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Brewer's Yeast and "Omics" Technologies

"Omics" technologies comprise genomics, transcriptomics, proteomics and metabolomics; the last three fields are pooled within the notion "functional genomics". In this review, these techniques which concentrate on aspects of the "course from gene to metabolites" are surveyed especially with regard to bottom-fermenting brewer's yeasts.
With the aid of these global methods it is possible to combine a collective knowledge of an investigated organism which is necessary to understand the details of its metabolic system. Hence, the challenge is to introduce the above mentioned studies for the determination of targets and approaches for the improvement of yeast organisms.
Herein, brewing yeasts are reviewed with concern to the determination of their "ome" levels. But because of the hybrid nature of the brewer's yeast's genome problems emerged regarding the "omics"-applicability which are depicted in this paper furthermore.
This Minireview is the first section of a two-part publication. The second part with the title "Reduction of diacetyl production by brewer's yeast" will be published in the next issue of Brewing Science in February 2010.

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1 Introduction

Yeasts have been used over centuries for the production of wines, bakery products and beer. But the fact that microorganisms such as yeasts are responsible for the fermentation and by-product formation has only been known for about 150 years. Today, these yeasts are used precisely. Especially for the beer production the selection of adequate yeasts is necessary to guarantee an optimal brewing process and taste of the beverage. In general, two types of beer can be classified: ale beers using top-fermenting yeasts and lager brews using bottom-fermenting yeasts which each require different fermentation conditions and as a consequence, show a diverse product character. But various yeast species do not combine every desired trait in one strain and therefore, these yeasts still need to be optimised. Hence, for the brewing industry it is indispensable that yeast improvement is the main focus of research purposes. But due to the fact that over 90 % of the worldwide produced beer are lager brews the research concentrates on lager brewing yeasts [1].

2 Brewer's yeast's hybrid genome

Brewer's yeasts of the *Saccharomyces sensu stricto* group differ from *Saccharomyces cerevisiae* regarding their genomes. Specifically, the size of the lager brewer's yeast's genome is approximately twice the size of *S. cerevisiae* and therefore reflects that lager brewer's yeasts vary from other brewing yeasts [1, 2]. Their genome is a combination of at least two species of which one was clearly identified as *S. cerevisiae*. The other ancestors were considered to be derived from another *Saccharomyces* species [1, 3]. Older results hypothesised that brewer's yeast is a hybrid of *S. cerevisiae* and *S. monacensis* or *S. bayanus* [4–6]. However, subtelomeric sequence hybridisation has suggested that *S. monacensis* is likely to be a closely related hybrid to brewer's yeast, rather than an ancestor [3]. Earlier, outcomes argued for *Saccharomyces uvarum* and/or *Saccharomyces bayanus* so that the lager brewer's yeast's genome could have been composed of three different ancestors. Furthermore, there were findings that lager yeasts consist either of the pure lines *S. uvarum* or *S. bayanus* or they are combinations together with *S. cerevisiae*. In addition, there could be a fourth part which may also originate from *S. uvarum* and was named "lager" according to its first revelation from lager brewer's yeast [7]. Moreover, *Gonzalez et al.* [8] and *Naumova et al.* [9] postulated new hybrids consisting of *S. cerevisiae* and other yeasts of the *Saccharomyces sensu stricto* group like *S. kudriavzevii* which constitute to the diversity and complexity of brewer's yeasts. But still it is unclear whether these hybrids are "real" species or may be only breedings of the same species. Recently, one widely used strain (W34/70) had been fully sequenced in order to finally clarify the origin of the brewer's yeasts. The outcome was brewer's yeast being a hybrid of *S. cerevisiae* and *S. bayanus* with two sub-genomes and a *S. bayanus*-based mitochondrial genome thus giving the possibility for the application of new technologies and basic research [10].

The type strains of bottom fermenting brewer's yeast have formerly been named *S. carlsbergensis* or *S. monacensis* but now the hybrid genome lines which contain a *S. cerevisiae* genome part should be named *S. pastorianus* [7].

