Session VII. New methods in microbiology

VII.O.1

New molecular method for typing clinical strains of Streptococcus Anginosus Group

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Streptococcus Anginosus Group, SAG (formerly milleri), consists of three species, which gained reputation of underestimate, unrecognized and misidentified bacteria. All that is because of their various phenotypes, different presented Lancefield antigens and also various life niches. SAG strains are generally regarded as human commensal flora and are isolated from oral cavity, gastrointestinal and genitourinary tracts. However, more and more clinical reports showed their importance in severe infection resulting in liver and brain abscesses and bacteremia. Moreover, SAG is rather poorly known bacterial group and their mechanism of pathogenesis is not well understood. In addition, there are no molecular typing methods for SAG, like in case of other pathogenic streptococci such as *Streptococcus pyogenes* or *Streptococcus agalactiae*. Molecular typing methods are especially useful in diagnostic laboratories to determinate relationship between strains and investigate population structure.

In this study we investigated SAG genomic sequences to identify loci, which could be targets for PCR based typing method called **M**ulti **L**ocus **V**ariable Number Tandem Repeats **F**ingerprint (MLVF). The target loci were examined for size polymorphisms of PCR products in a pool of clinical SAG strains. Nine loci, which were characteristic for SAG but not for all other streptococci, were chosen. MLVF based on typing of these nine loci is able to generate over half a million different patterns and to detect similarities and differences between SAG strains comparable to PFGE analysis.

Key words: *Streptococcus anginosus*, MLFV

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VII.O.2

Rapid culturing, enumeration and analysis of bacterial cells using droplet microfluidics

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We demonstrate a droplet digital microfluidic technology for rapid cultivation and enumeration of bacterial cells. Our system allows analyzing metabolically active aerobic microorganisms one-by-one within five hours of incubation.

Droplet microfluidics is a technology to manipulate and analyze small volumes (from picoliter to microliter scale) of liquid confined in immiscible oil phase. Each droplet can be considered as a single tiny test tube due to their stability and isolation from other droplets. Main advantages of droplet microfluidics include convenient handling of miniature liquid volumes, possibility to carry out single-cell studies and suitability for high-throughput experiments. Over the last years, droplet microfluidics has increasingly been demonstrated as a suitable tool in different fields of microbiology: for monitoring bacterial growth, detection of bacteria, determination of susceptibility to antibiotics, enzyme activity screening and bacterial community interaction studies.

Precise quantification of bacteria is important for analysis of pathogen populations in environmental and medical samples as well as in microbiological studies, for example testing the toxicity of different antibiotics and their impact to bacterial adaptation and evolution of resistance. Traditionally, bacteria have been quantified by the plate count method. This gold standard approach demands at least an overnight incubation, often longer in the case of slowly growing bacteria.

We demonstrate for the first time the usage of dodecylresorufin as a fluorescent marker for viable bacteria in droplet microfluidic setup. Resorufin-based assays can theoretically be adapted for all aerobic organisms and are therefore suitable for vast number of pathogens and model microorganisms. Future applications of our technology may include, but is not limited to: bacterial quantification, antibiotic susceptibility tests, investigation of antibiotic action mechanisms, *etc*.

Key words: bacterial detection and quantification, droplet microfluidics

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VII.O.3

Use of *k*-Nearest Neighbors algorithm in viral host prediction

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The advent of new sequencing techniques allows cheap and rapid sequencing of environmental samples. Availability of the sequence data poses new challenges for scientists. For example, we do not know anything about the potential host or hosts of the newly discovered virus isolated from sewage. First approach to infer the phylum of a viral host was presented by Kapoor *et al.* [1] The group, representing each viral sequence by its mono- and dinucleotide frequencies, successfully employed discriminant analysis to distinguish between *Picorna*-like viruses infecting mammals, plants or insects.
Here we report a similar analysis based on *k*-Nearest Neighbors (*k*-NN) algorithm. Applying this algorithm we were able to properly ascribe above 99% viruses to one of the two groups: viruses infecting *Eukaryota* and those infecting *Bacteria* or *Archaea*. Our analysis was not restricted to any taxonomic group of viruses, but covers all non-segmented viral sequences.
Biological samples (like ocean water or sewage), could potentially contain viruses infecting a wide range of hosts, from bacteriophages to human. Similar problems may be encountered while studying feces or respiratory fluids, since viruses found in these samples might have been ingested or inhaled earlier. We believe that our discovery may facilitate the analysis of viral sequences found in such samples.

