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The first head- tailed virus, MFTV1, produced by a hyperthermophilic methanogenic deep- sea archaea

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Abstract :

Deep-sea hydrothermal vents are inhabited by complex communities of microbes and their viruses. Despite the importance of viruses in controlling the diversity, adaptation and evolution of their microbial hosts, to date, only eight bacterial and two archaeal viruses isolated from abyssal ecosystems have been described. Thus, our efforts focused on gaining new insights into viruses associated with deep-sea autotrophic archaea. Here, we provide the first evidence of an infection of a hyperthermophilic methanogenic archaea by a head-tailed virus, Methanocaldococcus fervens tailed virus 1 (MFTV1). MFTV1 has an isometric head of 50 nm in diameter and a 150 nm-long non-contractile tail. Virions are released continuously without causing a sudden drop in host growth. MFTV1 infects Methanocaldococcus species and is the first hyperthermophilic head-tailed virus described thus far. The viral genome is a double-stranded linear DNA of 31 kb. Interestingly, our results suggest potential strategies adopted by the plasmid pMEFER01, carried by M. fervens, to spread horizontally in hyperthermophilic methanogens. The data presented here opens a new window of understanding on how the abyssal mobilome interacts with hyperthermophilic marine archaea.

INTRODUCTION

Deep-sea hydrothermal environments are described as extreme habitats but also as "oasis of life" (Marteinsson *et al.*, 1997; Prieur, 1997; Flores and Reysenbach, 2011). These ecosystems are characterized by steep physicochemical and temperature gradients, high hydrostatic pressures and darkness. Some of these parameters can change quickly at both temporal and spatial scales (Flores and Reysenbach, 2011). Abyssal ecosystems host a large diversity of bacteria and archaea which harbor a wide panel of mobile genetic elements, such as viruses and plasmids (Geslin *et al.*, 2003a; Soler *et al.*, 2011; Gorlas *et al.*, 2012; Gorlas *et al.*, 2013; Krupovic *et al.*, 2013; Lossouarn *et al.*, 2015a; Lossouarn *et al.*, 2015b). This suggested that the evolution of microorganisms inhabiting hydrothermal vents is largely driven by mobile genetic elements facilitating DNA exchanges.

Most archaeal viruses have thus far been isolated from hyperthermophilic or extreme halophilic terrestrial hosts belonging to the phyla *Crenarchaeota* and *Euryarchaeota* (Krupovic *et al.*, 2018). Very few viruses have been isolated from marine archaea (Kim *et al*., 2019). To date, only two viruses, PAV1 and TPV1, have been isolated from archaea inhabiting deep-sea hydrothermal vents, both infecting members of hyperthermophilic *Euryarchaeota* of the order *Thermococcales* (Geslin *et al.*, 2003b; Geslin *et al.*, 2007; Gorlas *et al.*, 2012). PAV1 and TPV1 have lemon-shaped virions and infect *Pyrococcus abyssi* and *Thermococcus prieurii,* respectively. Viruses isolated from hyperthermophilic archaea appear to be morphologically more diverse than their mesophilic counterparts. This diversity extends to the genes they encode, with a majority of genes without or only few homologs in public databases (Krupovic *et al.*, 2018). No head-tail viruses infecting hyperthermophilic archaea have been isolated thus far.

Methanogens are a major component and key drivers of microbial carbon cycling in deep-sea hydrothermal vents (Dick, 2019). They are autotrophic producers that play a crucial ecological role in anaerobic environments by removing excess hydrogen and fermentation products to produce CH_4 (Lyu *et al.*, 2018). Within the *Euryarchaeota* phylum, to date 12 species of hyperthermophilic methanogens were classified into 4 genera, *Methanocaldococcus*, *Methanopyrus, Methanotorris* and *Methanothermus* (Huber *et al.*, 1982; L'Haridon *et al.*, 2003; Takai *et al.*, 2004; Lauerer *et al.*, 1986). All strains belonging to the genus *Methanocaldococcus* have been isolated and described from deep-sea environments such as hydrothermally heated sediments (Zhao *et al.*, 1988) or hydrothermal chimneys (Jones *et al.*, 1983; Jeanthon *et al.*, 1998; Jeanthon *et al.*, 1999; L'Haridon *et al.*, 2003; Bellack *et al.*, 2011; Stewart *et al.*, 2015).

Prior to this study, no virus infecting hyperthermophilic methanogens have been isolated. Only, four thermophilic head-tail viruses (ψM1, ψM2, ΦF1 and ΦF3) (Meile *et al.*, 1989; Nölling *et al.*, 1993; Pfister *et al.*, 1998)**,** and two mesophilic viruses (MetSV, Drs3) (Weidenbach *et al.*, 2017; Wolf *et al.*, 2019) have been isolated from methanogens (*Methanobacterium* and *Methanosarcina* genera) recovered from anaerobic sludge digesters.

However, comparative genomics analysis has revealed the presence of proviruses derived from head-tail viruses in several hyperthermophilic deep-sea *Methanocaldococcus* species*: M. jannaschii* strain JAL-1^T (Mjan-Pro1, NC_000909), *M. vulcanius* strain M7^T (Mvul-Pro1, NC_013407) and *M. fervens* strain AG86^T (Mfer-Pro1, NC_013156) (Krupovic *et al.*, 2010). Here, we describe the induction of one of these proviruses and the infectivity of the corresponding virions. The newly discovered virus, siphovirus MFTV1, is produced by *Methanocaldococcus fervens* AG 86^T, a strain isolated in Guaymas basin, Gulf of California, at a depth of ~2000 m (Zhao *et al.*, 1988). MFTV1 is released continuously, with a basal viral production of ~ 3.5×10^8 virions per ml. Its double-stranded DNA genome of 31 kb is identical to the provirus integrated in *M. fervens* strain AG 86^T. We demonstrate its infectivity on phylogenetically related strains of *Methanocaldococcus* sp. which were also isolated from deep-sea hydrothermal vents. We also show horizontal transfer of a plasmid, pMEFER01, carried by *M. fervens* to these hyperthermophilic methanogens. This illustrates the remarkable possibilities of horizontal propagations of mobile genetic elements in abyssal ecosystems at high temperature. The discovery of MFTV1, the first described hyperthermophilic head-tailed virus, isolated from a deep-sea methanogen, provides new data and insights to better understand the

interactions between viruses and marine archaea and their significant ecological and biogeochemical implications.

RESULTS

Virion isolation and morphology

Culture supernatants of more than 70 strains of hyperthermophilic methanogens were screened for the presence of virus particles (Table S1). These strains, that included both reference strains and strains from our laboratory culture collection, were isolated from deep-sea hydrothermal vents on the East Pacific Rise (EPR 9 °N and 13 °N; Guaymas basin) and Mid Atlantic Ridge. Cultures with a positive viral signal in epifluorescence microscopy were further analysed for the presence of virus-like particles by transmission electron microscopy. This analysis led to the identification of the virus MFTV1 (Figure 1) which was produced by *Methanocaldococcus fervens* AG 86^T (UBOCC-M-3290). The strain producing MFTV1 virions was isolated from a 30 cm top layer of deep-sea sediment close to a hydrothermal vent in Guaymas basin, Gulf of California, at a depth of 2 003 m (Zhao *et al.*, 1988). MFTV1 had a density of 1.15 $g.ml^{-1}$, as determined by a linear iodixanol density gradient used in the process of purification. MFTV1 is a head-tail virus, with a hexagonal head of 50 nm in diameter and a non-contractile and flexible tail of approximately 150 nm in length (Figure 1). These morphological features are typical for siphoviruses within the order *Caudovirales*. It is worth noting that during the purification process and all subsequent experiments, virions were exposed to oxygen and a wide range of temperatures (from 4 °C to 80 °C) without apparent adverse effect on their stability and infectivity.

