BaMa-P01

Master's Specialisation Microbiology at Radboud University

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The Radboud University international Master's Specialisation Microbiology ranges from environmental to medical microbiology. At Radboud University and the Radboud University Medical Center we investigate the functioning of microorganisms and their potential for improving our health and the environment. As a Master's student you will be part of a vibrant microbiology research team that is at the forefront of the areas of environmental, medical and molecular microbiology. Think of working on energy-efficient societal waste processing or on next generation vaccines. As the program contains more than one year of academic research, you will gain extensive hands-on experience in microbial research supervised by excellent, dedicated staff members and PhD students.

With a maximum class size of 24 students, we can provide customized programs that meet your individual demands. The educational program is being developed according to the Microbiology Learning Framework developed by the American Society for Microbiology (ASM). The program focuses on 'understanding by design' in which linking theoretical knowledge to real world issues is the guiding principle. By ensuring the continuum of the entire Specialisation, there will be a clear progression in the two-year program covering evolution, biochemistry, 'omics', physiology, virology, cell and systems functioning. This broad educational base, one of the pillars of the Radboud University, is an excellent preparation for a wide range of careers in both academia and industry, covering pharmaceutical research, public health authorities, policy making and teaching at academic level.

Microbiology master students of previous years have won 'The Darwin' thesis award and the Unilever Research Prize for best master thesis of the year, indicating the high level and quality of colleague students. The lecturers have been bestowed with the Best Supervisor of the Year Award in the Netherlands and best teacher of the biology curriculum, showing the quality and dedication of our staff.

BaMa-P02

Physiological characterization of a novel thermophilic sulfate-reducing Desulfotomaculum species isolated from acidic volcanic soils of Pantelleria Island

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Terrestrial volcanic areas are characterized by high gas release and contribute to global warming by emission of the greenhouse gasses CO_2 and CH_4. Extremely acid terrestrial mud volcanoes contain high amounts of sulfate (SO_4^{2-}) and emit mainly hydrogen sulfide (H_2S).

Pantelleria is a small island, south of Sicily, Italy. In the middle there is a geothermal active area, characterized by high geothermal gas emission, high temperature (50-100°C), low pH (2-4.5) and low oxygen concentration. Despite the harsh conditions, previous research showed a diverse community and focused mainly on the involvement in the carbon cycle. Geochemical analysis revealed high sulfate concentration in the soil. High sulfate concentration and low oxygen concentration are conditions where sulfate reducing bacteria (SRB) can thrive. SRB are anaerobic microorganisms that use SO_4^{2-} as an electron acceptor. Some can grow autotrophically using H_2 as an electron donor and carbon dioxide (CO_2) as a carbon source in their metabolism. Some species depend on organic molecules as energy source.

In this study, a thermoacidophilic sulfate reducing bacterium is enriched and isolated from geothermal soil samples, collected on the volcanic island Pantelleria in Italy. Soil samples are dissolved into medium of pH 4.5 and incubated at 50°C H_2 served as an electron donor and CO2 as carbon source. Serial dilution to extinction is performed to isolate the strain. Active cultures show an increase in optical density, H_2 consumption and H_2S production. 16S rRNA gene analysis reveals that the isolated bacterium is a new species of the genus Desulfotomaculum.

Different growth media are tested and the optimum pH is determined. The optimum pH is determined by calculating the bacterial growth rates at different pH values within a range from pH 3 to 6.5 at 50°C. This resulted in no growth at pH 3, poor growth at pH 3.5 and pH 4, moderate growth at pH 4.5 to 5.5 and optimal growth at pH 6.5 for the new Desulfotomaculum species.

This study shows that the isolated Desulfotomaculum sp. is capable to adapt to acidic conditions at high temperature. Together with the ability to reduce sulfate, this microorganism could be applied in biotechnical applications, such as sustainable cleanup for acidic mining water, since sulfate reduction

will increase the pH.

BaMa-P03
Anoxic organic matter utilizers from the Black Sea
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The Black Sea (BS) serves as a natural laboratory for anoxic microbiology as it harbours the largest permanently anoxic and stratified sulfidic water column of the world. Stable chemical and biological stratification is caused by the inflow of more dense and heavier saline water that hinders vertical mixing with oxygenated surface water. These stratified layers allow for a stable redox gradient over tens of meters and hence largely defines the structure of the microbial community. The microbial communities in the suboxic and anoxic zones are highly specialized, but their metabolic strategies remain largely unknown.
Particulate organic matter (POM) is often described as microbial 'hotspots' as they facilitate several microhabitats. Furthermore, particle associated microbes appear to have higher activity compared to free living microbes. Thus, POM might play an important role in defining the community composition. This research focuses on how the type of complex POM influences the particle attached microbial community composition across a redox gradient.

During the BS cruise 2018 (R/V Pelagia), incubations were prepared with carbon coated magnetic beads and black sea water from different depths (100, 1000 and 2000m). 6h in-situ incubations were also performed at 1000 and 2000 m. Incubations were harvested at several time points (7, 35 and 75 days) and used for microscopy, 16S rRNA gene amplicon Illumina sequencing and nutrient measurements.

