Session 3: Biochemistry of microorganisms

Lectures

L.03.1

Developing an animal infection model for pathogenic algae of the *Prototheca* genus

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Prototheca spp. are unicellular, achlorophyllous algae, which are the only known plants capable of causing opportunistic infections (protothecosis) in both animals and humans. The aim of the study was to explore the virulence of the *Prototheca* algae *in vivo* using a murine model of infection.
Type strains of three pathogenic (*P. wickerhamii*, *P. bovis*, and *P. ciferrii*) and one saprophytic (*P. stagnora*) species were used to experimentally inoculate mice of both immunocompetent (wt) and immunodeficient phenotype. The study was carried out on 54 groups of 10-week-old, female mice (6 individuals per each) depending on the inoculum (algae or PBS as a control), the challenging dose (i.e. $5x10^6$ or $5x10^7$ CFU/mL), and inoculation route (subcutaneous, intramammary, and intraperitoneal). Six weeks post-infection, the animals were euthanized, and their organs were explanted, weighted, homogenized, plated on Sabouraud agar, and incubated for 72 h at 30°C.
Almost a third (29.9%) of wt mice and half (45.8%) of immunodeficient animals showed signs of infection. *P. ciferrii* accounted for the majority (40.4%) of infections, followed by *P. bovis* (30.3%), *P. wickerhamii* (22%), and *P. stagnora* (2%). Among healthy mice, the bulk of infections were due to *P. ciferrii* (53.5%), mostly as a result of intramammary inoculation (41.5%). Infections among immunosuppressed animals were chiefly (40.9%) induced by intraperitoneal inoculation with *P. bovis* and *P. ciferrii* being equally pathogenic (31.8%).

L.03.2

Transcriptional profile of *Leptospira interrogans* serovar hardjo using a sheep as host-adaptation model

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Leptospirosis is a bacterial disease caused by the genus *Leptospira*. The infection is widespread in animals and is also one of the most common zoonotic diseases in humans. Endemic infection of cattle and sheep with serovar Hardjo is of economically and clinically importance, especially with regard to reproductive problems. This type of infection is often attributed to abortions, stillbirths, and death of weak newborn . Two genotypes of serovar Hardjo, belonging to different Leptospira species, *L. interrogans* and *L. borgpetersenii*, have been recognized.
Comparative genomics of pathogenic and saprophytic *Leptospira* strains identified nearly 900 pathogen genes unique to *L. interrogans* or *L. borgpetersenii*. These genes potentially encode virulence-related proteins. In this subset 78% have no known function, suggesting the presence of a pathogenic mechanism unique to *Leptospira*.
To better understand the pathogenesis we performed transcriptomic studies using sheep as an animal model. The genes altered by entering the host environment were detected by pairwise comparisons of *in vitro* culture vs. bacteria incubated 24h and vs. 72h *in vivo*. After initial 24h of incubation in the host, 773 altered genes have been detected, and 331 (42,8%) were upregulated. After 72h of incubation in the host, 525 genes were altered, of which 242 (46%) were upregulated. Characterization of the transcriptome provides insight into factors that may correlate with infection and host adaptation.

Acknnowledgements

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L.03.3

The phenylbutyrate affects the proliferation and expression of selected enterotoxin genes in food-derived strains of *Bacillus cereus* sensu lato

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Bacillus cereus sensu lato comprises closely related aerobic gram-positive bacteria with wide distribution and significant implications for industry and health. Due to their toxin-producing ability, they can cause food poisoning, sometimes with severe symptoms. They are commonly isolated from diverse food sources, including milk products and ready-to-eat food. Phenylbutyrate (PBA) has potential as an inhibitory agent against these bacteria.
This study aims to assess the effect of sodium PBA on the proliferation rate of *B. cereus*, the expression of selected toxin genes, and changes in susceptibility to specific antibiotics in strains isolated from animal-derived food under different conditions.
Results demonstrate that a moderate concentration of PBA (10 mM) reduces the growth rate of *B. cereus* under typical and microaerophilic conditions (5% CO_2). It also decreases the relative expression of *nhe*, *hbl*, and *cytK* operons encoding enterotoxins, without affecting cereulide synthetase formation. While *B. cereus* exhibits widespread resistance to selected antibiotics, particularly β-lactams, it remains susceptible to aminoglycosides, lincosamides, macrolides, and chloramphenicol. The supplementation of PBA has no noticeable impact on bacterial susceptibility to the tested antibiotics.
PBA holds potential as a food preservative and supportive therapy for human food poisoning cases.

