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Facts, myths and legends on the prime industrial microorganism

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SUMMARY

Archaic speculations and firmly established legends regarding the origin of the yeast *Saccharomyces cerevisiae* and related species are revisited in light of past and recent ecological evidence pointing to a strict association with artificial, man-made environments such as wineries and fermentation plants. The nomenclature within this industrially important group is also discussed in view of the modifications imposed from application of molecular techniques to classification.

INTRODUCTION

The production of ethanol by fermentation of juices extracted by simple pressure from fruits and other plant parts (wines) or by hydrolytic breakdown of cereal starch (beer and saké) has been the most prosperous of mankind's industries since time immemorial. It is well recognized that the main and invariable actor of these *ante litteram* applications of biotechnology is a yeast of the genus *Saccharomyces* whose species name, *cerevisiae*, comes directly from the Latin name for beer.

As a consequence of this important role, *S. cerevisiae, the* yeast species by definition, the winning protagonist of millenia of bread, wine and beer making, probably the first living being domesticated by man, is one of the best known organisms on Earth, be it physiologically, genetically, morphologically or technologically. Unfortunately, in spite of the fact that its genome has been almost completely sequenced, two less sophisticated and advanced aspects of its biology are still surrounded by much confusion: ecology in natural environments and classification by means of conventional procedures.

The scope of this presentation is to increase the ecological and taxonomical awareness of those physiologists, biochemists, geneticists and fermentation technologists, who presently use *S. cerevisiae* as a model eukaryotic organism for their studies, most often referring to it simply with the restrictive informationless expression 'the yeast'. We hope to do this by clarifying those dark sides of the biology of *S. cerevisiae* that may still perpetuate old prejudices based on antiquated, inaccurate or completely wrong information, some dating back to Pasteur's time. This will be done by recounting two tales, with our yeast hero as the main protagonist. Both are utterly sad accounts: in the first one we witness a major relocation of our wine yeast from the glory of open spaces of natural environments to the obscure confinement of winery cellars and machinery. In the second story, we mourn the taxonomic death of most of the names of long known and renowned wine and beer yeasts.

THE ECOLOGY OF *SACCHAROMYCES CEREVISIAE*

The interest in a thorough revisitation of the ecology of *S. cerevisiae* in natural environments was reawakened in one of us (A.M.) by the reading of *The Life of Yeasts* by Phaff, Miller and Mrak [34]. More precisely, the trigger was a series of statements summarizing the views of experienced yeast ecologists. First of all (page 96), the routine use of the enrichment culture in liquid media was strongly questioned because '... minority types could easily outgrow majority types if the former found life in the artificial medium more advantageous' and consequently '... a completely erroneous picture would be obtained of the actual yeasts present in the material under study'. Another opinion was also expressed in relation to fresh, sugary fruits and fruit juices (page 128): 'It is interesting that *Saccharomyces cerevisiae* is not common as a natural spoilage organism.'

Then, in the second edition of *The Life of Yeasts* [35] the heretic view was explicitly expressed (pp. 230–231) that '*S. cerevisiae* (wine yeast) is a minority organism during the early stages of natural wine fermentation'. Finally, the whole ecological edifice of wine microbiology was torn to pieces (page 230). 'In our experience typical strains of *S. cerevisiae* (excluding *S. cerevisiae* var. *tetrasporus*) are rarely if ever present on the fruits and berries of wild species of plants'.

The impact of these unconventional opinions on yeast ecology was dramatic because it was directly in contrast to the firmly established view implying that *S. cerevisiae* is a dweller of natural habitats. Louis Pasteur [30] was the first to prove that the conversion of must into wine is a spontaneous process brought about by the resident yeasts of the grape surface. The occurrence of *S. cerevisiae* and its many taxonomic relatives

This paper is dedicated to Professor Herman Jan Phaff in honor of his 50 years of active research which still continues.

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(*S. bayanus*, *S. chevalieri*, *S. oviformis*, *S. pastorianus*, *S. italicus*, *S. fructuum*, and others still unknown) in fermenting grape musts was successively confirmed by an endless series of ecological surveys by several authors in many countries. These studies also confirmed that the wine yeast *S. cerevisiae* is not exclusively and strictly associated with vineyard and orchard soils where supposedly it feeds on the sugary fruits falling from plants, being also widespread in many natural environments. At the beginning of this century, it was commonly believed [14] that yeast cells are resident on the surface of sugary fruits: from there they reach the soil, either washed off by rain or along with the fallen fruits. At the onset of the summer these cells are conveyed back to the fruits by the carrying activity of winds, air currents or insects.

