

Biodiversity of Yeasts in Man-made Environments

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Summary

*Agroecosystems and foods are based on natural habitats which have become partially or largely artificial by human interventions. Nevertheless, foods can be treated in terms of microbial ecology, and vineyards and wineries provide examples of man-made ecosystems in which the biodiversity of yeasts can be studied. Molecular techniques have made it possible to characterize the intraspecies population of the wine yeast, *Saccharomyces cerevisiae* at the genetic level, to follow its distribution, variation and dynamics from the grapes through spontaneous and induced fermentation, as well as to study the speciation and domestication of *Saccharomyces sensu stricto* yeasts.*

Keywords: *Saccharomyces sensu stricto*, yeast biodiversity, microbial ecology

Introduction

Biodiversity means the total variability among living organisms in all ecological systems on Earth, the functioning and maintenance of which is essential for human life. In our time there is growing concern about a global biodiversity crisis that created much interest from scientific, economic and social aspects alike (1). These relate almost exclusively to natural ecosystems such as tropical forest, oceans, lakes and rivers, and the loss of plant and animal species inhabiting them as a consequence of increasing human impact with the intensification of agriculture, urbanization and industrialization. Far less is spoken of, and in fact known, about microorganisms which occur in all niches where life is possible and about their biodiversity which is crucial for functioning of every ecosystem on the Earth.

Global biodiversity matters are of fundamental and vital importance. In the context of the present paper I shall confine myself to aspects of biodiversity within the broad conceptual framework of food microbial ecology, taking yeasts as examples.

Given the definition of the ecosystems, it is easy to see that ecological concepts are applicable to food microbiology. It is now increasingly recognized and appreciated that foods form an ecosystem regardless how artificial and man-made it may be (2). The borderline between natural and artificial ecosystems is not clear. Agroecosystems are based on natural habitats which have become partially or largely artificial through the

introduction of selected crop plants and livestock animals by man replacing natural flora and fauna (Fig. 1). The artificiality of these systems varies enormously depending upon the intensity of human intervention from agricultural produces to the fully processed foods. Understanding foods as functioning ecosystems that are

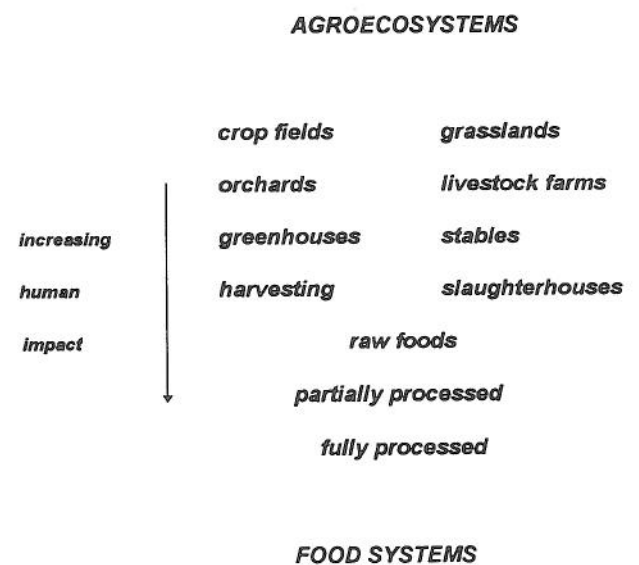


Fig. 1. Man-made agroecosystem

elements of microbial biodiversity is a relatively new field of research. Nevertheless, the microbial ecology of foods has become a recognized and increasingly accepted concept that has greatly contributed to our understanding of the structural and functional properties of food ecosystems (3).

Yeasts occur widely in natural and artificial ecosystems and the principles of food microbial ecology can be applied to food-borne yeasts. Foods can be described as ecological niches whose colonization by yeasts as well as their growth and the selection of specific spoilage association are governed by the intrinsic and extrinsic ecological factors prevailing in foods as opposed to the implicit properties of yeasts (4). Sharp differences exist between yeast communities of various foods since yeast species display specific preferences for certain ecological conditions prevailing in foods and develop highly specific spoilage associations. Correlations can be found between the population and diversity of yeast species and the type of foods (5).

