Int Microbiol (2003) 6: 175–182
DOI 10.1007/s10123-003-0131-2

REVIEW ARTICLE

Ann Vaughan-Martini

Reflections on the classification of yeasts for different end-users in biotechnology, ecology, and medicine

Received: 17 February 2003 / Accepted: 5 June 2003 / Published online: 29 July 2003
© Springer-Verlag and SEM 2003

Abstract The approach to yeast identification has significantly changed in just a few decades due to the rapid increase in basic biological knowledge, increased interest in the practical applications and biodiversity of this important microbial group, and enormous technological advances. While some conventional methods can still be validly applied, many molecular techniques have been developed that allow for strain classification on all taxonomic levels. A critical evaluation of the actual scope of each identification procedure will in the end determine the most appropriate use of the many protocols now available. Nonetheless, the oldest tool of microbiology, the microscope, is still a fundamental accessory for studies involving yeast biology, biodiversity and taxonomy.

Keywords Yeast identification · Identification · Yeast biotechnology · Conventional and molecular taxonomy

Introduction

The approach to microbial classification has undergone profound changes during the twentieth century due to the thousands of studies that have explosively expanded our knowledge of biological systems. In the meantime, taxonomists are often considered to be a dying breed of ruthless, picky, and boring fanatics who concentrate more on changing microbial "name tags" than on anything useful for the real world. In addition, more rather than less confusion currently characterizes the taxonomic world, as users of the innumerable molecular methods now available join the ranks of microbial "classifiers". Many, on the basis of minor genomic "quirks", are confusing biodiversity with species and creating invalid taxa as they disregard such "obsolete" notions as ecological origin, strain physiology, and phylogenetic relationships. By contrast, yeast taxonomists (including Herman Phaff) have always known that small to large intraspecific differences are the norm rather than the exception, and that it takes a lot more than simple physiological or genomic variations to define a species. In addition, in spite of the enormous increase in valid descriptions of new species in recent years, a general taxonomic ignorance prevails as many researchers still believe that *Saccharomyces cerevisiae* is the only (or the most representative) yeast on the face of the Earth. So, either yeast taxonomists (together with their life work) are destined to extinction, or they are only communicating among themselves while the rest of the world is turning at a different speed!

This review proposes to illustrate the usefulness of taxonomy (and of taxonomists) by briefly evaluating the past and present "state of the art" in recognition of the increasing appreciation for and importance of correct classification. Valid identification schemes are essential in industry, biotechnology, medicine, and ecological studies. Keeping in mind these diverse needs, it must be emphasized that the actual procedures employed will necessarily vary in response to each classification scenario. As a result, one could even use a modified "journalistic" approach of "who, what, when, where, and how":

- Where did the strain come from?
- How was it isolated?
- Who requested the identification?
- What is it for? What level is required? What methods are most appropriate?
- When is an answer necessary?

The responses to the above questions will be fundamental in determining a correct, timely, and cost-effective identification procedure. As anyone with experience in microbial classification knows, there is no universal

A. Vaughan-Martini
Dipartimento di Biologia Vegetale e Biotecnologie Agroambientali, Università di Perugia, Sez. Microbiologia Applicata e Collezione dei Lieviti Industriali DBVPG, Perugia, Italy
E-mail: avaughan@unipg.it
Tel.: +39-0755856479
Fax: +39-0755856470

approach. In addition, while the job can occasionally be straightforward, in other instances obtaining a satisfactory answer can be quite laborious. Regardless of the protocol eventually chosen (some of which are proposed later in this review), one can work effectively thanks to the vast array of methods that have been developed over 150 years of study and classification of yeasts and yeast-like organisms.

Conventional taxonomic procedures, which analyze strain phenotypic characteristics (ecological origin, morphology, physiology, and sexual aspects), are well known and clearly described in the latest edition of the monograph *The yeasts, a taxonomic study* [51]. Nevertheless, consideration of results obtained using those techniques has changed radically in the last 30 years, since elucidation of the structure and function of informational macromolecules (DNA and RNA) has shown that phenotypic variations do not always correspond to genomics. While we should definitely not throw out microscopes or abolish all traditional tests, the development of molecular techniques has significantly widened the tools available for understanding and documenting species designations and phylogenetic relationships. Finally, as already mentioned, the course of action and the level of classification will be determined by the goals of each project, some of which could be, for example:

