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An abyssal mobilome: Viruses, plasmids and vesicles from deep-sea hydrothermal vents

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Abstract :

Mobile genetic elements (MGEs) such as viruses, plasmids, vesicles, gene transfer agents (GTAs), transposons and transpovirions, which collectively represent the mobilome, interact with cellular organisms from all three domains of life, including those thriving in the most extreme environments. While efforts have been made to better understand deep-sea vent microbial ecology, our knowledge of the mobilome associated with prokaryotes inhabiting deep-sea hydrothermal vents remains limited. Here we focus on the abyssal mobilome by reviewing accumulating data on viruses, plasmids and vesicles associated with thermophilic and hyperthermophilic Bacteria and Archaea present in deep-sea hydrothermal vents.

Keywords : Deep-sea hydrothermal vent, Bacteria, Archaea, (Hyper-)thermophiles, Mobilome

1. Introduction

Deep-sea hydrothermal vents represent one of the most extreme environments on Earth. These ecosystems are characterized by steep physicochemical gradients, high hydrostatic pressures, high temperatures, obscurity and the prevalence of chemosynthesis. These extreme environments are home to a vast diversity of mesophilic and (hyper-)thermophilic prokaryotes belonging to the Bacteria and Archaea [1-3]. Although our knowledge of deep-sea hydrothermal vent microbial communities is progressing, the impact of mobile genetic elements (MGEs) on microbial ecology and evolution largely remains overlooked in these abyssal ecosystems [2]. MGEs such as viruses, plasmids, membrane vesicles, gene transfer agents (GTAs), transposons and transpovirons, which collectively represent the mobilome, interact with cellular organisms from all three domains of life, including those thriving in extreme environments [4, 5]. Many reviews have highlighted how MGEs and especially viruses are powerful agents that affect not only the diversity and evolution of microbial communities but also the global biochemical cycles in marine environments [6-12]. Evidence was recently reviewed supporting the hypothesis that MGEs could also play a key role in deep-sea hydrothermal vents notably by facilitating horizontal gene transfers [2]. MGEs have a potential to be powerful drivers of cellular host adaptations to the extreme marine environments [2]. Here, we propose the first review focused on deep-sea hydrothermal MGEs associated to (hyper-)thermophilic prokaryotes, collectively denoted as the abyssal mobilome.

2. Viruses in deep-sea hydrothermal vents

2.1. Evidence for viral activity

Only a few viral ecological studies have been performed on deep-sea hydrothermal vents [13-17]. Viral abundance and viral production were notably investigated in diffuse flow hydrothermal vent fluids. In these samples, collected from vents within the Endeavour Ridge system [13] and the East Pacific Rise [14], average VLP (Virus Like Particles) abundances were estimated at ~ 10^7 VLPs per milliliter and were ~ 10-fold higher than the prokaryote abundances. In comparison, VLP abundances in productive coastal waters were estimated at ~ 10^8 VLPs/mL and exceed those of prokaryotes by ~ 15-fold [6, 7]. These VLPs can represent *bona fide* viral particles (virions) but also membrane vesicles containing cellular, plasmid or viral DNA (viral membrane vesicles) (see section 3).

Viral activities occurring in these extreme ecosystems are also highlighted by genome sequence analyses of deep-sea hydrothermal Bacteria and Archaea [2]. The presence of clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) systems has been reported in many thermophilic bacterial and archaeal genomes [18, 19]. These systems provide acquired, yet heritable, sequence-specific “adaptive” immunity against viruses and other horizontally-acquired elements, such as conjugative plasmids [19]. CRISPR loci consist of several noncontiguous direct repeats separated by stretches of variable sequences called spacers, which correspond to fragments derived from invading DNAs such as viruses and plasmids [18]. CRISPR regions therefore act as a record of past viral infections that occurred in the history of the prokaryotes [2, 19, 20]. Interestingly, it was reported that thermophilic strains harbored a higher number of CRISPR loci in their genomes than mesophilic and psychrophilic strains [2, 21]. This may illustrate that viral infections play a major role in the ecology and evolution of thermophilic communities, notably those inhabiting deep-sea hydrothermal vents [2].

A viral metagenomic study, using CRISPRs, indicated that a diffuse flow sample collected from Hulk vent on the Juan de Fuca ridge in the Pacific ocean contained a range of viruses with the potential to infect mesophilic and thermophilic hosts from both the archaeal and bacterial domains [22]. Like in other marine viral metagenomes, most of the viral reads belonged to the *Myoviridae*. Other tailed viruses frequent in marine virome, the *Podoviridae* and the *Siphoviridae*, were also recovered. Archaeoviral reads belonging to the *Rudiviridae*, *Fuselloviridae* and *Lipothrixviridae*, which are frequently found in hot terrestrial spring viral assemblages, were largely absent from this marine vent virome [22]. The abundance of Archaea in deep-sea hydrothermal vents strongly suggests that archaeoviruses were, however, present in the marine vent virome. The problem is that little is known about the virosphere of hydrothermal marine environments, which is more the consequence of insufficient screening than low virus abundance. Indeed, to date only two viruses have been isolated from described marine hyperthermophilic Archaea [23-25], in addition to proviruses and plasmids (see next sections). Therefore, deep-sea hydrothermal systems may play host to novel archaeoviruses.

