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Polysaccharides of green alga *Caulerpa lentillifera*

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It is known that polysaccharides of seaweed possess various biological activity. Biologically active polysaccharides were isolated from brown, red and green seaweed. Polysaccharides of the seaweed *Caulerpa lentillifera* were investigated for the first time. *C. lentillifera* was cultivated in laboratory conditions. Polysaccharides were extracted with hot water (fr K-1), 1 M (fr K-2) and 4 M KOH (fr K-3) successively and the yield of the polysaccharide fractions of the dry alga weight was 2.7%, 4.0% and 3.5%, respectively. The molecular weights of polysaccharides of fractions K-1 and K-2 determined by gel filtration are 20-60 kDa and 20-40 kDa, respectively. Fraction K-3 is the high-molecular polysaccharide (>70 kDa). Monosaccharide composition of all fractions contained glucose, galactose, mannose and xylose in a different ratio. Monosaccharides were identified in the form of polyol acetates using GLC-MS. The ¹³C NMR spectrum of fractions K-2 and K-3 show signals which are characteristic of α -1 \rightarrow 4-glucan. Using ¹³C NMR spectroscopy, it was shown that fraction K-1 included α -1 \rightarrow 4-glucan and laminaran (1 \rightarrow 3;1 \rightarrow 6- β -D-glucan). In IR spectra of all investigated fractions absent characteristic for sulfate groups absorption at 1230-1255 cm⁻¹, 822 and 849 cm⁻¹. These data indicate that *C. lentillifera* polysaccharides under study are unsulfated.

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Immunomodulatory properties of cucumariosides

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The medicine Cumaside was created from a base of triterpene oligoglycosides, cucumariosides, from the Far-Eastern holothurian *Cucumaria japonica*, and its immunomodulatory properties were studied. The influence of Cumaside in low doses on mouse macrophages was accompanied by a more than two-fold stimulation of lysosomal activity and induced a rapid increase in cytosolic Ca²⁺ content. This preparation was shown to significantly increase the animal resistance against bacterial infections elicited by various pathogens. Cumaside stimulates phagocytosis, ROS, IL6 and TNF- α production in lymphocytes, increases the number of antibody producing cells, and amplifies the expression some of preliminary suppressed CD-markers. Cumaside was found to inhibit Ehrlich carcinoma tumor growth in the initiatory stages and increase the mouse survival after irradiation. At the same time the preparation did not affect the delayed-type hypersensitivity, proliferate activity of lymphocytes, cytotoxic activity of NK-cells and cytokine IFN γ and IL12p70 release. The mechanism of Cumaside action is discussed.

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Screening of biologically active marine natural products in PIBOC

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The search for biologically active compounds in the laboratory of Bioassay in PIBOC consists mainly of cell-based screening, image technology and ion channels as intracellular targets, and several *in vivo* models. This work includes application of various methods such as the technique of radioisotopes, ion-selective electrodes, fluorescent spectroscopy, cytofluorimetry, BLM technique and cell image analysis, which afford evaluation of interaction of substances with various types of cells, biological and model lipid membranes. We perform our

investigations using some cell cultures, embryos of marine invertebrates (Echinodermata), microorganisms including two-hybrid transgenic yeasts with human estrogen receptors, and lipid liposomes. We screen for chemicals with antimicrobial, antiviral and anticancer activity, immunomodulatory activity, inhibitors of cell adhesion, and compounds with antioxidant properties. High resolution MRI-tomography with 7T magnet (PharmaScan 70/16US, Bruker) allows us to search *in situ* for compounds with anticancer, antistroke and antiischemia activity, inhibitors of angiogenesis and hepatoprotective substances. The structures of complexes of some testing compounds with viral and cellular proteins and ion channels as potential therapeutic targets are theoretically predicting in experiments *in silico* by the methods of molecular modeling and the docking approach using Linux cluster of interdepartmental supercomputer center of FEBRAS. Examples of each type of mentioned above methods will be presented, and the problems and difficulties of each technique will be discussed.