1. Studies of biodiversity in nature
    a. Elucidation of the species (known or novel) present in a habitat
    b. Study of interrelationships between the micro- and macro-flora
    c. Survey of the genomic characteristics of all species (cultivable and non-cultivable) in an ecosystem
2. Investigations regarding physiological biodiversity
    a. Screening for useful properties
    b. Classification, fingerprinting, and patent protection of promising strains
3. Industrial applications of yeast
    a. Exploitation of microbial physiology for useful processes
    b. Quality control of fermentation microflora (inoculum and/or contaminants)
4. Health sciences
    a. Rapid identification of pathogens
    b. Application of microbial antagonistic properties

Yeast classification: getting started and avoiding dangerous pitfalls

The type strain

One of the most important parameters that must be respected when initiating any scientific investigation (and taxonomy is no exception) is that of using appropriate standards. For yeast classification, this involves the inclusion of internationally recognized type strains of known species in all comparisons. These can be obtained from one of several official culture collections, some of which are listed in Table 1. Nevertheless, a quick look at the literature shows us that this very simple, "obvious" aspect is often ignored, even in investigations that profess to systematically investigate the yeast world. One extraordinary oversight in this regard was the sequencing of the *S. cerevisiae* genome [11]. First of all, the project was carried out using one of the least representative of the over 800 known yeast species if we consider that *S. cerevisiae* (the absolute champion of alcoholic fermentation) is one of a handful of osmo- and ethanol-tolerant taxa [51]. In addition, the type strain (ATCC 18824, CBS 1171, DBVPG 6173, IFO 10217, NCYC 505, NRRL Y-12,632), was not used for the project probably because it has essentially no sexual cycle as a result of another peculiarity of this species: aneuploidy [2]. To make matters worse, the more genetically "cooperative" strain studied, YGSC S288C (DBVPG 6820), is really only a representative of itself since it is a very slow grower (ATCC note), a poor fermenter (Vaughan-Martini et al., unpublished data), and a probable hybrid of two different osmophilic, fermenting species: *S. cerevisiae* and *S. paradoxus* (Vaughan-Martini et al., unpublished data). As a result, the affirmation that the results of that investigation reveal everything about *the yeast* genome [7] could not be less true!

Table 1 Some culture collections useful for obtaining type strains and taxonomic information

Institution	Acronym	Location	Website
American Type Culture Collection	ATCC	Manassas, Va., USA	http://www.atcc.org/
Centralbureau voor Schimmelcultures	CBS	Utrecht, The Netherlands	http://www.cbs.knaw.nl/
Industrial Yeasts Collection	DBVPG	Perugia, Italy	http://www.agr.unipg.it/dbvpg/home.html
Institute for Fermentation, Osaka	IFO	Osaka, Japan	http://www.ifo.or.jp/index_e.html
National Collection of Yeast Cultures	NCYC	Norwich, UK	http://www.ifr.bbsrc.ac.uk/ncyc/
National Center for Agricultural Utilization Research	NRRL	Peoria, Ill., USA	http://nrrl.ncaur.usda.gov/
Herman J. Phaff Culture Collection	UCD	Davis, Calif., USA	http://www.phaffcollection.org/home.asp

The pure culture, strain maintenance, and conventional or molecular taxonomic procedures

A fundamental aspect of valid classification is the use of pure cultures, which can be obtained by one of several basic microbiological protocols. Although these procedures are sometimes underestimated, the erroneous use of mixed cultures has led to many unnecessary losses of time, energy and money. It should also be emphasized that a single isolation step is very often insufficient, particularly in the case of samples of very high microbial density, where the possibility of having a mixed culture after the first isolation is high. Finally, it goes without saying that once procured, pure cultures should be correctly maintained at least until the end of the identification process. Culture conservation techniques, which include maintenance on fresh slants with periodic transfer, lyophilization, and freezing at ultra-low temperatures are clearly illustrated in Hunter-Cevera and Belt[21] and Yarrow [51]. Whatever conservation method is used, the scope is universal: that of maintaining the genetic, physiological, and phenotypic characteristics of strains.

Universally recognized methods of yeast strain identification can be found in taxonomic monographs [6, 51], or in manuals of molecular biology [14, 21]. Although at times considered old and obsolete, conventional taxonomic techniques can reveal useful and important information. For example, certain morphological characteristics can give a good indication of the genus, particularly if a sexual cycle is detected. [39]. In addition, strain origin can be an important indicator of the probable species. Ecological niches, such as fermenting fruit juices or milk products [49], decaying cactus [31], the diseased human body [1], and insect intestines [40], often yield a characteristic array of species.

