THE INTIMATE RELATIONSHIP BETWEEN MAN AND YEAST: IT'S COMPLICATED

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ABOUT THE AUTHOR

Alfred Botha was born in Uitenhage on 30 January 1961 and spent most of his youth at Worcester in the Boland. After matriculating from High School Montana in 1978, he studied at Stellenbosch University where he completed his BSc Agric in biochemistry, followed by a BSc Agric honours also in biochemistry. In 1986 he joined the CSIR in Pretoria, where he was first introduced to the field of yeast biology by Prof JP van der Walt, himself a student of the well-known Dutch microbiologist, Prof Albert Jan Kluyver.

While working at the CSIR as a researcher, Alfred completed his MSc dissertation on the siderophores of lipomycetaceous yeasts at the University of Pretoria under the joint supervision of Prof Albert Eicker and Prof van der Walt. In 1990 he joined the University of the Orange Free State as researcher and later as lecturer, where he completed his PhD thesis on the lipid metabolism of lipomycetaceous yeasts under the supervision of Prof Lodewyk Kock. In July 1998 he took up an appointment as senior lecturer at Stellenbosch University and was promoted to associate professor in 2003.

Under Prof Botha's supervision, twenty-two students completed their MSc dissertations, while eight students obtained their PhDs. The findings of his research, mostly on the ecology and physiology of yeasts, have been published in over eighty peer-reviewed conference proceedings, journal articles and book chapters. He currently serves on the editorial board of the Canadian Journal of Microbiology and FEMS Yeast Research. Prof Botha is married to Nicolene and they have two children, Marco (15) and Nina (18).

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THE INTIMATE RELATIONSHIP BETWEEN MAN AND YEAST: IT'S COMPLICATED

INTRODUCTION

Yeasts have been associated with mankind's welfare for a long time. For millennia these unicellular fungi were cultivated by man as a source of food and drink. However, it was only some 140 years ago that Pasteur demonstrated that live yeast is essential for beer and wine fermentations (Pasteur, 1866; 1876). Since then, these organisms were also found to have biotechnological potential in the production of vitamins (Roman, 1957), fine chemicals (Botes et al., 2005; Miao et al., 2011), enzymes (Steyn & Pretorius, 1990), biofuel (Lynd et al., 2002) and even single-cell proteins (Du Preez, 1990; Roman, 1957). However, yeasts are not only important for industrial biotechnology, but these fungi also have potential uses in agriculture. Some yeasts were found to be beneficial for mycorrhizal interactions during which crop performance is enhanced (Fracchia et al., 2003), others inhibit growth of post harvest pathogens on damaged fruit (Chand-Goyal & Spotts, 1997; Roberts, 1990), while a few species are currently being included in biological fertilisers that are claimed to enhance soil quality. However, yeasts may also be detrimental to mankind since a number of species may act as opportunistic pathogens of humans (Ikeda et al., 2002; Lamagni et al., 2001). This phenomenon is of great importance to an ever-increasing immunocompromised human population suffering from HIV/Aids.

The extraordinary progress made in yeast biology may largely be ascribed to decades of studying the intrinsic characteristics of these organisms while growing in pure culture (Kurtzman & Fell, 1998; Lodder, 1971). Thus, during the last two hundred years yeast morphology, metabolism, as well as classical and molecular biology, was always at the frontiers of the biological sciences of the time. This review takes a closer look at the reasons for man's fascination with this versatile group of organisms and focuses on one of the new frontiers in yeast biology, that of its ecology in natural environments. However, before we can explore this realm of science, we first need to obtain a better understanding of the general characteristics of these microscopic eukaryotes that have captured the imagination of so many biologists over the decades.

WHAT ARE YEASTS?

Yeasts belong to a polyphyletic group of fungi, some of which are able to form sexual spores within an ascus, typical of the Ascomycetes, or on a basidium, characteristic of the Basidiomycetes (**Figure 1; Figure 2;** Kurtzman & Fell, 1998). However, these unicellular eukaryotes primarily proliferate via budding (**Figure 3**) or, in some cases, cell fission. This characteristic is seen as an adaptation to growth in aqueous environments, where yeasts may either reproduce while in suspension or within biofilms (**Figure 4**), attached to submersed surfaces (Branyik et al., 2004; Joubert et al., 2003; Lachance & Starmer, 1998).

