ABSTRACTS POSTER PRESENTATIONS BaMa SYMPOSIUM 2018

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 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 department 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 dedicated staff 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
A new tool to determine antimicrobial activity against *Staphylococcus aureus* biofilm
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To survive stressful environments *Staphylococcus aureus* (*S. aureus*) is able to form a shield-like layer which together with the bacterial cells is called a biofilm. The matrix of this layer is composed of exopolysaccharides, extracellular DNA, proteins and lipids. A *S. aureus* biofilm infection is extremely hard to treat since the matrix shields the bacteria off from the immune system and antimicrobial compounds, such as antibiotics, increasing the change for the infection to become chronic.
The aim of this study is to look for new antibiotics to fight *S. aureus* infections and in particular biofilm related infections. Herbal extracts described in traditional Chinese medicine with suspected activity against *S. aureus* will be selected and anti *S. aureus* activity will be detained by microdilution, agar diffusion and in a biofilm inhibition assay. Three strains of *S. aureus* are used, two of which are multi resistant to antibiotics. Furthermore, we will assay the expression of proteins essential for biofilm formation and study whether the addition of herbal extracts can alter this, using green fluorescent protein (GFP) technology.
So far nine Chinese herbal extracts and five mixtures of herbal extracts have been tested using microdilution and agar diffusion. Seven herbal extracts and all five mixtures show growth inhibition of the three *S. aureus* investigated. The extracts that cause most inhibition are selected and the effect of these compounds on *S. aureus* biofilm production will be monitored.
It can be concluded that some Chinese herbs have antimicrobial activity against *S. aureus*. It is yet unknown whether the antimicrobial activity is bacteriostatic or bacteriolytic, and whether these herbs also inhibit *S. aureus* biofilm formation. Further research is needed to determine this.

BaMa-P03
Sensitization of methicillin resistant *Staphylococcus aureus* to human Group-IIA Secreted Phospholipase A2 through interference with lipoprotein maturation
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Introduction Human antimicrobials are crucial in the host defense against Gram-positive bacteria, such as *Staphylococcus aureus* (*S. aureus*). However, more serious infections often require the administration of antibiotics. The increasing use of antibiotics contributes to the development of antibiotic resistant bacteria, of which methicillin resistant *S. aureus* (MRSA) is a prime example. MRSA infections present a huge burden to health care and especially hospital acquired infections form a threat due to the already diminished health of patients. Different treatment strategies are therefore needed to treat MRSA infections.
Recently, our group showed that MRSA with defects in the lipoprotein maturation pathway become more susceptible to daptomycin and the human antimicrobial Group-IIA Secreted Phospholipase A_2 ($sPLA_2$-IIA). $sPLA_2$-IIA is a potent antimicrobial that kills Gram-positive bacteria by hydrolyzing the phospholipids of the bacterial membrane. We set out to

determine whether we could sensitize MRSA to $sPLA_2$-IIA-mediated killing using globomycin and pepstatin A, which are known inhibitors of lipoprotein signal peptidase A (LspA) .
Methods Bacterial strains were grown to exponential phase (OD_{600} = 0.4) in Todd Hewitt broth supplemented with or without 100 µg/ml globomycin or 50 µM pepstatin A. Subsequently, bactericidal killing assays with recombinant $sPLA_2$-IIA were performed to determine whether the treated bacteria were more susceptible than the untreated counterparts. The LspA deletion mutant was used as a control.
Results Globomycin and pepstatin A both sensitizes MRSA Wild-Type (WT) to $sPLA_2$-IIA-mediated killing. The susceptibility of globomycin and pepstatin A treated MRSA to $sPLA_2$-IIA was comparable to that of MRSA Δ*lspA*.
Conclusion New therapeutic strategies are required to combat MRSA infections. We show that MRSA can be sensitized to the potent human antimicrobial molecule $sPLA_2$-IIA through specific protease inhibitors that target the lipoprotein maturation pathway. These inhibitors act as antibiotic adjuvants for endogenous antibiotics and possibly also for the last resort antibiotic daptomycin.

BaMa-P04
Urinary antibacterial activities of fosfomycin and nitrofurantoin in healthy volunteers
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Introduction: In the context of emerging resistance of uropathogens against current antibiotics, old antibiotics such as fosfomycin (FOS) and nitrofurantoin (NFT) should be reevaluated. We aimed to investigate the urinary inhibitory titers (UIT) and urinary bactericidal titers (UBT) against the most important uropathogens after therapeutic dosages of 1x3 grams FOS and 4x50 mg or 3x100 mg NFT for the treatment of uncomplicated urinary tract infections.
Methods: 20 healthy, female volunteers were included in the FOS study. After intake of 3 grams FOS-trometamol, urine samples were collected during the following 48 hours with every voiding. Urine samples were taken after administration of NFT with food for an 8 hour period. The mean maximum FOS concentration was 2381.4 ±1196.7 mg/L after 7.1 ±4.4 hours. FOS concentrations were quantified with a validated UPLC-MS/MS system. NFT was administered to another group of 12 volunteers in a crossover design. NFT concentrations were 94.4 ±47.8 mg/L and 94.1 ±49.9 mg/L for the 50 mg and 100 mg dose, respectively and were quantified using a UPLC-UV system. UIT represents bacteriostatic activity in urine and was defined as the highest 2-fold dilution of sample that inhibits visible growth after overnight incubation. UBT was defined as the highest 2-fold dilution that exhibits bactericidal activity. UIT-UBT values were determined for three *E. coli* strains (MIC_{FOS} 0.25, 1 and 2 mg/L and MIC_{NFT} 8 and 16 mg/L) and two *K. pneumoniae* strains ($MIC_{FOS,NFT}$ 8 and 32 mg/L).
Results: UIT-UBT values for *E. coli* were higher for both FOS and NFT (median (range) of 1:4 (<1:1–1:256), 1:4 (<1:1-1:16)) compared to those for *K. pneumoniae* (<1:1 (<1:1–1:64), 1:1 (<1:1-1:8)) and were as expected based on higher MIC's of *K. pneumoniae*.
Maximum FOS UIT-UBT values of 1:256 were found after 4-6h after administration for all strains and declined with time together with FOS sample concentrations. Maximum NFT UIT-UBT values of 1:16 were found after 0-2h after administration for 50 mg dosage and after 2-4h for 100 mg dosage of NFT and declined with time. FOS Titers exceeded 1:1 for at least 48h in *E. coli* and for 24h in *K. pneumoniae*. NFT Titers exceeded 1:1 for at least 6-8h in *E. coli* and for 4-6h in *K. pneumoniae*, regardless of dosage. The expected correlation between sample concentrations and UTI-UBT values was found in *E. coli* (R^2 0.99 ±0.01) and *K. pneumoniae* (R^2 0.94 ±0.04) for FOS. Correlation of NFT was reasonable for *E. coli* and *K. pneumoniae* MIC_8 (R^2 0.94 ±0.03), but questionable for *K. pneumoniae* MIC_{32} since the majority of the titers was (<)1:1.
Conclusion: Both FOS and NFT exhibit mainly bactericidal activity against *E. coli* and are more active against this uropathogen compared to *K. pneumoniae* based on higher UIT-UBT values for *E.coli*. Bacteriostatic activity and lower UIT-UBT values were found for *K. pneumoniae* reflecting the higher MIC_{FOS} of these strains. NFT is significantly less active in *K. pneumoniae,* reflecting the higher MIC_{NFT}. This questions NFT-use for this pathogen in these dosages.

