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ABSTRACT

This investigation lies in the domain of environmental mycology that clearly characterizes the laboratory of mycology in the Department of Botany and Microbiology, Assiut University. It is an extension of the extensive surveys performed in different environments in Egypt: in soil, air, phyllosphere and phylloplane, rhizosphere and rhizoplane, carposphere and carpoplane, seeds and grains, food materials etc., which extended for more than 40 years. The achievements were very fruitful with regard to the unprecedented broad knowledge of fungi in Egypt, which was culminated by the establishment of a large Culture Collection of fungi embraced by the Mycological Centre, Assiut University. If we remember that only about 6–7 % of the total fungal species on earth suggested to be 1.5 million are known (Hawksworth, 1991, 2001, 2004, and others), mycologists everywhere are strongly urged to work very hard to search for new species in the different ecosystems around them.

The present investigation focused for the first time in this laboratory on yeast mycobiota in the environments of two economically-important plants citrus (orange) and grapevine plantations in Sahel-Saleem City, Assuit Governorate, Egypt. The study focused on the incidence and biodiversity of yeasts from air, soil, phyllosphere, phylloplane, carposphere, and carpoplane, in citrus and grapevine plantations, in addition to fruit juice of the two plants, air, soil, surfaces of leaf, and fruit in addition to fruit juice, in a 12-month experiment during the period from April 2008 to February 2009, employing two media of isolation: [yeast extract malt extract agar supplemented with dichloran (DYM) and dichloran rose bengal chloramphenicol agar (DRBC)].

It should be mentioned that identification of yeast genera and species was performed using the morphological, microscopical and biochemical characteristics. In suspected isolates, molecular techniques [Internal transcribed spacer (ITS) sequences of nuclear ribosomal DNA were amplified using primers ITS1, ITS4] were employed that either confirm the previous methods or disagree with them and the latters are registered as unidentified.

A total of 38 species, in addition to 5 unidentified species, related to 20 genera were isolated, of which 22 species are new records to Egypt. The broadest spectra of species were recorded in the following order: *Cryptococcus* (7 species), *Pichia* (4 species), *Pseudozyma* (3 species and 1 unidentified), *Candida* (3 species), *Rhodotorula* (3 species), and *Sporidiobolus* (3 species). The broadest spectra of genera and species were recorded in

citrus air (12 genera and 18 species on DRBC), citrus phyllosphere (11 and 16 on DYM), and grapevine phyllosphere (10 and 16 on DRBC) and carposphere (10 and 15 on DYM), while the narrowest was recorded in grapevine soil (2 and 2 on DYM) and (4 and 4 on DRBC). The highest counts of yeasts were recorded from the juice of both fruits (almost more than 95 % of total fungi), followed by citrus carposphere and carpoplane where they constituted about one-third of total fungi. The lowest percentage counts (less than 1 %) of was recorded in soil of both plantations and citrus phyllosphere. Also, the current study reveals four patterns of correlation between dominance (counts) of certain groups of yeasts and the different studied sources:

1) Soil pattern in which the basidiomycetous yeasts e.g. *Cryptococcus* and *Rhodotorula* were isolated from grapevine soil only, while ascomycetous yeasts were reported mainly from citrus soil but also from that of grapevine,
2) Air, phyllosphere, and phylloplane pattern where basidiomyceteous yeasts were dominant over ascomyceteous yeasts in these environmrnts,
3) Carposphere and carpoplane pattern where yeast fungi were fairly dominant over filamentous fungi and ascomyceteous yeasts were also dominant over basidiomyceteous ones, and
4) Fruit juice pattern: where yeasts were extremely dominant over filamentous (almost over 95 % of total fungi) and ascomyceteous yeasts were dominant over basidiomyceteous ones. Finally, photos are provided for most species treated.

HISTORICAL REVIEW

Microscopic fungi are distributed worldwide. They are important component of the ecosystems and play important biological roles in recycling of nutrients in natural and modified ecosystems, soil formation, providing nutrition to plants through their roots, and transformation of waste materials into useful products (Christensen 1989, Tuomela *et al.* 2000, Gadd 2004). They are of great importance in various industries, such as in the making of cheese, the manufacture of alcohol and the rotting of flax, as well as in medicine and agriculture. Also they cause diseases of plants and animals, including man (Díaz Muňoz 2006).

Fungi are the major decomposers of dead organic matter and contribute significantly. The species richness of a fungal community and relative abundance of individual species have been considered as measures of functional activities of the group in the particular habitat (Kjøller and Struwe 1982, 1987).

Yeasts are important members in many ecosystems and form a significant contribution to the biodiversity (Fleet 1998). The soil is the ultimate repository for storage and an even development of certain species of yeasts. Most of the yeast species possess a wide spectrum of metabolic abilities, enabling them to utilize many of the hydrolytic products of plant materials generated by fungal and bacterial activities (Phaff and Starmer 1987). Some species (e.g. *Cryptococcus*) also produce extracellular polysaccharides. These compounds bind soil particles together and thus they may establish a physical protection of a fraction of the soil organic matter (Killham 1994). The yeast cells are considered to be tolerant of unfavourable conditions and nutritionally undemanding (Slavikova *et al.* 2003).

The yeasts differ from filamentous fungi by showing themselves, predominantly under unicellular form. The majority is classified as ascomyceteous and show themselves as spherical, oval or cylindrical cells, with cellular division through budding (Pelczar *et al.* 1980, Madigan *et al.* 2004). The distribution of yeasts “in nature” is done by insect vector and wind. The flowers and fruits are important habitats to their development due to the high concentration of simple sugars and low pH (Pelczar *et al.* 1980). Many authors have isolated yeasts with fermentative capacity from fruits, citric concentrates and other sugar substrates (Brannon and Pollit 1935, Trindade *et al.* 2002).