A definite pattern was even recognized [23] in the natural fermentation of sugary juices: lemon-shaped, slow-fermenting, apiculate cells (*Kloeckera apiculata*) always dominate the initial phase of fermentation whereas faster growing, elliptical cells (*S. cerevisiae*) take over after 3–4 days. The adjective 'natural' is still used in wine microbiology to designate a grape must fermentation occurring spontaneously when grapes are pressed into juices. Conversely, a guided fermention is that obtained by using a selected strain of *S. cerevisiae* as a fermentation starter which takes over the natural yeast flora.

This ecological ubiquitousness promptly gave rise to the doctrine that the 'abundant' *S. cerevisiae* flora of natural environments associated with wine-making must presumably conceal some particularly gifted strain to profitably utilize as a starter in wine-making. A logical consequence of this was the belief in the existence of a mythical 'superselected' yeast starter, able to make a 'superwine' even under extreme environmental conditions and from bad grapes as well. Its discovery was only a question of hard, screening work under the form of repeated ecological surveys on naturally fermenting grape musts.

A staunch supporter and leader of the above doctrine from 1930 to 1970 was Tommaso Castelli from the University of Perugia in Italy who in the 40–50s had involved several laboratories of different countries in and outside the Mediterranean basin in the quest for the superselected grape must fermentation superstarter. An endless series of surveys [7] on naturally fermenting musts and vineyard soils coming from innumerable different countries, regions, zones or microclimates confirmed the accepted pattern characterized by the predominance of *Kloeckera apiculata* and *S. cerevisiae* escorted by a few other, numerically limited, more or less occasional inhabitants. At that time, almost every wine and beer drinker was proud to know that the marvelous old friend of mankind was living everywhere in nature, ready every year to work the miracle of improving a normally tasting fruit juice to a sensational beverage.

The reading of these nonconformistic opinions of Herman Phaff and colleagues [34] was taking place while A.M. was working as a postdoctoral fellow in his laboratory in Davis in 1969. A.M. originally came from the same laboratory at the University of Perugia in Italy where Castelli was still teaching. Probably concerned about A.M.'s feelings due to his past involvement in some of those ecological surveys on wine yeasts, Herman Phaff touched this point gently, offering every now and then indirect clues and casual hints at the danger of transforming the enrichment culture into the main and only philosophy in ecological studies. The seed of doubt was evidently sown because, upon returning to Italy for a teaching appointment to the same chair Castelli had left after retirement, he decided to reconsider the origin of *S. cerevisiae*.

An initial literature search revealed (Table 1 A) that *S. cerevisiae* had been consistently found on grape skins, on the surface of other sugary fruits or in vineyard soils only in those ecological surveys of the past 100 years performed by using an enrichment culture in grape must. Incidentally, yeasts may be isolated from natural environments either by direct streaking on agar media such as malt agar or by using an enrichment culture in liquid media before the actual direct isolation. The rationale of this approach is to overcome the minority presence of some species by providing them with selective nutritive and/or growth conditions, suitable only for them while depressing other yeasts.

Evidently, the founding fathers of wine microbiology had failed to remember that grape must is already a powerful differential medium because its low pH (ca. 3.5) prevents the growth of most bacteria; that it only allows the growth of yeasts tolerating relatively high sugar concentrations (often >20%, w/v), and among them, only those able to ferment the substrate, and among the fermenting ones, only those capable of tolerating high concentrations of ethanol. In other words, the spontaneous fermentation of grape juices is definitely an enrichment culture, expressly tailored to *S. cerevisiae*.

On the other hand, all the investigations carried out on grape surfaces by direct isolation (without enrichment) constantly showed that *Kloeckera apiculata* is always the predominating inhabitant of the grape surface (ca. 75% of the cells); this numerical supremacy may explain its initial domination in natural fermentations. *Metschnikowia pulcherrima* is often present, followed by a group of film-forming (*Hansenula*, today included in the genus *Pichia*) or pigmented (*Rhodotorula*) species and by the ever present yeast-like organism *Aureobasidium pullulans*. Finally, the most significant finding of these studies was that *S. cerevisiae* is practically absent from grapes and vineyard soils (Table 1 B).

