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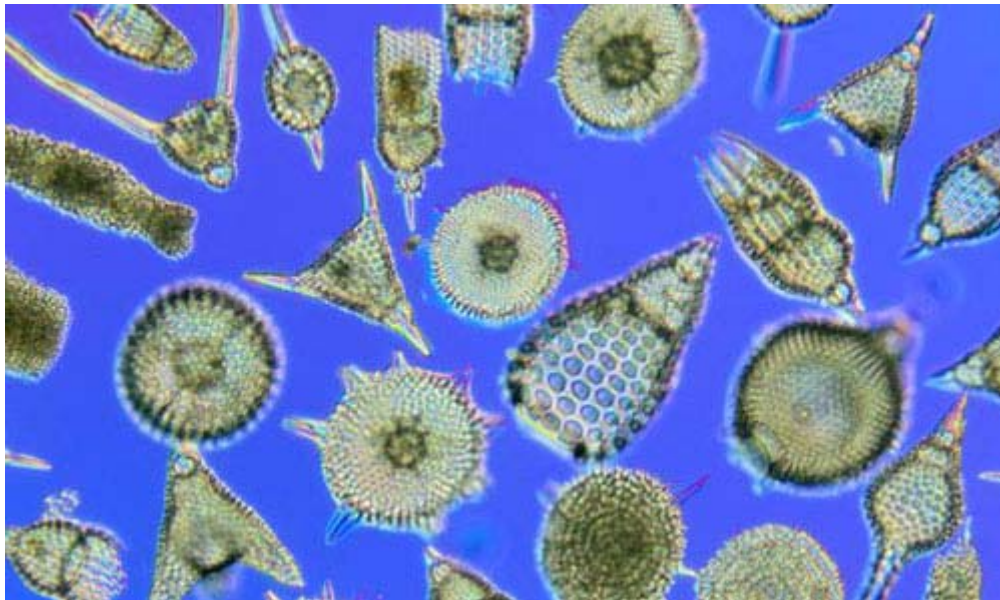## Asia-Pacific Economic Cooperation

"Marine Microorganisms: Capacity Building for a Broader Cooperative Research and Utilization"

Final Report

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APEC PROJECT

«Marine Microorganisms: Capacity Building for a Broader Cooperative Research and Utilization»

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INTRODUCTION

The development of marine microbiology is very important for the ATR. Microorganisms are an important part of marine ecosystems. Health of the marine environment and self-healing capacity depends on the communities of marine bacteria that are involved in the cycle of substances. An anthropogenic effect on the marine environment affects all living things. The only living organisms able to cope with this threat - it is the microorganisms. They are able to dispose of toxic and complex flavors to simple and safe. In addition, microbial cell is very sensitive to the effects of pollutants and is therefore it is a very accurate tool in the bioindication of pollution.
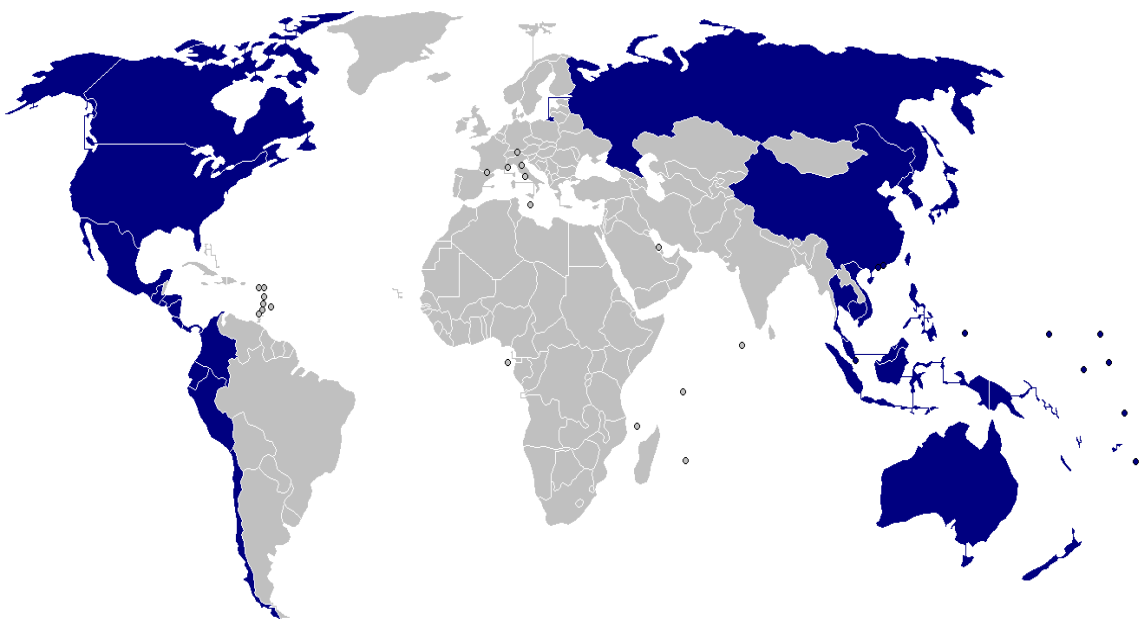
Of course it is important not only to environment but also marine animals and plants inhabiting it. The big problem mariculture farms, acquiring great importance in the Asia-Pacific economies, are the fact that *in vitro* breeding of animals often get sick. And here the problem is not in the detection of pathogen, but also in using microorganisms as probiotics for the treatment of debilitated animals. Features of marine organisms are badly investigated. They are the source of many biologically active compounds, which analogues were found in animals or plants, but often surpass them in their properties. Simple conditions of cultivation, a high growth rate and excellent characteristics of product are attractive to many producers. Marine microbiology is the science of the future. Using

microorganisms, we can save the environment, produce useful materials and products and raise on the high technological level of industrial production.

1. AUS Australia

Estuaries are among the most productive marine ecosystems on earth; however, they are also subject to the greatest pressures from coastal human populations. On the east coast of Australia, several estuaries have become eutrophied as consequence of human activity, and consequently, they have strong gradients in productivity and nutrient availability (Bowen et. al., 1996). In these, as in other estuaries and adjacent waters, bacteria (including cyanobacteria) are abundant and productive components of plankton, with typical abundances of 10^5 to 10^7 cells ml, and typically represent 70% of the carbon biomass in upper surface waters (Ducklow, Carlson, 1992). Despite their biogeochemical importance, little is known of the composition, and consequently, of the patterns of diversity, of bacterioplankton communities in most marine environments (Giovannoni, Rappe, 2000). The study of bacterioplankton communities has traditionally been limited by dependence upon culture-based techniques (Giovannoni et al., 1990), but only a small fraction of bacterioplankton species are culturable.

Thus, modern study of these communities has focused upon molecular techniques that circumvent the limitations of cultivation. Advances in DNA technology have allowed detailed investigation into the diversity and species richness of bacterioplankton communities. Early studies of the molecular diversity of bacteria in the ocean focused upon the cloning and sequencing of amplified conserved genes, such as 16S rRNA (Giovannoni et al., 1990). This method allows high phylogenetic resolution of bacterioplankton communities, because identity is based upon sequence information. However, recent study has demonstrated that a very large number of clones need to be processed to accurately estimate the absolute species richness of bacterioplankton communities by this approach (Hughes et al., 2001), making it both time-consuming and expensive. Thus, while the approach is useful for describing bacterioplankton communities, it is often impractical to use clone libraries quantitatively, especially for relatively rare components that require especially large libraries. In recent years, whole-community fingerprinting approaches have been used to study complex bacterial communities and to estimate the diversity and relative representation of individual bacterial taxonomic units within the total detectable bacterial communities. Three common fingerprinting methods are terminal restriction fragment length polymorphism (TRFLP) of universally conserved genes (Avaniss-Aghajani et al., 1994), denaturing gradient gel electrophoresis (DGGE) (Troussellier et. al.,

2002), and automated rRNA intergenic spacer analysis (ARISA). In TRFLP, 16S rRNA is amplified by PCR with a 5' fluorescent primer, and amplicons are digested into fragments using restriction enzymes, resulting in terminal restriction fragments of distinctive lengths (Avaniss-Aghajani et al., 1994). DGGE relies on melting-point variations in variable portions of target molecules, often 16S rRNA. ARISA amplifies the region between 16S and 23S rRNAs using a fluorescent primer; this portion of the operon is highly variable in length (from 150 to 1,200 bp), and therefore, digestion of amplicons is not necessary (Borneman, Triplett, 1997).

Ian Hewson and Jed A. Fuhrman (2004) from Department of Biological Sciences, University of Southern California, Los Angeles, California, USA chose to use ARISA in their study for a few reasons. TRFLP provides less phylogenetic resolution because it relies upon only a few sequence heterogeneities in a generally conserved molecule. DGGE, on the other hand, offers high phylogenetic resolution but has less sensitivity than ARISA or TRFLP for minor taxa; ARISA and TRFLP typically use a laser detection system that can detect bands and peaks containing 0.1% of the total loaded DNA, while the images or scans of DGGE gels often require 0.5%. Also, DGGE relies on gel band positions that cannot easily be converted into standard data points (unlike digital fragment lengths in ARISA or TRFLP); therefore, it is difficult to standardize or to compare between laboratories. Additionally, DGGE gels are typically shorter than ARISA or TRFLP sequencing gels and therefore permit fewer possible operational taxonomic units (OTU). With respect to speed and cost of analysis, these approaches are all advantageous compared to clone library approaches, since mixed DNA from an entire bacterial community is amplified and visualized within a single assay. However, these methods are not as sensitive to taxonomic differences as clone library approaches, and it has been argued that they distinguish near the genus (TRFLP) and species (ARISA) levels (Avaniss-Aghajani et al., 1994; Fisher, Triplett, 1999). Analytical methods of sampling bacterial diversity, such as clone libraries and whole-community fingerprinting, have been criticized, since inherent biases are introduced by using PCR to amplify community DNA. Like all ecological techniques, fingerprinting is not perfect, since inevitably components of communities are not accounted for and some components may be overestimated. Biases can be reduced by limiting the number of PCR cycles, which prevents overamplification of minor peaks (Fisher, Triplett, 1999).

Maintaining high stringency can also prevent the formation of spurious products which could be misinterpreted as phylotypes during fingerprinting analysis (Fisher, Triplett, 1999). Another possible complication that arises from the use of ARISA for analyzing bacterioplankton communities is that some species (especially fast-growing ones which have multiple rRNA operons (Klappenbach et al., 2000) have more than one internally transcribed spacer (ITS) length, since it is not as conserved as 16S rRNA sequence and there is heterogeneity in operon copy numbers within cells (Klappenbach et al., 2000) However, within slowgrowing bacterial communities, the copy number of the rRNA operon is low, and thus, heterogeneity is less likely to affect fingerprinting analysis in slow-growing marine bacterioplankton communities(Avaniss-Aghajani et al., 1994, Borneman, Triplett, 1997; Klappenbach et al., 2000). Fingerprinting is reproducible and suitable for displaying clear differences between communities (Fisher, Triplett, 1999). Furthermore, since the information generated on bacterial communities by fingerprinting can indicate the rank abundance of microbial communities, it indicates the coverage of each microbial community (Hughes et al., 2001). It can be argued that fingerprinting is the most cost-effective alternative for comparing multiple bacterial communities. The aim of American researchers was to determine patterns of bacterioplankton diversity along a subtropical estuarine gradient of Moreton Bay, Australia. Such an estuary provides an ideal model system in which a sharp gradient of environmental conditions, yet without physical barriers, can be studied in a short time. Their results demonstrate that bacterioplankton communities vary significantly along the estuarine gradient and that diversity as measured by ARISA may be related to habitat and resource availability. They also demonstrate that ARISA, with its high phylogenetic resolution, is a useful technique for characterizing estuarine and marine bacterioplankton communities. Bacterioplankton community diversity was investigated in the subtropical Brisbane River-Moreton Bay estuary, Australia (27°25_S, 153°5_E). Bacterial communities were studied using automated rRNA intergenic spacer analysis (ARISA), which amplifies 16S-23S ribosomal DNA internally transcribed spacer regions from mixed-community DNA and detects the separated products on a fragment analyzer. Samples were collected from eight sites throughout the estuary and east to the East Australian Current (Coral Sea).
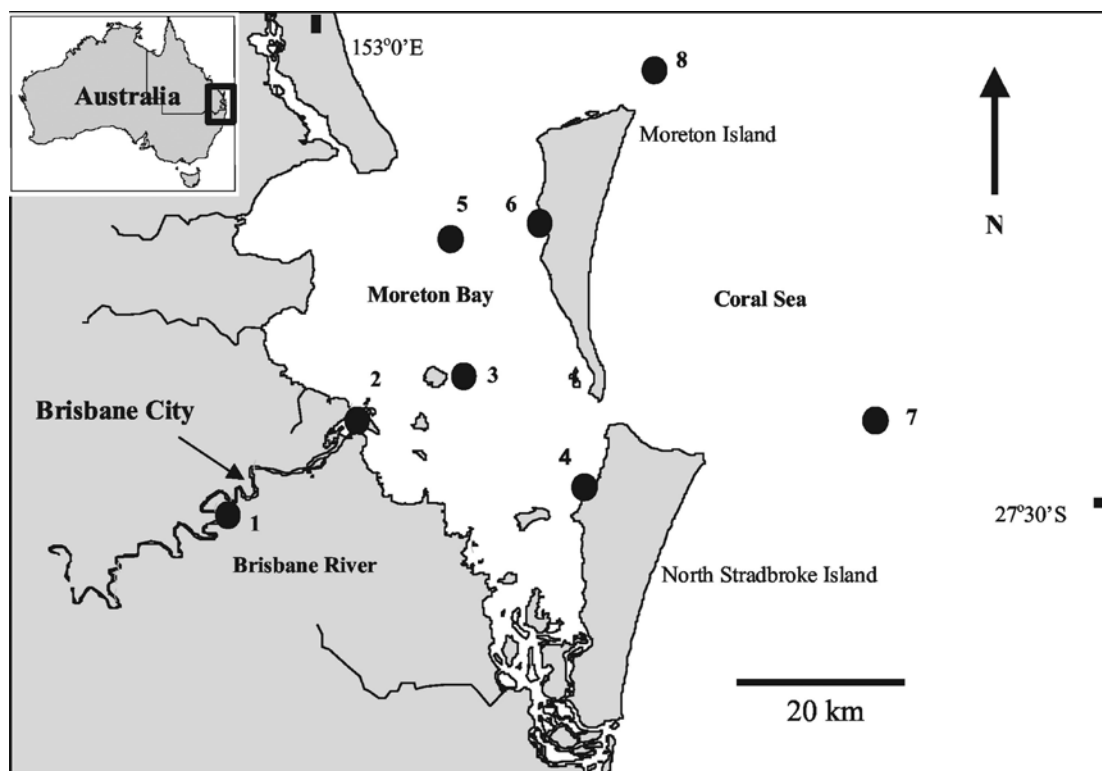
Map of Brisbane River-Moreton Bay estuary showing sampling sites.

Bacterioplankton communities had the highest operational taxonomic unit (OTU) richness, as measured by ARISA at eastern bay stations (S [total richness] _ 84 to 85 OTU) and the lowest richness in the Coral Sea (S _ 39 to 59 OTU). Richness correlated positively with bacterial abundance; however, there were no strong correlations between diversity and salinity, NO_3 and PO_4 concentrations, or chlorophyll a concentration. Bacterioplankton communities at the riverine stations were different from communities in the bay or Coral Sea. The main differences in OTU richness between stations were in taxa that each represented 0.1% (the detection limit) to 0.5% of the total amplified DNA, i.e., the "tail" of the distribution. Ian Hewson and Jed A. Fuhrman found that some bacterioplankton taxa are specific to distinct environments while others have a ubiquitous distribution from river to sea. Bacterioplankton richness and diversity patterns in the estuary are potentially a consequence of greater niche availability, mixing of local and adjacent environment communities, or intermediate disturbance. Furthermore, these results contrast with previous reports of spatially homogeneous bacterioplankton communities in other coastal waters. The centre of marine microbiology and genetic of Australia Institute of marine sciences studies reasons of coral death in the coastal water of Austalia.Researchers found that reson of death is not microoorganisms, But is change of environment(Мартин, Сато, Карин, Дэвид Борн, 2012).

2. CAN Canada

Fisheries & Oceans Canada, Pacific Biological Station take have a part in study deseases of salmons.Viral hemorrhagic septicemia virus (VHSV) infects over 70 fish species inhabiting marine, brackish or freshwater environments throughout the Northern Hemisphere. Over its geographic range, 4 VHSV genotypes and multiple subtypes exist. Here, we describe the development and validation of a rapid, sensitive and specific real-time reverse transcription quantitative PCR assay (RT-qPCR) that amplifies sequence from representative a isolates of all VHSV genotypes (I, II, III and IV). The pan-specific VHSV RT-qPCR assay reliably detects 100 copies of VHSV nucleoprotein RNA without cross-reacting with infectious hematopoietic necrosis virus, spring viremia of carp virus or aquatic birnavirus. Test performance characteristics evaluated on experimentally infected Atlantic salmon Salmo salar L. revealed a diagnostic sensitivity (DSe) > or = 93% and specificity (DSp) = 100%. The repeatability and reproducibility of the procedure was exceptionally high, with 93% agreement among test results within and between 2 laboratories. Furthermore, proficiency testing demonstrated the VHSV RT-qPCR assay to be easily transferred to and performed by a total of 9 technicians representing 4 laboratories in 2 countries. The assay performed equivalent to the traditional detection method of virus isolation via cell culture with the advantage of faster turnaround times and high throughput capacity, further suggesting the suitability of the use of this VHSV RT-qPCR in a diagnostic setting(Garver, Hawley, McClure, Schroeder, Aldous, Doig, Snow, Edes, Baynes, Richard, 2011).

Dalhousie University studies community of new marine microorganisms.Proteorhodopsins are light-energy-harvesting transmembrane proteins encoded by genes recently discovered in the surface waters of the world's oceans. Metagenomic data from the Global Ocean Sampling expedition (GOS) recovered 2674 proteorhodopsin-related sequences from 51 aquatic samples. Four of these samples were from non-marine environments, specifically, Lake Gatun within the Panama Canal, Delaware Bay and Chesapeake Bay and the Punta Cormorant Lagoon in Ecuador. Rhodopsins related to but phylogenetically distinct from most sequences designated proteorhodopsins were present at all four of these non-marine sites and comprised three different clades that were almost completely absent from marine samples. Phylogenomic analyses of genes adjacent to those encoding these novel rhodopsins suggest affiliation to the Actinobacteria, and hence we propose to name these divergent, non-marine rhodopsins

'actinorhodopsins'. Actinorhodopsins conserve the acidic amino acid residues critical for proton pumping and their genes lack genomic association with those encoding photo-sensory transducer proteins, thus supporting a putative ion pumping function. The ratio of recA and radA to rhodopsin genes in the different environment types sampled within the GOS indicates that rhodopsins of one type or another are abundant in microbial communities in freshwater, estuarine and lagoon ecosystems, supporting an important role for these photosystems in all aquatic environments influenced by sunlight (Sharma, Zhaxybayeva, Papke, Doolittle, 2008).

Québec-Océan, Département de Biologie, and Institut de Biologie Intégrative et des Systèmes, Université Laval, Québec studies phylogenetic changes of microbial communities depending on environmental conditions. Increasing global temperatures are having a profound impact in the Arctic, including the dramatic loss of multiyear sea ice in 2007 that has continued to the present. The majority of life in the Arctic is microbial and the consequences of climate-mediated changes on microbial marine food webs, which are responsible for biogeochemical cycling and support higher trophic levels, are unknown. We examined microbial communities over time by using high-throughput sequencing of microbial DNA collected between 2003 and 2010 from the subsurface chlorophyll maximum (SCM) layer of the Beaufort Sea (Canadian Arctic). We found that overall this layer has freshened and concentrations of nitrate, the limiting nutrient for photosynthetic production in Arctic seas, have decreased. We compared microbial communities from before and after the record September 2007 sea ice minimum and detected significant differences in communities from all three domains of life. In particular, there were significant changes in species composition of Eukarya, with ciliates becoming more common and heterotrophic marine stramenopiles (MASTs) accounting for a smaller proportion of sequences retrieved after 2007. Within the Archaea, Marine Group I Thaumarchaeota, which earlier represented up to 60% of the Archaea sequences in this layer, have declined to <10%. Bacterial communities overall were less diverse after 2007, with a significant decrease of the Bacteroidetes. These significant shifts suggest that the microbial food webs are sensitive to physical oceanographic changes such as those occurring in the Canadian Arctic over the past decade (Comeau, Li, Tremblay, Carmack, Lovejoy,2011) .