Traditionally, the next series of tests in a conventional classification scheme investigates 50–60 metabolic activities of the culture. While a complete series of physiological tests can take up to 3 weeks, commercial identification kits that yield data on several aerobic characteristics in about 3 days are now available [e.g., API ID 32 C system, Biomérieux SA, Marcy-Etoile, France, (http://www.biomerieux.com)]. Although these kits have the advantage of being highly standardized, they are not appropriate for slow-growing cultures or for those with limited physiological capabilities. Nevertheless, they are useful for the elucidation of species of medical or industrial importance.

Once the data on phenotypic characteristics have been obtained, diagnostic keys, available in monographs on yeast taxonomy [6, 28], can be used to determine the species. Today, this task has been facilitated by the introduction of computerized keys [5; CBS yeast identification guide: http://www.cbs.knaw.nl/] that can more rapidly elucidate possible taxonomic designations. All systems present inherent problems. however, since a strain can exhibit enough physiological variations from the standard description to key out as an entirely different species. As a result, considerable experience is required to effectively interpret conventional taxonomic data and avoid the possibility that minor physiological differences will lead to an erroneous species designation. This is particularly important when using computer programs that tend to yield a redundant number of possible species, many of which are completely wrong.

Finally, the biggest problem with classification based upon conventional taxonomic criteria is the hierarchical system of biological classification by which the progressive assignation of organisms into each descending category (i.e. order → family → genus → species) is based upon one or a very few phenotypic differences [23]. As a result, "splitters" (those who establish new species on the basis of minimal physiological differences) dominated yeast taxonomy until molecular studies showed that many apparent differences have no genetic basis.

Some useful phenotypic indicators

As the number of species described and the persons studying yeast increased, many new methods of investigation were developed. Several of these, based upon the study of informational macromolecules (DNA or RNA), gave birth to the discipline now known as molecular taxonomy which will be briefly discussed below. Other techniques, based upon various cellular components or characteristics, can be applied in particular identification situations.

The use of electron microscopy in microbiology immediately demonstrated a distinct difference between ascomycetous and basidiomycetous yeast cell wall structure [37]. As a result, the determination of this characteristic can be a good beginning to a classification protocol, especially since a relatively fast and simple method, i.e., testing a reaction to diazonium blue B (DBB) [19, 45], can be used to differentiate the two groups. The importance of this criterion is confirmed by the fact that the DBB reaction is the first test listed in the taxonomic key to species in the most recent edition of *The yeasts, a taxonomic study* [28]. Although the actual mechanism of the reaction is still not completely understood [51], it is very useful for rapidly revealing the status of imperfect (anamorphic) yeasts.

Although not really pertinent for taxonomic studies per se, and now partially replaced by molecular methods, some relatively easy tests are useful for the rapid recognition of the three most important yeast pathogens: *Candida albicans, Cryptococcus/Filobasidiella neoformans*, and *Candida tropicalis* [1]. For example, antigenic testing for specific receptors on the yeast cell wall [20], particular growth media [24], color reactions [38], and kits for rapidly testing enzymatic activities [32] can yield a presumptive identification within 24–48 h.

Lastly, while it has been shown that yeast killer character, discovered and described in the 1960s [34], cannot be used for the designation of taxa; it was found that sensitivity to killer toxins is strain specific [42]. As a result, strain reactions to a panel of different killer toxins can be a relatively easy, rapid, and inexpensive non-molecular system for fingerprinting individual industrial or patent strains [8, 9].

Molecular taxonomy

The interpretation of conventional taxonomic data changed radically when it was demonstrated that all characters, both observed and not expressed, are "written" in the sequences of informational macromolecules. From the moment that Marmur, Doty and colleagues began their pioneering work on the physical-chemical characteristics of DNA [36, 43], molecular taxonomy was born and has been growing ever since. The vast array of methods examining the various classes of DNAs and RNAs allow for the classification of microorganisms at different taxonomic levels, permitting us to understand microbial evolution and to arrange groups according to ancestral relationships. The reader can obtain information regarding the history of molecular applications to yeast taxonomy from several sources [26, 29, 44].

The first molecular studies, such as the determination of the guanine + cytosine (mol% G + C) content of nuclear DNA (nDNA) and the comparison of base sequence relatedness employing one of various techniques of nDNA/nDNA hybridization, immediately revealed one huge advantage over traditional taxonomic methods: consistency. In fact, independent of cultural status or growth conditions, genomic data are constant because DNA or RNA sequences normally do not change even though their expression (phenotype) can vary. This offered taxonomists enormous opportunities for establishing stable microbial groupings and for setting up more effective classification schemes by confronting genomic and phenotypic characteristics. As a result, new species descriptions now require conventional phenotypic data [17, 28] as well as various molecular parameters (see below).

