symposium MEP 2012

Symposium on Marine Enzymes and Polysaccharides

Abstract book & Scientific Programme

Nha Trang, 10-17, December 2012

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This book composes the abstracts of the presentations for the plenary lectures, oral communications and and poster sessions of the 1th Symposium on Marine Enzyme and Polysaccharides, conducted at the Nha Trang , Vietnam 10-17, December 2012.

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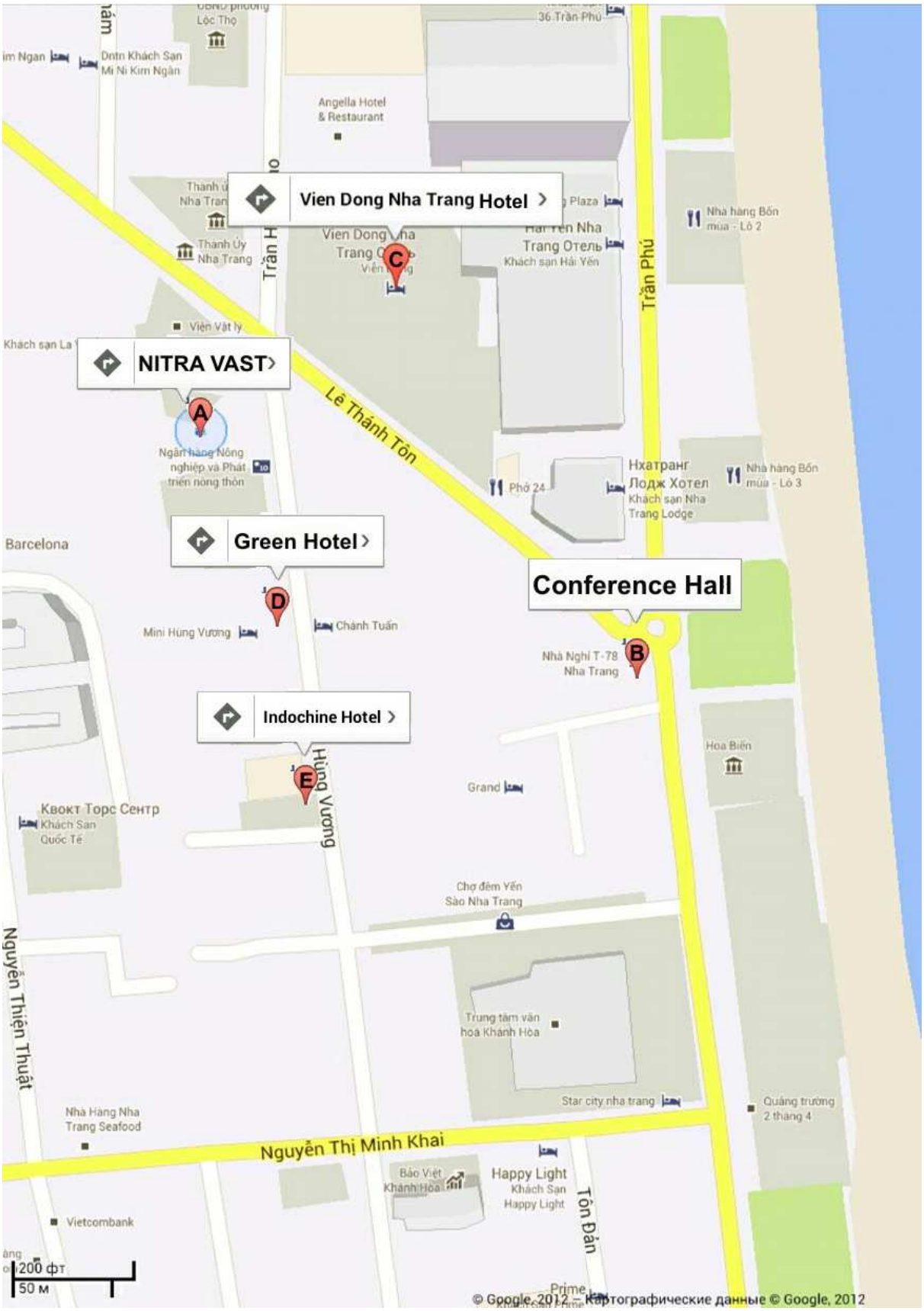
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Scientific Programme

MONDAY, DECEMBER 10, 2012

09:00-12:00 Registration

Location: Ground floor in the NITRA (02, Hung Vuong st.). **A** in the map of Nha Trang

14:00-14:40 Opening Ceremony

Location: Conference hall, NHA NGHI T78 (44, TRAN PHU). **B** in the map of Nha Trang

18:00-20:00 Welcome party

Location: Ground floor in the NITRA (02, Hung Vuong st.). **A** in the map of Nha Trang

TUESDAY, DECEMBER 11, 2012

09:00-11:00 Session 1

Location: Conference hall, NHA NGHI T78 (44, TRAN PHU) **B** in the map of Nha Trang

Structure and Biological Activity of Polysaccharides
Chair: Nikolay Nifantiev

9:00 **PL1** Tatyana Zvyagintseva
Bioactive brown algae polysaccharides: aspects of supplements and drugs creation

9:40 **PL2** Anatoly Usov
Structural diversity of brown algal fucoidans

10:20 **PL3** Svetlana Ermakova
Biological activities of fucoidans

11:00-11:20 Coffee break

11:20-13:00 Session 1 (continued)

Location: Conference hall, NHA NGHI T78 (44, TRAN PHU). **B** in the map of Nha Trang

Structure and Biological Activity of Polysaccharides
Chair: Anatoly Usov

11:20 **PL4** Nadezhda Ustyuzhanina
Seaweed fucoidans – a platform for new drug discovery

12:00 **OC1** Olesya Vishchuk
The fucoidans are biologically active polysaccharides induced apoptosis in cancer cells

12:20 **OC2** Anna Kravchenko
Sulfated polysaccharides and phycobiliproteins from red alga *Ahnfeltiopsis flabelliformis* (rhodophyta, phyllophoracea)

12:40 **OC3** Maxim Kokoulin
Structural study of the O-specific polysaccharide from a marine bacterium *Cobetia pacifica* KMM 3879^{t}

12:40-16:00 Lunch time

WEDNESDAY, DECEMBER 12, 2012

9:00-11:00 Session 2

Location: Conference hall, NHA NGHI T78 (44, TRAN PHU). **B** in the map of Nha Trang

Biodegradation of Polysaccharides
Chair: William Helbert

9:00 **PL5** Antonio Trincone
Biocatalytic processes using marine biocatalysts: cases in point
9:40 **OC7** Olga Senko
Immobilized biocatalyst for conversion of polysaccharide-containing renewable sources for industry
10:00 **OC5** Ilya Lyagin
Conversion of renewable resources into products useable for chemical and fuel industries
10:20 **OC6** Barindra Sana
Biofuels from marine microalgae and seaweed polysaccharides: prospects & challenges
10:40 **OC15** Van-Tinh Nguyen Apoptosis induction of gliotoxin isolated from marine fungus *Aspergillus* sp. In human cervical cancer cell and human chondrosarcoma cell *in vitro*

11:00-11:20 Coffee break

11:20-13:00 Session 3

Location: Conference hall, NHA NGHI T78 (44, TRAN PHU). **B** in the map of Nha Trang

Marine Enzyme Structure, Functionality Screening and Regulation
Chair: Antonio Trincone

11:20 **PL8** Trond Ø. Jørgensen
Bioprospecting in the arctic; screening for bioactive compounds in cold adapted organisms
11:40 **PL7** Nils Peder Willassen
Bioprospecting – hunting for novel marine enzymes
12:00 **PL6** William Helbert
CRAZY Polysaccharides: sCReening of Active enZYmes on a collection of polysaccharides having known and unknown structure
12:40 **OC13** Alexander Zakharenko 1,3-β-D-glucanases of bivalves of the South China sea

13:00-16:00 Lunch time

THURSDAY, DECEMBER 13, 2012

9:00-12:20 Session 3 (continued)

Location: Conference hall, NHA NGHI T78 (44, TRAN PHU). **B** in the map of Nha Trang

Marine Enzyme Structure, Functionality Screening and Regulation
Chair: Nils Peder Willassen

9:00 **OC9** Shin-ichiro Suye
Purification and properties of alginate lyase from *Stenotrophomonas maltophilia* No.43

9:20 **OC10** Irina Bakunina
Marine bacteria α-N-acetylgalactosaminidase and α-galactosidase, modifying structure of cell antigens

10:00 **OC11** Maria Pesentseva
Features of passing transglycosylation and hydrolysis reactions of polysaccharides catalyzed by endo-1,3-β-D-glucanases from marine mollusks of Sea of Japan and South China Sea

10:20 **OC12** Vasily Golotin
Optimization of a highly active recombinant alkaline phosphatase *Cm*AP expression and purification

10:40-11:00 Coffee break

11:00-12.00 Session 3 (continued)

Location: Conference hall, NHA NGHI T78 (44, TRAN PHU). **B** in the map of Nha Trang

Marine Enzyme Structure, Functionality Screening and Regulation

Chair Trond Ø. Jørgensen

11:00 **OC8** Alexander Dreshchinskii
Distribution of transparent exopolymer particles (tep) in the world ocean

11:20 **OC17** Alexandra Seytkalieva Salt resistant alkaline phosphatase from the eggs of sea urchin *Strongylocentrotus intermedius*

11:40 **OC4** Vo Thanh Trung
Research to produce ethanol from seaweed biomass *Cladophora* sp.

12:00 **OC16** Evgeny Pislyagin Pharmacokinetic properties of cucumarioside A_2-2 determined by MALDI- TOF-MS and MALDI-IMS

12:00-16:00 Lunch time

16:00-17:30 Round table "Problems of the structure elucidation and biological activity investigation of the seaweeds polysaccharides"
Location: Library of NITRA. **A** in the map of Nha Trang

FRIDAY, DECEMBER 14, 2012

Excursion on Monkey Island (free for participants and accompanying person)

SATURDAY, DECEMBER 15, 2012

10:00-12:00 Poster Session
Location: Ground floor in the NITRA (02, Hung Vuong st.). **A** in the map of Nha Trang

16:00-18:00 Round table "Structure and specificity of enzymes"
Location: Library of NITRA. **A** in the map of Nha Trang

SUNDAY, DECEMBER 16, 2012

09:00-17:00 Excursion on Vinperland (30 USD)
18:00-21:30 Farewell Party
Location: Ground floor in the NITRA (02, Hung Vuong st.). **A** in the map of Nha Trang

MONDAY, DECEMBER 17, 2012

09:00-10:00 Closing ceremony of Symposium
Location: Conference hall, NHA NGHI T78 (44, TRAN PHU) **B** in the map of Nha Trang

PLENARY LECTURES

PL1

BIOACTIVE BROWN ALGAE POLYSACCHARIDES: ASPECTS OF SUPPLEMENTS AND DRUGS DEVELOPMENT

Tatyana Zvyagintseva, Svetlana Ermakova, Mikhail Kusaykin, Natalia Shevchenko, Tatyana Imbs

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Brown seaweeds synthesize unique bioactive polysaccharides: laminarans, alginic acids and fucoidans. A wide range of biological activities has been attributed to fucoidans. It was shown now that there are a great variety of structures of fucoidans. However, their role with respect to structure-activity relationship is still under debate.

Points of our peculiar interest are anticoagulation, immunomodulatory and antitumor activities of fucoidans, relation structure-activity and possibilities of standardization of structure and biological activity of brown algae polysaccharides for creating of supplements and medicine on their base.

In recent years we focused on development of methods of processing seaweeds, establishment of structure of polysaccharides, enzymatic and chemical modifications of native algae polysaccharides. To standard preparations we studied influence of life cycle stage and environmental factors of brown seaweeds on content and structural characteristics of polysaccharides and revealed that seaweed reproductive status is determinant.

As results we have the rich collections of algae polysaccharides to investigate relationship of algae polysaccharides structure-bioactivity in cell cultures and animal models. For example we showed that the antithrombin activity of fucoidan fractions depends on the monosaccharide relation Man/Fuc and Gal/Fuc. Fucoidans with smaller quantity of xylose have more anticoagulant activity and are the most perspective for creation on their basis of new anticoagulants - medical products. Also we demonstrated importance of sulfate/acetyl groups for the immunostimulatory activity of fucoidans: partial removal of sulfate and/or acetate groups from native fucoidan decreases the activity.

The investigations are necessary for functional food, supplements and medicine development.

PL2

STRUCTURAL DIVERSITY OF BROWN ALGAL FUCOIDANS

Anatoly Usov

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Sulfated polysaccharides of brown algae (fucoidans) are known as biopolymers having several promising biological activities (anticoagulant, antitumor, antiviral, antiinflammation, etc.). Depending on the algal species, some fucoidans may have linear backbones built up of α-L-fucopyranose residues with 1→3 or alternating 1→3,1→4 linkages. These backbones are usually substituted by numerous sulfate, acetate and various monosaccharide residues, giving rise to extremely complex heterogeneous mixtures of molecules. In addition, crude fucoidan preparations may contain polysaccharide components of absolutely different structures, as it was shown for sulfated polysaccharide extracts obtained from *Kjellmaniella crassifolia* and *Saccharina latissima*. The algae belonging to the family Sargassaceae seem to be the sources of especially complex polysaccharides, a good examples are fucoidans from *Sargassum stenophyllum* and *Hizikia fusiforme*. Chemical methods of structural analysis, as well as NMR spectroscopy of native fucoidans give only partial information on their structures. As the result, the relationships between structure and biological action of fucoidans are not clearly established.

Application of high-performance anion-exchange chromatography makes it possible to isolate polysaccharide fractions suitable for investigation of chemical structure. Several specific procedures of structural analysis, such as mild desulfation methods and partial degradation by bacterial enzymes giving rise to sulfated oligosaccharide fragments, are needed for successful elucidation of fucoidan structures. Several very interesting and unusual polysaccharides were described recently in the species belonging to the genera *Fucus, Saccharina, Undaria, Chordaria, Analipus,* etc. According to the present knowledge, every species of brown algae seems to have its own unique composition of a mixture of sulfated polysaccharides.

PL3

BIOLOGICAL ACTIVITIES OF FUCOIDANS

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Various species of marine algae occur in the ocean, they are classified into three divisions: Rhodophyta (red algae), Phaeophyta (brown algae), and Chlorophyta (green algae). Fucoidans are sulfated polysaccharides found widely in the cell walls of brown seaweeds. They were identified in the first half of the last century by Kylin. In recent years, different brown algae were analyzed for content of sulfated polysaccharides. Fucoidans are highly heterogeneous in both monosaccharide composition and molecular structure. They all comprise a family of heteromolecules based on L-fucose, D-galactose, D-xylose, D-glucuronic acid, and D-mannose. The amount of fucoidans in a brown alga varies by species of seaweed, place of location, developing stage and harvesting season. Published research on fucoidans increased three fold between 2000 and 2010.

They have been extensively investigated for their anticoagulant and antitrombotic activity. The subjects of investigations are antivirus, antitumor and immunomodulatory activities. Lots of studies show that fucoidan presents significant antioxidant activity in experiments *in vitro*. It can also remarkably reduce blood lipids.

Anticomplementary and anti-inflammatory activities are also attractive for studying. The intensity of biological activities of fucoidan varies with species, molecular weight, composition, structure and the route of administration.

This presentation discusses the role of fucoidans in these bioactivities and includes several aspects.

PL4

SEAWEED FUCOIDANS – A PLATFORM FOR NEW DRUGS DISCOVERY

N.E. Nifantiev,[1] N.E. Ustyuzhanina,[1] N.A. Ushakova,[2] M.E. Preobrazhenskaya,[2] M.I. Bilan,[3] A.I. Usov,[3] D.O. Croci,[4] A. Cumashi,[5] A. Piccoli,[6] L. Totani,[6] A.A. Grachev,[1] G.E. Morozevich,[2] A.E. Berman,[2] C.J. Sanderson,[7] M. Kelly,[7] P. Di Gregorio,[8] C. Rossi,[5] N. Tinari,[5] S. Iacobelli,[5] G.A. Rabinovich,[4] A.S. Shashkov,[9] A.G. Gerbst[1]

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[8] S.S. Annunziata Hospital, University G. D'Annunzio, School of Medicine, 66100 Chieti, Italy

Natural polysaccharides fucoidans were found in many species of brown seaweeds and in some echinoderms. Fucoidans represent an intriguing class of fucose-enriched sulfated polysaccharides found in the extracellular matrix of brown algae. These polysaccharides have been tested in a vast array of experimental models showing anti-coagulant, anti-tumor, immunomodulatory, anti-inflammatory, and anti-complement properties. Detailed chemical structures of fucoidans depend primarily on the algal species used as source of polysaccharides. However, even a sulfated polysaccharide isolated from a given species of brown algae may be a mixture of structurally different polymers. Thus, in spite of increasing efforts, the structure-activity relationship of fucoidans is still an unresolved issue.

Fucoidans often consist from selectively O-sulfated α-L-fucopyranose residues linked through (1→2)-, (1→3)- and (1→4)-linkages with the presence of branched points that makes difficult their detail structural characterization. At the same time these biopolymers exhibit very interesting profile of biological activity. Recently we have investigated the anti-inflammatory, anti-coagulant, anti-angiogenic, and anti-adhesive activities of nine different fucoidans isolated from *Laminaria saccharina* (renamed as *Saccharina latissima*) *L. digitata, Cladosiphon okamuranus, Fucus evanescens, F. vesiculosus, F. serratus, F. distichus, F. spiralis,* and *Ascophyllum nodosum* as pool samples. We found that the different profiles of biological activities exhibited by these polysaccharides depend on variations of their structural features. Interestingly, among the most active compounds studied, those extracted from *L. saccharina* have been characterized by their prominent anti-angiogenic and anti-coagulant activities *in vitro* as well as their ability to block selectin-mediated inflammation *in vivo*.

Particularly, they may effectively inhibit microbial and viral adhesion to host cells, angiogenesis development, P- and L-selectin mediated inflammation, blood coagulation and some other biological processes. It should be noted that the structure of fucoidans differ absolutely from human ligands involved in the development of above listed diseases and processes, thus the pharmacophore fucoidan fragments are the efficient mimetics of these human ligands and thus can be considered as potential drugs.

To assess structures of pharmacophore fucoidan fragments we conduct systematic synthesis of corresponding short and long chain linear and branched selectively and per-O-sulfated oligofucosides and study their NMR and conformational characteristics and biological properties. Obtained results will be summarized in this lecture.

