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1-6 December
2017

2nd International Symposium
Marine Enzymes and Polysaccharides

1-6 December
Nha Trang, Vietnam

Symposium on Marine Enzymes and Polysaccharides

Abstract Book and Scientific Program

Nha Trang, December 1-6, 2017

NITRA ТИБОХ V.A.S.T

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The abstracts are reproduced as accepted by the scientific committee of the symposium and appear in alphabetical order by the name of reporter, divided by the types of report.

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The Symposium is financially supported by Nafosted fund.

SCIENTIFIC PROGRAM

Friday, December 1 Location: NITRA Institute, 02 Hùng Vương, thành phố Nha Trang					
8:00-18:00	Registration of participants				
18:00-20:00	Welcome party				
Saturday, December 2 Location: NITRA Conference Hall, Hòn Chồng, Vĩnh Phước, Tp. Nha Trang					
Session 1 – Fucoidan and other bioactive polysaccharides. Chairman – Prof. Zvyagintseva Tatyana					
8:00-8:15	Forewords, prefaces				
8:15-8:55	Ermakova Svetlana, DrSc.	Russia	PIBOC FEB RAS	Radioprotective effect of fucoidans	Plenary
8:55-9:35	Maria Dalgaard Mikkelsen, PhD	Denmark	Technical University of Denmark	Discovery of new fucoidan modifying enzymes	Plenary
9:35-10:15	Mukhamejanov Emil	Kazakhstan	Fucoidan World Ltd	Fucoidan – natural geroprotector	Plenary
10:15-10:45 - coffee break					
Session 2 - Fucoidan molecular properties and modification. Chairman – Drsc. Ermakova Svetlana					
10:45-11:25	Zvyagintseva Tatyana, Prof., DrSc.	Russia	PIBOC FEB RAS	α-N-acetylgalactosaminidases in nature, biotechnology and medicine	Plenary
11:25-11:40	Pham Duc Thinh, PhD	Vietnam	NITRA, VAST	Structural characteristics of fucosylated chondroitin sulfate isolated from sea cucumber *Holothuria spinifera*	Oral
11:40-11:55	Jong-Ki Kim, Prof.	South Korea	Catholic University of Daegu	Thrombolytic fucoidan activates plasma tissue-type plasminogen activator by inhibiting tPA-PAI-1 complexation: molecular mechanism of fucoidan-mediated thrombolysis	Oral
11:55-12:10	Huynh Hoang Nhu Khanh, PhD	Vietnam	NITRA, VAST	Hydrolysis characterization of enzyme isolated from trisidos semitora-the marine	Oral

				invertebrate with fucoidans of vietnamese sea cucumbers	
12:10-12:25	Malyarenko Olesya, PhD	Russia	PIBOC FEB RAS	Cancer-preventive effect of water-soluble polysaccharides from brown *alga Fucus evanescens*	Oral
12:25-12:40	Usoltseva Roza, PhD	Russia	PIBOC FEB RAS	Galactofucans from brown algae of Russia and Vietnam	Oral
12:40-12:55	Cao Thi Thuy Hang, PhD student	Vietnam	NITRA, VAST	Sulfated galactofucan modification by using fucoidan degrading enzymes	Oral
Sunday, December 3 Location: NITRA Conference Hall, Hòn Chồng, Vĩnh Phước, Tp. Nha Trang					
Session 3 - Polysaccharide-modifying enzymes. Chairman – Prof. Kim Jong-Ki					
8:00-8:40	Bakunina Irina, DrSc.	Russia	PIBOC FEB RAS	O-glycoside hydrolases of psychrotolerant bacteria from microbial community of the pacific red alga *Ahnfeltia tobuchiensis*	Plenary
8:40-8:55	Belik Alexey	Russia	PIBOC FEB RAS	Recombinant alginate lyases of marine bacteria: substrate specificity and products analysis	Oral
8:55-9:10	Dubrovskaya Yuliya, PhD	Russia	PIBOC FEB RAS	Producer of alginate lyases and fucoidanases found among epiphytes of the brown alga Sargassum polycystum of Vietnam coast	Oral
9:10-9:25	Kalitnik Aleksandra, PhD	Russia	FEFU	Immunotropic properties of κ/β-carrageenan from red alga *Tichocarpus crinitus*	Oral
9:25-9:40	Zueva Anastasiia	Russia	FEFU, PIBOC FEB RAS	Novel recombinant fucoidanase from marine bacterium *Wenyingzhuangia fucanilytica*	Oral
9:40-9:55	Rasin Anton, PhD student	Russia	PIBOC FEB RAS	Structure, enzymatic transformation, anticancer activity of fucoidan and sulphated fucooligosaccharides from *Sargassum horneri*	Oral
9:55-10:30 - coffee break					
Session 4 - Biologically active substances from marine and terrestial sources and their enzymic modification. Chairman – Dr. Maria Dalgaard Mikkelsen					
10:30-11:10	Fedoreev Sergey, PhD	Russia	PIBOC FEB RAS	The relict tree *Maackia amurensis* is a rich source of biologically active polyphenolic compounds for	Plenary

				the new medicines creation	
11:10-11:25	Mishchenko Natalia, PhD	Russia	PIBOC FEB RAS	Biologically active quinones from sea urchins of Central Vietnam coast	Oral
11:25-11:40	Nguyen Duy Nhut, PhD	Vietnam	NITRA, VAST	Cytotoxicity properties of hydrolyzed alginate from vietnam brown algae	Oral
11:40-11:55	Kokoulin Maksim, PhD	Russia	PIBOC FEB RAS	Sulfated O-polysaccharides from some marine Gram-negative bacteria: structural diversity and biological activity	Oral
11:55-12:10	Pham Thu Thuy, PhD	Vietnam	Nha Trang University	Biodiversity and amylase production activity of marine fungi isolated from coastal regions of Khanh Hoa province, Vietnam	Oral
Monday, December 4 Location: NITRA Conference Hall, Hòn Chồng, Vĩnh Phước, Tp. Nha Trang					
Session 5 - Biologically active substances from marine and terrestial sources and their enzymic modification. Chairman – Dr. Pham Duc Thinh					
8:00-8:15	Truong Hai Bang, master	Vietnam	NITRA, VAST	Effect of extraction methods on the chemical structure of ulvan from *Ulva reticulata*	Oral
8:15-8:30	Pham Nam Thang, PhD. student	Vietnam	Institute of Materials Science, VAST; University of Science and Technology, VAST	Nano-extraction from spirunlina and potential application in integrative medicine	Oral
8:30-8:45	Bui Van Nguyen	Vietnam	University of Khanh Hoa	Structure and intestinal immunomodulating activity of fucoidan from two brown seaweed species *Sargassum crassifolium* and *Padina australis*	Oral
8:45-9:00	Surits Valerii	Russia	FEFU, PIBOC FEB RAS	Polysaccharides from brown alga *Sargassum duplicatum*	Oral
9:00-9:15	Tarbeeva Darya, PhD	Russia	PIBOC FEB RAS	Prenylated polyphenolic compounds from *Maackia amurensis* root bark	Oral
9:15-9:30	Volodko Aleksandra, PhD	Russia	PIBOC FEB RAS	Carrageenan/chitosan soluble complexes and films for controlled release of drugs	Oral
9:30-11:30	Poster Session				

Tuesday, December 5 Spare time
Wednesday, December 6 Farewell Party

FOREWORD FROM VALENTIN A. STONIK, DIRECTOR OF PIBOC FEB RAS

Dear Colleagues and Friends,

It is my privilege and pleasure to heartily greet participants of the 2nd Symposium on Marine Enzymes and Polysaccharides (MEP-2017). The main goal of MEP-2017 is to provide a new level of collaboration between the scientists over the world working in this field. During this meeting everybody has a good chance to exchange scientific results and ideas.

This year's symposium will include such topics as studying radioprotecting, geroprotecting and thrombolytic effects of fucoidans; characterization of new fucooligosaccharides, galactofucans, carrageenans, alginates, chondroitin sulfates, fucoidanases, alginate lyases, α-N-acetylgalactosaminidases, amylases, lectins, quinones, florotannins, polyphenolic compounds and other subctances with direct or indirect biological activity.

Enzymatic studies for the past 25 years blew away the fluffy clouds obscuring the view of earlier generations of enzymologists, revealing enzymes' 3D structures and molecular dynamics. Much of the drudgery of obtaining enzymes for study has been removed and the accessibility of enzymes has expanded enormously. Molecular biology allows one to select essentially any enzyme for study and produce large quantities of pure protein with minimal effort. On another hand, theoretical structural diversity of carbohydrates exceeds those of any other class of biomolecules, making this field extremely promising for searching active compounds.

It's becoming a good tradition to organize MEP-2017 in Nha Trang. It is a quickly growing city, especially if compared with the situation I personally evaluated 30 years ago. Due to persistence and cooperation, Vietnamese nation has restored its ruined country after a terrible war and now is overcoming the consequences of the last terrible typhoon. I positively believe in the prospering future of Vietnam and Nha Trang.

For many years G.B. Elyakov Pacific Institute of Bioorganic Chemistry of the Far-Eastern Branch of the Russian Academy of Sciences (PIBOC) has been collaborating with scientific institutes of the Vietnamese Academy of Science and Technology, including Nha Trang Institute of Technology Research and Application, which is the host of MEP-2017. Our scientific relations with this Institute are developing from year to year and have good prospects for the future.

I wish you productive and enjoyable days of dialogue, collaboration, and learning. On behalf PIBOC FEB RAS, I genuinely thank all of our distinguished speakers, moderators, panelists, sponsors, and attendees who will add great substance to this vital discussion. Working together with you, I hope to inspire, energize, and empower attendees to take action to make algal and invertebrate mariculture, discovering of new enzymes and introduction of drugs of carbohydrate origin become the new standard of world's healthcare.

I would also like to thank President of Vietnamese Academy of Science Prof. Chau Van Minh and Director of Nha Trang Institute of Technology Research and Application Dr. Pham Trung San for the opportunity to hold this Symposium.

Thank you for participating.

Prof. Valentin Stonik

Director of G.B. Elyakov Pacific Institute of Bioorganic Chemistry, FEB RAS

α-N-ACETYLGALACTOSAMINIDASES IN NATURE, BIOTECHNOLOGY AND MEDICINE

Bakunina I.Y.

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α-N-Acetylgalactosaminidases (EC 3.2.1.49) are exo-glycosidases. They catalyze the hydrolysis of the terminal α-bound N-acetylgalactosamine from the non-reducing ends of various complex carbohydrates and glycoconjugates. Glycolipids, glycopeptides, and glycoproteins, blood group A erythrocyte antigens, lipopolysaccharides of the cell walls and capsules of bacteria are physiological substrates for α-N-acetylgalactosaminidases. These enzymes are found in the organs and tissues of terrestrial mammals, birds, invertebrates, and worms. Interesting to note the enzymes are not found in plants. The enzymes take part in the catabolism of complex oligosaccharides. It was found that the deficiency of lysosomal α-N-acetylgalactosaminidase in the human body causes a dangerous hereditary Schindler/Kanzaki disease, which stimulated an intensive, comprehensive study of this enzyme. It is known that α-N-acetylgalactosaminidase is produced by cancer cells and influenza virus, and accumulates in the blood plasma of patients. To date, α-N-acetylgalactosaminidases were revealed in the anaerobic terrestrial bacteria of phylum *Firmicutes* and *Bacteroidetes*. In the marine environment, α-N-acetylgalactosaminidases have been found in the liver and digestive organs of the marine invertebrate and in marine bacteria of the genus *Arenibacter*.

These enzymes found in 27, 36, 109 and 129 (GH) families of glycoside hydrolase according to modern carbohydrate active enzymes classification (CAZy), which is based on homology of the amino acid sequence and secondary protein structure.

The report provides an overview of past and current studies of α-N-acetylgalactosaminidases. Along with the physiological function of these enzymes in organisms of eukaryotes and prokaryotes, the biochemical properties and structures of α-N-acetylgalactosaminidases from different origins are shown. The evolution of the amino acids sequences, 3D structures and mechanisms of these enzymes action are discussed. Prospects for the use of the enzymes are considered. Particular attention is paid to examples of marine α-N-acetylgalactosaminidases, especially to enzyme from marine bacteria of the genus *Arenibacter.* The growing interest in α-N-acetylgalactosaminidases is stimulated with the possibility of its practical application in biotechnology and biomedicine.

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RADIOPROTECTIVE EFFECT OF FUCOIDANS

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The search of substances increasing the resistance of the organisms against radiation is one of the purposes of radiology. Unfortunately, a currently known radioprotectors are far from ideal and can hardly be recommended for personal application. Substances from terrestrial and marine organisms are promising for investigation as radioprotective agents due to their multi-functional effects on the living organism. They can be used also in areas with a hard ecological situation. Marine algae contained a number of substances that possessed a biological activity. The most valuable are the polysaccharides of algae, especially brown algae. The amount of polysaccharides can be from 40 to 80% of dry weight of algae depending of species of algae and time and place of their harvest. Sulfated polysaccharides of brown algae - fucoidans, showed a wide range of biological activities, which is described in numerous research articles and reviews. However, only few publications related to the ability of these unique polysaccharides to protect against radiation are available [1-7].

The literature data and the results of the investigations devoted radioprotective activity of fucoidans from brown algae conducted at the PIBOC FEB RAS will be presented.

The work was financially supported by the Russian Science Foundation (grant №16-14-10131).

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THE RELICT TREE *MAACKIA AMURENSIS* IS A RICH SOURCE OF BIOLOGICALLY ACTIVE POLYPHENOLIC COMPOUNDS FOR THE NEW MEDICINES CREATION

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Natural phenolic metabolites such as isoflavonoids are found in plants of the Leguminosae family and in cell cultures derived from them both as free compounds and as various glycoconjugates. Some of these isoflavonoids play an important role in the prevention of cardiovascular and coronary heart diseases as well as breast and prostate cancers. Amur maackia (*Maackia amurensis* Rupr. et Maxim.) is the only woody plant, a representative of the Leguminosae family in the flora of the Russian Far East. This species is a relict tree of the tertiary flora. The polyphenolic complex from the heartwood of *M. amurensis* (PHW), called as Maksar® preparation, is registered in the Russian Federation as a hepatoprotective drug. The main components of this preparation are eleven isoflavones, two pterocarpans, monomeric and dimeric stilbenes. Composition of this preparation also includes the polyphenolic compounds belonging to isoflavanones, isoflavans, flavanones and dimeric stilbenes. Maksar is recommended as a hepatoprotector for medical application. The normative and technical documentation for the production of the substance and "Maxar® preparation tablets, coated with a film membrane, 60 mg" has been developed. This medicine was shown to contribute to the correction of blood lipid spectrum disorders and fatty liver dystrophy. It possesses antioxidant effect and prevents the occurrence of alimentary hyperlipoproteinemia in animals. Maxar® reduces the intensity of lipid peroxidation products formation and regulates the body antioxidant protection system. The drug has antiaggregative and anti-inflammatory properties and is effective for the treatment of viral hepatitis.

