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REVIEW ARTICLE

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Molecular evolution in yeast of biotechnological interest

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Abstract The importance of yeast in the food and beverage industries was only realized about 1860, when the role of these organisms in food manufacture became evident. Since they grow on a wide range of substrates and can tolerate extreme physicochemical conditions, yeasts, especially the genera *Saccharomyces* and *Kluyveromyces*, have been applied to many industrial processes, Industrial strains of these genera are highly specialized organisms that have evolved to utilize a range of environments and ecological niches to their full potential. This adaptation is called "domestication". This review describes the phylogenetic relationships among *Saccharomyces* and *Kluyveromyces* species and the different mechanisms involved in the adaptive evolution of industrial yeast strains.

Keywords Yeasts biotechnology · Adaptive evolution · Molecular phylogeny

Introduction

Food biotechnology is mainly concerned with the use of food-grade microorganisms, such as yeasts, filamentous fungi, and some bacteria, in industrial processes. The importance of yeasts in the food and beverage industries was only realized about 1860, when their role in food manufacture became evident. However, the use of yeasts dates back to ancient days. Before 7000 B.C., beer was produced in Sumeria. Wine was made in Assyria in 3500 BC and ancient Rome was making bread by 100 BC. Milk has been made into kefir and koumiss using *Kluyveromyces* species in Asia for many centuries [12].

Yeasts are unicellular fungi that can be classified into two phylogenetic groups: teleomorphic and anamorphic ascomycetous yeasts and teleomorphic and anamorphic basidiomycetous yeasts [12]. Differentiation between the taxa is usually achieved by comparison of morphological traits and physiological features [1, 11]; however, in some cases this can lead to an incorrect classification of species or a false identification of strains. Recent progress in molecular biology has led to the development of new techniques for yeast identification and characterization, and the specificity of nucleic acid sequences has resulted in several methods for rapid species identification. Comparison of RNA (rRNA) and its template ribosomal DNA (rDNA) has been used extensively in recent years to assess both close and distant relationships among many kinds of organisms including yeast species identification. Some of these methods are based on sequence analysis, primarily of the 26S rDNA D1/D2 domain [13] and of the 18S subunit [10]. Esteve Zarzoso et al. [7] proposed a rapid and easy method for routine yeast identification based on restriction analysis of the 5.8S rRNA gene and the internal transcribed spacers (ITS1 and ITS2). This database has been improved to allow identification of more than 300 yeast species (http://motor.edinfo.es/iata). Using the same methodology, but amplifying a different region, i.e. 18S rRNA and ITS1, Dlauchy et al. [6] constructed a database of restriction fragment patterns of 128 species associated mainly with food and fermented drinks.

Yeast are used in many industrial processes, such as the production of alcoholic beverages, baked goods, ethanol, CO_2, proteins, vitamins, pigments, and flavoring compounds. They grow on a wide range of sub-

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strates and can tolerate extreme physicochemical conditions. Although many genera and species of yeasts are used for these processes, ascomycetous yeasts of the genera *Saccharomyces* (particularly *S. cerevisiae*) and *Kluyveromyces* are the most important. However, while these two genera have been the subject of many recent studies, their phylogenetic relationships remain unclear.

Phylogenetic relationships among yeast species of biotechnological interest

The genus *Saccharomyces* has undergone innumerable taxonomic changes over the years. At present, 14 species of the genus *Saccharomyces* are accepted according to the latest taxonomic reviews [10, 30]. The 14 species are currently classified into three groups previously established by van der Walt [29]. The *Saccharomyces sensu lato* complex includes *S. barnettii*, *S. castellii*, *S. dairenensis*, *S. exiguus*, *S. rosinii*, *S. servazzii*, *S. spencerorum*, *S. transvaalensis* and *S. unisporus*. The second group comprises *Saccharomyces kluyveri*. The third group, which includes species of biotechnological interest, consists of the *Saccharomyces sensu stricto* complex, comprising four different taxa: the domesticated *S. bayanus*, *S. cerevisiae*, and *S. pastorianus,* all associated with the fermentation industry, and *S. paradoxus*, the only *Saccharomyces sensu stricto* species typically isolated from natural habitats (insects, tree exudates, etc.). Recently, three new species isolated from natural habitats, *S. cariocanus*, *S. kudriavzevii,* and *S. mikatae*, have been established as new taxa included in the *Saccharomyces sensu stricto* group [21]. The other genus with biotechnological interest is *Kluyveromyces*, containing 18 species, including the new *K. sinensis* [17], *K. hubeiensis* [18], *K. piceae* [31] and *K. bacillisporus* [15]. The genetics, ecology, and evolution of several species belonging to this group have been the subject of extensive investigations.