Preliminary results show densely colonized magnetic beads and a more diverse community on peptidoglycan beads with the phyla Firmicutes, Cloacimonetes andProteobacteria, in comparison to chitin beads that were generally colonized by members of theProteobacteria.

This indicates promising results that might help explain the effect of complex POM on stratified marine microbial anoxic systems and may further deepen our understanding of microbial functions in naturally occurring redox-gradients.

BaMa-P04
De novo ester synthesis using Eat1 in Clostridium beijerinckii
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Esters are commodity chemical compounds applied within the food industry primarily because of their strong aromas as well as within other types of industries mainly for their use as industrial solvent. Nowadays esters are mainly produced from petrochemical sources which is undesirable in terms of the finite nature and environmental pollution. The natural ability of microorganisms to produce the esters used in industry can be utilized to develop biobased processes that can replace todays unsustainable production methods. In this thesis research has been done on improving the production titer of ethyl and butyl esters in Clostridium beijerinckii fermentations by introducing wildtype, harmonized and truncated Eat1 gene types originating from yeast species Kluyveromyces marxianus and Wickerhamomyces anomalus. Results have shown that the K30 truncated harmonized Eat1 version from K. marxianus increases butyl acetate formation compared to the W. anomalus wildtype version and that ethyl acetate and ethyl butyrate formation can be induced by cultivating in a medium supplemented with ethanol. Ester production titers are still low, quite probably because of native esterase activity by beijerinckii which has also been confirmed in this study.

BaMa-P05
Interlaboratory comparison of the Oxford Nanopore's MinION® Sequencing Device for Microbial WGS Applications.

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The applicability of Oxford Nanopore Technologies (ONT) MinION sequencing for the identification and characterization of pathogenic micro-organisms has increased substantially. This low-cost portable device can sequence the whole genome of micro-organisms in a real-time fashion, thus huge potential in areas such as antimicrobial resistant genes detection, species analysis, and public health investigations. To build a portable and real-time sequencing laboratory the reproducibility of the method needs to be guaranteed. As an emerging technique MinION sequencing needs to be tested on various angels.

The aim of this study is to compare and evaluate the reproducibility of multiplex sequencing performed by ONT MinION sequencing at two distinct institutions (Avans University of Applied Science and Maastricht University Medical Center (MUMC+)). A harmonized protocol was used to sequence 12 clinical relevant micro-organisms using barcoding at Avans and MUMC+, respectively. Different DNA isolation methods have been evaluated, phenol-chloroform DNA extraction was used because of its ability to produce longer fragments. Library preparation was performed according to the 1D library protocol with the SQK-RBK004 rapid barcoding kit. Sequence library was loaded onto an R9.5 SpotON flow cell and sequenced on an MK1B MinION device. The subsequent, base-calling and demultiplexing was performed by Albacore and Porechop. At this moment, *de novo* assemblies are performed using a Galaxy-based pipeline. This workflow contains FastQC (v.0.72), Filtlong (v.0.2.0), Unicycler (v.0.4.6.0), minimap2 (v.2.12), QUAST (v.4.6.3), and bedtools(v.2.27.0.0). Furthermore, GoSeqIT Tools will be used for genetic characterization and SNP detection will be performed to determine an interlaboratory comparison between the two institutions using the CLC Genomic Workbench (v.12.0) software. Afterward, the results from Avans and MUMC+ will be compared to evaluate the reproducibility of the method.
Altogether, this research will give new insights into the laboratory workflow of ONT MinION sequencing for future implementation with clinical research and educational purposes.

BaMa-P06
Microencapsulation for improved gut microbiome
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A disturbed microbiome is a major cause of intestinal problems in the Western world. A reason for this can be a poor distribution of the different microorganisms living in the gut. Treatment by ‘Fecal Microbiota Transplants’ is very invasive, whereas oral administration of probiotics is ineffective due to harsh gastric conditions. Additionally, most gut bacteria are anaerobic and require special protective measures. The aim of this research is to investigate a more effective, non-invasive delivery system. *Akkermansia muciniphila* is used as a model for probiotic microorganisms, since this bacterium could be greatly beneficial in promoting gut health.

To protect *A. muciniphila*, a mild state of the art double emulsion technology was used in combination with oxygen scavenging. The bacteria were encapsulated in a thin oil film, creating water in oil droplets. This technology was optimized to encapsulate the bacteria so that the double emulsion stays intact. By measuring droplet size, it was safely assumed that the double emulsions are sufficiently stable to allow bacteria to survive the osmotic pressure differences found in the stomach. In vitro testing over a series of concentrations of the oxygen scavenger cysteine, led to the conclusion that cysteine has a positive effect on the viability of *A. muciniphila*. Simulated digestive tests proved that in the gastric phase the double emulsions droplets remained stable, but that they collapsed during the intestinal phase. Consequently, the bacteria are protected during passage of the stomach and released at the targeted position in the intestine, which substantiates the principle of this delivery system.

These results are promising for future use of double emulsions as a delivery system of the probiotic *A. muciniphila*, and the same principle can most likely be applied to other components that need to be released in the intestine.

