Thirty years ago, a remarkable microorganism was described for the first time by Karl Stetter and coworkers (Fiala and Stetter, 1986) (Fig. 1). Its name, meaning furious fireball, was special and over the years may have fuelled the imagination of many, but it also held a promise for the future. Now, three decades later, we can conclude that Pyrococcus kept that promise and that it indeed became 'furious' in many respects.

Although Pyrococcus furiosus was not the first hyperthermophile described to be able to thrive above the boiling point of water, it soon became one of the best studied representatives. One of the reasons was its vivid growth with a doubling time of \(~37\) min, which was together with its strong motility the reason for the name 'furiosus'. Another reason was that in contrast to many other hyperthermophiles, it preferred sugars (starch) over amino acids (proteins) for its anaerobic metabolism. Whereas efficient growth on proteins required elemental sulfur as electron acceptor, producing hydrogen sulfide, growth on oligosaccharides could do without sulfur, which made culturing and harvesting of cells much easier. In particular, starch-derived sugars were rapidly fermented to acetate, \(\text{CO}_2\) and \(\text{H}_2\) as end-products.

Soon after its discovery in 1986, the first metabolic discoveries came to the light of day. \(P.\) furiosus was one of the first archaea whose sugar metabolism was investigated in detail and it appeared that it was unlike the classical glycolytic pathways in many respects. Sugar kinases were shown to require ADP instead of ATP (Kengen et al., 1994) and glyceraldehyde-3-phosphate oxidation was not coupled to ATP synthesis and required ferredoxin instead of \(\text{NAD}^+\) (Mukund and Adams, 1995). Conversion of phosphoenolpyruvate to pyruvate was AMP and PPi dependent, catalysed by phosphoenolpyruvate synthase (Imanaka et al., 2006). Conversion of acetyl-CoA to acetate occurred by a one-step reaction, not involving acetyl-P as intermediate (Glasmacher et al., 1997). Disposal of reductant to hydrogen involved a novel type membrane-bound hydrogenase complex (Mbh) composed of 14 proteins (Sapra et al., 2000). It showed a primitive type of respiration, because it enabled the build-up of a proton gradient (Sapra et al., 2003). \(P.\) furiosus was also shown to contain several aldehyde oxidoreductases, which appeared to contain tungsten, an element rarely used in enzymes (Roy et al., 2001). These are just a few early examples, but the list of novel and unusual metabolic discoveries kept on growing and is now extensive.

Pyrococcus furiosus not only revealed a multitude of unprecedented metabolic reactions, it also was a source of thermostable enzymes, with potential applications in various industrial processes. Probably, the most famous example is the DNA polymerase I that was already described in 1991 (Lundberg et al., 1991), and which...
possessed an associated 3’-to-5’ exonuclease activity. Due to this proofreading ability, this Pfu-DNA polymerase had a much lower error rate in PCRs, compared with the Taq-DNA polymerase. The Pfu polymerase is now used in thousands of PCRs all over the world. Various other P. furiosus enzymes have been isolated in the past three decades, some exhibiting extreme thermostability. For example, a β-glucosidase had a half-life of 85 h at 100°C (Kengen et al., 1993) and an α-amylase had a half-life of 2 h at even 120°C (Jorgensen et al., 1997).

