MEETING REPORT

Thermophiles 2007*

Among extremophilic microbes, thermophiles that are capable of growth at elevated temperatures are a well-established group that comprises some viruses, prokaryotes (Archaea and Bacteria), fungi, algae and protozoa. These are well known to play an important and critical role in the environment and are the source of an array of thermostable enzymes that are used in the industry as well as in molecular biology. Thermophiles 2007, the four-day conference on thermophiles was held at Bergen, Norway, to deliberate on the advances in understanding the microbial life at elevated temperatures. The recent developments on various aspects of thermophiles were presented and discussed through 57 lectures and over 100 informative posters. In order to provide a basic understanding of the subject and stimulate research interest in this area, high-school students and teachers were invited to a public lecture on 'Thermophile hunters' by Frank Robb (USA) and to view the posters. In addition, a tutorial was organized by Todd Lowe on the Archaeal and Extremophile genome browser to help participants access on-line information from the database.

The conference began with the inaugural address by Nils-Kare Birkeland, convener of the conference. Briefly describing the history of conferences on thermophiles, he emphasized their importance in updating our knowledge on this group of microbes. The German microbiologist, Karl Stetter, in his lecture entitled 'Going deep – hyperthermophiles and the history of life', talked about hyperthermophilic bacteria that grow optimally above 80°C with an upper temperature limit of 113°C for growth and emphasized that hyperthermophiles could have existed already on the Early Earth, about 3.9 Gyr ago, due to their unique growth characteristics. *Nanoarchaeota* and *Korarchaeota* form the deepest archaeal phylogenetic lineages and *Korarchaeota* perhaps diverged very early from the archaeal lineage. He further mentioned about the sequencing of the genome of *Nanoarchaeum equitans* by the Diversa Corporation, and that a *Nanoarchaeum* sp. has also been reported in association with *Pyrobaculum* sp. in the Kamchatka hot springs of Russia. He expressed some doubts on the report by Kashefi and Lovely (USA) on the upper temperature limit of 121°C for the bacterial strain S121, and further stated that it is closely related to *Pyrococcus fumarii* that has a T_{opt} of 116°C for growth. Also, members of the genera *Pyrodictium* and *Pyrolobus* can survive at least an hour of autoclaving, though their optimum growth temperatures are lower.

The session on ecology and biodiversity of thermophiles included ten lectures. Anna-Louise Reysenbach (USA) talked about the diversity of novel thermophilic chemolithoautotrophic and heterotrophic microbes in deep-sea thermal vents. Her group has cultivated the first member of the endemic euryarchaeotal lineage, DHVE2, that makes up around 15% of the total archaeal community. The newly isolated *Aciduliprofundum boonei* forms a new order in Archaea and probably can grow fermentatively. It is an obligate thermoacidophilic, sulphur and iron-reducing heterotroph and is capable of growing at pH from 3.3 to 5.8, between 60°C and 75°C, and its genome sequencing is almost complete. Rachel Whitaker (Germany) described work on understanding the evolution of genome diversity using comparative genomics among eight complete genome sequences of *Sulfolobus islandicus* from four geographically isolated populations from the Mutnovsky Volcano and Geyser Valley in Kamchatka, and Yellowstone and Lassen National Parks in the western United States. Horizontal gene transfer was shown to be responsible for the divergence of unique ecotypes within a single population. The genome evolution in these geothermal environments could be through a balance of selection, neutral mutation and gene flow. Stephen Pointing (Hong Kong) presented work on the diversity within the thermophilic microbial mats from five similar locations along a 380 km transect in the Daggyai Tso geothermal field, Tibet. The dominant microflora included *Roseiflexus castenhlozii* and *Synechococcus* sp., which along with an unidentified alphaproteobacterium were used for the identification of various functional genes. The distribution of thermophilic crenarchaeotes in hot springs of Kamchatka, Baikal lake region and Iceland was described by Anna Perevalova. Majority of the detected sequences were related to the 'uncultured' *Crenarchaeota* reported previously from the Yellowstone National Park and Iceland, while some of the sequences resembled the cultivated organisms – *Desulfurococcales*, *Thermoproteales* and *Sulfolobales*. A novel strain representing a new phylogenetic group of *Crenarchaeota*, earlier known as uncultivated organisms, was isolated from Caldera Uzon/Kamchatka. Based on the genomic features, this isolate was assigned a new genus – *Fervidococcus*, the type species being *Fervidococcus fontis*. This is a hyperthermophilic and anaerobic organotroph that grows optimally at a pH of 6.0–6.5 and in the temperature range between 60°C and 85°C. R. Rachel (Germany) presented work on understanding the interaction between *Nanoarchaeum equitans*, the smallest archaeal cell known, and *Ignicoccus hospitalis* that serves as a host for the former. The ultrastructure of the site of interaction between these two archaea indicated the involvement of a number of molecules in cell–cell contact and interaction. The membrane proteins appeared to play a possible role in mediating metabolite transport to *N. equitans*, while the *Ignicoccus* flagella help in adhesion rather than motility.

