Session VI. Environmental microbiology

VI.O.1

Role of microorganisms in bioremediation of oil polluted soils

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The comparative analysis of microbial communities of pure and oil – polluted soils of Ukraine conducted by us has shown the considerable predominance in the latter the representatives of *Rhodococcus* and *Gordonia* genera. Among them the most part of strains belonged to the species of *Rhodococcus erythropolis* and *Gordonia rubripertincta. Rhodococcus fascians*, *Rhodococcus ruber* and *Dietzia maris* species were also characterized by active hydrocarbon – oxidizing properties. Among isolated and collection strains of hydrocarbon-oxidizing bacteria we selected the most active strains, which belong to *Rhodococcus erythropolis*, *Gordonia rubripertincta* and *Acinetobacter calcoaceticus* species. They don't possess pathogenecity and phytotoxicity, that permits to use them in natural conditions. It was shown by chromato-mass-spectrometry method, that the selected strains were capable to utilize almost all oil components.

In *Gordonia rubripertincta*, which forms only S-variants of colonies, cells hydrophobicity was increasing in 3.9 times. In contrast to *Rhodococcus* and *Gordonia*, *Acinetobacter calcoaceticus* cells possessed high initial hydrophobicity index – 99%, which during the growth process on hydrocarbons was reducing to 70%.

Cell suspensions of *Rhodococcus erythropolis* and *Gordonia rubripertincta* have the high (51–54%) of emulsification index. And supernatants of their cultural broth have the low index of emulsification. It testifies the fact that they produce associated with cells emulgators.

Presence of small quantities of biosurfactants in supernatant is explained by lipid nature of these substances. They are easily dissolved in hydrocarbons and can be extracted by them from cells.

During of these strains growing process on the hydrocarbons the surface tension in the cultivation medium was slightly reduced (from 70 to 46–50 milliNewton per meter). The investigated strains differed between themselves by the type of emulsion they formed. *Rhodococcus* and *Gordonia* strains formed the stable emulsion "oil in water", and *Acinetobacter* – "water in oil".

Study of chemical nature of tested strains biosurfactants has shown, that in *Actinobacteria* they are presented by glycolipids, identificated as trehalose monomycolate and trehalose dimycolate. They are the main glycolipid components of their walls cells. Carbohydrate and protein components prevail over in the composition of extracellular biosurfactants of *Acinetobacter calcoaceticus* strain. These biosurfactants contain small lipids quantity. As to the structure, these substances are close to "emulsans", which are typical for this species. They represent heteropolysaccaride, containing fatty acids and noncovalently bound with them proteins.

On the base of investigated strains we have created biopreparations RODOIL and EKOLAN for purification of soil and water from oil and oil products. The preparations include hydrocarbon-oxidizing bacteria which are immobilized on oil absorbed sorbents and mineral fertilizers.

Keywords: oil polluted soil, *Rhodococcus*, *Gordonia*, biosurfactants, biopreparation

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VI.O.2

Biological diversity of the Black Sea deepwater soil and surface water bacteria

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The Black Sea microbiota is the foundation and integral part of ecosystem and plays key role in biochemical processes occurring there. It functions as "immune system" and forms conditions for other organisms' existence. Last decades are characterized by increasing interest to World Ocean microbiology investigation, however yet little is known about the Black Sea bottom and surface water inhabitants.

The aim of research was investigation of Black Sea microbiota's biodiversity of hydrogen sulphide bathyal deepwater bottom sediments and seawater using metagenomic analysis, molecular-genetic, molecular-biological methods.

First, typical for surface water aerobic microorganisms were detected in Black Sea hydrogen sulphide bathyal deepwater soil samples in amount up to 10^4 CFU. Fatty acids profile and PCR analyses identified studied strains as: *B. cereus, B. pumilis, B. megaterium, B. licheniformis, B. subtilis, B. atrophaeus, B. mycoides, B. viscosus, B. thuringiensis, B. thuringiensis israelensis, Paenibacillus macerans, Paenibacillus polymixa, Paenibacillus alvei, Brevibacillus choshinensis, Brevibacillus parabrevis, Lysinibacillus sphaericus, Virgibacillus pantothenticus.* The question appears: are these microorganisms resident bathyal deepwater soils inhabitants or they come from surface water falling into dormancy and thus are saved? The marine soil samples PCR analysis revealed unique specific nucleotide sequences of anammox bacteria: *Candidatus Brocadia, Candidatus Kuenenia*, *Scalidua wagneri* and *Scalidua sorokinii.*