In the Bedford Institute of Oceanography studies diversity of microorganisms.In the Gulf of Maine area (GoMA), as elsewhere in the ocean, the organisms of greatest

numerical abundance are microbes. Viruses in GoMA are largely cyanophages and bacteriophages, including podoviruses which lack tails. There is also evidence of Mimivirus and Chlorovirus in the metagenome. Bacteria in GoMA comprise the dominant SAR11 phylotype cluster, and other abundant phylotypes such as SAR86-like cluster, SAR116-like cluster, Roseobacter, Rhodospirillaceae, Acidomicrobidae, Flavobacteriales, Cytophaga, and unclassified Alphaproteobacteria and Gammaproteobacteria clusters. Bacterial epibionts of the dinoflagellate Alexandrium fundyense include Rhodobacteraceae, Flavobacteriaceae, Cytophaga spp., Sulfitobacter spp., Sphingomonas spp., and unclassified Bacteroidetes. Phototrophic prokaryotes in GoMA include cyanobacteria that contain chlorophyll (mainly Synechococcus), aerobic anoxygenic phototrophs that contain bacteriochlorophyll, and bacteria that contain proteorhodopsin. Eukaryotic microalgae in GoMA include Bacillariophyceae, Dinophyceae, Prymnesiophyceae, Prasinophyceae, Trebouxiophyceae, Cryptophyceae, Dictyochophyceae, Chrysophyceae, Eustigmatophyceae, Pelagophyceae, Synurophyceae, and Xanthophyceae. There are no records of Bolidophyceae, Aurearenophyceae, Raphidophyceae, and Synchromophyceae in GoMA. In total, there are records for 665 names and 229 genera of microalgae. Heterotrophic eukaryotic protists in GoMA include Dinophyceae, Alveolata, Apicomplexa, amoeboid organisms, Labrynthulida, and heterotrophic marine stramenopiles (MAST). Ciliates include Strombidium, Lohmaniella, Tontonia, Strobilidium, Strombidinopsis and the mixotrophs Laboea strobila and Myrionecta rubrum (ex Mesodinium rubra). An inventory of selected microbial groups in each of 14 physiographic regions in GoMA is made by combining information on the depth-dependent variation of cell density and the depth-dependent variation of water volume. Across the entire GoMA, an estimate for the minimum abundance of cell-based microbes is $1.7 \times 10(25)$ organisms. By one account, this number of microbes implies a richness of $10(5)$ to $10(6)$ taxa in the entire water volume of GoMA. Morphological diversity in microplankton is well-described but the true extent of taxonomic diversity, especially in the femtoplankton, picoplankton and nanoplankton--whether autotrophic, heterotrophic, or mixotrophic, is unknown (Li, Andersen, Gifford, Incze, Martin, Pilskaln, Rooney-Varga, Sieracki, Wilson, Wolff, 2011).

3. CHL Chile

In the coastal district of Chile was found the biggest virus. Mimivirus, a DNA virus infecting acanthamoeba, was for a long time the largest known virus both in terms of particle size and gene content. Its genome encodes 979 proteins, including the first four aminoacyl tRNA synthetases (ArgRS, CysRS, MetRS, and TyrRS) ever found outside of cellular organisms. The discovery that Mimivirus encoded trademark cellular functions prompted a wealth of theoretical studies revisiting the concept of virus and associated large DNA viruses with the emergence of early eukaryotes. However, the evolutionary significance of these unique features remained impossible to assess in absence of a Mimivirus relative exhibiting a suitable evolutionary divergence. Here, we present Megavirus chilensis, a giant virus isolated off the coast of Chile, but capable of replicating in fresh water acanthamoeba. Its 1,259,197-bp genome is the largest viral genome fully sequenced so far. It encodes 1,120 putative proteins, of which 258 (23%) have no Mimivirus homologs. The 594 Megavirus/Mimivirus orthologs share an average of 50% of identical residues. Despite this divergence, Megavirus retained all of the genomic features characteristic of Mimivirus, including its cellular-like genes. Moreover, Megavirus exhibits three additional aminoacyl-tRNA synthetase genes (IleRS, TrpRS, and AsnRS) adding strong support to the previous suggestion that the Mimivirus/Megavirus lineage evolved from an ancestral cellular genome by reductive evolution. The main differences in gene content between Mimivirus and Megavirus genomes are due to (i) lineages specific gains or losses of genes, (ii) lineage specific gene family expansion or deletion, and (iii) the insertion/migration of mobile elements (intron, intein)(Van Etten, Lincoln, 2011).
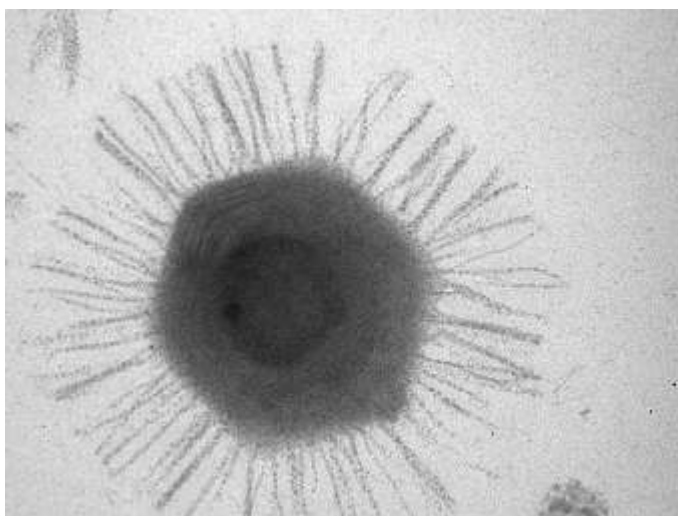
Megavirus

A vast carpet of underwater microbes that covers an area as big as Greece has been discovered on the seabed off the west coast of South America. Scientists believe the microbes could be directly descended from some of the earliest life forms to have evolved on Earth.

Microbial mat" lives in a deep layer of seawater that is deprived of both light and oxygen and seems to have survived by "eating" hydrogen sulphide and "breathing" nitrates. It could represent a present-day community of organisms descended from primitive microbes which first evolved about 3 billion years ago, when there was no oxygen on the planet.

Initial tests showed the microbial mat is composed of a community of micro-organisms adapted to growing under extreme hypoxia, when there is little or no oxygen. It is the same kind of conditions that existed before the evolution of the first photosynthetic algae, which were able to convert carbon dioxide into oxygen.

 Scientists estimate that the mat extends over vast areas of the seabed in this region of the ocean, covering a territory roughly the size of Greece. Explorers have found them off the central and northern parts of both Chile and Peru, and they have also been detected in sulphur-rich waters off the Galapagos islands, Ecuador and Panama.

The largest filaments of the mats are about half the width of a human hair and are composed of individual microbial cells organised into long multicellular strands that are white because of a build-up of sulphate salts in the living tissue. The bacteria within the mats are some of the biggest known(Galardo,2010).

4. PRC People's Republic of China

China has some of the world's worst water pollution. All of China's lakes and rivers are polluted to some degree. According to a Chinese government report, 70 percent of rivers, lakes and waterways are seriously polluted, many so seriously they have no fish, and 78 percent of the water from China's rivers is not fit for human consumption. In a middle class development near Nanjing call Straford a polluted river has buried underground in giant pipe while a new ornamental river, rally a lake, has been built above it.

The most polluted rivers are in the east and south around the major population centers with the pollution getting worse the further downstream one goes. In some cases each city along a river dumps pollutants outside their city limits, creating increasingly more pollution for the cities downstream.

Many rivers are filled with garbage, heavy metals and factory chemicals. Suzhou Creek in Shanghai stinks of human waste and effluence from pig farms.

The pollution has taken its toll on aquatic life. Fish catches from the river declined from 427,000 tons in the 1950s to 100,000 tons in the 1990s. The Yangtze is in danger of becoming a "dead river" unable to sustain marine life or providing drinking water. According to report by the Chinese Academy of Sciences released in April 2007 the Yangtze is seriously and largely irreversibly polluted. More than 600 kilometers of its length and almost 30 percent of its major tributaries are in critical condition.

Water pollution caused primarily by industrial waste, chemical fertilizers and raw sewage accounts for half of the $69 billion that the Chinese economy loses to pollution every year. About 11.7 million pounds of organic pollutants are emitted into Chinese waters very day, compared to 5.5 in the United States, 3.4 in Japan, 2.3 in Germany, 3.2 in India, and 0.6 in South Africa.

Jianlong Wanga with coauthors from Tsinghua University, State Key Joint Laboratory of Environment Simulation and Pollution Control, Tsinghua University, Beijing are working on problems of environmental pollution by heavy metals. In investigations they pay much attention to biosorption of heavy metals by yeasts *Saccharomyces cerevisiae*. Heavy metal pollution has become one of the most serious environmental problems today. Biosorption, using biomaterials such as bacteria, fungi, yeast and algae, is regarded as a cost-effective

biotechnology for the treatment of high volume and low concentration complex wastewaters containing heavy metal(s) in the order of 1 to 100 mg/L. Authors established *Saccharomyces cerevisiae* has received increasing attention due to the unique nature in spite of its mediocre capacity for metal uptake compared with other fungi. *S. cerevisiae* is widely used in food and beverage production, is easily cultivated using cheap media, is also a by-product in large quantity as a waste of the fermentation industry, and is easily manipulated at molecular level.

Metal-binding capacity for various heavy metals by *S. cerevisiae* under different conditions is compared. Lead and uranium, for instances, could be removed from dilute solutions more effectively in comparison with other metals. The yeast biosorption largely depends on parameters such as pH, the ratio of the initial metal ion and initial biomass concentration, culture conditions, presence of various ligands and competitive metal ions in solution and to a limited extent on temperature. An assessment of the isotherm equilibrium model, as well as kinetics was performed. The mechanisms of biosorption are understood only to a limited extent. Elucidation of the mechanism of metal uptake is a real challenge in the field of biosorption. Various mechanism assumptions of metal uptake by *S. cerevisiae* are summarized.

The problem of oil contamination of seas is quite topical for China too. Natural disasters, shipping activities, accidental spillage of fuels and other petroleum products cause serious ecological damage to coastal fauna and flora. Hydrocarbons present in the fuel are major pollutants of marine environment. Once the oil leaks into the natural environment, it transforms due to evaporation and photo-oxidation. The conventional methods used to handle petroleum pollution are restricted to physical and chemical suppression. However, these are expensive and helpful measures only to clean-up high concentrations of oil releasing secondary pollutants into the environment (Pritchard et al., 1992). Generally, ultimate and complete degradation is accomplished mainly by bio-degradation due to the activities of marine micro flora.

Microbial degradation is considered to be the best method for the breakdown of hydrocarbons. It has been claimed to be an efficient, economic, and versatile alternative to physicochemical treatment. The main components of diesel are medium and long-chain alkanes, and most of them degraded naturally by the water circulation. However, the residual part of the long-term alkanes stays in the environment for a long time and causes toxicity hazards to the marine life and environment. Some marine bacteria, capable of degrading

petroleum hydrocarbons have been isolated, which include the genera *Pseudomonas*, *Cycloclasticus* (Dyksterhouse et al., 1995)*, Alcanivorax*, *Neptunomonas* (Hedlund et al., 1999), Oleiphilus, *Oleispira,* etc. Unfortunately, natural microbial degradation occurs relatively slowly in marine environments due to limited availability of nitrogen and phosphorous salts, and due tolow temperatures. It was reported that the assimilation/ degradation process that occurs in bacteria is mediated by enzymes and the degradation by genes.

Laboratory of Marine Biogenetic Resources, Third Institution of Oceanography State Oceanic Administration isolated *Novosphingobium indicum* from deep-sea hydrothermal environment, degrading a polycyclic aromatic hydrocarbons (PAHs).

A PAH-degrading strain TVG9-VII was isolated from the hydrothermal chimney sample of the Lau basin in Southwest Pacific Ocean. It showed 99.7% similarities with 16S rRNA gene of Novosphingobium indicum strain H25(T). The degradation rates of this strain against phenanthrene, fluoranthene and pyrene were 95.2%, 57.3% and 69.6% in 21 days, respectively. A gene cluster, containing PAHs initial dioxygenase genes pheA1a and pheA1b, was obtained from genomic fosmid library, with the insertion size of 12.522 kb. The gene pheA1a was enhanced by 4.2 folds in mRNA expression in presence of phenanthrene, but expression enhancement was not observed in other tested PAHs including naphthalene, pyrene and fluoranthene(Dong , Chen , Liao , Shao, 2011).

In recent years, bioremediation technology has been popular for low investment with no secondary pollution and for good results of the basic advantages controlling marine oil pollution. There must be efficient degrading micro flora as executives of biological repair. The purpose of this study was to isolate diesel oil degrading bacteria, screening and identification. Also to obtain efficient diesel-degrading bacteria with degrading enzyme genes like alkane hydroxylase (*alk*B), P450 etc. The aim of this study was to describe the phenotypic characteristics of strain Y9 isolated from the sea water and to study its phylogenetic placement, based upon the analysis of its 16S rRNA sequences. In order to exploit further applications of the effective bioremediation technology, the distribution of degrading gene was also analysed.

Sun M. et al. (2012) conducted a study to characterize and to detect the alkane degradation capacity of the bacterial strain (strain Y9) isolated from the sea mud of the crude oil-polluted Dinghai area in China. A Gram-negative, strictly aerobic, oxidase negative, and

catalase positive bacterium, strain Y9 was isolated and identified as *Acinetobacter* sp. based on its physiological characteristics and its 16S rRNA gene sequence analysis. The 16S rRNA of strain Y9 sequence is 99.8% identical with *Acinetobacter venetianus* RAG-1T. In recent years, bioremediation technology has been popular for low investment with no secondary pollution and for good results of the basic advantages controlling marine oil pollution. So the results of this study are useful for searching of efficient degrading microflora.

Red tides have increased in their numbers and severity in coastal areas of China, particularly in Bohai Bay off eastern China, the East China Sea and the South China Sea. Large red tides have occurred around the Zhoushan Islands near Shanghai.

Jiaozhou Bay is a large semienclosed water body of the temperate Yellow Sea in China. Eutrophication has become its most serious environmental problem, along with red tides (harmful algal blooms), species loss, and contamination with toxic chemicals and harmful microbes. Due to different sources of pollution and various levels of eutrophication across Jiaozhou Bay (mariculture, municipal and industrial wastewater, crude oil shipyard, etc.), a wide spectrum of environmental conditions may contribute to a widely varying community structure of anammox (ammonium oxidation) bacteria. The study of Hongyue Dang with coauthors (2010) used both 16S rRNA and *hzo* genes as targets to measure their abundance, diversity, and spatial distribution and assess the response of the resident anammox bacterial community to different environmental conditions. Environmental factors with potential for regulating the sediment anammox microbiota are discussed.

Abundance and distribution of *hzo* genes revealed a greater taxonomic diversity in Jiaozhou Bay, including several novel clades of anammox bacteria. In contrast, the targeting of 16S rRNA genes verified the presence of only "*Candidatus* Scalindua," albeit with a high microdiversity. The genus "*Ca*. Scalindua" comprised the apparent majority of active sediment anammox bacteria. Multivariate statistical analyses indicated a heterogeneous distribution of the anammox bacterial assemblages in Jiaozhou Bay. Of all environmental parameters investigated, sediment organic C/organic N (OrgC/OrgN), nitrite concentration, and sediment median grain size were found to impact the composition, structure, and distribution of the sediment anammox bacterial community. Analysis of Pearson correlations between environmental factors and abundance of 16S rRNA and *hzo* genes as determined by fluorescent real-time PCR suggests that the local nitrite concentration is the key regulator of the abundance of anammox bacteria in Jiaozhou Bay sediments.

Because ongoing eutrophication of coastal bays contributes significantly to the formation of low-oxygen zones, monitoring of the anammox bacterial community offers a unique opportunity for assessment of anthropogenic perturbations in these environments.

One is very important district of microbiology is producing secondary metabolites. In the Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, Yantai it has been isolated a series of angucyclinone antibiotics from marine Streptomyces sp. strain W007 and identified (Qin, Zhang, Li, Zhu, Zheng ,2012).

Marine environment is the sources of new microorganisms strains. Strains YIM M 10366T, YIM M 10378T and YIM M 10400T were isolated from marine sediments, collected from the Xisha Islands in the South China Sea. All three isolates were able to grow optimally at pH 7.0, 28°C and NaCl concentrations between 0-3 % (w/v). Comparison of 16S rRNA gene sequences showed that these strains are members of the genus Streptomyces, exhibiting moderately high 16S rRNA gene sequence similarities to 97.0-98.8% with the most closely related Streptomyces spp. Morphological characteristics, physiological characteristics, compositions of the whole-cell sugars and phospholipids are consistent with the diagnostic characteristics of the genus Streptomyces, but still allowed for differentiation amongst the three strains and their neighbours. Based on the 16S rRNA gene sequence analysis, DNA-DNA relatedness, phenotypic characteristics and chemotaxonomic data, strains YIM M 10366T, YIM M 10378T and YIM M 10400T were identified as three novel species of the genus Streptomyces(Xu , He , Tian , Li , Yang , Xie , Tang , Chen , Zhang , Li ,2011).

One of the microbial research is deseases of fish. Orange-spotted grouper (Epinephelus coioides) is an economically important marine fish cultured in China and Southeast Asian countries. The emergence of infectious viral diseases, including iridovirus and betanodavirus, have severely affected food products based on this species, causing heavy economic losses. Limited available information on the genomics of E. coioides has hampered the understanding of the molecular mechanisms that underlie host-virus interactions. In this study, we used a 454 pyrosequencing method to investigate differentially-expressed genes in the spleen of the E. coioides infected with Singapore grouper iridovirus

This study provided abundant ESTs that could contribute greatly to disclosing novel genes in marine fish. Furthermore, the alterations of predicted gene expression patterns reflected possible responses of these fish to the virus infection. Taken together, our data not only

provided new information for identification of novel genes from marine vertebrates, but also shed new light on the understanding of defense mechanisms of marine fish to viral pathogens(Huang, Huang, Yang, Tsai, Ouyang, Cui, Wang Qin, 2011)

5. HKC Hong Kong, China

With a population of 5.5 million and a limited amount of land (1046 km^2), Hong Kong is one of the most densely populated areas in the world. Due to the decentralization scheme to ease the densely populated centers, the rural area has been urbanized rapidly. The so-called 'satellite' cities in the New Territories have developed and expanded tremendously. As a result, housing estates are interspersed with agricultural and industrial areas. The discharge of untreated industrial effluent and sewage is the cause of higher lead concentrations in the coastal waters and in marine sediment leading to the higher level of lead in aquatic organisms.

Combustion of leaded gasoline from automobiles is believed to be the major lead contamination in the environment. This is of concern for those who live next to traffic-congested streets. However, the airborne lead levels range from 0.6 to 3.7 μg/m^3 and the mean value of lead in human blood samples is *15*μg/ 100 ml. These values are comparable to lead levels in other parts of the world. The measurement of lead in dust has also been used to indicate the extent of lead contamination in several studies. In general, they all found that the level of lead in street dust (mean =1627 μg/gm in one study) was of the same magnitude as values obtained in different cities throughout the world. As reported elsewhere, the lead content in soil samples fell off rapidly with increasing distance from highways. Vegetables grown near roadways were found to contain higher lead concentrations than those growing at remote areas. Since many households and farms are close to traffic, and since vegetable growing for family consumption is traditional, a more detailed investigation of lead levels in food and drink consumed locally seems important.

Some reports showed that the aqueous extracts of roadside dust and soil inhibited root growth of crops. It has also been discovered that the roadside population of two grass species, *Eleusine indica* and *Cynodon daclylon* had a higher tolerance to lead than their normal counterparts. This suggests that the lead levels in roadside soils are sufficiently high as to act as a relative factor in determining the survival of plants. There is a common practice in Hong Kong, China; Chinese Taipei;, China and other Asian economies of recycling waste materials such as night-soil (human excreta), sewage sludge and animal manure for land application and as fishpond fertilizer or supplementary feeds. In general various investigations demonstrated an increase in productivity with the appropriate application rates of these wastes. Nevertheless, the rather high concentrations of various

heavy metals, including lead in the treated crops or fish, especially those treated with sewage sludge, might impose a health hazard.

Hong Kong, China is located south of Kwantung Province, China, and consists of Hong Kong Island with its surrounding 200 smaller islands, Kowloon Peninsula, and part of mainland China named the New Territories. Out of the total land area of 1046 km^2, about 82% consists mainly of hill slopes. A large portion of land in Hong Kong was reclaimed from the sea, for example, the commercial areas of the north coast of Hong Kong and the south of Kowloon Peninsula. Recently, a large scale land reclamation has been completed at Shatin New Town aiming to house over half a million people. Reclamations on a smaller scale have also been underway in other areas of the New Territories, e.g., Tai Po. This decentralization scheme of easing the densely populated areas led to rapid urbanization in the rural areas (the New Territories). Although the number of registered vehicles had decreased to 327 803 by the end of 1983, a reduction of 3.5% compared with that at the end of 1982 after the introduction in May, 1982 of fiscal measures to restrain the growth in ownership of motor vehicles, there is a high traffic density of over 200 vehicles per kilometer of road surface in Hong Kong with most of these vehicles concentrated in the small area of Hong Kong Island and also in 'metropolitan' Kowloon. Lower density traffic occurs in the New Territories.