BaMa-P05
Antimicrobial effects of fruit and flower anthocyanins
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Introduction: As antibiotic-resistance is rising worldwide, there is an urgent need for new antimicrobials. Anthocyanins may be the antimicrobials of the future. Anthocyanins are water-soluble pigments found in fruits and flowers of higher plant species. All anthocyanins are composed of an anthocyanidin core bound to different glycosidic moieties and to date more than 600 different anthocyanins have been described. These pigments provide coloration to attract animal pollinators, act as antioxidants and protect the plants against abiotic stresses. Further, there is evidence that anthocyanins have antimicrobial activity. However, for future application as new antimicrobials, the full potential of these substances should be further investigated. In the current project, the antimicrobial potential of anthocyanin-extracts from different plant and fruit species is further investigated.
Methods: Anthocyanins were extracted from grapes, red cabbage and roses using HCL. After solid phase extraction, the anthocyanin content of the different extracts was determined by High Pressure Liquid Chromatography (HPLC) followed by Diode-Array Detection (DAD). The antimicrobial efficacy of the anthocyanin extracts was determined against *E. coli*.

S. aureus, *S. epidermidis*, *S. pyogenes* and *K. pneumoniae* using agar well diffusion assays and minimal inhibitory concentration (MIC) assays.
Results: The HPLC chromatograms showed that the composition of the different anthocyanin-extracts differed widely in terms of composition as well as concentration of the different anthocyanins entities present. Red cabbage contained at least six different entities, whereas the grape extract contained three prominent polar anthocyanins, and an abundance of smaller peaks. Rose extracts contained only one anthocyanin.
All anthocyanin extracts demonstrated antimicrobial properties. However, to what extent differed between the extracts. Both grape and rose-extracts were much more potent as compared to red cabbage anthocyanins. The bacterial sensitivity to anthocyanin-induced inhibition appeared to be very much strain-dependent. *S. aureus, S. epidermidis,* and *S. pyogenes* appeared to be very sensitive to anthocyanin-induced inhibition, whereas *E. coli* was more resistant. Also *K. pneumoniae* growth was inhibited by anthocyanins.
In order to investigate if inhibitory anthocyanin-extracts are bacteriostatic or bacteriocidal, *E. coli* and *S. aureus* inhibited by red cabbage and grape anthocyanins were recultured in the absence of anthocyanins. Cultures that were inhibited by high concentrations of cabbage anthocyanins showed growth after the anthocyanins were removed, whereas cultures that were inhibited by grape anthocyanins demonstrated no growth even after removal of the anthocyanins.
Conclusion: 1) Our results show that the effects are highly dependent of the anthocyanin-extract used, suggesting that one or several specific entities are responsible for the observed antimicrobial effects. 2) Not all bacterial strains appear to be equally sensitive to anthocyanin-induced growth inhibition. Gram-positive strains appear to be more sensitive as compared to gram-negative strains, albeit that within these groups strains-specific differences are likely to exist. 3) Grape-anthocyanins appear to poses bacteriocidal properties, whereas cabbage anthocyanins appear to be more bacteriostatic in nature. Altogether, our results demonstrate the antimicrobial potential of anthocyanins, but future research efforts should focus on the specific anthocyanin entities responsible for the observed effects, as well as the inhibitory mechanism of action.

BaMa-P06
In vivo detection of ammonia oxidizing bacteria
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Nitrification, the oxidation of ammonia to nitrate via nitrite, is a two-step process and was considered to be catalyzed by two functionally distinct clades of microorganisms. However, the discovery that members of the genus *Nitrospira* are capable to oxidize ammonia completely to nitrate (comammox) overturned this nitrification paradigm. The enzyme catalyzing the first step of nitrification is the ammonia monooxygenase (AMO). This enzyme oxidizes ammonia to hydroxylamine, an intermediate in the nitrification process, in both canonical ammonia-oxidizing and comammox bacteria. Alkynes are well-known inactivators of the AMO, with acetylene being the most common one. AMO converts acetylene or any other alkyne to a ketene, which subsequently binds covalently to histidine residues in the substrate channel, thus blocking the access for ammonia and inactivating the enzyme. The alkyne 1,7-octadiyne (1,7OD) has been reported to act as a mechanism based, irreversible inactivator of the AMO enzyme and has been successfully employed as a bifunctional probe for the activity-based protein profiling (ABPP). The aim of this project is to optimize the application of an AMO-based ABPP protocol to active and living biomass, in order to be able to *in vivo* detect AMO-containing microorganisms present in a variety of microbial communities. To achieve that, planktonic and active cells of the canonical ammonia oxidizer *Nitrosomonas europeae* were inactivated by 1,7OD. Subsequently, the cells were fluorescently and covalently labelled via a Cu-catalyzed alkyne azide cycloaddition (“click”) reaction. Observation of the labelled biomass using an epifluorescent microscope indicated that approximately 20% of the cells were stained, whereas no unspecific labelling was observed. Application of the same protocol to a floc-forming comammox *Nitrospira* enrichment culture resulted also in the partial labelling of the biomass. In more detail, the characteristic dense floc formation of the culture led to a low dye incorporation. Consequently, the fluorophores used in these experiments were incorporated only by cells present in smaller flocs indicating that an efficient floc disruption protocol must be used prior to the application of the AMO-based ABPP protocol. In conclusion, these preliminary results indicate that optimization and application of this technique to living biomass will have a huge impact on the way that the slow growing ammonia oxidizers can be identified and, in combination with a cell sorting system, isolated based on their activity. Furthermore, this technique will provide an excellent tool to identify novel ammonia-oxidizing bacteria in complex environmental samples without the need for sophisticated enrichment techniques.

