The main objective of this part of work package 7 was to understand and develop methods and protocols to overcome the hurdles to successful industrial exploitation of promising bio-activities, enzymes and interesting compounds from natural isolates from marine environments. Thus, an isolated microorganism that showed a particular activity of interest based on de-replication and fractionating studies (WP5 – Task 5.2, 5.3) and bioassay activities (WP3 – D3.2-D3.4) was passed onto WP7 for further investigation.

The key strategic aim was to generate an improved understanding of how to routinely process (cultivate) such isolates in such a manner that unwanted changes in metabolomes are minimised. This involved, fermentation and culturing processes, microbiological analysis, metabolomic analysis across scales and statistical analysis of process factors linked to metabolome change. .Methodologies on purification of compounds and the use of spectroscopic techniques were also employed. Table 1 shows a list of the species for which protocols have been developed. The Process Handbook for SeaBioTech provides the relevant protocols.

Table 1 – Source microorganisms received by WP7

Strain	**Phylum**	**Source**	**Potential products**
Colwellia sp. DG1864**	Proteobacteria	SAMS	Exopolysaccharides, exopolymers, enzymes
*Labrenzia sp. 1229****	Proteobacteria	SAMS	Cryptic secondary metabolite (NRPS-PKS) operon
*Nodularia sp.****	Cyanobacteria	SAMS	NA****
Synechococcus bacillaris	Cyanobacteria	SAMS	NA
Rhodella maculata	Rhodophyta	SAMS	NA
Vibrio splendidus	Proteobacteria	NPMG [WP5] – Orkney samples	Turbomycin B
Bacillus licheniformis	Firmicutes	MBL	Exopolysaccharides
Muricauda ruestringensis	Cytophaga-Flavobacterium-Bacteroides	Matis	Aseanostatin P6 and derivatives
Algoriphagus marincola	Bacteroides	Matis	Aseanostatin P6 and derivatives

One species in particular drew early attention. The *Colwellia* sp., produced a novel polymer with very interesting rheological characteristics. Protocols were developed for reproducible cultivation of the organism, maximising polysaccharide production and this was selected for further development in a new programme. Work is continuing post SeaBiotech via PhD programme with Unilever and SAMS.

Factors affecting process physiology

Factors affecting process physiology, and consequent process efficiency, were investigated for all the key organisms studied in work package 7. Process parameters such as salinity, temperature, pH, dissolved oxygen, agitation rate as well as medium

components were all studied. For those organisms which came to Strathclyde, the effect of different process parameters on the metabolomics of the organism were also studied, and the results used to help the design of subsequent experiments. The results are detailed in the deliverables of WP 7 and 5. As might be expected, the factors affecting the process physiology varied from organism to organism.

Scaled industrial fermentation protocols for *construct* microorganisms

Genes of interest were selected from marine species and transferred into *Escherichia coli.* The innovative inABLE® technology provided by Ingenza was utilised as it is used for DNA recombination and has been widely shown to increase the efficiency with which diverse genetic constructs can be combined . The resultant constructs are stable and thus provided a good basis for the development of constructs for SeaBiotech.

Novel biocatalysts (Ingenza)

To address opportunities in chemical, polymer and pharmaceutical manufacture identifying, adapting and introducing novel biocatalysts with specific activities and properties is key to the success of companies like Ingenza and other bio-based industries. A number of these catalysts were selected as targets in this project. Ingenza initially aimed to identify and evaluate novel activities from the marine metagenomic sources accessed in SeaBioTech. The programme provided opportunities to study a range of enzymes as work package partners screened their sequence database according to a target list which included esterases, transferases, reductases, hydrolases and cyclases with a particular focus on thioesterases, aminotranferases, carboxylic acid reductases and a number of different cyclases. During the second period of the project, further exploitation opportunities emerged for Ingenza to apply mutase and, in particular, thioesterase and carboxylic acid reductase enzymes in the biosynthesis of chemical building blocks. Additionally, Ingenza identified opportunities in drug discovery where marine derived biocatalysts are highly relevant to the biosynthesis of novel classes of peptides with potential applications in oncology treatment.

Ingenza have developed a generic fermentation process based on commercial enzymes of interest from alternative metagenomic and strain repository sources which were used to prepare expression constructs, develop screens (WP6) and subsequent production processes (WP 7). IN The current phase of work to fully complete this deliverable some of the new enzymes sourced from marine metagenomic libraries can be incorporated within the benchmark processes which have been developed by Ingenza which should see improved yields for the commercial targets.

Alginate lyases

A collection of alginate lyases, a total of 10 enzymes from genomes of different marine bacteria (WP4 & WP6), have been studied by Matis and Lund for potential use in biorefineries utilizing seaweed biomass. Various applications are envisaged e.g. in pre-processing and development of feedstocks for fermentation and further enzymatic bioconversions, as well as in production of oligosaccharides for feed, food and cosmetic usage. The enzymes were compared using relevant industrially important properties, stability, and various optima. The four most thermostable enzymes were selected for production development, and demonstration purposes. These genes are derived from *Rhodothermus marinus 378* (completely sequenced in WP4) and are termed AlyRm1 to AlyRm4. These alginate lyases are the most thermostable alginate lyases discovered to date and have diverse properties as regards substrate specificities and product formation. In combination, they are capable of near complete degradation of alginate into unsaturated mono-uronates. Thermostable alginate lyases were successfully overexpressed and purified. They can subsequently be produced in high quantities for various applications for degradation of alginate. Appropriate protocols have been developed and cultivation has been successful at both small scale and pilot scale.

Cloning and expression of recombinant beta-glucosidase

A secreted thermostable β-glucosidase (GH3 family) from *Rhodothermus. marinus* has been identified and recombinantly expressed in *E. coli* (Lund) . The enzyme degrades

	cello-oligos (β-1,4) and laminari-oligos (β-1,3) and has been selected for incorporation and expression in the genome of an ethanologenic production organism, *Thermoanaerobacterium* sp. AK17, for engineering of increased substrate range. An intracellularly expressed β-glucosidase was successfully inserted into the genome of AK17 and its function was verified. Further research and engineering is needed to develop successful secretion of the β-glucosidase to increase the metabolic range of the AK17 ethanol production strain. Such work in currently being undertaken in parallel projects. ***Novel biocatalysts (Ingenza)*** To address opportunities in chemical, polymer and pharmaceutical manufacture identifying, adapting and introducing novel biocatalysts with specific activities and properties is key to the success of companies like Ingenza and other bio-based industries. A number of these catalysts were selected as targets in this project. Ingenza initially aimed to identify and evaluate novel activities from the marine metagenomic sources accessed in SeaBioTech. The programme provided opportunities to study a range of enzymes as work package partners screened their sequence database according to a target list which included esterases, transferases, reductases, hydrolases and cyclases with a particular focus on thioesterases, aminotranferases, carboxylic acid reductases and a number of different cyclases. During the second period of the project, further exploitation opportunities emerged for Ingenza to apply mutase and, in particular, thioesterase and carboxylic acid reductase enzymes in the biosynthesis of chemical building blocks. Additionally, Ingenza identified opportunities in drug discovery where marine derived biocatalysts are highly relevant to the biosynthesis of novel classes of peptides with potential applications in oncology treatment. Ingenza have developed a generic fermentation process based on commercial enzymes of interest from alternative metagenomic and strain repository sources which were used to prepare expression constructs, develop screens (WP6) and subsequent production processes (WP 7). IN The current phase of work to fully complete this deliverable some of the new enzymes sourced from marine metagenomic libraries can be incorporated within the benchmark processes which have been developed by Ingenza which should see improved yields for the commercial targets. ***Physiology of Rhodothermus marinus*** *Rhodothermus marinus is a potential* source of several products, e.g. carotenoids and thermostable enzymes with potential use in many fields. Carotenoids are tetraterpenoids, produced from 8 isoprene molecules and contain 40 carbon atoms. Medical literature suggests that carotenoids have a positive effect on health. In addition, the compounds are used in the food industry as pigments and in fragrances and perfumes. Exopolysaccharides (EPSs) are found in many marine bacteria and are thought to protect the bacteria from the severe environments. They are high molecular weight carbohydrate polymers which are containing different monosaccharides. As a result, they have shown potential in food, pharmaceutical, biomedical industries. Two strains of *Rhodothermus marinus (DSM 4252) and*, *strain 493),* were selected and investigated for the production of exopolysaccharides. Production was small scale and protocols for growth and product formation determined. During the work it has been shown that the pigment production was dependent on the cultivation conditions, and different media have been constructed resulting in pigmented or non-pigmented cells (reported in WP6). To better exploit the organism and provide materials for further studies, protocols were developed through a Lund /Strathclyde collaboration to maximise the production of the carotenoid pigment. This has also resulted in a set of standard operating procedures, securing reproducible collection of material from *R. marinus*.
Potential impacts	**Scientific breakthrough:** Combining the novel gene technologies, metabolomics and ability to rapidly scale

