Session III. Virology and Phages

Opening Lecture

III.OL.1

Express-analysis of bacteria-phage interaction

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For successful reproduction bacteriophage have to deliver genome into bacterial cytoplasm without destroying the host cell envelope, and to lyse the host in a controlled manner at the end of infection cycle. Viral genome delivery as well as effective lysis are dependent on energy state of the cell. We obtain information on virus entry and phage-induced lysis from permeability changes of the host membranes and the energy state of cells using electrochemical methods of analysis. The entire viral one-step growth cycle could be analyzed directly in the infection medium and electrochemical monitoring of the infection process directly reflects the state of cells without introduction of biases related to the process of sampling, e.g., changes of oxygen level in the medium during filtration or centrifugation, delay of the data acquisition. Electrochemical on-line monitoring of the infection allows additional analyses of samples taken directly from the vessel, i. e., O.D. measurements, determinations of ATP level, counting of bacteria and phages. Aeration of the infection mixture can be controlled by the rotation rate of a magnetic stirrer and/or using different stirring bars. Accumulated amount and the rate of leakage of intracellular K^+ reflects the permeability of the plasma membrane (PM), tetraphenylphosphonium (TPP^+) ions are used to follow the PM polarization and to evaluate the selectivity of phage-induced channels, as TPP^+ accumulation in the cytoplasm is membrane voltage ($\Delta\Psi$)-dependent. Monitoring of pH allows to evaluate the intensity of energy metabolism and to detect changes in PM permeability to H^+ ions during the infection. Phenyldicarbaundecaborane (PCB^-) ions are used to determine the amount of inactivated cells, as the lipophilic anions accumulate in membranes of metabolically inactivated bacteria. Such electrochemical system of express-analysis allows to evaluate the sensitivity of bacteria to virus, to characterize the infection cycle, to detect factors affecting the efficiency of infection.

Keywords: phage entry, infected cell lysis, electrochemical analysis, membrane voltage

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Oral presentations

III.OP.1

Bacteriophages in fight against *Salmonella enterica* – does it work? Evaluation of phage effectiveness in eradication of *S. enterica* in liquid cultures and biofilm

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Salmonella enterica is a pathogen responsible for one of most common foodborne disease – salmonellosis. The main source of infection are contaminated poultry meat and eggs. This bacteria is also able to create a biofilm on surfaces such as glass or steel. Therefore it can cause a problem in food industry. The main mean for prevention against *S. enterica* colonisation of birds intestines are antibiotics added to chicken feed or water. However, this causes a rapid development of antibiotic resistant strains. Alternative ways of preventing *S. enterica* outbreaks are being researched. One of such methods may be use of bacteriophages, viruses that infect bacteria. However, due to the fact that *S. enterica* is subdivided into more that 2500 serovars, that can vastly differ from one another it is hard to find a phage that will be equally effective against all of them.

Our group has created a collection of phages isolated from chicken faces samples, infecting *S. enterica* serovars commonly found in poultry. The aim of our work was to evaluate the effectiveness of a single phage and an experimental phage cocktails in eradication of various *S. enterica* serovars in liquid cultures as well as in biofilm. We have observed that not all the phages were equally effective against tested serovars, even though the previous tests suggested otherwise. Interestingly, we have also observed that some of the phages were more effective in eradication of biofilm formed by *S. enterica* compared to their use in liquid cultures.

Keywords: Bacteriophages, *Salmonella enterica*, biofilm, phage cocktail

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III.OP.2

Three bacteriophage-originated rod-shaped structures in yeast: a new tools for nanotechnology?

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Virus-like particles (VLPs) have evolved and became widely accepted tools, especially in the field of vaccinology. Some VLP-based vaccines are currently used as a commercial products, while others are at different stages of clinical studies. While icosahedral VLP platforms have been studied in detail, but rod-shaped VLPs have been mostly forgotten. Many icosahedral VLPs are synthesized in bacteria and there is no information regarding the generation of tailed-bacteriophage rod-shaped structures in yeast.

