MEETING REPORT

Thermophiles 2005*

The first international conference on 'Thermophiles' was held in Italy in 1990 followed by those in Iceland (1992), New Zealand (1993), USA (1996), France (1998), India (2001) and England (2003). About 120 delegates attended the 'Thermophiles 2005' conference from various countries. The next thermophile conference will be organized by Nils-Kare Birkeland (University of Bergen, Norway) in 2007. The conferences are aimed at: (i) deliberating on the progress achieved on various aspects of thermophiles, (ii) understanding the recent trends in thermophile research, and (iii) setting priorities for future research.

In his opening plenary lecture, Paul Davies (Australian Centre for Astrobiology, Macquarie University, New South Wales, Australia) reiterated the claim of astrobiologists that life is a 'cosmic imperative' bound to arise under earth-like conditions, and likely to spread across the galaxy and beyond. Further, he raised a question: Are we alone in the universe? The answer for this question needs extensive search for life in space.

Moreland Gibbs (Macquarie University, Sydney, Australia) explained the utility of random drift mutagenesis for directed evolution of β-glucosidase of *Caldicellulosiruptor saccharolyticus* using the substrate Imagene Green. David Saul (University of Auckland, New Zealand) explained the applicability of thermostable proteinase of *Bacillus* sp. EA1 in an easily–automated, closed-tube forensic DNA extraction procedure. Solvent extraction is not required for removing residual proteinase, and the reaction could be controlled by temperature regime programmed into a thermal cycler. Kunihiko Watanabe and coworkers (Kyoto Prefectural University, Kyoto, Japan) have isolated a thermophilic bacterium *Geobacillus collagenovorans* that exhibited collagenolytic protease activity. The enzyme is a homodimer with a molecular mass of 210 kDa. The gene encoding this protease was cloned. Asmita Prabhune and coworkers (NCL, Pune) isolated a novel thermophilic bacterium *Geobacillus caldoxylosilyticus* from hot-water springs near Pali that secreted thermostable penicillin V acylase as well as bile salt hydrolase. Mala Rao (NCL, Pune) dwelt on the production of carboxymethylcellulase by *Thermomonospora* sp. with a single active site that finds application in biofinishing of denim. The enzyme has negligible activity on crystalline cellulose. Garo Antranikian (Hamburg University of Technology, Institute of Microbiology, Hamburg, Germany) presented their work on glucoamylase of the acidophilic euarychaebacterium *Picrophilus torridus* that thrives at pH 0.0 and 65°C. The extracellular enzyme is optimally active at 90°C and pH 2.0, while the intracellular one at 50°C and pH 5.0. The enzyme hydrolyses amylopectin and glycogen efficiently. V. S. Junior Te'o (Macquarie University, Sydney) described efforts in producing thermostable xylanase B of *Dictyoglomus thermophilus* and β-glucosidase of *Caldicellulosiruptor saccharolyticus* using recombinant *Trichoderma reesei*. His group is trying to study pathways involved in protein modification and degradation. Stefan Janecek (Institute of Molecular Biology, Slovak Academy of Sciences, Bratislava, Slovakia) dwelt on starch binding domain (SBD) of amylolytic enzymes of Archaea. The SBD of *Haloferax mediterranei* appeared to be closely related to the bacterial SBDs of cyclodextrin glucanotransferases originated from Bacilli. Tulasi Satyanarayana (University of Delhi South Campus, New Delhi) presented work on hyperthermostable and Ca^{2+}-independent α-amylase of an extreme thermophile *Geobacillus thermoleovorans*. The native enzyme contains 25% α-helix and 21% β-sheet, and it exhibits activity on raw as well as soluble starches. The enzyme integrates well into an ideal starch saccharification process with other thermostable amylases. Kwan-Hwa Park (Seoul National University, Seoul, South Korea) described their work on thermostable amylase of *Pyrococcus furiosus* that opens cyclodextrin rings to produce maltooligosaccharides. The enzyme also hydrolyses starch into maltooligosaccharides. Satya Singh and coworkers (Saurashtra Univ., Rajkot) presented their work on thermostable alkaline proteases from haloalkaliphilic bacteria. The enzymes are highly resistant to chemical denaturation. He also presented the structure and function analysis of the thermostability of β-glucosidase of a hyperthermophilic bacterium, *Thermotoga maritima.*