In order to confirm this absence, a large-scale search for *S. cerevisiae*, repeated for two consecutive years, was organized for more than 2000 single berries aseptically removed at different stages or ripening from casually selected clusters coming from two vineyards [37]. Each grape was inserted into a test tube with sterile grape must in order to realize a small-scale natural fermentation. Yeasts were present on 5% of grapes three weeks before vintage but increased to 60% during vintage. Fermenting yeasts such as *Kloeckera apiculata* appeared only during the last week while *S. cerevisiae* was isolated from only one of the 2016 berries examined (Table 1 B).

The logical conclusion was that *S. cerevisiae* must be associated with some other ecological niche. A literature search for some alternative location produced an old study carried out by two leading French enologists, Peynaud and Domerq [32], who found that *S. cerevisiae* was the main and

TABLE 1

Microecology of *Saccharomyces cerevisiae* in natural and technological environments

A) Presence of *S. cerevisiae* in natural environments as affected by the isolation procedure

Isolation source	Number of samples	Number of isolates	Isolation procedure	*S. cerevisiae* in all samples	Reference
Soil	517	1122	enrichment	83%	[5]
"	26	811	direct	0%	[9]
Wild fruits	26	415	enrichment	73%	[10,11]
"	6	180	direct	>1%	[12,13]
Grapes	55	877	enrichment	69%	[7]
"	31	3996	direct	0%	[1]

B) Direct isolation of *S. cerevisiae* from environments associated with grapes without using enrichment cultures in grape must or malt

Environment	Samples	Yeast flora	Reference
English vineyard	all vine parts, soil, air, animal vectors, other plants	fermenting yeasts (*S. cerevisiae*) were rarely found	[8]
Italian vineyards	grape surface during ripening (2016 sampled berries)	one colony of *S. cerevisiae* from one grape sample	[40]

C) Yeast flora of winery surfaces

Environment	Samples	Yeast flora	Reference
French cellar	various surfaces: walls, ceilings, floors, vats, machinery, hoses	*S. cerevisiae* predominates; a few non fermenting, film-forming species are always present	[32]
French cellars	same surfaces	*S. cerevisiae* predominates; same film-forming species	[3]
Italian cellars from different regions	same surfaces	*S. cerevisiae* predominates, same film-forming species	[37] [41]

constant colonizer of all the possible surfaces of a winery such as equipment, floors, walls, ceilings, vats, utensils, and the hands of people working in the plant (Table 1 C). Evidently, this new piece of ecological information had remained isolated, possibly because its implications patently contrasted with the dogmatic view of the time.

Additional investigations (Table 1 C) carried out by Bélin [4] and Rosini [38, unpublished data] confirmed the predominance of *S. cerevisiae* in wine cellars and the occurrence of several film-forming species of the genera *Pichia* (ex *Hansenula*) and *Candida* and as well as of some other sporadic 'non-wine' yeasts. On the assumption that the primary and exclusive habitat of *S. cerevisiae* is the various surfaces of the wine plant that are exposed at each vintage to billions and billions of its cells, Rosini [37,38] also followed the take over of a newly established plant by a labelled yeast starter (*S. cerevisiae* H_2S-negative, DBVPG[1] 1883) which is unable to produce H_2S during fermentation and forms white colonies on Nickerson agar medium whereas H_2S-positive wild yeasts give black colonies. Wine-making was carried out for two consecutive vintages with the H_2S-negative yeast as a starter and the result was that all surfaces of the winery were readily colonized by the labelled *S. cerevisiae* strain during the first year. The third year, fermentation was allowed to go on spontaneously with the result that the grape must was immediately occupied by the winery-resident labelled yeast that almost completely inhibited the growth of wild yeasts such as *Kl. apiculata* coming from the surface of the berries. Additional evidence was provided by Rosini et al. [41] on the role of the winery resident yeasts on fermentations carried out with selected starters. At this moment, on the basis of incontrovertible experimental support from the numerous surveys carried out on the yeast ecology of various natural and man-made environ-

[1] DBVPG = Industrial Yeasts Collection, Dip. Biologia Vegetale, Univ. Perugia, Italy.

ments associated with grape must fermentation, we must exclude a natural origin for *S. cerevisiae*.