PICES XIV MEQ_Poster-2384 **O-glycosyl hydrolases of marine bacteria**

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The present report summarizes knowledge on the different O-glycosyl hydrolases, involved in cell wall antigens and brown seaweed polysaccharide degradation produced by marine bacteria. The data on distribution of α -galactosidases, α -N-acetylgalactosaminidases and fucoidanases among symbiotic and free-living marine bacteria are shown. The same enzymes are isolated, purified and characterized in detail (MW, pH optimum, T optimum, *etc.*). Inhibition of α -galactosidase with natural and synthetic compounds is investigated. The elements of structure, hydrolytic properties and substrate specificity of these enzymes are studied. α -galactosidase of the marine bacterium *Pseudoalteromonase* sp. KMM 701 is immobilized into hybrid polysaccharide-silica nanocomposite materials. Possible biotechnological application of these enzymes is discussed.

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The content of selenium in a number of marine organisms from the upwelling zone off the Kuril Islands

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Selenium is unevenly distributed in seawater, and there are zones of high and low concentrations of this element in the world ocean. Upwellings are active hydrologic structures, which provide saturation of seawater with various microelements, including selenium. The content of selenium was studied in a number of free-living and sessile marine organisms, caught in the upwelling zones off the central and northern Kuril Islands. Liver tissues have been analyzed in two squids (*Berryteuthis magister* and *Moroteuthis robusta*), seven bonefishes (*Theragra chalcogramma*, *Sebastes alutus*, *Sebastes borealis*, *Albatrossia pectoralis*, *Reinhardtius hippoglossoides*, *Hexagrammos octogrammus* and *Pleurogrammus monopterygius*) and one cartilaginous fish (*Bathyraja maculata*). Soft tissues have been analyzed in two bivalves (*Crenomytilus grayanus* and *Modiolus kurilensis*). The content of selenium has been determined on the AAS AA 6800 Shimadzu. Concentrations of this element in the liver of all examined species did not exceed 1.0 $\mu\text{g Se per one gram } (\mu\text{g/g})$ of dry weight, ranging between 0.108 and 0.998 $\mu\text{g/g}$, and the highest concentrations were found in walleye pollock (*Theragra chalcogramma*) and robust clubhook squid (*Moroteuthis robusta*). Soft tissues of bivalve mollusks contained 0.97-1.7 $\mu\text{g/g}$ of selenium. Our data revealed rather low total content of selenium in various marine organisms (including sedentary residential, demersal and actively migrating species) occurring in the Kuril upwelling zone, which is not consistent with normally higher selenium content in organisms inhabiting upwelling regions in some other areas of the world ocean.

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O-glycosylhydrolases of marine fungi inhabiting in sea of Okhotsk bottom deposits

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Marine fungi are less studied than other ecological groups. Obligatory marine fungi are still main objects. However, recently interest in studying marine facultative fungi has been growing since many new metabolites have been isolated from them which have not been found in terrestrial fungi. Among the metabolites of marine fungi, enzymes degrading polysaccharides are of a special interest. The purpose of our work is to study the distribution of some glycosylhydrolases in filamentous fungi inhabiting in sea of Okhotsk bottom deposits. Content of some O-glycosylhydrolases (glycosidases and polysaccharide hydrolases) was determined in cultural filtrates from 57 marine facultative strains. It has been established that glycosidases are widely distributed in cultural filtrates of these strains: β -D-glucosidases (in 55 samples), N-acetyl- β -D-glucosaminidases (in 29 samples), β -D-galactosidases (in 17 samples), α -D-mannosidases (in 9 samples), sulfatase (in 1 sample). Amylases (in 55 samples), 1,3- β -D-glucanases (in 47 samples), pustulanases (in 33 samples) are the most widespread among enzymes degrading polysaccharides, whereas the enzymes splitting CM-cellulose (in 4 samples), fucoidan from *Laminaria cichorioides* (in 4 samples), galactan (in 2 samples) and guluronic acid (in 2 samples) are rare. The enzymes hydrolyzing fucoidan from *Fucus evanescens*, agar and *p*-Nph- α -D-fucoside were absent. It has been revealed that the habitat affects level of enzyme activity and the enzyme composition of facultative fungi. Thus our studied allowed us to reveal a series of strains promising as producers both of individual enzymes and of a set of enzymes splitting carbohydrate-containing compounds.