Plant tissues and surfaces are colonized by microbial communities consisting of mycelial fungi, yeasts, bacteria, actinomycetes, and algae (Last and Warren 1972, Dickinson 1976). Plant-associated microbes include symbionts, pathogens, saprobes, or casual inhabitants. Different zones along the plant axis provide a multitude of topographical features, sources of nutrients and water, and a range of microclimatic conditions for correspondingly diverse communities of microbes, which in turn establish varied relationships with their hosts (Andrews and Harris 2000).

Phytopathogens have long been identified and studied owing to the economic impact of the diseases they cause on agricultural crops (Leben 1965, Morris 2001) but for many years much less was known about the identity or properties of the numerous saprophytic microbes that inhabit plant surfaces. However, the last few decades have witnessed a renewed interest in microbial epiphytes that apparently play important roles in nutrient cycling or in modulating population size of deleterious microbes, and some are being exploited as biological control agents for disease or frost control (Windels and Lindow 1985, Fokkema 1991, Andrews 1992, Lindow and Leveau 2002).

Examples of plant habitats that have been extensively investigated for their yeast inhabitants include the nectar of flowers (Golonka 2002, Herzberg 2004), tree exudates or slime fluxes (Phaff and Starmer 1987) and the necrotic tissues of cacti (Starmer *et al.* 1991). The yeast communities found therein and whose composition was specific for each type of habitat were generally dominated by ascomyceteous species and insects were identified as the major vectors for the introduction and/or dispersal of those yeasts (Phaff and Starmer 1987, Babjeva and Chernov 1995). In contrast, communities found on plant surfaces such as leaves, flowers (excluding nectaries), immature or intact fruits and bark were dominated by basidiomyceteous yeasts and the species composition of those communities was generally considered more uniform (Last and Price 1969, Phaff and Starmer 1987, Babjeva and Chernov 1995).

Significant variations in relative sizes of populations of different yeast species on different plants in the same geographic area were demonstrated in the studies by Inacio *et al.* (2002) and Maksimova and Chernov (2004).

Citrus is the most economically important tree fruit crop in the world (Spiegel-Roy and Goldschmidt, 1996). *Citrus* species are small to medium-size shrubs or trees that are cultivated throughout the tropics and subtropics. They are native to parts of India, China, Northern Australia, and New Caledonia. All species are aboriginal, early European, or modern introductions throughout Oceania. *Citrus* is primarily valued for the fruit, which is

either eaten alone (sweet orange, tangerine, grapefruit, etc.) as fresh fruit, processed into juice, or added to dishes and beverages (lemon, lime, etc.). All species have traditional medicinal value (Manner *et al.* 2006).

The orange (*Citrus aurantium* var. *sinensis* L. = *C. sinensis* Osbeck) is unknown in the wild state; is assumed to have originated in Southern China, Northeastern India, and perhaps Southeastern Asia (formerly Indochina). It was carried to the Mediterranean area possibly by Italian traders after 1450 or by Portuguese navigators around 1500. Up to that era, citrus fruits were valued by Europeans mainly for medicinal purposes, but the orange was quickly adopted as a luscious fruit and wealthy persons grew it in private conservatories, called orangeries. By 1646 it had been much publicized and was well known (Morton 1987). The orange has become the most commonly grown tree fruit in the world. It is an important crop in the Far East, the Union of South Africa, Australia, throughout the Mediterranean area, and subtropical areas of South America and the Caribbean. The United States leads in world production, with Florida, alone, having an annual yield of more than 200 million boxes, except when freezes occur, which may reduce the crop by 20 or even 40 %. California, Texas and Arizona follow in that order with much lower production in Louisiana, Mississippi, Alabama and Georgia. Other major producers are Brazil, Spain, Japan, Mexico, Italy, India, Argentina and Egypt (Morton 1987).

The orange tree, reaching 7.5 m or, with great age, up to 15 m, has a rounded crown of slender branches. The twigs are twisted and angled when young and may bear slender, semi-flexible, bluntish spines in the leaf axils. There may be faint or conspicuous wings on the petioles of the aromatic, evergreen, alternate, elliptic to ovate, sometimes faintly toothed "leaves" – technically solitary leaflets of compound leaves. These are 6.5-15 cm long, 2.5-9.5 cm wide; borne singly or in clusters of 2 to 6, the sweetly fragrant white flowers, about 5 cm wide, have a saucer-shaped, 5-pointed calyx and 5 oblong, white petals, and 20 to 25 stamens with conspicuous yellow anthers. The fruit is globose, subglobose, oblate or somewhat oval, 6.5-9.5 cm wide; dotted with minute glands containing an essential oil, the outer rind (epicarp) is orange or yellow when ripe, the inner rind (mesocarp) is white, spongy and non-aromatic. The pulp (endocarp), yellow, orange or more or less red, consists of tightly packed membranous juice sacs enclosed in 10 to 14 wedge-shaped compartments which are readily separated as individual segments. In each segment there may be 2 to 4 irregular seeds, white externally and internally, though some types of oranges are seedless. The sweet orange differs physically from the sour orange in having a solid center (Morton 1987). Some common postharvest fungus diseases of citrus are stem-end rot (*Lasiodiplodia theobromae* or

Diaporthe citri), green mould (*Penicillium digitatum*), blue mould (*P. italicum*), sour rot (*Galactomyces citri-aurantii*), anthracnose (*Colletotrichum gloeosporioides*), *Alternaria* stem-end rot (*Alternaria citri*), and brown rot (*Phytophthora palmivora* and *P. nicotianae*) (Manner *et al.* 2006).