PL5

BIOCATALYTIC PROCESSES USING MARINE BIOCATALYSTS: CASES IN POINT

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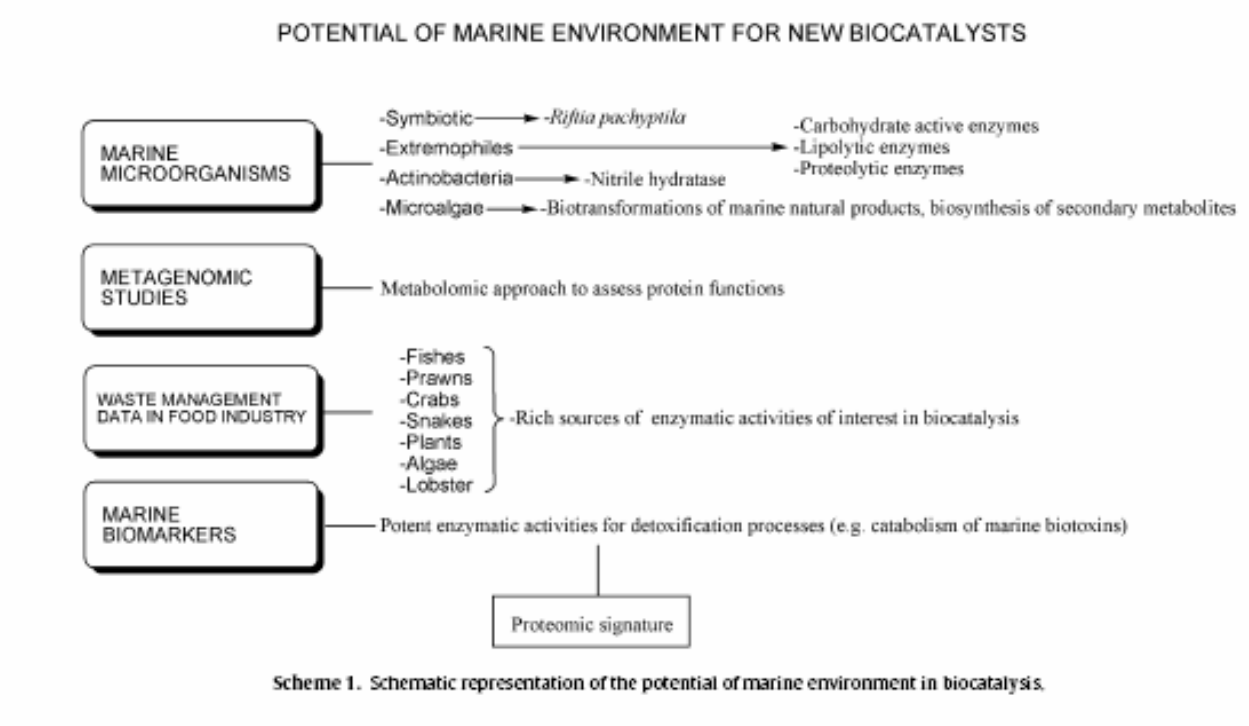
The adaptation of marine organisms to a wide range of environmental conditions in the specific environment (temperature, salinity, tides, pressure, radiation, light, etc.) has made them an enormous reservoir of interesting biological material for both basic research and biotechnological improvements. This evolutionary richness and the knowledge of enzyme action including the comprehension of interactive effects of the environmental factors are of key importance to exploit marine biocatalyst's potential.



Scheme 1. Schematic representation of the potential of marine environment in biocatalysis.

On the top of that a marine enzyme may carry novel chemical and stereochemical properties, thus biocatalytically oriented studies (testing of suitable substrates, appropriate checking of reaction conditions, study of stereochemical asset of catalysis) should be performed to appropriately reveal this "chemical biodiversity" which increases interest for these enzymes.

In this presentation, cases in point will be depicted for a comparison of enzymes from terrestrial and marine environments. Each case was selected for its importance related to biocatalysis, showing practical details to value the concept of potential usefulness of marine enzymes for organic chemists.

From this analysis a foresight regarding the strategic potential of marine habitat is clear. Sustainability of collection methods and availability of commercial fresh organisms are two important aspects, also in relation to international policy on biodiversity. As early as scientific interest arise possibly the way to access useful biocatalysts should avoid destructive large-scale collections of marine biomass, then recombinant biocatalysts could be desirable and their preparation should be possible after gene identification.

PL6

CRAZY Polysaccharides: sCReening of Active enZYmes on a collection of polysaccharides having known and unknown structure

William Helbert

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Polysaccharides are the most abundant and the most diverse renewable polymers found on land and in oceans. Except polysaccharides used traditionally in food and non-food industries, the structure and the functionality of most of them are unknown and unexplored. Structural analyses of complex polysaccharides benefit strongly from the use of polysaccharides depolymerising enzymes: glycoside hydrolases (GH) and polysaccharide lyases (PL).

Aiming at crossing the chemical diversity of terrestrial and marine polysaccharides with the biological diversity of bacteria as source of new GH and PL, we have designed and implemented a medium throughput screening strategy polysaccharides having known and unknown structure. The detection of active enzymes is achieved by colorimetric method and by mass spectrometry using protocol allowing detection of anionic and neutral oligosaccharides [1, 2]. In parallel, a collection of more than 150 polysaccharides substrates was collected. This includes polysaccharides extracted from land plants, marine macroalgae (red, brown and green) and, land and marine bacteria.

The screening was successfully applied to complex bacterial extracts allowing highlighting different profiles of GH or PL production according to culture conditions (i.e. induction by polysaccharides). As examples, the comparison of predicted enzyme activities from the sequenced genome of *Pseudalteromonas atlantica* with the experimentally detected activities or the screening of the microbiome of *Aplysia sp.* allows us evidencing new polysaccharides degrading enzymes [3].

The screening program involved a consortium of French laboratories interested in understanding polysaccharides/protein interaction, resolving the structure of complex polysaccharides as well as ascribing function of putative GH and PL (INRA: M. Lahaye, D. Ropartz; IFREMER: C. Boisset, CNRS: R. Daniel; CEVA: J.-F. Sassi, CNRS: B. Henrissat, G. Michel)

1. M. Fer, A. Préchoux, J.-F. Sassi, M. Lahaye, C. Boisset, P. Nyvall-Collén, W. Helbert. *J. Microbiol. Meth.* 2012, in press

2. D. Ropartz, P.-E. Bodet, C. Przybylski, M. Fer, F. Gonnet, R. Daniel, D. Bertrand, W. Helbert, H. *Rapid Communications in Mass Spectrometry* 2011, 25, 2059-2070

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PL7

BIOPROSPECTING – HUNTING FOR NOVEL MARINE ENZYMES

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Microorganisms have evolved and accumulated remarkable physiological and functional heterogeneity and are believed to constitute the major reserve for genetic diversity on earth. Current methods of cultivation reveal less than 1% of the microbial diversity. This hidden genetic diversity contains a vast array of novel biocatalysts (enzymes), metabolic pathways and secondary metabolites, which could potentially be useful to many biotechnology applications, and lead to e.g. development of new antimicrobial and anticancer drugs. Using metagenomics, namely to recover genetic material directly from environmental samples, this biogenetic diversification can be accessed in a cultivation independent approach. However, despite the contributions from metagenomics technologies consequent upon the discovery of novel enzymes, the new field requires major improvements. In this presentation, one will compare function-based screening, functional metagenome screening and sequence-based metagenome data mining, discussing the advantages and limitations of the methods, and how these methods can be combined with other omics technologies to increase the output.

PL8

BIOPROSPECTING IN THE ARCTIC; SCREENING FOR BIOACTIVE COMPOUNDS IN COLD ADAPTED ORGANISMS

Trond Ø. Jørgensen

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As a marine environment, the high Arctic is unparalleled with respect to combination of temperature and light regimes. This implies an evolution of a variety of organisms with unique physiological and biochemical adaptations and with a correspondingly good prospect for finding novel bioactives and lead compounds. The MabCent integrates various disciplines and partnerships of academics at the University of Tromsø (UiT) and experts at SMEs, acting in a consortium. Thus the center covers the pipeline from biology of marine resources/species through screening and characterization / optimization of bioactive molecules to commercialization of drugs, biotech and nutraceutical products.

The resources to be focused are marine bacteria harvested on the surface of the ice pack or deep water sediments, various marine biota and marine microalgae sampled under different blooming conditions, in addition to a huge variety of benthic invertebrates found in the Arctic seas. The national marine biobank, Marbank, is organizing the sampling and produce extracts to the “high-through-put” screening platform, Marbio.

Compounds and molecules active against bacteria, tumour cells and inflammation as well as immuno stimulants, anti-oxidants and various enzymes and inhibitors are further isolated and characterized by their structure and eventually optimized (homologs) when synthesized. All the technological platforms involved are state-of-the-art equipped for the tasks and targets running in the MabCent operation.

The four commercial MabCent partners are acting in an R&D synergy although at different levels and areas (pharmaceuticals, nutraceuticals, research tools etc.). Through their interaction with the interdisciplinary expertise at UiT and academic partners, the MabCent initiative intend to nurse research and innovation and setting standards for future marinebased discovery and development within the field of marine bioprospecting biotechnology in Norway.

ORAL COMMUNICATIONS

OC1

THE FUCOIDANS ARE BIOLOGICALLY ACTIVE POLYSACCHARIDES INDUCED APOPTOSIS IN CANCER CELLS

Olesya Vishchuk

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Cancer is known to be the leading cause of death in economically developed countries and the second leading cause of death in developing countries [1]. Chemotherapy is one of the most frequently used therapeutic modalities for the treatment of cancer, but it does not achieve a satisfactory result, because of high toxicity and side effects of drugs. Novel approach for cancer therapy is the induction of apoptosis (programmed cell death), which has been assumed to be critical for cancer prevention.

Among marine organisms, brown algae are rich sources of structurally diverse polysaccharides with various biological activities. There is accumulating evidence to support the proposal that the use of fucoidans as a supplement provides protection against various cancers [2]. In vivo studies performed using mouse xenograft models have demonstrated that fucoidans suppresses tumor growth of A20-derived lymphoma [3], inhibits metastasis of Lewis lung adenocarcinoma, and has anti-angiogenesis activity against Lewis lung adenocarcinoma and B16 melanoma [4, 5]. In vitro, several mechanisms have been postulated to underlie the anticancer activity of fucoidan, including induction of apoptosis in cells derived from human lymphoma, colon carcinoma, breast carcinoma, and hepatoma and prevention of angiogenesis and invasion in fibrosarcoma cells [6, 7]. However, the molecular mechanisms involved in the anticancer action of fucoidan are complex, and the targets and molecular mechanisms by which it initiates death of cancer cells are incompletely understood.

In the present study we investigated the anti-tumor activity, including induction of apoptosis by the fucoidans from brown algae of the Sea of Japan, the Sea of Okhotsk and the South China Sea, elucidated molecular mechanism of fucoidans action and determined their structural-activity relationship.

The work was supported RFBR grant № 12-04-00669.

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OC2

SULFATED POLYSACCHARIDES AND PHYCOBILIPROTEINS FROM RED ALGA *AHNFELTIOPSIS FLABELLIFORMIS* (RHODOPHYTA, PHYLLOPHORACEA)

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Red algae are a source of many biologically active substances, which are unique in their structure and physico-chemical properties. There are phycobiliproteins (PBPs) (phycoerythrin (PE), phycocyanin (PC), allophycocyanin (APC)), which possess bright color and intensive fluorescence through which they are used in fluorescence immunoassay and in cosmetic and food industries as natural dyes. The main components of red seaweeds - sulfated polysaccharides (agar and carrageenan) possess the properties to form viscous solutions and strong gels, which determine their wide practical application. Red alga *A. flabelliformis* forming extensive population in Russian Far Eastern Seas can be a new potential source of sulfated polysaccharides and PBPs therefore it's investigation is actual. The influence of water temperature, salinity and photosynthetic active radiation (PAR) on seasonal changes of polysaccharide and pigment compositions from this alga was investigated. Seaweeds presented by carposporophyte were collected in February-September and November-December 2009 in Amursky Bay (Sea of Japan) at 2 m depth. In addition, comprehensive analysis of PBPs and polysaccharides from *A. flabelliformis* collected in July 2012 in Troitsa Bay, Cape of Andreeva (cystocarps) and in Risovaya Bay (sterile form) was carried out.

According to the results of analysis, the highest PE and PC yields were observed in June and APC – in May while the lowest PBPs amounts were recorded in the second half of the April and in July-September, that is likely to concern with increased irradiance in that period. Amount of isolated polysaccharides varied from 5,9 to 26,7 % during the whole year. Maximal polysaccharide yields were found in February and November-December under decreased water temperature and PAR and minimal – in September. A significant negative correlation between PAR and PBPs and polysaccharide contents was established. In addition, a significant negative correlation between the polysaccharide content and water temperature was found. The major monosaccharides of polysaccharides were galactose and 3,6-anhydrogalactose. Galactose content was rather high during the research period while the highest 3,6-anhydrogalactose amount was registered in June. No environmental factors influenced on the contents of these monosaccharides were observed. The highest amounts of sulfate groups in polysaccharide were observed in April-May and July-August at a high PAR level.

PBPs from *A. flabelliformis* with cystocarps were extracted with 0,1 M phosphate buffer (method 1) and 1,5 % sodium nitrate (method 2), but those from sterile alga – 1,5 % sodium nitrate. After isolation of pigments polysaccharides were sequentially extracted with hot water three times. According to the results of analysis, all PBPs contents from algal samples collected in July 2012 were significantly lower than that harvested in July 2009 irrespective of algal life cycle. Probably, observed discrepancies were connected with algal growth conditions. It should be noted that PBPs extraction with sodium nitrate doubled PE yield as compared with phosphate buffer irrespective of life cycle. However, contents of PE and PC isolated from alga with cystocarps by method 2 were higher than those from sterile seaweeds. Polysaccharide yields after pigments isolation were higher in 2 times than that without previous PBPs extraction. This phenomenon can be connected with supplementary cell wall destruction during pretreatment with buffer solution. Polysaccharides content from *A. flabelliformis* harvested in 2009 without previous PBPs extraction was lower in 2 times than in 2012.

OC3

STRUCTURAL STUDY OF THE O-SPECIFIC POLYSACCHARIDE FROM A MARINE BACTERIUM *COBETIA PACIFICA* KMM 3879^T

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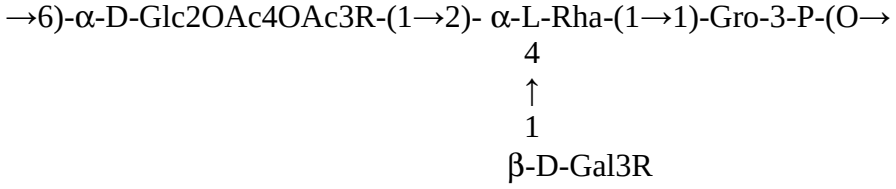
Strain KMM 3879^T was isolated from a sandy sadiment sample collected from the Sea of Japan seashore, Russia, and classified as «*Cobetia pacifica*» sp. nov. [1].

The O-polysaccharide of *C. pacifica* (OPS) was obtained by mild acid degradation of the lipopolysaccharide (LPS), isolated from wet bacterial cells by the phenol-water procedure. The OPS was studied by sugar analysis and NMR spectroscopy. GLC and GLC-mass spectrometry analysis of the acetylated alditols after hydrolysis and acetylated methyl glycosides after methanolysis of the OPS revealed resiudes of rhamnose (Rha), glucose (Glc), galactose (Gal) and glycerol (Gro). ^{1}H and ^{13}C-NMR spectra showed, *inter alia*, signals for three anomeric carbons and signals of methyl groups of 6-deoxy sugar and O-acetyl groups. ^{31}P spectra showed signal charactaristic for phosphodiester linkage. Rhamnose and glucose were found to be α-linked, galactose was found to be β-linked. ^{1}H-NMR spectrum was interpreted using 2D homonuclear COSY and TOCSY experiments. With the ^{1}H-NMR spectrum assigned, the ^{13}C-NMR spectrum was interpreted using 2D heteronuclear ^{1}H, ^{13}C HSQC experiment. Linkage and sequence analysis of the polysaccharide was performed using a 2D ^{1}H , ^{1}H NOESY,^{1}H, ^{13}C HMBC and ^{1}H, ^{31}P HMBC experiment. The presence of sulfate groups was determined by turbidimetric method and confirmed by method of IR spectroscopy. The sites of attachment of the sulfate groups, O-acetyl groups and the absolute configurations of monosaccharides were established on the basis of the values of chemical shifts and α- and β-effects of the ^{13}C glycosylation. Thus, based on all our data obtained, we suggest the following structure for repeating unit of the O-specific polysaccharide:



Where R is -SO_3H

An anomeric phosphodiester linkage is found in many glycopolymers of the outer memrane in gram-negative bacteria (LPS and capsular polysaccharides) [2]. The OPS from *C. pacifica* is distinguished by the presence of sulfate groups, which have been previously found in nature only in a few bacterial glycopolymers. In addition, the simultaneous presence of sulfate and a phosphate groups was found in the first time.

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OC4

RESEARCH TO PRODUCE ETHANOL FROM SEAWEED BIOMASS *Cladophora* sp.

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Biomass seaweed *Cladophora sp.* (BSC) included *Cladphora socialis, C. flexuosa, C. crupila* which was hydrolyzed in dilute sulfuric acid and enzymatic hydrolysis of cellulase. BSC was treated by dilute sulfuric acid at concentration of 0; 0.5%; 1%; 2%; 3%; 4%; 5%; 6%(v/v) (v: volume) in autoclave respectively at 121^0C at time 20, 40 and 60 minute, with the rate between seaweed and solution acid of 1/10(w/v) (w: weight). On order hand, BSC was pretreated in dilute sulfuric acid at 0,5%(v/v) at 121^0C at time 20, after it was treat at concentrations 0; 0.2; 0.4; 0.6; 0.8; 1 ml/g with the enzymatic hydrolysis consisted of cellulase in control (50°C, pH 5.0, 24h).

The results showed that, total carbohydrate contents of two methods is the same, but Carbohydrate content was varied by treatment time of heat. Rates of acid 4%; 5%, 6% (v/v) and 0,8;1 ml/g have high Carbohydrate content within the range of 42.38 – 48.51mg/ml. 400ml of hydrolysis solution was fermented by *Saccharomyces cerevisiae* at 30°C for 72 h.

After fermentation, 5% ethanol solution was identified for the first 100 ml evaporate solution. BSC is easily hydrolyzed and fermented ethanol, it shows excellent prospects as a potential feedstock for the production of bioethanol.

OC5

CONVERSION OF RENEWABLE RESOURCES INTO PRODUCTS USEABLE FOR CHEMICAL AND FUEL INDUSTRIES

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Conversion of renewable sources (parts of marine phototrophic microorganism's biomass) into various products for chemical and fuel industries (organic acids, solvents, hydrogen, methane) under action of bacterial, yeast and filamentous fungus cells was demonstrated in with work.

The effectiveness of different pretreatment methods of phototrophic microorganism's biomass (thermolysis, acid hydrolysis, hydrolysis by enzymatic complexes secreted by filamentous fungi) for conversion of raw materials into target products was revealed.

The use of immobilized cells of producers (bacteria, yeast and filamentous fungi) as compared to the free cells of the same microorganisms appeared to be preferred in the investigated processes owing to increased tolerance of immobilized cells to various negative factors accompanying cell cultivation with various sources of raw materials as substrates. The possibility of the organization of semi-continuous processes using immobilized biocatalysts was established. A comparative analysis of the characteristics process producing same target products from a variety of renewable raw materials with the same immobilized biocatalysts was realised.

OC6

BIOFUELS FROM MARINE MICROALGAE AND SEAWEED POLYSACCHARIDES: PROSPECTS & CHALLENGES

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High carbon foot-print of fossil fuels and their diminishing reserve are major concern in recent years. Several renewable resources are being studied as feasible alternatives and biofuel shows vast potential. Plant derived feed-stocks are most widely used for current biofuel production. However, plant derived raw materials may not be sustainable because energy crops compete with food crops for land and other resources and, deforestation for profitable energy crops production has negative environmental impacts [1]. Algal oils could be used for biodiesel production whereas seaweed polysaccharides are potential feedstock for bioethanol fermentation. Biofuels from marine microalgae and seaweed biomass may substitute plant-derived biofuels but several challenges need to be overcome before their large scale production at competitive price.