To make more effective use of a unique relic tree in the pharmaceutical industry, the possibility of using other plant organs, for example roots, as an alternative source of raw materials for the creation of pharmaceutical preparations, was evaluated. We showed that the *M. amurensis* roots, in contrast to the wood contained more than 78% of the glycoside forms of isoflavones and pterocarpans and possessed pronounced antioxidant and hepatoprotective properties.

The ability of 7-*O*-gentibiosylformononetine (GBF) and other related isoflavonoids, isolated from the roots of *M. amurensis*, to inhibit platelet and coagulation hemostasis indices has been demonstrated for the first time. This fact is of great practical importance, since it opens the perspective of creating a new oral medication that can reduce the likelihood of thrombosis in various cardiovascular diseases.

Financial support was provided by the FEBRAS 15-I-5002

Plenary lecture

DISCOVERY OF NEW FUCOIDAN MODIFYING ENZYMES

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Fucoidan is a highly sulfated polysaccharide produced by brown algae. Fucoidan designates a group of certain fucose-containing sulfated polysaccharides (FCSPs) that have a backbone built of (1→3)-linked α-L-fucopyranosyl or of alternating (1→3)- and (1→4)-linked α-L-fucopyranosyl residues, but also include sulfated galactofucans with backbones built of (1→6)-β-D-galacto- and/or (1→2)-β-D-mannopyranosyl units with fucose or fuco-oligosaccharide branching, and/or glucuronic acid, xylose or glucose substitutions. The fine structure of fucoidan has not yet been determined, although some oligosaccharide structures in several fucoidans have been described. Fucoidan has in several studies been proposed to have beneficial properties against diseases like cancer and anti-inflammatory (Ermakova *et al.* 2011, Vishchuk *et al.* 2011, Li *et al.* 2008). In order to determine the fine structures of fucoidan and to obtain oligosaccharides of a certain size and chemical structure, novel enzymes modifying and/or partially degrading fucoidan are an important prerequisite for future studies and further use in the medical industry.

To date, only a few fucoidan degrading enzymes have been described including a group of fucoidanase sequences containing predicted T9SS secretion system domains (Colin *et al.* 2006, Silchenko *et al.* 2017; Takayama *et al.* 2002). T9SS domain containing proteins are known to be involved in cell adhesion to and motility over surfaces (McBride and Zhu 2013). The T9SS containing sequences are to date however only found in Bacteroidetes species (Abby *et al.* 2016), which suggests that in other bacterial species the enzyme hydrolyzing fucoidan has evolved by convergent evolution. This can be suggested through the findings of different fucoidan degrading enzymes two containing LamininG domains (Takayama *et al.* 2002) and others containing predicted fucosidase domains (Woo-Jung *et al.* 2008, Descamps *et al.* 2006).

We have analyzed several fucoidan degrading enzymes for their specific activities through recombinant expression and purification. Several of the enzymes seem targeted for degradation, primarily from the C-terminal end. The C-terminal end of several of the fucoidan degrading enzymes seem not to be important for enzymatic activity and can hence be deleted, as previously reported for the fucoidanase FcnA (Colin *et al.* 2006).
We have optimized the expression of several fucoidan degrading enzymes through different means in order to obtain higher amounts and more robust enzymes. Furthermore, we have found that the preference towards different substrates exists between the different fucoidan degrading enzymes, which might reflect the differences in the preference for, not only fucose linkages but perhaps also the level of sulfatation and other substitution with galactose and other sugar residues as described above. We have also found that a very important aspect of analyzing fucoidan degrading enzymes are the purity of the fucoidan substrates used as well as the optimal conditions of the reaction pH.

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FUCOIDAN – NATURAL GEROPROTECTOR

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The ageing process in the world has dramatically climbed, which leads to increase age-related illnesses and effects to the quality of life and independence of the old folks, an increase the cost of their medication, therefore the development of methods for the preventive measure and the treatment of these diseases has the great social and practical importance.

In the recent years, more and more interest has been devoted in the search for nutraceuticals with the high bioactivity. Life has come out of the sea and all the bioactive compounds of the land are flowing back into the sea, so the animal and vegetal life of the sea reveals the high bioactivity. The most interesting substance in this regard is the sulfated polysaccharide of brown seaweed- Fucoidan. The number of the scientific publications and studies on Fucoidan has increased in the arithmetical progression in recent years.

Fucoidan shows its bioactivity mainly at the stage of metabolic regulation and regulatory systems. Let’s stop on the impact on Fucoidan on the age-related illnesses.

Fucoidan prevents the death of cholinergic and dopamine neurons, which contributes to the prevention of Alzheimer's and Parkinson's diseases.

Fucoidan, as an antioxidant, prevents the cells damage by the excess of the free radical’s level, and also as a pro-oxidant saves the ability of the cells to grow and to adapt.

Fucoidant helps restore the protective link of immunity (antibacterial, antiviral and anti-cancer effects) and reduce the attacker (antiallergic).

Fucoidan improves the processes of bone synthesis, helping to negate the manifestations of osteoporosis, and compound with the anti-inflammatory effect reduces the symptoms of osteoarthritis.

Fucoidan helps maintain the homeostasis of the energy sources (glucose and fat), which provides the prevention and treatment of diabetes, obesity and cardiovascular diseases.

Thus, fucoidan helps maintain the homeostasis of regulatory and metabolic systems that are most affected by the older persons. Therefore, fucoidan can be equated to the natural geroprotectors. In the report will be discusses the mechanisms of the positive effects of fucoidan in the various age-related illnesses.

FLOROTANNINS OF BROWN ALGAE – INHIBITORS OF FUCOIDANASES. INTERRELATION OF STRUCTURE AND INHIBITORS ACTION

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Polyphenols of two brown algae inhibit fucoidanases isolated from mollusks and microorganisms.

For the first time the inhibitors of fucoidanases of mollusks and microorganisms were found among the metabolites of brown algae *Fucus evanescens* and *Costaria costata*. Substances with inhibiting effect were isolated and studied with help of recombinant fucoidanase FFA2 from the marine bacterium *Formosa algae* KMM 3553T. An irreversible type of inhibition was established. NMR spectroscopy and mass spectrometry analysis showed that polyphenols of various structures and molecular weights from both algae were inhibitors of fucoidanases. It has been suggested that the effectiveness of inhibition increases with increasing molecular weight of inhibitory substances. It was suggested that algae are protected from eating by marine organisms, synthesizing substances that inhibit fucoidanases.

The work was supported by RFBR grant № 15-04-01004.

RECOMBINANT ALGINATE LYASES OF MARINE BACTERIA: SUBSTRATE SPECIFICITY AND PRODUCTS ANALYSIS

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For the recent two years there has been achieved a significant progress in studying of therapeutical properties of alginate oligosaccharides, obtained by the action of alginate lyases. They show antioxidant properties *in vitro*, inhibit growth and differentiation of adipocytes and absorbtion of saturated fatty acids, exhibit antiallergic properties by suppressing IgE and stimulate immune system by increasing of production of cytokines (G-CSF, TNF-α, interleukins) in murine macrophages, inhibit growth of osteosarcoma MG-63 cells by 60-70%. Alginate oligosaccharides have advances due to their low toxicity, availability of raw materials, environmentally safe production. Biological activity of alginates strongly depends on monosaccharide composition and polymerization degree, that increases the importance of screening of suitable raw materials and main instruments of their modification – alginate lyases. Alginate lyases are found in all groups of living organisms, exhibit moderate thermostability, and their activity can be significantly increased by directed mutagenesis. In the Laboratory of Enzyme Chemistry PIBOC FEB RAS there were isolated and studied different alginate lyases from marine bacteria and invertebrates. These enzymes can be used for producing biologically active alginate oligosaccharides [1-4].

We expressed recombinant forms of five alginate lyases of marine bacterium *Formosa algae* KMM 3553 and two alginate lyases of marine bacterium *Pseudoalteromonas issachenkonii* KMM 3549(T) (one of them – in two forms). By using the method of assaying of reducing sugars it was shown that these enzymes are active against polymannuronic, polyguluronic and mixed type of alginic acids. Five enzymes were classified as polymannuronate lyases (EC 4.2.2.3) and three as polyguluronate lyases (EC 4.2.2.11). 30.6 kDa recombinant alginate-lyase ALFA3 from *Formosa algae* appeared to be polyspecific. We measured its basic properties and analysed products of action.

It was shown that ALFA3 rapidly digests all types of alginic acids, leading to formation of range of alginate oligodsaccharides with degree of polymerization from two and higher. But the amount of high-molecular fraction, remaining after exhaustive hydrolysis depends on the ratio of mannuronic and guluronic residues in the molecules of substrate. 1H NMR analysis showed formation of 4-deoxy-L-erythro-hex-4-enepyranosyluronate residues jointed with mannuronic residue (ΔM) in the case of polymannuronic acid digestion as well as ΔM and ΔG in case of polyguluronate enriched substrate.

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STRUCTURE AND INTESTINAL IMMUNOMODULATING ACTIVITY OF FUCOIDAN FROM TWO BROWN SEAWEED SPECIES *SARGASSUM CRASSIFOLIUM* AND *PADINA AUSTRALIS*

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We studied the structure of fucoidans extracted from two brown seaweed species, *Sargassum crassifolium* and *Padina australis,* and their intestinal immunomodulating activity via Peyer's patch cells of C3H/HeJ mice. ESI-MS analysis indicated that the dominant structure of both fucoidans has a backbone of α-(1→4)-linked and α-(1→3)-linked L-fucose residues and sulfate groups are attached at the C-2 and C-4 positions; branches of fucoidan from *S. crassifolium* are galactose residues with (1→4)- linkage and branching points are at C-4 of fucose, while fucoidan from *P. australis,* branches are sulfated galactose-fucose disaccharides and sulfated galactose monosaccharides attached to the main chain through (1→3)- or (1→4)-linkages. According to small angle X-ray scattering (SAXS) measurements, the two fucoidans have a branched structure. We simulated them with molecular models based on our proposed primary structure. These fucoidan samples have the ability to stimulate intestinal immunological activity via Peyer's patch cells.

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SULFATED GALACTOFUCAN MODIFICATION BY USING FUCOIDAN DEGRADING ENZYMES

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Fucoidans designate a family of sulfated, fucose-rich polysaccharides uniquely produced by brown marine macroalgae (seaweeds) and certain marine invertebrates such as sea cucumbers. In general, fucoidans, or fucose-containing sulfated polysaccharides, FCSPs, consist of a backbone of α-L fucosyl residues linked together by (1→3) and/or (1→4)-glycoside bonds which are organized in stretches of α(1→3) or of alternating α(1→3)- and α(1→4)-glycoside bonds. The L-fucosyl residues may be sulfated ($–SO_3^-$) at positions C2 and/or C4 (rarely at C3). Some fucoidans may have fucose, galactose, glucuronic acid or other mono- and oligosaccharides as short branches. In this study, we search for fucoidanases and sulfatases that are able to modify fucoidans, in particular galactofucans from *Sargassum mcclurei*, brown algae found in the Vietnamese sea. The results showed that *S. mcclurei* fucoidan was degraded by the enzymes included three endo-fucoidanases (EC 3.2.1.-) belonging to glycosyl hydrolase family 107, referred to as FcnA2, Fda1, Fda2, and two unclassified enzymes previously reported to be endo-fucoglucuronomannan lyases, called FdlA and FdlB. Furthermore 20 different bacterial strains isolated from the sea cucumber gut were able to produce fucoidanases or sulfatases capable of modifying fucoidan from *S. mcclurei*. The genomes of eight bacteria with highest fucoidan modifying activities have been identified to genus level and 6 sulfatases have been synthesized from one of the strains showing the highest level of sulfatase activity.

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STUDIES OF THE LECTINS FROM THE MUSSELS *CRENOMYTILUS GRAYANUS* AND *MYTILUS TROSSULUS*

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Lectins are multivalent carbohydrate-binding proteins that are widely presented among all the organisms and are believed to act as specific receptors of oligosaccharides (glycans) of glycoconjugates (glycoproteins, glycolipids and proteoglycans). There is considerable interest in glycobiology relating with marine organisms, because they have rich resource of glycoconjugates and lectins. Based on the structural similarity of carbohydrate recognition domain lectins are classified into a number of structurally distinct families. In recent years, many lectins from marine invertebrates have been identified. These lectins have been demonstrated to play key roles in various immune events, like cytotoxic effects, antibacterial activity. However, molecular features and functional studies of such lectins are still sparse compared with those in insects and vertebrates.

A new lectin family has been proposed as a consequence of the isolation by us of the first member from the mussel *Crenomytilus grayanus* (CGL), which binds specifically to Gal and GalNAc and which amino acid sequence does not match with any lectin family that have been reported to date. The second member of this novel Gal-specific lectin family was isolated by Japanese scientists from the bivalve *Mytilus galloprovincialis* (MytiLec) of which 3 isoforms have been reported when analyzing the gene. Recently we have purified and characterized the third member from the mussel *Mytilus trossulus* (MTL). All three lectins consist of about 150 residues with three tandem-repeat domains, having 83-84% homology with each other.

It has been established that these lectins adopted beta-trefoil fold that was characteristic of ricin and another 13 protein families such as cytokines, agglutinins, etc. Such structure can form oligomers owing to the presence of internal symmetry. Oligomerization and variable valences and domains are important for carbohydrate-binding proteins such as lectins. SDS electrophoresis reducing and non-reducing conditions showed that the lyophilized lectins formed oligomers upon storage. The same effect was observed upon increasing the lectins solution concentration.

Oligomerization of several lectins is known to occur only upon reaction with carbohydrate ligands. We confirmed experimentally that the CGL and MTL self-associated directly in solution. Gel filtration and mass spectrometry indicated that lectins existed entirely as the monomers in the solution of low concentration (0.001 mg/mL). Dimers appeared in the solution of high concentration (1 mg/mL). Thus, the fraction of the dimer in solution increased as the lectins concentration increased.

Besides it has been established that CGL and MTL oligomerization was important for several if not all of them biological properties. Lectins oligomers in solution retained their carbohydrate binding properties. This was evident in the hemagglutination reaction, ELISA and in an experiment on CGL inhibition of tumor cells growth.

