# Horizontal Gene Transfer in *Thermus* spp.

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**Abstract**

The small amount of genetic content in thermophiles generally limits their adaptability to environmental changes. In *Thermus* spp., very active horizontal gene transfer (HGT) mechanisms allow the rapid spread of strain-specific adaptive gene modules among the entire population. Constitutive expression of a rather particular and highly efficient DNA transport apparatus (DTA) is at the center of this HGT-mediated enhanced adaptability. The function of the DTA is dependent on the integrity and longevity of the extracellular DNA (eDNA) being transformed, which can be improved by the production of extracellular vesicles (EV) through lysis of a fraction of the population. The DTA must also contend with the recipient cell's defensive barriers, namely restriction enzymes, a panoply of CRISPR-Cas systems, and the argonaute-like protein TtAgo, which may be bypassed by transjugation, a new class of bidirectional transformation-dependent conjugation. Efficient transjugation depends on the presence of the ICETh1, an integrative and conjugative element which promotes simultaneous, generalized DNA transfer from several points in the genome. Transjugation shows preference for genes located within a megaplasmid replicon, where the main strain-specific adaptive modules are located. Contribution of transformation, vesicle-mediated eDNAs, and transjugation to HGT in this genus is discussed.

**Introduction**

Horizontal gene transfer (HGT) and symbiosis constitute a basis to explain the explosive evolution and dramatic adaptability to new environments shown by both prokaryotes and eukaryotes. Among extreme thermophilic bacteria, the remnants of HGT events have been discovered through bioinformatics analysis of the large number of genome sequences currently available. The most accepted analyses support that 20-25% of the genes present in organisms belonging to ancient clades such as *Thermotoga* or *Aquifex* were acquired from *Archaea* (Aravind *et al.*, 1998; Deckert *et al.*, 1998; Nelson *et al.*, 1999), likely thermophilic ones, which may have played relevant roles in the thermal adaptation of these genera (Aravind *et al.*, 1998). This is the case for several isolates of the genus *Thermus*, whose genomes harbor several genes of archaeal origin in addition to others shared by their phylogenetic neighbors. Members of the genus *Thermus* are widespread among very different thermal environments, with optimal growth temperatures ranging from moderately high (50 °C) to more extreme (80 °C) thermal environments, and in locations as diverse as thermal effluents on earth and in the sea to compost (Da Costa *et al.*, 2006).

At the time of writing (March 2018), there are 33 public genome projects involving Thermus spp. (https://gold.jgi-psf.org/index), 23 of which are kept as permanent drafts. The average genome size is about 2.3 Mbp, which in most strains is divided between a chromosome of around 2 Mbp (containing the core genome conserved among all strains in the species) and one or more megaplasmid ranking from 60 to 440 kbp that harbors strain-specific genes and thus, showing lower synteny among isolates (Bruggemann and Chen, 2006). Small plasmids <20 kbp are also found in different strains (Tripathi et al., 2017). Most T. thermophilus strains contain a megaplasmid of around 200 kbp named pTT27 in the strains HB27 and HB8 (Ohtani et al., 2012). Although the pTT27-related megaplasmids of T. thermophilus and other Thermus spp concentrate “dispensable” genes, they also encode complete or partial housekeeping pathways, including the synthesis of relevant compounds such as carotenes, coenzyme B12, dATP, dGTP and dUTP, and siroheme (Henne et al., 2004)

(Tripathi et al., 2017). Those Thermus isolates lacking a pTT27-like megaplasmid encode these housekeeping genes within different regions of the chromosome (Brumm et al., 2015; Fujino et al., 2017; Gounder et al., 2011). Remarkably, 4 or 5 chromosomal copies per cell have been reported, implying a polyploid nature at least for T. thermophilus HB8 (Ohtani et al., 2010), with copy numbers of the pTT27 megaplasmid similar to that of the chromosome.

One of the factors that likely contributes to strain diversity and differences in chromosomal and megaplasmid synteny is the presence of several insertion sequences (IS) distributed throughout the genome, concentrated to a larger extent in the megaplasmid (Henne *et al.*, 2004). These IS have been ascribed to different families according to their differences in transposition mechanism, and though in some cases there is evidence of recent transposition activity (Gregory and Dahlberg, 2008; Swarts, 2014), in others only a pseudogene for the transposase remains (Henne *et al.*, 2004). Regardless of activity, the presence of several copies of these elements and their conservation among different species and isolates contributes to the diversity found between otherwise close species, and likely facilitates the integration of DNA sequences flanked by conserved IS through recombination.

Among the genes present in the core genome and the pangenome of *Thermus* spp., there is a collection of homologs that are frequently found in thermophilic archaea and bacteria. Several of these genes are also located in the pTT27 megaplasmid, including defense-related adaptive CRISPR-Cas (Staals *et al.*, 2014), Argonaute proteins (Swarts, 2014), and reverse gyrases (Brochier-Armanet and Forterre, 2007). In addition to genes of archaeal origin, genes encoding specific environmental adaptations can be found in the pTT27-like megaplasmid, including genes for the use of specific sugars or for the use of nitrogen oxides in anaerobic respiration (Alvarez *et al.*, 2011). Some of these genes are associated with IS, and several of these strain-specific capabilities have been proven to easily transfer between strains, for example allowing a formerly aerobic strain to grow anaerobically by denitrification (Alvarez *et al.*, 2011).

Thus, the genes mainly found in the megaplasmid of different *Thermus* spp. seem to constitute an evolutionary strategy, in which the sharing of a large pangenome allows rapid population adaptation without requiring individual maintenance of a large genome that would be risky to replicate under high temperature conditions. For this to be an effective adaptive strategy, the genus has developed very active DNA acquisition mechanisms that promote a dynamic gene exchange flux.

Regardless, such DNA acquisition mechanisms must be compatible both with the high environmental temperatures that partially melts extracellular DNA (eDNA), and with the formidable barrier posed by the cell envelope. *Thermus* spp. have a complex envelope that combines features of both Gram-negative and Gram-positive microorganisms (Cava *et al.*, 2009), and electron microscopy studies have revealed the presence of an outer membrane (OM) surrounding the cell wall. However, the peptidoglycan layer (as thin as those found in Gram-negatives) includes dipeptide bridges between the D-Alanine at the fourth position in one muropeptide, and the L-ornithine at the third position in another chain, as in Gram-positives (Quintela *et al.*, 1995). Moreover, the cell wall includes secondary cell wall polysaccharides (SCWP) covalently bound to the peptidoglycan, as found in some Gram-positives, and even rare modifications such as the presence of phenyl-acetate in the peptidoglycan of some isolates (Quintela *et al.*, 1999). Actually, the OM is anchored to pyruvylated sugars of the SCWP through the N-terminal domain of a regular protein layer (SlpA) (Cava *et al.*, 2004) that constitutes a class of membrane scaffold reminiscent of the OmpA porin in *Escherichia coli*. Thus, for eDNA to efficiently cross this multi-layer barrier, a similarly complex DNA transport apparatus (DTA) must be in place.

In reality, very efficient horizontal gene transfer (HGT) mechanisms exist in *Thermus* spp., studied extensively in the model *T. thermophilus* strain HB27, to which most of the specific aspects of HGT described in this chapter will be addressed. The main HGT mechanisms in *T. thermophilus* involve a DTA that can act directly on free eDNA (Averhoff, 2009) as described in other bacteria, or uptake eDNA from extracellular vesicles (EV) (Blesa and Berenguer, 2015), or

directly transfer from donor cells in intimate contact in a process called transjugation (Blesa *et al.*, 2017).