Virus-host relationship

Virions were produced throughout the logarithmic growth phase of *M. fervens* with no addition of stressors and were spontaneously released into the medium. After 6 h 30 min of cultivation at 80°C, the concentration of MFTV1 and *M. fervens* reached 3.5×10^8 virions per ml and 1.6×10^8 cells per ml, respectively (corresponding to a ratio of 2.2 viruses per cell). During growth of the infected host, no decrease in cell density was observed, suggesting that only a small subpopulation of cells lysed to release the virions (Figure 2).

Viral induction assays

Attempts were made to increase the viral production by induction using different chemical or physical treatments and physiological stresses (see experimental procedures for details) (Figure 2; Figures S1, S2, S3 and S4). In most cases no induction of virus production was observed when applying the stress. The viral production followed cellular growth. Nevertheless, a slight stimulation of virus production was observed when variations of growth temperature were tested on cultures during the log growth phase (Figure 2). The maximal viral concentration of 3.5×10^8 virions/ml was reached after 6 h 30 min of cultivation without stress whereas with cold stress (4°C during 30 min) the maximal viral concentration of 3.5×10^8 virions/ml was reached earlier after 4 h 30 min of cultivation (Figure 2). Moreover, at 2 h 30, the viral production was found to be significantly different for cold stress compared to the optimal growth condition at 80 °C, as a result of stress applied (Student test, $p=0.047$). Thus, under stresses, we did not observe a substantial virus induction, the maximal virus titer was not higher but it was reached faster.

Metals are naturally present in the deep sea vent systems. *M. fervens* was isolated from Guaymas basin which contains Cu as trace elements. Only two vents areas had concentrations above the detection limit (about 1.1 µmoles/kg and 0.1 µmoles/kg) (Von Damm *et al.*,1985). We investigated the effect of copper (30 µM) on viral production (Figure S2). Again, we did not observe induction but rather a decrease of the viral production. Viral preparations from cultures, with or without copper, were observed by TEM and Cryo-EM. While some vesicles were observed in copper-free cultures, copper-exposed cultures showed greater production of membrane vesicles (MVs) together with a very low level of viral production (Figure 3). This observation of membrane vesicles production by *M. fervens* shows that this archaeon has different mechanisms for the transfer of a variety of molecular cargoes.

Host range

In order to confirm the stability of MFTV1 virions in hyperthermophilic condition, where the host thrives, as well as the infectivity of MFTV1 on strains of *Methanocaldococcus,* the kinetic of infection was investigated in two steps. We first investigated the stability of MFTV1 virions subjected to high temperatures. This was done by incubating MFTV1 virions for 10 hours at 80 °C in DSM141 modified medium, without any host (Figure 4C, 16S at 10h and MCP at 10h). The infectivity of these MFTV1 virions were then tested on a co-culture of 4 *Methanocaldococcus* strains. Based on 16S rRNA comparison, these 4 strains share more than 99 % of identity with *Methanocaldococcus bathoardescens* and *Methanocaldococcus jannaschii* (Table 1 and Table S2). The reference strains of *M. bathoardescens and jannaschii*

are closely related to *M. fervens* (average nucleotide identity of 84.7 % and 83.9 %, respectively) (Rodriguez and Konstantinidis, 2016).

Infection assays were monitored by qPCR. The copy number of the virus encoded MCP (Major Capsid Protein) gene was about 3.4×10^5 at 10 h, just before the addition of the *Methanocaldococcus* cells (Figure 4C, MCP at 10h). After addition of the *Methanocaldococcus* co-culture to the virions, an increase in the MCP gene copy number was observed and reached 1.2×10^7 at 15 h when the virions had been in the presence of potential host cells for 5 h (Figure 4C, MCP). After 36 h, the copy number of the MCP gene was 4.1×10^6 copies per ml. This increase of MCP gene copies suggests that *Methanocaldococcus* strains were successfully infected with MFTV1. Moreover, these in vitro experiments showed that MFTV1 virions are stable in hyperthermophilic condition and keep their infectivity at 80 °C. Throughout the 36 h experiment, *M. fervens*, the positive control, showed a stable copy number of the 16S rDNA gene (10^8 copies per ml), the viral MCP (Major Capsid Protein) gene (10^{10} copies per ml) and the plasmid gene (10^{10} copies per ml) (Figure 4A). As expected for the negative control, the pool of non-infected *Methanocaldococcus* strains expressed the 16S rDNA gene (10^8 to 10^9 copies per ml) but no expression for the MCP gene and the plasmid gene was detected (Figure 4B).

Unexpectedly, a rise in the copy number of a hypothetical gene (WP-012795053), encoded by the plasmid pMEFER01 carried by *M. fervens* (Soler *et al.*, 2011), was also observed when the *Methanocaldococcus* strains were infected with MFTV1 virions. At 10 h, 1.3×10^6 copies of the plasmid gene per ml were observed, the maximum number of 1.5×10^7 copies per ml was

observed at 15 h and 6.6×10^6 copies per ml were observed after 36 h (Figure 4C). To investigate if the plasmid is packaged by virions, PCR amplification of a 599 bp plasmid fragment was obtained on DNA extracted from purified MFTV1 virions pretreated with benzonase 1U/µl. In addition, we obtained an amplification of a 599 bp plasmid fragment, from total DNA extracted after infection of a pool of *Methanocaldococcus* by virions previously treated with benzonase. The signal was lost when naked plasmid was treated with benzonase (1U/µl) (Figure S5). The observation of a plasmid band by PCR confirmed that the plasmid was protected inside viral capsids. We did not observed smaller MFTV1 capsids, in TEM, that are likely to contain a single copy of the plasmid (22.1 kb).

DNA content of MFTV1 and viral genome analysis

To compare the MFTV1 genome extracted from purified virions to the 31.2 kb provirus integrated in the *M. fervens* chromosome, experimental and *in silico* digestions with EcoRV were performed. Lengths of the fragments obtained for the provirus *in silico* and those obtained experimentally for MFTV1 were identical. To confirm that the DNA packaged in MFTV1 virions corresponds to the proviral sequence, the viral DNA genome extracted from purified virions was sequenced with Ilumina MiSeq. The reads were assembled into a linear contig of 31.2 kb (Figure 5). The viral DNA sequence obtained was identical, except for one 2 bp deletion, to the provirus sequence on *M. fervens* chromosome (Krupovic *et al.*, 2010). The viral genome contains 44 open reading frames (ORFs), 27 of which could be functionally annotated (for details, see corresponding annotation Table S5 in Supplementary Information).

