BaMa-O01

Enrichment of novel comammox Nitrospira from a Dutch drinking water treatment plant

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Enrichment of novel comammox Nitrospira from a Dutch drinking water treatment plant

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Nitrification, the aerobic oxidation of ammonia to nitrate via nitrite, was discovered as a two- step process mediated by two distinct groups of microorganisms, the ammonia-oxidising bacteria (AOB) and nitrite-oxidising bacteria (NOB). Only recently it has been shown that complete ammonia oxidation to nitrate (comammox) is catalysed by members of the genusNitrospira, which were previously considered as strict nitrite-oxidising microorganisms. Comammox Nitrospira form two divergent clades, referred to as clade A and B based on phylogenetic analysis of the ammonia monooxygenase (AMO) gene. Both clade A and B comammox Nitrospira were identified in engineered and natural environments. Despite the presence of clade B comammox Nitrospira in many natural and man-made environments, all available comammox enrichment cultures to date contain clade A comammox bacteria and only one comammox pure culture is available.

Metagenomic analyses indicate that Dutch drinking water plant (DWTP) samples harbour comammox Nitrospira with clade B being the most abundant, especially in the wall biofilm. This study aims to enrich novel comammox Nitrospira from DWTP samples using three parallel trickling biofilter systems that are designed to mimic the sand filtration system. Each system will be inoculated with samples collected from filters sand-beds and wall biofilm. The microbial community will be examined with an integrated approach of molecular techniques including fluorescence in situ hybridisation and clone library analyses of the amoA, nxrB, and 16S rRNA genes. Additionally, an ammonium-limited sequencing batch reactor was used to obtain a highly enriched culture of a novel clade A comammox Nitrospira from the wall biofilm. amoA-Targeted PCRs indicated that comammox Nitrospira were the only ammonia- oxidising bacteria in this enrichment and thus will be used to obtain a comammox Nitrospirapure culture.

In conclusion, this study shows that DWTPs are an excellent source for the enrichment of novel comammox Nitrospira. Enrichment and isolation of these enigmatic newly discovered organisms are necessary to better understand their physiology and contribution to nitrification in engineered and natural ecosystems.

BaMa-O02

Uptake of sialic acid by non-typeable Haemophilus influenzae increases complement resistance through decreasing IgM-dependent complement activation

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Introduction:Non-typeable Haemophilus influenzae (NTHi) is a member of our normal upper respiratory tract microbiome, but is also a major cause of mucosal infections of the upper and lower respiratory tract resulting in for instance otitis media in children and pneumonia in the elderly. Uptake of sialic acid by NTHi has been shown to be a major determinant for its pathogenic potential whereby resistance to complement-mediated killing is increased. However the mechanism of action is thus far not elucidated. In this study, we provide evidence that growth of NTHi in the presence of sialic acids decreases complement-mediated killing through abrogating the classical pathway of complement activation, largely by preventing IgM antibody binding to the bacterial surface.

Methods: Four different NTHi strains were collected from patients with different disease aetiologies and grown with and without supplementation of sialic acid. C5b9 pore formation was determined using serum in a Sytox assay. Serum survival was determined using different concentrations human serum. Binding of complement C3, C5b9 complex, IgG, IgM, CRP and factor H to the bacterial surface was determined using flow cytometry.

Results: Flow cytometric analysis revealed decreased binding of complement C3 and C5b9 complex for all NTHi strains grown in medium supplemented with sialic acid. C5b9 pore formation was lower for all NTHi strains grown with sialic acid. In accordance with decreased pore formation, uptake of sialic acid increased complement resistance for all NTHi strains, although the magnitude was strain dependent. In a search to identify the mechanism by which sialic acids increase complement resistance, no differences in binding of factor H were found, while clear differences in IgM binding to the bacterial surface were

detected between strains grown with or without sialic acid. Sialic acid-mediated decrease in IgM binding is required for increased complement resistance demonstrated in IgA/IgM deficient serum with or without IgM supplementation.
Conclusion:This study shows that (1) uptake of sialic acid by NTHi results in increased complement resistance of NTHi, (2) sialic acid-mediated complement resistance in NTHi is not factor H dependent and (3) sialic acid-mediated complement resistance in NTHi is associated with decreased binding of IgM to the bacterial surface. These findings could be used in the design of strategies against NTHI infections.