Characterizing Yeast Biodiversity

The biodiversity of yeasts in agroecosystems and foods can be assessed on taxonomic, ecological and genetic levels (Fig. 2). Organismal biodiversity in various foods has been studied using the traditional physiological and biochemical tests of classification. Ecological surveys have provided an overview of the distribution and frequencies of food-borne yeasts (Table 1). Of these studies the picture has emerged showing that some 60 to 90 species can occur in foods rich in nutrients whereas 30 to 50 species can thrive in commodities providing a more restrictive habitat. Nevertheless, only 10 to 20 species form a specific association with any food item where only a few of these are predominant (Table 2).

It is the population at interspecific and intraspecific levels that brings the three approaches characterizing biodiversity to a common denominator. It has, however, become accessible for study only recently by the use of modern molecular techniques. Methods used to distinguish between strains include killer activity (6), profiles of total soluble cell protein or their isoenzyme fraction (7,8), cellular fatty acid patterns (9), as well as various methods of nucleic acid fingerprinting and typing such as PFGE, RFLP, and PCR-based techniques (10–17). Of

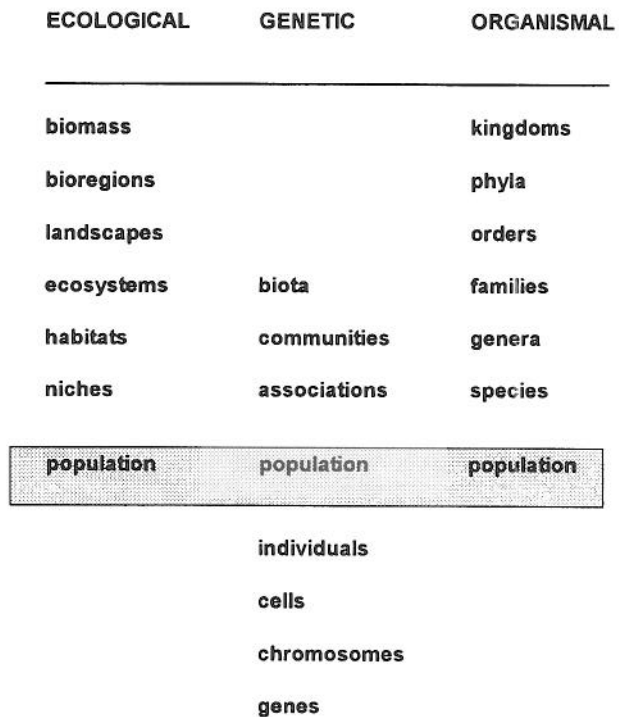


Fig. 2. The composition and levels of biodiversity

Table 1. Distribution of yeasts in various foods

Food	Number of species		
	Total	Dominant	Minor
Fruits	18	1	14
Sweet corn	20	2	13
Orange concentrate	12	3	5
Spoiled wine	7	1	4
Pickles	5	1	3
Cheese brine	17	2	0
Vienna sausage	22	2	12
Cassava flour	10	1	9
Sugar cane	8	1	7
Vegetable salad	5	2	1

Date from Deak and Beuchat (5)

Table 2. Fractions (%) of some food-borne yeast species

Species	All foods	Fruits, beverages	Meat, dairy	Low a_w products	Low pH products
<i>Debaryomyces hansenii</i>	6.72	4.61	8.65	5.64	4.73
<i>Dekkera anomala</i>	0.21	0.65	–	–	–
<i>Hanseniaspora uvarum</i>	1.35	3.20	–	0.59	–
<i>Pichia anomala</i>	4.56	4.25	4.19	3.53	5.52
<i>Rhodotorula mucilaginosa</i>	3.78	3.45	3.63	0.94	2.10
<i>Saccharomyces cerevisiae</i>	7.04	6.40	6.70	7.53	7.37
<i>Zygosaccharomyces bailii</i>	3.05	4.76	1.14	4.94	5.52
<i>Zygosaccharomyces rouxii</i>	1.14	0.37	2.09	9.41	4.21

Data from Deak and Beuchat (5)

these, the restriction analysis of mitochondrial DNA and the karyotyping of chromosomal DNA have proved very successful and are widely used (18–21).

In addition to characterizing population structure, other questions relating biodiversity composition and function can now be addressed by using modern methods. The species of the *Saccharomyces sensu stricto* group is an excellent model for the dynamics of biodiversity in vineyard and winery ecosystems and also for showing the process of speciation and domestication.

The Vineyard and Winery Ecosystem

Production of wine implies a number of operations of increasing intervention by man from the vineyard to bottling (Fig. 3). The accompanying microbiological processes have been the subject of numerous studies and the yeast microbiota of grapes, the changes in yeast population and the succession of species in the course of must fermentation are well known (22).