**Natural competence**

Natural competence in *T. thermophilus* has been reported as one of the most efficient systems described to date in terms of rate (up to 40 kbp/s per cell) and promiscuity, as internalization of DNA from members of all three domains of life occurs with similar efficiency (Schwarzenlander and Averhoff, 2006). It is also noteworthy that the system functions during all growth phases (Hidaka *et al.*, 1994). However, transformation frequency varies greatly from strain to strain (Koyama *et al.*, 1986) despite the high degree of conservation of natural competence genes among strains, possibly due to barriers against HGT such as restriction-modification systems.

At least 16 genes have been implicated in the DTA of *T. thermophilus* HB27 through a combination of bioinformatics and mutational analysis (Averhoff, 2009), many of which play a dual role in natural transformation and biogenesis of type IV pili (T4P) (Averhoff, 2009; Friedrich *et al.*, 2001; Friedrich *et al.*, 2002; Friedrich *et al.*, 2003), and they have been assigned to three different groups.

The first group includes the highly conserved competence proteins ComEA, ComEC and DprR proteins. ComEA was found to bind double-stranded DNA (dsDNA), thereby contributing to the transport of eDNA through the OM (Salzer *et al.*, 2016b). ComEA is exclusively present in the inner membrane (IM). This finding was quite surprising, since all ComEA orthologues in Gram-negative bacteria are soluble. ComEC is a polytopic IM protein whose orthologues are also widespread in many bacteria. The IM localization together with the finding that *comEC* deletion mutants still take DNA into a DNAse-resistant state suggests that ComEC mediates DNA transport from the periplasm to the cytoplasm (Schwarzenlander *et al.*, 2009). ComEC also modulates the transcriptional regulation of DNA translocator and T4P components, thereby mediating a response to extracellular stimuli (Salzer *et al.*, 2014c). The function of DprA has not been addressed in detail, but the finding that DNA binding and uptake in *dprA* knockout mutants are unaffected, together with functions

conveying incoming single-stranded DNA (ssDNA) to RecA in orthologues (Mortier-Barriere *et al.*, 2007; Yadav *et al.*, 2014) is in line with the idea that DprA stabilizes incoming DNA and is important for strand exchange during recombination.

The second group of competence proteins consists of proteins which are very similar to components of the T4P biogenesis system, including four pilin-like proteins (PilA1, PilA2, PilA3 and PilA4), a leader peptidase (PilD), a AAA-ATPase (PilF), an IM protein (PilC), a PilM-homologue and a secretin-like protein (PilQ). Individual deletion of PilA1, PilA2 and PilA3 abrogated competence without affecting piliation, suggesting distinct roles for the individual pilins in DNA uptake but not in pilus assembly. In contrast, PilA4 plays an essential role in DNA uptake and is also the major subunit of T4P (Friedrich *et al.*, 2003; Schwarzenlander *et al.*, 2009). PilF is a unique zinc-binding AAA-ATPase essential for the transport of DNA through the OM and polymerization of T4P (Rose *et al.*, 2011; Salzer *et al.*, 2013; Salzer *et al.*, 2014b; Schwarzenlander *et al.*, 2009). It has a tripartite structure: a unique N-terminus containing three general secretory domains; a central region; and a C-terminal ATPase and zinc-binding domain. PilF assembles into hexameric complexes that have a disk and ring-like structure separated by a short stem-like structure (Rose et al., 2011, Collins et al., 2013). The C-terminal region encodes a tetracysteine motif which mediates zinc binding (Rose *et al.*, 2011; Salzer *et al.*, 2014a). Individual cysteine residues are also important for complex stability, probably beneficial for the functionality of PilF under high temperature environmental conditions (Salzer *et al.*, 2014a). Analogous to PilA4 and PilF, the competence proteins PilC, PilM, PilN and PilQ were also found to be absolutely essential for T4P assembly (Friedrich *et al.*, 2002). Structural analyses suggest that the IM protein PilC forms dimers which might interact with cytoplasmic and periplasmic proteins (Karuppiah *et al.*, 2010). However, further work is needed to identify interaction partners and elucidate the function of PilC. PilM interacts with the IM protein PilN, forming dimeric PilMN complexes that initiate interaction with two PilO monomers, generating a transmembrane platform for the assembly of pilins (Karuppiah *et al.*, 2013; Karuppiah and Derrick, 2011). The secretin PilQ has a unique modular organization and forms homopolymeric

complexes, which are essential for the passage of DNA through the OM (Burkhardt *et al.*, 2011; Schwarzenlander *et al.*, 2009). The PilQ complex has a unique structure consisting of a “cone”, a “cup” and six rings with a large central channel. The non-conserved N-terminus of PilQ exhibits a modular architecture comprised of domains with alternating α-helices and β-sheets that assemble into the six ring system underneath the cup-like structure and are important for pilus extrusion and function but are dispensable for natural transformation (Burkhardt *et al.*, 2012; Salzer *et al.*, 2016a). The structural features together with the unprecedented length (34 nm) of the secretin complex suggests that the PilQ complex spans the entire cell periphery, thereby mediating DNA transport across the OM and periplasmic space in a single step, which might be beneficial for DNA uptake in high temperature environments.

The similarities of competence proteins to components of T4P biogenesis led to the hypothesis that T4P plays a role in natural transformation. However, our findings that non-piliated *Thermus* mutants still take up free DNA, some with even higher frequencies than wild-type cells, led to the conclusion that piliation and natural competence, despite sharing some common genes, are separate systems (Burkhardt *et al.*, 2012; Salzer *et al.*, 2014b).

In addition to these conserved competence proteins, DTA depends on several unique proteins, such as ComZ, PilN, PilO and PilW (Friedrich *et al.*, 2002; Friedrich *et al.*, 2003). The role of ComZ remains to be determined, though it seems to be implicated in DNA uptake through the OM (Schwarzenlander *et al.*, 2009). PilN and PilO are both part of an IM platform for initiation of the biogenesis of the DTA and T4P (Karuppiah *et al.*, 2013; Rumszauer *et al.*, 2006). The non-conserved competence protein PilW is also implicated in both DTA and T4P, and is essential for assembly and/or stability of the OM-spanning PilQ complexes (Rumszauer *et al.*, 2006). Based on the numerous structural and functional data, a model of the DTA in *T. thermophilus* is presented in Figure 1.

The next question is: What is the fate of the incoming DNA? So far it is not clear whether one or both strands of the eDNA are taken up by *T. thermophilus*. In

most natural competence systems, only one strand of the eDNA enters the cell, thus this pattern has also been proposed for *Thermus* spp. In any case, eDNA is incorporated into the genome by homologous recombination, with >90% similarity and sizes above 500 nt required for successful integration.