Today, there are many techniques for obtaining a partial base sequence evaluation that require much smaller quantities and less purified DNA. Nevertheless, "conventional" nDNA/nDNA hybridization by an optical [30], colorimetric [10] or radioactive [48] method is still the only way to compare all expressed and non-expressed genes of two strains in a single experiment. In addition, highly purified nDNA samples can be maintained at −18 °C for several years and serve as a useful library for eventual hybridization experiments with unidentified strains. For these purposes, the DBVPG Industrial Yeasts Collection (Università di Perugia, Italy) currently conserves over 800 samples of high-molecular-weight DNA ready for use in optical hybridizations.

Estimating phylogenetic relationships by comparison of rRNA and rDNA sequences

In spite of the important impact that DNA/DNA hybridization has had on yeast systematics, it does not allow for elucidation of relationships above the species level. For this purpose, many techniques for analyzing different rRNA sequences, or its template ribosomal DNA (rDNA), are now standard in molecular systematics [22, 25, 27]. These studies have made it possible to construct phylogenetic trees of all known species, and to better understand interspecific and intergeneric relationships [50]. As a result, it is now common practice to deposit the sequences of key molecular regions, such as the 600-nucleotide variable region D1/D2 of LSU (large subunit) (26S) rDNA and the ITS1 and ITS2 (internal transcribed sequences) of 18S rRNA, with database servers such as Genbank (http://www.ncbi.nlm.nih.gov/Genbank/GenbankSearch.html) when new species are described (Table 2).

Methods for rapid yeast strain identification or fingerprinting

Today, due to the huge databases compiled as a result of sequencing studies, together with the diffusion of personal computers and the development of polymerase chain reaction (PCR) technology, a vast array of molecular methods are now available for rapid presumptive yeast strain identification. (It goes without saying that this is something that many of us, including probably Herman Phaff, often dreamed about when a long and detailed conventional classification procedure

Table 2 Useful websites for obtaining taxonomic or sequence data, or for information regarding culture collections or patent protection

Institution	Website
World Federation of Culture Collections (WFCC)	http://wdcm.nig.ac.jp/wfcc/
European Culture Collection Organization (ECCO)	http://www.eccosite.org
World Intellectual Properties Organization (WIPO)	http://www.wipo.int/
NIH- taxonomy page	http://www3.ncbi.nlm.nih.gov/htbin-post/Taxonomy/wgetorg
Genbank sequence data	http://www.ncbi.nlm.nih.gov/Genbank/GenbankSearch.html
WFCC-MIRCEN World Data Centre for Microorganisms (WDCM)	http://wdcm.nig.ac.jp

brought us to an absolute dead end!) While not all of these techniques are appropriate for the official designation of a new species, they can be extremely useful in other identification scenarios when a more "practical" application is required or when the range of species possibilities is relatively limited. This can be the case in efforts such as identification of the agent of a human disease, quality control of an industrial fermentation, or in some ecological surveys. While it is obviously impossible to list all of the techniques currently available, a few which offer interesting possibilities will be briefly illustrated.

Rapid identification of pathogenic yeast

Much effort has been devoted to abbreviating the time necessary for identifying the causative agent of an active disease in order to allow for a timely adoption of specific therapeutic regimens. Serious problems still prevail since results from serological tests (see above) still take 24–48 h and do not always give unequivocal responses [47]. In addition, the isolation of pure cultures for conventional identification involves at least 2 days for sufficient colony development. Nevertheless, once pure cultures are obtained, several interesting techniques have been developed that can give an accurate same-day identification of key species. For example, a PCR system that involves a 1-h DNA extraction procedure was developed for the identification of 14 species of human pathogenic yeast. The use of two universal and two species-specific primers derived from the D1/D2 region of 26S rDNA allows for a rapid species identification even using mixed cultures [35]. Another method, involving a multiplex PCR amplification using four universal ITS primers (ITSs1–4) followed by agarose gel or microchip electrophoresis (denominated PCR-AGE or PCR-ME, respectively), reportedly allows for the detection of pathogens in under 6 h [15]. It is obvious that the end goal of these and similar studies will be that of direct analysis of clinical specimens for a same-day diagnosis [47]. Some possible identification scenarios in a medical situation are outlined in Table 3a.

