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The genus *Thermotoga*: Recent developments

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Abstract

The genus *Thermotoga* comprises extremely thermophilic ($T_{\text{opt}} = 70^{\circ}\text{C}$) and hyperthermophilic ($T_{\text{opt}} = 80^{\circ}\text{C}$) bacteria that have been extensively studied for insights into the basis for life at elevated temperatures and for biotechnological opportunities (e.g., biohydrogen production, biocatalysis). Over the past decade, genome sequences have become available for a number of *Thermotoga* species, leading to functional genomics efforts to understand growth physiology as well as genomics-based identification and characterization of novel high temperature biocatalysts. Discussed here are recent developments along these lines for this novel group of microorganisms.

Keywords

Thermotoga; extreme thermophiles; genomics; microbial physiology

1. Introduction

The genus *Thermotoga* consists of some of the most thermophilic bacteria known, with optimum growth temperatures up to 80°C [1–3]. Their ability to degrade a wide range of simple and complex carbohydrates, produce fermentative hydrogen at high yield, and catalyze a variety of high-temperature reactions has been the basis for numerous biotechnological applications [4]. These bacteria are also viewed as model systems for studying adaptation to high temperature and microbial evolution, as they present a challenge to conventional classification [5, 6]. A number of genome sequences for *Thermotoga* species have become available in the past few years [7], offering additional insights into the biology of these interesting bacteria and suggesting biotechnological opportunities.

Members of the order Thermotogales are anaerobic, rod-shaped bacteria encapsulated by a unique “toga”-like outer membrane. Reported substrates for *Thermotoga* growth include hexoses, pentoses, disaccharides, glucans, xylans, glucomannan, galactomannan, pectin, chitin, and amorphous cellulose [1, 8]. This diversity of carbon sources correlates with the unusually large fraction of *Thermotoga* genes involved in carbohydrate degradation and utilization [9]. The primary products of fermentation are acetate, CO_2 , and H_2 , although lactate, ethanol, alanine, and α -aminobutyrate have also been detected [1, 10, 11]. Some *Thermotoga* species can also use thiosulfate, sulfur, and Fe(III) as electron acceptors [12].

Thermotoga species have been isolated from geothermally heated environments across the globe, including oil reservoirs, submarine hot springs, and continental solfataric springs (Table 1). When the *Thermotoga maritima* genome sequence was completed, 24% of the genes were found to be most similar to archaea, suggesting that significant lateral gene

transfer has occurred between these two groups [13]. However, when this issue was revisited recently with more genome sequence data available, only 7.7–11% of genes were found to be most similar to archaea, while 42.3–48.2% of genes were most similar to Firmicutes [7]. The decrease in genes most similar to archaea is probably related to disproportionate expansion in the number of bacterial, compared to archaeal, genome sequences. Nevertheless, evidence for archaea-bacteria gene transfer remains, and several studies have provided evidence for frequent gene transfer within the *Thermotoga* genus [5, 6, 14]. These studies of genus-level diversity revealed that genes involved in essential processes and central metabolism are more highly conserved than those responsible for utilization of specific carbohydrates, especially polysaccharides.

The formally identified *Thermotoga* species (Table 1) can essentially be split into two groups based on optimum growth temperature and the ability to reduce elemental sulfur. Four species have optimum growth temperatures of 77°C and above (*T. maritima*, *T. petrophila*, *T. neapolitana*, and *T. naphthophila*), while the other five species grow optimally at 70°C and below (*T. elfii*, *T. thermarum*, *T. subterranea*, *T. hypogea*, and *T. lettingae*). With one exception (*T. lettingae*), sulfur reduction is limited to the group of higher temperature species. Based on 16S rRNA, the higher temperature group is very closely related compared to the remaining species (Fig. 1). Of the lower temperature group, *T. subterranea*, *T. elfii*, and *T. lettingae* appear to be more related, while *T. hypogea* and *T. thermarum* do not cluster with any members of the genus.