Industrial activities. The discharge of the relatively untreated effluent from the 20 000 factories in Hong Kong resulted in the lead concentration (0.66 μg/ml) of harbour waters being 160 times higher than those found in the open sea (Chan et at., 1974). An analysis of 12 drain samples had lead concentrations on average 500 times higher and in one case 1000 times higher than those found in the open sea (Chan et at., 1973). Rather high concentrations of lead (20-77 μg/gm) were found in the sediments receiving runoff from several streams entering Tolo Harbour, an almost landlocked sea (Wong et at., 1980).

Sewage dumping. When analysing the marine sediments of Hong Kong for heavy metals it has been found that lead is one of the best indicators of human activities. The rather high values of lead in the sediments of Victoria Harbour are due to the fact that the harbour is one of the busiest in the world. The major source of pollution is due to the scraping off of the red lead-containing paints used in rust-proofing ships. This is emphasized as a source by the rather high concentrations of lead at Junk Bay where the shipbreaking industry is located. Furthermore, the discharge of 56 000 m^3 of sewage per

day from the 18 sewage outfalls, which receive no pre-treatment apart from screening to remove large solid particles in the larger discharge, into Victoria Harbour also accounted for the elevated lead content in the sediment of the harbour. Higher concentrations of various heavy metals including lead were also obtained in marine organisms such as the seaweed *Viva lactuca* (green alga) sampled along the coastal areas within Tolo Harbour, than those collected from remote areas south of Hong Kong Island. In addition, the rather high concentrations of various heavy metals in the tissue of the Pacific oyster *(Crassostrea gigas),* cultured at Deep Bay, which receives water from rural areas where industries are interspersed with agricultural activities, have received much attention. Another report indicated that although cadmium and arsenic were enriched in the Pacific oysters purchased from retail markets in Hong Kong, lead, zinc, and copper concentrations were within the range reported elsewhere. Significantly elevated concentrations of cadmium and arsenic in Hong Kong seafood (fish, mollusks, lobsters, prawns, shrimps and crabs) were also noted (Phillips et at., 1982). However, the present limit for lead in Hong Kong seafood of 6 μ*g/*gm wet weight was not exceeded by the various seafoods (including oysters) analysed in these reports. An attempt has been made to use the rock oysters *(Saccostrea glomerata)* as an indicator of trace metals in Hong Kong (Phillips, 1979). Unfortunately, the detection of lead was omitted.

Agricultural wastes. Agricultural wastes are the most serious cause of pollution in Hong Kong. Pig effluents and poultry droppings contribute more than two-thirds of the total quantity of readily putrescible matter entering streams. Some of this is used by fish farmers and vegetable growers, some is used to feed back to the chickens after being passed through a drum-drier, some is dumped on land, and more than 50% is dumped into streams. It has been revealed that waste materials also possess a substantial amount of lead (total lead of chicken manure 20 μ*g/*gm, pig manure 8.3 μ*g/*gm*),* copper, zinc and manganese (Cheung and Wong, 1981) which might hinder their usage.

Iron ore tailings. The environmental impact of the iron ore tailings deposited along the coastal area of Tolo Harbour has been extensively studied. It is generally found that the comparatively higher level of various metals, including lead in seawater and sediment, results in higher concentrations of these metals in living organisms (Wong et at., 1979).

Growing algae. The aqueous extracts of sewage sludge and animal manure were found to be excellent culture media for growing *Chiorella pyrenoidosa,* C. *salina* and *Viva*

iactuca at suitable concentrations, even better than the artificially enriched media. However, the rather high levels of various heavy metals, including lead, in the sludge was believed to be the most important factor in inhibiting algal growth when the concentrations of sludge extracts exceeded 10% *(*v/v*)*. Additions of 10^{-4} and 10^{-3} M EDTA greatly increased algal growth in activated sludge extract which possessed a rather high level of heavy metals (Wong et at., 1984). Since the public has reacted swiftly against food grown on wastes containing potentially objectionable inputs, such as those cultivated in sewage sludge, it is envisaged that algal products derived from food processing wastes would face less consumer resistance since these wastes, such as carrot, coconut, sugar-cane and soybean wastes, possess higher plant nutrients but a lower concentration of lead and other heavy metals.

Although the data on the concentrations of lead in different ecological compartments of Hong Kong are fragmented, the preliminary studies indicated that lead contamination of air, soil, dust, marine sediment and living organisms are connected mainly to the combustion of leaded gasoline and the dumping of untreated sewage.

Knowledge of the sources of the environmental exposure and the path-ways of this metal in the environment are urgently needed before any effective control strategy can be recommended. This is especially important in a densely populated area such as Hong Kong where a large portion of the population is exposed to the congested traffic as well as various industrial activities. Furthermore, surveys on lead emissions from industrial origins, for example, combustion of fossil fuel and incineration of municipal wastes, are required. The common practice in the area of recycling waste materials such as night-soil (human excreta), animal manure, and sewage sludge which contain a substantial amount of heavy metals should not be ignored. Reports elsewhere have shown concern that continued use of sewage materials might lead to a hazardous accumulation of metals in soil and therefore restrictions on their uses are suggested. However, investigation on the usage of these waste materials as fishpond fertilizer and feed supplements should also be initiated in order to trace the fate of the various metals derived from the wastes.

6. INA Indonesia

Gadjah Mada University studies microorganisms as producers of biological active substances. Pseudomonas is a genus of non-fermentative gram-negative Gammaproteobacteria found both on land and in the water. Many terrestrial isolates of this genus have been studied extensively. While many produce bioactive substances, enzymes, and biosurfactants, other Pseudomonas isolates are used for biological control of plant diseases and bioremediation. In contrast, only a few marine isolates of this genus have been described that produce novel bioactive substances. The chemical structures of the bioactive substances from marine Pseudomonas are diverse, including pyroles, pseudopeptide pyrrolidinedione, phloroglucinol, phenazine, benzaldehyde, quinoline, quinolone, phenanthren, phthalate, andrimid, moiramides, zafrin and bushrin. Some of these bioactive compounds are antimicrobial agents, and dibutyl phthalate and di-(2-ethylhexyl) phthalate have been reported to be cathepsin B inhibitors. In addition to being heterogeneous in terms of their structures, the antibacterial substances produced by Pseudomonas also have diverse mechanisms of action: some affect the bacterial cell membrane, causing bacterial cell lysis, whereas others act as acetyl-CoA carboxylase and nitrous oxide synthesis inhibitors. Marine Pseudomonas spp. have been isolated from a wide range of marine environments and are a potential untapped source for medically relevant bioactive substances (Isnansetyo, Kamei,2009).

Japanese researchers isolated from seawater obtained from Semarang Port in Indonesia.

An aerobic, Gram-negative, motile bacterium, strain C02(T), was Cells of strain C02(T) were peritrichously flagellated and rod-shaped. Strain C02(T) was able to degrade naphthalene, alkylnaphthalenes and phenanthrene. 16S rRNA gene sequence analysis revealed that this strain was affiliated with the family Rhodobacteraceae in the class Alphaproteobacteria and was related most closely to Marinovum algicola FF3(T) (95.7 % similarity) and Thalassobius aestuarii JC2049(T) (95.2 %). The DNA G+C content of strain C02(T) was 64.6 mol%. The major cellular fatty acids were C(18 : 1)omega7c (50.9 % of the total), C(16 : 0) (17.9 %), 11 methyl C(18 : 1)omega7c (14.7 %), C(18 : 1)omega9c (2.9 %) and C(19 : 0) cyclo omega8c (2.4 %), and the predominant respiratory lipoquinone was ubiquinone-10. Based on physiological, chemotaxonomic and phylogenetic data, strain C02(T) is suggested to represent a novel species of a new genus, for which the name Tropicibacter naphthalenivorans gen. nov., sp. nov. is proposed. The

type strain of Tropicibacter naphthalenivorans is C02(T) (=JCM 14838(T)=DSM 19561(T))(Harwati, Kasai, Kodama, Susilaningsih, Watanabe, 2009).

7. JPN Japan

Petroleum pollution has been chronic in seas and oceans. Although many bacteria and allied microorganisms are known to decompose and utilize this pollutant, there have been only a few reports on the precise distribution of these microorganisms in marine environment. One of the reasons might be the absence of a desirable method for the determination of their population densities in seawater. Humitake Seki (1973) developed silica gel medium for the enumeration of petroleumlytic microorganisms in the marine environment. The medium was satisfactorily used for the investigation of the vertical distribution of bacteria in seawater from the surface to 1,000 m depth of western north Pacific central water as well as the neritic region of Japan. Another scientists (Harayama, et al., 2004) using culture-independent rRNA approaches analyzed changes in the structure of microbial communities in marine environments contaminated by a real oil spill and in micro- or mesocosms that mimic such environments. *Alcanivorax* and *Cycloclasticus* of the gamma-*Proteobacteria* were identified as two key organisms with major roles in the degradation of petroleum hydrocarbons. *Alcanivorax* is responsible for alkane biodegradation, whereas *Cycloclasticus* degrades various aromatic hydrocarbons. This information will be useful to develop in situ bioremediation strategies for the clean-up of marine oil spills.

Petroleum-hydrocarbon-degrading bacteria were obtained after enrichment on crude oil (as a 'chocolate mousse') in a continuous supply of Indonesian seawater amended with nitrogen, phosphorus and iron nutrients. They were related to Alcanivorax and Marinobacter strains, which are ubiquitous petroleum-hydrocarbon-degrading bacteria in marine environments, and to Oceanobacter kriegii (96.4-96.5 % similarities in almost full-length 16S rRNA gene sequences). The Oceanobacter-related bacteria showed high n-alkane-degrading activity, comparable to that of Alcanivorax borkumensis strain SK2. On the other hand, Alcanivorax strains exhibited high activity for branched-alkane degradation and thus could be key bacteria for branched-alkane biodegradation in tropical seas. Oceanobacter-related bacteria became most dominant in microcosms that simulated a crude oil spill event with Indonesian seawater. The dominance was observed in microcosms that were unamended or amended with fertilizer, suggesting that the Oceanobacter-related strains could become dominant in the natural tropical marine environment after an accidental oil spill, and would continue to dominate in the environment after biostimulation. These results suggest that Oceanobacter-related bacteria

could be major degraders of petroleum n-alkanes spilt in the tropical sea (Teramoto , Suzuki , Okazaki , Hatmanti , Harayama , 2009).

The monitoring of sanitary condition of coastal sea waters is important task for investigations of Japanese researches. The work of Venkateswaran K. with coauthors (1989b) was conducted to examine the association between zooplankton and enteric pathogens like *V. cholerae* and *Salmonella* spp. in an aquatic environment. In addition, they have correlated various environmental parameters and the pollution indicator bacterial population with the isolation of *V. cholerae* and *Salmonella* spp. Researchers studied seasonal variation of human pathogens such as *Vibrio cholerae* non-O1 and *Salmonella* spp. in Fukuyama coastal waters and the role of zooplankton in their distribution for a period of 1 year. Comparison of two established methods, viz., the elevated temperature method and the two-step enrichment method of enumerating *V. cholerae*, showed that the former is superior in the recoveries of *V. cholerae* non-O1. Isolation of this pathogen on a wider range of salinities (0.4 to 32.5‰) revealed that these organisms are apparently an autochthonous component of the aquatic environment. Temperature appears to be the most crucial element in governing the distribution of *V. cholerae* non-O1. Among the 69 isolates serotyped, 22 different serovars were identified, while one isolate failed to react with any of the known Louisiana State University antisera tested. Zooplankton samples did not harbor more *V. cholerae* non-Ol than the water column did. Better isolation of an allochthonous pathogen, viz., *Salmonella* spp., was noticed from the water samples when swabs were employed. Of the 251 isolates serotyped, 18 serotypes with three variants of *Salmonella* spp. were identified. A high amount of nutrients in the water column increased the survival rate of these pathogens in saline waters as evidenced by a higher incidence of various serotypes in polluted Fukuyama port than in clean marine waters. *Salmonella* spp. association with zooplankton remained below detectable levels in most of the sampling periods. No significant association between *V. cholerae* non-O1 or *Salmonella* spp. with zooplankton could be noticed as influencing their seasonal distribution.

Mariculture in Japan includes commercially important fish, shellfish, as well as seaweed. About 30% of all mariculture products are from the Seto Inland Sea. Oysters are the major mariculture product and account for 80% of the total mariculture industry in Japan; the coastal area of Hiroshima prefecture alone accounts for 85% of the Seto Inland Sea oyster culture production. Next in importance in the marnculture of this area after oysters are shrimp (34%), as well as seaweeds, such as nori (*Porphira* sp. [39%]) and wakame (*Unidariia pinnatifida* [20%]), which are frequently eaten raw. Therefore, the recovery of

pathogenic vibrios, listonellas, and *Clostridiium botulinlutm* from regions where shellfish are harvested has become a great concern from the public health viewpoint. The investigation of Venkateswaran K. with coauthors (1989a) was carried out to understand the ecology and distribution of the predominant genus of marine heterotrophic bacteria, i.e., Vibrio, and the influence of abiotic factors on their abundance in Seto Inland Sea. Furthermore, an alarmingly increasing incidence of infant botulism in Japan makes it important to know the ecology of *C. botlulinium* in various environments. A simple technique using a membrane filter and short preenrichment in alkaline peptone water was developed to resuscitate the injured cells, followed by plating them onto TCBS agar. In addition, a survey was conducted to determine the incidence of *Clostridium botulinum* in sediment samples. Large populations of heterotrophs were found in surface water, whereas large numbers of total vibrios were found in bottom water. In samples from various water sampling regions, high counts of all bacterial populations were found in the inner regions having little exchange of seawater when compared with those of the open region of the inland sea. In the identification of 463 isolates, 23 *Vibrio* spp. and 2 *Listonella* spp. were observed. *V. harveyi* was prevalent among the members of the Vibrio genus. Vibrio species were categorized into six groups; an estimated 20% of these species were in the so-called "pathogenic to humans" group. In addition, a significant proportion of this group was hemolytic and found in the Bisan Seto region. *V. vulnificus, V. fluvialis*, and *V. cholerae* non-O1 predominated in the constricted area of the inland sea, which is eutrophic as a result of riverine influence. It was concluded that salinity indirectly governs the distribution of total vibrios and analysis of variance revealed that all bacterial populations were distributed homogeneously and the variance values were found to be significant in some water sampling regions. Of 26 sediment samples (12%), 3 harbored *C. botulinum*; one was typed as C, while the toxin type of the other two could not be determined. All *C. botulinum*-positive samples were collected from inshore regions and riverine effluents would have influenced the higher incidence of clostridial spores. The present investigation concludes that there is considerable contamination with pathogenic organisms, which will challenge the inhabitants of the Seto Inland Sea, as well as their dependence on natural resources.

The tropical seas covered by the report lie between the Asian mainland and the Australian continent, interspersed with some 20,000 islands. These islands plus ti.e sections of the Asian mainland that include Trial land and part of Malaysia and Hong Kong, harbour a population of about 300 million people, the majority of whom are coastal dwellers.

8. ROK Republic of Korea

Yeongil Bay is located on the east coast of Korea. Due to its proximity to heavy industrial and commercial activity, Yeongil Bay is considered to be one of the most contaminated coastal areas in Korea (Koh et al., 2004, 2006a). Series of studies aimed at characterizing organic chemical contamination in Korean coastal areas were conducted by different authors (Khim et al., 2001; Koh et al., 2002, 2004). The study of Chul-Hwan Koh et al. (2006b) examined concentrations and distribution of persistent organic pollutants (POPs), polycyclic aromatic hydrocarbons (PAHs), and selected endocrine disrupting compounds (EDCs) in Yeongil Bay sediment. In addition to the chemical analyses, presented elsewhere (Koh et al., 2006a), in vitro bioassays were applied to facilitate a more complete understanding of the mixture of biologically active contaminants present in Yeongil Bay sediment. Mechanism-based in vitro bioassays have several attributes that make them a useful complement to routine quantitative chemical analysis. First, in vitro bioassays are capable to detecting unknowns for which there are no established analytical methods, standards, etc. Second, in vitro bioassays target only those chemicals that contribute to an interaction with one or more biomolecules known to mediate a biological response through a defined mechanism of action. As a result, analyses are focused on biologically relevant compounds. Finally, bioassays integrate the response of complex chemical mixtures, providing an indication of the potential biological potency and/or relevance of an entire mixture. However, unlike instrumental analytical methods, bioassays generally cannot identify the chemical(s) causing the response, nor can they precisely quantify the concentrations of chemical(s) present, as bioassay response is a function of concentration, potency, and interactions. Thus, in vitro bioassays serve as a useful complement, although not a substitute, for chemical analysis.

Heavy metal pollution is also important problem of Korean coastal areas. Mining is one of the most important sources of heavy metals in the environment. Mining and milling operations together with grinding, concentrating ores and disposal of tailings, along with mine and mill waste water, provide obvious sources of contamination (Adriano, 1986). Therefore, large areas of agricultural land can be contaminated, including paddy fields. Korea, for example, has a long history of metalliferous mining and the most extensive activities occurred during the early twentieth century. As a result, over 1000 metalliferous mines were distributed along mineralized zones; most of the mines were abandoned due to a

lack of ore minerals. However, these abandoned mines can become an important point source of toxic elements including As, Cd, Cu, Pb and Zn in the surface environment. Numerous studies have been undertaken into trace element contamination derived from mining activities, in soils, plants, waters and sediments in various countries (Merrington and Alloway, 1994; Pestana et al., 1997). Limited studies of heavy metal concentration derived from mining activities, however, have been carried out in Korea (Jung and Thornton, 1996; Chon et al., 1997). The objectives of Churl Gyu Lee and coauthors' work (2001) were (1) to investigate the extent and degree of trace element contamination of soils, plants, waters and sediments influenced by mining activity of the Daduk mine; (2) to examine the variations of trace elements in paddy fields throughout the rice growing season and those in waters and sediments between the dry and wet seasons; (3) to find out the differences in element concentrations in soils by a chemical decomposition method.

Elevated levels of Cd, Cu, Pb and Zn were found in tailings with averages of 8.57, 481, 4,450 and 753 mg/kg, respectively. These metals are continuously dispersed downstream and downslope from the tailings by clastic movement through wind and water. Thus, significant levels of the elements in waters and sediments were found up to 3.3 km downstream from the mining site, especially for Cd and Zn. Enriched concentrations of heavy metals were also found in various plants grown in the vicinity of the mining area, and the metal concentrations in plants increased with those in soils. In a study of seasonal variation on the heavy metals in paddy fields, relatively high concentrations of heavy metals were found in rice leaves and stalks grown under oxidizing conditions rather than a reducing environment ($P<0.05$).

Oil contamination control and searching for ways of sea water purification is another important problem for Korean scientists. In study of Chung et al. (2009), a marine bacterium isolated from crude-oil-contaminated seawater was subjected to a polyphasic taxonomic analysis and allocated to the genus Muricauda. Crude-oil-contaminated seawater was collected from the west coast of Korea. A Gram-staining-negative, rod-shaped and non-motile bacterium, designated CL-SS4T, was isolated from crude-oil-contaminated seawater from the west coast of Korea and was investigated by means of a polyphasic taxonomic approach. Strain CL-SS4T grew optimally at 25–30 6C, at pH 6.8–7.7 and in the presence of 2–3% sea salts. The major fatty acids were iso-C17 : 0 3-OH, iso-C15 : 0 and iso-C15 : 1 G. The major isoprenoid quinone was MK-6. The DNA G+C content was 50.7 mol%. Analysis of the 16S rRNA gene sequence of strain CL-SS4T revealed that it was a member of the genus Muricauda, sharing 95.7–96.9% sequence similarity with the type strains of Muricauda species and being most closely related to *Muricauda aquimarina* SW-63T.

Phylogenetic analyses based on 16S rRNA gene sequences confirmed that strain CL-SS4T formed a deep lineage related to the genus Muricauda. Strain CL-SS4T was distinguishable from recognized Muricauda species on the basis of differential phenotypic characteristics and DNA G+C content. Based on these results, it is proposed that strain CL-SS4T represents a novel species of the genus Muricauda, for which the name Muricauda olearia sp. nov. is proposed. An emended description of the genus Muricauda is also proposed. The type strain is CL-SS4T (5KCCM 90075T5JCM 15563T). Obtained results can be useful for further investigations in searching of active strains for bioremediation purposes.

Blooms of the cyanobacterium *Microcystis aeruginosa* are ubiquitous phenomena in eutrophic lakes and reservoirs in Korea and many other countries of the world. Many strains of *Microcystis* are known to produce cyanobacterial hepatotoxins called microcystins (MC). These toxins are soluble peptides and are lethal to many kinds of aquatic organisms. MC are found in strains of the genera *Microcystis, Oscillatoria, Anabaena*, and *Nostoc* (Skulberg et al., 1993). To date, at least 69 MC have been structurally characterized (Kaya, 1999).

Therefore, the ability to detect and predict MC in water resources is very important.

Normally, a high-performance liquid chromatography (HPLC) analysis is used for the detection and qualification of MC in water (Kaya, 1999). However, this method has certain weaknesses in that it usually requires a concentration process and is only feasible in a laboratory equipped with an HPLC system. Recently, a protein phosphatase (PP) inhibition assay was introduced for detecting MC in water and algal samples. The PP inhibition assay for MC consists of measuring the release of acid-soluble 32P from 32P-labeled glycogen phosphorylase or a colorimetric assay utilizing the ability of PP-1 to dephosphorylate *p*-nitrophenyl phosphate. Although some research has been carried out to compare the results from the HPLC and PP inhibition assays (Wirsing et al., 1999), a clear relationship between these methods has not yet been established.

