O01

Metagenomic global survey and in-depth genomic analyses of Ruminococcus gnavus reveal differences across host lifestyle and health status

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Ruminococcus gnavus is a prevalent gut bacterium found in >90% of healthy individuals, but its increased abundance is associated with chronic inflammatory diseases, particularly Crohn's disease. Nevertheless, its global distribution and intraspecies genomic variation remain understudied. Through a large-scale survey of 12,791 gut metagenomes, we recapitulated known associations with metabolic diseases and inflammatory bowel disease. We uncover a higher prevalence and abundance of R. gnavus in Westernized populations and observe relative abundances up to 83% in newborns. Next, we built a resource of R. gnavus isolates (N = 45) from healthy individuals and Crohn's disease patients and subjected these to PacBio circular consensus sequencing to generate complete R. gnavus genomes. Analysis of these genomes and publicly available high-quality draft genomes (total > 300 genomes) revealed multiple clades which separated Crohn's-derived isolates from healthy-derived isolates. Functional analyses of genes predicted to constitute R. gnavus virulence factors could not explain this separation. Bacterial genome-wide association study (GWAS) revealed that Crohn's-derived isolates were enriched in genes related to mobile elements and mucin foraging. Together, we present a large R. gnavus resource that will be available to the scientific community and provide novel biological insights into the global distribution and genomic variation of R. gnavus.

O02

Age-Related Decline in IgM Responses Associate with Reduced Bactericidal Activity Following Vaccination with Conjugated Pneumococcal or Meningococcal Polysaccharides

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Background: Vaccination is a key strategy in preventing Streptococcus pneumoniae and Neisseria meningitidis infections. However, vaccine responses often decline with age. We studied the functionality of humoral vaccine responses in different age groups by comparing IgG, IgM and functional antibody concentrations following vaccination with either the 13-valent pneumococcal conjugate vaccine (PCV13) or the quadrivalent meningococcal conjugate vaccine (MenACWY-TT).

Methods: In separate phase IV open-label studies, participants aged 10–15 years (n=204), 50–64 years (n=100), and 65-85 years (n=222) received MenACWY-TT, and participants aged 26–49 years (n=44), 50–64 years (n=71) and 65-98 years (n=141) received PCV13. Serum samples were collected pre-vaccination, at four weeks and one year post-vaccination. Polysaccharide (Ps)-specific (Ps) IgG and IgM concentrations were measured using a multiplex immunoassay, and functional antibodies were determined using the opsonophagocytosis assay (OPA) for pneumococci and the serum bactericidal assay rSBA) for meningococci as measure for bactericidal activity.

Results: Post-vaccination serotype-specific OPA and serogroup-specific rSBA were significantly lower in adults aged 50-64 years and ≥65 years compared to younger age groups (10–15 y or 26-49 years). Ps-specific IgG responses were generally less affected by age, whereas Ps-specific IgM responses showed a pronounced decline with increasing age. Moreover, strong correlations were observed between either OPA or rSBA responses and Ps-specific IgM responses across all age groups. In contrast, in case of several serogroups/serotypes, no or weak correlations between OPA or SBA responses and IgG levels were seen. Remarkably, rSBA titers significantly decreased upon IgM depletion but remained largely unaffected following IgG depletion.

Conclusion: These observations suggest that lower IgM, rather than IgG concentrations, could explain reduced conjugate vaccine-induced bactericidal activity in aging adults. As such, Ps-specific IgM responses could be evaluated alongside IgG to better understand vaccine-induced functional responses following pneumococcal and meningococcal conjugate vaccination.

O028

An Rcs stress-based high-throughput screen reveals novel gyrase inhibitors as indirect inducers of cell envelope stress in E. coli.

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The highly impermeable cell envelope of Gram-negative bacteria is an important hurdle to the development of novel antibacterials. However, compounds that disrupt the integrity of the cell envelope can act as potent antibiotics by directly inhibiting cell growth and viability or by enhancing the penetration of larger antibiotics. To identify such novel compounds, we used the European Lead Factory compound libraries to screen >500.000 small molecules for inducing the Rcs cell envelope stress response in E. coli. We identified a series of novel 2-quinolones and 4-quinolones that target gyrase and topoisomerase IV, suggesting unforeseen effects of such compounds on the bacterial cell envelope. Here, we show that the quinolones induce a structure-dependent profile of specific cell envelope stress responses. These response profiles were not only observed for quinolone-type but also for other gyrase inhibitors and occurred independently of SOS stress activation. Importantly, DNA damage and SOS response activation alone were insufficient to explain the high levels of cell envelope stress, underscoring gaps in our understanding of the interplay between gyrase function and maintenance of cell envelope integrity. Microscopy showed structural changes that are likely related to the observed stress. Importantly, cell elongation, associated with quinolone-induced SOS stress response, also occurred in SOS-deficient bacteria. These serendipitous findings highlight both the complexity of gyrase-associated bactericidal mechanisms and the challenges in antibiotic discovery. Nevertheless, this study supports the utility of stress-based assays as sensitive phenotypic tools for identifying new antimicrobial agents.

O03

Respiratory syncytial virus (RSV) incidence estimates in primary care among adults aged 50 years and older in the Netherlands

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Introduction: The burden of respiratory syncytial virus (RSV) in adults is mostly derived from hospital studies. To support decision making for preventive measures in older adults, this study aims to estimate the RSV incidence among adults aged 50+ in primary care in the Netherlands.

Methods: Seasonal RSV-associated incidence rates in patients needing medical attention in primary care, were calculated for respiratory seasons 2011/2012-2018/2019 by combining data from (1) Nivel Primary Care Database (Nivel-PCD): the weekly number of new acute respiratory infection (ARI) episodes, recorded by the general practitioner (GP), with (2) the weekly proportion of specimens that tested positive for RSV, derived from sentinel surveillance in 40 GPs that collected naso-/oropharyngeal swabs among patients with ARI. Seasonal number of contacts with the GP was calculated based on claims data. Analyses were conducted for all persons aged 50+, as well as patients considered to be at increased at risk for RSV infection due to ≥1 prevalent chronic condition.

Results: Across all respiratory seasons studied, the estimated RSV incidence rates increased with age ranging from 2.3-12.6/1,000 persons aged 50-54 years, to 4.1-18.6/1,000 persons aged 85+. Average RSV incidence rates were 30% higher for persons with chronic pulmonary disease when compared to other comorbidities, and almost twice as high when compared to the 50+ population. Number of contacts with the GP also increased with age, and was 50% higher among patients with ≥1 prevalent chronic condition.