Quality control of an industrial fermentation

The possibility of monitoring and immediately intervening during an industrial fermentation has long been the dream of biotechnologists. This is perhaps becoming a reality thanks to novel methodologies developed for specific industrial processes. For example, a PCR-RFLP (restriction fragment length polymorphisms) analysis of the rDNA ITS region allows for detection and quantification of different yeast species typically present during spontaneous grape-must fermentation [16]. With this method, enologists can effectively monitor the succession of active species during wine production, even though time limitations exist as DNA extractions must be made from isolated colonies. Another technique requiring pure cultures, the determination of electrophoretic karyotypes by pulsed field gel electrophoresis (PFGE), has been proposed for wine starter identification [18]. Although this is quite useful for strain identity control in culture collections or for classification procedures [46], its validity for starter fingerprinting is limited since it has been shown that chromosomal profiles of industrial strains of *S. cerevisiae* can vary after a number of generations (Vaughan-Martini et al., unpublished data, [41]). Others have proposed a PCR method for recognition of species or even individual production strains that involves producing arbitrary DNA sequences by RAPD (randomly amplified polymeric DNA) using single primers of arbitrary nucleotide sequences [4].

Finally, a possible real-time approach for monitoring starter domination throughout the vinification process could be a method based upon the known polymorphism in the number and position of introns in the mitochondrial *COX1* gene in strains of *S. cerevisiae*. For this purpose, oligonucleotide primers homologous to regions flanking *COX1* introns were designed for verifying strain frequency. The authors report that results can be obtained quite rapidly (in approximately 8 h) and that DNA isolation is not required as grape must samples can be used directly for the PCR reaction [33].

In spite of the promise of the above techniques, it is still generally accepted that no single PCR-mediated typing technique allows for 100% discrimination at the strain level, and that this is only possible by combining results obtained from a series of typing techniques [3]. Some possible identification scenarios for wine or food fermentations are outlined in Table 3b, c.

Ecological investigations

One of the biggest obstacles to monitoring yeast impact in an ecological niche is the difficulty of revealing the total microflora. This is due to the minority status of yeasts in many habitats, the difficulty of effectively separating all cells from surfaces, and/or the fact that many species are obligate symbionts and as such are not cultivable in pure culture [31]. Some of these problems have been partially overcome by the introduction of more vigorous isolation procedures, such as fast shaking and mild sonication which allow for a more efficient liberation of viable cells from surfaces [12]. However, the problem of revealing non-cultivable or extremely minority species remains. The recently developed cDNA microarray techniques could potentially allow for an in situ evaluation of a global microbial community by way of mini-hybridizations of total sample DNA with various classes of oligonucleotide probes. Because of their high-density and high-throughput capacity, microarray-based genomic technologies could revolutionize the analysis of microbial community structure, function, and dynamics. The potential exists to assess simultaneously in a single

Table 3a, b, c, d Possible yeast identification or characterization schemes

Where	**a. Active human disease**		
How isolated	Clinical specimen?	Conventional isolation	Conventional isolation
Who	Physician	Physician	Medical microbiologist/taxonomist
What for	Verification and control of disease agent	Verification and control of disease agent	Epidemiological or taxonomic study
What level	Species/serotype	Species/serotype	Species/serotype
What methods[a]	PCR of D1/D2 or ITS; serology?[b]; KIL[c]	API ID 32 C; serology; PCR of D1/D2 or ITS; KIL	Conventional and molecular taxonomy; Serology; DNA/DNA; mol% G+C; KIL PCR of D1/D2 or ITS; PFGE
When	ASAP[d]	ASAP	NSTF[d]
Where	**b. Fermenting grape must**		
	Inoculated	Inoculated or natural	Non-inoculated
How isolated	Direct must sample	Conventional isolation	Conventional isolation
Who	Starter or wine producer	Wine producer	Wine biotechnologist
What for	Verification of starter	Verification of all species present	Search for novel/local starters
What level	Strain	Species	Strain
What methods	PCR-COX1; RAPD?	PCR-RFLP; PFGE?	Technological evaluation; killer; PCR-COX1; PFGE?; RAPD?
When	ASAP	ASAP	NSTF
Where	**c. Food fermentation**		
How isolated	Conventional isolation	Conventional isolation (with vigorous treatments)	
Who	Quality control analyst	Quality control analyst	
What for	Verification of starter dominance	Verification of contaminants	
What level	Strain	Species	
What methods	Killer; RAPD? PFGE; PCR-RFLP	Conventional and molecular taxonomy PFGE; PCR-RFLP?; RAPD?	
When	ASAP	ASAP	
Where	**d. Soil-plant ecosystem**		
How isolated	Total DNA isolation	Conventional isolation (with vigorous treatments)	Conventional isolation (with vigorous treatments)
Who	Microbial ecologist	Biotechnologist	Microbial ecologist/taxonomist
What for	Survey of all possible taxa	Screening for useful properties	Search for novel cultivable species
What level	Species	Strain/species[e]	Species
What methods	PCR-RFLP?; microarrays?; RAPD?	Metabolic screening initially + conventional and molecular taxonomy; killer; PFGE; RAPD?of promising strains for patent purposes	Conventional and molecular taxonomy; DNA/DNA; PFGE; mol% G + C PCR of D1/D2 or ITS
When	NSTF	NSTF	NSTF