The aim: to expand the knowledge of yeast-expressed bacteriophage tail tube and tail sheath protein self-assemblage into rod-shaped structures and characterize their morphology.

Methodology: DNA sequences coding tail tube or tail sheath proteins of bacteriophages NBD2, FV3 as well as RaK2 were cloned into yeast protein expression vector. Synthesis of phage proteins was confirmed by protein electrophoresis and rod-shaped structures were analyzed by transmission electron microscopy.

Findings: Our work has focused on synthesis of tail sheath proteins from RaK2, FV3 as well as the tail tube protein from NBD2 bacteriophage in yeast. It was found that in vivo recombinant bacteriophage structural tail proteins in the absence of other phage proteins, self-assemble into tubular structures with different surface morphology. Yeast-expressed tail sheath proteins from RaK2 and FV3 self-assemble into non-flexible comparatively short rod-like structures. However, yeast-expressed tail tube protein from NBD2 bacteriophage sell-assembles into highly-organized extremely long and flexible structures. We demonstrated that rod-shaped structures formed by gp29 from NBD2 bacteriophage are extremely stable at different environmental conditions. To our knowledge, it is the first attempt to produce bacteriophage-originated rod-shaped structures in yeast and characterize their structural morphology.

Conclusion & Significance: This work intends to show the suitability of yeast protein synthesis system to generate high-yields of stable, long and flexible rod-shaped structures originated from *Escherichia coli* infecting bacteriophages NBD2 and FV3 as well as *Klebsiella* sp. bacteriophage RaK2.

Keywords: bacteriophage, rod-shaped structures, yeast protein synthesis system, self-assembly

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III.OP.3

Raman spectroscopy as a method of HPV infection identification and cervical cancer diagnosis

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Raman spectroscopy is a label-free tool to investigate chemical composition of components in samples, based on measurement of inelastic scattering of light. Raman spectra give molecule-unique fingerprints, so provide the possibility of chemical analysis of both inorganic and organic samples. Measurement by Raman spectroscopy enable detection of even subtle differences in the chemical structure of compounds, such as epigenetic changes in DNA methylation [1]. Combining Raman spectroscopy with microscopy gives opportunity to study biological samples with the subcellular resolution. For this reason, this method is very often used for cell or tissue analysis. In this work, Raman microscopy was used to study differences between human cervical epithelial cells acquired from women with cervical cancer, women with cervical dysplasia, as well as women without cervical dysplasia (a control group). Before spectroscopic analysis, samples were subjected to a cytological examination and also tested for HPV infection. Based on these studies, authors divided samples into 4 groups: without dysplasia and HPV, with dysplasia and without HPV, without dysplasia and with HPV and cancer cells with HPV. The aim of the work was finding spectroscopic markers of HPV and dysplasia related to DNA/protein methylation. The preliminary results have showed differences in chemical composition of the control, HPV positive and cervical cells. These results, although require deeper examination, give hope for the possibility of using Raman spectroscopy to diagnose HPV infection and cervical pathology.

References:

1. Brozek-Pluska *et al.* Development of a new diagnostic Raman method for monitoring epigenetic modifications in the cancer cells of human breast tissue. DOI: 10.1039/C6AY02559E

Keywords: human papillomavirus, Raman spectroscopy, cervical neoplasia

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III.OP.4

Double antiviral combinations applied by consecutive alternating administration against Coxsackievirus B1 infection in mice

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Human enteroviruses, distributed worldwide, are causative agents of a broad spectrum of diseases with enormously high morbidity, including a series of severe illnesses that affect the CNS, heart, β-cells of pancreas, skeletal muscles, and so on. Unfortunately, there is no specific treatment or vaccine available for these infections, and the patients' treatment is mainly supportive. In the last few years our team has developed an experimental alternative treatment strategy based on consecutive alternating application (CAA) of inhibitors with different modes of action. This work represents the antiviral activity of double combinations of anti-enteroviral compounds applied *via* CAA course against the Coxsackievirus B1 neuroinfection in mice.