MC production by cyanobacteria results from cyanobacterial blooms caused by an abundance of nutrients and favorable conditions for cyanobacterial growth. Changing environmental factors in a water system will have an impact on the MC concentration. Chlorophyll-*a* would appear to be useful for an initial estimate of a MC concentration in field situations dominated by potentially MC-producing genera. The relationships between MC concentration and the N and P concentrations in water have already been studied (Rapala et al., 1997; Rapala J., Sivonen, 1998). However, the development of proper parameters, including the ratios of particulate N to P and particulate to dissolved N or P, to estimate MC concentrations is still needed to improve the ability to manage water quality.

Physicochemical and biological water quality, including the microcystin concentration, was investigated from spring to autumn 1999 in the Daechung Reservoir, Korea. The dominant genus in the cyanobacterial blooming season was *Microcystis*. The microcystin concentration in particulate form increased dramatically from August up to a level of 200 ng liter21 in early October and thereafter tended to decrease. The microcystin concentration in dissolved form was about 28% of that of the particulate form. The microcystins detected using a protein phosphatase (PP) inhibition assay were highly correlated with those microcystins detected by a high-performance liquid chromatograph (r 5 0.973; $P < 0.01$). Therefore, the effectiveness of a PP inhibition assay for microcystin detection in a high number of water samples was confirmed as easy, quick, and convenient. The microcystin concentration was highly correlated with the phytoplankton number (r 5 0.650; $P < 0.01$) and chlorophyll-*a* concentration (r 5 0.591; $P < 0.01$). When the microcystin concentration exceeded about 100 ng liter21, the ratio of particulate to dissolved total nitrogen (TN) or total phosphorus (TP) converged at a value of 0.6. Furthermore, the microcystin concentration was lower than 50 ng liter21 at a particulate N/P ratio below 8, whereas the microcystin concentration varied quite substantially from 50 to 240 ng liter21 at a particulate N/P ratio of >8. Therefore, it seems that the microcystin concentration in water can be estimated and indirectly monitored by analyzing the following: the phytoplankton number and chlorophyll-*a* concentration, the ratio of the particulate and the dissolved forms of N and P, and the particulate N/P ratio when the dominant genus is toxigenic *Microcystis*.

Also in Korea found a new species.A novel species belonging to the genus Grimontia is described in this study. A Gram-negative, chemoheterotrophic, obligately aerobic, catalase- and oxidase-positive, motile by a single polar flagellum, and rod-shaped bacterium, designated IMCC5001(T), was isolated from surface seawater of the Yellow Sea. Strain IMCC5001(T) grew optimally at 30°C in the presence of 3.5% NaCl. Phylogenetic analysis based on 16S rRNA gene sequences showed that the isolate was related most closely to Grimontia hollisae with a sequence similarity of 95.8%, and formed a robust phyletic lineage with Grimontia hollisae. Differential physiological characteristics between the new strain and Grimontia hollisae KCCM 41680(T) and chemotaxonomic characterization including determination of DNA G+C content, fatty acid methyl esters, quinone composition, and polar lipid profiles justified the assignment of strain IMCC5001(T) to the genus Grimontia as a novel species. In conclusion, strain IMCC5001(T) represents a new species, for which the name Grimontia marina sp. nov. is

proposed, with the type strain IMCC5001(T) (=KCTC 22666(T) =NBRC 105794(T))(Choi , Kim , Kang , Youn , Suh , Lee , Cho ,2012).

9. MAS Malaysia

Heavy metal contamination of the coastal environment continues to attract the attention of environmental researchers due to its increasing input to the coastal waters, especially in the developing countries. As for other contaminants, heavy metals could be introduced into the coastal environments by several pathways including disposal of liquid effluents, runoff carrying chemicals originating from a variety of urban, industrial and agricultural activities as well as atmospheric deposition.

Due to their toxicity, persistence and bioaccumulation problems, heavy metals become one of the more serious pollutants in our natural environment. Heavy metals in natural waters and their corresponding sediments have become a significant topic of concern for scientists in various fields associated with water quality, as well as a concern of the general public. The focus of this concern is direct toxicity to man and aquatic life as well as indirect toxicity through accumulations of metals in the aquatic food chain.

The main aim of Prof. Dr. Ahmad Ismail's work (2009) from School of Graduate Studies, Universiti Putra Malaysia were to determinate the concentrations of Cd, Cu, Pb, Zn, Ni and Fe in the surface seawater, suspended particulate matter, algal mat, sediments and gastropod *Nerita lineata* to assess the status of heavy metal pollution in Dumai coastal waters. Concentrations of heavy metal in sediments varied from 0.88, 6.08, 32.34, 53.89, 11.48 μg/g dry weight and 3.01 % for Cd, Cu, Pb, Zn, Ni and Fe, respectively. These concentrations were comparable to metal concentrations in non polluted coastal waters and even lower than concentrations found in the sediments from the west coast of Peninsular Malaysia which is located just at the opposite side of the study area by the Malacca Straits. Generally, higher metal concentrations in the surface seawater, suspended particulate matter, algal mat, sediment and gastropod *Nerita lineata* were found at the stations with more industrial and anthropogenic activities near Dumai city center (eastern and central regions). Metal pollution index (MPI) of *N. lineata* further confirm higher level of heavy metal contaminations at the eastern region of Dumai coastal waters. Cd in sediments from the eastern region was the only metal that slightly higher than effective range low (ERL) but still below effective range medium (ERM) values. The enrichment factor (EF) and pollution load index (PLI) values for Cd and Pb in the eastern region were also higher than other regions. Index of geoaccumulation (Igeo) indicated that most of the stations were categorized as class

1 (unpolluted to moderately polluted environment) and only Cd in Cargo Port was in class 2 (moderately polluted).

More than 50 % of Cd, Cu, Pb, Zn, Ni and Fe in sediments were accumulated in the 'resistant' fraction which indicated that the mobility of these metals in Dumai coastal waters were quite low. The eastern and center regions of Dumai coastal waters accumulated higher percentages of 'nonresistant' fraction of metals, especially Pb. However, only Pb in the eastern region was dominated by 'nonresistant' fraction (> 60 %). For overall stations, Pb also showed the highest percentage (45.32 %) of 'non-resistant' fraction, suggesting more anthropogenic inputs of Pb in Dumai coastal waters in comparison with other metals (43.22, 40.85, 37.34, 25.93 and 18.03 % for Cd, Zn, Ni, Cu and Fe, respectively).

All biomonitor organisms analyzed in the present study showed their ability to accumulate metals from their environment. *T. telescopium* accumulated the highest concentrations of Cd, Cu and Pb, whilst *N. lineata* accumulated the highest concentration for Zn. However, the difference between concentrations of Cd, Cu and Pb in both species was not significant and *T. telescopium* was not widely distributed in Dumai coastal waters as for *N. lineata*. Furthermore, among the studied biomonitors, *N. lineata* has wider range of distribution in the study area and it also fulfilled some of the prerequisites for being indicator organisms for heavy metal pollution.

The mean concentrations of Cd, Cu, Pb, Zn, Ni and Fe in *N. lineata* were 4.14, 5.90, 44.43, 3.74, 20.73, 24.91 μg/g in shell; 4.16, 7.31, 51.78, 17.63, 23.52, 30.60 μg/g in operculum and 0.71, 15.16, 9.34, 94.69, 5.08, 397.96 μg/g d.w in the total soft tissue, respectively. Concentrations of Cd, Pb and Ni decreased in the order: operculum > shell > soft tissue whilst Cu, Zn and Fe in the order of soft tissue > operculum > shell. Metal concentrations, especially Cd in the shells and Pb in the soft tissues of *N. lineata* were significantly correlated with some of the respective geochemical fractions of metal concentrations in sediment as well as with metal concentrations in algal mat, suspended particulate matter and seawater which suggest that *N. lineata* could be used as biomonitoring agent for heavy metals pollution in Dumai coastal waters.

Geochemical fractionation analysis and calculated pollution indices using surface sediments and gastropod *N. lineata* revealed that Dumai coastal waters can be classified as unpolluted to moderately-polluted coastal environment. Therefore, although Dumai coastal waters is still not seriously polluted, a continuous environment monitoring program should be implemented as the ever increasing human activities and rapid developments in many infrastructures and industry sectors are continuously expanding.

Other scientists Ong M.C., Kamaruzzaman B.Y. (2009) conducted assessment of metals contamination in bottom sediment from South China Sea coastal waters, Malaysia. The coastal waters in South China Sea are dissected by many meandering and slow flowing rivers and streams. The largest river if the Pahang river which slightly over 434 km length and has a catchments area of about 29,137 km^2. The main economic yield of this coastal water includes fishery resources with an utilizable mangrove forest resources and swamp peat areas along the back-shore of the coast. This waters is shared to three developing economies namely Thailand, Indonesia and Malaysia were important as an international shipping route especially for the northern countries such as Thailand, Vietnam, China, Chinese Taipei, Japan and Korea which particular on logistic purpose.

The approach most often used to determine the sources of the pollutant is through the normalization of geochemical data to reference metal. The reference metal must therefore be an important constituent of one or more of the major fine-grained trace metal carriers reflect their granular variability in the sediment. The most often used reference metal is Al, which represents a chemical tracer of Al-silicates, particularly the clay minerals. For a better estimation of anthropogenic input, an enrichment factor was calculated for each metal by dividing its ration to the normalizing element by the same ration found in the chosen baseline. EF values are applied to evaluate the dominant source of the sediments and as indicators for pollution and describe as EF = [(E/Al).sub.sed]/[(E/Al).sub.crust] where [(E/Al).sub.sed]/[(E/Al).sub.crust] are the relative concentrations of the respective element E and Al in the sediment and in the crustal material, respectively. EF close to 1 point to a crustal origin, while those with a factor more than 10 are considered to have a non-crustal source. In this study, the elements studied were proven to be deficiency to minimal enrichment, with EF values were ranged from 0.05-3.82 for Pb and 0.06-2.75 for Cu. Authors established generally metals concentration in the sediment was much influenced by natural processes. The EF and [I.sub.geo] values indicates all metal studied occurrence in both lithogenous and non-lithogenous fractions. The relatively high concentration of studied metals at some sampling point in the study are clearly indicates that the main sources of pollution were probably come from urban sewage, industrial effluents and shipping transportation.

Heavy metal contamination of ocean undoubtedly influences on biorecourses's quality as a foodstuff. In work of Titik Budiati (2010) from Food Technology Division , School of Industrial Technology, Universiti Sains Malaysia, Penang, Malaysia determined the risk assessment of salmon due to the presence of arsenic as heavy metal contaminant.

In spite of the beneficial effects that fish consumption provides, fish can harbor a number of biological and chemical hazards, such as toxic compounds, POPs, pathogenic bacteria, and viruses, which can survive the processing steps. Unfortunately the chemical contaminants are stored within the lipid component of the fish so they are well protected when entering the human body. In general, food of animal origin contributes to about 80% of the overall human exposure to dioxins. The contamination can vary widely depending on the origin of the foodstuff. Meat, eggs, milk, farmed fish and other food products may be contaminated above background levels from feedingstuffs. Such contamination may be due to a high level of local environmental contamination, for example from a local waste incinerator, to incidents, such asin 1999 in Belgium, or to a high content of dioxins in fishmeal and fish oil. Wild fish from certain polluted areas may be highly contaminated. Fish can indeed contribute significantly to the human dietary exposure to many contaminants, but in this risk assessment author focused in heavy metal contaminant such as arsenic. The maximum level of arsenic in fish feed is 6 mg/kg and for inorganic arsenic is 2 mg/kg. The provisional tolerable weekly intake (PTWI) for inorganic arsenic of 15 μg/kg b.w./week and noted that organic forms of arsenic present in sea foods need different consideration from the inorganic arsenic in water. There are no reports of toxicity in man or animals from the consumption of organoarsenicals in seafood. Organic arsenic compounds such as arsenobetaine and arsenocholine seem not to be converted to inorganic arsenic *in vivo* and not genotoxic in mammalian cells *in vitro*. Therefore, arsenobetaine and arsenocholine from fish and sea food consumption is not considered to represent a significant health risk.

The monitoring of organic and faecal contamination of coastal sea waters is important part of environmental condition control. In study of Owens J.D. (1978) from School of Biological Sciences, Science University of Malaysia, Penang, Malaysia the concentrations of coliform and *Escherichia coli* bacteria were determined in inshore seawater from 15 different areas, embracing more than three quarters of the coastline of Penang Island. Each area included from 3 to 20 sampling sites, each of which was sampled on at least two occasions, once at high tide and once at low tide. Samples were collected from water 100–150 mm below the surface and where, in most cases, the total water depth was about 0.6 m.

Logarithmic mean concentrations in samples from areas with hinterlands having small or large human (and/or domestic animal) populations ranged from 493 to 712,000 coliform bacteria and from 72 to 234,700 *E. coli* bacteria per 100 ml. Samples from an area with a hinterland of natural forest and very few dwellings contained logarithmic mean concentrations of 31 coliform and less than 7 *E. coli* bacteria per 100 ml. The highest

concentrations found in any sample from this area were 1100 coliform and 93 *E. coli* bacteria per 100 ml. It is suggested that the bacterial concentrations in seawater from this area may approximate to 'base-line' contamination levels. The overall results are considered in relation to standards applied elsewhere to bathing waters and to those applied to waters for shellfish culture.

10. MEX Mexico

One of the direction of marine microbiology is studing of micro algae .It was done collection which was open to the worldwide scientific community as a source of organisms in controlled conditions that can be used as a useful tool for microalgae research work.

Some microalgae are economically important in Mexico and the world because they can be potentially toxic. Algal explosive population growths are named harmful algal blooms and are frequently recorded in Mexico. The authors set up potentially toxic microalgae cultures from the Gulf of Mexico (Garrapatas tideland, Barberena river, Carpintero lagoon in Tamaulipas State; Chalchoapan and Catemaco lakes in Veracruz State), from the Mexican Pacific Ocean, Guerrero, Colima and Michoacán States, and from interior water bodies such as Vicente Aguirre dam, Chapultepec lake and several waste water treatment plants. This research is about the diversity and abundance of phytoplankton in relation a specific site because of harmful algal bloom events. Microalgae cultures are useful in order to solve taxonomic problems, to know life cycles, molecular studies, for the study of toxic species, and the isolation of useful metabolites (Espinoza-Valles, Soto-Rodríguez, Edwards, Wang, Vora, Gómez-Gil, 2012).

In the Molecular Microbiology Laboratory, Department of Marine Biotechnology, Centro de Investigación Científica y de Educación Superior de Ensenada there is so direction as using microorganisms as probiotcs. Functional feed supplemented with alternative-economic nutrient sources (protein, carbohydrates, lipids) and probiotics are being considered in shrimp/fish aquaculture production systems as an option to increase yield and profits and to reduce water pollution. In this study the probiotic potential to formulate functional feeds have been evaluated using four dietary treatments: Treatment 1 (B + Bs); Bacillus subtilis potential probiotic strain was supplemented to a soybeanmeal (SBM)-carbohydrates (CHO) basal feed. Treatment 2 (B + Bm); Bacillus megaterium potential probiotic strain was supplemented to the same SBM-CHO basal feed. In Treatment 3 (B); SBM-CHO basal feed was not supplemented with probiotic strains. Treatment 4 (C); fishmeal commercial feed (FM) was utilized as positive control. Feeding trials evaluated the survival, growth, and food conversion ratio and stress tolerance of juvenile Litopenaeus vannamei (Boone) Pacific white shrimp. Best overall shrimp performance was observed for animals fed with Treatment 1 (B+Bs); additionally, stress tolerance and hemolymph metabolites also showed the best performance in this treatment.

SBM-CHO basal feed not supplemented with probiotic strains (B) presented smaller growth and lower feed conversion ratio (FCR). Shrimps fed with the fishmeal commercial feed (C) presented the lowest stress tolerance to high ammonia and low oxygen levels. Specifically selected B. subtilis strains are recommended to formulate functional and economical feeds containing high levels of vegetable; protein and carbohydrates as main dietary sources in L. vannamei cultures (Olmos, Ochoa, Paniagua-Michel, Contreras, 2011).

Instituto Politécnico Nacional, La Paz, specializes in obtaining biolgicheskih substances from marine algae. having antibiotic activity for pathogenic bacteria.

Sixty ethanol extracts of marine flora of Baja California Sur (Mexico) were screened to evaluate the reversing effect of the bacterial resistance to antibiotics in combination with a sublethal concentration of ampicillin or erythromycin.

The activity was assayed by using a modification of the classical agar-diffusion method against 3 resistant, pathogenic bacteria; Escherichia coil (ATCC BAA196), Staphylococcus aureus (ATCC BAA42), and Streptococcus pyogenes (ATCC BAA946).

From the 60 ethanolic extracts, 12 (20%) of them in combination with ampicillin were able to reverse the resistance of Staphylococcus aureus and 8 (13%) with erythromycin yielded the same reversal with Streptococcus pyogenes. An extract from Sargassum horridum was the only one that reversed the resistance to antibiotics against both Staphylococcus aureus and Streptococcus pyogenes.Our findings suggest that some algae may be source of compounds with the potential to reverse the antibiotic resistance of some bacteria. In addition, of the assayed extracts, 35 (57%) showed inhibitory activity against Staphylococcus aureus, 48 (78%) were active against Streptococcus pyogenes, but none was active against Escherichia coil. The most active extracts were from Laurencia spp., Gelidium robustum, Chnoospora implexa, Padina mexicana, Gracilaria subsecundata, and Dictyopteris undulata.

11. NZ New Zealand

The main direction is symbiotic attitude in this economy.Marine sponges often contain diverse and abundant communities of microorganisms including bacteria, archaea and eukaryotic microbes. Numerous 16S rRNA-based studies have identified putative 'sponge-specific' microbes that are apparently absent from seawater and other (non-sponge) marine habitats. With more than 7500 sponge-derived rRNA sequences (from clone, isolate and denaturing gradient gel electrophoresis data) now publicly available, we sought to determine whether the current notion of sponge-specific sequence clusters remains valid. Comprehensive phylogenetic analyses were performed on the 7546 sponge-derived 16S and 18S rRNA sequences that were publicly available in early 2010. Overall, 27% of all sequences fell into monophyletic, sponge-specific sequence clusters. Such clusters were particularly well represented among the Chloroflexi, Cyanobacteria, 'Poribacteria', Betaproteobacteria and Acidobacteria, and in total were identified in at least 14 bacterial phyla, as well as the Archaea and fungi. The largest sponge-specific cluster, representing the cyanobacterium 'Synechococcus spongiarum', contained 245 sequences from 40 sponge species. These results strongly support the existence of sponge-specific microbes and provide a suitable framework for future studies of rare and abundant sponge symbionts, both of which can now be studied using next-generation sequencing technologies (Simister, Deines, Botté, Webster, Taylor,2012).

Diversity in guilds of primary producers enhances temporal stability in provision of organic matter to consumers. In the Antarctic ecosystem, where temporal variability in phytoplankton production is high, sea ice contains a diatom and microbial community (SIMCO) that represents a pool of organic matter that is seasonally more consistent, although of relatively small magnitude. The fate of organic material produced by SIMCO in Antarctica is largely unknown but may represent an important link between sea ice dynamics and secondary production in nearshore food webs. We used whole tissue and compound-specific stable isotope analysis of consumers to test whether the sea ice microbial community is an important source of organic matter supporting nearshore communities in the Ross Sea. We found distinct gradients in delta13C and delta15N of SIMCO corresponding to differences in inorganic carbon and nitrogen acquisition among sites with different sea ice extent and persistence. Mass balance analysis of a suite of consumers demonstrated large fluxes of SIMCO into the nearshore food web, ranging from 5% to 100% of organic matter supplied to benthic species, and 0-10% of organic matter to

upper water column or pelagic inhabitants. A delta13C analysis of nine fatty acids including two key biomarkers for diatoms, eicosapentaenoic acid (EPA, 20:5omega3), and docosahexaenoic acid (DHA, 22:6omega3), confirmed these patterns. We observed clear patterns in delta13C of fatty acids that are enriched in 13C for species that acquire a large fraction of their nutrition from SIMCO. These data demonstrate the key role of SIMCO in ecosystem functioning in Antarctica and strong linkages between sea ice extent and nearshore secondary productivity. While SIMCO provides a stabilizing subsidy of organic matter, changes to sea ice coverage associated with climate change would directly affect secondary production and stability of benthic food webs in Antarctica (Wing, McLeod, Leichter, Frew, Lamare, 2012).

12. PE Peru

Universidad Nacional Mayor de San Marcos shows that Marine sediments of the central coast of Peru, are a source of actinomycetes strains showing high capacity to produce bioactive compounds able to inhibit pathogens classified as multi-drug-resistant such as methicillin-resistant S. aureus and vancomycin-resistant E. faecalis.