Posters

P.03.1

Yeast DNA topoisomerase II targeting: the search for an antifungal compound with a novel mechanism of action

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The COVID-19 pandemic has highlighted the problem of fungal drug resistance and worldwide ineffectiveness in combating comorbid invasive fungal infection. In 2022, WHO prepared the first fungal priority pathogens list to emphasize the problem of growing fungal resistance and to strengthen the global response to fungal infections. Clinically available antifungals are highly effective in some cases, however, have limitations. Therefore, there is an urgent need for development of novel drug with a different mechanism of action. DNA topoisomerases (Topos) are enzymes that catalyze changes in the spatial structure of DNA and play an important role in cell. Topos are significant molecular target in antibacterial and anticancer chemotherapy but very little is known about the possibilities to target fungal enzyme. We undertook research to confirm the hypothesis that inhibition of yeast DNA Topo II may result in antifungal activity and that human and fungal enzymes exhibit differences in sensitivity to inhibitors. We analyzed three group of compounds: anthracyclines, bisacridines and anthracenediones. Our studies showed that well known anticancer drugs: idarubicin (IDA), daunorubicin (DAU), pixantrone and mitoxantrone, exhibit antifungal activity and inhibit yeast DNA Topo II (IC_{50} IDA 1.18 μM, DAU 1.35 μM). Some newly synthetized bisacridines target yeast Topo II (IC_{50} IKE18 18.8 μM) and are furthermore active against fluconazole resistant *C. albicans* strains (MIC_{90} 8-16 μg mL^{-1}).

P.03.2

Candida albicans O-acetyl-L-homoserine sulfhydrylase (Met15) characterisation with the use of a novel RP-HPLC-MS method

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Fungal infections are a serious threat to public health as they are becoming increasingly frequent. Advancements in medicine and accessibility to therapies causes the expansion of at-risk population including immunosuppressed patients. A major problem is a rising fungal resistance to currently available antifungal therapies, therefore novel molecular targets are highly desirable. Exploration of enzymes participating in the biosynthesis pathways of essential amino acids such as L-methionine (L-Met) may provide new insight into pharmaceuticals development. We have overproduced and purified putative *O*-acetyl-L-homoserine sulfhydrylase from *C. albicans* (orf19.13090) as N-terminus hexa-histidine tagged (Met15NH) fusion protein in *E. coli*. Basic molecular properties of the purified protein were determined, including molecular weight of 48 kDa and a homotetrameric structure. Met15NH catalyses the sulfhydrylation reaction of L-homoserine to L-homocysteine in L-Met biosynthesis pathway and exhibited optimal activity at pH 8. A new RP-HPLC-MS method using derivatization with 5,5-dithio-bis-(2-nitrobenzoic acid) was developed and used to quantify L-homocysteine. Presence of end pathway product (L-Met) in the assay mixture up to 10 mM had little effect on the enzyme activity whereas 10 mM (S)-4-acetamido-2-aminobutanoic acid exhibited 70% inhibition. Above mentioned method allowed for the biochemical characterisation of the Met15NH and determination of its kinetic parameters.

P.03.3

Phage-antibiotic synergy (PAS) in *Staphylococcus aureus/ Candida albicans* dual-species community

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Alternative therapies against drug-resistant microorganisms are still widely searched. The solution might be phage-antibiotic synergy (PAS) therapy. However, we must extend knowledge of how phages and drugs cooperate. Also, it is important to find an antibiotic that acts synergistically with phages.

In this study, we examined how a combination of ciprofloxacin, caspofungin and phages eradicate S. *aureus*/*C. albicans* in broth culture and biofilm. Overnight cultures of both pathogens were mixed. Then ciprofloxacin (CIP) 1 mg/L (1×MIC), caspofungin (CASP) 0,2 mg/L (1×MIC) and phage cocktail (vB_SauM-A (A); vB_SauM-D (D) (MOI=0,1)) were added separately or together. Also, mature *S. aureus*/*C. albicans* biofilm was treated (CIP 8 mg/L (1x $MBEC_{80}$); CASP 0,2 mg/L (1×$MBEC_{80}$); phage cocktail (AD) 1×10^7 PFU/mL. Afterward, plate count, biofilm staining, and qPCR were performed.