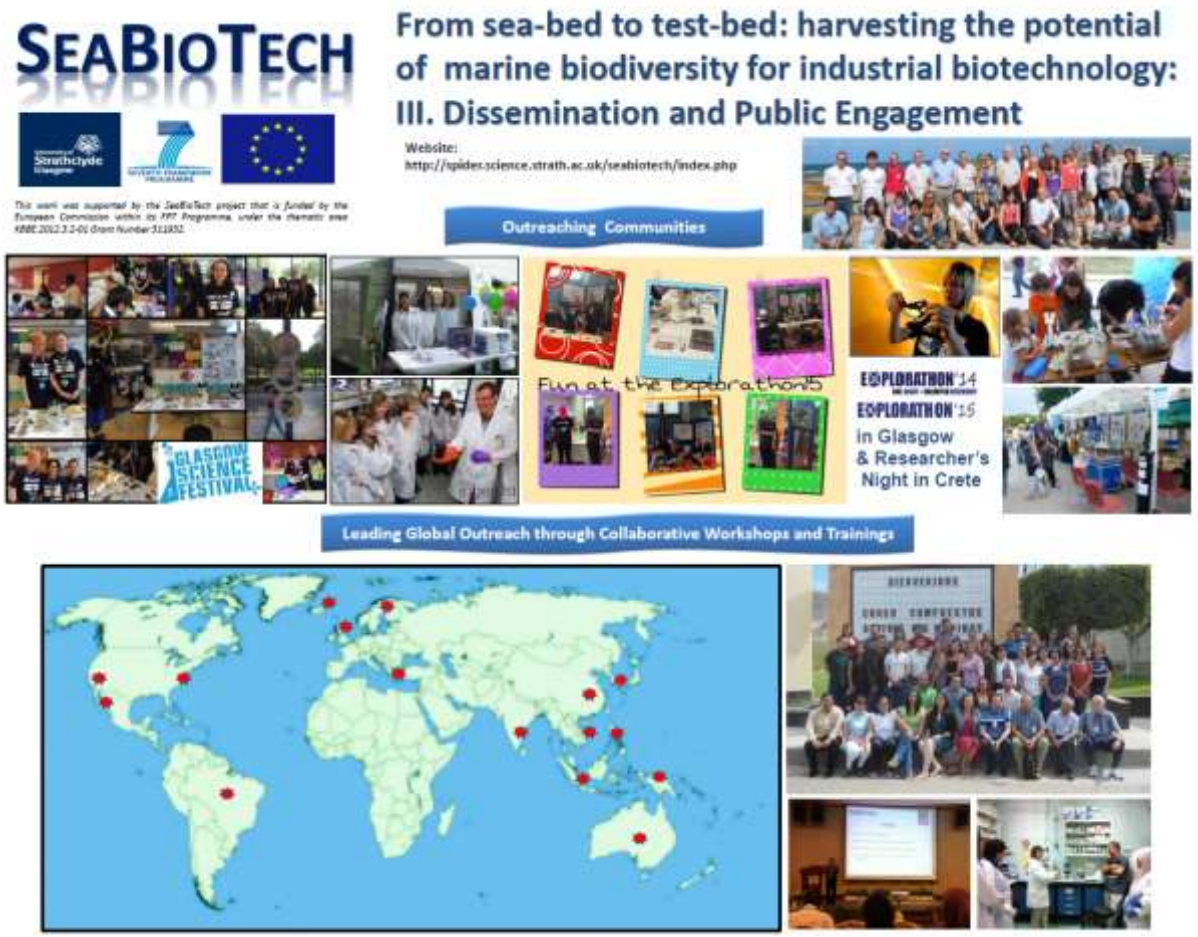
	processes, using clearly defined standard operating procedures, is the unique aspect of the programme. This is of particular interest to industrial partners and significantly benefits both the companies involved in SeaBiotech and the scientific community in general. Many of the techniques can now be regarded as **generic** and could be exploited elsewhere on other projects and processes. Genes from source organisms, expressing novel enzymes, have been successfully inserted into industry work horse organisms and have been successfully scaled up. Such enzymes have novel capabilities and are being successfully used by on customer projects (D1.6, section 6). Generating new construct microorganisms allowed the exploitation of enzymes, e.g. alginate lyases & thioesterases to name two, capable of utilising different kinds of feedstocks and which allow processes which previously suffered from bottlenecks to work effectively and efficiently. This is a significant scientifc breakthrough as the potential for industry is great. A novel polymer was isolated from *Colwellia* sp. And found to have rheological characteristics which are unusual enough to have immediate interest from a major international company. The organism has been successfully grown at scale in WP7 and a spin off project has developed between SAMS and Unilever . New bioactive compounds have been identified (WP3) and tested at scale in WP7. Initial trials have shown the organisms from which the bioactives are isolated can be grown at scale but research to improve the productivity of the bioactives continues. **Economic impact (health costs, market,…):** The generation of new enzymes & polysaccharides will have considerable influence on the economies of the consortium partner companies and on the economy of the EU and also on global markets. The enzymes in particular have significant industrial capability and applications will be numerous. The ability to use new substrates , previously un-useable either because it was not scientifically possible or because process economics were not favourable, will have significant impact on increased process efficiency, improved supply chains (substrate choice increases) & reduction in upstream costs . As seen above impact will not just be industrial, Ingenza see significant potential in the health care market where opportunities in drug discovery from marine derived biocatalysts are highly relevant to biosynthesis of compounds for treatment of disease. The market share for companies who use SeaBiotech derived enzymes and compounds could expand rapidly. **Increased competiveness.** Potential impact on EU and global markets: markets could include food , beverage, health, chemical and associated industries. The outcomes of SeaBioTech will give a competitive edge to companies involved in the programme. **Societal impact (quality of life, health, education, employment, citizen awareness,…):** Quality of life will be improved as products derived from the processes described above come to market. Potential benefits include: New industrial products; New health care products ;New food and beverage products, all of which will hopefully improve health & lifestyle . **Education and awareness:** Seabiotech already has a considerable profile & has contributed greatly to the education of the general public . Events have had citizens from toddlers to pensioners! Opportunities have allowed us to present at scientific conferences & to the general public via radio & television appearances, open days & at science museums. **European competitiveness / standards and policies:** Some of the products will no doubt lead to an increase in the competitiveness of the EU markets. New products attract investment!
Main dissemination / exploitation	The main dissemination of WP7 has been via public scientific and public presentations, listed in the appropriate section of WP1. The nature of WP7 means that publications can be commercially sensitive. Publications fall in the crossover area with WP6. The most significant exploitation is likely to be from the application potential

	thioesterases and the alginate lyases. The polysaccharide from *Colwellia* could also be of significance but there are aspects of the work still to be researched.

WP8 – Marine policy, legal and ethical issues related to marine biodiscovery pipelines	
Main objectives	- To ensure the respect of the Convention of Biological diversity. - To ensure that (i) all experiments performed in the project comply with national, European and international rules and (ii) all ethical issues arising from the SeaBioTech research are properly addressed. - To identify relevant laws, to see how they apply to particular projects and to recommend proposals for harmonizing and improving the effectiveness of the legal system. - To pool efforts with other projects including MICROBIO3, BlueGenics and PharmaSea
Contributing partners	**SIPBS**, **SAMS**, IGZ, PKZ, MBL, PHARMAQ, AXXAM, MATIS, LU, UWUERZ, HCMR, NOVAMEN, VTT
Main results	A relatively modest resource (7.25 PM) was allocated to this WP. A significant amount of this allocation was used by individual partners to ensure that all those involved were fully aware of, and conformed to, developments of the Convention of Biological Diversity (CBD). In addition, the WP liaised closely with, and contributed to, common areas of activity dealing with legal/ethical aspects being undertaken in the parallel EU funded projects: MICROB3, BlueGenics and PharmaSea. An overarching group of experts was formed, i.e. the Advisory panel of policy and legal experts (APPLE). APPLE, an advisory board brought together the breadth of experience, legal, scientific and commercial, necessary to address the critical policy and legal barriers which currently hinder progress in innovative marine biotechnology in Europe. The projects have worked together on these aspects to avoid duplication of effort and enable a wider-reaching and more global approach of benefit to these consortia and beyond. During the lifetime of the project the legal implications to bioprospecting have changed status with the implementation of the Nagoya protocol, which became legally binding from the 12th of October 2014. An overarching, generic Material Transfer Agreement (MTA), conforming to the requirements of the Nagoya Protocol has been developed by Microbio3. This has, with minor adjustments, been applied across the projects. SeaBioTech contributed to the development, structure and content of the PharmaSea Deliverable on development of web-based, interactive, toolkit to assist Marine Genetic Resource (MGR) practitioners in navigating the different legal and policy regimes involved in access to MGR and associated benefit sharing. This area has rapidly developed and on-line resources associated with the CBD Clearing House are available to users/ potential users of biological resources. Work undertaken by APPLE, particularly the PharmaSea legal team, has resulted in considerable progress with respect to the developing of possible solutions to the implications of the collection of materials in areas beyond the Economic Exclusive Zone (EEZ) i.e. in Areas Beyond National Jurisdiction (ABNJ). These were presented at the UN HQ, New York on16-20th June 2014 for consideration for possible future proposed changes to the UN Common Law of the Sea (UNCLOS). SeaBioTech has input into the PharmaSea case studies: Role of biorepositories and impact of proposed EU regulation on ABS; the European blue biotech community's preparedness and response to the implementation of the Nagoya Protocol. During the project lifetime, there has been some clarification with respect to the issue of retrospectively. There are no specific implications in the context of CBD, or the Nagoya protocol. However, individual national legislation could include retrospectivity, particularly with respect to new applications using biological materials collected pre CBD. In addition to the close liaison maintained with the other KBBE Bioprospecting projects, SAMS, acted as a link between SeaBioTech and the ESFRI road map Research Infrastructures (RIs): EMBRC and MIRRI (MIcrobial Resource Research