Grape (*Vitis vinifera* L.) is believed to have originated in Armenia near the Black and Caspian seas in Russia. An independent and recent origin of grapes is also traced to North America. Its leaves and seeds were discovered in North America and Europe in fossil deposits of the Tertiary period of geological time. Seeds were also found in the refuse mounds of the pile dwellers of lakes in South Central Europe belonging to the bronze age. From Armenia grapes spread westwards to Europe and Eastwards to Iran and Afghanistan. Grape was introduced into India in 1300 AD by the Monghul invaders. The total area under grape cultivation in the world is 7,399,546 hectares with the production of 68,952,793 tonnes resulting in a yield of 9.32 (tonnes/ha). Spain covers the largest area of harvest of 1,200,000 hectares for grapes in the world, which makes a share of 16.22 % of total area of harvest for grapes in the world. After Spain, France (842,026), Italy (754,987), Turkey (550,000), China (483,200), USA (320,000), Iran (314,547), Portugal (222,528), Argentina (218,991), Romania (187,094), Chile (178,000), Australia (158,167) are the other important grape producing countries. Grape yield in Egypt (one of the leading countries in the world) was estimated by 21.67 (tones/ha) (www.icare.org.in/ Indian Council of Agricultural Research).

The fruit of the grape is a berry. Berries are attached to the stem. Many berries make up the cluster or bunch of grapes. The essential parts of the berry include the skin, pulp, and seeds. The skin consists of an outer layer covering the berry. It is made up of six to ten layers of thick-walled cells. The outer surface of the skin is covered with a wax-like coating called the cuticle, which renders the berry waterproof. The main components in the skin are: coloring matter (red and yellow pigments), tannins, aromatic substances, and potassium and other minerals. Below the skin layer lies flesh or pulp which makes up most of the berry volume. Cells in the pulp have large vacuoles containing the cell sap or juice. When the berry is gently crushed, the fragile cells in the pulp are broken and the juice is released. This juice is commonly referred to as the free run. The seeds are localized in the center of the flesh. The berry contains two to four seeds (Dharmadhikari, www.iastate.edu).

1. Air-borne yeast fungi

In the atmosphere many microbioparticles are present called as air spora. These are fungal spores, pollen grains, insect parts. The study of aeromycology is important in plant pathology and in disease forecasting of plant diseases.

It is well known that fungi require certain optimum conditions for each phase of their growth. In this regard associations with temperature and moisture have been well documented in the mycological literature. It has also been established that spore concentrations in the atmosphere fluctuate with changes in weather; temperature, humidity and rainfall in particular play an important role in this regard. However, the air spora also fluctuate for biological reasons such as growth and differentiation of spores or pollen-producing organs (Gregory 1973).

Aeromycological research from the Middle East area is limited and scattered; in Kuwait (Moustafa 1975, Moustafa and Al-Musallam 1975, Khan *et al.* 1999), in Qatar (Al-Subai 2002), in Saudi Arabia (Abdel-Hafez 1984, Abdel-Hafez and Shoreit 1985, Hasnain *et al.* 2005), in Yemen (El-Essawy *et al.* 1992), in Turkey (Colakoglu 1996, Sarica *et al.* 2002, Sakiyan and Inceoglu 2003, Asan *et al.* 2004, Ozkara *et al.* 2007), in Iran (Hedayati *et al.* 2005, Nourian *et al.* 2007) and in Jordan (Al-Eisawi and Dajani 1987, 1988, Shaheen, 1992, Al-Qura'n 2008). In Egypt, air-borne fungal spores were studied using the sedimentation method at Assuit (Moubasher and Moustafa 1974, Abu-El-Souod 1974), Qena (Moubasher *et al.* 1981), Wadi Qena (Abdel-Hafez and El-Said 1989), Wadi Bir-El-Ain, Eastern Desert (Moubasher *et al.* 1986), Western desert (Ismail *et al.* 2002), El-Minia (Mazen and Shaban 1983, El-Gendy 1988), Ismailia (Abdul Wahid *et al.* 1996), Zagazig (El-Sherbeny 1982), and Cairo (Zaky 1960, Ali *et al.* 1973) and Alexandria (Saad 1958). They showed regular periodicities and exhibited their peaks in spring and autumn, and the trough in summer. Moubasher (1993) reported that *Cladosporium* was almost the commonest organism in the air of Egypt, as is the case in many temperate and tropical zones.

It is suggested that fungal spores are dislodged from soil by air currents. A part of them remains suspended in air and the others alight or are sedimented on vegetation surface where a new substrate or niche is initiated. In this niche, the conditions are substantially different from those in soil. Competition for the colonization of this substrate is less severe. Atmospheric conditions are more drastic, high light intensity, and deep diurnal fluctuations of temperature and humidity. Consequently, the mycobiota developing in this niche has a

basically different pattern from that of soil. The dark-colored fungi, or the melanin-containing, are predominated over the hyaline ones, contrasting the pattern in the soil (Moubasher 1995).

Ben-Meir-Glueck (1952) isolated more than thirty different species from the air of orange groves and packing sheds and from the skins of fruits. These included *Penicillium italicum* and *P. digitatum*, which are the main incitants of citrus rot. Barkai-Gollan (1961) studied the air-borne fungi in citrus fruit packing houses and reported that *P. digitatum* and *P. italicum* predominated, whereas *Fusarium*, *Trichoderma*, *Colletotrichum*, *Diplodia* were encountered only occasionally.

There are several reports on the occurrence of yeasts in the air (Di Menna 1955, Hamilton 1959, Turner, 1966, Voros-Felkai 1966, 1967, Al-Doory 1967, Gregory 1973).

Al-Doory (1967) found that species of *Cryptococcus*, *Rhodotorula*, *Sporobolomyces*, and *Debaryomyces* were the most dominant species from the air in San Antonia, Taxas, U.S.A.

