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Life with yeasts during retirement

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In the summar of 1994 I received a wonderful letter from four of my colleagues—Sally Meyer, Arnold Demain, Don Ahearn and Clete Kurtzman. Together they initiated the idea of publishing a special issue of the Journal of Industrial Microbiology with articles by my former students and postdoctoral visitors to celebrate my fifty years of research on yeasts.

Since an introductory article by me was to be part of the plan, I decided that rather than writing a research paper, it would be more interesting to update my earlier autobiography, which was written in 1985 as the prefatory chapter in the Annual Review of Microbiology [9]. This approach would enable me to tell my readers how, following mandatory retirement in 1983, I spent these years as a Professor Emeritus. Although mandatory retirement was later abolished at the University of California, departmental policy on emeriti at the time of my retirement varied considerably across campuses with respect to office space and laboratory facilities. The fact that I had an NSF research grant until 1991, justified a modest office as well as laboratory space sufficient to carry out my grant obligations. Two other factors also contributed to my need for space in a crowded department. The first one involved my associate editorship of the International Journal of Systematic Bacteriology, which I have maintained to the present, while the second dealt with the supervision of a collection of thousands of yeast strains, isolated over many years from numerous natural sources and about which I shall say more later.

My formal classroom teaching, which I did in the Department of Bacteriology, was discontinued shortly after retirement. With the advent of molecular biology in microbiology, organismic microbiology began losing its appeal and my courses were phased out at UC Davis as well as at many other universities. However, because of the rapidly growing interest in the broad aspects of biodiversity, one may hope that the teaching of organismic microbiology will make a come-back at universities. A recent NSF announcement 'Special competition in systematic biology: Partnerships for enhancing expertise in taxonomy (PEET)' is an encouraging step in this direction.

RESEARCH

The focus of my research activities changed periodically during my academic career [9]. Early research dealt with yeasts involved in various aspects of food spoilage and food fermentations, primarily from the taxonomic point of view, which, at that time, was still in its embryonic stage of development. My work then went in an ecological direction, emphasizing the role of insects in the transmission of spoilage yeasts to food products. Later my students and I became interested in yeasts that insects themselves require under natural conditions to complete their life cycle successfully. The main emphasis was on wild species of *Drosophila* and on various bark beetles. Suspected feeding places of *Drosophila*, such as tree exudates, were also investigated extensively. These studies, carried out over wide geographic areas [10,21], led to the discovery of many new yeast species.

Parallel with the above activities, a number of biochemical aspects of yeasts were pursued. These included the polysaccharide composition of yeast cell walls and their enzymatic degradation by various β-glucanases of bacterial as well as endogenous yeast origin, capsular polysaccharides, types of carotenoid pigments, and hydrolytic enzymes of yeasts, such as polygalacturonase, amylases, inulinase, trehalase, and transferases.

In the seventies, yeast taxonomy started to become molecular as a supplement to traditional taxonomy. As it became possible to determine nuclear DNA base competition routinely and methods to do DNA pairing were being developed, revolutionary changes in taxonomic criteria resulted [16]. Our laboratory has been active in this area thanks to grants from the National Institutes of Health. At about the same time a collaboration began with colleagues from the University of Arizona in Tucson on the yeast biota found in rotting tissue of various cactus species, which is the habitat of desert-adapted species of *Drosophila*. Initially the yeasts found in these cactus necroses appeared by traditional phenotypic criteria to represent well-known species from other habitats. Molecular criteria were

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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essential in recognizing most of them as new species with highly specific habitats. In 1976, a more extensive survey of yeasts in cactus necroses was conducted in the North American Sonoran Desert, including the Baja California Peninsula and the State of Sonora, Mexico [19]. More than 700 strains of yeasts were collected and identified in Davis, resulting in the recognition of a total of nine new cactus-specific yeasts.

Also in the late seventies, we began collaborating with Stuart Barker from the University of Sydney, Australia in a study of the yeast biota of *Opuntia* species found in Australia. Cacti are not native in Australia but were introduced during the last century. Prickly pears ultimately overgrew large areas of the continent until during the 1920s a biological control program was instituted. It involved the introduction of rotting cactus tissue from the Americas, containing yeasts, bacteria and eggs of the destructive moth *Cactoblastis cactorum*. This program kept the spread of the *Opuntia* species under control, but introduced at the same time the microbial biota associated with American opuntias. Samples of rotting *Opuntia* species were collected by Barker and colleagues over a vast area of eastern Australia and airmailed to the Davis laboratory where they were analyzed for the yeast communities present [1]. Most of the 950 strains studied were cactus-specific and similar to those found in the southwestern US. However, two new species were identified and described: *Pichia opuntiae* [17] and *Clavispora opuntiae* [11]. The last species is heterothallic and occurs only as haploid mating types in nature, one of the mating types being extremely rare in Australia and present in only one narrow area. *Pichia opuntiae* has not been found during surveys of cactus necroses in North America and it remains a challenge to find the origin of this species in the Americas.