Large amount of phycocolloids such as alginate, agar and carrageenan are produced by seaweeds [2]. They are high molecular weight polysaccharides mainly composed of simple sugars, which are potentially fermentable. Ethanol production from seaweed biomass is a multistep procedure that includes polysaccharide isolation from seaweed biomass, hydrolysis of polysaccharide to simple fermentable sugar and ethanol production by microbial fermentation. Isolation of several polysaccharides and their hydrolysis are streamlined from different seaweeds but these processes are energy consuming, need harsh chemical treatments and the production cost is higher than conventional biofuels [3]. Seaweed biomass contains high concentration of salts that needs to be removed for development of enzymatic hydrolysis and easy fermentation process. Long-term sustainability of large-scale seaweed biofuel production may is questionable due to potential depletion of marine ecosystem. All these problems need to be carefully addressed. In addition, improved polysaccharide production by genetic modification and screening of efficient strain may increase gross productivity of seaweed biofuel.

Algal cells accumulate oil that can be potentially used for biodiesel production [4]. The idea of biofuel production from photosynthetic microorganism is relatively new but attracts significant attention due to less land requirements. Many marine algae were reported to grow at relatively low light intensity, in presence of slats or organic solvents that may be an added advantage. Some natural marine algae were studied for biofuel production but productivity is the major concern due to their slow growth rate and low oil/biomass ratio [5]. Large scale production is also complicated for their light-dependent growth, which need large surface area for harvesting the sunlight. Competitiveness of algal biofuel is yet questionable but there is room for development. Productivity of algal biofuel could be increased by enhancing microbial growth rate, by diverting nutrients towards oil production or by developing more efficient industrial processes. Metabolic pathway can be engineered for extracellular secretion of oil in culture media, it can bypass complicated oil extraction technique. Algal biofuels may emerge as fuel of future only after enhancement of productivity and development of easy industrial processes.

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OC7

IMMOBILIZED BIOCATALYST FOR CONVERSION OF POLYSACCHARIDE-CONTAINING RENEWABLE SOURCES FOR INDUSTRY

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Immobilization is a powerful factor stabilizing microbial cells and increasing the efficiency of their functional activity in various biotechnological processes. Development of long functioning immobilized biocatalysts based on cells of various microorganisms (bacteria, yeasts, filamentous fungi) made it possible to increase the overall productivity of the process of conversion of renewable raw materials, together with technological simplification of production processes of important metabolites.

Several approaches to production of immobilized forms of producers of various commercially important products useful for industry were developed. At the same time different sources of renewable raw materials (polysaccharides of phototrophic microorganism's biomass) and plant biomass were used as the main substrates for cultivation of cells of various microorganisms. A comparative analysis of the characteristics of free and immobilized cells was carried out.

It was shown that developed immobilized biocatalysts based on cells of different microorganisms could be used for conversion of biomass components of marine phototrophic microorganisms with high efficiency to a wide spectrum of target products (organic solvents, organic acids, hydrogen, methane), and also can be applied to obtain of hydrolytic enzymes in media with various polysaccharides.

OC8

DISTRIBUTION OF TRANSPARENT EXOPOLYMER PARTICLES (TEP) IN THE WORLD OCEAN

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Since the development of methods to quantify transparent exopolymer particles (TEP) in early 90th, it has been shown that TEP represent a pool of gel-like particles, abundant and ubiquitous, that play a significant role in the biogeochemical cycling of organic carbon in the ocean. It was revealed that TEP production generally couples to phytoplankton, although a number of variations in particular regions of the ocean were detected as well. Based on the local information on TEP abundance, herein we essay to introduce the distribution of TEP in the entire World Ocean. Consequently, all available data derived from literature sources were collected and generalized to uniform appearance. The calculations were made with the help of the existing empirical relationships developed in the present study. Applying the designed database, a map of the TEP distribution in the World Ocean was created. The data analysis conducted in this study revealed low and very similar concentrations of TEP in various oligotrophic parts of the ocean as well as in certain coastal regions during winter periods. Furthermore, the data have shown an only weak correlation between concentrations of TEP and chlorophyll *a* suggesting more complex temporal and spatial relationships between gel particles and ecosystem dynamics.

OC9

PURIFICATION AND PROPERTIES OF ALGINATE LYASE FROM STENOTROPHOMONAS MALTOPHILIA No.43

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Marine biomass, such as seaweeds is potential resource for bioenergy production. We screened sodium alginate degrading microorganism from seaweeds and mud in Japan coastal area (e.g., Fukui, Chiba, and Okinawa) for utilization of marine biomass. At first, microorganisms which grew on the medium containing 2.0% salt were obtained from sample. The obtained culture were used an the source for isolation of alginate utilizing microorganism. The medium contained (%, w/v): Sodium alginate, 0.1; NaCl, 2.0; Yeast extract, 0.2; MgSO4･7H2O, 0.05; and agar, 2.0 (pH 6.6-6.8). Several strain showed clear halo in the medium containing sodium alginate as sole carbon source with Congo Red staining. Then, the alginate lyase activity of culture broth was assayed by measuring optical density at 235 nm which indicates double bonds of the sugar units in the substrate. Six strain showed alginate lyase activity in extracellular. Strain No. 28 produced the most enzyme. Strain No. 28 was taxonomically identified with *Stenotrophomonas maltophilia* on a phylogenetic analysis of 16S rDNA-based 500-bp gene sequence. The optimal cultivation conditions for alginate lyase production obtained with yeast extract as nitrogen source at 30°C. The alginate lyase activity was four times compared with standard cultivation conditions. Under these conditions, the maximum alginate lyase activity showed 47.5 h cultivation.

Purification and characterization of alginate lyase from *S. maltophilia* were also investigated. The enzyme was purified about 321-fold with a yield of 9.0% from the culture broth of *S. maltophilia*. The enzyme preparation was homogeneous on slab gel

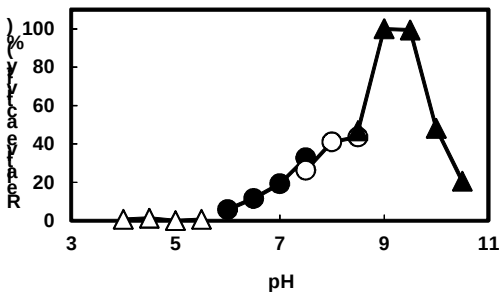

Fig.1 pH Activity of alginate lyase

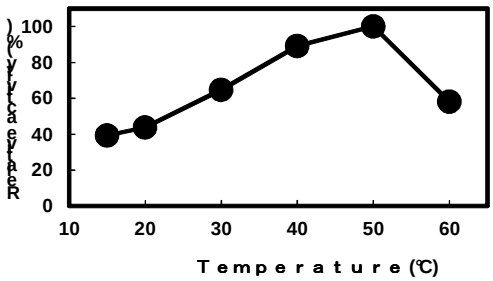

Fig. 2 Temerature activity

electrophoresis. The purified enzyme had an optimal pH from 9.0 to 9.5 and was stable in the range 8.0–10.5. Its optimal temperature of reaction was 50°C, and it was stable below 50°C for 10 min heat treatment. The retained 50% its initial activity at 60°C for 1.0 h. Its molecular weight was 38,000 by gel filtration chromatography and 47,000 by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. These results indicate that the enzyme is monomer. N-Terminal amino acids sequence was clarified as ASWLVHDAEFATAASCLQP. The homology of amino acids sequence was analyzed using BLAST serach, the enzyme has 90% homology with alginate lyase of *S. matophilia* R551-3 and 80% with alginate lyase of S. mal_2066. These enzymes belong to the family 6 of polysaccharide lyase. Alginate lyase from *S. maltophilia* No.43 also might belong to the Family 6 enzyme.

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OC10

MARINE BACTERIA α-N-ACETYLGALACTOSAMINIDASE AND α-GALACTOSIDASE, MODIFIING STRUCTURE OF CELL ANTIGENS

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O-glycoside hydrolases are of great interest in the study and modification of an antigen structure of cells. A special case of this enzymatic modification is the directional change of the group specificity of human A, B and AB red blood cells in O red blood cells, which has been used in biotechnology to obtain "universal blood." The report summarizes the results of the study of marine bacterial glycosidases inactivating serological activity A and B red blood cells: α-N-Acetylgalactosaminidase (EC 3.2.1.49) isolated from the type strain of the marine bacterium *Arenibacter latericius* KMM 426^T and α-galactosidase (EC 3.2.1.22) isolated from the marine bacterium *Pseudoalteromonas* KMM 701. The report provides information on the distribution of these enzymes in marine bacteria, as well as their properties, substrate specificity, primary, 2D- and 3D-structures, and classification by family of glycoside hydrolases in CAZy database. The mechanism of enzymes action is being discussed. The studies were performed with application of traditional methods of enzyme chemistry and modern methods of molecular biology, bioinformatics and computer modeling.

The work was supported by the Russian Foundation for Basic Research (project no. 11-08-01200), the program ”Molecular and Cell Biology” of the Russian Academy of Sciences, and grants of the Far Eastern Branch of the Russian Academy of Sciences (project nos. 12-III-A-05-057, 12-III-A-05-019 and 12-III-A-06-105).

OC11

FEATURES OF PASSING TRANSGLYCOSYLATION AND HYDROLYSIS REACTIONS OF POLYSACCHARIDES CATALYZED BY ENDO-1,3-β-D-GLUCANASES FROM MARINE MOLLUSKS OF SEA OF JAPAN AND SOUTH CHINA SEA

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Laminarinases of known specificity and mode of action are important tools for studies of brown algae polysaccharides (laminarans) structure. Furthermore, enzymatic transformation of laminarans yields a new biologically active (1→3); (1→6)-β-D-glucan. It has been shown that marine invertebrates, represented by a great number of types and species at various stages of evolution are important sources of laminarinases with interesting specificity.

The features of transglycosylation and hydrolysis reactions were studied for five endo-1,3-β-D-glucanases from marine mollusks: *Littorina sitkana*, *Pseudocardium sachalinensis*[1], *Mezuhopecten yessoensis* [2] (Sea of Japan), *Perna viridis* [3] and *Tapes literata* [4] (South China Sea) using MALDI TOF and colisionally-induced dissociation (CID) tandem electrospray ionization mass-spectrometry (ESIMS/MS) and HPLC methods. The (1→3)-β-D-glucanases from marine mollusks catalyzed hydrolysis *Laminaria cichoriodes* laminaran at high rates. However the accumulation of hydrolysis products was differed. All of enzymes possessed transglycosylation activities, which were studied for different acceptors according to their structure and concentration. For one of them the structure of the products of transglycosylation reaction was established. The significant distinctions of catalytic properties of LIV and GI were revealed. The results have shown the some differences in the rates of hydrolysis and transglycosylating reactions The ratio of *K*tr,gly (transfer to glycerol) to *K*hydr (transfer to water) has been estimated for some enzymes. The study and compare of simultaneously passing reactions of transglycosylation and hydrolysis catalyzed by the enzymes of marine mollusks allow us to understand correlation between structure-specificity and use them for synthesis of new biologically active products.

This work was supported by the Russian Foundation for Basic Research grant

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OC12

OPTIMIZATION OF A HIGHLY ACTIVE RECOMBINANT ALKALINE PHOSPHATASE *Cm*AP EXPRESSION AND PURIFICATION

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Alkaline phosphatase (AP) are widespread in nature enzyme. It catalyzes the removal of the 5'-phosphate groups of DNA or RNA. AP from the strain of marine bacteria *Cobetia marina* (KMM 296 PIPOC FEB RAS) is a very promise enzyme in the field of biotechnology and genetic engineering demonstrating unusual physical and chemical properties. Now it is the most highly active alkaline phosphatase in the world (11000-14000 units/mg) compared to other commercially available APs. Previously we have developed a method of producing a recombinant protein AP of marine bacterium *Cobetia marina* (*Cm*AP) with the use the recombinant plasmid 40Ph. For optimization of the expression and the yield of the purified enzyme the recombinant plasmid 40Ph was modified and the cultivation conditions of transgenic strain *E.coli E.coli* Rosetta (DE3)/40Ph were matched.

The colonies of *E.coli* Rosetta (DE3)/40Ph were grown in LB medium containing 25 μ/ml of kanamycin at 37 °C up to an optical density OD_{600} = 0,6 - 0,8. Then the *Cm*AP gene expression was induced by adding IPTG to a final optimal concentration of 0,2 mM and incubated on a thermostatic shaker incubator for 12 hours at 16°C.

After harvesting the microbial cells were resuspended in a buffer containing 0.05 M Tris-HCl buffer, pH 8.6, and 0.01% NaN_3 (buffer A) and disintegrated by ultrasonic treatment. The *Cm*AP was eluted with a gradient concentration of NaCl (0.05 M–0.35 M) with buffer A from a DEAE-cellulose column (Whatman). The fractions with the enzyme activity were collected and loaded onto Ni-resin column (Qiagen). The enzyme was eluted with buffer A containing 50 mM EDTA. Eluted fractions were desalted with DEAE-toyeperl 650M (TOYO SODA). At the final purification stage of gel permition chromatography (Sephacryl S-100 HR, Sigma), a 34%-yield enzyme preparation was 385-fold purified and a single 55 kDa band of *Cm*AP was detected by SDS-page electrophoresis.

The new scheme of isolation and purification of the recombinant *Cm*AP enables increase the yield of the biotechnologically important enzyme up to 1 mg per 1 liter of bacterial cell culture with a specific activity of *Cm*AP reached 6000 U/mg of protein. In diethanolamine (DEA) buffer the specific activity of the enzyme was 20400 units per 1 mg that exceeded the value of specific activity of the native *Cobetia marina* AP twofold.

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OC13

1,3-b-D-GLUCANASES OF BIVALVES OF THE SOUTH CHINA SEA

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1,3-b-D-Glucanases (laminarinases) belong to O-glycosyl hydrolases, the key enzymes of carbohydrate metabolism. They cleave O-glycoside bonds in b-1,3-glucans. These enzymes are widely distributed in various organisms, from archebacteria to eukaryotes, and are involved in many physiological processes [1]. In particular, 1,3-b-glucanases participate in the degradation of polysaccharides, which are utilized by bacteria as an energy source. In fungi, these enzymes catalyze the lysis of the intrinsic cell matrix during the development of cells. In plants, they take part in cell differentiation, the defense of cells against pathogenic fungi, and the degradation of the b-1,3-glucan layer of the envelope of seeds during their germination. 1,3-b-Glucanases play an important role in the digestion of sea invertebrates. They are assumed to be involved in the fertilization and early embryogenesis of sea urchins. Sea invertebrates, which are represented by a great number of taxons being at different stages of evolution and differing from one another in the mode of nutrition and living, are rich and relatively accessible sources of various O-glycosyl hydrolases. Some sea invertebrate species serve as objects of mariculture. O-Glycosyl hydrolases are most often found in the alimentary tracts of sea animals, which are brought upon industrial processing of animals into wastes.

Some 1,3-b-D-glucanases of the South China sea were established. The role of certain functional groups for catalytic activity of this enzyme was evaluated by inhibitor analysis. Were established correlation between amino acid sequences and physicochemical properties of enzyme [2].

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OC14

SUBSTRATE SPECIFICITY AND CATALYTIC PROPERTIES OF THE FUCOIDANASE FROM *FORMOSA ALGAE* KMM 3553

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The strain KMM 3553 from the collection of marine microorganisms of PIBOC FEB RAS was chosen as a producer of fucoidanase on the basis of screening results and identified as *Formosa algae*. The study of fucoidanase properties was carried out with using of the highly purified fucoidanase from the marine bacterium *F. algae*. To detect sulfated oligosaccharides which were produced during enzymatic hydrolysis of the fucoidan carbohydrate polyacrylamide gel electrophoresis was used. Fucoidanase catalyzed the hydrolysis of *Fucus evanescens* fucoidan in a wide range of pH from 6.5 to 9.1 (Fig. 1 A). The presence of Mg^{2+}, Ca^{2+} and Ba^{2+} cations strongly activated the enzyme, cations of Cu^{2+} and Zn^{2+} has possessed by the evident inhibitory effect on enzymatic activity (Fig. 1 B). Sulfated oligosaccharides were detected after 12 hours of incubation and maximum yield of the enzymatic hydrolysis products of fucoidan was observed after 48 hours. Fucoidanase catalyzed hydrolysis of the fucoidans from *F. evanescens* and *Fucus vesiculosus* containing 1→3 and 1→4 glycosidic links but not fucoidan from *Saccharina cichorioides* consist of sulfated 1→3 linked α-L-fucopyranose only (Tabl. 1). For detailed investigation of the specificity of fucoidanase the products of enzymatic transformations *F. evanescens* fucoidan were obtained and analyzed by ^{1}H NMR spectroscopy. We have estimated the integral intensity of signals at 1.24-1.32 area and 1.38-1.41 area and compare the ratio of 1→3 and 1→4 glycosidic bounds in products of enzymatic hydrolysis. Using this calculation ratio 1→3:1→4 in fucoidan before enzyme action was approximately equal and after one was about 3:1 respectively. Accumulation of 1→3 linkages in the enzymatic hydrolysis products indicates that fucoidanase specific to the splitting of 1→4 glycosidic bound in fucoidan molecules.

Table 1 Specificity of FFA action on different substrates

Substrate	**Degree of hydrolysis, %**
Fucus evanescens	7
F. dAc*	9.4
F. dS*	0.8
Fucus vesiculosus	5.6
Saccharina. cichorioides	0

F. dAc* – Deacetylated derivative of *F. evanescens* fucoidan
F. dS* - Desulfated derivative of *F. evanescens* fucoidan

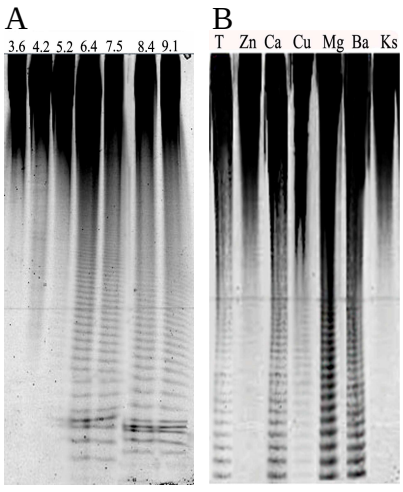

Fig. 1 (**A**) Electrophoregram of the hydrolysis products of fucoidan by FFA with different pH. Numbers above the line refer to a buffer with different pH range. (**B**) Influence of the different bivalent cations on fucoidanase was monitored by C-PAGE. Cations of bivalent metal are indicated over the lines. (T)- Control of enzymatic activities without of bivalent cations addition. (Ks)- unhydrolysed fucoidan.

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OC15

OPTOSIS INDUCTION OF GLIOTOXIN ISOLATED FROM MARINE FUNGUS *ASPERGILLUS* SP. IN HUMAN CERVICAL CANCER CELL AND HUMAN CHONDROSARCOMA CELL *IN VITRO*

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Gliotoxin, a secondary metabolite produced by marine fungus *Aspergillus* sp., possesses several bioactivities including anti-cacer [1]. However, the molecular mechanism of gliotoxin on human cervical cancer cells (Hela) and human chondrosarcoma cells (SW1353) has been unknown. To explore the effect of gliotoxin on apoptosis-related proteins, RT-PCR and Western blotting were used to detect the expression of caspase-3, caspase-8, caspase-9, Bax, Bcl-2, p53 activities and release of mitochondrial cytochrome c (cyt c) into cytosol. The results indicated that gliotoxin induced apoptosis of Hela and SW1353 cells confirmed by DAPI staining and flow cytometry analysis. The mitochondrial pathways were mediated by down-regulation of Bcl-2 and up-regulation of Bax, release of cyt c to cytosol, and subsequent activation of caspase-3, caspase-8 and caspase-9 followed by down-stream events leading to apoptotic mode of cell death.