Lysogeny is actually presumed to be a more common viral cycle in deep-sea hydrothermal vents than those listed in other environments. Comparative analysis of a cellular and viral metagenome obtained from a Hulk vent diffuse flow sample revealed a higher enrichment of proviruses in the vent cellular fraction than in a range of other aquatic and terrestrial cellular metagenomes [26]. This result complements the high proportion of inducible lysogenic microorganisms previously reported in deep-sea hydrothermal vents [14]. Furthermore, a relatively high abundance of auxiliary metabolic genes was found in the vent viral fraction compared to the cellular fraction. All these results suggest the prevalence of temperate viruses in deep sea vent habitats, expressing or facilitating horizontal transfer of genes that may notably enhance the metabolic flexibility of the host cells [26]. Indeed, proviruses could contribute to the fitness of the host strains whereas for the virus, the integrated state represents a means to avoid the harsh conditions of these ecosystems [14].

2.2. Virus-host systems characterized

Despite obvious viral activity in deep-sea hydrothermal environments, few viruses have been isolated and characterized to date.

Within the Bacteria, 6 bacterioviruses (i.e. bacteriophages) have been isolated from deep-sea hydrothermal vents (Table 1). These bacterioviruses have head and tail morphotypes like the majority of those isolated from terrestrial hot springs [27]. Head and tail viruses affiliated to the *Caudovirales* order actually constitute the predominant group of prokaryotic viruses [28], which consequently also seems to be well represented in the most extreme environments on the Earth.

Four of these deep-sea hydrothermal bacterioviruses are lytic and were discovered following viral plaque assays observation during the cultures of *Bacillales* strains isolated from deep-sea hydrothermal fields in the Pacific (19°24'08"N, 148°44'79"E, 5060 m depth and 12°42’29’’S, 102°02’01’’W, 3083 m depth) [29-31]. These bacterioviruses infect aerobic, thermophilic and heterotrophic strains belonging to *Bacillus* and *Geobacillus* genera, with optimal growth temperatures around 65°C. **BVW1** is unclassified**, GVE1** and **GVE2** are affiliated to the *Siphoviridae* family whereas **D6E** belongs to the *Myoviridae* family. These viral particles contain double stranded linear DNA genomes with sizes ranging from 18 to 49.3 kb [29-31]. GVE2 and D6E were more extensively studied. The analyses revealed an extensive mosaicism of D6E viral genome with other mesophilic and thermophilic bacteriovirus genomes. D6E replication and transcription functional modules are notably highly similar to those of GVE2. The mosaic nature of these thermophilic viral genomes

highlighted that mobile elements contributed to a substantial dispersion of DNA sequences even in genomes of deep-sea vent communities [31].

NrS-1 is a temperate siphovirus associated with a member of the *Epsilonproteobacteria* [32]. Members of this bacterial phylum have frequently been found to dominate microbial communities inhabiting deep-sea hydrothermal environments [3]. NrS-1 infects the chemolithoautotrophic, anaerobic and microaerobic moderately thermophilic strain *Nitratiruptor* sp. SB155-2 cultivated at 55°C [33]. This lysogenic strain was isolated from a hydrothermal chimney sample collected in the Ibeya North field (27°47'N, 126°53'E, 1000 m depth). NrS-1 virions contain a double-stranded linear DNA genome of 37.1 kb, which is circularly permuted and terminally redundant. NrS-1 is affiliated to the *Siphoviridae* family, but its genome composed of 51 ORFs and its sequence organization are distinct from those of any other previously characterized siphoviruses. Homologues of NrS-1 genes were found to be widely distributed among the bacterial genomes of *Epsilonproteobacteria*. The large distribution of these genes may illustrate an early coevolution between *Epsilonproteobacteria* and their temperate bacterioviruses, prior to the divergence of *Epsilonproteobacteria* habitats and consequently to their physiological adaptations [32].

MPV1 is the first virus isolated and described amongst the *Thermotogales*, an order well represented in deep biosphere ecosystems and deep-sea hydrothermal vents in particular [34]. Its host, *Marinitoga piezophila* is a thermophilic (growing at 65°C), anaerobic, heterotrophic and piezophilic strain isolated from a deep-sea hydrothermal chimney located in the East Pacific Rise (12°48721'N, 103°56351'W, 2630 m depth) [35]. MPV1 is a temperate *Siphoviridae*-like virus with a 43.7 kb genome (Fig. 1 A). This double-stranded viral DNA reveals a connection to genomes of *Firmicutes* and bacterioviruses known to infect them. Surprisingly, MPV1 virions carry not only the viral DNA but preferentially package a plasmid of 13.3 kb (pMP1). This plasmid is the second mobile genetic element carried by *M. piezophila*. The system described in *M. piezophila* is the first virus-mediated plasmid exchange reported in deep-sea hydrothermal vents and could correspond to a new example of molecular piracy (Fig. 2) [34].

To date, only two viruses have been isolated and described from Archaea living in deep-sea hydrothermal vents, both infecting *Euryarchaea* of the order of *Thermococcales* (Table 1). *Thermococcales* have been frequently isolated from marine thermal environments, notably from deep-sea vents [1]. This order is represented by 3 genera: *Pyrococcus*, *Thermococcus* and *Palaeococcus* and includes obligate anaerobic, fermentative, sulfur-metabolizing hyperthermophiles. *Thermococcales* are widely studied model microorganisms in various fields of investigation including microbial adaptation to extreme temperature and ionizing radiation, DNA replication mechanisms, metabolism, phylogeny and genome evolution [1, 36, 37]. Accordingly, intensive effort has been made to sequence diverse genomes of members of this order, resulting in the availability of 14 genomes of *Thermococcus*, 6 genomes of *Pyrococcus*, and 1 of *Palaeococcus*. The availability of all these data makes *Thermococcales* a good model to explore the hyperthermophilic and marine virosphere that currently remains largely uncharacterized. Proviruses have been detected in several genomes of *Thermococcales* (TKV1 to TKV4 in *Thermococcus kodakaraensis* [38], TGV1 and TGV2 in *Thermococcus gammatolerans* [39], PHV1 in *Pyrococcus horikoschii* genome [40]).