Rhodotorula mucilaginosa and *Cryptococcus albidus* were the most dominant species followed by *Debaryomyces hansenii* isolated from the air of El-Minia city, Egypt while *Rhodotorula rubra*, *R. aurantiaca*, *Kluyveromyces marxianus*, *Torulaspora delbrueckii*, *Saccharomyces kluyveri*, and *Hansenula polymorpha* were of less frequency (Haridy 1992).

2. Yeast fungi recovered from soils

Soil fungi were extensively studied in Egypt by Moubasher and his collaborators (1965-2017) and several other investigators (Sabet 1935, Ragab 1956, Besada and Yusuf 1968a, b, Salama *et al.* 1971, Ali *et al.* 1975). Soil fungi showed seasonal periodicities. The months with moderate temperature are regularly the richest (in counts and species spectra), while the summer months are the poorest (Moubasher and El-Dohlob 1970, Moubasher and Abdel-Hafez 1978). In summer, fungi are subject to unfavourable conditions. The soil dries up quickly and the temperature becomes so high as to affect severely the inhabitants of soil. *Aspergillus* tends to prevail in months with average temperature, exceeding those of *Penicillium*. Also, *Aspergillus* species predominate in substrates temperate (Moubasher and El-Dohlob 1970, Moubasher *et al.* 1972). Fungi classified in *Aspergillus* Section *Nigri* (the black aspergilli) are ubiquitous saprophytes in soils around the world, particularly in tropical

and subtropical regions (Klich and Pitt 1988, Pitt and Hocking 1997, 2009). Naim (1967) isolated fungi from soil under citrus trees. Moubasher *et al.* (1971) found that the fungus flora of soil under five varieties of citrus was not specific, but almost similar to that in other Egyptian cultivated soil and the basic components were *Aspergillus*, *Fusarium* and *Penicillium.*

Yeasts are widely distributed in nature. They have been found in soil of widely different texture, chemical composition, humidity, and pH value at various geographic locations and diverse climatic conditions, in bare soils as well as in soil that support natural vegetation or are cultivated by man (Carmo-Sousa 1969). In most cases, especially agricultural soils, the soil should be regarded more as a reservoir for yeasts from sources above it than as a specific habitat. Although in some instances, there are many yeast species that are typical soil inhabitants and for which no obvious surface sources are known (Phaff *et al.* 1978).

The distinctive nature of epiphytic (non-pathogenic) yeasts received further support from comparisons with the communities found in the rhizosphere, ensuing from the early work of di Menna (1959) with pasture plants in New Zealand (reviewed in Carmo-Sousa, 1969 and Last and Price, 1969) and later confirmed by other workers (Kvasnikov *et al.* 1975, Fokkema and Schippers 1986, Maksimova and Chernov 2004). The results of those studies showed that although basidiomyceteous yeasts were also dominant, the species found in soils near the roots of plants (e.g. *Cryptococcus albidus*, *Cr. diffluens*, *Cr. humicola*, *Cr. curvatus*) did not coincide with those isolated from the aerial surfaces of the same plants (*Cryptococcus laurentii*, *Rhodotorula ingeniosa*, *Rh. graminis*, *Rh. mucilaginosa*, *Sporobolomyces roseus*) (di Menna 1959). Another relevant observation by di Menna was that while the composition of the soil yeast communities varied with soil type but not with season, the phyllosphere populations changed with season but not with locality or plant (Babjeva and Chernov 1995).

Haridy (2002) found that *Trichosporon beigelii*, *Kluyveromyces marxianus* and *Torulaspora delbrueckii* were the dominant species in rhizosphere and non-rhizosphere areas of potato, maize, vegetable marrow, and cabbage plants in El-Minia City. *Cryptococcus humicola* and *Candida tropicalis* were represented by considerable numbers of strains, while *Saccharomyces cerevisiae* and *Candida blankii* were of low occurrence.

3. Phyllosphere and phylloplane yeast fungi

The term "phyllosphere" was proposed for the environment provided by the leaf surface and enabling microbial development (Ruinen 1956, Last 1965). The phyllosphere is the living leaf as a whole and includes the surface (phylloplane) and internal tissues colonized by a variety of epiphytic and endophytic microorganisms respectively, thereby occupying two distinct habitats on the leaf (Carroll *et al.* 1977, Petrini 1991, Andrews 1996). The interest shown in the last few years in the study of phyllosphere microbes is due principally to their interactions with plants, herbivores and pathogens on living leaves which may be involved in the plant immunity system, reabsorption of organic and mineral matters from leachates, redistribution of nutrients prior to leaf fall and participation in the primary degradation of plant tissues (Carroll *et al.* 1977, Cabral 1985, Lindow and Brandl 2003, Osono 2006). Another aspect of colonization ecology of phylloplane and/or phyllosphere fungi principally relates to the prevailing microenvironmental conditions on the leaf surfaces and their physical, chemical and phenological properties which affect the fungal establishment thereon (Pandey 1990, Dix and Webster 1995).

The epiphytic (non-phytopathogenic) microbial communities of leaves are very diverse and their best-studied components have been bacteria and fungi, including yeasts (Andrews and Harris 2000, Hirano and Upper 2000, Morris 2001, Lindow and Brandl 2003). Cuticle composition and topographic features (stomata, trichomes, veins, etc.) are also highly variable both within a leaf and among different plant species (Baker 1971, Hallam and Juniper 1971) and may influence the composition and distribution of phylloplane communities (Kinkel 1997). Molecules leached from plant leaves include a variety of organic and inorganic compounds, such as sugars, organic acids, amino acids, methanol and various salts (Blakeman 1971, Tukey 1971, Morris 2001). The abundance of such nutrients varies with plant species, leaf age and growing conditions. Exogenous nutrient sources, such as aphid honeydew and pollen, have been associated with dramatic increases in the microbial carrying capacities of some leaves (Diem 1974, Fokkema *et al.* 1983, Stadler and Muller 1996).