BaMa-O03
A love and hate relationship: Nitrogen removal by a co-culture of comammox and anammox bacteria
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Complete nitrogen removal from wastewater is crucial to prevent eutrophication. To achieve this, wastewater treatment plants mostly combine nitrification and denitrification. Firstly, ammonia is aerobically oxidized to nitrite by ammonia-oxidizing microorgansims and subsequently to nitrate by nitrite-oxidizing bacteria. Secondly, anaerobic reduction of nitrate and nitrite to dinitrogen gas is performed by heterotrophic denitrifiers. This classical approach has a high oxygen demand for the nitrification step. In addition, the high organic carbon demand of denitrification make it unsustainable. With the discovery of anaerobic ammonium-oxidizing (anammox) bacteria it became possible to achieve completly autotrophic nitrogen removal and this process is increasingly employed in wastewater treatment.
Recently, our understanding of the nitrogen cycle has been further challenged by the discovery of the complete ammonia oxidation (comammox) by a single microorganism. While the termodinamic feasability of complete nitrification was well known, these microorganisms were only discovered recently. Interestingly, these comammox Nitrospira were obtained in a hypoxic enrichment culture from an aquaculture biofilter that also contained anammox bacteria. This finding was puzzling, as Nitrospirahave an aerobic lifestyle, while anammox bacteria require anoxic conditions. Thus, understanding the interactions between commammox and anammox bacteria might prove critical to apply these in sustainable wastewater treatment.
We hypothesize that comammox bacteria perform a novel type of reaction, nitrite comproportionation. Here, they would couple ammonia oxidation to nitrate reduction, thereby providing anammox with additional nitrite and thus enhancing anammox activity. In order to investigate this hypothesis, we established an anammox-comammox coculture on synthetic medium. We used fluorescence in situ hybridization (FISH) and polymerase chain reaction (PCR) to characterize the composition of this co-culture and to ensure its stable composition during physiological characterisation experiments. To better understand the interactions occuring and to test for nitrite comproportionation, we performed activity assays using stable isotopes (^{15}N-labelled ammonia or nitrate). The isotopic composition of the nitrogen gas produced by anammox will be analysed and used to infer the activity and reaction rates for both interaction partners.
In conclusion, this research investigates a novel metabolic link between two key nitrogen cycle organisms. The knowledge obtained here can be used to better comprehend nitrogen cycling in engineered systems, and for the optimization of efficient and sustainable wastewater treatment.

BaMa-O04
Differential expression of nutrient transporters in the compartmentalized anammox bacterium Kuenenia stuttgartiensis
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Anaerobic ammonium oxidizing (anammox) bacteria play an important role in the nitrogen cycle as they are responsible for about half of the oceanic nitrogen loss to the atmosphere through conversion of ammonium to N_2 with nitrite as the terminal electron acceptor (the anammox reaction). Anammox bacteria contain a cell organelle, the anammoxosome, where the anammox reaction takes place.

However, it is unclear how the anammox substrates enter the anammoxosome or how end products are excreted. The genome sequence of the model anammox organism Kuenenia stuttgartiensis revealed the presence of no less than seven ammonium, six nitrite, and two nitrate transporters. A reason for the high redundancy in nutrient transporters could be that different transporters are expressed under different growth conditions. We investigated this hypothesis by using comparative transcriptomics on K. stuttgartiensis, grown in a continuous flow single cell membrane bioreactor under nitrite- versus ammonium limited conditions. Triplicate RNA samples per condition were sequenced by Illumina MiSeq RNA sequencing. Data was analysed using CLC genomics and statistical analysis was performed using DESeq2. Of the six amtB ammonium transporters, Amt-1, -2 and -3 were significantly upregulated under ammonium-limited conditions and amt-4 was upregulated under nitrite-limited growth conditions. Of the six focA nitrite transporters, focA_2 showed significant induction under nitrite-limited growth conditions. Nar1/FocA_6 showed low expression but significant upregulation under ammonium-limited conditions. The nark nitrate transporters did not show differential expression. In addition, several genes involved in the anammox reaction were also differentially expressed: nitrite reductase (nir), nitrite oxidoreducase (nxr) and hydroxylamine oxidoreductase (hao) were upregulated under nitrite-limited condiotions, and hydrazine synthase (hzs) and hydrazine dehydrogenase (hdh) were upregulated under ammonium-limited growth conditions. The electron transport chain associated genes were not significantly up- or downregulated. We conclude that 1) part of the redundancy in nutrient transporters in K. stuttgartiensis can indeed be explained by differential expression under different growth conditions, 2) availability of ammonium and nitrite also affects the expression of enzymes involved in the anammox reaction, but 3) not the expression of genes involved in the electron transport chain.