In the statement of Phaff and colleagues [35] reported at the beginning of this section, a clear hint was introduced that only one member of the *Saccharomyces cerevisiae* group, the ex variety *tetrasporus*, is actually found in natural environments. Today this has been shown to be a separate species from *S. cerevisiae* [44] and these strains are classified under the epithet of *S. paradoxus* originally isolated and described by the Russian mycologist Anna Batschinkaya [2]. Since that time, strains of *S. paradoxus* have been isolated on at least three continents, usually associated with the exudates of oak trees [27,28,33] and never in fermentation environments as in the case of *S. cerevisiae*. This species will be further discussed in the following section dedicated to the classification of *S. cerevisiae* and related yeasts.

We would like to imagine the moment, buried in the night of time, when an unknown cave dweller, perhaps an obscure benefactor of human kind from Mesopotamia, happily discovered that fruit juices may become much more palatable if left to stand for a while. Perhaps at about the same time some unknown fermenting ancestor of *S. cerevisiae* realised that its survival chances were to be much improved by the association with Man and decided to leave the uncertain natural environments and become the first domesticated microorganism.

THE CLASSIFICATION OF *SACCHAROMYCES CEREVISIAE*

It is without doubt that our interest in the taxonomy of *S. cerevisiae* began as a consequence of the strict association with the laboratory of Herman Phaff. At about the same time that he and Sally Meyer, who was working on her Ph.D., were trying to extend to yeast the molecular approach already profitably used in bacterial systematics, A.E.V., then an undergraduate student, was working as a part-time laboratory technician. A.M., at UCD as a postdoctoral fellow, was developing together with H.J.P. an optical method for nDNA/nDNA reassociation in yeasts [25]. This procedure, that does not require labelling of reference DNA by a radioactive tracer, allows for the complete cross hybridization of all the DNA samples from a given group. This technique was preliminarily used to study species relationships within the genus *Kluyveromyces* [24,26].

Upon returning to Italy in 1972, we were eager to apply the newly developed procedure (as well as the expertise acquired in H.J.P.'s laboratory) to other yeast groups and genera. Due to previous ecological and enological experience of workers in the laboratory in Perugia, the choice of studying the 'sensu stricto' group of species of the genus *Saccharomyces*, was essentially an automatic decision. As a result, the 'massacre' began of innumerable well-known epithets which had been part of fermentation technology's vocabulary long before its development as an applied science.

At this point it would be appropriate to open a small parenthesis for a historical briefing on *S. cerevisiae*. Even though present on the alcoholic beverages scene for at least 10 000 years, our yeast superstar was actually seen only in 1684, when Antonie van Leeuwenhoek sent one of his letters to the Royal Academy in London with the first schematic representation of 'animalcula' living in fermenting malt. In the following 150 years, the low efficiency and scarce availability of microscopes prevented workers from reaching the logical conclusion that these oval bodies were actually responsible for the fermentation of beer. In fact, it was only in the 1820–30s that the biological nature of the process was recognized, when a team of young biologists composed of not-yet-famous scientists was advancing the then absurd theory that a specific living organism was responsible for the transformation. They were fiercely opposed by the powerful, prestigious and dogmatic Chemical School led by Justus von Liebig and Friedrich Wohler who strongly asserted that fermentation was no more than a simple, spontaneous chemical reaction. The fungal nature of van Leewenhoek's round bodies was first recognized in 1838 when Meyen [26] described the first yeast species assigning it a dual name according to the rules established by von Linné for animals and plants: *Saccharomyces cerevisiae* or 'sugar fungus of the beer'.

Then, in the 1860s, it was once again the Father of Microbiology, Louis Pasteur, who had the last word in this controversy, as he already had done in the dispute on spontaneous generation. He elegantly demonstrated that only when these oval, budding bodies, now called yeasts, are inoculated, multiply and grow in sugary liquids, can ethanol be recovered by distillation [29].

