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Induction and purification of a virus from a novel Lokiarchaea strain

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Abstract

Asgard archaea are considered key organisms for understanding the evolutionary origin of eukaryotes; however, the diversity as well as biology of their associated viruses remains largely unexplored. Although recent metagenomic studies have identified several viruses linked to Asgard archaea, their experimental characterization is still limited, as the cultivation of their hosts remains challenging.

This project focused on a virus-host system including *Fylgjavirus 1* (FylV1), an integrated 16.1 kbp provirus, and its host, a novel lokiarchaeal strain *Ca. Lokiarchaeum ossiferum* B36, which is maintained in enrichment culture. The integrated provirus is able to excise and replicate independently, which is associated with the formation of viral particles. However, only very few particles have so far been discovered in culture supernatants.

The aim of this thesis was to investigate whether FylV1 can be induced by different potential stress conditions, including exposure to ambient air, elevated salinity and mitomycin C treatment, and whether these conditions influence viral DNA abundance and particle production. For this purpose, FylV1 dynamics were assessed by quantifying viral DNA and the abundance of *Ca. Lokiarchaeum ossiferum* B36 cells over time. In addition, virus particle purification was performed from culture supernatants for further characterization of FylV1, while microscopy-based analyses were used to examine potential changes in host cell morphology and distribution.

Among the tested stress conditions, the addition of 10% ambient air to the gas phase of the anaerobic cultures resulted in an increase in circular FylV1 DNA, suggesting that oxygen-related stress may influence FylV1 induction.

Virus particle purification further enabled the isolation of icosahedral virus-like particles with a diameter of ~ 55 nm, probably representing candidate FylV1 particles.

Overall, this study provides initial experimental insights into an Asgard archaeal virus-host system, represented by the provirus *Fylgjavirus 1* and its host, *Ca. Lokiarchaeum ossiferum* B36, and establishes a foundation for future investigations into Asgard archaeal virology.

Zusammenfassung

Asgard-Archaeen gelten als Schlüsselorganismen für das Verständnis des evolutionären Ursprungs von Eukaryonten; jedoch sind die Diversität und Biologie ihrer assoziierten Viren bislang weitgehend unerforscht. Obwohl in aktuellen metagenomischen Studien mehrere mit Asgard-assoziierte Viren identifiziert wurden, bleibt ihre experimentelle Charakterisierung nach wie vor begrenzt, da die Kultivierung ihrer Wirte eine Herausforderung darstellt.

Dieses Projekt fokussiert sich auf ein Virus-Wirt-System, bestehend aus dem Fylgjavirus 1 (FylV1), einem integrierten 16,1-kbp-Provirus, und seinem Wirt, einem neuen lokiarchaealen Stamm, *Ca. Lokiarchaeum ossiferum* B36, welcher in einer Anreicherungskultur gehalten wird. Das integrierte Provirus ist in der Lage, aus seinem integrierten Zustand hervorzutreten und sich unabhängig zu replizieren, was mit der Bildung viraler Partikel einhergeht. Jedoch wurden bisher nur sehr wenige Partikel in den Kulturüberständen entdeckt.

Ziel dieser Arbeit war es zu untersuchen, ob FylV1 durch unterschiedliche potenzielle Stressbedingungen induziert werden kann, darunter die Exposition gegenüber Umgebungsluft, ein erhöhter Salzgehalt und eine Behandlung mit Mitomycin C, welches sich auf die Bildung viraler DNA und Partikelproduktion auswirken sollte. Infolgedessen wurde die Dynamik von FylV1 durch Quantifizierung der zirkulären, viralen DNA und der Zellzahl von *Ca. Lokiarchaeum ossiferum* B36 über die Zeit untersucht. Zudem wurde zur weiteren Charakterisierung von FylV1 eine Viruspartikelaufreinigung aus Kulturüberständen durchgeführt, während mikroskopie-basierte Analysen verwendet wurden, um mögliche Veränderungen in der Morphologie sowie Verteilung der Wirtszellen zu untersuchen.

Unter den getesteten Stressbedingungen führte die Zugabe von 10% Umgebungsluft zur Gasphase der anaeroben Kulturen zu einem Anstieg zirkulärer FylV1-DNA, was darauf hindeutet, dass sauerstoffbedingter Stress die Induktion von FylV1 beeinflussen könnte.

Weiters ermöglichte die Viruspartikelaufräumung die Isolierung von ikosaedrischen virusähnlichen Partikeln mit einem Durchmesser von ~ 55 nm, welche vermutlich Kandidaten für FyLV1-Partikel darstellen.

Insgesamt liefert diese Studie erste experimentelle Einblicke in ein Asgard-archaeales Virus-Wirt-System, repräsentiert durch das Provirus Fylgjavirus 1 und seinen Wirt, *Ca. Lokiarchaeum ossiferum* B36 und schafft eine Grundlage für zukünftige Untersuchungen zur Virologie von Asgard-Archaeen.

Introduction

Asgard archaea play an essential role in the evolution of complex cellular life (Zaremba-Niedzwiedzka *et al.*, 2017; Liu *et al.*, 2021); nevertheless, viruses which are able to infect these organisms are poorly described. Recent metagenomic studies have characterized six double-stranded DNA (dsDNA) viral genomes that infect two Asgard archaeal phyla, including Lokiarchaeia and Helarchaeia. Furthermore, these viruses are suggested to contain eukaryotic as well as prokaryotic-like features and to interact with their hosts (Rambo *et al.*, 2022).

More broadly, archaeal viruses are viruses that can infect members of the domain “Archaea”. However, these viruses remain one of the most enigmatic areas of the virosphere (Krupovic *et al.*, 2018). The reason for this is largely due to their unusual morphological and genomic diversity. In particular, a lot of archaeal viruses display unique virion morphologies which have not been observed in viruses of either bacteria (i.e. bacteriophages) or eukaryotes (Krupovic *et al.*, 2018). This morphological diversity includes a wide range of virion morphotypes, such as coil-shaped, ovoid, head-tailed, icosahedral, filamentous, spindle- or lemon-shaped, bottle-shaped, droplet-shaped, rod-shaped, spherical and ellipsoid virions. Among these, coil-shaped, spindle- or lemon-shaped, bottle-shaped and droplet-shaped virions appear to be particularly characteristic of archaeal viruses and highlight their distinct position within the virosphere (Krupovic *et al.*, 2018; Baquero *et al.*, 2020; Kuiper *et al.*, 2024).

Additionally, some archaeal viruses are part of the cosmopolitan fraction of the archaeal virosphere, meaning that they resemble viruses from other domains of life (Krupovic *et al.*, 2018; Kuiper *et al.*, 2024). Selected archaeal virus groups further illustrate this diversity through their genome size ranges and representative virion morphotypes (Figure 1) (Krupovic *et al.*, 2018). However, since this overview was published, archaeal virus taxonomy has expanded substantially, and following the 2025 ICTV taxonomic update, archaeal viruses are officially classified into 163 species, 94 genera and 62 families (Krupovic *et al.*, 2025). Therefore, Figure 1 should be considered a representative overview instead of a complete current classification.

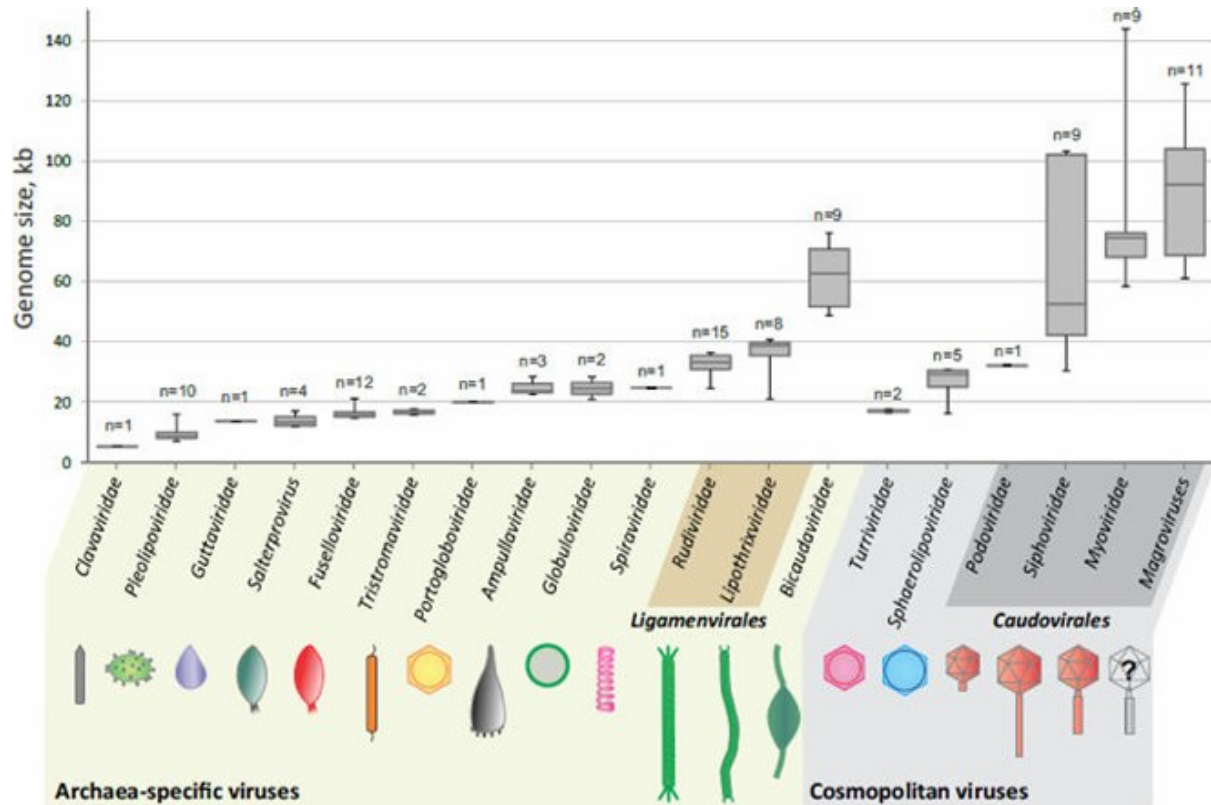


Figure 1: Representative morphological and genomic diversity of archaeal viruses. Selected archaeal virus groups are shown with their genome size ranges and representative virion morphotypes. Genome size variation within each family is shown as box plots, and the number of genomes included is indicated above the corresponding plot. Different background colours distinguish the archaea-specific and cosmopolitan fractions of the archaeal virosphere. Reprinted from Virus Research, Vol. 244, Krupovic *et al.*, Viruses of archaea: Structural, functional, environmental and evolutionary genomics, pp. 181–193, Copyright 2018, with permission from Elsevier.

Beyond their morphological diversity, archaeal viruses also exhibit distinct gene contents, genome architectures and features of their encoded proteins, which further underscore their uniqueness (Prangishvili *et al.*, 2017; Krupovic *et al.*, 2018). Archaeal viral genomes contain many genes with no recognizable homologues in bacterial or eukaryotic viruses, and the functions of many encoded proteins often remain unknown (Wirth and Young, 2020; Kuiper *et al.*, 2024). They also interact with the archaeal cell envelope through diverse and still only partly understood mechanisms of viral entry and egress, further highlighting their distinct virus-host interaction strategies (Kuiper *et al.*, 2024). In addition, most characterized archaeal viruses infect hosts which inhabit extreme environments (Krupovic *et al.*, 2018) and many archaeal host systems, including Asgard archaea, remain challenging to isolate and maintain in culture (Maslać *et al.*, 2026; preprint).

Virus production in archaeal systems can also be influenced by environmental and cellular stress conditions. For lysogenic archaeal viruses, it has been reported that induction can be triggered by stimuli including UV radiation, addition of mitomycin C, nutrient depletion or changes in oxygen levels (Quemin and Quax, 2015). However, compared to bacteriophage induction, molecular mechanisms underlying archaeal virus induction remain largely unexplored. Nevertheless, one of the few characterized examples is the temperate haloarchaeal virus SNJ2, in which mitomycin C-induced DNA damage activates a virus-encoded regulatory module controlling the switch from lysogeny to an induced state (Chen *et al.*, 2023). In bacterial systems, similar stress conditions can also trigger the induction of temperate bacteriophages, including exposure to DNA-damaging stimuli such as UV radiation or mitomycin C. These agents can activate the SOS response, as shown for phage λ , where RecA-mediated cleavage of the phage repressor promotes the switch from lysogeny to the lytic cycle (Zhang *et al.*, 2022).

Within the still largely unexplored virosphere of archaea, viruses that infect members of the superphylum Asgard archaea have been poorly described (Rambo *et al.*, 2022). Current knowledge of Asgard-associated viruses is mainly based on metagenomic data by matching viral sequences to Asgard CRISPR spacers, followed by the classification of these into distinct groups using phylogenomic analysis (Medvedeva *et al.*, 2022). These analyses revealed three family-level groups: putative tailed Caudoviricetes-like viruses, skuldviruses with predicted icosahedral capsids and wyrdviruses related to spindle-shaped viruses previously known from other archaea (Medvedeva *et al.*, 2022).

Despite previous advances in cultivation and cellular characterization of Asgard archaea (Imachi *et al.*, 2020; Rodrigues-Oliveira *et al.*, 2023), experimental insights into virus-host interactions in Asgard archaea in laboratory cultures of Lokiarchaea are still lacking. This limited experimental understanding is particularly relevant because Asgard archaea are widely regarded as the closest archaeal relatives of eukaryotes (Eme *et al.*, 2023).

The evolutionary relevance of Asgard archaea is central, because most recent models of eukaryogenesis propose that the first eukaryotic cell arose from a symbiotic scenario between an archaeal host and an alphaproteobacterial endosymbiont (Baum and Baum, 2014; López-García and Moreira, 2023). Whereas mitochondria are derived from the endosymbiotic partner, the identity of the archaeal host remained unclear until the discovery of Asgard archaea (Zaremba-Niedzwiedzka *et al.*, 2017).

Asgard archaea belong to an archaeal superphylum and currently include 16 recognized distinct lineages, such as Lokiarchaeia or Heimdallarchaeia, which represent the closest known archaeal relatives of eukaryotes (Eme *et al.*, 2023; Maslać *et al.*, 2026; preprint). This association is further supported by the Asgard genomes, which encode numerous eukaryotic signature proteins (ESPs), including cytoskeleton components (e.g. actin homologues), ESCRT complex proteins and small GTPases, which are involved in regulatory processes such as cytoskeleton formation, vesicular trafficking and signal transduction (Zaremba-Niedzwiedzka *et al.*, 2017; Liu *et al.*, 2021).

Among the representatives of the Asgard superphylum, Lokiarchaeia was the first lineage which was identified. They were discovered through metagenome-assembled genomes (MAGs) reconstructed from metagenomic analyses of deep-sea sediments collected near Loki's Castle hydrothermal vent field in the Arctic Ocean (Jorgensen *et al.*, 2012; Spang *et al.*, 2015). For many years, these organisms remained uncultivated and were known only from environmental metagenomic data until strain MK-D1 was isolated through long-term enrichment and cultivation (Imachi *et al.*, 2020). This organism is considered the first cultured representative of Asgard archaea and was later formally described as *Promethearchaeum syntrophicum* (Imachi *et al.*, 2024). Based on 16S rRNA gene sequence analysis, *Candidatus* Lokiarchaeum ossiferum was identified as its closest relative detected in enrichment cultures (Imachi *et al.*, 2024).

Moreover, *Ca. Lokiarchaeum ossiferum* has been described as an anaerobic lokiarchaeal archaeon characterized by a coccoid central cell body that gives rise to multiple, frequently branched protrusions of variable number and length (Rodrigues-Oliveira *et al.*, 2023; Radler *et al.*, 2025; preprint).

In this study, enrichment cultures of *Ca. Lokiarchaeum ossiferum* strain B36 (Loki-B36) were used. These enrichments contain several co-cultured microbial partners, including members of *Halodesulfobrio*, *Spirochaetes*, and bacteria belonging to the group Izmoplasmataceae (Radler *et al.*, 2025; preprint; Maslać *et al.*, 2026; preprint). *Ca. Lokiarchaeum ossiferum* strain B36 was selected because, in contrast to the closely related strain B35, it carries a 16.1 kbp integrated provirus, which can replicate independently, thus forming virus particles, making Loki-B36 the first available cultured Asgard archaeal strain with an active virus-host system to date (Maslać *et al.*, 2026; preprint).