[a]Methods: *COX1*, Survey of *COX1* regions of mtDNA; *D1/D2*, analysis of signature sequences of LSU (large subunit) (26S) rDNA; *DNA/DNA*, conventional nDNA hybridization; *killer*, sensitivity to a panel of different yeast killer toxins; *mol% G+C*, determination of the guanine+cytosine content of nuclear DNA;*PFGE*, pulsed field gel electrophoresis; *RAPD* random amplified polymorphic DNA; *RFLP* restriction fragment length polymorphisms; *ITS*, internal transcribed sequences of 18S rRNA

[b]Questionable applicability or utility of a method

[c]*KIL* Yeast killer toxin antagonistic properties could be potentially useful for topical treatment against some yeast infections [12]

[d]*ASAP* As soon as possible, *NSTF* no specific time frame

[e]A species designation may not be important until a useful character or application is identified

assay all, or most, of the constituents of a complex natural community [52]. In spite of these potential applications, certain problems will have to be overcome before this technology can be routinely applied to ecological analyses. For example, the target and probe sequences are very diverse in environmental studies, and it is still not clear whether the performance of microarrays with mixed samples will be similar to that obtained with pure cultures. In addition, natural samples are generally contaminated with substances such as humic matter, organic contaminants, and metals, which may interfere with DNA hybridization on microarrays. There are also questions regarding sensitivity since the retrievable biomass in environmental samples is generally low, and it remains to be seen whether microarray hybridization is sensitive enough to detect microorganisms in all types of environmental samples. Finally, it is uncertain whether microarray-based detection can be quantitative and it currently is very expensive [13]. (Nevertheless, if he were still around, it is likely that Herman Phaff would have been among the first to apply this technology during one of his many jaunts in nature for collecting his favorite microorganisms). Some possible identification schemes for ecological studies are outlined in Table 3d.

Concluding remarks and recommendations

Yeast identification has undergone significant transformation in the space of a few decades due to the rapid increase in basic biological knowledge, increased

interest in the practical applications and biodiversity of this important microbial group, and enormous advances in technology. Many choices are available, but we must never forget to look in the microscope and to continue to marvel at that wonderful organism, the yeast, which Herman Phaff was instrumental in teaching generations of young biologists to know and love. In addition, we must always keep in mind that instant answers to taxonomic questions are rare, and a that a thorough knowledge and appreciation of yeast biology, ecology, genetics, and phylogeny will always be an important asset in our continuing quest for understanding our single-celled friends. Those of us who were lucky enough to know and work with Herman will always be grateful for his teachings and his example.