To investigate the diversity of virus-like particles (VLPs) from deep-sea vents, a screening was carried out on enrichment cultures obtained from samples collected in various geographically distant hydrothermal sites located on the East Pacific Rise (EPR 9°N and 13°N) and the Middle Atlantic Ridge (MAR 36°N and 37°N). These enrichment cultures were performed at 85°C, which favored *Thermococcales* growth and led to the detection of a vast morphological diversity of VLPs by transmission electron microscopy [41]. Among the different morphotypes observed, the lemon-shaped type prevailed, but rods and new pleomorphic morphologies were also reported. Additionally, this study highlighted the striking

similarity between VLP morphologies in deep-sea hydrothermal vents and those from terrestrial hot environments [41]. Thereafter, two viruses associated with *Thermococcales* strains were isolated and characterized. They represent the only two marine hyperthermophilic viruses reported to date.

The virus **PAV1** was discovered in *Pyrococcus abyssi* strain GE23, isolated from a deep-sea vent sample collected in the North Fidji Basin (White Lady Site; 16°59''S, 173°55''W, 2000 m depth), and cultivated at 85°C [23]. PAV1 is lemon-shaped (120 x 80 nm) with a short tail terminated by fibers (Fig. 1 B). PAV1 virions contain a double-stranded circular DNA of 18kb which is also present in high copy number in a plasmid form in the host cystoplasm. In genome size and virion morphology, PAV1 strikingly resembles the SSVs fuselloviruses isolated from aerobic, acidic hot spring *Crenarchaeota* belonging to both *Sulfolobus* and *Acidianus* genera [23]. However, none of its 25 predicted proteins, except one (MCP), exhibit similarity to SSV proteins. Instead, PAV1 is evolutionarily related to plasmids of *Thermococcales*, with three genes having homologues in the plasmid pTN2, two in the plasmid pP12-1 and one in pRT1. In addition, PAV1 shares one gene with the provirus TKV4 of *T. kodakaraensis* [24, 36, 40]. PAV1 persists in the host strain in a stable carrier state. Indeed, PAV1 virions are spontaneously and continuously released from the host cell without causing lysis or growth delay of this one [23]. The infectivity of PAV1 has been demonstrated by the inverted spot test method which indicates that PAV1 particles can infect *Pyrococcus glycovorans* leading to growth retardation, but does not infect any of the *Thermococcus* strains tested [42].

The virus **TPV1** was discovered in *Thermococcus prieurii* isolated from a hydrothermal chimney sample collected from the East Pacific Rise ("Sarah Spring" area, 7°25′24 S, 107°47′66 W, 2700 m depth) and cultivated at 80°C [43]. TPV1 is lemon-shaped (140 x 80 nm) with a short tail terminated by fibers (Fig. 1 C), similarly to PAV1 [25]. TPV1 contains a double-stranded circular DNA of 21.5 kb which is also present in high copy number in a free form in the host cell. The TPV1 genome encompasses 28 predicted genes. Proteins and transcriptional regulators predicted to be involved in genome replication were identified. TPV1 encodes also a predicted integrase (SSV-type) of the tyrosine recombinase family. The only two genes that are homologous between TPV1 and PAV1 encode proteins containing a concanavalin A-like lectin/glucanase domain that might be involved in virus-host recognition. TPV1 shares more homologous genes with proviruses, (TKV2, TKV3, TKV4, TGV1) and plasmid (pT26-2) from *Thermococcus* species, than with PAV1 [25]. The infection with TPV1 does not cause host lysis, and viral replication can be induced by UV irradiation. TPV1 can infect several reference species belonging to the *Thermococcus* genus leading to growth retardation [42]. Interestingly, TPV1 shares its host with two plasmids pTP1 (3.1 kb) and pTP2 (2.0 kb). These 3 mobile elements do not have a single gene in common and stably propagate in infected cells, without any apparent antagonistic effect on each other [25, 44].

3. Plasmids in deep-sea hydrothermal vents

Plasmids, including those of (hyper-)thermophiles, are abundant in the biosphere. They actively participate in horizontal gene transfer, which plays a major role in microbial plasticity, adaptation and evolution [45-47]. To date, only 6 bacterial plasmids and 24 archaeal plasmids have been isolated and described from deep-sea hydrothermal vents.

3.1. Bacterial domain

Among the few plasmids isolated from deep sea vents bacteria, 1 is associated to a mesophilic strain [48] while the other 5 are carried by thermophilic strains [49-53]. Only these 5 bacterial plasmids are given in Table 2. These latter have generally not been described in details, most have only been annotated and briefly mentioned in the genome description of their hosts. We will briefly focus on two of them.

pDF308 is a 308.54 kb megaplasmid associated with the thermophilic, anaerobic and heterotrophic strain *Deferribacter desulfuricans* SSM1 cultivated at 65 °C [52, 54]. This megaplasmid, whose two-thirds of the CDSs have no apparent similarity with any CDSs in the database, interestingly encodes 17 copies of a gene cluster encoding two transposases to the *IS200* and *IS605* family whereas none was found in the host chromosome [52]. Very similar CRISPR/Cas systems have also been identified on both pDF308 and *D. desulfuricans* genomes [52].

pMP1 is a plasmid recently described within the *Thermotogales* order [34, 53]. This 13.3 kb genetic element, carried by *Marinitoga piezophila* (previously introduced in 1.2 section), is unrelated to the only known plasmids amongst *Thermotogales*: two cryptic miniplasmids in the genus *Thermotoga* [55, 56] and a 1,724 bp miniplasmid in *Mesotoga prima* [57]. pMP1 encodes a site-specific recombinase XerD (primase family) and a protein with both a DnaB (helicase) and a DnaG (primase) domain. A large fraction of the encoded proteins might actually be involved in DNA binding, replication and propagation of pMP1. The plasmid, which shares its host with the temperate siphovirus MPV1 (mentioned in section 1.2), is induced by mitomycin C, along with the provirus, and responds by replicating and escaping from the bacterial hosts by using helper viral capsids [34] .