BaMa-P07

Optimization of a viability-qPCR for live-dead discrimination of S.aureus

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Date: 01 March 2019

Ensuring microbiological safety is imperative for food producers such as Huijbregts Groep. The consequences for consumers when safety standards are not met are potentially immense. Microbiological culture methods are commonly used to confirm compliance with these standards. The disadvantages concerning these methods are its considerable time-consumption and inaccuracy. Furthermore, the emergence of molecular techniques makes the method less future-proof.

The aim of this study was to investigate the suitability of viability-qPCR (vPCR) as a replacement for microbiological culture methods for live-dead discrimination of *S.*aureus. To achieve this, various aspects of an established vPCR protocol have been optimized. Through extensive optimization, a qPCR was developed which reached a limit of detection of least 7 CFU/ml with a standard deviation of 0.07 log CFU/ml, an efficiency of 98.59% and an R^2 of 0.9996, making it a solid foundation for vPCR. Next, the level of qPCR suppression on DNA of several 'viability-chemicals' (propidium-monoazide-xx (PMAxx), (dichloro(η-cyclooctane-1,5,-diene)Pd (Pd) and cis-diamminedichloroplatinum(II) (Pt)) was determined. Pd proved the most potent, although, all chemicals could be used as a basis for vPCR.

The large number of unknown variables surrounding Pt and Pd were compelling reasons for designing a PMAxx 'reference' vPCR. Optimization of the PMAxx-vPCR demonstrated that 25 µM PMAxx had a minimal effect on the qPCR signal of living bacteria and that 10 µM PMAxx led to significant suppression of the signal of dead bacteria. This indicated that the optimal concentration for live-dead discrimination is around 10-25 µM. In its current form, however, the vPCR technique proved inconsistent.

As a concluding remark, it can be stated that qPCR is unsuitable as a direct replacement of microbiological culture methods, but forms a solid basis for vPCR. Likewise, the current PMAxx-vPCR protocol is not suitable as a replacement for microbiological culture methods and demonstrates the requirement for further optimization.

BaMa-P08

Optimizing real-time PCR for Aphanomyces astaci detection

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E.J.M Knooren, N.M.Groenen and J.E. Maduro

In Europe, the populations of indigenous European crayfish species are decreasing massively. One of the main reasons for this is the presence of the oomycete *Aphanomyces astaci*, which is transmitted by North American crayfish species that have been brought to Europe. This oomycete is the cause of the crayfish plague. North American species do not perish from the infection, but for European species an infection is almost always lethal. Early detection of this pathogen is of great importance for the preservation of the European species, for which a real-time PCR (Vrälstad et al. 2009) is used. However, the current standard test is not reliable and sensitive enough to detect low loads of *Aphanomyces astaci*.

The aim of this research is to optimize the real-time PCR, making it more sensitive and specific, in order to identify a low degree of infection. Sequence analysis of PCR products is used to ensure the reliability of the test during this process and to gain insight into the optimization process. Furthermore, a ddPCR test is being developed for more reliable quantification.

In addition to determining the most efficient real-time PCR program for amplification, buffer composition and additives were also tested. As a result, false-negative results are greatly reduced and the robustness of the test is increased. This has led to a better detection method for *Aphanomyces astaci*, where the sensitivity of the test is greatly improved. In order to validate the test, there is further collaboration with partners from Germany and Finland to detect infections of local crayfish populations with *Aphanomyces astaci.* The ddPCR test is currently under development.

BaMa-P09
The role of interspecies-interaction on the denitrifying and growth phenotypes of rhizosphere bacteria
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Rhizosphere, the narrow zone of soil surrounding plant roots, harbors unique microbial populations. The composition and function of this microbial community is influenced by both plant-microbe and microbe-microbe interactions. Investigating microbial inter-species interactions is essential to better understand processes occurring in the rhizosphere. Activity of the rhizosphere community affects not only plant growth and health, but is important in ecosystem functions, such as nutrient cycling. Microbial denitrification is an important part of the global nitrogen cycle. In this manner, fixed nitrogen is returned from the biosphere to the atmosphere, mainly as N_2, but several intermediates are produced such the greenhouse gas N_2O. N_2O has a global warming potential almost 300 times greater than that of CO_2. Under anoxic conditions, rhizosphere microbiota are both a source and a sink of N_2O. Therefore, it is important to investigate the community's ability to denitrify and how individual's capacity to denitrify is influenced by interactions among community members. In this project, we will focus on bacterial species isolated from the rhizosphere of *Arabidopsis thaliana*. The aim is to examine if these bacteria interact and how their phenotypes could contribute to the total functioning of the community. In the first part, we will analyze the influence of co-culturing on aerobic and anaerobic growth. Furthermore, we will investigate if these strains compete for electron acceptors (NO_3^-, NO_2^-, NO and N_2O) and if presence of inter-species competition influences denitrification of the whole community. In addition, existing knowledge of metabolites produced by these strains will be integrated with their interaction phenotypes to develop hypothesis that will improve our understanding of plant-microbe interactions.