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OC16

PHARMACOKINETIC PROPERTIES OF CUCUMARIOSIDE A_2-2 DETERMINED BY MALDI-TOF-MS AND MALDI-IMS

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Pharmacokinetic studies are an important stage in the new drug discovery. The rates of delivery of biologically active preparations into different organs and tissues as well as the excretion rates can be estimated by this approach, and both optimal single and course doses and the protocol of treatment can be determined. The medical lead, so-called Cumaside, was created on the basis of triterpene oligoglycosides (mainly cucumarioside A_2-2) from the Far-Eastern edible sea cucumber *Cucumaria japonica* for the prevention and treatment of human immunodeficiency states. However, the pharmacokinetic behavior of the drug in target organs is still largely unclear.

The aim of this research was to determine the pharmacokinetic properties of cucumarioside A_2-2 by mass-spectrometry techniques. For this purpose the stability and dynamics of glycoside content changes over time in Balb/c mouse spleen tissue homogenate as well as the study of the cucumarioside A_2-2 spatial distribution on the surface of tissue sections were investigated using MALDI-TOF-MS and MALDI-IMS, correspondingly.

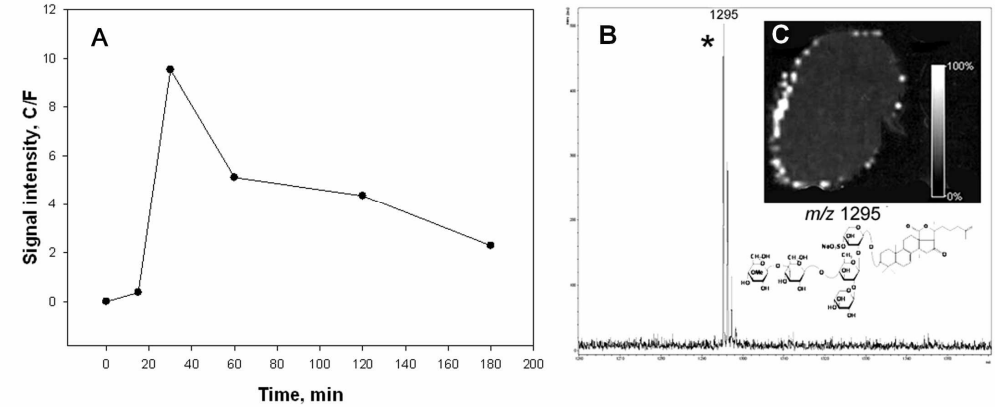

Fig. 1. The dynamics of cucumarioside A_2-2 content changes in the mouse spleen homogenate (A), MALDI-TOF spectrum of the glycoside and its spatial distribution determined by MALDI-IMS in the spleen tissue (B, C) after single *i.p.* administration.

It was shown that cucumarioside A_2-2 is reliably detected by MALDI-TOF-MS in the mouse spleen tissue after single intraperitoneal (*i.p.*) injection at a dosage of 5 mg/kg. It was established that the glycoside is stable in the spleen and does not undergo metabolic transformation both in tissue homogenates and in the intact organ after *i.p.* injection within 24h. It was found that cucumarioside A_2-2 was absorbed quite quickly: the glycoside maximum concentration (C_{max}) in tissue homogenate was observed in the first 30 minutes after injection; the minimum values were registered in 3 hours (Fig. 1A, B). These results are in agreement with those obtained in the pharmacokinetic study of ^{3}H-cucumarioside A_2-2. Glycoside was mainly located in the tunica serosa part of spleen and was not observed within red and white pulp of the organ by MALDI-IMS (Fig.1C). It was detected in the tissue at high concentrations and found in tissue sections obtained both from the spleen surface, and from the middle part.

Acknowledgments

The authors very much appreciated Dr. Avilov S.A. and Dr. Silchenko A.S., PIBOC FEBRAS (Vladivostok, Russia), for providing with cucumarioside A_2-2. This work was supported by Grant of RFBR N°11-04-01084-a, Grants of FEBRAS N°12-III-B-05-022 and Federal Program N° 2012-1.3.2-12-000-1001.

OC17

SALT RESISTANT ALKALINE PHOSPHATASE FROM THE EGGS OF SEA URCHIN *STRONGYLOCENTROTUS INTERMEDIUS*

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Alkaline phosphatases (AP, EC 3.1.3.1) are widely distributed in nature and play an important role in the metabolism of phosphate. These enzymes are involved in the processes of biomineralization within the marine invertebrates. AP belong to the class of hydrolases and are non-specific zinc-containing metalloenzymes catalyzing the alkaline hydrolysis of various phosphomonoesters with the formation of inorganic phosphate.

We have previously shown that the AP from the eggs of sea urchin *Strongylocentrotus intermedius* (StAP) to be stable within range of pH from 6.5 to 9.0, and its optimum pH is located at pH 8.1-8.5. Calcium and magnesium ions have been shown to increase the stability and activity of phosphatase that confirmed our suggestion about metallodependence of our enzyme. StAP has the unique ability to hydrolyze the substrates in solutions with the high ionic strength and in natural seawater without loss of enzyme activity. This unusual property of StAP from homogenates of sea urchin eggs allowed us to developed enzymatic test system for assaying the quality of marine and fresh water [1]. According to data obtained by gel filtration, SDS PAAG-electrophoresis and mass spectrometry MALDI MS VP the enzyme, studied, is a homodimer with a molecular mass of 160 kDa, but in contrast to the other AP our enzyme was found to reveal the activity not only in dimeric form but also in monomeric form.The purpose of this work was to study the spatial structure of the StAP in solutions of high ionic strength by optical spectroscopic methods. Fluorescence and circular dichroism (CD) spectra of StAP were recorded. The presence of a distinct fine structure of the CD spectrum of StAP in the near UV region indicates that there is a significant asymmetry in the environment of the side groups of the aromatic amino acid residues, that is, they are constrained, and, consequently, the studied enzyme has highly organized tertiary structure. The percentage of canonical elements secondary structures of the enzyme was calculated by the method of Srirama and Wood using the CD spectrum in the far UV region. The protein content of α-helices - 5.1 %, β-structures – 39.7 % (27.1 – regular and 12.6 – distorted), β-loops – 21.7 %, and unordered structure – 33.5 %. Thus, β-structures predominate in the StAP molecule.

Fluorescence spectroscopy is one of the most convenient and versatile methods to determine the relationship between activity and the spatial structure of the protein. It is shown that the StAP maintains the highest level of activity in the presence of NaCl concentrations up to 0.75 M, as well as in natural and artificial seawater with a salinity of 33 ‰ (0.50 M NaCl). The influence of NaCl and natural seawater on the conformational stability of the enzyme has been studied by methods of CD- and fluorescence spectroscopy. The slight change of the tertiary structure of the phosphatase has been found at the concentrations of NaCl 0,75 and 1.0 M. The same distortions of the enzyme conformation was shown to be after adding sea water. Further increasing the concentration of salt (up to 1.5 and 2.0 M) leads to a restructuring of the tertiary structure of the protein. Secondary structure of the StAP was affected by ionic strength in less degree than the tertiary. At 1 M concentration of NaCl the CD-spectrum of enzyme in the far UV region does not change, some changes in the secondary structure of the protein will be shown only after increasing the concentration of NaCl higher than 1.5 M.

Thus, it is shown that the StAP has a high conformational stability and keeps the enzymatic activity in solutions with a high ionic strength. As far as we have known the salt resistance alkaline phosphatase in marine animals was not found.

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OC18

PROPERTIES OF HIGH-MANNOSE N-GLYCAN-SPECIFIC LECTINS FROM THE RED ALGAE, CARRAGEENOPHYTES

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The red algae, carrageenophytes including *Kappaphycus alvarezii*, *Kappaphycus striatum* and *Eucheuma serra*, not only are as source of carrageenans, but also as source of lectins. The isolated lectins from these algae shared the same properties in hemagglutination activity, hapten-inhibition profile of hemagglutination, oligomannoside binding specificity and equivalent molecular mass of a monomeric protein having identical 20 N-terminal amino acid sequences to each othe. The hemagglutination activities of lectins were inhibited strongly by glycoproteins bearing high-mannose type N-glycan. In the binding experiment with pyridylaminated oligosaccharides by centrifugal ultrafiltration-HPLC assay, lectins exclusively bound to different high-mannose type N-glycans, but not to other glycans, indicating that they recognized the branched oligomannosides. The binding activity of these lectins have high affinity for oligosaccharides having the exposed (α1-2)Man in the D2 arm. The results indicated that lectins appear to recognize the extended carbohydrate structure with a minimal length of a tetrasaccharide, Man(α1-3)Man(α1-6)Man(α1-4)GlcNAc. This study showed that red algae, carrageenophytes, are a good source of a valuable lectin(s) that is strictly specific for high-mannose N-glycans.

POSTER COMMUNICATIONS

P1

GLYCOSIDASES OF *BACTEROIDETES* ISOLATED FROM MARINE ALGAE

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Glycosidase activities of 177 strains of the phylum *Bacteroidetes*, belonging to 18 genera and isolated from the algae *Chondrus* sp., *Polysiphonia* sp., *Neosiphonia japonica, Saccharina crassifolia, Saccharina japonica, Chorda filum, Acrosiphonia sonderi,* and *Ulva fenestrata* collected in the littoral zones of the Sea of Okhotsk and the Sea of Japan, Pacific ocean, were studied. According to the data obtained, glycosidases catalyzing hydrolysis of the β-glycoside bond were present in over 70% epiphytes of marine algae. It should be noted that α-galactosidases and the extremely rare enzymes, α-N-acetylgalactosaminidases, were more frequent in the *Bacteroidetes* than in the *Proteobacteria* analyzed previously. It was found that the overwhelming majority of the bacteria of the dominant genera *Zobellia* and *Maribacter* contained the complete set of the tested glycosidases involved in degradation of algal polysaccharides. Apparently, the presence of the wide range of glycosidases in bacterial strains of these genera makes it possible for them to occupy diverse ecological niches under extreme conditions of the tidal zone. However, such important enzymes of the microbial lytic complex as α-galactosidases, β-galactosidases, or β-xylosidases, were not detected in the numerically important genus *Winogradskyella*. The noted difference in the metabolic profiles of the strains of these genera suggests the assumption that *Winogradskyella* strains play a unique role in the microbial communities, unrelated to the hydrolysis of such polysaccharides as agar and carrageenan. Significant differences in production of glycosidases among the different taxonomic groups were revealed which is of importance for directed search of promising enzymes for biotechnology.

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P2

DISTRIBUTION OF MARINE BACTERIUM *PSEUDOALTEROMONAS* SP. α-GALACTOSIDASE INHIBITORS AMONG MARINE ENVERTIBRATES IN THE KURIL ISLAND'S WATER

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Compounds affected the functioning of enzymes are important for the development of theoretical and applied aspects of enzymology. Such compounds are fine tools in biochemical and pharmacological studies. In particular, the modification or blocking of biochemical processes involving glycosidases with potent selective inhibitors is the basis for treating a variety of infectious diseases, cancer and genetic disorders.

α-Galactosidases [EC 3.2.1.22] play an important role in the vital functions of many micro-and macro-organisms. They are widespread among marine bacteria of the Okhotsk Sea, as free-living in the water, and associated with sponges and sea squirts [2, 3]. Information about the natural inhibitors of α-galactosidase from the marine bacterium *Pseudoalteromonas sp.* KMM 701 is still unknown. The inhibition of the enzyme by natural 5-hydroxy- and 5,8-dihydroxy-1,4-naphthoquinones from sea urchin and the effect of the substituents nature, their number and position in the structure on inhibitory properties of these compounds have been previously studied [4].

This work presents the results of the search for inhibitors of α-galactosidase of 541 water-ethanol extracts of sea sponges and sea squirts, collected during the expedition vessel "Academic Oparin" in July and August 2012 in the Kuril Islands. Ninety-three extracts inhibited the enzyme. The analysis of the distribution of inhibitors of α-galactosidase activity among animals revealed the dependence on their habitat environmental conditions such as depth, bottom. Most of the animals, which a potential source of inhibitors, were collected in the water area of the Urup and Rasshua islands.

RFBR (11-08-01200), the program "Molecular and Cell Biology" of the RAS, and grants of the FEBRAS (12-III-A-05-019, 12-III-A-05-057).

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P3

DIVERSITY AND HYDROLYTIC ACTIVITY OF MARINE BACTERIA OF GENUS *ARENIBACTER*

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Bacteroidetes represents one of the main branches of the phylogeny domain *Bacteria* and one of the dominant components of the marine ecosystem microbiota. Due to multienzyme complexes, facilitating to dispose of any natural substrates as sources of carbon and energy, marine *Bacteroidetes* are considered as a promising source of these unique biologically active compounds.

Representative of marine *Bacteroidetes* the genus *Arenibacter* have been shown previously are not able to degrade high molecular weight natural biopolymers. However, they synthesize a wide range of glycosidases, which may facilitate the adaptation of microorganisms to different habitats in a constantly changing environment of inter tidal flat zone. *A. latericius* KMM 426^T is the first representative of this kind of bacteria. α-N-Acetylgalactosaminidase (EC 3.2.1.49) isolated from the type strain of the marine bacterium *A. latericius* KMM 426^T had a rare substrate specificity and capable to inactivate serological activity of human A red blood cells, efficiently removing A antigens at neutral pH.

A total of 16 bacterial strains belonging to the genus *Arenibacter* were isolated from diverse microbial communities associated with the various marine habitats including seaweeds, invertebrates and bottom sediments and collected from different location. All strains demonstrated phenotypic characteristics, typical for the genus. Phylogenetic analysis revealed that strains KMM 6372, KMM 6684 and KMM 6685 presented potentially three new species within the genus *Arenibacter*. None strains contained enzymes-depolymerising polysaccharide and other high molecular weight substances but the isolates synthesized a wide spectrum of glycosidases. Highly active β-N-acetylhexosaminidases were the main glycoside hydrolases for all *Arenibacter* species irrespective of the isolate source and biogeography. However, a level of the enzyme activities varied in depends on the taxonomic and ecophysiological characteristics. Degenerate oligonucleotide primers synthesized on the base of genes of 20, 27 and 36 glycoside hydrolases families were matched to amplify predominant genes of glycoside hydrolase apparatus of *Arenibacter*. The results of the present study showed that glycosidase-encoding genes analysis together with phylogenetic information were suggested for providing a characterization of natural populations of *Arenibacter*, their diversity prediction in the environmental samples and elucidation of their possible ecological role in marine environment.

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P4

DETERMINATION OF STRUCTURE OF cDNA CODING ENDO-1→3-β-D-GLUCANASE FROM MARINE MOLLUSK *LAMBIS* SP.

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Glycoside hydrolase family 16 (GH-16) is one of the most polyspecific due to uniting different enzymes, able to catalyze the hydrolysis of entire specter of carbohydrates. At the same time, 1→3-β-d-glucanases of marine origin, in particular, marine mollusks, are the lest researched. The distinctive feature of the endo-1→3-β-D-glucanases of marine mollusks in compare with glycoside hydrolases of land-based sources (lysozymes, alpha-amylases, cellulases etc.) is their increased capacity for transglycosylation, which resultes a set of oligosides "labeled" by reducing end residue of the acceptor molecule to be found in the reaction products. The practical usage for production of biologically active 1→3- or mixed 1→3;1→4- and 1→3;1→6-β-D-glucans having immunomodulating properties is based on this property of endo-1→3-β-D-glucanases of marine mollusks. For today the amino acid sequences of endo-1→3-β-D-glucanases from 9 mollusk species are determined. The study of structural and functional properties of endo-1→3-β-D-glucanase from marine mollusk *Lambis* sp. compared with endo-1→3-β-D-glucanases from other marine mollusk species will help to specify a relationship between enzymes' structure and properties in this glycoside hydrolase family.

At this point the work to determine the cDNA sequence encoding this enzyme was done, and the work on its isolation was begun. The determination of the primary structure of endo-1→3-β-D-glucanase was carried by cloning and sequencing of cDNA encoding the enzyme. For today it is most effective approach to determine the amino acid sequences of eukaryotic proteins. On the first stage total RNA was isolated from the hepatopancreas of *Lambis* sp. On the second stage, the first strand of cDNA was synthesized from the obtained RNA, and then was amplified by PCR. For the amplification of cDNA fragments we used degenerate oligonucleotide primers made on the base of analysis of conserved regions of amino acid sequences of known endo-1→3-β-D-glucanases. As a result of PCR there were obtained cDNA fragments of about 400 b.p. molecular weight. On the base of determined nucleotide sequence of internal cDNA fragment the oligonucleotide primers for amplification of cDNA end fragments were designed. Amplification was carried out using the RACE method (rapid amplification of cDNA ends). Thus, we determined the nucleotide sequence of full-length cDNA of endo-1→3-β-D-glucanase *Lambis* sp. It has a length of 1781 b.p. and encodes a polypeptide chain of 451 amino acid residues.

This work was supported by RFBR No. 12-04-32110 grant.

P5

DEVELOPMENT OF A HEMOLYTIC HEMAGGLUTININ DETECTION METHOD

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The hemolytic hemagglutinin and non-hemolytic hemagglutinin are two major hemagglutinins. The hemolytic hemagglutinin exhibits in many marine invertebrates such as lobster (*Panulirus argus*)and shrimp (*Litopenaeus vannamei*). With both hemolysis and coagulation properties, the bad interference of the results by Traditional Hemagglutination Test Method will occur while using hemolytic hemagglutinin. To improve the accuracy of test, we collected human blood type as A, B, AB and O as well as dog blood red blood cells to undergo hydrolysis by Papain, frozen rupture and staining treatment to obtain the red blood cell fragment (RBCF) in blue color. Using the RBCF to test both the hemolytic hemagglutinin and non-hemolytic hemagglutinin activities in serum of Litopenaeus vannamei, we can observe the great accuracy of measurements in above two hemagglutinins. It is feasible to use the RBCF to substitute the traditional Hemagglutination Test Method. It is worthy to mention that the RBCF remain great titer as the same as freshly collected red blood cells after 30 days storage.

P6

THE EFFECTS OF *GRACILARIA LEMANEIFORMIS* EXTRACTS ON ANTIOXIDANT CAPACITY AND ON THE FUNCTIONAL PROPERTIES IN SKIN-CARE PRODUCTS

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The *Gracilaria lemaneiformis* is the major feeds for artificially cultivation of Taiwanese abalone (*Haliotis diversicolor*) in Taiwan , however, is rich in polysaccharides and algae pigments, which exhibit great hydration, anti-oxidation and even brightening effects to make it the potential utility for cosmetic application. In our study, the different extracting procedures for whole algae body to isolate various active components rather than single one can compose the optimal formula. The results have shown that the extract of algae pigments at 25mg/ml exist the best clearance capacity up to the extent of 82.1%, and that, the second one is the extract of algae polysaccharide and the third one is the algae proteins. After the determination of the clearance capacity, anti-oxidation ability and brightening effect, the most optimal formula was obtained to develop the jelly facial mask. The three types of combination of optimal formula in algae and basal materials for facial mask were underwent high temperature at 50℃ , centrifugation for 30 minutes and sonic vibration over 5 minutes to test whether the syneresis occurred . The observation over longer term at pH 4,8 to 6.0 kept stable status can confirm the stability in our products. The greater hydration ability in algae also shown the feasible to application of *Gracilaria lemaneiformi.*

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P7

USE OF CHITOSAN FOR PURIFICATION OF AQUEOUS SOLUTIONS FROM ENDOTOXINS

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Chitosan is a widely known non-toxic polysaccharide, with a variety range of physiological activities, including antibacterial, antiviral, anti-tumor, etc, that provides its attraction for widespread use in medicine and pharmacy.