T. lettingae was isolated from a sulfate-reducing bioreactor, where methanol was the only carbon source. In addition to *T. lettingae*, it was reported that *T. subterranea*, *T. elfii*, *T. thermarum*, and *T. maritima* are all able to use methanol [11]. Methanol is produced by pectin degradation, a known capability of *T. maritima* [15, 16]. During growth of *T. maritima* on pectin, expression of a Zn-dependent alcohol dehydrogenase (TM0436) is up-regulated, raising the possibility that this enzyme may catalyze the oxidation of methanol to formaldehyde [16]. Of the six *Thermotoga* species with complete genome sequences, the presence of this specific alcohol dehydrogenase correlates with the reported ability to degrade methanol in four instances. In the other two instances, *T. petrophila* has this gene, but was reported to not use methanol [10]; while *T. sp. RQ2* has this gene, and has not been tested for methanol use. *T. lettingae* also has a catalase/peroxidase enzyme (Tlet_1209), which could catalyze hydrogen peroxide-dependent oxidation of methanol, but this presumably would only occur in the presence of oxygen.

2. Formation of molecular H₂ by carbohydrate fermentation by *Thermotoga* species

Thermotoga species have been targeted for biohydrogen production due to reported yields approaching the Thauer limit for anaerobic fermentation [17–24]. This limit (4 mol H₂/mol glucose) can only be attained if all of the reducing equivalents from glucose oxidation are used to reduce protons to H₂ [25]. In practice, these reducing equivalents are also used for biosynthetic purposes and the formation of other fermentation products, including acetate, lactate, ethanol, butyrate, and butanol. In particular, NADH produced by glyceraldehyde 3-phosphate dehydrogenase (GAPDH) is usually not used for hydrogen production, as reduced ferredoxin and formate are stronger reducing agents [25, 26]. Therefore, model fermenters like *Escherichia coli* and *Clostridia* species often yield 1–2 mol H₂/mol glucose, where the hydrogen formed is a result of pyruvate oxidation [26, 27]. In *E. coli*, pyruvate is converted to acetyl-CoA, H₂, and CO₂ by the combined action of pyruvate formate lyase and formate hydrogen lyase. In *Clostridia* species, pyruvate ferredoxin oxidoreductase (PFOR) produces reduced ferredoxin (Fd), which is re-oxidized by hydrogenases to form H₂.

High hydrogen yields have been observed for the hyperthermophilic archaeon *Pyrococcus furiosus* as a result of a modified Embden-Meyerhof pathway [28]. In *P. furiosus*, glyceraldehyde 3-phosphate:ferredoxin oxidoreductase (GAPOR) converts glyceraldehyde 3-phosphate directly to 3-phosphoglycerate, replacing the typical EMP enzymes (GAPDH and phosphoglycerate kinase) [29]. This mechanism utilizes ferredoxin, not NADH, as electron carrier. Therefore, a yield of 4 H₂ per glucose is attainable when all reduced ferredoxin from GAPOR and PFOR is used for hydrogen production via the membrane bound hydrogenase [28, 30, 31]. It should also be noted that this modified EM pathway lacks the formation of ATP by phosphoglycerate kinase. Unlike *P. furiosus*, *T. maritima* has classical Embden-Meyerhof, pentose phosphate, and Entner-Doudoroff pathways [29]. The high H₂ yields attained with *T. maritima* are related to the heterotrimeric [Fe-Fe] hydrogenase, which has 73, 68, and 19 kDa subunits (α , β , and γ , respectively). Sequence analysis suggests that the β subunit is a flavoprotein that accepts electrons from NADH, and the γ subunit transfers electrons from the β subunit to the catalytic α subunit. Initial work was unable to demonstrate the use of ferredoxin or NADH as an electron donor for hydrogen production [32, 33]. However, it has since been demonstrated that the *T. maritima* hydrogenase requires both reduced ferredoxin and NADH. This enzyme has been called a “bifurcating” hydrogenase, and it has been proposed that energy from the oxidation of ferredoxin drives the unfavorable oxidation of NADH [34]. Thus, *T. maritima* has the ability to use the NADH from glycolysis for hydrogen production, allowing yields to approach the Thauer limit. Because this hydrogenase uses both NADH and reduced ferredoxin as electron donors, H₂ production may become unfavorable at lower H₂ partial pressures than if reduced ferredoxin alone was used [26]. Genome sequence analysis indicates that such “bifurcating” hydrogenases may be present in several *Clostridia* species. The relatively high H₂ yields and low biomass yields observed for *Thermotoga* may be related to the apparently limited options for pyruvate metabolism, discussed below.

