Extremophiles (2016) 20:351–361
DOI 10.1007/s00792-016-0826-x

ORIGINAL PAPER

Analysis of the complete genome sequence of the archaeon *Pyrococcus chitonophagus* DSM 10152 (formerly *Thermococcus chitonophagus*)

Konstantinos Papadimitriou[2] · Panagiotis K. Baharidis[2] · Anastasios Georgoulis[1] · Marion Engel[4,5] · Maria Louka[1] · Georgia Karamolegkou[1] · Aggeliki Tsoka[1] · Jochen Blom[7] · Bruno Pot[8] · Piotr Malecki[6] · Wojciech Rypniewski[6] · Harald Huber[3] · Michael Schloter[4] · Constantinos Vorgias[1]

Received: 21 January 2016 / Accepted: 15 March 2016 / Published online: 25 March 2016

Abstract Here we analyze the first complete genome sequence of *Pyrococcus chitonophagus*. The archaeon was previously suggested to belong to the *Thermococcus* rather than the *Pyrococcus* genus. Whole genome phylogeny as well as whole proteome comparisons using all available complete genomes in *Thermococcales* clearly showed that the species belongs to the *Pyrococcus* genus. *P. chitonophagus* was originally isolated from a hydrothermal vent site and it has been described to effectively degrade chitin debris, and therefore is considered to play a major role in the sea water ecology and metabolic activity of microbial consortia within hot sea water ecosystems. Indeed, an obvious feature of the *P. chitonophagus* genome is that it carries proteins showing complementary activities for chitin degradation, i.e. endo- and exo-chitinase, diacetylchitobiose deacetylase and exo-β-D glucosaminidase activities. This finding supports the hypothesis that compared to other *Thermococcales* species *P. chitonophagus* is adapted to chitin degradation.

Keywords Genome analysis · *Pyrococcus chitonophagus* · Chitinolytic activity · Hyperthermophilic archeon

Communicated by H. Atomi.

Electronic supplementary material The online version of this article (doi:10.1007/s00792-016-0826-x) contains supplementary material, which is available to authorized users.

✉ Constantinos Vorgias
cvorgias@biol.uoa.gr

[1] Department of Biochemistry and Molecular Biology, National and Kapodistrian University of Athens, Panepistimiopolis Zografou, 15701 Athens, Greece

[2] Department of Food Science and Human Nutrition, Agricultural University of Athens, Iera Odos 75, 11855 Athens, Greece

[3] Lehrstuhl für Mikrobiologie und Archäenzentrum Universität Regensburg, Universitätsstrasse 31, 93053 Regensburg, Germany

[4] Research Unit for Environmental Genomics, Helmholtz Zentrum München, Deutsches Forschungszentrum für Gesundheit und Umwelt (GmbH), Ingolstädter Landstr. 1, 85764 Neuherberg, Germany

[5] Research Unit for Scientific Computing, Helmholtz Zentrum München, Deutsches Forschungszentrum für Gesundheit und Umwelt (GmbH), Ingolstädter Landstr. 1, 85764 Neuherberg, Germany

[6] Institute of Bioorganic Chemistry, Polish Academy of Sciences, Noskowskiego 12/14, Poznan 61-704, Poland

[7] Bioinformatics and Systems Biology, Justus Liebig University, Giessen, Germany

[8] Institut Pasteur de Lille, U1019, UMR 8204, CIIL, Centre d'Infection et d'Immunité de Lille, Univ. Lille, CNRS, Inserm, CHU Lille, 59000 Lille, France

Abbreviations

ORBs	Origin recognition boxes
HGT	Horizontal gene transfer
CRISPRs	Clustered regularly interspaced short palindromic repeats
Cas	CRISPR-associated sequences
GIs	Genomic islands
$GlcNAc_2$	*N,N*'-Diacetylchitobiose
GlcNAc	*N*-Acetylglucosamine
Dac	Diacetyl-chitobiose diacetylase
GlmA	Exo-β-D-glucosaminidase
GlcN-GlcNAc	*N*-Acetylchitobiose
Glk	D-Glucosamine kinase
Pfk	Phosphofructokinase
GAP	D-Glyceraldehyde 3-phosphate
Fba	Fructose-biphosphate aldolase
TpiA	Triose-phosphate isomerase
GAPOR	Glyceraldehyde-3-phosphate-ferredoxin oxidoreducatse
Pps	Phosphoenolpyruvate synthase
Pyk	Pyruvate kinase
POR	Pyruvate:ferredoxin oxidoreductase
ACS	Acetyl-CoA synthetase
MBH	Membrane-bound hydrogenase
Nsr	NADPH S^0 reductase

Introduction

Thermococcales have attracted consistent attention from researchers because of their evolutionary significance as well as their biotechnological potential, linked to the production of thermostable enzymes (Atomi 2005). *Thermococcales* are strictly anaerobic and hyperthermophilic archaea belonging to the Euryarchaeota phylum. In this order, three genera are distinguished: *Pyrococcus* (Fiala and Stetter 1986), *Thermococcus* (Zillig et al. 1983) and *Palaeococcus* (Takai et al. 2000). Most of these hyperthermophilic archaea are organoheterotrophs that utilize proteins, starch and maltose with elemental sulfur (S^0) or protons as electron acceptors (Sapra et al. 2003).

The members of *Pyrococcus*, with higher optimum growth temperature (95–103 °C) than *Thermococcus* (75–93 °C) are ubiquitously present in natural high-temperature environments, and are therefore considered to play a major role in the ecology and metabolic activity of microbial consortia within hot-water ecosystems. Complete genome sequences are available for six *Pyrococcus* species (Cohen et al. 2003; Jun et al. 2011; Jung et al. 2012; Kawarabayasi et al. 1998; Lee et al. 2011; Robb et al. 2001).

Pyrococcus chitonophagus is a hyperthermophilic anaerobic archaeon, isolated from a deep sea hydrothermal vent site off the Mexican west coast at a depth of 2600 m (Huber et al. 1995). In its original description, *P. chitonophagus* was shown to grow chemoorganoheterotrophically by fermenting chitin as a sole carbon source or peptide mixtures like yeast extract, meat extract, etc. (Huber et al. 1995). It can also perform S^0 respiration producing H_2S. *P. chitonophagus* seems to be unable to utilize simple sugars like glucose, *N*-acetylglucosamine, maltose or to degrade complex polysaccharides like pectin, glycogen, agar, chitosan, cellulose some of which can be catabolized by other pyrococci and thermococci. *P. chitonophagus* was misclassified at the genus level and it has now been suggested to belong to the *Pyrococcus* rather than the *Thermococcus* genus (Lepage et al. 2004). Here we describe the analysis of the first complete genome sequence of *P. chitonophagus*, as well as a comparison with all available complete genome sequences in *Thermococcales*.

Materials and methods

Sequencing and assembly

The library of *P. chitonophagus* was prepared using Nextera DNA Sample Preparation Kit (Illumina) following the manufacturer's user guide. The initial concentration of gDNA was measured using the Qubit® dsDNA HS Assay Kit (Life Technologies). The sample was then diluted accordingly to achieve the recommended DNA input of 50 ng at a concentration of 2.5 ng/μL. Subsequently, the sample underwent the simultaneous fragmentation and addition of adapter sequences. These adapters are utilized during a limited-cycle (5 cycles) PCR in which unique index was added to the sample. Following the library preparation, the final concentration of the library was measured (6.52 ng/μL) using the Qubit® dsDNA HS Assay Kit (Life Technologies), and the average library size (1020 bp) was determined using the Agilent 2100 Bioanalyzer (Agilent Technologies). The library (12.5 pM) was sequenced using 600 Cycles v3 Reagent Kit (Illumina) in MiSeq (Illumina). Genome was assembled using NGEN (DNAstar) by Molecular Research LP (MR DNA Shallowater, TX USA).