Antiviral combination effects were examined by relying on double combinations of pleconaril, guanidine hydrochloride, MDL-860 and oxoglaucine put through CAA treatment scheme on CVB1 neuroinfection in ICR newborn mice infected s.c. with 20 MLD_{50}. Cumulative mortality (percentage), mean survival time (MST) (days) and weight (in grams) of suckling mice were recorded.

The results of these analyses indicate markedly improved efficacy of PG, PO and PM combinations administered according to the CAA treatment schedule in CVB1 infected mice - decreased mortality rate and lengthening of the mean survival time (MST). MG and MO applied consecutively were ineffective. In comparison with placebo groups the monotherapeutic course with pleconaril demonstrated some independent antiviral effect. It was found that MDL-860, oxoglaucine and guanidine.HCl monotherapies were without a marked antiviral effect.

Keywords: double combinations, *in vivo*, Coxsackievirus B1

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III.OP.5

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

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Uncertainties in hantavirus taxonomy result from the paucity of full-length genomes and the dearth of hantavirus isolates. Although referred to as novel viruses, nearly all of the more than 30 hantaviruses (family Hantaviridae) identified recently in shrews and moles (order Eulipotyphla) and bats (order Chiroptera) exist only as viral sequences. Because Nova virus (NVAV), harbored by the European mole (*Talpa europaea*), represents a highly divergent hantavirus lineage which is widespread across Europe, its isolation has been a high-priority. Lung tissue homogenates, prepared from four NVAV-infected European moles captured in Huta Dłutowska in central Poland in 2013, were inoculated onto Vero E6 cell monolayers, then subcultured at two- to four-week intervals, at which time cells and culture media were analyzed for NVAV RNA by RT-PCR. After several failed attempts, NVAV RNA was detected in cells and culture media at 34 days after inoculation with tissues from one of four European moles. Subsequently, NVAV RNA was detected following inoculation of fresh Vero E6 cells with culture supernatant, indicating virus replication, and typical hantavirus-like particles, measuring 80–120 nm in diameter, were found by transmission electron microscopy. Genomic sequences of the isolate, designated NVAV strain Te34, were identical to that amplified from the original lung tissue, and phylogenetic analysis of the full-length L, M and S segments, using maximum-likelihood and Bayesian methods, showed identical topologies, with NVAV clustering with the highly divergent bat-borne hantaviruses. Infant Swiss Webster mice, inoculated with NVAV by the intraperitoneal route, developed weight loss and hyperactivity, beginning at 16 days, followed by hind-limb paralysis and death. NVAV RNA was detected in lung, liver, kidney, spleen and brain tissues by conventional and quantitative real-time RT-PCR. The long-awaited isolation of the first mole-borne hantavirus will accelerate the acquisition of new knowledge about its pathogenic potential.

Keywords: hantavirus, mole, Poland

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III.OP.6

Giant viruses and virophages – how tiny „giants" revolutionized the view of the world of microbes

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Viruses, since they have been defined, are understood as ultramicroscopic (20–450 nm), potentially infectious agents with only one nucleic acid, replicating only within the cells of living hosts, using their enzyme apparatus. The discovery of giant viruses has rocked the state of knowledge about viruses, because giant viruses can be seen in light microscopy, and have features that are unparalleled to viruses, e.g. they code genes related to translational machinery, have DNA repair genes, transcription factors, polysaccharides and jumping elements, and thus features of the Prokaryota, Archea and Eukaryota. Hence, such a situation triggered discussions over their classification, leading to the proposal of existence in the world of the "fourth domain of life", that giant viruses could represent. Giant viruses are inhabiting in particular the aquatic environment, mainly protozoa, algae, sponges, corals, and have been registered in mammals (humans, sheep, cattle), in which they cause pathological states. Giant viruses are also hosts and carriers of virophages – "virus-eaters", which are parasites themselves, unable to replicate alone, having additional "parasites" - virophages, which are now registered 20 and, like giant viruses, have unprecedented features.

Keywords: giant viruses, virophages

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