BaMa-P07

Understanding intrinsic pneumococcal growth characteristics and the contribution to the disease manifestation

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Introduction

Streptococcus pneumoniae asymptomatically colonizes the upper respiratory tract and is transmitted from this site or can become invasive and cause life-threatening diseases such as pneumonia, septicemia, and meningitis. The pneumococcal vaccine is effective at preventing invasive pneumococcal diseases (IPD), but pneumococcal meningitis continues to cause high morbidity and mortality. Many have attempted to uncover the pneumococcal meningitis pathogenesis mainly focusing on host defense factors. However, the role of bacterial factors is scarcely explored. Our hypothesis was: there are differences in maximum optical density (OD), growth rate, lag phase, lysis rate, and time-to-lysis of IPD-causing pneumococcus cultured in the rich medium; and these differences contribute to disease manifestation of meningitis.

Methods

A total of 378 strains of *S. pneumoniae* (32 serotypes) were retrieved from the Pneumococcal Bacteraemia Collection *Nijmegen* cohort. They were collected from patients with a pneumococcal bacteremia admitted to two Dutch hospitals (January 2000-June 2011), thirty of which caused meningitis.

Strains were inoculated on blood agar plate and incubated overnight at 37^{o}C and 5% CO_2. After 16-18 hours, colonies were collected and sub-cultured in a pre-warmed liquid medium (50% glucose-supplemented M17 broth [GM17]; 50% CAT) to mid-log OD_{620} of 0.3 ($\pm$ 0.01). When the designated OD was reached, aliquots were made in 15% glycerol and were stored at -80^{o}C. Rich medium (50% GM17; 50% CAT; 10% Fetal Calf Serum [FCS]) supplemented with catalase (0,75 µL/mL) was used for the growth kinetics measurement. Growth was monitored in a sterile flat-bottomed 48-well plate. A bacterial subculture of 15 µl was inoculated to the well, and OD_{620} was measured every 10 minutes using Tecan microplate reader (15 hours).

Results

Growth curves and derived values (i.e. maximum OD, growth rate, lag phase, lysis rate, and time-to-lysis) showed clear differences between isolates and indicated that the results were highly reproducible. Growth characteristics analysis revealed that there was no association between maximum OD, growth rate, lag phase, lysis rate, and time-to-lysis to the clinical manifestation of meningitis (*t-test*, $p > 0.05$). There were significant associations between maximum OD, growth rate, lag phase, lysis rate and time-to-lysis with the capsular serotype of pneumococcus (one way ANOVA, $p<0.05$). When serotype was taken into account, meningitis-causing strains from serotype 1, 6B, and 7F exhibited a distinguished growth pattern compared to the pneumonia-causing counterpart. Serotypes 1 and 19F, serotypes that are known to be associated with resistance to complement killing and opsonophagocytic activity, showed a significantly increased maximum OD as compared to the other serotypes. Regression analysis indicated that there was no significant associations between the maximum OD, growth rate, and lag phase with odds ratio of pneumococcal invasive disease potential.

Conclusions

Clear differences were measured in growth characteristics (maximum OD, growth rate, lag phase, lysis factor, and time-to-lysis) of pneumococcus cultured in rich medium.

No association was found between growth characteristics of pneumococcus with clinical manifestation of meningitis.

Within the same capsular serotype, meningitis-causing strains showed different growth patterns.

Further research is needed to confirm this finding, preferably using a different method to quantify bacterial growth, such as qPCR.

BaMa-P08

Dietary microbial exposure assessment in adults from the Netherlands and China

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Introduction According to the hygiene hypothesis, exposure to microorganisms might have a protective effect against the development of allergic diseases (Strachan, 1989). Earlier studies have quantified the microorganism intake through diet for the Dutch. Daily dietary microbial intake can be estimated based on the food type and its intake amount and condition. However, dietary microbial intake for Chinese has not been assessed yet. The objective of this study is to evaluate the microbial intake from the diet for the Chinese population and compare the intake between China and the Netherlands in order to find out how the dietary pattern can affect the individual intake.

Methods

The microbial load of three dominant microbial groups in foods, being the total aerobic bacteria, lactic acid bacteria and yeasts/moulds are estimated taking the various processing or storage condition for consumers into account (Grijseels,

2012). The China Health and Nutrition Survey 2011 provides a 3 consecutive days' 24-hour recall survey for 1250 interviewees living in Shanghai. The estimated microbial load is multiplied with the consumption data of each food. The foods that have important microbial intake contribution are identified for the design of a food frequency questionnaire as another method to assess the microbial exposure.

Result

This research shows that the total dietary microbial exposure for Chinese ranges between 7.3 to 10.6 log cfu/day, which is mainly determined by the total aerobic bacteria while the main microbial exposure contributor in the Netherlands is lactic acid bacteria. The best estimate of the mean daily dietary exposure for Chinese of aerobic spoiler bacteria is 7.6 log cfu/day, for lactic acid bacteria is 6.5 log cfu/day and for yeast/moulds is 5.8 log cfu/day. With high microbial load estimation, mushroom plays an important role in the aerobic spoiler bacteria (best estimation:7 log cfu/g) intake in the Chinese diet, attributing to 95% of the total exposure. The intake variance of aerobic spoiler bacteria and yeast/moulds between China and Netherlands has no significant difference. The mean dietary exposure of lactic acid bacteria for Chinese is over 2 log cfu/day less than Dutch due to the low cheese popularity in Chinese diet. Yogurt acts as the dominant lactic acid bacteria contributor in China, which accounts for 93% of the total intake.

Conclusions

The difference in dietary pattern between China and Netherland gives a large difference in microbial intake but this is mainly determined by a significant different level of lactic acid bacteria intake.

References

1. Strachan, D. P. (1989). Hay Fever, Hygiene, And Household Size. *BMJ: British Medical Journal, 299*(6710), 1259-1260.
2. Grijseels, W. (2012). *Assessment of dietary exposure to micro- organisms: towards a validated instrument.* Wageningen University.