Recently, electron cryo-tomography and reconstruction has provided the first three-dimensional *in situ* structure of the secretin (Gold *et al.*, 2015). The secretin channel was found to be highly dynamic, switching between closed and open states. Two gates safely close the channel in the absence of pili but open

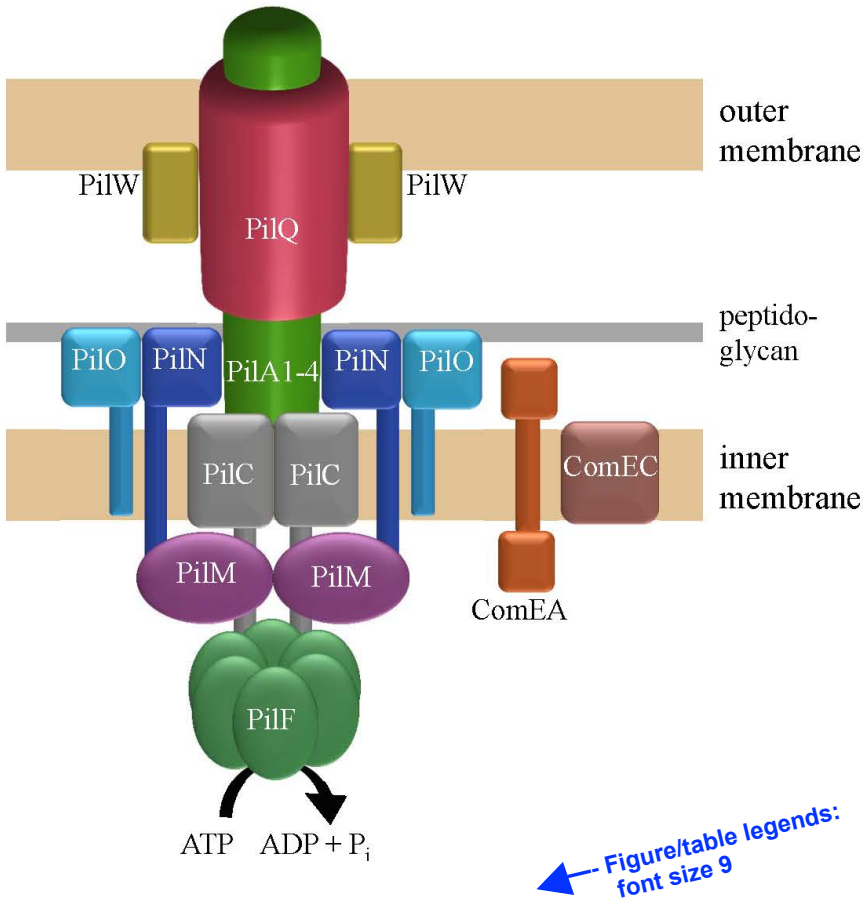


**Figure 1.** Model of the DNA translocator. The secretin forms a channel spanning the outer membrane (OM) and the periplasm. This channel mediates extrusion of a pilus-like structure comprised of PilA1-4. PilW is important for the assembly of the secretin complex, and the AAA-ATPase (PilF) powers the polymerization of the pilus-like structure. The dimeric PilC complex links the energy released from PilF-mediated ATP hydrolysis to the assembly of a pilus-like structure. PilM, PilN and PilO form the inner membrane (IM) assembly platform, while ComEA binds incoming DNA and delivers it to the IM channel formed by ComEC.

when pili are extruded. Moreover, the opening of the channel to make way for pilus extrusion is accompanied by major conformational changes of the N-terminal domains of the central secretin. Interestingly, only ~20% of the secretin channels extruded pili, and it is tempting to speculate that the idle complexes may be active in DNA uptake. Taken together, these *in situ* studies open avenues for further protein structure-function analyses, shedding more light onto the mechanisms of DNA translocation.

**Vesicle-protected HGT**

Efficient HGT through natural competence is limited by the size of the eDNA, as effective integration into the chromosome requires a rather long DNA fragment. This is especially relevant in high-temperature environments, where the stability of the DNA is further compromised (Soler *et al.*, 2008). One of the mechanisms that helps alleviate problems with eDNA stability in high temperatures is protection by attachment to or enclosure within extracellular vesicles (EVs) or

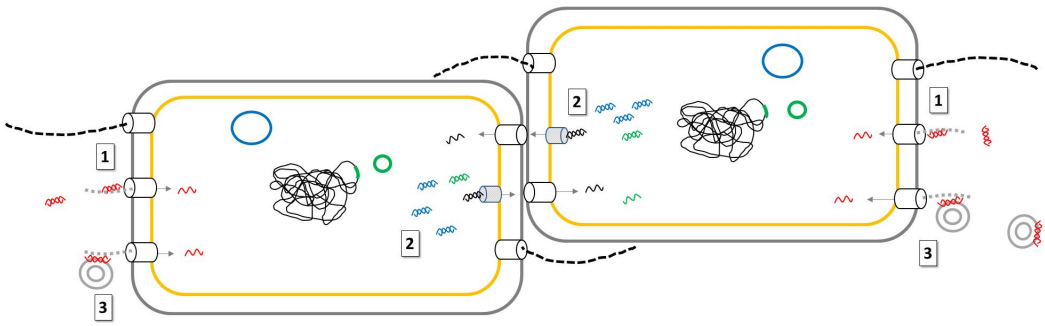


**Figure 2.** Scheme of HGT processes detected in *T. thermophilus*. *T. thermophilus* cells in close contact are represented, with the chromosome and pTT27 megaplasmid shown in black and blue lines, respectively. Production of polar type IV pili (broken black lines) involved in twitching motility is also shown. 1) Both cells can take in unprotected DNA (red) from the environment through the natural competence apparatus, likely via the PilQ protein (white cylinder) localized at the cell poles and shared by the type IV pili. Internalized DNA is shown as single-stranded, but this has not yet been demonstrated. 2) A transjugation model is depicted in which the ICEth1 (green), represented both as an inserted and circular element, generates the enzymes involved in the recognition of origins of transfer in the genome at different frequencies, in a distributive seemingly random manner. The fragments generated from the chromosome (black), the megaplasmid (blue), and the ICETh1 (green) are transported through the cell envelope ("push" step) in a TdtA-dependent manner (gray cylinders), and the secreted DNA is captured by the competence apparatus and internalized ("pull" step), in a DNAse-protected process. 3) Both cells can capture DNAse-protected DNA from membrane vesicles generated from lysis of distant donor cells. The natural competence apparatus is required for such internalization, and no clear-cut conclusions can be made regarding the requirement of vesicle fusion to liberate trapped DNA. Mechanisms 1 and 3 are subject to the TtAgo surveillance defense system based on DNA-DNA interference, whereas mechanism 2 is not.

phage-like capsids called gene transfer agents (Lang *et al.*, 2012). Vesicle-protected eDNA can withstand time and nuclease activity, allowing displacement of eDNA over long distances. EVs stored for more than a year at 4 °C maintained their ability to be taken up by *T. thermophilus*.

EVs are membrane-enclosed structures that are produced by members all three domains of life under different names (membrane vesicles, exosomes, shedding microvesicles, etc.) which are frequently generated by bacteria during the growth phase or when integrated within a biofilm (Turnbull *et al.*, 2016). In Gram-negative bacteria, the production of OM vesicles (OMVs) is quite common, driven by different mechanisms in response to diverse trigger signals. In addition to the expected OM and periplasmic components, these OMVs frequently contain cytoplasmic enzymes and/or DNA, playing a great diversity of roles, such as in cell communication, immune system evasion, and stress response, among others (Schwechheimer and Kuehn, 2015). Indeed, it is evident that OMV biogenesis diverges among bacteria and is influenced by the environmental context (Deatherage and Cookson, 2012; Orench-Rivera and Kuehn, 2016).

