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review

Yeasts – What Reactions and Interactions Really Occur in Natural Habitats

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Summary

Yeasts are important players in many ecosystems and make a significant contribution to the biodiversity of nature. This biodiversity has given species that have significant practical and commercial importance. Traditional concerns have focused on their roles as spoilage organisms, agents of desirable fermentations and on their medical significance. Recent interests include their potential use as biocontrol agents and probiotic species, and more intensive screening for biocatalytic activities that may have commercial benefit.

Keywords: yeast, isolation, properties, interactions

Introduction

To understand, manage and exploit the growth and activities of yeasts in their habitats, the following fundamental information and data are required:

- Accurate and reliable methods for the isolation and enumeration of yeasts from their habitats.
- Identification of the yeast species and strains present.
- A topographical picture of the spatial location and arrangement of the yeast cells in their habitat.
- Quantitative data on the growth of the yeast species within the ecosystem over a defined time-scale.
- The chemical and physical properties of the eco-substrate and eco-environment that impact on the growth and activities of the associated species.
- The biochemical and physiological properties of the species, that permit their growth and activity in the ecosystem.
- The specific interactions that occur between the different yeast species and strains and between yeasts and other microorganisms (bacteria, fungi, algae) which occur in the habitat.

This presentation addresses the above needs, revealing significant gaps in present knowledge, and demonstrating the need for greater systematic research which more precisely describes the influence of environmental factors on yeast growth, and the diversity and nature of yeast interactions with other microorganisms.

Methods for Isolation

Methods for the isolation of yeasts from natural habitats and the limitations associated with them have been described by Phaff and Starmer (*1,2*) and Beuchat (*3*). The approach remains classical, namely, suspending and homogenising a sample of the habitat in a diluent, preparing serial dilutions and plating them onto an appropriate agar medium. However, there are still a few traps in this approach which, if ignored, could give less than an accurate picture of the yeasts in the ecosystem.

Martini and co-workers (*4*) have been emphasising for some time now the need for using physically aggressive methods such as blending and sonication to release the yeast cells from their habitat into a suspension from which they can be isolated and enumerated. Mild shaking or rinsing of samples with the suspension medium are insufficient to detach yeast cells embedded in crevi-

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ces and pores of the habitat substrate or adhering to the substrate surfaces. Failing such considerations, there is a significant risk of underestimating the number of yeast species present and their true populations.

The diluent in which the sample is suspended and diluted for subsequent agar plating is another significant consideration often overlooked. In the field of bacteriology it is well known that a small concentration of nutrient (*e.g.* 0.1% peptone) should be included in the diluent to prevent losses in cell viability. Suspensions and dilutions prepared in distilled water, saline or buffers can lead to rapid and significant losses in cell viability and gross underestimations of the true population. The same concept applies to yeasts where, unfortunately, many workers still use distilled water and saline as diluents. Mian *et al.* (*5*) have clearly shown that some yeast species are particularly sensitive to dilution in water or saline and that 50–60% or more of the population may be lost if these diluents are used (Table 1). Longer times between dilution and plating lead to greater losses in cell viability. Incorporation of 0.1% peptone or 0.1% malt extract into the diluent will prevent this problem.

Table 1. The effect of diluent on the viable counts of various yeast species (*5*)

Diluent	Viable count / %		
	a	b	c
Peptone (0.1%)	100	100	100
Malt extract (0.1%)	96	90	95
Distilled water	84	55	65
Saline	87	78	60
Phosphate buffer	94	78	61

a – *Sacch. cerevisiae*, b – *Hanseniaspora uvarum*, c – *Zygosaccharomyces bailii*

The field of bacteriology has also revealed that, in most natural environments, bacterial cells are surviving in a stressed or sub-lethally injured state. These cells do not yield colonies when cultured directly onto selective isolation media, but are readily recovered when cultured under non-selective conditions. However, given appropriate conditions for resuscitation and repair, these sub-lethally injured cells will grow on selective media (*6*). This same concept equally applies to yeast cells, although very much ignored in many ecological studies where samples of habitat are directly plated onto acidified agar media. Yeast cells become stressed and injured when exposed to sub-lethal conditions of heating or cooling and a good proportion of them may not be recovered when plated directly onto acidified media but are fully recoverable if plated onto non-acidified media (Table 2). Other initiatives to make the isolation medium selective, such as inclusion of NaCl, can also lead to significant underestimation of the stressed population (Table 2).