BaMa-O05

The optimization of the in vitro cultivation of four strains of Plasmodium falciparum from ring stage to sporozoite

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“The optimization of the in vitro cultivation of four strains of Plasmodium falciparum from ringstage to sporozoite”
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Graduation internship at:
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Introduction:
Despite decades of control efforts, malaria is still a global problem, with an estimated of 216 million people infected and 500.000 to 600.000 deaths each year (2017 WHO malaria report). Understanding the biology of human malaria infections and how the mosquito-salivary gland sporozoites are able to migrate in the human or rodent host from the site of mosquito inoculation is important to ultimately control the parasite. Particularly sporozoites expressing fluorescent markers such as MC(Mcherry), GFP (Green fluorescent protein) or bioluminescent markers (e.g. Luciferase) are useful for following the migratory paths of sporozoites and their interaction with different immune cells. To do such experiments, sporozoites need to be harvested from mosquito salivary glands 2-3 weeks after feeding gametocytes to the mosquitoes. The purpose of this study is to optimize the in vitro cultivation of four strains of P. falciparum from ringstage to gametocyte to enable the production of high numbers of sporozoites in the mosquitoes.
Methods:
Four strains of P. falciparum are cultured in flasks in a semi-automated shaker and during the sexual stages P. falciparum produces gametocytes. To assess the difference in parasite development between the strains, gametocyte cultures were followed over time for two weeks. Light microscopy was used to count three fields containing the asexual stages, the sexual stages and the uninfected erythrocytes. Subsequently, we changed the starting concentrations of gametocyte cultures to 0,6%-0,8% and also compared development of gametocyte cultures that were started with fresh blood and those started with 7 days old blood. Results were gathered by dissecting the mosquitoes and obtaining oocysts and the sporozoites.
Results:
Comparing the development of the different strains showed that the WT(Wild Type) strain produces higher numbers of the sexual stages and gametocytes than the other strains. Increasing the starting concentrations of the WT with a parasitemia of 0,6% resulted in an average of 60k sporozoites per

mosquito. The MC had a parasitemia of 0,7% and an average of 1,2k sporozoites per mosquito. GFP had a parasitemia of 0,8% with very low numbers of sporozoites.
Stage V gametocytes which had been started on 7 days old blood looked deformed as compared to their counterparts which were started on fresh blood.
Conclusion:
Screening the different strains revealed differences between the WT, MC and GFP strain, whereby the WT parasites produced significantly more of the sexual stages.
The start concentration of the gametocyte cultures and the age of the red blood cells are important parameters that determine the production of gametocytes and ultimately sporozoites.