In general, the indigenous yeasts from grapes, mainly species of *Hanseniaspora/Kloeckera*, as well as some *Candida* and *Pichia* grow during the early stages of fermentation but eventually die off leaving *Saccharomyces cerevisiae* as the predominant wine yeast which completes the fermentation. Some insight into yeast biodiversity has been obtained by classical studies as summarized by Vaughan-Martini (23). *Hanseniaspora guilliermondii* is most abundant on grapes and early must in southern Italy whereas *H'spora uvarum* is more frequent in the north; *Metschnikowia pulcherrima* is relatively frequent in Italian and Spanish wine regions while *C. stellata* is widespread in the Bordeaux region of France. *P. mem-*

branifaciens may survive long in low temperature fermentations, *Torulaspota delbrueckii* and *Kluyveromyces thermotolerans* can in some cases compete with *Sacch. cerevisiae* in the mid-phase of fermentation, but eventually only *Sacch. cerevisiae* is found at the end of fermentations, all over the world.

A number of questions have, however, remained open or answers to them are still controversial. Since the time of Pasteur it has been claimed that *Sacch. cerevisiae* wine strains colonize the grapes and are introduced into must when grape clusters are crushed. Hansen formulated the idea that *Sacch. cerevisiae* survives the winter in vineyard soil falling to the ground with ripe grapes and grapes which become colonized again in summer by insect vectors. However, later studies have asserted that *Sacch. cerevisiae* is very infrequent on grapes or does not even occur at all, leading to claims that it is introduced into must from winery equipments and from the walls of fermentation tanks (23). So, what is the origin of wine yeast *Sacch. cerevisiae*? If it comes from the grapes, are there characteristic strains specific for a wine region, vineyard or cultivar? How it is influenced by locality, climate, vintage and stage of ripening? If it comes from the winery, again, are there characteristic strains for a cellar? How it is influenced by the type of wine and the method of fermentation? What is the dynamics of wine yeast strains? If a starter is inoculated, how does it compete with native/indigenous strains? What is the interaction between strains possessing different killer characteristics?

Molecular Approaches

Answers to these and other questions have become available only recently with the use of molecular techniques. Both electrophoretic karyotyping and mtDNA analysis have revealed that high polymorphisms exist within *Sacch. cerevisiae* strains and that this serves as a means to study their biodiversity (24), to establish correlation between specific strains and ecological factors (18), to follow the population dynamics during spontaneous fermentation and the competition between indigenous and inoculated strains (25,26).

Using a primer from the 2 μ m plasmid for PCR-amplifying and CHEF karyotyping, Török *et al.* (27) have demonstrated that *Sacch. cerevisiae* does occur on grapes, although in very low population (usually less than 0.1% of the resident yeast biota the total population of which may be up to 10^5 to 10^6 g⁻¹). Strains found on the grapes can be followed through the fermentation process. On the other hand, PFGE data have also given evidence for the winery origin of strains, some of them becoming predominant during fermentation from the same location over consecutive years (28,29). Querol *et al.* (25) showed high similarity between mtDNA patterns in one winery where a single strain was predominant in successive years. A small number of strains of *Sacch. cerevisiae* dominated the fermentation in all vats in the same winery independently of cultivar and the time of harvest (30). There are conflicting data as to the geographic diversity of wine yeasts. Versavaud *et al.* (31) found no correlation between genetic relatedness and the locality of wineries, and the finding that predominant strains

Vineyard	climate, soil
	agrotechnique
	cultivar
	harvest
Winery	crushing, pressing
	SO₂ addition
	fermentation
	inoculation
Wine	racking
	fining
	aging
	bottling

Fig. 3. Factors influencing vineyards and winery ecosystems

were found over consecutive years in the same cellars was interpreted as a consequence of their prevalence in the natural microbiota present on grapes. On the contrary, Guillamón *et al.* (18) established close relationships among strains not only from the same regions but also according to the type of wine (white and red) and cultivars. Data are more consistent demonstrating that during spontaneous fermentation several different strains of *Sacch. cerevisiae* occur simultaneously and these follow each other successively until the end of fermentation when only one or few become predominant (25,26,30,31). Inoculated strains will sooner or later replace native yeasts which, however, may survive and contribute to the fermentation (25,32). A commercial wine strain can establish itself in the winery, become predominant and can continue to be found after several years (29,33).