3.2. Archaeal domain

The huge majority of archaeal plasmids isolated from deep-sea hydrothermal vents are associated with hyperthermophilic strains, with optimal growth temperatures between 80 and 95°C, belonging to *Thermococcales* and *Methanoccocales* orders; these are given in Table 3.

Plasmids are common in *Thermococcales* strains (approx. 40% were found to harbour at least one extrachromosomal element) [1, 36, 58-60], however only 16 of them have been sequenced and described. They have recently been classified according to their different types of replication proteins [60].

The pTN1 family comprises 3 small plasmids of almost 3 kb: **pTN1** isolated from *Thermococcus nautili* [61], **pGT5** isolated from *Pyrococcus abyssi* GE5 [62] and **pTP1** recently isolated from *Thermococcus prieurii* [44]. They encode a new family of rolling-circle replication initiator proteins (Rep74, Rep75 and RepTP1), which contain the three characteristic motifs of the RC-Rep superfamily II, with a single tyrosine in motif 3 [63], as well as a fourth motif conserved with RC transposases, mainly encoded by insertion elements [61]. pGT5 and pTN1 were used to construct shuttle-vectors in *Thermococcales*: pYS2 [64] and pYS3 [65] are respectively available for *P. abyssi* and *P. furiosus* and pLC70 [66] for *Thermococcus* species.

The pTP2 family could be represented by the 2.0 kb plasmid **pTP2** which propagates in *T. prieurii* cells with pTP1, mentioned above. pTP2 is the smallest known plasmid of the hyperthermophilic archaea and is unrelated to other *Thermococcales* plasmids [44]. It encodes a RC-Rep containing two tyrosines in motif 3 (superfamily I), which shares significant sequence similarity with RC-Rep of plasmid pGS5 isolated from *Archaeoglobus*

profundus AV18 [67]. Futhermore, it has been shown that pGS5 is negatively supercoiled due to the presence of a gyrase introducing negative supercoiling [68], in contrast with other plasmids isolated from hyperthermophiles which are relaxed or slightly positively supercoiled [69].

The pRT1 family contains **pRT1**, a small 3.4 kb plasmid isolated from *Pyrococcus* sp. JT1 [70] and a much larger plasmid of 20.5 kb, **pAMT11**, isolated from *Thermococcus* sp. AMT11 [71]. They encode the homologous replication proteins Rep63 and Rep72, respectively, unrelated to RC-Rep proteins and could replicate via a theta mechanism [72]. Interestingly, pAMT11 genome revealed homology and synteny with the genome of TKV1 a virus-like integrated element of *T. kodakaraensis* [38].

The pT26-2 family constitutes a new large family of archaeal plasmids and integrated elements that probably predate the separation of *Thermococcales* and *Methanococcales* [40]. The plasmid **pT26-2**, from *Thermococcus sp.* 26-2, encodes several proteins including a SSV-type integrase and a new DNA replicative helicase related to the superfamily AAA+ ATPases probably involved in theta-type replication. pT26-2 shares homologues with virus-like integrated elements of *Thermococcales* (TKV2 and TKV3 present in *T. kodakaraensis* genome, TGV1 present in *T. gammatolerans* genome and PHV1 present in *P. horikoschii* genome) and of *Methanococcales* (MMPV1, MMC7V1, MMC7V2, MMC6V1 in *Methanococcus maripaludis* strains genomes; MVV1 in *Methanococcus voltae* genome) and with the plasmid pMEFER01 isolated from *Methanocaldococcus fervens*. The pT26-2 family is identified by the presence of nine core genes [40, 60] and phylogenetic analyses have shown that these "pT26-2 core genes" have co-evolved with cellular hosts [40].

The pTBMP1 family includes only **pTBMP1** which is the largest plasmid of *Thermococcales* known to date. This 54.2 kb element, sequenced in the framework of the *Thermococcus barophilus* genome project [73], is not related to other *Thermococcales* plasmids. Interestingly, pTBMP1 encodes a homologue of archaeal replication initiator protein Cdc6/Orc1 and thus replicates via a theta mechanism [73].

The pTN2 family, comprising 7 plasmids from *Thermococcales* and one from *Methanococcales*, with sizes ranging from 8.5 kb to 13.3 kb, is divided into two subfamilies based on the gene content [60]. The pTN2-like subfamily comprises **pTN2** isolated from *T. nautili*, **pP12-1** from *Pyrococcus* sp, **pCIR10** and **pIRI48** both from *Thermococcus* sp [40, 60], whereas the subfamily pEXT9a-like is represented by **pEXT9a**, **pAMT7** and **pIRI33**, all isolated from *Thermococcus* sp [60] and **pMETVU01** isolated from *Methanocaldococcus vulcanius* M7. In addition to the superfamily 1 helicase [40], these plasmids encode proteins distantly related to the archaeo-eukaryotic primase superfamily, PriS and PriL, strongly suggesting that they replicate via a theta mechanism [40]. pTN2-like (except for pIRI48 which encodes only the PriS domain) and pEXT9a-like proteins have the PriL domain in common, whereas the PriS domain is missing in the pEXT9a-like proteins [60]. The pTN2-like plasmids also encode several proteins that have homologues with the virus PAV1 [23, 24].