BaMa-P10
Antibiotic resistance in exotic anaerobes

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Anaerobic bacteria are part of the normal flora of human skin and mucosal membranes. They can cause a spectrum of infections, ranging from local abscesses to life-threatening infections. Resistance to antimicrobial agents increases in both aerobic and anaerobic bacteria. Antimicrobial resistance has been studied for commonly encountered clinically relevant anaerobic bacteria, but not in the less commonly encountered ones. Due to the introduction of Matrix Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS), species of the latter group are now suddenly identified. This makes it even more important to assess their antibiotic susceptibility profile.
The objective of this study was to determine the antibiotic susceptibility profiles of 79 previous collected and identified anaerobic isolates to 12 antibiotics using the Etest method.
Before determining antibiotic susceptibility profiles the MALDI-TOF MS was used to re-identify these isolates. The 79 isolates belonged to 25 genera and 30 species. All strains were susceptible to amoxicillin/clavulanic acid, ertapenem, meropenem and tigecycline. Twenty-nine (36,7%) of the isolates were fully susceptible, 44 (55,7%) were resistant to at least one antibiotic and 6 (7,6%) isolates were multi-drug resistant (MDR), defined as acquired resistance for three or more classes of antibiotics. With the exclusion of vancomycin, due to intrinsic resistance of gram-negative anaerobic bacteria, 21 of the 25 genera/species showed resistance to at least one antibiotic. Gram-negative bacteria were mainly resistant to benzylpenicillin, amoxicillin, clindamycin and moxifloxacin, while gram-positive bacteria were mainly resistant to metronidazole, moxifloxacin and benzylpenicillin. Six isolates were found to be MDR; one isolate of *Flavonifractor plautii, Odoribacter splanchnicus, Robinsoniella peoriensis* and three isolates of *Sutterella wadsworthensis.*

BaMa-P11
Characterization of a novel cytoplasmic heterodisulfide reductase complex from *Methermicoccus shengliensis*
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Methane is a potent greenhouse gas and most of the methane on Earth is produced by methanogenic archaea. Recently, a novel methanogenic pathway has been discovered in the thermophilic

methanogen *Methermicoccus (M.) shengliensis*[1]. This archaeon can use methoxylated aromatic compounds as substrates for methane production[2]. However, the metabolism of this organism has not been studied in detail so far. During methanogenesis reduction equivalents such as reduced ferredoxin and reduced $F_{420}H_2$ are produced which are then used to generate a proton- or sodium motive force for ATP generation. Genomic analysis of *M. shengliensis* revealed that there are two soluble heterodisulfide reductase (Hdr) complexes present which are associated with proteins resembling the formate dehydrogenase subunit alpha (FdhA) and the F_{420} hydrogenase subunit beta (FrhB). It also revealed that there is a F_{420} dehydrogenase reoxidizing F_{420}, but not the genes encoding complexes like Ech or Rnf which usually reoxidize ferredoxin. Therefore, the question rises how ferredoxin is reoxidized. The aim of this study is to characterize one of these Hdr complexes (HdrABC-MvhD-FrhB/FdhB-FdhA) by use of bioinformatics and enzyme assays. The six genes encoding this enzyme complex are cloned in expression vectors and expressed in *Escherichia coli* strain BL21(DE3) ΔiscR. The enzymes will be produced and purified anaerobically via Strep tag affinity chromatography, the cofactor content will be analyzed and the catalytic activity is measured. With this we aim to elucidate more about the role of this complex in *M. shengliensis.*

1. Cheng L (2007) DOI: 10.1099/ijs.0.65049-0
2. Mayumi D (2016) DOI:10.1126/science.aaf8821.

BaMa-P12

The soluble heterodisulfide reductase complex of the coal-degrading methanogen Methermicoccus shengliensis

A.X.Q. Le, , M. S. M. Jetten , C. U. Welte, J. M. Kurth Radboud University, Department of Microbiology, Nijmegen, Netherlands Methanogenic archaea are responsible for the vast majority of methane production contributing to greenhouse gas emissions on Earth. The thermophilic, methylotrophic methanogen, Methermicoccus (M.) shengliensis [1] has been recently revealed to be capable of using plenty of different methoxylated aromatic compounds as substrates for methane production[2]. This discovery indicates that the role of methoxydotrophic methanogens in methane generation and the global carbon cycle might be more important than previously thought due to the prevalence of methoxy compounds as the main component of coal and lignin on Earth. However, the metabolism of this organism has not been studied in detail so far. Heterodisulfide reductase enzymes are well known to link carbon dioxide (CO2) fixation and methane formation in methanogenesis through electron bifurcation activity. Methermicoccus shengliensis genome displays two gene clusters encoding cytoplasmic heterodisulfide reductase (Hdr) complexes, but they do not exhibit high similarity of sequences among other related known complexes and also have an odd subunit composition. Therefore, the aim of this study is the characterization of one of the cytoplasmic Hdr complexes. To investigate its enzyme characteristics , the genes encoding the heterodisulfide reductase HdrABC subunits, the two F420-non-reducing hydrogenase subunits MvhDG and the coenzyme F420-reducing hydrogenase subunit FrhB from M. shengliensis were cloned and heterologously expressed in E.coli for subsequent protein characterization and activity assays. [1] Cheng L (2007) DOI: 10.1099/ijs.0.65049-0. [2] Mayumi D (2016) DOI:10.1126/science.aaf8821.