A few decades later, Emyl Christian Hansen from the Carlsberg Brewery in Copenhagen applied to yeast the recently introduced methodology for the acquisition of pure cultures and was the first to propose the use of selected yeast strains in brewing. He also did extensive research on yeast ecology in natural environments and described two species, *S. cerevisiae* and *S. ellipsoideus*, isolated respectively from fermenting malt and fermenting grape must [15].

At the beginning of this century, almost every investigator interested in alcoholic fermentation was busily occupied in isolating and classifying new species of yeasts. Many new epithets, well known to fermentation technologists, such as *S. pastorianus*, *S. carlsbergensis*, *S. chevalieri*, *S. uvarum* and several others were introduced.

The abundance of new species from these ecological investigations created the need for an efficient system of classification. The first attempt at establishing a coherent procedure dates back to 1912, when the Frenchman Alexandre Guilliermond proposed yeast classification based on a few physiological tests such as ability to ferment four or five monosaccharides. Interestingly, this was the first as well as the last application of such tests to fungal taxonomy since even today all fungi except yeasts are still classified only on a morphological basis. On the basis of fermentation tests of a few sugars [14], 20 different species were separated among the oval-elliptical-shaped, highly fermenting cultures isolated from grape musts and breweries.

This pioneering work was soon taken over by Albert Kluyver at the Technical University of Delft in Holland, who established the Dutch School of yeast taxonomy. As a matter of fact, the current classification of yeasts is the result of a joint effort of several taxonomists throughout the past 80

years. Most of them were women and many came from the Dutch School of yeast taxonomy that later generated the culture collection of the Centraalbureau voor Schimmelcultures (CBS), that was responsible for the series of volumes published on yeast taxonomy, starting in the 1930s [21–22,43]. The last monograph appeared in 1984 [19] while the new edition edited by C.P. Kurtzman and J.W. Fell is due to appear in 1995 with a chapter on the genus *Saccharomyces* prepared in our laboratory.

According to this bible for yeast taxonomists, from 55 to 70 different tests are considered necessary to classify an unknown yeast. Most of these tests are based on the assimilative (oxidative) utilization of different compounds as sole carbon or nitrogen sources for growth, a practice introduced in the 1950s by Lynferd Wickerham and coworkers [48] of NRRL, Peoria, Illinois, USA (still today a well known center for yeast taxonomy).

From now on we will refer to this procedure, based on these 55–70 taxonomic tests, with the name of Conventional Taxonomy as opposed to another series of procedures introduced later on, based on the comparison of informational macromolecules of the cell, that will be called Molecular Taxonomy.

In spite of the redundancy of the conventional approach [42], the separation of two species was often arbitrarily established on one or a few differences of single genes coding for hydrolytic enzymes. Herman Phaff was probably the first yeast taxonomist to emphasize the many classification problems created by conventional taxonomy due to the failure to establish clear-cut separations between species [36]. The main reason for the confusion caused by conventional taxonomy is the fact that, for the passage from genus to species, the discrimination between two taxa is established on the basis of only one or two characters, while all remaining phenotypic properties are essentially identical.

An example of this situation is the old classification of many wine species where the ability to ferment one single monosaccharide often permitted the separation of species, otherwise identical for all remaining characters. Genetic analysis of these strains has shown that the variable phenotypic expression of these fermentation characters may be the result of the presence of multiple gene loci which can be active, silent or missing from one strain to the other instead of the expression of genetically distinct species [6]. It has also been demonstrated that these same species are often polyploid or aneuploid. This explains why conventional mating as a tool for taxonomy, genetic studies and strain improvement has always been arduous and also why definite identification of a strain of *S. cerevisiae* or some other related yeasts is often an almost impossible task. This unstable physiological behavior was observed many times and by many authors for all the species related to *S. cerevisiae* and collectively associated with the fermentation of sugary juices.

On the basis of the preceding evidence, a few years ago many leading yeast taxonomists reached the conclusion that the separation of species on the basis of differences in single phenotypic characters, often governed by a single or at the most very few genes, can no longer be accepted. In other words, another approach to classification, more discriminating than conventional taxonomy, was needed.