	Infrastructure).This has involved relevant CBD related input to the development of the H2020 EMBRIC project. SAMS has also been responsible for providing advice to the government of the Republic of the Seychelles on building a Blue economy, including the need for managing access to MGR. In conclusion, the high-level legal frame-work/ the implications of the CBD to the SeaBioTech consortia are much clearer than at the beginning of the project. The SeaBioTech sample submission portal ensures tracking of samples and transfer of data between partners ensuring CPD compliance. The detail mechanisms to ensure access to the biological resources, and their associated data, beyond the lifetime of the project will agreed and implemented over the next 6-10 months.
Potential impacts	***Scientific breakthrough:***This WP was not specifically science orientated, but significant developments include the implementation of common legal standards across a widely diverse range of partners in universities, independent research institutes and SMEs. ***Economic impact (health costs, market…):***Since the implementation of the Nagoya Protocol, a legal instrument under the Convention on Biological Diversity (1992), in October 2014, the legal landscape surrounding the access to and utilization of genetic resources has changed. This has inevitably impacted working procedures for scientists having turned pre-existing ethics into legal obligations. Work undertaken on the developing of possible solutions to the implications of the collection of materials in areas beyond the EEZ i.e. in Areas Beyond National Jurisdiction (ABNJ) has been presented at the UN for consideration for the proposed changes to the UNCLOS. ***Societal impact (quality of life, health, education, employment, citizen awareness…):*** WP8 gave the opportunity to: >disseminate relevant information to practitioners including young researchers, PhD students, technical staff and PIs. >raising citizen awareness by diverse outreach activities to pupils, students and the interested public through face to face events and via radio and TV coverage. - ***European competitiveness / standards and policies:*** Compliance to the CPD, with adherence to the Nagoya protocol is no longer an option, it is a legal requirement. Whilst most EU Member States grant free access to their genetic resources, others do not. This moves the focus from access to measures enabling tracking to utilisation triggering benefit sharing and the need for tracking of samples, and transfer of data between partners, as is the case in the SeaBioTech database.
Main dissemination / exploitation	European Conference on Marine Natural Products (ECMNP) 30th August – 2nd Sept 2015 Glasgow science Festival 2015; Explorathon Glasgow 2015 & 2014 Articles in local (Scottish) and national (UK) Newspapers; BBC presentations on the legal aspects of bioprospecting involving Univ Strathclyde, SAMS & MBL, on: BBC TV Breakfast News, BBC TV News 24, BBC Radio 4, BBC Scotland, BBC World Service and CBBC Newsround.

1.4. Selection of promotion and illustration supports



Organising Joint European-wide Symposium with Other FP7 Consortia

Press Releases

The original document is available upon request.

1.5. Beneficiaries / Principal Investigators and WP leaders contact details

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2. USE AND DISSEMINATION OF FOREGROUND

2.1. Section A (public)

This section describes the dissemination measures, including any scientific publications relating to foreground.

INTERNAL TO THE CONSORTIUM FACE TO FACE AND ON REMOTE MEETINGS			
Date	**Location**	**Participants**	**Comments**
10 & 11/10/2012	Glasgow (UK)	All partners	Kick-off meeting
17 & 18/10/2013	Heraklion (GR)		1st annual meeting
18 & 19/08/2014	Reykjavik (IC)		2nd annual meeting
27 & 28/08/2015	Glasgow (UK)		3rd annual meeting, with SAB member (Ronald Quin)
27 & 28/06/2016	Lyon (FR)		Final meeting, with SAB member (Joanne Porter)
04/12/2013	teleconference	4 members, COO, NOVAMEN	SAB meetings
03/11/2014		3 members, COO NOVAMEN	
31/01/2013	teleconference	IP members	IP meeting
30/01/2013 06/02/2013 08/02/2013 16/04/2013 18/10/2013 21/05/2014 10/10/2014	teleconferences	WP leaders, COO, WP partners	WPs meetings
27 & 28/02/2013	Brussels (BE)	EU, consortium and external	Workshop with MICRIOB3 consortium

INTERNAL TO THE CONSORTIUM FACE TO FACE AND ON REMOTE MEETINGS			
Date	**Location**	**Participants**	**Comments**
07 & 08/05/2014	Leuven (BE)		Workshop with PharmaSea consortium
29/07/2014	teleconference	PO, COO, NOVAMEN	redistribution of HDL budget

PUBLICATIONS	
2013	
1	Polymenakou, PN, Nomikou P, Mandalakis M, Kilias SP, Christakis C, Kyrpides N, Ivanova N, Oulas A, Dailianis T, Carey S, Kotoulas G, Magoulas A, Papanikolaou D. *"Microbiological exploration of a unique CO2-rich shallow submarine hydrothermal vent field (Kolumbo, Santorini island, Aegean Sea)"* Conference Proceedings. Mediterranean Marine Biodiversity Conference, Heraklion Crete, Greece, 2013.
2	Oulas, A, Polymenakou PN, Mandalakis M, Nomikou P, Carey S, Christakis C, Kotoulas G, Magoulas A, Tripp HJ, Espino DPA, Ivanova NN, Kyrpides NC. *"Metagenomics of microbial communities inhabiting the Kolumbo volcano shallow-sea hydrothermal vent field and Santorini (caldera)."* Conference Proceedings. The 8th conference of the Hellenic Society for Computational Biology and Bioinformatics - HSCBB13
2014	
3	Macintyre, L.; Zhang, T.; Viegelmann, C.; Martinez, I.J.; Cheng, C.; Dowdells, C.; Abdelmohsen, U.R.; Gernert, C.; Hentschel, U.; Edrada-Ebel, R. *"Metabolomic Tools for Secondary Metabolite Discovery from Marine Microbial Symbionts".* Mar. Drugs 2014, 12, 3416-3448.
4	Viegelmann, C.; Margassery, L.M.; Kennedy, J.; Zhang, T.; O'Brien, C.; O'Gara, F.; Morrissey, J.P.; Dobson, A.D.W.; Edrada-Ebel, R. *"Metabolomic Profiling and Genomic Study of a Marine Sponge-Associated Streptomyces sp."* Mar. Drugs 2014, 12, 3323-3351. (A joint publication with PharmaSea)

5	Macintyre, L., Zhang, T., Viegelmann, C., Martinez, I.J., Cheng, C., Dowdells, C., Abdelmohsen, U.R., Gernert, C., Hentschel, U., Edrada-Ebel, R. *Metabolomic tools for secondary metabolite discovery from marine microbial symbionts.* Mar. Drugs, 12: 3416-3448.
2015	
6	Cheng Cheng, Lynsey MacIntyre, Usama Ramadan Abdelmohsen, Hannes Horn, Paraskevi N. Polymenakou, RuAngelie Edrada-Ebel, Ute Hentschel. *"Biodiversity, anti-trypanosomal activity screening, and metabolomics profiling of actinomycetes isolated from Mediterranean sponges."* PLOS ONE 2015 Sep 25;10(9):e0138528. doi: 10.1371/journal.pone.0138528
7	Harvey, A.L.; Edrada-Ebel, R.; Quinn, R.J. *"The re-emergence of natural products for drug discovery in the genomics era".* Nature reviews. Drug discovery 2015, 14, 111-129.
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11	Abdelmohsen UR, Grkovic T, Balasubramanian S, Kamel MS, Quinn RJ, Hentschel U. *"Elicitation of secondary metabolism in actinomycetes"* Biotechnol Adv. 2015 Nov 1;33:798-811.
12	Horn H., *et al* An abundance of CRISPR and other defense-related features in marine sponge-associated microbial metagenomes. Frontiers in Microbiol: submitted
13	Green DH, et al *Bacterial diversity associated with the coccolithophorid algae Emiliania huxleyi and Coccolithus pelagicus f. braarudii.* BioMed Research Intern. 15.

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14	Thomas T, *et al* . Diversity, structure and convergent evolution of the global sponge microbiome. Nat Commun. 11870. doi: 10.1038/ncomms11870
15	PURVES, K., MACINTYRE, L., BRENNAN, D., HREGGVIOSSON, G. O., KUTTNER, E., ASGEIRSDOTTIR, M.E., YOUNG, L.C., GREEN, D.H. EDRADA-EBEL, R, DUNCAN, K.R. "*Using Molecular Networking for Microbial Secondary Metabolite Bioprospecting"* Metabolites 6(1): 2. doi:10.3390/metabo6010002
16	BUCHHOLTZ, H. AND DUNCAN, K. R. *"The chemical ecology of microbial communities associated with Antarctic sponges.* Current Organic Chemistry, accepted for publication
17	RIZZO, A.L., CARACAUSI, A., CHAVAGNAC, V., NOMIKOU, P., POLYMENAKOU, P.N., MANDALAKIS, M., KOTOULAS, G., MAGOULAS, A., CASTILLO, A., LAMPRIDOU, D. "*KOLUMBO SUBMARINE VOLCANO (GREECE): AN ACTIVE WINDOW INTO THE AEGEAN SUBDUCTION SYSTEM"* NATURE SCIENTIFIC REPORTS, 6:28013, DOI: 10.1038/SREP28013.
18	Abdelmohsen UR, Balasubramanian S, Oelschlaeger TA, Grkovic T, Pham NB, Quinn RJ, Hentschel U *Potential of marine natural products against drug-resistant pathogens.* Lancet Infect Dis: in press
19	Cheng C, Balasubramanian S, Fekete A, Krischke M, Mueller MJ, Hentschel U, Oelschläger TA, Abdelmohsen UR. *Inhibitory potential of strepthonium A against Shiga toxin production in EHEC strain EDL933.* Int J Med Microbiol: in revision
20	Abdelmohsen UR, Cheng C, Sholkamy E, Stopper H, Edrada-Ebel R, Hentschel U. *Metabolomics-based isolation of petrocidin A, a new cyclic dipeptide from the marine sponge-derived bacterium Streptomyces sp. SBT348.* J Nat Prod: in revision
21	Cheng C, Othman EM, Fekete A, Krischke M, Stopper H, Edrada-Ebel R, Mueller MJ, Hentschel U, Abdelmohsen UR *"Strepoxazine A, a new cytotoxic phenoxazin from the marine sponge-derived bacterium Streptomyces sp. SBT345"* Tet Lett 57(37), 4196-4199
22	Cheng C, Othman EM, Reimer A, Gruene M, Kozjak-Pavlovic V, Stopper H, Hentschel U, Abdelmohsen UR *"Ageloline A, new antioxidant and antichlamydial quinolone from the marine sponge-derived bacterium Streptomyces sp. SBT345."* Tet Lett 57(25), 2786-2789