Oral report

PRODUCER OF ALGINATE LYASES AND FUCOIDANASES FOUND AMONG EPIPHYTES OF THE BROWN ALGA *SARGASSUM POLYCYSTUM* OF VIETNAM COAST

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A search for enzymes involved in the degradation of alginic acid and fucoidan was conducted among epiphytic bacteria of *Sargassum* growing in the territorial waters of the Socialist Republic of Vietnam. Two resistant bacterial strains F10 and F14 have been isolated from the algal microflora, which degradated the thallus of the algae under laboratory conditions. These bacterial strains have a different morphological, physiological, biochemical characteristics and composition of enzymes. For standardization of the cultivation conditions, the effects of yeast extracts and peptones from various manufacture firms on the growth and development of the strain F14 were investigated. The yeast extract from the manufacture firm "France CNRS" proved to be the most suitable for use as a supplement for obtaining alginate lyase, while Bacto-peptone "Difco" is the best for obtaining fucoidanase. Since we do not know the optimum pH for the sought-for enzymes, we tested the activity in biomass extracts at pH 5.2 and 7.2. According to our data the fucoidanase was more active at a pH of 5.2 and polymannuronate lyase at a pH of 7.2. Fucoidan from *S. mcclurei* was most effectively destructed at a pH of 5.2 under the action of extract from F14 that was grown on the HiMedia Lab medium with peptone. The partially purified alginate lyase was stable between 0 and 40 ºC and has an optimal pH and temperature at pH 6.0 and 35 °C, respectively.

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Oral report

HYDROLYSIS CHARACTERIZATION OF ENZYME ISOLATED FROM *TRISIDOS SEMITORA*-THE MARINE INVERTEBRATE WITH FUCOIDANS OF VIETNAMESE SEA CUCUMBERS

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Fucoidans are a group of sulfated polysaccharides that exhibit various biological activities including anti-viral, anti-bacterial, anticoagulant and anti-tumoral activities. The biological activities of fucoidans vary depending on the sources. Fucoidan isolated from sea cucumbers has also been shown to possess some useful functions such as anticoagulant and osteoclastogenesis although reports on sea cucumber fucoidan are few. Despite their diverse pharmacologic activities, the structural variation, high molecular masses and viscous nature of polysaccharides including marine-derived ones may limit their successful applications. Bioactive oligosaccharides with lower-molecular weights would help overcome these problems. Therefore, searching for new enzyme activities that degrade the fucoidans from sea cucumber becomes increasingly important area for both academic researchers and pharmacological activities.

In this study, we report on the isolation enzyme and characterization hydrolysis of a marine invertebrate *Trisidos semitora* which have the ability to utilize and degrade fucoidans from different Vietnamese sea cucumbers *Stichopus vagiegatus (Sv)* and *Holothuria spinifera* (Hsp). The identification of enzymatic hydrolysis products of fucoidan using the most representative method is carbohydrate polyacrylamide gel electrophoresis (C-PAGE).This enzyme could be a valuable tool for the structural analysis of fucoidans from sea cucumber and production of bioactive fuco-oligosaccharides.

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IMMUNOTROPIC PROPERTIES OF κ/β-CARRAGEENAN FROM RED ALGA *TICHOCARPUS CRINITUS*

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Sulfated polysaccharides of red algae – carrageenans consisting of D-galactose residues linked by alternating regularly β-(1-4) and α-(1-3) glycosidic bonds refer to soluble dietary fibers and relate in the list of foodstuffs (Food and Drugs, 2008). Also carrageenans are known as nontoxic biopolymers with different biological activity. In particular, carrageenans possess a number of immunotropic effects and have an ability to stimulate of immune mediators biosynthesis including different cytokines, pro-inflammatory as well as anti-inflammatory.

Earlier, we have shown the gelling polysaccharide from red alga *Tichocarpus crinitus* (Gulf of Peter the Great, Sea of Japan, Vladivostok, Russia) – κ/β-carrageenan induces *ex vivo* synthesis of cytokines in human blood immune cells in dose-depend manner. Also we have studied the *in vivo* effect (under oral administration) of κ/β-carrageenan on cytokine production in mice blood cells. It was established that κ/β-carrageenan given orally stimulates the induction of cytokines, such as interleukin-12 (IL-12), IL-1β, IL-4, IL-10 and also significantly stimulates the production of interferon-γ (INF-γ) and besides stronger than lipopolysaccharide (LPS). Furthermore we have demonstrated the carrageenan has a protective effect in LPS-induced endotoxemia in mice. Pretreating mice with carrageenan before injecting LPS promotes to increase the levels of IL-10 and reduction of TNF-α and IL-1β production compared with control.

We also have studied the influence of κ/β-carrageenan on murine peritoneal macrophages activity, under oral administration of carrageenan alone or before LPS injection. The cellular activity was comparatively estimated using a number of linear and non-linear morphological parameters which describes the cell morphology changing during different activation processes. According our results κ/β-carrageenan introduced orally, alone and in combination with LPS promotes to stimulating the macrophage adhesion and spreading steps that's on the one side confirmed by higher Area and lacunarity and on the other – low Density in all experimental groups compared with control that correlates with general non-specific complication of cells shape, their activation and also changing cells behavior. However the macrophage Area was significantly higher in groups injected LPS only and in group treated with carrageenan before injection LPS compared with control group and group getting carrageenan alone. So, carrageenan alone activates cells in less degree compared with LPS. It is necessary to note that the both substances are also contribute to reduce of Density values, however, LPS reduces of it more than carrageenan, at the same time the combined effect of LPS and carrageenan gives the average value between LPS and carrageenan. This fact could be explained by that carrageenan in some extent promotes to reducing of intensive activation of macrophages by such stronger inflammatory agents as LPS, thus exhibiting some protective effect.

Thus our data have demonstrated the carrageenan possesses an immunomodulatory effects due to their influence on cytokines production and activation of immune cells and furthermore carrageenan have a protective effect in LPS-induced endotoxemia in mice.

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THROMBOLYTIC FUCOIDAN ACTIVATES PLASMA TISSUE-TYPE PLASMINOGEN ACTIVATOR BY INHIBITING TPA-PAI-1 COMPLEXATION: MOLECULAR MECHANISM OF FUCOIDAN-MEDIATED THROMBOLYSIS

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Identifying a pharmacological means for increasing the production of t-PA is always desirable to cure impaired production of this enzyme in various atherothrombotic diseases. An fucoidan from Korean *Undaria pinnatifida sporophylls* (UF) has been shown to exhibit novel thrombolytic effects, and demonstrated enhancement of the plasma levels of active t-PA in mouse arterial thrombus models. Thrombolytic activity and binding affinity with PAI-1 of various fucoidan fractions from Russian marine alga were examined in mouse arterial thrombus models and in tPA-PAI-1 coated well, respectively. Thrombolytic activities comparable with UF were found among various fucoidan fractions with relatively difference in sensitivity and the time for reperfusion between 30-90 min. More importantly, competitive binding of fucodian with t-PA-complexed plasminogen activator inhibitor-1 (PAI-1) enabled releasing free t-PA, in which relative amount was estimated reciprocally among various fucoidan fractions by measurement of remained tPA-PAI 1 complex. The tPA-activating properties of fucoidan fractions were found accordingly with the result of thrombolytic activities *in vivo*.

SULFATED O-POLYSACCHARIDES FROM SOME MARINE GRAM-NEGATIVE BACTERIA: STRUCTURAL DIVERSITY AND BIOLOGICAL ACTIVITY

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Gram-negative bacteria are an important component of marine ecosystems where they occupy diverse habitats including deep-sea and hydrothermal vents, sea ice as well as open and coastal water areas. Lipopolysaccharide (LPS) molecules are the major component of the outer membrane of Gram-negative bacteria. These characteristic and vital molecules maintain the contact between the bacterial cell and the surrounding environment; therefore, it is plausible that many of the functional changes induced by the harsh habitats can target LPS structure.

In the past few years, we have studied the O-polysaccharides (OPS) of LPS from some marine Gram-negative bacteria that belong to genera *Cobetia*, *Idiomarina* and *Poseidonocella*. The chemical structure of the carbohydrate moiety of LPS of these marine Gram-negative bacteria is diverse and includes acidic monosaccharides and non-carbohydrate substituents. A common feature of these OPS is the presence of rare for Gram-negative bacteria polysaccharides sulfate groups.

Bacteria of genus *Cobetia* (*C. pacifica* KMM 3789^T and KMM 3878) produce sulfated OPS composed of trisaccharide repeating units. The type strain contains D-glucose 3-sulfate and D-galactose 3-sulfate. A distinctive feature of the OPS of KMM 3878 is the presence of D-galactose 2,3-disulfate. OPS of marine bacterium *C. litoralis* KMM 3880^T consists of trisaccharide repeating units and includes 2-keto-3-deoxy-D-*manno*-octulosonic acid 5-sulfate. The same sugar residue was found in the disacharide repeating unit of OPS from *Poseidonocella pacifica* KMM 9010^T. Besides higher sugar, this polysaccharide includes D-rhamnose 2-sulfate. The OPS of another bacterium of genus *Poseidonocella* – *P. sedimentorum* KMM 9023^T, also consists of disacharide repeating units and contains 2-keto-3-deoxy-D-*glycero*-D-*galacto*-nonulosonic and D-glucoronic acid 2-sulfate. One more sulfated OPS was found in the LPS of deep-sea marine bacterium *I. abyssalis* KMM 227^T. It consists of pentasaccharide repeating units and includes 3-(4-hydroxybutyramido)-3,6-dideoxy-D-glucose 2-sulfate.

Well known, that sulfated polysaccharides can possess either a direct inhibitory action on cancer cells and tumors or influence different stages of carcinogenesis and tumor development, recover the broken balance between proliferation and programmed cell death and are useful for cancer prophylactics.

From biological point of view, we demonstrated that the LPS and O-deacylated LPS from *C. litoralis* KMM 3880^T, *P. pacifica* KMM 9010^T and *P. sedimentorum* KMM 9023^T inhibit colony formation of different human cancer cell lines, including melanoma SK-MEL-5 and SK-MEL-28, colorectal carcinoma HT-29 and HCT-116 and breast adenocarcinoma MCF-7. We showed that sulfated O-polysaccharides retain anticancer properties that open up new prospects for studying the antitumor activity of sulfated LPS and OPS from marine Gram-negative bacteria.

Finally, the structural information about carbohydrate-containing biopolymers may be useful in classification of marine Gram-negative bacteria and elaborating current concepts regarding the organization and mechanisms of functioning of their cells.

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CANCER-PREVENTIVE EFFECT OF WATER-SOLUBLE POLYSACCHARIDES FROM BROWN ALGA *FUCUS EVANESCENS*

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Carcinogenesis is a multistage process in which numerous genes important in the regulation of cellular functions may be prime targets for cancer preventive agents. Prevention and therapeutic intervention by natural compounds is a newer dimension in cancer management. Administration of nontoxic natural compounds was shown to prevent initiation, promotion, and progression events associated with carcinogenesis in different animal models, and has been suggested to effectively reduce cancer mortality and morbidity [1].

The brown algae are truly one of the most valuable gifts of the Ocean, because they produce bioactive polysaccharides, namely alginic acids, laminarans, and sulfated polysaccharides (fucoidans). The algal water-soluble polysaccharides are of great interest, because they possess anticancer, anticoagulant, antiviral, immunostimulatory, antioxidant, and radioprotective activities without toxicity for organisms [2].

The aim of the present study was to investigate cancer-preventive activity of laminaran and its sulfated derivative as well as fucoidan isolated from brown alga *Fucus evanescens* and elucidate molecular mechanism of their action.

It was found that the native and sulfated laminarans inhibited proliferation, colony formation of human colon carcinoma, melanoma and breast adenocarcinoma cells *in vitro* and effectively prevented migration of breast adenocarcinoma cells by inhibiting of the Matrix Metalloproteinases 2 and 9 activities.

The fucoidan from brown alga *F. evanescens* was found to effectively suppress EGF-induced neoplastic cell transformation of mouse epidermal cells and colony formation of human colon carcinoma cells through regulation of TOPK/ERK1/2/MSK 1 signaling axis. In xenograft animal model, oral administration of the fucoidan suppressed colon carcinoma growth.

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Oral report

BIOLOGICALLY ACTIVE QUINONES FROM SEA URCHINS OF CENTRAL VIETNAM COAST

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Class Echinoidea comprises more than 1000 species, and its representatives inhabit all the oceans of the world. Echinoids are divided into regular and irregular sea urchins. Regular sea urchins are symmetrical, almost spherical; irregular sea urchins include sand dollars, which are extremely flattened, and heart urchins, which have a heart shape, as the name indicates. Many of them are of commercial interest because the gonads of sea urchins are edible in various regions such as in North America, Mediterranean and Asian countries. After the removal of the gonads, a large amount of sea urchin shells remains. These are rich in bioactive compounds, which can be used for many biomedical applications. In traditional Chinese medicine, sea urchin shells were used for heart diseases treatment and for the resolution of phlegm. Korean researchers showed the anti-diabetic effect of powdered sea urchin shells on type 2 diabetic rats. Minerals from the shells can be used for replacing and reconstruction of damaged or defective hard tissue; polysaccharides from the shells possess an anti-inflammatory effect; and proteins from the shells and spines demonstrate antitumour activity. Secondary metabolites specific to sea urchins – polyhydroxynaphthoquinones (PHNQs) – also exhibit a wide range of pharmacological activities. For example, the extract of *Strongylocentrotus droebachiensis* shells, which contain PHNQ pigments, demonstrated antiallergic effects. The most well-known sea urchin pigment, echinochrome A, also showed antiallergic activity. Echinochrome A protected mitochondrial functions from cardiotoxic agents such as doxorubicin, sodium nitroprusside and *tert*-butyl hydroperoxide. Echinochrome A treatment enhanced the oxygen consumption rate and the mitochondrial ATP level in rat cardiomyoblast cells and up-regulated the biogenesis of transcription genes. Egyptian scientists demonstrated the potential of echinochrome A in the treatment of rats with experimental type 1 and type 2 diabetes. In addition, echinochrome A is the active substance in the cardioprotective and antioxidant drug Histochrome, produced in Russia from the sand dollar *Scaphechinus mirabilis*.