MFTV1 genome encodes proteins conserved across bacterial and archaeal members of the order *Caudovirales*, including those responsible for virion morphogenesis and structure. In particular, it contains the three essential components of genome packaging machinery (Sun *et al.*, 2012) (ORFs 1, 2, 3): the portal protein as well as the small and large subunits of the terminase complex. The portal protein is located at the fivefold vertex of the capsid through which the DNA is threaded into the head. Notably, the portal protein of MFTV1 is fused to the phage Mu gpF-like minor capsid protein (PF04233; Table S5). The large terminase provides energy for packaging with its ATPase activity and also has nuclease activity for packaging initiation and termination. The small subunit of the terminase recognizes the viral DNA and brings it to the large terminase subunit for the initial cleavage (Sun *et al.*, 2012). The capsid maturation protease and MCP are encoded by ORF9 and ORF11, respectively. The MFTV1 MCP is homologous to the HK97-like MCPs conserved in all members of the orders *Caudovirales*, including archaeal viruses, and *Herpesvirales* (Duda and Teschke, 2019). ORF4, ORF14, ORF17, ORF20 and ORF21 code for proteins predicted to be involved in the assembly and structure of the tail.

MFTV1 encodes a homolog of the minichromosome maintenance (MCM) helicase (ORF41), the main replicative helicase in archaea and eukaryotes (Bell and Botchan, 2013). Notably, helicases are the most commonly encoded genome replication proteins in viruses with dsDNA genomes (Kazlauskas *et al.*, 2016). Presumably, MFTV1 helicase recruits the host replisome to the replication origin of the viral genome. Indeed, previous phylogenetic analysis has suggested that MCM helicases encoded by *Methanocaldococcus* proviruses have been horizontally acquired from the host (Krupovic *et al.*, 2010). Notably, virus TPV1 isolated form deep-sea

Thermococcales also encodes an MCM helicase, which apparently has been independently acquired from its respective host. ORF42, which is located immediately downstream of the *mcm* gene, encodes an exonuclease homologous to 3'-5' proofreading exonucleases found in family B DNA polymerases (PF10108) and thus might also participate in the MFTV1 DNA replication.

MFTV1 ORFs 7, 28 and 39 encode putative transcription regulators with various DNA-binding domains (Table S5), whereas ORF27 encodes a histone homolog and might be involved in the condensation and regulation of transcription of the viral genome, as is the case with archaeal histones (Sanders *et al.*, 2019). Other ORFs encoding proteins involved in genome transactions include ORF24 and ORF44. ORF24 encodes a homolog of the RecA/RadA /Rad51-family recombinase and may be involved in homologous DNA recombination. Homologous recombination plays an important role during reparation of double-strand DNA breaks, the restart of stalled replication forks and may participate in the initiation of replication (Hogrel *et al.*, 2018). ORF44 encodes a XerC/D-like integrase of the tyrosine recombinase superfamily which is likely to be responsible for the integration of the MFTV1 genome into and excision from the host chromosome.

Interestingly, ORF35 and ORF36 represent a toxin–antitoxin (TA) system of the HicA-HicB family in which HicA functions as a translation-independent mRNA interferase and HicB is the inhibitor of HicA (Turnbull and Gerdes, 2017). Different TA systems are commonly encoded by plasmids, including those of hyperthermophilic archaea (Krupovic *et al.*, 2013), but are far less frequent in viral genomes. We hypothesize that the TA system of MFTV1 stabilizes the proviral state. Indeed, in the case of bacteriophage P1, toxin causes cell death upon curing of

the episomal prophage, whereas the antitoxin prevents host death when prophage is retained (Lehnherr *et al.*, 1993). Finally, ORFs 33, 34, 40 and 43 encode proteins with domains of unknown function (DUF). Many DUFs are highly conserved in all three domains, suggesting an important role.

Some of our analyses, based on PCR, suggest that the plasmid pMEFER01 (22.1 kb), harbored by *M. fervens*, may be packaged in viral particles. It has been reported that the frequency of plasmid incorporation into virus particles increases with the DNA similarity between the plasmid and the corresponding virus (Bravo and Alonso, 1990). We have not detected any homology between MFTV1 and pMEFER01. Thus, the mechanism of plasmid incorporation into MFTV1 virions remains unclear.

DISCUSSION

It is worth noting that most of the archaeal viruses isolated from hot environments display diverse and original morphotypes and genomic content not observed among viruses of bacteria or eukaryotes (Prangishvili *et al.*, 2017). Therefore, it has been suggested that their greater diversity and uniqueness is an adaptation to the extreme physicochemical conditions under which these viruses and their hyperthermophilic hosts thrive. Until now, head-tail viruses had not been isolated from hyperthermophilic archaea, raising questions regarding the thermostability of this morphotype. The characterization of MFTV1 puts this discussion to rest by unequivocally demonstrating, for the first time, that head-tail viruses can propagate at very hot temperatures more than 90°C. Moreover, the viral genome presents a typical array of genes found in head-tailed viruses of bacteria and archaea.

In nature, interactions between viruses and their hosts can take multiple forms that cannot always be classified into the 3 main viral cycles: lytic, lysogenic or chronic cycles. Under the influence of environmental factors, such as temperature variations, the mode of infection cycle may vary. Hydrothermal vents are known to be environment with fluctuating physicochemical conditions. To cope with an environment with so many potential stressors, viruses and hosts must adopt several strategies to live. In a non-extreme marine habitat, it was observed for algal viruses that a minor change of the temperature (+1 °C) had impacts on viral infection strategy (switch from lytic cycle to carrier state) and the development of resistance in algae against viral infection (Demory *et al.*, 2017).

Lysogeny has been suggested to be common in hydrothermal deep-sea vents (Anderson *et al.*, 2013), i.e. in the integrated state the viruses avoid being exposed to the harsh environmental conditions. From a cellular point of view, carrying a virus could increase the host fitness by enhancing, via introduced novel genes and/or mutations, survivability and adaptation to fluctuating environmental conditions (Anderson *et al.*, 2011).

MFTV1 is a temperate virus with a particular lysogenic cycle, where MFTV1 virions have not been shown to be inducible and do not cause a sudden drop in host growth. Several explanations are possible, including that the stresses applied to induce viral production were not the right ones or were not carried out under optimal conditions. Another possibility is that the culture conditions commonly used in the laboratory to grow *M. fervens*, were already non-optimal or stressful, and induced basal viral production resulting in low-level induction.

MFTV1 possesses different strategies to maintain itself. We suggest that a fraction of the

infected microbial population maintains the integrated viral genome within it, thus ensuring its stability, while another fraction produces virions that will have a role as "explorers" to ensure dispersion. MFTV1 might in this way be an example of the spontaneous induction phenomenon, where the excision of the provirus is not triggered by an external element such as a stressor (Howard-Varona *et al.*, 2017). Under this scenario a part of the microbial population is lysed and a part continues to grow to maintain host and viral populations. We hypothesize that the TA system (HicA/HicB) carried by MFTV1 stabilizes its proviral state. Further analyses will be necessary to better understand the MFTV1 viral cycle (entry and release mechanisms for example). Moreover, it is important to note that virions are stable and remain infectious even if they have been exposed to aerobic or anaerobic conditions, to low (4 °C) or high temperatures (80-90°C). These conditions can naturally be encountered in the deep-sea hydrothermal environment. Study the structure of MFTV1, by an atomic force microscopy approach for example, may reveal particular adaptations in response to extreme physico-chemical conditions.