23	Roya R.R. Sardari, Evelina Kulcinskaja, Emanuel Y.C. Ron, Snædís Björnsdóttir, Olafur H. Fridjónsson, Gudmundur Oli Hreggvidsson, Eva Nordberg Karlsson *Evaluation of the production of exopolysaccharides by two strains of the thermophilic bacterium Rhodothermus marinus.* Carbohydrate Polymers OI:10.1016/j.carbpol.2016.08.062
24	Emanuel Y. C. Ron, Merichel Plaza , Thordis Kristjansdottir , Roya R. R. Sardari, Steinn Gudmundsson, Snaedis Bjornsdottir, Gudmundur Oli Hreggvidsson, Charlotta Turner, Ed W. J. van Niel, Eva Nordberg-Karlsson *Characterization of carotenoids in Rhodothermus marinus strains DSM 4252T, DSM 4253 and PRI 493*. Submitted to Plose One.

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25	CHRISTAKIS, C., POLYMENAKOU, P.N., MANDALAKIS, M., OULAS, A., KOTOULAS, G., GONTIKAKI, E., NOMIKOU, P., MAGOULAS, A. 2015. *Culture-independent studies shed light in the prokaryotic diversity of microbes in the Kolumbo volcano and Santorini caldera, Greece.*
26	CHRISTAKIS, C., POLYMENAKOU, P.N., MANDALAKIS, M., KOTOULAS, G., SMYRLI, M., KATHARIOS, P., MAGOULAS, A. *Diversity of culturable bacteria in Santorini volcanic complex, Greece.*
27	CHRISTAKIS, C., POLYMENAKOU, P.N., MANDALAKIS, M., KOTOULAS, G., MAGOULAS, A. *Metagenomic exploration of Santorini volcanic complex, Greece. Under preparation*
28	CHRISTAKIS, C., POLYMENAKOU, P.N., MANDALAKIS, M., KYRPIDES, N.C., KOTOULAS, G., MAGOULAS, A. 2016. *Metagenomic exploration of Kalisti lakes, Santorini caldera, Greece. Under preparation.*
29	CHRISTAKIS, C., POLYMENAKOU, P.N., MANDALAKIS, M., KYRPIDES, N.C., KOTOULAS, G., MAGOULAS, A. 2016. *Metagenomic exploration of three different microbial mat layers of a polymetallic gas chimney, Kolumbo volcano, Hellenic Volcanic Arc. Under preparation*
30	Margrét Eva Ásgeirsdottir, Eva Kuttner, Hörður G. Kristinsson, Guðmundur Óli Hreggviðsson. *ANTI-DIABETIC PROPERTIES OF SEAWEED EXTRACT USING THE ADIPOCYTE CELL MODEL 3T3-L1.*
31	Varsha Kale, Olafur Fridjonsson, Sesselja Omarsdottir, Solveig Petursdottir, Bryndis Bjornsdottir, Brynjar Ellertsson, Solveig Olafsdottir, Gudmundur Hreggvidsson. *IDENTIFICATION, EXPRESSION AND CHARACTERIZATION OF MARINE POLYSACCHARIDE DEGRADING ENZYMES FROM NOVEL BACTERIA ISOLATED FROM INTERTIDAL BIOTOPES.*

32	Cheng Cheng , Srikkanth Balasubramanian, Agnes Fekete, Markus Krischke, Martin J. Müller, Ute Hentschel, Tobias A. Oelschläger,, and Usama Ramadan Abdelmohsen, *Inhibitory potential of strepthonium A against Shiga toxin production in EHEC strain EDL933*
33	Cheng Cheng , Eman M. Othman, Anastasija Reimer, Matthias Grüne, Vera Kozjak-Pavlovic, Helga Stopper,Ute Hentschel, Usama R. Abdelmohsen. *Ageloline A, new antioxidant and antichlamydial quinolone from the marine sponge-derived bacterium Streptomyces sp. SBT345*
34	Usama Ramadan Abdelmohsen, Srikkanth Balasubramanian, Tobias A. Oelschlaeger, Tanja Grkovic, Ronald J. Quinn, Ute Hentschel. *Potential of Marine natural products against drug resistant pathogens.*
35	Bela Sanches , Ahmed Tawfike, Christina Viegelmann, Grainne Abbott, Louise Young, Solveig Olafsdottir, Gudmundur Hreggvidsson, Brian McNeil, Linda Harvey, RuAngelie Edrada-Ebel. *Alpha-glucosidase active metabolites from Geothermal Flavobacteriaceae.*
36	MacIntyre, L. Zhang, T., Fazenda, M. L., Viegelmann, C., Dowdells, C., Young, L., Clements, C., Abbott, G., Duncan, K.R., Green, D., Harvey, L.M., McNeil, B. and Edrada-Ebel, R. *Metabolomics-assisted Isolation of Indolic Antimycobacterial Compounds from a Sponge-associated Strain of Vibrio splendidus. Under preparation.*
37	Katherine Duncan, Lynsey MacIntyre, Christina Viegelmann, David Green, Brian McNeil, Linda Harvey, RuAngelie Edrada-Ebel. *Molecular networking the scale-up production of anti-mycobacterial bisindoles.*
38	Abdelmohsen UR, Balasubramanian S, Oelschlaeger TA, Grkovic T, Pham NB, Quinn RJ, Hentschel U *Potential of marine natural products against drug-resistant pathogens.* Lancet Infect Dis: in press
39	Cheng C, Balasubramanian S, Fekete A, Krischke M, Mueller MJ, Hentschel U, Oelschläger TA, Abdelmohsen UR. *Inhibitory potential of strepthonium A against Shiga toxin production in EHEC strain EDL933.* Int J Med Microbiol: in revision
40	Abdelmohsen UR, Cheng C, Sholkamy E, Stopper H, Edrada-Ebel R, Hentschel U. *Metabolomics-based isolation of petrocidin A, a new cyclic dipeptide from the marine sponge-derived bacterium Streptomyces sp. SBT348.* J Nat Prod: in revision

41	Wang Y, Seppänen-Laakso T, Rischer H, Wiebe M. Euglena gracilis growth and cell composition under different temperature and light conditions. Manuscript
42	Nygren H, Seppänen-Laakso T, Risher H. *Liquid chromatography-mass spectrometry (LC-MS)-based analysis of molecular lipids in algae samples.* Methods in Molecular Biology. Manuscript
43	Seppänen-Laakso T, Nygren H, Rischer H. *Fatty acid and lipid class analyses by GC, GC-MS and UPLC-ELSD.* Methods in Molecular Biology. Manuscript.
44	Thordis Kristjansdottir, Snædís Björnsdóttir, Emanuel Y. C. Ron, Eva Nordberg-Karlsson, Birkir Reynisson, Ólafur Friðjónsson, Gudmundur Oli Hreggvidsson, Steinn Gudmundsson. Working title: *A metabolic reconstruction of the thermophilic bacterium Rhodothermus marinus* Manuscript under preparation 2016
45	Kazi Zubaida Gulshan Ara, Marek Gabriško, Javier Linares-Pasten, Olafur H. Fridjonsson, Gudmundur O. Hreggvidson, Štefan Janeček, Eva Nordberg Karlsson. *Diversity in a single family: Biochemical characterization of six GH family GH3 enzymes from Rhodothermus marinus.* Manuscript under preparation 2016
46	Javier Linares-Pastén, Lilja Björk Jonsdottir, Gudmundur O. Hreggvidsson, Olafur H. Fridjonsson, Hildegard Watzlawick, Eva Nordberg Karlsson.. *Insights into the structure of proteobacterial non-Leloir beta- glucosyltransferases from glycoside hydrolase family GH17: A computational study*. Manuscript under preparation 2017

DISSEMINATION ACTIVITIES			
Date	**Event, Location**	**Participants / Authors**	**Comments / Titles**
2013			