BaMa-P09

Twin arginine translocation subunit A: Intermembrane and membrane attached protein

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In every living organism proteins need to be transported across the cytoplasmic membrane to fulfil their function. Bacteria and Archaea mainly use two systems: The secretory (sec) system and the Twin arginine translaction (Tat) system that transports folded proteins. The Tat system is absent in humans and plays a role in the virulence of bacteria, therefore an interesting drug target. The system consist of four parts: TatA, TatB, TatC and TatE. Proteins targeted to the Tat system are recognized by a TatBC complex and bind in the membrane on the N-terminal side. The binding triggers the proton motive force for the recruitment and oligomerization of TatA protomers to activate the translocation side to create a pore. The aim is to demonstrate that the protein TatA with a GFP fusion (TatAeGFP), from the strain Escherichia coli MC4100, occurs as a membrane attached protein and not only as an intermembrane bound. Are TatA complexes coming out of the cell during protein transport? The hypothesis is that large TatA complexes are also membrane attached and come out of the cell.

TatA is a hydrophobic protein with an transmembrane helix, amphipathic helix and a tail. Washing the membrane of the bacteria with sodium carbonate (pH 11,5) will remove loosely bound peripheral membrane proteins. This principle is used to detect if TatA is also a membrane attached protein. When the protein is attached, TatA will be detectable in carbonate wash fractions. Four fractions were collected during this experiment: PBS (pH 7,5) membrane, PBS wash, carbonate (pH 11,5) membrane and carbonate wash. All four fraction were further prepared for Western blot, Dot blot and Mass spectrometry experiments.

The Western blot experiment showed bands in both membrane fractions and the carbonate wash fraction, using primary antibody GFP, around 40 kDa. The combination of GFP (30 kDa) and TatA (10 kDa). When using primary antibody TatA, only the membrane fractions were detectable. Similar results were found with mCherry as a different fluorescent fusion protein and Dot blot experiments. Mass spectrometry was done to get a better inside of what is detected by Western blotting. Peptides of GFP and TatA were found in both membrane fraction and a limited number of GFP peptides in PBS wash. The carbonate wash was empty.

To conclude, the Western blot and Dot blot results show that TatAeGFP complexes can be found in membrane samples and carbonate washing samples. With this result an answer on the question can be given: yes, some TatA complexes are membrane attached and come out of the cell. However this is only detectable with antibody GFP or mCherry and not with TatA. Apparently the antibody TatA does not recognise the TatAeGFP protein when it is placed in a hydrophilic environment with a high pH. In both situations, Western blot and Mass spectrometry, a more comfortable environment needs to be created for a hydrophobic protein and a good antigen to antibody binding. This will be the next step in finding a reliable answer and greater understanding of the Tat system.

BaMa-P10
Mycelial material
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Introduction
The exponentially increasing production system that we find ourselves in nowadays does not match with the production rhythm of our main resource supplier: nature. A sustainable strategy can only be reached by mimicking the way materials are recycled in the natural environment. Given the ability of fungi to grow on dead organic matter, these organisms may prove of great importance in the development of novel biobased materials. Namely, fungi can colonize low quality plant waste streams and turn them into high quality mycelial materials. Although there are already a few companies dedicated to production of fungi-derived materials, increasing feasibility is a requirement for the imperative paradigm shift.
Materials and Methods
79 wild type *Schizophyllum commune* strains were grown in triplicate in rapeseed straw in order to find potential candidates for material creation. Cultures were grown at 30 ºC for two weeks in the dark. Pictures were taken on day 3, 4, 5, 6, 7, 10 and 14. Next, growth of 7 selected strains was assessed through 10 minute-length CO_2 production measurements, quantified in ppm. Strains were grown in quadruple in plastic microboxes using rapeseed straw as substrate. Cultures were grown at 30 ºC for two weeks in the dark. Mycelial activity was measured on day 3, 4, 5, 6, 7, 10, and 14 using the CO_2 production method developed in our lab.
Results
Screening 79 strains of *S. commune* showed remarkable diversity in mycelium growth. The selection of the 10 best performing strains was based on the colonization rate and the mycelium density. Afterwards, the CO_2 measurements revealed that the maximum mycelial activity was reached in the fourth day of growth for every strain. The strains 295 and 4-8AxB showed the highest values in these measurements [6622 Δppm ± 478 (SEM) and 6475 Δppm ± 185 (SEM), respectively]. These strains also showed the densest mycelium by visual examination of growth.
Discussion
2 out of 79 strains have shown to perform best regarding CO_2 production after growth in rapeseed straw, which was in accordance to visual examination. The development of a new protocol for testing mycelial activity is of great potential in further screening of wild type strains for biobased materials production. In previous experiments this method has already given insight in choosing the right water content for different fungal species. Moreover, different growth conditions can be tested to create an overview of the right growth conditions for the right fungal strains. Further experiments will focus on mechanical testing of wood-like pressed materials that are produced using fungi. To conclude, fungal materials result in a very promising alternative not just due to their biodegradability, but also because they would harness low quality waste streams while creating a high quality raw material.
Conclusions
1- Different *S. commune* strains show differences in growth in similar growth conditions.
2- The maximum mycelial activity is reached in the fourth growing day in every analysed strain.
3- The strains 4-8AxB and 295 are two outstanding biomass-producers among the tested strains.

BaMa-P11
Validation of the FtsZ domain, a C-terminus, of *E. coli* as an antibiotic target
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In *E. coli*, cell division is one of the fundamental processes to propagate themselves by binary fission. The recruitment of the divisome proteins at mid-cell ensure proper cell division through the synthesis of new cell envelope and formation of viable daughter cells. Among these proteins, FtsZ is a cytoskeletal protein that forms the Z-ring at the nascent division site, which is the initial step of the cell division process[1]. Besides it is also essential for the recruitment of late cell division proteins. The Z-ring is formed by the polymerization of FtsZ monomers. The C-terminus of FtsZ, anchors the protofilaments to the membrane through membrane bound proteins, FtsA and ZipA. Initial studies suggest that inhibition of Z-ring formation leads to bacteria to filament[2]. With the present study, we attempt to prevent Z-ring formation by overexpression of C-terminus FtsZ. Excess of C-terminus FtsZ would be responsible to obstruct the anchoring of native FtsZ to FtsA and ZipA, since both plasmid-expressed and native C-terminus FtsZ would compete to bind to the same proteins. This would subsequently prevent bacterial from fully septation and would lead to filament formation and finally cell death.
References:
1. Harry, E., Monahan, L. and Thompson, L. (2006). Bacterial Cell Division: The Mechanism and Its Precison. International Review of Cytology, pp.27-94.
2. Chen, Y., Milam, S. and Erickson, H. (2012). SulA Inhibits Assembly of FtsZ by a Simple Sequestration Mechanism. Biochemistry, 51(14), pp.3100-3109.