BaMa-P13

Localization of nutrient transporters in the compartmentalized anammox bacterium Kuenenia stuttgartiensis

(Sakthipriya Shanmughanathan, Marjan Smeulders, Stijn Peeters, Mike Jetten, Laura van Niftrik) Department of Microbiology, Institute for Water & Wetland Research, Faculty of Science, Radboud University, Nijmegen, the Netherlands. Abstract Anaerobic ammonium oxidizing (anammox) bacteria convert ammonium and nitrite to dinitrogen gas in the absence of oxygen. These organisms are responsible for the removal of ammonium from the environment and they are also applied for effective removal of ammonium from wastewater treatment systems. The Gram-negative anammox bacteria have an unusual cell plan: they contain a prokaryotic organelle, the anammoxosome, where the energy-conserving anammox reactions take place. This means that the nutrients ammonium and nitrite, as well as the waste product nitrate, need to cross both the outer and cytoplasmic membrane as well as the anammoxosome membrane. We are interested in how anammox bacteria can direct the nutrients to the anammoxosome. The genomes of sequenced anammox bacteria reveal the presence of multiple nutrient transporter genes. Model organism Kuenenia stuttgartiensis contains 7- ammonium, 6- nitrite and 2- nitrate transporter genes. To localize these different transporters in the cell, we aim to separate the three different anammox membranes to obtain three different membrane proteomes. First, we isolated anammoxosomes from intact cells as previously established. Next, we

will perform proteome analysis of the isolated anammoxosome membranes in order to obtain the exact protein composition, providing us with an overview of the location of the anammox nutrient transporters.

BaMa-P14

The influence of neutrophils on persister induction.

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Introduction:

Staphylococcus aureus (*S. aureus*) is known to cause recalcitrant and recurrent infections. Its recalcitrance could be caused by the presence of persister cells. Persisters are cells that can survive high concentrations of antibiotics. It is yet not very well known what induces this persister state. Persister formation has been linked to the bacterial stress response[1], which is for example induced by environment acidity, and to being phagocytosed by immune cells[2]. We set out to determine whether *S. aureus* forms persister bacteria upon phagocytosis by neutrophils as well.

Methods:

We compared the amount of persisters induced by neutrophil phagocytosis to that occurring naturally. We tested this for antibiotics from six different classes in the following assay: Bacteria were opsonized using pooled human serum and added to the isolated neutrophils (at about 10X multiplicity of infection (MOI)). After half an hour this mixture was washed and the neutrophils were lysed using triton (t=0 h). The remaining bacteria were incubated for 20 hours at 37° Celsius and exposed to 10 times the minimal inhibitory concentration (MIC) of the different antibiotics. After 20 hours the antibiotics were washed away and the surviving bacteria enumerated by plating. We compared the number of colony forming units (CFU) before and after the antibiotic exposure. To determine the number of persisters already present in the inoculum, samples were treated identically, except the incubation with neutrophils was left out.

To test which triggers could contribute to persister formation, we also tested the effect of neutrophils degranulated with tumour necrosis factor alpha and N-formyl-methionyl-leucyl-phenylalanine (TNFa/fMLP) and of triton-lysed neutrophils on tolerance to a single antibiotic (linezolid).

Results:

Phagocytosis always (n=4) resulted in more persisters than naturally occurring in the inoculum, independent of the antibiotic used. Also the degranulate of neutrophils induced persister formation.

Conclusion:

Neutrophils induce *S. aureus* to form persisters. It is quite ironic that the immune cells required to contain staphylococcal infections simultaneously induce tolerance to antibiotic treatment. Dissecting the molecular mechanism of persister induction by neutrophils will help us to device treatments aimed at eradicating persister bacteria.

References:

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[2] Helaine S, Cheverton AM, Watson KG, Faure LM, Matthews SA, Holden DW. 2014. Internalization of *Salmonella* by macrophages induces formation of nonreplicating persisters. Science. 343(6167):204-8

BaMa-P15

Isolation and characterization of diclofenac-degrading bacteria from activated sludge.

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Over the past years, the concentration of pharmaceutically active compounds has increased in aquatic habitats. These contaminants of emerging concern (CECs) have an effect on the behaviour of aquatic organisms and the information related to their removal is insufficient. Biodegradation is one of the main removal routes of these compounds during wastewater treatment (WWT). However, the knowledge about which bacteria can degrade them and what genes and metabolic pathways are involved is limited. In order to close this knowledge gap, the aim of this project is to isolate, identify and characterize bacteria present in WWT plants able to degrade pharmaceuticals. Isolation experiments were carried out under aerobic conditions at room temperature. Samples from the activated sludge of Groesbeek WWTP were incubated in mineral medium with diclofenac as the only carbon source. After several days, an aliquot was plated onto agar plates with the same composition as the medium used previously. The growth of colonies with different morphologies was observed. Further isolation of axenic cultures and subsequent degradation assays will follow together with the identification of bacterial strains and diclofenac catabolic genes. Knowing the different metabolic

pathways involved in diclofenac degradation in active sludge will help to design new WWTPs that more efficiently remove pharmaceuticals to protect the environment.