From an ecological point of view, the *Methanocaldococcus* strains which we successfully infected by MFTV1 *in vitro* were isolated from Pacific Ocean (East Pacific Ridge 13 °N and from Juan de Fuca 45 °N). *M. fervens*, the natural infected host, was isolated from the Guaymas basin located also in Pacific Ocean, and geographically close to 13°N and Juan de Fuca hydrothermal vent fields. The infection described in our study therefore seems to be possible in natural environments.

Our results suggest potential strategies adopted by the plasmid pMEFER01 to spread

horizontally in the population. The plasmid could be packaged into the MFTV1 virions and transferred to new cells by transduction. Similar molecular piracy has been described for virus ΨM1, which infects a methanogen *Methanothermobacter marburgensis* (Meile *et al.*, 1989). The capsids of ΨM1 package multimers of pME2001, a plasmid of 4.4 kb carried by *M. marburgensis*, with a total size identical to the size of the viral genome. As observed here for MFTV1 and pMEFER01, no sequence homology has been detected between ΨM1 and pME2001 (Meile *et al.*, 1989; Meile *et al.*, 1990). The amount of plasmid packaged into virus capsids may vary. Nevertheless, the plasmid may display more efficient transfer, even at low abundance, since it will not kill its host.

Such a strategy of capsid hijacking by a plasmid has also been observed in the case of a bacteriophage MPV1 which was isolated from a hydrothermal vent and infects *Marinitoga piezophila* UBOCC-M-2489 (Lossouarn *et al.*, 2015b). The MPV1 viral capsids were shown to predominantly package a plasmid of 13.3 kb (pMP1). This was the first reported example of molecular piracy in deep-sea hydrothermal vents.

Another possibility would be plasmid transfer via membrane vesicles (MVs). Indeed, *M. fervens* produces membrane vesicles that could represent a potential vehicle for the plasmid. For example, the plasmid, pR1SE, carried by an haloarchaea, uses MVs as a vehicle for infecting new cells. pR1SE encodes proteins that are found in regularly shaped MVs, and the MVs enclose the plasmid DNA (Erdmann *et al.*, 2017). Moreover, MVs have been shown to protect DNA against thermodenaturation, and can be involved in DNA transfer between cells at high temperature (Gaudin *et al.*, 2013).

Our analyses do not allow us to differentiate the mode of transfer used by the plasmid (virions, vesicles, or, less likely, naked DNA). However, the data presented here illustrate horizontal transfer of the plasmid among methanogens at high temperature.

The virus and plasmid dynamics observed illustrate an intricate network of interactions in the mobilome dedicated to horizontal transfer of genes with potential to drive adaptation and evolution of the cellular hosts. More generally, this type of relationship between several mobile genetic elements has been described for all tree domains of life (Christie and Dokland, 2012).

CONCLUDING REMARKS

MFTV1 and pMEFER01 are mobile genetic elements interacting with hyperthermophilic methanogens which play a key role in the carbon biogeochemical cycles. Important impacts on the life cycle of these microorganisms can result in more global feedbacks (Danovaro *et al.*, 2016). Indeed, methanogens play an important role in the global carbon cycle by producing methane. Viral infections are able to dynamically alter the composition and metabolic potential of these infected methanogens. Indeed, viral infections can lead to a shift in methanogen populations with a direct impact on other prokaryotic community compositions (Anderson *et al.*, 2014). More globally, the potentially cascading effects caused by possible shifts in viral life-cycle strategies (lysogenic versus lytic) in response to naturally encountered stressors in deep-sea hydrothermal vents could have important effects on global biogeochemical cycles.

EXPERIMENTAL PROCEDURES

Culture condition

Cultivation of *Methanocaldococcus* spp. was performed in modified DSM141 medium containing NaCl (20 g/l), $MgCl_2.6H_2O$ (4 g/l), $MgSO_4.7H_2O$ (3.45 g/l), $CaCl_2.2H_2O$ (0.14 g/l), NH_4Cl (0.25 g/l), KCl (0.34 g/l), K_2HPO_4 (0.14 g/l), $NaHCO_3$ (1 g/l), $Fe(NH_4)_2(SO_4)_2$, $7H_2O$ (2 mg/l), resazurin (1 mg/l) and cysteine-HCl (0.5 g/l). This medium has been depleted in iron to avoid adsorption and precipitation of virions during the purification process. The pH was adjusted at 6.5 using 3 M HCl. After autoclaving the medium was reduced by addition of $Na_2S.9H_2O$ at a final concentration of 0.05 % w/v. Supplementary solutions were added to the medium, for 1 l: 1 ml of element traces mixture (Widdel and Bak, 1992), 1 ml of vitamins mixture (Widdel and Bak, 1992), 1 ml of selenite tungstate solution (10 mM NaOH, 0.02 mM $Na_2SeO_3.5H_2O$ and 0.02 mM $Na_2WO_4.2H_2O$). The gas phase was replaced by a mixture of H_2/CO_2 (80:20; 1.7 bars) and the pH was adjusted at 6.3 by adding carbonate buffer. Cultures were incubated with shaking (100 rpm) at 80 °C according to their optimal temperature of growth in 1 l bottle with 250 ml of medium or in 125 ml bottle with 25 ml of medium.

Concentration and purification of virions

A 1 l of culture of *M. fervens* was stopped at the last part of log-phase of growth and supernatant was used for purification as described previously with slight modifications (Mercier *et al.*, 2018) (see Supplementary information). Following ultracentrifugation in a linear Iodixanol gradient (OptiPrep, 15–45 % diluted in a buffer (10 ml Tris-HCL, 100 mM NaCl, 5 mM $CaCl_2.5H_2O$ and 20 mM $MgCl_2$, pH 8)), the opaque virus band was recovered and stored at 4 °C until use. These steps were performed under aerobic conditions. To maximize the amount of virions, for infection analysis, the purification step was not carried out. Instead, the concentrated viruses' solutions, prepared as described in the supplementary information, were

exposed to oxygen at high temperature (80°C) to inactivate the metabolism of the remaining cells and kill them. Moreover, to prevent cellular nucleic acids contamination, the concentrated virions suspensions were treated with Benzonase® Nuclease (Sigma-Aldrich), at a final concentration of 1 U per µl, to destroy all forms of free DNA.

Viral suspensions (purified or concentrated) were stained with 2 % (wt/vol) uranyl acetate and observed using a JEOL JEM 100 CX II transmission electron microscope as previously described (Geslin *et al.*, 2003b; Gorlas *et al.*, 2012).

Extraction of chromosomal, viral and plasmid DNA

Total DNA from *Methanocaldococcus* strains, listed in Table 1, and viral DNA from concentrated or purified MFTV1 virions were prepared as previously described (Geslin *et al.*, 2003b; Gorlas *et al.*, 2012). Plasmid DNA was extracted from cells by alkaline lysis method (Bimboim and Doly, 1979; Geslin *et al.*, 2003b). Before viral DNA extraction, viral fractions were treated with benzonase as described above or with DNase (Promega, mixture of endo- and exo-nuclease at a final concentration of 50 µg/l) and RNase (Promega, 100 µg/l) to avoid extracellular DNA contaminations, followed by DNAse inhibition at 75 °C for 10 min.