Results show that the best effectiveness was reached when all factors were added both in broth cultures and biofilm. Interestingly, when only drugs or only phages were added the effect of the curation was ineffective in tested environments. That implies a strong synergy between selected drugs and phages.

To sum up, PAS is more effective than monotherapy in eliminating pathogens from polymicrobial communities. This aspect of phage therapy should be considered during planning alternative curation based on phages.

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P.03.4

Can lipid profile be an indicator of increased polyene tolerance in *Malassezia pachydermatis* strains?

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Antibiotic resistance is a significant challenge in medicine and veterinary science. *Malassezia sp.* are widespread among people and warm-blooded animals. *M. pachydermatis* is a natural part of skin and mucous membranes of most mammals and birds, but it can also cause surface inflammations. In the mid-20th century, polyenes emerged as a widely used group of antifungal drugs that target the integrity of the fungal cell membrane.

The study aimed to compare the lipid profiles of *M. pachydermatis* strains that were initially susceptible to nystatin (NYS) and natamycin (NAT) with the same strains after 75 weeks of exposure to subliminal concentrations of NYS (4ug/ml) or NAT (8ug/ml) during 7-day passaging. Thin-layer chromatography and gas chromatography coupled with mass spectrometry were employed to determine the lipid profiles of the tested strains.

The results showed that as the minimum inhibitory concentration (MIC) value increased, the total content of fatty acids (FA) decreased by 30% in the case of NYS and by 2 times in the case of NAT. Both antifungals caused an increase of polyunsaturated FA content.

The analysis of lipid profiles and FA content in strains with increased tolerance to polyenes suggests that a reduction in FA content accompanied by an increase in the proportion of unsaturated FA may be one of the mechanisms involved in the development of resistance to polyenes in yeast-like fungi.

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P.03.5

Changes in the lipid profile of *Candida albicans* during the acquisition of polyene tolerance

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Fungal infections caused by *Candida* sp. are often encountered in cases of surface, organ, and systemic mycoses. Antibiotic resistance is becoming more prominent, with the growing number of individuals susceptible to fungal infections. A valuable approach for studying this problem is to model resistance using initially susceptible strains, allowing for the observation of changes throughout the development of resistance.

Polyenes represent a major class of antifungal drugs used in the treatment of superficial and systemic candidiasis. Therefore, the study aimed to compare the lipid profiles of *Candida albicans* strains that were initially susceptible to nystatin (NYS) and natamycin (NAT) with the same strains after undergoing 75 weekly passages with subliminal concentrations of NYS (4 μg/ml) and NAT (4 μg/ml).

The lipid profiles were determined using thin-layer chromatography and gas chromatography-mass spectrometry (GC-MS). As the minimum inhibitory concentration (MIC) value increased, the total content of fatty acids (FA) decreased by approximately 2.5 times in the NYS-treated strains and by 30% in the NAT-treated strains. NYS treatment increased in the proportion of monounsaturated FA at the expense of saturated FA.

The analysis of lipid profiles and FA content in the strains during the development the resistance suggests an accelerated rate of FA desaturation. This may represent one of the mechanisms underlying the development of tolerance to polyenes.

P.03.6

Different fates of pathogenic *Leptospira* serovar Hardjo species within *in vivo* and *in vitro* model

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The spirochetal bacteria *Leptospira* spp is one of the most widespread zoonotic diseases. Theoretically, any *Leptospira* serovars may infect any animal species, but fortunately, each serovar tends to be maintained in specific animal hosts. It is known that these pathogens possess unique virulence mechanisms, that allow them to escape from the host's innate immune system and established prolonged infection in the kidney and reproductive tracks of the maintenance host. Cattle and sheep are recognized maintenance hosts for serovar Hardjo. Two genotypes of this serovar, located in different *Leptospira* species: *L. interrogans* (Hardjoprajitno, HP) and *L. borgpetersenii* (Hardjobovis, HB) have been identified. Their host-parasite relationship has been a conundrum. The aim of the study was to examine host-bacterial interactions, particularly with regard to host body liquids and macrophages. Cultures of HP and HB strains, using dialysis membrane chambers, were implemented in the peritoneal cavities of sheep. Furthermore, sheep peripheral blood macrophages (BMs) were used to evaluate intracellular trafficking of pathogens. During the study, we found different survival rates in body fluids for two genotypes. HB did not survive in body fluids. However, successful infection of macrophages, together with changes in the expression of sphingomyelinase gene-virulence factor produced by pathogenic Leptospires, may explain the establishment of successful intracellular bacterial infection.