The provirus found in Loki-B36 was identified as Fylgjavirus 1 (FylV1), representing the first described member of the proposed novel viral family Fylgjaviridae. Its genome encodes 32 putative proteins, including genes that encode proteins associated with capsid formation, genome packaging and recombination, such as a double jelly-roll (DJR) major capsid protein (MCP), a genome packaging NTPase and a tyrosine recombinase-like protein. At the same time, several additional open reading frames (ORFs) encode putative proteins with no clearly assigned function, reflecting the still limited understanding of Asgard-associated viruses. Furthermore, although FylV1 is related to skuldviruses, genomic comparisons indicate only limited overall similarity, supporting its classification as a separate viral family, Fylgjaviridae (Figure 2) (Maslać *et al.*, 2026; preprint).

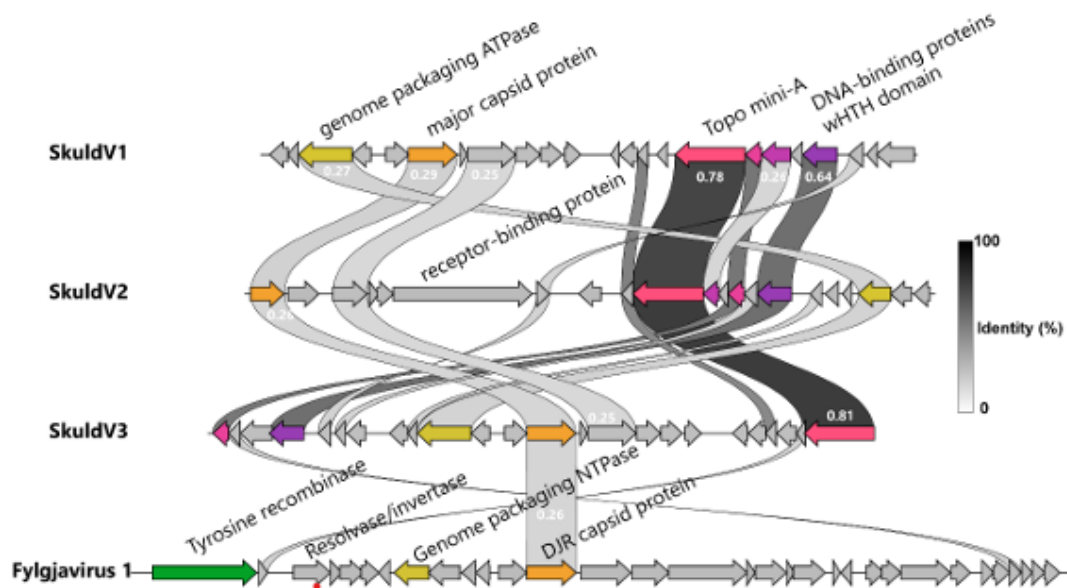


Figure 2: Genomic alignment of related skuldviruses and Fylgjavirus 1. Similarity values between proteins based on BLASTp are indicated in grayscale. Homologues of conserved genes are color-coded as follows: major capsid protein in orange, genome packaging NTPase/ATPase in yellow, Topo mini-A and ATPase in magenta, DNA-binding proteins in lilac, and tyrosine recombinase-like protein in green. Adapted from Maslać *et al.* (preprint; 2026).

The predicted icosahedral morphology of FylV1 is based on its encoded DJR MCP, which is a hallmark of viruses assigned to the realm *Varidnaviria* and is often associated with an icosahedral virion shape (Koonin *et al.*, 2020; Krupovic *et al.*, 2022; Maslać *et al.*, 2026; preprint).

Furthermore, related members of this viral group infect hosts throughout the tree of life, and related varidnaviruses of the family *Skuldviridae* have been proposed to infect members of the Lokiarchaeia (Medvedeva *et al.*, 2022; Maslać *et al.*, 2026; preprint).

However, in the present study, these putative viral particles could not be unambiguously identified in Loki-B36 cultures.

The aim of this study was first to increase the number of viral particles by applying different growth conditions, followed by purification of the provirus-derived particles, and finally, to visualize virus and host by microscopy.

Our hypothesis was that the provirus excision and multiplication could be triggered by stress conditions, as these are known to trigger virus production in other prokaryotic and eukaryotic systems. Consequently, Loki-B36 cultures were exposed to different stress conditions, including elevated salinity, addition of oxygen and the cytotoxic antibiotic

mitomycin C (Williamson and Paul, 2006; Zhang *et al.*, 2022; Chen *et al.*, 2023) which is well known to trigger viruses. The provirus Fylgjavirus 1, was successfully induced only upon exposure to oxidative stress, i.e. exposure to air. In addition, provirus-derived particles were purified by ultracentrifugation and imaged by negative staining.

Overall, this study establishes a foundation for future investigations into virus-host interactions in Asgard archaea, the virology of Asgard archaeal systems, and further characterization of these viruses.

Materials and Methods

Growth conditions and medium composition of *Ca. Lokiarchaeum ossiferum* strain B36

Ca. Lokiarchaeum ossiferum strain B36 was grown under anoxic conditions in MLM medium with a 0.3 bar overpressure of N₂:CO₂ (80%:20%). Cultures were grown in 120 mL serum flasks sealed with rubber stoppers and aluminium crimps in final volumes of 50 mL, or in 1 L Schott bottles with a final volume of 500 mL, using a 1:10 inoculum. Cultures in exponential phase were used as an inoculum. Serum flasks with medium were made anoxic prior to inoculation through five consecutive vacuum (1 min) and gas sparging cycles (30 s). The cultures were incubated at 20 °C without light exposure or shaking.

The sterile MLM medium was prepared as described in Rodrigues-Oliveira *et al.*, 2022.

The acid trace element solution (2000-fold concentrated) contained (per litre): 491 g FeCl₂·4H₂O, 0.062 g H₃BO₃, 0.068 g ZnCl₂, 0.017 g CuCl₂·H₂O, 0.099 g MnCl₂·4H₂O, 0.119 g CoCl₂·6H₂O, 0.024 g NiCl₂·6H₂O and 4.106 mL of HCl (37%). The alkaline trace element solution (2000-fold concentrated) contained (per litre): 0.017 g Na₂SeO₃, 0.033 g Na₂WO₄, 0.021 g Na₂MoO₄ and 0.4 g NaOH. The Se/W solution (1000-fold concentrated) contained (per litre): 6 mg Na₂SeO₃·5H₂O, 8 mg Na₂WO₄·2H₂O and 0.5 g NaOH.

The MLM medium was supplemented with vitamins thiamine (B1), riboflavin (B2), biotin (B7) and cobalamin (B12) in every second round of cell passaging (final concentration of 1 mg/mL). The vitamin stocks were sterile filtered (0.22 µm pore size; Whatman TM, Germany) and stored at 4 °C.

Induction experiments of FylV1

In order to trigger provirus induction, Loki-B36 cultures were exposed to various stress conditions, including the introduction of ambient air into the gas phase, alteration of the salinity of the medium and antibiotic treatment with mitomycin C.

Introduction of ambient air into the gas phase

Two 50 mL Loki-B36 cultures, which were in exponential growth phase, were combined and then used as an inoculum (1:10) to set up the cultures for testing the effect of different amounts of ambient air in the gas phase. Ambient air was added to the gas phase at volumes corresponding to 5%, 10% and 20% of the culture volume. Cultures without any changes to the gas phase composition were used as controls. Each condition (5%, 10%, 20% and control) was set up in triplicate (Figure 3). Time point 0 (T0) was taken right after the addition of air, followed by sampling on days 2, 4, 7, 21, 32 and 46 after air introduction.

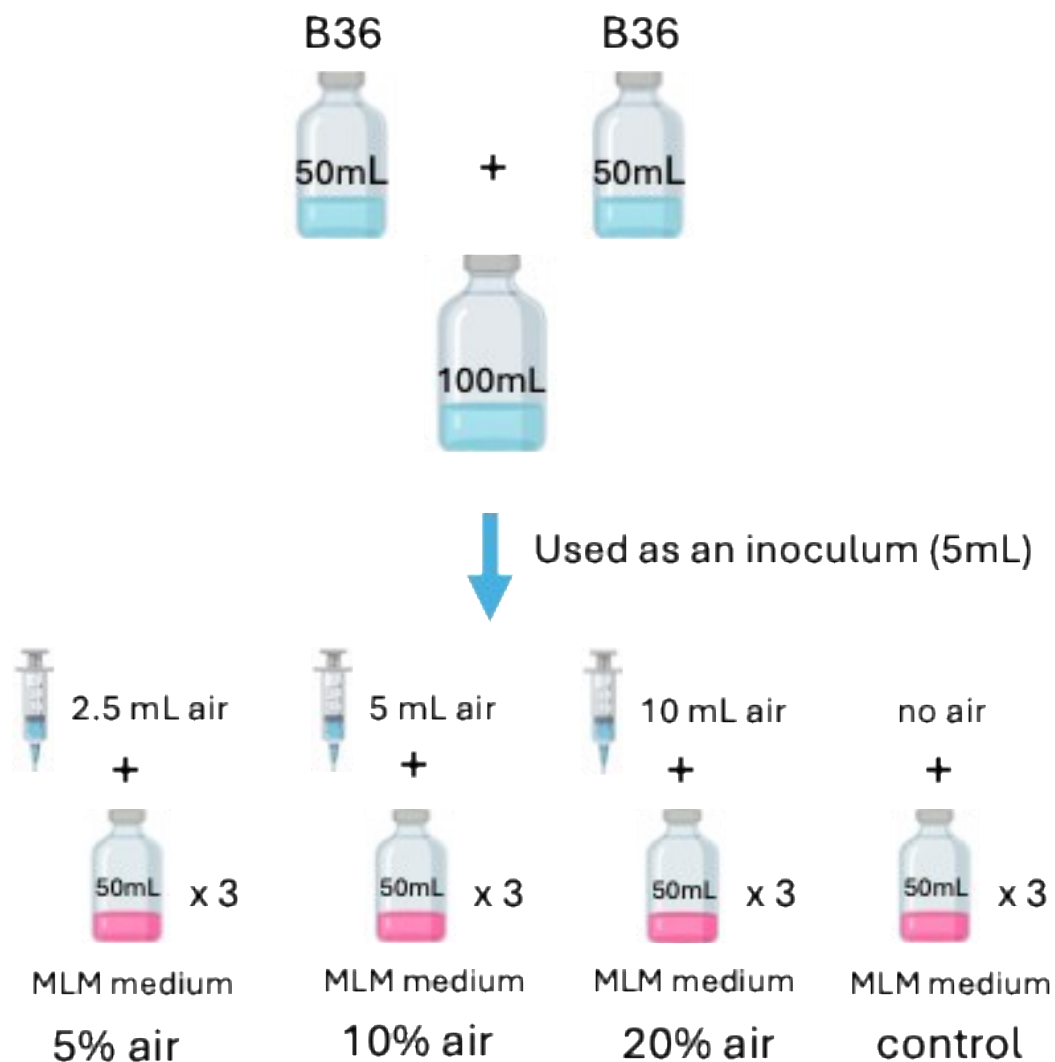


Figure 3. Experimental setup for ambient air supplementation - approach 1. Created with BioRender.com.

Alternatively, when the experiment was repeated, a 500 mL Loki-B36 culture in exponential growth phase was divided into nine anaerobic, sterile 50 mL serum flasks. The addition of 5% and 10% air to the gas phase was done immediately afterwards, while the 20% air condition was not included. All conditions were tested in triplicate (Figure 4). Sampling time points were at 7, 14, 21 and 28 days after incubation, with time point 0 (T0) directly after air introduction.

In both cases, the growth of the cultures and the number of circularized viral genomes present in both the cell pellet and culture supernatant fractions were followed via quantitative polymerase chain reaction (qPCR, see below).

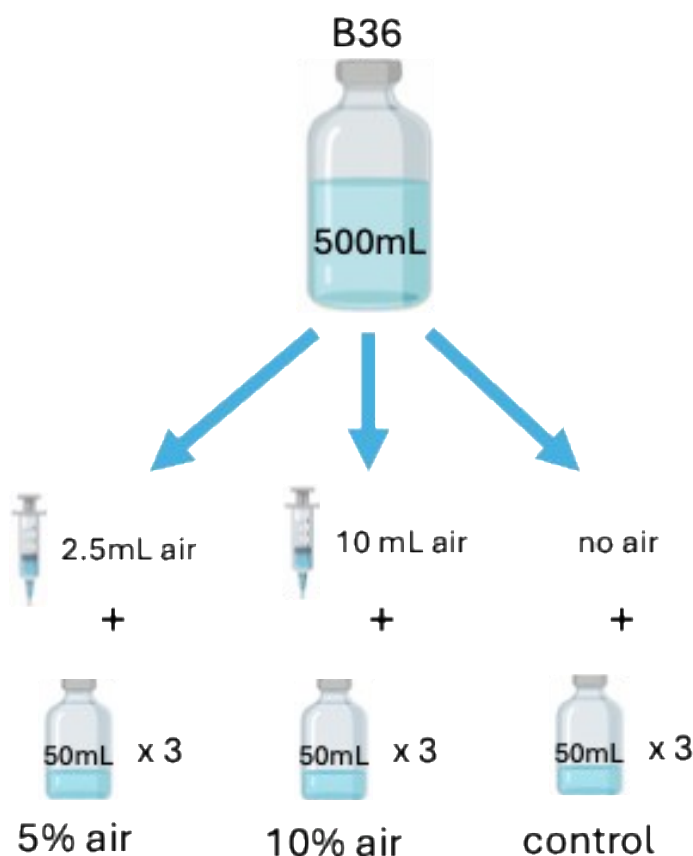


Figure 4. Experimental setup for ambient air supplementation – approach 2. Created with BioRender.com.

Salinity variations

In order to test whether the provirus could be triggered by varying the salinity in the medium, a gradient of sodium chloride (NaCl) (2% – 6% (w/v)) was established. For this, NaCl solutions were aliquoted into nine 50 mL serum flasks and autoclaved.

Afterwards, the headspace atmosphere was exchanged, and the sterile serum flasks were anaerobized. Following this, subsamples of a 500 mL Loki-B36 culture in exponential growth phase were added on top to achieve the final concentrations of 4.5% (w/v) NaCl and 5.5% (w/v) NaCl. In the control cultures, an NaCl stock matching the initial NaCl concentration of the MLM medium (2.07% (w/v)) was used to keep the final volume constant across all conditions.

The experiment was performed in triplicate and sampling was done 7, 10 and 15 days after inoculation, with time point 0 (T0) taken immediately (Figure 5). The number of Loki-B36 cells and circularized viral genomes in the cell pellet and culture supernatant fractions was quantified via qPCR (see below).

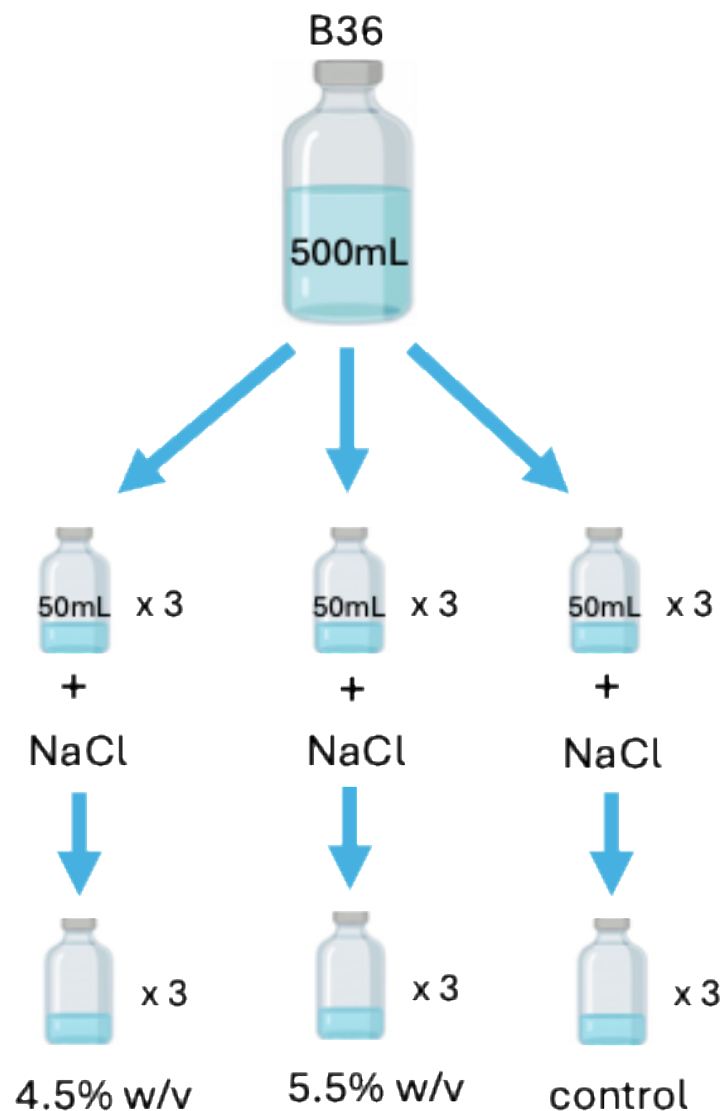


Figure 5. Experimental setup for salinity (NaCl) variations supplementation. Created with BioRender.com.