The ability of chitosan to form specific complexes with polyanions opens up broad opportunities for its application as a drug and gene delivery vector as well as a constituent component of biospecific adsorbents and composites. By virtue of its polycationic origin, chitosan can bind to endotoxin (LPS) and can be selected as a specific endotoxin ligand.

Endotoxins (lipopolysaccharides - LPS), are the major component of cell walls of gram-negative bacteria, circulating not only in macroorganisms but also being presented in enviroments that creates serious difficulties for the food and pharmaceutical industries. Unlike bacteria, endotoxin can not be removed by standard methods such as autoclaving or sterile filtration.

We have previously demonstrated high specificity of binding endotoxins of gram-negative bacteria with chitosan. Hence development of highly efficient chitosan-based sorbents for clearing of biological fluids from endotoxins is of great important.

The ability of chitosan use for the treatment of zeolites and subsequent using of samples obtained for purification of aqueous solution from endotoxins have been studied. Chitosan bound with zeolites preserved ability to bind LPS. The efficiency of adsorption of LPS *Escherichia coli* and *Yersinia enterocolitica* from solutions in a concentration of 2 mg/ml is presented in the Table 1. Amount of endotoxin in solution after its incubatin (10 minutes) with modified zeolites dramatically increased in comparison with raw zeolite. Sorbtion activity of zeolites treated with chitosan depended on the modification type and LPS structure.

Table 1. Sorption efficiency (%).

№	Sorbent	Sorption efficiency, %	
		Y. enterocolitica	*E. coli*
1	Zeolite	42,47±6,02	52,50±5,75
2	Zeolite +chitosan	81,73±5,56	90,94±0,66
3	Zeolite +chitosan+$Cu(Fe(CN)_6$	96,08±0,52	83,44±1,10

According to the dates of the effect of temperature and incubation time on the sorption the optimal conditions for the effective endotoxin removing from the model solutions have been chosen.

P8

MARINE BACTERIAL POLYSACCHARIDES AS GLYCOSAMINOGLYCAN-MIMETICS

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In the biotechnological challenge for the discovery of original biomolecules and biocatalysts, the bacteria from marine ecosystems are a highly valuable bio-resource. In particular, polysaccharides from marine prokaryotes offer a source of safe, biocompatible, biodegradable and valuable renewable products with specific biological functions emphasized by a significant structural chimiodiversity with novel material and biological properties. The bacteria that produce polysaccharides are also a source of key enzymes for the production of tailor-made polysaccharides.

We have described more than 15 bacterial strains from hydrothermal deep-sea origin, belonging to three main genera (*Vibrio, Alteromonas and Pseudoalteromonas*) and producing exopolysaccharides (EPS) with both unusual structural features and innovative properties. They are high-molecular-weight carbohydrate polymers, either linear or highly branched. Most of them have a high uronic acid content, and bear different substituing groups (sulphate, pyruvate, lactate).

The biological activity of glycopolymers derives from their molecular structure including molecular size, polydispersity and repeating unit features varying in size, in osidic residues, in linkages and in carried substituents. Therefore, to maximize polysaccharide applications as glycosaminoglycan-like (GAG-like) molecules and generate new biological functions, they may be suitably chemically or enzymatically engineered (depolymerisation and sulphation). The characteristics of enzymatic methods to get oligosaccharides of biological relevance meet well the needs of better control of the modification process and of environmentally safer processing steps. Enzymes capable of generating targeted modifications (glycoside hydrolases or polysaccharide lyases, carbohydrate sulphotransferases) may be obtained by screening of enzyme extracts or commercial preparations, purifying from an entire metabolising microorganism or gene mining.

In the case of the polysaccharide produced by *Vibrio diabolicus* (Fig. 1.) [1, 2] exhibiting structural features similar to hyaluronic acid, enzymes are searched with the aim to find a new efficient and selective depolymerisation enzymatic way. In other respects, studies are carried out to understand the biosynthetic machinery leading to a better control of the polysaccharide production and to the identification of carbohydrate active enzymes.

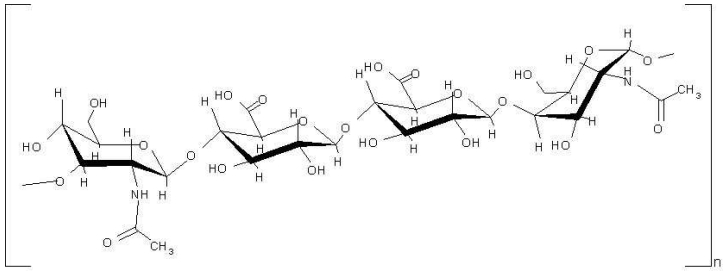

Fig. 1. Osidic sequence of the repeating unit of the polysaccharide HE800

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P9

DISTRIBUTION OF O-GLYCOSYDE HYDROLASES IN MARINE FUNGI: CHARACTERIZATION OF EXOCELLULAR β-D-GLUCOSIDASE AND N-ACETYL-β-D-GLUCOSAMINIDASE OF THE MARINE FUNGUS *PENICILLIUM CANENSIS*

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The fungi live in the ocean as saprophytes, parasites, and symbionts on various substrates: bottom sediments, water, sea sand, remains of trees, algae, marine organisms, etc. The marine fungi constitute a little known ecological group. At present, principal attention is paid to the investigation of low molecular biologically active compounds produced by marine fungi while the capacity of fungi to degradation of a wide variety of polymers in the marine environment remains little studied [1]. However, even scarce data obtained by authors from India, South Korea, Japan, Russia, etc. indicate that marine fungi produce highly active enzymes stable to various factors, urgently needed in modern industries. The marine fungi attract great interest as producers of O-glycosylhydrolases catalyzing hydrolysis of O-glycoside bonds in various carbohydrate-containing compounds [2-5].

The present study is aimed at the investigation of distribution of O-glycosylhydrolases in marine fungi collected in various regions of the Pacific Ocean and characterization of properties of individual N-acetyl-β-D-glucosaminidase and β-D-glucosidase, involved in a complex of extracellular O-glycosylhydrolases of the marine fungus *Penicillium canescens* Sopp.

The capacity to produce exocellular enzymes was studied for 92 samples of fungi from various marine habitats in the Sea of Okhotsk (78 strains) and the Sea of Japan (14 strains). Strains producing highly active glycanases and glycosidases were found. Synthesis of O-glycosylhydrolases was stimulated by addition of laminaran to the nutrient medium.

Extracellular β-D-glucosidase was isolated in a homogeneous state from the marine fungus *Penicillium canescens*. According to SDS-electrophoresis, the molecular weight of the enzyme was 64 kDa and the maximal activity was observed at pH 5.2 and 70°C. Glucosidase catalyzed the hydrolysis of β-glycosidic bonds both in glycosides and in glucose disaccharides and had transglycosylation activity.

Highly purified N-acetyl-β-D-glucosaminidase of the marine fungus *Penicillium canescens* had molecular weight 68 kDa. The enzyme displayed maximum activity at pH 4.5 and temperature 45°C. Inactivation half-time of the enzyme at 50°C was 25 min. N-acety-β-D-glucosaminidase hydrolyzed both β-glucosaminide and β-galactosaminide bonds. Enzyme possessed a high transglycosylating activity.

The enzymes can be used for the ascertaining of the structure of carbohydrate component in biopolymers and for the enzymatic synthesis of new carbohydrate-containing compounds.

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P10

THE EXPRESSION OF RECOMBINANT ALPHA-GALACTOSIDASE OF MARINE BACTERIUM *PSEUDOALTEROMONAS* SP. KMM 701

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α-Galactosidase (α-D-galactoside galactohydrolase; EC 3.2.1.22) catalyzes the hydrolysis of α-1,6-linked galactose residues in oligosaccharides and polymeric galactomannan. α-Galactosidases are of particular interest in view of their many potential biotechnological and medical applications.

α-Galactosidase (α-Gal) from the marine bacterium *Pseudoalteromonas* sp. KMM 701 was found to be able to transform the B-blood antigens into H-antigens in the absence of the red blood cell aggregation and hemolysis and it showed maximum activity at the physiological range pH and temperature from pH 6,7 to 7,7 and 20 to 22ºC, respectively. The enzyme showed apparent psychrophilic properties that is why it can be easily removed from the reaction of blood group conversion at 37 °C. Universal donor blood group O is the most used group in emergencies in the case of impossibility to determine the blood group of the recipient by several reasons. In addition, it is useful for pediatric transfusions and when the unusual or rare phenotypes of blood groups are required as well as in the cases of the deficiency of healthy donors.

We have developed a method of producing a highly active dimeric form of the recombinant α-Gal of marine bacterium *Pseudoalteromonas* sp. KMM 701 with the use of recombinant plasmid 40Gal and transgenic strain of *E. coli* Rosetta (DE3)/40Gal providing induced stable synthesis with the high yield of soluble and active enzyme into the cell periplasm.

The method resulted in a stable synthesis of the recombinant α-Gal and led to the heterologous expression of the enzyme at 500 U/mg of total extracellular protein after optimization of process. Furthermore, the expression level in the study represented up to 30% of the total extracellular soluble protein produced by the host organism, which was comparatively higher than those reported for the native strain of *Pseudoalteromonas* sp. KMM 701.

This work was supported by RFBR # 11-08-01200-a, FEB RAS 12-III-A-05-019 and the RAS Presidium Program "Molecular and Cell Biology" 09-I-P22-05.

P11

GLYCOLIPIDS FROM BROWN ALGA *FUCUS EVANESCENS* INHIBIT INVERTEBRATE *O*-GLYCOSYDE HYDROLASES

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Marine invertebrates feed on alga and use specific enzymes, such as glycanases and glycosidases, to decompose algal polysaccharides. Marine algae contain some substances, which inhibit or activate these enzymes and possibly defend the plant against invertebrates.

We studied the effect of an ethanol extract from brown alga *Fucus evanescens* on the activities of endo-(1→3)-*β*-D-glucanase and *β*-D-glucosidase from marine gastropod *Littorina sitkana*. This extract showed the 100% inhibitory effect on the *O*-glucosyl hydrolases at the concentration of 0.25 µg/ml. The monogalactosildiacylglycerol (MGDG) was isolated from the ethanol extract of *F. evanescens* and tested on the same enzymatic activities.

The *β*-D-glucosidase loosed 80% activity while the endo-(1→3)-*β*-D-glucanase kept its activity when MGDG was applied at the concentration of 0.25 µg/ml. The value of I_{50} was about 75 and 280 µM for the *β*-D-glucosidase and the endo-(1→3)-*β*-D-glucanase, respectively. These data are in the relation with the inhibitory effect of gluconolactone - the known inhibitor of the *O*-glucosyl hydrolases from plant, which showed I_{50} values of 50 and 300 µM for the β-D-glucosidase and the endo-(1→3)-β-D-glucanase, respectively. The inhibition of invertebrate *O*-glycosyl hydrolases by MGDG from brown alga *F. evanescens* show that this algal lipid may be important for a regulation of alga/invertebrate interaction in marine ecosystems and food webs.

This study was supported by the Russian Foundation for Basic Research (projects no 11-04-93009-Viet_a and 12-04-0069) and the RAN Program Molecular and Cell Biology.

P12

ANTI-INFLAMMATORY EFFECTS OF EXTRACT OF MARINE RED ALGAE, *CALLOPHYLLIS JAPONICA* IN LPS INDUCED CYTOKINE PRODUCTION IN RAW264.7 MACROPHAGE

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The red algae, *Callophyllis japonica* is distributed in South Korea and Japan and it has been used for eaten in orient area. In recent study, solvents extracts of *Callophyllis japonica* is reported to possess bioactive properties such as antioxidant and radioprotective activity. However, there are few studies about anti-inflammatory effects of methanol extracts of *Callophyllis japonica* on LPS-induced Raw264.7 macrophage. To investigate anti-inflammatory effect of methanol extract of *Callophyllis japonica* (MECJ) on lipopolysaccharide (LPS)-stimulated RAW264.7 macrophages and elucidated the molecular mechanism. The results show that the MECJ suppresses LPS-induced production of nitric oxide (NO) via inducible prostaglandin E2 (PGE2) expression in a dose-dependent manner. It also significantly reduced the gene transcription and protein expression, such as NO synthase (iNOS) and cyclooxygenase-2 (COX-2). Furthermore, MECJ significantly suppresses phosphorylation of mitogen-activated protein kinases (MAPKs). Therefore, potent anti-inflammatory effects of the MECJ might suggest possibility for high valuable utilization and application for therapeutic substances.

P13

THE NEURO-PROTECTIVE EFFECT OF DIECKOL ISOLATED FROM MARINE BROWN ALGAE, *ECKLONIA CAVA* ON EXPRESSION OF COX-2 AND iNOS IN MURINE MICROGLIAL CELLS

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To identify neuro-protective effect of dieckol and phloroglucinol, isolated from marine brown alga, *Ecklonia cava*, we investigated the anti-inflammatory effect of dieckol on lipopolysaccharide (LPS)-stimulated murine BV2 microglia and elucidated the molecular mechanism. The results showed that dieckol suppress LPS-induced production of nitric oxide (NO) and prostaglandin E_2 (PGE_2), and expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) in a dose-dependent manner, without causing cytotoxicity. It also significantly reduced the generation of proinflammatory cytokines, such as interleukin (IL)-1β and tumor necrosis factor (TNF)-α. Moreover, dieckol significantly reduced LPS-induced nuclear factor-κB (NF-κB) and p38 mitogen-activated protein kinases (MAPKs) activation, as well as reactive oxygen species (ROS) production. Taken together, the inhibition of LPS-induced NO and PGE_2 production might be due to the suppression of NF-κB, p38 MAPK signal pathway, and, at least in part, by inhibiting the generation of ROS. Hence, these effects of dieckol might help to therapeutic treatment for neurodegenerative diseases that are accompanied by microglial activation.

P14

ISOLATION OF ANTICOAGULANT ACTIVITIES OF AN OLIGOPEPTIDE FROM MARINE BIVALVIA, BLUE MUSSEL (*MYTILUS EDULIS*)

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A novel oligopeptide with high affinity to blood coagulation factors (FII, FIX, FX) was isolated from blue mussel (*Mytilus edulis*). *M. edulis* anticoagulant peptide (MEAP) was purified using anion exchange and gel filtration chromotography. It was revealed that MEAP has 2.46 kDa of molecular mass and its amino acid sequence, EADIDGDGQVNYEEFVAMMTSK, has high identity (67%) with that of the EF-hand domain of CaM from scallop adductor muscle by ESI-QTOF analysis and database research. MEAP could potently prolong thrombin time (TT) and the activated partial thromboplastin time (APTT). Binding affinity assay using SPR analyzer showed that MEAP could specifically interact with blood coagulation factors; FIX, FX and FII. Dissociation equilibrium constant (*K*D) values of MEAP for FIX, FX and FII was determined as 11.28 nM, 21.35 nM and 65.56 nM, respectively, in the present of Ca^{2+}. Amidolytic assay showed that MEAP could potently inhibit proteolytic activation of FX by the intrinsic FXase (IC_{50}; 13.6 μg/ml) and formation of FIIa by prothrombinase complex (IC_{50}; 42.9 μg/ml) in dose-dependant manners. The present study elucidated that MEAP can prolong blood clotting time by inhibiting activation of FX in the intrinsic tenase complex (FIXa/VIIIa/PLs) and conversion FII to FIIa in the prothormbinase complex (FXa/FVa/PLs). These results provide a fundamental data for the potential anticoagulant effect to prevent cardiovascular or haemostatic disorder.

P15

STUDY ON CALCIUM BIOAVAILABILITY OF FISH-BONE PHOSPHOPEPTIDE IN OSTEOPOROSIS-MODELING RATS

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Fish-bone peptide (FBP) with a high affinity to Ca was isolated using hydroxyapatite affinity chromatography, and FBP II with a high ratio of phosphopeptide was fractionated in the range of molecular weight 5.0-1.0 kDa by ultramembrane filtration. *In vitro* study elucidated that FBP II could inhibit the formation of insoluble Ca salts in neutral pH. In vivo effects of FBP II on Ca bioavailability were further examined in the ovariectomised rat. During the experimental period, Ca retention was increased and loss of bone mineral was decreased by FBP II supplementation in ovariectomised rats. After the low-Ca diet, the FBP II diet, including both normal level of Ca and vitamin D, significantly decreased Ca loss in faeces and increased Ca retention compared with the control diet. The levels of femoral total Ca, bone mineral density, and strength were also significantly increased by the FBP II diet to levels similar to those of the casein phosphopeptide diet group (no difference; $P < 0.05$). In the present study, the results proved the beneficial effects of fish-meal in preventing Ca deficiency due to increased Ca bioavailability by FBP intake.

P16

BIODEGRADABLE ACTIVITY OF MARINE ALGAE *(LAMINARIA JAPONICA)* BY ABALONE (*HALIOTIS DISCUS HANNAI*) INTESTINAL CRUDE ENZYME (AICE)

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Abalone (*Haliotis discus hannai*) distributed in south-western coastal areas are known to feed preferentially on marine brown algae. Their digestive system with various enzymes includes highly bio-degradable potentials on marine algae. In the present study, we isolated and charaterized crude enzymes from guts of abalone *H. discus hannai*, was provides by aqua-culture company in Wando, Jeollanam-do, S. Korea. The digestive ability of abalone intestine crude enzyme (AICE) was examined on by-products (roots and spores) of *Laminaria japonica* (Goheung, Jeollanam-do S. Korea) and the optimal condition of AICE was determined pH 7.5~8.0 (50%), temperature 50℃, substrate concentration 3%, ratio of substrate and enzyme (50:1), incubation time through 12 hours. In the optimal condition, the results showed that the degree of *L. japonica* by-products hydrolysis by AICE could be increased up to 70%.

P17

PREPARATION OF CHITOOLIGOSACCHARIDE (COS) DERIVATIVES WITH HYDROXYETHYL SULFATE GROUPS AND ITS ANTICOAGULANT ACTIVITY

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Chitooligosaccharide (COS) with sulfated hydroxyethyl groups (hydroxyethyl, 6-O-desulfation of hydroxyehyl, quaternozed amino hydroxyethyl, and carboxylated hydroxyehtyl COS sulfates) were prepared by chemical synthesis, and chemical structures of the sulfated COS derivatives were confirmed using elemental analyzer, FT-IR, and ^{13}C NMR. Their inhibitory activities on hyman blood clot formation were studied in terms of the activated partial thromboplstin time (APTT), prothrombin time (PT), and thrombin time (TT) assays. In the results, the hydroxyethyl COS sulfate (SCOS1) with similar structure of heparin could increase the blood clotting times of APTT and TT.