We investigated composition and content of PHNQ pigments of ten sea urchin species from Nha Trang bay: *Tripneustes gratilla* (Linnaeus, 1758), *Diadema setosum* (Leske, 1778), *D. savignyi* (Audouin, 1829), *Paracentrotus lividus* (Lamarck, 1816), *Echinothrix calamaris* (Pallas, 1774), *E. diadema* (Linnaeus, 1758), *Astropyga radiata* (Leske, 1778), *Toxopneustes pileolus* (Lamarck, 1816), *Stomopneustes variolaris* (Lamarck, 1816), *Phyllacanthus imperialis* (Lamarck, 1816). Rapid structural characterization of the PHNQ compounds existing in the pigment extracts of sea urchins was carried out using HPLC-DAD-MS. The content of PHNQ pigments was determined spectrophotometrically. Main sea urchins pigments were tested for their ability to scavenge the stable DPPH radical and to inhibit lipid peroxidation. It was found that sea urchins *A. radiata* and *D. setosum* are rich sources of echinochrome A. A method for obtaining an echinochrome substance has been developed. The potential of PHNQs should be explored more detail for development of new medicinal preparations.

The study of antioxidant activity of quinonoid pigments was supported by the program "The Far East", Grant No. 15-I-5-030.

CYTOTOXICITY PROPERTIES OF HYDROLYZED ALGINATE FROM VIETNAM BROWN ALGAE

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Mixture of sodium alginate after hydrolysis with ascorbic acid / H_2O_2, include of β-mannuronic acid (M-block), α-guluronic acid (G-block), and alternating sequences of both β-mannuronic and α-guluronic acid (MG-block), was tested for cytotoxic activity by A. Monk method, which showed that hydrolyzed alginate was inhibited 87.46% of LNCaP prostate cancer cells, 82.61% of LU-1 lung cancer cells, 85.76% of cells HL-60 blood at concentrations of 100µg / ml.

Alginate has the highest content of brown algae polysaccharide, and is safe for use as food, so this result gives a new promise in the production of medicinal products that support cancer treatment in Vietnam.

Oral report

STRUCTURAL CHARACTERISTICS OF FUCOSYLATED CHONDROITIN SULFATE ISOLATED FROM SEA CUCUMBER *HOLOTHURIA SPINIFERA*

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In this study, fucosylated chondroitin sulfates were isolated from sea cucumber *Holothuria spinifera* by papain enzymatic digestion. Two fractions of fucosylated chondroitin sulfate (fCS1, fCS2) and fucan sulfate fraction (FS3) were obtained by using anion-exchange chromatography on DEAE-cellulose column. Structural characteristics of fCS1 and fCS2 fractions were eludated using chemical and IR, NMR spectroscopic methods. The resuls indicated that the compositions of fCS1 and fCS2 consist of N-Acetyl-Galactosamine, D-glucuronic acid and fucose residues with different molar ratios, sulfate contents were 24,58% and 30,49% respectively, beside of sulfate content of FS3 was 32,92%. fCS1 and fCS2 fractions were different in partten of sulfation of N-Acetyl-Galactosamine and fucose residues. Sulfate groups were primarily found in C4 and/or C6 positions of N-Acetyl-Galactosamine residues, and C2 and/or C4 positions of fucose residues.

The work was supported by Vietnam Academy of Science and Technology (project no. VAST06.05/15-16), the program “Marine Science and Technology” of Vietnam Academy of Science and Technology.

NANO-EXTRACTION FROM SPIRUNLINA AND POTENTIAL APPLICATION IN INTEGRATIVE MEDICINE

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Spirulina platensis strain with good quality was cultured under the requirements for functional foods according to Vietnam national food standards. The extract product from *Spirulina* was treated with friendly solution of only ethanol and water as a green chemistry process. The obtain particles of the extraction were characterized by dynamic light scattering, SEM, FT-IR, absorption spectra. The particle size distribution is almost around 90 nm was measured by dynamic light scattering. *Spirulina* has high protein, minerals and also antioxidants which go in the daily foods and popular using. The nano-extraction of *Spirulina* could be combine with other traditional medicines to get higher effect and easy to use. This work present the obtained results of nano-extraction from *Spirulina* and potential application in integrative medicine or as special supplement.

Oral report

BIODIVERSITY AND AMYLASE PRODUCTION ACTIVITY OF MARINE FUNGI ISOLATED FROM COASTAL REGIONS OF KHANH HOA PROVINCE, VIETNAM

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It was estimated that there were more than 10.000 marine fungi species but many of them have not been described yet. Moreover, marine fungi strains have been attracting more and more attention as a resource for novel hydrolytic enzymes. Certain marine fungal strains present enzymes with high potential for industrial application in comparison with their terrestrial counterparts such as more tolerance toward extreme conditions of temperature, pH, and salinity. Therefore, it is required to discover new marine fungal strains with new enzyme production activity. This present study aims to explore the biodiversity of marine fungi strains isolated from the coastal regions of Khanh Hoa province in Vietnam and screen for their amylase production activity. A total of 112 yeast and 48 mold strains were isolated from 10 seawater samples collected in Van Phong Bay and Nha Trang Bay at 50 cm depth by culture plate method. A preliminary screening for the production of extracellular amylase using starch hydrolysis plate assay and measurement of enzyme activity revealed 10 mold strains with high amylase activities. Three of which, TB8M, DM12M and DW7M, have been shown with the highest activities. They were identified as *Aspergillus flavus, Aspergillus aculeatus* and *Fusicolla acetilerea*, respectively, using sequencing of intergenic transcribed space (ITS) regions of ribosomal DNA. The results provide data about the biodiversity, taxonomy and amylase activity of mycoplankton in this area, thereby allowing assessment of their positive role in the biogeochemical cycle of carbon in coastal ecosystems and the development of new bioactive compounds for potential industrial applications.

The work was financially supported by Vietnam National Foundation for Science and Technology Development (NAFOSTED) under a grant no. 106-NN.02-2016.70.

STRUCTURE, ENZYMATIC TRANSFORMATION, ANTICANCER ACTIVITY OF FUCOIDAN AND SULPHATED FUCOOLIGOSACCHARIDES FROM *SARGASSUM HORNERI*

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Fucoidans are fucose-rich sulphated polysaccharides with complex structures and assorted biological activities. Fucoidans extracted from brown algae of *Sargassum* genus are extremely heterogenic, which complicates the determination of their fine structure. In this study structure and anticancer activity of fucoidan from *Sargassum horneri* (ShF) and of products of its enzymatic transformation were investigated. The purificated fucoidan's monosaccharide composition includes fucose (90%), galactose (9%) and sulphate groups (23%). The fucoidan's NMR spectrum was unclear. The enzyme treatment resulted in the mixture of oligosaccharides with various polymerization degrees (LMP) and fucoidan fragments of high molecular weight (HMP). It yielded 52% of HMP and 40% of LMP. The LMP fraction was divided by ion-exchange chromatography on Q-sepharose. Structure of 6 sulphated oligosaccharides with polymerization degree 4-10 was established by NMR-spectroscopy. The HMP fraction was treated by fucoidanase from marine bacteria *Wenyingzhuangia fucanilytica*, which allowed for NMR spectra sufficiently fine to determine its structure as well.

The fucoidan extracted from *S. horneri* is almost pure fucan. The main chain of the fucoidan is established to consist mostly of the repeating →3-α-L-Fuc*p*(2SO_3^-)-1→4-α-L-Fuc*p*(2,3SO_3^-)-1→ fragment, with insertions of →3-α-L-Fuc*p*(2,4SO_3^-)-1→ fragment. Unsulphated side chains with the α-L-Fuc*p*-1→2-α-L-Fuc*p*-1→ structure connect to the main one at the C4 of monosaccharide residue.

Human colorectal DLD-1 cell line (8000 cells) was treated with the native fucoidan, HMP and four oligosaccharides in the concentration 100 μg/mL, incubated for 24 h. The investigated poly- and oligosaccharides were non-cytotoxic at concentrations less than 200 μg/mL for the investigated cell line. ShF and HMP suppressed colony formation of DLD-1 cells on about 50% compared with control, while oligosaccharides were non-effective on the inhibition of cancer cells colony formation. Some differences in the anticancer activities of fucoidans and oligosaccharides can be explained by their structural features. The degree of polymerization of fucoidan's fragments apparently influences antitumor activity heavily. Short fragments of fucoidan with 4-10 polymerization degree are obviously unable to interact with the target tumor cells effectively.

The study was supported by Grant No. 16-13-10185 from the RSF (Russian Science Foundation).

FUCOIDAN SULFATASES: CLONING, EXPRESSION AND BIOCHEMICAL CHARACTERIZATION

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Sulfatases play a key role in the catabolism of various sulfated polysaccharides of marine origin (ulvans, carrageenans, agarans, fucoidans, etc.). Most characterized carbohydrate sulfatases catalyze the cleavage of sulfate groups through the hydrolytic mechanism and belong to the S1 family (SulfAtlas classification) [1]. The variety of sulfated polysaccharide structures implies a large amount of sulfatases with different substrate specificity. Interest in carbohydrate sulfatases does not weaken and is associated with the possibility of using them to establish the structure of sulfated polysaccharides [2]. Sulfatases can be used to produce sulfated polysaccharides with a sulfation pattern which are not presented in nature. Such modifications can be used to establish the relationship between the sulfate position in biologically active polysaccharides and their physiological effects. To date, only a few carrageenan sulfatases and agaran sulfatases have been biochemically characterized [3]. There are only fragmentary data about fucoidan sulfatases [3]. Amino acid sequences, specificity, mode and mechanism of action of fucoidan sulfatase are still unknown.

In this work, the preparation of two recombinant sulfatases able of catalyzing the cleavage of sulfate groups from fragments of fucoidan molecules is described for the first time. Two genes of sulfatases of the marine bacterium *Wenyingzhuangia fucanilytica* CZ1127^T, named by us as *swf1* and *swf4*, were cloned and the proteins were produced in *Escherichia coli* cells. Some biochemical characteristics of recombinant fucoidan sulfatases have been studied. The predicted molecular weights of gene products were 55.94 kDa (495 amino acid residue) for SWF1 and 57.08 kDa (496 amino acid residue) for SWF4. The analysis of amino acid sequences allowed to classify sulfatases SWF1 and SWF4 to subfamilies S1_17 and S1_25 of the S1 family of sulfatases. Specificity and some catalytic features of sulfatases were determined using various sulfated fucooligosaccharides (Fig. 1). Based on the substrate specificity, the enzymes are classified by us as fucoidan exo-2O-sulfatase (SWF1) and fucoidan exo-3O-sulfatase (SWF4).

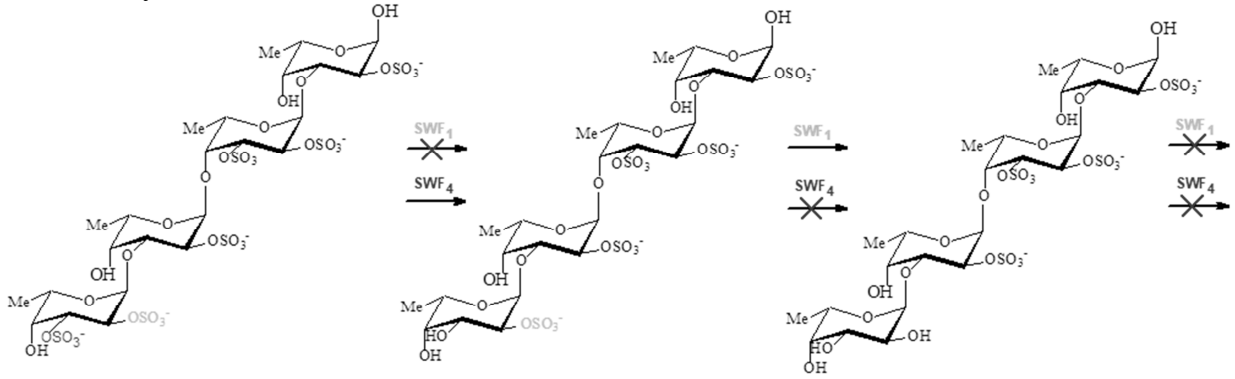

Figure 1 - Scheme of sequential action of fucoidan sulfatases SWF1 and SWF4 on 2,3-O-sulfated tetrasaccharide. ⤫ - sulfatase is not able to catalyze desulfation of this substrate.

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Oral report

POLYSACCHARIDES FROM BROWN ALGA SARGASSUM DUPLICATUM

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Brown algae are a renewable, easily cultivated source of interesting in structure and biological activity polysaccharides: laminarans, fucoidans and alginic acids. The immunomodulating, radioprotective, antiviral actions of these polysaccharides, as well as anticoagulant, thrombolytic, antitumor and other activities, characteristic of fucoidans, are widely studied. These properties make promising their use in medicine, agriculture, fish farming, cosmetic and food industries [1, 2]. The aim of this work is to obtain highly-purified polysaccharides from Vietnamese brown alga *Sargassum duplicatum*, to study their structures and anticancer activity *in vitro*.

The laminaran and fucoidan fractions were obtained from sample of alga by complex scheme of isolation, including extractions by 70% ethanol and diluted HCl, anion exchange and hydrophobic chromatography. The yields of high-purified laminaran SdL and fucoidan SdF were 0.28 and 0.15% of defatted alga weight, respectively.

Analysis of monosaccharide composition of laminaran and fucoidan showed that SdL was pure glucan, SdF - galactofucan (Fuc:Gal ~ 1:1). The sulfate content of fucoidan was 31.7 %. By the data of NMR spectroscopy, laminaran SdL contained a main chain of 1,3-linked residues of β-D-glucopyranose with branching points, to which the single glucose residues were attached at C6 (ratio of bonds 1,3:1,6 = 6:1). The galactofucan was deacetylated and desulfated to obtain more regular fraction SdFDADS. According to data of NMR analysis, SdFDADS contained a main chain of alternating 1,4-linked α-L-fucopyranose and β-D-galactopyranose residues with a small amount of single α-L-fucopyranose in branches. Also it was shown, that fucoidan could be sulfated in position C2 and C3 of both fucose and galactose residues, and C6 of galactose residues.

We performed partial depolymerization of the fucoidan by autohydrolysis in heavy-oxygen water and mild acid hydrolysis. The structural features of the fucoidan fragments were investigated by mass spectrometry and confirmed by NMR data. It was shown that the main chain of the polysaccharide was built up of the repeating unit of →4)-α-L-Fuc-(1→4)-β-D-Gal-(1→ fragments. Side chains were represented by extensive (DP = 5, or, probably, more) chains, built up of 1,3-linked 2,4-disulfated fucose residues, having some branching points at C2. The branching points of the main chain were, probably, at C6 of Gal residues, since corresponding 6-linked disaccharides were found. The sulfates occupied positions C2, C4 and less at C3 of fucose residues and C2, C3 and less at C4, C6 of galactose residues.

Thus, the investigated fucoidan SdF from *S. duplicatum* had unusual structure. The fucoidans with the main chain of alternating 1,4-linked residues of fucose and galactose were not described previously in literature.