To compare the MFTV1 genome extracted from purified virions to the provirus integrated in the *M. fervens* chromosome, experimental and *in silico* digestions with EcoRV, were performed. Digestions were performed for the provirus *in silico* using Restriction Mapper software (http://www.restrictionmapper.org) and those obtained experimentally were done in accordance with the manufacturer's instructions (Promega®).

To confirm the nature of the DNA packaged in the viral capsids and to check potential chromosomal contamination, PCR amplifications were performed using primer sets for 16S rDNA gene (*M. fervens* chromosomal DNA marker), major capsid protein encoding gene (ORF11, virus marker) and hypothetical protein encoding gene (WP-012795053, pMEFER01 plasmid marker). The primers and PCR protocol are described in supporting information (Table S3).

16S rRNA genes of the strains used in this study (FS 438-32; PH24A, PH24B and PH3) were sequenced and compared with sequences available in the NCBI nr database (results detailed in supplementary data). They share > 99 % of identity on 16S rRNA with *Methanocaldococcus bathoardescens* strain JH146^T and *Methanocaldococcus jannaschii* strain JAL-1^T, and were therefore classified as *Methanocaldococcus* strains (Table 1) (Table S2).

Viral titration by epifluorescence microscopy

Viral particles were titrated using epifluorescence microscopy. Viral fraction was first filtered through a 0.45 μm pore size filter (membrane Tuffryn, syringe filter, PALL) and then collected on a 0.02 μm pore size filter (Anodisc 25, Whatman). After a staining with SybrGreen I (10 X, Invitrogen-Molecular Probes), viral particles were counted under an Olympus microscope BX60 (WIB filter) according to Noble and Fuhrman (Noble and Fuhrman, 1998).

Flow cytometry counts of archaea and virions

Archaeal and viral abundances were monitored by flow cytometry using a FACS Canto II flow cytometer (BD Bioscience) equipped with a laser with an excitation wave-length of 488 nm (15

mW). Samples were fixed with glutaraldehyde (0.5 % final concentration, electron microscopy grade, Sigma Aldrich) and stained with the nucleic acid specific dye SYBR Green I (Invitrogen-Molecular Probes) (more details in supplementary information).

Viral induction assays

Attempts were made to increase the viral production using different treatments as physiological stresses [variation of the growth temperature; metabolic limitation: H_2 deficiency; physical (ultraviolet exposure) or chemical stresses (metal exposure: copper (20-50 µM); Bromoethane sulfonate (BES) exposure (1 mM) (as an inhibitor of the methanogenesis)]. All stresses were applied on cultures of *M. fervens* at the beginning of the log growth phase (5.10^6 to 1.10^7 cells/ml on Thoma cell counting chamber). For temperature assays, variation of growth temperatures (4 °C, room temperature, 75 °C or 90 °C) were applied for 30 min, 1 h or 2 h. Then the cultures were moved back to their optimal growth temperature at 80 °C with shaking for 24 h. In parallel, control cultures were conducted at 80 °C with shaking.

Detailed information on the other stress treatments are given in the supplementary information. The different induction conditions were compared based on the number of virions released. All viral counts were performed by epifluorescence microscopy and flow cytometry (see above).

Cryo-electron microscopy (Cryo-EM)

To prepare samples for Cryo-EM, 10 ml cultures (stationary phase) were centrifuged at 5 000 x g for 20 min. The pellets were resuspended with 50 µl of buffer (10 mM Tris-HCl, 100 mM NaCl, 5 mM CaCl2). 10 µl droplets of preparations were adsorbed onto a grid covered with a perforated fine layer of carbon (QUANTIFOIL®, R2/4). After removing the excess liquid with Whatman® paper, the grids were quickly immersed in liquid ethane and transferred under

liquid nitrogen into the microscope using a side entry nitrogen-cooled (Gatan, 626-DH cryotransfer system) cryoholder. The observations of vesicles were performed with a Cryo-EM JEOL 2100 LaB6 transmission electron microscope with an accelerating voltage of 200 kV, at the nominal magnification of 10 000. Images were recorded under low dose conditions with ultra-scan 1 000 camera (Gatan, 2k x 2k pixels CCD).

Infection assays monitored by qPCR

A preliminary infection assay was performed on 22 *Methanocaldococcus* strains, and 10 of these produced a virus band, corresponding to the MCP gene, when checked by PCR after incubating the archaeon with virions (ratio virions/cell: 10/1) for 36h (data not shown).

A more detailed infection assay, by qPCR, was then performed on a pool of 4 of these potentially infected strains, listed in Table 1. These 4 strains had no evidence of viral signature: no PCR amplification of capsid and tail genes, and no evidence of viral production observed by TEM or epifluorescence microscopy. The use of a pool of strains enhances the probability of infection of one of them, at one time. First, to assess virions' stability under hyperthermophilic conditions, MFTV1 virions, at a final concentration of 5×10^8 virions per ml (estimated by epifluorescence microscopy) were incubated for 10 hours without any host, in DSM141 modified medium at 80°C. After 10 hours of incubation of the virions at high temperature, the pool of *Methanocaldococcus* cells (4 strains), at a final concentration of 1×10^6 cells per ml (on Thoma cell counting chamber), was added to test the infectivity of MFTV1. A culture of the pool of non-infected *Methanocaldococcus* strains was used as negative control, and a culture of *M. fervens* producing MFTV1 virions was used as positive control. For these controls, cells

were inoculated at the beginning of the experiment (0 h). For the infection, at 80°C, cells were added to the virions after 10 hours.

Infection assays were monitored by qPCR. Primers sets target: MCP (Major Capsid Protein) gene of MFTV1, a hypothetical gene of the plasmid pMEFER01 and 16S rDNA of *Methanococcales* (see Table S4 and details in supplementary information).

Sequencing and assembly of MFTV1 genome

DNA extracted from purified MFTV1 was sequenced at The Applied Genomics Core (http://tagc.med.ualberta.ca/) using the Nextera kit on an Ilumina MiSeq (Bentley *et al.*, 2008) as 1/8 of a run. The sequences were assembled in CLC Genomics Workbench version 7.0.1 using default trimming option, word and bubble size. One contig of 31 202 bp with 3800-fold coverage was identified as the virus genome. Four additional contigs showed > 100-fold coverage, of which two had higher than 400-fold coverage. The remaining contigs had coverage values in the range 2-34. The four high-coverage contigs all mapped to the chromosome of *M. fervens*. The two contigs with > 400 fold coverage were 26.7kb and 36.6kb suggesting these could have been packaged in the virus capsid, while the two 100-200 fold coverage contigs were > 100 kb.

The genbank accession number of the viral genome extracted from capsids of MFTV1 (this study) is MT711370.

Accession numbers of the chromosome with the proviral sequence: NC_013156 and of the plasmid : NC_013157 were already available.

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TABLE AND FIGURES LEGENDS

Table 1: List of *Methanocaldococcus* strains used in this study.

Figure 1: Electron micrographs of MFTV1 virions negatively stained with 2% uranyl acetate.