P18

A SULFATED HYDROXYETHYL CHITOOLIGOSACCHARIDE INHIBIT HUMAN BLOOD CLOTTING VIA ANTITHROMBIN ACTIVATION

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Sulfated hydroxyethyl chitooligosaccharide (SHECOS) potently prolonged the activabed partial thromboplastin time (APTT) corresponding to inhibition of an endogenous blood coagulation factor in the intrinsic pathway and thrombin time corresponding to inhibition of thrombin and fibrin formation pathway. Binding affinity assay using a surface plasmon resonance (SPR) spectometer showed that antithrombine III (AT III) bound to SHECOS could cersatilely inhibit activated blood coagulation factor; FXIIa, FXIa, FIXa, FXa, and FIIa (thrombin). It is possible to provide health benefits of a novel cutraceutical of pharmaceutical material with an anticoagulant activity.

P19

A GASTROINTESTINAL DIGEST OF ABALONE, *HALIOTIS DISCUS HANNAI* INTESTINAL PROMOTES OSTEOBLASTIC DIFFERENTIATION ON MG-63 CELLS

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Osteoblast differentiation and bone formation are accompanied in bone health. Some human bone diseases such as osteoporosis, osteoarthritis and rheumatoid arthritis are caused by imbalance between bone formation and bone resorption. Abalone is one of the important marine resources and have a high commercial value in the fishery industry. In this investigation, abalone intestines, one of the by-products of abalone processing, abalone intestine gastro-intestinal (AIG) and abalone intestine gastro-intestinal digests (AIGIDs) were used as bioactive substances by using invitro gastrointestinal digestion and an ultrafiltration system for testing osteoblast differention and bone formation. Our results indicated that AIG and AIGIDs significantly increased activity of alkaline phosphatase (ALP), a phenotypic markers for early-stage osteoblastic differentiation, and amount of hydroxyapatite as expression of mineralization processing. Interestingly, stimulation of AIG and AIGIDs have positive effect on BMP expression and promoted osteoblastic differentiation via MAPK pathway. The investigation may provide new insights in stimulating bone formation, contributing to regain balance of bone homeostasis for supplement in bone health.

P20

STRUCTURAL INVESTIGATION OF BIOGLYCAN FROM MARINE BACTERIUM *GLACIECOLA MESOPHILA* KMM 241 ^{T}

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Strain KMM 241^{T} was isolated from internal liquar of a specimen of the ascidian *Halocynthia aurantium*, collected from coastal sea water at a depth of 5 m in Troitsa Bay, Peter the Great Bay, Sea of Japan, Russia and classified as «*Glaciecola mesophila*» sp. nov. [1].

Cell wall glycopolymer of *G. mesophila* was isolated from wet bacterial cells by the phenol-water procedure. Bioglycan was purified from concomitant glucan by ion-exchange chromatography. Purified glycopolymer was studied by sugar analysis and NMR spectroscopy. GLC and GLC-mass spectrometry analysis of the acetylated alditols after hydrolysis and acetylated methyl glycosides after methanolysis of the bioglycan revealed resiudes of rhamnose (Rha), galactose (Gal) and glycerol (Gro). ^{1}H and ^{13}C-NMR spectra showed, *inter alia*, signals for two anomeric carbons and signal of methyl group of 6-deoxy sugar. ^{31}P spectra showed signal charactaristic for phosphodiester linkage. All two sugars were found to be α-linked. ^{1}H-NMR spectrum was interpreted using 2D homonuclear COSY and TOCSY experiments. With the ^{1}H-NMR spectrum assigned, the ^{13}C-NMR spectrum was interpreted using 2D heteronuclear ^{1}H, ^{13}C HSQC experiment. Linkage and sequence analysis of the polysaccharide was performed using a 2D ^{1}H , ^{1}H NOESY, ^{1}H, ^{13}C HMBC and ^{1}H, ^{31}P HMBC experiments. The absolute configurations of monosaccharides was established on the basis of the values of chemical shifts and α- and β-effects of the ^{13}C glycosylation. Thus, based on all our data obtained, we suggest the following structure for repeating unit of the glycopolymer:

→3)-α-L-Rha-(1→3)- α-D-Gal-(1→2)-Gro-3-P-(O→

It was shown that the main component of the cell wall of the bacterium was not lipopolysaccharide characteristic for Gram-negative bacteria, but teichoic acid-like glycopolymer. As known these glycopolymers are primarily components of the cell walls of gram-positive microorganisms [2]. Teichoic acid-like glycopolymer composed of rhamnose, galactose, and glycerophosphate to the best of our knowledge has been identified as part of the cell wall of gram-negative bacteria in the first time.

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P21

STRUCTURE OF O-SPECIFIC POLYSACCHARIDE OF THE MARINE BACTERIUM *RHEINHEIMERA PACIFICA* KMM 1406^T

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Gram-negative bacteria are an essential component of marine environments where they occupy diverse habitats, including coastal and open water areas, deep-sea and hydrothermal vents, bottom sediments as well as marine plants and animals. Marine Gram-negative bacteria of different genera produce superficial antigenic glycopolymers having unique structures. These bioglycans contain unusual acidic monosaccharides, 6-deoxyamino- and keto-sugars, higher sugars as well as non-carbohydrate substituents.

We studied the lipopolysaccharide of bacterial strain *Reinheimera pfcifica* KMM 1406^T isolated from a seawater sample taken at a depth of 5000 m from the northwestern Pacific Ocean [1]. The O-specific polysaccharide was obtained by mild acid degradation of the lipopolysaccharide isolated from bacterial cells by hot phenol-water extraction followed by gel chromatography. It was found that the polysaccharide contains 2-acetamido-2-deoxy-D-galactose (D-GalNAc), 2-acetamido-2-deoxy-D-galacturonic acid (D-GalNAcA), 2,4-diacetamido-2,4,6-thrideoxy-D-glucose (D-QuiNAc4NAc) and 4,6-dideoxy-(4-N-acetyl-alanine)-D-glucose (D-Qui4NAlaAc). Amino acid analysis confirmed the presence of GalN and showed the availability of alanine (Ala).

Solvolysis of the polysaccharide with anhydrous trifuoromethanesulfonic acid to give a disaccharide containing D-GalNAc and D-QuiNAc4NAc. The initial polysaccharides and the resultant disaccharide were studied by sugar analysis and ^{1}H and ^{13}C NMR spectroscopy, including COSY, TOCSY, ROESY and HSQC experiments. Based on the data obtained, we suggest the following structure of the repeating unit of the O-specific polysaccharide:

→4)-α-L-Gal*p*NAcA-(1→3)-α-D-Gal*p*NAc-(1→3)-β-D-Qui*p*NAc4NAc-(1→3)-β-D-Qui*p*4NAlaAc-(1→

This work was supported by the Russian Foundation for Basic Research (grant No. 12-04-00938-a).

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P22

BENEFICIAL EFFECT OF A OLIGOPEPTIDE FROM BIODIESEL BYPRODUCTS OF MARINE MICROALGAE, *NANNOCHLOROPSIS OCULATA* ON MG-63 AND D1 CELLS DIFFERENTIATION

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Osteoblasts are important bone cells responsible for bone formation, and these cells keep bone homeostasis with osteoclasts responsible for bone absorption. The homeostatic imbalance between osteoblasts and osteoclasts lead to the bone disease such as osteoporosis, osteoarthritis, or rheumatoid arthritis. Thus, development of the osteoblasts-active natural products could find out a desire therapy for treating severe bone diseases. To utilize biodiesel by-products of marine microalgae *Nannochloropsis oculata* and evaluate their beneficial effects, enzymatic hydrolysis was carried out using commercial enzymes such as alcalase, flavourzyme, neutrase, PTN, and protamex. Among the enzymatic hydrolysates of *N. oculata*, the alcalase hydrolysate exhibited the highest osteoblastic differentiation activity. To purify and identify a bioactive substance from the hydrolysates, consecutive HPLC purification system and tandem mass analysis for the amino acid sequence was performed. The results show that the osteoblast-differentiatory peptide from *N. oculata* (OPNO) has amino acid sequence as MPDW and low molecular weights of 601 Da. In osteoblast differentiation *in vitro* assays, the results showed that OPNO promotes osteoblast differentiation by increasing expression of several osteoblast phenotype markers such as alkaline phosphatase (ALP), osteocalcin, type I collagen, and bone mineralization in both human osteoblastic cell (MG-63) and murine mesenchymal stem cell (D1). In addition, the peptide induced phosphorylation of MAPK and Smad pathway in both cells. These results suggest that OPNO possesses positive effects on osteoblast differentiation and may provide possibility for treating bone diseases.

P23

NEURITOGENIC AND NEUROPROTECTIVE EFFECTS OF POLAR STEROIDS FROM THE FAR EAST STARFISHES *PATIRIA PECTINIFERA* AND *DISTOLASTERIAS NIPON*

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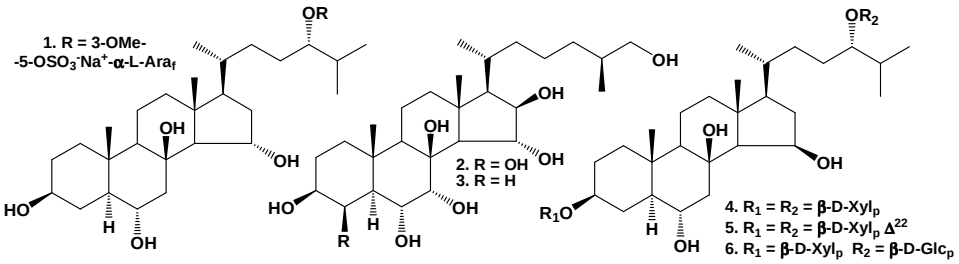
Neurotrophic factors (NTFs), such as nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF), are essential for modulation of neuron cell survival, promotion and maintenance of neurite networks. NTFs have potential as remedial drugs against brain ischemia and different neurodegenerative diseases. However, the large sizes of their molecules produce considerable difficulties in passing through the blood-brain barrier. Therefore, neurotrophin small molecule mimetics might be interesting as candidate therapeutic agents against neurological disorders.

Earlier the polar steroids (polyhydroxysteroids and related glycosides) from different species of starfish have been reported as natural compounds which can induce neurite outgrowth on mouse neuroblastoma C-1300 (NB) cells [1-3]. The present investigation is devoted to more profound study of neuritogenic activities of six starfish steroidal substances: asterosaponin P_1, octaol, heptaol (1-3) from *Patiria pectinifera* and distolasterosides D_1-D_3 (4-6) from *Distolasterias nipon* in the culture of NB cells. The neuritogenic effects of these compounds appeared on day 2 after the treatment. The lifetime observation and experiments with silver impregnation showed that the substances 1-3 beginning from a dose of 0.05 μM and up as well as substances 4-6 beginning from a dose of 0.005 μM and up increased the percentage of neuronal differentiation (percentage of cells bearing neurites longer than two cell diameters or bearing more than two processes). The dose-dependent response of the neuritogenic activity of the compounds 1-6 was also observed. Moreover, all tested substances exhibited notable synergistic effects on the NGF- or BDNF-induced neurite outgrowth of NB cells.



Neuroprotective activity of starfish steroidal substances against oxygen-glucose deprivation (OGD) was elucidated in the culture of NB cells and organotypic hippocampal slice cultures. It was shown that the treatment of both cultures exposed to OGD with compounds 1-6 increased the percentage of living cells and the found effects were comparable to action of a vascular endothelial growth factor (VEGF).

It may be assumed that both the NGF-like neuritogenic and neuroprotective activities are common properties of starfish polyhydroxysteroids and related glycosides, although the level of these effects depends upon peculiarities of their structures. The obtained data showed that the tested steroidal compounds are promising candidates for further investigation as potential neurotrophic and neuroprotective preparations.

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P24

AMINO ACID SEQUENCE OF TYROSYLPROTEIN SULFOTRANSFERASE FROM THE MARINE GASTROPOD *LITTORINA SITKANA*

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Tyrosine sulfation of proteins and peptides is one of the most common post-translational modifications in multicellular eukaryotes. Tyrosylprotein sulfotransferase (TPST, EC 2.8.2.20) are localized in the trans Golgi network and catalyse transfer of a sulfuryl group (SO_3^{2-}) from universal sulphate donor PAPS to the hydroxyl group of the tyrosyl residues in peptides and proteins.

Tyrosine-sulphated proteins have been observed not only in many tissues in mammals, but also in all vertebrates and invertebrates and also in lower multicellular organisms [1]. The known functions of tyrosine sulfation in mammals are various and multiple such as: maintenance of haemostasis, triggering inflammatory responses, strengthening leucocyte adhesion, defining specificity of chemokine receptors and enhancement of potency of bioactive peptides [2]. In spite of importance of the processes in which TPSTs are involved, almost all known TPSTs are mainly of mammal origin. Only few enzymes from terrestrial invertebrates have been studied. It is well known that marine habitats are a rich sources of unique enzymes very useful for original biotechnological applications. Hence the present study was devoted to the search for the tyrosylprotein sulfotransferases among the marine molluscs.

The gastropod mollusk *L. sitkana* was chosen for tyrosylprotein sulfotransferase search. The obtained cDNA sequence of 714 bp encoded 238 amino acids long fragment of TPST. TPST sequence comparisons have shown 78-84% similarity and 65-67% identity with human TPST-2 and other orthologues. The search for TPST genes expressed in other species of marine mollusks were also carried out. According to obtained PCR results only the bivalve *Mizuhopecten yessoensis* had cDNA encoded TPST and the other 8 species had not.

This raises several questions about the role of TPST genes in invertebrates and also about their physiologic alternatives in organisms, in which they are absent.

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P25

SCREENING OF POTENTIAL HIV-1 INHIBITORS/REPLICATION BLOCKERS USING SECURE LENTIVIRAL IN VITRO SYSTEM

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1. The development and usage of safe cell systems for testing agents which possess anti-HIV activity is a very important factor in the design of new drugs. We have described in detail a system we designed that is based on lentiviral vectors for swift and completely safe screening of potential HIV-1 replication inhibitors. The system enables one to test the efficiency of the inhibitory activity of compounds whose action is directed towards either wild-type HIV-1 reverse transcriptase or integrase, or mutant enzymes corresponding to the drug-resistant virus form. Testing results of a number of already known drugs, which correlate well with published data as well as data on newly synthesized compounds, were obtained. Application of this system substantially broadens the possibilities of preclinical anti-HIV drugs testing.

2. The effect of sulfated polysaccharides on the efficiency of infection of mouse embryonic fibroblast cell lines SC-1 and NIH 3T3 by replication competent recombinant Moloney murine leukemia virus (Mo-MuLV) carrying the eGFP gene was investigated. It was shown that used polysaccharides have no cytostatic and cytotoxic effects on SC-1 and NIH 3T3 cells in the concentrations from 0.01 to 100 μg/ml and have virucidal activity against Mo-MuLV.
Polysaccharides in the indicated concentrations inhibit cell infection by Mo-MuLV, that prevents further expansion of viral infection. It was detected that sulfated polysaccharides are effective inhibitors of other retroviruses, including lentiviruses, that use heparan sulfate as cell receptors for nonspecific binding

P26

AN ANTI-INFLAMMATORY PEPTIDE ISOLATED FROM INTESTINE OF MARINE MOLLUSK, *H. DISCUS HANNAI* ON LPS-INDUCED CYTOKINE PRODUCTION VIA THE P-P38/P-JNK PATHWAYS IN RAW 264.7 MACROPHAGES

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A marine mollusk, abalone (*Haliotis discus hannai*) is one of the important fishery species in aquaculture industry, but nutraceutical and pharmaceutical benefits of *H. discus hannai* have been rarely identified and studied [1]. To evaluate beneficial effects of *H. discus hannai*, an anti-inflammatory peptide (AAIP, abalone anti-inflammatory peptide) was purified from abalone intestines using consecutive HPLC purification system. In tandem MS analysis, the fragmentation results illustrate that the AAIP responsible for the nitric oxide (NO) inhibitory activity (IC_{50}= 55.8 uM) has amino acid sequence as Pro-Phe-Asn-Glu-Gly-Thr-Phe-Ala-Ser (1175.2 Da). To investigate anti-inflammatory effect of AAIP on lipopolysaccharide (LPS)-stimulated RAW264.7 macrophages and elucidated the molecular mechanism. The results show that the AAIP peptide suppresses LPS-induced production of nitric oxide (NO) via inducible nitric oxide synthase (iNOS) expression in a dose-dependent manner. It also significantly reduced the gene transcription of proinflammatory cytokines, such as interleukin (IL-1β), tumor necrosis factor (TNF-α), and IL-6. Furthermore, AAIP significantly suppresses phosphorylation of mitogen-activated protein kinases (MAPKs) such as p-p38 and p-JNK. These results indicated that abalone intestine-derived anti-inflammatory peptide, AAIP inhibits LPS-induced inflammatory response via blocking of MAPK pathway in murine macrophages. Therefore, potent anti-inflammatory effects of the natural peptide from abalone might suggest possibility for high valuable utilization and application as nutraceutical and therapeutic substances.

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P27

ISOLATION OF ANGIOTENSIN I CONVERTING ENZYME (ACE) INHIBITOR FROM BIODIESEL BYPRODUCTS OF MARINE MICROALGAE, *NANNOCHLOROPSIS OCULATA*

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To utilize biodiesel byproducts massively discarded from the lipid extraction step of marine alga *Nannochloropsis oculata* and investigate its biomedical and nutraceutical benefits, we examined an angiotensin I converting enzyme (ACE-I) inhibitory activity of various enzymatic hydrolysates of biodiesel byproducts of *N. oculata*. Among the enzymatic hydrolysates prepared using various commercial enzymes such as Alcalase, Neutrase, Flavourzyme, PTN, and Protamex, the biodegradable hydrolysate produced by Alcalase showed the highest ACE-I inhibitory activity (IC_{50}= 89.2 ug/ml). Using consecutive purification using a Hiprep 16/10 DEAE FF anion exchange and an octadecylsilane (ODS) C18 reversed phase liquid chromatographic techniques, a novel ACE-I inhibitory oligopeptide from *N. oculata* (NAIP) was purified and identified to be a pentameric peptide (LVTVM, 561 Da) by the tandem MS analysis. Lineweaver–Burk plots illustrate that the purified peptide, NAIP play a role as a non-competitive inhibitor against ACE-I. MTT assay showed no cytotoxicity on human embryonic lung fibroblasts cell line (MRC-5). Hence, this study suggests that the ACE-inhibitory peptide derived from biodiesel byproducts of *N. oculata* could be applied in nutraceutical and pharmaceutical applications as potential candidates.

P28

PROTECTIVE EFFECTS OF EMODIN AND CHRYSOPHANOL ISOLATED FROM MARINE FUNGUS *ASPERGILLUS. SP* ON ETHANOL-INDUCED TOXICITY OF IN HEPG2/CYP2E1 CELLS

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Alcohol-induced liver injury progresses from fatty infiltration and follows a harmful course of inflammation leading to irreversible damage. In this study, we isolated two compounds (emodin and chrysophanol) from marine fungus *Aspergillus* sp and examined whether they could protect against ethanol-induced toxicity *in vitro*. When the ethanol-induced HepG2/CYP2E1 cells were treated with compound at various concentrations, there was a dose-dependent decrease of Gamma-Glutamyl transpeptidase (GGT) activity and increase glutathione (GSH) in the media and loss of cell viability. Furthermore, the protect effects of compound were evaluated by protein expression levels of GGT, GSH and CYP2E1 using western blot. Among the compounds, emodin attenuated the ethanol cytotoxicity effectively compared to the chrysophanol. It could be suggested that emodin from this genus would be more potential candidates for attenuating ethanol induced liver damage for further industrial applications as functional food and pharmaceuticals.