Annotation and comparative analysis of the *P. chitonophagus* genome

The genome sequence of *P. chitonophagus* was annotated using the RAST server (Overbeek et al. 2014). Full chromosome alignments were performed using Kodon (Applied Maths) and ProgressiveMauve (Darling et al. 2010). Core genome and singletons determination as well as whole genome phylogeny were performed with the EDGAR platform (Blom et al. 2009).

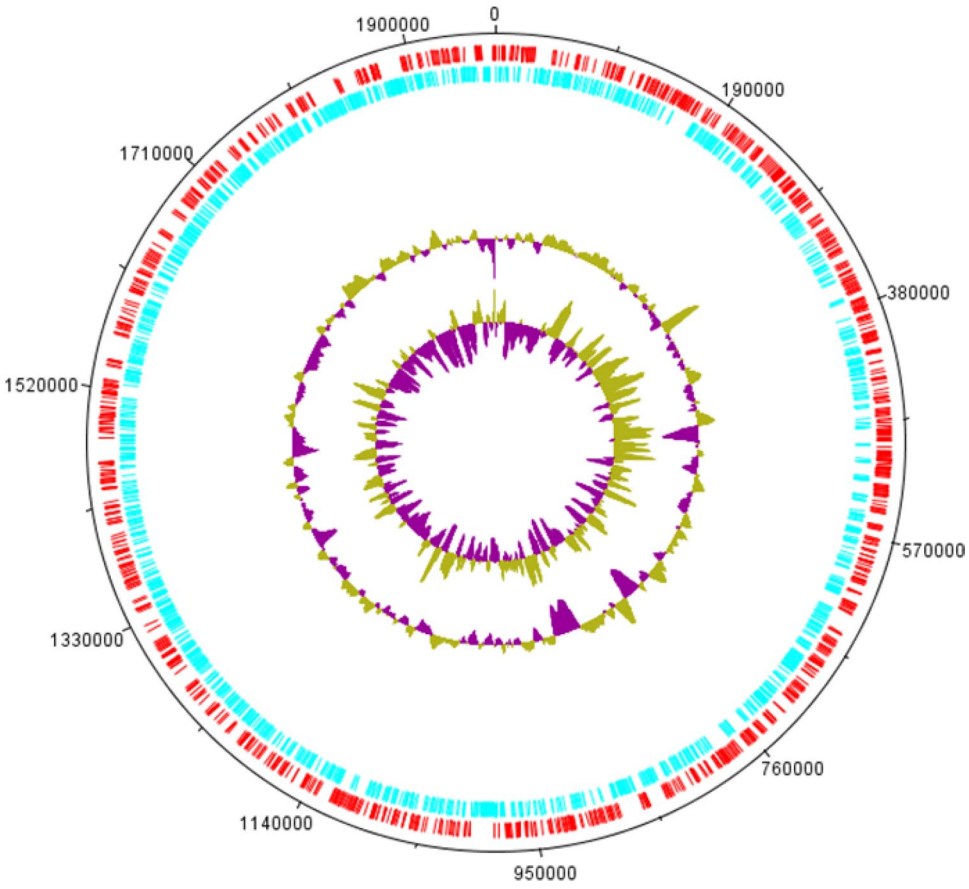

Fig. 1 Genome map of *P. chitonophagus*. Tracks from the periphery of the map towards the center: CDSs in the forward strand (*red*), CDSs in the reverse strand (*blue*), GC plot, GC skew

Additional analysis

The map of the *P. chitonophagus* genome was constructed using DNAPlotter software (Carver et al. 2009). The OriC of *P. chitonophagus* was predicted using the Ori-Finder 2 tool (Luo et al. 2014). Clustered regularly interspaced short palindromic repeats (CRISPRs) were analyzed within the CRISPRcompar web-service (Grissa et al. 2008). Whenever necessary, the BLAST suite was used for sequence similarity searches (Altschul et al. 1990). Functional annotation was performed using the eggNOG database (Powell et al. 2012). Metabolic networks of the archaeon were automatically constructed using the KAAS (Moriya et al. 2007). The pathways presented in this study were also manually curated. PanFunPro was employed for pan-genome analysis (Lukjancenko et al. 2013).

Results and discussion

General properties of the *P. chitonophagus* genome

The map of the *P. chitonophagus* genome is shown in Fig. 1. The genome is a circular molecule of 1,969,648 bp with a GC content of 44.92 %. The archaeon contains 2,153 protein coding genes covering more than 90 % of the genome sequence. The genome also carries 48 tRNA and 4 rRNA genes (2 copies of 5S rRNA, 1 copy of 16S rRNA and 1 copy of 23S rRNA genes). A single OriC was identified using the Ori-Finder 2 tool at regions spanning positions 247,894 and 248,700, right upstream of the *cdc6* and DNA pol II genes. Manual investigation indicated the presence of two origin recognition boxes (ORBs) inverted repeats showing high similarity to ORBs described previously for *Pyrococcus abyssi* (Matsunaga et al. 2003) (Supplementary Fig. 1).

Comparative and evolutionary genomics

Thermococcus chitonophagus was suggested to belong to the *Pyrococcus* rather than the *Thermococcus* genus (Lepage et al. 2004). To further investigate this matter we performed full chromosome alignments using all available complete genome sequences in *Thermococcales.* The genome did not align with *Thermococcus* spp. (Supplementary Fig. 2), but it showed extensive regions of alignment solely with some of the *Pyrococcus* genomes, especially with *Pyrococcus* sp. ST04 (Fig. 2a). The relatedness of *P. chitonophagus* to other *Pyrococcus* strains was also supported based on whole proteome comparisons generated by

