Session IV. Environmental Microbiology I

Opening Lecture

IV.OL.1

Antibiotic resistome in environmental technology and management

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The emergence of antibiotic resistance has become a global health concern of the 21st. Although antibiotic resistance is of natural origin, numerous anthropogenic sources contribute to the spread of resistance, including antibiotic use in clinical and agricultural settings. Municipal, agricultural and hospital wastewaters in particular are characterized by high abundances of antibiotic resistance determinants. Wastewater treatment systems have been recognized as hotspots for antibiotic resistant bacteria and antibiotic resistant genes (ARGs) acting as reservoirs, horizontal gene transfer stations and dissemination nodes between anthropogenic and natural environments. The common practice of soil fertilization with animal manure facilitates the dissemination of antibiotic residues, ARGs and associated mobile genetic elements (i.e. plasmids, integrons) from the farm to the environment. The use of antibiotics in farm animals is considered to be one of the factors behind the emergence of multidrug –resistant bacterial infections in humans. Although the resistomes of conventional wastewater treatment plants (WWTPs) and its impact on the environment have received due attention, the fate of antibiotic resistance determinants in other environmental technology systems and agroecosystems has remained less studied thus far.

Keywords: agroecosystem, antimicrobial resistance, environmental biotechnology, resistome

Oral presentations

IV.OP.1

Diversity and antagonistic activity of endospore-forming bacteria of bottom sediments of the Black Sea

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Deep-sea bottom sediments of the Black Sea are an example of a bacteriologically poorly-studied habitat. The quantitative and qualitative parameters of the microbiota of the sediment data are of scientific interest because of a combination of a number of unique physico-chemical characteristics such as anoxygenity, moderate salinity, great depth, etc. The question of the presence and, if any, the taxonomical composition of an facultatively-anaerobic microbiota in these sediments is not clear. The growing need for antimicrobial compounds of new types and classes is a practical motivation to expand the search for microbial strains for screening. The aim of the work was to detect, in deep-sea bottom sediments of the Black Sea, facultative-anaerobic mesophilic endospore-forming bacteria and to identify their ability to produce antibiotic compounds. As a result of research on samples of bottom sediments from depths in the range of 888–2080 meters located in the zone of deep anaerobiosis, 150 strains of facultative-anaerobic endosporeforming bacteria have been isolated. Isolation was carried out by pasteurization followed by scattering on meat-peptone agar. The strains were identified by analyzing fatty acid spectra. All of them belonged to the families *Bacillaceae* and *Paenibacillaceae*. Among them, representatives of the genera *Bacillus*, *Brevibacillus*, *Paenibacillus*, *Lysinibacillus* were identified. Screening of antagonists was carried out by the method of radial strokes on the medium of Gauze-2. As antagonistic activity indicators, strains of *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Salmonella enterica, Candida albicans*, *Klebsiella pneumoniae*, *Bacillus cereus*, *B. subtilis*, *Staphylococcus aureus* were used. The results indicate that 60 of 150 isolates exhibit antagonistic activity against at least one of the test strains, of which 30 exhibit a high level of antagonistic activity. Further identification of antagonistic factors by metabolomic methods is carried out.

Keywords: sea, sediments, endosporeformers, antagonism

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IV.OP.2

Diversity and role of plasmids in adaptation of psychrotolerant bacteria to hostile Arctic and Antarctic environment

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Plasmids, as self-replicating, extrachromosomal mobile genetic elements, are the key players responsible for horizontal gene transfer between bacteria. These replicons have been poorly studied in bacteria inhabiting Arctic and Antarctic regions, and the knowledge about their diversity and ecological role is rather scarce. In our studies, over 250 bacterial strains were isolated from polar areas and their taxonomic position was determined by comparative analysis of 16S rRNA gene sequences. Vast majority of the isolates are bacteria belonging to *Arthrobacter*, *Psychrobacter*, *Polaromonas*, *Pseudomonas* and *Variovorax* genera. Specific growth experiments revealed that these are psychrotolerant and metalotolerant strains. Moreover, it was shown that about 30% of isolates carry at least one plasmid. Over 100 of these plasmids were sequenced *de novo* and their complete nucleotide sequences were obtained. These are mostly small, cryptic plasmids, including really miniature replicons (not exceeding 1 kb) found in *Variovorax* spp. Detailed analysis of large (>10 kb) plasmids revealed the presence of numerous genes and modules which seem to affect on bacterial adaptation to the surrounding environment. These genes encode proteins involved in numerous processes, e.g. energy production and overall metabolism, protection against reactive oxygen species and UV radiation, resistance to heavy metal ions, fimbriae synthesis and biofilm formation, assembly of iron-sulfur [Fe-S] clusters, as well as transport and metabolism of osmoprotectants and cryoprotectants. Comparative analysis of the identified plasmids revealed that their plasticity and diversity most probably result from recombinational shuffling of their genetic modules. Interestingly, closely related modules were found also within plasmids of bacteria belonging to various taxonomic groups, which exemplify the possible directions of horizontal gene transfer in polar environments.