The most interesting lecture perhaps in the session was from Ken Takai (Japan), who spoke on his work on methane production using a newly isolated hyperthermophilic methanogen, *Methanopyrus kandleri* strain 116. An increase in hydrostatic pressure was shown to extend the temperature maximum for growth from 116°C at 0.4 MPa to 122°C at 20 MPa, which further extends the earlier reported highest temperature limit for life, i.e. 121°C for bacterial strain S121. *M. kandleri* survived 3 h at 130°C. Tatiana Slepova (Russia) delivered a talk on the CO transformation by anaerobic microbial

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communities of Kamchatka hot springs. The microbial communities comprise up to 10^6 cells of phylogenetically and physiologically diverse CO-oxidizing anaerobes per cubic cm of sediment, and several of them were found to be hydrogenogens. Another development in this area included the invention of 'high temperature compost' by a Japanese company Sanyu, where the inside temperature increased up to 95°C or even higher. T. Oshima (Japan) reported isolation of a new Gram-negative, rod-like, non-spore-forming extreme thermophile YMO81 from the high-temperature compost that grows aerobically up to 83°C. A genus nov. and species nov. *Caldaterra satsumae* was proposed for this thermophile.

In the gene expression session, several scientists presented their works on understanding the regulation of transcription in hyperthermophiles like *Pyrococcus furiosus*, *Thermococcus kodakaraensis*, *Methanocaldococcus jannaschii* and *Sulfolobus solfataricus*. John Reeve (USA) presented work on gene expression in *T. kodakaraensis*. *In vitro* studies revealed that *T. kodakaraensis* RNA polymerase initiates transcription from a wide variety of promoters, and transcription factors TFB1 and TFB2 could have unique roles in facilitating activated transcription from different promoters.

Roger Garret (Denmark) and David Prangishvili (France) presented work on DNA viruses of *Sulfolobus* and *Acidianus*. These viruses are included in Rudiviridae and Lipothrixviridae. Aspects such as their comparative genomics, genome replication and structure and function of viral proteins are being understood.

Peter Schonheit (Germany) discussed about the modified ED pathway in the thermoacidophilic *Picrophilus torridus* and the reaction mechanism of ADP-forming acetyl CoA synthetase in *P. furiosus*. Michael Danson (England) explained about the discovery of a multienzyme complex 2-oxoacid dehydrogenase complex in *Thermoplasma acidophilum*. His team has been able to produce components by cloning and expression, and assemble them into an active multienzyme complex. Tadayuki Imanaka (Japan) presented recent findings on pathways involved in carbon metabolism of *T. kodakaraensis* KOD1. The Type III Rubisco was shown to be involved in the pathway for retrieving pentose carbon and redirecting it to glycolysis. Jeurgen Weigel and coworkers (USA) have been able to isolate *Natranaerobius thermophilus*, an anaerobic, halophilic and thermophilic bacterium from sun-heated salt lakes of Wadi Natrum, North Egypt.

