

Conference report

The 27th International Symposium Specialized in Yeasts (ISSY27) "Pasteur's legacy: yeasts for health and biotechnology", Paris, August 26-29, 2009

The 27th International Symposium Specialized in Yeasts (ISSY27) recently took place at the Conference Centre of the Institut Pasteur in Paris. Over a period of four days (August 26-29, 2009), 256 scientists from 44 different countries gathered to discuss and exchange their views on "Pasteur's legacy: yeasts for health and biotechnology". There were 49 oral presentations from which 22, mostly given by young scientists, were selected from over 220 abstracts by an international scientific committee.

The conference was opened by an address of A. Sibirny, chair of the International Commission on Yeasts (ICY) which hosts ISSY meetings. A. Sibirny presented ICY and the Federation of European Microbiology Societies (FEMS), a major sponsor of this meeting who awarded Meeting Grants to 17 young scientists and 5 invited speakers.

As its title indicates, the aim of the ISSY27 meeting was to cover recent advances in yeast research relevant for both biotechnological applications and human health issues. Three conference sessions and one poster session (over 220 posters in total) were devoted to each topic.

The first session focused on advances in yeast population structure and in the origin of their biodiversity. Global analysis of intraspecific variability has recently exploded thanks to the introduction of massive high throughput genomic and post-genomic approaches. Two talks in this session as well as several others in following sessions illustrated this novel trend. Ed Louis reported on the genetic structure of yeast populations based on low coverage sequencing of 36 strains from each two *Saccharomyces* species, *S. paradoxus* and *S. cerevisiae*. Contrary to *S. paradoxus* which has evolved into clearly differentiated geographical subgroups, *S. cerevisiae* strains are much more closely related regardless of their geographical or environmental distribution. They exhibit however a large phenotypic diversity which is even increased in their offspring: using SNPs and linkage analysis appears as a promising way to identify major QTL underlying this diversity, a hot theme that was also illustrated by several other communications (e.g. S. Dequin, C. Ambroset). Through a combination of DNA hybridization on tiling arrays and of transcriptomic analysis, Josef Schacherer presented impressive results on a genome-wide analysis of variations in 63 *S. cerevisiae* strains from different origins. An association study of nucleotidic polymorphisms and of global expression profiles evidenced clear differences in linkage disequilibrium values between strains according to their environmental origins, and thus in their selection history. Clearly, such approaches may pave the way to new strategies for understanding molecular evolution of yeast populations as well as for harnessing genetic variability for strain improvement. The other talks were devoted to the recent recognition of the major contribution of interspecific hybridisation events in strain evolution. Beer yeasts, as well as a few wine yeasts, have been long known to be natural hybrids between *S. cerevisiae* and sibling species of the *S. bayanus* or *S. uvarum* type. S. Rainieri proposed lab made hybrids between *S. uvarum* and *S. cerevisiae* strains isolated from non fermented beverages as a mean to incorporate new traits in starter cultures. These hybrids may undergo multiple recombination events resulting in new phenotypic traits as exemplified by Ursula Bond in the case of beer yeast hybrids (*S. pastorianus*) and these events were shown to be induced (or selected) after exposure to environmental stresses. Recently, the contribution of horizontal transfer to the evolution of yeast species has been extended to many other cases: it now emerges as a rapid solution to the adaptation of strains to a new biotope. Several examples were discussed in the case of *S. cerevisiae* derived wine yeasts. A. Querol described strains that had acquired part of

the genome of *S. kudriavzevii*, a more cryotolerant species than *S. cerevisiae*, and reported on the hitherto unsuspected presence of *S. kudriavzevii* wild strains in rather warm, wine making associated, climates which may have permitted hybrid formation. S. Dequin (see below) reported events involving even more distant partners.