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The experimental evaluation of toxic effect of lead on the holothurian immunity

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Toxic effects of lead on echinoderms are little studied. However, the studies are important both for the ecological forecast and aquaculture. We studied the influence of $\text{Pb}(\text{NO}_3)_2$ (2 and 4 mg/L) on apoptosis and expression of receptors to mitogen concanavalin A (con A) of coelomocytes and two phagocyte fractions of holothurian *Eupentacta fraudatrix*. After 48 h, apoptosis increased only in coelomocytes and one of the phagocyte (P1) fractions (92% of purity) of holothurians treated with lead at both concentrations, as assessed with DNA electrophoresis. Hoechst 33342 staining revealed that apoptosis increased 2- and 1.7-fold in coelomocytes of animals treated with 2 and 4 mg/L $\text{Pb}(\text{NO}_3)_2$, correspondingly. In P1, apoptosis increased by 36 % at 2mg/L and was not significantly changed at 4 mg/L $\text{Pb}(\text{NO}_3)_2$. FITC-conjugated con A binding to coelomocytes and P1 increased 2 and 1,7 times, correspondingly in animals treated with 2mg/L $\text{Pb}(\text{NO}_3)_2$, but not in another phagocyte fraction. The 4 mg/L concentration did not affect con A binding to any type of cells studied. However, viability of coelomocytes decreased only at 4 mg/L concentration. Cell viability decrease in P1 was shown already at 2 mg/L, and was higher at 4 mg/L concentration. The data obtained indicate that one of the phagocyte fractions and nonseparated coelomocytes are especially sensitive to lead, and both apoptosis and expression of receptors to con A increase mainly at 2mg/L concentration, but cell viability decrease is more significant at 4 mg/L concentration. We suggest that 2mg/L is a threshold $\text{Pb}(\text{NO}_3)_2$ concentration damaging holothurian immunity.

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Use of 1,3;1,6- β -D-glucans for detection of the some infection in potato and tobacco

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Among the substances inducing plant resistance in plants to the pathogens, significance is attached to carbohydrate-containing polymers, in particular the glucans having β -configuration of the glucoside linkages. Similar glucans can act as amplifier of the protective mechanisms of the plants or as suppressor, speeding up penetration of the pathogens in plant-host. The results of study on biological action of the 1,3;1,6- β -D-glucans have been obtained by enzymatic transformation of the laminaran are presented. We studied the influence of the different β -D-glucans (I, II, III, IV) on the development of some diseases (fungi, viruses, viroid) in potato and tobacco. The relationship between biological action of the β -D-glucans and their chemical structure had been shown. The effect of the β -D-glucans on the speeding up of the penetration hyphal material of fungi *Phytophthora infestans* in the tissue tubers potato have also been determined. However, glucan IV, with degree of polymerisation (DP) 35 have decreased expansion of mycelium fungi in the tissues leaf and tubers potato. The β -D-glucans IV had some inhibitory affect on tobacco mosaic virus and X-virus potato infection. The action of the β -D-glucans on the development of caused by potato spindle tuber viroid (PSTVd) had been evaluated. The effect of β -D-glucan with DP 20-25 was optimum for the detection of the maximum number of the plants infected by this viroid. The β -D-glucans with DP less 15 caused the very early acceleration of the PSTVd symptoms of the leaf potato.

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Characterization of HABs in China and management actions

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Red tides (HABs) are an important environmental problem in China's coastal areas and can do great harm to coastal economies. The first record of red tides in China commenced in 1933, and from then on, more than 600 red tides have been recorded. There were only 14 records of red tides before 1979, but subsequently, 75 cases were reported in the 80s, 226 cases in the 90s and 399 cases from 2000 to 2004. The characteristics of marine red tides were described as being highly concentrated, and occurring over increasingly large areas, and over long periods of time. In Chinese coastal waters, red tides are believed to occur during all the 12 months of the year. In addition, poisonous and harmful species have increased over the last several decades. The dominant red tide organisms are *Prorocentrum donghaiense*, *Karenia mikimotoi*, *Phaeocystis* spp. in recent years. For the purpose of controlling and mitigating the damage of red tides, 18 red tide monitoring zones have been established. These routine monitoring programs have enhanced the discovery of red tides and have facilitated emergency response systems that play an important role in mitigating the impacts of red tides.

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Review on the basin-scale survey in the Yellow Sea between Korea and China

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The Yellow Sea is extremely important to the economy and to the health and well being of surrounding countries, Korea and China. Recently the Yellow Sea is under constantly increasing threat of degradation due to the increase of environmental pollution and over-fishing. A major part of the threat is due to the release of large

quantities of pollutants to the Yellow Sea that may be transported to near shore and even to the open ocean.