To determine the antimicrobial potential of marine actinomycetes against drug-resistant pathogens represented by strains of methicillin-resistant Staphylococcus aureus (MRSA) and vancomycin-resistant Enterococcus faecalis (VRE).

Strains of actinomycetes (29) isolated from marine sediment were evaluated by their characteristics in two culture media and by testing their inhibitory capacity by in vitro antagonism against multi-drug resistant (MDR) pathogenic bacteria for MRSA and VRE. Organic extracts of 3 selected actinomicetes were processed to determine the minimum inhibitory concentration (MIC) of the active compound.

Most isolated actinomycetes belong to a homogeneous group of write-gray actinomycetes with a good growth in Marine Agar. The inhibitory rates of the isolates were above 85% for both pathogens with inhibition zones greater than 69 and 78 mm in diameter for MRSA and VRE respectively. Dichloromethane extracts of 3 isolates (I-400A, B1-T61, M10-77) showed strong inhibitory activity of both pathogens, M10-77 being the highest actinomycete strain with antibiotic activity against methicillin-resistant S. aureus ATCC 43300 and vancomycin-resistant E. faecalis ATCC 51299 with a minimum inhibitory concentrations (MIC) of 7.9 and 31.7 μg/ml respectively. Phylogenetic analysis of M10-77 strain showed 99% similarity with the marine species Streptomyces erythrogriseus (León, Aponte, Rojas, Cuadra, Ayala, Tomás, Guerrero, 2011).

The next direction is deseases of fish.This investigation realizes Peruvian Marine Institute.In the institute studies following agents::Lumphocystis viral disease in Amphiprion ocellaris,Coccidia Eimeria affecting the pelagic marine fish Trachurus murphyi,Coccidia in liver of Trachurus murphyi, Eimeria sp. In Paralabrax humeralis. The leader of this research is proffessor Enrique Mateo.

13. RP The Republic of the Philippines

The waters of Southeast Asia occupy a crossroad position between the Indian and Pacific oceans on the trade routes of Europe, Africa, the Middle East, Japan and other Far Eastern nations. The major transportation route for oil imported into the region is from the Middle East and Africa through the Straits of Malacca and the South China Sea, most of it in transit to Japan, with offshoots to Thailand, Chinese Taipei and the Philippines. Hence, the water along this route is constantly at a risk of being contaminated by oil, either from accidental spills or routine ship operations such as loading, discharging and bunkering. The various sources from land and marine-based oil pollution of the ASEAN marine environment have been studied (WHO/PEPAS, 1981). Hydrocarbon concentrations were measured in both the open seas and coastal waters. Concentrations vary widely in the region, but coastal areas are generally more than 1000 times higher than the open sea baseline easurement (Bilal and Kuhnhold,1980). This study deals mainly with part of the South China Sea off the western coast of the Philippines to provide an information on the levels of petroleum hydrocarbons generally present in this region. The study is a joint cooperation between the Bureau of Fisheries and Aquatic Resources (BFAR) of the Philippines, the Marine Fishery Resources Development and Management Department (MFRDMD) of Malaysia, Southeast Asian Fisheries Development Center, Training Department (SEAFDEC TD) and Chulalongkorn University, Thailand.

Saramun S., Wattayakorn G study on petroleum hydrocarbon concentrations in seawater from the South China Sea off the western coast of the Philippines was conducted during April to May 1998. The concentrations of dissolved/dispersed petroleum hydrocarbons (DDPH) in seawater samples were measured at 31 stations, using Ultraviolet Fluorescence (UVF) Spectroscopy technique. The DDPH concentrations were found to be in the range of 0.02 Ð 1.47 mg/l as chrysene equivalent, with an average of 0.25 mg/l. An attempt was made to compare between petroleum hydrocarbons in seawater samples from the near-shore area (8 stations) and the offshore area (23 stations). It was found that the DDPH concentrations of the near-shore stations were in the range of 0.03 Ð 0.47 mg/l, with an average of 0.12 mg/l, whereas the DDPH concentrations of the offshore stations were in the range of 0.02- 1.47 mg/l, with an average of 0.29 mg/l. However, the studentÕs t-test of the two data groups indicated that the two means were not significantly different at a = 0.05.

Water samples were taken from 31 stations along the western coast of the Philippines. Sample collection took place on board the M.V.SEAFDEC between the months of April to May 1998. The study area is located between 117o and 121o E longitude and 11o and 20o N latitude. These included 8 stations from the near-shore area and 23 stations from offshore area .

Seawater samples were collected at 1 meter depth below sea surface, using 4 -liters

amber coloured glass bottles mounted on a weighted frame, the design of which is in accordance with IOC standard procedure (IOC/UNESCO,1984). There after 100 ml of each sample was discarded and immediately replaced with 50ml nano-grade hexane. The samples were thoroughly shaken before storage in a dark, cool place. Analysis for dissolved/dispersed petroleum hydrocarbon (DDPH) concentration was conducted in the laboratory upon returning to shore.

Each water sample was extracted three times with nano-grade hexane in a separatory funnel. The combined hexane volume was dried by an addition of anhydrous Na2SO4 and concentrated to 5 ml using a rotary evaporator. Ultraviolet fluorescence (UVF) intensity of the reduced samples was measured at an emission wavelength of 360 nm and excitation wavelength of 310 nm, using a Perkin Elmer Model 3000 Spectrofluorometer. The measurement of UVF intensity obtained from the samples was calibrated against standard chrysene. Statistical analysis for this study was a simple studentÕs t-test.

Analysis of the seawater samples taken from 31 stations in the South China Sea yields the total of DDPH concentration values shown : The obtained DDPH concentrations are within the range of 0.02-1.47 mg/l as chrysene equivalent, with a mean value of 0.25 mg/l. DDPH concentrations in seawater can be grouped into two general areas, namely the near-shore and the offshore stations . The DDPH concentrations of the near-shore stations were in the range between 0.03-0.47 mg/l, with the mean value of 0.11 mg/l. Meanwhile, the offshore concentrations were found in the range of 0.02-1.47 mg/l, with an average value of 0.29 mg/l. However, studentÕs t-test of the data indicated no significant difference between the mean of the near-shore area as compared to that of the offshore area, within the 95% confidence interval .

Generally the concentrations of DDPH in this part of the South China Sea are lower than that in the Gulf of Thailand and the east coast of Peninsular Malaysia (Wattayakorn et al., 1998; Wongnapapan et al., 1997). This finding indicates that coastal areas and semi-

enclosed marine embayment, like the Gulf of Thailand, have higher levels of petroleum contamination in water as compared to the open sea areas since the biggest contributions of oil come from terrestrial sources (National Research Council, 1985) .

The analysis shows that contamination of DDPH in this area is likely to be the result of maritime and shipping activities as well as offshore oil exploration and production.

The mean value of DDPH concentrations from near-shore and offshore areas is not significantly different from each other within the range of the 95% confidence interval.

Next direction is study of communities of microorganisms with marine aquatic life.It was done attempt to find stimulus of coral diseases in Bolinao Bay.But phylogenetic research shows there is no differences between healthy and ill coralls. (Arboleda , Reichardt W, 2009).

14. RUS Russia

Prymorskiy krai in the most degree connected with economies of Pacific-Asian Region.The main centres of research in Vladivostok are Far Eastern Federal, University Pacific Institute of Bioorganic Chemistry, Institute of Marine Biology.

In Primorye Marine Microbiology represents in the following areas:

Microbiological monitoring of marine pollution, especially the coastal zone, which occurs as a result of human impacts and environmental bioremediation by microorganisms. These researches conducted in Far Eastern Federal University.

The study of biologically active properties of marine organisms is in the Pacific Institute of Bioorganic Chemistry, which was created the unique collection of marine organisms and which is included in World Federation of Culture Collection.

Sanitary microbiology and the study of survival of pathogenic bacteria in sea water - Institute of Microbiology and Epidemiology, Russian Medicine Academy of science.
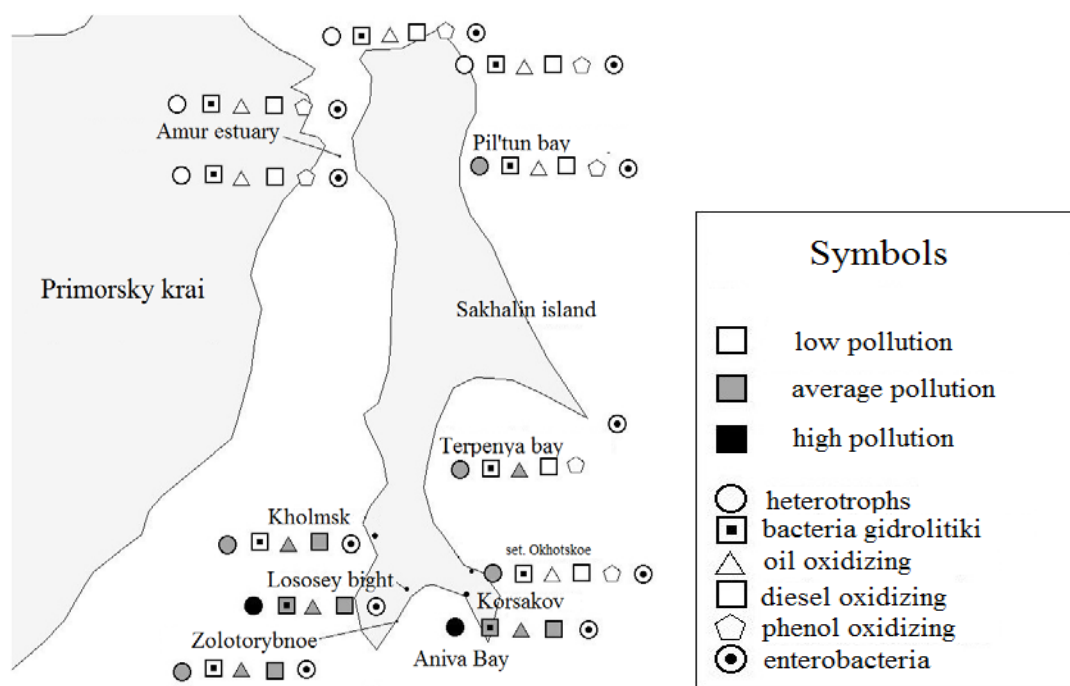
IBM collaborates with Vietnamese center. They work on the study evaluation of sea water biocorrossion activity and solve the following problems: fouling biomass, adhezion surface, number of microorganisms, activity of enzymes (multisubstrate testing method). A result of research, it was found that *Pseudomonas* have anticorrosion activity, large number of *Listeria*. and *Staphylococcus* attached in the composition of biofilms on the substrate.
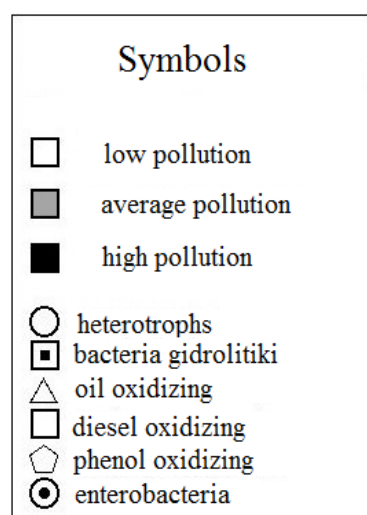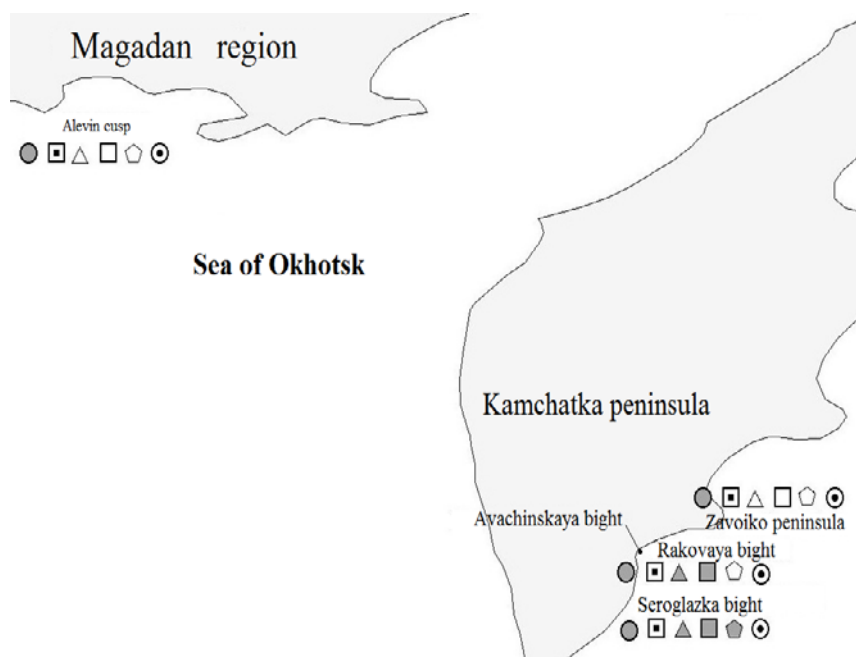
 The laboratory of Marine Microbiology Research and Education Center "Marine Biota, "Far Eastern Phederal University for 6 years created a collection of strains of sea microorganisms which was isolated from various waters of the Far Eastern seas with high anthropogenic stress. Collected strains have a unique ability to destroy the various oil hydrocarbons, phenols, and some other organic substances and accumulate high concentrations of heavy metals (Cd, Cu, Co, Zn, Ni, Pb).
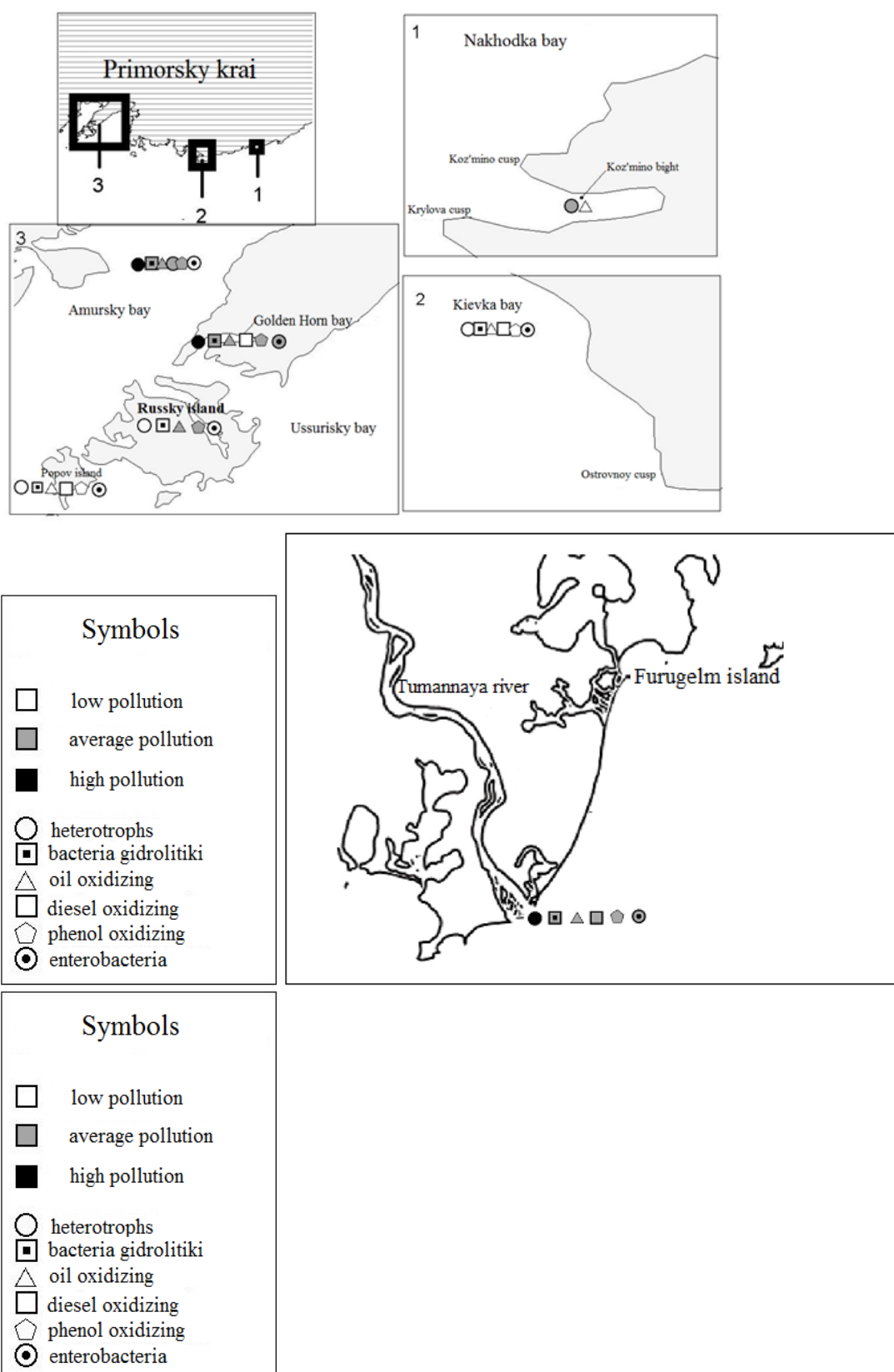
The material was selected by students during the summer practice and during two expeditions on the PUS "Hope" (11.08-21.09 2001) on the research vessel

It was done a comparative study of microorganisms number of different ecological-trophic groups in the sea waters of the Far Eastern Seas in a complex of organic pollution. It is shown that although marine microorganisms have polyresistance to a variety of contaminants, but their reaction is individual for each type of pollutant. Based on the

results of the microbial indication it was assessed the quality of the environment in ecologically stressed areas of the Pacific coast. It were the coast of Sakhalin in the region of oil production ,the Kamchatka near the Avacha Bay, southern Primorye in waters near river Misty and bay Golden Horn which located near the center of Vladivostok..

Magadan region

Alevin cusp

Sea of Okhotsk

Kamchatka peninsula

Avachinskaya bight

Zavoiko peninsula

Rakovaya bight

Scroglazka bight

Symbols

□ low pollution

◼ average pollution

■ high pollution

○ heterotrophs
⊡ bacteria gidrolitiki
△ oil oxidizing
□ diesel oxidizing
⬠ phenol oxidizing
⊙ enterobacteria

Mapping of different areas of pollutions :1-island Sachalin, 2-Kamchatka, 3- Primorsky krai, 4-Tumannaya river.

Using the methods of the microbial indication it confirmed that the Far Eastern seas are heavily polluted. At each of geochemical provinces microorganisms reflect it's specific.

Along the coast of south-western and south-eastern Sakhalin was also high nutrient pollution, while in the waters of the north- eastern shelf of the island's most numerous groups were oxidizing bacterias, indicating the appropriate levels and types of pollution associated with the impact areas are located in oil production, terrigenous and river runoff containing oil.

In Kamchatka in Avacha Bay microbial indication in general revealed a dominant industrial pollution (diesel, fuel oil, oil, etc.)

in the south of Primorye - a high level of readily degradable and difficult to decompose organic substances.

Selected strains can produce biologically active substances (enzymes, vitamins, hormones, growth factors) in destroying of pollutants of microorganisms.

With the help of test systems it is identified strains that have the most highly resistant to pollutants and heavy metals. It was study the biological diversity of the studied physiological groups of microorganisms. Identification of the obtained strains that are resistant to pollutants and heavy metals was carried out by PCR and the API-tests. A collection includes identified548 strains (468 still in the process of the work).

*T*here is the fact of living pathogenic bacteria in seawater of the coastal zone of Primorye: *Pseudomonas, Listeria, Enterobacteria, Vibrio cholerae*. It is shown the high contamination of marine waters in the summer-autumn period of time. It is shown the fact of dwelling bacterium *Listeria monocytogenes* in sea water, ice and in the western part of the bottom sediments of the Okhotsk Sea. It is shown that a low positive temperature cultivation and pre-adapted strains of *Listeria monocytogenes* contributes to long-term preservation of the viability of *Listeria monocytogenes* in sea water in the reproductive state.

*1.*This unique ecological collection may be required for scientific objectives for the study of natural remediation of the environment, of the role of microorganisms in the cycle of matter in marine ecosystems which subject to anthropogenic stress. The reference strains can be used in the performance of candidate and doctoral work. Receive similar collection of strains from other regions is expensive and doesn t often appropriate in carrying out environmental research in the Far East. 2. The collection can be used for teaching and educational process in the course of the program "Ecology" "Ecology of Microorganisms" "Microbiology", "Biotechnology". Collection of Microorganisms at the University will enhance its status and increase international relations.
For the official registration of the collection and its subsequent development it is necessary establish an environment for storing collections of microorganisms and need to purchase low-temperature refrigerator -70 ^{0}C, krio test tubes and reagents.