3 "Omics" technologies

The availability of complete genomes for an increasing number of organisms brought about a strong need for comprehensive methods of analysis to take advantage of these complete inventories of genes

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[11]. Because only sequencing an organism's genome does not give any details of its enzymes and their encoding genes the field of "omics" technologies was created to understand the sequence information better [12]. These techniques have become important tools in many *metabolic engineering* strategies and furthermore have facilitated the understanding of organisms' metabolism [13]. These are mainly genomics, transcriptomics, proteomics and metabolomics. Genomics concentrate on the study of genomes of an organism. Within functional genomics (FG), the levels of the transcriptome, the proteome and the metabolome reveal the complete set of the mRNA molecules, the proteins or the metabolites of a cell. These techniques have allowed the identification of genetic differences (transcripts) and gave insight into their cellular effects (proteins, metabolites). Furthermore, they have provided an insight into the cellular response to genetic alterations or environmental changes. The accumulation of such in-depth knowledge is highly advantageous for elucidating functions of novel genes and gives a cause for thoughts about their *genetic engineering* with regard to organisms' improvement [12].

3.1 Determination of the 'ome' levels of brewer's yeast

As previously mentioned lager brewer's yeast strains are hybrids between *S. cerevisiae* and *S. bayanus* (see section 2). As the genome sequence of brewer's yeast has not been publicly available until lately the application of "omics" technologies in brewer's yeast studies has mostly been performed by employing the current knowledge of the *S. cerevisiae* genome sequence. Nevertheless, recent approaches regarding the 'ome' levels of brewer's yeast studies can be found in the literature [13].

For the diversity and complexity studies of brewer's yeast genomes comparative genomic hybridisation (CGH) using microarrays was carried out. The results showed the diversity of genome composition and possibly occurred hybridisation events between different lager brewer's yeast strains suggesting their hybrid genomes [14]. In addition, large segments of the *S. cerevisiae* DNA were found to be absent in lager brewer's yeast [15]. *Pope et al.* [16] combined both microarray and amplified fragment-length polymorphism (AFLP) techniques for the discrimination between various lager strains which resulted in their relatively good characterization.

Transcriptional profiles of brewer's yeast during the fermentation process highlighting different points in time were studied by the application of DNA microarrays [17–20]. So far, these studies have been performed with *S. cerevisiae* gene chips. As the information for the lager genome part is not available using *S. cerevisiae* chip technology two-species microarrays for the detection of the brewer's yeast transriptome level have been developed [14]. Microarray approaches have provided valuable insights according to the involvement of genes in various cellular processes and even for the detection of genes which were expressed under certain conditions or involved in particular pathways and therefore could help to understand the yeast physiology during brewing processes [21, 22].

For the detection of the translated proteins (proteomics) in brewer's yeast strains two-dimensional (2D) gel electrophoresis as well as MS techniques had been used. The first proteome maps of lager brewer's yeast were presented by *Joubert et al.* [23] and *Kobi et al.* [24]. Herein, the relatedness between different brewer's yeasts and *S. cerevisiae* and the co-migration of the proteins could be observed. Furthermore, different fermentation stages as the lag and early exponential phase were investigated by proteomics studies to determine the early-induced proteins [25]. These proteome analyses allowed the identification of many novel non-*S. cerevisiae* proteins of lager brewer's yeast [26]. Although the results obtained via transcriptome analyses concerning the complexity and relation of investigated brewing yeast strains could be confirmed by proteome analyses, the expression levels of the genes and their encoded proteins differed [25]. Once again, these findings indicate that the amount of the gene product (protein) cannot be predicted with the aid of the expression signals of the activated genes.

Metabolic footprinting methods such as GC/MS have also been applied for the discrimination between brewer's yeasts besides transcriptomics and proteomics [16]. Moreover, the researcher is able to characterise various strains concerning their metabolic profile using these powerful tools. The obtained information helped to design genetic changes for improving brewer's yeasts and accordingly monitoring the adjustment of industrial fermentation conditions [27]. Numerous attempts are described for *metabolic engineering* strategies [28-32].