Figure 2: Virus-host kinetics monitored by flow cytometry. Typical growth curve of the host *M. fervens* is shown as filled squares on a continuous black line. The production of MFTV1

particles, reaching 3.5×10^8 virions per ml after 6 h 30 of culture, is shown as empty squares on a dotted black line. The growth curve under stress condition of the host *M. fervens* is shown as filled triangles on a continuous grey line. The production of MFTV1 particles under stress condition, reaching 3.5×10^8 virions per ml after 4 h 30 of culture, is shown as empty triangles on a dotted grey line. The period of induction by a cold stress (4 °C during 30 min) is outlined by a black frame.

Figure 3: Electron micrographs of membrane vesicles produced by *M. fervens* exposed to copper (at a final concentration of 30 µM). Cryo-EM (A) and TEM (B).

Figure 4: qPCR analyses were performed to assess the replication rates of chromosomal gene (by targeting the 16S rRNA gene), viral gene (by targeting the gene encoding the major capsid protein (MCP, ORF11) and plasmid gene (by targeting a gene encoding a hypothetical protein specific to pMEFER01, WP-012795053), of *M. fervens* (as positive control), a co-culture of *Methanocaldococcus* strains (as negative control), and a co-culture of *Methanocaldococcus* strains infected with MFTV1. 16S rRNA is shown with black bars, MCP with dark grey bars and plasmid with light grey bars. For controls (A and B), cells were inoculated at the beginning of the experiment (0 h). For the infection (C), cells were inoculated just after 10 hours.

Figure 5: Genome organization of MFTV1 genome. Arrows in grey with number (1 to 44) indicate predicted ORFs. TerS and TerL, small and large subunits of the terminase, respectively; portal-MuF, portal protein; MCP, Major Capsid Protein; TMP, Tail Major Protein; RBP, receptor-binding protein; RadA, homolog of the RecA/RadA/Rad51-family recombinase; DBP, histone-like dsDNA-binding protein; hicA hic B, Toxin-Antitoxin system; wHTH, winged-

helix transcription regulator; MCM, Minichromosome maintenance helicase; Exo, exonuclease; Int, integrase. The scale bar represents 2 000 bp.

Supporting Information

Figure S1: MFTV1 induction assays performed with BES (Bromoethane sulfonate at 1 mM) on *M. fervens* and checked by flow cytometry. Typical growth curve of the host *M. fervens* is shown as filled squares on a continuous black line. The production of MFTV1 particles is shown as empty squares on a dotted black line. The growth curve under stress condition of the host *M. fervens* is shown as filled triangles on a continuous grey line. The production of MFTV1 particles under stress condition is shown as empty triangles on a dotted grey line. The period of induction by a stress is depicted by an arrow.

Figure S2: MFTV1 induction assays performed with copper (30 µM) on *M. fervens* and checked by flow cytometry. Typical growth curve of the host *M. fervens* is shown as filled squares on a continuous black line. The production of MFTV1 particles is shown as empty squares on a dotted black line. The growth curve under stress condition of the host *M. fervens* is shown as filled triangles on a continuous grey line. The production of MFTV1 particles under stress condition is shown as empty triangles on a dotted grey line. The period of induction by a stress is depicted by an arrow.

Figure S3: MFTV1 induction assays performed with H_2 deficiency on *M. fervens* and checked by flow cytometry. Typical growth curve of the host *M. fervens* is shown as filled squares on a continuous black line. The production of MFTV1 particles is shown as empty squares on a dotted black line. The growth curve under stress condition of the host *M. fervens* is shown as

filled triangles on a continuous grey line. The production of MFTV1 particles under stress condition is shown as empty triangles on a dotted grey line. The period of induction by a stress is surrounding by a black frame.

Figure S4: MFTV1 induction assays performed with UVs exposition on *M. fervens* and checked by flow cytometry. Typical growth curve of the host *M. fervens* is shown as filled squares on a continuous black line. The production of MFTV1 particles is shown as empty squares on a dotted black line. The growth curve under stress condition of the host *M. fervens* is shown as filled triangles on a continuous grey line. The production of MFTV1 particles under stress condition is shown as empty triangles on a dotted grey line. The period of induction by a stress is surrounding by a black frame.

Figure S5: PCR amplification results, on triplicate, targeting a plasmid gene (a 599 bp fragment): from DNA extracted from benzonase-treated virions; from total DNA extracted after infection of *Methanocaldococcus* strains with benzonase-treated virions; and from naked plasmid DNA treated with benzonase 1U/µl. MT: 1 kb DNA ladder, Promega.

Table S1. List of methanogens screened in this study for the presence of viral particles.

Table S2. 16S rRNA comparison of *Methanocaldococcus* spp. used in this study.

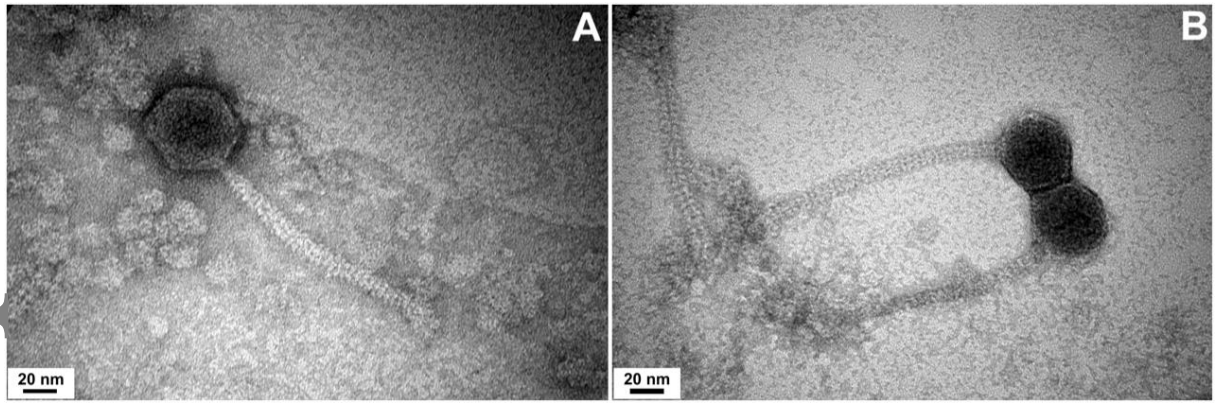
Table S3. Sequences of primers, designed for PCR, targeting viral, plasmid and chromosomal genes.