Peter Schonheit (Univ. Kiel, Germany) reported that *Thermotoga* catabolizes glucose and glucose polymers to acetate or CO_2 using O_2 and sulphur or nitrate as electron acceptors. In Archaea, conversion of acetyl-CoA to acetate is catalysed by a novel enzyme ADP forming acetyl-CoA synthetase. In contrast, in hyperthermophilic bacterium *Thermotoga*, glucose is fermented to acetate via EM pathway. Michael Danson (Univ. of Bath, Bath, UK) focused on the central metabolism in thermophilic Archaea. In *Sulfolobus acidocaldarius*, glucose is metabolized by non-phosphorylative ED pathway, in which glucose dehydrogenase, gluconate dehydratase and 2-keto-deoxygluconate aldolase convert glucose to pyruvate and glyceraldehydes. This single set of enzymes has been shown to catabolize both glucose and galactose to pyruvate. Besides this pathway promiscuity, unusual redundancy in the subsequent catalysis of pyruvate was seen; more than one enzyme appears to catalyse the same reaction. Michael Adams (Univ. of Georgia, USA) described the pathways that are operating in *Pyrococcus furiosus* for metabolizing inorganic compounds, hydrogen, sulphur and iron. The genome of this archaebacterium appears to contain four different types of hydrogenase; two are in cytoplasm and the other two are integral membrane proteins. John Oost (Wageningen University, The Netherlands) used proteomics analysis to show that several new proteins are induced, which convert arabinose in a step-wise manner in *Sulfolobus solfataricus*. Donald Cowan (University of Western Cape, Cape Town, South Africa) reported that a number of commercial organizations have acquired substantial libraries of gene products from metagenomic resources. Metagenome is defined as the sum total of all genomes present in any environment. Yutaka Kawarabayasi

*A report on 'Thermophiles 2005: From Evolution to Revolution' organized by Bharat Patel, Griffith University, Brisbane, Australia during 18–22 September 2005 at Crowne Plaza, Gold Coast.

(National Institute of Advanced Industrial Science and Technology, Ibaraki, Japan) presented work done in his laboratory on the construction of 380 expression vectors using genomic information of *Sulfolobus tokodaii.* Over 150 recombinant proteins were expressed in *Escherichia coli* as soluble thermostable proteins. The analysis of recombinant enzymes suggested that archaeal enzymes are capable of utilizing many kinds of materials as substrates and many kinds of metal ions as cofactors.

Tadayuki Imanaka (Kyoto Univ., Japan) presented genome analysis of *Thermococcus kodakaraensis*. The gene disruption technology has been recently developed utilizing a pyrF-deficient mutant KU25 as a host strain, and this has been used to clarify physiological roles of fructose 1,6-biphosphatase, and genes involved in the interconversion of C_3 and C_4 carbon metabolites. When reverse gyrase gene was disrupted, the growth rate of the stain was low at high temperatures. Seiki Kuramitsu (Osaka Univ., Japan) discussed whole-cell project for *Thermus thermophilus* HB8. More than 80% of the proteins were purified. Structural analysis predicted the functions of more than 60% proteins. Functions of the other hypothetical proteins were characterized by mRNA analysis, gene disruption, etc. Bharat Patel (Griffith Univ., Brisbane, Australia) announced that the genome of *Halothermothrix orenii* has been sequenced. Biochemical evidence has suggested that this bacterium may possess a novel adaptation strategy to enable it to grow in a moderately saline environment. Gregory Cook (Univ. of Otago, Dunedin, New Zealand) presented unique structural and functional features acquired by ATP synthase in thermoalkaliphilic *Bacillus* sp. strain TA2.A1. Keiko Watanabe and his associates (Tokyo University of Pharmacy and Life Sciences, Japan) have developed mutant enzymes of 3-isopropylene dehydrogenase from *T. thermophilus*. The thermal stability analysis of mutant enzymes supported hyperthermophilic universal ancestor hypothesis. Comparing the sequences of aminoacyl-tRNA synthetases as well as t-RNAs of about 60 different genomes, Jeffrey Wong (Hong Kong Univ. of Science and Technology, China) has arrived at the conclusion that *Methanopyrus kandleri* is closely located to the last universal common ancestor.

Sun Bok Lee (Pohang University of Science and Technology, Korea) emphasized on biochemical and genetic characterization of glyceraldehyde, dehydrogenase and glycerate kinase, which is expected to advance our understanding of the glycolysis mechanism in thermoacidophilic archaea. Ulrike Jahn (Univ. of Regensburg, Germany) presented his work on analysis of lipids of *Nanoarchaeum equitans* and *Igniococcus*. Both organisms contained identical lipids, and the isoprenoid hydrocarbon chains of the former are most probably synthesized in the latter.