Yeasts were isolated from leaf surfaces of five species of fruit trees (apple, cherry, apricot, peach, and plum) located in southwest Slovakia. Seventeen yeast species were identified, but only three occurred regularly: *Aureobasidium pullulans*, *Cryptococcus laurentii*, and *Metschnikowia pulcherrima*. Species such as *Hanseniaspora uvarum*, *Pichia*

anomala, *Rhodotorula glutinis*, and *Saccharomyces cerevisiae* were isolated less frequently (Slavikova *et al.* 2009). The red yeast species *Sporobolomyces roseus* also belongs to the yeasts frequently occurring on leaf surfaces (Phaff and Starmer 1987, Nakase 2000). Other studies revealed that phylloplane communities usually comprise deeply pigmented species belonging to the genera *Rhodotorula* and *Sporobolomyces* (collectively referred to as 'pink yeasts' in many studies) and non-pigmented *Cryptococcus* species ('white yeasts') (Hislop and Cox 1969, McBride and Hayes 1977, Fokkema *et al.* 1979, McCormack *et al.* 1994b).

Ascomyceteous yeasts are usually rare on the phylloplane but the species *Debaryomyces hansenii* was found with high frequency on plants from the Canary Islands (Middelhoven 1997) and on sugarcane in Brazil (Azeredo *et al.* 1998) and was also reported to occur on leaves of forest plants in Russia (Babjeva *et al.* 1999, Glushakova and Chernov 2004, Maksimova and Chernov 2004). On apple fruit skin (Beech and Davenport 1970, Bizeau *et al.* 1989) species of *Hanseniaspora* and *Metschnikowia* are commonly present together with the basidiomyceteous species (*Aureobasidium*) that are also found on the leaves, on which the formers are absent (Pennycook and Newhook 1981).

4. Carposphere and carpoplane yeast fungi

Grape berries, especially the interface between soluble nutrients and the septic world, are common niches for yeasts. Nevertheless, the yeast biota of grapes is surprisingly poorly documented (Loureiro and Malfeito-Ferreira 2003, Ribereau-Gayon *et al.* 2005). As determined so far, the physiognomy of the grape microbiota may change in response to various factors such as: the climate, grape variety and geographical region (Sabate *et al.* 2002, Combina *et al.* 2005, Raspor *et al.* 2006). *Botrytis* infection resulted in a larger population and greater diversity of yeasts enriched with fermentative or spoilage species (Nisiotou and Nychas 2007).

Several species of *Aspergillus* in section *Nigri* are common in vineyards and are often associated with bunch rots (Amerine *et al.* 1980). *A. niger* is reported to be the primary cause of *Aspergillus* rot in grapes before harvest (Nair 1985, Snowdon 1990), while *A. aculeatus* (Jarvis and Traquair 1984) and *A. carbonarius* (Gupta 1956) have also been reported. Black aspergilli are important as ochratoxin-producing organisms which contaminate several agricultural products, including grape-derived products (Cabaňes *et al.* 2002, Samson *et al.* 2004, Battilani *et al.* 2006). *A. carbonarius* and *A. niger* have been shown to produce

ochratoxin A (OA) (Abarca *et al.* 1994, Téren *et al.* 1996, Heenan *et al.* 1998, Abarca *et al.* 2001), have been isolated from grapes in France (Sage *et al.*, 2002), South America (Da Rocha Rosa *et al.*, 2002), Spain (Cabañes *et al.* 2002), Italy (Battilani *et al.* 2003), Portugal (Serra *et al.* 2003) and Greece (Tjamos *et al.* 2004).

Melchers (1931) and Jones (1935) reported *P. italicum* and *P. digitatum* as causal agents of citrus-rot in Egypt, however Moubasher *et al.* (1971) and Elnaghy *et al.* (1973) reported that *P. italicum* was the sole incitant of *Penicillum*-rot in the Assuit area. Moubasher *et al.* (1971) found also that *Cladosporium herbarum* followed by *A. niger* and *Alternaria* species were the basic components on citrus fruits.

Fungal spoilage of citrus fruit attributed to *Alternaria citri, Fusarium, Penicillium digitatum, Penicillium italicum, Aspergillus, Geotrichum* as well as to *Botrytis* was also reported (Splittstoesser 1987, Ritenour *et al.* 2003).

In Egypt, Haridy (1994) found that the most common spoilage yeast species of soft sound and unsound fruits (apple, grapes, dates, figs, strawberries, peach, apricot, plum, and guava) was *Hanseniaspora valbyensis* followed by *H. vineae* and *Saccharomyces cerevisiae. Metschnikowia pulcherrima, Torulaspora delbrueckii* and *Kluyveromyces marixianus* were represented by considerable numbers of strains.

Joly (1955) studied the microbiota of yeasts of ripe fruit and obtained three genera of yeasts, *Klceckera, Pichia, Candida,* with the apiculates predominating (41 %).

De la Torre *et al.* (1999) reported that yeasts such as *Sporobolomyces roseus, Cryptococcus albidus, Rhodotorula rubra* and *Candida* were part of the natural microbiota of certain varieties of grapes in southern Spain.

Hanseniaspora species (anamorph *Kloeckera*) are common yeast constituents on grapes (Phister *et al.* 2007), and on grapes and musts in Europe (Bioletti and Cruess 1912).