The **pTN3 family** includes pTN3 (18.3 kb), isolated from *T. nautili* sp. 30-1 [74]. pTN3 encodes an SSV-type integrase and is closely related to the virus-like integrated element TKV4 from *T. kodakaraensis* [74]. pTN3 and TKV4 actually represent genomes of defective viruses that encode proteins common to viruses belonging to PRD1-adenovirus viral lineages: the capsid protein which contains the double-jelly roll fold and the putative packaging ATPase [74, 75]. Interestingly, pTN3, which is present in *T. nautili* in both an integrated and episomal form, is also harbored in membrane vesicles produced by its host [74, 76] (see next section).

Seven plasmids have been isolated from deep sea hyperthermophilic methanogens to date. They are almost exclusively associated with the *Methanocaldococcus* genus and have sizes

ranging from 4.7 kb for **pMETVU02**, isolated from *M. vulcanius*, to 58.4 kb for **ECE1,** isolated from *M. jannaschii* [77]. Most have only been sequenced and annotated, such as pMETVU02, pFS01, pMEFER01, pMETOK01. pMETVU02 encodes a putative MCM helicase suggesting a replication via a theta mechanism. Interestingly, the plasmid **pMETVU01** also isolated from *M. vulcanius* has homologues in the genomes of pEXT9a-like plasmids isolated from *Thermococcales* and described above. This suggests possible horizontal transfers from *Thermococcales* to *Methanococcales* [60, 72].

4. Membrane Vesicles in deep-sea hydrothermal vents

4.1. General features of the membrane vesicles in the three domains of life

The production of membrane vesicles (MVs) is a universal physiological phenomenon shared by cells from the three domains of life [74, 78]. MVs were recently reported in deep-sea hydrothermal vents by studying *Thermoccocales* strains [74, 79] (see below section 3.3). In the open ocean, MVs may reach up to 6.10^6 to 3.10^5 MVs per mL of seawater and notably play a key role into the carbon cycling [80].

MVs are spherical closed compartments between ~ 50 to 200 nm in diameter with differing production depending on the domain of life [81, 82] (Fig. 1 D). In *Eukarya*, different types of MVs have been described, exosomes, which originated from the extrusion of intracellular multivesicular bodies and ectosomes (also called microparticles) directly formed by budding from the cytoplasmic membrane [81, 82]. In Bacteria with double-membranes, outer membrane vesicles (OMVs) are formed by budding of the outer membrane and can contain not only periplasmic and outer-membrane proteins [83] but also proteins characteristic of the inner membrane and cytoplasmic compartments [84]. In Archaea, and more precisely in *Thermococcales*, MVs are produced by budding of the cytoplasmic membrane, similar to eukaryotic ectosomes and are frequently surrounded by a glycoproteic S-layer [85].

4.2. Roles of MVs

MVs are involved in many exchanges between cells in the three domains of life, e.g., transporting toxic molecules, quorum-sensing agents, pathogenicity factors or metabolites [74]. Protection of MVs against viral infection has also been reported [86]. Lastly, MVs can carry different type of nucleic acids (Fig. 2).

Notably, some archaea produce proteinic or peptidic toxins against competing species [87, 88]. Archaeal MVs were first described in the terrestrial and thermophilic order *Sulfolobales* as a conveyor of proteinaceous toxins [88-90]. Similarly, *Thermococcus* MVs were recently found to display toxicity towards competing *Thermococcales* strains, suggesting the existence of “Thermococcins” (A. Gorlas *et al*, unpublished work).

A recent study reports that marine bacterial extracellular vesicles are enriched in outer membrane components, suggesting that they harbor viral receptors that can act to directly bind viral particles [84]. As reported for mesophilic bacteria [86], protection of MVs against viral infection could also be possible in hot deep-sea environments. *Thermococcales* virions were sometimes observed attached to MVs which may result to lesser exposure to the viral infection [23, 79, 85] and could increase the survival of the host population.

Interestingly, MVs have been universally reported to be able to carry and transfer different type of nucleic acids by fusing with recipient cells. Transfers between cells of chromosomal, plasmid or viral DNAs packaged into MVs have been reported in Bacteria [91- 94]. All these

results lead us to consider that MVs could be involved in horizontal gene transfer **(**HGT) and thus represent another type of MGE. The ability of archaeal MVs to carry and potentially transfer different types of DNA was also recently reported in *Thermoccocales* strains isolated from deep-sea hydrothermal vents.