Roberto Anitori (Macquarie Univ., Sydney) reported the emission of radon (^{222}Rn; alpha radiation) from Paralana hot spring that supports a flourishing microbial community with a variety of bacteria and archaea. Richard Castnholz (Univ. of Oregon, USA) discussed the diversity of acidophilic and thermophilic, unicellular eukaryotic algae of Yellowstone National Park that contained *Cyanidium caldarium*, *Galdieria sulphuraria* and *Cyanidioschyzon merolae*. Stephen Pointing (Univ. of Hong Kong, China) presented molecular diversity of microflora of geothermal location in central Tibet. All models demonstrated that diversity is not related to thermal stress in a linear fashion. Hiroyuki Yamamoto (Japan Agency for Marine-Earth Science and Technology, Japan) has attempted to distinguish between volcanic and non-volcanic terrestrial geothermal environments and marine hydrothermal vent system. The typical microbial mat formation found in hot springs has not been found in vent areas. He further informed that the deep subsurfaces are being investigated under Integrated Ocean Drilling Program.

Jeurgen Wiegel (University of Athens, USA) presented work on the diversity of alkalithermophiles. His group has isolated three novel facultatively aerobic, halophilic and alkalithermophilic bacteria from Wadi Natrum (Egypt) and salt flats located in Bufala Spring Area, Nevada. These have been identified as *Bacillus natrunensis*, *Caloramator halophilus* and *Thermococcus alcaliphilus*. Hugh Morgan (Univ. of Waikato, Hamilton, New Zealand) isolated *Pyrobaculum* sp., *Aeropyrum pernix* and *Ignisphaera aggregans* from hot pools of central North Island of New Zealand. Anna Reysenbach (Portland State University, USA) dealt with deep-sea and terrestrial hydrothermal ecosystems. Members of Aquificales are often prevalent in terrestrial hydrothermal systems. Distribution of *Perspephonella* and other Aquificales in deep-sea vent systems does not appear to be uniform. She said that the genome sequences of *Sulfurihydrogenibium azorense* and *Perspephonella marina* have been completed. Subrata Das (Institute of Life Sciences, Bhubaneswar) isolated several thermophilic bacterial isolates from hot springs in Orissa. On further analysis, the isolated strains appeared to belong to new species of *Comamonas* and *Thiomonas*.

Anthony Greene (Griffith Univ.) stated that thermophilic Mn (IV)-reducing bacteria might have future roles in heterotrophic leaching of manganese ores and tailings. Bernard Ollivier (Marseille, France) presented diverse microbial populations of oilfield reservoirs. It is, however, difficult to comment on the origin of these microbes. All these microbes (species of *Petrotoga*, *Geotoga*, *Thermosipho*, *Thermotoga*, *Archaeoglobus*, *Desulfacinum*, *Thermodesulfobacterium*, etc.) are believed to participate in the overall complex biogeography of oil reservoirs, as they possess a wide range of metabolic features. He further stated that knowledge of microflora of oil wells is so little that it is not possible to exploit them in microbially enhanced oil recovery at the moment. Alexander Slobodkin (Winogradsky Institute of Microbiology, Russian Academy of Sciences, Moscow, Russia) presented work on the reduction of more than 90% of technetium (VII) to Tc(IV) by *Tepidibacter thalassicus* and *Thermoterrabacterium ferrireducens*. *T. thalassicus* was also able to reduce Se(IV) using hydrogen as an electron donor. Tatyana Sokolova (Winogradsky Institute of Microbiology) isolated several thermophilic anaerobes such as *Carboxydothermus hydrogenoformans*, *Thermolithobacter carboxydivorans* and *Carboxydocella ferrireducens,* which exhibited hydrogenogenic CO oxidation.

Jennifer Littlechild (Univ. of Exeter, UK) spoke on structure–function relationships of cysteine protease and L-aminoacylase of *Thermococus littoralis*. Cysteine protease was subjected to site-specific mutagenesis. Hydrophobic disulphide bridges and ion interactions have been shown to play an important role in stabilizing the enzyme. Nils-Kare Birkeland and his coworkers (Univ. of Bergen, Norway) have resolved the three-dimensional structure of isocitrate dehydrogenase of *Thermotoga maritima.* The high thermostability of the enzyme was explained by four five-membered ionic networks, more inter-subunit ion pairs, higher percentage

of charged residues at the accessible surface, more hydrophobic and charged residues at the subunit interface, slightly more hydrogen bonds per residue in the overall structure and a reduced number of residues per monomer due to loop deletions.

Akihiko Yamagishi and coworkers (Tokyo Univ. of Pharmacy and Life Sciences) have developed a new way of designing thermostable mutant enzymes using phylogenetic tree. Mutant enzymes of 3-isopropylmalate dehydrogenase exhibited higher thermostability than the enzyme from *Sulfolobus tokodaii*. A similar observation was recorded with glycyl-transfer RNA synthetase from *T. thermophilus*. The investigators have concluded that these results support the hyperthermophilic common ancestor hypothesis.