In contrast to the apparently sophisticated mechanisms involved in OMV generation in some bacteria, the generation of EVs in *Thermus* spp. seems to be a consequence of cell lysis, with fortuitous capture of DNA fragments from the genome within membrane fragments (Blesa and Berenguer, 2015). This lytic origin is also seen in other bacteria where it is linked to stressful conditions, suggesting it is either a consequence of a specific program to induce lysis or the result of irreversible cell damage (Orench-Rivera and Kuehn, 2016).

Very little is known about how EV-associated eDNA can enter the recipient cell, though a fusion mechanism has been proposed for OMVs that would allow eDNA access to the periplasm, with other internalization mechanisms required for entry to the cytoplasm. Regarding the EVs of lytic origin, access is likely related to the presence of a functional natural competence apparatus in the recipient (Fulsundar *et al.*, 2014).

Different *Thermus* isolates produce DNAse-protected eDNA associated with EVs in a growth medium and phase-dependent manner, in such a way that fast-growing cells generate larger amounts of eDNA than cells in stationary phase (Blesa and Berenguer, 2015). This eDNA was acquired by the recipient cells at similar frequencies independently of its integration site in the chromosome or in the megaplasmid. Actually, the analysis of the eDNA fraction associated with EVs and protected from the DNAses revealed a size of around 20 kbp, similar to that obtained for genomic preparations of the strain following conventional methods, and further analysis showed that the whole genome was randomly represented in the EV-protected eDNA fraction (Blesa and Berenguer, 2015).

Proteomic assays of the EV fraction also revealed the presence of proteins from all the cell compartments, with the majority of proteins from the IM (20%), OM (12%), and periplasmic (27%) fractions, but with a significant presence of cytoplasmic proteins (14%). The presence of random genomic fragments and proteins clearly supports a lytic origin for *Thermus* EVs, similar to other bacteria. Interestingly, the production of EVs was highest during the adaptive (latency) and exponential phases of *T. thermophilus* cultures, lower when the cells entered stationary phase, and almost undetectable when the cells were grown in minimal media, where the growth rate was much lower than in rich media. These data suggest that the production of EVs in laboratory conditions is a consequence of unbalanced growth in a non-natural medium with nutrient excess, but also reveals that cell lysis is a normal event, and that cell populations in natural environments may generate EVs as vehicles for HGT. In this sense, the possibility of a programmed lytic process in parts of the population, such as those described in *Pseudomonas aeruginosa* biofilms (Turnbull *et al.*, 2016), cannot be excluded.

The mechanism underlying the entry of EV-associated eDNA into *T. thermophilus* is not clear, but it depends on the natural competence of the recipient cell, as mutants lacking different competence genes are unable to acquire such eDNA. Therefore, recipient cells are somehow capable of directly contacting the EV-protected eDNA: either the EV fuses to the OM, or the eDNA is actually adsorbed to surface of the EV and is thus accessible to the

competence machinery, but hidden enough to avoid degradation by DNAses. Keeping in mind the great complexity of the multilayered *T. thermophilus* cell envelope and its intermediate structure between that of Gram-negative and Gram-positive bacteria (Cava *et al.*, 2009), external display of eDNA on the EV surface seems to be a more likely option.

Whatever the mechanism of eDNA acquisition is, it is relevant to place it within the general context of DNAse-resistant HGT in *Thermus* spp., where much higher transfer frequencies are detected for other mechanisms such as transjugation, as described below.

**Transjugation**

Genes encoding proteins homologous to components of the classical conjugation machinery (including type 4 secretion systems,T4SS, relaxases and relaxosome elements involved in *oriT* recognition and transfer) have been identified in plasmids from *T. thermophilus* JL-18 (accession CP003254), *T. thermophilus* SGO, 5JP17-16 (accession CP002778) and *T. aquaticus* Y51MC23 (Brumm *et al.*, 2015), though a conjugation phenotype has not yet been proven in these strains.

However, a conjugation-like cell-to-cell transfer process has been described in *T. thermophilus* strains that lack obvious homologs to these conjugation genes. For example, conjugation between a nitrate respiring strain of *T. thermophilus* (NAR1) and the aerobic strain HB27 was described that allowed for isolation of a nitrate-respiring HB27 derivative (Ramirez-Arcos *et al.*, 1998). Sequencing of the transferred DNA identified an apparently mobile element encoding the nitrate respiration capability (NCE) (Cava *et al.*, 2009). Additional conjugation experiments with other denitrifying strains of *T. thermophilus* that had no apparent conjugative apparatus (based on genome sequences with 98% coverage, laboratory results) also led to the isolation of denitrifying derivatives of HB27 (Alvarez *et al.*, 2011). All these data supported the existence of a new mechanism for conjugation independent of T4SS in these *T. thermophilus* strains.

This unconventional conjugation-like mechanism was subsequently identified in the aerobic *T. thermophilus* HB27 (César *et al.*, 2012), and a deeper analysis revealed transfer efficiencies similar to transformation, although the mechanism differed significantly from classical conjugation (Blesa *et al.*, 2014). Firstly, it didn't involve inhibition or incompatibility barriers for DNA donation, in such a way that the same cell could function both as donor and recipient, making parenthood analysis of the progeny a difficult task. No homologues to T4SS components were identified in the genome, and genes seemed to transfer without maintenance of their order in the chromosome, in a process more akin to generalized transduction than to conjugation. Finally, it was also noted that the genes encoded by the pTT27 megaplasmid were transferred at a rate one order of magnitude more efficiently than chromosomal genes, despite similar copy numbers per cell.

Still more surprising was the requirement of a fully active natural competence system in the recipient cells, in such a way that mutants deficient in virtually any described component of the DTA could serve only as DNA donor, a property that led to proposal of the term *transjugatio*n (transformation-dependent conjugation) (Blesa *et al.*, 2017). Further parenthood analysis experiments, in which combinations of non-competent mutants were used, confirmed that the DNA-donation machinery was independent of the competence apparatus, both present in the same bacteria, implying a two-step ("push and pull") model for transjugation.

The search for putative components of the DNA-pushing system led to the identification of transjugation donor translocase A (TdtA), a protein belonging to the FtsK-HerA family of DNA translocase-helicases. Its mutation impaired the ability of the cell to act as a donor in transjugation, without significant effect on natural competence or any other phenotypic trait (Blesa *et al.*, 2017). Purified recombinant TdtA was subsequently shown to form hexameric rings in the presence of ATP, with a central pore wide enough to accommodate dsDNA, resembling the TraB protein encoded by conjugative plasmids of *Streptomyces* spp. In fact, TraB seems to be the only protein required for conjugative transfer of plasmids between *Streptomyces* spp., with additional proteins encoded by

conjugative plasmids acting at the level of intramycelium spreading (Thoma and Muth, 2012) (Thoma and Muth, 2015).

Interestingly, the TdtA protein is only present in a few of the sequenced strains of *T. thermophilus*, encoded by a mobile genetic element (ICETh1) reminiscent of the integrative and conjugative elements (ICE) found in many conjugative bacteria (Blesa *et al.*, 2017). This 14 kb element encodes a typical phage-like recombinase of the XerC family and a four-gene operon encoding: a type IIG restriction endonuclease identical in sequence to *Tth111*II (Zhu *et al.*, 2014); a putative nuclease of the NurA family; TdtA; and a putative DNA methylase. Additional genes in the ICETh1 include a transposase for which two more copies exist in the genome, and a putative hydrolase of unknown function. The whole ICETh1 is integrated within the chromosome flanked by 46-bp direct repeats belonging to the 3'-end of a tRNA gene, and its exogenous origin is supported by a much lower G+C content (58%) than that of the chromosome (68%).