The second session, "From yeast genomes to functions", illustrated different spin-offs of genomic knowledge at strain level. It was opened by P. Baret who stressed the necessity to develop new approaches to overcome the current limitations of automatic annotation as the number of sequenced genomes increases exponentially whereas the manpower dedicated to manual curation remains constant. Relying on a small set of well chosen and manually curated genomes, a semi-automatic method was proposed to minimize bioinformatic propagation of nomenclature errors and a plea was made for a digital designation of gene families, alike the one used by the Enzyme Commission. S. Dequin reported on the sequence of a commercial diploid, heterozygous wine yeast strain (EC111) which resulted from the recent transfer of three large fragments of DNA from three species, two of them being quite distant. One of the contributors was indeed identified as *Zygosaccharomyces bailii*, a spoiling yeast that colonizes wine niches as does *S. cerevisiae*. Interestingly, this appears not to be an isolated event, since many wine yeast strains could be shown to harbour one or several of these same fragments. Unravelling the advantage conferred by these foreign DNAs remains critical if we want to understand why these events, as most other cases of horizontal transfer, were initially selected and then expanded. In a different field, Jens Nielsen nicely illustrated why building models for regulatory circuits cannot rely on a single -omic approach, but must integrate the results of different levels of observation (mRNA, protein, metabolites...), using two extreme cases as examples: one where transcription and metabolic fluxes showed no correlation (mainly metabolic and post-transcriptional regulation) and one where the connection was tight. Other regulatory networks were discussed by N. Soontorngun (non fermentable carbon source utilization) and K. Tyo (Unfolded Protein Response). Control of mRNA stability is obviously one level of transcript regulation, although not a much considered one. J. Perez-Ortin used different global approaches to get genome wide values for transcription and degradation rates. He showed that the degradation of some mRNAs was coregulated in a pathway-specific manner, suggesting existence of degradation regulons. The session was closed by C. Boone, who described robotized methods for identifying pathways through exhaustive search of synthetic interactions in *S. cerevisiae*, even in the case of essential genes. The methodology could be readily extended to the screening of new drugs that would affect these interactions. Interestingly, an offer was made to invite participants to take advantage of these facilities for developing their own target-specific screens.

The third session was dedicated to advances in genetic engineering of strains for specific purposes. Several talks concerned the development of new *S. cerevisiae* strains able to produce metabolites from non-food carbon sources like lignocellulosic materials, particularly from xylose or arabinose which are poorly fermented by *S. cerevisiae*. Organic acid production from xylose was discussed by M. Pentillä as well as the interest of introducing new pathways oxidising xylose to xylonic acid rather than reducing it to xylitol or isomerising it directly to xylulose. Although significant conversion rates were obtained, further engineering is needed. J. Pronk's group is working on strains expressing a fungal xylose isomerase. He insisted on the need to avoid successive fermentation of mixed substrates (e.g. glucose and then xylose or galactose), and discussed evolutionary engineering by serial flask transfer as a tool to shorten fermentation times. One bottleneck could be identified as the ATP supply under acidic conditions when glycolysis is slowed down on non preferred substrates and thus prevents efficient pumping out of protons. Another strategy was presented by E.

Boles who developed strains expressing a score of heterologous, codon optimized genes: a new bacterial xylose isomerase, a bacterial arabinose pathway and a *Pichia stipitis* arabinose transporter. Current efforts of his group are currently directed at engineering strains able to produce 1-butanol or isobutanol, biofuels which are energetically more attractive than ethanol. Finally, several talks illustrated current developments in the use of non *S. cerevisiae* strains for biotechnologies. Advances in the use of *Candida famata* for the production of riboflavin and derived flavin coenzymes were presented by A. Sibirny. Elaborate genetic engineering resulted in a very substantial increase of production yields, although there is still room for improvement if the process is to be more competitive than the current bacterial process. Manipulation of the *Kluyveromyces lactis* stress responses (oxidative stress, calcium/calmodulin pathway) was shown by D. Uccelletti to increase heterologous protein production in this yeast, whereas current developments of expression platforms in *Yarrowia lipolytica* and *Zygosaccharomyces bailii* were summarized by C. Madzak and P. Branduardi, respectively.

The three next sessions were devoted to yeast and human health. The first one, "Yeast for medicine", highlighted recent breakthroughs in three directions: vaccination, drug making and drug screening. There has been a recent rise of interest for therapeutic vaccines in the field of cancer treatment, using either patient cells or virus delivered tumour antigens, with two such vaccines being in the process of approval in the US. Use of a heat-killed yeast, genetically engineered to express specific tumour proteins, was proposed by J. Hodge as a promising and safer alternative. Yeast cells expressing CEA (carcinoembryonic antigen) were shown to elicit strong specific anti-tumour immune responses in mice. Vaccination extended overall survival and reduced tumour size in mice that have been injected. Results on human cells were promising enough to launch a phase I trial, and phase II is to start next year. Two examples of drug elaboration were presented. Fighting malaria, for which no effective vaccine exists, has become problematic in many countries due to the selection of *Plasmodium* resistant to current drugs (e.g. chloroquine). Artemisin is a novel and very effective drug extracted from plant, which is however in short supply. C. Paddon presented a non profit project funded by the Gates foundation to engineer expression of the artemisin pathway in *S. cerevisiae*. Sophisticated genetic engineering associated to the selection of a good yeast strain (CEN.PK2 appeared superior to S288c) resulted in the production of over 40 g/l of the precursor amorphadiene, which can then easily be chemically converted to artemisin. Another example was presented by B. Dumas who described the genetic tour de force that was carried out to have *S. cerevisiae* producing hydrocortisone, a process still under improvement. Using yeast to discover new drugs was discussed by two speakers. T. Oteiro explained that alpha-synuclein, the main protein aggregating in Parkinson's disease, causes growth arrest when overexpressed in *S. cerevisiae*. This permitted screening of the yeast deletion collection for synthetic effects and of a chemical library for drugs alleviating the phenotype. This pointed to genes involved in vesicle trafficking and lipid metabolism and may hopefully help in designing new therapies. M. Blondel used a yeast colony assay to screen for drugs inhibiting formation of a yeast prion. Some of these were also effective in blocking the PrP^C to PrP^{Sc} transition both in vitro and in an in vivo in mouse model. Two of these drugs were used in an affinity assay on yeast cell extracts to fish out the targets. These turned out to be the 25S rRNA, and more specifically its chaperone function, raising the intriguing possibility that rRNA might be involved in prion folding in both yeast and mammals.