BaMa-O06

Collateral Sensitivity Network of Antibiotic-Resistant Streptococcus pneumoniae

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Collateral Sensitivity Network of Antibiotic-Resistant treptococcus pneumoniae
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Introduction: Collateral sensitivity (CS) is the phenomenon in which a bacterial population with a developed resistance to one antibiotic displays increased sensitivity to a second antibiotic. This is a promising strategy in combating antimicrobial resistance in different bacterial species. Previous work has found that CS and CR responses are shown in various bacterial species, such as Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus. The aim of this study was to assess the presence of collateral responses in S. pneumoniae. Given that collateral responses are observed, we will decipher the collateral networks of S. pneumoniae in order to combat antibiotic resistance in this species.
Methods: All experiments were conducted using isogenic antimicrobial-resistant mutants of the S. pneumoniae R6 strain selected at antibiotic (ciprofloxacin, co-trimoxazole, fusidic acid, linezolid, penicillin, rifampicin or trimethoprim) concentrations higher than or equal to the epidemiological cut-off value set by the European Committee on Antimicrobial Susceptibility Testing (EUCAST). Species identification of antibiotic-resistant mutants was confirmed by amplifying the species-specific lytAgene and/or using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF).
To determine the minimal inhibitory concentration (MIC) of both wild-type (WT) and mutant derivatives of S. pneumoniae R6, broth dilutions were performed according to EUCAST and ISO 20776-1:2003 guidelines with minor modifications. Briefly, Mueller Hinton II (MHII) cation-adjusted broth was supplemented with 100 U of catalase instead of 5% defibrinated lysed horse blood. MIC values for ciprofloxacin, co-trimoxazole, daptomycin, fusidic acid, linezolid, penicillin, rifampicin, sulfamethoxazole, tetracycline, trimethoprim and vancomycin were determined in triplicate on a 1.5-fold testing scale. A WT S. pneumoniae R6 strain was used as quality controls.
Results: CS was observed among all selected antibiotic-resistant mutants towards fusidic acid, linezolid, rifampicin and tetracycline. All linezolid-resistant mutants (n = 11) showed CS towards vancomycin, varying between a 1.5-fold and 12-fold decrease. Additionally, collateral resistance (CR) responses were observed among the majority of rifampicin (7 out of 11), linezolid-resistant mutants (8 out of 11) and high-level resistant ciprofloxacin-mutants (5 out of 11) towards daptomycin. All trimethoprim-resistant mutants (n = 8) showed CR towards ciprofloxacin.
Furthermore, reciprocal CS responses were detected for 3 out of 10 rifampicin-resistant mutants towards linezolid, 6 out of 11 linezolid-resistant mutants towards rifampicin and 4 out of 11 towards ciprofloxacin, 6 out of 11 ciprofloxacin-resistant mutants towards linezolid, 4 out of 10 rifampicin-resistant mutants towards co-trimoxazole and 6 out of 6 co-trimoxazole-resistant mutants towards rifampicin.
Conclusion: In conclusion, the presence of collateral responses in pneumoniae is assessed, as assumed at the start of this research project. Given that collateral responses are observed, a part of the collateral networks of S. pneumoniae in order to combat antibiotic resistance in this species is deciphered. Our results indicate that antibiotic cycling is a potentially interesting therapeutic strategy for treatment of S. pneumoniae, however, follow-up studies using strains of different genetic backgrounds and in vivo studies are essential.

BaMa-O07

The effect of the CO:H2 ratio on the ethanol flux in a co-culture of Clostridium kluyveri and Clostridium autoethanogenum

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The effect of the CO:H_2 ratio on the ethanol flux in a co-culture of Clostridium kluyveri and Clostridium autoethanogenum

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Syngas fermentation is a promising technology in the transition to a bio-economy. However, the product spectrum of most known carboxydotrophs, is limited to ethanol and acetate. Previously, it was shown that co-cultivation of Clostridium autoethanogenum and Clostridium kluyveri fed on CO produced chain-elongated products[1]. autoethanogenum converts CO to ethanol and acetate, C. kluyveri uses these products for chain elongation. The conditions that regulate the predicted ethanol flux from autoethanogenum to C. kluyveri are poorly understood. Here we studied the effect of the CO:H_2 feed ratio on the ethanol flux in pure C. autoethanogenum cultures and in co-cultures with C. kluyveri. Monocultures of autoethanogenum and co-cultures with ”kluyveri fed on a reference feed of CO were exposed to increasing supplemental H_2 feed fractions in gas transfer limited continuous stirred tank reactors. The lowest H_2 feed fraction tested in the monoculture was 1%(v/v). This corresponds to the predicted H_2 production by kluyveri in the co-culture. H_2 was increased stepwise up to 62%.
A 1% H_2 feed fraction was insufficient to trigger ethanol production in monoculture. Albeit, acetate production increased from 4.1±0.1 to 5.5±0.1 mmol.$gDW^{-1}.h^{-1}$ - between 0% and 28% H_2 feed fractions. At a 40% H_2 feed fraction ethanol production (0.41±0.01 mmol.$gDW^{-1}.h^{-1}$--) was triggered, albeit qEthanol remained 2-fold lower than the minimum predicted qEthanol in the co-culture (0.97±0.07 mmol.$gDW^{-1}.h^{-1}$). In co-culture, C.kluyveri always maintained a lower ethanol concentration than the CO and H_2 feed rates implied on C. autoethanogenum based on monoculture results. This trend was broken upon CO_2 depletion, excess H_2 was used for solvent production.
The results narrowed down to the suggestion of a thermodynamic sink for ethanol maintained by ”kluyveri as driving force the predicted flux to ethanol. It is proposed that understanding this effect can be exploited to further increase the productivity of the co-culture.

[1] Diender M, Stams AJ, Sousa DZ (2016) Production of medium-chain fatty acids and higher alcohols by a synthetic co-culture grown on carbon monoxide or syngas. Biotechnol Biofuels. 2;9:82.

BaMa-O08

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-O09
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