Metagenomic analysis of Odesa coast seawater revealed the presence of 1006 operational taxonomic units (OTU) from 8 main phyla of Bacteria Domain: Proteobacteria 408 OTU, (40.4)%, Cyanobacteria 185 OTU (18.3%), Bacteroidetes 82 OTU (8.1%), Actinobacteria 54 OTU (5.3%), Verrucomicrobia 43 OTU (4.2%), Firmicutes 19 OTU (1.9%), Planctomycetes 7 OTU (0.7%), Acidobacteria 2 OTU (0.2%) and unassigned 206 OTU (20.4%). The correlation between functional gene clusters responsible for biological processes, molecular-biological functions and cell component synthesis was established.

The carried research improved knowledge about Black Sea prokaryotic biodiversity in water and bathyal deepwater soil, showed that its ecosystem is inexhaustible source of active microorganisms available for biotechnology investigations.

Key words: Black Sea, bathyal, plankton, microbiota, biological diversity, 16S rRNA analysis, metagenomics

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VI.O.3

Integrons in the marine environment

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Nowadays, antibiotic resistant bacteria and antibiotic resistance genes are considered as novel emerging pollutants in the environment and wastewater treatment plants are regarded as key players in global resistance gene dissemination. In this process mobile genetic elements play the crucial role. Among them, integrons are suspected to be the most important since they are often associated with other mobile genetic elements, such as plasmids or transposons, and are detected in various environments and matrices.
Our study site – highly anthropogenically impacted Gulf of Gdansk and its shallow western branch – Puck Bay due to limited water exchange between these water bodies and open Baltic Sea is ideal region to compare the distribution of antibiotic resistance patterns and integrons among bacteria from wastewater and marine waters.
For this purpose we isolated bacteria associated with the human intestine from two local wastewater treatment plants (Gdańsk-Wschód and Gdynia-Dębogórze) and their receiving waters: Gulf of Gdańsk (southern Baltic Sea). Bacteria were isolated according to the procedure dedicated for fecal coliforms. In all samples *Escherichia coli* was a dominant species. However, members of other genera (*Aeromonas*, *Citrobacter*, *Enterobacter*, *Klebsiella*, *Shigella*, *Plesiomonas* and *Vibrio*) were also isolated. All isolates were biochemically identified and their drug susceptibility was determined. *Escherichia coli* isolates resistant to at least one antibiotic were tested for prevalence of integrons and antibiotic resistance genes using PCR. The presence of integrons was associated with increased frequency of resistance to fluoroquinolones, trimethoprim/sulfamethoxazole, amoxicillin/clavulanate, piperacillin/tazobactam, and presence of multidrug-resistance phenotype. In conclusion, human-associated bacteria, even potential pathogens and bacteria carrying antibiotic resistance genes of clinical significance can survive in wastewater and marine water conditions. These findings highlight that further studies are needed to understand the dissemination, stability and transmission of resistance genes in marine ecosystem. Mobile genetic elements play crucial role in spreading antimicrobial-resistance genes.

Key words: antibiotic resistance, integrons, marine environment

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VI.O.4

Confronting the challenges of the brackish water environment – bacterial adaptations to osmotic stress analyzed by OMIC approaches

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The Baltic Sea is one of the world's largest brackish water environments with dynamic, regional shifts in salinity, mainly due to varying freshwater inflows. Unicellular organisms are particularly affected by these challenging conditions. Thus, the aim of this study was to identify physiological and molecular strategies employed by bacteria to confront the salinity shifts characteristic of the Baltic Sea (from 2‰, through 7‰ to 20‰).

Three bacterial Baltic Sea representatives, identified as *Shewanella baltica*, *Flavobacterium* sp. and *Paracoccus* sp. on the basis of 16S rRNA sequencing and MALDI-TOF analyses, were cultured under controlled growth conditions in a chemostat system. The complete genome sequence of *S. baltica* is available, while for the other two species we carried out genome-wide *de novo* sequencing and genome assembly using Illumina HiSeq 2000 and SOAPdenovo2 platforms, respectively. To analyze the dynamics of the bacterial proteomes in response to osmotic challenges, we employed a combined approach of two-dimensional electrophoresis, multiplex fluorescent staining of proteins in gels and LC-ESI-MS/MS and/or MALDI-TOF/TOF-driven protein identifications.