Mitomycin C treatment

To investigate viral induction through the addition of the cytotoxic antibiotic mitomycin C (MMC) (Roche, Germany), a 500 mL Loki-B36 culture in exponential growth phase was divided into nine anaerobic, sterile 50 mL serum flasks. MMC was introduced at final concentrations of 0.5 $\mu\text{g/mL}$ and 1 $\mu\text{g/mL}$, respectively. As controls, untreated cultures were used. All conditions, as well as the control set up in triplicate (Figure 6).

Samples for cell and circular virus quantification as described below were taken at 1, 4, and 24 h after adding MMC, time point 0 (T0) was taken right after the addition of MMC.

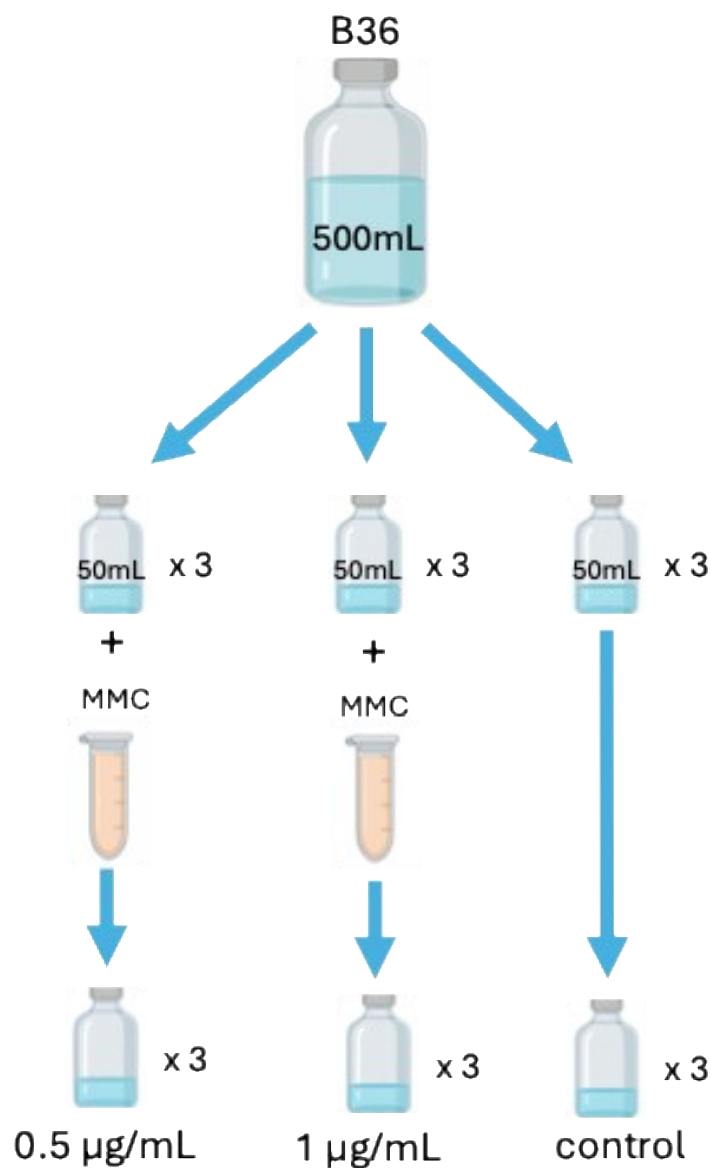


Figure 6. Experimental setup for mitomycin C (MMC) supplementation. Created with BioRender.com.

Culture sub-sampling and DNA extraction

For DNA extraction, a total of 2 mL of each culture was sampled and centrifuged at 20,000 rpm at 4 °C for 30 min. The cell pellets were used immediately for DNA extraction, while the supernatants were concentrated using Amicon Ultra-4 Centrifugal Filters (10 kDa MWCO; Sigma-Aldrich, Ireland) via centrifugation at 3,220 rpm at room temperature (RT) for 20 min to a final volume of 100 µL.

DNA extraction from both cell pellet and concentrated supernatant fractions was performed for all samples using the NucleoSpin® Soil Kit (MACHEREY-NAGEL GmbH & Co. KG, Germany). The manufacturer's protocol was followed with the following modifications: the FastPrep-24™ Classic homogeniser (MP Biomedicals, Germany) was used for 30 s at 5 m/s for homogenisation in the lysis step; the samples were placed on ice for 10 min during the precipitation step and washed with SW2 without vortexing. Finally, the elution buffer (SE buffer) was heated up to 80 °C, the samples were incubated with 30 µL pre-warmed SE buffer and eluted by centrifugation at 11,000 rpm for 30 s at RT. The elution step was performed twice.

DNA quantification

After the extraction, the amount of DNA was measured with the Qubit® 2.0 Fluorometer (Thermo Fisher Scientific Inc., USA). For this, the Invitrogen Qubit™ dsDNA HS Assay Kit (Thermo Fisher Scientific Inc., USA) was used following the manufacturer's protocol.

quantitative Polymerase Chain Reaction (qPCR)

In all experiments, both lokiarchaeal cells and circularized viral genomes were quantified via quantitative polymerase chain reaction (qPCR). The Lokiarchaea-specific 16S rRNA gene primers (LkF, 5'-ATCGATAGGGGCCGTGAGAG and LkR, 5'-CCCGACCACTTGAAGAGCTG) and primers targeting the circularized viral genome (Circ19F, 5'-TCT CAC TAA GCA AAA CCC TTA CTT and Circ349R, 5'-ACC TCC GTT TAA TAA GAT TTT TCG TGA) were used. Loki-B36 abundance was estimated based on the Loki-specific 16S rRNA gene copy numbers determined by qPCR. Each sample was run in

triplicate on a CFX Connect Real-Time PCR Detection System (Bio-Rad Laboratories Ges.m.b.H, USA) and fluorescence data were acquired with CFX Maestro software (v2.3). Every reaction (20 μ L total volume) contained: 10 μ L Luna Universal qPCR Master Mix (BioLabs, New England), 0.5 μ M of each primer and 5–10 ng of DNA or 10 μ L ChamQ Universal SYBR qPCR Master Mix (Neo Biotech, France), 0.25 μ M of each primer and 5–10 ng of DNA. The qPCR cycling was carried out with an initial denaturation at 95 °C for 1 min, followed by 40 amplification cycles of 95 °C for 15 s and 60 °C for 60 s for the reactions prepared with Luna Universal Master Mix and initial denaturation at 95 °C for 30 s, followed by 40 cycles of 95 °C for 10 s and 60 °C for 30 s for the reactions prepared with ChamQ Master Mix. The annealing and extension phases were combined, and the fluorescence signal was measured after each cycle in both cases.

Melting curve analyses were performed by increasing the temperature from 60 °C to 95 °C in 0.5 °C intervals with each step held for 5 s and fluorescence measured after each increment. Before implementing the ChamQ Universal SYBR qPCR Master Mix, comparative tests with the Luna Master Mix were carried out and showed no differences in the obtained values. Previously amplified lokiarchaeal 16S rRNA gene fragments as well as circular viral DNA from Loki-B36 cultures were used as quantification standards. Standard curves were created with a dilution series in the range from 10 to 10⁸ copies and were quantified in triplicate. The reaction efficiencies were between 90% – 95%, with R² values of >0.99.

Polymerase Chain Reaction (PCR) and gel electrophoresis

To assess whether circular viral DNA was present in the individual gradient fractions, polymerase chain reaction (PCR) followed by agarose gel electrophoresis was carried out. Every PCR reaction (25 μ L total volume) consisted of 13.75 μ L Milli-Q water, 5 μ L Q5 reaction buffer (New England BioLabs, USA), 0.125 μ L forward primer (10 μ M final concentration), 0.125 μ L reverse primer (10 μ M final concentration), 0.5 μ L deoxynucleotide (dNTP) solution mix (10 μ M stock concentration), 0.25 μ L bovine serum albumin (BSA; 0.5 mM stock concentration), 0.25 μ L Q5® High-Fidelity DNA Polymerase (New England BioLabs, USA), and 5 μ L template DNA. Amplification was performed with a TProfessional Standard Thermocycler (Biometra, Germany) under the following cycling

conditions: initial denaturation at 98 °C for 5 min, 35 cycles of denaturation at 98 °C for 30 s, annealing at 60 °C for 30 s and extension at 72 °C for 30 s, followed by a final extension at 72 °C for 10 min.

PCR products were analysed by gel electrophoresis with a PowerPac™ Basic Power Supply (Bio-Rad Laboratories, USA). A 1% agarose gel was prepared with 1 x TAE buffer (ROTIPHORESE®; Carl Roth, Germany) and supplemented with SYBR Safe DNA Gel Stain (1:10,000 dilution) (Thermo Fisher Scientific Inc., USA). For each sample, 10 µL of PCR product was mixed with 2 µL gel loading dye (New England BioLabs, USA) and loaded onto the gel. GeneRuler 100 bp Plus DNA Ladder (Thermo Fisher Scientific Inc., USA) was used as a molecular size marker. Gel electrophoresis was performed at 120 V for 30 min with a constant current of 400 mA. The gels were visualized and documented via a Universal Hood II imaging system (Bio-Rad Laboratories, USA).

Isolation and purification of viral particles

Four 500 mL Loki-B36 cultures in exponential growth phase were used for provirus isolation. Each of the four cultures was split in half, resulting in a total of eight cultures. In half of them, the virus was induced by introducing ambient air into the headspace at a volume corresponding to 10% of the final culture volume, while the other half remained untreated and served as a control. All eight cultures were incubated for 18 days at 20 °C without light exposure or shaking.

qPCR was used to determine whether virus induction occurred in the cultures exposed to ambient air (see below). Lokiarchaeal B36 cell numbers were quantified in all eight cultures in the same way. Although the virus induction was not observed in any of the four air-exposed cultures, all eight cultures (air-exposed and untreated control) were nevertheless used for further virus purification.

All centrifugation steps were performed in a benchtop 5810 R centrifuge (Eppendorf, Germany) equipped with a swing-bucket rotor (A-4-18). Ultracentrifugation was carried out in an Optima XPN-80 ultracentrifuge (Beckman & Coulter, USA) using an SW 41 Ti swing-bucket rotor. For sample concentration, Amicon Ultra-15 Centrifugal Filters (30 kDa MWCO; Sigma-Aldrich, Ireland) were used. All cultures were centrifuged at $11,300 \times g$ at RT for 40 min. Afterwards, the pellets were resuspended in 1 mL of the

respective supernatant, merged into four samples (corresponding to the starting four cultures), centrifuged again at $21,130 \times g$ at 4°C for 20 min, flash-frozen in liquid nitrogen and stored at -80°C . The supernatants were pooled into four samples as well (corresponding to the starting four cultures) and precipitated by adding 40% PEG 6000 (Sigma-Aldrich, Germany) to a final concentration of 10% PEG 6000. Following this, the samples were thoroughly shaken and incubated overnight at 4°C .

The next day, all four samples were centrifuged at $15,000 \times g$ at 4°C for 90 min. The resulting supernatants were stored at 4°C , whereas the resulting pellet samples were resuspended in 1 mL of salt buffer (100 mM Tris pH 7.4, 0.4 M NaCl, 14 mM $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 14 mM $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 9.3 mM KCl, 50 mM $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$) and merged.

The merged sample was filtered through a $0.65 \mu\text{m}$ pore size filter (Minisart® NML; Sartorius AG, Germany), transferred into a new Falcon tube and diluted to a final volume of 4 mL with the salt buffer. The sample was concentrated to 1 mL using 30 kDa Amicon filters by centrifugation at 3,220 rpm at RT for 28 min, split into two and loaded onto a 60% – 15% OptiPrep (Sigma-Aldrich, Germany) density gradient. The gradient consisted of 2 mL layers of 60%, 35%, 30%, 25%, 20% and 15% OptiPrep. 500 μL of the concentrated sample was carefully layered on top of the gradient and ultracentrifuged at $288,000 \times g$ at 4°C for 22 h.

After ultracentrifugation, the gradient was sampled in 1 mL fractions, yielding 12 gradient fractions in total (two fractions per gradient layer). The subsamples from two technical replicates were merged, mixed by pipetting up and down and then split into two 1 mL aliquots. One aliquot was stored at 4°C for electron microscopy analysis, while the other aliquot was used for DNA extraction followed by PCR and gel electrophoresis as previously described.

As no virus was detected in any of the gradient fractions, indicating that the PEG precipitation was not successful in precipitating the wanted virus particles, the amount of circularized viral genomes in the supernatant from the PEG precipitation step was quantified.

From each supernatant sample, 10 mL was taken, combined with the same type of sample (control or induced) and concentrated with 30 kDa Amicon filters by centrifugation at 3,220 rpm at RT for 2 h until a final volume of 1 mL per filter was reached. From these concentrated samples, DNA was extracted, followed by PCR targeting the circular viral DNA and gel electrophoresis as previously described.

As the provirus could also not be detected in the supernatant, qPCR was performed on both the gradient layer samples and these supernatants to confirm the absence of viral DNA and validate the PCR results; no circular viral DNA was detected by qPCR either.

The virus purification was repeated without induction by adding ambient air or PEG precipitation. Two 500 mL Loki-B36 cultures in exponential growth phase were used. Both cultures were concentrated using two 30 kDa Amicon filters (one filter per culture) to reach a final volume of 500 μ L in total (250 μ L per culture). Concentration was performed by centrifugation at 3,220 rpm at RT until the wanted final volume was reached (~ 9 h).

The concentrated samples were loaded on 60% – 15% OptiPrep gradients and subsequently ultracentrifuged at $152,000 \times g$ at 4 °C for 21 h. The concentrations as well as the volumes of the layers were identical to those used previously (see above).

The respective layers from the two samples were merged, resulting in a final volume of 4 mL per layer for each of the six layers in total. While 1 mL was reserved for microscopy, the remaining 3 mL was further concentrated through 30 kDa Amicon filters to achieve a final volume of 200 μ L by centrifugation at 3,220 rpm at RT for 6 min. These six concentrated samples were used for DNA extraction, followed by PCR and gel electrophoresis as previously described.

Microscopy Analysis

To assess whether differences in cell morphology and distribution occurred between cultures which were exposed to 10% ambient air and untreated cultures, a 500 mL Loki-B36 culture in exponential growth phase was used for phase-contrast and fluorescence microscopy.

Six 50 mL aliquots were transferred into anaerobic, sterile 50 mL serum flasks. To three of these six, 10% ambient air relative to their total culture volume was introduced into the gas phase, whereas the other three cultures remained untreated and no air was added, serving as controls. The cultures were sampled 7 and 21 days after adding air, with time point 0 (T₀) taken immediately after the introduction of 10% air into the headspace. At the respective time points, 1 mL was sampled from each culture. For cell fixation, 20 µL of a 25% glutaraldehyde stock solution (Sigma-Aldrich, Germany) was added to each sample, thus a final concentration of 0.5% glutaraldehyde was reached. Subsequently, the samples were stained with one drop of NucBlue (Thermo Fisher Scientific Inc., USA). The samples were then incubated for 20 min at RT, protected from light exposure. After incubation, cells from each sample were spun down by centrifugation at 21,000 × *g* at RT for 5 min and 900 µL of the resulting supernatant was carefully aspirated, while avoiding disturbance of the cell pellet. The cells were resuspended in 900 µL fresh Milli-Q water and pelleted again under the same centrifugation conditions as before. The supernatant was aspirated again, and the cells were resuspended in 450 µL Milli-Q water to reach twofold-concentrated samples. Afterwards, 10 µL of each of the six samples was transferred into a well of an epoxy microscope slide (Paul Marienfeld GmbH & Co. KG, Germany) and dried at 60 °C for 8 min. After drying, 2 µL of VectaShield (Vector Laboratories, Inc., USA) was applied to the dried cells, covered with a coverslip (Fisher Scientific GmbH, Austria) and sealed with nail polish. Microscopy was performed with a Nikon Eclipse Ni-U microscope (Nikon Corporation, Japan). For every sample, five phase-contrast images and five fluorescence images were acquired, resulting in a total of 10 images per sample.