3.2. Emerging problems in brewer's yeast's genetics

In comparison to euploid yeasts *genetic engineering* of brewer's yeast is much more difficult because of its hybrid genome and aneuploidy respectively. Although overexpressions of specific genes are feasible, gene deletions for inactivation of metabolism functions are complicated due to the fact that usually two copies of each genomic part (*S. cerevisiae* and non-*S. cerevisiae*) have to be disrupted. Here, for the selection of correct clones either four dominant drug markers have to be applied [1]. When the marker shall be removed for further use still three selection markers are needed. To facilitate genetics for brewer's yeasts and to get a deeper knowledge of cellular regulations, sequencing of their genomes is required. Recently, the genome of one lager brewer's yeast strain (WH 34/70) used in the brewing industry had been sequenced and is now publicly available [1, 10].

Gene expression analyses for brewer's yeast strains are in general complicated because so far only the *S. cerevisiae* chip technology had been deployed for determining brewer's yeast's transcriptome level [18, 33]. Array-based CGH approaches failed to differentiate between studied strains as the information for non-*S. cerevisiae* genome part cannot be obtained. For a better opportunity to precisely discriminate between lager brewer's yeast strains a two-species microarray has been developed. It is based on the genome sequence of one *S. cerevisiae* strain and contig sequences of one *S. bayanus* var. *uvarum* strain [14]. But the exploitation of this two-species microarray could not fully evaluate the genotype of lager brewer's yeast. This is caused by the estimation that the *S. bayanus* sequence which contributed to the lager brewer's yeast genome was about 10% divergent to the sequence of the *S. bayanus* var. *uvarum* strain which provided the basis for the microarray [14]. Another possibility for the detection of brewer's yeasts transcripts is the specific construction of bottom-fermenting yeast oligoarrays [34].

The major challenge will be the construction of a multi-species-array for the detection of the sum of brewer's yeast genes.

Also, proteome studies have been hampered due to the lack of the non-*S. cerevisiae* sequences. Only *S. cerevisiae* databases are available which have been used for the identification of found proteins after application of separating gelelectrophoresis and following MS techniques. But, of course, the non-*S. cerevisiae* proteins cannot be distinctly identified with this common methods. The identification of non-*S. cerevisiae* proteins requires methods such as tandem MS or nano-electrospray tandem MS/MS and has to be based on sequence homologies [25, 26].

Still there are certain limitations detecting metabolite structures of brewer's yeasts. The interpretation of metabolic studies will be more difficult because one has to consider both the *S. cerevisiae*-derived and the non-*S. cerevisiae* genes having an impact on the translation into functional proteins. The regulation of the different genomes still remains unclear and thus, the research shall proceed with this goal.

4 Conclusion

Applying the global "omics" technologies has already enabled the accumulation of in-depth knowledge about cellular activities of yeast organisms since these technologies have been applied frequently. Combining these methodologies one is able to get desirable insights from the genotype to the phenotype [12].

Implementing "omics" for further investigations and improvements of brewer's yeasts is much more challenging because of the diversity of *S. cerevisiae* and brewer's yeast's genomes. Since these analyses are based on the current knowledge of the *S. cerevisiae* genome sequence they cannot be simply applied to lager brewer's yeast studies. Nevertheless, the now accessible whole brewer's yeast sequence will afford the scientists to carry out comprehensive expression analyses and genome structural analyses [1, 10]. Furthermore, the construction of the bottom fermenting yeast DNA microarray will strongly facilitate the enlargement of the basic knowledge. Both the progress of *genetic engineering* and the application of 'ome' analyses have led to the creation of numerous novel brewer's yeast strains with high benefit for the brewing industry including possibilities to control beer processes and their sustainability.

But as long as the connections of the brewer's yeast's *S. cerevisiae* and non-*S. cerevisiae* genes with their accompanying proteins and metabolites are not entirely clarified the knowledge about the cell as a whole system will remain incomplete.

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