Tairo Oshima and coworkers (Institute of Environmental Microbiology, Tokyo, Japan) demonstrated *T. thermophilus* to produce 16 different polyamines (PAs), including long, branched and standard PAs. Long PAs effectively stabilize nucleic acids. When genes encoding PA biosynthesizing enzymes were knocked out, the mutants failed to grow at elevated temperatures, and supplementing with PAs restored growth. Investigations using pure enzymes supported a new pathway for PA biosynthesis.

Daniel Prieur and coworkers (Univ. of Occidental Bretagne, Plouzane, France) reported isolation of barophilic *Thermococcus barophilus* and ionizing radiation-resistant *T. gammatolerans*. They have also isolated a virus (PAV1) from *Pyrococcus abyssi* that has a genome of 18098 bp with 24 ORFs.

Philip Hendry and coworkers (CSIRO Molecular and Health Technologies, Australia) have isolated *Sulfolobus* sp. JP2, that showed minor differences with *S. solfataricus* at the genomic level. Microarray analysis suggested the expression of genes related to sulphur oxidation, electron transport and carbondioxide fixation. The strain was able to efficiently leach copper from chalcopyrite.

Radhey Gupta (McMaster Univ., Hamilton, Canada) presented work on the identification of many conserved signatures in widely distributed proteins that are unique to members of Aquificales that are not found in any other organisms. Further evidence was provided that Aquificales phylum is a late branching lineage within bacteria.

The conference also included 72 poster presentations on aspects such as diversity of thermophilic bacteria and archaea from various terrestrial and marine thermal environments, physiology and biochemistry, cloning and overexpression of thermostable proteins and enzymes, structure–function relationships of enzymes, biotechnological application of enzymes, genomics and proteomics, genetics and molecular biology. The conference gave ample opportunity to understand recent trends in research on thermophilic microorganisms, and investigations that are in progress all over the world on basic as well as applied aspects of thermophilic microbes.

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MEETING REPORT

Linking Indian rivers vs Bay of Bengal monsoon activity*

In a popular article on 'Interlinking rivers: Is it the solution?' (*The Hindu*, 26 August 2005), V. Rajamani brought out a possible consequence of the proposed interlinking of Indian rivers on the SW monsoon. The connection is that the run-off of Indian rivers to the Bay of Bengal (BOB) plays a critical role in the process of monsoon intensification by creating and sustaining a low-salinity layer on top of the BOB. The President of India, A. P. J. Abdul Kalam, as an advocate of the river-linking project showed keen interest in Rajamani's article, particularly to the link between run-off to the BOB and the SW monsoon. In view of the importance of the project, the interest of the scientist-President and the scientific importance of the problem, a one-day meeting of earth scientists interested in the air–sea–land interactions was arranged for a focused discussion on the following: If the proposed river linkages were to reduce the total annual run-off into the BOB or the rate at which run-off is released into it, then do river linkages have any significant consequence on the Indian monsoon rainfall? This report is an outcome of the discussion meeting and the opinion expressed is the prevailing and collective knowledge of the participants (listed at the end) on the issue.

Owing to its unique geographic location, the BOB receives large quantities of freshwater from both local precipitation and river discharge. The estimated freshwater influxes into the BOB from local precipitation and through river discharge are 4700 and 3000 km^3/yr respectively. Ganga, Brahmaputra, Mahanadi and Eirawaty are the major rivers that discharge in the head BOB. The loss due to evaporation is about 3600 km^3/yr. Thus, on an annual scale, freshwater input exceeds the loss due to evaporation substantially. This tends to make the water of the BOB relatively less saline compared to other oceans and the low salinity is confined to the top ~ 10–20 m layer. There is a strong seasonal dependence, as the major fraction of the freshwater comes during the summer monsoon period, i.e. during June–September. During this time, southwesterly winds tend to accumulate the freshwater in the head BOB and the salinity decreases further, resulting in the occurrence of a quasi-permanent, low salinity layer north of 17°N. Once the southwest monsoon ends (in October), water starts flowing southwards. Eventually, BOB water spreads out over the equatorial and south Indian Ocean and also over the east Arabian Sea.

Density of sea water increases with salinity. The low-salinity layer at the BOB, is therefore a layer of less dense water floating on denser water below as a stratified layer. This inhibits vertical mixing and even strong monsoonal winds have limited success in inducing vertical mixing. Most of

*A report on one-day meeting of earth scientists held at the premises of the Indian Academy of Sciences, Bangalore on 15 October 2005.