GENETIC DIVERSITY AND EVOLUTION OF FERMENTATION YEASTS FROM SACCHAROMYCES SENSU STRICTO COMPLEX -review-

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Abstract

The Saccharomyces genus includes two groups of species: Saccharomyces sensu stricto (designated van der Walt as the Saccharomyces species strictly associated with the fermentation industry) and Saccharomyces sensu lato. In this work we'll focus on the Saccharomyces sensu stricto yeasts. Over the years, the grouping of Saccharomyces sensu stricto yeasts has changes, name of species have also undergone changes and that caused confusion for yeast scientists and fermentation technologists. The present work evidences the importance of genetic variation and explores the importance of artificial inter- and intraspecific hybridization to obtain yeast strains targeted for specific industrial application.
Keywords: *genetic variation, evolution, Saccharomyces sensu stricto*

Introduction

Saccharomyces sensu stricto is a species complex that includes most of the yeast strains relevant in the fermentation industry as well as in basic science. The taxonomy of these yeasts has always been controversial, particularly at species level. Over the years, the grouping of Saccharomyces sensu stricto yeasts has undergone changes in accordance with the system employed in classifying yeast cultures. Names of species and single isolates have also undergone changes that have caused confusion for yeast scientists and fermentation technologists.

Yeasts represent a quite divergent group of fungi that exist predominantly as unicellular organisms. They include important industrial organisms that have been used for many centuries, pathogens and popular laboratory organisms that serve as general models to understand the eukaryotic cell.

The genus Saccharomyces includes strains commonly used in the fermentation industry as well as species of scientific relevance. Studies on the genus Saccharomyces date back to 1838. Saccharomyces cerevisiae was the first yeast species to be described.

In 1870, M. Reess differentiated and named Saccharomyces ellipsoideus as the yeast fermenting fruit juices and Saccharomyces pastorianus as the brewing yeast.

In 1912, A. Guilliermond established the first system for yeast classification, based on cell morphology and on a few physiological tests such as the ability of yeasts to ferment a number of monosaccharides.

The system for yeast classification was progressively enlarged. The number of physiological tests (mainly based on fermentation and assimilation of different compounds as sole carbon or nitrogen sources) increased and evaluation of biochemical features was also included. Table 1 show, as an example, the different grouping and naming of Saccharomyces sensu stricto according to the most relevant classifications between 1912 an 1998. (Guilermond, 1912; Lodder,1952, 1970; Barnett, 1983; Kurtzaman, 1998).

The paucity of traditional criteria gave rise to more accurate systematics established on nucleic acid analysis. Introduction of these methods reshuffled some yeast species between genera and other species were reclassified as identical species. Initially, the advent of nucleic acid analysis appeared to be like a salvation. In fact, detailed studies showed the usefulness of species classification but revealed complications in deciding whether the given species are identical or only closely related. It is because the shape of the phylogenetic three depends (i) on the calculation approach, (ii) selection of analyzed sequence and (iii) tricks depicting statistical confidence. Therefore, fertility of progeny, a long known simple criterion, is the final evidence to prove identity of yeast species. Recently an alternative approach taking advantage of nucleo-mitochondrial compatibility has been suggested.

Genetic Diversity and Evolution

A biological species is a group of organisms defined by their inability to mate successfully and produce viable offspring with other species. Barriers that prevent successful mating can be either prezygotic, blocking fertilization, or post-zygotic and their origin in nature is still poorly understood (Piskure, 2004). Yeasts with their well-characterized genomes

now represent ideal models to understand the molecular background underlying speciation.

Table 1: Changing of the Saccharomyces sensu stricto species according to the most relevant taxonomic monographies (Rainieri, Zambonelli and Kaneko, 2003)

1912	1952	1970	1984	1998
S. cerevisiae S. ellipsoideus S. turbidans S. ilicis S.vordermanni S. sake S. cartilaginosus S. batatae S. tokyo S. yeddo	S. cerevisiae	S. cerevisiae	S. cerevisiae	S. erevisiae S. bayanus S. pastorianus S. paradoxus
S. willianus S. intermedius S. validus	S. willianus			
S. coreanus	S. coreanus	S. coreanus		
S. carlsbergensis S. monacensis	S. carlsbergensis S. uvarum S. logos	S. uvarum		
S. uvarum S. logos S. bayanus S. pastorianus	S. bayanus S. pastorianus S. oviformis S. beticus	S. bayanus		
	S. heterogenicus	S. heterogenicus		
	S. chevalieri S. fructuum	S. chevalieri		
	S. italicus S. steineri	S. italicus		
	S. globosus	S. globosus		
		S. aceti S. prostoserdovi S. oleaginosus S. olaceus		

The *Saccharomyces sensu stricto* yeasts, including S. bayanus, S. cerevisiae, S. paradoxus, S. cariocanus, S. kudriavzevii and S. mikatae, represent an isolated and well-supported monophyletic group with overall phenotypic similarity (Kurtzman and Robnett, 2003). These species can mate with each other , but interspecific pairings result predominantly in sterile hybrids (Naumov, 1996; Marinoni et al., 1999), and interactions between the

nuclear and mitochondrial genome might also be impaired (Sulo et al., 2003). Rearrangements within nuclear genome have been common during yeast evolution. In the Saccharomyces complex, chromosome numbers and size have been changed (Langkjaer et al., 2000), gene loss has taken place (Blandin et. Al., 2000).