When the H₂ partial pressure builds up, *T. maritima* must dissipate electrons from glycolysis in other ways to continue growing. Fermentation products detected during growth of *Thermotoga* species include acetate, CO₂, H₂, ethanol, and lactate (Figure 2). Acetate production involves the action of pyruvate ferredoxin oxidoreductase (PFOR), phosphate acetyltransferase (TM1130), and acetate kinase (TM0274). These reactions are motivated by the formation of ATP by acetate kinase, which allows cells to yield 4 ATP/mol glucose. H₂ yield is optimized when all glucose is converted to acetate, because the steps from pyruvate to acetate do not involve oxidation of NADH. When H₂ accumulates and the hydrogenase no longer oxidizes NADH, pyruvate would be diverted away from acetate production, possibly towards lactate production. Lactate is produced from pyruvate by lactate dehydrogenase (TM1867) with the concomitant reoxidation of NADH (Fig. 2). However, lactate has not been documented as a significant product of *Thermotoga* growth, since the initial isolation report [1, 17]. In *Clostridia* species, NADH can be oxidized during the production of butyrate and butanol, but these pathways are not apparent in *Thermotoga*. On first glance, *T. maritima* lacks the pyruvate decarboxylase activity necessary for ethanol formation. However, this reaction has been catalyzed *in vitro* by the PFOR from *P. furiosus* [35], suggesting that the *T. maritima* homolog (TM0015–18) may have the same ability. Acetaldehyde generated from pyruvate decarboxylation could be converted to ethanol by the product of TM0820, which is apparently a NADPH-dependent primary alcohol dehydrogenase [36]. Therefore, ethanol production may be more favourable, if excess NADPH is produced by carbon flux through the oxidative stage of the pentose phosphate pathway.

Although hydrogenases are inactivated by oxygen, have reported increased hydrogen production by *T. neapolitana* in the presence of oxygen has been reported with yields of 8.5 $\pm$ 2.9 mol H₂/mol glucose [18, 19]. These high yields and the depletion of headspace oxygen

suggested that *T. neapolitana* utilizes oxygen for a more energy-efficient catabolic process. Extra-cytoplasmic oxygen-resistant hydrogenase activity has been detected in *T. neapolitana* [37], but the physiological role is unknown and H₂-evolving hydrogenases are generally cytoplasmic [38]. Structural modelling of the [FeFe] hydrogenase α subunits suggests that the catalytic site of *T. neapolitana*'s enzyme may be less accessible to oxygen than *T. maritima*'s enzyme, accounting for increased resistance to inactivation [39]. However, subsequent studies on *T. neapolitana* [20, 21] have provided evidence that growth and hydrogen production are inhibited by oxygen. In Van Ooteghem's experiments, the hydrogen yield may have been overestimated if any carbon sources in other media components (2 g/L yeast extract and 2 g/L trypticase) were utilized in addition to glucose. Also, Eriksen *et al.* [20] demonstrated that oxygen is depleted from the headspace of cell-free medium, and that a lack of agitation may allow anaerobic conditions to be established in the bottom of the culture where the cells settle. Thus, the level of oxygen actually encountered and consumed by *T. neapolitana* may be much less than it initially appears, especially in unshaken cultures.