We determined the cytotoxicity of fucoidan SdF from *S. duplicatum* for colorectal carcinoma cells HT-29, HCT 116, and DLD-1and their ability to inhibit the colony formation of listed cell lines. We established that SdF was non-toxic for tested cells under 400 μg/mL and in concentration of 200 μg/mL inhibited the colony formation of DLD-1, HCT 116 and HT-29 cells on 70, 43 and 23%, respectively.

The work was supported by RFBR grant № 16-33-60023.

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PRENYLATED POLYPHENOLIC COMPOUNDS FROM *MAACKIA AMURENSIS* ROOT BARK

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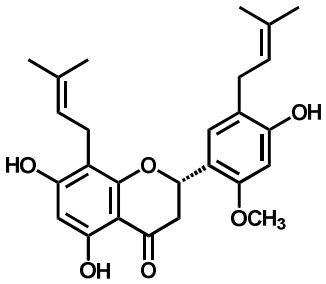
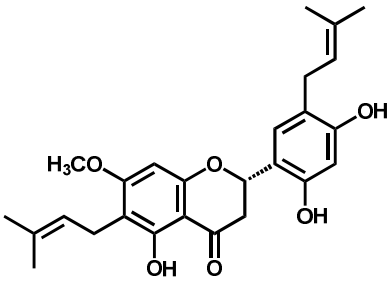
Maackia amurensis is widespread in the southern part of the Russian Far East and is a rich source of polyphenolic metabolites possessing significant hepatoprotective, antioxidant and antiplatelet properties. Previously, the roots of this tree have been shown to contain gentiobiosides, primverosides and glucosides of isoflavones and pterocarpans [1]. However, prenylated polyphenolic compounds of *M. amurensis* roots have not been investigated so far.

In this study eight polyphenolic compounds, including two new prenylated flavanones **1** and **2** and a new prenylated stilbene **3** have been isolated from *M. amurensis* roots using column chromatography on silicagel, LH-20 and C-18.



1



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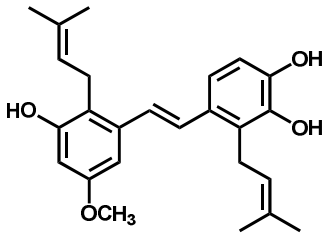

3

The structures of these compounds were established as (2*S*)-4',5,7-trihydroxy-2'-methoxy-5',8-di-(3-methylbut-2-enyl)-flavanone (**1**) (*2S*)-2',4',5-trihydroxy-7-methoxy-5',6-di-(3-methylbut-2-enyl)-flavanone (**2**) and (*E*)-3,3',4'-trihydroxy-5-methoxy-2,2'-di-(3-methylbut-2-enyl)-stilben (**3**) by NMR, CD, HPLC–PDA–MS and HR-ESI-MS data analyses.

Along with the new compounds, five known prenylated flavanones, maackiaflavanone (**4**), maackiaflavanone A (**5**), maackiaflavanone B (**6**), abyssinone V (**7**) and 5-hydroxysophoranone (**8**) have been isolated and identified by comparison of their physical and spectroscopic data reported previously [2]. We showed that these polyphenols are present not only in *M. amurensis* stems, but also in the root bark of this plant.

The cytotoxicity of compounds **1**, **2** and **4**–**8** against two human cancer cell lines HeLa and SK-MEL-5 was detemined by MTS method. Cisplatin was used as a reference compound. All tested polyphenols inhibited tumor cell growth. Compounds **4**, **6** and **8** showed the strongest cytotoxic activity among the compounds tested with IC_{50} values of 6.5, 8.8 and 7.7 μM, against SK-MEL-5 cells and 8.2, 18.8 and 12 μM against HeLa cells, respectively.

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EFFECT OF EXTRACTION METHODS ON THE CHEMICAL STRUCTURE OF ULVAN FROM *ULVA RETICULATA*

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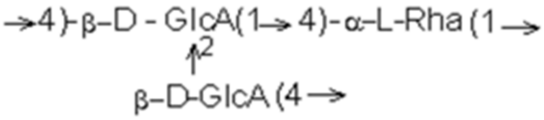
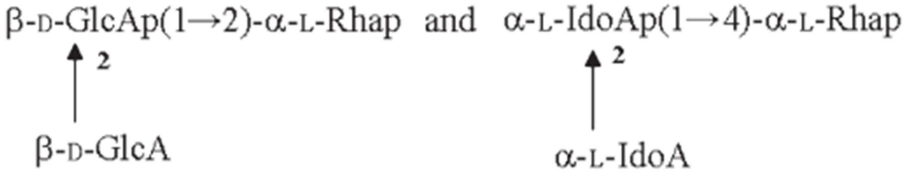
Ulvans are water-soluble sulfated polysaccharides derived from marine green seaweed. They were reported to exhibit a wide range of physiological and biological activities. The structural complexity of ulvans may be derived from the differences in seaweed species, extraction method and place of cultivation.

In this study, IR, NMR, SEC-MALLS techniques were applied to determine chemical constituents and structures of ulvan extracted by water and HCl 0,1 N from seaweed *Ulva reticulata* collected in Nhatrang bay. The results showed a significant effect of extraction conditions on structure of the ulvan.

Ulvan extracted from water was composed of rhamnose, galactose, xylose, manose and glucose (mole ratio Rha: Gal: Xyl: Man: Glu = 1: 0.12: 0.1: 0.06: 0.03), uronic acid (22.5%), sulfate (17.6%). This ulvan structure was built up [→4)β-D-GlcA(1→4)α-L-Rha3S-(1→] branching at C-2 of glucuronic acid.



Ulvan extracted by acid contained monosaccarides with mole ratio Rha: Gal: Xyl: Man: Glu = 1: 0.12: 0.1: 0.06: 0.03, uronic acid (22.5%), sulfate (17.6%). Chemically structural determination showed that the ulvan mainly composed of two main chains β-D-GlcA(1→2)α-L-Rhap and α-L-IdoAp(1→4)-α-L-Rhap. Branching point was at O-2 of uronic acid. Sulfate groups were attached at C-2, C-3 and C-4 of rhamnose residues.



GALACTOFUCANS FROM BROWN ALGAE OF RUSSIA AND VIETNAM

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The brown algae produce valuable biologically active sulfated polysaccharides – fucoidans. The fucoidans can have different structures: from pure fucans (built from fucose residues) to fucoidans – heteropolysaccharides. The algal polysaccharide built predominantly from fucose and galactose residues (galactofucans) are one of types of fucoidans.

In this work we studied a fucoidans from six species of brown algae of Far East of Russia (*Alaria angusta*, *Alaria marginata*, *Sacharina gurjanovae*) and coast of Vietnam (*Sargassum duplicatum*, *Sargassum mcclurei*, *Turbinaria ornata*). The individual fractions of galactofucans were obtained from listed algae by extraction with 0.1 M HCl, further fractionation of extracts by hydrophobic and anion exchange chromatography and thorough purification of obtained fraction. We applied a structural modification of galactofucans, including methylation, desulfation, deacetylation, and depolymerization of native and modified polysaccharides, autohydrolysis and mild acid hydrolysis in different conditions. Also, the instrumental methods: 2D NMR of fucoidans and their derivatives; MALDI and ESIMS mass spectrometric analysis of oligosaccharide fragments of fucoidans were used for investigation of fucoidan structures [1-7].
It was shown, that the galactofucans from *A. angusta*, *A. marginata*, and *S. gurjanovae* (Fuc:Gal = 1:0.9, 1:1 and 1:0.3, respectively), belonging to order Laminariales, contained a main chain build up from 2,4-disulfated 1,3-linked α-L-fucose residues. Chains of galactose residues were found in fucoidans from *A. marginata* and *S. gurjanovae*. Fucoidan from *T. ornata* (Fuc:Gal = 1:0.2) contained a main chain build from 1,3-linked fucose residues with branches at C2 and C4 in the form of single residues or short chains of fucose and galactose. Single HexA residues could be in branches at C2 of the main chain of this fucoidan. The brown alga *S. mcclurei* produced fucoidan (Fuc:Gal = 1:0.6) with the main chain build from 1,3-linked 2,4-disulfated fucose residues, including residues of 1,4-linked 3-sulfated fucose, and 6-linked galactose on the reducing ends. The branches were represented by galactose residues or chains of alternating fucose and galactose residues. Fucoidan from *S. duplicatum* (Fuc:Gal = 1:1) had a main chain build up from 1,4-linked alternating α-L-fucose and β-D-galactose residues. Side chains were represented by chains of 1,3-linked fucose residues with branching points at C2. All investigated galactofucans exhibited no cytotoxicity under 500 μg/ml and were effective against colony formation of cancer cell lines *in vitro*.

The work was supported by RFBR grants № 16-54-540004.

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CARRAGEENAN/CHITOSAN SOLUBLE COMPLEXES AND FILMS FOR CONTROLLED RELEASE OF DRUGS

A.V. Volod'ko[1]*, V.N. Davydova[1], E.A. Chusovitin[2], N.P. Mischenko[1], I.M. Yermak[1]

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The capacity of oppositely charged natural polymers to form polyelectrolyte complexes (PECs) offers the ability to prepare various kinds of bioactive composites that has increasingly become important in the formulation of pharmaceuticals for controlling the release of drugs. Carrageenans (CGs) are complex families of water-soluble, linear, sulfated galactans. They mainly consist of alternating 3 –linked β-D –galactopyranose and 4-linked α-D galactopyranose or 4-linked 3,6 anhydro-α-D- galactopyranose, forming the disaccharide repeating unit of CG. They possess a wide spectrum of biological activities, such as antiviral, anticoagulant, antitumor, and immunomodulatory activities. Chitosan (CH) is a linear polysaccharide polymer chain constructed from β-1,4-linked residues of D-glucosamine and N-acetyl-D-glucosamine. It has also shown potential pharmaceutical properties including hypolipidemic, hepatoprotective, radioprotective, immunostimulatory, antibacterial and antiviral activity. CG:CH PEC in the form of gels, solutions and films can be used as a basis for controlled release of the drug.

The purpose of the work is to obtain soluble CG:CH complexes and multilayer films as matrices that include the natural polyhydroxynaphthoquinone echinochrome (Ech) (registered in the Russian Federation as a medicine "Histochrome") with a wide range of pharmacological effects.

CG:CH complexes were obtained and the initial components ratio effect on their formation was studied by dynamic light scattering (DLS), atomic force microscopy (AFM) and electrokinetic measurements. It was shown that the PEC formation mechanism is determined by polymer present in excess in the complex. According obtained dates CG:CH complexes with incorporated Ech represent a charge particle of two populations in size in size: 128-150 nm (95%) and 3000 nm – 5 %, for complex with an excess of CH; 200 nm (92%) and 4000 nm – 8%, with an excess of CG. More stable complexes were obtained by adding Ech to CG. The results of AFM and SEM show that the addition of Ech to CG:CH leads to a change in the supramolecular structure of the complex. CG:CH complex represents CG fibers with CH particles on the surface, while for a complex with incorporated ECH on the surface irregularly shaped particles are visible.

With variation of preparation methods (sequence of layering, concentration of polysaccharides, as well as concentration and solution for inclusion of ECH), a series of films of CG:CH with included ECH was obtained. The defect-free, smooth, thin films were formed by layer-bylayer deposition of polyion solutions. A 1% aqueous solution of CG (with Ech) was deposited onto a balanced substrate and dried to the gel-like state; afterwards, a 1% CH solution was deposited on the surface of the layer and the films were dried at room temperature. The obtained PEC films (with a thickness of 25–30 μm) consisted of three layers (i.e., CG, CS and the insoluble PEC layer formed between two polyion layers) and Ech. Some physical and technical characteristics of the films were obtained.

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NOVEL RECOMBINANT FUCOIDANASE FROM MARINE BACTERIUM *WENYINGZHUANGIA FUCANILYTICA*

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Worldwide accessibility, abundance and diversity make algae an important source of biologically active metabolites. Among the high-molecular compounds of brown algae the most interesting are fucoidans - sulfated heteropolysaccharides. A wide range of their biological activity attracts more and more attention of researchers [1]. In addition, fucoidans can be used for targeted drug delivery [2]. These properties make promising their use in medicine. There are difficulties in establishing the structure of these biopolymers, characterized by a large structural diversity of fucoidan molecules, which makes it difficult to identify the relationship between their structure and physiological action. The most promising tools for establishing the structure and standardization of complex polysaccharides is the use of enzymes with a precisely established substrate specificity and mechanism of action. Such enzymes are fucoidanases, which remains one of the least studied hydrolase class enzymes.

Information about the nucleotide sequences of genes encoding fucoidanase was obtained by sequencing the genome of the marine bacterium *Wenyingzhuangia fucanilytica* (GenBank: GCA_001697185.1) [3]. Recombinant forms of fucoidanases FWF1 and FWF2 were obtained by heterologous expression in *Escherichia coli* cells. The recombinant enzymes were partially purified and used to study some of the catalytic properties. We studied the optimum pH values for the manifestation of enzymatic activity, the temperature optimum and the effect of divalent metal salts solutions on fucoidanase activity. Both fucoidanases showed the highest activity in a wide pH range from 6 to 8.4. Temperature optimum of FWF1 and FWF2 ranged from 20^{o}C to 35^{o}C. Activity of both fucoidanases was inhibited by Al^{3+}, Cu^{2+}, Fe^{3+} ions and in lesser extent by Co^{2+}. The addition of Na^{+} ions (NaCl) to the reaction mixture dramatically increased activity of FWF1 and FWF2. The presence of a solution of EDTA (10 mM) in the reaction mixture inhibited the activity of both enzymes. Studying of the kinetics of substrate hydrolysis revealed that fucoidanases FWF1 and FWF2 are endo-acting enzymes. It was confirmed by the formation of a large variety of sulfated oligosaccharides in the early stages of enzymatic hydrolysis. Earlier, using the classical methods of carbohydrate chemistry, the structure of fucoidan from *Fucus evanescens* was established. It was shown that the polysaccharide consists of alternating 1 → 3-; 1 → 4-linked fucose 2-sulfate residues. Additional sulfate groups occupies the 4 position of a part of 1→3-linked fucose residues [4]. Fucoidan from *Fucus evanescens* was hydrolyzed using recombinant fucoidanases FWF1 and FWF2. The resulting mixture of sulfated oligosaccharides was separated by ion exchange chromatography. Homogeneous oligosaccharides were analyzed by NMR spectroscopy (1H, 13C, COSY, HSQC, HMBC). The NMR spectra analysis of the obtained oligosaccharides revealed the presence of additional sulfate groups at C3 in 1→4-linked α-L-fucose residues. In this way, we found that fucoidan from *F. evanescens* contains fragments of structures with sulfation at the 3 position.