BaMa-P12

Evaluation of *Mycoplasma hominis* transfer by different swabs to Sigma-VCM™ transport medium

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Background

Mycoplasma hominis (*M.hominis*), a fastidious opportunistic bacteria, of the class *mollicutes*, causes genitourinary infections in women. With rising rates of *M.hominis* antibiotic resistance, it is becoming more imperative to carry out antimicrobial susceptibility testing. Currently all diagnositic samples must be transported to a central London reference laboratory; therefore, this study evaluates bacterial capture and release of various clinical swabs used for *M.hominis* isolation and transport.

Methods

Sigma-VCM™ transport medium with either foam tip swabs (∑-Swab®) or with PurFlock® swabs, were compared to transfer to Sigma-VCM™ by Copan Rayon dry tip or Cobas PCR female swab. Two low-passage clinical isolates were examined, following propagation in CPM SAS *M.hominis* specific broth. *M.hominis* growth was titred in broth and inoculation of Mycoplasma Experience Mycoplasma selective agar. Growth was evaluated by broth colour change (yellow to red) and microscope-assisted colony counting on agar following incubation at 37^{o}C for 48 h under aerobic conditions. Variables examined: 1) bacterial concentration in droplet; 2) droplet volume; 3) immediate versus 30 min drying prior to placement in transport media; 4) addition of 2 minute vortex to assist release from swab in transport medium. Droplets of varying volumes and concentrations were placed on a non-absorbent sterile surface, each swab was used to absorb the droplets and then transfer bacteria to Sigma-VCM® transport medium. Release from swab was standardised to 2 minute incubation at room temperature, prior to disposal of swab and immediate measurement of bacterial load in transport medium. Maximum transfer value was set as the amount of *M.hominis* present when bacterial droplet was directly inoculated into transport medium (no swab). Results were analysed by Graphpad Prism using ANOVA with Bonferroni's correction for multiple post-hoc comparisons.

Results

Swab transfer of *M.hominis* inoculums ranging from $6x10^4$-$8.5x10^5$ CCU/mL in droplet volumes of 5-500µL were examined. ∑-Swabs® appeared to absorb more than the other swabs, but all swabs left some residual inoculum behind for 500µL droplets. Following a 2 min incubation, the foam ∑-Swab® also transferred 30.79 ± 6.15% of the bacteria to the Sigma-VCM™ transport medium on average and the Purflock® tip transferred 18.14 ± 2.21% (no significant difference), while the Copan Rayon dry tip only transferred 3.48 ± 1.29% ($P<0.01$) and the Cobas PCR swab only transferred 2.66 ± 1.55% ($P<0.001$). No difference was observed between the two clinical strains of *M.hominis*, or between droplets of 5µL and 50µL, but yield of bacteria transferred decreased by 24-62% for ∑-Swab® and Purflock® tips for droplet volumes of 500µL ($P<0.01$; matched t-test). Vortexing did not significantly increase the release of bacteria from any of the swab types, and allowing the swab to dry for 30 min did not reduce the viable bacterial transfer.

Conclusions

- MWE Sigma-VCM™ with ∑-Swab® performed slightly better than Purflock® swab for capturing and releasing viable *M.hominis* into transport medium; both were significantly better than Copan Rayon and Cobas PCR swabs.
- Percentage transfer was better for pick-up of <500µL, but not influenced by bacterial concentration, vortexing, or 30 min delay in adding swab to transport medium.

BaMa-P13

Making a 3D model of the polygonal anaerobic methanotroph *Methylomirabilis* sp. and localization of the nitric oxide dismutase enzyme

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Methylomirabilis bacteria perform the anaerobic oxidation of methane (AOM) coupled to denitrification. It is proposed that these bacteria denitrify nitrite to nitric oxide and use a novel enzyme "nitric oxide dismutase" (NOD) to convert nitric oxide to nitrogen gas and oxygen. This internally produced oxygen is then used for the oxidation of methane to carbon dioxide. Next to their unusual metabolism, the ultrastructure of *Methylomirabilis* is also remarkable: the cells have an atypical polygonal cell shape with sharp edges that run along the cell length and end in a cap-like structure at the cell poles. It is hypothesized that this polygonal cell shape is formed by a surface protein layer (S-layer).

With this study we aim to gain more insight into the unusual polygonal cell structure of *Methylomirabilis* sp. by performing electron tomography on serial sections to obtain a complete 3D image and model of a cell. In addition, we will investigate the location of the novel NOD enzyme in the cell through immunogold localization.

BaMa-P14
Nitrogen removal by a co-culture of comammox and anammox bacteria
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Nitrification is a key step in the biogeochemical nitrogen cycle and is greatly impacted by anthropogenic activities. It is a two-step process, carried out by different microorganisms. First, ammonia is oxidized to nitrite by aerobic ammonia-oxidizing bacteria or archaea, and subsequently to nitrate by nitrite-oxidizing bacteria. Although it would be energetically advantageous to perform complete nitrification, no microorganism was known to catalyze this until recently, when complete ammonia oxidation (comammox) carried out by bacteria of the genus *Nitrospira* was reported. The comammox enrichment described in 2015 had been derived from biomass of a recirculating aquaculture system in a hypoxic bioreactor. The biomass consisted of a co-culture of *Nitrospira* and anammox bacteria, a puzzling combination given the fact that *Nitrospira* spp. are aerobic and anammox bacteria need anoxic conditions to be active. In addition, the oxidation of ammonia by the comammox bacteria would require oxygen for ammonia activation. Two different hypotheses were contemplated to explain the activity of this co-culture. In the first one, *Nitrospira* was considered to act as an ammonia oxidizer or a canonical nitrite-oxidizing bacterium, oxidizing either ammonia or nitrite to nitrate and thus competing with anammox for its substrates. In the second and more plausible conjecture *Nitrospira* would act as nitrite reducer, using the nitrite oxidoreductase in the reverse direction, coupled to ammonia oxidation. The produced nitrite would be used together with ammonium by anammox to generate dinitrogen gas.
The aim of this project is to characterize the microbial community in a novel anammox/comammox enrichment culture sustained on synthetic medium, and to elucidate the metabolic link between the two nitrogen cycle microorganisms. In order to answer these questions, activity assays were performed using stable isotopes (^{15}N-labelled ammonium and nitrate) to test for anammox and denitrification activity. The isotopic composition of the produced nitrogen gas was analyzed, showing a stable increase of $^{29}N_2$ from ^{15}N-ammonium, characteristic for the anammox reaction. In addition, an increase in $^{30}N_2$ was detected, indicating the production of ^{15}N-labelled nitrite from ammonia through aerobic ammonium oxidation. Furthermore, by employing fluorescence *in situ* hybridization (FISH), we observed that the culture was dominated by anammox (>40% of the total bacterial biomass), and *Nitrospira* contributed the majority of the remaining population. The unraveling and better understanding of the comammox/anammox interaction will help to better comprehend nitrogen cycling in engineered systems and change established perceptions about the roles of ammonia-oxidizing microorganisms.