In 1981, NSF support was obtained for three years for 'A Collaborative Investigation of the Yeast Flora Associated with *Opuntia* Cacti in the Southwestern United States'. The two principal investigators were Tom Starmer and myself. Tom, coming from the University of Arizona, had been a postdoctoral visitor in my lab and was at that time on the faculty of Syracuse University. Besides isolating more than one thousand yeast strains from cactus necroses in southern Arizona and Texas, two cruises of the NSF research vessel 'Cape Florida' were made in 1982 and 1983, respectively, to explore the yeast biota associated with cactus necroses on islands in the Caribbean Sea and the Bahamas. The following islands or countries were included: The Dominican Republic; Haiti; Jamaica; Monserrat; Little Conception and Great Inagua (Bahamas); Tortola, Virgin Gorda, and Prickly Pear Island (British Virgin Islands); Cayman Brac, Little Cayman and Grand Cayman (Caymand Islands); Navassa (United States); and St Maarten (Netherlands).

In part, the survey was intended to compare the yeast biota of cacti in a subtropical climate with that of the Sonoran Desert. *Pichia antillensis* [20], *Pichia barkeri* [12], and *Pichia kluyveri* var. *cephalocereana* [13] represented three new species not found in the Sonoran Desert or other areas sampled. Conversely, a number of cactus-specific species common in the Sonoran Desert were absent in the Caribbean area. We isolated nearly 1100 strains during these cruises, most of which were purified en route since we had laboratory facilities on board [21].

Two additional NSF grants were awarded for the period 1984–1991. Both dealt with 'Population Biology and Systematics of Yeasts Associated with Cactus: a Collaborative Investigation'. During this period the original team was joined by André Lachance, one of my former PhD students and then a faculty member at the University of Western Ontario, Canada. During this time, some further collections of yeasts were made from *Opuntia phaeacantha* in the southwestern United States for the purpose of determining seasonal changes in the yeast community structure and to establish the role of various insects in the distribution of the microbiota responsible for cactus necrosis from plant to plant. A limited survey was also made in 1990 of yeasts found in opuntias and columnar cactus necroses in the Tucuman area of northern Argentina by Lachance and myself. Most of the cosmopolitan cactus-specific yeasts identified in North America were also found in Argentina, but several other common species appeared to be absent. In particular, *Pichia opuntiae*, not present in North America but common in Australian opuntias, was not isolated, even though the rotting opuntias used in the biological control program were shipped in part from Buenos Aires to Australia. Clearly, a wider more inclusive future survey is needed in Argentina to discover the origin of *Pichia opuntiae*. Close relatives of this species, *Pichia thermotolerans* [4] and *Pichia antillensis* [20] are found in North America.

Most efforts from 1984–91 in the Davis laboratory were devoted to work on taxonomic problems by molecular approaches to cactus-specific yeasts, that resulted in the recognition of two additional new species, *Pichia caribaea* [14] and *Candida caseinolytica* [15]. Molecular taxonomic evidence also revealed a new species of the genus *Sporopachydermia*, which shows no significant DNA complementarity with *Sporopachydermia cereana*, another cactus-specific yeast, although it is phenotypically very similar. André Lachance has recently found some differentiating characteristics of this species, which is common in the Caribbean area.

It has become clear from molecular and phenotypic evidence that yeasts from cactus necroses classified in earlier papers as *Candida ingens* [21], which is not a cactus-specific yeast, do not conform to that species. Instead, they represent at least three separate new species belonging to the genus *Dipodascus* or *Geotrichum* (the asexual state of *Dipodascus*). Up to now, the sexual state of only one of these species has been discovered through mating of complementary haploid isolates. A paper describing this new species is in preparation. We assume that through evolutionary convergence all three of these species have acquired a common phenotype that is similar to that of *Candida ingens* (sexual state *Dipodascus ingens*) [2]. Traditional differentiating characteristics in yeast taxonomy, including morphology, did not distinguish these three species from *C. ingens* when studied initially [8]. We later found that DNA relatedness between *C. ingens* and the three putative new species was very low. Moreover, *C. ingens* is urease-positive and lipase-negative in contrast to the cactus isolates. The latter can now be separated by criteria such as sensitivity to triterpene glycosides, utilization of 2-propanol as

sole carbon source and ability to utilize L-lysine as sole nitrogen source. Altogether, NSF support has contributed to the recognition of at least 25 new cactus-specific yeast species, in addition to several from other sources [3,5,6].