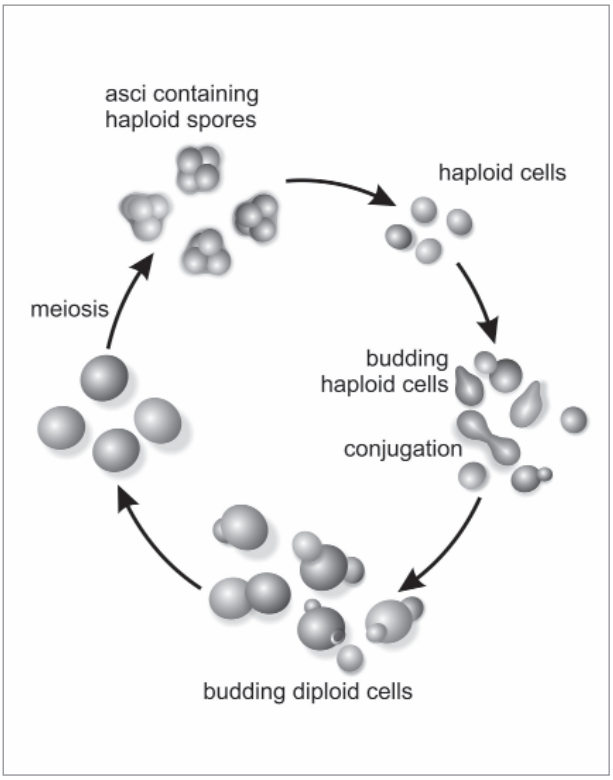

Figure 1: *An illustration representing the ontogenic stages in the life cycle of the ascomycetous yeast* Saccharomyces cerevisiae. *Although this yeast may produce rudimentary pseudomycelia, it usually reproduces asexually via budding. Sexual reproduction occurs via the formation of a sac-like ascus containing the haploid meiospores, called ascospores. Usually, these ascospores are dispersed via watery currents or insects to colonise fresh substrates.*

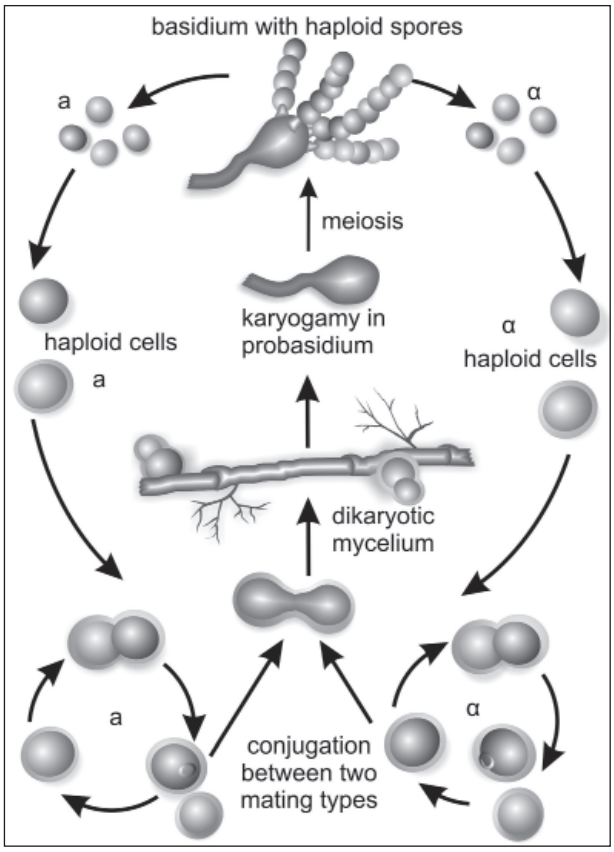

Figure 2: *An illustration representing the ontogenic stages in the life cycle of the basidiomycetous yeast* Filobasidium neoformans *(anamorph;* Cryptococcus neoformans*). This yeast produces a distinctly filamentous ontogenetic stage following conjugation between two different mating types. The filamentous stage gives rise to the sexual fruiting structures called basidia, on which the haploid meiospores called basidiospores are produced. Usually, these basidiospores are dispersed via air currents to colonise fresh substrates.*

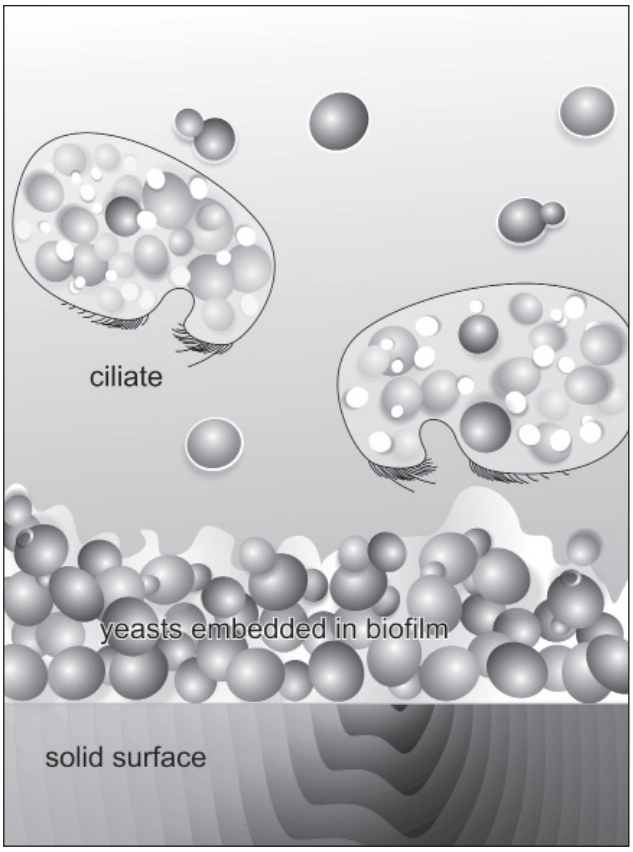

Figure 4: *An illustration showing yeasts growing in an extracellular polymeric matrix of a biofilm that is being grazed upon by ciliates. The ciliates may prey upon planktonic yeasts released from the biofilm, as well as graze upon the acellular biofilm matrix. Biofilm formation is a known mechanism whereby microbes sequester and concentrate nutrients while growing in low nutrient watery environments, which occur in many natural habitats such as soils, wetlands and oceans (Decho, 1990).*