P29

ANTIOXIDANT ACTIVITY OF FERMENTED MARINE MICROALGAE, *PAVLOVA LUTHERI* (HAPTOPHYCEAE) WITH *HANSENULA POLYMORPHA* IN MACROPHAGE CELL

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Microalgae are major primary producers of organic matters in aquatic environments through their photosynthetic activities. Fermented microalgae (*Pavlova lutheri*) preparation (FMP) is product of yeast fermentation of *Hansenula polymorpha*. It was tested for their antioxidant activities including lipid peroxidation inhibitory activity, free radicals scavenging activity, inhibition of reactive oxygen species on mouse macrophages, RAW264.7 cell and inhibited myeloperoxidase (MPO) activity in human myeloid cells (HL60). FMP exhibited the highest antioxidant activity and inhibitory intracellular ROS such as hydroxyl and superoxide radicals. And MTT assay showed no cytotoxicity on mouse macrophages cell (RAW264.7), human myeloid cells (HL60) and human fetal lung fibroblast cell line (MRC-5). Further, the antioxidant mechanism of FMP was evaluated by protein expression levels of antioxidant enzyme (superoxide dismutase and glutathione) using western blot. The results obtained in the present study indicated that the FMP is a potential source of natural antioxidant.

P30

PURIFICATION OF PEPTIDE FROM ABALONE *HALIOTIS DISCUS HANNAI*, THAT INHIBITS MMP-2 AND MMP-9 EXPRESSION THROUGH NF-KB ACTIVATION IN HUMAN FIBROSARCOMA CELLS

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Abalone (*Haliotis discus hannai*), which is one of the most commercially important gastropods in aquaculture. Abalone intestine gastrointestinal digests III (AIGID III) with below 10 kDa molecular weight, which fractionated using ultrafiltration (UF) membrane bioreactor systems. AIGID III was purified by recycle chromatography on JAI W253 silica-gel column and reverse-phase high-performance liquid chromatography (HPLC) on a OHpak SB-803 HQ column. A fraction V exhibited the highest inhibitory effect on MMP-2 and MMP-9 activity in HT1080 and amino acid sequence of peptide was identified as Ala-Glu-Leu-Pro-Ser-Leu-Pro-Gly with a molecular size of 907.0 Da. Further, the purified peptide could attenuate protein expression of p50 and p65, a part of nuclear transcription factor kappa B (NF-κB). These results suggest that purified peptide down-regulates MMP-2 and MMP-9 expression in HT1080 cells through the NF-κB-mediated pathway.

P31

AN OLIGOPEPTIDE ISOLATED FROM FERMENTED MICROALGAE, *PAVLOVA LUTHERI* THAT INCREASE ALP ACTIVITY THROUGH MAPK ACTIVATION, AND INDUCES OSTEOBLASTIC DIFFERENTIATION

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In the present work, fermented microalgae (*Pavlova lutheri*) preparation is product of yeast fermentation by *Hansenula polymorpha*. And purified and characterized a alkaline phosphatase (ALP) activity peptide from fermented microalgae. After its separation from the fermented microalgae by several purification steps, the peptide responsible for the ALP activity was isolated and its sequence was identified as Glu-Pro-Gln-Trp-Phe-Leu (908.9 Da). Our results indicate that a non-toxic peptide can increase activity of ALP as phenotypic markers for early-stage osteoblastic differentiation. Furthermore, the results showed positive effects of peptide on alkaline phosphatase and bone morphogenic protein-2 (BMP-2) as important factors for bone formation, remodeling and mineralization. To elucidate the mechanisms by which the peptide acted, we examined its effects on TPA-induced MAPKs/NF-κB activation and determined that the peptide treatment significantly reduced p-p38 (p-ERK) kinase/NF-κB (p65) in MG-63 cells. The present study may provide new insights in the osteoblastic differentiation of purified peptide and possibility for its application in bone health supplement.

P33

COMPOSITION AND CONTENT OF THE SULFATED POLYSACCHARIDES IN BROWN ALGAE AT DIFFERENT STAGE OF ALGAE ONTOGENESIS

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The brown seaweeds are the rich and renewable source of biological active polysaccharides: fucoidans, lainarans and alginic acids. Russian Far East seas are rich with brown algae. The main problem of medical application of polysaccharides to obtain of pure polysaccharides possessing standard properties. The content and composition of polysaccharides are known to change depending on species, stage of algae ontogenesis, location, methods of extraction. The investigation of monthly changes in the composition and content of the polysaccharides, physiological age where algae synthesized maximum quantity of polysaccharides with a fixed structure and the highest biological activity are necessary for determining of the optimal time for collecting algae in order to standardize preparation of these polysaccharides.

Sterile and reproductive tissue of seven brown algae (*Alaria ochotensis, Costaria costata, Fucus evanescens, Sargassum pallidum, Silvetia babingtonii, Saccharina japonica* and *Undaria pinnatifida)* from Russian Far East seas were comparatively for the contents and monosaccharide compositions of fucoidans. It was proved that reproduction stages have an apparent effect on fucoidan content and its' monosaccharide composition. Fucoidan content in fertile tissue was pointed out to be 1.3 – 5.0 times as high as in sterile ones. Fertile plants of *S. babingtonii* yielded the highest fucoidan content (25 % dw).

Structural changes of the polysaccharide are species-specific and perhaps, depend on the type of the synthesized polysaccharide. It was determined that the sterile algae synthesized a low-sulfated heteropolysaccharide and the fertile plants – highly-sulfated polysaccharide containing are fucose and galactose as main components. Based on the present and previously reported data we believe that fucoidan accumulation during reproductive structure development is a common trend for brown seaweeds. Reproduction caused changes in the biosynthesis of the polysaccharide. It has been suggested that the content and composition of structural polysaccharides are determined to a large extent by life cycle stage rather than by effects of environmental factors.

P34

BIOACTIVE METABOLITES FROM A MARINE-DERIVED FUNGUS *PENICILLIUM THOMII* KMM 4645

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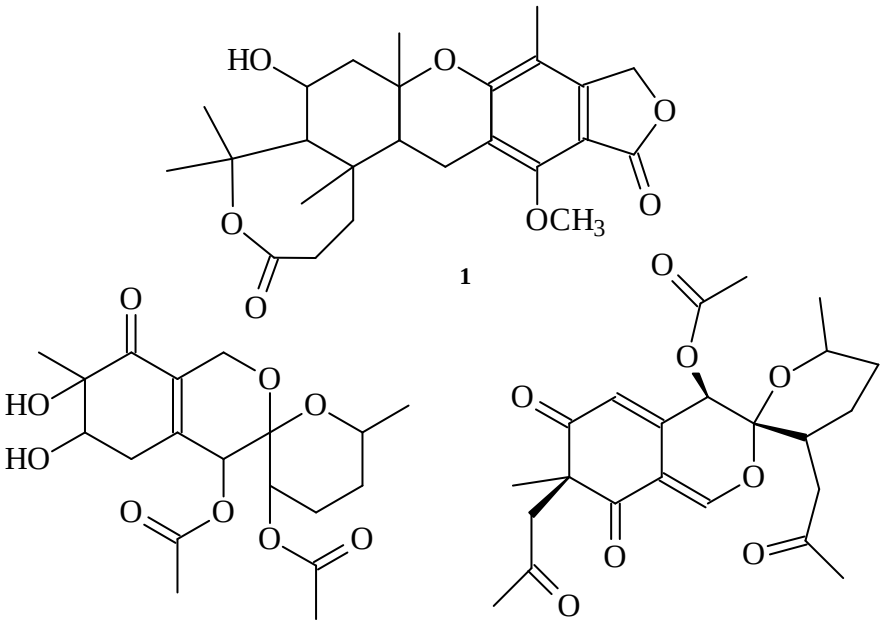
In our search for the biologically active substances from marine-derived fungi, we found that *Penicillium thomii* KMM 4645, associated with *Sargassum miyabei* (Lazurnaya Bay,the sea of Jupan), produce two new substances Austalide J-1 (1) and Pestafolide B (2) together with known Daldinin D (3). The structures of these compounds were determined mainly by analysis of their NMR spectroscopic data. The Pestafolide B (2) and Daldinin D (3) don't show cytotoxic activity against HL-60 and HeLa human cancer cell lines (IC_{50} >200 μM, evaluated with MTS-test), at the same time the substances able to inhibit the growth of human cancer HeLa cell colonies in soft agar at non-cytotoxic concentrations of 15.6 and 12.0 μM respectively. Compounds 2 and 3 do not effect on action of the 1→3-*β*-D-glucanase (laminarinase) from *Spisula* (*Pseudocardium*) *sacchalinensi*s and *Perna viridis*. The biological activity of 1 not examined.



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3

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STRUCTURE AND ANTI-HIV ACTIVITY OF AN UNFRACTIONATED FUCOIDAN FROM BROWN SEAWEED *TUBINARIA ORNATA*

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1. Introduction: Fucoidans are sulfated polysaccharides derived from marine brown seaweed. They were reported to exhibit a wide range of biological activities. The structure of fucoidan isolated from Turbinaria Ornata is still poorly investigated in contract to other fucoidan The aims of this study are to elucidate structure of fucoidan extracted from brown seaweed Turbinaria Ornata collected at Vietnam coast by tandem ESI-MS and to investigate its anti-HIV activity.

2. Experimentals

Extraction: Turbinaria Ornata species was collected at Nha Trang Sea in April, 2008 and identified by Dr. Le Nhu Hau. Extraction was followed the method of Bilan et al (Carbonhydr. Res., 339, 511-517, 2004)

Chemical analysis: Neutral monosaccharide compositions were elucidated by the method of Bilan et al. (Carbohydr. Res., 337, 719-730, 2002) .Uronic acid content was determined by following the carbazole method (Anal. Biochem., 4, 330-334, 1962. Sulfate content was estimated using gelatin/BaCl2 method (Biochem. J., 78, 312-319, 1961).

Acid hydrolysis: Acid hydrolysis of fucoidan was carried out using TFA (0.75 M, 1 h, 60oC).

ESI-MS: ESIMS experiments were performed on a Xevo TQ MS, Waters-USA. The analyses were carried out in negative mode.

Anti-HIV activity: MTT assays were realized at Retrovirology Laboratory – CRP Santé – Luxembourg, on human T lymphocyte cell line (MT4) which had been transferred by VLTH-1 (Virus lymphotropique T humain). (Vandamme et al. 2000 and Reed L.J., Muench H 1938).

3. Results and Discussion

Sugar composition of un fractionated fucoidan is simple, it contains mainly two kinds of sugar with the molar ratio Fucose: Galactose = 1.0:0.3. Figure 1 shows the mass spectrum of hydrolysed fucoidan. The assignments represented are anionised fragments (Table 1). The MS2 of base peak at m/z 243.07 corresponding to the monosulfated fucose was fragmented. Signals from C-4 (m/z 183.02) and C-2 (m/z 138.86) sulfation of α-L-Fucp residues were detected The ion at m/z 138.86 was the major fragment indicating that the fucosyl units are mainly sulfated at position 2. The MS2 of ions at m/z 307.06; 389.09; 405.01; 491.02 and 535.14 also have been done. All of the results indicated that unfractionated fucoidan from brown seaweed Turbinaria Ornata is predominantly (1→3) α-L-fucose residue and sulfate groups occupied mostly the C-2 and partly the C-4 positions of the fucose residues.

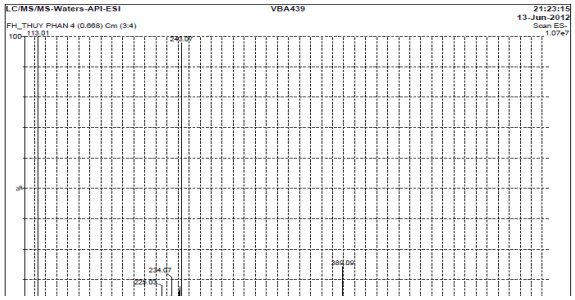

Figure 1: ESI-MS of sulfated oligosaccharides derived from the hydrolysis of fucoidan

m/z	composition
225.03	$[Fuc_1SO_3Na\text{-}H_2O\text{-}Na]^-$
234.07	$[Fuc_2(SO_3Na)_2\text{-}2Na]^{2-}$
243.07	$[Fuc_1SO_3Na\text{-}Na]^-$
298.15	$[Fuc_3(SO_3Na)_2\text{-}H_2O\text{-}2Na]^{2-}$
307.06	$[Fuc_3(SO_3Na)_2\text{-}2Na]^{2-}$
389.09	$[Fuc_2SO_3Na\text{-}Na]^-$
405.01	$[Fuc_1Gal_1SO_3Na\text{–}Na]^-$
453.06	$[Fuc_5(SO_3Na)_7\text{-}2Na]^{2-}$

Table 1: Fragmentation of fucoidan by negative ESI-MS

Fucoidan sample was investigated for anti-HIV activity. The results indicated that our fucoidan has protection to cell against HIV-1 with IC50 3.63µg/ml**.**

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STRUCTURE OF THE O-SPECIFIC POLYSACCHARIDE FROM A MARINE BACTERIUM *ECHINICOLA PACIFICA* KMM 6172^T CONTAINING 2,3-DIACETAMIDO-2,3-DIDEOXY-D-GLUCURONIC ACID

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The genus *Echinicola* was proposed to accommodate heterotrophic, Gram-negative, gliding and pigmented bacteria belonging to the family *Cyclobacteriaceae* of the phylum *Bacteroidetes*. *Echinicola pacifica* strain KMM 6172^T (=KCTC 12368^T =LMG 23350^T) from the Collection of Marine Microorganisms of the Pacific Institute of Bioorganic Chemistry, Far East Branch of the Russian Academy of Sciences was isolated from the sea urchin *Strongylocentrotus intermedius* collected in Troitsa Bay, Gulf of Peter the Great, East Sea (also known as the Sea of Japan) [1].

The O-specific polysaccharide *E. pacifica* was obtained by mild acid degradation of the lipopolysaccharide isolated from dried bacterial cells by the extraction with aqueous phenol and studied by chemical methods and ^{1}H and ^{13}C NMR spectroscopy, including 2D COSY, TOCSY, ^{1}H,^{13}C HMQC and HMBC experiments. Sugar analysis and NMR spectroscopy revealed that the polysaccharide contains D-galactose (D-Gal), L-rhamnose (L-Rha), 2-acetamido-2-deoxy-D-galactose (D-GalNAc), 2,3-diacetamido-2,3-dideoxy-D-glucuronic acid (D-Glc*p*NAc3NAcA) and O-acetyl group in nonstoichiometric amount (80%).

In addition, the polysaccharide was subjected to the solvolysis to confirm the full structure of repeating unit and receive the additional information. The resultant fragment was analyzed by 1D and 2D NMR spectroscopy as the initial OPS. It was found that this fragment is the disaccharide consisted of two (1→4)-linked 2,3-diacetamido-2,3-dideoxy-D-glucuronic acid residues. Based on the data obtained the following structure of the branched pentasaccharide repeating unit was established:

β-D-Glc*p*NAc3NAcA4Ac-(1→4)-β-D-Glc*p*NAc3NAcA
1
↓
3
→6)-β-D-Gal*p*-(1→3)-β-L-Rha*p*-(1→4)-β-D-Gal*p*NAc-(1→

This work was supported by the Russian Foundation for Basic Research (grant No. 12-04-00938-a).

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BLACK SEA BACTERIA – PRODUCERS OF GLYCOSIDASES AND PROTEASES

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The marine microorganisms are the major contributors of vital important organic substances in complex ecosystem of World Ocean due to expression of unique enzymes spectrum, including hydrolyses which modify the biopolymers [1]. The enzymes from marine microorganisms are characterized by higher activity, low temperature optimum, the resistance to chemical modifications and prolonged storage [2, 3]. On the contrary to microorganisms isolated from Pacific and Indian Oceans, the Black Sea microorganisms studied insignificantly up to present. Therefore the aim of the present investigation was to identify bacteria, isolated from Black Sea and study their ability to produce enzymes with glycolytic and proteolytic action. Sixty four strains, isolated from water and invertebrates of Black Sea were identified as representatives of the following genus: Alteromonas, Halomonas, Marinomonas, Oceanimonas, Pseudoalteromonas and Shewanella. It was shown that 64% of the studied strains displayed the capacity to the synthesis enzymes with α-L-rhamnosidase activity which varied from 0.01 to 0.20 U/ml depending on the strain. The greatest number of the enzyme producers was found in representatives of Alteromonas macleodii. Other investigated glycosidase activities: amylase, β-N-acetyl-D-glucosaminidase, α-N-acetyl-D-glucosaminidase, α-N-acetyl-D-galactosaminidase, β-N-acetyl-D-galactosaminidase, β-D-glucoronidase, β-D-galactosidase, α-D-galactosidase, β-D-glucosidase, KM-cellulase activities though have been found, but mainly with inconsiderable indices. α-D-Glucosidase, α-D-mannosidase, α-L-fucosidase, β-D-xylosidase and α-D-xylosidase activities were found in neither of the strains tested.

Strains with rather high proteolytic activity were found among marine species of bacteria. It has been established that 18 strains (28%) of 64 marine isolates were characterized by rather high level of total proteolytic activity (from 0.1 to 0.5 U/ml), 43.75% of them displayed inconsiderable (up to 0.1 U/ml) or only trace (up to 0.01 U/ml) activity, 18.75% did not display any hydrolytic activity in respect of casein. Investigations of substrate specificity to a number of fibrillar and globular proteins of nine strains studied, which displayed considerable general (caseinolytic) activity has shown that eight of them displayed fibrinolytic activity from 0.15 to 2.175 U/ml. All nine strains were characterized by gelatin activity. Collagenase and keratinase activity was also revealed. Neither of nine strains exerted elastase activity.

So we showed that Black Sea as Pacific Ocean inhabit a number of microorganisms which may be effective producers of important for practice enzymes such as α-L-rhamnosidases and proteases in particular with fibrinolytic activity. These enzymes may be perspective in using for medicine and various biotechnological processes.

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RED SEAWEEDS FROM PACIFIC COAST AS SOURCE OF BIOLOGICAL-ACTIVITY SUBSTANCE. PERSPECTIVES OF CARRAGEENANS APPLICATION

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Red algae are a source of interest on the structure, physico-chemical properties and biological activity of polysaccharides and pigments. Polysaccharides – carrageenans belonging to food fibers have been safely consumed as constituents of food products for many years and have pronounced properties useful in the biomedical field. Carrageenophyte seaweeds are abundant on Pacific coast and species from family *Gigartinaceae, Tichocarpaceae, Phyllophoraceae* were studied.