Chromosome translocations found in Saccharomyces sensu stricto have been mapped previously and, surprisingly, the number of translocations relative to S. cerevisiae does not correlate with the sequence-based phylogeny (Fisher et al., 2000). Therefore, chromosomal translocations in yeast might contribute to the reproductive isolation among sensu stricto species, but are not the only cause as speciation. This hypothesis has been tested recently by Delneri et al. (2003) by re-engineering the yeast chromosome sets. Based on the availability of the genome sequences and the development of a method for generating precisely opted strains of S. cerevisiae for studies on reproductive isolation. The imposed collinearity allowed the generation of interspecific hybrids, in crosses among otherwise different species, which could produce viable but aneuploid spores (Delneri et al., 2003).

Another important point in the above work (Delneri et al., 2003) is the existence of a mechanism for chromosome quality control following inter-species crosses (Wolfe, 2003). While hybrid zygote formed among sensu stricto species preserve both parental chromosome sets, hybrid zygotes obtained from crosses among less related Saccharomyces species tend to eliminate most of the chromosomes from one of the parents (Marinoni et al., 1999). Similarly, spores produced by sensu stricto interspecies hybrids often retain the complete set of chromosomes from one parent and about half the chromosomes from the other (Delneri et al., 2003). Apparently, the presence of the complete genome of only one parent (aneuploidy) provides a way to overcome the meiotic problems caused by translocation differences. New genome sequences from Saccharomyces and other related yeasts will now increase the opportunities for future experiments on chromosome stability and species barriers, such as searching for specific genes that are directly involved in these processes.

Moreover, a significant complication appears to be the frequent interspecies mating, since many technological *Saccharomyces* species are infertile hybrids. However, genome duplication and tetraploidy seems to be an important source of evolutionary novelty.

During the past few years, intensive research has been focused on elucidating molecular mechanisms involved in yeast adaptation to industrial

process, and on reshaping the genomic characteristics, selected over billions of generations, of industrial yeast One of the most interesting mechanisms observed in the adaptation of members of Saccharomyces sensu stricto to industrial processes is the formation of hybrids. For example, it has been demonstrated that S. pastorianus is a partial allotetraploid resulting from the hybridization of S. cerevisiae and S. bayanus . A hybrid nature has also been postulated for some strains of S. bayanus, including the type strain .These hybrid strains had been already proposed as a separate group based on previous physiological and molecular characterizations of S. bayanus strains

The results led to retention of the S. bayanus epithet for these hybrid strains, and assignment of the the nonhybrid strains to the S. uvarum taxon. However, Naumov and Naumova suggested that the partial reproductive isolation of these two subgroups, as indicated by the semisterility of their hybrids, make them varieties within S. bayanus.

Another important mechanism of yeast evolution is through mobile genetic elements (Kellis 2003). Mobile genetic elements such as transposons, retrotransposons, and viruses have had a profound effect on the organization of host genomes. Their activity has led to rapid increases in eukaryotic genome size and has contributed to the stratification of eukaryotic genomes into euchromatic and heterochromatic compartments. The presence of homologous mobile elements at multiple sites in the genome provides fodder for intragenomic recombination events, leading to rearrangements, duplications, and deletions within the genome. Finally, because many mobile elements encode transcriptional regulatory sequences, introduction of such elements into a new genomic location can potentially change the transcriptional profile of nearby host genes. Although some innovations in genes or gene expression may be selectively beneficial to the host genome, most mobile element insertions are likely to be deleterious (Wilke, 1992). Because mobile elements have the potential to reduce host fitness, it is useful to think of mobile elements and host genomes as being locked in long-term ''genetic conflict.''(Sawyer, 2006). Under this model, host genes are under constant selective pressure to limit the success of mobile elements, whereas mobile elements are under constant selective pressure to evade these limitations. The outcome of such a scenario is the rapid evolution of genes encoded by both the mobile element and the host. Two types of host genes are relevant to this conflict. First, many host genomes encode intracellular restriction factors, recently dubbed ''intrinsic immunity'' proteins, whose sole purpose is to limit mobile genetic elements such as viruses (Sawyer, 2006).