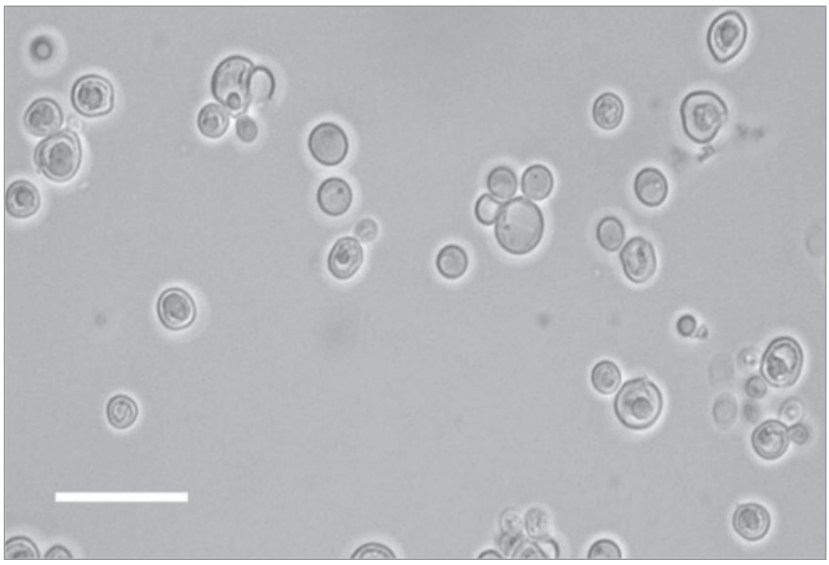

Figure 3: *Photomicrograph of a budding yeast cells representing the opportunistic human pathogen,* Candida albicans. *Bar = 10 µm (Photo: Marnel Mouton).*

It is estimated that fungi, including yeasts, have existed on earth for a long time. Molecular evidence exists that the fungal lineage diverged from that of animals approximately one billion years ago, while nucleic acid variation and fungal fossil records suggest that the split between the Ascomycete and Basidiomycete lineages occurred approximately 400 million years ago (Taylor & Berbee, 2006). This event occurred long after the emergence of photosynthesis and coincided roughly with the period associated with the fossil records of the earliest land plants. Although it was hypothesised by some scientists that the first fungi were unicellular, molecular data also indicated that the emergence of the present yeast lineages occurred relatively recently (James et al., 2006). Fossil records of fungal yeast stages were uncovered in amber that range in age from 15 to 100 million years (Rikkinen & Poinar, 2001, 2002; Schmidt et al., 2008; Veiga-Crespo, 2004). At present, these unicellular fungi, representing a wide diversity of unrelated taxa, occur in many different habitats (Rosa & Péter, 2006). Although these fungi may notably differ regarding the morphology of their sexual stages, nutrient utilisation profiles, oxygen and temperature requirements, as well as the ability to withstand detrimental compounds in their environment, they all are characterised by a unicellular vegetative growth stage containing a ridged permeable cell wall able to accumulate heavy metals (Brady & Duncan, 1994; Kurtzman & Fell, 1998). The above therefore indicates that through the course of time, the yeast form proved to be successful in the presence of many other extinct and extant organisms.

Species according to Kurtzman and Fell (1998)	***[a]F***	***[b]Carbohydrate assimilation***			***[c]Aromatic compound assimilation***		
		xyl	ara	cel	Phb	Van	Fer
Cryptococcus albidus	-	+	+	+	+	+	+
Cryptococcus curvatus	-	+	v	+	+	-	-
Cryptococcus gastricus	-	+	+	+	+	-	-
Cryptococcus gilvescens	-	-	+	+	nd	nd	nd
Cryptococcus humicolus	-	+	+	+	+	-	-
Cryptococcus laurentii	-	+	+	+	+	-	-
Cryptococcus podzolicus	-	+	+	+	-	-	-
Cryptococcus terreus	-	+	+	+	+	+	v
Filobasidium uniguttulatum	-	+	+	-	-	-	-
Cystofilobasidium capitatum	-	+	+	+	-	-	-
Leucosporidium scottii	-	+	v	+	+	+	v
Mrakia frigida	+	+	+	+	-	-	-
Rhodotorula aurantiaca	-	+	v	v	+	-	-
Rhodotorula glutinis	-	v	v	v	+	+	v
Rhodotorula mucilaginosa	-	+	-	+	+	+	+
Schizoblastosporion starkeyi-henricii	-	-	-	-	+	-	-
Sporobolomyces roseus	-	v	v	v	+	+	+
Trichosporon cutaneum	-	+	+	+	+	-	-

Table 1: *The most abundant soil yeast species are able to aerobically assimilate the degradation products of lignocellulosic compounds originating from plants (Botha et al., 2006). These compounds include carbohydrates such as L-arabinose, D-xylose and cellobiose, as well as aromatic compounds such as ferulic acid, p-hydroxybenzoic acid, and vanillic acid. In some cases carbohydrates may also be fermented by these yeasts."*

[a] Ability of species to ferment carbohydrates according to Kurtzman and Fell (1998); + = able to ferment at least glucose, - = unable to ferment carbohydrates.

[b] Ability of species to aerobically assimilate carbohydrates according to Kurtzman and Fell (1998); xyl = D-xylose, L-arabinose, cel = cellobiose, + = able to assimilate, - = unable to assimilate, v = some strains unable to assimilate.