Before introducing the procedures of molecular taxonomy, it could be interesting to give an example of the confusion caused by the above somewhat redundant and often unstable taxonomic approach originated and perpetuated throughout the past 90 years. A classical example is the 'sensu stricto' complex of the genus *Saccharomyces* so designated by a leading yeast taxonomist, the South African van der Walt [47], to include all species of the genus strictly associated with the fermentation industry.

Figure 1 shows schematically what happened to the species of this group over the span of 72 years from the beginning of yeast taxonomy to the latest edition of *The Yeasts, A Taxonomic Study* [19]. The 20 species described by Guilliermond in 1912 [14], were reduced to eight in the 1952 [22] edition, while eight more new species described after 1912, were added. The 1970 edition [47] reduced to eight the 16 species of 1952 but introduced 13 new taxa bringing to 21 the number of species associated with the fermentation industry. These were actually grouped in the especially created subdivision of the genus *Saccharomyces*, called 'sensu stricto' group.

Finally, the 1984 edition, on the basis of the evidence discussed before, included all 'sensu stricto' species in one omnicomprehensive taxon, *S. cerevisiae* [49]. This decision was unquestionable and mostly correct because the 21 species included in the *Saccharomyces* 'sensu stricto' group were and still are ecologically, physiologically and technologically identical.

But the adventure is far from over. When these species, unified under a single taxon because of identical phenotypic characters, were reidentified with procedures of molecular taxonomy such as nuclear DNA/DNA reassociation, it was found that *S. cerevisiae*, as defined according to the 1984 taxonomic monograph [49], includes at least three different taxa: *S. bayanus*, *S. cerevisiae* and *S. pastorianus* [39,45,46]. Needless to say, many of the renamed strains had been characterized by very famous epithets, such as *S. carlsbergensis*, *S. diastaticus*, *S. ellipsoideus*, *S. oviformis*, *S. uvarum*, and were deeply rooted in the subconscious mind of all yeast technologists. The choice of the names for the three taxa re-established by means of molecular taxonomic methods may make a few workers uncomfortable, but was dictated by the rules of the International Code for Botanical Nomenclature calling for priority of the 'oldest validly published epithet'.

At about the same time, a series of genetic studies by Naumov [27,28] on *S. cerevisiae*-like yeast strains isolated exclusively from nature came to light. These, together with two strains isolated by Phaff et al. [33] from *Drosophila* in the Californian mountains were studied by Vaughan-Martini [44] for their macromolecular relationship to the other three species of *Sacharomyces* 'sensu stricto'. Results of nDNA/nDNA hybridization revealed that this species is yet another, intermediately related member of the group, and the epithet *S. paradoxus* Batschinskaya [2] was reinstated.

These results were in part confirmed by studies of chromosomal and gene loci polymorphisms [18,31] as well as by an investigation of signature sequences of ribosomal RNA of type