BaMa-P15
Communication in the rhizosphere; deciphering the conversation
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As the interface between root and soil, the rhizosphere is composed of unique microbial communities which directly affect plant physiology. Amongst the most prominent advantages provided by rhizospheric microbes are: increased accessibility to nutrients such as nitrogen, phosphate and iron; and protection against pathogenesis by mechanisms including inhibition of quorum sensing and biofilm formation, anti-fungal activity and, the induction of systematic resistance . Whilst some methods of rhizospheric communication have been characterized the vast majority remain unidentified, reflected by the number of recognized rhizospheric compounds of unknown function.
In order to further our understanding of metabolite exchange in the rhizosphere, we made a selection of 6 Arabidopsis thaliana rhizosphere strains to construct a synthetic rhizospheric community . From the known Arabidopsis rhizosphere 6 bacterial strains were selected and a comparison of genomes conducted; overlap and differences in known genetic functionality were identified. These data will be used to direct further investigation. Genomic analyses highlighted variation in the strains' known genetic functionality including the presence or absence of genes associated with flavonoid, indole and glycan biosynthesis; estrogen and adipocytokine organismal signaling pathways; and environmental signal processing Wnt and notch environmental signaling pathways, highlighting differences in known communication strategies of the selected strains. Together chosen strains represent 51.75% of total known Arabidopsis rhizosphere function, with each individual strain representing between 29.2% to 19.9%. These areas of differentiation provide opportunity to attribute compounds to genetic functionality in future studies.

BaMa-P16
Inhibition of germination and growth by antimicrobial peptides on *Aspergillus fumigatus*
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Background
Aspergillus fumigatus is one of the opportunistic fungal pathogens that is able cause infections after inhalation of conidia by immunocompromised patients. Invasive aspergillosis is nearly 90% of the cases caused by A. fumigatus. At present, antifungal therapies using amongst others azoles are used to eliminate the fungi but unfortunately, these are losing their effectiveness due to development of resistance.
Antimicrobial peptides (AMPs) are small molecular weight proteins with a broad spectrum antimicrobial activity against bacteria, fungi, and viruses. Given their potent broad-spectrum antimicrobial properties, AMPs are considered as a promising new class of antibiotics with several major advantages as compared to conventional antibiotics. Derivatives

of the AMPs cathelicidin were made and are named PepBiotics (patent pending). We investigate the mechanism of action of PepBiotics and determine sensitivity of conidia and/or germinating conidia and hyphal forms in time course experiments. Furthermore, we test sensitivity of mutants lacking important conidial surface components for PepBiotics.
Methods
Using metabolic assays with 106 CFU/mL of A. fumigatus spores in minimal medium with 2% glucose and resazurin conversion (∆ OD 570 nm), the antifungal activity of AMPs against A. fumigatus wildtype Af293, CEA10 and its derivatives lacking either RodA, RodB or DHN-melanine was determined. Propidium iodide staining is used to determine whether PepBiotics mediate killing of A. fumigatus at different stages of germination. Confocal microscope with fluorescent labeled PepBiotics will be used to determine interactions with spores and hyphal forms.
Results
A. fumigatus mutants lacking surface components DHN melanine or either of the rodlet proteins did not show increased sensitivity for the tested peptides, instead, they had a slightly reduced sensitivity for the peptides tested.

BaMa-P17
Evaluation of aerobic methanotrophic activity in arctic thermokarst lake sediments
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Permafrost stores twice the amount of carbon currently present in the atmosphere. Due to global warming permafrost thaws, forming thermokarst lakes. These lake formations have an influence on organic matter degradation, potentially affecting the microbial community and the methane (CH4) emissions in the sediment. Methane-oxidizing bacteria (MOB) uses CH4 as sole energy and carbon source, playing an important role in the oxidation of CH4. The effect of climate change on the aerobic methanotrophic activity and community structure in thermokarst lake sediment is not yet well explored.
Previous studies showed that increase in temperature (4 to 10ºC) stimulate the activity of the aerobic methanotrophic community in thermokarst lake sediment from Barrow, Alaska. Community analyses of enriched cultures revealed the coexistence of type I methanotrophs with methylotrphs, Flavobacteriales and Shingobacteriales. A bioreactor system will be set up to study the interactions in these enrichments at 10ºC under controlled conditions with different CH4 and oxygen concentrations. However, the presence of eukaryotes in the incubations forced us to prioritize their elimination. Several techniques were used: inoculum of the enrichment cultures was filtered through 5, 1.5 and 0.8 µm pores or frozen at -20ºC for 2, 5, 8 and 11 days prior transferring to 120 mL serum bottles containing 50mL of mineral medium. Activity was monitored by gas chromatography (GC) and GC-mass spectrometry, and the presence of eukaryotes was visualized by microscopy. The effect of these methods on the microbial community will be analyzed by 16S rRNA gene amplicon sequencing and qPCR. This study will help to better understand the effect of global warming on the carbon cycle in Arctic ecosystems and the global budget of CH4.

BaMa-P18
Saliva as a specimen tested in surveillance of meningococcal carriage
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Neisseria meningitidis (meningococcus) is a major causative agent of invasive bacterial disease, which is usually manifested by septicemia and/or meningitis. Meningococci are also commensals of the human respiratory tract. Available vaccines target strains of only selected serogroups from 13 reported to be clinically relevant. Vaccine effects are monitored in surveillances of invasive meningococcal disease (continuous) and of carriage (periodical). The common method to detect meningococcal carriage is selective culture of oropharyngeal swab. Recently Manigart et al. showed that using molecular methods, increases the sensitivity of carriage detection. Carriage of meningococci can spread from person to person via saliva, suggesting that oral fluids could be a specimen to replace oropharyngeal swabs. Collection of saliva is simpler than oropharyngeal swab.
The overall aim of this study is to find the optimal sampling method for meningococcal carriage detection. Different sample types from various carriage studies across all age groups are used. DNA is isolated from polymicrobial samples with Qiagen DNeasy Blood & Tissue kit and tested with quantitative PCR for the presence of sequences unique for meningococcal genes ctrA, metA, natC and ackA1.
Up to now, we tested 63 saliva samples collected from 26 schoolchildren in 2015-2016 for the presence of metA. Of these, 11 (17,5% of 63) samples collected from 7 (26.9% of 26) students were positive. In one of the students, five samples collected over a period of 8 months were positive for metA. We also observed high concordance between metA and ctrA results. We are currently optimizing natC and ackA1 qPCR assays. These preliminary results suggest to us that saliva can effectively be used as diagnostic specimen to detect meningococcal carriage. The low invasiveness of taking saliva samples make for a better alternative for meningococcal carriage surveillance studies in all age groups.

