Proc. KSAM International Symposium
June 20-22, 2001, Chonan, Korea

Expression Profiling and Functional Genomics of Common ancestral Genes in the Energy Pathway of *Thermotoga maritima*

Bharat K.C. Patel
Microbial Discovery Research Unit, School of Biomolecular & Biomedical Sciences,
Faculty of Science, Griffith University, Brisbane Australia

rRNA phylogeny indicates that there are 3 distinct lines of evolution or descent in the Universal Tree of Life which are termed domains (super kingdoms): Domain *Bacteria* includes all eubacteria, domain *Archaea* (formerly archaebacteria) consists mainly of extremophiles and domain *Eucarya* houses the complex eucaryotic cells (Woese, 2000). Rooting the tree with paralogous duplicated protein genes indicates that the 3 domains may have arisen from a hypothetical unicellular ancestor, the progenote (Doolittle, 2000; Woese, 1998). It has been suggested that the prognote may have evolved under anaerobic conditions in a hot soup containing inorganic compounds (e.g. sulfur, thiosulfate, pyrite) as energy sources for growth and hence could have been a thermophile (Stetter *et al.,* 1990; Di Giulio, 2000). Therefore, the deep and slow evolving thermophilic lineages of domains *Bacteria* and *Archaea* could hold the key to unlocking our understanding of universal ancestral structures and their functions. We have chosen *Thermotoga maritima*, (order *Thermotogales*) whose complete genome sequence is available, as a model bacterium, and propose to use gene profiling and functional genomics to understand the role of thiosulfate/sulfur in energy generation as an ancestral shared trait.

The order *Thermotogales* is one of the deepest branches of domain *Bacteria* and its members are thermophilic anaerobes (Patel *et al.,* 1985, 1986, 1987, 1991, 1991; Huser *et al.,* 1986; Huber *et al.,* 1990; Andrews and Patel, 1996; Ravot *et al.,* 1995; Fardeau *et al.,* 1997). The order currently consists of the genera *Fervidobacteria*, *Thermotoga*, *Geotoga*, *Petrotoga* and *Thermosipho*. *Fervidobacterium* and unclassified bacterium resembling *Thermotoga* were first described by Patel *et al.,* (1985a) and Huser *et al.,* (1986) and subsequently ascribed to the order *Thermotogales* based on rRNA sequence analysis (Huber *et al.,* 1990). Members of the order are not confined to volcanic hot springs and have also been isolated from underground geothermal aquifers such as the Great Artesian Basin of Australia (Andrews and Patel, 1996) and oil fields (Ravot *et al.,* 1995; Fardeau *et al.,* 1997). It is thought that their presence in oil fields leads to corrosion of oil field pipelines and in oil souring. The most studied member of the order *Thermotogales* is *T. maritima*, a hyperthermophile which grows optimally at 80 °C, was isolated from a volcanically heated marine sediments (Stetter *et al.,* 1990). A similar bacterium was isolated from Fiji hot springs (Huser *et al.,* 1986) where its presence could also be demonstrated *in situ* (Patel *et al.,* 1989). Approximately 225 papers that have been published on *T. maritima* deal with cloning and characterisation of genes and their products with a view to understanding the basis of thermostablity, evolution, and their biotechnological potential (Blamey and Adams, 1994; Dairmont and Sterner, 1994; Brown *et al.,* 1994; Beaucamp *et al.,* 1997; Ding *et al.,* 2000).