The next direction in the microbiological research is sanitary and microbial research of infecting of Ballast Waters.
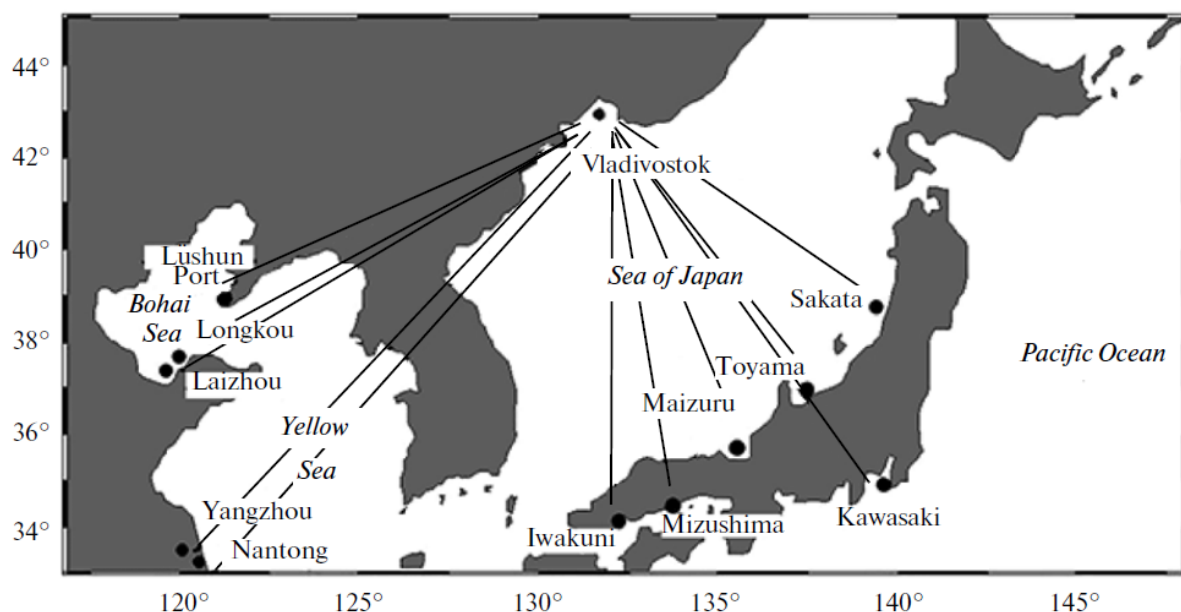
The major routes of ships arriving in the port of Vladivostok from Chinese and Japanese ports.

In ballast water (BW) of the *Minotaur* arriving from the Chinese ports of Longkou and Laizhou (Lairouwan Bay, Yellow Sea), the total number of heterotrophic bacteria (TNHB) was fairly stable; that is, it was in the range from 10^3 to 10^4 CFU/ml. This fact points to a moderate bacterial pollution of water (*Gidrokhimicheskie pokazateli…*, 2007). The average number of heterotrophic bacteria in BW from the port of Longkou changed depending on the season of the year.

The number of heterotrophic and sanitary indicator microorganisms (CGB, *Escherichia coli*, *Enterococcus faecalis*), CFU/mL, in BW of the tanker *Minotaur*, which arrived from the Chinese port of Longkou (2008–2009)

Date of sampling /CFU/mL	TNHB (surface layer/bottom)	CGB	*Escherichia coli*	*E. faecalis*
May 29, 2008	$\dfrac{(3.5 \pm 0.2) \times 10^3}{(2.0 \pm 0.4) \times 10^4}$	0	0	0
June 10, 2008	$\dfrac{(1.7 \pm 0.3) \times 10^4}{(3.4 \pm 0.5) \times 10^4}$	0	0	0

July 29, 2008	$\dfrac{(3.8 \pm 0.4) \times 10^5}{(4.5 \pm 0.5) \times 10^5}$	$\dfrac{(3.2 \pm 0.1) \times 10}{(5.1 \pm 0.1) \times 10}$ 0	0
August 23, 2008	$\dfrac{(3.2 \pm 0.3) \times 10^3}{(2.2 \pm 0.3) \times 10^4}$	$\dfrac{(1.2 \pm 0.1) \times 10}{(0.9 \pm 0.1) \times 10}$ 0	0
January 12, 2009	0	0 0	0
February 22, 2009	0	0 0	0
April 24, 2009	$\dfrac{(4.0 \pm 0.1) \times 10^2}{(2.5 \pm 0.2) \times 10^2}$	$\dfrac{10 \pm 1}{0}$ 0	0
June 2, 2009	$\dfrac{(2.1 \pm 0.1) \times 10^2}{(4.1 \pm 0.5) \times 10^2}$	$\dfrac{5 \pm 1}{10 \pm 3}$ 0	0
September 24, 2009	$\dfrac{(5.8 \pm 0.1) \times 10^6}{(6.1 \pm 0.4) \times 10^6}$	$\dfrac{(2.2 \pm 0.3) \times 10^6}{(2.0 \pm 0.2) \times 10^6}$ 0	0
October 18, 2009	$\dfrac{(1.9 \pm 0.1) \times 10^2}{(1.4 \pm 0.2) \times 10}$	$\dfrac{0}{(1.0 \pm 0.1)}$ 0	0

In warm months, BW was characterized as dirty (July and August 2008—10^4–10^5 CFU/ml) or very dirty (September 2009—10^6 CFU/ml) in accordance with the indexes of organic pollution. In cold months (January, February, and October), the total number of heterotrophic bacteria was low. It should be noted that coliform bacteria were found in BW in the warm season. These bacteria are indicative of the sanitary state of the water and can be evidence of the pollution with household water wastes. The highest number of these bacteria was observed in September 2009; it exceeded significantly the sanitary norms for wastewater released into water bodies (*Sanitary rules* …, 2000) and was 40 times higher than the standards for pollution by ballast water of ships (*Mezhdunarodnaya konventsiya* …, 2005). As for the *Enterococcus faecalis* bacteria, they were not found in any samples. In BW from the port of Laizhou, the total number of heterotrophic bacteria was about one or two orders of magnitude lower than that from the port of Longkou.

The number of heterotrophic and sanitary indicator microorganisms (CGB, *Escherichia coli*, *Enterococcus faecalis*), CFU/mL, in BW of the tanker *Minotaur*, which arrived from the Chinese port of Laizhou (2008–2009)

Date of sampling /CFU/mL	TNHB (surface layer/bottom)	CGB	*Escherichia coli*	*E. faecalis*
July 19, 2008	$\dfrac{(8.9\pm0.2)\times10^3}{(9.2\pm0.3)\times10^4}$	$\dfrac{(5.0\pm0.1)\times10}{(7.1\pm0.2)\times10}$	5	0
March 8, 2009	$\dfrac{0}{32\pm4}$	0	0	0
May 6, 2009	$\dfrac{(6.0\pm0.4)\times10}{(1.0\pm0.2)\times10^2}$	$\dfrac{0}{20\pm3}$	0	$\dfrac{0}{10\pm0}$
August 29, 2009	$\dfrac{(6.8\pm0.2)\times10^3}{(2.3\pm0.1)\times10^3}$	$\dfrac{4.0\pm0.1}{7.0\pm0.1}$	0	0
September 11, 2009	$\dfrac{(3.2\pm0.2)\times10^3}{(0.8\pm0.1)\times10^2}$	0	0	$\dfrac{6\pm0.1}{0}$

One can characterize such BW as moderately polluted in the warm season and as clean in the other seasons. Among all the isolates, enterococci, not *E. coli,* were the most numerous against the background of the low CGB number in BW. This fact is indicative of recent pollution of the water under study (*Ekologiya* …, 2004). The number of heterotrophic bacteria in the BW samples from the Chinese ports on the Yangtze River was higher—from 10^5 to 10^6 CFU/ml.

According to the TNHB rates, this BW can be characterized as dirty (July and August 2008) or moderately polluted (September 2008, May 2009). In addition, it was highly infected with CGB (from 10^2 to 10^3 CFU/ml), the number of which exceeded the sanitary norms. At the same time, such sanitary indicator microorganisms as colon bacilli and enterococci were not dominant in the samples. In BW sampled in September, the number of enterobacteria was stably high—from 10^2 to 10^3 cells/ml—under the general decrease in CFU of heterotrophic bacteria to the level

number of heterotrophic and sanitary indicator microorganisms (CGB, *Escherichia coli*, *Enterococcus faecalis*), CFU/mL, in BW of the tanker *Minotaur*, which arrived from the Chinese ports of Nantong and Yangzhou (Yangtze River), 2008–2009

Date of sampling /CFU/mL	TNHB (surface layer/bottom)	CGB	*Escherichia coli*	*E. faecalis*
July 4, 2008	$\dfrac{(8.8 \pm 0.5) \times 10^4}{(1.2 \pm 0.2) \times 10^5}$	$\dfrac{(4.2 \pm 0.3) \times 10^3}{(9.1 \pm 0.3) \times 10^2}$	20	0
August 11, 2008	$\dfrac{(4.2 \pm 0.3) \times 10^6}{(4.5 \pm 0.5) \times 10^5}$	$\dfrac{(1.5 \pm 0.1) \times 10}{(8.0 \pm 0.3) \times 10^2}$	0	0
September 5, 2008	$\dfrac{(3.7 \pm 0.5) \times 10^3}{(5.2 \pm 0.4) \times 10^3}$	$\dfrac{(2.2 \pm 0.1) \times 10^3}{(3.1 \pm 0.3) \times 10^3}$	0	0
December 2, 2008	$\dfrac{(1.0 \pm 0.1) \times 10}{(8.1 \pm 0.2) \times 10^2}$	0	0	0
December 19, 2008	$\dfrac{(2.0 \pm 0.2) \times 10}{(3.0 \pm 0.12) \times 10}$	0	0	0
April 12, 2009	$\dfrac{(2.1 \pm 0.2) \times 10^3}{(1.5 \pm 0.3) \times 10^3}$	$\dfrac{(1.04 \pm 0.12) \times 10^?}{(1.47 \pm 0.2) \times 10^3}$	0	0
May 20, 2009	$\dfrac{(4.5 \pm 0.3) \times 10^2}{(3.3 \pm 0.2) \times 10^3}$	$\dfrac{(1.5 \pm 0.1) \times 10^2}{(2.3 \pm 0.4) \times 10^2}$	0	0

corresponding to moderate water pollution. In BW of the *Sunrise Wisteria* sailing from the Japanese ports, the number of heterotrophic bacteria was one or two orders of magnitude lower than that in the samples from the *Minotaur* (from the Chinese ports) and ranged from 102 to 103 cells/ml.

The number of heterotrophic and sanitary indicator microorganisms (CGB, *Escherichia coli*, *Enterococcus faecalis*), CFU/mL, in BW of the tanker *Sunrise Wisteria*, which arrived from the Japanese ports.

Date of sampling /CFU/mL	TNHB (surface layer/bottom)	CGB	*Escherichia coli*	*E. faecalis*
August 17, 2008 Kawasaki	$\dfrac{(3.7 \pm 0.3) \times 10^2}{(2.5 \pm 0.4) \times 10^2}$	0	0	0
September 5, 2008 Mizushima	$\dfrac{(2.9 \pm 0.4) \times 10^3}{(4.8 \pm 0.3) \times 10^2}$	0	0	0
September 12, 2009 Iwakuni	$\dfrac{(1.8 \pm 0.1) \times 10^3}{(7.4 \pm 0.2) \times 10^2}$	0	0	0
November 17, 2008 Kawasaki	$\dfrac{(2.7 \pm 0.1) \times 10^2}{(3.6 \pm 0.3) \times 10^2}$	0	0	0
December 2, 2008 Kawasaki	$\dfrac{(3.0 \pm 0.3) \times 10}{(8.1 \pm 0.2) \times 10}$	0	0	0

During the period from August through November, TNHB in BW from the port of Kawasaki (Japan) was fairly stable and ranged within 102 cells/ml. In BW sampled from other Japanese ports, this index was one order of magnitude higher: the port of Mizushima— (2.9 ± 0.4) × 103 cells/ml (surface layer), 4.8 ± 0.3) × 102 cells/ml (near bottom layer); the port of Iwakuni—(1.8 ± 0.1) × 103 cells/ml (surface layer), (7.4 ± 0.2) × 102 cells/ml (near bottom layer). It should be emphasized that coliform bacteria and fecal streptococci were not found in any BW samples from the ports of Japan. Almost all the BW samples, except those taken in winter, contained CGB. At the same time, the number of enterococci and colon bacilli was singular. It should be emphasized that, in the surface layer,

the number of bacteria, including the opportunistic pathogenic ones, was lower than or comparable to that in the bottom layers. For this reason, it is evident that only the surface layer can be used for further monitoring. The monitoring of heterotrophic bacteria in the Pervaya Rechka water area of Amur Bay (the dumping area) showed that in 2008–2009 their number varied from 102 to 106 CFU/ml depending on the season of the year.

The CFU number of heterotrophic and sanitary indicator microorganisms in the water area of Amur Bay (CGB, *E. coli*, *Enterococcus faecalis*), cells/mL

Date of sampling /CFU/mL	TNHB (surface layer/bottom)	CGB	*Escherichia coli*	*E. faecalis*
January 12, 2009	-	-	-	
February 22, 2009	-	-	-	
April 24, 2009	$\dfrac{(1.54 \pm 0.32) \times 10^3}{(1.35 \pm 0.18) \times 10^3}$	$\dfrac{0}{30 \pm 5}$	$\dfrac{20 \pm 2}{0}$	$\dfrac{0}{20 \pm 0}$
May 20, 2009	$\dfrac{(2.0 \pm 0.3) \times 10^3}{(5.5 \pm 0.2) \times 10^3}$	$\dfrac{200 \pm 5}{50 \pm 7}$	0	$\dfrac{40 \pm 3}{0}$
June 2, 2009	$\dfrac{(1.7 \pm 0.2) \times 10^3}{(5.1 \pm 0.4) \times 10^2}$	$\dfrac{80 \pm 7}{120 \pm 10}$	$\dfrac{0}{120 \pm 10}$	0
August 29, 2009	$\dfrac{(8.2 \pm 0.1) \times 10^4}{(9.0 \pm 0.2) \times 10^3}$	$\dfrac{(1.3 \pm 0.1) \times 10}{(2.4 \pm 0.2) \times 10^2}$	0	$\dfrac{0}{(4.3 \pm 0.1) \times 10}$
September 24, 2009	$\dfrac{(3.4 \pm 0.1) \times 10^6}{(1.5 \pm 0.4) \times 10^5}$	$\dfrac{(2.4 \pm 0.2) \times 10^6}{(1.1 \pm 0.2) \times 10^5}$	$\dfrac{(7.0 \pm 0.2)}{0}$	0
October 18, 2009	$\dfrac{(3.8 \pm 0.1) \times 10^2}{(2.3 \pm 0.2) \times 10}$	$\dfrac{(1.8 \pm 0.1) \times 10^2}{(1.4 \pm 0.3) \times 10}$	$\dfrac{(1.5 \pm 0.2) \times 10}{0}$	0

In the winter months, no microflora was found in the water samples under typical inoculation. Nevertheless, the colony_forming cells were isolated through the filtration of the water (100 ml) and the subsequent inoculation of the bacteria remained on the surface of the filter. Undoubtedly, BW contributes to the number and pathogenicity of the native microflora cenoses inhabiting the coastal areas of Vladivostok. At the same time, Japanese ports cannot be considered a source of the spread of infectious diseases, because the number of heterotrophic bacteria coming into Amur Bay with BW from Japan does not exceed the number of microflora in this water area. A more considerable problem is BW from the Chinese ports. In this water (for the most part from the ports of the Yangtze River

and Longkou), CGB are dominant over *Escherichia coli* and enterococci. On the basis of this fact, this BW is characterized as chronically polluted. In BW from the port of Laizhou, there is mainly a fresh fecal pollution, which can be judged from the presence of enterococci in isolates. The characterization of physical and biochemical features of the strains isolated from BW allows the estimation of the ecological state of the water body from which the samples were taken. All in all, 43 morpho_ types were isolated in the culture. According to the type of cell wall, 23 of them were gram_positive, and all the others were gram_negative. Among the gram_negative microflora, enterobacteria were dominant (94%). Up to 84% of the isolates had a fermentative type of metabolism. The hydrolytic activity of bacteria is indicative of their ability to break up simple organic compounds used as a substrate. It was found that the strains of the collection had low hydrolytic activity: 28% were amylolytic bacteria (synthesize and release amylase, which is an enzyme hydrolyzing starch, into the medium); 26% were lipolytic bacteria (synthesize and release lipase into the medium); 14% were proteolytic bacteria (break up protein substrates). The analysis of the bacterial resistance to metals showed that nine strains (21%) were the most tolerant to the whole list of metals. In the concentrations used for the study, all the isolates were resistant to Pb. Twenty_five percent of the isolates were resistant to antibiotics. In addition, half of the isolates were insusceptible to oleandomycin and polymyxin. Almost all the isolates were insusceptible to ceftazidime. The presence of sanitary indicator microorganisms in marine water is a real threat to human health, because many of them display pathogenic features. Enterobacteria coming into coastal waters of Vladivostok through BW make up almost 50% of the total number of microorganisms. For this reason, their identification was made, and their pathologic features were studied. It was found that *Hafnia* (37%) was the most numerous among the isolates. According to the number in BW, other enterobacteria can be presented in the following order: 22%—*Enterobacter liqueface*; 15%—*Citrobacter*; 7%—*Shigella*; 7%—*Enterobacteraerogenece*; 4%—*Salmonella*; 4%—*Escherihia*; 4%—*Serracia*. The study of the pathogenic features of bacteria on the culture of Hella cells showed that the cytolysis was positive in all strains of the collection, but for two. In addition, the complete disruption (100%) of susceptible cells was favored by all strains from the *Shigella* and *Hafnia* genera. The strains of the *Citrobacter* and *Salmonella* genera destroyed 75% of the cell culture. According to the results of the study, the highest number of heterotrophic bacteria in BW released into coastal waters of Vladivostok was not more than 106 CFU/mL, which is probably typical of this zone with its climatic and geographic peculiarities. The volume of transport and the

survival rate of bacteria in a new environment can be significant. Thus, the analysis of data from the microbiological study of BW and sediments of 69 ships arriving in the Chesapeake Bay (USA) showed that up to 1018–1019 bacterial cells transported by the ballast survive in the Chesapeake Bay every year (*Drake et al., 2007*).

In our case, the highest epidemiological threat is during the summer traffic of BW. In winter, the number of heterotrophic and, particularly, opportunistic pathogenic bacteria was not high to a large extent. The application of a bacterial filter allowed a more effective isolation from the winter samples. It was able to be lowered via an uncultivated state of the cells or the for mation of biofilms. It is common knowledge that microorganisms have special adaptation skills. High ecological plasticity is typical of them. They remain viable under the influence of many abiotic factors—humidity, temperature, organic composition, pH, and so on (*Somov and Buzoleva, 2004*). In winter, microorganisms, in particular, some enterobacteria and listeria, persistent in an uncultivated form, L_form, and biofilms are able to divide actively and intensify their virulent features under more favorable conditions (*Buzoleva and Terekhova, 2002*). In this connection it is necessary to consider the number of microorganisms in the colder seasons of the year. All in all, gram_negative mobile rod_shaped bacteria with the enzymatic type of metabolism were dominant in BW from the Chinese ports, which allows characterizing their habitats as insufficiently sated with oxygen (which occurs when water is polluted by household wastes) (*Buzoleva et al., 2006*). Low hydrolytic activity and high resistance to antibiotics and metals indicate that the strains are tolerant to highly concentrated pollutants and enable them to exist in polluted coastal waters of Vladivostok for a long time (*Vashchenko, 2000*). Quite evident is a threat of pollution of native marine cenoses by allochthonic bacteria (*Thomson et al., 2003*). As a result, there is a risk that the water areas of ports will be liable to microbiological infection. According to the number and biological features of heterotrophic organisms, BW from the Chinese ports can be characterized as dirty and very dirty. Despite the fact that water from the port of Laizhou was significantly infected by sanitary indicator microorganisms typical of a high level of fecal pollution, BW from the ports of Longkou and the Yangtze River poses the highest infection risk to Amur Bay. The BW samples from the ports of the Yangtze River with a relatively high number of heterotrophic bacteria (from 10^5 to 10^6 CFU/mL) are of special interest. Unlike the water from the ports of Longkou and Laizhou, this water was highly polluted with CGB. The number of CGB was significantly higher than the value accepted by sanitary norms. This fact can be indicative that the strains of

these bacteria survive in BW or invade when the tanker reballasts (additional ballasting while sailing). We consider the first variant to be the most probable. It is common knowledge that reballasting offshore is not a very efficient procedure, because it is impossible to completely remove the sediment in the ballast tanks of ships when reballasting (*Williams et al., 1988*). It is also noteworthy that, among opportunistic pathogenic bacteria, we found bacteria from the *Hafnia* and *Shigella* genera, which were not mentioned in the literature before. They are a considerable epidemiological threat, because they are the agents of enteric infections and pneumonia (*Pozdeev, 2002*). According to data from cytolytic tests, these bacteria are highly pathogenic: the disruption of 100% of cells takes place even under large culture cultivations. Such highly virulent strains are a threat not only to humans but also to marine organisms in planktonic and bottom cenoses.