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O-GLYCOSIDE HYDROLASES OF PSYCHROTOLERANT BACTERIA FROM MICROBIAL COMMUNITY OF THE PACIFIC RED ALGA *AHNFELTIA TOBUCHIENSIS*

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The study of O-glycoside hydrolases (EC 3.2.1.-) is an actual problem. These enzymes are most in demand from modern biotechnology and are widely used for the needs of the food, pharmaceutical, light industry and other fields. Marine bacteria are of greatest interest as sources of O-glycoside hydrolases. Bacteria play a key role in the metabolic processes of marine ecosystems. Quick response of microorganisms to environmental changes and their important roles in ecosystems are provided by various enzyme systems. Heterotrophic, aerobic obligate marine bacteria are the sources of enzymes with original properties and unusual specificity.

In the Far East Russia seas, the red alga-agarofit *Ahnfeltia tobuchiensis* forms extensive algal fields representing monodominant community unattached seaweed. It is of great environmental importance, contributing to the purification of eutrophicated coastal waters prone to anthropogenic contamination. In addition, algae are the source of the gel-forming polysaccharide of agar, which has found wide application in microbiology and various branches of the food industry. Agarose is the basis of agar-agar. The agarose molecule has a regular structure built from 3-linked β-D-galactopyranose and 4-linked 3,6-anhydro-α-L-galactopyranose, which may contain methyl, sulfate, and carboxyethylene substituents. The purpose of this work was to study the diversity of O-glycoside hydrolases in bacteria isolated from the microbial community of this alga growing in the coastal zone of Paramushir Island (Kuril Islands) of the Sea of Okhotsk, as well as comparison of various glycosidase activities with the taxonomic position of algal isolates. Identification of bacteria was carried out on the base of standard phenotypic, chemotaxonomic and genotypic characteristics. Affiliation to genus and species was determined from the sequence of nucleotides in the 16S rRNA gene and by the combination of physiological and biochemical characteristics of the strains.

As a result of the work done, the phylogenetic diversity of bacteria from the microbial community of *Ahnfeltia tobuchiensis* was shown. New genera and species of bacteria are found in this community. The distribution of polysaccharide hydrolases, polysaccharide-lyase and glycosidases among members of the microbial community, especially new bacteria was shown. Profiles of O-glycoside hydrolases of bacteria associated with red alga *Ahnfeltia* sp., playing an important role in trophic relationships of organisms of the host and bacteria were characterized. The results can be used for characterization both the metabolic potential and for selecting of biotechnologically promising strains of marine bacteria.

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SEARCH OF MARINE BACTERIUM *PSEUDOALTEROMONAS* SP. ALPHA-GALACTOSIDASE INHIBITIRS AMONG MARINE INVERTEBRATES INHABITED IN THE KURIL ISLAND'S WATER

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The marine environment is considered as attractive source of inhibitors of various enzymes. Inhibitors are compounds that reduce or entirely suppress the activity of enzymes. Such compounds are fine tools in biochemical and pharmacological studies. 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 [1].

α-Galactosidases [EC 3.2.1.22] play an important role in the vital functions of many micro- and macroorganisms. 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]. A large-scale screening of extracts of marine invertebrates, especially sponges and ascidians, is carried out during several recent expeditions of the NIS "actinic oparin". The results of such studies obtained in the 47-th expedition were already reported at the 1st Symposium "Marine Enzymes and Polysaccharides" in 2012 [5].

This work presents the results of the screening for inhibitors of α-galactosidase of 383 water-ethanol extracts of sea sponges and sea squirts collected during the 51-th expedition of vessel "Academic Oparin" in May and June 2017 in the Kuril Islands.

The recombinant enzyme solution (0.05 ml, 0.02 units of α-galactosidase in 0.05 M sodium phosphate buffer solution, pH 7.0) was placed into each well of 96-well plate, than mixed with 0.025 ml of the solution of the extract water solution (4 mg / ml) and held for 30 minutes. Then 0.004 ml of the substrate was added to this mixture to a final concentration of 0.002M and incubated for 10 min at room temperature. The reaction was stopped by the addition of 0.150 ml of a solution of 0.5M NaOH. The hydrolytic activity of the enzymes was determined from the n-nitrophenol released during the enzymatic reaction. The enzyme residual activity (%) after the addition of various extracts was calculated by the formula: $I = U / U_0 * 100$, where U_0 is the activity of the enzymes without addition of the extracts (control).

As the result, 105 extracts inhibited the enzyme activity. 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 sediments. Most of the animals, which are a potential source of inhibitors, were collected in the water area of the Itirup, Urup and Onekotan Islands.

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THE EFFECT OF FUCOIDAN FROM THE BROWN ALGA *FUCUS EVANESCENCE* ON THE ACTIVITY OF α-N-ACETYLGALACTOSAMINIDASE OF HUMAN CANCER CELLS

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α-N-acetylgalactosaminidase (EC 3.2.1.49.) catalyzes the hydrolysis of N-acetamido-2-deoxy-α-D-galactoside residues from the non-reducing ends of various complex carbohydrates and glycoconjugates. This enzyme is found in the organs and tissues of mammals and birds, in invertebrates *Helix pomatia*, earthworm. The enzyme takes part in the catabolism of complex oligosaccharides. In the marine environment, α-N-acetylgalactosaminidases have been found in the liver and digestive organs of the marine gastropod *Turbo cornitus*, the starfish *Asterina amurensis*, the squid *Todarodes pacificus*, the mollusc *Patella vulgate*, the shrimp *Solenocera melantho*, the striped tuna *Katsuwonus pelamis*, as well as in marine bacteria of the genus *Arenibacter* [1].

Previously, it was established that α-N-acetylgalactosaminidase was produced by human cancer cells and accumulated in the blood plasma of patients [2]. The ability of α-N-acetylgalactosaminidase to inhibit macrophage activity in patients with developing tumors, acting as an immunosuppressor is of a great interest. Compounds inhibiting the activity of this enzyme could serve as a base of the creation of an immunotherapeutic drug that is why it is necessary to find a source of α-N-acetylgalactosaminidase.

A method for screening α-N-acetylgalactosaminidase activity in cancer cells has been developed. Cancer cell lines HT-29, HCT-116, SK-Mel-28, RPMI-7951, DLD-1 were tested for the ability to express α-N-acetylgalactosaminidase. It has been shown that almost all cells express the enzyme. The highest specific activity of the enzyme was found in human intestinal carcinoma cells DLD-1. α-N-acetylgalactosaminidase was isolated from the biomass of these cells. Its biochemical and catalytic properties are characterized. The enzyme exhibits maximum activity at the pH 5.2 and temperature 55 ºC. The Michaelis-Menten constant for commercial p-NP-α-D-galactosaminide is 1.28 mM, V max = 0.046 μmol/min/mg at 37 °C, pH 5.2.

The effects of fucoidan from the brown alga *Fucus evanescence* on the activity of α-N-acetylgalactosaminidase in human colon carcinoma DLD-1 cells and on the biosynthesis of this enzyme after the action of the polysaccharide were investigated. It was shown that fucoidan did not inhibit free α-N-acetylgalactosaminidase, however, it reduced the expression of the enzyme in the cells.

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INFLUENCE OF ANTHROPOGENIC FACTORS ON THE LEVEL OF LECTINS FROM MARINE BIVALVE

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Bivalve mollusks that are near-bottom filter feeders have proved to be adequate bioindicators of sea contamination. It is no mere coincidence that mussels have been chosen as an object for the monitoring of coastal waters. Among bivalve, there are many commercially important species that are used as food and cultivated. Therefore, it is necessary to estimate the level of health of these animals depending on the quality of the natural environment.

Unlike vertebrates, invertebrates lack antibody-mediated humoral immunity in their system. However, they possess innate immunity: by virtue of the innate immune response, they are believed to possess host-defense mechanisms which protect them from various pathogenic infections in the aquatic environment. The governing factor in distinguishing foreign tissues and cells ("non-self" objects) is an interaction between humoral factors, hemocytes, and target cells that leads to agglutination, opsonization, phagocytosis, or incapsulation of the foreign material. Lectins are the main factors performing protective functions.

Lectins are proteins or glycoproteins with specific binding affinity for carbohydrate moiety of glycoproteins or glycolipids on cell surface. Many lectins also possess various biological activities in vitro and in vivo, and some lectins bind to specific carbohydrate receptors on cells, which can activate the receptors and thereby induce intracellular signaling cascades leading to alterations in cellular behavior.

In our previous study, some novel lectins were identified and characterized from the sea invertebrates: from the mussels *Mytilus trossulus* (MTL) and *Crenomytilus grayanus* (CGL), from the scallop *Patinopecten yessoensis* (PYL) and mollusk *Glycymeris yessoensis* (GYL). Using the methods of the hemagglutination assay and enzyme-linked immunosorbent assay we investigated the lectins activity in response to anthropogenic contaminants. These study were conducted at the Marine Experimental Station of G.B. Elyakov Pacific Institute of Bioorganic Chemistry (PIBOC) FEB RAS in Troitsy Bight (Possjet Bay, Sea of Japan). In the experiments on the effects of cadmium acetate we revealed significant changes in lectins activity as a response to the effects of Cd^{2+}. Evidently, similar changes reflect the adaptive compensatory processes that occur in mollusks subjected to intoxication. Variations in the level of lectins activity with a dependence on the concentration of the toxic agent or on the duration of the exposure had a phase character. The experiments with mollusks subjected to the impacts of the detergent and diesel fuel showed that the content of lectins changed with the dependence on the time of exposure of the animals to the contaminating agents. These compounds very probably affect different links in the protective system of the animals. Each of the tested compounds is characterized by its own dynamics of activation of components of the mollusk immune system, which obviously depends both on the nature of the toxic agent (especially its biotoxic effect) and on the strength (concentration and duration) of the traumatic effect.

Thus the determination of level of lectins can be used both for early diagnostics of environmental pollution, and for assessment of the state of the immune system of animals, especially along with other characteristics of the defense systems of marine invertebrates.

THE TECHNOLOGY OF ALGAE HARVESTING AND POLYSACCHARIDE EXTRACTION – OPPORTUNITY AND CHALLENGE

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Polysaccharide exists in all living plants on the earth, and their bioactive are very diversity. The bioactive of algae polysaccharide are best evaluated inside of plant polysaccharide. However, the content and bioactive of algae polysaccharide are depended on various factors, for example: age and size of algae, grown algae local (temperature, the impact of people etc), algae season, algae species, harvesting technology, post-harvest technology (algae preservation, polysaccharide extraction, application of polysaccharide). In actual production, many factors are not identified or controlled. At the same time, the comsumption trend of seaweed polysaccharide is increasing more and more. Thus, the review will emphasizes Opportunity and challenge for algae harvesting and polysaccharide extraction technology.

STUDY TECHNOLOGY OF PRODUCTION SOLUBLE PROTEIN FROM SCRAP OF SHRIMP USING ENZYME FLAVOURZYME

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In Vietnam, seafood processing is one of the leading sectors of the economy, bringing a large export turnover to the country. Vietnamese exports of shrimp in 2015 reached $ 3 billion, representing 2% of the value of exports compared to the whole country and 44% of the value of exports of seafood [1]. The main exports of shrimp are HOSO (head on shell-on shrimp), HLSO (headless shell-on), PTO (peeled tail-on). A large amount of scrap of shrimp in the production process is not considered and is often used as low-grade products. However, when analyzing the chemical composition of these shrimps, high enough protein content was shown: 19 ÷ 21%, lipid: 0.7 ÷ 1.7%, water: 74.5 ÷ 7.0%, ash: 1.14 ÷ 1.63%. In addition, in this protein there are many kinds of non-replaceable amino acids.

In the laboratory of food technology faculty, Nha Trang University, the initial technological scheme for the production of dissolved protein from scrap of shrimp using the Flavourzyme enzyme is proposed, as shown in Figure 1 [2]:

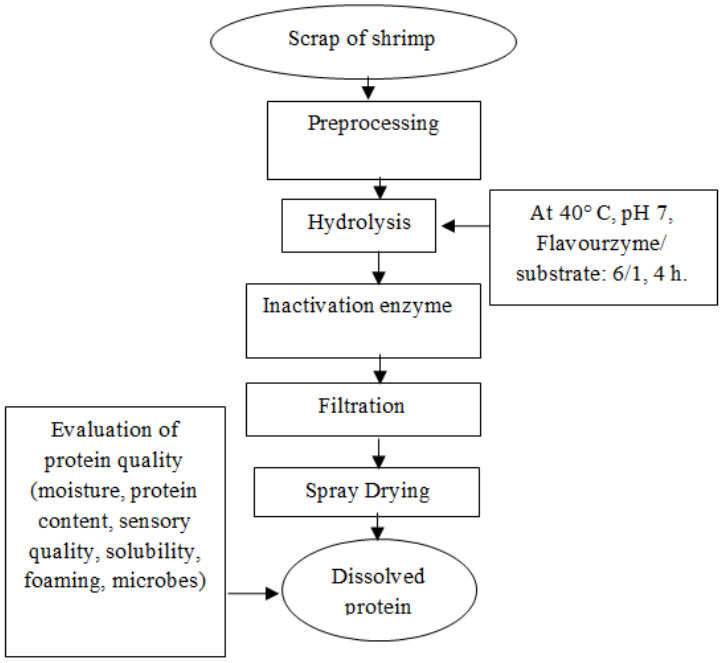

Figure 1. Initial technological scheme for the production of dissolved protein from scrap of shrimp using the enzyme Flavourzyme

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ENZYMATIC MODIFICATION OF BROWN ALGAE POLYSACCHARIDES

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Enzyme bioprospecting is a basic research activity devoted to the search for novel biocatalysts. Remarkable or unusual bioprocesses are performed by marine biocatalysts due to habitat-related characteristics such as salt tolerance, hyperthermostability, barophilicity and cold adaptivity, which can be desirable features recognized from a general biotechnological perspective. The knowledge of marine enzymes is of key importance to exploit an enzyme's potential. 91 marine invertebrates samples, 200 marine bacteria strains and 128 marine fungi strains were collected and isolated for screening enzymes which degrade fucoidan and laminaran. The research results showed that the percentage of marine invertebrates samples, marine bacteria strains and marine fungi strains have hydrolytic activity fucoidan from *Sargassum mcclurei* were 53,8 %, 34,5% and 27,3% respectively. Meanwhile, only 35,2% samples of marine invertebrates have laminarnase active.