[c] Ability of species to aerobically assimilate aromatic compounds; Phb = p-hydroxybenzoic acid, Van = vanillic acid, Fer = ferulic acid, + = able to assimilate, - = unable to assimilate, v = some strains unable to assimilate, nd = not determined.

The cornerstone of survival: adaptable physiology

Pivotal to the survival of yeasts in the natural environment is the ability of these heterotrophs to utilise a wide diversity of carbon and nitrogen sources at temperatures ranging from about 4 °C to 40 °C (Kurtzman & Fell, 1998). For example, the majority of soil yeasts discovered so far are saprotrophs able to utilise organic carbon compounds associated with plants (**Table 1**; Botha, 2006; Kurtzman & Fell, 1998). Depending on temperature, redox potential, water activity and nutrient source availability, some yeasts have the ability to ferment carbohydrates, whereas others are able to respire both carbohydrates and non-fermentable organic compounds. In soils these compounds mostly originate from plant litter (Hättenschwiler et al., 2005) including fruit (Phaff et al., 1966) and root exudates (De Ruiter et al., 1998). While some physiological characteristics are being used to delineate yeast species, many yeast species show intraspecific diversity when comparing certain physiological traits, such as assimilation of specific carbon or nitrogen sources. The latter emphasises the adaptability of these unicellular eukaryotes.

The adaptability of yeasts may be brought about by a number of genetic mechanisms, such as genetic recombination occurring during sexual reproduction (Evans, 1990; Spencer & Spencer, 1997). However, asexual chromosomal instability and chromosomal alterations were also found to affect physiological functions in yeasts (Rustchenko et al., 1994; Rustchenko et al., 1997). For example, when 100 spontaneous mutants of a single *Candida albicans* strain were tested for their ability to utilise 21 carbon and three nitrogen sources at three different temperatures, it was found that the nutrient utilisation profiles of the mutants differed significantly from those of the parent strain (Rustchenko et al., 1997). In addition to such spontaneous chromosomal alterations, it was demonstrated that the parasexual cycle, characteristic of *C. albicans* during conjugation, is less stable than meiosis (Forsche et al., 2008). A similar mechanism that plays a significant role in *Candida albicans*' asexual method of adaptation is non-disjunction (Perepnikhatka et al., 1999). Negative and positive regulators control beneficial genes, and are in turn controlled by changes in chromosome numbers. It would seem, therefore, that yeasts may utilise several mechanisms through which genetic diversity is increased to cope with changing environmental conditions. However, the survival and growth of a yeast strain in its natural environment seems to depend not only on the intrinsic abilities of the particular strain to maintain itself within its habitat (Botha, 2006), but also on other factors, such as interactions with other living members in the ecosystem.

Interactions with others improve survival

A substantial body of evidence exists indicating that, within their natural habitat, yeasts may intimately coexist with a wide diversity of organisms (Botha, 2006; 2011). These symbioses that are briefly discussed in the following paragraphs are essential to yeast survival, since such interactions may be employed to overcome nutrient limitation, disperse the progeny and even evade predation.

Amensalism

It is known among plant pathologists that when the basidiomycetous yeast *Cryptococcus laurentii* is applied onto fruit, it may reduce the effect of postharvest pathogens, such as filamentous fungi growing on wounded fruit (Chand-Goyal & Spotts, 1997; Roberts, 1990). While some researchers ascribed this antagonistic effect to competition for nutrients, others stated that an amensalistic relationship may exist between some yeasts and filamentous fungi (Fredlund et al., 2002; Masih & Paul, 2002; Roberts, 1990). Amensalism, based on chemical interference, may include the production of enzymes, such as glucanases that hydrolyse the cell walls of competing fungi. Amensalism may also occur as a result of killer activity, where the yeast produces extracellular glycolipids or glycoproteins with fungicidal or fungistatic activity (Golubev, 2006). Yeast killer activity was first studied to understand problems encountered during beer and wine fermentations (Young, 1987). Later, it was also studied to find potential organic methods to control plant pathogens (Roberts, 1990), and to be used against medically important *Candida* species (Vadkertiova & Sláviková, 2007).

Competition

It has been known since the beginnings of modern microbiology (Chung & Ferris, 1996) that microbes, including yeasts (Van der Walt, 1971), can be isolated from different habitats by using enrichment cultures. These methods are based on the principle that specific microbes or yeast species within a diverse microbial community are enriched for when growth conditions, such as an utilizable nutrient source, are favourable to them. The same principle applies when a nutrient such as a potential carbon source is added to the ecosystem, either on purpose or unintentionally as a pollutant. For example, *Candida tropicalis* emerged as a dominant

n-alkane utilising microbe when soil microcosms containing inocula from petroleum-contaminated soil were treated with an *n*-alkane mixture (Schmitz et al., 2000).