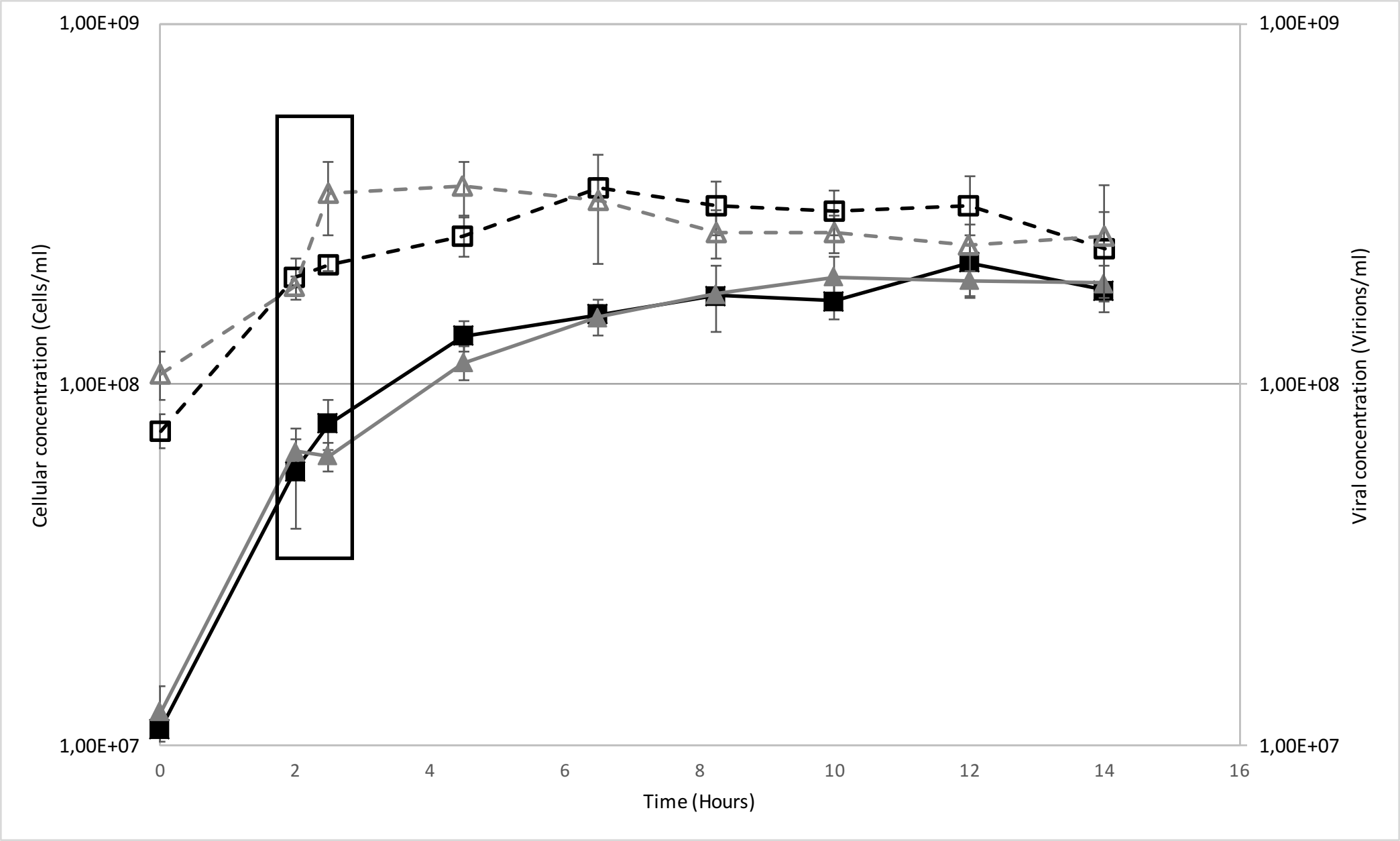
Table S4. Sequences of primers, designed for qPCR, targeting chromosomal, viral and plasmid genes.

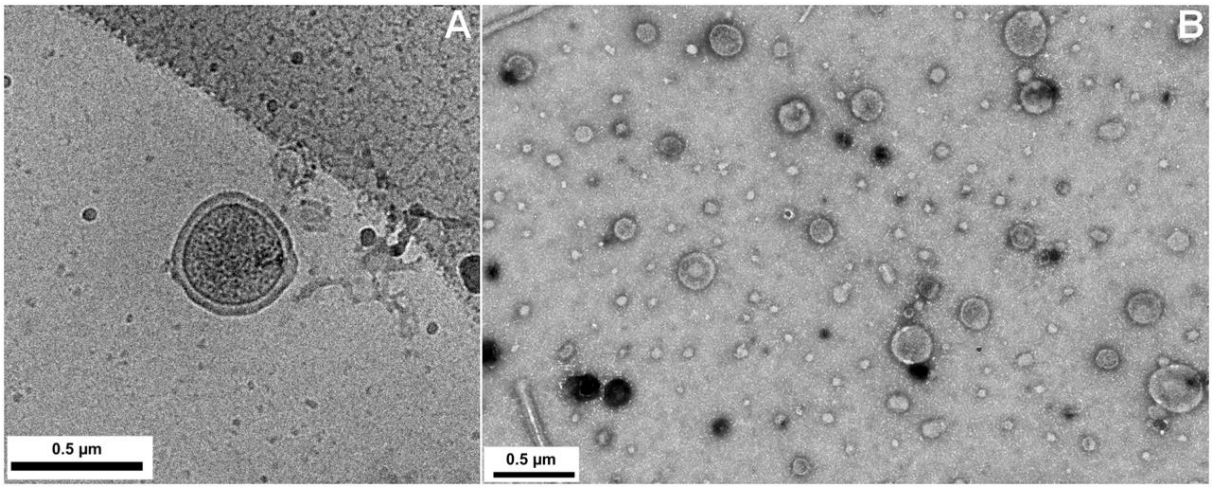
Table S5. Genome annotation of MFTV1. The predicted ORFs in the MFTV1 genome were translated to amino acid sequences and compared to Genbank by BlastP searches. The top scoring match for each ORF outside the genus *Methanocaldococcus*, if available, is reported.

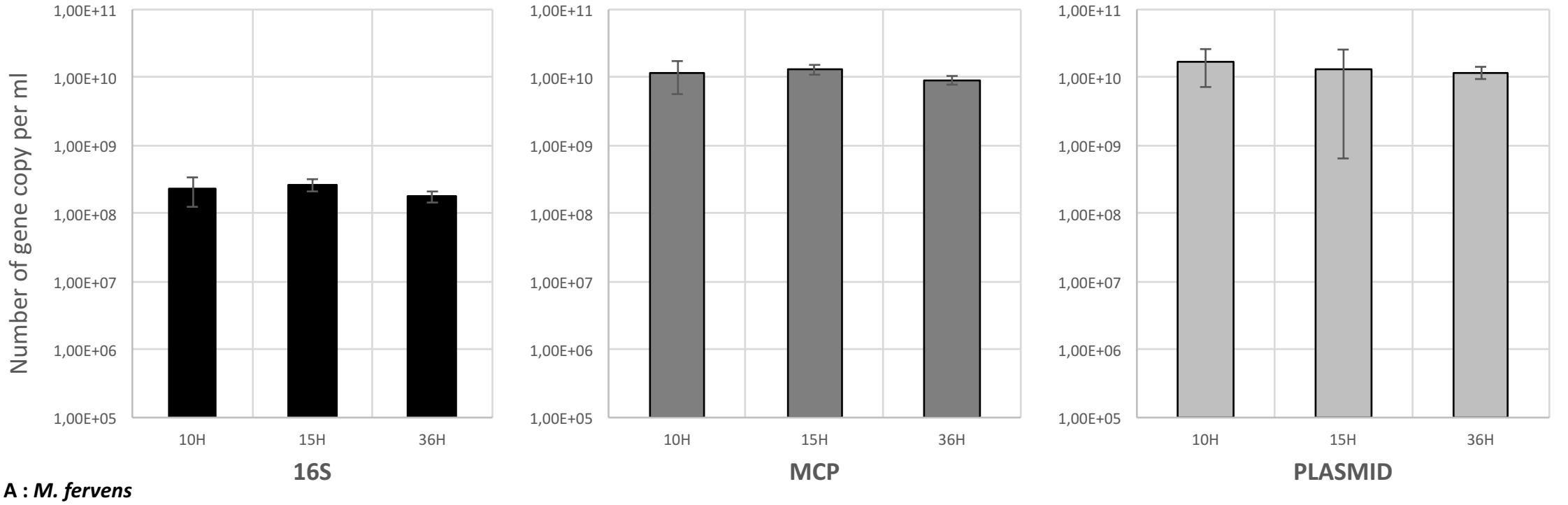
Table 1: List of *Methanocaldococcus* strains used in this study