This analysis allowed the identification of a subset of proteins, both of the general stress response group and of other functional categories, differentially expressed under osmotic challenges tested. Three bacterial strains differed in their adaptations to osmotic stress: *Paracoccus* sp. required a significantly less number of proteins to survive in the stress conditions in comparison to *Shewanella baltica* and *Flavobacterium* sp.

The study presented here provides a characterization of highly regulated protein inventories in the marine bacteria studied. The identification of a large number of novel candidates for stress response players lays a groundwork for deciphering the complexity of microbial adaptive processes in the specific ecosystem of the Baltic Sea.

Key words: marine bacteria, genomics, proteomics, stress response, osmotic stress, Baltic Sea

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VI.O.5

Comparative transcriptome analysis of wood decay fungus *Cerrena unicolor*

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Cerrena unicolor belongs to the group of white-rot fungi and is growing mainly on live or dead logs of many hardwood trees: *Fraxinus excelsior*, *Betula sp.*, *Salix sp.*, *Acer sp.*, *Malus sylvestris*, *Sorbus sp.*, *Aesculus hippocastanum*. To elucidate the number of genes involved in lignocellulosic cell wall attack as well as their regulation, the white-rot fungus *Cerrena unicolor* FCL139 growing on birch-tree, ash, maple and control liquid nutrient medium containing glucose was submitted for whole transcriptomes analysis using next generation sequencing (NGS) approach on Solid™ platform. Two-hundred-ninety-four million of forward reads were obtained in total and for each culture variant to at least 47% of these reads were mapped to 12966 gene model from *C. unicolor*. Amongst detected gene transcripts 1827 were at least four times more abundant during growth on one of the sawdust medium when compared to nutrient medium. 366 of these transcripts were present in all trascriptomes isolated from fungus growing on sawdust medium. However each of the sawdust medium was characterized by different number of unique transcripts that were expressed fourfold higher in comparison to nutrient medium, namely – birch (320); ash (533) and mapple (196). In the *C. unicolor* transcriptome 51 transcripts coding for enzymes engaged in wood decomposition were found. 21 one of them were common for all three sawdust medium (14 of them have been annotated as coding for cellulases). Only 7 genes were found to be transcribed during growth on two of the sawdust and 23 were uniquely used by fungus to decompose specific wood. These data allowed us to conclude that *C. unicolor* use various and unique set of lignolytic enzymes during growth on each sawdust.

Key words: *Cerrena unicolor*, wood decay, whole transcriptome analysis, NGS

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VI.O.6

Partial characterization of a highly divergent hantavirus isolated from the European mole (*Talpa europaea*) in Poland

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Hantaviruses (family Bunyaviridae, genus *Hantavirus*), the etiologic agents of hemorrhagic fever with renal syndrome and hantavirus pulmonary syndrome, were once believed to be harbored exclusively by rodents. Recently, however, genetically distinct hantaviruses have been discovered in multiple species of shrews and moles (order Eulipotyphla) and insectivorous bats (order Chiroptera). In Poland, we previously demonstrated the co-circulation of Boginia virus in the Eurasian water shrew (*Neomys fodiens*) and Seewis virus in the Eurasian common shrew (*Sorex araneus*). In addition, we found high prevalence rates of Nova virus (NVAV) infection in the European mole (*Talpa europaea*), with evidence of widespread virus dissemination in visceral organs. We now report the successful isolation of NVAV in Vero E6 cells, from lung tissues of an adult male European mole, captured in Huta Dłutowska in August 2013. Typical bunyavirus-like particles, measuring 80–120 nm in diameter, were found by thin-section transmission electron microscopy in infected Vero E6 cells. Whole-genome sequences of the isolate, designated NVAV strain Te34, were identical to that amplified from the original lung tissue, and phylogenetic analyses, using maximum-likelihood and Bayesian methods, showed that NVAV formed a highly divergent lineage with hantaviruses in insectivorous bats, consistent with an ancient evolutionary origin. In animal experiments, two-day old Swiss Webster mice inoculated intraperitoneally with NVAV developed weight loss, ruffled fur and neurological signs at 14 days. Moribund mice had IgG antibodies against NVAV, and NVAV RNA was detected in lung, spleen and brain tissues by RT-PCR. The long-awaited isolation of a highly divergent mole-borne hantavirus will accelerate the acquisition of new knowledge about its infectivity and pathogenicity in humans. Because European moles often reside near human habitation, physicians and health care workers should be vigilant for possible NVAV infection in individuals, who develop febrile illnesses or unusual clinical syndromes following known or suspected exposures.

Key words: Nova hantavirus, European mole, Central Poland

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