Conclusion: This study shows that the incidence of RSV among adults aged 50+ in primary care in the Netherlands is considerable, especially among those aged 75+, and those with chronic pulmonary disease. Care demand was also increased in those groups. High-quality estimates of the RSV disease burden are needed to assess the public health impact of new prevention strategies for older adults and to improve patient outcomes.

O04

Unveiling the spectrum of Respiratory Syncytial Virus disease in Adults: from Community to Hospital

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Background: Respiratory syncytial virus can cause severe disease in the older adult population. Three vaccines for RSV are currently market approved but the risk of RSV-hospitalization in (older) adults from a community level remains elusive. We aimed to estimate the risk of RSV-hospitalization and characterize the patients that end up in hospital.
Methods: We manually analyzed records of adults aged ≥20 with RSV-infection between 2022-2024 in three hospitals in the Netherlands. These hospitals implemented routine RSV-testing at emergency departments. Using population-based data in combination with the in-hospital data, we estimated the population risk of RSV-hospitalization. Hospital records were analyzed to characterize the role RSV played in their course of disease.
Results: We analyzed 709 RSV cases of whom 503 (70.9%) were hospitalized. 526 patients were ≥60, and 183 were <60 years of age. The population risk of RSV-hospitalization was 0.006-0.02% for patients aged 20-59 years and 0.04-0.24% for those ≥60. The highest risks were seen in older patients with congestive heart disease (0.14-5.0%) and COPD (0.17-1.76%). RSV caused clinically relevant infection in 88% of hospitalized cases but was only mentioned using specific ICD-codes in 4.4%. Comorbidity was prevalent (88.5%) and exacerbation of underlying disease caused of 46.3% of RSV-related hospital admissions. ICU admittance was 11.2% and in-hospital mortality was 8.1%.
Conclusion: The risk of RSV-hospitalization from the community is low but is increased substantially in those with underlying disease. RSV is often clinically relevant in hospitalized patients by causing exacerbation of underlying disease but is infrequently mentioned in specific ICD-codes.

O05

Acute hepatitis B infection in a fully vaccinated person: a vaccine-escape strain in the Netherlands?

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Introduction

Hepatitis B virus (HBV) is an important cause of infectious hepatitis globally. Vaccines against HBV lead to life-long protection against infection. Vaccine failure due to mutations has rarely been reported. Here we describe an acute HBV infection in an adequately vaccinated person due to a D144A mutation in the (surface) S-gene.

Case

A 72-year-old man was seen by his general practitioner with gastrointestinal complaints. His liver function tests were abnormal. Serological and molecular testing indicated an acute HBV infection. These included positive HBV surface antigen (HBsAg), antibodies against HBV surface and envelop antigen (anti-HBs, anti-HBe), IgG and IgM antibodies against HBV core antigen (anti-HBc), and HBV DNA (1,4*10^4 copies/mL) in plasma. However, the patient had been fully vaccinated against HBV in 2003 with an anti-HBs control titer of >1000 IU/l. He denied risk behavior for contracting HBV infection.

The strain was a genotype A-2, which is an endemic strain in the Netherlands. Whole genome sequencing was unsuccessful, but the surface gene was sequenced and a mutation was found at location D144A in the S-gene, a mutation that has previously been described in relation to vaccine escape. This mutation has only been found in 4 other HBV strains that have been sequenced in the Netherlands between 2004 and 2024 by the National Institute for Public Health and the Environment.

Conclusion

This case shows that despite full vaccination, acute HBV infection may still occur. Physicians should not rule out this possibility in a vaccinated patient presenting with acute hepatitis. Surveillance of HBV strains is important to keep track of strains harboring vaccine escape mutations.

O06

Baseline immunity to highly pathogenic avian influenza viruses in a healthcare worker cohort

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Highly pathogenic avian influenza (HPAI) viruses from the H5 subtype increasingly spill over into mammals, including dairy cows and humans. Although there is no sustained transmission in humans, and only approximately 900 human infections have been reported, this increased spillover rate has raised concerns about future outbreaks. The relatively low number of human cases could be attributed to two factors: (1) poor adaptation to the new host, and (2) pre-existing immunity in the population. Humans are frequently exposed to seasonal influenza viruses through natural infection or (yearly) vaccinations. H5-specific antibodies in the population are uncommon, but specific T-cell responses have been reported in small studies. Based on in silico data, these T-cells are likely H1-specific and cross-reactive with H5, due to conserved epitopes in HPAI.

Here, we assessed baseline population immunity to HPAI from the H5 subtype in a large cohort of healthcare workers (HCW). We collected blood, nasosorptions, and nasal brushes. Binding antibodies in serum and nasosorptions were measured by protein microarray (PMA). Functional antibodies were measured through hemagglutinin inhibition assays performed on serum. T-cell responses were determined by interferon-gamma release assays performed on whole blood and confirmed via activation-induced marker assays.

Functional antibodies targeting recent seasonal influenza viruses were highly prevalent in HCWs, unlike HPAI-specific functional antibodies, which were rarely detected. However, both seasonal- and HPAI-reactive T-cells were detected frequently. Solid correlations between HPAI and seasonal responses indicate exposures to seasonal H1N1 viruses induced T-cells targeting both the HA and NA protein from HPAI.

In conclusion, we demonstrate that a cohort of HCWs frequently exposed to seasonal influenza viruses possesses baseline T-cell immunity to HPAI from the H5 subtype. The clinical relevance of these T-cells is yet to be fully understood; while virus-specific T-cells cannot provide sterilizing immunity upon infection, they could blunt the severity of human HPAI.

