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Highlight

Cold is cool, the human microbiota and taking multiple SIPs

Craig Daniels,[1] Carmen Michán[2] and Juan-Luis Ramos[3]*

[1]*Structural Proteomics in Toronto, UHN and University of Toronto, Banting and Best Department of Medical Research; C.H. Best Institute 112 College Street, M5G 1L6, Toronto, Ontario, Canada.*

[2]*Universidad de Córdoba, Campus de Rabanales, Dept. of Biochemistry and Molecular Biology, Edificio Severo Ochoa C-6, 2ª Planta, 14071, Córdoba, Spain.*

[3]*Estación Experimental del Zaidín, Consejo Superior de Investigaciones Científicas, C/Prof. Albareda, 1, E-18008 Granada, Spain.*

Cold is cool

Cavicchioli and colleagues (2011) report in *Microbial Biotechnology* on enzymes from cold places; they start indicating that the temperature of the bulk of the Earth's biosphere is below 6°C (e.g. 90% of the ocean's waters), and that this cold water sustains a broad diversity of microbial life. Permanently cold environments are also found on land, high mountains and polar regions. The exploration of those environments is revealing an unexpected microbial biodiversity (Campanaro *et al.*, 2010; Collins *et al.*, 2010). As a result Cavicchioli and colleagues propose that indigenous psychrophilic microorganisms having enzymes that function effectively in the cold can be exploited in biotechnology. The catabolic potential of these enzymes derives from their flexible structure which compensate for the low kinetic energy present in cold environments (Cavicchioli *et al.*, 2002). They highlight some of the key features of psychrophilic enzymes: decreased core hydrophobicity, increased surface hydrophobicity, lower arginine/lysine ratio, weaker inter-domain and inter-subunit interactions, more and longer loops, decreased secondary structure content, more glycine residues, less proline residues in loops, more proline residues in α-helices, less and weaker metal binding sites, a reduced number of disulfide bridges and fewer electrostatic interactions (Gerday *et al.*, 2000; Cavicchioli *et al.*, 2002; Siddiqui and Cavicchioli, 2006; Margesin and Feller, 2010). In their review article Cavicchioli and colleagues (2011) have re-examined in depth the most valuable psychrophilic enzymes. Among these are the DNA-modifying alkaline phosphatase that molecular biologists use to dephosphorylate DNA vectors before cloning to prevent self-ligation (re-circularization), and for the removal of phosphates at the 5′ termini of DNA strands before end-labelling by T4 polynucleotide kinase. Another more industrially relevant protein is the cellulase complex from an earthworm living in a cold environment that has both endo-β-1,4-D-glucanase and β-1,4-glucosidase activity properties which are useful for ethanol production (Ueda *et al.*, 2010). A cold-adapted xylanase is in use in baking (Collins *et al.*, 2006); the authors also point out that there is a high demand for lipases to use in biofuel production (Tuffin *et al.*, 2009) and in fine chemical synthesis (Aurilia *et al.*, 2008; Joseph *et al.*, 2008). A cold-adapted lipase isolated by screening libraries generated from oil-contaminated soil exhibited a high preference for esters of primary alcohols and a high selectivity for (R) enantiomers of pharmaceutically important substrates (Elend *et al.*, 2007).

Hydrolyses substrates have proven useful for cleaning applications in a wide range of industries, including laundry, food, dairy and brewing (Lowry, 2010). The link between a reduced wash temperature and improved energy conservation has been recognized by detergent manufacturers, with a reduction in wash temperature from 40°C to 30°C reported to produce a 30% reduction in electricity used, equating to a reduction of 100 g of CO_2 per wash (Nielsen, 2005). Proteases, amylases, lipases and cellulases, such as alcalase, natalase and lipolase ultra from Novozymes, have been used for low temperature (≥ 20°C) washing (Aehle, 2007).

Exploitation of biotechnological potential of psychrophiles is still modest and advances in the biology of psychrophile microbes may be achieved by studying specific models. One of these model organisms is the cold-adapted *Archeae Methanococcoides burtonii*. Cavicchioli's group have taken a proteomic approach aimed at identifying the differential abundance of proteins from cells grown at different temperatures (–2°C to 28°C). The

*For correspondence. E-mail juanluis.ramos@eez.csic.es; Tel. (+34) 958 181608; Fax (+34) 958 135740.

analysis enabled three largely distinct physiological states to be described: cold stress (–2°C), cold adaptation (1°C, 4°C, 10°C and 16°C) and heat stress (23°C and 28°C). The authors indicated that the rationalization for their observation is that psychrophiles are naturally adapted to growth at low temperature – particularly at 1°C, 4°C and 10°C, the protein profiles are indicative of cold adaptation and a physiological state well suited to a cold environment. Above 16°C cells grow faster but a stress response is induced at –2°C when *M. burtonii* exhibits a very low growth rate, decreased biomass yield, increased aggregation and EPS production. At this low temperature there is increased production of oxidative stress proteins, including ROS scavengers.

Perhaps the most unanticipated finding was the evidence for oxidative stress occurring during growth at both physiologically suboptimal (T_{min}) and supra-optimal (T_{max} and T_{opt}) growth temperatures. Oxidative stress appeared to manifest in distinct ways at each temperature extreme, and a number of aspects of the response were linked to methanogen-specific pathways. An interesting future research area would involve the investigation of other psychrophiles to evaluate if they demonstrate an equivalent oxidative stress response to these thermal extremes.