Microbes not only compete for carbon or nitrogen sources, but competition for ferric iron, mediated by microbial siderophore production, is quite common in the microbial world. Siderophores are iron-chelating compounds, of low molecular weight (500–1000 daltons), that are produced by many different microbes to acquire iron from the environment (Neilands, 1981). Yeasts are no exception. It was found, for example, that *Rhodotorula glutininis,* a yeast isolated from plants and soil, produces a siderophore called rhodotorulic acid, which sequesters ferric iron. Consequently, conidial germination of moulds occurring in the same habitat, such as *Penicillium expansum* and *Botrytis cinerea,* may be inhibited by the iron-chelating activity of rhodotorulic acid (Calvente et al., 1999; Sansone et al., 2005). Similarly, *Metschnikowia pulcherrima* (anamorph: *Candida pulcherrima*), a yeast isolated from soil and the rhizosphere, and studied since 1918 for its iron-binding pigment (Kluyver et al., 1953; Kvasnikov et al., 1975; Vadkertiova & Sláviková, 2007), inhibits growth of a wide diversity of microbes by producing pulcherriminic acid, which complexes iron and renders it unavailable for the yeast's microbial competitors (Sipiczki, 2006).

Predation

Within microbial ecosystems, predators feeding on primary saprotrophs, such as bacteria, prevent accumulation of microbial biomass and ensure the transfer of the sequestered nutrients into living biomass through subsequent trophic levels (Adl & Gupta, 2006). Similarly, in natural habitats yeasts are preyed upon by a number of other organisms, including micro-arthropods, nematodes, and protista (Bardgett & Griffiths, 1997). The millipede *Pachyiulus flavipes* (Byzov et al., 1998), as well as the collembolans *Protaphorura armata* and *Vertagopus pseudocinereus*, are known to consume a wide diversity of yeast species (Men'ko et al., 2006). Interestingly, such grazing may not necessarily lead to consumption of yeast biomass. Grazing upon yeasts by *Drosophila* may also aid in yeast dispersal, since it was found that ascospores of *S. cerevisiae* may survive passage through the gut of *Drosophila melanogaster* (Coluccio et al., 2008). Similarly, grazing by protista may not always result in the consumption of yeast cells. For example, when biofilms of the soil yeasts *Cryptococcus laurentii* and *Cryptococcus podzolicus* were exposed to ciliates, the ciliates preferred grazing on the noncellular biofilm matrix instead of the yeasts (**Figure 4;** Joubert et al., 2006). Using fluoromicroscopy and photometric quantification, it was demonstrated that protistan grazing enhanced yeast metabolism in biofilms, whereas biofilm biomass and viability increased simultaneously.

The predators of yeasts may not always be larger than their prey, since bacteria may also act as predators of yeasts. It was found that a number of Gram-positive bacteria cause lysis of *S. cerevisiae* (Goto-Yamamoto et al., 1993). Similarly, extracellular enzymes that cause lysis of a wide diversity of ascomycetous and basidiomycetous yeast species are produced by Myxobacteria (Yamanaka et al., 1993). These bacteria are Gram-negative soil bacteria characterised by gliding motility and the formation of thin film-like swarms while moving on solid surfaces in search of prey (Reichenbach, 1999).

Antagonism against predators

An interesting phenomenon is that a yeast may sometimes exert a lethal effect on its predator. For example, it was found that when the soil nematode *Caenorhabditis elegans* ingests the opportunistic human pathogen, *Cryptococcus neoformans,* the yeast causes distention of the nematode's intestine (Mylonakis et al., 2002). Capsule formation, as well as a range of other virulence factors, was found to play a role in the killing of the nematode. This yeast may also exert an antagonistic effect on other predators when phagocytosed by them. Studies indicated that the capsule of *C. neoformans* may act as a virulence factor against the protist *Acanthamoeba castellanii* and the slime mold *Dictyostelium discoideum* (Fuchs & Mylonakis, 2006). Consequently, it was stated that the virulence this opportunistic pathogen exerts on man may originally have evolved by the yeast to ensure its survival in the natural environment in the face of many microbial predators (Mylonakis et al., 2002). However, yeasts do have some allies that help them to survive in the presence of many dangers in the natural world.

Beneficial interactions with plants

Plants are known to be the best allies of yeasts in the natural environment. As previously highlighted in this review, yeasts are saprotrophs able to utilise many organic compounds originating from plants.

Roots

In soil the majority of yeasts occur on plant roots and in the narrow zone surrounding these roots, i.e. the rhizosphere (Moawad et al., 1986). As a result of root metabolism, the chemical characteristics of the soil in the rhizosphere, for instance pH, redox potential, as well as the profile of organic compounds, may differ from that of the bulk soil away from the roots (Huang & Germida,