O07

Horizontal acquisition of the serotype 2 capsule locus and antimicrobial resistance genes drives the recent emergence of novel zoonotic Streptococcus suis lineages in Thailand

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Background: Streptococcus suis is a global emerging zoonotic porcine pathogen that causes sepsis and meningitis with high mortality rates in humans. It has become one of the leading causes of adult bacterial meningitis in Thailand due to widespread consumption of traditional raw pork products. While the majority of global zoonotic infections are caused by strains from lineage CC1 carrying a serotype 2 capsule, zoonotic infections in Thailand are caused by an unusually diverse set of lineages with ~40% of reported infections caused by two Thai endemic lineages, CC104 and CC233/379. In this study, we have characterised the emergence and recent evolution of these two lineages.
Methods: We whole genome sequenced 140 Thai S. suis zoonotic and porcine isolates and combined them with a curated global dataset of 2762 published S. suis genomes. We used comparative genomics, Bayesian evolutionary modelling and multi-dimensional scaling to investigate the emergence of multi-drug resistance and zoonotic potential in CC104 and CC233/379.
Findings: We identified multiple antimicrobial resistance acquisition events in the Thai endemic zoonotic lineages, with some strains carrying up to 12 resistance determinants against eight classes of antibiotics. Most importantly, CC104 and CC233/379 have both acquired increased resistance to penicillin and ceftriaxone, which are commonly used to treat S. suis infections, through recombination in their penicillin binding proteins. We estimated recent emergence dates for CC104 (1990; 95% posterior: 1987-1992) and CC233/379 (2002; 95% posterior: 2000-2004), which follow a period of intensification of pig farming in Thailand in the 1960s during which commercial pig breeds were imported from the UK and US. The emergence of both lineages was associated with two acquisitions of the serotype 2 loci from CC1.
Interpretation: Our study shows how horizontal transfer of AMR and capsular loci can lead to the emergence of novel zoonotic multi drug resistant S. suis strains

O08

Genomic features of Mycobacterium avium complex are associated with disease manifestation

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Introduction
Mycobacterium avium complex (MAC) bacteria are the most frequent nontuberculous mycobacteria (NTM) to cause opportunistic infections in humans. The MAC consists of 12 species, but M. avium, M. intracellulare and M. chimaera are most frequently isolated. MAC disease has multiple manifestations, including pulmonary disease, skin and soft tissue infections, disseminated disease and lymphadenitis. It is not known whether the distinct disease manifestations are caused by specific subgroupings of M. avium and other MAC bacteria. We aimed to associate phylogenetic groupings with specific disease manifestations.

Methods
Clinical isolates of MAC (unique MAC per patient) obtained from the Dutch NTM reference unit at Radboudumc were included in a retrospective study (2020 – 2023). Whole-genome sequencing was performed using Illumina short-read technology. Mycobacterial reads were extracted using an in-house produced mycobacterial database and Kraken2. Fastq files were run through the 'Bactopia' pipeline (version 3.0.1) to create fasta files. Next, phylogenetic relationships were inferred using core single-nucleotide polymorphisms (SNP) consensus parsimony. Detailed clinical characteristics obtained from consultations of the NTM reference unit were associated with genomic data.

Results
Overall, 382 isolates from 354 patients were included. While most patients had one species within MAC (92.4%), 26 patients had two species (7.3%) and one patient had three species within MAC (0.3%). The majority was genotyped as M. avium (48%), M. chimaera (21%) and M. intracellulare (11%). Isolates of patients associated with fibro-cavitary or nodular MAC pulmonary disease were widely distributed throughout the phylogenetic tree. Isolates of patients with lymphadenitis, skin and soft tissue infections and disseminated disease were only seen in the branch of M. avium and predominantly in one subbranch.

Conclusion
Extra-pulmonary MAC disease manifestations were specifically seen in M. avium and in specific subbranches thereof, suggesting that genotype is correlated with clinical manifestation.

O09

The role of human MPEG1 in severe Staphylococcus aureus infections

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Introduction: The molecular basis of interindividual clinical variability upon infection with Staphylococcus aureus is unclear. Life-threatening staphylococcal disease can result from single-gene inborn errors of immunity. A 1.5-year-old boy from a consanguineous family was hospitalized for sepsis due to a necrotizing pneumonia and concurrent bacteremia with S. aureus. Whole exome sequencing of the patient revealed two homozygous, ultra-rare, missense variants in macrophage expressed gene 1 (MPEG1), encoding Perforin-2. Perforin-2 is involved in the intracellular killing of bacteria by phagocytes and in endosomal antigen leakage by cross-presenting cells. We hypothesize that human autosomal recessive MPEG1 deficiency may predispose to life-threatening S. aureus infections.

Methods: To investigate the functional impact of the patient's alleles, CRISPR/Cas9 was employed to either introduce the patient's mutations or create a complete MPEG1 knockout in THP-1 cells. Endogenous MPEG1 expression was quantified in the patient's dermal fibroblasts and gene-edited THP-1 cells using qPCR and western blotting. Intracellular bacterial killing assays were conducted on PMA-stimulated THP-1 cells, to assess the role of MPEG1 in bacterial clearance. Additionally, a saporin-puromycin pulse-chase assay was used to evaluate the role of MPEG1 in facilitating antigen leakage from endosomes.

Results: qPCR analysis revealed strongly reduced MPEG1 mRNA expression in both the patient's dermal fibroblasts and gene-edited THP-1 cells compared with those of healthy controls. Consequently, Perforin-2 expression was markedly reduced in the patient's cells. Intracellular killing assays demonstrated a marked reduction in bacterial clearance in MPEG1 knockout THP-1 cells. The saporin-puromycin assay confirmed the role of MPEG1 in efficient endosomal escape of antigens.

Conclusion: Our findings highlight two key functions of MPEG1 in immune responses: intracellular bacterial killing and antigen leakage. Further functional characterizations of the patient's alleles are currently performed to elucidate the molecular mechanism by which autosomal recessive MPEG1 deficiency underlies life-threatening S. aureus infection in humans.

O10

Streptococcus pyogenes evades antibody recognition through decoration of the Group A Lancefield antigen with glycerol phosphate

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Introduction: Streptococcus pyogenes (or Group A Streptococcus, GAS) presents a frequent cause of mild to life-threatening infections. A recent study designated GAS as a priority pathogen for vaccine development based on its global morbidity and mortality. Several GAS vaccines are in development that include the species-defining Group A Carbohydrate (GAC), a conserved surface glycan which has been used in clinical diagnostics for decades. Three different GAC glycoforms are expressed on the bacterial surface: linear polyrhamnose, polyrhamnose carrying GlcNAc modifications, and a glycoform where approximately 25% of GlcNAc moieties are modified with glycerolphosphate (GroP). Preclinical vaccines include either the polyrhamnose or GlcNAc-containing variant but not the GroP-modified structure. However, the GroP modification affects susceptibility to host defenses such as sPLA2-IIA and Zn2+ toxicity. We therefore aimed to investigate how the presence of GroP modifications impacts antibody recognition of GAC.
Methods: Using a multiplexed bead-based flow cytometry assay, we measured systemic antibody responses (IgM, IgG1-3 and IgA) against the three different GAC glycoforms in human plasma samples from 16 healthy donors and 13 patients with invasive GAS infection using fully-defined synthetic structures. Additionally, we isolated single GAC-specific B cells from healthy donors to produce recombinant GAC-specific monoclonal antibodies.
Results: We observed the universal presence of GAC-specific IgM and IgG antibodies in plasma of healthy individuals and GAS bacteremia patients. Antibody reactivity was highest against the polyrhamnose-GlcNAc epitope. In both groups, the presence of GroP significantly decreased antibody binding to GAC-GlcNAc. We isolated GAC-specific B cells of different specificities and produced recombinant monoclonal antibodies with different epitope specificities as confirmed by binding to fully-defined synthetic structures.
Conclusion: Here, we provide insight in the human glycoform-specific antibody repertoire against GAC, an important vaccine antigen. Our findings suggest that the presence of GAC-GroP interferes with antibody recognition, which may affect GAC-related vaccine efficacy.