Anaerobic microbes have been found to consume small levels of oxygen, although they do so to protect themselves from its deleterious effects. NADH oxidases which can convert O₂ to H₂O₂ have been detected in *T. hypogea*, *T. neapolitana*, and *T. maritima* [40, 41]. Such enzymes are useless for oxygen detoxification unless they are accompanied by a peroxidase to convert H₂O₂ into H₂O. This peroxidase activity is presumed to be the work of rubrerythrin (TM0657), which is upregulated in *T. maritima* in response to oxidative stress [42] and has been shown to function as a NADH peroxidase in *Pyrococcus furiosus* [43]. In the presence of oxygen, microbes also have to deal with reactive oxygen species like superoxide, which results from the reduction of oxygen. In *P. furiosus*, a system for superoxide reduction has been identified which involves transfer of electrons from NADPH with the involvement of NADPH:rubredoxin oxidoreductase, rubredoxin, and superoxide reductase [44, 45]. Homologs of these proteins have been identified in *T. maritima* (TM0754, TM0659, and TM0658, respectively), and the putative superoxide reductase is upregulated in response to oxidative stress [42]. Other genes upregulated by oxygen exposure include TM1368, which encodes a protein involved in iron-sulfur cluster assembly and repair, as well as TM0755. The recombinant protein encoded by TM0755 was characterized as a rubredoxin oxygen oxidoreductase. This enzyme is capable of converting O₂ to H₂O without the formation of H₂O₂, a favorable property for efficient oxygen detoxification.

3. Microbial ecology of Thermotoga species

Growth in cell communities is the predominant mode in natural microbial environments [46]. Functional genomics and proteomics studies on microorganisms to date have largely focused on metabolism and physiology based in the cytosol. Evidence from molecular microbial ecology studies over the past 15 years has established that bacteria utilize a chemical language, based on a variety of small signalling molecules/peptides/proteins [47, 48]. Also clear is that microbial communities are not merely a collection of unicellular and isolated individuals, but rather a cooperative society capable of acting on a multi-cellular level [49]. A wide spectrum of molecules are known to be released in its surroundings by the bacteria, and their ability to sense the concentration of these molecules through receptors in the cell membrane forms the basis of this chemical language. Quorum sensing is defined as the ability of a single cell to sense the number of bacteria in its proximity based upon the accumulation of signalling molecules [50]. Although not extensively studied, it appears that *Thermotoga* species also participate in community behaviour, although with some differences in certain cases from less thermophilic bacteria.

Functional genomics approaches have been used to understand community-behaviour associated responses in *T. maritima*, including cell density-dependent processes, such as biofilm formation during growth in mono- and co-culture. Previous studies revealed significant wall growth when *T. maritima* cells were grown alone in continuous culture [51], suggesting the formation of exopolysaccharide (EPS)-associated sessile cell communities. Mixed culture experiments have also shed light on syntrophic interactions between hydrogen-producing *T. maritima* cells and the cells of methanogen *Methanocaldococcus jannaschii* [52]. Transcriptomic tools, in association with statistical bioinformatics analyses, have given new insights into the ecological interactions of *Thermotoga* species by mimicking conditions that might arise in natural habitats.

Transcriptional differences were measured between sessile and planktonic *T. maritima* cells from a continuous culture reactor. The sessile state of cells in the biofilms was found to be associated within rope-like structures, which formed on the reactor walls, polycarbonate filters and nylon mesh [53] (Fig. 3). Transcripts from heat shock (e.g. *dnaK*, *smHSP*) genes were found to be higher in biofilm cells, while transcripts of a cold shock gene [54] were lower in biofilms genes similar to general observations made from transcriptional studies of mesophilic biofilms [55–57].

Pure culture studies can provide information about the growth physiology; however, mixed culture studies can offer further understanding of intricate mechanisms relevant to growth in cells communities, conditions more akin to the natural state of many microorganisms. *T. maritima* grew to extremely high densities in a co-culture with methanogenic archaeon *M. jannaschii*, which uses the growth-inhibitory hydrogen produced by *T. maritima* to produce methane [58] (Fig. 4A). Full genome transcriptional comparisons between *T. maritima* cells in pure culture and in high density co-culture with *M. jannaschii* showed changes in sugar utilization and transport genes, including glycosyl transferases and genes encoding known glucomannan (TM1752) and α -glucan (TM1834) hydrolases, which correlated with the appearance of EPS in the culture.