The ICEth1 can excise from the chromosome but apparently does not replicate, as the circular form of the element is much more difficult to detect than the corresponding scar left in the chromosome after its excision (Blesa *et al.*, 2017). Actually, the ICEth1 is apparently lost at high frequencies (up to 8% of cells) during exponential growth, supporting its non-replicative nature, and raising questions regarding its maintenance within the population.

In contrast to classical ICEs that have evolved to promote their self-transfer, ICETh1 stimulates the generalized and parallel transfer of all the genes in the genome, especially of those genes localized in the megaplasmid, at similar or higher frequencies than the ICETh1 itself. This apparently altruistic nature of the ICETh1 might constitute a sophisticated strategy for spreading, using traits encoded by the genome for co-selection, though a putative advantage of those cells harboring the ICETh1 cannot be discarded at this point.

One of the less understood aspects of transjugation is the way in which the origins of transfer (*oriT*) are selected. As the TdtA protein itself does not contain

DNA-binding motifs similar to those found in the TraB protein (Thoma and Muth, 2012), a very attractive hypothesis involves action of the restriction endonuclease *Tth111*II, which is itself encoded by the ICETh1, at multiple targets in the genome. Genes located near regions with a high density of *Tth111*II recognition sequences seem to be transferred at higher frequencies than genes located in regions with fewer recognition sites, and mutants defective in this restriction enzyme have transfer frequencies three orders of magnitude lower than the wild-type. Further efforts to analyze the frequency of transfer of plasmids with or without restriction sites should shed more light on the process.

The role of the NurA nuclease also remains to be elucidated, though its relevance seems clear as *nurA* null mutations abolish transjugation (Blesa *et al.*, 2017). Archaeal homologs of NurA have been shown to act in coordination with the HerA helicase in repair of dsDNA-breaks involving homologous recombination. NurA-HerA complexes have been proposed in which the helicase pushes DNA through a NurA dimer that digests the DNA in a trimming-like process (Rzechorzek *et al.*, 2014). In *Thermus*, the formation of a putative NurA-TdtA complex would likely be involved in DNA donation, although its exact role must be studied further.

**The role of innate and adaptive immunity as barriers against HGT**

Several classes of barriers have been described that protect cells against infection and replication of viruses (Seed, 2015). In addition to the DNA restriction barriers present in most bacteria, and likely as a consequence of the great promiscuity and constitutive nature of its natural competence system, *T. thermophilus* wields an arsenal of adaptive and innate defensive systems. Adaptive defenses rely on a collection of 10-12 CRISPR sequences, most of them located in the megaplasmid, and the corresponding Cas protein systems belonging to three different families (IE, IIIa, IIIb) that function as crRNA-DNA and crRNA-RNA interference systems against invading DNA (Staals *et al.*, 2014). The role of these systems against intragenus or intraspecies HGT is not likely to be significant as these genes cannot be recognized by the CRISPR

spacers of the recipient strains, for the same reasons that the donor strain does not target its own genome.

In addition to this adaptive system, *Thermus* spp. contain a homolog to the eukaryotic argonaute protein (TtAgo), whose role in defense against both plasmidic (Swarts, 2014) and genomic DNA (Blesa *et al.*, 2014) acquired by natural competence has been well established. The protein somehow recognizes the entering eDNA and uses an ssDNA guide to screen for complementarity and degradation after DNA-DNA interference, in what seems to be an additional innate defense mechanism. In contrast to its activity against eDNA acquired by natural competence, TtAgo does not limit access of DNA acquired by transjugation, despite the involvement of natural competence in both processes (Blesa *et al.*, 2014). The possibility that its discrimination is based on a lower G+C content of the target DNA, as proposed after *in vitro* assays (Swarts *et al.*, 2017; Swarts, 2014), seems not to be the case *in vivo*, as interference was also detected when isogenic DNA was transferred by transformation, but not by transjugation (Blesa *et al.*, 2014). Therefore, the possibility exists for an activation mechanism of TtAgo that is somehow triggered by eDNA.

**Transduction**

Most *Thermus* genomes sequenced to date do not harbor integrated prophages, supporting that temperate phages are not common in the biology of *Thermus* spp. Nevertheless, there are some exceptions to this general observation. For example, the genome of *T. aquaticus* Y51MC23 contains two complete and two residual prophages (Brumm *et al.*, 2015), whereas *Thermus* strains RL, 2.9, and CCB_US3_UF1 each contain a prophage (Dwivedi *et al.*, 2012; Navas *et al.*, 2015; Teh *et al.*, 2012). However, the ability of these prophages to excise and replicate has not been studied, and their involvement in specialized transduction is yet to be assayed.

In contrast, a great variety and number of lytic phages have been described infecting different *Thermus* spp. (Yu *et al.*, 2006), although sequences are only available for a few of them (phiYS40, TMA, phiOH2, P23-77, P23-45, p74-26,

In93). This fact, together with the abundance of CRISPR sequences and Cas systems found in the genomes of most *Thermus* isolates (Staals *et al.*, 2014), strongly supports frequent infection by environmental viruses, suggesting that generalized transduction may be a contributing factor to HGT gene flow in these bacteria.

**Concluding remarks**

The genus *Thermus* is ubiquitous and widespread in diverse thermal environments revealing an apparent metabolic flexibility which contrasts its small genome size. In what seems to be a strategy to cope with limitations on genetic content, most strains contain a genetically plastic megaplasmid that allocates many specific adaptation modules and very efficient HGT mechanisms that allow the rapid acquisition and spread of such traits among the population in response to a sudden change in the environment.

The natural competence machinery is central to all of the transfer mechanisms demonstrated so far: transformation, EVs and transjugation. Putative involvement of viral transduction or classical donor-recipient conjugation systems has yet to be proven, but the abundance of *Thermus*-specific phages and the existence of gene clusters reminiscent of the T4SS and relaxases in a few of the sequenced strains indicate that they also may contribute to HGT in the species.

Natural competence itself is demonstrated to be a very efficient method of HGT for some *T. thermophilus* strains, and it has been proposed as the major mechanism involved in strain adaptation. Acquisition of traits such as new S-layers and the capability to grow anaerobically with nitrate are laboratory examples of how natural competence affords such adaptability. The DTA is a highly complex and very dynamic system composed of several unique proteins, highlighting the features which might have been triggered by the extreme environment and/or the complex cell envelope. It exhibits extraordinarily broad substrate specificity and a very high efficiency, which may be of major importance for thermoadaptation of *T. thermophilus* and interdomain DNA transfer in hot environments. However, in its natural environment, the stability of

naked eDNA is always compromised by the presence of DNAses and high temperatures, suggesting that eDNA transport in a protected manner is more likely. In this sense, EVs produced in laboratory conditions during exponential phase may mimic the natural process in which fragmented cell envelopes generate a protective coat that adsorbs and engulfs large genome fragments, allowing for their environmental persistence until capture by a recipient cell's natural competence apparatus.