The reconstruction of evolutionary trees either by 18S rRNA gene sequences [4, 10], 26S rRNA gene partial sequences [14], or restriction analysis of the 5.8S rRNA gene and the two internal ribosomally transcribed spacers [2, 19, 27] results in intermixing these two genera with other genera of the Saccharomycetaceae family. Thus, comparison of rRNA genes is presently used to assess phylogenetic relationships among yeast belonging to the Saccharomycetaceae family, in which all the above genera are included [4, 13, 14]. However, there are few studies of other genes that corroborate the conclusions obtained from the rRNA phylogenetic analyses.

Mitochondrial DNA is widely employed in evolutionary studies of higher eukaryotes because it is a relatively small molecule, consisting mainly of coding sequences, and lacks the genetic complexity that complicates interpretation of nuclear data. However, yeasts show a paucity of mitochondrial genes. This is compensated in some species by the structural complexity of intergenic regions resulting from the distribution of optional introns, rearrangements, and/or insertions-deletions that encompass entire genes, and a complex pattern of changes from the universal code of codon usage [5]. Our group has sequenced the mitochondrial gene *COII* (also *COX2*), which encodes subunit II of the cytochrome *c* oxidase complex, from species of the genera *Kluyveromyces, Saccharomyces, Torulaspora* and *Zygossacharomyces*. These *COII* gene sequences have been used to determine the phylogenetic relationships among species of the genus *Kluyveromyces* and to other ascomycetous yeasts.

According to the phylogenetic reconstruction using the *COII* sequences, *Kluyveromyces* is a polyphyletic genus consisting of three groups (Fig. 1): one, which is monophyletic, is made up of the species *K. lactis*,

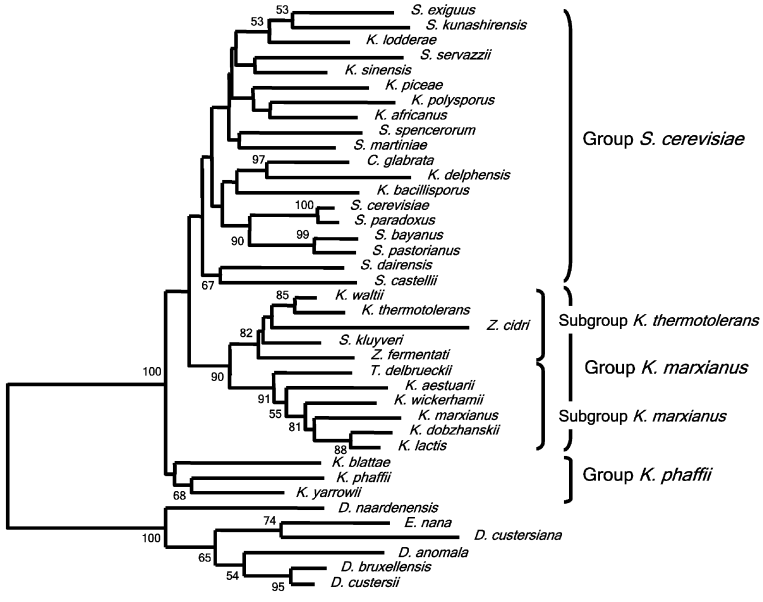

Fig. 1 Neighbor-joining tree based on total nucleotide divergences between pairs of *COII* sequences. Branch lengths are proportional to the scale given in substitutions per nucleotide. Percentage bootstrap values based on 1,000 pseudoreplicates are given on the nodes (see [2])

K. marxianus, *K. aestuarii, K. dobzhanskii* and *K. wickerhamii,* which appear to be closely related to species of the genera *Torulaspora* (*T.delbrueckii*), *Zygosaccharomyces* (*Z. fermentati*) and *Saccharomyces* (*S. kluyveri*). A second group includes *K. africanus*, *K. delphensis*, *K. lodderae*, *K. polysporus* and *K. yarrowii*, which appear intermixed with species belonging to the genus *Saccharomyces* (*S. exiguus*, *S. kunashirensis*, *S. servazii*, *S. spencerorum*, *S. martiniae*, *S. cerevisiae*, *S. paradoxus*, *S. bayanus*, *S. dairensis* and *S. castelli*) as a polyphyletic group. The species *K. blattae* and *K. phaffii* form distinct lines quite separated from each other and from the other two groups. These three groups, obtained from analysis of the *COII* sequence data, are congruent with the classification based on ascus morphology and their chromosomal patterns (Fig. 2).

Recently, these results have been confirmed by Kurtzman and Robnett [14] using different genes of the rDNA transcription unit (18S, 26S, ITS), single copy nuclear genes (translation elongation factor 1α, actin-1, RNA polymerase II) and mitochondrially encoded genes (small-subunit rDNA and cytochrome oxidase II). These authors proposed a reclassification of these species into different genera.