47	20 – 22 February	Tromso, Norway	RuAngelie Edrada-Ebel, Lynsey MacIntyre	BIOPROSP_13, The 6th international BIOPROSP Conference on Marine Bioprospecting from cold marine environment. Lynsey MacIntyre et al. POSTER http://mabit.no/sites/mabit.no/files/P17%20RuAngelie%20Edrada-Ebel_0.pdf)
48	April	Kong Lyngby, Denmark	Eva.Nordberg Karlsson	Workshop on carbohydrate bioeng. Functional Characterisation GH3 Enzymes Isolated from Rhodothermus marinus
49	May	Prague, Tchech Republic	Eva.Nordberg Karlsson	Conference CBM10. The GH3 family in Rhodothermus marinus: biochemical and structural characterization
50	7 June	Glasgow, UK	RuAngelie Edrada-Ebel, Lynsey MacIntyre	University's annual Research Day on 7 June 2013 as part of the Glasgow Science Festival 2013. The poster was presented to visiting 12 secondary schools from East Renfrewshire & Glasgow.
51	26 June	Glasgow, UK		Visit by schoolchildren to SIPBS. A one day programme of events for the visit of senior pupils from Springburn & St.Mungo's academies to the Strathclyde Institute of Pharmacy & Biomedical Sciences (SIPBS).
52	1-4 July	Glasgow, UK	RuAngelie Edrada-Ebel, Lynsey MacIntyre	Metabolomics 2013, July 1st – 4th 2013 in Glasgow, UK. Oral Presentation. In Session 6A: Drug Discovery. O6A-1. Lynsey MacIntyre.
53	9 September	University of Edinburgh, UK		The New Genomics: Knowledge, Health & Society. Research Symposium. www.sulsa.ac.uk
54	15-20 September	La Toja, Spain	RuAngelie Edrada-Ebel, Lynsey MacIntyre	Joint Meeting of the 14th International Symposium on Marine Natural Products (MaNaPro XIV) 2013 & the 8th European Conference on Marine Natural Products. Poster Presentation. Lynsey MacIntyre et al.
55	30 Sept. – 02 October	Brussels, Belgium	Ian Fotheringham	European Forum for Industrial Biotechnology & the Biobased Economy (EFIB) 2013 (http://www.efibforum.com/workshops.aspx)
56	7-9 October	*Mediterranean Marine Biodiversity Conference* *Heraklion Crete, Greece*	Polymenakou, P.N., Nomikou, P., Malakis, M., Kilias, S., Christakis, C., Kyrpides, N.C., Ivanova, N., Oulas, A. Dailianis, T., Carey, S.,	Microbiological exploration of a unique CO2-rich shallow submarine hydrothermal vent field (Kolumbo, Santorini isl&, Aegean Sea).

			Kotoulas, G., Magoulas, A., Papanikolaou, D.	
57	14 October	DeOosterpoort, Groningen, The Netherlands	Sólveig Petursdottir1, Sigmar K. Stefánsson, Bryndis Bjornsdottir,Snaedís Bjornsdóttir, Brynjar Orn Ellertsson, Varsha Kale, Elisabet Guðmundsdottir, Edda Olgudóttir1, Solveig Olafsdóttir, Olafur Fridjonsson and Gudmundur Hreggvidsson.	Prospecting extreme costal environments for carbohydrate enzymes and polysaccharides applying genomic and metagenomic sequencing. Harvesting Environmental Genomes for the Development of Biocatalysts A joint initiative of the MicroB3 and MetaExplore EU/FP7 projects
58	22-24 November	*The 8th Conference of the Hellenic Society for Computational Biology & Bioinformatics - HSCBB13* *Thessaly, Greece*	OULAS, A., Polymenakou, P.N., Malakis, M., Nomikou, P., CAREY, S., Christakis, C., Kotoulas, G., Magoulas, A., TRIPP, H.J, ESPINO, D.P.A., Ivanova, N., Kyrpides, N.C.	Metagenomics of microbial communities inhabiting the Kolumbo volcano shallow-sea hydrothermal vent field & Santorini caldera
2014				
59	8-10 September	Metagenomics Bioinformatics course, European Bioinformatics Institute, CB10 1SD,	CHRISTAKIS, C., POLYMENAKOU, P.N., NOMIKOU, P., MANDALAKIS, M.,	Prokaryotic diversity in the hydrothermal chimneys of the Kolumbo submarine volcano (Aegean Sea).

		UK	OULAS, A., KOTOULAS, G., MAGOULAS, A.	
60	1st October	Reims, France	RuAngelie Edrada-Ebel	7th Annual European Forum for Industrial Biotechnology and the Biobased Economy Harnessing the potential of marine biodiversity for industrial biotechnology. SeaBioTech – Year 2 and onwards.
61	8th October	Hong Kong University of Science and Technology	RuAngelie Edrada-Ebel	Marine Biotechnology in SeaBioTech
62	13-18 October	Padang, West Sumatera, Indonesia	RuAngelie Edrada-Ebel	The 7th International Seminar of The Indonesian Society for Microbiology Metabolomic Strategies in Dereplication for Targeted Cultivation and Isolation of New Bioactive Secondary Metabolites from Fungal Endophyes and Marine Microbial Symbionts (Plenary Speaker)
63	22-24 October	Kolumbo, Santorini. University of Thessaloniki, Greece	Polymenakou, P.N	10th International Congress of the Hellenic Geographical Society. In-situ CTD scans as a probe for submarine volcano's morphology: the distinct case.
64	25-30 October	Baltimore, USA	Ute Hentschel	2nd International Sponge Conference Phylogeny, -omics, and visualisation of poribacteria
65	18 – 20 November	Natural products meeting Inverness, Scotland, UK	Stanley MS, Slocombe SP and Day JG.	Microalgal lipids: not just for burning. (Selected for oral presentation, presentation delivered by S. Slocombe)
			Lynsey MacIntyre	Natural Product Biotechnology 2014. Metabolomic tools to target and accelerate the isolation of bioactive compounds from the marine microbial symbiont Vibrio splendidus
			Mariana Fazenda	Natural Product Biotechnology 2014. From sea-bed to test-bed: Industrial bioprocessing of microbial marine products
			Katherine Duncan	Natural Product Biotechnology 2014. Identifying secondary metabolites and their biosynthetic pathways using GnPS molecular networking
66	20. Feb	EU Workshop Brussels	J. Kristjansson	Innovative SMEs under Horizon 2020: Towards market breakthroughs on bio-based industries
67	18 March 2014 3 March	Lectures for the course "Marine Biotechnology" (organized by Prof. M.	POLYMENAKOU, P.N.	Microbial diversity and ecosystems, the case of the Eastern Mediterranean extreme environments.

	2015	Kentouri), Department of Biology, University of Crete, Greece		
68	29 May	Micro-B3 Summer School, HCMR-IMBBC, Heraklion Crete, Greece, 29	POLYMENAKOU, P.N.	Microbial diversity and ecosystems: the case of the Eastern Mediterranean sea extreme environments.
2015				
69	5 March	University of Iceland	ÆVARSSON, A	Exploring microbes and enzymes for fundamental research and applied biotechnology
70	15 July	Ukraine.	KRISTJANSSON, J.K.:	Use of Geothermal Bacteria: From Discovery to Applications, Institute of Cell Biology,
71	May	Helsinki, Finland	Kazi Zubaida Gulshan Ara	Conference CBM11. Evaluation of microbial production of exopolysaccharide by Rhodothermus marinus strains: potential for industrial biotechnology
72	May	National University of Ireland, Galway, Ireland	Ruangelie Edrada-Ebel	Utilisation of Metabolomics to Dereplicate the Production of Secondary Metabolites in Microbial Symbionts and Endophytes
73	18 - 19 May	Edinburg, UK	Louise Young, Grainne Abbott	SULSA – SLAS Conference: Cutting-edge Technologies for Drug Target Validation. SEABIOTECH: FROM SEA-BED TO TEST-BED: Harvesting the potential of marine biodiversity for industrial biotechnology.
74	April 14-16	Tampere, Finland	Nygren, Heli Seppänen-Laakso, Tuulikki	1st Finnish Symposium on Biological Mass Spectrometry. Effect of light on lipidomic profile and fatty acid content of microalgae Euglena gracilis
75	3-5 April	Athens, Greece	CHRISTAKIS, C., POLYMENAKOU, P.N., NOMIKOU, P., MANDALAKIS, M., OULAS, A., KOTOULAS, G., MAGOULAS, A.	6th National Mikrobiokosmos Conference: New Horizons in the Micro World. Culture-dependent and culture-independent studies shed light in the prokaryotic diversity of microbes in the Kolumbo volcano and Santorini caldera. Metagenomic investigation of the geologically unique Hellenic Volcanic Arc reveals a distinctive ecosystem with unexpected physiology.
76	11- 13 March	Kyungpook National University, Korea	Carol Clements	Brain Korea 21 PLUS Project; Innovative Youth development Programme for Revolutionary 4-H Agricultural Bio-Industry. SeaBioTech: From Sea-Bed to Testbed: Harvesting the Potential of Marine Biodiversity for Industrial Biotechnology

77	5 March	University of Iceland	ÆVARSSON, A	Exploring microbes and enzymes for fundamental research and applied biotechnology
78	22-27 February	Granada, Spain	POLYMENAKOU, P.N.	ASLO Meeting, Aquatic sciences: global and regional perspectives - North meets South Carbon sink capacity of seagrass meadows in naturally acidified CO2 vents.
79	7-11 February	Washington USA	Carol Clements	Society for Laboratory Automation and Screening (SLAS) Conference . SEABIOTECH: FROM SEA-BED TO TEST-BED: Harvesting the potential of marine biodiversity for industrial biotechnology.
80	26-28 September	11th International Sea Lice Conference Westport, Ireland	C. Wiik-Nielsen, L. Macintyre, L. Stucchi, L. Young, G. Abbott, R. Andersen, E. Aksnes, R. Edrada-Ebel, D. Carettoni.	A screening program within SeaBioTech to address major parasitic problems in aquaculture
81	12-16 September	University of Sao Paolo, Riberao Preto	Ruangelie Edrada-Ebel	Workshop on Application of Metabolomics in Natural Products Research
82	August	BBSRC NIBB NPRONET Manchester, UK	Katherine Duncan	Comparative omics approaches to natural products discovery.
83	27 – 30 June	Marine Microbiome – Discovery & Innovation' , MaCumBa in Berlin	RuAngelie Edrada-Ebel, Louise Young, Christina Viegelmann	3-Part Poster Seabiotech :: From sea-bed to test-bed: harvesting the potential of marine biodiversity for industrial biotechnology: I. Industrial Partnership; II. Prospects and Products; II. Dissemination and Public Engagement.
84	18-20 April	MBI Modular training course in Industrial Biotechnology, UCL, London, UK	Alison Arnold (lecture)	Biocatalysis through to Synthetic Biology