15. SIN Singapore

Since near Singapore found transportation routes, it is necessary to found stains of remediators. A taxonomic study was carried out on strain A-11-3(T), which was isolated from an oil-enriched consortia from the surface seawater of Hong-Deng dock in the Straits of Malacca and Singapore. Cells were aerobic, Gram-negative, non-spore-forming irregular rods. The strain was catalase- and oxidase-negative. It grew on a restricted spectrum of organic compounds, including some organic acids and alkanes. 16S rRNA gene sequence comparisons showed that strain A-11-3(T) was most closely related to the type strains of Alcanivorax jadensis (96.8 % sequence similarity), Alcanivorax borkumensis (96.8 %), Alcanivorax dieselolei (94.8 %), Alcanivorax venustensis (94.2 %) and Alcanivorax balearicus (94.0 %). The predominant fatty acids were C(16 : 0) (31.2 %), C(18 : 1)omega7c (24.8 %), C(18 : 0) (9.6 %), C(12 : 0) (8.3 %), C(16 : 1)omega7c (8.3 %) and C(16 : 0) 3-OH (5.1 %). The G+C content of the genomic DNA was 54.7 mol%. Moreover, the strain produced lipopeptides as its surface-active compounds. According to physiological and biochemical tests, DNA-DNA hybridization results and sequence comparisons of the 16S-23S internal transcribed spacer, the gyrB gene and the alkane hydroxylase gene alkB1, strain A-11-3(T) was affiliated with the genus Alcanivorax but could be readily distinguished from recognized Alcanivorax species. Therefore strain A-11-3(T) represents a novel species of the genus Alcanivorax for which the name Alcanivorax hongdengensis sp. nov. is proposed. The type strain is A-11-3(T) (=CGMCC 1.7084(T)=LMG 24624(T)=MCCC 1A01496(T))(Wu, Lai, Zhou, Qiao, Liu, Shao,2009).

The next direction is fight with corrosion of ships. Microbiologically influenced corrosion (MIC) of stainless steel 304 by a marine aerobic Pseudomonas bacterium in a seawater-based medium was investigated by atomic force microscopy (AFM) and X-ray photoelectron spectroscopy (XPS). AFM was used to observe in situ the proliferation of a sessile Pseudomonas cell by binary fission. The development of a biofilm on the coupon surface and the extent of corrosion damage beneath the biofilm after various exposure times were also characterized by AFM. Results showed that the biofilm formed on the coupon surface increased in thickness and heterogeneity with time, and thus resulting in the occurrence of extensive micro-pitting corrosion; whilst the depth of pits increased linearly with time. The XPS results confirmed that the colonization of Pseudomonas bacteria on the coupon surface induced subtle changes in the alloy elemental composition in the outermost layer of surface films. The most significant feature resulting from

microbial colonization on the coupon surface was the depletion of iron (Fe) and the enrichment of chromium (Cr) content as compared to a control coupon exposed to the sterile medium, and the enrichment of Cr increased with time. These compositional changes in the main alloying elements may be correlated with the occurrence of extensive micropitting corrosion on the surface(Yuan, Pehkonen,2007).

Nanyang Technological University investigated the aerobic and anoxic biodegradation of four estrogens [estrone (E1), estradiol (E2), estriol (E3), and the synthetic 17 alpha-ethinylestradiol (EE2)] in microcosms constructed with marine sand and ulftrafiltered (UF) secondary effluent. Three estrogen-degrading bacteria, LHJ1, LHJ3, and CYH, were isolated. Based on gram-stain morphology and 16S rRNA sequence homology, LHJ1 and LHJ3 belong to the genus Acinetobacter and Agromyces, respectively; CYH matched to 95% with the genus Sphingomonas. Aerobically LHJ3 degrades E3, CYH degrades E1, and all three isolates oxidize E2 to E1. Under anoxic conditions, CYH degrades E1 and LHJ3 degrades E2, whereas E3 and EE2 were not degraded by the three isolates; EE2 was transformed in microcosms incubated with site ground water. The degradation kinetics of E1 and E2 by CYH and E2 by LHJ3 under aerobic conditions was linearly correlated with the initial concentration, which ranged from 50 to 2,000 microg/l. The degradation of E1 by CYH under anoxic conditions followed Michaelis-Menten kinetics. 16 alpha-Hydroxyestrone was found to be a transient transformation product of E3 under aerobic conditions (Ke, Zhuang, Gin, Reinhard, Hoon, Tay, 2007).

Department of Microbiology, National University of Singapore studys the immobilization and performance of a hydrocarbon-degrading microorganism on polyurethane foam (PUF) in the bioremediation of petroleum hydrocarbons. The ability of four different microorganisms to immobilize on PUF and to degrade various petroleum products (Arabian light crude (ALC), Al-Shaheen crude (ASC), diesel and oil slops) was assessed by measuring the n-alkane fraction remaining in the petroleum products over time. A Rhodococcus sp. (designated as F92) had the highest number of immobilized viable cells (10(9) cells per cm3 PUF) and a maximum attachment efficiency of 90% on PUF of a density of 14 kg/m3. Scanning electron microscopy showed the presence of extracellular structures that could play an important role in the immobilization of F92 on PUF. Analysis by GC-MS revealed that both free and immobilized F92 cells were able to degrade approximately 90% of the total n-alkanes in the petroleum products tested within 1 week at 30 degrees C. Rhodococcus sp. F92 was efficiently immobilized onto PUF and the

immobilized cells were able to degrade a variety of petroleum products such as ALC, ASC, diesel and oil slops. The results suggest the potential of using PUF-immobilized Rhodococcus sp. F92 to bioremediate petroleum hydrocarbons in an open marine environment (Quek, Ting, Tan,2005).

16. THA Thailand

The Gulf of Thailand has been a major marine resource for Thai people for a long time. However, recent industrialization and community development have exerted considerable stress on the marine environments and provoked habitat degradation. Untreated waste water discharged directly and indirectly to the waterways are the most serious problems of the country. Eutrophication of coastal waters is an emerging problem. By contrast, few problems have been documented from trace metals discharged by industries, and public health threat from seafood contamination does not appear to be significant. Oil pollution has not been a problem, although occasional spills from oil tankers have been recorded and fears of a major spill exist. Although many efforts have been undertaken to solve the degradation of marine habitats, problems of habitat degradation are still an important issue to be addressed. The problem is agreeing a sustainable management plan for natural coastal resources conservation and utilization. Thailand has implemented a program on marine pollution control during the past three decades. Such a program includes basically four components i.e., 1. Baseline and monitoring studies, 2. Water quality criteria establishment, 3. Identification of sources, pathways and quantity of pollutants and 4. Pollution control, abatement, rehabilitation. So far Thailand has implemented such a program, but certain components need to be emphasized (Cheevaporn, Menasveta, 2003).

Thus, Kitja Chitpirom with coauthors (2009) isolated highly arsenic resistant bacteria (27 isolates), which had a minimum inhibitory concentrations (MICs) for arsenite and arsenate of $\geq$ 40 mM and > 400 mM, respectively from tannery wastes and agricultural soils collected in Central Thailand. On the basis of the morphological, cultural, physiological and biochemical characteristics, and on the principal ubiquinone component and 16S rRNA gene sequence analyses, they were identified as nine isolates each of Klebsiella (Groups 1 and 8) and Acinetobacter (Groups 2, 3 and 7), four isolates each of Pseudomonas (Groups 4 and 6) and Comamonas (Group 5), and one isolate of Enterobacter (Group 9). From these 27 isolates, only one isolate, A3-3 from the genus Comamonas, appeared potentially capable of oxidizing arsenite to arsenate, as determined by silver nitrate staining of arsenite agar plates after colony growth.

Other researchers (Panwichian et al., 2010) in order to determine whether waters used for the shrimp cultivation contained toxic levels of heavy metals (HMs) and sodium (Na), carried out analysis on 31 shrimp ponds in areas of southern Thailand. Purple nonsulfur bacteria (PNB) were also isolated from the same ponds to investigate if they could be used for bioremediation of the above contaminants. The highest HMs concentrations of the sediment samples in mg/kg dry weight were found as follows: 0.75 cadmium (Cd), 62.63 lead (Pb), 34.60 copper (Cu) and 58.50 zinc (Zn). However, all sediment samples met Hong Kong standards for dredged sediment. In contrast, contamination of Cu (9-30 µg/L) and Zn (140-530 µg/L) exceeding the standard guidelines for marine aquatic animal set by the Pollution Control Department, Thailand, were found in 32 and 61% of water samples, respectively. Two metal resistant PNB isolates, NW16 and KMS24, were selected from the 120 PNB isolates obtained. Both isolates reduced the levels of HMs by up to 39% for Pb, 20% for Cu, 7% for Cd, 5% for Zn and 31% for Na from water that contained the highest levels of HMs found and 3% NaCl when cultured with either microaerobic-light or aerobic-dark conditions. The isolate NW16 removed a greater percentage of the HMs than the isolate KMS24, but the isolate KMS24 was able to survive better under a greater variety of environmental conditions. Both strains were therefore suitable to use for further investigating their abilities to remediate water contaminated with HMs and Na.

17. USA United States

For nearly 100 years, indicator bacteria have been used successfully to monitor the bacteriological quality of water. In recent years, however, it has been realized that counts of indicator bacteria can be influenced markedly by stressors which can injure or kill indicator organisms and thus give low false estimates of their numbers. Injured organisms-those which have been subjected to sublethal stresses so that they will not grow under conditions which support the growth of untreated cells-occur in almost all aquatic environments. Among heavy metals, tin and organotin compounds can inhibit or kill aquatic microorganisms (Hallas, Cooney. 1981), including E. coli. Tin compounds are present in fresh, estuarine, and marine waters, and the use of tin compounds is increasing. Moreover, tins can be methylated in the aquatic environment, which can increase their toxicity after they enter a body of water. It is possible that organotin compounds in aquatic systems can affect the results of standard microbiological assays for water quality. Therefore, Pettibone G. W. and Cooney J. J. the University of Massachusetts at Boston, USA examined the effect of several organotin compounds on the enumeration of fecal coliforms and fecal streptococci by using standard plating media (Pettibone, Cooney, 1986). Methyltin compounds were selected because they can be formed in aquatic systems, and a tributyltin compound was chosen because tributyltins are components of some antifouling paints used on boat hulls and can be found in waters and sediments in areas impacted by human activity. Researchers reported that $(CH_3)_2SnCl_2$ and $(CH_3)_3SnCl$ decreased viable counts at about 10 to 100 mg of Sn liter-' (8.4 x 10-5 to 8.4 x 10-4 mol of Sn liter-'), and tributyltin chloride was effective at about 0.1 to 1.0 mg of Sn liter-' (8.4 x 10-7 to 8.4 x 10-6 mol of Sn liter-'. These concentrations, particularly for the methyltin compounds, are greater than the concentrations reported to date for these compounds in aquatic ecosystems. Thus, organotin compounds alone would not be likely to cause reductions in counts of indicator organisms measured by standard methods. However, it is suggested that, when combined with other environmental stressors or upon long exposure, organotins such as butyltins may contribute to the injury of indicator organisms.

Molecular methods are useful both to monitor natural communities of bacteria, and to track specific bacterial markers in complex environments. Length-heterogeneity polymerase chain reaction (LH-PCR) and terminal restriction fragment length

polymorphism (T-RFLP) of 16S rDNAs discriminate among 16S rRNA genes based on length polymorphisms of their PCR products. With these methods, Field K.G. with coauthors (2003) developed an alternative indicator that distinguishes the source of fecal pollution in water. They amplified 16S rRNA gene fragments from the fecal anaerobic genus *Bacteroides* with specific primers. Because *Bacteroides* normally resides in gut habitats, its presence in water indicates fecal pollution. Molecular detection circumvents the complexities of growing anaerobic bacteria. Researchers identified *Bacteroides* LH-PCR and T-RFLP ribosomal DNA markers unique to either ruminant or human feces. The same unique fecal markers were recovered from polluted natural waters. The unique markers were cloned and sequenced; marker sequences were used to design specific PCR primers that reliably distinguish human from ruminant sources of fecal contamination. This approach is more sensitive than fecal coliform assays, is comparable in complexity to standard food safety and public health diagnostic tests, and lends itself to automation and high-throughput. In other work these scientists (Bernhard A.E., Field K.G., 2000) described a new PCR-based method for distinguishing human and cow fecal contamination in coastal waters without culturing indicator organisms, and showed that the method can be used to track bacterial marker sequences in complex environments. They identified two human-specific genetic markers and five cow-specific genetic markers in fecal samples by amplifying 16S ribosomal DNA (rDNA) fragments from members of the genus *Bifidobacterium* and the *Bacteroides-Prevotella* group and performing length heterogeneity PCR and terminal restriction fragment length polymorphism analyses. Host-specific patterns suggested that there are species composition differences in the *Bifidobacterium* and *Bacteroides-Prevotella* populations of human and cow feces. The patterns were highly reproducible among different hosts belonging to the same species. Additionally, all host-specific genetic markers were detected in water samples collected from areas frequently contaminated with fecal pollution. Ease of detection and longer survival in water made *Bacteroides- Prevotella* indicators better than *Bifidobacterium* indicators. Fecal 16S rDNA sequences corresponding to our *Bacteroides-Prevotella* markers comprised closely related gene clusters, none of which exactly matched previously published *Bacteroides* or *Prevotella* sequences. The described method should be widely applicable for monitoring spatial and temporal fluctuations in specific bacterial groups in natural environments.

Marine mammals are at risk for infection by fecal-associated zoonotic pathogens when they swim and feed in polluted nearshore marine waters. Because of their tendency to consume 25-30% of their body weight per day in coastal filter-feeding invertebrates, southern sea otters (Enhydra lutris nereis) can act as sentinels of marine ecosystem health in California. Feces from domestic and wildlife species were tested to determine prevalence, potential virulence, and diversity of selected opportunistic enteric bacterial pathogens in the Monterey Bay region. We hypothesized that if sea otters are sentinels of coastal health, and fecal pollution flows from land to sea, then sea otters and terrestrial animals might share the same enteric bacterial species and strains. Twenty-eight percent of fecal samples tested during 2007-2010 were positive for one or more potential pathogens. Campylobacter spp. were isolated most frequently, with an overall prevalence of 11%, followed by Vibrio cholerae (9%), Salmonella spp. (6%), V. parahaemolyticus (5%), and V. alginolyticus (3%). Sea otters were found positive for all target bacteria, exhibiting similar prevalences for Campylobacter and Salmonella spp. but greater prevalences for Vibrio spp. when compared to terrestrial animals. Fifteen Salmonella serotypes were detected, 11 of which were isolated from opossums. This is the first report of sea otter infection by S. enterica Heidelberg, a serotype also associated with human clinical disease. Similar strains of S. enterica Typhimurium were identified in otters, opossums, and gulls, suggesting the possibility of land-sea transfer of enteric bacterial pathogens from terrestrial sources to sea otters (Oates, Miller, Byrne, Chouicha, Hardin, Jessup, Dominik, Roug, Schriewer, Jang, Miller , 2012).

In University of Massachusetts studies microorganisms in marine foods.We previously demonstrated the widespread presence of enterotoxigenic *Bacillus cereus* in marine foods. In view of the widespread consumption of raw fish, we sought to determine the ability of this organism to grow on the surface of wild Alaskan salmon at abusive temperatures (12, 16, and 20°C), using an isolate able to produce elevated levels of hemolysin BL enterotoxin and nonhemolytic enterotoxin. An incubation temperature of 37°C for colony formation was found to be selective for B. cereus grown on salmon held for up to 24 h at each temperature. A fivefold increase in log CFU per gram was observed after 26 and 22 h at 16 and 20°C, respectively, while a >4-log CFU/g increase occurred on salmon held at 12°C for 48 h. Generation times of 169.7, 53.5, and 45.6 min were observed at 12, 16, and 20°C. Nonhemolytic enterotoxin was detected when levels of B. cereus were in excess of $10^{(8)}$ CFU/g. Nisin, at concentrations of 1 and 15 m g/g of salmon, reduced levels of B.

cereus 2.5- and 25-fold, respectively. Our results indicate that fresh salmon can serve as an excellent substrate for enterotoxigenic B. cereus and that this organism can reach levels associated with foodborne illness following moderate temperature abuse(Labbé, Rahmati ,2012)

18. VN Viet Nam

Petroleum-based products are the major source of energy for industry and daily life. Petroleum is also the raw material for many chemical products such as plastics, paints, and cosmetics. The petroleum transport across the world is frequent, and the amounts of petroleum stocks in developed countries are enormous. Consequently, the potential for oil spills is significant, and research on the fate of petroleum in a marine environment is important to evaluate the environmental threat of oil spills, and to develop biotechnology to scope with them since half of world oil production is transported by sea (Shigeaki et al. 1999).

The Interdepartmental Collaborative Research Program has been carried out since 1995 with the main objective of collecting and analyzing data and necessary information for the management of fishery resources and the protection of the environment through collaborative research member countries and organization concerned. With the agreement of Vietnamese Government, the Collaborative Research Program in Vietnamese waters, as area IV, has been carried out from 29th April to 30th May 1999. The project of researches from Institute of Biotechnology, Hanoi, Vietnam (Dang et al., 1999) in the program is to conduct petroleum hydrocarbon pollution study with the following objective:

· Detection of total petroleum hydrocarbon level.

· Investigation of number of HDM.

· Study of biodegradative capacity of isolated hydrocarbon-degrading microbial communities in laboratory condition.

After finishing the cruise, 97 samples have been collected that including 39 sediment samples and 58 surface water samples. In general, the sediment samples can be divided into four groups such as mud, sandy mud, muddy sands, and sand.

Total hydrocarbon concentration. Only 11 samples from 58 research stations were investigated. Among them only in two stations oil concentration 0.095 mg/L and 0.017 mg/ L respectively was detected. In other research stations the oil concentration was under detected level of IRS detector (< 0.01 mg/L).

These results indicated that in these samples no oil contamination was observed. This data can not be used for the conclusion of oil pollution level in seawater because of several technical reasons that concerning to the method used for these chemical analysis.

Microbial investigation. According to obtained results HDM number is in the range from 10^1 to 10^5 cell/ml or cell/ g of sample. Number of microorganisms in sediment samples was normally higher than in surface water samples. Number of HDM in the surface water samples was higher than in the sediment samples, it was found only in two stations. There are 12 stations in which the number of hydrocarbondegraders was equal in both sediment and surface water samples. In 18 samples (6 from surface and 12 from sediment) number of HDM was 10^5 cell/ml, in 22 samples (8 from surface and 14 from sediment) bacterial number was 10^4 cell/ml, in 32 samples (23 from surface and 9 from sediment) number of HDM was 10^3 cell/ml and in the last 23 sample (19 from surface and 4 from sediment) number of HDM 10^2 cell/ml was detected only. According to colony morphology of all isolated bacteria, they can be belonged to about 20 groups.

According to the number of HDM (indirect indication), oil contamination was not detected in 30 stations, and light oil contamination was detected in other 28 stations. In almost stations in the South Sea light oil contamination was observed.

Microbial community of hydrocarbon- degraders in different research stations showed different oil degradative capacity. The strongest community in diesel oil degradation was found in two stations, about 94 % oil was reduced during 7 day shaking cultivation. Surfactant producing bacteria were isolated from 8 stations. Isolated bacteria and their products play an important role in the process of cleaning up oil contamination (Oberbremer A. et al., 1990).

Study of polycyclic aromatic hydrocarbon degradative capacity by purified culture was also carried out by Vietnamese scientists. The result showed that some bacterial strains isolated from different stations degraded rapidly phenanthrene. For example, after 4 day cultivation, strain I-572 that isolated from sediment could degrade 99 % of added phenanthrene in MSM.

Enumeration of hydrocarbon-degrading microorganisms (HDM) and their degradative capacity studies were carried out in area IV of South China Sea (Vietnamese water). Microbial most probable number (MPN) varied from 10^1 to 10^5 cell/ ml of surface seawater or gram of sediment. Some microbial communities and bacterial strains isolated from 97 collected samples show high hydrocarbon degradation and surfactant producing abilities. Preliminary results of our microbial study indicated that oil pollution in IV area

was not found in 30 research stations. Slight oil contamination was observed in 28 survey stations.

19. Cambodia

Japanese Center for Marine Environmental Studies (CMES) make research on the terriitory of Cambodia.They studies influence tributylin on the resistance on this substances to geterotrofic microorganisms. Tributyltin (TBT) is organotin compound that is toxic to aquatic life ranging from bacteria to mammals. This study examined the concentration of TBT in sediment from and near the Mekong River and the distribution of TBT-resistant bacteria. TBT concentrations ranged from <2.4 to 2.4 ng/g (dry wt) in river sediment and <2.4-15 ng g(-1) (dry wt) in harbor sediment. Viable count of total bacteria ranged from 2.0 x 10(4) to 1.4 x 10(7)cfu/g, and counts of TBT-resistant bacteria ranged <1.0 x 10(2) to 2.5 x 10(4)cfu/g. The estimated occurrence rate of TBT-resistant bacteria ranged from <0.01 to 34% and was highest in upstream sites in Cambodia. The occurrences of TBT in the sediment and of TBT-resistant bacteria were unrelated, and chemicals other than TBT might induce TBT resistance. TBT-resistant bacteria were more abundant in the dry season than in the rainy season. Differences in the selection process of TBT-resistant bacteria between dry and rainy seasons were examined using an advection-diffusion model of a suspended solid (SS) that conveys chemicals. The estimated dilution-diffusion time over a distance of 120 km downstream from a release site was 20 days during dry season and 5 days during rainy season, suggesting that bacteria at the sediment surface could be exposed to SS for longer periods during dry season. (Suehiro, Mochizuki, Nakamura, Iwata, Kobayashi, Tanabe, Fujimori, Nishimura, Tuyen, Tana, Suzuki,2007).