Transjugation is a newly identified mechanism that promotes HGT between isogenic and non-isogenic strains of *T. thermophilus* bearing the ICETh1. The presence of this element results in the contact-mediated transfer of any marker in the genome from donor to recipient, and especially favors those genes located within the megaplasmid, which may be more useful for environmental adaptation. The mechanism is similar to the distributive transfer found in *Mycobacterium,* as it involves parallel transfer of genes from multiple sites in the genome in an apparently simultaneous manner, though it is still unknown if transjugation produces mosaic progenies, as in mycobacteria. The mechanism depends on the activity of a DNA translocase protein that can accommodate dsDNA, but also a nuclease and a restriction enzyme that are likely involved in selection of the origins of transfer. The most exciting property of transjugation, however, is that although it depends on the natural competence apparatus in the recipient cell, the transferred DNA avoids the interference mechanisms mediated by TtAgo, which degrades at least 90% of eDNA molecules that enter the cell by transformation. Considering the 10-fold preference for the transfer of megaplasmid-associated genes over chromosomal ones, all the evidence clearly points to this mechanism as a major tool for environmental adaptation in *Thermus* spp.

*Open questions*

Despite being the best known model among extreme thermophiles, several questions remain to be answered regarding the HGT mechanisms reviewed here, such as: How is the simultaneous expression of distantly located competence genes regulated? How is the signal relayed from outside to inside? Which form of energy powers the uptake of DNA? How does the competence

apparatus recognize and transport eDNA? Where is the apparatus located within the cell? What is the link between the competence apparatus and pili? How can the bacteria distinguish between eDNA acquired by transformation and DNA acquired by transjugation while using the same competence proteins?

The competence-dependence of the transjugation process itself continues to be a puzzle itself, with such fundamental questions as: How does the system recognize regions in the chromosome or megaplasmid as the origin of transfer? Which proteins in addition to those encoded by the ICEth1 are necessary for donor cells to secrete DNA through the complex cell wall? How is such DNA protected from DNAses before the competence proteins of the recipient cells are able to hide it within the periplasm? Additional questions regarding the relevance of eDNA transfer through vesicles or the putative relevance of viruses as vehicles of HGT also await future research.

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**References**

Alvarez, L., Bricio, C., Jose Gomez, M., and Berenguer, J. (2011). Lateral Transfer of the Denitrification Pathway Genes among *Thermus thermophilus* Strains. Appl Environ Microbiol *77*, 1352-1358 DOI: 10.1128/aem.02048-10.

Aravind, L., Tatusov, R.L., Wolf, Y.I., Walker, D.R., and Koonin, E.V. (1998). Evidence for massive gene exchange between archaeal and bacterial hyperthermophiles. Trends Genet *14*, 442-444.

Averhoff, B. (2009). Shuffling genes around in hot environments: the unique DNA transporter of *Thermus thermophilus*. FEMS Microbiol Rev *33*, 611-626 DOI: 10.1111/j.1574-6976.2008.00160.x.

Blesa, A., Baquedano, I., Quintans, N.G., Mata, C.P., Caston, J.R., and Berenguer, J. (2017). The transjugation machinery of *Thermus thermophilus*:

Identification of TdtA, an ATPase involved in DNA donation. PLoS genetics *13*, e1006669 DOI: 10.1371/journal.pgen.1006669.

Blesa, A., and Berenguer, J. (2015). Contribution of vesicle-protected extracellular DNA to horizontal gene transfer in *Thermus* spp. Int Microbiol *18*, 177-187 DOI: 10.2436/20.1501.01.248.

Blesa, A., César, C.E., Averhoff, B., and Berenguer, J. (2014). Non canonical cell-to-cell DNA transfer in *Thermus* spp. is insensitive to Argonaute-mediated interference. J Bacteriol DOI: 10.1128/jb.02113-14.

Brochier-Armanet, C., and Forterre, P. (2007). Widespread distribution of archaeal reverse gyrase in thermophilic bacteria suggests a complex history of vertical inheritance and lateral gene transfers. Archaea *2*, 83-93.

Bruggemann, H., and Chen, C. (2006). Comparative genomics of *Thermus thermophilus*: Plasticity of the megaplasmid and its contribution to a thermophilic lifestyle. J Biotechn *124*, 654-661 DOI: 10.1016/j.jbiotec.2006.03.043.

Brumm, P.J., Monsma, S., Keough, B., Jasinovica, S., Ferguson, E., Schoenfeld, T., Lodes, M., and Mead, D.A. (2015). Complete Genome Sequence of *Thermus* aquaticus Y51MC23. PloS one *10*, e0138674 DOI: 10.1371/journal.pone.0138674.

Burkhardt, J., Vonck, J., and Averhoff, B. (2011). Structure and function of PilQ, a secretin of the DNA transporter from the thermophilic bacterium *Thermus thermophilus* HB27. J Biol Chem *286*, 9977-9984.

Burkhardt, J., Vonck, J., Langer, J.D., Salzer, R., and Averhoff, B. (2012). Unusual N-terminal alphaalphabetaalphabetabetaalpha fold of PilQ from *Thermus thermophilus* mediates ring formation and is essential for piliation. J Biol Chem *287*, 8484-8494 DOI: 10.1074/jbc.M111.334912.

Cava, F., de Pedro, M.A., Schwarz, H., Henne, A., and Berenguer, J. (2004). Binding to pyruvylated compounds as an ancestral mechanism to anchor the outer envelope in primitive bacteria. Mol Microbiol *52*, 677-690 DOI: 10.1111/j.1365-2958.2004.04011.x.

Cava, F., Hidalgo, A., and Berenguer, J. (2009). *Thermus thermophilus* as biological model. Extremophiles *13*, 213-231 DOI: 10.1007/s00792-009-0226-6.

César, C.E., Álvarez, L., Bricio, C., van Heerden, E., Littauer, D., and Berenguer, J. (2012). Unconventional lateral gene transfer in extreme thermophilic bacteria. Int Microbiol *14*, 187-199.

Da Costa, M.S., Rainey, F.A., and Nobre, F. (2006). The genus *Thermus* and relatives. In The Prokaryotes, a handbook on the biology of bacteria, M. Dworkin, S. Falkow, E. Rosenberg, K.-H. Schleifer, and E. Stackebrandt, eds. (Singapore: Springer), pp. 797-812.

Deatherage, B.L., and Cookson, B.T. (2012). Membrane vesicle release in bacteria, eukaryotes, and archaea: a conserved yet underappreciated aspect of microbial life. Infect Immun *80*, 1948-1957 DOI: 10.1128/IAI.06014-11.

Deckert, G., Warren, P.V., Gaasterland, T., Young, W.G., Lenox, A.L., Graham, D.E., Overbeek, R., Snead, M.A., Keller, M., Aujay, M.*, et al.* (1998). The complete genome of the hyperthermophilic bacterium Aquifex aeolicus. Nature *392*, 353-358 DOI: 10.1038/32831.

Dwivedi, V., Sangwan, N., Nigam, A., Garg, N., Niharika, N., Khurana, P., Khurana, J.P., and Lal, R. (2012). Draft genome sequence of *Thermus* sp. strain RL, isolated from a hot water spring located atop the Himalayan ranges at Manikaran, India. J Bacteriol *194*, 3534 DOI: 10.1128/JB.00604-12.