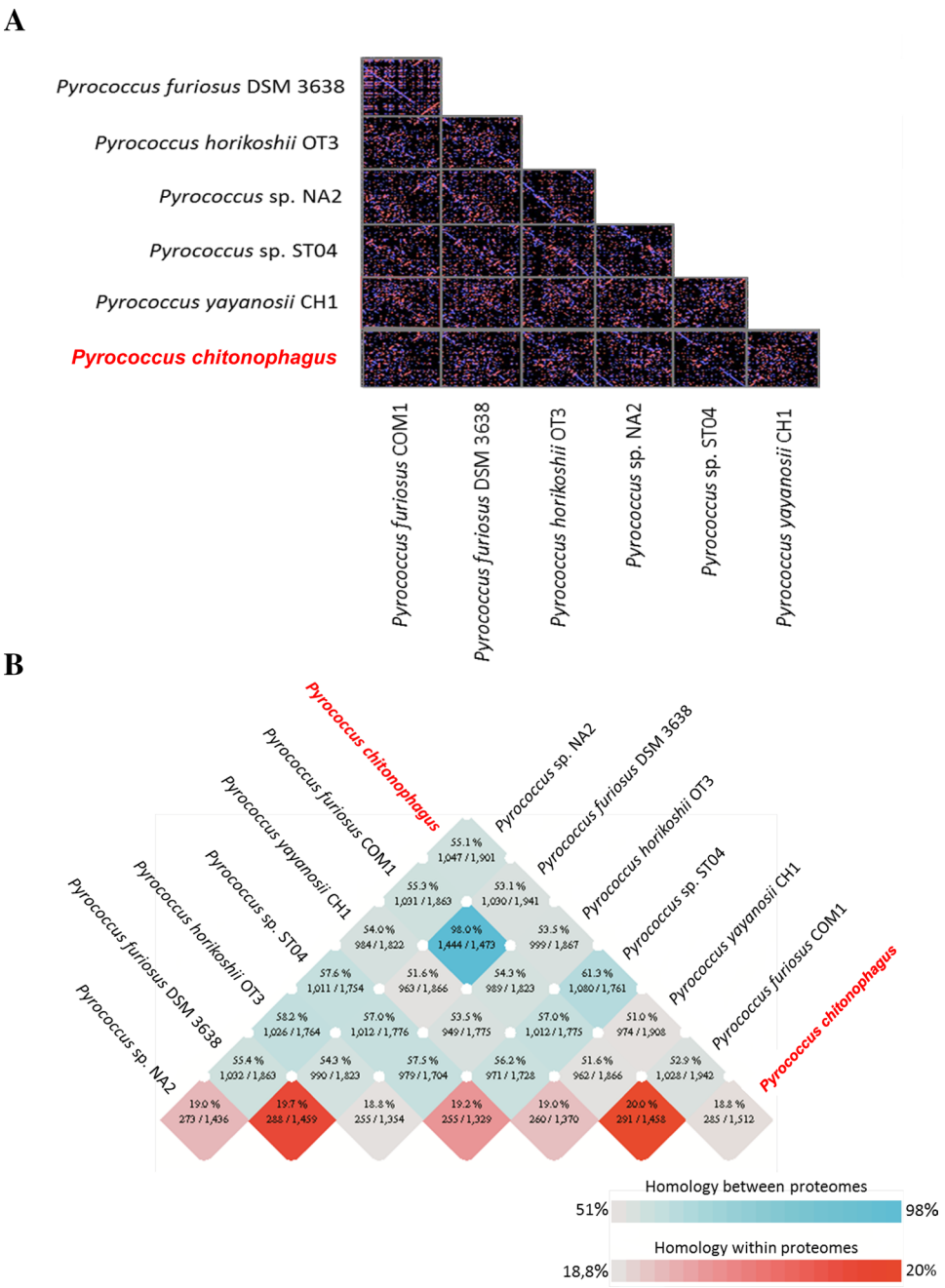

Fig. 2 Full chromosome alignments of *P. chitonophagus* against other *Pyrococcus* genomes with complete genome sequences using the Kodon tool (**a**). The comparative chromosome mapping in Kodon allows alignment of homologous regions between pairs of chromosomes. The mapping of each pair of chromosomes is presented as a dot plot based on the DNA sequence homology. *Blue* stretches represent homology between both sequences in the direct orientation, whereas *red* stretches represent homology with one sequence inverted. Comparison of similarity at the proteome level among the aforementioned strains (**b**). The *triangle shaped matrix* represents pairwise comparisons between or within genomes. *Each blue box* shows the number of shared protein families in the two different genomes under comparison as a percentage and as an absolute number. The number of total protein families found in the two genomes is also indicated. *Red boxes* correspond to pairwise genome comparisons of strains against themselves and the numbers represent protein families with more than one members

the PanFunPro (Fig. 2b). Whole genome phylogeny using all available complete genomes in *Thermococcales* showed that indeed *P. chitonophagus* belongs to the *Pyrococcus* genus (Fig. 3). Interestingly though, thermococci were split in two branches one of which also included the *Palaeococcus pacificus* DY20341. Similar results were also obtained by 16S rDNA phylogenetic analysis suggesting that reassessment of the current taxonomy within *Thermococcales* may be necessary (data not shown).

Comparative analysis of *P. chitonophagus* to its closest relative available today, i.e. *Pyrococcus* sp. ST04 indicated an intermediate degree of conservation that varied along the two chromosomes (Fig. 4a). According to the orientation of the two genomes as deposited in the databases, the highest degree of conservation was evident at the two extremities of the genomes while the central part was less conserved. The two archaea share around 72 % of *P. chitonophagus* genes, i.e. 1558 genes (Fig. 4b). Comparison of all complete *Pyrococcus* genome sequences revealed a core genome of 1072 genes (Supplementary Table 1), while the singletons of *P. chitonophagus* accounted to 282 genes (Supplementary Table 2). The *Pyrococcus* core genome

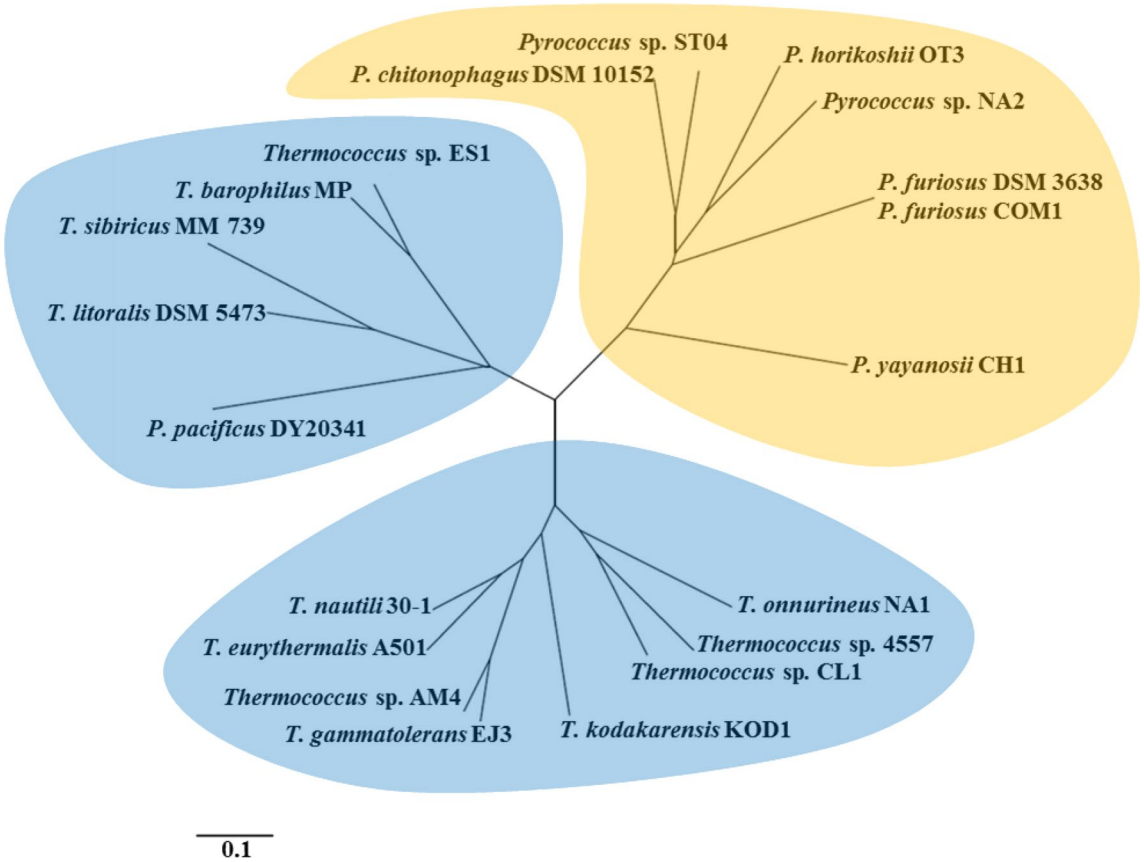

Fig. 3 Whole genome phylogeny of the *Thermococcales* spp. with complete available genome sequences. The whole genome phylogenetic tree is constructed based on the concatenated alignment of the core genes of the genomes under investigation (Blom et al. 2009). The three main clades were pseudocolored manually

consisted of genes involved in central processes (Table 1). The majority of the singletons of *P. chitonophagus* were mostly of unknown function, however, some of them could be identified as glycosyltransferases, type I restriction modification system proteins, OppAB oligopeptide ABC transporter components, DNA repair proteins (single-stranded exonuclease associated with Rad50/Mre11 complex, HerA, Mre11), etc.