In 1992, when I presented some lectures on yeast at the Culture Collection and Research Center of the Food Industry Research and Development Institute in Hsinchu, Taiwan, ROC, there was an opportunity to collect more yeast strains from various natural sources with the help of Mr Ching-Fu Lee. The isolates were studied in part in Hsinchu as well as in Davis during Mr Lee's visit here and led to the discovery of an interesting new species of the genus *Arthroascus* [7]. It is evident from the foregoing that as a result of the many ecological forays, numerous cultures accumulated in the Department of Food Science and Technology in the form of mini-collections that ultimately grew into a major collection.

THE YEAST CULTURE COLLECTION

The collection was started by Professor W.V. Cruess at UC Berkeley in the mid thirties to maintain yeasts associated with wine production and spoilage. It was extended by my colleagues Emil Mrak and Reese Vaughn to include both yeasts and bacteria involved with the fermentation of pickles and olives. After the Food Science Department moved to Davis in the early fifties, the yeast collection expanded greatly by the addition of thousands of strains collected during various expeditions and research projects described earlier [10] and in this article.

Within the constraints of available facilities, the only practical way to preserve so many strains was storage on malt agar slants covered with sterile mineral oil. Only the most valuable cultures were lyophilized additionally as a back-up. Storage under mineral oil, although convenient for access, has many disadvantages as well. Some species do not survive well due to the production of toxic metabolites (such as acetic acid produced by *Brettanomyces* spp. and others), or are intrinsically fragile (e.g. *Pichia amethionina* and close relatives). Mutation of strains constitutes another problem with this type of storage, especially serious with haploid strains. It is also labor-intensive (special media for osmophilic species and those with unusual growth requirements) and the need for transfer to fresh medium every three to ten years. Contamination is also a factor during long storage, as well as drying out if the agar is not fully covered with oil. As a result we have lost a significant part of the collection which now totals approximately 6000 strains. During the past years we have received several small grants from the Genetic Resources Conservation Program on campus, that enabled me with undergraduate student help to transfer cultures in critical need of fresh medium.

The question of why save so many strains may be asked as long as the type strains of new species have been deposited in an internationally recognized culture collection as is required by the Botanical Code that governs yeasts and other fungi. The Davis Yeast Collection is unique because in addition to the type strains, a large number of other strains are preserved that differ in habitat and geographic origin. This allows for a broader and more meaningful delimitation of species than those based on a single strain. Multiple strains are useful when studies are to be done to determine genetic variation caused by allopatry or host plant variation [18]. The importance of special research collections has also been recognized by the Directorate for Biological Sciences, Division of Environmental Biology of the National Science Foundation. The recently announced program on 'Research Collections in Systematics and Ecology' offers potential support in collection improvement and collection computerization. I have applied for such a grant and if approved the Davis yeast collection can be permanently conserved by storage at low temperatures and accessible by computer. This will not only involve the physical relocation of the collection but also the renaming of many strains which have become synonyms as a result of the many reclassifications in recent years. A new (4th) multi-authored edition of *The Yeasts—a Taxonomic Study* under the editorship of Clete Kurtzman and Jack Fell, scheduled for publication in 1995, should facilitate bringing the older nomenclature up to date.

CONCLUDING REMARKS

In addition to my principal activities mentioned above, I have kept busy in a number of other areas. Since emeritus professors remain members of the academic senate, committee service is a normal activity for emeriti. I served on the Davis Faculty Welfare Committee, first as a member and later as its chair. Chairmanship also entails membership of the university-wide Faculty Welfare Committee, that meets regularly with representatives of the system-wide administration in Oakland. This has been one of the most interesting and enjoyable assignments as the Committee works on a broad range of issues affecting the well-being of faculty and emeriti.

My associate editorship of the International Journal of Systematic Bacteriology has also been a very satisfying experience. I have been handling manuscripts dealing with Actinomycetes, yeasts, and bacteria related to foods throughout my retirement. It has been a wonderful and stimulating learning experience in spite of frustrating moments when a reviewer loses a manuscript or when one receives two opposing recommendations: accept and reject! With the strong growth of the journal in recent years, editorial work has become increasingly demanding on my time, and I feel that it is appropriate for someone else to take over.

I very much appreciate the Special Issue of the Journal of Industrial Microbiology initiated by my friends and colleagues, and I thank them warmly for their efforts in realizing it. I am thankful too that my overall health during the years of retirement has permitted me to do the many things I have written about and enjoy doing. A real inspiration in these activities has been my wife Diane, whom I married in 1987 and who has created a happy new life for me.

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