O11

Virulence traits of Mannheimia haemolytica targeting the bovine respiratory mucosal surface

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Bovine Respiratory Disease (BRD) is a critical disease with high mortality that particularly affects young calves. The pathogenesis in BRD is poorly understood, but coinfections of viral and bacterial pathogens are often observed. Mannheimia haemolytica is a commensal bacterium of the upper respiratory tract in healthy cows, but is found in the lower respiratory tract in diseased animals. Its pathogenic properties are poorly characterized. The aim of this study was to gain more insight into the capacity of M. haemolytica to establish infection on the bovine bronchial mucosal surface. Analysis of the genomes of multiple M. haemolytica clinical isolates demonstrated the presence of genes encoding for mucus O-glycan degrading enzymes, including fucosidase and sialidase enzymes. Sialidases and fucosidases are important virulence factors that catalyze the first steps of mucus degradation. In addition, sialic acids play important roles in viral interactions with mucosal surfaces. Enzyme assays demonstrated that all M. haemolytica isolates had sialidase activity whereas only one isolate showed fucosidase activity. M. haemolytica also showed enhanced growth in the presence of mucins. Next, bovine bronchial material was taken from calves and primary air-liquid cultures on Transwell plates were established. The bovine bronchial cultures were infected with M. haemolytica for 24 hours. In this time frame, the bacteria efficiently removed α2,3- and α2,6-linked sialic acids from the bovine mucosal surface. Using confocal spinning disk microscopy we observed that M. haemolytica was preferentially invading non ciliated-, potential mucus secreting cells. We conclude that M. haemolytica has unique virulence traits that aid colonization of the respiratory mucosal surface. In future studies, we will address whether these M. haemolytica traits such as sialic acid removal contribute to the bacterial-viral coinfections that are often observed in BRD.

O12

Endogenous viral elements can bias virus discovery

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Introduction: Large-scale metagenomic and -transcriptomic studies have revolutionized our understanding of viral diversity and abundance, allowing us to generate an unprecedented amount of viral genomic sequences useful to all kinds of studies, from virus discovery to phylodynamics/molecular epidemiology, especially in less studied organisms. In contrast, endogenous viral elements (EVEs), remnants of viral sequences integrated into host genomes, have received limited attention in the context of virus discovery. EVEs resemble their original viruses, making distinguishing between active infections and integrated remnants difficult, affecting virus classification and biases downstream analyses.

Methods: In our study, we assessed the effects of EVEs on a prototypical virus discovery bioinformatic pipeline. We examined EVEs (as detected by our screening tool detectEVE) and exogenous viral sequences linked to Orthomyxoviridae, a diverse family of negative-sense segmented RNA viruses, in 13 genomic and 538 transcriptomic datasets of Culicinae mosquitoes.

Results: Our analysis revealed a substantial number of viral sequences in transcriptomic datasets. However, a significant portion appeared not to be exogenous viruses but transcripts derived from EVEs. Distinguishing between transcribed EVEs and exogenous virus sequences was especially difficult in samples with low viral abundance. For example, three transcribed EVEs showed full-length segments, devoid of frameshift and nonsense mutations, exhibiting sufficient mean read depths that qualify them as exogenous virus hits.

Conclusion: Our study highlights that our knowledge of the genetic diversity of viruses can be altered by the underestimated presence of EVEs in high-throughput sequencing datasets, leading to false positives and altered or missing sequence information, which can affect downstream analyses, especially phylodynamics/molecular epidemiology. We also provide potential solutions to mitigate the risk.

O13

MicrobTiSDA: An R package for longitudinal microbiome data analysis

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Longitudinal analysis of microbiomes is crucial for understanding the temporal dynamics of host-associated or environmental microbiomes, laying the foundation for exploring ecological patterns and mechanisms of host-microbiome interactions. Sampling microbiota at multiple time points allows researchers to uncover community trends, stability, and responses to external interventions such as therapies. Thus, developing efficient and accurate methods for microbiome time-series analysis has become increasingly urgent.

We developed MicrobTiSDA, an R package designed for microbiome time-series analysis. It employs regression models to explore the temporal dynamics of microbial communities and integrates Learning Interactions from Microbial Time-Series (LIMITS), which uses the discrete-time Lotka-Volterra model to infer interspecies interactions. These interactions could be incorporated into the regression models to enhance their explanatory power. MicrobTiSDA simultaneously evaluates species abundance trends over time and the impact of interspecies interactions. Additionally, it provides tools for analyzing temporal dynamic similarities within microbial communities, offering new perspectives on functional relationships.

MicrobTiSDA was applied to two datasets: one focused on the individual-level gut microbiota of 12 infants during their first year of life, and the other on the group-level gut microbiota of septic preterm infants. At the individual level, MicrobTiSDA revealed significant differences in microbial interaction networks and the highly specific dynamics of gut microbiota. At the group level, MicrobTiSDA identified operational taxonomic units (OTUs) with potential disease associations, displaying highly similar temporal patterns that correlated with disease onset. For example, in septic preterm infants, the abundance of the Escherichia-Shigella genus in the gut increased significantly prior to disease onset, whereas the non-septic preterm group exhibited an opposite trend. These findings align with the detection of Escherichia coli pathogens in the peripheral blood of affected septic patients.

MicrobTiSDA significantly enhances the toolkit for longitudinal microbiome analysis, providing robust support for advancing microbiome research.