A possible involvement of cyclic-di-GMP in regulating EPS formation and maintenance was also suggested by the transcriptional patterns observed. Cyclic-di-GMP, a second messenger, is also known to regulate cellulose synthesis in *Gluconacetobacter xylinum* [59], *S. enterica* serovar *Typhimurium* [60] and, biofilm formation in *V. cholerae* [61], *Staphylococcus aureus* [62], *Yersinia pestis* [63], *Pseudomonas aeruginosa* [64], among others. Several GGDEFdomain containing proteins displayed different expression patterns between the pure culture and co-culture, including a putative diguanylate cyclase (TM1163), subsequent characterization for which confirmed its diguanylate cyclase activity [65], and a putative cyclic-di-GMP phosphodiesterase (TM1184). Transcriptional response data led to the identification of a small, unknown open reading frame (TM0504) co-localized with an ABC transporter lacking a substrate binding protein, suggesting a possible role in peptide export, a processing mechanism for which was proposed prior to the secretion of the TM0504 peptide (Fig. 4B,C). In the *T. maritima* genome, the permeases (TM0503, TM0502) and ATP-binding subunits (TM0501, TM0500) (Fig. 4C), comprising an oligopeptide ABC transporter, are located immediately upstream of gene encoding for TM0504; anti-microbial peptide transporter domains existed in both TM0500 and TM0501 (COG4167 and 4170). As a periplasmic-binding protein was seen to be missing from this operon, this cluster of genes was predicted to export a small peptide derived from the protein product of TM0504. In addition, TM0504 contained a GG motif (Fig. 4B), similar to the cleavage point for the active form of autoinducing peptides found in *S. pneumonia* and lactic acid bacteria [66]. Due to lack of a genetic system developed for *T. maritima*, knock out mutants lacking TM0504 could not be constructed and so, to understand the function of this polypeptide, a truncated version of the mature form of TM0504 peptide was dosed into pure

low-density *T. maritima* cultures. EPS formation was triggered in the pure cultures dosed with the synthetic peptide whereas, no EPS formed in the undosed control cultures (Fig. 4D). This report was the first indication of the importance of peptide-based quorum sensing in hyperthermophilic habitats.

Further, analysis of the region around TM0504 revealed that this it was co-located, on the opposite strand with the gene encoding *ssrA*, a hybrid of tRNA and mRNA (tmRNA), which is involved in a *trans*-translational process related to ribosome rescue and, is ubiquitous in bacteria [67]. Specific DNA probes were designed and used in real time PCR assays to follow separate transcriptional responses of the co-located ORFs during transition from exponential into stationary phase, chloramphenicol challenge, and syntrophic co-culture with *M. jannaschii*. No significant change in either TM0504 or tmRNA transcription was observed under normal growth conditions in both pure cultures. However, an eight-fold increase in transcription of the tmRNA gene and thirty-fold decrease in TM0504 transcription levels compared to pure culture, were noted for high density co-cultures with *M. jannaschii*. The down-regulation of the TM0504 gene was thought to be a result of the cells no longer participating in quorum sensing behaviour due to aggregation with *M. jannaschii*. Chloramphenicol challenge resulted in a forty-fold increase after five minutes and a twenty three-fold increase after thirty minutes, in tmRNA gene transcription, from dosing. A two-fold increase was noted for TM0504 transcription. The effects were attributed most likely to disruptions of translational processes caused by the addition of antibiotic. The possibility that a biologically active peptide was encoded on, and independently transcribed from, the strand opposite tmRNA in most bacterial genomes was found to be intriguing. Although, no direct inferences were drawn from these observations as to a definite reason for such an arrangement, but it has been suggested that overlapping genes may compress genome size in species subject to reductive evolution [68].