4.3. A focus on MVs isolated from Thermococcales and implicated in horizontal gene transfer

MV production actually appears to be widespread in *Thermococcales* isolated from deep sea hydrothermal vents. 26 out of 34 *Thermococcales* strains, observed by transmission electron microscopy, revealed the production of MVs [79]. Some of these MVs were strongly associated with cellular DNA and consequently appeared as fluorescent dots by epifluorescence microscopy. Experiments showed that these MVs can protect exogenic DNA against DNase treatments and heat denaturation, suggesting an intravesicular localization of the DNA [79]. Transformation with naked DNA might actually not be an option in deep-sea hydrothermal vents due to thermo-denaturation. It was therefore hypothesized that MVs, by protecting DNA against thermo-degradation, could play an important role in horizontal gene transfer (HGT) in these deep-sea hot environments [79]. The production of nanospheres or nanotubes was recently observed at the surface of MVs from *T. kodakaraensis* and *T. gammatolerans.* It has been speculated that this mechanism could notably be used in order to facilitate DNA transfers from MVs to cells [95]. The idea that MVs produced by *Thermococcales* can be considered as MGEs was recently strengthened when plasmid incorporation and transfer through MVs into a plasmid-free cell was demonstrated using the genetically tractable species, *T. kodakaraensis* [85]. MVs containing endogenous plasmids were also observed in *Thermococcus nautili* strains [36, 74]. MVs produced by *T. nautili* sp. 30-1 harbored the pTN3 plasmid, which corresponds to a viral genome, carries genes from a defective virus but has ineffective virion production [74]. The name “viral membrane vesicles” (vMVs) was proposed to specify these particular vesicles containing viral/plasmid genomes, considering that virus and plasmid spaces often overlap [96]. These results reinforce the hypothesis of an evolutionary connection between MVs and viruses. This presumption was previously made by considering that some viruses produce virions that strikingly resemble MVs. For example, some archeoviruses and bacterioviruses produce pleomorphic virions consisting of lipid vesicles into which viral proteins and corresponding nucleic acids are embedded [97]. It has been postulated that MVs produced by ancient cells or proto cells, anterior to LUCA, could have played a key role in the apparition of some of the first viral lineages [98].

Despite copious evidence that MVs are produced by (hyper-)thermophilic prokaryotes in deep-sea biosphere, their abundance and functions remain largely presumed to date. The study of MVs thus emerges as a new fundamental field of research in cell and evolutionary biology. Further work is now needed to better understand the global impact of MVs on deep-sea hydrothermal vents.

5. Conclusion

Mobile genetic elements (MGEs) could have been key players in the evolution of the biosphere since the very beginning. The mobilome still is a driving force for the diversity and evolution of prokaryotes and eukaryotes. Although the real impact of MGEs is not clearly known in deep sea hydrothermal vents, as reviewed in figure 2, they have a potential to be

powerful agents that drive adaptation of their cellular hosts. For example, temperate viruses and their lysogenic hosts seek to coexist so as to enhance their intertwined fitness faced with the extreme conditions of the deep sea vents. Evidence of this, given by comparative analysis of cellular and viral metagenome [26], is the relative enrichment of genes related to energy metabolism in the proviral fraction by providing their hosts with new or supplemental features to enable adaptation to a challenging environment. These proviruses boost host fitness, and in turn, enhance their own survival.

Comparative studies of MGEs from Archaea and Bacteria will help us understand the dynamic genetic network of the microbial communities in the deep biosphere. The study of how microbial populations are modulated by these genetic interactions will become a keystone area.

Although the ecological and evolutionary effects of MGEs is not clearly known in deep sea vents, their productions probably play a major role in the lifestyle of extremophile microorganisms, making analysis of these processes a prerequisite to fully understanding their physiology and rationally exploit their biotechnological potential (transfer of toxins, nucleic acids, etc.).

Conflict of interest

The authors declare no conflict of interest.

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Figures and tables

Figure 1: Electron micrographs of some viruses and membrane vesicles isolated from deep-sea hydrothermal vents, negatively stained with 2% uranyl acetate.

A: MPV1, a virus isolated from *Marinitoga piezophila* KA3
B: PAV1, a virus isolated from *Pyrococcus abyssi* GE23
C: TPV1, a virus isolated from *Thermococcus prieurii*
D: Membrane vesicles from *Thermococcus prieurii*

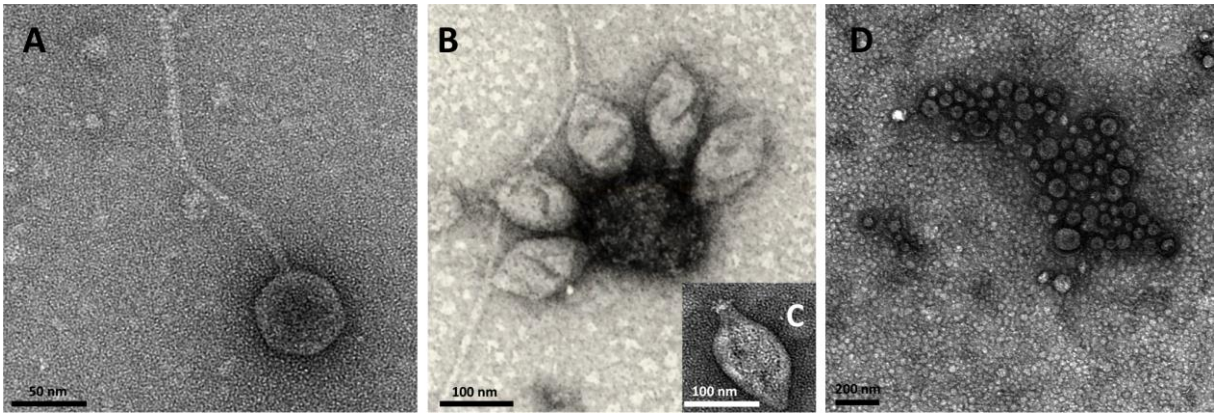

Figure 2: Multipurpose mobilome within deep-sea hydrothermal vents. Possible roles of viruses (via their viral cycles: lytic, lysogenic, carrier state or chronic), vesicles and plasmids in horizontal gene transfers of cellular, viral or plasmid DNA (in blue, red or green, respectively); a specific role of vesicles in defense against viral attack, and many other roles in cell-cell communication. This is a schematic presentation and is not to scale. The arrows with solid lines indicate demonstrated results; arrows with dotted lines show more speculative ones.