Keywords: plasmid, Antarctica, Arctic, adaptation

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IV.OP.3

Bacterial community dynamics in the biofilm of an experimental domestic greywater treatment system

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Water is one of the most essential resource on Earth. Large quantity of fresh water could be saved if wastewater could be reused. The source-separation of wastewater into greywater (water from laundry, bath and kitchen) and toilet water, allows on-site treatment of greywater with biological treatment systems and can be reused in many purposes where water quality is not a priority.

Objectives of this study were: 1) determine the microbial activity and community structure of the greywater treatment filters; 2) assess the bacterial community succession in the system; 3) relate system treatment efficiency to the bacterial community parameters.

The study was carried out in a newly established experimental domestic greywater treatment system (three vertical flow filters followed by horizontal flow filter) over ten-month long study period. The bacterial community abundance and structure was assessed by 16S rRNA gene copy numbers and sequencing, respectively. In addition, dehydrogenase and potential nitrification activity was measured.

The filter system demonstrated good performance in TOC, TSS, TN and organic matter removal. Rapid increase in bacterial community abundance and activity occurred during the first 100 days. Dominant bacterial phyla and genera differed between vertical and horizontal filters. Bacterial genera related to nitrification process were detected in vertical flow filter units and they were strongly correlated to potential nitrification activity. Bacterial community temporal dynamics exhibited large changes till day 160, after which the community structure stabilized. The beta diversity partitioning results showed that the temporal change in the bacterial community structure in both filters was dominated by species replacement. Bacterial community diversity was mostly affected by influent organic carbon and nitrogen compounds in vertical and ammonia and organic carbon in horizontal filters. System treatment efficiency was related to bacterial community diversity, abundance and certain bacterial genera.

Keywords: greywater, biological treatment, microbial community diversity, dehydrogenase activity

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IV.OP.4

ESBL-producing *Aeromonas* spp. isolated from wastewater and marine environment

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Aeromonas spp. are widely distributed in natural environments, especially in aquatic habitats, they are both pathogenic to humans and animals. Clinical isolates have shown an increasing incidence of antimicrobial drug resistance, but the data about resistance profiles in environmental *Aeromonas* is still limited.

The purpose of this study was to determine the distribution, prevalence, antimicrobial resistance and presence of extended-spectrum beta-lactamases (ESBL) among isolates belonging to *Aeromonas* genus in marine environment and in treated wastewater coming from wastewater treatment plant discharging final effluents directly to the Baltic Sea, Poland.

Bacteria were isolated on MacConkey agar with/or without addition of cefotaxime. Samples were obtained from 'Gdynia-Dębogórze' wastewater treatment plant (treated wastewater) and from receiving waters: Gulf of Gdańsk, southern Baltic Sea (marine water and sand). All isolates were biochemically identified and their drug susceptibility was determined. Seventy two *Aeromonas* isolates were collected, belonging to seven species: *A. sobria*, *A. veronii* (isolated only from marine sand), *A. caviae*, *A. hydrophila*, *A. salmonicida* (isolated only from marine waters) and *A. hydrophila/caviae*. Among them 15 isolates produced ESBLs. All ESBL-producing bacteria carried class 1 integron. Most of ESBL phenotypes were coded with *bla*CTX-M-3 gene. Genotyping of those isolates revealed that ESBL-positive isolates from marine waters were the same as those identified in treated wastewaters.

These findings highlight that further studies are needed to understand the dissemination, stability and transmission of *Aeromonas* carrying antibiotic resistance genes of clinical significance in marine ecosystems.

Keywords: *Aeromonas* spp., antimicrobial resistance, integrons, extended-spectrum beta-lactamases (ESBL)

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