The genome of *T. maritima* was hypothesized to have undergone extensive gene transfers [13] and this was validated in the case of *Thermotogales* using comparative genomic hybridization carried out to investigate genome plasticity and Lateral Gene Transfer (LGT) in these species [5]. To date, the genome of *T. maritima*, has been found to contain eight distinct CRISPR sequences [69]. The genomes of *T. maritima* MSB8 and *T. neapolitana* NS-E have been compared where, a whole-genome alignment revealed numerous large scale DNA rearrangements, most of which are associated with CRISPR DNA repeats and/or tRNA genes [70]. This study suggested that the *Thermotogales* species, including *T. maritima* and *T. neapolitana*, favoured inversion/translocation events within a replicore and as examples proposed different paths consisting of such events to achieve the same DNA segment found in both the species.

4. Biocatalysis using Thermotoga enzymes

T. maritima's xylanolytic abilities have attracted interest for food, paper, and biofuel-related applications. Xylooligosaccharides resulting from xylan degradation have been shown to increase the numbers of beneficial gut bacteria, such as *Bifidobacterium* species [71]. Production of xylooligosaccharides, especially xylobiose, has been demonstrated using immobilized xylanase B (XynB) from *T. maritima* (TM0070), as well as a combination of arabinofuranosidase (TM0281) and XynB [72, 73]. The combination of both enzymes resulted in twice as much xylan degradation as XynB alone. The rationale for this is that arabinofuranosidase cleaves modified groups of the xylan backbone, allowing XynB more access [73]. XynB has also been used as an additive for breadmaking, as it improves oven spring, volume, shape, and texture. In comparison to lower temperature xylanases, the thermostable version from *T. maritima* performs better due to extended activity during the baking process. Xylanases may improve bread quality by solubilizing arabinoxylans and

changing the water distribution in the dough [74]. Xylanases can also be useful to the pulp and paper industry, as they can reduce the amount of chlorine necessary for pulp bleaching. XynB from *T. maritima* was shown to be effective under conditions appropriate for this application, namely high temperature and alkaline pH [75].

T. maritima XynB is a 40 kDa single domain protein that is located in the periplasm. *T. maritima* also has a larger 120 kDa multi-domain protein, xylanase A (TM0061), which is located primarily in the outer membrane [76]. Xylanase A (XynA) has two carbohydrate binding modules (CBMs) on each side of the catalytic domain. One of the N-terminal domains binds xylan, while one of the C-terminal domains binds cellulose [77, 78]. The thermostability of XynA decreases when the N-terminal modules are removed. The thermostability is not affected when the C-terminal modules are removed, suggesting that the N-terminal modules are more important for the thermostability of the enzyme [78]. A fusion protein combining one of these N-terminal domains with the xylanase 2 from *Trichoderma reesei* exhibited greater thermostability and substrate binding ability than the wild-type xylanase 2, providing further evidence that these N-terminal domains are involved in thermostability [79]. XynA hydrolyzes 90–95% of xylohexaose (X₆) to xylobiose (X₂)/xylotetraose (X₄) and 5–10% to xylotriose (X₃). When the C-terminal domains of XynA are removed, X₂/X₄ production decreases significantly, while X₃ production appears relatively unaffected. In this case, about 50 % of X₆ is hydrolyzed to X₂/X₄ while the other 50 % is hydrolyzed to X₃. This variant also has about 60 % higher activity on insoluble wheat arabinoxylan compared to the wild-type enzyme. These modulations in substrate specificity and hydrolysis products may be useful for optimization of a biomass conversion process [80].