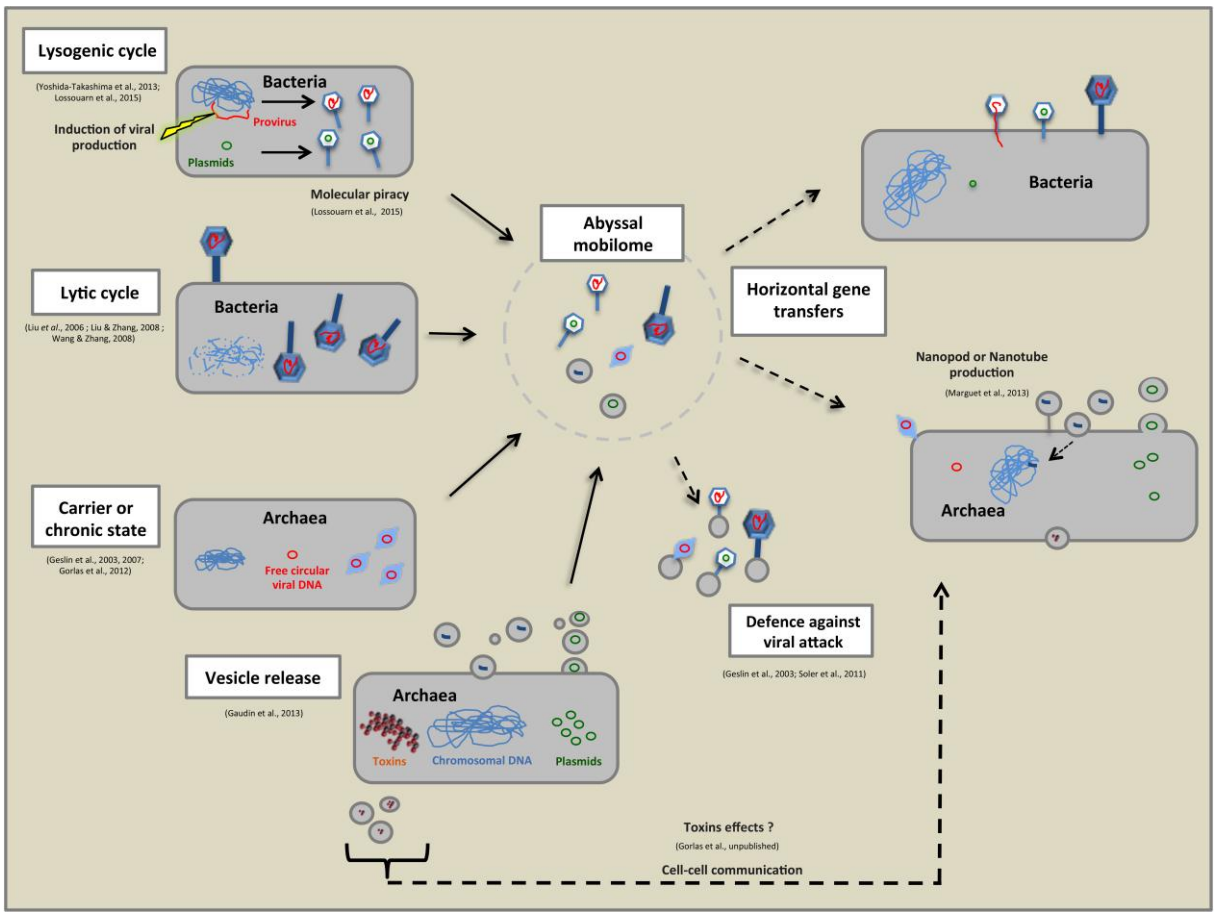

Table 1. Bacterial and archaeal viruses isolated from deep-sea hydrothermal vents.

Bacterioviruses References	Host strains (Growth temperature)	Family	Virions morphology	Virus-host relationship	Genomes
BVW1 [29]	*Bacillus* sp. w13 **(65°C)**	Unclassified	Long flexible tail, 300 nm Hexagonal head, 70 nm diameter	**Lytic**	Double-stranded linear DNA 18 kb
GVE1 [29]	*Geobacillus* sp. E26323 **(65°C)**	*Siphoviridae*	Flexible tail, 180 nm Hexagonal head, 130 nm diameter	**Lytic**	Double-stranded linear DNA 41 kb
GVE2 [30]	*Geobacillus* sp. E263 **(65°C)**	*Siphoviridae*	Not described Probably similar to GVE1	**Lytic, potentially lysogenic**	Double-stranded linear DNA 40.9 kb
D6E [31]	*Geobacillus* sp. E263 **(65°C)**	*Myoviridae*	Contractile tail 60 nm Hexagonal head, 60 nm diameter	**Lytic**	Double-stranded linear DNA 49.3 kb
Nsr-1 [32]	*Nitratiruptor* sp. SB155-2 **(55°C)**	*Siphoviridae*	Flexible tail, 210 nm Hexagonal head, 64 nm diameter	**Lysogenic**	Double-stranded linear DNA 37.1 kb
MPV1 [34]	*Marinitoga piezophila* KA3 **(65°C)**	*Siphoviridae*	Flexible tail, 200 nm Hexagonal head, 50 nm	**Lysogenic**	Double-stranded linear/circular DNA 43.715 kb
Archeoviruses References	**Host strains (growth temperature)**	**Family**	**Virions morphology**	**Virus-host relationship**	**Genomes**
PAV1 [23, 24]	*Pyrococcus abyssi* GE23 **(85°C)**	*Fuselloviridae*	Lemon-shaped 120 nm length, 80 nm width	**Carrier state**	Double-stranded linear DNA 18 kb
TPV1 [25]	*Thermococcus prieurii* **(80°C)**	*Fuselloviridae*	Lemon-shaped 140 nm length, 80 nm width	**Carrier state**	Double-stranded linear DNA 21. 5 kb

Table 2. Bacterial plasmids (associated to thermophilic strains) isolated from deep-sea hydrothermal vents.