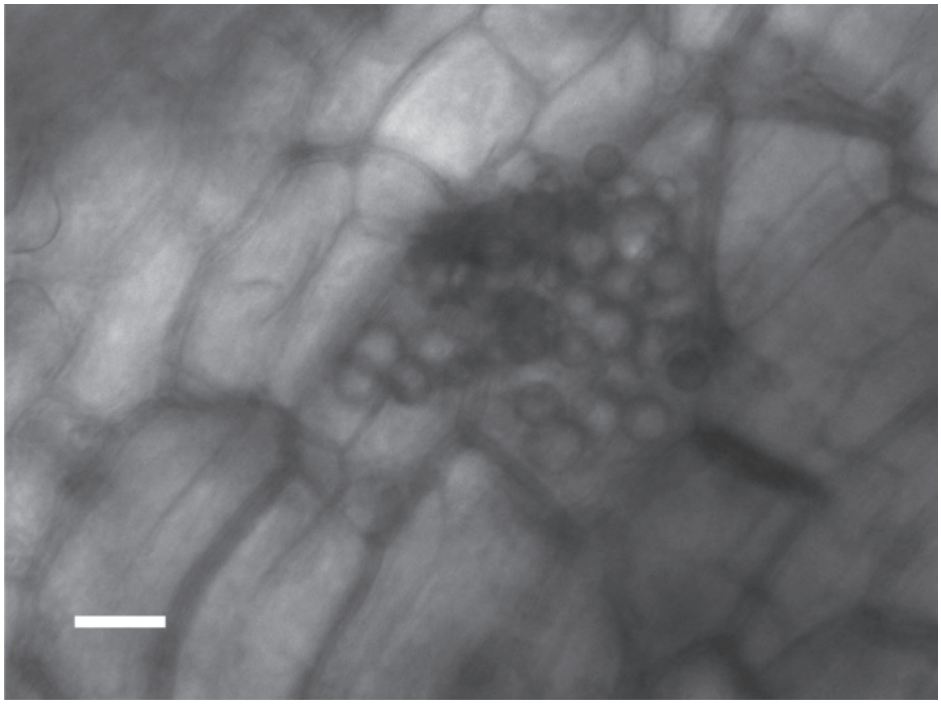

Figure 5: . *Photomicrograph of a colony of the soil yeast Cryptococcus laurentii growing on the root surface of a well-known Fynbos plant, named buchu (Agathosma betulina [Berg.] Pillans). It was found that this yeast increases root growth, as well as the concentration of nutrients such as phosphorous and iron in the roots. In addition, compared to control plants not inoculated with the yeasts, the presence of the yeasts on the roots resulted in higher photosynthetic resource-use efficiencies for water, nitrogen and phosphorous (Cloete et al., 2009, 2010a, 2010b) Bar = 10 µm*

(Photo: Karen Cloete).

2002; Lynch 1990). The clustering of yeasts and other soil microbes around the roots may be ascribed to the fact that up to 40% of the photosynthate of plants is released via the rhizosphere into the surrounding soil as potential nutrients for the soil microbial community. These root exudates may include amino acids, carbohydrates, organic acids and phenolic compounds (Bertin et al., 2003; Botha, 2006). In turn, the soil microbial community holds many benefits to plants. The interactions occurring within the roots and in the rhizosphere have subsequently been studied in depth, since these are of great importance to crop production (Sen, 2003). It was found that mutualistic symbioses between mycorrhizal fungi and plant roots may facilitate uptake of up to 80% of the phosphorus and 25% of the nitrogen requirements of the host plant (Marschner & Dell, 1994).

Since many different yeasts are known to occur in the rhizosphere (Botha, 2006; Zachow et al., 2009), interactions between mycorrhizal fungi and soil yeasts are inevitable, and a number of studies were conducted to investigate these interactions. It was found that inoculation of legumes with *S. cerevisiae* increases nodulation as well as arbuscular mycorrhizal (AM) fungal colonization (Singh et al., 1991). Also, hyphal growth of the AM fungus *Glomus intraradices* colonising cucumber roots was enhanced by the presence of baker's yeast (Ravnskov et al., 1999), but phosphorus uptake by the AM fungus was unaffected by the yeast. A co-inoculum consisting of the ascomycetous yeast *Yarrowia lipolytica* and an AM fungus, *Glomus deserticola*, resulted in higher levels of mycorrhizal-root colonisation of tomato plants, compared to plants receiving only the AM fungus (Vassilev et al., 2001).

Soil yeasts are also capable of directly enhancing plant growth. However, the mechanism by which growth is increased may differ depending on the yeast species involved. *Yarrowia lipolytica* enhances plant growth as a result of its ability to solubilise rock phosphate, thereby increasing the acquisition of plant phosphorus (Medina et al., 2004). Other yeasts, however, may enhance root growth via the production of plant growth regulators (Cloete et al., 2009; El-Tarabily & Sivasithamparam, 2006). The latter compounds may include molecules such as indole-3-acetic acid, indole-3-pyruvic acid, gibberellins and polyamines. Interestingly enough, it was found that similar to AM fungi, soil yeasts may be able to increase phosphorus, as well as iron uptake, and are able to positively affect photosynthetic resource-use efficiency of the plant (**Figure 5**; Cloete et al., 2010a, 2010b).

In addition to the mutually beneficial interactions between yeasts and plant roots described above, some soil yeasts are antagonistic towards the growth of fungal root pathogens, thereby increasing plant health. For example, it has been demonstrated that *Candida glabrata*, *Candida maltosa*, *Rhodotorula rubra* and *Trichosporon cutaneum* reduce the incidence of late wilt disease of maize caused by *Cephalosporium maydis* (El-Mehalawy et al., 2004). Similarly, yeasts such as

Candida valida, Rhodotorula glutinis and *Trichosporon asahi* protected sugar beet against *Rhizoctonia solani* diseases during glasshouse trails (El-Tarabily, 2006). However, the mechanism of this antagonistic effect seems to differ among yeast species. The antagonistic effect of *C. valida* was ascribed to b-1,3-glucanase activity, that of *R. glutinis* to the production of inhibitory volatiles, while *T. asahii* seems to be antagonistic as a result of the production of diffusible antifungal metabolites. It was found that these three yeasts act synergistically on disease suppression and that they enhance plant growth, through the production of indole-3-acetic acid and gibberellic acid.