AI based tools

ChatGPT v. GPT 5.2 and Deepl DL were used to correct grammatical as well as language errors and providing suggestions for improving phrasing and style in certain passages of the text.

Results

This study aimed to gain insights into virus-host interactions in laboratory cultures of *Ca. Lokiarchaeum ossiferum* strain B36 by investigating the induction of its integrated provirus. This contributes to a broader understanding of the virology of Asgard archaea as well as to the further characterization of their associated archaeal viruses. To induce the provirus and thus increase the number of viral particles, different stress conditions were applied to the cultures, including exposure to ambient air (5%, 10% and 20%), elevated sodium chloride (NaCl) concentrations (4.5% (w/v) and 5.5% (w/v)) and mitomycin C treatment (0.5 µg/mL and 1.0 µg/mL). In all induction experiments, the mean fold increase was determined by dividing each replicate value by the mean control value at time point 0. In addition, provirus-derived particles were purified, and their morphology was analysed by microscopy in collaboration. The influence of ambient air was also examined through microscopic comparison between 10% air-exposed cultures and untreated cultures for potential differences in cell morphology and distribution.

Induction of FyIV1 by ambient air

The highest increase in detected circularized viral genomes was observed in cultures to which 5% ambient air was added to the gas phase, with circular viral DNA increasing continuously and reaching its peak on day 46 (Figure 7A). In contrast, cultures which were exposed to 10% and 20% ambient air remained largely unchanged throughout the whole experiment and no clear provirus induction was detected under these conditions (Figure 7A). The untreated control cultures indicated an increase in viral particles after 4 days, which continued until day 7, followed by a decline on days 21 and 32 and a subsequent increase towards day 46, when the maximum was reached. However, the control cultures at day 46 showed a comparatively high standard deviation (Figure 7A).

In parallel, Loki-B36 cell numbers per mL, estimated based on 16S rRNA gene copy numbers, were also determined at the last three sampling points (days 21, 32 and 46) to exclude the possibility that the observed increase in detected circular viral DNA was not solely due to the increase in cell numbers (Figure 7B). In cultures where 5% ambient air was added to the gas phase, an increase in Loki-B36 cell numbers was observed from day

21 to day 32, reaching a maximum of approximately 3.7×10^5 cells per mL, followed by a slight decrease on day 46. In contrast, in cultures exposed to 10% and 20% ambient air, no Loki-B36 cells were detected during the experimental period (Figure 7B). In the untreated control cultures, Loki-B36 cell numbers remained stable on days 21 and 32 and showed a slight increase on day 46 (Figure 7B).

Due to limited culture growth and generally low cell numbers observed in the initial ambient air introduction experiment, the experiment was repeated with modifications to the experimental setup.

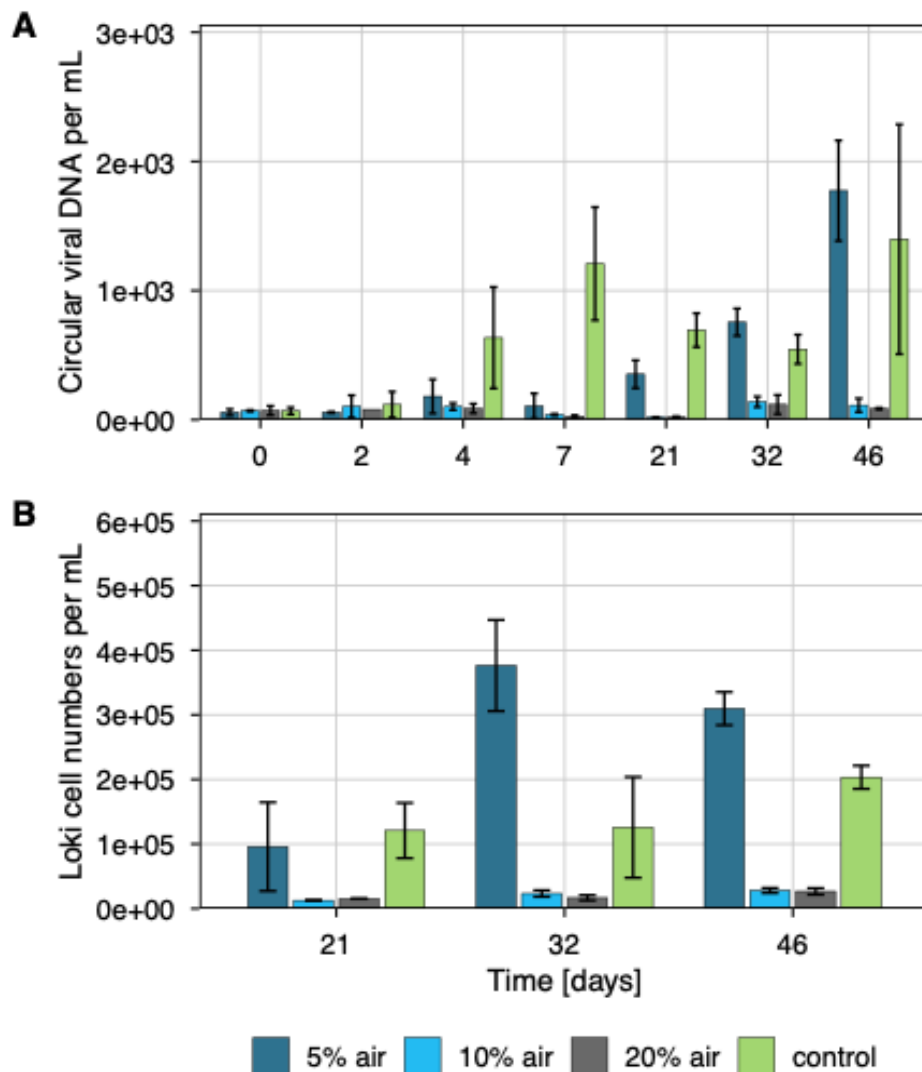


Figure 7: Circular viral DNA and *Ca. Lokiarchaeum ossiferum* strain B36 abundance after exposure to ambient air. **A**, Circular FyIV1 DNA copies per mL measured in cell pellets at 0, 2, 4, 7, 21, 32 and 46 days after exposure to ambient air corresponding to 5%, 10% and 20% of the culture volume, compared with untreated control cultures (C). **B**, *Loki-B36* cell numbers per mL measured in the same experimental cultures at 21, 32 and 46 days after exposure to ambient air. Error bars represent ± 1 standard deviation (SD) of three biological replicates (n=3).

When the experiment was repeated, a Loki-B36 culture in exponential growth phase was directly divided and 5% and 10% ambient air were added to the gas phase of the resulting cultures alongside untreated control cultures. The numbers of viral genomes and Loki-B36 cells were determined in the cell pellet as well as in the concentrated culture supernatant fractions over the course of 28 days (Figure 8).

In the cell pellet fractions, the highest fold increase in circular viral DNA was detected 21 days after adding 10% ambient air to the gas phase of the cultures, corresponding to a mean fold increase of 1.26 (Figure 8A; Supplementary Table S1). In cultures where 5% ambient air was added to the gas phase, an increase in the number of viral genomes was observed from day 7 to day 21, followed by a decrease on day 28 (Figure 8A). In contrast, the control cultures remained largely unchanged during the experiment, with only a slight increase towards day 21 (Figure 8A).

In the concentrated culture supernatant fractions, the highest fold increase in circular viral DNA was detected in cultures exposed to 10% ambient air, where a continuous increase was observed from day 14 onwards and reached a maximum mean 2.4-fold increase on day 28 (Figure 8C; Supplementary Table S2). In cultures where 5% ambient air was added to the gas phase, a slight decrease in the number of viral genomes occurred between days 7 and 14, followed by an increase on day 21 and a subsequent decrease again on day 28 (Figure 8C). In the control cultures, circular viral DNA remained largely unchanged throughout the experiment (Figure 8C).

A comparison between the cell pellet fractions and the concentrated culture supernatant fractions revealed that the increase in circular viral DNA was higher in the concentrated supernatant of cultures in which 10% ambient air was added to the gas phase (Figures 8A and 8C). A maximum mean fold increase of 2.4 was reached on day 28, whereas the corresponding cell pellet fractions showed a lower maximum mean fold increase of 1.26 on day 21 under the same condition. In cultures exposed to 5% ambient air, only moderate changes were detected in both the concentrated supernatant and cell pellet fractions, with both fractions reaching their maximum mean fold increase on day 21,

corresponding to 1.62 in the concentrated supernatant fractions and 1.06 in the cell pellet fractions (Figures 8A and 8C).

However, high standard deviations across the samples indicated high variation between them; thus, the individual samples driving this variability were determined and additional graphs were created presenting each replicate separately, as the error bars represent ± 1 standard deviation (Figures 8B and 8D). Furthermore, the specific fold-change values are provided in the appendix (Supplementary Tables S1 and S2).

Notably, in the cell pellet fractions, a large error bar in the cultures exposed to 10% ambient air on day 21 (Figure 8B) could be attributed to the high value of replicate 3, which reached a fold increase of 1.72 and resulting in an increased standard deviation (Supplementary Table S1). The remaining replicates in cultures to which 5% ambient air was added as well as in the untreated control cultures showed comparable values at the corresponding time points (Figure 8B).

In the concentrated culture supernatant fractions, high standard deviations were observed across several samples (Figure 8D). This variability was driven by individual replicates and varied depending on the experimental condition or control as well as on the time point. In cultures to which 5% ambient air was added to the gas phase, replicate 2 showed a higher value than the other two replicates on day 7, reaching 1.97, whereas on day 21, replicate 1 contributed most to the high standard deviation, with a value of 2.34 (Figure 8D; Supplementary Table S2). However, on day 28, the replicate values of these cultures largely converged. In cultures exposed to 10% ambient air, the three replicates showed comparable values on day 7 and the same pattern was observed on day 14, but on both days 21 and day 28, replicate 1 and replicate 3 displayed higher values, reaching 1.96 and 2.65 on day 21 and 3.21 and 2.51 on day 28 (Figure 8D; Supplementary Table S2). In the control cultures, replicate values were largely consistent at all time points, except on day 28, when replicate 1 reached a value of 2.44 (Figure 8D; Supplementary Table S2).

Compared with the cell pellet fractions, variability among replicates in the concentrated culture supernatant fractions was observed more often in the experimental conditions and the control as well as across sampling time points. The outliers did not totally overlap between the two fractions, suggesting a heterogeneous and partly stochastic provirus

induction pattern. Nevertheless, the higher values in replicates 1 and 3 on days 21 and 28 in the concentrated supernatant of cultures exposed to 10% ambient air indicate that this variability was not completely random (Figures 8B and 8D).

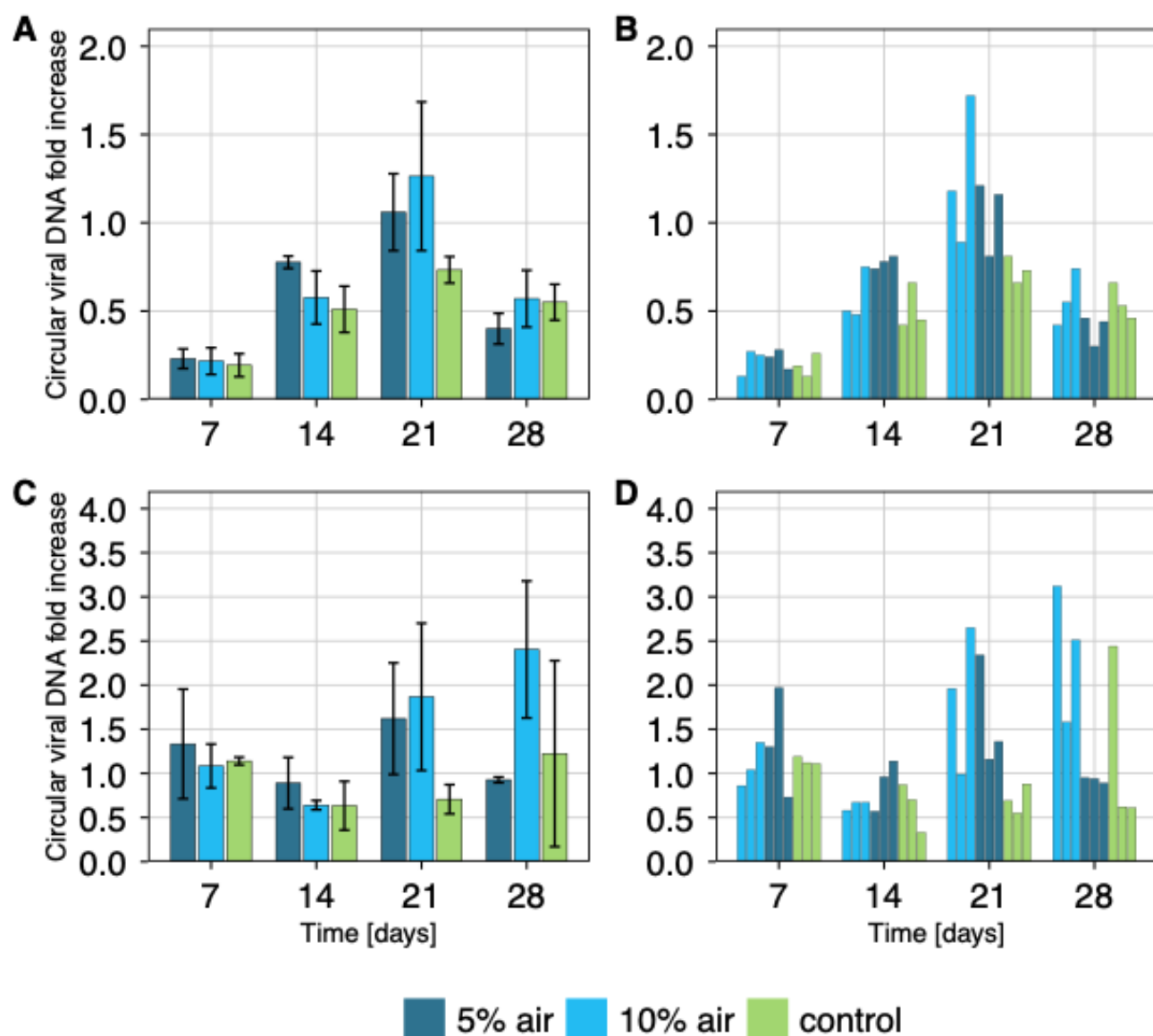


Figure 8: Circular viral DNA fold increase in the cell pellet and concentrated culture supernatant fractions after exposure to ambient air. **A**, Mean circular viral DNA fold increase measured in the culture cell pellet fraction at 7, 14, 21, and 28 days after exposure to ambient air corresponding to 5% and 10% of the culture volume, compared with untreated control cultures (C). **B**, Circular viral DNA fold increase values in the culture cell pellet fraction for each experimental condition and time point. **C**, Mean circular viral DNA fold increase measured in the concentrated culture supernatant fraction at 7, 14, 21, and 28 days after exposure to ambient air corresponding to 5% and 10% of the culture volume, compared with untreated control cultures (C). **D**, Circular viral DNA fold increase values in the concentrated culture supernatant fraction for each experimental condition and time point. Error bars in panels A and C represent ± 1 standard deviation (SD) of three biological replicates ($n=3$). In panels B and D, bars represent individual biological replicates ($n=3$), shown from left to right as replicate 1, replicate 2 and replicate 3.

In parallel to the quantification of circular viral DNA copies in the cell pellet and supernatant fractions, Loki-B36 cell numbers per mL were also determined in the same samples to assess whether the observed increase in circular viral DNA was due to an increase in host cell abundance. The Loki-B36 cell numbers followed the same trend in all experimental conditions, reaching their maximum on day 7 after the introduction of ambient air, followed by a decline on day 14 and reached their minimum on day 28 (Figure 9). Since the cultures were already in exponential growth phase when ambient air was introduced into the gas phase of the cultures, an early decline in Loki-B36 cell numbers is likely related to the growth dynamics of the cultures (Figure 9).