20. Eucador

In 1977 a group of geologists, geochemists, and arrived at the Galapagos Rift, located west of Ecuador, the first time to explore the active hydrothermal outputs in marine glubinas..On the depth of about 2.5 km, the rifts - the representatives of the specific type of Pogonophora, up to 1.5 m thick with an adult's hand person), giant clams Galapagos Rift - is the boundary between the individual plates, the crust on the bottom of the ocean, which from west to east, spewing lava tear cracks. Cold sea water penetrates into the cracks and become hot, highlights some elements, in return for others - such as magnesium and silicon - from rocks of the crust. Going through the cracks, hot water is poured along the sea bottom, and metal oxides are deposited.

Under the influence of extreme temperatures and pressures of sulfate in sea water turns into hydrogen sulfide. In the fragrant compound, as explained later, microbiologists, contains the secret underwater life in the oases. Certain bacteria recycle hydrogen sulfide and multiply. These microorganisms, in turn, serve as food for giant clams, which reach the size of soup plates. Thus, in the dark energy source is not sunlight, and chemicals produced from the earth's crust of the ocean floor. It is they who set in motion the chain of life. This process is called chemosynthesis, first discovered in the depths of the ocean (National Geographic).

21. Panama

The coastal waters of Panama have municipal and domestic pollution.That way one of the direction of marine microbiology is sanitary and microbial control for water and aquatic microorganisms. The first confirmed case of Vibrio parahaemolyticus gastroenteritis in Panama is reported. This marine organism, causing food poisoning in some countries and isolated only recently from seawater in Panama, was recovered from the stools of a patient with acute gastroenteritis, as well as from fresh shrimp used in preparing seafood dishes. This person and 39 other individuals became acutely ill a few hours after eating shrimp at the same restaurant (Montgomery, Lee, Charity, Riveiro, Peralta,1984).

Conclusion

The marine pollution problem is still actual in all   economics of the Asian-Pacific region, including economies with developed economies. The main pollutants are heavy metals, oil and fecal contamination.

Oil pollution is a result of accidents during transportation of oil and its production. In the Pacific Ocean are the major transportation routes. As a result of accidents suffered to a greater extent in Hong Kong, the Philippines, Malaysia and Japan. Oil spills resulting from accidents cause enormous economic damage and disrupt the ecological conditions of existence.   Only during 16 years in this region occurred following a major accident:

1. Singapore in 1995. As a result of collision of the tanker "Evoikos" a huge amount of oil spilled. Formed the largest oil spill in the history of Singapore.
2. January 2, 1997 Tanker "Nakhodka" went to Kamchatka with a cargo of 19.000 tons of heavy oil. The rear of the building "Findings" sank in deep water area (2,500 m), and the front drifted to the west coast of Japan, causing the pollution. 6400 kiloliters of oil spilled. It was contaminated with 200 km. coast
3. October 1997 . The tanker "Pacific Tower" collided with another vessel. 15 October 1997 The EVOIKOS spilled some 28,500 tonnes of heavy marine fuel oil and polluted a large portion of Singapore port waters.
4. 3 October 2000 The 51,096 gross ton Panamanian tanker NATUNA SEA carrying some 70,000 tonnes of Nile Blend crude, was en-route from Middle East to China. The tanker went aground at the Batu Berhanti Reef about 7,000 tonnes of oil escaped into the sea.

As for oil, you can remember recent events on the 20th of April, 2010, an explosion aboard the Deepwater Horizon in the Gulf of Mexico spewed millions of gallons into the water.

Before chemical suppression on petroleum pollution left part of the long-term alkanes stays in the environment for a long time and causes toxicity hazards to the marine life and environment. Generally, ultimate and complete degradation is accomplished mainly by bio-degradation. Microbial degradation is considered to be the best method for the breakdown of hydrocarbons.

Different kind of bacterias can break down oil to carbon dioxide and water. Certain microbes highly prefer oil hydrocarbons over other energy sources and their numbers will increase faster than others in the community in response to an oil spill. Some marine bacterias degradate petroleum hydrocarbons have been isolated, which include the genera *Pseudomonas*, *Cycloclasticus* (Dyksterhouse et al., 1995)*, Alcanivorax* (Yakimov et al., 1998), *Neptunomonas* (Hedlund et al., 1999), Oleiphilus (Golyshin et al., 2002), *Oleispira* (Yakimov et al., 2003) etc.

Bioremediation is one of the best solutions to environmental problems. Interest in the oil of bacteria is growing. Over the past five years, the biologically active strains of bacteria that are isolated and should be useful for the economies of the Asia-Pacific region.

Oil oxidizing microorganisms isolated at research institutions the economies of Asian-Pacific region

Economies	Research institution	The study area	Species of microorganism
China	Second Military Medical University and Naval Medical Research Institute Shanghai Laboratory of Marine Biogenetic Resources, Third Institution of Oceanography State Oceanic Administration	(crude oil) Dinghai Lau basin in Southwest Pacific Ocean	*Acinetobacter venetianus* *Novosphingobium indicum*
Japan	Biological Resources and Functious, National institute of Advanced Industrial Science and Technology, Tsukuba Department of Biotechnology, National Institute of Technology and Evaluation	In sea water after the Nakhodka tanker oil spill coastal waters of the Sea of Japan	*Cycloclasticus pugetii* *Alcanivorax borkumensis* *Alcanivorax, Cycloclasticus*
Vietnam	Institute of	South China Sea	Enumeration of hydrocarbon-

	Biotechnology, Hanoi	(Vietnamese water)	degrading microorganisms (HDM) - 26 isolates
Korea		west coast of Korea	*Muricauda aquimarina* SW-63T
Singapore	Department of Microbiology, National University of Singapore Nanyang Technological University	Since near found transportation routes	*Alcanivorax jadensis Rhodococcus sp* *Acinetobacter Agromyces Sphingomonas*
Russia	Far East		

investigated the aerobic and anoxic biodegradation of four estrogens [estrone (E1), estradiol (E2), estriol (E3), and the synthetic 17 alpha-ethinylestradiol (EE2)] in microcosms constructed with marine sand and ulftrafiltered (UF) secondary effluent. Three estrogen-degrading bacteria, LHJ1, LHJ3, and CYH, were isolated. Based on gram-stain morphology and 16S rRNA sequence homology, LHJ1 and LHJ3 belong to the genus and, respectively; CYH matched to 95% with the genus. Aerobically LHJ3 degrades E3, CYH degrades E1, and all three isolates oxidize E2 to E1. Under anoxic conditions, CYH degrades E1 and LHJ3 degrades E2, whereas E3 and EE2 were not degraded by the three isolates; EE2 was transformed in microcosms incubated with site ground water. The degradation kinetics of E1 and E2 by CYH and E2 by LHJ3 under aerobic conditions was linearly correlated with the initial concentration, which ranged from 50 to 2,000 microg/l. The degradation of E1 by CYH under anoxic conditions followed Michaelis-Menten kinetics. 16 alpha-Hydroxyestrone was found to be a transient transformation product of E3 under aerobic conditions (Ke, Zhuang, Gin, Reinhard, Hoon, Tay, 2007).

In contrast to earlier conducted studies now of microbial communities in oil-polluted sea water is estimated on the molecular level, using the developed oligonucleotide zondes.

In economies of Asian-Pacific region a large part of the nutritional makes up seafood, which is used in its raw form. Water pollution affects on aquatic resources adversely. This

situation has forced many economies to do aquaferms for breeding and cultivation of aquatic organisms under controlled conditions. The modern mariculture valuable aquatic food is a rapidly developing branch of science and technology.

China and Japan are leaders in breeding of mariculture.In unhappiness, it is very difficult to content mariculture in the clean conditions. Waste from industrial production is one of the sources of water pollution by heavy metals, sewage. These problems for the Asia-Pacific region is also crucial, because die or become unfit for aquatic food and mariculture. There is damage to public health and huge economic losses

The actual problem is outbreaks of diseases caused by pathogenic bacteria, Vibrio, Salmonella, and others in Japan, Thailand, China, Malaysia , the Philippines. Elevated levels of fecal Escherichia be found near human settlements. Some pathogenic bacteria have been reported time, which leads to the closure of beaches. Chronic problems in public health, and there are outbreaks of hepatitis A in Hong Kong (and China) has been associated with the consumption of shellfish.

The monitoring of organic and faecal contamination of coastal sea waters is important part of environmental condition control.

Economies	Research institution	The study area	Species of microorganism
Malaysia	School of Biological Sciences, Science University of Malaysia, Penang 2003	coastline of Penang Island	*coliform and Escherichia col*
		Straits of Malacca	*Coliform 10^4 -10^5*
Philippines	E.D. Gomez et al.:State of the marine environment in the East Asian Seas Region UNEP Regional seas Reports and Studies №126	Manila Bay	*Coliform 10^4 -10^5*

Russia	Research Institute of Microbiology and Epidemiology, Siberian Branch, Russian Academy of Medical Sciences (2002 -2011)	of coastal sea waters of Primorsky Krai shellfish	*Yersinia pseudotuberculosis* *Salmonella* *Shigella* *Klebsiella* *E.coli* *Listeria monocytogenes*
Thailand	E.D. Gomez et al.:State of the marine environment in the East Asian Seas Region UNEP Regional seas Reports and Studies №126	Shellfish Gulf of Thailand	*Klebsiella Enterobacter* *Vibrio parahaemolyticus, V. cholerae* *Shigella boydii S. flexneri,* *E.coli*
Japan	Deparotmenit of Food Microbiology an,d Hvgiene' anid Depar-tmenit oJ Aquatic En ironinmental Biology,- Faculty of Applied Biological Science, Hiroshimat University (1989-2006)	Seto Inland Sea of coastal sea waters of Fukuyama	*Listonella, Clostridiium botulinlutm* *Vibrio cholerae* *Salmonella* *Vibrio cholerae non-O1*
Hong Kong	E.D. Gomez et al.:State of the marine environment in the East Asian Seas Region UNEP Regional seas Reports and	Shellfish Tolo Harbour , Victoria	*Hepatitis* *Coliform 10^5 -10^6*

	Studies №126	Harbour	
China	E.D. Gomez et al.:State of the marine environment in the East Asian Seas Region UNEP Regional seas Reports and Studies №126	shellfish	Hepatitis *Salmonella* *E.coli*
Indonesia	E.D. Gomez et al.:State of the marine environment in the East Asian Seas Region UNEP Regional seas Reports and Studies №126	Jakarta Bay	*Salmonella* *Shigella* *Staphylococcus* *E.coli*
USA	University of Massachusetts	in marine foods	*Bacillus cereus*
Canada	Fisheries & Oceans Canada, Pacific Biological Station	salmons	Viral hemorrhagic septicemia virus (VHSV
Panama		shrimp , seawater	*Vibrio parahaemolyticus*

As pathogenic bacterias as mariculture polluted by heavy metal. For example, arsenic was found in salmon and shrimp in Malaysia, in Thailand.

Industry growth in China, Thailand, Japan is caused the contamination their rivers and coast of the seas .

Scientists from different economies are working on problems of environmental pollution by heavy metals. In investigations they pay much attention to biosorption of heavy metals. As a biosorbent used in China the yeasts *Saccharomyces. cerevisiae* for the sorption of uranium and lead (Jianlong Wanga with coauthors from Tsinghua University, State Key

Joint Laboratory of Environment Simulation and Pollution Control, Tsinghua University, Beijing), in central Thailand - Klebsiella, Acinetobacter, Pseudomonas Comamonas, Enterobacter for the sorption of arsenic, as well as nonsulfur purple bacteria Rhodobium Marinum NW16 и Rhodobacter Sphaeroides KMS24 for the sorption of Cd, Zn, Pb, Cu(Department of Microbiology Faculty of Science and   National Center of Excellence for Environmental and   Hazardous Waste Management-Satellite Center   Prince of Songkla University)

Chronic pollution of water bodies leads to eutrophication. Red tides have increased in their numbers in coastal areas of China, particularly in Bohai Bay off eastern China, the East China Sea and the South China Sea (June 2004 2007)., near the coast of Korea, Gulf of Thailand. Eutrophication has become the most serious environmental problem along with red tides (harmful algal blooms), species loss, and contamination with toxic chemicals and harmful microbes.

In order to prevent loss of water is necessary to conduct monitoring studies. The scientists from Chinese University showed that anammox bacteria as the test on organic pollution. Korean research offer original methods for the determination of organic contaminants research using concentration of microcystins in water. Microcystins are hepatotoxins which produce by cyanobacterias. In 2003 the American researchers Field K.G. with coauthors offered a method of indication of faecal pollution using specific primers for determine anaerobic genus Bacteroides . This experience has been successfully applied researcher from Hong Kong University of Science and Technology in 2010 year.

At present time bioremediation is one of the perspective solutions of ecological problems. Interest to petroleum oxidizing bacteria steadily grows. Biologically active bacteria strains are isolated and supposed to be useful for economies of Asian Pacific Region. All economies in the region are now taking measures to protect the marine environment. .Measures include pollution control at source, environmental impact studies, national and regional legislation to prevent and respond to oil spills, and adherence to international conventions on the protection of the marine environment. More and more economies try to prevent it, because of cleaning from pollutants is very expensive . In this connection the permanent collaboration of Asian Pacific Region's economices and exchange of experience and information are necessary to achieve positive results in solvation of topical ecological and economical problems.

How did microbiology change in the next few years?

1. We are actively searching new strains, including the producers of biologically active substances. It creates a collection of strains that can be claimed for industrial production.

2. Microbial communities and relationship between them studies. Using these knowledge, it is conducted the search of substances with anti-corrosion properties.

3. Mikroorganisms are one of the main tools for displaying and remediation of contaminated marine environment.

4. Diagnosis of diseases of marine animals and their treatment with probiotics improved for the development of mariculture farms.

5. Methods of indication and identification of marine microorganisms improve. Molecular genetic methods used widely.

6..Sanitary and microbiological investigations of coastal waters are a reliable measure of prevention of infectious diseases.

It is impossible to say that the marine microbiology developed to the same extent in all economies of Asia-Pacific region. Certainly, leaders in almost all areas of research are the U.S., Canada, Japan, Korea and Russia. In some economies have been developed only to the areas of microbiology, which are urgently needed. So, in Singapore the coastal zone which suffers from frequent spills, mostly developing direction is remediation of the marine environment. In China, Malaysia, Thailand, where mariculture develops, also looks for sought strains that can clean up the environment from heavy metals. Acute illness is a topic of aquatic organisms and fish. This is a problem for Peru also. In Thailand and Korea, where suffering from red tide, focusing on indication of eutrophic pollution. In addition, much attention gives to microorganisms as producers biological active substances. Of particular concern is the contamination of the coastal area of the industrial cities of China, Russia, Vietnam, Thailand and Hong Kong.

Of course, this review does not purport to address information in the field of marine microbiology in the Asian-Pacific economies. In the available literature it was found little information about such economies as Colombia, Chile, New Zealand, Singapore, Mexico,

Philippines, Australia and Indonesia. And almost there isn᾽t data on the development of marine microbiology in Cambodia, Macau, Salvador, Nicaragua.

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The Workshop Agenda   **Annex 1**

12, July, 2012

10.00– 10:30	**Registration**
10:30 – 11:00	**Welcome Remarks** Moderator – **Ms. Anastasia Filichkina, Borlas Security Systems (BSS), Business Development Director** Welcome Remarks – **Ms. Irina Vasilkova, Vice-Governor of Primorsky Region;** Welcome Remarks – **Mr. Vyacheslav Bairamov, Head of Division Of Life Sience of the Ministry of Education and Science of the Russian Federation, Russia;** Welcome Remarks – **Professor Lubov Buzoleva (School of Natural Science, Far Eastern Federal University, Head of Laboratory of Pathogenic bacteria ecology, Institute of Epidemiology and Microbiology, Siberian Branch of Russian Academy of Medicine), Russia;** "APEC Life Science Innovation Forum – Necessity of Environment Protection and Related Activities" - **Mr. Anastasia Filichkina, Project Leader, BSS, Russia.** GROUP PHOTO
11:00 – 11:15	**Coffee Break**
11:15 - 12:30	**International Collaborations in Area of Marine Microbiology Asia Pacific Region** Moderator **- Ms. Anastasia Filichkina, BSS, Russia** 1. "Taxonomy, Biotechnological Potential and Ecological Properties of Obligately Marine Heterotrophic Bacteria"- **Mr**. **Valeryi Mikhailov, Professor of Pacific Ocean Institute Bioorganic Chemistry of Far Eastern Branch of Russian Academy of Science, Russia;** 2. "Bio Prospecting of Marine Microbial Symbioses of Reef Invertebrates: Exploitation of Under-Explored Marine Microorganisms" – **Mr. Ocky Karna Radjasa, Ph.D, Professor of Marine Microbiology Department, Marine**

	Science Diponegoro University, Semarang, Indonesia, Russia; 3. "Microbiological researches in Maritime division of Russian-Vietnamese Tropical Center"- **Uliana Kharchenko, Ph.D. Institute of Chemistry FEB RAS, Beleneva I.A., Ph.D. A.V.Zhirmunsky Institute of Marine Biology FEB RAS, Karpov V.A., Ph.D. A.N.Severtzov Institute of Ecology and Evolution RAS, Russia;** 4. "Marine Microorganism Biodiversity Around Srichang Island, Thailand" **– Dr. Suthep Thaniyavarn, Head Dept. of Microbiology, Chulalongkorn University, Thailand.** Q&A SESSION
12:30 – 13:30	**Luncheon**
13:30 – 14.30	**Microbiological Evaluation of Conditions of Sea Environment and it's Influence on Marine Microorganism - II** Moderator - **Ms. Anastasia Filichkina, BSS, Russia** 5. "New Method for Improving Cultivability of Bacteria from Natural Environment" **– Mr. Tae Seok AHN, Department, of Environmental Science, Kangwon National University, Korea**; 6. Water purity monitoring of the Sea of Okhotsk and the Sea of Japan as the providers of bio resources for Asian Pacific Region countries" - **Ms**. **Elena Bogatyrenko, Research Scientists, Laboratory of Marine Microbiology Far Eastern Federal University, Russia;** 7. "The Influence of Anthropogenic Pollution on Pathogenic Properties Forming of Marine Microorganisms" – **Ms. Alexandra Dvornik, Research Scientists School Natural Science of Far Eastern Federal University, Russia;** Q&A SESSION
14:30 – 14:45	**Coffee Break**
14:45 – 15:45	**Sea Environment in Asia Pacific - II** Moderator - **Ms. Anastasia Filichkina BSS, Russia** 1. "Microbial infections in marine fishes from Perú" – **Dr.**

	Enrique C. Mateo Scientist Researcher Peruvian Marine Research Institute (IMARPE), Peru; 2. "Microbial diseases in trout culture and their impact on productivity in Peru" - **M.V. Veronica A. Sierralta, Scientist Researcher Peruvian Marine Research Institute (IMARPE), Peru.** 3. "Research on marine microorganism by Research Institute for Marine Fisheries and allied institutes" **– Dr. Nguyen Van Nguyen, PhD, Deputy Director, Research Institute for Marine Fisheries, Viet Nam;** Q&A SESSION

12:30– 13:30	**Luncheon**

14, July, 2012

10.00	**Visit to the Far Eastern Federal University**
12.00	**Visit to Institute of Epidemiology and Microbiology (Russian Academy of Medical Science)**
14.00	**Closing Remarks**

The Workshop Recommendations   Annex 2

1.   To join efforts of APEC economies and all the interested parties – United Nations, Business companies and Associations for creating a foundation to support scientific researches in the field of marine microorganisms monitoring and utilization;

2.   To study more carefully bacteria acting as bioremediation of ecocides;

3.   To create a common database of marine microorganisms of Asia-Pacific region, to provide the Internet support of database and free accept for experts, scientists, representatives of related business and institutions and all the interested parties;

4.   To continue projects in APEC in Life Science Innovation Forum, dedicated to research, monitoring and use of Ocean and its potential for mutual benefits of all the APEC economies: to share experience, to hold workshops, seminars, conferences and trainings in this field, to involve business companies for commercialization of scientific researches results;

5.   To implement projects in collaboration network with other related APEC – Ocean and Fisheries Working Group, Health Working Group, APEC Industrial Science and Technology Working Group, as well as with relevant international organizations (like biodiversity and biotechnology working groups) and leading scientific institutions;

6.   To provide in the frames of APEC capacity building of collaborative scientific work in APEC economies;

7.   Exchange of students for training in the Asia-Pacific countries and share their guidance on qualification work will develop a close relationship and fruitful cooperation;

8.   Standardize methods in monitoring studies for the exchange of information with colleagues of different economies and comparative studies in order to predict environmental hazards in the region.