The governments of Korea and China have been aware of the importance of the Yellow Sea and reached an Environmental Agreement between Korea and China at the governmental level (November, 1993). According to this environmental agreement, the Yellow Sea Environmental Cooperative Research between Korea and China has been undertaken since 1997. The first research activity of the research between Korea and China was conducted by the KORDI in Korea and the Offshore Environmental Monitoring Network Center (OEMNC) of the State Environmental Protection Administration (SEPA) in China 1997. Since 1998, in accordance with the agreement of the 1998's Yellow Sea Environmental Cooperative Research Agreement between the Ministry of Maritime Affairs and Fisheries (MOMAF) in Korea and SEPA in China, the Korean host institution for this research has been changed from the KORDI to the West Sea Fisheries Research Institute (WSFRI) in Korea.

Up to now, research has been performed 8 times. The joint cruise had been conducted once a year at 33 stations in the 4 lines of the Yellow Sea where the 9 stations of the D line was newly added in the 7th cruise in 2003. The research parameters were temperature, salinity, dissolved oxygen, transparency, chlorophyll-*a*, pH, suspended solids, chemical oxygen demand, oil and grease, total organic carbon, nitrite, nitrate, ammonia, phosphate, silicate, and heavy metals (Hg, Cd, Cu, Pb, Zn, and As), grain size and sediment type, total nitrogen, total phosphorus, and polychlorinated biphenyls for sediment; phytoplankton and zooplankton. Additionally, 2 pilot studies, namely the distribution of dinoflagellate cysts and the vertical profiles of sediment chemistry using core samples, were examined in 2003. The samples were analyzed by scientists of both countries at the WSFRI, Korea and the OEMNC of the SEPA, China in turn. The annual report has been published every year during 1998-2004.

The scientific efforts to fix the cruise time in October and to extend research frequency, to twice a year, should be considered, and this requires the governmental supports such as research funds and other related administrative assistance on both sides. Finally, scientists should also pay a concentrated attention to standardize the analytical methods including quality control and to improve this Yellow Sea research as one of the most represented international projects in the Yellow Sea where sharing additional information available, if exist, such as dumping sites and material content, and of the freshwater quality will be of great help to broaden the output of this joint research project.

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Ecosystem-based management of fisheries resources in Wangdol-cho in the East Sea, Korea

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The Wangdol-cho ecosystem (approximately 13.66km²) consists of three reefs and is located in the East Sea in Korea. Wangdol-cho is an important area for habitat and spawning of organisms inhabiting the East Sea. In recent years the fishing ground surrounding Wangdol-cho has become overexploited, and underscores the necessity for the management of fisheries resources. We introduced an ecosystem-based approach in Wangdol-cho ecosystem to manage fisheries resources by considering ecological interactions of organisms in the ecosystem. We conducted a survey using gillnet and trap fisheries during 2002-2004 and analyzed the annual and seasonal changes in biomass. The dominant species in the ecosystem were *Gadus macrocephalus* and *Pleurogrammus azonus*. We collected the ecological information on all organisms in the ecosystem (including biomass, production, consumption, mortality, catch and diet composition by species). Using the ecosystem structure model (Ecopath), we constructed the structure of the ecosystem and analyzed ecotrophic levels of functional groups in Wangdol-cho ecosystem. The ecosystem dynamic model (Ecosim) was employed to conduct dynamic simulation of the ecosystem on the various levels of the fishing mortality or environment changes.

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Impacts from 500hPa circulation systems on algal blooms outbreak in spring of East China Sea

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The latitude change of position where algal blooms frequently break out corresponds with the seasonal oscillation of Pacific Northwest Subtropical High. Algal blooms usually occur in northern or southern edge areas of the western ridge of subtropical high, where the cyclone systems are active. A hypothesis is suggested that the ocean upwelling is enhanced under the convergent wind fields in these cyclone systems. Nutrients are brought upwards by the ocean upwelling from the deeper ocean water. This process is regarded as the forming phases of algal bloom outbreaks. Then, with this area being controlled by the subtropical high, algal blooms break out when the appropriate environmental conditions (such as water temperature, light and salinity) are available.

A prediction method for algal blooms is developed based on the hypothesis above. Sixteen typical algal bloom events in spring of 2001, 2002 and 2003 were selected to investigate the impacts from 500hPa circulation system on algal bloom outbreaks in spring in the East China Sea. By analogue analysis four typical favorable patterns for algal blooms were concluded. With these patterns, eight experimental predictions of algal blooms in 2004 were made, which gave four correct predictions, two empty predictions, and two predictions with 48 hours deviation (earlier than the actual occurrences).