References

1. Ahearn DG (1998) Yeasts pathogenic to humans. In: Kurtzman CP, Fell JW (eds) The yeasts, a taxonomic study, Elsevier, Amsterdam. pp 9–12
2. Bakalinsky AT, Snow R (1990) The chromosomal constitution of wine strains of *Saccharomyces cerevisiae*. Yeast 6:367–382
3. Baleiras Couto MM, Eijsma B, Hofstra H, Huis in't Veld JH, van der Vossen JM (1996) Evaluation of molecular typing techniques to assign genetic diversity among *Saccharomyces cerevisiae* strains. Appl Environ Microbiol 62:41–46
4. Baleiras Couto MM, van der Vossen JM, Hofstra H, Huis in't Veld JH (1994) RAPD analysis: a rapid technique for differentiation of spoilage yeasts. Int J Food Microbiol 24:249–260
5. Barnett J (1994) Yeast identification PC program, Norwich, UK
6. Barnett JA, Payne RW, Yarrow D (1990) Yeasts: characteristics and identification, 2nd edn. Cambridge University Press, Cambridge
7. Botstein D, Chervitz SA, Cherry JM (1997) Yeast as a model organism [comment]. Science 277:1259–60
8. Buzzini P, Martini A (2000) Utilisation of differential killer toxin sensitivity patterns for fingerprinting and clustering yeast strains belonging to different genera. Syst Applied Microbiol 23:450–457
9. Buzzini P, Martini A, (2001) Large-scale screening of selected *Candida maltosa, Debaryomyces hansenii* and *Pichia anomala* killer toxin activity against pathogentic yeasts. Med Mycol 39:479–482
10. Cardinali G, Liti G, Martini A (2000) Non-radioactive dot-blot DNA reassociation for unequivocal yeast identification. Int J Syst Evol Microbiol 50:931–936
11. Cherry JM, Ball C, Weng S, Juvik G, Schmidt R, Adler C, Dunn B, Dwight S, Riles L, Mortimer RK, Botstein D (1997) Genetic and physical maps of *Saccharomyces cerevisiae*. Nature 387 Suppl:67–73
12. Diriye FU, Scorzetti G, Martini A (1993) Methods for the separation of yeast cells from the surfaces of processed, frozen foods. Int J Food Microbiol 19:27–37
13. Epstein CB, Butow RA (2000) Microarray technology-enhanced versatility, persistent challenge. Curr Opin Biotechnol 11:36–41
14. Evans I (ed) (1996) Yeast protocols. Methods in yeast molecular biology, vol 53. Humana Press, New Jersey
15. Fujita S, Senda Y, Nakaguchi S, Hashimoto T (2001) Multiplex PCR using internal transcribed spacer 1 and 2 regions for rapid detection and identification of yeast strains. J Clin Microbiol 39:3617–3622
16. Granchi L, Bosco M, Messini A, Vincenzini M (1999) Rapid detection and quantification of yeast species during spontaneous wine fermentation by PCR-RFLP analysis of the rDNA ITS region. J Appl Microbiol 87:949–956
17. Greuter W, Barrie FR, Burdet HM, Chaloner WG, Demoulin V, Hawksworth DL, Jørgensen PM, Nicolson DH, Silva PC, Trehane P, McNeill J (1994) International code of botanical nomenclature. Koeltz, Koenigstein, Germany
18. Guillamón JM, Barrio E, Querol A (1996) Characterization of wine yeast strains of the *Saccharomyces* genus on the basis of molecular markers: Relationships between genetic distance and geographic or ecological origin. Syst Appl Microbiol 19:122–132
19. Hagler AN, Ahearn DG (1981) Rapid diazonium blue B test to detect basidiomycetous yeasts. Int J Syst Bacteriol 31:204–208
20. Hamajima K, Nishikawa T, Shinoda T, Fukazawa Y (1988) Detection and specificity of a new antigen in *Candida tropicalis* and its evaluation by taxonomic DNA analysis. Microbiol Immunol 32:1013–1024
21. Hunter-Cevera JC, Belt A (eds) (1996) Maintaining cultures for biotechnology and industry. Academic Press, San Diego
22. James SA, Colins MD, Roberts IN (1996) Use of an rRNA internal transcribed spacer region to distinguish phylogenetically closely related species of the genera *Zygosaccharomyces* and *Torulaspora*. Int J Syst Bacteriol 46:189–194
23. Jeffery C (ed) (1977) Biological nomenclature, 2nd edn. Edward Arnold, London
24. Kitch TT, Jacobs MR, McGinnis MR, Appelbaum PC (1996) Ability of RapID yeast plus system to identify 304 clinically significant yeasts within 5 hours. J Clin Microbiol 34:1069–1071
25. Kurtzman CP (1992) rRNA sequence comparisons for assessing phylogenetic relationships among yeasts. Int J Syst Bacteriol 42:1–6
26. Kurtzman CP (1998) Nuclear DNA hybridizations: quantitation of close genetic relationships. In: Kurtzman CP, Fell JW (eds) The yeasts, a taxonomic study, Elsevier, Amsterdam. pp 63–68
27. Kurtzman CP, Blanz PA (1998) Ribosomal RNA/DNA sequence comparisons for assessing phylogenetic relationships. In: Kurtzman CP, Fell JW (eds) The yeasts, a taxonomic study, Elsevier, Amsterdam. pp 69–74
28. Kurtzman CP, Fell JW (eds) (1998) The yeasts, a taxonomic study. 4 edn. Elsevier, Amsterdam
29. Kurtzman CP, Phaff HJ, (1987) Molecular taxonomy. In: Rose AH, Harrison AJS (eds.) The yeasts: biology of yeasts. Academic Press, London. pp 63–94
30. Kurtzman CP, Smiley MJ, Johnson CJ (1980) Emendation of the genus I*ssatchenkia* Kudriavzev and comparison of species by deoxyribonucleic acid reassociation, mating reaction, and ascospore ultrastructure. Int J Syst Bacteriol 30:503–513
31. Lachance MA, Starmer WT (1998) Ecology and yeasts. In: Kurtzman CP, Fell JW (eds.) The yeasts, a taxonomic study, Elsevier, Amsterdam. pp 21–30
32. Land GA, Salkm IF, El-Zaatari M, McGinnis M, Hashem G (1991) Evaluation of the Baxter-Microscan 4-hour enzyme-based yeast identification system. J Clin Microbiol 29:718–722
33. Lopez V, Fernandez-Espinar MT, Barrio E, Ramon D, Querol A (2002) A new PCR-based method for monitoring inoculated wine fermentations. Int J Food Microbiol 81:63–71
34. Makower M, Bevan EA, (1963) The inheritance of killer character in yeast (*Saccharomyces cerevisiae*). In: Goerts (ed) Proc Int Cong Genet. The Hague, pp 127–133
35. Mannarelli BM, Kurtzman CP (1998) Rapid identification *of Candida albicans* and other human pathogenic yeasts by using short oligonucleotides in a PCR. J Clin Microbiol 36:1634–1641
36. Marmur J, Doty P (1961) Thermal renaturation of deoxyribonucleic acids. J Mol Biol 3:585–594
37. Moore RT (1987) Micromorphology of yeasts and yeast-like funghi and its taxonomic implications. In: de Hoog GS, Smith MT, Weijman ACM (eds) The expanding realm of yeast-like fungi. Elsevier, Amsterdam, pp 203–226