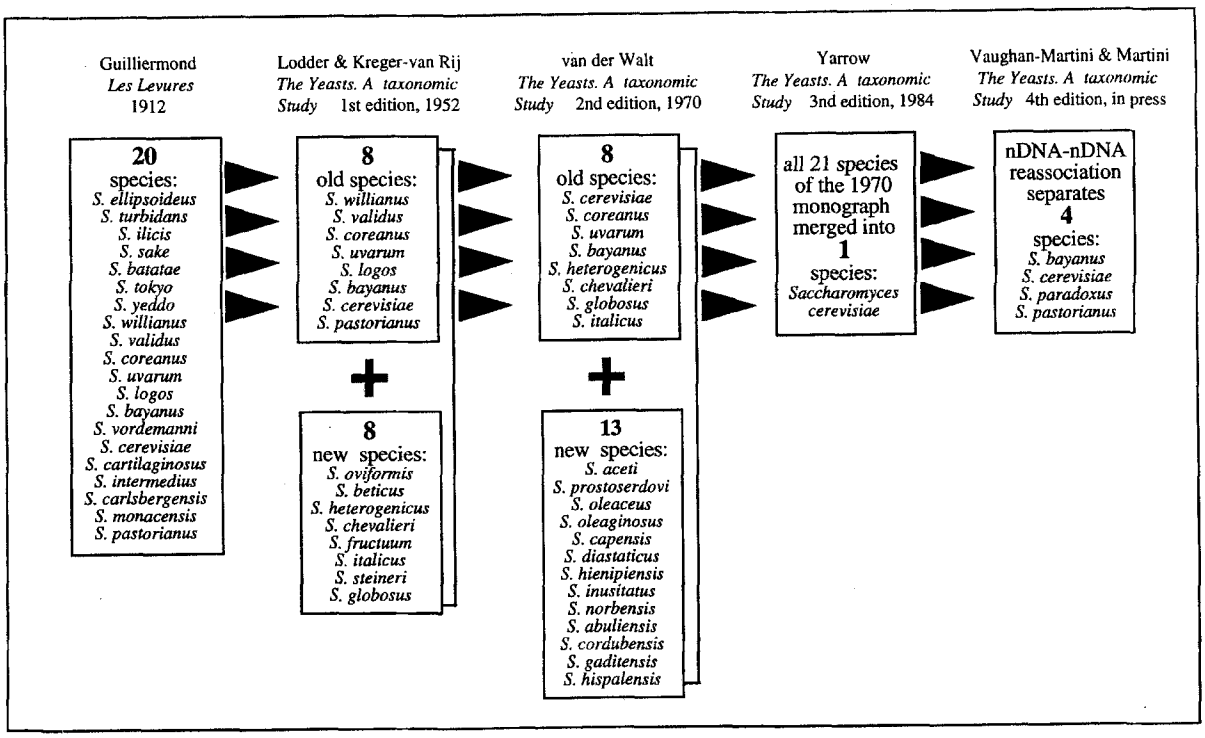

Fig. 1. Evolution of the nomenclature of *Saccharomyces cerevisiae* and related species from 1912 to the present.

strains [20]. Macromolecular relationships in this group of yeasts, very likely in the process of a separation into species, can be seen in Fig. 2.

These findings caused many apparent nomenclature contradictions, very hard to understand and accept by non-yeast taxonomists, which means more or less the majority of those who study and utilize yeast. A few examples of these perplexities are summarized in Table 2 where some traditional epithets of the wine and beer industries are reviewed. The third example, 'wine yeast for refermentation processes' is emblematic. In 1970 van der Walt [47] included the epithet *S. oviformis* under the species *S. bayanus*. It is still today believed

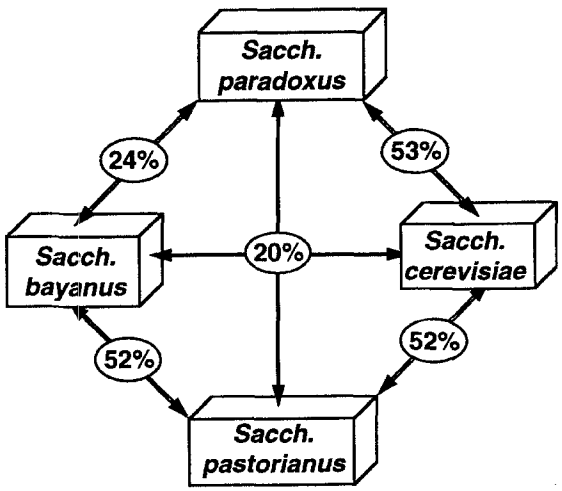

Fig. 2. Molecular relationships between the species of *Saccharomyces*, 'sensu stricto'[1].

by wine technologists that strains known as *S. oviformis* are particularly resistant to high ethanol concentrations, and therefore are most useful as starters for refermentation processes. Studies in recent years in Perugia revealed two fundamental incongruencies with this credence. DNA/DNA reassociations demonstrated that the type strain of *S. oviformis* does not belong to the species *S. bayanus*, but rather is synonymous to *S. cerevisiae* [46]. In addition, those cultures actually classified in *S. bayanus* are characterized by a relatively low resistance to ethanol, with maximum concentrations produced via fermentation never exceeding 9% in volume (unpublished data) as opposed to real refermenters of the *S. cerevisiae* group that can reach levels of 16–17%. It is easy to imagine that the new terminology may create misunderstandings with wine-makers.

It is also interesting to note that in spite of the 'gold standards' introduced by nDNA comparison, there are still some workers who obstinately call strains, *S. bayanus*, because they are able to ferment and/or assimilate melibiose. This phenotypic character has been shown to be extremely variable within the strains of *Saccharomyces* 'sensu stricto' [46].