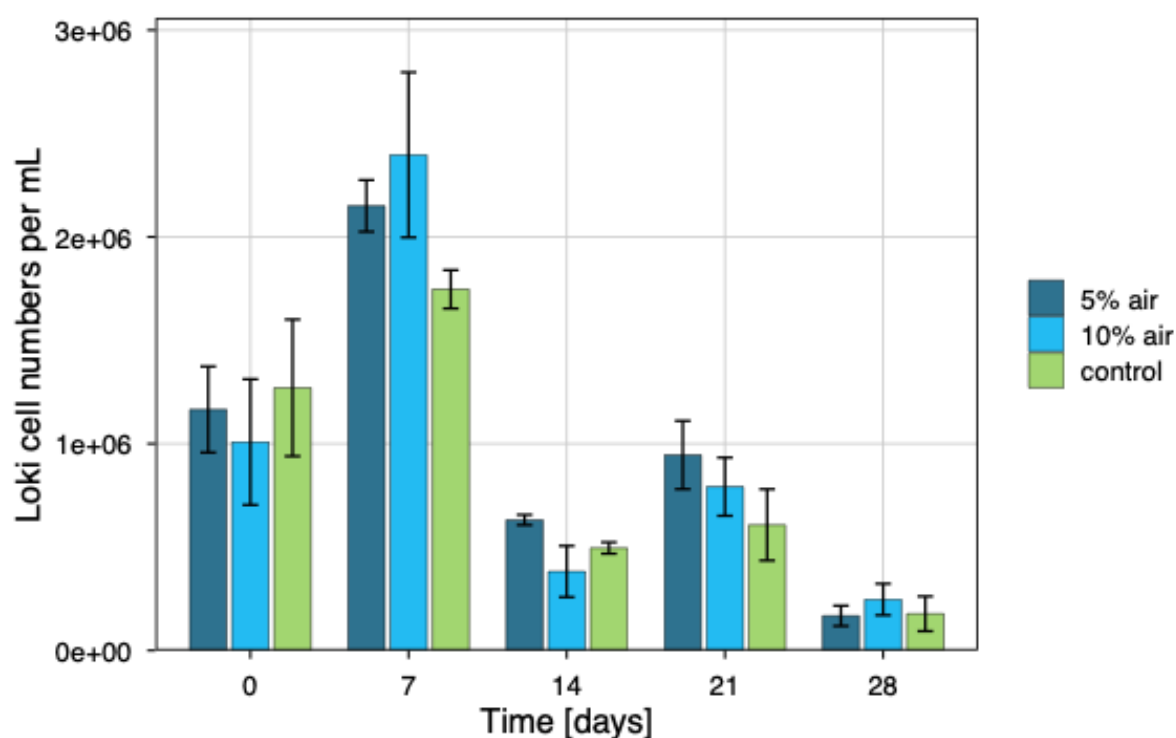


Figure 9: *Ca. Lokiarchaeum ossiferum* strain B36 abundance after exposure to ambient air. Loki-B36 cell numbers per mL measured in cell pellets from the same experimental cultures at 0, 7, 14, 21 and 28 days after exposure to ambient air corresponding to 5% and 10% of the culture volume, compared with the untreated control cultures (C). Error bars represent ± 1 standard deviation (SD) of three biological replicates (n=3).

Increased medium salinity does not reproducibly induce FylV1

Another approach tested in *Ca. Lokiarchaeum ossiferum* strain B36 was increasing the sodium chloride (NaCl) concentration to assess whether elevated salinity affected provirus induction, as NaCl is already a component of the cultivation medium and changes in salinity have been reported to influence virus-host dynamics in other systems (Williamson and Paul, 2006; Zhang *et al.*, 2022).

In order to test this, the NaCl concentration was increased from the standard 2.07% (w/v) to 4.5% (w/v) and 5.5% (w/v), while the control cultures remained at the standard NaCl concentration. Circular viral DNA was quantified after 7, 10 and 15 days in the cell pellet fractions as well as in the concentrated culture supernatant fractions.

No increase in viral genomes was detected in cell the pellet fractions after 7 days of NaCl addition, neither in the 4.5% (w/v) and 5.5% (w/v) treatments nor in the untreated control cultures. The highest fold increase in circular viral DNA was observed in the control cultures on day 15. However, no mean fold increase above 1 occurred in any experimental condition throughout the experiment (Figure 10A), indicating that increased salinity concentrations did not trigger the induction of the provirus.

In the concentrated culture supernatant fractions, cultures with a NaCl concentration of 4.5% (w/v) showed an increase from day 7 to day 10, reaching a maximum mean fold increase of 2.29, followed by a decrease on day 15 (Figure 10B; Supplementary Table S4). Similarly, the mean fold increase in circular viral DNA in the 5.5% (w/v) NaCl-treated cultures increased from day 7 to day 10 with a maximum mean fold increase of 2.26 and decreased again by day 15 (Figure 10B; Supplementary Table S4). In the untreated control cultures, the same overall pattern was observed, but with a lower maximum mean fold increase of 1.55 on day 10 (Figure 10B; Supplementary Table S4).

The concentrated culture supernatant fractions showed a higher fold increase in viral DNA in cultures treated with 4.5% (w/v) and 5.5% (w/v) NaCl than the cell pellet fractions, regardless of the sampling time point. The untreated control cultures from the concentrated culture supernatant fractions reached a maximum mean fold increase of 1.55 on day 10, while the control cultures from the cell pellet fractions had a maximum

mean fold increase of 0.91 on day 15 (Supplementary Tables S3 and S4). Thus, the concentrated culture supernatant fractions and the cell pellet fractions showed different patterns during the experiment (Figures 10A and 10B).

Furthermore, compared with the cell pellet fractions, in the concentrated culture supernatant fractions large error bars in the graphs were observed, indicating higher variation among replicates. Therefore, an additional graph was generated to show each replicate separately in order to identify which samples contributed to this variation, as the error bars represent ± 1 standard deviation (Figure 10C).

In cultures to which 4.5% (w/v) NaCl was added, replicate 1 showed a circular viral DNA fold increase of 2.79, while replicate 3 exhibited a value of 0.21 on day 7, leading to a high standard deviation and a correspondingly large error bar, since replicate 2 was excluded from the analysis (Figure 10C; Supplementary Table S4). In the 5.5% (w/v) NaCl-treated cultures, large error bars were observed on days 7, as well as day 10. At both time points, this variability was driven by replicate 3, which showed a fold increase of 1.75 on day 7 and 3.59 on day 10 (Figure 10C; Supplementary Table S4). In the untreated control cultures, a large error bar was visible on day 10 due to replicate 1 with a fold increase of 2.99, whereas replicates 2 and 3 exhibited values < 1 (Figure 10C; Supplementary Table S4).

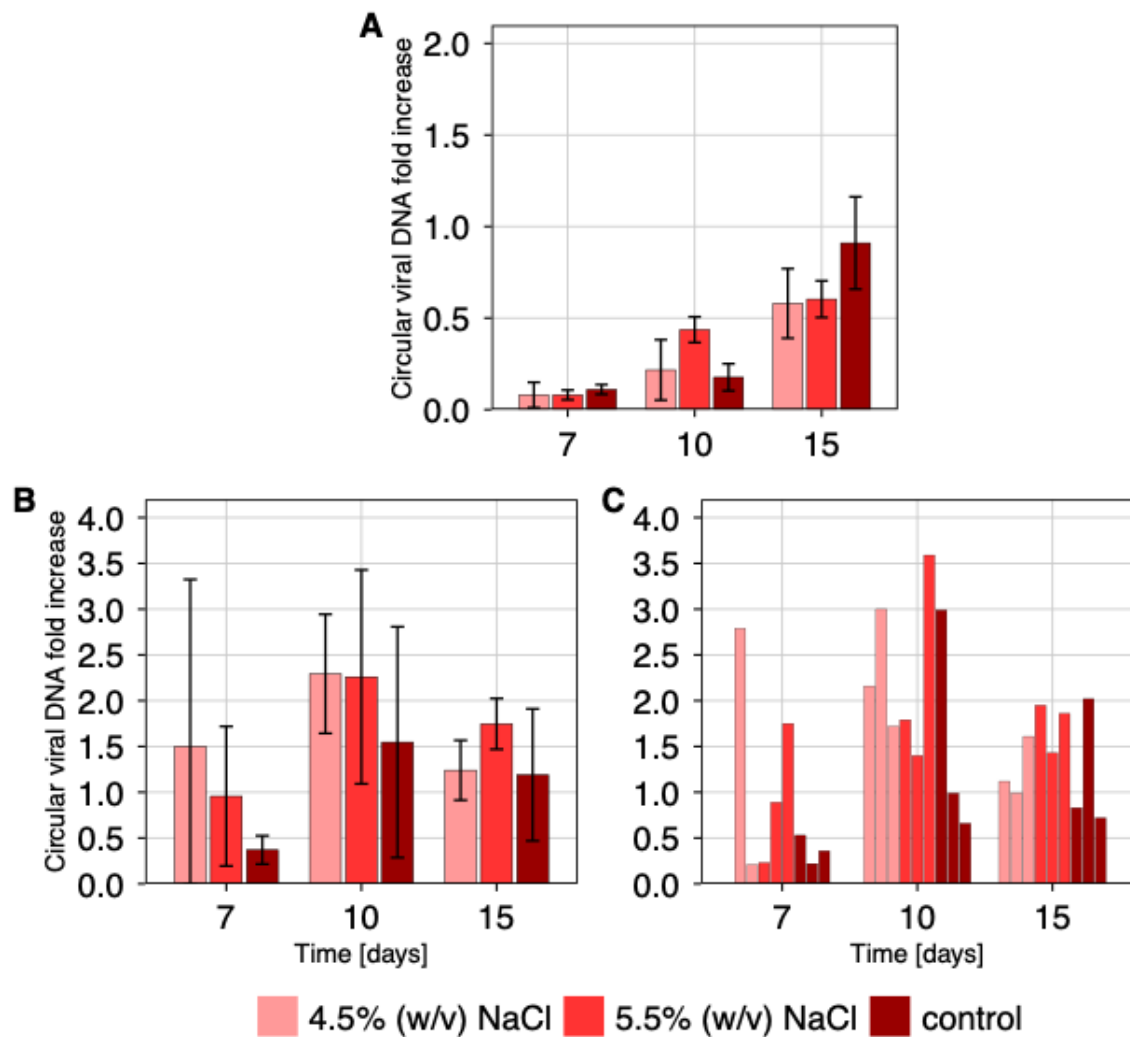


Figure 10: Circular viral DNA fold increase in the cell pellet and concentrated culture supernatant fractions under NaCl stress conditions. **A**, Mean circular viral DNA fold increase measured in the culture cell pellet fraction at 7, 10, and 15 days under NaCl stress conditions corresponding to 4.5% and 5.5% (w/v) NaCl, compared with untreated control cultures (C). **B**, Mean circular viral DNA fold increase measured in the concentrated culture supernatant fraction at 7, 10, and 15 days under NaCl stress conditions corresponding to 4.5% and 5.5% (w/v) NaCl, compared with untreated control cultures (C). **C**, Circular viral DNA fold increase values in the concentrated culture supernatant fraction for each experimental condition and time point. Error bars in panels A and B represent ± 1 standard deviation (SD) of three biological replicates (n=3), except for cultures exposed to 4.5% (w/v) NaCl at day 7 in panel B, for which replicate 2 was excluded from the analysis due to a value >19 (Supplementary Table S4). In panel C, bars represent individual biological replicates (n=3), shown from left to right as replicate 1, replicate 2 and replicate 3.

The growth of Loki-B36 cells was also monitored in parallel over the course of the experiment. After NaCl addition to the cultures at the start of the experiment, Loki-B36 cell numbers increased in the 4.5% (w/v) and 5.5% (w/v) NaCl-treated cultures as well as in the untreated control cultures until day 7. This was followed by a further slight increase until day 10 and a subsequent decline on day 15 in both NaCl-treated cultures and the untreated control cultures. Overall, Loki-B36 cell numbers showed a similar pattern in the NaCl-treated cultures and the untreated control cultures (Figure 11).

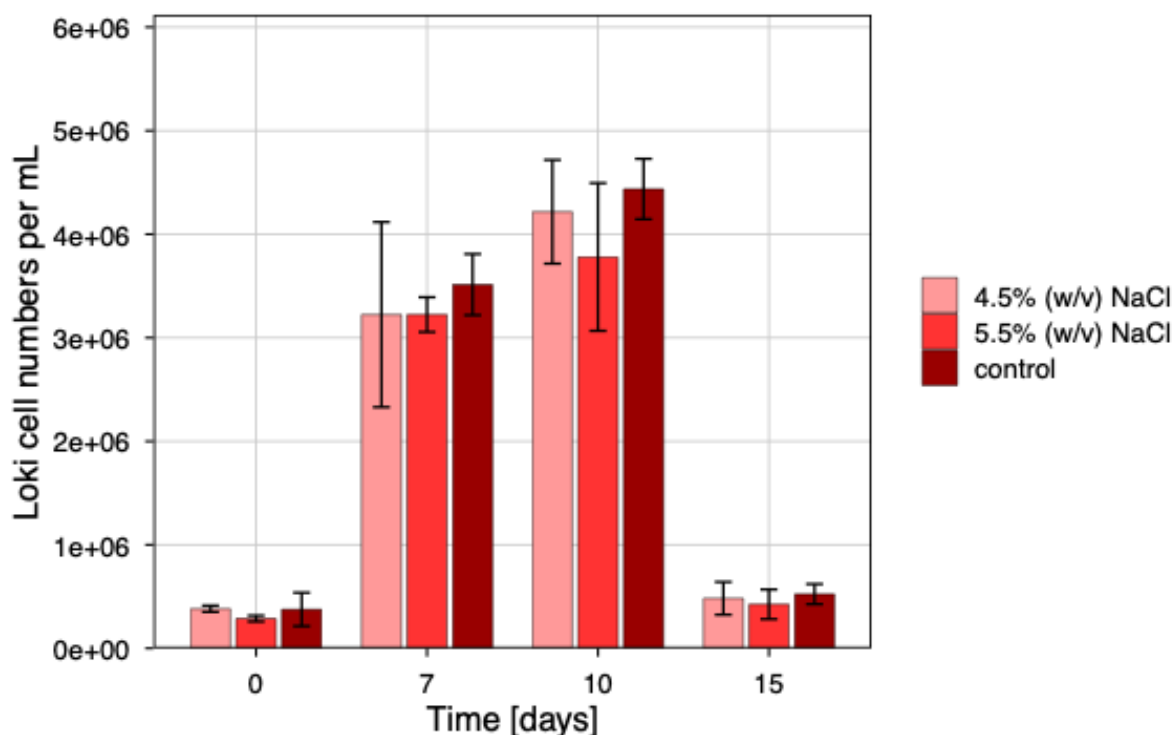


Figure 11: *Ca. Lokiarchaeum ossiferum* strain B36 abundance under NaCl stress conditions. Loki-B36 cell numbers per mL measured in cell pellets from the same experimental cultures at 0, 7, 10 and 15 days under NaCl stress conditions corresponding to 4.5% and 5.5% (w/v) NaCl, compared with untreated control cultures (C). Error bars represent ± 1 standard deviation (SD) of three biological replicates (n=3).

Mitomycin C does not induce FylV1

Next, the effect of mitomycin C (MMC), a cytotoxic antibiotic, on the induction of the integrated provirus of *Ca. Lokiarchaeum ossiferum* strain B36 was tested.

In this experiment, cultures were treated with 0.5 $\mu\text{g/mL}$ or 1.0 $\mu\text{g/mL}$ MMC, while untreated cultures served as the control. Samples were collected 1, 4 and 24 hours after the introduction of MMC at the start of the experiment, and circular viral DNA was quantified only in the cell pellet fractions.

In cultures to which 0.5 $\mu\text{g/mL}$ MMC was added, the fold increase remained largely unchanged over the entire experiment, reaching a mean fold increase of around 1. In contrast, in cultures treated with 1.0 $\mu\text{g/mL}$ MMC, a slight continuous increase in circular viral DNA was observed, reaching a maximum mean fold increase of 1.45 at 24 hours after adding MMC to the cultures. In the untreated control cultures, a slight increase in viral genomes was observed from 1 to 4 hours and the values subsequently remained largely unchanged at 24 hours. However, the mean fold increase remained < 1

across all sampling time points during the experiment (Figure 12A; Supplementary Table S5).

Furthermore, Loki-B36 cell numbers per mL were determined in parallel to examine whether cytotoxic MMC treatment caused short-term changes in host abundance. In both the 0.5 $\mu\text{g/mL}$ and 1.0 $\mu\text{g/mL}$ MMC-treated cultures as well as in the untreated control cultures, the mean Loki-B36 cell numbers remained within a range of approximately 1×10^6 to 1.6×10^6 cell numbers per mL, indicating that no clear effect of MMC treatment on Loki-B36 cell abundance was observed throughout the experiment (Figure 12B).