O14

euk-mOTUs: a universal marker gene-based profiling tool to study eukaryotes in microbial communities

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Eukaryotic microorganisms play critical roles in microbial communities, but much remains to be learnt about how they shape community composition and dynamics, even for well-studied human microbiomes. To enable such studies, we developed a software tool, euk-mOTUs, designed to facilitate the detection and quantification, i.e. taxonomic profiling, of eukaryotic microorganisms in shotgun metagenomes. This tool is based on a database featuring diverse fungi, flatworms, nematodes, and protists. From their genomes 20 universal single copy marker genes were selected for their suitability as taxonomic markers, allowing for profiling of 2507 operational species-level taxonomic units (mOTUs).

The euk-mOTUs profiler was benchmarked against previously published tools for eukaryotic profiling using simulated metagenomes designed to closely resemble real metagenomes derived from fecal samples. Results from this benchmark indicate that euk-mOTUs achieves precision and recall, performing similar to the best-performing other eukaryotic profilers, with very good in quantification accuracy.

Additionally, we compared euk-mOTUs profiles generated from fecal metagenomes to qPCR-derived data on the presence or absence of eukaryotic parasites in a small study population from a low-/middle income setting. This showed euk-mOTUs to outperform other eukaryotic profilers in accuracy as compared to qPCR-derived estimates.

We applied euk-mOTUs to a large collection of fecal metagenomes to gain an overview of the most prevalent and abundant micro-eukaryotes in the human gut across geographically diverse human populations. Currently we are exploring if the presence of micro-eukaryotes is associated with prokaryotic community composition and ecology. Detailed information on eukaryota-bacteria interactions across diverse life-style and disease contexts will add an intriguing facet to our understanding of the human gut microbiome.

O15

Localization and functional analysis of spore germination proteins in Bacillus spores

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Cells of bacteria of Bacillus and Clostridium orders are well known for producing dormant, highly resistant spores capable of surviving extreme environments, including high temperatures, desiccation, radiation, and antibiotic exposure. This resistance is primarily attributed to the spores' unique structure, which includes multiple protective layers, a low-water-content core (25–40%) with high levels of dipicolinic acid (DPA) chelated with calcium ions (Ca^{2+}) in a 1:1 ratio (CaDPA). When nutrients appear in environment, spores are triggered to germinate, and lose their resistance and ultimately outgrow into vegetative cells. In spore-forming bacteria that cause disease, germination is often trigged by L-alanine, a crucial early step in disease progression, making it a promising target for interventions. Previous studies with the pathogenic spore-former Bacillus cereus demonstrated that the L-alanine binding GerRB clusters in foci, similar to the germination protein complex (germinosome) in Bacillus subtilis. GerRB and the germinosome scarffold protein GerD exhibit Förster Resonance Energy Transfer (FRET) indicating a proximity of < 10Å. In addition, GerRB and SpoVAEa colocalize, indicative of putative interactions of the germinant receptor (GR) GerR and at least one protein that is a part of spores' CaDPA channel.

In this study, we have investigated spore germination in B. subtilis, as it is a model organism and has genetic tractability. In B. subtilis, GRs GerA, GerB, and GerK, along with the scaffold protein GerD, form germinosomes, and previous study on GerKB show its dynamic changes during germination. GerAB is one of the three proteins of the GerA GR, and functions as an L-alanine sensor and probably a water channel. Using fluorescence microscopy, live imaging, and FRET, we have visualized GerAB in B. subtilis spores, studied its colocalization, and interaction with GerD during germinosome formation, and dynamic changes in interactions with SpoVA proteins during germination.

O16

The novel tiny regulator WerA of Mycobacterium marinum controls expression of the ESX-4 secretion system and putative substrates implicating ESX-4 in interbacterial warfare

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Mycobacteria produce up to five type VII secretion systems (ESX1-5) to export protein substrates for virulence and nutrient uptake to the cell surface and environment. The ancestral ESX-4 system is not well understood, and is often not even expressed in pathogenic mycobacteria like Mycobacterium marinum. We hypothesized that we might learn more about ESX-4 function by studying ESX-4 regulation.

Therefore, we generated mCherry reporter strains for the ESX-4 substrates EsxU and EsxT and performed a transposon screen. We found insertions in a previously unknown tiny ORF coding for a 47 aa protein, which we dubbed “Wee ESX regulator A” (WerA). We could show that WerA is a tiny regulatory protein and not regulatory RNA, because only in frame mutations disrupted function. Knockout strains of werA produced 200-fold more ESX-4 mRNA than wildtype controls. Remarkably, ESX-4 protein levels were undetectable, indicating another layer of regulation. By quantifying individual bacteria with flow cytometry, we find that werA mutants uniformly express ESX-4, while in the wildtype a small subpopulation (<1%) does.
Furthermore, by performing RNA-seq on the werA mutant, we could show that 50 unknown genes are upregulated, many of which are organized in a three gene operon: encoding for a helix-turn-helix protein, followed by structural homologues of known bacteriocins and putative antitoxins. By co-incubating with other mycobacteria, we see interspecies growth restriction and ESX-4 expression in the colony interface, implying a role for ESX-4 in competition.

We propose a mechanism where WerA negatively regulates the ESX-4 master regulator alternative sigma factor SigM by inhibiting WhiB5 mediated activation of SigM. Furthermore, WerA regulates expression of ~50 potentially secreted proteins, although the role of ESX-4 and the function of these proteins remains to be elucidated. However, these toxin-antitoxin pairs could indicate a function of ESX-4 in interbacterial warfare, which is unusual for pathogenic mycobacteria.

O17

A Gift from Asgard: The Prokaryotic Origin of Eukaryotic Immune Mechanisms

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All life on Earth deals with viral infections daily, from prokaryotes to eukaryotes. Defending themselves against viruses is crucial for survival. While the immune system serves this function in eukaryotes, its origin remains a topic of debate.

Recently, there has been growing discussion about a potential bacterial origin of innate immune mechanisms. Our study focuses on positioning archaeal defense systems in this debate, particularly those in the Asgardarchaeota group, commonly referred to as 'Asgard.' Asgard is the closest prokaryotic group to eukaryotes and is proposed as a descendant of the host cell involved in eukaryogenesis (the origin of eukaryotes).

In this study, we identify 2610 defense systems in Asgard genomes. By combining a well-established technique (protein sequence homology) with protein structure prediction, we shed light on the origins of proteins that can be linked to the eukaryotic immune system, focusing specifically on two shared by all forms of life: viperins and argonautes.