Bark, stem, twigs and leafs

Yeasts are not only associated with plant roots, but together with other microbes these unicellular fungi are known to occur as epiphytes on the phyloplane and endophytes within the plant (Fonseca and Ináco, 2006; Gai et al., 2009). Although much is still to be learnt from the role of yeasts in these habitats, a number of ecological studies of yeasts associated with the above-ground organs of plants produced some significant results.

Some of the studies conducted in our own laboratory revealed that yeasts, within a syntrophic relationship with filamentous lignocellulolytic fungi, may grow on the woody phyloplane of trees (Van Heerden et al., 2011). In this symbiosis, the filamentous fungus produces extracellular wood-degrading enzymes and the yeast utilises the simple degradation products resulting from the action of the enzymes. In another study it was found that the pathogenic yeast *C. neoformans* is able to grow on wood by itself, since it possesses the necessary genes and enzymes systems (Botes et al., 2009).

Fermenting the sugars from plants

By far the most studied interactions of plant-associated yeasts are the microbial interactions occurring during malting in beer production (Flannigan, 1996; Laitila et al., 2006), as well as the interactions occurring on the grape surface and the fermentation process once the grape is crushed during wine production (Jolly et al., 2003a, 2003b; Pasteur, 1866; Pretorius et al., 1999).

In essence, this fermentation process represents a series of consecutive, but overlapping fermentations of different yeast and bacterial populations (Pretorius et al., 1999). The metabolic products of each of these microbial populations, starting with the apiculate ascomycetous yeasts and other "wild yeasts" dominating the yeast populations on the grape's surface, the lactic acid bacteria, as well *S. cerevisiae* populations dominating the later stages of the fermentation process, all contribute to the unique flavour of wine.

Yeast fermentations not only contribute to the well-being of man, but also to that of other organisms. Recently, it was found that nectar yeasts warm the flowers of a winter-blooming herb *Helleborus foetidus* that occur in Europe (Herrera & Pozo, 2010). Thus, it was suggested that whilst the yeast populations within the nectar utilises the sugars provided by the plant, their metabolic activity warms the flowers, resulting in enhanced plant reproduction through mechanisms such as increased pollinator visitation, pollen germination, pollen tube growth, fertilisation success, fruit development and seed size.

From the above it is evident that the ability of yeasts to adapt to their physico-chemical and biological environment has ensured their evolutionary success in many natural and man-made environments. The most obvious of these being bakeries, breweries and wineries. One may even argue that man's interest in eukaryotic biology, and suitability of yeasts to act as experimental subjects, may contribute to the survival of these organisms, especially the alcohol-producing ascomycetous yeasts, for many years to come, at least for as long as man is there to study and care for these "domesticated" organisms. However, man's presence also resulted in the unintended proliferation of some other yeasts, with more sinister consequences.

YEASTS AS BIOLOGICAL POLLUTANTS

Many examples exist where anthropogenic activities have caused extensive unimpeded proliferation of harmful organisms in new habitats. Such biological pollution was well studied by plant pathologists aiming to curb the spread of diseases and pests among crops (Britton, 2004). Recently, the effects of biological and chemical pollution on mammals were studied in relation to a colony of harbour seals subjected to sewage pollution (Mos et al., 2006). However, one could argue that the pathogens in sewage may not be true biological pollutants since they are unable to proliferate without a suitable host. Nevertheless, some opportunistic pathogens may be able to proliferate in the environment, from where they are able to infect susceptible hosts. It is known that the fungal domain contains a number of such opportunistic pathogens (De Hoog et al., 2000).

Research conducted in our laboratory indicated that *C. albicans* is able to grow in polluted rivers away from its mammalian host, the latter long thought to be the natural habitat of this notorious opportunistic fungal pathogen (Stone, unpublished results). During these experiments, the fate of *C. albicans* strains were monitored in pot cultures prepared with mud and plants originating from wetlands. In addition, the fate of this yeast was monitored in rivers using both classical and molecular identification methods. The findings point to the potential establishment of clinical strains of *C. albicans*, originating from HIV/Aids-infected individuals, in South African river systems. The possibility of a similar phenomenon regarding *Cryptococcus neoformans* is also being studied. During the past decade this basidiomycetous yeast emerged as a notable opportunistic pathogen among South Africans (Govender, 2008). It causes cryptococcosis in humans, especially in immuno-compromised patients (Casadevall & Perfect, 1998; Kwon-Chung, 1998). Cryptococcosis is especially relevant to South Africa, given the large population of HIV positive individuals in this country. Susceptible individuals probably obtain the disease via infection with airborne basidiospores or desiccated cells originating from the environment, including avian droppings, soil and vegetative debris such as decaying wood (Ellis & Pfeiffer, 1990; Feldmesser et al., 2001; Hull and Heitman, 2002; Sorrell & Ellis, 1997).