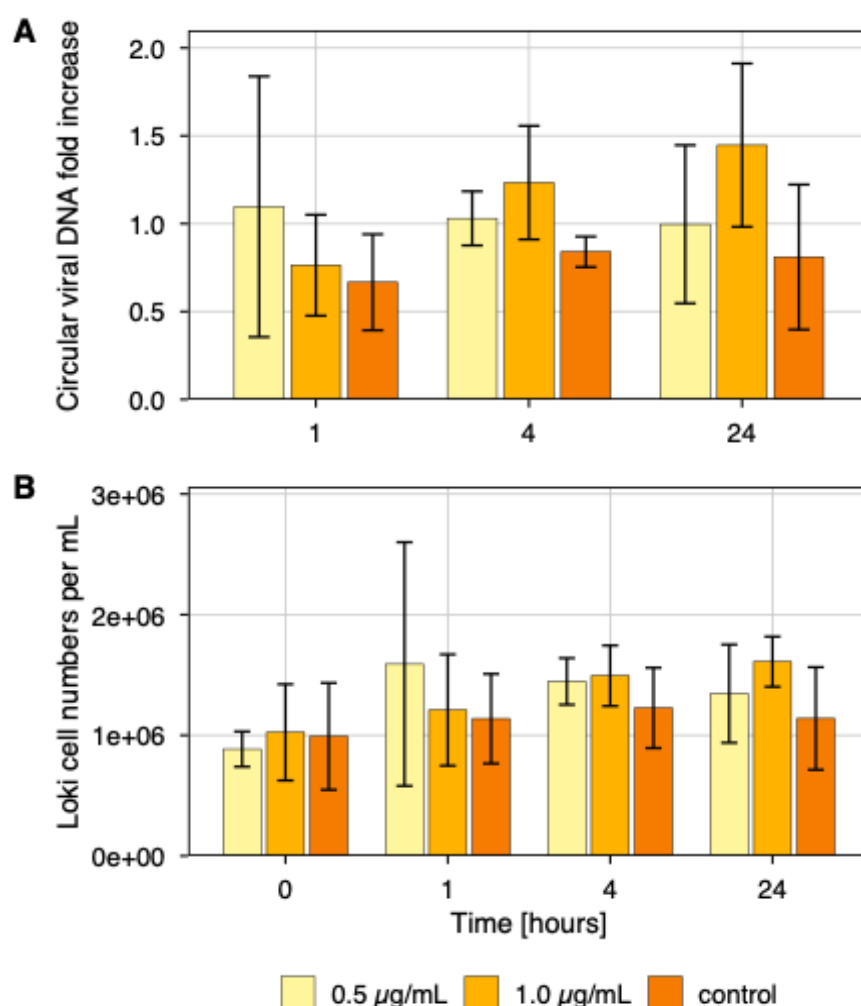


Figure 12: Circular viral DNA and *Ca. Lokiarchaeum ossiferum* strain B36 abundance after mitomycin C (MMC) treatment. **A**, Mean circular viral DNA fold increase measured in cell pellets at 1, 4, and 24 hours following MMC treatment corresponding to 0.5 $\mu\text{g/mL}$ and 1.0 $\mu\text{g/mL}$, compared with untreated control cultures (C). **B**, Loki-B36 cell numbers per mL measured in the same experimental cultures at 1, 4, and 24 hours following MMC treatment. Error bars represent ± 1 standard deviation (SD) of three biological replicates (n=3).

Microscopy analysis of Loki-B36 cultures exposed to ambient air and untreated control cultures

Microscopy analysis was performed to assess whether exposure to 10% ambient air affected cell morphology and distribution in Loki-B36 cultures compared with untreated control cultures. The 10% condition was selected because a twofold increase in circular viral DNA was detected under this treatment.

The enrichment culture of Loki-B36 contains at least three microbial partners, including spirochaetes (spiral-shaped), *Halodesulfovibrio* (rod-shaped), and members of the order *Izempiasmatales* (coccoid) (Radler *et al.*, 2025; preprint; Maslač *et al.*, 2026; preprint). Lokiarchaeal cells exhibit a variable morphology characterized by a cell body and several cellular protrusions, which can vary in number and length (Radler *et al.*, 2026; preprint).

All samples from cultures to which 10% ambient air was added to the gas phase as well as untreated control cultures were collected immediately after air introduction (T0) and after 7 (T1) and 21 (T2) days. Afterwards, these samples were imaged by phase-contrast and fluorescence microscopy, and representative micrographs are shown below (Figure 13). The corresponding fluorescence images displayed detectable signals at all three time points for both treated and untreated cultures and their respective replicates. This confirmed the presence of DNA-containing cells in the cultures and allowed clearer visualization of the Loki-B36 cells rather than only detached cellular protrusions (Figure 13). However, quantification of Loki-B36 cell abundance from microscopy images was not performed due to high cell density as well as the presence of cell clusters.

At time point 0 (T0), both cultures exposed to 10% ambient air and the control cultures showed cells which were distributed across the field of view (Figure 13).

At time point 1 (T1), more Loki-B36 cells were qualitatively visible in the images of both treated and untreated cultures than at time point 0 (Figure 13).

At time point 2 (T2), both treated and untreated cultures displayed a higher apparent overall cell density than at the previous time points, and clustered cell arrangements were visible in both treatments (Figure 13).

Across all three time points, *Loki-B36* cells with the characteristic *Ca. Lokiarchaeum ossiferum* morphology were observed in both, 10% air-treated and untreated samples and no clear qualitative treatment-specific difference in cell distribution or morphology was apparent in the representative micrographs. (Figure 13).

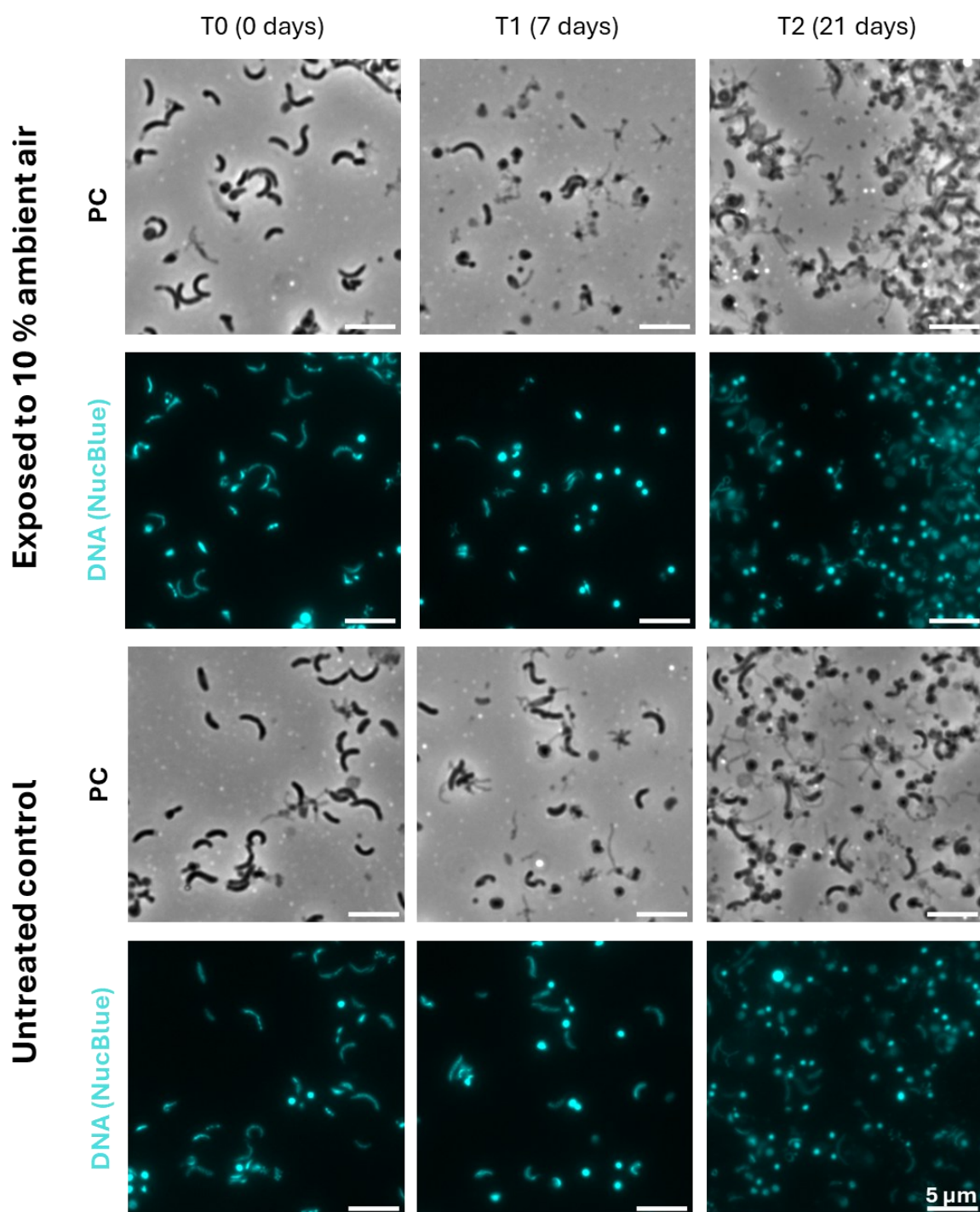


Figure 13: Phase-contrast and fluorescence microscopy of *Ca. Lokiarchaeum ossiferum* strain B36 enrichment cultures. *Loki-B36* cultures exposed to 10% ambient air and untreated control cultures were sampled immediately after air addition (T0) and after 7 (T1) and 21 (T2) days. The upper two rows show cultures exposed to 10% ambient air. The lower two rows show untreated control cultures. For each condition and time point, phase-contrast images and the corresponding fluorescence images are shown, with NucBlue used as DNA stain. Scale bars represent in 5 µm

Isolation and purification of FylV1

In order to isolate viral particles from Loki-B36 cultures for further characterization, a purification procedure including PEG precipitation and OptiPrep density gradient centrifugation was performed with cultures to which 10% ambient air was added to the gas phase. To assess whether FylV1 particles had been successfully isolated, the gradient fractions obtained after ultracentrifugation were analysed by PCR followed by agarose gel electrophoresis.

The expected size of the PCR product corresponding to the circularized FylV1 genome was 320 base pairs (bp), representing a fragment across the circularization junction rather than the complete viral genome (16.1 kbp) (Maslać *et al.*, 2026; preprint).

However, no viral DNA was detected in any of the gradient fractions. Faint bands were observed in both positive controls (PC), whereas no band was visible in the negative control (NC) (Figure 14).

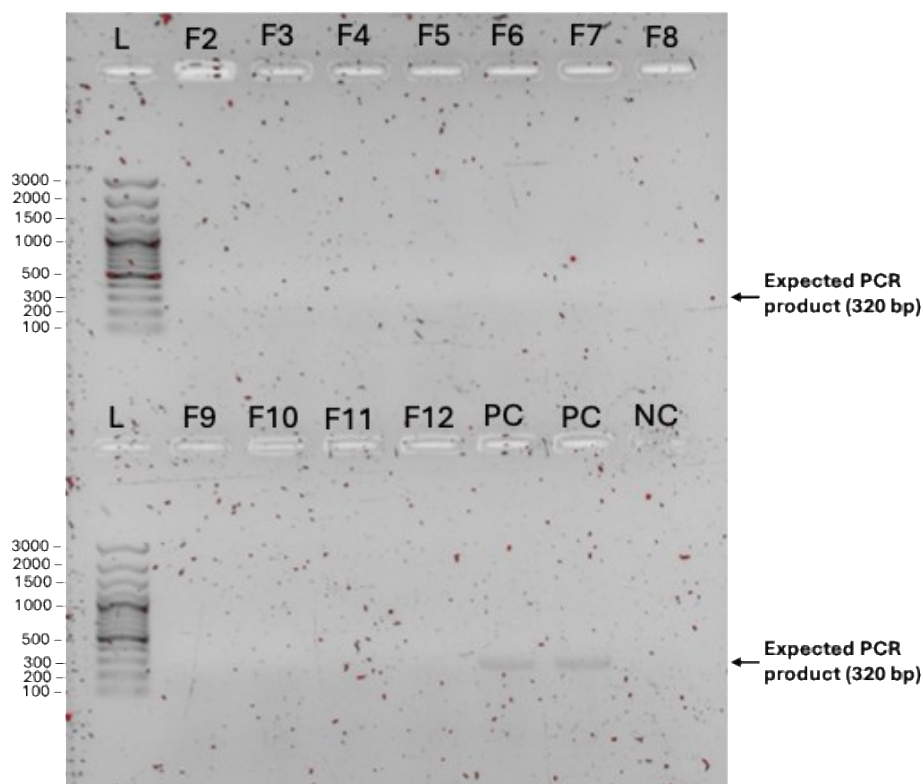


Figure 14: PCR screening of OptiPrep gradient fractions for circularized FylV1 DNA after virus purification. Gradient fractions were screened for the presence of circularized FylV1 DNA by PCR followed by agarose gel electrophoresis (L = Ladder, F2-F12 = corresponding OptiPrep gradient fractions: F2 = 15%, F3-F4 = 20%, F5-F6 = 25%, F7-F8 = 30%, F9-F10 = 35%, F11-F12 = 60%, PC = positive control, NC = negative control).

Since no viral DNA was detected in the OptiPrep gradient fractions, the supernatants obtained from the PEG precipitation step were combined, concentrated and screened in the same way as the OptiPrep gradient fractions to determine whether viral particles had remained in the supernatant instead of being precipitated. Nevertheless, no circularized viral DNA corresponding to the FyIV1 genome was detected in any of the four samples. A faint band was visible in the positive control (PC), while no band was observed in the negative control (NC) (Figure 15).

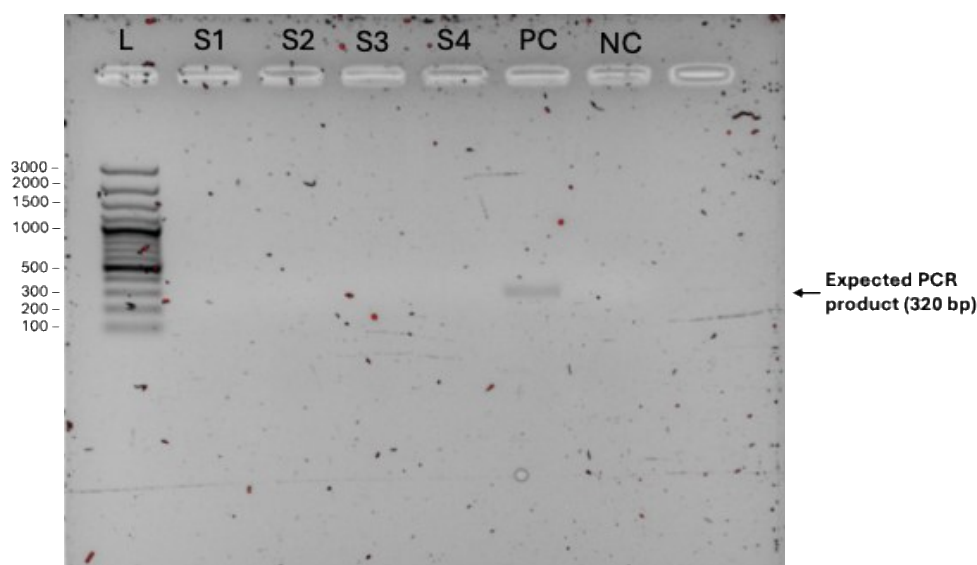


Figure15: PCR screening of PEG-precipitation supernatants for circularized FyIV1 DNA after virus purification. Supernatants from the PEG-precipitated samples used for virus purification were screened for the presence of circularized FyIV1 DNA by PCR followed by agarose gel electrophoresis (L = Ladder, S1-S4 = corresponding samples, PC = positive control, NC = negative control).

As no circularized viral DNA from FyIV1 was detected in either the OptiPrep gradient fractions or the supernatant from the PEG-precipitated samples, the virus purification was repeated using a modified protocol in which 10% ambient air was not added to the gas phase of Loki-B36 cultures and PEG precipitation was omitted. Instead, Loki-B36 cultures in exponential growth phase were directly concentrated, followed by OptiPrep density gradient ultracentrifugation.

In this modified purification, circularized FyIV1 DNA was detected in the 30% and 35% gradient fractions, corresponding to the expected PCR product size of 320 bp. A band was visible in the positive control (PC), whereas no band was observed in the negative control (NC).

In addition, qPCR was performed on the PCR products of the 30% and 35% gradient fractions, showing circularized FylV1 DNA copy numbers of 5.47×10^4 copies per mL for the 30% OptiPrep gradient fraction and 6.16×10^5 copies per mL for the 35% OptiPrep gradient fraction, confirming the PCR and gel electrophoresis data (Figure 16).