Asgard viperins (asVip) are sister proteins to eukaryotic viperins, suggesting that both evolved from a common ancestor. Moreover, we experimentally proved that asVip are functional and capable of protecting cells against viral infections. Regarding argonautes, by positioning Asgard proteins in both sequence and structural contexts, we uncovered compelling evidence linking eukaryotic and prokaryotic argonautes to ancient Asgard versions of this protein, showing a remarkably high degree of conservation. Asgard archaea have undoubtedly influenced the complexity of eukaryotic cells, and our findings provide evidence that the same applies to their defense strategies.

Additionally, our computational predictions have been recently validated by two independent research groups, highlighting the power of dual-homology (sequence and structural) approaches to elucidate the functions and origins of proteins found across the tree-of-life. Overall, this work highlights the need to include archaea in discussions surrounding the origins of immune system mechanisms and other eukaryotic features.

O18

Impact of Early Antibiotic Exposure on Gut Microbiome Diversity and Taxa Composition in Preterm Infants

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Antibiotics are frequently administered to preterm infants in their first days of life under suspicion of early onset sepsis (EOS). However, only <5% turns out to have confirmed EOS. This policy exposes many preterm infants unnecessary to antibiotics, yet the impact on the gut microbiome remains poorly studied. This study aims to investigate the association between antibiotic-exposure-duration during the first week of life and the gut microbiome colonization during the first month.

A cohort of 127 very preterm infants (<30 weeks gestational age) without confirmed sepsis or necrotizing enterocolitis were enrolled in nine Neonatal Intensive Care Units in the Netherlands and Belgium. Participants were divided into three groups: no (0h), short (48–96h), and long (96–192h) antibiotic-exposed group. Antibiotic types varied according to the local standard clinical practices. Stool samples were collected on days 7, 14, 21, and 28 of life, and bacterial profiles were determined using 16S amplicon sequencing.

In this cohort, antibiotic exposure was associated with increased alpha-diversity, with significant differences between the no-antibiotic-exposed group and both short- (21 days: $p = 0.014$; 28 days: $p = 0.0002$) and long-antibiotic-exposed groups (21 days: $p = 0.006$; 28 days: $p = 0.009$). Beta-diversity was driven by four clusters, dominated by Enterococcus, an unidentified genus of the Enterobacteriaceae family, Staphylococcus and Escherichia-Shigella. Infants clustered initially to the Enterococcus or the Staphylococcus cluster, the latter one persisted until week 4 in antibiotic-exposed groups, but disappeared by week 3 in non-antibiotic-exposed infants. By week 4, non-antibiotic-exposed infants fit often in the Escherichia-Shigella cluster, while antibiotic-exposed infants fit more frequently in the Enterococcus cluster.

Antibiotic exposure in the first week of life in very preterm infants is associated with increased alpha-diversity and altered gut microbiome development, with longer exposure amplifying these effects. These effects related to long-term health requires further investigation.

O19

MRSA on the rise in neonatal care departments

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Introduction
A relatively high number of methicillin-resistant Staphylococcus aureus (MRSA) outbreaks in neonatal care departments were reported to the Healthcare-associated Infections and Antimicrobial Resistance Monitoring Group (SO-ZI/AMR). Over the past two years, 48% (14/29) of all reported MRSA hospital outbreaks occurred in these settings. The national enhanced MRSA surveillance program receives isolates from laboratories for molecular typing. This study aims to identify MRSA clusters in neonatal care.
Methods
We analysed isolates from patients under one year old, collected since 2016. Results of multilocus variable-number tandem repeat analysis (MLVA) were analysed together with epidemiological data, to identify clusters (≥three neonates) based on similar MLVA type, time (within three months) and geographic proximity (two or more hospitals in the same region or linked by patient transfer). Additionally, isolates from non-neonates were screened for potential epidemiological links to these MLVA-clusters. Whole genome sequencing (WGS) was available from a semi-random sample set of all surveillance isolates from each year.
Results
A total of 29 clusters were identified, encompassing 189 neonates, of which 14 clusters had been previously reported to SO-ZI/AMR. The largest cluster involved 19 neonates, with a median cluster size of five. WGS had been conducted for isolates from 10 clusters (34%). We identified 448 isolates from non-neonates that may be linked. Most clusters remained localised within neonatal care regions, although some spread to multiple areas.
Discussion
Analysing pathogen surveillance data identified 15 additional MRSA clusters in neonatal care beyond the 14 already voluntarily reported to SO-ZI/AMR. The primary mode of transmission between hospitals may be the transfer of colonised neonates to surrounding community hospitals for post-NICU care. Additionally, WGS has been initiated to include at least one isolate from each hospital per cluster, to elucidate relationships among isolates and enhance our understanding of MRSA transmission dynamics in neonatal settings.

O20

Fecal microbiota profiling as an early noninvasive predictive biomarker for Gram-negative late-onset sepsis in preterm infants: a longitudinal case-control microbiome study

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Introduction: Late-onset sepsis (LOS) by Gram-negative pathogens is an important cause of morbidity and mortality in preterm infants. Increasing evidence suggests that Gram-negative pathogens originate from the gut. We aimed to assess the potential of fecal microbiota profiling as a predictive biomarker for Gram-negative LOS.

Methods: Preterm infants born between 24-30 weeks of gestation in one of nine neonatal intensive care units in the Netherlands and Belgium with blood culture-proven LOS were included. Daily fecal samples collected within five days preceding clinical onset of LOS were analyzed by IS-PRO. This untargeted microbiota detection technique is capable of generating absolute and relative abundances (RA) on species level within six hours following sampling. Cases were 1:3 matched to controls based on gestational and postnatal age.

Results: 532 fecal samples of 58 LOS cases and 174 controls were retrospectively analyzed. Compared to controls, the RA of the causative pathogen was higher in LOS cases with Escherichia coli (n=11 cases, $p<0.001$), Serratia marcescens (n=6 cases, $p=0.009$), and Klebsiella spp. (n=5 cases, $p=0.005$), in the five days prior to LOS. Using an optimal cut-off value based on E. coli RA, E. coli cases could be discriminated from controls (AUC 0.90, sensitivity 100%, specificity 81%, $p<0.001$). The optimal cut-off value for Klebsiella spp. RA discriminated Klebsiella spp. cases from controls (0.77, 77%, 78%, $p=0.001$), while the optimal cut-off value for Serratia spp. RA discriminated S. marcescens cases (0.94, 92%, 97%, $p<0.001$).