BaMa-P19
Interactions of *Aspergillus niger* compounds with immune receptors
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Achtergrond: Aspergillus species are worldwide distributed and ubiquitous in the environment, among this genus relevant industrial and medical relevant species are present. For instance, A. niger is used in industrial production systems and has also been reported to cause infections. Extracellular compounds from Aspergillus spp. can play various roles during human infections, for example by modulation of immune system response. An Aspergillus niger strain (D15#26) with a mutation in the LaeA gene, a regulator for secondary metabolites, has been found to produce very small compounds that bind to certain immune receptors. This has been determined with a competition binding assay using a mixture of human neutrophils, leucocytes and monocytes. We want to identify these fungal compounds that interact with immune receptors.
Materiaal/methoden: Spores isolated from A. niger D15#26 strain will be transferred to a liquid transformation medium supplemented with glucose. After 16h at 30°C the mycelium will be transferred to a minimal medium supplemented with either xylose or maltose and grown for 72h at 30°C. The supernatant obtained from this culture will be filtered with Miracloth (Merck, Darmstadt, Germany). This filtered supernatant will be filter sterilized and small molecules are obtained via filtration through a Millipore (Merck, Amicon, Germany) 3 kDa filter. With this 3 kDa sample a competition binding assay will be performed. Small molecules will be extracted with organic solvents and analysed via Thin Layer Chromatography.
Resultaten: Tricolor analysis showed that 3kDa-filtrated supernatants contain molecules that bound to immune receptors. In particular CD181 and CD182 were bound by the D15#26 sample.

BaMa-P20

Saliva-based longitudinal study on *Streptococcus pneumoniae* carriage of in schoolchildren

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Streptococcus pneumoniae (pneumococcus) is the leading cause of bacterial pneumoniae and invasive bacterial disease. Carriage is a prerequisite for disease and young children are considered to be the main reservoir of pneumococci. The golden standard for pneumococcal carriage detection is conventional culture of a nasopharyngeal swab. However, there is evidence that testing saliva with molecular diagnostic methods is not inferior to this gold standard. Collection of saliva is much less invasive compared to a nasopharynx swab.
Here, saliva samples (n=508) were collected monthly between March 2015 and March 2016 from students (n=51, 5-13 years of age) of a single primary school in Utrecht. Pneumococcal carriage was detected by conventional culture and by testing DNA extracted from culture-enriched harvests using qPCRs targeting pneumococcal-specific genes *lytA* and *piaB*. Cultured pneumococcal strains were serotyped using the Capsule Sequence Typing (CST) method. Overall, 68 of 508 samples (13%) and 31 of 51 children (61%) were identified as carriers by culture whereas 392 (77%) of 508 of samples were positive for pneumococcal carriage by qPCR with period prevalence reaching 96% (49 of 51 students by the sixth month of the study). There were no differences in carriage prevalence between study time points. A capsular sequence type (serotype) was determined for 66 cultured pneumococcal isolates (97%) with serotypes 6C (15%), 19F (13%), and 24F (12%) being the most common.
To our knowledge, this study is the first longitudinal study on pneumococcal carriage in school-aged children in the Netherlands. The preliminary conclusion is that saliva can be efficiently used in carriage surveillance and provides accurate representation of carriage parameters in that age group. Currently, molecular methods are being applied to detect serotypes present in culture-enriched samples in order to accurately provide information regarding co-carriage of multiple serotypes.

BaMa-P21

***Aspergillus fumigatus* conidia and protection against oxidative stress**

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Aspergillus fumigatus is one of the most common invasive airborne fungal pathogens to be regularly inhaled. In healthy individuals, spores are eliminated by the immune system, but in immunocompromised individuals and in patients with several lung diseases, it can often lead to aspergillosis. The incidence rate of aspergillosis has been steadily rising due to increased employment of immunosuppressive agents for solid organ and stem cell transplants. Intracellular survival of the Aspergillus conidia in macrophages or epithelial cells requires protection against the oxidative burst which generates Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) intracellularly. This research project focuses on the protective role of conidial components of A. fumigatus against this oxidative burst.
We investigated if DHN-melanin could protect against hydrogen peroxide or hydroxyradicals by comparing a wild type A. fumigatus and pksP mutant which lacks this DHN-melanin. We did not observe a significant increase in sensitivity of the mutant. Research performed with the yeast Cryptococcus neoformans showed L-DOPA melanin as an important molecule for protection against hydroxyl radicals due to the presence of quinone structures in DHN-melanine. Also indol-containing compounds were shown to act as radical scavengers. We propose that Aspergillus conidia contain other protective compounds which have a similar structure and/or radical scavenging ability.

Two indol containing metabolites, fumigaclavine and fumitremorgin, are present in the vast majority of conidia from different A. fumigatus isolates. We will investigate if these compounds indeed have a protective function in conidia by firstly, as a proof of principal, look if they can protect Cryptococcus neoformans lacking L-DOPA melanin against ROS. Secondly we will generate A. fumigatus mutants lacking fumigaclavine and fumitremorgin and investigate whether protection for ROS and RNS is compromised. We propose that these indol containing secondary metabolites act as radical scavengers which ensure conidial survival in the macrophage.

BaMa-P22

Interaction between *Aspergillus fumigatus* and *Acanthamoeba castellanii*

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Background

In every inch of soil there is an ongoing battle taking place between microorganisms and free living amoebae. These amoebae, including Acanthamoeba castellanii, eat and digest bacteria, viruses and fungi. Some of these microbes possess mechanisms to evade the killing by amoeba's like A. fumigatus . This fungus is an opportunistic pathogen and can cause non-invasive and invasive infections in humans. Conidia of this fungus survive for a long time after ingestion by A. castellanii and other amoebae species and are able to escape and kill the amoeba at time of germination. The mechanisms enabling A. fumigatus to circumvent killing by amoebae may also facilitate survival in vertebrate macrophages and other phagocytic cells.