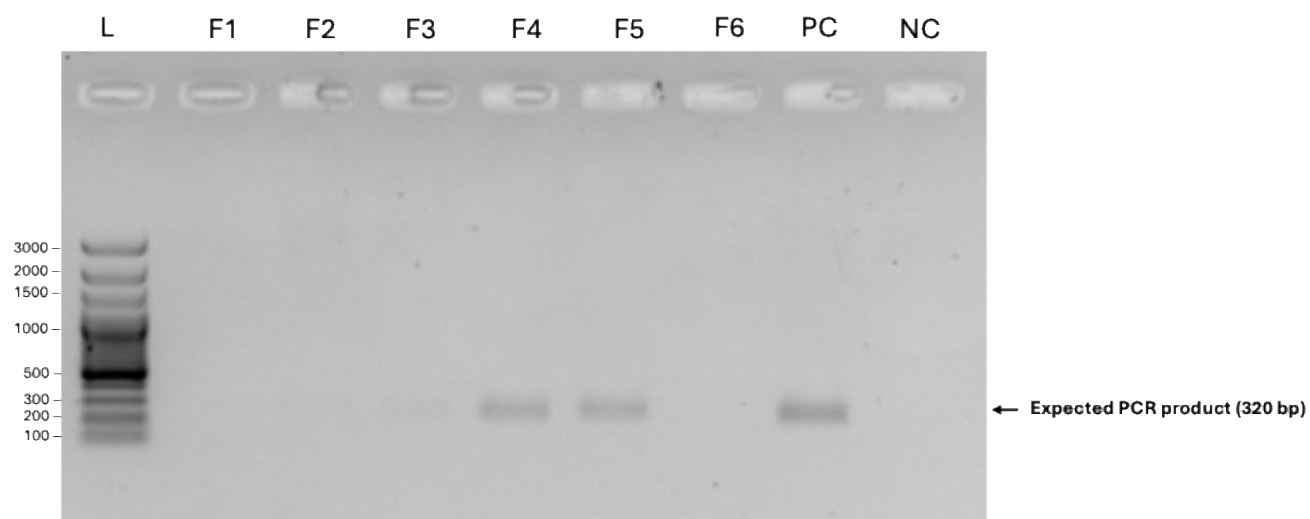


Figure 16: PCR screening of the OptiPrep gradient fractions for circularized FylV1 DNA after modified virus purification. Gradient fractions from the modified virus purification were screened for the presence of circularized FylV1 DNA by PCR followed by agarose gel electrophoresis (L = Ladder, F1-F6 = corresponding OptiPrep gradient fractions: F1 = 15%, F2 = 20%, F3 = 25%, F4 = 30%, F5 = 35%, F6 = 60%, PC = positive control, NC = negative control).

Following the successful virus purification, the two OptiPrep gradient fractions (30% and 35%) containing circularized FylV1 DNA were sent to collaborators at the Institute of Science and Technology Austria (ISTA) for negative staining electron microscopy. The micrographs showed multiple icosahedral virus-like particles, including particles with diameters between 50–60 nm as well as smaller particles of 30 nm. The ~ 55 nm particles were considered candidate FylV1 particles; however, their identity could not be confirmed unambiguously based on morphology alone (Figure 17).

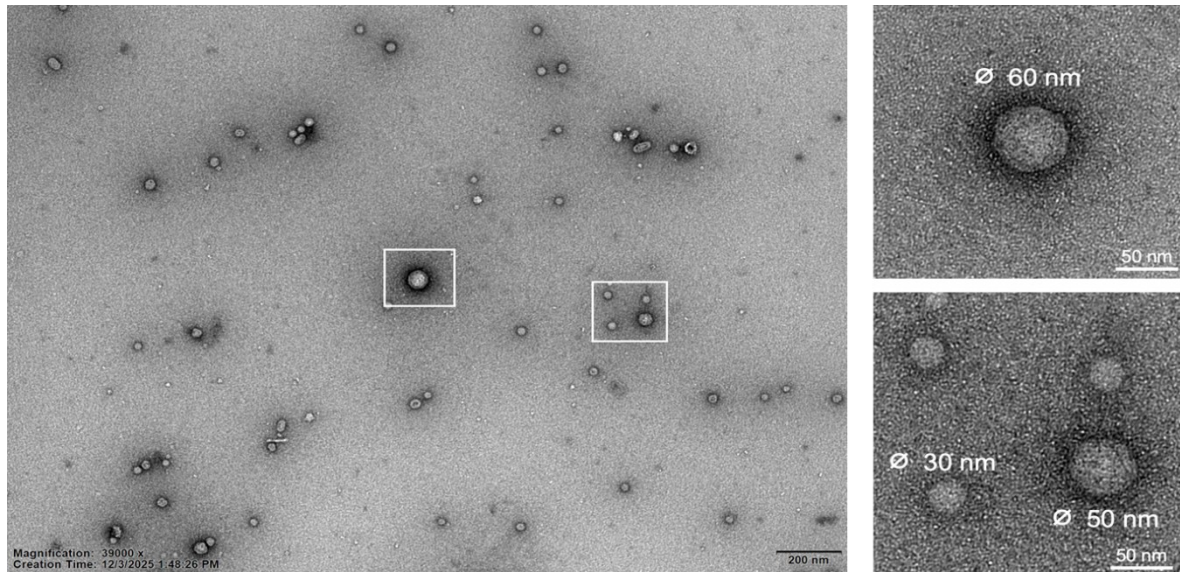


Figure 17: Negative staining electron micrograph of FylV1 candidate particles. Icosahedral virus-like particles with a diameter of ~55 nm are shown on the right (scale bar 50 nm) and their surrounding environment is shown on the left side (scale bar 200 nm). Image provided by collaborators at the Institute of Science and Technology Austria (ISTA).

Discussion and Conclusion

Ca. Lokiarchaeum ossiferum strain B36 (Loki-B36) and its integrated provirus Fylgjavirus 1 (FylV1) represent the first Asgard archaeal virus-host system that is available in culture.

Therefore, the goal of this study was to investigate the possibility of a successful FylV1 provirus induction which is triggered by different stress conditions (ambient air, salinity, mitomycin C), whether selected induction conditions affect Loki-B36 cell morphology and distribution, as assessed by microscopy, and whether viral particles can be purified from the Loki-B36 cultures.

Circular viral DNA of FylV1 was detectable in all induction experiments, including exposure to ambient air, increased sodium chloride (NaCl) concentrations and mitomycin C (MMC) treatment. Depending on the experiment, circular viral DNA was quantified in both cell pellet fractions and concentrated culture supernatant fractions, except for cultures treated with MMC, for which only cell pellet fractions were analysed.

The highest fold increase in circular viral DNA was observed in the ambient air experiments, in which ambient air (5%, 10% and 20%) was added to the gas phase of the cultures. In particular, exposure to 10% ambient air resulted in the highest fold increase in the concentrated culture supernatant fractions, with a maximum mean fold increase of 2.4 on day 28 (Figure 8C). This suggests that 10% ambient air may have been the most effective condition among the tested treatments for triggering FylV1 induction, since Loki-B36 is cultivated under anaerobic conditions and exposure to air represents an oxygen-related stress factor that can promote provirus induction.

This is supported by other studies on temperate bacteriophages, showing that oxidative stress can induce prophages, for example hydrogen peroxide-induced prophage induction of phage λ in *Escherichia coli* and prophages in *Staphylococcus aureus* (Selva *et al.*, 2009; Glinkowska *et al.*, 2010). In these bacterial systems, prophage induction is often associated with DNA damage, which can activate stress response pathways such as the SOS system (Selva *et al.*, 2009; Glinkowska *et al.*, 2010). During the SOS response, damaged DNA can lead to an accumulation of single-stranded DNA, which promotes the activation of RecA. Activated RecA then stimulates cleavage of the bacterial SOS

repressor LexA, thereby leading to the expression of SOS response genes. In the case of phage λ , RecA also promotes cleavage of the phage repressor CI, which can trigger the switch from lysogeny to the lytic cycle, resulting in viral replication and release from an endangered host cell (Lima-Noronha *et al.*, 2022; Zhang *et al.*, 2022).

For archaeal viruses, comparable mechanisms are much less understood. However, the induction of the archaeal proviruses APSV1 and APOV1 from the extremophilic host *Aeropyrum pernix* under limited oxygen supply indicates that changes in oxygen levels can affect archaeal provirus dynamics (Mochizuki *et al.*, 2011; Quemin and Quax, 2015). Thus, while adding ambient air to Loki-B36 cultures may have acted as an oxygen-related stress factor, further studies are needed to gain better insights into how changes in oxygen levels influence FylV1 induction at the molecular level.

Compared with the ambient air experiments, increased NaCl concentrations (4.5% (w/v) and 5.5% (w/v)), as well as MMC (0.5 $\mu\text{g/mL}$ and 1.0 $\mu\text{g/mL}$), also had an effect on circular FylV1 DNA, but it was less consistent across replicates and time points (Figure 10A–B; Figure 12A). Although elevated NaCl concentrations resulted in increased fold changes in circular viral DNA at specific time points in the concentrated culture supernatant fractions (Figure 10C), high variation was observed among the replicates, indicating that salinity stress did not trigger a reproducible induction pattern.

Furthermore, MMC treatment did not lead to a pronounced fold increase in circular FylV1 DNA obtained from the cell pellet fractions (Figure 12A). Since viral particle numbers were only quantified from these fractions, potential accumulation of circular viral DNA in the culture supernatant could not be assessed.

In parallel, Loki-B36 cell abundance, based on 16S rRNA gene copy numbers, was also determined during all induction experiments to assess whether changes in circular viral DNA were associated with changes in host cell numbers. Since the Loki-B36 cultures were already in exponential growth phase at the time when the potential stress conditions were introduced to the cultures, the observed early decline in host cell numbers was likely related to the natural growth dynamics of the enrichment cultures rather than being solely caused by FylV1 induction.

Overall, the induction experiments imply that the induction of FylV1 was not uniform across all treatments, replicates and time points. This was reflected by the high standard deviations observed in some experiments, especially under increased NaCl concentrations, where the variability was mainly driven by individual replicates. Instead of indicating a strong and reproducible induction pattern across the whole culture, this replicate-dependent variability suggests that FylV1 induction may occur in a heterogeneous and potentially stochastic manner. Thus, induction may be limited to only a small fraction of host cells (Maslać *et al.*, 2026; preprint). Similar heterogeneous induction patterns have been described for temperate phage systems, where induction can vary between individual cells and may be related to differences in host cell physiology and stress responses (Nanda *et al.*, 2014; Liang *et al.*, 2025). However, whether comparable mechanisms also apply to archaeal proviruses, particularly to Asgard archaeal viruses, remains largely unknown (Kuiper *et al.*, 2024).

To further assess the influence of the provirus induction condition which showed the strongest response, Loki-B36 cultures exposed to 10% ambient air were analysed by microscopy to determine whether ambient air exposure also affected Loki-B36 cell morphology or cell distribution within the enrichment cultures. Across all time points, Loki-B36 cells with the characteristic *Ca. Lokiarchaeum ossiferum* morphology were observed in both 10% air-treated and untreated control cultures (Radler *et al.*, 2025; preprint). In addition, an apparent increase in cell density and clustering was observed qualitatively over time in both treatments (Figure 13). Thus, 10% ambient air did not cause clear qualitative changes in Loki-B36 cell morphology or distribution under the condition examined.

Finally, virus purification was performed to further characterize FylV1-derived particles. Candidate FylV1 particles were isolated in two OptiPrep gradient fractions (30% and 35%); this was confirmed by qPCR, in which 5.47×10^4 copies per mL (30% gradient fraction) and 6.16×10^5 copies per mL (35% gradient fraction) were quantified. Negative staining electron microscopy of these fractions revealed icosahedral virus-like particles, including particles with a diameter between 50–60 nm, which were considered candidate FylV1 particles. However, their identity could not be confirmed based only on

morphology, especially since smaller particles with a diameter of 30 nm were also present. These smaller particles may represent other extracellular structures, such as extracellular membrane vesicles of various sizes. Thus, further molecular confirmation would be required to unambiguously link the observed particles to the FylV1 provirus (Figure 17).

Together, the induction, microscopy and purification experiments suggest that FylV1 is not strongly induced across the entire Loki-B36 population. Viral production appears to occur only at low levels, consistent with the low qPCR-based fold increases in circular FylV1 DNA observed in this study. In temperate virus-host systems, such low viral titers may suggest a life strategy in which viral induction occurs only at low frequency, so that only a small fraction of host cells enters a productive cycle at a given time, potentially under stress conditions such as oxygen exposure in the case of Loki-B36. A comparable low-level induction pattern has recently been described for the temperate archaeal virus MSTV1 infecting its host *Methanobrevibacter smithii*, where only a small fraction of host cells actively produced virions and the virus-to-host ratio remained low (Baquero *et al.*, 2024). Similar low-frequency induction is also known from temperate bacteriophage systems, in which spontaneous prophage induction can also occur only in a small fraction of host cells (Nanda *et al.*, 2014).

Furthermore, low particle numbers may suggest that the tested stress conditions promote viral excision or circular DNA replication, but do not necessarily result in efficient virion assembly, release or spread through the whole culture. This is important to consider, since qPCR-based detection of circular viral DNA does not directly show the production of high numbers of infectious virions. In the case of FylV1, low qPCR-based fold increases may therefore point towards a heterogeneous, potentially stochastic induction pattern rather than a strong, synchronized induction across the entire host population.

Such a strategy may be beneficial for both the virus and the host, since FylV1 can persist together with its host without causing a strong reduction in host cell abundance, while Loki-B36 may be protected from other viruses. This might be especially advantageous in a slowly growing host, such as Loki-B36, where extensive host lysis would likely be a disadvantage for long-term virus maintenance (Maslać *et al.*, 2026; preprint).

To summarize, these findings provide experimental insights into the first Asgard archaeal virus-host system available in culture. While previous knowledge of Asgard archaeal viruses has largely been based on metagenomic predictions, the Loki-B36/FylV1 system enables the experimental investigation of provirus induction and putative viral particle production under laboratory conditions.

Like other Asgard-associated viruses described so far (Tamarit *et al.*, 2022; Medvedeva *et al.*, 2022; Rambo *et al.*, 2022), FylV1 does not appear to be closely related to eukaryotic viruses. Although FylV1 is related to Asgard-associated skuldviruses, its overall similarity to these viruses is limited and several predicted proteins have no clearly assigned function. This suggests that FylV1 represents a distinct archaeal viral lineage (Maslać *et al.*, 2026; preprint). Therefore, the discovery and characterization of further novel Asgard archaeal viruses remain essential to gain better insights into the diversity of viruses associated with Asgard archaea (Tamarit *et al.*, 2022; Medvedeva *et al.*, 2022).

As an outlook, future studies should combine optimized induction conditions with particle-associated DNA sequencing, microscopy-based approaches such as cryo-TEM, and biochemical characterization. This would help to confirm the identity of the observed candidate FylV1 particles and to further investigate FylV1 interactions with its host Loki-B36. Furthermore, the closely related virus-free strain *Ca. Lokiarchaeum ossiferum* B35 could serve as a valuable reference strain for future experiments with the aim of establishing a genetic system for Asgard archaea, for example through transfection experiments using viral DNA.

