Session VI. Environmental Microbiology III

Oral presentations

VI.OP.1

Genotypic and phenotypic diversity of cyanobacteria classified to *Lyngbya genus*

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Genus *Lyngbya* constitutes a highly polyphyletic group composed of many phylogenetically unrelated taxa that were clustered together based on morphological similarities. In this study we analyzed seven strains belonging to *Lyngbya* genus, isolated from the Baltic Sea, the North Sea and one lake in northern England. The phenotypic analysis comprise cell and filament morphology and the profile of metabolites, mainly peptides (LC-MS/MS). The molecular phylogenetic analyses were based on sequences of the 16S rRNA gene and the adjacent intergenic transcribed spacer (ITS), phycocyanin intergenic spacer region (PC-IGS) and gene from cyanobactin-encoding cluster (*lynA*). Additionally, crude extracts and fractions from selected strains were tested for their biological activity.

Four strains of Baltic *Lyngbya* and the one isolated from English lake exhibited 100% similarity in case of 16S rRNA partial sequence. Those isolates were also similar based on their morphological features. Genetic variability was much higher when other investigated sequences were considered and it correlated well with the determined peptide profiles. Baltic strains from *Lyngbya* genus exhibited antimicrobial and cytotoxic activity. Further studies are essential to recognize their ecological significance and biotechnological potential.

Keywords: *Lyngbya*, microbial diversity, polyphasic approach, blue biotechnology

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VI.OP.2

Bioleaching of pyrite by mixed cultures of iron and/or sulphur oxidizing bacteria isolated in Armenia

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Bioleaching of pyrite (FeS_2) by pure and mixed cultures of new isolated iron-and sulfur- oxidizing *Acidithiobacillus* sp. 13Zn, *Leptospirillum ferriphilum* CC, *Acidithiobacillus albertensis* SO-2 and heterotrophic *Acidiphilium* spp. bacteria has been studied. According to data obtained the highest activity in pyrite oxidation among studied bacteria showed pure culture of new isolated thermotolerant iron-and sulfur-oxidizing *Acidithiobacillus* sp. 13Zn. It was shown that the efficiency of *Acidithiobacillus* sp. 13Zn in oxidation of pyrite was increased 1.8 times in association with *L.ferriphilum* CC. The association constructed on the base of *Acidithiobacillus* sp. 13Zn, *At.albertensis* SO-2 and *L.ferriphilum* CC allowed to increase twice the amount of total iron extracted from pyrite. Pyrite is acid insoluble and consequently according to sulfide minerals oxidation mechanisms can be dissolved only by ferric ion. Thus, the presence of *Leptospirillum* sp. bacteria in mixed cultures resulted in oxidation of ferrous ion (Fe^{2+}) and regeneration of ferric iron (Fe^{3+}), which in its turn accelerated pyrite oxidation. However, the highest activity in pyrite oxidation showed the association of *Acidithiobacillus* sp. 13Zn with heterotrophic *Acidiphilium* spp. bacteria. It is assumed that heterotrophic *Acidiphilium* spp. bacteria can utilize organic compounds contained in exudate or lysate of cells and thus reduce their toxic effect on autotrophic bacteria. Besides, heterotrophic bacteria excrete CO_2 during respiration that can be assimilated by autotrophic bacteria in their constructive metabolism. Thus, synergetic interaction between different species of autotrophic chemolithotrophic bacteria and acidophilic heterotrophic bacteria in association leads to the enhancement of metal extraction from pyrite.

Keywords: Bioleaching of pyrite; iron and/or sulphur oxidizing bacteria; pure and mixed cultures; ferric iron

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VI.OP.3

Bacterial and chemical qualities of potable water in the Al Ain City – United Arab Emirates

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Providing a safe drinking water in a distribution systems is a top priority goal of health agencies in the United Arab Emirates (UAE). Water Quality testing in the UAE relies mostly on testing for indicator microorganisms. However some pathogenic microbial species may exist but they escape the control testing methodology. Most of water contamination comes from the level of hygiene in the distribution network notably, mixing clean water with sewage. In order to address the problem scientifically, the current study is conducted to assess potable water quality and suitability for human consumption within the UAE. For this, 48 water samples were collected from 16 different sources. Water samples were filtered using nitrocellulose membranes and plates were incubated at 30°C and 37°C. Pure bacterial cultures were prepared and characterized morphologically and by colorimetric and enzymatic essays using Vitek 2 technology.