In this project, we investigate the interaction between A. castellanii and A. fumigatus. We will compare the behavior of A. fumigatus to its non-pathogenic cousin A. niger . Previously, we showed that A fumigatus is internalized more efficiently in epithelial lung cells and has a prolonged dormant state as compared to A niger.. Also mutants of A fumigatus lacking DHN-melanine, pyomelanine, RodA or RodB will be compared to the wild type. DHN melanine and RodA mutants are less virulent in animal models.

Our data may provide further strength for the use of this amoeba model as a macrophage model.

Methods

By co-culturing A. fumigatus and A. castellanii, differences in the interaction between the different fungal strains and the amoeba will be investigated. Confocal microscopy will be used to monitor attachment, internalization and germination of the conidia. For this cause, strains expressing fluorescent protein will be constructed. Extracellular and intracellular conidia can be distinguished using calcofluor white chitin stain. Moreover, survival and growth of the amoeba and fungus will be investigated. Propidium iodide staining will be used to determine killing of amoeba.

BaMa-P23

Competition and coexistence of nitrifying bacteria in a lab-scale membrane bioreactor as affected by changing oxygen and ammonia concentrations

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Excess nitrogen in the form of ammonium (NH4+), nitrite (NO2-) and nitrate (NO3-) is toxic to aquatic life and leads to eutrophication of inland and costal water bodies. Ammonium removal in engineering systems is accomplished through the activity of nitrifying microorganisms, which oxidize ammonia to nitrate. Nitrification can be mediated either by two distinct groups of chemolithoautotrophic microorganisms, the ammonia-oxidizing prokaryotes (AOP) and nitrite-oxidizing bacteria (NOB), or, as recently discovered, by some Nitrospira species capable of complete nitrification (comammox). All comammox bacteria known to date belong to Nitrospira sublineage II and can be highly abundant in engineered environments, but their exact role in these systems remains unclear. Comammox Nitrospira appear to out-compete canonical AOP under highly oligotrophic conditions. In engineered systems nitrifying microorganisms are often exposed to oxygen-limited conditions. Based on genome analysis, comammox Nitrospira appears to be much adapted to microaerophilic conditions and may have a competitive advantage over canonical AOP at low oxygen concentrations. This study aims to investigate the influence of ammonium and oxygen availability on the competitive success of different nitrifying microorganisms. A stable co-culture of nitrifying bacteria was established under oxygen-limited conditions in a lab-scale membrane bioreactor. The microbial population in the reactor system was examined with an integrated approach of molecular techniques including fluorescence in situ hybridization (FISH) and polymerase chain reaction (PCR). Nitrosomonas and Nitrospira-like species were found to dominate the microbial community. An amoA-targeted PCR approach was used to confirm the presence of comammox Nitrospira in the enrichment culture. Furthermore, FISH and PCR-based quantification of comammox Nitrospira and AOB amoA gene copy numbers, together with 16S rRNA and nxrB genes, will be used to monitor changes in the nitrifying community at different concentrations of oxygen and ammonium. This approach is optimally suited to elucidate how comammox Nitrospira contributes to nitrification in oxygen-limited nitrogen removal systems.

BaMa-P24

Genetic and physiological characterisation of novel methanotrophs discovered on the volcanic island of Pantelleria (Italy)

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Methane is an important greenhouse gas contributing to global warming, and is produced by abiotic and biotic processes. An important biological sink for the released methane are methanotrophs, organisms able to use the oxidation of methane as their energy source. Methane oxidisers were previously thought to be only present within the phylum Proteobacteria, but in 2007 methanotrophs were also discovered within the phylum Verrucomicrobia. This study aims at expanding our knowledge on the role of Verrucomicrobia in methane oxidation, by enriching and characterising novel methanotrophic species. In order to detect new genomes, DNA was extracted from soil samples excavated on the volcanic island of Pantelleria (Italy), and used for metagenomics analysis. Additionally, these soil samples acted as a starting material to inoculate a minimal medium containing methane as the only energy source. Here, we present the genome and metabolic capacity of a novel Verrucomicrobia species, for which we propose the genus Ca. Methylacidithermus pantelleriae. Furthermore, we describe an enrichment culture actively consuming methane at pH 3 and 50 °C, in which 16S rRNA analysis revealed an uncharacterised Verrucomicrobium species belonging to the genus Methylacidimicrobium. Taken together, our results add new insights to the world of Verrucomicrobia by describing the genetic and physiological characteristics of two novel methanotrophs able to thrive in the extreme temperatures and pH values of volcanic environments.

BaMa-P25

Implementation of automatic disk diffusion using WASP/WASPlab and Adagio.

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Urinary tract infections (UTI) are the most common type of infection in the world. Stichting PAMM does diagnostics for hospitals and practitioners is south-east Brabant. After a patient is diagnosed with a UTI the resistancepattern of the pathogen has to be determined. The current methods are expensive and time-consuming. To reduce costs and hands-on time, PAMM strives to automate susceptibility testing (AST) by implementing automatic disk diffusion with WASPlab and Adagio for gram negative rods (GNR), staphylococci and enterococci. Also it is researched if incubation time can be shortened to six hours to determine a suitable treatment faster.
914 GNR and 48 staphylococci were tested by Vitek®2 and 40 enterococci were tested by manual disk diffusion and agar dilution. These strains were also tested with automatic disk diffusion and read by the Adaio software. Three discrepancies could be detected when comparing the methods: minor, major or very major. According to the International Organization for Standardization document 20776-2:2007 each tested antibiotic has to show an overall agreementpercentage of ≥90%. Major and very major percentages have to be ≤3%.
After normal incubation (18±2 hours) all results for the three groups lie within the set margins except for amoxicillin-clavulanate. For amoxicillin-clavulanate the overall agreement is 83,5% for GNR. The percentage very major discrepancies is 15,5%. After six hours the staphylococci and enterococci didn't grow enough to read automatically. For GNR almost all results lie within the margins. Amoxicillin-clavulanate has an overall agreement of 85,1%. The percentage very major discrepancies is 12,2%. For co-trimoxazole the percentage very major discrepancies is 3,7%. The method can be implemented for all three groups barring amoxicillin-clavulanate for GNR. For GNR the incubation time can be shortened barring amoxicillin-clavulanate and co-timoxazole. For amoxicillin-clavulanate and shorter incubation times more research for the most optimal breakpoints is necessary.