References

- Baum, D. A., & Baum, B. (2014). An inside-out origin for the eukaryotic cell. *BMC Biology*, 12, Article 76. <https://doi.org/10.1186/s12915-014-0076-2>
- Baquero, D.P., Liu, Y., Wang, F., Egelman, E. H., Prangishvili, D., & Krupovic, M. (2020). Structure and assembly of archaeal viruses. *Advances in Virus Research*, 108, 127-164. <https://doi.org/10.1016/bs.aivir.2020.09.004>
- Baquero, D. P., Medvedeva, S., Martin-Gallausiaux, C., Pende, N., Sartori-Rupp, A., Tachon, S., Pedron, T., Debarbieux, L., Borrel, G., Gribaldo, S., & Krupovic, M. (2024). Stable coexistence between an archaeal virus and the dominant methanogen of the human gut. *Nature Communications*, 15, 7702. <https://doi.org/10.1038/s41467-024-51946-x>
- Chen, Z., Liu, Y., Wang, Y., Du, X., Deng, X., Xiang, J., Wang, Y., Wang, J., Krupovic, M., Du, S., & Chen, X. (2023). A virus-borne DNA damage signaling pathway controls the lysogeny-induction switch in a group of temperate pleolipoviruses. *Nucleic Acids Research*, 51(7), 3270–3287. <https://doi.org/10.1093/nar/gkad125>
- Eme, L., Tamarit, D., Caceres, E. F., Stairs, C. W., De Anda, V., Schön, M. E., Seitz, K. W., Dombrowski, N., Lewis, W. H., Homa, F., Saw, J. H., Lombard, J., Nunoura, T., Li, W.-J., Chen, L.-X., Banfield, J. F., St John, E., Reysenbach, A.-L., Stott, M. B., ... Ettema, T. J. G. (2023). Inference and reconstruction of the heimdallarchaeal ancestry of eukaryotes. *Nature*, 618(7965), 992–999. <https://doi.org/10.1038/s41586-023-06186-2>
- Glinkowska, M., Łoś, J. M., Szambowska, A., Czyż, A., Całkiewicz, J., Herman-Antosiewicz, A., Wróbel, B., Węgrzyn, G., Węgrzyn, A., & Łoś, M. (2010). Influence of the *Escherichia coli* oxyR gene function on λ prophage maintenance. *Archives of Microbiology*, 192, 673–683. <https://doi.org/10.1007/s00203-010-0596-2>
- Imachi, H., Nobu, M. K., Nakahara, N., Morono, Y., Ogawara, M., Takaki, Y., Takano, Y., Uematsu, K., Ikuta, T., Ito, M., Matsui, Y., Miyazaki, M., Murata, K., Saito, Y., Sakai, S., Song, C., Tasumi, E., Yamanaka, Y., Yamaguchi, T., ... Takai, K. (2020). Isolation of an archaeon at the prokaryote–eukaryote interface. *Nature*, 577, 519–525. <https://doi.org/10.1038/s41586-019-1916-6>
- Imachi, H., Nobu, M. K., Kato, S., Takaki, Y., Miyazaki, M., Miyata, M., Ogawara, M., Saito, Y., Sakai, S., Tahara, Y. O., Takano, Y., Tasumi, E., Uematsu, K., Yoshimura, T., Itoh, T., Ohkuma, M., & Takai, K. (2024). *Promethearchaeum syntrophicum* gen. nov., sp. nov., an anaerobic, obligately syntrophic archaeon, the first isolate of the lineage ‘Asgard’ archaea, and proposal of the new archaeal phylum Promethearchaeota phyl. nov. and kingdom Promethearchaeati regn. nov. *International Journal of Systematic and Evolutionary Microbiology*, 74, 006435. <https://doi.org/10.1099/ijsem.0.006435>
- Jorgensen, S. L., Hannisdal, B., Lanzén, A., Baumberger, T., Flesland, K., Fonseca, R., Øvreås, L., Steen, I. H., Thorseth, I. H., Pedersen, R. B., & Schleper, C. (2012). Correlating microbial community profiles with geochemical data in highly stratified sediments from the Arctic Mid-Ocean Ridge. *Proceedings of the National Academy of Sciences of the United States of America*, 109(42), E2846–E2855. <https://doi.org/10.1073/pnas.1207574109>

- Koonin, E. V., Dolja, V. V., Krupovic, M., Varsani, A., Wolf, Y. I., Yutin, N., Zerbini, F. M., & Kuhn, J. H. (2020). Global organization and proposed megataxonomy of the virus world. *Microbiology and Molecular Biology Reviews*, 84(2), e00061-19. <https://doi.org/10.1128/MMBR.00061-19>
- Krupovic, M., Cvirkaite-Krupovic, V., Iranzo, J., Prangishvili, D., & Koonin, E. V. (2018). Viruses of archaea: Structural, functional, environmental and evolutionary genomics. *Virus Research*, 244, 181–193. <https://doi.org/10.1016/j.virusres.2017.11.025>
- Krupovic, M., Makarova, K. S., & Koonin, E. V. (2022). Cellular homologs of the double jelly-roll major capsid proteins clarify the origins of an ancient virus kingdom. *Proceedings of the National Academy of Sciences of the United States of America*, 119(5), e2120620119. <https://doi.org/10.1073/pnas.2120620119>
- Krupovic, M., Baquero, D. P., Liu, Y., Wang, F., Prangishvili, D., & ICTV Taxonomy Summary Consortium. (2025). Summary of taxonomy changes ratified by the International Committee on Taxonomy of Viruses (ICTV) from the Archaeal Viruses Subcommittee, 2025. *Journal of General Virology*, 106(7), 002117. <https://doi.org/10.1099/jgv.0.002117>
- Kuiper, B. P., Schöntag, A. M. C., Oksanen, H. M., Daum, B., & Quax, T. E. F. (2024). Archaeal virus entry and egress. *microLife*, 5, 1–13. <https://doi.org/10.1093/femsml/uqad048>
- Liang, X., Yang, S., Radosevich, M., Wang, Y., Duan, N., & Jia, Y. (2025). Bacteriophage-driven microbial phenotypic heterogeneity: Ecological and biogeochemical importance. *npj Biofilms and Microbiomes*, 11, 82. <https://doi.org/10.1038/s41522-025-00727-5>
- Lima-Noronha, M. A., Fonseca, D. L. H., Oliveira, R. S., Freitas, R. R., Park, J. H., & Galhardo, R. S. (2022). Sending out an SOS - the bacterial DNA damage response. *Genetics and Molecular Biology*, 45(3 Suppl. 1), e20220107. <https://doi.org/10.1590/1678-4685-GMB-2022-0107>
- Liu, Y., Makarova, K. S., Huang, W.-C., Wolf, Y. I., Nikolskaya, A. N., Zhang, X., Cai, M., Zhang, C.-J., Xu, W., Luo, Z., Cheng, L., Koonin, E. V., & Li, M. (2021). Expanded diversity of Asgard archaea and their relationships with eukaryotes. *Nature*, 593(7860), 553–557. <https://doi.org/10.1038/s41586-021-03494-3>
- López-García, P., & Moreira, D. (2023). The symbiotic origin of the eukaryotic cell. *Comptes Rendus Biologies*, 346, 55–73. <https://doi.org/10.5802/crbiol.118>
- Maslač, N., Ponce-Toledo, R. I., Rodrigues-Oliveira, T., Alarcón Schumacher, T., Homola, M., Luo, Z.-H., White, M. F., Schur, F. K. M., Erdmann, S., & Schleper, C. (2026). Active virus-host system in a Lokiarchaeon culture [Preprint]. *bioRxiv*. <https://doi.org/10.64898/2026.02.13.705702>
- Medvedeva, S., Sun, J., Yutin, N., Koonin, E. V., Nunoura, T., Rinke, C., & Krupovic, M. (2022). Three families of Asgard archaeal viruses identified in metagenome-assembled genomes. *Nature Microbiology*, 7, 962–973. <https://doi.org/10.1038/s41564-022-01144-6>
- Mochizuki, T., Sako, Y., & Prangishvili, D. (2011). Provirus induction in hyperthermophilic archaea: Characterization of *Aeropyrum pernix* spindle-shaped virus 1 and *Aeropyrum pernix* ovoid virus 1. *Journal of Bacteriology*, 193(19), 5412–5419. <https://doi.org/10.1128/JB.05101-11>

- Nanda, A. M., Heyer, A., Krämer, C., Grünberger, A., Kohlheyer, D., & Frunzke, J. (2014). Analysis of SOS-induced spontaneous prophage induction in *Corynebacterium glutamicum* at the single-cell level. *Journal of Bacteriology*, 196(1), 180–188. <https://doi.org/10.1128/JB.01018-13>
- Prangishvili, D., Bamford, D. H., Forterre, P., Iranzo, J., Koonin, E. V., & Krupovic, M. (2017). The enigmatic archaeal virosphere. *Nature Reviews Microbiology*, 15, 724–739. <https://doi.org/10.1038/nrmicro.2017.125>
- Quemin, E. R. J., & Quax, T. E. F. (2015). Archaeal viruses at the cell envelope: Entry and egress. *Frontiers in Microbiology*, 6, 552. <https://doi.org/10.3389/fmicb.2015.00552>
- Radler, P., Viehboeck, T., Luo, Z.-H., Maslać, N., Schmidt, K., Hauschild, R., Nobu, M., Bulgheresi, S., Stradal, T. E. B., Rottner, K., Imachi, H., Sixt, M., & Schleper, C. (2025). Dynamic protrusions mediate unique crawling motility in Asgard Archaea (Promethearchaeota) [Preprint]. *bioRxiv*. <https://doi.org/10.1101/2025.11.30.690169>
- Rambo, I. M., Langwig, M. V., Leão, P., De Anda, V., & Baker, B. J. (2022). Genomes of six viruses that infect Asgard archaea from deep-sea sediments. *Nature Microbiology*, 7, 953–961. <https://doi.org/10.1038/s41564-022-01150-8>
- Rodrigues-Oliveira, T., Wollweber, F., Ponce-Toledo, R. I., Xu, J., Rittmann, S. K.-M. R., Klingl, A., Pilhofer, M., & Schleper, C. (2023). Actin cytoskeleton and complex cell architecture in an Asgard archaeon. *Nature*, 613, 332–339. <https://doi.org/10.1038/s41586-022-05550-y>
- Selva, L., Viana, D., Regev-Yochay, G., Trzcinski, K., Corpa, J. M., Lasa, I., Novick, R. P., & Penadés, J. R. (2009). Killing niche competitors by remote-control bacteriophage induction. *Proceedings of the National Academy of Sciences of the United States of America*, 106(4), 1234–1238. <https://doi.org/10.1073/pnas.0809600106>
- Spang, A., Saw, J. H., Jørgensen, S. L., Zaremba-Niedzwiedzka, K., Martijn, J., Lind, A. E., van Eijk, R., Schleper, C., Guy, L., & Ettema, T. J. G. (2015). Complex archaea that bridge the gap between prokaryotes and eukaryotes. *Nature*, 521, 173–179. <https://doi.org/10.1038/nature14447>
- Tamarit, D., Caceres, E. F., Krupovic, M., Nijland, R., Eme, L., Robinson, N. P., & Ettema, T. J. G. (2022). A closed Candidatus Odinarchaeum chromosome exposes Asgard archaeal viruses. *Nature Microbiology*, 7, 948–952. <https://doi.org/10.1038/s41564-022-01122-y>
- Williamson, S. J., & Paul, J. H. (2006). Environmental factors that influence the transition from lysogenic to lytic existence in the 7HSIC/*Listonella pelagia* marine phage–host system. *Microbial Ecology*, 52, 217–225. <https://doi.org/10.1007/s00248-006-9113-1>
- Wirth, J., & Young, M. (2020). The intriguing world of archaeal viruses. *PLoS Pathogens*, 16(8), e1008574. <https://doi.org/10.1371/journal.ppat.1008574>
- Zaremba-Niedzwiedzka, K., Caceres, E. F., Saw, J. H., Bäckström, D., Juzokaite, L., Vancaester, E., Seitz, K. W., Anantharaman, K., Starnawski, P., Kjeldsen, K. U., Stott, M. B., Nunoura, T., Banfield, J. F., Schramm, A., Baker, B. J., Spang, A., & Ettema, T. J. G. (2017). Asgard archaea illuminate the origin of eukaryotic cellular complexity. *Nature*, 541, 353–358. <https://doi.org/10.1038/nature21031>

Zhang, M., Zhang, T., Yu, M., Chen, Y.-L., & Jin, M. (2022). The life cycle transitions of temperate phages: Regulating factors and potential ecological implications. *Viruses*, 14(9), 1904. <https://doi.org/10.3390/v14091904>

Appendix

Table S1: Circular viral DNA fold change of each culture measured in the cell pellet fractions at 7, 14, 21, and 28 days after exposure to ambient air (5% and 10% of the culture volume) compared to the control (C). Fold change values were calculated by dividing the qPCR value at each time point by the mean qPCR value of the control at day 0. Values represent individual biological replicates, with mean values calculated for each condition.

Condition	Replicate	Day 7	Day 14	Day 21	Day 28
5%	1	0.24	0.74	1.21	0.46
5%	2	0.28	0.78	0.81	0.30
5%	3	0.17	0.81	1.16	0.44
Mean		0.23	0.78	1.06	0.40
10%	1	0.13	0.50	1.18	0.42
10%	2	0.27	0.48	0.89	0.55
10%	3	0.25	0.75	1.72	0.74
Mean		0.22	0.58	1.26	0.57
Control	1	0.19	0.42	0.81	0.66
Control	2	0.13	0.66	0.66	0.53
Control	3	0.26	0.45	0.73	0.46
Mean		0.19	0.51	0.73	0.55

Table S2: Circular viral DNA fold change of each culture measured in the concentrated culture supernatant fractions at 7, 14, 21, and 28 days after exposure to ambient air (5% and 10% of the culture volume) compared to the control (C). Fold change values were calculated by dividing the qPCR value at each time point by the mean qPCR value of the control at day 0. Values represent individual biological replicates, with mean values calculated for each condition.

Condition	Replicate	Day 7	Day 14	Day 21	Day 28
5%	1	1.30	0.57	2.34	0.95
5%	2	1.97	0.96	1.16	0.94
5%	3	0.73	1.14	1.36	0.89
Mean		1.33	0.89	1.62	0.93
10%	1	0.86	0.58	1.96	3.12
10%	2	1.04	0.67	0.99	1.58
10%	3	1.35	0.67	2.65	2.51
Mean		1.08	0.64	1.87	2.40
Control	1	1.19	0.87	0.69	2.44
Control	2	1.12	0.70	0.55	0.62
Control	3	1.11	0.33	0.88	0.61
Mean		1.14	0.63	0.71	1.22

Table S3: Circular viral DNA fold change of each culture measured in the cell pellet fractions at 7, 10 and 15 days under salt stress conditions (4.5% (w/v) and 5.5% (w/v)) compared to the control (C). Fold change values were calculated by dividing the qPCR value at each time point by the mean qPCR value of the control at day 0. Values represent individual biological replicates, with mean values calculated for each condition.

Condition	Replicate	Day 7	Day 10	Day 15
4.5%	1	0.16	0.17	0.74
4.5%	2	0.04	0.08	0.37
4.5%	3	0.04	0.40	0.63
Mean		0.08	0.22	0.58
5.5%	1	0.07	0.51	0.68
5.5%	2	0.11	0.37	0.49
5.5%	3	0.06	0.43	0.64
Mean		0.08	0.44	0.60
Control	1	0.13	0.12	1.20
Control	2	0.12	0.26	0.79
Control	3	0.08	0.15	0.74
Mean		0.11	0.18	0.91

Table S4: Circular viral DNA fold change of each culture measured in the concentrated culture supernatant fractions at 7, 10 and 15 days under salt stress conditions (4.5% (w/v) and 5.5% (w/v)) compared to the control (C). Fold change values were calculated by dividing the qPCR value at each time point by the mean qPCR value of the control at day 0. Values represent individual biological replicates, with mean values calculated for each condition. The value 19.56 (4.5% (w/v), replicate 2, day 7) was excluded from the calculation of the mean, as it was considered an outlier.

Condition	Replicate	Day 7	Day 10	Day 15
4.5%	1	2.79	2.16	1.12
4.5%	2	19.56	3.00	0.99
4.5%	3	0.21	1.72	1.61
Mean		1.50	2.29	1.24
5.5%	1	0.23	1.79	1.95
5.5%	2	0.89	1.40	1.43
5.5%	3	1.75	3.59	1.86
Mean		0.96	2.26	1.75
Control	1	0.53	2.99	0.83
Control	2	0.22	0.99	2.02
Control	3	0.36	0.66	0.72
Mean		0.37	1.55	1.19

Table S5: Circular viral DNA fold change of each culture measured in the cell pellet fractions at 1, 4 and 24 hours following mitomycin C (MMC) treatment (0.5 µg/mL and 1.0 µg/mL) compared to the control (C). Fold change values were calculated by dividing the qPCR value at each time point by the mean qPCR value of the control at day 0. Values represent individual biological replicates, with mean values calculated for each condition.

Condition	Replicate	Day 7	Day 10	Day 15
0.5 µg/mL	1	0.73	0.90	1.35
0.5 µg/mL	2	0.61	1.20	1.15
0.5 µg/mL	3	1.95	0.99	0.49
Mean		1.10	1.03	1.00
1.0 µg/mL	1	0.55	1.79	1.23
1.0 µg/mL	2	0.65	1.40	1.13
1.0 µg/mL	3	1.09	1.59	1.98
Mean		0.76	1.23	1.45
Control	1	0.44	0.89	0.72
Control	2	0.59	0.74	1.26
Control	3	0.97	0.89	0.45
Mean		0.67	0.84	0.81