According to Skinner *et al.* (1980) and Phaff (1990), the natural microbiota of fruits is commonly composed of yeasts and yeast-like organisms such as *Aureobasidium, Rhodotorula, Sporobolomyces, Cryptococcus, Candida, Pichia, Kloeckera, Hanseniaspora,* more rarely *Saccharomyces* and *Schizosaccharomyces,* and also the terrestrial species of *Metschnikowia*. The microbiota associated with widely commercialized fruits from temperate zones was extensively studied, such as strawberries (Buhagiar and Barnett 1971) and cherry fruits (Stollarova 1982). Several studies of the occurrence of yeasts in grapes have already been done (Goto and Yokotsuka 1977, Goto 1980), many of which were frequently carried out in association with must fermentation (Longo *et al.* 1991, Yanagida *et al.* 1992). Studies have also been done on the processing of citrus fruits and juices from fruit concentrates

(Parish and Higgins 1989, 1990, Deak and Beuchat 1993). However, studies with yeasts in tropical environments have been rare, and most of the time they have focused on medical concerns (Hagler *et al.* 1995). Ivo (1982) and Robbs *et al.* (1989) studied the association of yeasts in pineapple plantations in Brazil. The genus *Candida* predominated in all types of samples analyzed by Ivo (1982), with 78 % frequency. Robbs *et al.* (1989) verified that the species of *Candida guilliermondii*, *C. krusei* and *Hanseniaspora guilliermondii* were associated with rotting fruit.

From fruits of twenty different species of angiosperms located along the coast of the State of Säo Paulo, Southeastern Brazil, yeasts and yeast-like fungi were isolated, of which 74 % showed ascomycetic affinity. *Candida* was the predominant genus, followed by (in descending order of occurrence): *Cryptococcus*, *Kloeckera*, *Sporobolomyces*, *Pichia*, *Hanseniaspora* and *Bullera*. Black yeasts and other strains showing basidiomycetic affinity were also isolated while *Saccharomyces* and *Schizosaccharomyces* were not found in the fruits collected (Prada and Pagnocca 1997).

5. Yeast fungi recovered from juice

Fruit juices are popular soft drinks with an important role in human nutrition. They are advertised as very healthy food supplements containing a variety of vitamins necessary for the good bodily function, and of the immune system in particular.

Of freshly squeezed juices, citruses are the most popular (Arias *et al.* 2002). In general, the acidity (pH) of orange or grapefruit juices ranged between 3.5 and 3.9 and high sugar content (Bibek and Bhunia 2004) creates favourable conditions for the growth of acidolactic bacteria, moulds, and yeasts. Sugar favours the development of a microbial biofilm. In addition, the fruit surface can contain different contaminants that end up in the freshly squeezed juice offered in markets. Inadequate cleaning of fruit processors can pose a risk for consumers (Hatcher *et al.* 2001).

Lactic acid bacteria are the primary spoilage bacteria in fruit beverages; however, their numbers are greatly reduced after pasteurization, concentration, and refrigeration. Moulds and yeasts tolerate high-osmotic and low-pH conditions and grow at refrigeration temperatures and can therefore cause spoilage in the processed product (Arias *et al.* 2002).

Before pasteurization, fruit juices contain a microbial load representative of the organisms normally found on fruits during harvest plus contaminants added post-harvest

(during transport, storage and processing). Pasteurization will rid juice of pathogens and other heat-sensitive microbes; therefore, it will reduce the microbial load substantially and extend the shelf-life of the product. Some investigations regarding fungal contamination of pasteurized fruit juice are also available (Recca and Mrak 1952, Mendoza *et al.* 1982, Kurtzman *et al.* 2001, Abdel-Sater *et al.* 2001). Most of these reports have shown yeasts to be the predominant fungi involved in juice spoilage (Parish and Higgins 1989, Hatcher *et al.* 2000). Yeast spoilage of fruit juice can result in formation of haze, production of CO_2 and off-odors, and changes in color (Grinbaum *et al.* 1994). *Candida* and *Saccharomyces* spp. have often been reported as spoilage-causing organisms in citrus juices (Hays 1951, Grawmlich *et al.* 1986, Parish and Higgins 1989, Teller and Parish 1992).

Many other yeast fungi such as *Candida, Rhodotorula, Kluyveromyces, Pichia, Trichosporon, Kloeckera, Zygosaccharomyces* have been isolated from natural food such as fruit juices, honey, milk and others, as well as from industrialized food (Cook 1958, Jay 1970, Ivo 1982, Magalhães and Queiroz 1991).

Strains isolated from fresh-squeezed, unpasteurized orange juice (FSOJ) and contaminated pasteurized orange juice (PSOJ) differed in species composition. Fourteen different species were identified in PSOJ whereas only six species were found in FSOJ. Predominant species of PSOJ isolates were *Candida intermedia* (22 %) and *C. parapsilosis* (19 %). The main species isolated from FSOJ was *Hansenula uvarum*, representing more than 46 % of the total FSOJ isolates, followed by *H. occidentalis* (27 %) and *P. kluyveri* (17 %). The remaining isolates were ascribed to *C. stellata*, *P. fermentans*, and *Saccharomycopsis crataegensis* and totaled less than 10 % of the FSOJ strains. At the genus level, *Hanseniaspora* spp. constituted more than 73 % of the FSOJ isolates, whereas *Candida* spp. represented more than 53 % of the PSOJ isolates (Arias *et al.* 2002).

Cryptococus neoformans, Candida guilliermondii, C. famata, C. sphaerica, C. krusei, C. colliculosa, C. albicans, Kloeckera spp., and *Trichosporon mucoides* were the most yeast species identified in the orange juice from Zagreb, Croatia (Uhitil *et al.* 2009). In contrast, Arias *et al.* (2002) isolated completely different yeast species in orange juice from Florida (*Candida stellata, Hanseniaspora occidentalis, H. uvarum, Pichia fermentas, P. kluyveri,* and *Saccharomycopsis crataegensis*) dominated by *Hanseniaspora uvarum* and *H. occidentalis*.