CRISPRs and horizontal gene transfer (HGT)

Sequence analysis of the *P. chitonophagus* genome revealed the presence of 8 CRISPRs. Such a high number of CRISPRs has been previously predicted in at least some other pyrococci (Portillo and Gonzalez 2009). The largest CRISPR contained 29 spacers, while all the rest contained 18 down to 5 spacers in a total of 105 spacers. The distribution of the CRISPR systems was scattered around the genome (Supplementary Fig. 3). Arrays with more than one Cas (CRISPR-associated sequences) proteins were found only next to CRISPR2 [Cas1 (Chiton_0201), Cas2 (Chiton_0202), Cas4a (Chiton_0203), Cas3 (Chiton_0204)] and CRISPR5 [Cas2 (Chiton_1030), Cas1 (Chiton_1031), Cas4a (Chiton_1032), Cas3 (Chiton_1033), Cas5t (Chiton_1034)].

IslandViewer identified eight putative genomic islands (GIs). In four of them we could not identify the potential donor (GIs 1, 2, 6 and 7) based on nucleotide sequence similarity searches. In the remaining half potential donors were *Thermococcus gammatolerans* EJ3 (GI 3) and *Thermococcus barophilus* (GIs 4 and 5) and *Pyrococcus* sp. ST04 (GI 8) for at least part of the GIs. Analysis of the proteins in these GIs did not reveal any significant biological trait. Most of them were hypothetical. We could also identify some glycosyltransferases, a number of transporters and some glycolytic enzymes.

Chitin degradation

The preference of *P. chitonophagus* for chitin is a clear indication of the adaptation of this archaeon to the hydrothermal vent sites for recycling this polysaccharide. An

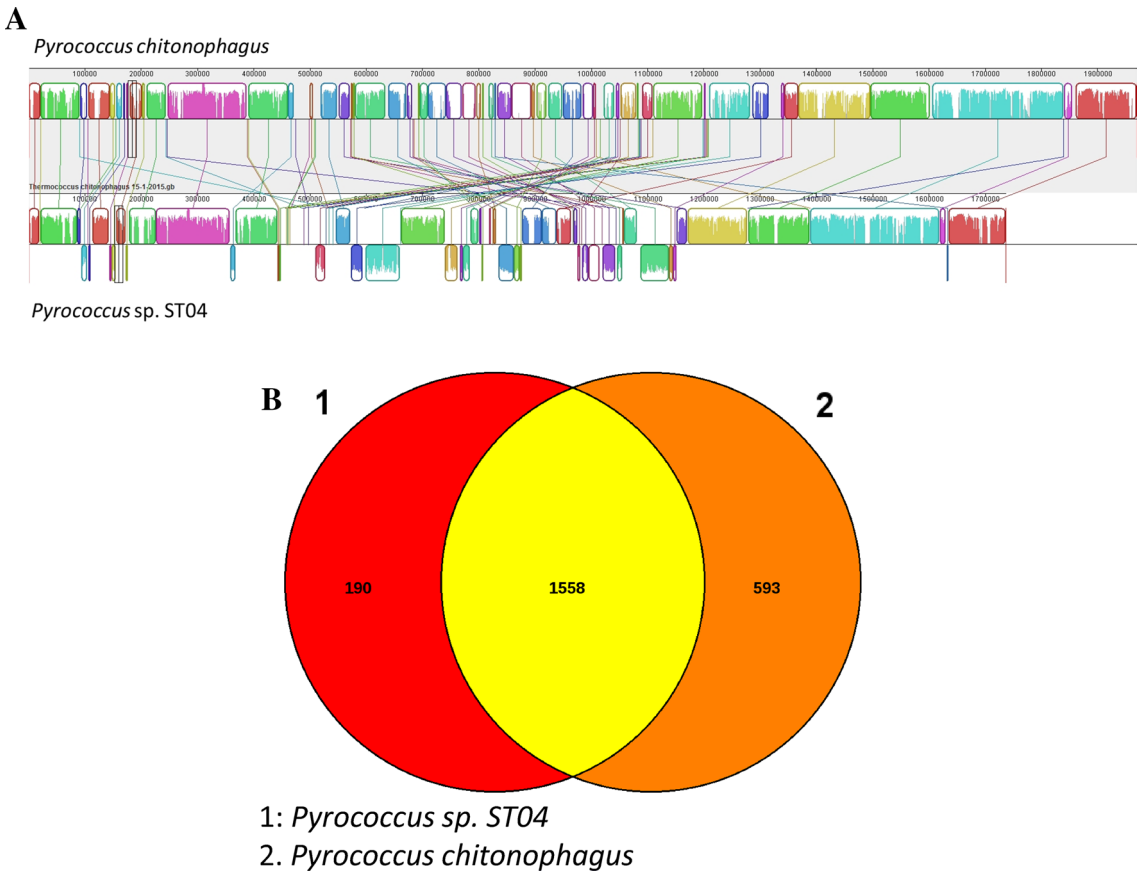

Fig. 4 Full chromosome alignments of *P. chitonophagus* DSM 10152 against *Pyrococcus* sp. ST04 using ProgressiveMauve (**a**). *Colored blocks* interconnected with *lines* represent stretches of homology between the two chromosomes. Venn diagram analysis for common genes and singletons of the two genomes mentioned above (**b**)

archaeal specific pathway for chitin catabolism has also been proposed for *Thermococcus kodakarensis* (Tanaka et al. 2004). Previous studies with *P. chitonophagus* suggest a chitinolytic chitonoclastic process (Huber et al. 1995). Experimental evidence suggested the presence of a number of chitinolytic enzymes in *P. chitonophagus* including three chitinases. Chi70 a membrane bound endochitinase was the first to be studied (Andronopoulou and Vorgias 2003), followed by Chi90 a potential periplasmic chitobiase, and Chi50 an extracellular not bound exochitinase (Andronopoulou and Vorgias 2004). The synergistic function of these three enzymes seems to be sufficient for complete chitin degradation down to *N,N'*-diacetylchitobiose ($GlcNAc_2$) and *N*-acetylglucosamine (GlcNAc). The chitin degrading system was induced by chitin but it was repressed by chitin monomers indicating a negative feedback regulation (Andronopoulou and Vorgias 2004).