It is now well established that the majority of cryptococcal meningitis among sufferers from HIV/Aids are being attributed to *C. neoformans* variety *grubii* (Kwon-Chung & Bennett, 1984; Mitchell & Perfect, 1995). Infections caused by *C. neoformans* var. *neoformans* or *Cryptococcus gattii* are rarely observed among the South African population (Govender, 2008). *Cryptococcus gattii* occurs predominantly in subtropical areas. However, recently it has been recognised that it also occurs in temperate and Mediterranean climatic zones in Europe (Bovers et al., 2008). It has been suggested that the large-scale colonisation reported in these climatic zones could reflect a change in the distribution of *C. gattii* as a result of global warming, a favourable microclimate in the colonised area (Kidd et al., 2004), as well as anthropogenic dispersal of the pathogen (Kidd et al., 2007). Our own research revealed the presence of both *C. neoformans* variety *grubii* and *Cryptococcus gattii* on decaying tree stumps in recreational areas in South Africa. Moreover, we could not detect these pathogens in pristine forests and also found that these pathogenic cryptococci are able to grow, mate and produce potential infectious basidiospores on wood alone (Botes et al., 2009). Given the above, these pathogenic cryptococci may also be considered biological pollutants, able to invade and occupy new geographical areas as a result of anthropogenic activities. Research to understand and curb the spread of pathogenic yeasts is therefore important to maintain public health. Typically, a first step towards reaching such a goal would be to determine which environmental variables affect the numbers of these yeasts in their natural environment.

CURRENT AND FUTURE RESEARCH IN YEAST BIOLOGY

As is evident from the many studies on yeast interactions mentioned in this review, yeasts are being studied as potential agents for the biological control of plant pathogens that are important to agriculture. This is imperative where environmentally friendly methods in crop protection are being considered as alternatives to toxic synthetic fungicides. An exciting new field of research is the use of yeasts as biofertilisers (Eman et al., 2008; Gomaa and Mohamed, 2007; Mohamed and Gomaa, 2005). Fertilisers consisting of yeasts, organic and inorganic components are already commercially available, with claims that some of the products are capable of re-establishing the sustainability of ecosystems, as well as enhancing the productivity of farmland for various crops (Pang et al., 2003; Zhang, 2002). Since it is known that different yeast species exert different levels of enhancement on mycorrhizal colonisation and root growth (Gollner et al., 2006; Sampedro et al., 2004), the challenge will be to develop compatible yeast/crop combinations to be used in sustainable agriculture. It is envisaged that such research in enhanced crop performance will include studies in plant physiology as well as yeast molecular biology.

From current literature it is evident that research on the classical and molecular biology of biotechnologically important yeasts, such as the ascomycetous alcohol-producing yeasts, will continue as long as man is able to consume and enjoy the products of these ancient organisms. It is also foreseeable that most of the research in yeast biology will be on industrially important yeasts, with applications in the bakery, beer, biofuel and wine industries.

The interactions of medically important yeasts, such as the pathogenic cryptococci and *Candida* species, with man and antifungal drugs will continue to be investigated. It is also envisaged that the virulence factors of these yeasts will remain an active study field in future. It should be noted that most of the research entails studies of

binary interactions under controlled conditions within a laboratory. Studying the fate of medically important yeasts outside the laboratory in the natural environment would require a paradigm shift.

Correlating yeast population sizes with environmental variables

In contrast to studying microbial pure cultures in a laboratory, investigations into the fate of microbial populations in the natural environment are much more complex. In nature, a plethora of interactions affect the survival of microbes, including that of yeasts (Botha, 2006). Thus, to obtain an indication of factors affecting the survival of a particular yeast species within an ecosystem, a shotgun approach may be used to correlate a series of physico-chemical environmental variables with yeast numbers (Cornelissen et al., 2003; Vreulink et al., 2007, 2010). Correlations that are being observed between environmental variables and yeast numbers could also be as a result of complex interactions within the ecosystem.

Correlations between the numbers of a particular yeast species and that of other organisms are more challenging to obtain than correlations between the numbers of a yeast and physico-chemical variables, for instance moisture content, nutrient concentrations and temperature. The main reason for this is that technology for quantitative chemical analyses used on a routine basis for soil, plant nutrient and pollutant analyses is more established than techniques to determine concentrations of genomes from different species sharing the same habitat. New technological advances in the field of rDNA sequence analyses of total community DNA (Gomes et al., 2007; Hunt et al., 2004; Lim et al., 2010; Zachow et al., 2009) show much potential to be used in the ecology of yeasts. However, these studies should be conducted taking into account the limitations of modern molecular analyses (Ellis et al., 2003; Gomez-Alvarez et al., 2009; Spiegelman et al., 2005), including the bias of screening only for selected taxonomic informative gene sequences. Also, it was demonstrated that within an ecosystem the functional diversity among microbes may outweigh the diversity among taxonomic entities (Dinsdale et al., 2008). Future studies on the survival of pathogenic yeast populations in natural environments should also be aimed at finding correlations between yeast numbers and the abundance of different functional genes in the metagenome. As a result, finding explanations for the survival and growth of a particular yeast species in nature suddenly becomes very complex.

CONCLUSION

To adequately explain the growth and the effects of a yeast colony on decaying wood, on root surfaces and riverbeds, or in the ocean, the complex interactions of physical, chemical and biological factors should be considered. Whether this is possible using current scientific thought based on reductionism remains to be seen. Alternatively, the answer may be to search for evidence of ontological emergence (Silberstein & McGeever, 1999) to explain the success of a particular yeast species in its natural habitat. This type of scientific thought, however, is not generally accepted in biology as yet.

Despite the complexities associated with yeast ecology, scientists will continue studying these organisms, which on the one hand benefit man in so many ways, while on the other pose a real threat in the form of resilient environmental pathogens. The relationship between man and yeast is truly complicated.

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