85	May	SYMPOSIUM Industry and Innovation in the Algae sector, Lyon, France	Stanley MS	State of the art and innovation in progress in laboratories and industry. (Invited Keynote)
86	2 May	University of Duesseldorf, Germany	Ruangelie Edrada-Ebel	Utilisation of Metabolomics to Dereplicate the Production of Secondary Metabolites in Microbial Symbionts and Endophytes (Invited Speaker)
87	20 - 24 April	La Paz, Mexico	Ruangelie Edrada-Ebel	Marine Natural Products Workshop: Searching Active Compounds from Marine Organisms specifically on the application of metabolomics on marine microbial research
88	17-22 April	Vienna, Austria,	POLYMENAKOU, P.N.	European Geosciences Union EGU General Assembly: Kolumbo submarine volcano (Greece): An active window into the Aegean subduction system.
89	13-15 April	Nancy, France	POLYMENAKOU, P.N.	Developments in Noble Gas Understanding and Expertise, CRPG . Kolumbo active seamount (Greece): a window into the Aegean mantle.
90	15-17 March	Terengganu, Malaysia	Ruangelie Edrada-Ebel	International Conference on Natural Products 2016. Utilisation of Metabolomics to Dereplicate the Production of Secondary Metabolites in Microbial Symbionts and Endophytes (Plenary Speaker)
91	13-14 March	Terengganu, Malaysia	Ruangelie Edrada-Ebel	International Conference on Natural Products 2016 Pre-Conference Workshop (Workshop Leader)
92	February 2016	Ventura, CA	Katherine Duncan (Chair)	Networking Early Career Researcher Workshop. Comparative metabolomics of secondary metabolites from bacteria
93	10–12 February	Karur, India	Ruangelie Edrada-Ebel	BioNat 2016 Biodiversity and Bioactive Natural Products for Human Welfare 2016 Marine Microbial Natural Products
94	10-11 Feb	BioInnovation – Process and manufacturing strategies leaders'	Alison Arnold	

		summit, Berlin, Germany.		
95	29 June - 2 July	Chania, Crete, Greece	POLYMENAKOU, P.N.	6th European Bioremediation Conference Metagenomic analysis reveals anaerobic degradation of hydrocarbons in the Kolumbo volcano and Santorini caldera.
96	29 June	College of Pharmacy, University of the Philippines	Christina Viegelmann	Metabolomics and Marine-based Drug Discovery
97	17 Sept.	ALGET workshop, Grenå, Denmark	A. Ævarsson	Prokazyme - Research and Development
98	30 September - 2 October	9th European Marine Natural Products Conference. August; Glasgow, UK	C. Wiik-Nielsen, L. MacIntyre, L. Stucchi, L. Young, G. Abbott, M. Stanley, C. Cheng, U. Hentschel Humeida, J. Day, E. Aksnes, R. Edrada-Ebel, D. Carettoni.	An integrated lead discovery programme within SeaBioTech to address major parasitic infections in aquaculture
			C. Cheng, L. MacIntyre, U.R. Abdelmohsen, H. Horn, P. Polymenakou, D. Carettoni, R. Edrada-Ebel, U. Hentschel Humeida.	Discovery of biologically active compounds from marine sponge-associated actinomycetes
			Alison Arnold	Development of Synthetic Biology Tools to More Predictably Clone, Express and Select Biocatalytic Activities for Metabolic Pathway Optimization and High Yield Biomolecule Production

			Guðmundur Óli Hreggviðsson	Novel Enzymes and Organisms for Processing Polysaccharides from Brown Algae (Invited Lecture)
			Alison Arnold	Development of Synthetic Biology Tools to More Predictably Clone, Express and Select Biocatalytic Activities for Metabolic Pathway Optimization and High Yield Biomolecule Production (Short Lecture)
			Roya R.R. Sardari	Evaluation of microbial production of exopolysaccharide by Rhodothermus marinus strains: potential for industrial biotechnology (Short Lecture)
			Snaedis Bjornsdottir	Novel members of the phylum Bacteroidetes – a potent source of bioactive compounds (Short Lecture)
			Christina Viegelmann	Bioprocessing of Marine Microbes for Industrial Exploitation (Short Lecture)
			Lynsey MacIntyre	Metabolomic tools to target and accelerate the isolation of bioactive compounds from marine microbial symbionts (Short Lecture)
			Katherine Duncan	Comparative metabolomics of secondary metabolites from bacteria using GnPS molecular networking (Short Lecture)
			Usama Abdelmohsen	Diversity and Natural Products Repertoire of Marine Sponge-Associated Actinomycetes. (Short Lecture)
			Ruangelie Edrada-Ebel	Chair
99	26-29 October	BioProcess International (BPI), Boston, USA	Alison Arnold	

100	November	Institute for Chemistry and Biology of the Marine Environment, University of Oldenburg	Katherine Duncan	Marine microbial metabolomics – addressing big picture questions in the comparative –omics era
101	November	Marine Microbiology Research Unit, GEOMAR Helmholtz Centre for Ocean Research	Katherine Duncan	Marine Microbial Chemical Ecology – Understanding the balance of ecosystem settings on metabolite production
102	November	Lund, Sweden	Eva.Nordberg Karlsson	2nd symposium on biomass valorization The GH3 family in Rhodothermus marinus: biochemical and structural characterization
103	14-18 December	San Francisco, USA	POLYMENAKOU, P.N.	AGU Fall Meeting, Kolumbo active seamount (Greece): a window into the Aegean mantle

2016			
NC	SLAS	RuAngelie Edrada-Ebel	poster *"From sea-bed to test-bed: harvesting the potential of marine biodiversity for industrial biotechnology: Dissemination and Public Engagement 2014 – 2016"*
10-11 Feb	BioInnovation – Process and manufacturing strategies leaders' summit, Berlin, Germany.	Alison Arnold	

10–12 February	Karur, India	Ruangelie Edrada-Ebel	BioNat 2016 Biodiversity and Bioactive Natural Products for Human Welfare 2016 Marine Microbial Natural Products
February 2016	Ventura, CA	Katherine Duncan (Chair)	Networking Early Career Researcher Workshop. Comparative metabolomics of secondary metabolites from bacteria
13-14 March	Terengganu, Malaysia	Ruangelie Edrada-Ebel	International Conference on Natural Products 2016 Pre-Conference Workshop (Workshop Leader)
15-17 March	Terengganu, Malaysia	Ruangelie Edrada-Ebel	International Conference on Natural Products 2016. Utilisation of Metabolomics to Dereplicate the Production of Secondary Metabolites in Microbial Symbionts and Endophytes (Plenary Speaker)
12 April	LVIV, Ukraine	A. Ævarsson	Innovative enzyme production
13-15 April	Nancy, France	POLYMENAKOU, P.N.	Developments in Noble Gas Understanding and Expertise, CRPG . Kolumbo active seamount (Greece): a window into the Aegean mantle.
17-22 April	Vienna, Austria,	POLYMENAKOU, P.N.	European Geosciences Union EGU General Assembly: Kolumbo submarine volcano (Greece): An active window into the Aegean subduction system.
18-20 April	MBI Modular training course in Industrial Biotechnology, UCL, London, UK	Alison Arnold	(lecture) Biocatalysis through to Synthetic Biology

20 - 24 April	La Paz, Mexico	Ruangelie Edrada-Ebel	Marine Natural Products Workshop: Searching Active Compounds from Marine Organisms specifically on the application of metabolomics on marine microbial research
May	SYMPOSIUM Industry and Innovation in the Algae sector, Lyon, France	Stanley MS	State of the art and innovation in progress in laboratories and industry. (Invited Keynote)
31 May	SCI, Day of Science and Careers, University of Strathclyde, Glasgow, UK.	Alison Arnold	
27 – 30 June	Conference in Berlin	Louise Young	MaCuMBA poster
August	BBSRC NIBB NPRONET Manchester, UK	Katherine Duncan	Comparative omics approaches to natural products discovery.
12-16 September	University of Sao Paolo, Riberao Preto	Ruangelie Edrada-Ebel	Workshop on Application of Metabolomics in Natural Products Research
September 26-28,	11th International Sea Lice Conference Westport, Ireland	C. Wiik-Nielsen, L. Macintyre, L. Stucchi, L. Young, G. Abbott, R. Andersen, E. Aksnes, R. Edrada-Ebel, D. Carettoni.	A screening program within SeaBioTech to address major parasitic problems in aquaculture