In silico analysis of the *P. chitonophagus* genome (as shown in Fig. 5) revealed the presence of three putative chitinase encoding genes i.e. Chiton_1119, Chiton_1716 and Chiton_1717. The N-terminal of Chiton_1119 contains a cellulose-binding domain distantly related to the ChiA chitinase of *T. kodakarensis,* while the C-terminal was found to be related to clostridial chitinases (data not shown). Chiton_1716 and Chiton_1717 are practically identical to the ChiA of *T. kodakarensis.* Chiton_1716 is a truncated protein (593 out of 1289 amino acids) compared to ChiA probably deriving from a duplication of the original gene. As previously described for ChiA, Chiton_1717 has a dual catalytic domain resulting in exo- and endo-chitinase activities in the N- and the C-terminal of the enzyme, respectively (Tanaka et al. 1999). The two domains may work synergistically liberating *N*-acetyl-chitooligosaccharides (C-terminal) down to $GlcNAc_2$ (Tanaka et al. 2001). If Chiton_1119 and Chiton_1716 are functional chitinases, this is a clear indication of an advanced chilinolytic arsenal in *P. chitonophagus* compared to other *Thermococcales* species. Downstream of the first step of chitin degradation, two important archaeal enzymes act to complete the process and allow the entrance of products into glycolysis. Orthologues of the diacetyl-chitobiose diacetylase (Dac) and

Table 1 Functional characterization of the core genome of pyrococci and the singletons of *P. chitonophagus* DSM 10152

Description of orthologous group	Number of genes in the *Pyrococcus* core genome	Number of genes in the *Pyrococcus chitonophagus* singleton
Amino acid transport and metabolism	70	4
Carbohydrate transport and metabolism	53	14
Cell cycle control, cell division, chromosome partitioning	19	
Cell motility	14	2
Cell wall/membrane/envelop biogenesis	26	
Chromatin structure and dynamics, secondary metabolites biosynthesis, transport, and catabolism	1	13
Coenzyme transport and metabolism	64	5
Defense mechanisms	14	35
Energy production and conversion	79	6
Function unknown	259	140
Inorganic ion transport and metabolism	46	7
Intracellular trafficking, secretion, and vesicular transport	10	1
Lipid metabolism, cell wall/membrane/envelop biogenesis	5	1
Lipid transport and metabolism	18	1
Nucleotide transport and metabolism	41	2
Post-translational modification, protein turnover, and chaperones	39	7
Replication, recombination and repair	62	15
Secondary structure	12	1
Signal transduction mechanisms	13	3
Transcription	62	24
Translation, ribosomal structure and biogenesis	155	

exo-β-D-glucosaminidase (GlmA) originally identified in *T. kodakarensis* (Tanaka et al. 2004) are also present in the genome of *P. chitonophagus*, that is Chiton_0574 and Chiton_1401, respectively. Dac activity involves the deacetylation of $GlcNAc_2$ from the non-reducing end producing *N*-acetylchitobiose (GlcN-GlcNAc). GlmA hydrolyses GlcN-GlcNAc into GlcNAc and GlcN, while Dac can further deacetylate the GlcNAc monomer. Subsequently, GlcN is phosphorylated by the ADP-dependent D-glucosamine kinase (Glk, Chiton_1323) (Koga et al. 2000; Tanaka et al. 2005).

Central glycolysis

The D-glucosamine 6-phosphate product is further de-aminated by an archaeal glucosamine 6-phosphate deaminase enzyme (Chiton_1402), resulting in β-D-fructofuranose 6-phosphate, which can enter glycolysis through the modified Embden-Meyerhof pathway (Tanaka et al. 2005). β-D-Fructofuranose 6-phosphate is phosphorylated to fructose 1,6-bis-phosphate through the action of the ADP-dependent phosphofructokinase (Pfk, Chiton_1878). The preference for ADP over ATP by Pfk and Glk may reflect the higher thermostability of ADP under high temperatures prevailing in the niches of hyperthermophiles or it may provide a growth advantage after periods of starvation when ATP is relatively depleted compared to ADP (Kengen et al. 1996). The pathway proceeds normally to the production of glycerone-phosphate and D-glyceraldehyde 3-phosphate (GAP) through the action of the archaeal type class I fructose-biphosphate aldolase (Fba, Chiton_0237) (Siebers et al. 2001). The triosephoshates are interconverted by a triose-phosphate isomerase (TpiA, Chiton_0024). GAP is oxidized by a tungsten-containing Fe–S enzyme glyceraldehyde-3-phosphate-ferredoxin oxidoreducatse (GAPOR, Chiton_1291) through the reduction of ferredoxin (Mukund and Adams 1995). According to this reaction the formation of 1,3-bisphosphoglycerate does not take place and GAP is directly converted into 3-phospho-D-glycerate. The transformation of PEP to pyruvate proceeds through the action