Key words: *k*-Nearest Neighbors, virus discovery, machine learning, virus-host interactions

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References:

Kapoor A, Simmonds P *et al.* (2010) Use of nucleotide composition analysis to infer hosts for three novel picorna-like viruses. *J Virol* **84**: 10322-10328.

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VII.O.4

Can blue light be used against *Pseudomonas aeruginosa*?

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Abstract: *Pseudomonas aeruginosa* is a gram-negative pathogen responsible for wound, ears, lung, blood and urinary tract infections. Adding to the problems of high incidence and infection severity, its resistance to conventional antimicrobial treatment has increased over the past decade. Thus, the development of alternative therapeutic option seem to be required. One of promising method is a phototherapy, which acts mainly through reactive oxygen species (ROS) leading to photodestruction of target cells.
Objectives: Our studies were aimed to analyze blue light irradiation (λ=405 nm) efficacy in various treatment conditions, against *P. aeruginosa* strains.
Materials and methods: Current research focused on two possibilities of using blue light to improve its antimicrobial efficacy.
Several doses of blue light (λ=405 nm; 0–15 $J \cdot cm^{-2}$) have been used in combination with antibiotics towards clinical strains of *P. aeruginosa*. Antibiotic resistance was determined accordingly to CLSI protocol and Minimal Inhibitory Concentration (MIC) values were evaluated on EnVision Spectrophotometer (Abs OD_{600}).
Secondly, pathogen responses to various blue light irradiation profiles were estimated. Specific light dose (15 $J \cdot cm^{-2}$) administered with different light powers (7.875–63 $mW \cdot cm^{-2}$) and exposure times, was employed.
Results: Selected antibiotics revealed different effects where combined with blue light irradiation. Non-lethal light doses significantly increased susceptibility of bacteria to gentamycin, whereas other antibiotics showed unchanged efficacy.
Furthermore, we confirmed that one light dose (15 $J \cdot cm^{-2}$) can exhibit different efficacy against *P. aeruginosa*, when various light powers and irradiation time were employed. Survival reduction after treatment hesitated from 0 to 6 $\log_{10}$ of colony forming units ($CFU \cdot ml^{-1}$).
Conclusions: Administration of blue light can effectively support selective antibiotic therapy against *P. aeruginosa*.
Moreover, significant differences in bacterial response to various light profiles suggest that the mechanism of blue light action is more complex, and further investigation has to be considered.

Key words: *Pseudomonas aeruginosa*, photoinactivation, light-profiles

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VII.O.5

A rapid qualitateve assay for detection of *Clostridium perfringens* from canned food products

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Clostridium perfringens (MTC 1349) is a Gram-positive, anaerobic, endospore forming, and rod-shaped bacterium. This bacterium produces a variety of toxins. *C. perfringens* can grow at temperatures between 20°C to 50°C, it causes a wide array of diseases in both humans and animals such are gas gangrene, cellulitis, septicemia, necrotic enteritis and food poisoning.
Based on the ability of producing major four toxins, *C. perfringens* are classified into 5 types A–E . In many countries including the United States, *C. perfringens* food poisoning is ranked as one of the most common cause of food borne infections. There is no direct one step assay for the detection of *C. perfringens*, only few methods are known for accurate detection of *C. perfringens*, all those methods have their own pros and cons. In present studyon an easy, rapid reliable assay for detection of *C. perfringens* was developed. This assay is based on the production of phospholipase C (lecithinase), the presence of lecthinase is detected by the prob impregnated with Peranitrophenyl phosphotidyl choline ester (colorless), a substrate of lecithinase, which realese the yellow color p-nitrophenyl from the phosphetidyl choline conjugates. The reaction is extremly specific and sensitive. No cross reactions of lecithinase was observed with any other substrate. In addition to that this assay gave negetive results with other clostridium strains, no cross reactions were observed with other experimental strains like *C. titani, C. botulinum, C. acetobutyricum, Bacillus subtilis, E. coli.* The assay is extremely rapid and provides reliable and reproducible result with in one hour of incubation at 37°C.