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PARTIAL STRUCTURE AND IMMUNOLOGICAL ACTIVITY OF LIPOPOLYSACCHARIDE FROM MARINE BACTERIUM *PSEUDOMONAS GLAREAE* KMM 9500^T

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Gram-negative bacteria of *Pseudomonas* genus are highly adaptable towards an ever-changing environment and can colonize different ecological niches including marine habitats. Lipopolysaccharide (LPS) molecules, that compose a huge part of the outer membrane of Gram-negative bacteria, represent the contact between the bacterial cell and the surrounding environment and play an essential role in the adaptation of the organisms to the peculiar environmental conditions. In most bacteria, LPS displays a common structural architecture that includes three domains: a lipophilic moiety termed lipid A, a hydrophilic glycan called the O-polysaccharide (OPS), and a joining core oligosaccharide.

It is well known, that LPS of some Gram-negative bacteria is a very potent virulence factor that activates mammalian innate immune cells primarily through CD14/TLR4/MD-2 receptor complex. After the interaction with specific receptor complex on the host effector cells, LPS induces the synthesis of a large number of endogenous cytokines. The overproduction of the cytokines can lead to an uncontrollable inflammatory reaction, which is also known as «cytokine storm». In general, *Pseudomonas* LPS stimulate less inflammation and lower overall host responses compared with that of enterobacterial LPS, such as from *E. coli*, but the TLR4-mediated responses are highly dependent on the acylation level of the lipid A.

Many terrestrial isolates of *Pseudomonas* genus have been studied extensively, while *Pseudomonas* strains from marine sources are still poorly investigated. The chemical structure of the carbohydrate moiety of the marine Gram-negative bacteria LPS is highly diverse and includes an ever-extending number of rare and unusual monosaccharides and non-carbohydrate substituents. Recently, we have established the structures of the OPSs from marine bacteria *P. stutzeri* KMM 226 and *P. xanthomarina* KMM 1447^T. In this work, we presented the study of LPS from marine *P. glareae* KMM 9500^T, a bacterium isolated from a sediment sample collected from the Sea of Japan seashore.

The O-polysaccharide was built up of linear tetrasaccharide repeating units constituted by D-glucuronic acid (D-GlcA), L-rhamnose (L-Rha), D-glucose (D-Glc) and 5-N-acetyl-7,9-O-[(*S*)-1-carboxyethylidene]-3,5-dideoxy-L-*glycero*-L-*manno*-non-2-ulosonic acid (Sug*p*8Ac(~70%)7,9(S-Pyr), partially substituted by an O-acetyl group at position 8 (~70%):

→4)-α-D-Glc*p*A-(1→3)-β-L-Rha*p*-(1→4)-β-D-Glc*p*-(1→4)-β-(Sug*p*8Ac(~70%)7,9(S-Pyr)-(2→

The lipid A fraction consisted mainly of symmetric hexa-acylated, penta-acylated and tetra-acylated components in which 3-hydroxydecanoyl and 3-hydroxydodecanoyl residues were linked as primary acyl substituents to the classical bisphosphorylated D-glucosamine disaccharide, while secondary substitutions of N-acyl fatty acids were presented only as dodecanoyl residues. The immunology experiments demonstrated that *P. glareae* KMM 9500^T LPS displayed a low ability to induce TNF-α, IL-1β cytokines production and acted as an antagonist of hexa-acylated *Escherichia coli* LPS in human blood *in vitro*.

The work was supported by RFBR grant № 15-04-03207.

STUDY ON ALTERATION OF ALT, AST AND CREATININ VALUES OF VOLUNTEERS TAKING FUCOIDAN FROM VIETNAM BROWN SEAWEED

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192 patients volunteered, 95 who received placebo, and 97 patients, including 59 males and 38 females, had taken fucoidan from Vietnamese seaweed at a daily oral dose of 1 g / day. After 1, 2, 3 months, blood chemistry indices including ALT, AST, and Creatinin were not altered from baseline nor compared with placebo. This result shows that fucoidan from Vietnam seaweed is safe to use.

CHEMICAL METABOLITES ISOLATED FROM MARINE MICROALGAE

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Microalgae are a diverse group of photosynthetic microorganisms capable to convert solar energy to chemical energy via photosynthesis. Microalgae have recently gained a lot of attention as a new biomass source for the production of bio fuels and for the treatment of waste water. They contain numerous bioactive compounds that can be harnessed for commercial use. Microalgae can be used to produce a wide range of metabolites such as proteins, lipids, carbohydrates, carotenoids or vitamins for health, food and feed additives, cosmetics and for energy production.

Dunaliella tertiolecta, Nannochloropsis oculata and *Chlorella vulgaris* are eukaryotic microalgae, that were used in wide range of applications. Recently, the MeOH extracts of these microalgae were found to show cytotoxicity against several cancer cell lines.

In this presentation, 16 compounds including *β*-caroten (**1**), phytol (**2**), campesterol (3), campesterol β-glucoside (**4**), glucose lipid (**5**), allitol (**6**), (22E,24R)-Ergosta-7,9,22-trien-3β-ol (**7**), *(*22E,24*R*)-5α,8α-epidioxyergosta-6,22-dien-3β-ol (**8**), (22E,24*R*)-Ergosta-7,22-diene-3*β*,5*α*,6*β*-triol (**9**), α-D-glucosyl dipalmitoylglycerol (**10**), Saccharose (**11**), lutein (**12**), periferasterol (**13**), palmitic acid (**14**), glycerol (**15**), phytyl palmitate (**16**) from the microalgae *Dunaliella tertiolecta, Nannochloropsis oculata* and *Chlorella vulgaris*. Several compounds exhibited the moderate cytotoxicity and antimicrobial activity.

The work was supported by the Vietnam Academy of Sciences & Technology under project number *VAST.TD.DLB.07/16-18.*

PLATE SCREENING ASSAYS FOR ALGAL POLYSACCHARIDE – DEGRADING ENZYMES OF MARINE MICROORGANISMS

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Plate assays based on specific physicochemical properties of polysaccharide such as complex formation with dyes or with detergent salts provide a rapid, easily applicable detection of polysaccharide – degrading marine microorganisms. In this study, using plate assays more than 150 strains of marine bacteria and 100 strains of marine fungi had screened for fucoidanase, ulvan lysae and alginate lyase activities. After growth of marine bacteria on polysaccharide-containing medium, the colonies were removed and the plates were flooded with a hexadecyltrimethylammonium bromide (Cetavlon) solution in 30 min for detection of fucoidanase and ulvan lyase production or with Gram' iodine solution in 3 min for alginate lyase production. After washing agents, colonies with enzyme activities were recognized by clear zones. The medium containing intact polysaccharide showed a milky white (in case of fucoidan and ulvan) or bluish black (in case of alginate) background. Whereas clear zones appeared where polysaccharide has been degraded. Polysaccharide – degrading marine fungi were identified by the same plate methods. Fungal strains were grown in solid medium, then extracted with 0.01M phosphate buffer pH 7.2. The crude extracts had tested for enzyme activities.

Screening of algal polysaccharide degrading marine microorganisms is an important step for selecting active strain. Plate assays are suitable for screening of large numbers of strains. These methods are not only stool for detection enzyme activity, but also provide preliminary evaluation of quantity of enzyme by diameter of clear zones.

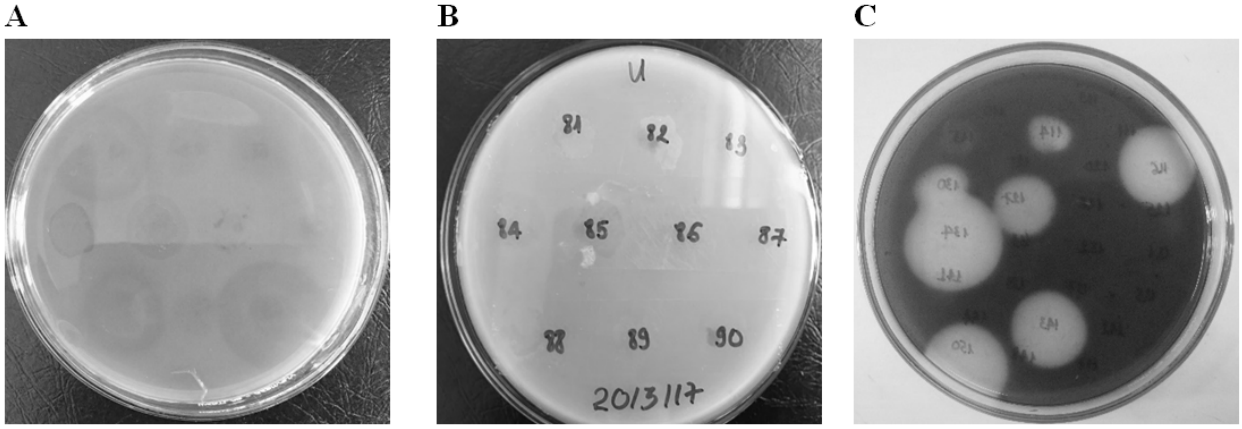

Figure 1. Plate assays for screening algal polyssacharide - degrading marine microorganisms.
A - for fucoidanase activity, B - for ulvanolytic enzyme, C - for alginate lyase

This study was supported by the "Construction of cultural marine microorganisms collection for exploitation and application" Program of the Vietnam Academy of Science and Technology, VAST.DA47.12/16-19.

CANCER TARGET THERAPEUTIC USE A FOLATE-MODIFIED CURCUMIN LOADED MICELLE DELIVERY SYSTEM

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Target delivery system use natural dugs for tumor cells is an appealing platform help to reduce the side effects and enhancing the therapeutic effects of the drug. In this study, we synthesized curcumin (Cur) loaded micelle (D,L Poly lactic – Poly ethylenglycol) (PLA-PEG_{2000}) with the ratio of PLA/PEG is 3/1 (w/w) and was conjugated with folate-modified (Cur/PLA-PEG-Fol) for cancer target therapy. The source material PLA-PEG_{2000} was synthesized by polymezation openring method, after the Cur loaded micelle to 0.73 $mg.ml^{-1}$. The folate-modified was conjugated with Cur/PLA-PEG_{2000} by the amide bond formation reaction. The average size of mixed micelle was 69 nm, the encapsulating effiency was 91.3%. Compared with the Cur/PLA-PEG solution, the in vitro release of Cur from Cur/PLA-PEG-Fol showed a sustained effectively. Futher more, the in vitro cellular uptake of Cur/PLA-PEG-Fol were significantly enhanced towards HepG2 cells. The results demonstrated that Folate-modified Cur micelle could serve as a potential nanocarrier to improve solubility and anti-cancer activity of Cur.

This work was financially supported by the National Foundation for Science and Technology development of Vietnam-NAFOSTED under Grant No. 106.99-2012.43 (HPT).

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THE EFFECT OF BACTERIA *YERSINIA RUCKERI* ON THE FUNCTIONAL ACTIVITY OF SCALLOP *MIZUHOPECTEN YESSOENSIS* HEMOCYTES

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Molluscs, a diverse group of animals some of them (scallops and shrimps) are economically important sources of food that can be grown in aquaculture. Innate immunity of invertebrates is a combination of reactions of nonspecific antimicrobial protection, which operates practically without a latent period, effectively and selectively recognizing "own" and "alien". Cell protective reactions of mollusks are carried out with the help of circulating hemolymph cells - hemocytes, capable to adhesion, phagocytosis, production of reactive oxygen species, intracellular accumulation of cationic proteins, synthesis and secretion of peroxidase in response to antigen stimulation.

Yersinia ruckeri is a Gram-negative bacterium that causes yersiniosis in fish. Every year this microorganism is responsible for large economic losses in the aquaculture industry. Recently *Y. ruckeri* outer membrane antigens were detected in organisms of commercial marine crustaceans and bivalves [1]. Presumably this fact may indicate the infection of these invertebrates, which leads to the activation of their immune system.

To study the effect of *Y. ruckeri* bacteria on the functional activity of *Mizuhopecten yessoensis* hemocytes, we used the culture of *Y. ruckeri* cells grown at 25^{o} C, and antibodies to outer membrane (OM) OmpF porin of *Y. ruckeri*. It was found that incubation of scallop hemocytes with bacterial cells within 1 h reduces their viability in comparison with the control. Preliminary treatment of *Y. ruckeri* cells with antibodies to OmpF porin increases the number of surviving hemocytes. It is possible that antibodies to porin, by binding to surface exposed regions of the protein molecule are able to block the full contact of bacteria with hemocyte surface and to inhibit of reaction cascade leading to the death of a eukaryotic cell.

It was shown that stimulation of hemocytes by *Y. ruckeri* cells causes synthesis of peroxidase and an increase in the number of cationic proteins. It was found that the amount of cationic proteins synthesized as a result of stimulation of hemocytes by Y. ruckeri cells depends on the incubation time. These data indicate that as a result of the interaction of Y. ruckeri with the receptors of the hemocyte membrane, both bactericidal systems of phagocytic cells of mollusks, oxygen-dependent and oxygen-independent, are activated.

In the presence of antibodies to *Y. ruckeri* OmpF porin, the stimulating action of bacterial cells is markedly reduced. This result indicates that this immunodominant protein is one of the pathogenicity factors of this *Yersinia* species, which participates in the processes of bacterial adhesion and activation of the innate immunity system of mollusks.

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STRUCTURE AND CYTOTOXICITY OF ULVAN FROM GREEN SEAWEED *ULVA LACTUCA*

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Ulvans are water-soluble sulfated polysaccharides derived from marine green seaweed. They were reported to exhibit a wide range of physiological and biological activities. The structural complexity of ulvans may be derived from the differences in seaweed species, extraction method and place of cultivation [1-3].

This work presents an investigation on the structure of ulvan obtained by water extraction from the green seaweed *Ulva lactuca* by using IR, NMR, SEC-MALL and ESI-MS methods. The results showed that the ulvan with a molecular weight of 347000 was composed of rhamnose (Rha), galactose (Gal), xylose (Xyl), manose (Man), glucose (Glu), with a mole ratio of 1: 0.03: 0.07: 0.01: 0.06, respectively. The uronic acid and sulfate content in the ulvan was found to be at 21.5% and 18.9%, respectively. This ulvan mainly consists of disaccharide [→4)-β-D-GlcA-(1→4)-α-L-Rha3S-(1→] and minor disaccharides β-GlcA-(1→2)-α-Xyl and β-GlcA-(1→2)-α-Rha. The ulvan was further evaluated for its cytotoxic effects on three human cancer cell lines and shown a significant cytotoxicity against the hepatocellular carcinoma (IC_{50} at 29.67±2.87 µg/ml), human breast cancer (IC_{50} at 25.09 ±1.36 µg/ml), and cervical cancer (IC_{50} at 36.33±3.84 µg/ml) in a dose dependent manner. The ulvan was shown a potential natural product as anti-cancer compound and will be further developed in our future work.