Strain	Origin	Growth temperature	Oceanographic cruise	Ref.
***Methanocaldococcus fervens* AG86^T (UBOCC-M-3290)**	Guaymas Basin	80-85 °C	-	Zhao et al, 1988
***Methanocaldococcus sp.* FS 438-32 (UBOCC-M-3326)**	Juan de Fuca 45 °N	80-85 °C	NEMO (1999)	This study
***Methanocaldococcus sp.* PH24 A (UBOCC-M-3338)**	East Pacific Ridge 13 °N	80-85 °C	PHARE (Le Bris, 2002)	This study
***Methanocaldococcus sp.* PH24 B (UBOCC-M-3339)**	East Pacific Ridge 13 °N	80-85 °C	PHARE (Le Bris, 2002)	This study
***Methanocaldococcus sp.* PH3 (UBOCC-M-3343)**	East Pacific Ridge 13 °N	80-85 °C	PHARE (Le Bris, 2002)	This study

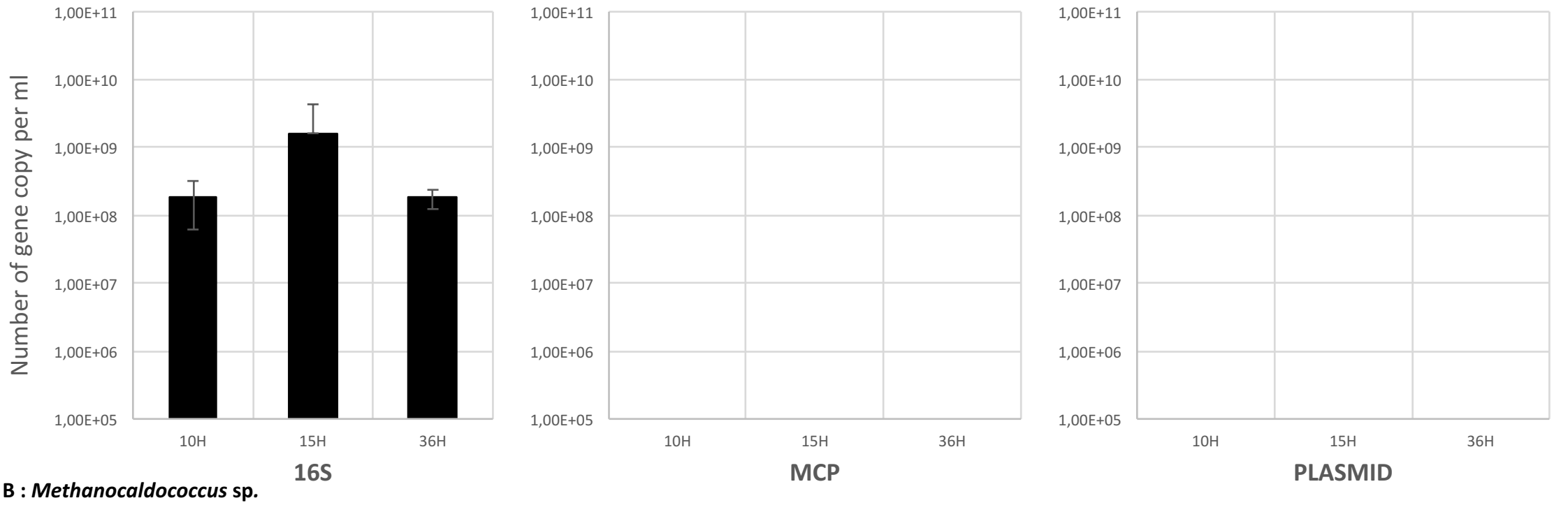



1,00E+09
1,00E+08
1,00E+07
Cellular concentration (Cells/ml)
Viral concentration (Virions/ml)
0
2
4
6
8
10
12
14
16
Time (Hours)

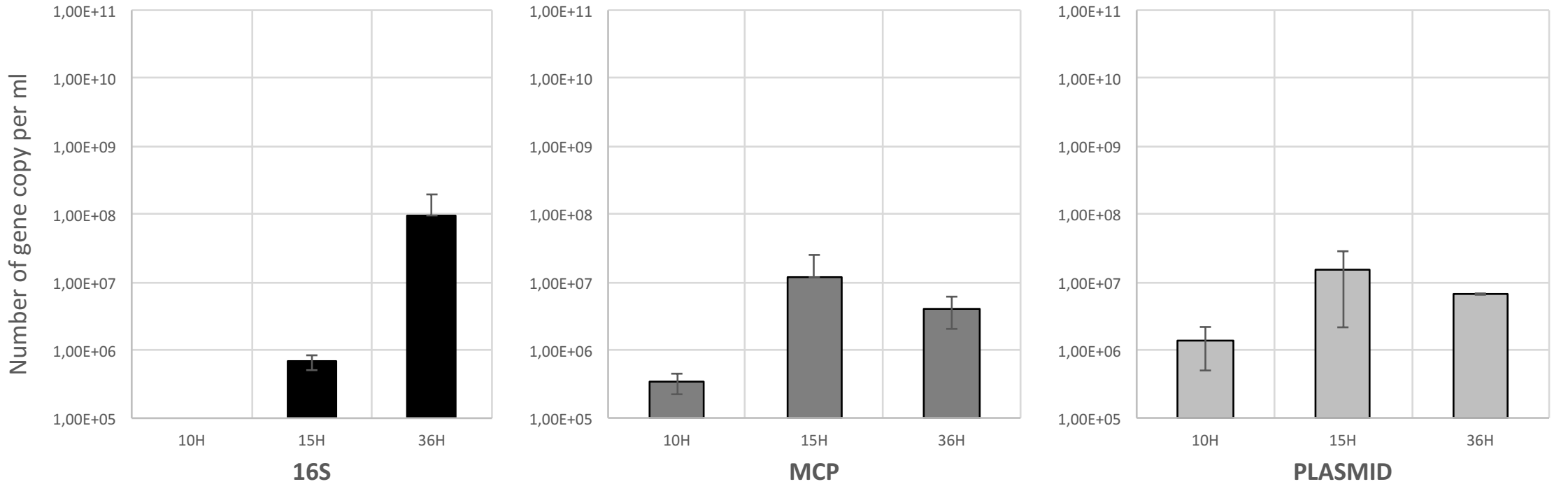




A : *M. fervens*



B : *Methanocaldococcus* sp.



C : *Methanocaldococcus* sp. + MFTV1


TerS
portal-MuF
TerL
MuG
protease
MCP
tail fiber
tail TMP
baseplate
RBP
RadA
histone
DBP
hicA
hicB
wHTH
MCM
Exo
Int
att
1
2
3
4
5
6
7
8
9
11
14
17
18
19
20
21
24
31
32
36
37
41
44
2 kbp