Key words: *Clostridium perfringens*, lecithinase, Peranitrophenyl phosphotidyl choline ester

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VII.O.6

Enhancement of oil pollution bioremediation by electric field application

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A research was conducted to investigate factors influencing biodegradation of petroleum hydrocarbons and to choose those of them, which can be altered to enhance bioremediation process creating more favourable conditions for its proceeding. Parameters that affect environment of oil products biodegradation include action of force fields, such as an electric one. Influence of the electric field on bioremediation process is less studied comparing to another factors, such as temperature, pH, medium composition, humidity, surfactants presence. At the same time there is a high potential of an electric field to be successfully applied for enhancement of oil products biodegradation.

This potential is proven by characteristics of an electric field and its action on hydrocarbons and microorganisms: 1) it increases mass transfer and mixing of substances in the reaction system by movement of charged particles; 2) promotes enzymatic activity of microorganisms and thus accelerates cell growth; 4) affects configuration and reactivity of hydrocarbon molecules.

To elaborate mechanism of how an electric field can be practically applied for enhancement of oil pollution bioremediation it is necessary to investigate its influence on ability of oil-degrading microorganisms to decompose hydrocarbons at different stages of biodegradation process. *Gordonia rubropertincta* Ac-5005, *Acinetobacter calcoaceticus* B-7013, *Rhodococcus erythropolis* Ac-5013 and *Pseudomonas sp.* B-7028 were chosen as model microorganisms. They were allocated from biosorbents that are used for cleanup of petroleum pollutants. Next step is exposure of cultural medium with microorganisms to an action of the electric field of different strength at (1) lag phase of bacterial growth and (2) during biodegradation process. All microorganisms are studied separately and in mixture as a consortium. Research shows at which moment of the biodegradation process it is better to apply electric field to achieve enhancement of the bioremediation by decrease of the hydrocarbon concentration in the medium after biodegradation comparing with control.

Key words: biodegradation, hydrocarbons, electric field, oil-degrading microorganisms.

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VII.O.7

Microbiocenosis in different type of manures and hygienisation methods

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The aim of the study was to determine the number of bacteria in poultry (laying hens, broilers and geese), cattle and swine manures as well as to perform hygienization of these wastes using microbial – mineral biopreparation.
Microbiological analyses of manures: the total number of aerobic and anaerobic bacteria, including bacteria of the genera *Enteroccocus*, *Clostridium*, coliforms and *Escherichia coli* and chemical analyses of manures: total solids, ash, organic solids, total organic carbon, total phosphorus, total Kjeldahl nitrogen were conducted.
Hygienization was performed by means of the biopreparation, which contained the strains of bacteria: *Bacillus subtilis subsp. spizizenii ŁOCK 0272*, *Bacillus megaterium ŁOCK 0963*, *Pseudomonas* sp. *ŁOCK 0961, Psychrobacter faecalis* ŁOCK 0965, *Leuconostoc mesenteroides ŁOCK 0964, Streptomyces violaceoruber ŁOCK 0967 (Patent No.* P393863) applied to the mineral carries – a mixture of perlite and bentonite (20:80 w/w).
The results showed the differences in chemical composition of the poultry manures. The highest content of total solids (711.06 g/kg), ash (110.63 g/kg) and organic carbon (33.37%) was determined in geese manure, whereas the broiler manure was abundant in dry organic solids (85.4%). With respect to the nutrient content, the highest concentrations of nitrogen (7.18%) and phosphorus (2.4 g/kg) were reported in the caged laying hen manure. The highest number of bacteria was recorded in swine manure (3.68×10^8–1.98×10^{11} cfu/g), while the lowest in cattle manure (4.67×10^5–1.22×10^7 cfu/g), and the cell number was dependent on the isolated group of bacteria. It was also shown that the coliforms and *Clostridium* genus in cattle manure; *Escherichia coli* in swine manure and total number of anaerobes in lying hen manure was reduced by one logarithmic unit after the application of the biopreparation. The activity of bacteria derived from the biopreparation allows to improve the sanitary conditions in poultry facilities.

Key words: microbial contamination, manure, hygienization

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