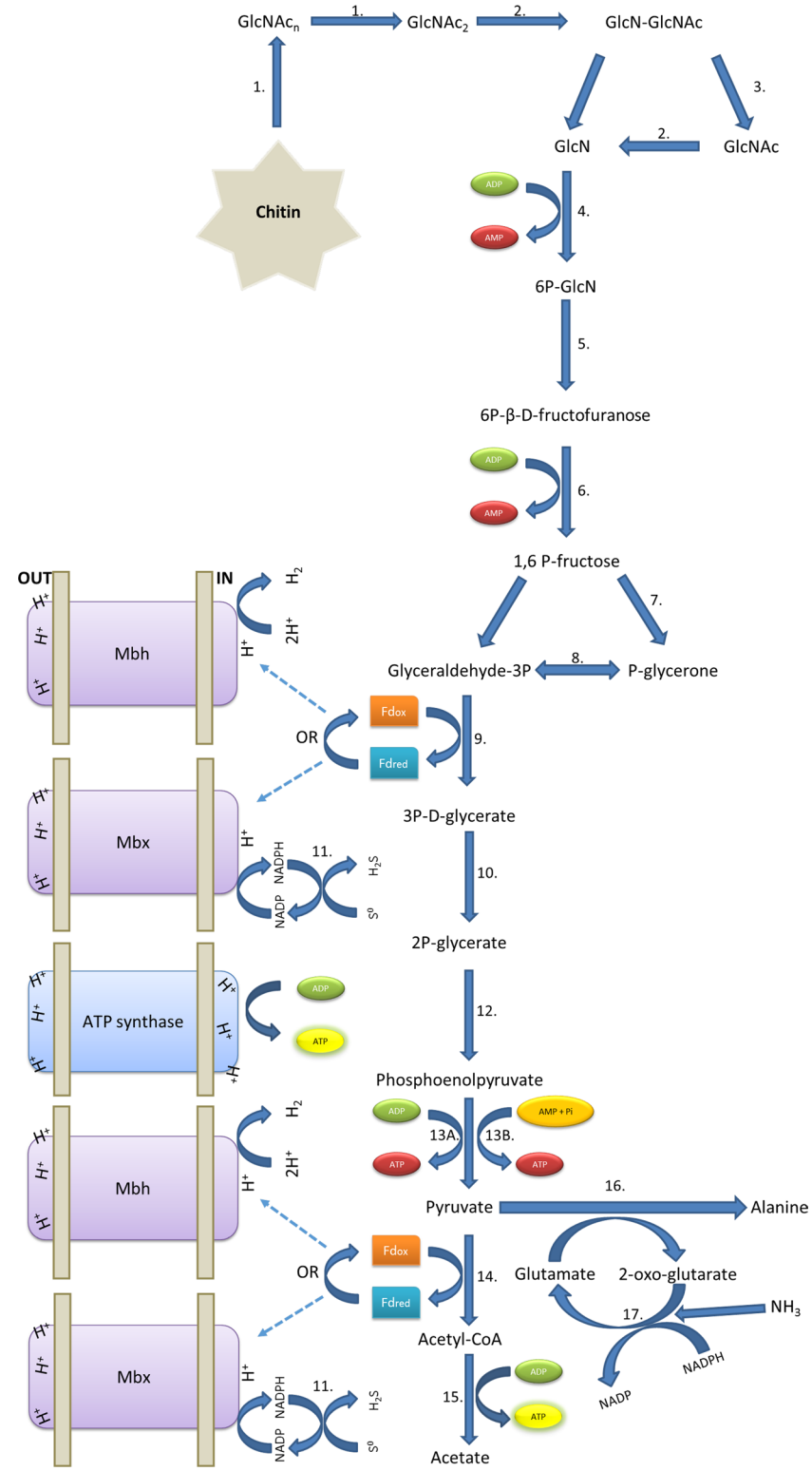

Fig. 5 Pathways for chitin degradation and central energy production of *P. chitonophagus*. Enzymatic activities are presented in the following order: chitinase (*1*), diacetylchitobiose diacetylase (*2*), exo-β-D-glucosaminidase (*3*), ADP-dependent D-glucosamine kinase (*4*), glucosamine 6-phosphate deaminase (*5*), ADP-dependent phosphofructokinase (*6*) fructose-biphosphate aldolase (*7*), triose-phosphate isomerase (*8*), glyceraldehyde-3-phosphate-ferredoxin oxidoreductase (*9*), phosphoglycerate mutase (*10*), CoASH-dependent NADPH S^0 reductase (*11*), enolase (*12*), pyruvate kinase (*13A*), phosphoenolpyruvate synthase (*13B*), pyruvate:ferredoxin oxidoreductase (*14*), acetyl-CoA synthetase (*15*), glutamate:pyruvate transaminase (*16*), NADP-dependent glutamate dehydrogenase (*17*)

of phosphoenolpyruvate synthase (Pps, Chiton_0228) and/or pyruvate kinase (Pyk, Chiton_0622). It has been previously suggested that these two enzymes act together to sustain energy production under different physiological states of *T. kodakarensis* cells (Imanaka et al. 2006).

The fate of pyruvate

Pyruvate can be oxidized to acetate or aminated to alanine. In the first case, ATP is produced while in the second $NADP^+$ is regenerated. Pyruvate is decarboxylated by the

pyruvate:ferredoxin oxidoreductase (POR, Chiton_0670, Chiton_0671, Chiton_0665, Chiton_0669) and oxidized to acetyl-CoA (Blamey and Adams 1993; Schäfer and Schönheit 1991). In turn ADP-forming acetyl-CoA synthetase (ACS) undertakes the unusual conversion of acetyl-CoA to acetate and ATP (Schäfer and Schönheit 1991). We found a number of potential ACS subunits scattered in the genome of *P. chitonophagus* including Chiton_1063, Chiton_1237, Chiton_1746 and Chiton_2077 (potential α subunits) and Chiton_2025 and Chiton_2076 (potential β subunits). The exact proteins forming the ACS enzyme in *P. chitonophagus* need experimental validation. Alternatively, alanine can be produced by the action of a glutamate:pyruvate transaminase (Chiton_1593) and glutamate must be continuously provided by the action of a NADP-dependent glutamate dehydrogenase (Chiton_1829). In *Pyrococcus furiosus* this pathway is active in the absence of S^0 but if S^0 is available almost no alanine is produced (Kengen et al. 1996).

The ferredoxin respiratory system

As mentioned above, the net yield of the modified Embden–Meyerhof pathway was thought to be zero. However, a study by Sapra et al. (2003) demonstrated a simple respiratory system in *P. furiosus* involving ferredoxin and a membrane-bound hydrogenase (MBH) complex (Sapra et al. 2003). In this anaerobic respiration scheme, the H^+ from ferredoxin are directly reduced to H_2 or exported by the MBH complex. The exported H^+ (or Na^+) generates a gradient that when used by an A_1A_0-ATPase can further generate ATP. The oxidation of ferredoxin coupled with the reduction of H_2 can take place either during the oxidation of GAP by GAPOR or during the oxidation of pyruvate to acetyl-CoA by POR (Sapra et al. 2003). These findings support the use of ferredoxin in the place of NAD as an electron acceptor in *P. furiosus*. The MBH complex is present in *P. chitonophagus* as well (Chiton_1642–Chiton_1654). For other cytosolic functions, reducing equivalents of NADPH may be generated by [NiFe] cytosolic hydrogenases SHI (Chiton_1017–Chiton_1020) and SHII (Chiton_0947–Chiton_0950) as previously suggested for *T. kodakarensis* (Kanai et al. 2011; Santangelo et al. 2011), but these pathways need further investigation (Schut et al. 2013).

P. chitonophagus also owns an MBX complex (Chiton_1625–Chiton_1637). In *P. furiosus* the MBX complex has FeS binding motifs similar to MBH, indicating that it can act as electron acceptor from ferredoxin and generate ion gradients like MBH. MBX has been shown to play an important role in the reduction of S^0 (Schut et al. 2007). Addition of S^0 in *P. furiosus* led to the up-regulation of a number of genes including those of the MBX complex, a CoASH-dependent NADPH S^0 reductase (Nsr) and others. It has been proposed that MBX oxidizes ferredoxin and reduces NADP to NADPH, allowing Nsr (Chiton_0620) to generate H_2S. However, deletion of Nsr did not show obvious growth defects on S^0 and only deletion of MBXL (the catalytic subunit of the MBX complex) significantly influenced the growth of the archaeon (Bridger et al. 2011). In general, it is believed that both MBH and MBX play a role in re-oxidizing ferredoxin produced during the modified Embden–Meyerhof pathway irrespective of the presence of S^0.

It has been shown in *P. furiosus* that SurR (Chiton_0314) regulates responses to the availability of S^0 (Schut et al. 2013). In a reduced state and in the absence of S^0, SurR upregulates the transcription of genes coding for hydrogenases SHI and SHII, MBH and other H_2 metabolism related proteins. Simultaneously reduced SurR inhibits the expression of proteins involved in the reduction of S^0 like MBX, Nsr, etc. Addition of S^0 to the system alters the situation. Reduced SurR is oxidized at its CXXC motif and alters its behavior by abolishing expression of the genes involved in H_2 metabolism mentioned above, but it now allows expression of the genes involved in S^0 metabolism (Lipscomb et al. 2009; Yang et al. 2010).