Taking multiple stable isotope probings

Stable isotope probing (SIP) methods are often used to identify microorganisms that use a particular growth substrate within a community. The methodology is primarily performed by adding isotopically labelled compounds to an environmental sample followed by the subsequent analysis of the isotope-labelled biomarkers that are produced in the target organisms. The approach has been applied to a myriad of molecules including lipids, nucleic acids and proteins. Although labelling of nucleic acids (DNA-SIP and rRNA-SIP) are well-established methods the techniques for mRNA-SIP are not well established. Dumont and colleagues present an interesting article in *Environmental Microbiology* describing the SIP of both mRNA and DNA (Dumont *et al.*, 2011). They utilized $^{13}CH_4$ to target aerobic methanotrophs, which are organisms that play an important role limiting the release of methane from numerous environments including lakes. Using their methodology they were able to label mRNA, DNA and rRNA and compare the results obtained from this simultaneous labelling. This combination of SIP approaches provided valuable information about the activity and growth of the methanotrophic populations and the cross-feeding of methanotroph metabolites by other microorganisms. Interestingly, the labelling of mRNA was quicker than the labelling of genes, demonstrating that mRNA-SIP is likely more sensitive than DNA-SIP. The authors also showed that labelling of *Betaproteobacteria* could be clearly seen by DNA-SIP and not by RNA-SIP, a result that suggests that cross-feeding of the ^{13}C was primarily detected by DNA-SIP.

This new methodology shows that DNA-SIP, rRNA-SIP and mRNA-SIP can provide complementary information, and that the relative abundance of labelled molecules can vary between phylotypes when using the different approaches. Using all three SIP methodologies will greatly improve the accuracy of conclusions drawn regarding the relative activity of a phylotype based on data from SIP.

A bacterial plant benefit

Bacteria associated with plants can be categorized as rhizobacteria, epiphytic bacteria and endophytic bacteria. Endophytic bacteria are defined as bacteria that can be detected within the tissues of apparently healthy plants (Schulz and Boyle, 2006). The majority of research on plant-associated bacteria has focused on rhizobacteria; however, interest in the diversity and role of endophytic bacteria is growing. The main reason for the attention to endophytes is the understanding that these bacteria can establish a plant-beneficial relationship. Thus, endophytes with plant beneficial traits are potentially excellent plant growth promoters or biological control agents for sustainable crop production. Malfanova and colleagues (2011) investigated 30 endophytic bacteria isolated from a variety of plant species growing near Saint-Petersburg in Russia. Using a broad screening technique they selected bacteria for various traits, which included properties beneficial to plants and DNA fragment patterns, subsequently reducing the pool of endophytes. The authors followed this by taxonomic identification in order to eliminate potential human and plant pathogens and then tested the remaining strains for their ability to promote radish root growth and protect tomato plants against tomato foot and root rot.

This extensive screening was rewarded with the isolation of *Bacillus subtilis* HC8, from the giant hogweed *Heracleum sosnowskyi* Manden, which was subsequently shown to significantly promote radish plant growth and protect tomato plants against foot and root rot. The putative compounds responsible for the plant growth promotion and antifungal activity were evaluated and the responsible metabolites were identified as the hormone gibberellic acid and (lipo)peptide antibiotics.

It is hoped that using endophytic bacteria that harbour the plant beneficial properties of natural compounds we can significantly contribute to the growth of plants on low-fertility soils and protect against pathogens without the continued use of petrochemical-based fertilizers and antifungals.

Human microbiome

The microbiota present in the human gastrointestinal tract has nutritional, metabolic, immunological and protective functions. Several studies have shown that in the early stages of life, the flora diversity is affected by diet, although the identification of the existing species displays contradictory results probably due to differences in the methodology used (Salonen *et al*., 2010). To avoid this, new studies are aiming to develop highly discriminative techniques that allow the differentiation of related species in easy culture-independent assays. The article presented by Boesten and co-workers in *Microbial Biotechnology* (2011) compares two of these methodologies (multi-species microarray versus qPCR) to study the presence of several *Bifidobacterium* in babies under six different feeding conditions including breastfeeding, and standard prebiotic or probiotic formulas. For the hybridization method, a platform from random DNA, specifically designed for detecting related bifidobacterial species, was used (described in Boesten *et al*., 2009). Both analyses gave comparative results although the qPCR analysis not only provided a more precise identification of the microorganisms but also gave quantitative information. Conversely, microarrays (and not qPCR) allowed one to draw a global picture that showed the sequential change in the species composition. Although the number of individuals tested in this study was very low, the results suggested that the sugar composition of the infants' diet directly affected the variety of their microbiota. This work is a clear example of how specifically designed microarrays can be a simple and affordable methodology to study uncultivable organisms in complex biological samples.

Another potential use of the human gastrointestinal flora is in the development of vaccines or drugs. Duong and colleagues (2011) present a study that brings this aspiration closer by facilitating the design of vectors that allow transcriptional regulation of target genes in response to different sugars in *Lactobacillus*. Mining the already published microarray data on *Lactobacillus acidophilus*, the authors selected three promoters for the construction of regulated expression vectors, those of the fructo-oligosaccharides, lactose and trehalose operons, and one additional promoter, from the phosphoglyceratemutase gene, for the construction of a constitutive expression vector. Vector efficiency was checked by placing the GUS reporter gene under the control of the various promoters in both *L. acidophilus* and *L. gasseri* species. The results were extremely positive, not only regarding the induction levels reached but also the specificity of the inducing sugar. Additionally, the oxalate-degrading operon was cloned into these constructs in order to generate strains with increased levels of oxalate degradation, a metabolic pathway not present in humans but desirable to prevent formation of urinary stones and gout. Apart from the obvious uses for the vectors described, it should also be emphasized that the inducers are cheap and readily available in the human diet. Further work needs to be completed to prove the newly generated vectors' effectiveness in other microorganisms and to eliminate the antibiotic resistance genes from the constructs.

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