Miscellaneous dissemination activities	
You Tube film https://www.youtube.com/user/santorinikolumbo.	Dr. Paraskevi Polymenakou uploaded brief videos summarizing the sampling event that took place in Crete in Submarine Kolumbo volcano, Santorini volcanic field, Greece (HCMR) 2 - Duration: 15 seconds
BBC http://www.bbc.co.uk/news/science-environment-27295159	*Ocean medicine hunt: A Wild West beneath the waves?* By Rebecca Morelle Science correspondent, BBC News, Oban, west Scotland (SIPBS/MBL/SAMS)
www.prokazyme.com/the-enzyme-blog	A. Ævarsson
http://www.clusteralisei.it/dal-mare-il-futuro-della-biotecnologia/	Daniele Carettoni (2016) Dai microrganismi marini ai farmaci del futuro con il progetto SeaBioTech. ALISEI (Advanced Life Sciences in Italy. Italian Association for the Development of Biotechnology.)
http://soapboxscience.org/?page_id=2851	Soapbox Science Edinburgh event 24/07: one of 13 UK Soapbox Science events in 2016 speaking about "medicines from the sea". It was the only soapbox science event in Scotland; Katherine Duncan was one of 12 speakers selected for the event which took place on The Mound.
Seabiotech : Marine Microbes (http://www.boys-day.de/)	Ute Hentschel, UWUERZ / GEOMAR, Open Day for Secondary Schools in Germany
BBC media (BBC TV Breakfast News, BBC TV News 24, BBC Radio 4, BBC Scotland, BBC World Service and CBBC Newsround)	Presentations on the legal aspects of bioprospecting involving Univ Strathclyde, SAMS & MBL
Public engagement with special focus on secondary schools	Glasgow Science Festival 2014 and 2015. SeaBioTech invited the public to experience the importance of marine life and research for our environment. Interactive exhibits will be open for kids of all ages at the Strathclyde Institute of Pharmacy and Biomedical Sciences

	Glasgow Science centre's Explorathon 2014 event (September 2014) Kirsty Black from MBL together with partners in SIPBS led by Lynsey MacIntyre, participated in.An exhibition table was set up showing various seaweeds, alginates and alginate containing products.
	Explorathon 2014 & 2015, Kelvingrove Museum in Glasgow Dr. Christina Viegelmann (SIPBS) with MBL
Strathclyde Images of Research 2014	Drs. Mariana Fazenda (SIPBS), Lynsey MacIntyre (SIPBS), and Kirsty Black (MBL) represented SeaBioTech. "Images of Research" is an annual competition and subsequent year-long exhibition brings the research conducted at Strathclyde to an audience of thousands of people visiting art galleries, museums and public spaces. The entry from Lynsey MacIntyre (SIPBS), and Kirsty Black (MBL) were among the commended entry under the Business and Industry category.
UK-India Seminar Lecture Series on Marine Natural Products and Biotechnology	Scientists from PharmaSea, Drs. RuAngelie Edrada-Ebel and Lynsey McIntyre. Department of Biotechnology Tanharil, Aizawl, Mizoram University: 15th-17th January 2014 sponsored by the Royal Society and the Indian Department of Science and Technology.

2.2. Section B

TEMPLATE B1: LIST OF APPLICATIONS FOR PATENTS, TRADEMARKS, REGISTERED DESIGNS, ETC.					
Type of IP Rights[1]:	**Confidential YES/NO**	**Foreseen embargo date dd/mm/yyyy**	**Application reference(s) (e.g. EP123456)**	**Subject or title of application**	**Applicant (s) (as on the application)**
Patent	NO		European Patent Application No. 15703635 & and USA Patent Application No. 15/110,132	THERMOSTABLE ALGINATE DEGRADING ENZYMES AND THEIR METHODS OF USE. 2016.	MATIS: Gudmundur Oli Hreggvidsson

Type of Exploitable Foreground[2]	**Description of exploitable foreground**	**Confidential Click on YES/NO**	**Foreseen embargo date dd/mm/yyyy**	**Exploitable product(s) or measure(s)**	**Sector(s) of application[3]**	**Timetable, commercial or any other use**	**Patents or other IPR exploitation (licences)**	**Owner & Other Beneficiary(s) involved**
Commercial exploitation of R&D results	*Polysiphonia extract (brominated compounds)*	*yes*	*End 2017*	*Agrochemicals*	*Pharmaceutical, Animal Health (Aquaculture)*		*End 2017 (for 2 years)*	*Promising performance on Sea Lice for Agrochem companies Pharmaq, MBL, Axaam, SIPBS*

[1] A drop down list allows choosing the type of IP rights: Patents, Trademarks, Registered designs, Utility models, Others.

[19] A drop down list allows choosing the type of foreground: General advancement of knowledge, Commercial exploitation of R&D results, Exploitation of R&D results via standards, exploitation of results through EU policies, exploitation of results through (social) innovation.

[3] A drop down list allows choosing the type sector (NACE nomenclature) : http://ec.europa.eu/competition/mergers/cases/index/nace_all.html

Type of Exploitable Foreground[2]	Description of exploitable foreground	Confidential Click on YES/NO	Foreseen embargo date dd/mm/yyyy	Exploitable product(s) or measure(s)	Sector(s) of application[3]	Timetable, commercial or any other use	Patents or other IPR exploitation (licences)	Owner & Other Beneficiary(s) involved
Exploitation of R&D results via standards	Seaweed Bark Extract (complex compound mix)	YES	early 2016 (for 3 years)	biostat/biocide	Natural biostat/biocide		**2019**	**MBL**
Commercial exploitation of R&D results	Small bioactives from Bacillus licheniformis SBT144 and SBT153	YES	End 2016	Pharmaceutical/ consumer healthcare	Pharmaceutical/ consumer healthcare		End 2016	**MBL**
Commercial exploitation of R&D results	Polysaccharide and small bioactives from *Celeribacter*	YES	**Q1 2017**	Cosmetics/ Personal Care, Biomedical Plastics	Cosmetics/ Personal Care, Biomedical Plastics		**Q1 2017**	**MBL, SIPBS**
Commercial exploitation of R&D results	High Value Saccharides Fucoidan, mannitol, Laminarin	**YES**	Q4 2016	Consumer Healthcare, Cosmetics	Consumer Healthcare, Cosmetics	**Q1 2017**	Q4 2016	**MBL**
Exploitation of R&D results via standards	*Small bioactives from Ulva lactuca*	YES	**Q1 2017**	Pharmaceutical	Pharmaceutical		**Q1 2017**	**MBL, SIPBS**
Exploitation of R&D results via standards	structurally-related compounds from Algoriphagus marincola against endothelial lipase,	YES	**Q1 2017**	Pharmaceutical treatment of atherosclerosis	Pharmaceutical treatment of atherosclerosis		**Q1 2017**	**AXXAM, MATIS, SIPBS**

3. REPORT ON SOCIETAL IMPLICATIONS

Replies to the following questions will assist the Commission to obtain statistics and indicators on societal and socio-economic issues addressed by projects. The questions are arranged in a number of key themes. As well as producing certain statistics, the replies will also help identify those projects that have shown a real engagement with wider societal issues, and thereby identify interesting approaches to these issues and best practices. The replies for individual projects will not be made public.

A General Information	
Grant Agreement Number:	311932
Title of Project:	From sea-bed to test-bed: harvesting the potential of marine microbes for industrial biotechnology
Name and Title of Coordinator:	Prof. Brian McNEIL

B Ethics	
1. Did your project undergo an Ethics Review (and/or Screening)? If Yes: have you described the progress of compliance with the relevant Ethics Review/Screening Requirements in the frame of the periodic/final project reports? Special Reminder: the progress of compliance with the Ethics Review/Screening Requirements should be described in the Period/Final Project Reports under the Section 3.2.2 *'Work Progress and Achievements'*	***YES*** ***YES***
2. Please indicate whether your project involved any of the following issues (tick box) :	***YES / NO***
RESEARCH ON HUMANS	
• Did the project involve children?	NO
• Did the project involve patients?	NO
• Did the project involve persons not able to give consent?	NO
• Did the project involve adult healthy volunteers?	NO
• Did the project involve Human genetic material?	NO
• Did the project involve Human biological samples?	NO
• Did the project involve Human data collection?	NO
RESEARCH ON HUMAN EMBRYO/FOETUS	
• Did the project involve Human Embryos?	NO
• Did the project involve Human Foetal Tissue / Cells?	NO
• Did the project involve Human Embryonic Stem Cells (hESCs)?	NO
• Did the project on human Embryonic Stem Cells involve cells in culture?	NO
• Did the project on human Embryonic Stem Cells involve the derivation of cells from Embryos?	NO
PRIVACY	
• Did the project involve processing of genetic information or personal data (eg. health, sexual lifestyle, ethnicity, political opinion, religious or philosophical conviction)?	NO
• Did the project involve tracking the location or observation of people?	NO
RESEARCH ON ANIMALS	
• Did the project involve research on animals?	NO
• Were those animals transgenic small laboratory animals?	NO
• Were those animals transgenic farm animals?	NO
• Were those animals cloned farm animals?	NO
• Were those animals non-human primates?	NO
RESEARCH INVOLVING DEVELOPING COUNTRIES	

• Did the project involve the use of local resources (genetic, animal, plant etc)?	**YES**
• Was the project of benefit to local community (capacity building, access to healthcare, education etc)?	**YES**
DUAL USE	
• Research having direct military use	NO
• Research having the potential for terrorist abuse	NO

C Workforce Statistics

3. Workforce statistics for the project: Please indicate in the table below the number of people who worked on the project (on a headcount basis).