Spatial Arrangement and Location of Yeast Cells

In many natural habitats, yeasts grow in association with a solid surface or substrate (*e.g.* surfaces of leaves and fruit, but consider also the confines of a fermenting bread dough or growth in block of cheese). In these cases, the cells do not get dispersed and remain in contact or close proximity to each other, leading to the development of microcolonies or biofilms. The concept of microcolony and biofilm formation has received significant attention in relation to bacteria (*7*) but has not been explored to any depth with yeasts.

Table 2. Populations of freeze-stressed yeast cells as determined on different media*

Species	Count / %			
	MEA	AMEA	YNB	MEA + 3% NaCl
Saccharomyces cerevisiae	100	44	66	60
Kluyveromyces marxianus	100	38	87	18

Yeast cells were frozen
MEA, malt extract agar; AMEA, acidified malt extract agar; YNB, yeast nitrogen base — 5% glucose

*M.A. Mian and G.H. Fleet, unpublished results

Scanning electron micrographs show that single yeast cells and microcolonies occur in crevices and pores on the surfaces of plants and fruits (*8,9*) and in the pores of wooden barrels used to store cider and wine (*10*). In the case of biofilm formation, a confluent mass of growth develops over the substrate surface and, presumably, there are many natural examples of this for yeasts but the reports are few. Douglas (*11*) has reported biofilm formation by *Candida albicans* in clinical situations, and velum formation by flor yeasts in the production of sherry could be another interesting example. Biofilms of yeasts, perhaps in association with bacteria, could possibly develop on the inner surfaces of wine barrels, when wine is stored for lengthy periods. In the fermentation of red wines, the juice is fermented in contact with the grape skins for the first few days. There is probably a film of yeast biomass firmly associated with the skins, the species or strains of which could be different from those growing in the juice. A similar situation could occur in the fermentation of Scotch whisky, where barley solids are part of the fermenting mash.

Biofilm formation involves unique cellular physiology and biochemistry. Cell surface hydrophobicity and the production of various extracellular gums and mucilages are important properties for the adherance and attachment of yeast cells to surfaces and maintenance of a cell mass (*7,11*). Moreover, the chemical composition and biochemical activities of yeast cells growing as a high-density mass could be significantly different from those growing as individuals in suspension (*12*).

Table 3. Viable populations of yeasts on different parts of broccoli before and after storage at 5 and 20 °C*

Broccoli parts	Initial	5 °C	20 °C
	Colony forming units/g		
Outer florets	4.1×10^4	8.2×10^4	6.7×10^4
Inner florets	6.1×10^3	5.9×10^3	1.0×10^4
Stem	1.8×10^3	6.3×10^3	1.4×10^3
Cut surface of stem	1.9×10^3	1.8×10^4	1.3×10^4

*M. Padaga, G. H. Fleet, J. E. Paton and G. M. Heard, unpublished data

Table 4. Effect of sodium chloride and sucrose concentrations on the growth response of yeasts at different pH values (*20*)

Yeast species	A				B			
pH	2	3	5	7	2	3	5	7
D. hansenii								
(3 strains)	NG	10.0	15.0	15.0	NG	60.0	60.0	60.0
(1 strain)	2.5	10.0	10.0	10.0	50.0	50.0	50.0	50.0
Y. lipolytica								
(5 strains)	2.5	10.0	12.5	12.5	50.0	50.0	50.0	50.0
P. anomala								
(1 strain)	2.5	15.0	15.0	12.5	70.0	70.0	70.0	70.0
P. membranaefaciens								
(2 strains)	2.5	12.5	5.0	5.0	50.0	50.0	50.0	50.0
Sacch. cerevisiae								
(2 strains)	NG	5.0	7.5	7.5	NG	50.0	50.0	50.0
K. marxianus								
(2 strains)	NG	7.5	5.0	2.5	NG	50.0	50.0	50.0
(2 strains)	NG	10.0	10.0	10.0	NG	50.0	50.0	50.0
Kl. apiculata								
(4 strains)	12.5	12.5	10.0	G	50.0	50.0	50.0	50.0
Z. bailii								
(6 strains)	2.5	12.5	5.0	NG	50.0	70.0	70.0	NG
Z. rouxii								
(2 strains)	NG	15.0	15.0	7.5	NG	70.0	70.0	70.0

$$A = \frac{\text{Maximum m(NaCl) / V(suspension)}}{\%}$$

$$B = \frac{\text{Maximum m(sucrose) / V(suspension)}}{\%}$$

G = growth in the absence of NaCl; NG = no growth in the absence of NaCl or sucrose.