A common physiological link exists between the phylogenetic deep branches of the domains Bacteria and Archaea:

The deep and slow evolving branches of domains *Bacteria* and *Archaea* are represented by thermophiles leading to suggestions that they could contain common ancestral cellular and/or functional

events. Consequently, physiological studies undertaken from 1990 to 1999 to determine common ancestral relationships established that members of domain *Archaea* used a diverse range of metabolic pathways and modifications in these pathways during growth (Adams, 1994; Selig *et al.*, 1997). Studies also showed that the archaeon, *P. furiosus* fermented glucose via a modified Embden-Meyerhof (EM) pathway (Serve *et al.*, 1994), but in the case of *T. maritima* both the Entner-Doudoroff (ED) and (EM) pathways were used (Selig *et al.*, 1997). However, despite this, both organisms produce fatty acids, H_2 and L-alanine as end-products (Ravot *et al.*, 1996; Serve *et al.*, 1994). The addition of sulfur or thiosulfate increases growth rates and cell yields of both organisms (Schicho *et al.*, 1993; Ravot *et al.*, 1996, 1997; Kengen *et al.*, 1996; Kelly and Adams, 1994; Stetter *et al.*, 1990) which suggests that common physiological traits exist in both organisms. Schicho *et al.*, 1993 concluded from their studies that this growth stimulation was either an energy-conserving mechanism (S° respiration) or an effect on a yield limiting process in *P. furiosus*. They further suggested that, in the case of *T. maritima*, the stimulation of growth in the presence of sulfur, may be due to the removal of H_2, a glucose fermentation inhibitory end product, by an alternative but unknown mechanism.

Our recent studies with *T. maritima* have shown similar metabolic end-product ratios and profiles are obtained during glucose fermentation in the presence or absence of thiosulfate. However, increased ATP yields were consistently obtained with cells grown in the presence of thiosulfate but not in its absence (Ravot *et al.*, 1997). This has lead us to suggest that thiosulfate acts as an electron acceptor, and therefore plays an important role in energy generation in *T. maritima* in a similar manner to that observed for *P. furiosus*. From these studies, it is reasonable to hypothesise that the reduction of sulfur compounds to sulfide and the production of L-alanine from carbohydrate fermentation may be a common metabolic feature in some of the deep and ancient members of *Archaea* and *Bacteria* and perhaps a remnant of ancestral metabolic pathways. If this was proven then it would provide evidence to support the notion that the common ancestor of domain *Bacteria* and *Archaea* was perhaps a thermophilic sulfidogen (Darimont and Sterner, 1994; Ma *et al.,1*993; Childers and Noll, 1994).

The inconsistency of the physiological data with that of the pathway data can be explained by the hypothesis of Woese (2000). He has suggested that during the very early stages of evolution cellular functions were in modular forms and these modules were loosely coupled in the cells. Extensive dispersion of functional modules occurred by vertical gene exchange and such acquisitions lead to innovations in the host. Once well-defined lines of descent were established lateral gene transfer mechanisms which improved functional complexity and specificity replaced the cellular innovation mechanism.

Of the 35 procaryotic genomes sequenced to date 10 are from extremophiles (7 *Euryarchaeota*, 1 *Crenarchaeota* and 2 *Bacteria*) and this demonstrates the significant research interest in extremophiles. Despite advances in genome sequencing, the physiology & metabolism of extremophiles have been poorly studied. The genome sequence of *T. maritima* published in 1999 shows that the single circular genome consists of 1,860,725 bp representing 1,877 coding regions. Of these 1,014 (54%) have been assigned functions but the remaining 863 (46%) could not be assigned any function. 71 genes, of which 64 are recorded as hypothetical, match exclusively the sequenced genomes of *Archaea* (Nelson *et al.*, 1999). The later suggests that either considerable lateral gene exchanges between the slow evolving members of domains *Bacteria* and *Archaea* has occurred or they possess genes from the last common ancestor transferred by vertical gene transfer mechanisms (Kyrpides and Olsen, 1999).

Prior to the genome era, coherent physiological studies of these organisms were not extensively undertaken due to handling difficulties of these microbes, a poor understanding of the properties of the

organisms and a general lack of high throughput techniques. The post genome era, however, has changed this equation considerably and it is now within the scope of relatively small laboratories to undertake physiological studies using gene expression by DNA arrays. We wish to capitalise on the complete genome information available to us and aim to study the energy generation pathway of *T. maritima* using DNA arrays. The paper will discuss the physiology of *Thermotogales* and provide information on some new approaches we are currently undertaking in our laboratory.

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