Practical Perspectives

Yeast have been widely used for millennia as cell factories. Some of the oldest products are alcoholic beverages, such as beer and wine, and bread. Later on, the processed food products were joined by vitamins, organic acids, lipids and recently also heterologous proteins, such as insulin, growth hormone, vaccines, etc. (Rose and Harison, 1993).

Yeasts belonging to the Saccharomyces complex have a number of unique characters not found in other yeast genera (Kurtzman and Fell, 1998). For example, Saccharomyces sensu stricto yeasts, as well as other Saccharomyces members, primarily degrade hexoses only to the C_3 and C_2 compounds pyruvate and ethanol, even in the presence of oxygen.

This phenomenon relies on a glucose repression circuit that represses the respiratory part in the presence of glucose (Johnston, 1999). The occurrence of fermentation under aerobic conditions is sometimes referred to as the Crabtree effect and the yeasts exhibiting it as Crabtree-positive yeasts (Pronk et al., 1996). While a majority of yeasts cannot grow in the absence of oxygen (aerobic yeasts), a majority of the Saccharomyces complex yeasts can also survive without any oxygen (Andreasen and Stier, 1953; Subik et al.., 1974; Pronk et al., 1996; Moller et al., 2001).

The life cycle of the Saccharomyces complex is also very unique. Saccharomyces sensu stricto yeasts are normally diploid cell that, under good nutritional conditions, reproduce by multilateral budding. Figure 1. shows diploid cells who can undergo meiosis generating one to four ascospores encapsulated into the modified mother cell (named an ascus). The ascus wall in Saccharomyces sensu stricto yeasts is persistent and does not spontaneously release the spores into the environment.

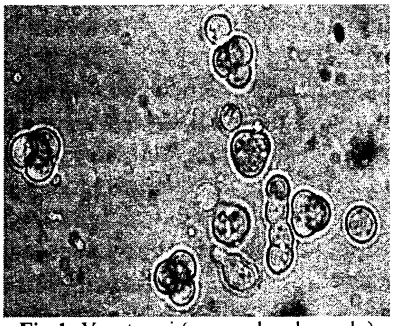

Fig 1: Yeast asci (www.phys.ksu.edu)

Meiosis occurs following the traditional scheme of eukaryotic cells; the ascospores representing the gametes.In Saccharomyces cerevisiae , starvation in a nitrogen-poor medium and the absence of a fermentable carbon source facilitate meiosis and the sporulation process. However, some Saccharomyces sensu stricto strains can also sporulate under good nutritional conditions.

According to the type of sexual cycle, Saccharomyces sensu stricto yeast can be heterothallic or homothallic. Spore sexuality is determined by gene occupying the MAT locus that is present in two allelic forms; MATa and MATα. In heterothallic strains, ascospores have a specific mating type (a or α) and cultures originating from such spores are maintained permanently in the haploid state (Rainieri and al., 2003). When two haploid cells with opposite mating type meet, they conjugate and produce a hybride that will be heterozygous for the mating type as well as for other characteristics. (Fig. 2)

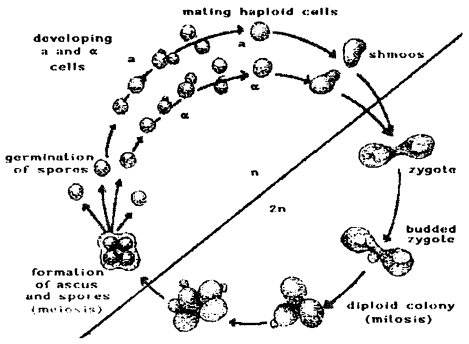

Fig.2: Life cycle of Saccharomyces sp. (www.bio.davidson.edu)

Lager - brewing yeast strains are natural hybrids between S. cerevisiae and another Saccharomyces sensu stricto species. Owing to the fact that more than half the genome has been unknown and because of the complicated genetic structure, application of genetic and molecular biology techniques has been challenging (Nilsson-Tillgren et al., 1981; Kielland-Brandt et al.,1995). The complex alloploid nature and co-existence of homeologous and hybrid chromosomes will continue to be a challenge for chromosome structure and recombination studies.

Strains with new properties, e. g. with mitochondrial markers, flocculation properties or expressing the killer toxin, have been generated by mutagenesis, hybridization or cytoduction (Barre et al.1993).Only a few of

these strains have been commercialized, including mitochondrial mutants, which have been very useful for implantation studies.

In conclusion, yeast has for millennia been one of man's favorite organisms, providing wine, beer and bread. During the last several decades, yeast has increasingly become a central model organism to understanding different biological aspects of the eukaryotic cell. Now yeasts are at the cutting edge of comparative genomics, providing a unique model and opportunity for the development of new tools to understand other eukaryotic and prokaryotic genomes.

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