Within the one product or substrate, yeast populations may be different, depending on the chemical and physical nature of the surface. Table 3 shows a ten-fold higher yeast population on the outer florets of broccoli compared with the inner florets. Cut surfaces of the stem have a ten fold higher population of yeasts than the non-cut surface. There are likely to be differences in the species associated with these different surfaces and parts of the one product.

Growth Profiles of Species and Strains

To fully understand the colonisation of habitats by yeasts, information is needed about the kinetics of growth of individual species and strains. Relevant information includes data about the rates of growth and death, the size of the cell populations formed, successional development of species and strains and, as noted already, whether or not the growth occurs as microcolonies, biofilms or dispersed cells. The gathering of such quantitative data requires extensive sampling and analyses over a defined period of time, the practical logistics of which can be extremely demanding and a deterrent to investigation. The vast majority of yeast ecological studies fall short of providing this information and, essentially, have focused on reporting identification of the most frequently isolated species. In a few cases, samples have been collected from the same habitat over a period of time so that climatic influences of temperature and rainfall could be correlated with the incidence of the dominating species. While generally short on the quantitative detail needed to fully understand the process of colonisation, these basic ecological studies have yielded a wealth of important information about the specificity of the association between some yeasts and their particular habitats (*1,2,13*).

In some industrial fermentations, which could be interpreted as natural habitats, there is relatively advanced understanding of the ecological process. The fermentation of grape juices into wine is one such example. Quantitative studies in many laboratories have clearly established that, in most grape juice fermentations, there is a successional development of yeast species within the genera *Kloeckera/Hanseniaspora*, *Candida*, and *Saccharomyces*. The fermentation involves varying quantitative contributions from the different species (*14*). Such understanding only evolves when growth curves of the individual species are constructed — thereby emphasising the importance of the quantitative approach. Moreover, using molecular techniques such as restriction analysis of mitochondrial DNA and pulsed field electrophoresis of chromosome profiles to differentiate strains within a species, it has been possible to demonstrate that there can be sequential growth of strains throughout the fermentation, further highlighting the complexity of the ecology (*15*).

The successional growth of *Kloeckera* spp., *Candida* spp., *Kluyveromyces* spp. and *Saccharomyces* spp. also occurs in the natural fermentation of cocoa beans. The ecology of this fermentation is further complicated by successional growth of fungi lactic acid bacteria, acetic acid bacteria and *Bacillus* species, and the selective effect of the temperature of the process which increases (naturally) from 25–30 to 50 °C (*16*).

Properties of the Habitat

The properties of the habitat can be considered in terms of the substrate upon which the yeast occurs and the conditions of the external environment. The interactive influences of these properties ultimately determine which yeast species grow in the habitat (*17*). With respect to the substrate, key factors are its chemical composition (available nutrients, antimicrobial components, pH, concentration of solutes such as sugar and salts, redox potential) and physical composition (water activity, viscosity, microstructure). For external factors, the relevant influences are temperature, relative humidity, gaseous atmosphere, pressure and radiation but this information is not known for many habitats.