BaMa-P16

The gut microbiota in nursing home residents: association with colonization by multidrug-resistant bacteria

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Introduction: Nursing homes constitute environments that can facilitate multidrug-resistant organism (MDRO) colonization and spread. MDROs can lead to severe, difficult-to-treat infections. The gut microbiota plays a protective role against colonization and infection by pathogens, including MDROs. Nursing home residents have different gut microbial compositions compared to community-living elders and these changes are linked with diminished health-statuses, possibly increasing the risk of MDRO colonization. The aim of this study is to associate the gut microbiota of nursing home residents with MDRO colonization.

Methods: This four-point-prevalence study was conducted amongst thirty nursing home residents (Leiden, NL). Fecal samples, socio-demographic and clinical data were collected at four time points, each two months apart. To detect colonization with extended spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae*, Vancomycin resistant enterococci (VRE) and Carbapenem-resistant *Enterobacteriaceae* (CRE), fecal samples were cultured on ESBL-, VRE- and McConkey tobramycin Chrom-ID agar plates after overnight incubation at 35°C in Tryptic Soy Broth. Identification and antibiotic susceptibility testing of MDRO isolates were performed with MALDI-TOF mass spectrometry and VITEK®2. ESBL production was confirmed with a double disk method. For microbiota analysis, DNA was extracted from fecal samples and 16S rRNA gene amplicon sequencing was performed. Raw sequencing data was processed using the bioinformatics pipeline NG-Tax and subsequently analyzed in R. Clinical risk factors for MDRO colonization were identified using multivariate logistic regression.

Results: Out of thirty nursing home residents, twelve (40%) were colonized with an MDRO at least at one point during the following 6 months period. Among the MDRO positive residents, eight (66.7%) were MDRO colonized at ≥ 2 different time points. Eleven (42.3%) of the identified MDRO strains were ESBL-producing *Escherichia coli*, two (7.7%) *Enterobacter cloacae* and one (3.8%) *Citrobacter non-koseri*. The twelve other strains (46.2%) were non-ESBL producing *Escherichia coli* with aminoglycoside and quinolone resistance. Hospitalization in the previous year was identified as risk factor for MDRO colonization ($p<0.01$). Preliminary microbiota analysis revealed no significant differences in richness and diversity between MDRO positive and negative samples. Differential abundance analysis showed an increase of *Eggerthella* in MDRO positive fecal samples ($p<0.001$).

Conclusion: MDRO colonization is prevalent among nursing home residents. We identified previous hospitalization as risk factor for MDRO colonization. Among these residents, MDRO colonization was not associated with reduced fecal microbiota richness or diversity. The increased abundance of *Eggerthella* among MDRO colonized subjects merits further evaluation.

BaMa-P17

Unraveling the mode of action of a new Gram-positive specific growth inhibitor

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Introduction:

Streptococcus pneumoniae is a Gram positive bacterium residing in the upper respiratory tract of humans and is transmitted from person to person. Colonization of the upper respiratory tract occurs asymptomatically. However, *S. pneumoniae* can also cause invasive diseases such as pneumonia, sepsis and meningitis leading to high morbidity and mortality world-wide. These infections can be treated with antibiotics, however, the rapid emergence of antibiotic resistance prioritizes the

development of new antibiotics. A new potential antibiotic is *N*-substituted pantothenamide, which is an analogue of pantothenate (vitamin B_5) that targets bacterial Coenzyme A biosynthesis showing an inhibitory effect on fungi, malaria parasites and other Gram positive bacteria. Here, we aim to elucidate more on the mode of action of this antibiotic and how it affects *S. pneumoniae.*

Methods:

Effect of *N*-substituted pantothenamide was assessed by determining growth curves with different concentrations of the inhibitor. This was performed for the laboratory strain TIGR 4 and multiple clinical isolates with different serotypes. Capsule switch mutants, strains with the same genetic background, but with a different capsule, were used to assess the influence of the capsule type on the diffusion of *N*-substituted pantothenamide. To dive more into the metabolism of this inhibitor, a vitamin B_5 rescue assay was performed by adding different concentrations of vitamin B_5 to the media.

Results:

Differences in maximal inhibitory concentration (IC50 value) were observed when comparing different serotypes. Certain capsule serotypes showed more resistance to the inhibitor compared to others. Furthermore, the IC50 value increased after supplementing the bacterium with vitamin B_5.

Conclusions:

Growth inhibition most likely depends on the pantothenate pathway and the enzyme pantotheinase kinase, which converts both pantothenate and pantothenamide to Coenzyme A, as supplementation with vitamin B_5 partly rescued the inhibition. Additionally, differences during the growth experiments were largely explained by variation in capsule type, where thickness or composition of the capsule could play a role in uptake or diffusion of the inhibitor.