Adaptive evolution of yeast species of biotechnological interest

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. For a review of this adaptation in wine yeast strains see [24].

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* [9, 28, 32]. A hybrid nature has also been postulated for some strains of *S. bayanus*, including the type strain [23]. These hybrid strains had been already proposed as a separate group based on previous physiological and molecular characterizations of *S. bayanus* strains [22, 25]. The results led to retention of the *S. bayanus* epithet for these hybrid strains, and assignment of the the non-hybrid strains to the *S. uvarum* taxon. However, Naumov and Naumova [20] 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 adaptation is the capability to ferment specific substrates, as in the case of *Kluyveromyces lactis*. This species has been divided into two varieties, *K. lactis* var. *lactis* and *K. lactis* var. *drosophilarum* [16], a result of the assembling of several strains sharing a common physiological type. This subspecies delineation was suggested by Sidenberg and Lachance [26], who proposed the subdivision of *K. lactis* into these two artificial but easily recognizable varieties. Separation of these varieties was based primarily on ecological grounds. The variety *drosophilarum* was proposed for lactose-negative strains isolated almost exclusively from plants and invertebrates, such as *Drosophila*, tree exudates, and fermenting plant extracts, and the variety *K. lactis* var. *lactis* for lactose-positive strains isolated from dairy products. However, Belloch et al. [3] recently analyzed the phylogenetic relationships within the species *K. lactis*, and the electrophoretic karyotypes and phenotypic characteristics of strains representative of *K. lactis* var. *lactis* and var. *drosophilarum*. From this study, they concluded that the *K. lactis* taxon comprises two groups of strains. The first, and ancestral, group comprises lactose-negative strains isolated from natural

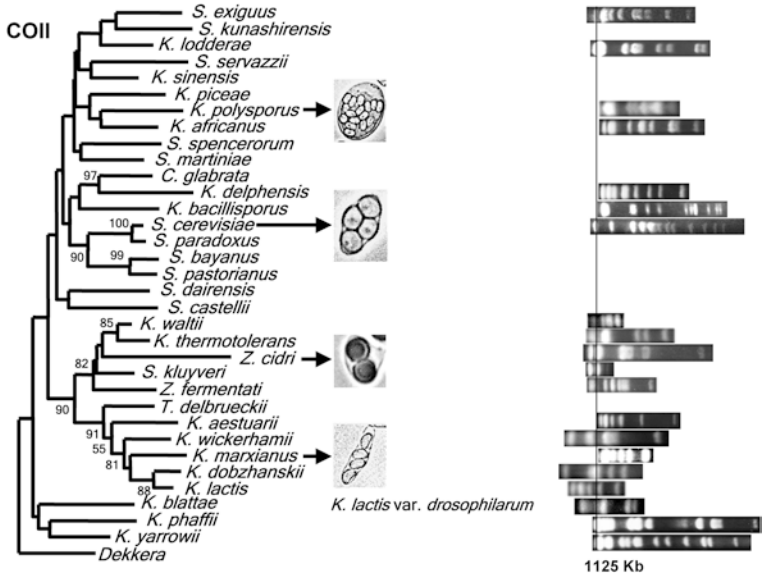

Fig. 2 Phylogenetic relationships according to the sequence of a mitochondrial gene (*COII*), and a comparison with ascus morphology and chromosomal patterns of some species of the Saccharomycetaceae family

habitats in North America, and the second, and derived, group includes both lactose-negative strains isolated from natural habitats in Europe and wine fermentations in South Africa, and lactose-positive strains associated with dairy products. This conclusion agrees with the analysis of reproductive isolation of these strains by Naumov and Naumova [20]. The large chromosomal rearrangement required to produce the very different chromosomal patterns exhibited by these two groups explains, at least in part, the reproductive isolation observed between American and European strains (including lactose-positive strains). Naumov and Naumova [20] proposed the subdivision of this *K. lactis* taxon into different varieties, var. *drosophilarum* and var. *phaseolosporus*, for the American lactose-negative strains, var. *krassitnikovii* for the European lactose-negative strains, and var. *lactis* for the lactose-positive strains. This last separation of the European lactose-negative and lactose-positive strains was also based on ecological grounds [26]. Hence, the present taxon *K. lactis* is a complex of different species and semispecies, or, at least, genetically structured populations, that lacks correspondence with the accepted subdivision into varieties. The close relationship between lactose-positive and European lactose-negative strains poses new and interesting questions as to the origin and evolution of the lactose fermenting capability with the *K. lactis* taxon.

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