Conclusions: Our findings reveal preclinical intestinal microbial alterations up to five days prior to Gram-negative LOS, characterized by an increased abundance of the causative pathogen. These results strongly support the potential of fecal microbiota profiling as a noninvasive predictive biomarker for Gram-negative LOS. This approach potentially offers clinicians a critical window for targeted microbiota-based interventions, which could improve health outcomes and reduce unnecessary antibiotic treatments.

O21

Implementation of probiotic administration in extremely preterm infants in Dutch NICUs: effect on clinical outcomes and gut microbiota colonization.

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Probiotic administration in preterm infants is associated with a reduction in necrotizing enterocolitis (NEC) and possibly late-onset sepsis (LOS). However, its efficacy in extremely preterm infants (EPIs; gestational age <28weeks) remains unclear due to limited data and potential safety risks. In Dutch Neonatal Intensive Care Units (NICUs), a three-strain probiotic product is implemented as standard of care. This study aims to evaluate the effect of implementation of probiotics on clinical outcomes in EPIs and the presence of these probiotic species in their gut microbiota.

Pre- and postimplementation cohorts of EPIs from 5 Dutch NICUs (2018-2023) participating in a fecal biomarker study were included. Clinical data and fecal samples from the first 28 days of life were collected. Incidence of NEC (modified Bell's stage ≥ IIA), LOS and mortality were compared with logistic regression, adjusting for birthweight. Time-to-full-enteral-feeds (tFEF) was analyzed through multiple regression, adjusting for feeding type and birthweight. Presence of probiotic species was assessed in week-2 fecal samples, using species- and strain-specific qPCR assays.

In total, 463 EPIs were included (202 pre-, 261 post-implementation). The adjusted odds ratio for NEC was 0.4 (95%CI 0.2-0.8; p<0.05); 0.8 (95%CI 0.5-1.2; p=0.28) for LOS and 0.6 (95%CI 0.3-1.1; p=0.12) for mortality in the probiotic-exposed group. The tFEF was 2.1 days shorter (p<0.001) in probiotic-supplemented infants. qPCR analysis on a subset of 136 probiotic-supplemented infants showed that 90% of the infants had all three probiotic species present.

This multicenter pre-postimplementation study provides real-world evidence that our used multi-strain probiotic product in EPIs is associated with a significant reduction in NEC and tFEF. This effect may be attributed to successful gastrointestinal colonization of probiotic species, as 90% of probiotic-supplemented EPIs showed presence of all three probiotic species. Further research is needed to determine whether EPIs with insufficient colonization are at higher risk for NEC.

O22

Innovative dashboard for early detection of outbreaks in the neonatal intensive care unit (NICU)

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Introduction: The prompt detection of and response to the colonization of neonates with (multi-drug resistant) microorganisms are critical for maintaining an effective antibiotic policy, ensuring reliable epidemiology, and implementing appropriate measures to prevent healthcare-associated infections. This study examined whether a real-time dashboard can identify elevated colonization and infection levels with pathogenic bacteria earlier and reduce the risk of outbreaks in a Dutch level 3 neonatal intensive care unit (NICU).

Methods: The conventional method consisted of a series of manual steps to enrich the reports from the laboratory information system (GLIMS, Clinisys) with a timeline in a conditionally formatted spreadsheet to highlight elevations in colonization and infection rates. Using their identification numbers, the laboratory information system and electronic patient records (HIX, ChipSoft) were queried to determine patients' ward locations and microbiological history to generate an epicurve.

In the new real-time dashboard approach, GLIMS and HIX were automatically queried and related daily by Microsoft's Power BI to augment microbiological data with bed locations and patients' admission tracks. The dashboard summarizes graphically laboratory and clinical findings over time.

The data of a past Serratia marcescens outbreak were used to compare the conventional with the dashboard method.

Results: The dashboard approach provided improved monitoring of pathogens with easy access to real-time data of all cultured microorganisms in the NICU. It features a heatmap of each pathogen, and when required, an epicurve for each patient can be shown.
The conventional method signalled an elevation of Serratia marcescens eight days later than the dashboard approach.

Conclusion: The dashboard approach enables earlier detection of pathogen emergence through real-time visualization of laboratory results at both the NICU and individual patient levels. In the S. marcescens test case, this method may have allowed for the implementation of additional infection prevention measures two to eight days earlier.

O23

A New Dawn for Methanotroph Cultivation: Bioelectrochemical Systems yield almost pure cultures of Methanoperedens Archaea

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Anaerobic methanotrophs, particularly ‘Candidatus Methanoperedens’ species, play a critical role in mitigating methane emissions from anoxic environments. These archaea oxidize methane to CO2 using various electron acceptors, thus mitigating the release of this potent greenhouse gas into the atmosphere. However, their "unculturable" nature has hindered in-depth studies of their physiology and metabolic capabilities.

This study showcases the power of bioelectrochemical systems (BES) to cultivate and characterize these elusive methanotrophs. By employing a poised electrode as an alternative electron acceptor, we achieved highly enriched cultures of ‘Ca. Methanoperedens’, with their relative abundance increasing from 11% to 96%.

Our BES setup allowed for the first experimental determination of growth rates for these archaea, revealing doubling times of 8.2 days at 23°C and an impressive 4.6 days at 30°C. This represents a significant increase in growth rate compared to previous estimates of ~30 days doubling time in bioreactor enrichment cultures. We confirmed methane as the primary electron source for current generation, with a 98% decrease in current observed upon replacing methane with argon. This methane-dependent current production remained consistent across varying anode potentials, suggesting a robust and adaptable electron transfer mechanism. Furthermore, our metatranscriptomic analyses revealed the upregulation of two distinct gene clusters potentially involved in extracellular electron transfer (EET). Notably, one cluster encodes proteins homologous to those found in Geobacter sulfurreducens, related to the formation of OmcZ nanowires. Transmission electron microscopy further supports this observation, revealing distinctive nanowire-like filaments protruding from archaeal cells.

This innovative approach not only unlocks the potential for cultivating previously "unculturable" methanotrophs but also provides a powerful tool for unraveling their unique metabolic capabilities and their role in greenhouse gas mitigation. Our findings pave the way for future research into the potential applications of these organisms in bioremediation and sustainable energy generation.