Typical yeast species found in citrus juices are *Candida parapsilosis, Candida stellata, Saccharomyces cerevisiae, Torulaspora delbrueckii*, and *Zygosaccharomyces rouxii*,

although species from the genus *Rhodotorula*, *Pichia*, *Hanseniaspora*, and *Metschnikowia* are also common (Hatcher *et al.* 2000).

Out of ten apple juice samples analysed by Uhitil *et al.* (2009), *Candida guilliermondii* was detected in six and *Cryptococus neoformans* and *Candida famata* in two.

The most common yeasts found in fruit salads were *Pichia* spp., *Rhodotorula* spp., *Candida pulcherrima, C. lambica, C. sake* and *Debaryomyces polymorphus*. Yeasts commonly found in fruit juices were *C. lambica, C. sake*, and *Rhodotorula rubra*. *Geotrichum* spp. and low numbers of *Penicillium* and *Fusarium* spp. were present in grapefruit juice in Washington (Tournas *et al.* 2006)**.**

OBJECTIVES OF THIS STUDY

Only about 6-7 % of total numbers of species of fungi on earth, suggested to be 1.5 million species, are known to science, and generations of mycologists are strongly urged to work very hard to discover the huge number of unknown ones. For this purpose the present investigation was designed with the following objectives:

1) Investigation, for the first time in this laboratory, of the diversity of yeast fungi associated with two economically-important plants, citrus (orange) and grapevine which includes air, soil, phyllosphere, phylloplane, carposphere and carpoplane, in addition to fruit juice.
2) Study of the seasonal fluctuations of yeast fungi associated with the two plants.
3) Evaluation of the pattern of dominance of yeast fungi in the environment of the two plants.
4) Enrichment of the Culture Collection of the Assuit University Mycological Centre with new and interesting strains of yeast fungi from Egyptian environment. This Culture Collection avails documented strains and other valuable services for researchers in the fields of plant, human and animal pathology, biotechnology, fungal physiology and mycotoxicology, and other branches of science related to fungi.
5) The work includes in details the methodology of isolation from different sources and characterization of yeasts (giving the details with photos in the experiments of physiological characterization).
6) Characterization of yeast isolates obtained on the basis of phenotypic, physiological and phenotypic features. Photos are provided for almost all species isolated.

METHODOLOGY

1. Sampling location

This study was carried out in Sahel-Saleem city at approximately 25 km south-east of Assiut city. Sampling was conducted bimonthly over a twelve-month period from April 2008 - February 2009. Three different plantations of citrus in the suburbs of Sahel-Saleem city and three of grapevine in El-Khawaled village (about 6 km to the east border of the river Nile), in the northeast of Sahel-Saleem city were selected.

2. Collection of samples

A total of 214 samples were collected from air, soil, leaves, fruits and fruit juices of citrus (114) and grapevine plantations (100). The numbers of samples of each source of both plantations are indicated in Table (1).

1. Soil samples were collected away from rhizosphere areas (soil particles attached to young roots) of soils cultivated with citrus and grapevine plants.
2. At least five samples were taken at random from each place, then the five or more soil samples from each replication were brought into one composite sample which was mixed thoroughly several times.
3. Soil samples were put directly each into a clean plastic bag.
4. Leaf and fruit samples were also collected at random from different plants at each farm and put directly each into a clean plastic bag.
5. Samples (soil, leaf and fruit) were brought into the laboratory and kept in a fridge (5°C) till fungal analysis.

Table 1. Number of samples collected from different sources in 3 farms of each of citrus and grapevine during the period from April 2008 – February 2009*.

Plant	Citrus							Grapevine						
Source	Air	Soil	Leaf		Fruit		Juice	Air	Soil	Leaf		Fruit		Juice
			Ps	Pp	Cs	Cp				Ps	Pp	Cs	Cp	
April 2008	3	3	3	3	3	3	-	3	3	3	3	3	3	-
June 2008	3	3	3	3	3	3	-	3	3	3	3	3	3	-
August 2008	3	3	3	3	3	3	-	3	3	3	3	3	3	3
October 2008	3	3	3	3	3	3	3	3	3	3	3	3	3	3
December 2008	3	3	3	3	3	3	3	3	3	3	3	2	2	-
February 2009	3	3	3	3	2	2	2	3	3	-	-	-	-	-
Total (214)	18	18	18	18	17	17	8	18	18	15	15	14	14	6

* Ps = phyllosphere; Pp = phylloplane; Cs = carposphere; Cp = carpoplane.

Figure 1. Citrus plantation at Sahel Saleem, Assiut City.

Figure 2. Development of citrus fruits: Primordial stage in April; immature stage in June and August; mature stage in October-December; and senescent stage in February.

Figure 3. Grapevine plantation at Sahel-Saleem, Assiut city.