Conclusions

Chitin, designated “animal cellulose” in the early literature, is the major component of the exoskeleton of insects and crustacea and this explains its wide distribution and great natural abundance. Only copepodes produce billions of tons of the polysaccharide annually, resulting in a continuous rain of chitin on the ocean floor (Gooday 1996; Muzzarelli 1977). It is estimated that, in the aquatic biosphere alone, more than 10^{11} metric tons of chitin are produced annually. If this enormous quantity of insoluble source of carbon and nitrogen was not converted to biologically useful material, the oceans would be depleted of these elements, in a few decades. Despite the continuous deposition of this highly insoluble polymer, marine sediments contain, in fact, only traces of chitin and the turnover of the polysaccharide is attributed primarily to marine bacteria, which are very abundant, widely distributed, can live under extreme conditions and are responsible for the bulk of the chitin recycling process (Gooday 1996; Keyhani and Roseman 1999; Muzzarelli 1977). We have decided to study the *P. chitonophagus* genome because it was reported that the species is an efficient chitinolytic archeon (Huber et al. 1995). Studies on archaeal or hyperthermophilic chitinases have been rather limited. Chitinases have been determined and studied in *T. kodakarensis* KOD1 (Tanaka et al. 1999, 2001), *P. furiosus* (Gao et al. 2003; Nakamura et al. 2005; Oku and Ishikawa 2006) and *P. chitonophagus* (Andronopoulou

and Vorgias 2003). In a previous work, we have presented data on the multiple components of the chitinolytic system, as well as, the inducing and repressing mechanisms regulating the production of chitinolytic enzymes of this archaeon (Andronopoulou and Vorgias 2004). The currently presented genome data clearly demonstrate that *P. chitonophagus* contains an exceptional chitinolytic system, similar to *T. kodakarensis* (Tanaka et al. 1999, 2001, 2004). The extensive, multi-component chitinolytic system of *P. chitonophagus* provides an interesting model to elucidate the biodegradation of chitin in environments of high temperatures. Modification of carbohydrates at elevated temperatures is of particular biotechnological interest. The existence of the complete genome of *P. chitonophagus* will certainly allow better functional characterization of its chitinolytic arsenal.

Data deposition

The genome sequence of *P. chitonophagus* DSM 10152 has been deposited in the DDBJ/EMBL/GenBank databases under the project accession number PRJEB12305.

Acknowledgments The project was funded by the Foundation for Polish Science.

References

Kawarabayasi Y et al (1998) Complete sequence and gene organization of the genome of a hyper-thermophilic archaebacterium, *Pyrococcus horikoshii* OT3 DNA research. Int J Rapid Public Rep Genes Genomes 5:55–76

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403–410. doi:10.1016/s0022-2836(05)80360-2

Andronopoulou E, Vorgias CE (2003) Purification and characterization of a new hyperthermostable, allosamidin-insensitive and denaturation-resistant chitinase from the hyperthermophilic archaeon *Thermococcus chitonophagus*. Extremophiles (life under extreme conditions) 7:43–53 doi:10.1007/s00792-002-0294-3

Andronopoulou E, Vorgias CE (2004) Multiple components and induction mechanism of the chitinolytic system of the hyperthermophilic archaeon *Thermococcus chitonophagus*. Appl Microbiol Biotechnol 65:694–702. doi:10.1007/s00253-004-1640-4

Atomi H (2005) Recent progress towards the application of hyperthermophiles and their enzymes. Curr Opin Chem Biol 9:166–173. doi:10.1016/j.cbpa.2005.02.013

Blamey JM, Adams MW (1993) Purification and characterization of pyruvate ferredoxin oxidoreductase from the hyperthermophilic archaeon *Pyrococcus furiosus*. Biochim Biophys Acta 1161:19–27

Blom J, Albaum SP, Doppmeier D, Puhler A, Vorholter FJ, Zakrzewski M, Goesmann A (2009) EDGAR: a software framework for the comparative analysis of prokaryotic genomes. BMC Bioinform 10:154. doi:10.1186/1471-2105-10-154

Bridger SL et al (2011) Deletion strains reveal metabolic roles for key elemental sulfur-responsive proteins in *Pyrococcus furiosus*. J Bacteriol 193:6498–6504. doi:10.1128/JB.05445-11

Carver T, Thomson N, Bleasby A, Berriman M, Parkhill J (2009) DNAPlotter: circular and linear interactive genome visualization. Bioinformatics (Oxford, England) 25:119–120. doi:10.1093/bioinformatics/btn578

Cohen GN et al (2003) An integrated analysis of the genome of the hyperthermophilic archaeon *Pyrococcus abyssi*. Mol Microbiol 47:1495–1512

Darling AE, Mau B, Perna NT (2010) ProgressiveMauve: multiple genome alignment with gene gain, loss and rearrangement. PLoS One 5:e11147. doi:10.1371/journal.pone.0011147

Fiala G, Stetter K (1986) *Pyrococcus furiosus* sp. nov. represents a novel genus of marine heterotrophic archaebacteria growing optimally at 100 °C. Arch Microbiol 145:56–61. doi:10.1007/BF00413027

Gao J, Bauer MW, Shockley KR, Pysz MA, Kelly RM (2003) Growth of hyperthermophilic archaeon *Pyrococcus furiosus* on chitin involves two family 18 chitinases. Appl Environ Microbiol 69:3119–3128

Gooday GW (1996) In: Muzzarelli RAA (ed) Chitin enzymology, pp 125–133

Grissa I, Vergnaud G, Pourcel C (2008) CRISPRcompar: a website to compare clustered regularly interspaced short palindromic repeats. Nucleic Acids Res 36:W145–W148. doi:10.1093/nar/gkn228

Huber R, Stöhr J, Hohenhaus S, Rachel R, Burggraf S, Jannasch H, Stetter K (1995) *Thermococcus chitonophagus* sp. nov., a novel, chitin-degrading, hyperthermophilic archaeum from a deep-sea hydrothermal vent environment. Arch Microbiol 164:255–264. doi:10.1007/BF02529959

Imanaka H, Yamatsu A, Fukui T, Atomi H, Imanaka T (2006) Phosphoenolpyruvate synthase plays an essential role for glycolysis in the modified Embden-Meyerhof pathway in *Thermococcus kodakarensis*. Mol Microbiol 61:898–909. doi:10.1111/j.1365-2958.2006.05287.x

Jun X, Lupeng L, Minjuan X, Oger P, Fengping W, Jebbar M, Xiang X (2011) Complete genome sequence of the obligate piezophilic hyperthermophilic archaeon *Pyrococcus yayanosii* CH1. J Bacteriol 193:4297–4298. doi:10.1128/jb.05345-11

Jung JH et al (2012) Complete genome sequence of the hyperthermophilic archaeon *Pyrococcus* sp. strain ST04, isolated from a deep-sea hydrothermal sulfide chimney on the Juan de Fuca Ridge. J Bacteriol 194:4434–4435. doi:10.1128/jb.00824-12

Kanai T, Matsuoka R, Beppu H, Nakajima A, Okada Y, Atomi H, Imanaka T (2011) Distinct physiological roles of the three [NiFe]-hydrogenase orthologs in the hyperthermophilic archaeon *Thermococcus kodakarensis*. J Bacteriol 193:3109–3116. doi:10.1128/jb.01072-10

Kengen SM, Stams AJM, de Vos WM (1996) Sugar metabolism of hyperthermophiles. FEMS Microbiol Rev 18:119–137. doi:10.1016/0168-6445(96)00006-X

Keyhani NO, Roseman S (1999) Physiological aspects of chitin catabolism in marine bacteria. Biochim Biophys Acta 1473:108–122

Koga S, Yoshioka I, Sakuraba H, Takahashi M, Sakasegawa S, Shimizu S, Ohshima T (2000) Biochemical characterization, cloning, and sequencing of ADP-dependent (AMP-forming) glucokinase from two hyperthermophilic archaea, *Pyrococcus furiosus* and *Thermococcus litoralis*. J Biochem 128:1079–1085