Friedrich, A., Hartsch, T., and Averhoff, B. (2001). Natural transformation in mesophilic and thermophilic bacteria: identification and characterization of novel, closely related competence genes in Acinetobacter sp. strain BD413 and *Thermus thermophilus* HB27. Appl Environ Microbiol *67*, 3140-3148 DOI: 10.1128/AEM.67.7.3140-3148.2001.

Friedrich, A., Prust, C., Hartsch, T., Henne, A., and Averhoff, B. (2002). Molecular analyses of the natural transformation machinery and identification of pilus structures in the extremely thermophilic bacterium *Thermus thermophilus* strain HB27. Appl Environ Microbiol *68*, 745-755.

Friedrich, A., Rumszauer, J., Henne, A., and Averhoff, B. (2003). Pilin-like proteins in the extremely thermophilic bacterium *Thermus thermophilus* HB27: implication in competence for natural transformation and links to type IV pilus biogenesis. Appl Environ Microbiol *69*, 3695-3700.

Fujino, Y., Nagayoshi, Y., Ohshima, T., Ogata, S., and Doi, K. (2017). Complete Genome Sequence of *Thermus thermophilus* TMY, Isolated from a Geothermal Power Plant. Genome announcements *5* DOI: 10.1128/genomeA.01596-16.

Fulsundar, S., Harms, K., Flaten, G.E., Johnsen, P.J., Chopade, B.A., and Nielsen, K.M. (2014). Gene transfer potential of outer membrane vesicles of

Acinetobacter baylyi and effects of stress on vesiculation. Appl Environ Microbiol *80*, 3469-3483 DOI: 10.1128/AEM.04248-13.

Gold, V.A., Salzer, R., Averhoff, B., and Kuhlbrandt, W. (2015). Structure of a type IV pilus machinery in the open and closed state. eLife *4* DOI: 10.7554/eLife.07380.

Gounder, K., Brzuszkiewicz, E., Liesegang, H., Wollherr, A., Daniel, R., Gottschalk, G., Reva, O., Kumwenda, B., Srivastava, M., Bricio, C.*, et al.* (2011). Sequence of the hyperplastic genome of the naturally competent *Thermus* scotoductus SA-01. BMC genomics *12*, 577 DOI: 10.1186/1471-2164-12-577.

Gregory, S.T., and Dahlberg, A.E. (2008). Transposition of an insertion sequence, ISTth7, in the genome of the extreme thermophile *Thermus thermophilus* HB8. FEMS Microbiol Lett *289*, 187-192 DOI: 10.1111/j.1574-6968.2008.01389.x.

Henne, A., Bruggemann, H., Raasch, C., Wiezer, A., Hartsch, T., Liesegang, H., Johann, A., Lienard, T., Gohl, O., Martinez-Arias, R.*, et al.* (2004). The genome sequence of the extreme thermophile *Thermus thermophilus*. Nature Biotech *22*, 547-553 DOI: 10.1038/nbt956.

Hidaka, Y., Hasegawa, M., Nakahara, T., and Hoshino, T. (1994). The entire population of *Thermus thermophilus* cells is always competent at any growth phase. Biosci Biotechnol Biochem *58*, 1338-1339 DOI: 10.1271/bbb.58.1338.

Karuppiah, V., Collins, R.F., Thistlethwaite, A., Gao, Y., and Derrick, J.P. (2013). Structure and assembly of an inner membrane platform for initiation of type IV pilus biogenesis. Proc Natl Acad Sci USA *110*, E4638-4647 DOI: 10.1073/pnas.1312313110.

Karuppiah, V., and Derrick, J.P. (2011). Structure of the PilM-PilN inner membrane type IV pilus biogenesis complex from *Thermus thermophilus*. J Biol Chem *286*, 24434-24442 DOI: 10.1074/jbc.M111.243535.

Karuppiah, V., Hassan, D., Saleem, M., and Derrick, J.P. (2010). Structure and oligomerization of the PilC type IV pilus biogenesis protein from *Thermus thermophilus*. Proteins *78*, 2049-2057 DOI: 10.1002/prot.22720.

Koyama, Y., Hoshino, T., Tomizuka, N., and Furukawa, K. (1986). Genetic transformation of the extreme thermophile *Thermus thermophilus* and of other *Thermus* spp. J Bacteriol *166*, 338-340.

Lang, A.S., Zhaxybayeva, O., and Beatty, J.T. (2012). Gene transfer agents: phage-like elements of genetic exchange. Nat Rev Microbiol *10*, 472-482 DOI: 10.1038/nrmicro2802.

Mortier-Barriere, I., Velten, M., Dupaigne, P., Mirouze, N., Pietrement, O., McGovern, S., Fichant, G., Martin, B., Noirot, P., Le Cam, E.*, et al.* (2007). A key presynaptic role in transformation for a widespread bacterial protein: DprA conveys incoming ssDNA to RecA. Cell *130*, 824-836 DOI: 10.1016/j.cell.2007.07.038.

Navas, L.E., Berretta, M.F., Ortiz, E.M., Benintende, G.B., Amadio, A.F., and Zandomeni, R.O. (2015). Draft Genome Sequence of *Thermus* sp. Isolate 2.9, Obtained from a Hot Water Spring Located in Salta, Argentina. Genome announcements *3* DOI: 10.1128/genomeA.01414-14.

Nelson, K., Clayton, R., Gill, S., Gwinn, M., Dodson, R., Haft, D., Hickey, E., Peterson, J., Nelson, W., Ketchum, K.*, et al.* (1999). Evidence for lateral gene transfer between Archaea and bacteria from genome sequence of *Thermotoga maritima*. Nature *399*, 323 - 329.

Ohtani, N., Tomita, M., and Itaya, M. (2010). An extreme thermophile, *Thermus thermophilus*, is a polyploid bacterium. J Bacteriol *192*, 5499-5505 DOI: 10.1128/JB.00662-10.

Ohtani, N., Tomita, M., and Itaya, M. (2012). The third plasmid pVV8 from *Thermus thermophilus* HB8: isolation, characterization, and sequence determination. Extremophiles *16*, 237-244 DOI: 10.1007/s00792-011-0424-x.

Orench-Rivera, N., and Kuehn, M.J. (2016). Environmentally controlled bacterial vesicle-mediated export. Cell Microbiol *18*, 1525-1536 DOI: 10.1111/cmi.12676.

Quintela, J.C., Pittenauer, E., Allmaier, G., Aran, V., and de Pedro, M.A. (1995). Structure of peptidoglycan from *Thermus thermophilus* HB8. J Bacteriol *177*, 4947-4962.

Quintela, J.C., Zollner, P., Garcia-del Portillo, F., Allmaier, G., and de Pedro, M.A. (1999). Cell wall structural divergence among *Thermus* spp. FEMS Microbiol Lett *172*, 223-229.

Ramirez-Arcos, S., Fernandez-Herrero, L.A., Marin, I., and Berenguer, J. (1998). Anaerobic growth, a property horizontally transferred by an Hfr-like mechanism among extreme thermophiles. J Bacteriol *180*, 3137-3143.

Rose, I., Biukovic, G., Aderhold, P., Muller, V., Gruber, G., and Averhoff, B. (2011). Identification and characterization of a unique, zinc-containing transport ATPase essential for natural transformation in *Thermus thermophilus* HB27. Extremophiles *15*, 191-202 DOI: 10.1007/s00792-010-0343-2.