However, the mechanism hypothesis mentioned above lacks evidence from field work during algal bloom events. Further research will be developed through coupled hydrodynamic-ecological modeling associated with quantified data from satellite results.

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Saxitoxins content in the Aniva Bay scallops caused by seasonal blooming of toxic phytoplankton

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We observed the seasonal changes of saxitoxin concentration in the tissues of scallop *Mizuhopecten yessoensis* caused by *Alexandrium tamarense*. The samples were taken in the coastal zone of Aniva Bay from May through October 2004. Scallop muscles and mantles were analysed for saxitoxin by the immunoassay method using the test-system «RIDASCREEN^R FAST Saxitoxin» on EIA (enzyme immunoassay) EL301. A sensitivity of method is 5 mkg/100g. Period of vegetation *A. tamarense* takes place from May through late July. A “blooming” of the toxic species was recorded in late May and then in early July. The *A. tamarense* numbers over 3400 cell/l were recorded in May. The second peak of this species development took place in early July (3550 cell/l). Saxitoxin content in scallop muscles and mantles in early July did not exceed the permissible level (PL - 80mkg/100g) for Russia. The toxin content in the mantle was always higher than in muscle. By the end of July and in August, the saxitoxin content, as a result of accumulation, had increased compared with early July. Saxitoxin in the mantle of scallops remains for several months after the blooming of *A. tamarense*. Maximum concentration was determined in two samples (97,95 mkg/100g in August and 107,48 mkg/100g in October). The average concentrations of saxitoxin in scallops' tissues did not exceed PC for Russia.

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A quantitative evaluation of an extent of anthropogenic disturbance of soft substrata macrobenthic communities

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A method for the quantitative evaluation of the disturbance of soft substrata macrozoobenthic communities due to anthropogenic contamination is proposed. The method is based on the definition of dependencies of different parameters describing the community abundance and structure - biomass, population density, number of species, Shannon-Wiener and Pielou indices, *etc.* – on the level of total sediment contamination. As particular characteristics of these disturbances the percent deflection of the values of biological parameters from the norm (initial almost horizontal section of model curves), and also magnitudes of ERL_q and ERM_q (Long *et al.*, 1995), which confine the area of the progressive degradation of a community (almost linear drop of the parameter values) is offered. The mapping of areas using these indices permits to estimate visually an extent and spatial scales of anthropogenic interference. The usage of the method displays that at the end of the past 20th century some water areas of the Peter the Great Bay, the Sea of Japan, were on the brink of ecological calamity. The results obtained by the method proposed and those derived by standard methods of the appraisal of ecological status of benthic communities are mainly adequate.

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Expression of 1,3- β -D-glucanase and α -D-mannosidase in sea urchin embryos *Strongylocentrotus intermedius*: Effect of nature compounds on developmental stages of fertilized eggs and enzyme activities

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It is known that the vitelline layer of sea urchin eggs consists of fucose, mannose, galactose, glucose, xylose, glucosamine, galactosamine and sialic acid by carbohydrate analysis. Therefore, enzymes under study in sea urchin embryos can be involved with metabolism of extracellular or cell surface glycoproteins during both the fertilization reaction and the cleavage period. The goal of the present work was to study composition and change of the activity O-glycosylhydrolases in the process of development sea urchin embryos *S. intermedius* and influence of compounds from brown seaweed at the same. High activities of the 1,3- β -D-glucanase and α -D-mannosidase were revealed in embryos at distinct developmental stages and unfertilized eggs. In contrast, low amounts of β -D-galactosidase and β -D-glucosidase were detected in the same conditions. After fertilization the high activity of α -D-mannosidase was revealed from blastula stage to mature larva and was increased twice after the pluteus stage. 1,3- β -D-Glucanase was decreased after gastrulation. The effect of different compounds (fucoidans from *Fucus evanescense* and *Laminaria cichorioides*; translam obtained by enzyme transformation of laminaran and 1,3- β -D-glucanase inhibitor from *L. cichorioides*) on enzyme activities and development of embryos were investigated. Fucoidans did not appear to change the development of embryos and expression of 1,3- β -D-glucanase and α -D-mannosidase. Translam (1,3;1,6- β -D-glucan) increased the speed of development of embryos in relation to control and respectively activity of the enzymes. In contrast, protein inhibitor of marine mollusk endo-1,3- β -D-glucanases decreased the speed of development of embryos and had no influence on enzyme activities.

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