BaMa-P18

The naked truth: shedding of the pneumococcal polysaccharide capsule

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Introduction

The pathogenic Gram positive bacterium *Streptococcus pneumoniae* is one of the major disease causing bacteria worldwide and is subdivided into over 95 serotypes based on differences in polysaccharide capsule. This capsule contributes to immune evasion by resistance to complement-mediated opsonophagocytosis. Recent findings suggests that this capsule can be shed from the surface upon interaction with human cells in which the human cationic antimicrobial peptide LL-37 plays an important role. Despite vaccination against serotype 19A, it is still able to cause invasive disease cases, which might suggest that capsule shedding by 19A is a vaccine escape mechanism using a decoy effect. Here we explored the differences in capsule shedding of 19A and 19F strains in the presence and absence of LL-37 .

Methods

Growth curves were made with different concentrations of LL-37 with a variety of 19A and 19F strains. Supernatant from liquid culture was used to determine the amount of capsule shedding by dot blot. Chemical fixation with PFA in phosphate buffer before lysine-acetate ruthenium red fixation was used for transmission electron microscopy (TEM).

Results

TEM showed that some 19A strains seem to have a very conservative capsule thickness while other strains showed more variation. Not only the presence of the capsule differed, but the density fluctuated as well. Growth curves showed different responses to LL-37. Capsule shedding by 19A strains was variable, whereas this seemed lower for 19F strains. A correlation between TEM measurements, capsule shedding and growth curves was not found.

Conclusions

Levels of capsule shedding were generally higher for 19A than for 19F strains, although this was highly variable within subtypes. The antimicrobial property of the LL-37 can have a different effect on the growth of the pneumococcus. Capsule shedding as decoy effect might be an explanation for the vaccine escape of 19A.

BaMa-P19

Production of secondary metabolites in a co-culture of Planctomycetes and other

microorganisms

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Abstract

The use of antimicrobials for treatment of microbial infections has led to an increasing emergence and spread of multi-resistant bacteria. According to the World Health Organization (WHO) the resistance to antimicrobials has reached alarming levels in many parts of the world. Therefore, there is a strong need to develop new drugs. Here, we aim at investigating the potential of yet untapped bacterial phyla for the production of novel bioactive secondary metabolites with potential antimicrobial activities. In particular *Planctomycetes*, a phylum of ubiquitous bacteria highly abundant in biofilm-forming communities on marine surfaces, are recognized as such promising producers of small bioactive molecules. Planctomycetal strains have large genomes and complex lifecycles, traits which are known from well-characterized microorganisms producing antibiotics (e.g. *Streptomycetes*). Indeed, in an *in silico* analysis of planctomycetal genomes a larger set of gene clusters putatively involved in the production of small bioactive molecules was identified. Previous studies indicated that phototroph-planctomycetal allelopathic interactions in bacterial communities can trigger the production of compounds with antimicrobial activity. Also, novel secondary metabolites were identified in algal-bacterial symbioses. Compounds secreted by microalgae can even be used as precursors for the production of algicides inhibiting their growth or facilitating their degradation. In parallel to co-cultivating Planctomycetes with different interaction partners, planctomycetal genes encoding enzymes putatively involved in the production of small bioactive molecules will be heterologously expressed in easily manipulable microbial hosts such as *Escherichia coli* for studying selected activities in more detail.

BaMa-P20

The soluble heterodisulfide reductase from the methanotrophic archaeon '*Candidatus* Methanoperedens sp.' with special focus on subunit HdrA

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The oxidation of methane by methanotrophs is an important process to reduce atmospheric concentrations of this potent greenhouse gas. Anaerobic oxidation of methane (AOM) is performed by methanotrophic Archaea and can be coupled to either the reduction of sulfate, by a bacterial partner, or the reduction of nitrate, catalyzed by '*Ca*. Methanoperedens sp.' alone. '*Ca.* Methanoperedens sp.' performs reverse methanogenesis, in which the electron carriers ferredoxin (Fd), coenzyme M (CoM), coenzyme B (CoB), and F_{420} are reduced.

Metagenomic data revealed a gene cluster that is hypothesized to be a soluble heterodisulfide reductase, catalyzing the confurcation of electrons from CoM-SH, CoB-SH, and Fd_{red} to F_{420}, thereby regenerating the electron acceptors essential for this metabolic pathway. The gene cluster consists of several genes, including *hdrABC* (heterodisulfide reductase subunit ABC), *frhB* (F_{420}-reducing hydrogenase subunit B), and *mvhD* (Methylviologen-reducing hydrogenase subunit D). While HdrABC and FrhB are thought to be involved in conversion of CoM-SH, CoB-SH, Fd_{red} and F_{420}, the role of MvhD is not clear. Interestingly, in our dataset in total five copies of *hdrA* could be identified, some of them fused together with *mvhD* (*hdrA2*).

In our study we aim to investigate the subunit composition and enzymatic activity of the functional heterodisulfide reductase complex. Therefore, we perform cloning and heterologous production of subunit HdrABC, as well as MvhD and FrhB. In addition, phylogeny and genomic surrounding of *hdrA* genes was analyzed in order to reveal possible functions of HdrA proteins. More knowledge on the function and origin of all five HdrAs is needed to give better insight in the metabolic activities of '*Ca.* Methanoperedens sp.' and other microorganisms containing Hdr genes.