Type of Position	Number of Women	Number of Men
Scientific Coordinator	2	1
Work package leaders	4	5
Experienced researchers (i.e. PhD holders)		
PhD Students		
Other		

4. How many additional researchers (in companies and universities) were recruited specifically for this project?	
Of which, indicate the number of men:	

D Gender Aspects

5.	**Did you carry out specific Gender Equality Actions under the project?**	●	Yes
		○	No

6. Which of the following actions did you carry out and how effective were they?

		Not at all effective				Very effective
❑	Design and implement an equal opportunity policy	○	○	○	○	○
■	Set targets to achieve a gender balance in the workforce	○	○	●	○	○
❑	Organise conferences and workshops on gender	○	○	○	○	○
❑	Actions to improve work-life balance	○	○	○	○	○
○	Other:					

7. Was there a gender dimension associated with the research content – i.e. wherever people were the focus of the research as, for example, consumers, users, patients or in trials, was the issue of gender considered and addressed?

○ Yes- please specify

● No

E Synergies with Science Education

8. Did your project involve working with students and/or school pupils (e.g. open days, participation in science festivals and events, prizes/competitions or joint projects)?

● Yes- please specify: Open days, science festivals

○ No

9. Did the project generate any science education material (e.g. kits, websites, explanatory booklets, DVDs)?

● Yes- please specify: Website, booklets, posters

○ No

F Interdisciplinarity

10. Which disciplines (see list below) are involved in your project?

● Main discipline[4]:

○ Associated discipline[4]:

○ Associated discipline[4]:

G Engaging with Civil society and policy makers

11a	**Did your project engage with societal actors beyond the research community?** *(if 'No', go to Question 14)*	●	Yes
		○	No

11b If yes, did you engage with citizens (citizens' panels / juries) or organised civil society (NGOs, patients' groups etc.)?

● No

○ Yes- in determining what research should be performed

○ Yes - in implementing the research

○ Yes, in communicating /disseminating / using the results of the project

[4] Insert number from list below (Frascati Manual).

11c	**In doing so, did your project involve actors whose role is mainly to organise the dialogue with citizens and organised civil society (e.g. professional mediator; communication company, science museums)?**	○ ○	Yes No

12. Did you engage with government / public bodies or policy makers (including international organisations)

- ○ No
- ○ Yes- in framing the research agenda
- ○ Yes - in implementing the research agenda
- ● Yes, in communicating /disseminating / using the results of the project

13a Will the project generate outputs (expertise or scientific advice) which could be used by policy makers?

- ● Yes – as a **primary** objective (please indicate areas below- multiple answers possible)
- ○ Yes – as a **secondary** objective (please indicate areas below - multiple answer possible)
- ○ No

13b If Yes, in which fields?

Agriculture		Energy		Human rights	
Audiovisual and Media		Enlargement		Information Society	
Budget		Enterprise		Institutional affairs	
Competition		Environment		Internal Market	
Consumers		External Relations		Justice, freedom and security	
Culture		External Trade		Public Health	
Customs		Fisheries and Maritime Affairs	●	Regional Policy	
Development Economic and Monetary Affairs		Food Safety		Research and Innovation	●
Education, Training, Youth		Foreign and Security Policy		Space	
Employment and Social Affairs		Fraud		Taxation	
		Humanitarian aid		Transport	

13c If Yes, at which level?

- Local / regional levels
- National level
- European level
- International level

H Use and dissemination

14. How many Articles were published/accepted for publication in peer-reviewed journals?	
To how many of these is open access[5] provided?	
How many of these are published in open access journals?	
How many of these are published in open repositories?	
To how many of these is open access not provided?	
Please check all applicable reasons for not providing open access:	
❑ publisher's licensing agreement would not permit publishing in a repository ❑ no suitable repository available ❑ no suitable open access journal available ❑ no funds available to publish in an open access journal ❑ lack of time and resources ❑ lack of information on open access ❑ other[6]: ……	

15. How many new patent applications ('priority filings') have been made? *("Technologically unique": multiple applications for the same invention in different jurisdictions should be counted as just one application of grant).*		
16. Indicate how many of the following Intellectual Property Rights were applied for (give number in each box).	Trademark	
	Registered design	
	Other	
17. How many spin-off companies were created / are planned as a direct result of the project?		**0**
Indicate the approximate number of additional jobs in these companies:		

18. Please indicate whether your project has a potential impact on employment, in comparison with the situation before your project:

■	Increase in employment, or	❑	In small & medium-sized enterprises
❑	Safeguard employment, or	❑	In large companies
❑	Decrease in employment,	❑	None of the above / not relevant to the project
■	Difficult to estimate / not possible to quantify		

[5] Open Access is defined as free of charge access for anyone via Internet.

[6] For instance: classification for security project.

19. For your project partnership please estimate the employment effect resulting directly from your participation in Full Time Equivalent (*FTE = one person working fulltime for a year*) jobs: Difficult to estimate / not possible to quantify	*Indicate figure:* *7* ❑

I Media and Communication to the general public

20. As part of the project, were any of the beneficiaries professionals in communication or media relations?

○ Yes ● No

21. As part of the project, have any beneficiaries received professional media / communication training / advice to improve communication with the general public?

○ Yes ● No

22 Which of the following have been used to communicate information about your project to the general public, or have resulted from your project?

❑	Press Release	■	Coverage in specialist press
❑	Media briefing	❑	Coverage in general (non-specialist) press
❑	TV coverage / report	■	Coverage in national press
❑	Radio coverage / report	■	Coverage in international press
■	Brochures /posters / flyers	■	Website for the general public / internet
❑	DVD /Film /Multimedia	■	Event targeting general public (festival, conference, exhibition, science café)

23 In which languages are the information products for the general public produced?

■	Language of the coordinator	■	English
❑	Other language(s)		

***Question F-10*:** Classification of Scientific Disciplines according to the Frascati Manual 2002 (Proposed Standard Practice for Surveys on Research and Experimental Development, OECD 2002):

FIELDS OF SCIENCE AND TECHNOLOGY

FIELDS OF SCIENCE AND TECHNOLOGY

1. NATURAL SCIENCES

1.1 Mathematics and computer sciences [mathematics and other allied fields: computer sciences and other allied subjects (software development only; hardware development should be classified in the engineering fields)]

1.2 Physical sciences (astronomy and space sciences, physics and other allied subjects)

1.3 Chemical sciences (chemistry, other allied subjects)

1.4 Earth and related environmental sciences (geology, geophysics, mineralogy, physical geography and other geosciences, meteorology and other atmospheric sciences including climatic research, oceanography, vulcanology, palaeoecology, other allied sciences)

1.5 Biological sciences (biology, botany, bacteriology, microbiology, zoology, entomology, genetics, biochemistry, biophysics, other allied sciences, excluding clinical and veterinary sciences)

2 ENGINEERING AND TECHNOLOGY

2.1 Civil engineering (architecture engineering, building science and engineering, construction engineering, municipal and structural engineering and other allied subjects)

2.2 Electrical engineering, electronics [electrical engineering, electronics, communication engineering and systems, computer engineering (hardware only) and other allied subjects]

2.3. Other engineering sciences (such as chemical, aeronautical and space, mechanical, metallurgical and materials engineering, and their specialised subdivisions; forest products; applied sciences such as geodesy, industrial chemistry, etc.; the science and technology of food production; specialised technologies of interdisciplinary fields, e.g. systems analysis, metallurgy, mining, textile technology and other applied subjects)

3. MEDICAL SCIENCES

3.1 Basic medicine (anatomy, cytology, physiology, genetics, pharmacy, pharmacology, toxicology, immunology and immunohaematology, clinical chemistry, clinical microbiology, pathology)

3.2 Clinical medicine (anaesthesiology, paediatrics, obstetrics and gynaecology, internal medicine, surgery, dentistry, neurology, psychiatry, radiology, therapeutics, otorhinolaryngology, ophthalmology)

3.3 Health sciences (public health services, social medicine, hygiene, nursing, epidemiology)

4. AGRICULTURAL SCIENCES

4.1 Agriculture, forestry, fisheries and allied sciences (agronomy, animal husbandry, fisheries, forestry, horticulture, other allied subjects)

4.2 Veterinary medicine

5. SOCIAL SCIENCES

5.1 Psychology

5.2 Economics

5.3 Educational sciences (education and training and other allied subjects)

5.4 Other social sciences [anthropology (social and cultural) and ethnology, demography, geography (human, economic and social), town and country planning, management, law, linguistics, political sciences, sociology, organisation and methods, miscellaneous social sciences and interdisciplinary , methodological and historical S1T activities relating to subjects in this group. Physical anthropology, physical geography and psychophysiology should normally be classified with the natural sciences].

6. HUMANITIES

6.1 History (history, prehistory and history, together with auxiliary historical disciplines such as archaeology, numismatics, palaeography, genealogy, etc.)

6.2 Languages and literature (ancient and modern)

6.3 Other humanities [philosophy (including the history of science and technology) arts, history of art, art criticism, painting, sculpture, musicology, dramatic art excluding artistic "research" of any kind, religion, theology, other fields and subjects pertaining to the humanities, methodological, historical and other S1T activities relating to the subjects in this group]

4. FINAL REPORT ON THE DISTRIBUTION OF THE EUROPEAN UNION FINANCIAL CONTRIBUTION

This report shall be submitted to the Commission within 30 days after receipt of the final payment of the European Union financial contribution.

Name of beneficiary	Final amount of EU contribution per beneficiary in Euros
Strathclyde University	
Ingenza	
Prokazyme	
Marine Biopolymers	
Pharmaq	
Axxam	
Horizon Discovery Ltd.	
Matis	
Lund University	
Wuerzburg University	
Hellenic Center for Marine Research	
Scottish Association for Marine Science	
Novamen	
VTT Technical Research Centre of Finland Ltd	
TOTAL	