The applications session included lectures by H. K. Kotlar, Johann Orylgsson, Dimitar Karakashev, Ross I. M. Wadsworth and Anita Skarstad on the use of thermophilic microorganisms in oil recovery, hydrogen, ethanol and biodiesel production. Hans Kotler (Norway) presented work on bioconversion of *n*-alkanes, waxes and cyclic compounds for generating less toxic diesel fraction. Johann Orylgsson (Iceland) described work on hydrogen and ethanol production by four saccharolytic moderate thermophiles. The isolates were shown to produce acetate, ethanol and H_2/CO_2 and lesser amount of lactic acid from glucose fermentation. One of the strains, AK_{17}, yielded up to 70% ethanol. In another presentation, Dimitar Karakashev (Denmark) reported that *Thermoanaerobacterium thermosaccharolyticum* PSU-2 yielded 2.53 mol H_2 mol^{-1} hexose at the rate of 12.12 mmol H_2 l^{-1} h^{-1} using an initial sucrose concentration of 20 g l^{-1} at pH 6.5 and at 60°C. The use of random drift mutagenesis (RNDM) for directed evolution of β-glucosidase from the thermophilic bacterium *Caldicellulosiruptor saccharolyticus* was described by Peter Bergquist (Australia). His talk was mainly focused on various advantages of using this technique over general methods of directed evolution. Continuing the discussion on thermophilic enzymes, the next lecture by Jenny Littlechild (England) concentrated on transaminase and dehalogenase from *Sulfolobus* spp. Both of these enzymes have been cloned and overexpressed, and the crystal structures have been established. Structural details to understand their thermostabilities, substrate specificities and reaction mechanisms were presented. The transaminase is a serine transaminase, while the dehalogenase is an L-2-haloacid dehalogenase that belongs to the super family of enzymes having a completely conserved aspartic acid residue important for catalytic activity. The production and characterization of phytase from the thermophilic fungus *Sporotrichum thermophile* was described by T. Satyanarayana (India). The phytase was shown to be insensitive to trypsin and pepsin, acid-stable and thermostable, and thus it suits application as animal feed supplement and dephytinization of soymilk. Rohit Sharma (India) presented work on the production of lipase by thermophilic bacteria. Some of the bacteria producing intracellular lipase were subsequently used for transesterification of long-chain fatty acid esters with methanol to yield methyl ester/s. The last lecture by Debananda Ningthoujam (India) was on protease production from an unidentified thermophilic *Bacillus* sp. isolated from garden soil in Manipur.

In parallel to the applications session, scientists presented work on thermophilic adaptations, and omics and systems biology. The role of disulphide bond in stabilization of the cellular proteins in some extremophiles has been well established. Neil King (USA) suggested that topological features of the protein chain such as bonding, linking and knotting might be important in some thermophiles. Cristian Danciulescu (Sweden), citing the example of glutamate dehydrogenase, emphasized that the placement of charged residues within the protein structure and their cooperative interactions contribute to stability of hyperthermophilic proteins. Coquelle Nicolas (France) presented a structural comparison of thermophilic, mesophilic and psychrophilic lactate dehydrogenases (LDHs) and revealed that amino acid substitutions, especially in the static core can affect thermal stability, whereas those in mobile regions affect the temperature dependence on enzymatic activity. F. T. Robb (USA) described the critical role of chaperones in protein folding and maintenance of unique conformation that contributes to their biological activity and stability. His group demonstrated that the combined expression of chaperones, heat shock proteins sHSP, HSP60 and prefoldin (pfd) from *Pyrococcus furiosus* and *Methanocaldococcus jannaschii* could protect *Escherichia coli* cells from high temperature, hypoosmotic shock and addition of ethanol to the culture medium.

Considering the pace at which microbial genomes are being sequenced, Hans-Peter Klenk (Germany) emphasized the potential advantages of using whole genome rather than just 16S rDNA sequences (that represent far less than 1% of the genomic information) and chemotaxonomical analyses in filling the deep phylogenetic gaps. With more than 500 prokaryotic genomes already published and more than 1000 projects ongoing on microbial genome sequencing, the future systematics would clearly be based on the analysis of whole genomes.