As a general conclusion, it appears that a broad intraspecific biodiversity exists within *Sacch. cerevisiae* wine yeast in that a monospecific but polyclonal population of this species develops during fermentation which is constituted of several genetically distinct strains growing simultaneously and successively, one or more of them becoming predominant.

Factors Influencing Yeast Biodiversity

One factor which can play a role in the competition between yeast strains is their killer property. This phenomenon has created much interest because killer strains could dominate over wine yeasts causing stuck fermentation. On the other hand, selected killer wine yeasts can be used to facilitate fermentation (6). Killer strains have been found differently distributed in various wine-producing areas, they may occur in 88% of spontaneous fermentations and make up 60 to 90% of the total population (34). The killer toxin producing phenotype is frequent among predominant wine yeast whereas the associated strains are usually sensitive or neutral. Nevertheless it has been shown that some sensitive strains may coexist with killer strains (31,35,36).

Speciation and Domestication

Two factors of general importance in influencing biodiversity, *i.e.* speciation and domestication, can be exemplified by the *Saccharomyces sensu stricto* group.

According to modern classification, three biological species, *Sacch. cerevisiae*, *Sacch. bayanus* and *Sacch. paradoxus*, and the hybrid taxon *Sacch. pastorianus* (between *Sacch. cerevisiae* and *Sacch. bayanus*) are recognized within the *Saccharomyces sensu stricto* species complex (37).

These sibling species cannot be reliably differentiated and identified by traditional tests (38), and their delineation rests on DNA/DNA homology and genetic hybridization. The degree of DNA/DNA reassociation between *Sacch. cerevisiae*, *Sacch. bayanus* and *Sacch. paradoxus* is 20 to 50% whereas between *Sacch. pastorianus* and its supposed parents is 50 to 70% (39,40). In accordance with the biological species concept, interspecific matings yield viable hybrids but these produce non-viable spores and are sterile (41). Recently, genetically

isolated populations of *Saccharomyces sensu stricto* yeasts have been found in Brasil and Japan that apparently represent three new species (42). However, no formal description of these has been made yet.

Sacch. cerevisiae is considered to be a domesticated species which is predominantly associated with grape must and wine and has also been isolated from fermenting fruit juices and various spoiled foods. A number of strains of this species are propagated as wine, baker's, distiller's and top brewing industrial yeasts (23). *Sacch. pastorianus* is the bottom brewer's yeast. The ecological niche for *Sacch. bayanus* is less clear. This species is represented by wine strains carrying out low temperature and secondary fermentations but also isolated as wild yeasts in breweries (strains earlier identified with the now synonym *Sacch. uvarum*) (19).

The wild (natural) populations of *Saccharomyces sensu stricto* group mostly belong to *Sacch. paradoxus* which, in turn, does not occur in man-made fermentations. This species is usually found in tree exudates, insects and uncultivated soils (43). Strains of *Sacch. cerevisiae* have very rarely been described from similar habitats, and only three strains of *Sacch. bayanus* are known of natural sources (38). It is suggested, that the domesticated forms of *Saccharomyces sensu stricto* yeasts had originated from wild populations of *Sacch. paradoxus* in the far East. Naumov *et al.* (42) also found that *Sacch. paradoxus* populations in Europe differ genetically from those in the far East and North America. These populations may represent an early stage of allopatric speciation.

With the application of molecular techniques the distribution and variation of yeasts in natural and artificial ecosystems can be studied and a clear picture is emerging on the biodiversity of yeasts in vineyard and winery ecosystems.

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Biolška raznolikost kvasaca u neprirodnom okolišu

Sažetak

Poljoprivredni ekosustavi i hrana zasnivaju se na prirodnim nalazištima koja su postala djelomično ili pretežno neprirodna zbog ljudske intervencije. Nasuprot tomu, hrana se može razmatrati s gledišta mikrobne ekologije, a vinogradi i vinarstvo pokazuju kako ljudi utječu na ekosustave u kojima se može proučavati biološka raznolikost kvasaca. Molekularni postupci omogućili su karakterizaciju unutar sojeva vinskog kvasca *Saccharomyces cerevisiae* na genskoj razini, kako bi se mogla pratiti njihova rasprostranjenost, varijacije i dinamika od grožđa do spontane i inducirane fermentacije, a ujedno proučavati specifičnost i uzgoj kvasaca *Saccharomyces sensu stricto*.