Rumszauer, J., Schwarzenlander, C., and Averhoff, B. (2006). Identification, subcellular localization and functional interactions of PilMNOWQ and PilA4 involved in transformation competency and pilus biogenesis in the thermophilic bacterium *Thermus thermophilus* HB27. FEBS Journal *273*, 3261-3272 DOI: 10.1111/j.1742-4658.2006.05335.x.

Rzechorzek, N.J., Blackwood, J.K., Bray, S.M., Maman, J.D., Pellegrini, L., and Robinson, N.P. (2014). Structure of the hexameric HerA ATPase reveals a mechanism of translocation-coupled DNA-end processing in archaea. Nature Comm *5* DOI: 10.1038/ncomms6506.

Salzer, R., D'Imprima, E., Gold, V.A., Rose, I., Drechsler, M., Vonck, J., and Averhoff, B. (2016a). Topology and Structure/Function Correlation of Ring- and Gate-forming Domains in the Dynamic Secretin Complex of *Thermus thermophilus*. J Biol Chem *291*, 14448-14456 DOI: 10.1074/jbc.M116.724153.

Salzer, R., Herzberg, M., Nies, D.H., Biukovic, G., Gruber, G., Muller, V., and Averhoff, B. (2013). The DNA uptake ATPase PilF of *Thermus thermophilus*: a reexamination of the zinc content. Extremophiles *17*, 697-698 DOI: 10.1007/s00792-013-0544-6.

Salzer, R., Herzberg, M., Nies, D.H., Joos, F., Rathmann, B., Thielmann, Y., and Averhoff, B. (2014a). Zinc and ATP binding of the hexameric AAA-ATPase PilF from *Thermus thermophilus*: role in complex stability, piliation, adhesion, twitching motility, and natural transformation. The J Biol Chem *289*, 30343-30354 DOI: 10.1074/jbc.M114.598656.

Salzer, R., Joos, F., and Averhoff, B. (2014b). Type IV pilus biogenesis, twitching motility, and DNA uptake in *Thermus thermophilus*: discrete roles of antagonistic ATPases PilF, PilT1, and PilT2. Appl Environ Microbiol *80*, 644-652 DOI: 10.1128/AEM.03218-13.

Salzer, R., Kern, T., Joos, F., and Averhoff, B. (2014c). Environmental factors affecting the expression of type IV pilus genes as well as piliation of *Thermus thermophilus*. FEMS Microbiol Lett *357*, 56-62 DOI: 10.1111/1574-6968.12506.

Salzer, R., Kern, T., Joos, F., and Averhoff, B. (2016b). The *Thermus thermophilus* comEA/comEC operon is associated with DNA binding and regulation of the DNA translocator and type IV pili. Environ Microbiol *18*, 65-74 DOI: 10.1111/1462-2920.12820.

Schwarzenlander, C., and Averhoff, B. (2006). Characterization of DNA transport in the thermophilic bacterium *Thermus thermophilus* HB27. FEBS Lett *273*, 4210-4218 DOI: 10.1111/j.1742-4658.2006.05416.x.

Schwarzenlander, C., Haase, W., and Averhoff, B. (2009). The role of single subunits of the DNA transport machinery of *Thermus thermophilus* HB27 in DNA binding and transport. Environ Microbiol *11*, 801-808.

Schwechheimer, C., and Kuehn, M.J. (2015). Outer-membrane vesicles from Gram-negative bacteria: biogenesis and functions. Nat Rev Microbiol *13*, 605-619 DOI: 10.1038/nrmicro3525.

Seed, K.D. (2015). Battling Phages: How Bacteria Defend against Viral Attack. PLoS Pathogens *11*, e1004847 DOI: 10.1371/journal.ppat.1004847.

Soler, N., Marguet, E., Verbavatz, J.M., and Forterre, P. (2008). Virus-like vesicles and extracellular DNA produced by hyperthermophilic archaea of the order *Thermococcales*. Res Microbiol *159*, 390-399 DOI: 10.1016/j.resmic. 2008.04.015.

Staals, R.H., Zhu, Y., Taylor, D.W., Kornfeld, J.E., Sharma, K., Barendregt, A., Koehorst, J.J., Vlot, M., Neupane, N., Varossieau, K.*, et al.* (2014). RNA targeting by the type III-A CRISPR-Cas Csm complex of *Thermus thermophilus*. Mol Cell *56*, 518-530 DOI: 10.1016/j.molcel.2014.10.005.

Swarts, D.C., Szczepaniak, M., Sheng, G., Chandradoss, S.D., Zhu, Y., Timmers, E.M., Zhang, Y., Zhao, H., Lou, J., Wang, Y.*, et al.* (2017). Autonomous Generation and Loading of DNA Guides by Bacterial Argonaute. Mol Cell *65*, 985-998 e986 DOI: 10.1016/j.molcel.2017.01.033.

Swarts, D.C.J., Matthijs M.; Westra, Edze R.; Zhu, Yifan; Janssen, Jorijn H.; Snijders, Ambrosius P.; Wang, Yanli; Patel, Dinshaw J.; Berenguer, José; Brouns, Stan J.; van der Oost, John (2014). DNA- guided DNA interference by a prokaryotic Arognaute. Nature DOI: 10.1038.

Teh, B.S., Abdul Rahman, A.Y., Saito, J.A., Hou, S., and Alam, M. (2012). Complete genome sequence of the thermophilic bacterium *Thermus* sp. strain CCB_US3_UF1. J Bacteriol *194*, 1240 DOI: 10.1128/JB.06589-11.

Thoma, L., and Muth, G. (2012). Conjugative DNA transfer in *Streptomyces* by TraB: is one protein enough? FEMS Microbiol Lett *337*, 81-88 DOI: 10.1111/1574-6968.12031.

Thoma, L., and Muth, G. (2015). The conjugative DNA-transfer apparatus of Streptomyces. Int J Med Microbiol *305*, 224-229 DOI: 10.1016/j.ijmm.2014.12.020.

Tripathi, C., Mishra, H., Khurana, H., Dwivedi, V., Kamra, K., Negi, R.K., and Lal, R. (2017). Complete genome analysis of *Thermus parvatiensis* and comparative genomics of *Thermus* spp. provide insights into genetic variability and evolution of natural competence as strategic survival attributes. Front Microbiol *8*, 1410 DOI: 10.3389/fmicb.2017.01410.

Turnbull, L., Toyofuku, M., Hynen, A.L., Kurosawa, M., Pessi, G., Petty, N.K., Osvath, S.R., Carcamo-Oyarce, G., Gloag, E.S., Shimoni, R.*, et al.* (2016). Explosive cell lysis as a mechanism for the biogenesis of bacterial membrane vesicles and biofilms. Nature Comm *7*, 11220 DOI: 10.1038/ncomms11220.

Yadav, T., Carrasco, B., Serrano, E., and Alonso, J.C. (2014). Roles of Bacillus subtilis DprA and SsbA in RecA-mediated genetic recombination. J Biol Chem *289*, 27640-27652 DOI: 10.1074/jbc.M114.577924.

Yu, M.X., Slater, M.R., and Ackermann, H.W. (2006). Isolation and characterization of *Thermus* bacteriophages. Arch Vir *151*, 663-679 DOI: 10.1007/s00705-005-0667-x.

Zhu, Z., Guan, S., Robinson, D., El Fezzazi, H., Quimby, A., and Xu, S.-y. (2014). Characterization of cleavage intermediate and star sites of RM. Tth111II. Scientific reports *4*, 3838.