The structure of kappa, lambda (*Chondrus armatus),* kappa/iota *(C. pinnulatus*) and kappa/beta and new type of carrageenan from *Tichocarus crinitus* were established. The dependence of the macromolecular organization of carrageenans on the features of their structure was shown by electron and atomic force microscopy, light scattering. On the basis of the data received in experiments *in vitro* and *ex vivo*, the dependence of some biological properties of polysaccharides on their structure was shown. The anticoagulant activity of carrageenans depends on the molecular weight of polysaccharides, sugar composition, sites of sulfation and the distribution of sulfated units along the galactan chain. The ability of carrageenans to influence on the cytokine production in the human blood cells largely dependences on the structure of polysaccharides and concentration. The carrageenans were able to increase the synthesis of anti-inflammatory interleukin-10 (IL-10) and at low concentrations their activity (kappa/beta-type) was higher than that of LPS alone. The activation of cells by carrageenans occurs through specific for LPS TLR4 receptors. Since cytokines play a critical role in regulating inflammatory and immunological processes of the host, *in vivo* administration of carrageenan may influence antibacterial host-defense systems and the infection process through modulation of cytokine production. The protective effect of carrageenans against damaging effect of endotoxin of gram-negative bacteria (LPS) was obtained *in vivo*. Pretreatment with carrageenans significantly prolonged survival of mice against LPS-challenge. The degree of polysaccharide protection depends on the structure of carrageenans, polysaccharide concentration and administration time and route. A nonspecific resistance of the organism to *E. coli* LPS induced by carrageenan was studied on laboratory animals by investigating the biochemical and pathomorphological parameters. The mechanism of the host resistance to endotoxin induced by carrageenan can be caused by an immunomodulating effect of these polysaccharides. At the same time, a transformation of the ultrastructure of LPS by action of carrageenans is observed. Moreover, carragenan changed both size and Zeta-potetntials of LPS from *E. coli* and *Y. pseudotuberculosis*.

The estimation of therapeutic action of food supplement (“Carrageenan-DV”) in complex therapy of patients by sharp intestinal infections was given. Carrageenan restored the system of the hemostatic and corrected parameters of immune system of organism in the course of treatment of patients with food toxic infection of Salmonella etiology more actively in comparison with a control. These results allow us to hope for a practical application of carrageenans for lowering the toxemia level observed under the development of the infectious process caused by Gram-negative bacteria. Comparative analysis of blood patients with coronary heart diseases receiving a favor therapy for a long time before and after application of “Carrageenan-DV” elicited statistically significant reduce in the levels of total cholesterol and low density lipoprotein cholesterol down to normal levels. The results of that investigation demonstrated that application of “Carrageenan-DV” as addititious food fiber source during complex therapy of patients with cardio vascular disorders contributed normalization of indices of lipid metabolism and chronic inflammatory process.

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STRUCTURAL CHARACTERIZATION OF MARINE POLYSACCHARIDES BY MEANS OF SMALL ANGLE X-RAY SCATTERING

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Introduction

We find many kinds of electrolyte polysaccharides in seaweeds, as exemplified with carrageenan, alginate, which have sulfate groups and carboxyl groups. They are applied for food additives as increasing viscosity or gelling agent, because their aqueous solution gives high viscosity or gels at various conditions. Their properties are affected with the concentration of metal salts and/or their kind. These behaviors are related with the solution structure of polysaccharide and their assembly structure at molecular level or nano-scale.

Recently the fucoidan, having many sulfate groups, reveals various physiological activities, as antiviral, antitumor, and so on. In order to this kind of mechanism, it is very important to elucidate the structure of association between protein and electrolyte polysaccharide.

In this study structural characterization of marine polysaccharide is performed by small angle X-ray scattering (SAXS).

Experimental

The fucoidan sample, derived from *Turbinaria Ornata* species, was collected along the central coasts of Vietnam. This chemical composition was analyzed as consisting of fluctose and galactose with high content of sulfate.

SAXS experiments were carried out at Photon Factory, Tsukuba, Japan. The X-ray beam from synchrotron radiation was used. The scattered X-ray was detected by Imaging Plate (IP) positioned at the distance of about 1 meter form sample holder.

Results and Discussion

Figure 1 shows the Kratky plots ($q^2I(q)$ vs q, where $I(q)$ is scattering intensity and q is the magnitude of scattering defined by $(4\pi/\lambda)\sin(\theta)$ with λ the wavelength of incident beam, 2θ scattering angle) for 1% fucoidan in water and in 0.5M NaCl. The weak peak can be found in 0.4 nm^{-1} of q, due to the repulsive electrostatic interaction. This means that this fucoidan sample contains much amount of sulfate groups. The addition of saline, the effect shielding against that force, made the peak disappear. The maxim in 0.75 nm^{-1} indicates the chain thickness of fucaidan as estimated by about 1 nm.

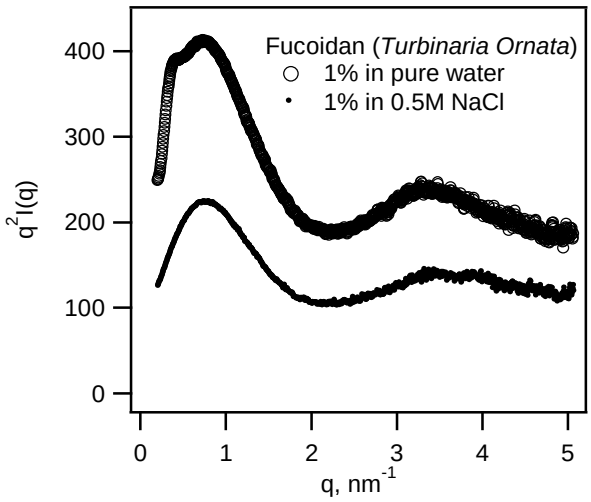

Figure 1. Kratky plots for SAXS from fucoidan 1% in water and in 0.5M NaCl

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SUBSTRATE SPECIFICITY AND CATALYTIC PROPERTIES OF THE FUCOIDANASE FROM *LAMBIS* sp.

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Fucoidanase from marine mollusk *Lambis* sp. was isolated from hepatopancreas to homogeneity with yield of 0.011 %. Molecular mass of the fucoidanase estimated by MALDI TOF was 38583 Da.

Fucoidanase was stable at high temperatures, half-live time inactivation was 20 minutes at 55° C and optima of temperature was 45° C. The maximum activity was observed at pH 5.0.

The presence of Mg^{2+}, Ca^{2+} and Ba^{2+} cations weakly activated the enzyme, cations of Cu^{2+}, $Zn^{2+}$and Hg^{2+} has possessed by the evident inhibitory effect on enzymatic activity.

Fucoidanase catalyzed hydrolysis of the fucoidans from *F. evanescens* and *Fucus vesiculosus* containing 1→3 and 1→4 glycosidic links but not fucoidan from *Saccharina cichorioides* consist of sulfated 1→3 linked α-L-fucopyranose only. It was noted that fucoidanase catalysed hydrolysis of desulfated fucoidan from *F. evanescens*

For detailed investigation of the specificity of fucoidanase the products of enzymatic transformations *F. evanescens* fucoidan were obtained. The products of enzymatic hydrolysis were oligosaccharides with a degree of polymerization *n*= 2-10. The major products of F. evanescens fucoidan transformation were di- and tetrasaccharides. It were analyzed by ^{1}H NMR spectroscopy. Accumulation of 1→3 linkages in the enzymatic hydrolysis products indicates that fucoidanase specific to the splitting of 1→4 glycosidic bound in fucoidan molecules.

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THE NOVEL ALGINATE LYASE WITH RARE SPECIFICITY FROM THE MARINE MOLLUSK *LAMBIS* SP.

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Alginate lyases or alginases catalyzes the degradation of alginate by a β-elimination mechanism, targeting the glycosidic 1→4 O-linkage between monomers with formation of 4-deoxy-L-eritro-hex-4-enopyranosyluronic acid as the nonreducing terminal moiety. At the moment alginate lyases have been isolated from many sources, including marine algae, some microorganisms and marine invertebrates. Mainly alginate lyases isolated from mollusks such as Haliotis, Dlabella, Littorina, Aplysia, and Turbo are classified into exo- or endo-type poly(M)-lyase. Only few G-specific lyase from the marine mollusk are found and classified as exo-polyguluronate lyase.

In present work we report about alginate lyases isolated from marine mollusk *Lambis* sp. and classified as endo-polyguluronate lyase (EC 4.2.2.11).

Marine mollusk *Lambis* sp. was collected from the South China Sea in the coastal area of Vietnam. Alginate lyase was purified from a hepatopancreas to homogeneous state with yield of 3.2 %. Molecular weigh of isolated alginate lyase was 31 kDa by SDS-PAGE and 25267 Da by MALDI TOF which is typical for alginases from marine invertebrates. Alginate lyase was stable at high temperatures; a half-inactivation time was 20 minutes at 64° C and optima of temperature was 45-50° C. The maximum activity was observed at pH 5.0. Cations are through to decrees the surface density of substrate charge, weakening the ionic interactions between alginate and enzyme. The presence of Mg^{2+}, Ca^{2+} and Ba^{2+} cations weakly activated the enzyme, cations of Cu^{2+}, $Zn^{2+}$and Hg^{2+} has possessed by the evident inhibitory effect on enzymatic activity. An inhibitory action of Hg^{2+} indicates that alginate lyase is SH-enzyme.

Alginate lyase from *Lambis* sp. catalyzed cleavage of polyguluronic acid with formation of unsaturated di-, tri- and tetraguluronate oligosaccharides as major products of enzymatic hydrolysis based on the ESI-MS mass-spectrometry. 1H NMR spectra of end products of enzymatic hydrolysis are revealed presence of double-bound at non-reducing end of guluronate oligosaccharides that are proving lyase mechanism of enzyme action.

So, alginate lyase isolated from *Lambis* sp. catalyzed cleavage of α-1→4 glycosidic bounds in alginates by end-type of action.

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ANTIOXIDANT AND ANTIHYPERLIPIDEMIC ACTIVITIES OF POLYSACCHARIDES FROM *LAMINARIA JAPONICA* AND *APOSTICHOPUS JAPONICUS*

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Polysaccharides from marine alga and invertebrate possess various bioactivities. The antioxidant activity of polysaccharides from sea cucumber (*Apostichopus japonicus*) as well as the fermentation products of kelp (*Laminaria japonica*) off-cut and mushroom was investigated in our group. At last, the functional food was developed and marketed, such as capsule, oral liquid using the polysaccharides.

Dietary fiber containing polysaccharides was prepared by fermentation products from mushroom and kelp off-cut. In order to study the radical scavenging capacity of the dietary fiber, the hydroxyl radical system and superoxide anion radical system was used. The radical scavenging capacity with dietary fiber made of kelp off-cut fermentation products was positively related to sample concentration. The capacity of hydroxyl radical scavenging of dietary fiber made of kelp off-cut fermentation products was higher than the capacity of superoxide anion radical scavenging.

Polysaccharides (AJP) were prepared from sea cucumber by protease hydrolysis method. Antioxidant activity *in vitro* and antihyperlipidemic activity *in vivo* was investigated. Chemical composition analysis indicated that AJP was mainly composed of glucosamine, galactosamine, glucuronic acid, mannose, glucose, galactose and fucose, with an average molecular weight about of 36.2 kDa. The antioxidant capacities of AJP were, respectively, evaluated by the assays of scavenging DPPH, hydroxyl and superoxide radicals, and reducing power *in vitro*. It showed potent free radical scavenging activities and reducing power. Serum total cholesterol (TC), triglyceride (TG) and low-density lipoprotein (LDL-C) decreased significantly and high-density lipoprotein cholesterol (HDL-C) increased significantly after treatment of hyperlipidemic Wistar rats with AJP. These results suggest that AJP may prove to be a potential candidate of the natural antioxidants as a therapeutic agent for hyperlipidemia.

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FUCOIDAN FROM BROWN SEAWEED *SARGASSUM MCCLUREI*: ISOLATION, CHEMICAL COMPOSITION AND ANTI-HIV ACTIVITY

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Fucoidan largely contains sulphated L-fucose residues. Hence fucose is the primary sugar in fucoidan. Sulfate groups also represent a large component of fucoidan. Many reports indicated that the biological activities of fucoidan is strongly related to its sulfate content. In this paper, crude fucoidan was extracted from brown seaweed *Sargassum mcclurei* collected at Nha Trang bay-Vietnam. The resulting crude fucoidan was purified and fractionated into 3 fractions by ion-exchange chromatography on DEAE-Mcro-prep column using aqueous sodium chloride of increasing concentration as eluant. The chemical compositon and position of sulfate groups of all fractions were determined. Anti-HIV activities of crude and fractionated fucoidans were investigated.

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SCREENING AND ISOLATION MARINE MICROORGANISMS AS FUCOIDANASE PRODUCERS

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Fucoidans, polysaccharides containing substantial percentages of L-fucose and sulfate ester groups, are constituents of brown seaweed and some marine invertebrates (such as sea urchins and sea cucumbers) [4]. Fucoidans have varied biological activities, including anticoagulant and antithrombotic, antivirus, antitumor and immunomodulatory, anti-inflammatory, gastric protective effects and therapeutic potential in suggery [1]. However, the detailed chemical structure of fucoidans remains unknown, expecially fucoidan from brown seaweeds in tropical region. Investigation of fucoidanases may provide insight into the structure and mechanism of the biological activity of fucoidans. Data available in the literature mainly concern the fucoidanases of molluscs [3],[5] and echinoderms [2] whereas information on microbial fucoidanases is scarce.

The aim of the present work was to screen marine bacteria for the efficient producers of fucoidanases. Since 2007, 800 strains including 600 strains of bacteria and 200 strains of fungi have been isolated from sea water, seaweeds, marine mollusks, bottom sediment. The Collection of Marine Microorganism was created at Nhatrang institute of Technology research and Application (NCMM – Nitra Collection of Marine Microorganism). Some of these strains are also stored at the Collection of Marine Microorganisms of PIBOC (KMM). More than 100 strains of marine bacteria and 20 strains of marine fungi were screened for producing fucoidan-degrading enzymes by Nelson method and C-PAGE method. 21 strains of marine microorganisms were found fucoidanase activity by Nelson method, 1 strain of bacteria NCMM 368 was investigated fucoidanase activity on fucoidan from *Sargassum polycystum* and *Laminaria cichorioides* by C-PAGE method.

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O-GLYCOSIDE HYDROLASE FROM VIETNAM MARINE INVERTEBRATES

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The distribution of O-glycoside hydrolase (fucoidanase, polymanuronic lyase, laminaranase) in 85 species of Vietnam marine invertebrates was studied. It is shown that O-glycoside hydrolase are widespread in the animals analyzed. Some mollusks, annelid and echinoderm species can probably serve as objects for isolation and detailed study of enzymes that degrade polysacchrides from seaweeds as well as O-glycoside hydrolase.

O-glycoside hydrolase are distributed widely and quite diverse in Vietnam marine invertebrates. These results provide the basis for further research in O-glycoside hydrolase which are tools for polysaccharides structure clearness and create bioactivity oligosaccharides service life. And it's also contribute to richness of the Vietnam marine resources. Especially, some of them such as *Chaetoptorus* sp. (Vermes), *Loricata* (Arthropoda), *Lambis* sp., *Pleuroploca filamentosa* (Gastropoda), *Septifer biocularis*, *Maleus maleus* (Bivalvia) are able to become objects for extracting enzyme fucoidan hydrolyzing; *Leporicypraea mappa*, *Laritus*, *Trochus maculatus* (Gastropoda), *Malleus regula*, *Previa penguin* (Bivalvia) for polymanuronic lyase, *Perna viridis* (Bivalvia), *Terebra maculata*, *Orula ovum* (Gastropoda) for laminaranase.

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SILAFFINS OF DIATOMS: APPLIED BIOTECHNOLOGY TO BIOMEDICINE

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Silaffins involved in the formation of cell walls of diatoms. It is known that silaffins able to precipitate silica in vitro, with the formation of nano-and micro particles in the form of spheres and plates containing many pores. It is important that the deposition of silica and particle morphology in the presence silaffins affect the chemical and physical agents (peptides, polyamines, phosphate, nitrogen, mechanical changes of the reaction mixture). It is believed that silaffins act as an organic matrix for silica-genesis and silica pore size should reflect the pattern of a matrix. Now, biotechnology silaffins discussed in the context of the "Hypothesis of silaffin matrix" and «LCPA-phosphate model."

We discuss the most perspective area of silaffins biotechnology - development of production of silicon structures with desired shapes and nanostructural properties for biocompatible materials and bioprostheses.

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BIOLOGICAL CHARACTERISTICS OF THE 3BT2 BACTERIA STRAIN ISOLATED FROM VUNG TAU OILFIELD PRODUCING THERMOSTABLE ALPHA-AMYLASE

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The 3BT2 bacteria strain isolated from the Vung Tau oilfield produces thermostable α-amylase which has pH_{opt} of 6.5 and t_{opt} of 80^{o}C. Because of the interesting thermostable enzyme, this strain has become a promising candidate for producing thermostable α-amylase strain source. According to biophysiological characteristics of this strain revealed the optimal temperature for growth at 45 - 50^{o}C and it is still able to survive at 6 – 10% NaCl concentration. Observation of colony resulted in round rough shape and purple red, its cells obtained short-rod-shape (0.5 µm × 1.4 µm), positive Gram, positive catalase. Continiously, this strain was classified based on Kit API 50/CHB as well as sequence of 16S rRNA. Based on the taxonomical results using APIWEB software, the 3BT2 strain is similar of 80 % as *Bacillus licheniformis* ATCC 19580, however the sequence of 1500 bp fragment coding for 16S rRNA indicated that the homologous sequence between this strain and *B. licheniformis* DSM 13 (≈ ATCC14580) strain raised up to 97.8 %, as hence its own named *B. licheniformis* 3BT2.

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OPTIMIZATION OF CULTURE CONDITIONS FOR A RECOMBINANT THERMOSTABLE ALPHA-AMYLASE GENE EXPRESSION IN *BACILLUS SUBTILIS* USING RESPONSE SURFACE METHODOLOGY

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A thermostable α-amylase gene from *Bacillus licheniformis* 3BT2 was cloned and expressed in *Bacillus subtilis* 168M. The open frame reading region of this α-amylase gene was fused with the promoter of α-amylase gene from *Bacillus subtilis* 168M by megaprimer method and thus, the cassette of two those components was introduced into the shuttle vector pHV33. The pHV33 vector containing both foreign parts was transformed subsequently into *Bacillus sublitis* 168 M. The recombinant strain produced extracellular α-amylase activity of 16 IU/ml, which showed 2 times higher than that of wild type. Moreover, optimization of each culture conditions such as medium, carbon source, phosphate and L-tryptophan concentrations and cultivated temperature lead an increase of activity up to 75 IU/ml. Among those fermentation factors we examined, the most significant variables influencing α-amylase expression were statistically elucidated for optimization and included medium (Luria Broth – LB), L-tryptophan and glucose. To determine the best culture condition for α-amylase expression, we used the response surface methodology. A 20-run Box-Behnken design (BBD) with two center points was employed in this work describing the relationships between the growth rate, α-amylase production and the medium components, two mathematical models were developed by the following second-order polynomial equation to fit data in RSM of BBD: The polynomial models for the growth rate and the α-amylase production were $Y_{OD}600$ = -5.65016 + 5.60753 * LB + 0.99613 * L-trytophan + 1.00898 * glucose - 1.86250 * LB * L-trytophan - 0.23700 * LB * glucose - 0.19600 * L-trytophan * glucose - 0.98876 * LB^2 + 1.46733 * $L\text{-trytophan}^2$ - 0.034014 * $glucose^2$ and $Y_{activity}$ = + 425.25868 - 513.13656 * LB + 206.94602 * L-trytophan - 12.55360 * glucose - 200.81015 * LB * L-trytophan - 30.41201 * LB * glucose + 5.93955 * L-trytophan * glucose + 355.78299 * LB^2 + 0.70427 * $trytophan^2$ + 1.87428 * $glucose^2$. Then the reliability of the model and the accuracy of the prediction were checked by verification experiment in triplicate. A maximal α-amylase production of 190 IU/ml was obtained with. This result agreed with the predicted value well and was 67.3% higher than that obtained with the basic medium.

AUTHORS' INDEX

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