Another unhappy group of non-taxonomists may be found in the beer industry as seen in the last three examples of Table 2. Of the strains used in brewing, the top yeast for the production of ale is traditionally considered to be *S. cerevisiae* while the bottom yeast used for lager, is said to be part of the species *S. carlsbergensis*.

While the epithet *S. cerevisiae* has remained intact since the beginning of yeast taxonomy, *S. carlsbergensis* clearly demonstrates typical nomenclature problems since this name is considered by many workers as synonymous to the bottom, lager yeast or to beer production itself. We have witnessed a

TABLE 2

Epithets of *Saccharomyces cerevisiae* in the wine and beer industries during the past 100 years

The wine years 'par excellence':

(1883) *S. ellipsoideus* → (1931) *S. cerevisiae* var. *ellipsoideus* → (from (1952) *S. cerevisiae*

The wine years in cold climates:

(1904 to 1952) *S. pastorianus* → (1970) *S. uvarum* → (1984) *S. cerevisiae* → (1992) *S. pastorianus*

The wine yeast for refermentations:

(1924) *S. oviformis* → (1970) *S. bayanus* → (1984) *S. cerevisiae* → (1987) *S. cerevisiae*

The brewer's top fermentation yeast:

(1883 to the present time) *Saccharomyces cerevisiae*

The brewer's bottom fermentation yeast:

(1908 to 1952) *S. carlsbergensis* → (1970) *S. uvarum* → (1984) *S. cerevisiae* → (1989) *S. pastorianus*

The brewer's worrisome contaminant:

(1970) *S. diastaticus* → (1984) *S. cerevisiae* → (1987) *S. cerevisiae*

series of changes over the years. This epithet disappeared for a period of 15 years when it was reduced first to synonymy with *S. uvarum* [47] and then included under *S. cerevisiae* [49]. It was briefly reinstated to species status by Vaughan Martini and Kurtzman [45] and remained so for two short years only to be again abolished [46] when it was found to have homologous nucleotide base sequences to the type strain of *S. pastorianus*, a species described by Hansen in 1904 [16], four years before the description of *S. carlsbergensis* [17].

From the numerous molecular studies done in recent years on the yeasts associated with *S. cerevisiae*, it is clear that different levels of genomic diversity exist between the four species of the complex. There is strong evidence indicating that those yeasts associated with the winery environment belong to *Saccharomyces cerevisiae*, while those utilized in brewing are probably strains of *S. pastorianus*. On the other hand, *S. parodoxus*, isolated so far only from *Drosophila* or tree exudates, is probably the only member of the complex to come from natural environments and could possibly represent the original ancestor of domesticated fermenting yeasts.

CONCLUDING REMARKS

A few suggestions would seem appropriate in consideration of the past and present confusion surrounding yeasts involved in processes of alcoholic fermentation. First of all, it may be possible that some of the so-called *S. cerevisiae* cultures that some workers are using or used in their investigations are incorrectly classified and actually belong to one of the other three species of the 'sensu stricto'. In other words, there is a possibility, perhaps remote, that results of relevant scientific importance may have been obtained with strains having genomes different from *S. cerevisiae*.

In order to exclude this possibility positively, what can and should be done? Ideally, culture collections should undertake the enormous task of verifying the classification of the 'sensu stricto' cultures they hold. It is obvious that taxonomists cannot preach sound taxonomy to other groups of scientists if they are not able to clean out their own stables.

An additional action should come from scientific societies grouping persons that use yeasts in their research, such as the International Commission on Yeasts, The Yeast Molecular Biologists and Geneticists, The International Society for Human and Animal Mycology, European Molecular Biology Organization and others, by officially establishing strict guidelines calling for the use of certified strains, labelled with unique numbers and acronyms of known culture collections. Finally, editorial boards of all scientific journals should adopt the policy of the journals of the American Society for Microbiology that rejects automatically manuscripts reporting results obtained with undefined microbial strains.

To conclude, a last recommendation to biotechnologists: do not overlook the fact that yeasts are a bottomless reservoir of biodiversity, with more to offer than the classical handful of species traditionally used or studied, such as *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Candida albicans* or *Kluyveromyces lactis*.

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