Already in the early years, P. furiosus was called the E. coli of the hyperthermophiles; however, the absence of a genetic system severely hampered its potential for biotechnological applications. Chromosomal modifications were possible in the closely related Thermococcus kodakarensis (Sato et al., 2003) and two Sulfolobus species (Wagner et al., 2009), but these all had lower temperature optima. It lasted until 2010 when a plasmid-based transformation system was developed (Waegeman et al., 2010), soon followed by chromosomal genetic manipulation of a naturally competent P. furiosus strain (COM1) (Lipscomb et al., 2011). Chromosome-encoded expression of heterologous genes now became possible, including genes of lower temperature origin or even from bacteria. This opened up many more possibilities for metabolic engineering, including the introduction of novel pathways for alternative product formation, to respond to the requests of the pharmaceutical industry or the biobased economy. In particular, the research groups of Michael Adams (University of Georgia) and Robert Kelly (North Carolina State University) were pioneering in this respect and several of their recent achievements were remarkable and opened up new avenues for sustainable production of chemicals and fuels. Synthetic pathways have been developed for lactate (Basen et al., 2012), CO₂ fixation (Keller et al., 2013; Hawkins et al., 2015), butanol (Keller et al., 2015), acetoin (Nguyen et al., 2015) and ethanol (Basen et al., 2014). Because most heterologous genes were derived from somewhat lower temperature ranges, a temperature shift approach was developed, in which optimal growth at ~95°C was followed by an expression phase at more moderate temperatures (Basen et al., 2012). Moreover, gene expression was controlled by a cold-shock promoter, avoiding the use of chemical inducers. By this method, the Adams group demonstrated production of lactate using a bacterial lactate dehydrogenase gene (Caldicellulosiruptor bescii). Another, more demanding task was to confer autotrophy to the typical heterotrophic Pyrococcus. This was accomplished by the heterologous expression of five genes of the carbon fixation cycle of the archaeon Metallosphaera sedula, which grows autotrophically at 73°C. The engineered P. furiosus strain was able to incorporate CO₂ into 3-hydroxypropionic acid using hydrogen gas as source of reductant (Keller et al., 2013; Hawkins et al., 2015). One of the special native features of P. furiosus is that it harbours a soluble hydrogenase (SHI) that is able to use hydrogen gas for the reduction of NADP, thereby providing a constant supply of reducing power for biosynthetic purposes (Keller et al., 2017). Another noteworthy engineering achievement is the work reported by Basen et al. (2014), which describes a completely novel pathway for ethanol formation (Basen et al., 2014). Whereas in conventional systems acetaldehyde is derived from pyruvate (yeasts) or acetyl-CoA (fermentative anaerobes), here acetate is used as source of acetaldehyde. Acetate reduction to acetaldehyde requires low-potential electrons (E₀’ = −580 mV), which cannot be provided by NAD(P)H (E₀’ = −320 mV). Therefore, the reaction requires the low-potential electron carrier ferredoxin (E₀’ = −500 mV) and is catalysed by an aldehyde:ferredoxin oxidoreductase (AOR), one of the native tungsten-containing enzymes present in P. furiosus. Reduced ferredoxin is produced at two positions in the pyrococcal glycolysis. Thus, by the insertion of a single gene, viz. an alcohol dehydrogenase gene (AdhA) from Thermoanaerobacter strain X514, the groups of Adams and Kelly accomplished the conversion of glucose (maltose) to ethanol, with a yield of 90% of the theoretical ethanol yield. The AdhA is NADPH-dependent, which may be produced from glyceraldehyde-3-phosphate (GAPN) or from hydrogen using the SHI. One of the benefits of the engineered strain was that it can use alternative carboxylic acids instead of acetate, like butyrate, propionate and isobutyrate, leading to the production of the corresponding alcohols. In addition, the authors accomplished the use of carbon monoxide as alternative source of reduced ferredoxin, by introducing the genes of the CO-dehydrogenase from Thermococcus onnurineus. The use of CO (via the introduced CO-dehydrogenase) and H₂ (via the native SHI) permits the use of syngas (a mixture of CO, CO₂ and H₂), which can be produced by gasification from renewable organics waste materials.

Recently, an alternative ethanol-producing pathway was established in P. furiosus (Keller et al., 2017). Here, acetaldehyde and ethanol were produced directly from acetyl-CoA by the introduction of a bifunctional alcohol dehydrogenase (AdhE), and acetaldehyde formation from acetate was blocked by deleting the AOR gene. It was shown that only AdhEs from two Thermoanaerobacter strains were functionally expressed and supported in vivo ethanol production. The other six AdhE homologues from different moderate thermophilic bacteria did not show significant activity. Highest ethanol levels were obtained, however, when a AdhE was combined with AdhA (61% of theoretical ethanol yield). Possibly, the activity of the AdhE, which relies solely on NADH, is insufficient and the NADPH-dependent AdhA can...
compensate for this. Nevertheless, the ethanol yield of this AdhE/AdhA system was still lower than the earlier system using AOR/AdhA.

From the viewpoint of sustainable biomass fermentation, *P. furiosus* may, however, not be the ideal platform organism, because it is rather limited in its substrate range. It efficiently grows on starch polymers and starch oligomers, but it barely grows on monomeric sugars and it cannot use lignocellulosic feedstocks. Its repertoire of sugar hydrodases is rather restricted, especially when compared to the thermophilic bacteria *Thermotoga maritima* or *Caldivcellulosiruptor saccharolyticus*. However, also these aspects may become targets for further metabolic engineering.

These examples show that *P. furiosus* is an excellent host for the expression of various synthetic pathways, involving genes of archaeal but also of bacterial origin and covering a broad temperature range. In addition to its native heterotrophic metabolism, it may also be used for an autotrophic metabolism, using CO₂ and reductant from hydrogen and/or carbon monoxide. Moreover, the high growth temperature can have various advantages for industrial application, like a reduced risk of contamination, improved mixing and diffusion and lower cooling costs. Moreover, the downstream processing of various alcohols may become easier at higher temperatures. Altogether, the recent engineering successes show that hyperthermophilic *P. furiosus* is well suited for establishing various biosynthetic pathways and that the atypical ferredoxin-based glycolysis, the unusual redox chemistry and the tungsten-containing AORs make it stand out amongst moderate bacterial fermentatives used in chemical and fuel production. *P. furiosus* is ready for the next decade, still being ‘the fast and the furious’.

**Conflict of interest**

None declared.

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