38. Palawal DK, Randhawa HS (1978) Evaluation of a simplified *Guizotia abyssinica* seed medium for differentiation of *Cryptococcus neoformans*. J Clin Microbiol 7:346–348
39. Phaff HJ, Miller MW, Mrak EM (1978) The life of yeasts. Harvard University Press, Cambridge, Massachusetts
40. Phaff HJ, Starmer WT (1987) Yeasts associated with plants, insects and soil. In: Rose AH, Harrison AJS (eds) The yeasts. Academic Press, London. pp 123–180
41. Puig S, Querol A, Barrio E, Pérez-Ortín JE (2000) Mitotic recombination and genetic changes in *Saccharomyces cerevisiae* during wine fermentation. Appl Environ Microbiol 66:2057–2061
42. Rosini G (1989) Killer yeasts: notes on properties and technical use of the character. In: Cantarelli C, Lanzarini G (ed) Biotechnological applications in beverage production, Elsevier, Amsterdam, pp 41–48
43. Schildkraut CL, Marmur J, Doty P (1962) Determination of the base composition of deoxyribonucleic acid from its buoyant density. J Mol Biol 4:430–433
44. Stackebrandt E, Goodfellow M (eds) (1991) Nucleic acid techniques in bacterial systematics. Modern microbiological methods, vol 5. Wiley, Chichester
45. van der Walt JA, Hopsu-Havu VK (1976) A colour reaction for the differentiation of ascomycetous and hemibasidiomycetous yeasts. Antonie van Leeuwenhoek 42:157–163
46. Vaughan-Martini A, Martini A, Cardinali G (1993) Electrophoretic karyotyping as a taxonomic tool in the genus *Saccharomyces*. Antonie van Leeuwenhoek 63:145–156
47. Velegraki A, Kambouris ME, Skiniotis G, Savala M, Mitroussia-Ziouva A, Legakis NJ (1999) Identification of medically significant fungal genera by polymerase chain reaction followed by restriction enzyme analysis. FEMS Immunol Med Microbiol 23:303–312
48. Warnaar SO, Cohen JA (1966) A quantitative assay for DNA-DNA hybrids using membrane filters. Biochem Biophys Res Commun 24:554–558
49. Wyder M-T (1998) M-T Wyder, Doctoral Thesis, Swiss Federal Institute of Technology, Zürich, Switzerland
50. Woese C (1998) The universal ancestor. Proc Natl Acad Sci USA 95:6854–6859
51. Yarrow D (1998) Methods for the isolation, maintenance and identification of yeasts. In: Kurtzman CP, Fell JW (eds) The yeasts, a taxonomic study. Elsevier, Amsterdam. pp 77–100
52. Zhou J, Thompson DK (2002) Challenges in applying microarrays to environmental studies. Curr Opin Biotechnol 13:204–207