Plasmids	Size (kp)	Host stains (Growth temperatures)	Replication	Informations	References
Unnamed	53.6	*Persephonella marina* Ex-H1 **(73°C)**	unknown	DNA replication protein DnaC DNA topoisomerase IA	[49]
pDF308	308.54	*Deferribacter desulfuricans* SSM1 **(60-65°C)**	unknown	CRISPR/cas system Transposases	[52]
pOCEPR01	135.3	*Oceanithermus profundus* 506 **(60°C)**	unknown	DNA helicase, DNA polymerase, DNA primase polymerase, bacteriolytic enzymes CRISPR/cas system	[50]
pTHEAM01	76.6	*Thermovibrio ammonificans* HB-1 **(75°C)**	unknown	DNA topoisomerase IA	[51]
pMP1	13.3	*Marinitoga piezophila* KA3 **(65°)**	unknown	DNA primase Integrase/recombinase	[34, 53]

Table 3. Archaeal plasmids isolated from deep-sea hydrothermal vents.

Plasmids	Family	Subfamily	Size (kb)	Host strains (Growth temperatures)	Replication	Informations	References
pTN1	**pTN1**		3.4	*Thermococcus nautili* 30-1 **(85°C)**	Rolling-circle type	Rep74	[61]
pGT5	**pTN1**		3.4	*Pyrococcus abyssi* GE5 **(95°C)**	Rolling-circle type	Rep75	[62]
pTP1	**pTN1**		3.1	*Thermococcus prieurii* **(80°C)**	Rolling-circle type	RepTP1	[44]
pTP2	**pTP2**		2	*Thermococcus prieurii* **(80°C)**	Rolling-circle type	RepTP2	[44]
pRT1	**pRT1**		3.4	*Pyrococcus* sp. JT1 **(95°C)**	Theta type	Rep63	[70]
pAMT11	**pRT1**		20.5	*Thermococcus* sp. AMT11 **(85°C)**	Theta type	Rep72, integrase SSV-type	[71]
pT26-2	**pT26-2**		21.6	*Thermococcus* sp. *26/2* **(85°C)**	Theta type	ATPase (replicative helicase) Integrase SSV-type	[40]
pTBMP1	**pTBMP1**		54.2	*Thermococcus barophilus* MP **(85°C)**	Theta type		[73]
pTN2	**pTN2**	**pTN2-like**	13	*Thermococcus nautili* 30-1 **(85°C)**	Theta type	Helicase protein (SFI) DNA primase-polymerase	[40]
pP12-1	**pTN2**	**pTN2-like**	12.2	*Pyrococcus* sp. 12/1 **(95°C)**	Theta type	Helicase protein (SFI) DNA primase-polymerase	[40]
pCIR10	**pTN2**	**pTN2-like**	13.3	*Thermococcus* sp. CIR10 **(85°C)**	Theta type	Helicase protein (SFI) DNA primase-polymerase	[40]
pIRI48	**pTN2**	**pTN2-like**	12.9	*Thermococcus* sp. IRI48 **(85°C)**	Theta type	Helicase protein (SFI) Primase-polymerase domain	[60]
pEXT9a	**pTN2**	**pEXT9a-like**	10.5	*Thermococcus* sp. EXT9 **(85°C)**	Theta type	Helicase protein (SFI)	[60]

pIRI33	**pTN2**	**pEXT9a-like**	11	*Thermococcus* sp. IRI33 **(85°C)**	Theta type	Helicase protein (SFI)	[60]
pAMT7	**pTN2**	**pEXT9a-like**	8.5	*Thermococcus* sp. AMT7 **(85°C)**	Theta type	Helicase protein (SFI)	[60]
pMETVU01	**pTN2**	**pEXT9a-like**	10.7	*Methanocaldococcus vulcanius* M7 **(80°C)**	Theta type	Helicase protein (SFI) Proteins homologous to proteins of pEXT9a-like plasmids	NC_013408.1 [60]
pTN3	**pTN3**		18.3	*Thermococcus nautili* 30-1 **(85°C)**	Theta type	DNA replicative helicase (MCM family) Integrase SSV-type	[74]
pGS5			2.8	*Archaeoglobus profundus* AV18 **(80°C)**	Rolling-circle type	RC-Rep	[68]
pMETVU02			4.7	*Methanocaldococcus vulcanius* M7 **(80°C)**	Theta type	Putative MCM family protein	NC_013409.1
pFS01			12.2	*Methanocaldococcus* sp. FS406-22 **(90°C)**	Theta type	Putative MCM family protein DNA polymerase domain	NC_013888.1
ECE1			58.4	*Methanocaldococcus jannaschii* **(85°C)**	Theta type	Putative MCM protein and ParA Restriction/modification system	[77]
ECE2			16.5	*Methanocaldococcus jannaschii* **(85°C)**	Theta type	Restriction/modification system	[77]
pMEFER01			22.2	*Methanocaldococcus fervens* AG86 **(80°C)**	Theta type	Putative MCM family protein	NC_013157.1
pMETOK01			14.9	*Methanothermococcus okinawensis* IH1^{t} **(60-65°C)**	Theta type	Helicase and nuclease domains	NC_015632