Results showed that 100% of samples were contaminated by a total of 46 Gram positive and Gram negative bacteria. The most frequent microorganisms found were *Staphylococcus lentus* and *Sphingomonas paucimobilis*. Anionic Chemical analyses was performed on the water samples to determine the concentration (PPM) of the following: chloride, Bromide, Nitrate, Sulfate, Chloride, Nitrite, Chlorate ($ClO_{3)}$, Chlorine Dioxide ($ClO_{2)}$, Bromate (BrO_{3}), and carbonates (CO_{3}). Total Dissolved Solids (TDS), Dissolved Oxygen and pH were also determined. Most of parameters appeared to be within the normal levels.

All water samples are generally found to be of acceptable level of quality according to the UAE current standards. However most water sources are being contaminated during the distribution process.

Our findings suggest the need by the UAE government authorities to intensify surveillance activities and enforce strict hygienic measures on potable water supply and distribution to improve water quality.

Keywords: Bacteria in water, UAE water

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VI.OP.4

Prevalence of multi-resistant bacteria in aquacultured and wild fish in Lithuania

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Aquatic environment is one of the most favourable settings for acquisition and dissemination of antimicrobial resistance. The aim of this study was to isolate, identify and characterize multi-resistant gram-negative bacteria prevalent in fish kept at aquaculture ponds as well as in wild fish in Lithuania.

Study subjects included 120 fish obtained from 5 fish farming open ponds as well as 120 wild fish caught at different freshwater lakes and rivers all over the country. Aquacultured fish species included Common carp (*Cyprinus carpio*), Rainbow trout (*Oncorhynchus mykiss*) and Bighead carp (*Hypophthalmichthys nobilis*) while 12 different wild fresh water species were included into experiments. Cloacal isolates resistant to at least three antimicrobial classes were treated as multi-resistant. Genes encoding antimicrobial resistance were investigated in bacterial isolates and in the total DNA from the cloacal samples of fish.

Thirty eight multi-resistant bacterial isolates were obtained from aquacultured fish with the highest prevalence being of *Pseudomonas*, Enterobacteriaceae, *Aeromonas* and *Chryseobacterium*. Only 6 multi-resistant isolates were obtained from wild fish. The isolates from aquacultured fish were most frequently resistant to beta-lactams, co-trimoxazole and nitrofuranoin. Some Enterobacteriaceae isolates demonstrated resistance to carbapenems, amikacin and tobramycin. Multi-resistant isolates from wild fish were detected only from river fish but not from the fresh water lakes. The most prevalent genes encoding resistance were *tetA*, *tetB*, *oxa-3*, *oxa-5*, *ctx-M*, *dfr1*, *dfr2* and *sul2* associated with the resistance to tetracyclines, beta-lactams, trimethoprim and sulphonamides.

The findings demonstrate that multi-resistant bacteria are prevalent in aquacultured fish as well as in wild fish from the rivers. Control measures regarding aquacultured farms should be considered with the aim to prevent spreading of resistant microbiota from open ponds into inland waters.

Keywords: aquatic environment, antimicrobial resistance, carp, wild fish

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VI.OP.5

Influence of iron, pH and biofilm formation on metal resistance of iron oxidizing bacteria

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The study of the resistance of iron- and sulfur-oxidizing bacteria to high concentrations of metals is of great scientific and practical interest. This interest is primarily caused by arisen from the problem of obtaining strains of leaching microorganisms resistant to high concentrations of copper for application in biogeotechnological processes. The tolerance of isolated *Leptospirillum ferriphilum* CC and *Acidithiobacillus* sp. 13Zn to copper and zinc was studied depending on concentration of the substrate (Fe^{2+}). The role of extracellular polymeric substances (EPS) in the resistance of the studied strains of bacteria was investigated as well. The influence of copper and zinc on the oxidation of Fe(II) by *Leptospirillum ferriphilum* CC and *Acidithiobacillus* sp. 13Zn was studied in the concentration range from 10 to 250 mM. It was revealed that oxidation of Fe (II) by *L.ferriphilum* CC was inhibited by 40% in the presence of 25 mM copper and zinc, whereas the inhibition of Fe(II) oxidation by *Acidithiobacillus* sp. 13Zn was only 45% in the case of 100 mM copper in the medium. Thus, the resistance of *Acidithiobacillus* sp. 13Zn to copper ions was considerably more than that of *L.ferriphilum* CC. Zinc ions inhibit Fe(II) oxidation by *Acidithiobacillus* sp. 13Zn in the concentration of 200 mM and higher. The results obtained have shown that the extents of inhibition of iron oxidation by the investigated strains in the presence of copper and zinc depends on the concentration of substrate.

Keywords: iron- and sulfur-oxidizing bacteria; high concentrations of metals; resistance; extracellular polymeric substances (EPS)

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