The fourth session was devoted to "Yeast as models for human diseases". G. Daum summarized recent advances in lipid metabolism in yeast, focusing on formation and function of lipid bodies. These were shown to be highly organized structures with a lipid core

surrounded by lipases and ester hydrolases. The metabolic activity of these enzymes is integrated within a complex network of lipid metabolic pathways ensuring storage and remobilisation of lipids. R. Wickner concentrated on the formation of yeast prions and prion variants. He showed that amyloid formation was independent of the primary sequence of prion domains, although dependent on their global amino acid composition. This leads to allele specific, register assembly of beta sheet structures, which in most case result in specific phenotypic defects reflecting faithful transmission of each prion variant. B. Arcangioli analyzed the effects in *Schizosaccharomyces pombe* of a defect in tyrosyl-DNA phosphodiesterase, a mutation which results in axonal apoptosis and ataxia in humans. *S. pombe* mutant cells accumulate unrepaired DNA breaks in stationary phase, as a probable consequence of oxidative damages resulting from mitochondrial respiration, suggesting that similar mechanisms could be involved in ataxia. R. Rachubinski recently identified Inp2 as a peroxisome-specific receptor for the myosin motor Mio2. Inp2 is required for peroxisome partitioning during mitosis, it is phosphorylated in a cell-cycle dependent manner and localises predominantly to peroxisomes directed to the bud, but not to those remaining in the mother cell. M. Bolotin-Fukuhara used mitochondrial genetics to recreate in *S. cerevisiae* mutations in mitochondrial tRNAs mimicking those found in human diseases. A nice correlation was often observed between the intensity of the phenotypes elicited by these mutations in human and yeast. A suppressor search identified several candidates, including a translation elongation factor which acted as a general suppressor. Identification of such suppressors may help understanding the basis of background effects resulting in e.g. tissue or individual variability of the phenotypic defects.

The last session was devoted to "Yeasts as pathogenic agents". J. Heitman discussed recent advances in the analysis of *Cryptococcus* population structure, based on the discovery that mating events could occur not only between strains of opposing mating type, but also of the same mating type without mating type switching. This may explain how largely unisexual populations in large geographic areas still exchange information and generate more virulent recombinants. The special case of the recent outbreak of *C. gattii* in the Vancouver Island, possibly originating from importation of an Australian isolate, provided a clear example of the rapid diversification which can occur within this species. F. Odds advocated the use of new tests to evaluate strain virulence more rapidly and more efficiently than current mouse lethal challenge tests. He could show, in the case of *C. albicans* injected intravenously to mice, that early measurement (12h after injection) of cytokine and neutrophil levels in the kidneys were robust indicators of the outcome of the infection. F. Muhlschlegel addressed environmental sensing in different yeast pathogens like *C. albicans*, *C. glabrata* and *C. neoformans*. He focused on a hitherto poorly studied pathway, CO₂ sensing mediated by carbonic anhydrase and adenylyl cyclase. CO₂ is a strong inducer of yeast to hyphae transition in *C. albicans*, a major virulence factor of this organism, and may be sensed directly through adenylyl cyclase. Finally, D. Poulain summarized recent work on beta-1,2 oligomannosides in *C. albicans*. These sugars are characteristic of pathogenic yeasts and are associated to surface phosphoproteins (PPM) and phospholipids (PLM) where they play a prominent role in host cell adhesion and modulation of the innate immune response. The biosynthetic genes (8 *BMT* genes) have been recently identified and their step wise action in beta-mannane biogenesis has been characterized. Independent transcriptional regulation of *BMT* genes was observed for PPM and PLM mannosylating activities.

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