Transgenic expression of carbohydrate-active enzymes in plants would be useful for large-scale production of enzymes for biomass conversion [81], as well as development of self-processing biomass. In either case, yields can suffer if the transgenic protein stunts plant growth. This can be avoided if the expressed protein is inactive at ambient temperatures, which is true for hyperthermophilic proteins. The endoglucanase Cel5A (TM1751) from *T. maritima* has been expressed in the chloroplasts of transgenic tobacco at levels up to 5.2 % of total soluble protein. The transgenic plants exhibited normal growth and development, which may be attributed to a combination of the thermophilic nature of the protein and the chloroplast localization [82]. *In planta* protein production could also benefit industrial starch liquefaction, where thermostable α -amylase is used for initial solubilization. Calcium is added to these processes, as it is a requisite cofactor for α -amylase function. The α -amylase (TM1840) from *T. maritima* has been expressed in tobacco cell cultures with a significant increase in thermostability compared to the *E. coli*-produced version, due to the intrinsic calcium levels in the tobacco cells [83]. Below 40°C, no activity was detected for this enzyme. Thus, self-processing plants can be envisioned. Such plants could grow normally while expressing thermophilic enzymes. After harvest, the plants would be heat treated to activate the enzymes, which would initiate degradation of the plant polysaccharides from within.

5. Conclusion

The genus *Thermotoga* will likely continue to be of interest to scientists and technologists because of the intriguing insights that can be obtained into life at extremely high temperatures, fundamentals of biomolecular function in thermal environments, and biotechnological applications, especially related to bioenergy. Still missing to complete the utility of these bacteria as model systems are versatile genetic systems. Such systems would allow for the testing of specific hypothesis related to physiological and ecological features and ultimately

lead to metabolic engineering of *Thermotoga* species to customize their function for certain applications.

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Figure 1.

Unrooted 16S rRNA tree of *Thermotoga* species created using the Mobyle portal (<http://mobyle.pasteur.fr/>).

Figure 2.
Central metabolism of *T. maritima* indicating open reading frames encoding enzymes involved in catabolic pathways.

Figure 3. Transcriptional analysis biofilm formation in *T. maritima* [53]; (A) biofilm cell samples were collected from a high-temperature continuous anaerobic bioreactor where the encrusted region shows biofilm formed on nylon mesh and reactor walls, (B) volcano plot showing differential gene expression in planktonic and biofilm *T. maritima* cells grown in chemostat culture at 80 °C (horizontal lines indicates Bonferroni correction).

Figure 4.

Overview of the proposed peptide-based (TM0504) signaling mechanism in *T. maritima* [58, 88]. (A) Epifluorescence images of *T. maritima* mono-culture (left) and *T. maritima* and *M. jannaschii* co-culture (right), where *T. maritima* displays rod morphology while *M. jannaschii* is the cocci bound up in the middle of the aggregate; (B) TM0504 putative signalling peptide with its predicted cleavage site after the GG motif; (C) schematic of the *T. maritima* cells in the co-culture summarizing the predicted export pathway for TM0504; (D) Acridine orange (top) and Calcofluor (bottom) stained cells of *T. maritima* 30 min after dosing with either buffer PBS (right), or synthetic TM0504 peptide (left).

Table 1

Thermotoga species.

Species	T _{opt} (°C)	Genome size (bp)	Isolation site	Country	Reference
<i>T. lettingae</i> TMO	65	2,135,243	sulfate-reducing bioreactor	Netherlands	[11]
<i>T. elfii</i> :SEBR 6459	66	N/A	oil field	Sudan	[84]
<i>T. hypogea</i> SEBR 7054	70	N/A	oil-producing well	Cameroon	[85]
<i>T. subterranea</i> SL1	70	N/A	continental oil reservoir	France	[86]
<i>T. thermarum</i> LA3	70	N/A	continental solfataric springs	Djibouti	[87]
<i>T. neapolitana</i> NS-E	77	1,884,562	shallow submarine hot springs	Italy	[3]
<i>T. petrophila</i> RKU-1	80	1,823,511	oil reservoir	Japan	[10]
<i>T. naphthophila</i> RKU-10	80	1,809,823	oil reservoir	Japan	[10]
<i>T. maritima</i> MSB8	80	1,860,725	geothermally-heated sea floors	Italy	[1]
<i>T. sp.</i> strain RQ2	76–82	1,877,693	geothermally-heated sea floors	Azores	[1]