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ANTIOXIDANT AND ANTICOAGULANT PROPERTIES OF DEGRADED CARRAGEENANS OF DIFFERENT SULFATION LEVELS

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Carrageenans are water-soluble linear sulfated galactans [1, 2], where repeating disaccharide unit is composed of 3-linked β-D-galactopyranose and 4-linked α-D-galactopyranose. Similarly to agarose, some carrageenans have 4-linked α-D-galactopyranose in the form of 3,6-anhydro cycle. Carrageenans are classified based on the amount and location of sulfate esthers and the existence of 4-linked α-D-3,6-anhydrogalactose [1] using Greek alphabet prefixes [2]. Carrageenan antioxidant and anticoagulant properties have been studied up to some extent [3] making them potentially valuable in therapeutic research and application.

Current research involved investigation of three different carrageenan samples (figure 1) – ι-carrageenan, κ-carrageenan and a sample containing various types of carrageenan (ι-, κ-, λ-, μ- and ν-carrageenan). Samples were degraded using both autohydrolysis and sonication separatedly. All samples were transformed to Na^+ form prior to each measurement.

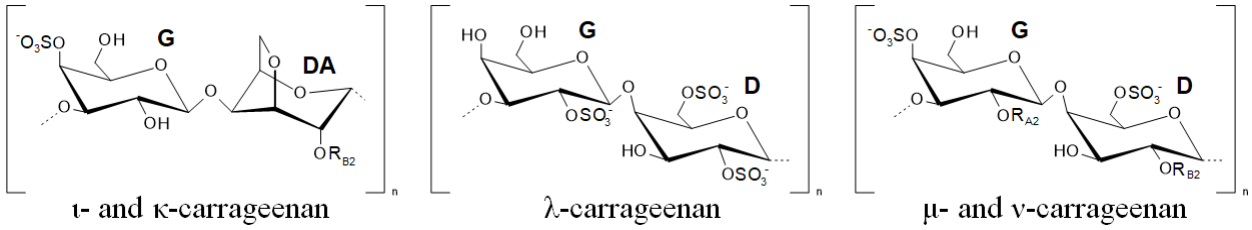

Figure 1. Molecular structure of ι- and κ-carrageenan, where $R_{B2}=SO_3^-$ for ι-carrageenan and $R_{B2}=H$ for κ-carrageenan; λ-carrageenan; and μ- and ν-carrageenan, where $R_{A2}=H$ and $R_{B2}=H$ for μ-carrageenan and $R_{A2}=H$ and $R_{B2}=SO_3^-$ for ν-carrageenan.

Molecular weights were determined using size exclusion chromatography. The antioxidant properties of hydrolysed carrageenan samples were compared to respective sonicated samples using different assays, including Folin-Ciocalteu reagent and ferric reducing/antioxidant power assay. The anticoagulant properties of degraded carrageenan samples were observed using a variety of tests, including prothrombin time and activated partial prothrombin time.

The main results indicate native, autohydrolysed as well as sonicated commercial carrageenan samples having antioxidant properties depending on molecular weight and level of sulfation. Anticoagulant assays prove carrageenan fractions having ability to inhibit clotting. Findings need further clarifying in method comparison as the initial screening has been a success.

The work was supported by Estonian Research Council project PUT1406 coordinated by Tallinn University School of Natural Sciences and Health as well as European Social Fund's Doctoral Studies and Internationalisation Programme DoRa, which is carried out by Foundation Archimedes.

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B/K-CARRAGEENAN VARIABILITY IN FURCELLARAN SAMPLES FROM *FURCELLARIA LUMBRICALIS*

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Furcellaria lumbricalis is a red algal species that grows in the epipelagic zone to a depth of up to 30 m attached to submerged rocks or as a drifting form in open situations (often on muddy or sandy shores) [1]. It grows in the colder parts of Atlantic Ocean, but also tolerates the brackish waters of the Baltic Sea [2]. It is mostly harvested from Canadian, Danish and Estonian waters for furcellaran production. Furcellaran (alternatively known as Danish agar) is an example of a hybrid carrageenan, mostly made up of β- and κ-carrageenan disaccharide units (Figure 1), which in turn consist of D-galactopyranosyl and 3,6-anhydro-D-galactopyranosyl units, where the first one is sulfated in κ-carrabiose units. The units are connected with alternating α-1,3 and β-1,4 glycosidic linkages. Carrageenans are widely used as gelling agents and the gel strength is primarily dependent on the molecular weight and the structure of the polymer.

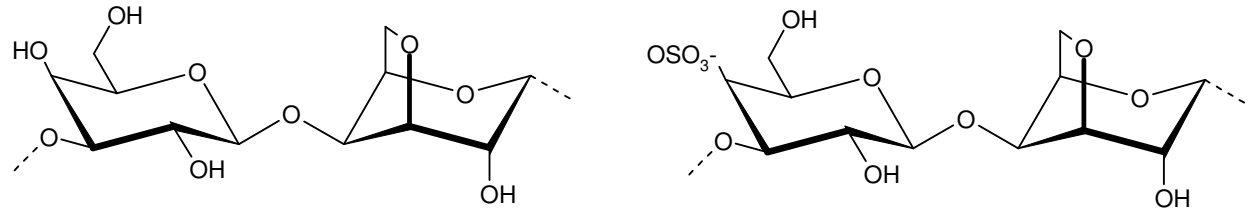

Figure 1. Idealized disaccharide units of β- (left) and κ-carrageenan (right).

The variation of β/κ-carrageenan ratios in furcellaran samples extracted from Canadian, Danish and Estonian *F. lumbricalis* samples were studied with ^{1}H NMR spectroscopy. The molecular weights, degree of methylation and the variability of polysaccharide content between attached and drifting form were also studied.

It was found that furcellaran has a β/κ disaccharide ratio of 0.7–1.4. Furcellaran extraction yields varied between 33–53% and the tested polysaccharide had up to one methyl group for every two disaccharide units. The peak-averaged molecular weights varied in the range of 1.3–2.4 MDa.

The work was supported by Estonian Research Council grant PUT1406.

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GLYCOSYL HYDROLASE AND POLYSACCHARIDE LYASE-DEGRADING POLYSACCHARIDE FAMILIES FROM MARINE MICROORGANISMS

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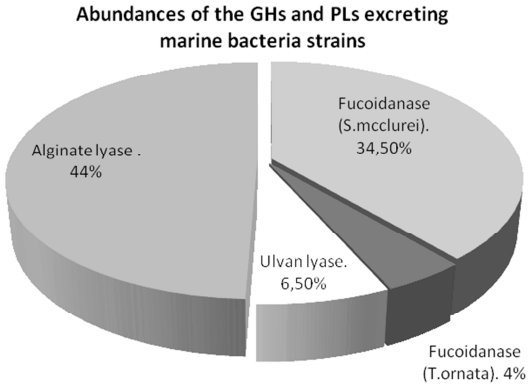
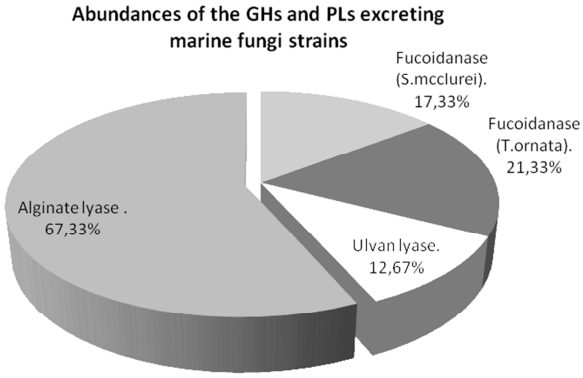
The most important reaction mechanism for polysaccharide degradation is hydrolysis, more than 145 different glycosyl hydrolase (GHs) families have been identified to date. The number of polysaccharide lyase (PLs) families is smaller than of glycosyl hydrolases with 27 PL families identified so far (CAZy Database), these enzymes cleave the bond between the carbohydrates, glycosidic linkage and uronic acid-containing polysaccharide chains, repectively. Here we report that 200 marine bacteria and 150 marine fungi growing on the surface of algae, sponge, soft coral, sediment were isolated and their capacity of excreting fucoidanases, ulvan lyase, alginate lyase was stydied. Fucoidans from *Sargassum mcclurei*, *Turbinaria ornata*; ulvan from *Ulva lactuca*; alginate from brown algae of Vietnam sea were used as enzyme substrates for testing glycosyl hydrolase activity and polysaccharide lyase activity. According to the result obtained, 38 % bacterial strains studied and 37 % fungal strains studied have been given glycosidases catalyzing hydrolysis of brown seaweed, which were lower 50% bacterial strains studied and 80% fungal strains studied have been given polysaccharides catalyzing lysis of brown seaweed. The abundance of GHs and PLs degrading polysaccharide families ability may be part of the role of fucoidanases, ulvan lyase, alginate lyase in the metabolic pathway of marine organisms.





The study was supported by the Grant of R/V "Akademik Oparin" expedition (VAST.HTQT.NGA.15-16/16-17).

STUDY ON CULTURE CONDITIONS OF MICROORGANISMS TO ENZYME PRODUCTION AND SURVEY FACTORS AFFECTING OF *CHAETOMORPHA LINUM* HYDROLYSATE BY ENZYME

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Seaweed *Chaetomorpha linum (Ch. linum)* have polysaccharide content 59% w. the polysaccharides have four types such as ulvan, cellulose, agar, starches. In this study we used the TRICHOMIX-DT products that have four strains of microorganisms as *Streptomyces* spp., *Pseudomonas* spp., *Bacillus subtilis*, and *Trichoderma* spp. It were cultured and enzyme product for *Ch. linum* hydrolysate. The results show that the cultured conditions were humidity of 65% and period of 14 days to obtained good active enzyme. The activity of the enzymes were 1,38 U/ml of cellulase, 0,82 U/ml of amylase, 0,53 U/ml of galactosidase, 1,84 U/ml of ulvanase. And the results of *Ch. linum* hydrolysis has created sugar content of 34.6 g/l from 100 g dry seaweed/l. The composition of solution sugar are 9,5 g/l of glucose, 2,6 g/l of galactose, 8,9 g/l of rhamnose. The results of this study are potential in green seaweed sachrification to the production of fermented products.

GLYCOSYL HYDROLASE FROM MARINE FUNGI IN CENTRAL VIETNAM SEA BY USING SIMPLE EXTRACTION METHOD

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Fungi produce a wide range of extracellular enzymes to break down plant cell walls, which are composed mainly of cellulose, lignin and hemicellulose. Among them are the glycoside hydrolases (GH), the largest and most diverse family of enzymes active on this group of polysaccharides. These enzymes with known are important tools for studies of the structural characteristic and their biological role. A variety of laboratory methods can be used to evaluate or screen the enzyme activities of extract solution marine fungi. The most know and basic method is culture fungi on liquid media to obtain crude extract however it is quite expensive and complicated to implement.

For the purpose of this study, the simple and sensitive method was used to screening glycosyl hydrolase from marine fungi on the major polysaccharides: ulvan, fucoidan and alginate from *Sarrgassum Mcclurei*, *Turbinaria ornata* and *Ulva lactuca* marine algae. More than 100 fungi strains were isolated from about 50 marine organisms in central Vietnam Sea most of which are from sponge and soft coral by cultured on slant medium and using 0.01M phosphate buffer pH 7.2 to extracting, then crude extracts were applied on a Sephadex G-25 column. Enzyme activities of these fungi were detected by Nelson method, ulvan–agarose plates and staining with hexadecyltrimethylammoniumbromide (Cetavlon) method and Carbohydrate–Polyacrylamide Gel Electrophoresis (C–PAGE) method.

This work was supported by the Grant of R/V "Akademik Oparin" expedition (VAST.HTQT.NGA.15-16/16-17) and 47 object from Vietnam Academy of Science and Technology (VAST.DA47.12/16-19).

PREPARATION OF LIPOSOMES CONTAINING CARRAGEENAN AND COMPOSITES ON THEIR BASIS

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The targeted delivery of biologically active substances into the mammalian organism is one of the topical areas of medicine and biological chemistry. One of the most common approaches is the application of liposomes – lipid vesicles containing in their internal space an aqueous solution of the necessary substance, or absorbing it into their shell. Liposomes coated with polysaccharides or lectins interact with certain types of cells more specifically [1].

Sulfated polysaccharides from seaweeds – carrageenans – exhibit a wide spectrum of biological activity and are used as a matrix for drug delivery in recent years [2]. The application of liposomes as a container for the polysaccharides delivery and the inclusion of vector molecules in their composition can significantly increase the effectiveness of their biological action.

The purpose of the study is to develop conditions for the incorporation of kappa-carrageenan polysaccharide from the alga Chondrus armatus into liposomes. As a vector, the liposome surfaces coated with a natural polycation - chitosan with an affinity to the glycoprotein mucin covering the surface of the epithelial cells of the stomach and some other organs of the body was used.

The work was carried out according to the following scheme:

Lipids (lecithin-cholesterol) + carrageenan (CRG) → liposomes (L-CRG) + chitosan (Ch) → L-CRG-Ch + mucin (M) → L-CRG-Ch-M

Fluorescently labeled derivatives of chitosan (F-Ch) and mucin (F-M) were synthesized to determine the incorporation of the investigated polymers into liposomes by means of the reaction of initial compounds with fluorescein isothiocyanate.

Liposomes were prepared by sonication of a lipid film (16 mg lecithin and 7 mg cholesterol) with a solution of carrageenan (100 mL, 1 mg/mL). After washing by centrifugation and lysis with butanol, the content of CRG in them was measured by the reaction with blue Taylor' blue, it was approximately 12% (all % were calculated as the initial compound loading). Further, the F-Ch solution (100 мL, 200 mg/mL) was added. After washing and lysis, the content of the included F-Ch by the fluorescence data was of 2%. Liposomes coated with Ch were obtained by replacing F-Ch with the original Ch. The F-M solution (100 мL, 200 mg/mL) was added to them, after washing and lysis, the content of F-M in the liposomes reached up to 16.3%. However, it should be noted that the F-M itself adhered on liposomes without chitosan – 10.5 %, which is apparently due to its hydrophobicity. Nevertheless, the coating of liposomes with chitosan may increase their binding to mucin-containing cells.

Thus, we prepared liposomes containing kappa-carrageenan, anionic polysaccharide of red algae in their internal space, and also we proposed a method for coating their surface with chitosan, mucin, or sequentially with chitosan and then mucin as vector molecules for interaction with cells.

The work was supported by the Russian Science Foundation grant № 16-14-00051.

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