Tamotsu Kanai (Japan) described a novel transcriptional regulator, Tgr (Thermococcales glycolytic regulator) that functions as both an activator and a repressor in hyperthermophilic archaeon *Thermococcus kodakaraensis* KOD1. In response to sugar availability, the Tgr was shown to simultaneously control both glycolytic and gluconeogenic metabolism via direct binding to the Thermococcales glycolytic motif (TGM). Phil Wright (UK) discussed the potential of alcohol metabolism in crenarchaeon *Sulfolobus solfataricus* that possesses 13 putative alcohol dehydrogenase (ADH) genes. This bacterium is able to metabolize and survive solely on ethanol or *n*-propanol as carbon source, and zinc-dependent alcohol dehydrogenases, ADH-10 and the putative ADH-2 were shown to be integral to ethanol and *n*-propanol metabolism. Patrick Forterre (France) presented an updated phylogenetic analysis of gyrase and the archaeal domain. Reverse gyrase was suggested to originate from a branch common to Crenarchaea and Euryarchaea, and the mesophilic archaeon *Cenarchaeum symbiosum* could form the third archaeal phylum.

The poster session provided a sufficient time for interaction with scientists working on various aspects of thermophiles ranging from isolation, identification and characterization of newer heat-loving microbes, to the structural determination and directed mutagenesis of their thermostable proteins/metabolites to improve their performance, and their potential applications in biodegradation, oil recovery, ethanol, hydrogen and enzyme production and others. The conference successfully highlighted the recent advances, but also pointed out that much about the microbial world in hot environments remains unexplored. The tenth and eleventh thermophiles conferences will be held at Beijing (China) and Montana (USA) in 2009 and 2011 respectively.

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MEETING REPORT

Science and technology in Karnataka*

A one-day State-level conference on 'Science and Technology in Karnataka: Past, Present and Future' was recently organized in Bangalore. Eminent scientists and technocrats of Karnataka who are internationally renowned in their fields were invited as resource persons to deliver special lectures. Achievements made in the State during the last 50 years in various fields of science and technology (S&T), the present status and future scenario were lucidly explained by each one of them during their presentations.

In his welcome address M. R. Gajendragad (Member, Karnataka Science and Technology Academy (KSTA)), explained in brief about the activities of the Academy. The State Minister Ramachandra Gowda, inaugurated the conference and gave an overview on S&T in ancient India and S&T initiatives and programmes of the Government of Karnataka. He also unveiled the website of the KSTA http://kstacademy.org.

C. N. R. Rao (JNCASR, Bangalore), Chief Guest at the inaugural function, released the first volume of the quarterly journal of the Academy edited by P. S. Shankar. In his keynote address, Rao delved on different aspects, including poverty, equity between the poor and rich and how to compete with Western countries. While providing the statistics of contribution to science by Indian scientists, he noted that it is 2.74% quantitatively and only 0.5% qualitatively. Contribution to scientific research by Indian universities was 60% in the 1950s, which has now come down to 10%, he revealed. India's contribution to science was far better in the 1980s as universities contributed a great deal and that trend needs to be revived, he said. Rao regretted to mention that India spends only about 3% of its GDP on education, while China and South Korea spend 6% each. India should at least spend 5%, which would hopefully help in the development of education in the State. 'We need to catch up with other countries which are ahead in education' Rao opined. He felt sorry to mention that there is no separate budget for research in our universities. Democracy and science are the two important cornerstones for development of any nation, and according to Rao, finding even a single new idea is a big contribution to science. His advice to the young researchers/scientists was to work, finish and publish their findings without wasting time. According to Rao, India is on a low profile in three fronts: (i) number of Ph Ds in science, (ii) number of papers submitted and published, and (iii) number of universities contributing to scientific research. He compared India with Brazil, China, South Korea and the US on science indicators and found that India was ranked below all of them. If India has to do well in science, we should have more resources, as well as commitment and dedication to the scientific spirit. Many more young people need to come into science, he urged.

U. R. Rao (Chairman, KSTA), in his presidential address, gave an account of various action plans taken up by the Academy since its inception in September 2005 under his Chairmanship. The Academy has taken up various activities to popularize S&T and to create awareness about the innovations in these fields among the students and public especially those living in rural areas. To mention a few – conducting science quiz competitions for high-school students, science exhibition for degree students, training for high school teachers/students, institu-

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