BaMa-P21

Predicting the disease severity of Shigellosis patients using genetic determinants of *Shigella* species and Enteroinvasive *Escherichia coli*

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Shigellosis is caused by the gram-negative bacterium *Shigella* species and can lead to dysentery. This disease belongs to category B2 of notifiable infectious diseases in the Netherlands. Enteroinvasive *Escherichia coli* (EIEC), a pathotype of *E. coli*, can also cause dysentery, however an infection caused by EIEC is not notifiable. To investigate the clinical relevance of infections like shigellosis, disease severity of the patient can be measured by using severity scales developed for assessment of disease severity of gastroenteritis. Genome-wide association studies (GWAS) are used to observe association between a phenotypic trait and genetic variants, and are applied in microbiology for associations of bacterial isolates with traits like antibiotic resistance and virulence. The purpose of this study is to investigate whether there is association between disease severity of the patients and genotypic determinants of the infecting *Shigella* and EIEC isolates resulting from the IBESS study that can be used to improve notification guidelines.
In total, 277 isolates were sequenced on an Illumina MiSeq® and assembled using an in-house assembly pipeline based on SPAdes. The genomes were annotated using Prokka, gene presence/absence for all genomes was determined using Roary and k-mer counting was performed using fsm-lite. Scoary, random forest classification and Pyseer were used to associate the k-mers and gene presence/absence with disease severity and symptoms of patients, like vomiting and blood in stool.
There was no association found between gene presence/absence and the tested traits using Scoary and random forest. Using Pyseer, there was one potential associated k-mer found, however, this k-mer was randomly distributed over the isolates of patients with different severity scores ($p = 0.604$), therefore it was a false positive result.
In conclusion, despite the use of several microbial GWAS methods, no genetic variants of *Shigella* species and EIEC were found that are associated with a higher disease severity.

BaMa-P22
Degradation of high molecular weight sugars by Planctomycetes
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Oceanic dissolved organic carbon (DOC) is the second biggest reservoir of organic carbon on earth. Heterotrophic bacteria in the ocean use this DOC for their metabolism and in effect produce CO_2. Since this process affects the atmospheric CO_2 concentration, which in turn affects global warming, understanding the cycling of DOC in the ocean is critical. Around 30% of DOC is stored in form of high molecular weight (HMW) compounds and it is generally assumed that this fraction is initially hydrolyzed by extracellular enzymes to sizes <600 Da. However, bacteria of the phylum Planctomycetes have been shown to be able to internalize macromolecules entirely, resulting in an competitive advantage. This process was previously proposed to be enabled by endocytosis-like uptake. However, along with other eukaryotic traits assigned to Planctomycetes, such as the lack of a peptidoglycan cell wall and a compartmentalized cell plan, the endocytosis-like uptake of macromolecules has recently been refuted. Instead, large crateriform structure-associated pit-fibers could be responsible for the internalization of HMW polysaccharides. Even though the degradation of HMW polysaccharides is important in marine habitats, both the uptake and subsequent degradation of these compounds is poorly understood. To tackle this lack of knowledge we characterized the sugar metabolism of *Planctopirus limnophila*, a planctomycetal model organism, employing a variety of computational and laboratorial techniques. Based on genomic data a reconstruction of the planctomycetal sugar metabolism will be made, which will be complemented by metabolic foot- and fingerprinting. The uptake of HMW sugars will be investigated by analyzing the transcriptome of *P. limnophila* grown with either glucose or dextran (a HMW sugar) as sole carbon source.

BaMa-P23
Identification of translationally active bacteria in complex microbial communities.
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Linking taxonomic identity with *in situ* activity to decipher the ecophysiological role of microorganisms in complex microbial communities is one major aim in microbial ecology. In the last decades, different methods have been developed allowing the combined detection of *in situ* activity and identification of

individual cells. However, most of these methods have low sample throughput and rely on the use of expensive and specialized instrumentations or radioactive substrates. An activity-based method that circumvents these limitations is Bioorthogonal Non-Canonical Amino acid Tagging (BONCAT). For this, biomass is incubated in the presence of an azide-modified amino acid analogue, which is taken up and incorporated into newly synthesized proteins by active microorganisms. Active cells are then fluorescently labelled by linking the azide group of the amino acid with an alkyne-modified fluorescent dye via copper-catalyzed azide-alkyne cycloaddition (“click” reaction). Subsequently, BONCAT-positives cells are visualized by epifluorescence microscopy. In addition, BONCAT can be combined with 16S rRNA-targeting fluorescence *in situ* hybridization (FISH) to taxonomically identify the active microorganisms.

In this study, we optimized the BONCAT technique to detect translationally active nitrifying microorganisms in complex communities. The potential of using BONCAT to determine metabolic activity of nitrifiers was confirmed by the detection of BONCAT-positive cells in a nitrite-oxidizing pure culture of *Nitrospira moscoviensis*. Furthermore, application of BONCAT on complex samples, such as activated sludge, revealed a substrate-dependent labeling of nitrifying bacteria. In addition, the active fraction of the microbial community was taxonomically identified by using 16S rRNA-targeting FISH. Taken together, BONCAT-FISH represents a promising tool for the identification and visualization of active nitrifiers in complex microbial communities, which is an important step towards a better understanding of these key players in the biogeochemical nitrogen cycle.