P.03.7

Bacterial microbiota of chromium-contaminated water biocenosis – resistance, biodiversity, biofilm and siderophore production

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The bacterial microbiota of the biocenosis formed in an extremely Cr(VI)-contaminated retention ditch was studied in terms of bacterial resistance to chromium, bacterial siderophores synthesis ability and biofilm production under varying Cr(VI) parameters conditions. Bacteria were obtained both from the wastewater and algae mat growing in. Bacterial isolates were identified and the most resistant to Cr(VI) were selected to test the chromium effect on: (i) siderophores production, (ii) their biofilm productivity under different conditions – originally adapted and sustained with Cr(VI) or cultured without Cr(VI) as unadapted, (iii) biodiversity of bacterial consortium associated with algae representative isolated from the mat.
Experimental results have shown differences in biofilm production amongst selected bacteria by calculated interpretation ratio, but no clear trend depending on Cr(VI) presence/absence or adaptation. In the case of at least one bacterial isolate, biofilm production was significantly higher when adapted to Cr(VI) concentration corresponding with Cr(VI) concentration in water. In the case of another strain, relatively high biofilm production was correlated with moderate to high siderophores production. Biodiversity of the microbiome determined morphologically and by CFU calculation has shown that in the presence of Cr(VI), the CFU value decreases, however biodiversity is high and higher amongst algae associated bacteria comparing to water.

P.03.8

New insights into diversity, adaptation strategies and bioprospecting of microbes living in Arctic deep-sea hydrothermal vent systems

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The deep sea biosphere represents the biggest living system on Earth, distinctive in its unparalleled complexity. This biosphere is often considered life's last frontier, characterized by a lack of light, high pressure, limited supply of nutrients, and temperatures ranging from low (2-4°C) in most habitats to extremely high (above 100°C) in the seabed hot hydrothermal vents.

The deep-sea biosphere's dimension, diversity, and functioning are almost beyond comprehension. Researchers are poised to discover unknown landscapes, resources, and organisms through deep-sea diving with submersibles equipped with advanced sensing, sampling, and analytical technologies. Today the deep sea becoming a frontier for resource exploration and bioprospecting. Our future relies on many aspects of sustainable use of marine resources, which become essential to support blue growth in Europe. The INDEPTH project aims to decipher the hidden reservoir of metabolic traits and biochemical/enzymological biodiversity of the vents found in the Arctic Mid-Ocean Ridge. Fundamentally important results can be expected with an impact on our understanding of the ecology, microbial metabolism, evolution, and the underlying molecular adaptation required to live under extreme conditions. Finally, within this project, novel enzymatic tools for biotechnology will be delivered and characterized.

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P.03.9

Characterization of LysTt72, a lytic endopeptidase from the extremophilic *Thermus thermophilus* MAT72 phage vB_Tt72

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This study presents the discovery and characterization of a novel endolysin from extremophilic *Thermus thermophilus* MAT72 phage vB_Tt72. Bioinformatic analysis of the vB_Tt72 phage genome revealed an open-reading frame with features characteristic of lytic endopeptidases. The enzyme (LysTt72) shows low sequence identity (<37%) to the members of the endopeptidase, except for two yet uncharacterized peptidases of *T. thermophilus* phages: φYS40 (87%) and φTMA (88%). The gene *LysTt72* encodes a 346-aa protein with a predicted molecular weight of 39,710 and an isoelectric point 8.06. Recombinant enzyme with His-tag at the *N*-terminus was overproduced in *E. coli*, purified by immobilized metal affinity chromatography, and biochemically characterized. The enzyme exists in solution in monomeric form. The Tt72 endopeptidase exhibits lytic activity in the pH range of 6 to 11 (optimum at pH 9.5) and is also active in up to 750 mM KCl. The LysTt72 shows high lytic activity against bacteria from the genus *Thermus*. Also, the enzyme demonstrated lytic activity against microbes such as *Escherichia coli, Serratia marcescens, Bacillus megaterium*, and *Staphylococcus aureus*. The enzyme is extremely thermostable (a midpoint of thermal denaturation Tm = 97,1°C ± 0,19°C) and retains approximately 60% of its lytic activity after 70 min of incubation at 99°C.