Lee HS, Bae SS, Kim MS, Kwon KK, Kang SG, Lee JH (2011) Complete genome sequence of hyperthermophilic *Pyrococcus* sp. strain NA2, isolated from a deep-sea hydrothermal vent area. J Bacteriol 193:3666–3667. doi:10.1128/jb.05150-11

Lepage E, Marguet E, Geslin C, Matte-Tailliez O, Zillig W, Forterre P, Tailliez P (2004) Molecular diversity of new *Thermococcales* isolates from a single area of hydrothermal deep-sea vents as revealed by randomly amplified polymorphic DNA fingerprinting and 16S rRNA gene sequence analysis. Appl Environ Microbiol 70:1277–1286

Lipscomb GL, Keese AM, Cowart DM, Schut GJ, Thomm M, Adams MW, Scott RA (2009) SurR: a transcriptional activator and repressor controlling hydrogen and elemental sulphur metabolism in *Pyrococcus furiosus*. Mol Microbiol 71:332–349. doi:10.1111/j.1365-2958.2008.06525.x

Lukjancenko O, Thomsen M, Voldby Larsen M, Ussery D (2013) PanFunPro: PAN-genome analysis based on FUNctional PROfiles (version 1; referees: 3 approved with reservations, vol 2), vol 265

Luo H, Zhang CT, Gao F (2014) Ori-Finder 2, an integrated tool to predict replication origins in the archaeal genomes. Front Microbiol 5:482. doi:10.3389/fmicb.2014.00482

Matsunaga F, Norais C, Forterre P, Myllykallio H (2003) Identification of short 'eukaryotic' Okazaki fragments synthesized from a prokaryotic replication origin. EMBO Rep 4:154–158. doi:10.1038/sj.embor.embor732

Moriya Y, Itoh M, Okuda S, Yoshizawa AC, Kanehisa M (2007) KAAS: an automatic genome annotation and pathway reconstruction server. Nucleic Acids Res 35:W182–W185. doi:10.1093/nar/gkm321

Mukund S, Adams MW (1995) Glyceraldehyde-3-phosphate ferredoxin oxidoreductase, a novel tungsten-containing enzyme with a potential glycolytic role in the hyperthermophilic archaeon *Pyrococcus furiosus*. J Biol Chem 270:8389–8392

Muzzarelli RAA (1977) Chitin. Pergamon Press, Oxford, pp 155–181

Nakamura T et al (2005) Crystallization and preliminary X-ray diffraction analysis of a chitin-binding domain of hyperthermophilic chitinase from *Pyrococcus furiosus*. Acta Crystallogr Sect F Struct Biol Crystal Commun 61:476–478. doi:10.1107/s1744309105010110

Oku T, Ishikawa K (2006) Analysis of the hyperthermophilic chitinase from *Pyrococcus furiosus*: activity toward crystalline chitin. Biosci Biotechnol Biochem 70:1696–1701. doi:10.1271/bbb.60031

Overbeek R et al (2014) The SEED and the Rapid Annotation of microbial genomes using Subsystems Technology (RAST). Nucleic Acids Res 42:D206–D214. doi:10.1093/nar/gkt1226

Portillo MC, Gonzalez JM (2009) CRISPR elements in the *Thermococcales:* evidence for associated horizontal gene transfer in *Pyrococcus furiosus*. J Appl Genet 50:421–430. doi:10.1007/BF03195703

Powell S et al (2012) eggNOG v3.0: orthologous groups covering 1133 organisms at 41 different taxonomic ranges. Nucleic Acids Res 40:D284–D289. doi:10.1093/nar/gkr1060

Robb FT et al (2001) Genomic sequence of hyperthermophile, *Pyrococcus furiosus*: implications for physiology and enzymology. Methods Enzymol 330:134–157

Santangelo TJ, Cubonova L, Reeve JN (2011) Deletion of alternative pathways for reductant recycling in *Thermococcus kodakarensis* increases hydrogen production. Mol Microbiol 81:897–911. doi:10.1111/j.1365-2958.2011.07734.x

Sapra R, Bagramyan K, Adams MW (2003) A simple energy-conserving system: proton reduction coupled to proton translocation. Proc Natl Acad Sci USA 100:7545–7550. doi:10.1073/pnas.1331436100

Schäfer T, Schönheit P (1991) Pyruvate metabolism of the hyperthermophilic archaebacterium *Pyrococcus furiosus*. Arch Microbiol 155:366–377. doi:10.1007/BF00243457

Schut GJ, Bridger SL, Adams MW (2007) Insights into the metabolism of elemental sulfur by the hyperthermophilic archaeon *Pyrococcus furiosus*: characterization of a coenzyme A- dependent NAD(P)H sulfur oxidoreductase. J Bacteriol 189:4431–4441. doi:10.1128/JB.00031-07

Schut GJ, Boyd ES, Peters JW, Adams MW (2013) The modular respiratory complexes involved in hydrogen and sulfur metabolism by heterotrophic hyperthermophilic archaea and their evolutionary implications. FEMS Microbiol Rev 37:182–203. doi:10.1111/j.1574-6976.2012.00346.x

Siebers B, Brinkmann H, Dorr C, Tjaden B, Lilie H, van der Oost J, Verhees CH (2001) Archaeal fructose-1,6-bisphosphate aldolases constitute a new family of archaeal type class I aldolase. J Biol Chem 276:28710–28718. doi:10.1074/jbc.M103447200

Takai K, Sugai A, Itoh T, Horikoshi K (2000) *Palaeococcus ferrophilus* gen. nov., sp. nov., a barophilic, hyperthermophilic archaeon from a deep-sea hydrothermal vent chimney. Int J Syst Evolut Microbiol 50 Pt 2:489–500. doi:10.1099/00207713-50-2-489

Tanaka T, Fujiwara S, Nishikori S, Fukui T, Takagi M, Imanaka T (1999) A unique chitinase with dual active sites and triple substrate binding sites from the hyperthermophilic archaeon *Pyrococcus kodakaraensis* KOD1. Appl Environ Microbiol 65:5338–5344

Tanaka T, Fukui T, Imanaka T (2001) Different cleavage specificities of the dual catalytic domains in chitinase from the hyperthermophilic archaeon *Thermococcus kodakaraensis* KOD1. J Biol Chem 276:35629–35635. doi:10.1074/jbc.M105919200

Tanaka T, Fukui T, Fujiwara S, Atomi H, Imanaka T (2004) Concerted action of diacetylchitobiose deacetylase and exo-beta-D-glucosaminidase in a novel chitinolytic pathway in the hyperthermophilic archaeon *Thermococcus kodakaraensis* KOD1. J Biol Chem 279:30021–30027. doi:10.1074/jbc.M314187200

Tanaka T, Takahashi F, Fukui T, Fujiwara S, Atomi H, Imanaka T (2005) Characterization of a novel glucosamine-6-phosphate deaminase from a hyperthermophilic archaeon. J Bacteriol 187:7038–7044. doi:10.1128/JB.187.20.7038-7044.2005

Yang H et al (2010) SurR regulates hydrogen production in *Pyrococcus furiosus* by a sulfur-dependent redox switch. Mol Microbiol 77:1111–1122. doi:10.1111/j.1365-2958.2010.07275.x

Zillig W, Holz I, Janekovic D, Schafer W, Reiter WD (1983) The archaebacterium *Thermococcus celer* represents, a novel genus within the thermophilic branch of the archaebacteria. Syst Appl Microbiol 4:88–94. doi:10.1016/s0723-2020(83)80036-8