O24

Synergistic mitigation of ammonia and methane emissions from livestock waste by a synthetic community

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Introduction:
Manure microbiota is a major source of ammonia and methane emissions in the global livestock sector. A synthetic community of lactic acid bacteria species (LAB SynCom) can be applied during manure storage, which has become a novel, effective, and promising strategy to mitigate gaseous emissions. However, efforts are needed to reduce mitigation costs, and the influences of LAB SynCom on the entire manure management chain remain unclear.
Methods: A headspace experimental setup was designed to simulate the manure management storage and composting. Nessler Reagent Spectrophotometer and Gas Chromatography were employed to measure ammonia and methane emissions in the manure management chain. 16S rDNA amplicon sequencing was employed to explore the influences of the LAB SynCom on taxonomic profile of manure microbiota during the manure storage and composting process.
Results: LAB SynCom acidified manure to a stable pH of 5.5 within one day, with 6% molasses proving to be the most economical option. During a 45-day storage period, the LAB SynCom treatment mitigated emissions of ammonia by 85% and methane by 91.2%. During composting using the acidified manure, ammonia emissions were notably mitigated at the early stages but were generated from the thermophilic phase. Furthermore, 16S rDNA amplicon sequencing found that the LAB SynCom treatment markedly decreased the alpha diversity and abundance of ammonia and methane-producing bacterial genera of manure microbiota during storage and the early stage of composting. However, the high temperature during the thermophilic phase considerably inhibited the growth of LAB SynCom and drove the generation of ammonia emissions.
Conclusion: This study provides a comprehensive evaluation of LAB SynCom on ammonia and methane emissions and microbial community throughout the manure management chain. These findings offer valuable knowledge for applying and optimizing LAB SynCom to achieve cleaner production of the livestock farming systems.

O25

Optimal Microbial Strategies in Dynamic Environments: Predictive Modelling and Experimental Validation

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Dynamic environments are the norm in ecology, yet much of our understanding of metabolic strategies stems from studies on static, pure-cultures grown in controlled conditions. Exploring how microbial communities adapt to recurrent dynamic conditions is crucial to advancing our knowledge of metabolic strategies and evolutionary trade-offs in nature. In our research, we investigate the metabolic strategies of slow-growing microbial communities in dynamic environments that mimic conditions during phosphorus removal in wastewater treatment plants.

We developed a Python toolkit for the implementation of conditional Flux Balance Analysis (cFBA) to model metabolic networks under dynamic conditions. Using cFBA, we predicted that temporal cycling of storage polymers is an optimal strategy during anaerobic-feast and aerobic-famine cycles. These predictions were validated in long-term enrichments using sequencing batch reactors (SBR) operated under repetitive, dynamic cycles. The resulting phenotypes of the SBR enrichments showed remarkable similarities and metabolic strategies as those predicted by cFBA.

Expanding on this approach, we demonstrated that temporal separation of biomass synthesis – a feature previously suggested for organisms in such environments – can also be predicted using cFBA. Finally, our toolkit was applied to study co-substrates uptake, identifying synergistic strategies shaped by metabolic network topology. We identified that concurrent uptake of different substrates can lead to optimized biomass yields, dependent on the entry point in the network. These predictions were then experimentally validated in microbial enrichments grown in SBRs.

Our findings reveal that cycling of distinct storage polymers and the temporal separation of metabolic activities are optimal strategies for microbial growth in dynamic environments. The presented cFBA toolkit not only predicts metabolic strategies, but also allows to gain mechanistic insights into the employed strategies by different organisms. Furthermore, it leads to the generation of testable, quantitative hypothesis, bridging the gap between predictive models and experimental validation in dynamic, mixed microbial cultures.

O26

Expanding metabolic horizons: Carbon monoxide is an alternative substrate for anaerobic methanotrophic archaea

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Anaerobic methanotrophic archaea (ANME) play a key environmental role in mitigating methane (CH_4) emissions in anoxic environments. Despite their pivotal ecological role, their physiology remains poorly understood due to the absence of axenic cultures and the complexities of ANME enrichments. A neglected key component in ANME physiology is electron donor flexibility and preference, which can have profound impact on understanding ANME physiology and ecology.
In this study, we coupled batch activity assays using granular biomass from an enrichment culture dominated by 'Ca. Methanoperedens' (37% relative abundance) with metagenomics and metatranscriptomics. Batch activity tests supplemented with nitrate as electron acceptor and multiple antibiotics showed that ANME can use carbon monoxide (CO) as alternative electron donor and that CO oxidation rates far exceed (up to ten-fold) CH_4 oxidation rates. Additionally, the genome of 'Ca. Methanoperedens' was circularized and found to encode six chromosomal carbon monoxide dehydrogenases (CODHs). Fascinatingly, also a 156 kbp mobile genetic element was identified, encoding two additional CODHs. In total, six of the CODHs were highly expressed under CO-oxidizing conditions, suggesting their involvement in the central metabolism of 'Ca. Methanoperedens'.
Here we demonstrate that ANME have a distinct physiology capable of CO utilization. Consequently, a non-methanotrophic lifestyle challenges the canonical view of ANME as strict methanotrophs and necessitate a re-evaluation of their physiology and ecology.

O27

Assessing the stability of using growth-coupled fumarate as a substrate for malate production in Synechocystis

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Cyanobacterial CO_2 conversion holds great promise towards sustainability while challenges remain. One of them is the instability of production. When production imposes a fitness burden on the cell, revertants that eliminate this burden via spontaneous mutations tend to take over the population by impairing the overall productivity. A strategy to overcome this instability is via growth-coupled production. In the cyanobacterium Synechocystis sp. PCC6803, this has been explored for acetate [1] and fumarate [2]. It has been hypothesized that growth-coupled products could be used as substrates to synthesize downstream compounds. This was first tested with photoautotrophic malate production by Synechocystis mutants. [3] Here, the enzymes responsible for recycling malate (ME and Mdh) are knocked out. Preliminary experiments suggested that such constructs would be stable even when higher production levels of malate would be achieved via the overexpression of fumarase (FumC). Here, we are testing malate production stability in much more stringent conditions. We use tightly controlled turbidostats where we cultivate during many generations with a small propagation bottle neck (5%). There, growth rate is applied as the selection pressure. Results show stable production in both the ΔmeΔmdh and ΔmeΔmdhΔNSI::fumC strains for > 55 generations. This suggests that drawing from a growth-coupled pool does not impose a hampering burden on the cells. We plan on extending the product range with additional thermodynamically favourable reactions and investigate the influence of dark metabolism. Our results show that this is a promising strategy to extend the list of alternative compounds to be stably produced.

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