Properties of the Yeast

Yeasts possess a diversity of physiological and biochemical properties. In order for yeasts to survive and proliferate, these properties must complement the chemical and physical conditions of the habitat. However, when one moves outside of *Saccharomyces cerevisiae*, and perhaps a few other species, there is very little precise information about how yeasts respond in terms of growth and biochemical activity to the chemical and

physical conditions of their environment (*18,19*). What are the upper and lower limits for growth with respect to temperature, pH, NaCl concentration, and sugar concentration? How do the different conditions interact to affect these limits? How do these conditions affect growth rates, and maximum cell populations? Similar questions can be asked about how these factors affect nutrient uptake and metabolite excretion, the pathways of metabolism, and the production and activity of extracellular hydrolytic enzymes (*e.g.* proteases, amylases, lipases). Table 4 shows how pH can affect the tolerance of various yeasts to the different concentrations of NaCl and sucrose. For some species (*e.g. Debaryomyces hansenii* and *Yarrowia lipolytica*) salt tolerance is best at pH = 5.0–7.0 while for others (*e.g. Kloeckera apiculata*), it is best at the lower values of pH = 2.0–3.0. In contrast, pH had less effect on the yeast growth response to sucrose concentrations (*20*). Environmental conditions affect the concentration of volatile end-products of *Debaryomyces hansenii*. Growth in the presence of high concentrations of NaCl but not high sucrose concentrations substantially decreases the production of ethyl acetate, isoamyl alcohol and acetoin (Praphailong and Fleet, unpublished data).

Interactions of Yeasts with Themselves and Other Microorganisms

Only in exceptional circumstances will a single species occupy a habitat. Generally, a mixture of species will be present, the composition of which will be determined by the outcome of species interactions. There are various possibilities for microbe–microbe interactions, all of which apply to yeasts. These possibilities are competition, antagonism/amensalism, commensalism, mutualism and parasitism/predation (*21*). In the case of yeasts, all of these possibilities exist for interaction between different yeast species and strains, interaction between yeasts and bacteria and interaction between yeasts and filamentous fungi (*22*).

The yeast-yeast interactions are largely typified by antagonism, where killer toxins produced by one species or strain inhibits or destroys another species or strain (*23*). Although there have been major advances in understanding the molecular biology of the killer phenomenon, there are still many uncertainties about the influence of environmental factors (*e.g.* pH, temperature, ethanol, metal ions, NaCl and sugar concentration) upon this type of interaction. A most recent and interesting observation has been the effect of NaCl on this reaction. Inclusion of NaCl in the medium significantly increases the sensitivity of cells to killer toxins and broadens the range of species that can be affected by the toxin (*24*). There are various reports in the literature where killer strains have dominated in a habitat, but this is not a consistent outcome (*25,23*). The production of ethanol by some species (*e.g. Saccharomyces cerevisiae*) to inhibit less ethanol tolerant species (*e.g. Kloeckera apiculata*) is another classical example of antagonism within yeasts. Lachance and Pang (*26*) have recently described predation between yeast species and it will be interesting to discover how widespread this type of interaction occurs.

Yeast-bacteria interactions cover a range of examples which include:

- Yeasts inhibit bacteria through the production of ethanol and antibiotics (*27*).
- Bacteria inhibit yeasts by the production of acetic acid, cell wall degrading enzymes and antibiotics (*28*).
- Bacteria and yeast co-sediment, aggregate and agglutinate (*22,29*).
- Yeast stimulate bacteria by release of nutrients from autolysis, by vitamin production and utilisation of organic acids (*30*).

With respect to yeast fungal interactions:

- Yeasts inhibit the growth of fungi by the production of ethanol, killer toxins and wall degrading enzymes (*31–33*).
- Certain fungal proteins inhibit yeast growth (*34*).
- Yeasts stimulate the growth of fungi.
- Fungi stimulate the growth of yeasts by excretion of hydrolytic enzymes that produce substrates for yeast growth.

However for most of these interactions, there are only scattered casual reports, and a more systematic approach to their study is required.

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Koje se reakcije i interakcije doista odvijaju u prirodnom okolišu?

Sažetak

Kvasci su važni sudionici mnogih ekoloških sustava i bitno pridonose biološkoj raznolikosti prirode. U prirodi postoje određeni sojevi koji imaju veliku praktičnu i komercijalnu važnost. Ranije se istraživalo njihovo djelovanje kao uzročnika kvarenja, agensa poželjnih fermentacija i njihova medicinska primjena. Danas se ispituje moguća uporaba kvasaca kao biokontrolnih agensa i probiotičkih vrsta te vrlo intenzivno istražuje njihova biokatalitička aktivnost koja bi se mogla komercijalno iskoristiti.