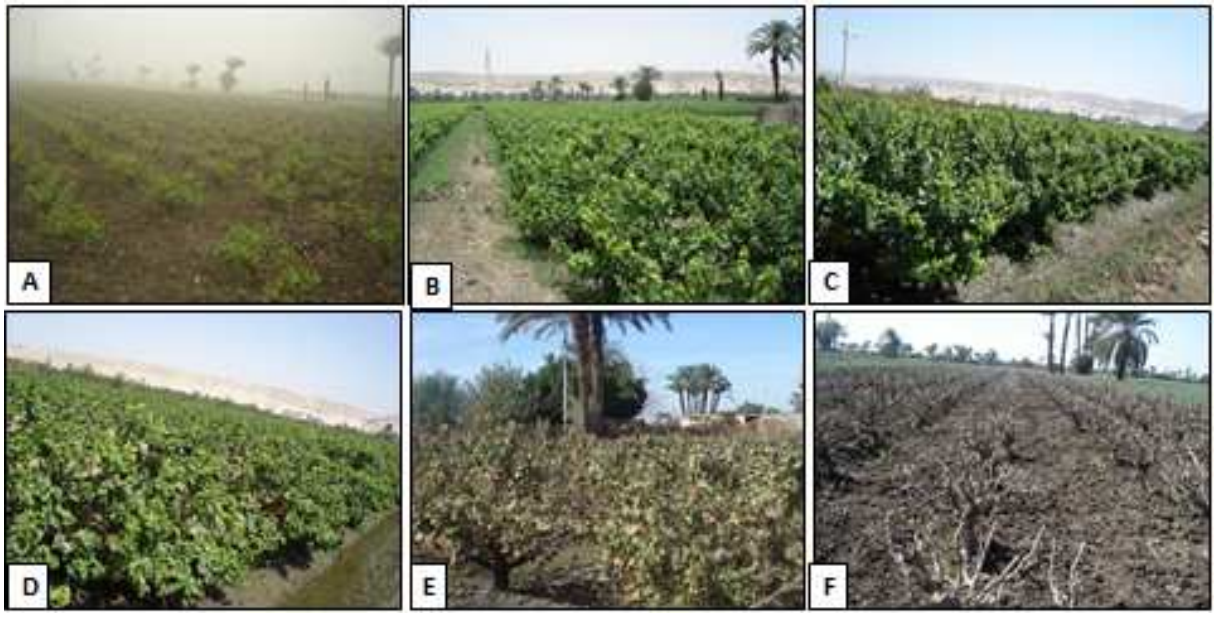

Figure 4. Grapevine leaves: A, juenvile leaves in April; B, immature leaves in June; mature leaves in August and October; E, senescent leaves in December; F, complete deciduous leaves in February in grapevine plantations.

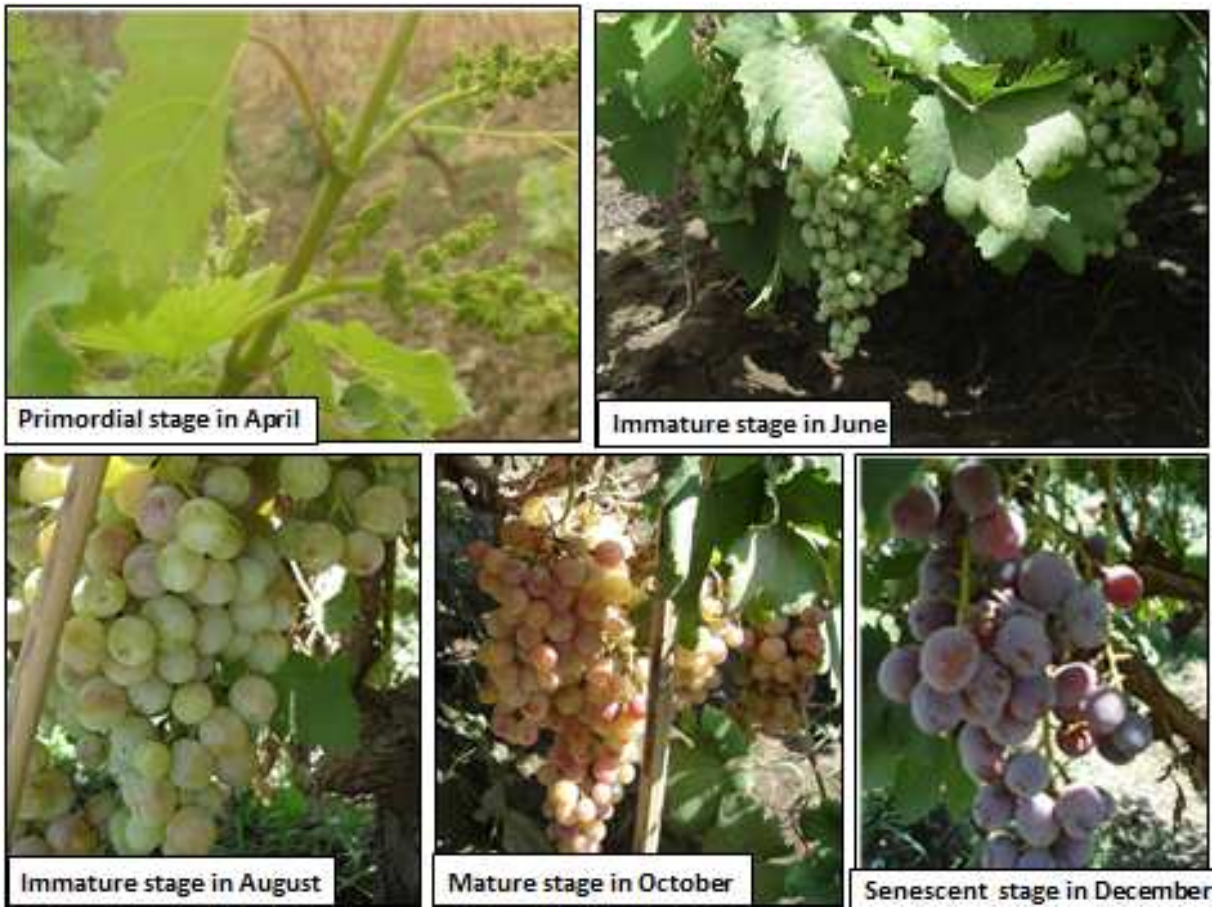

Figure 5. Different stages of grapevine fruits.

3. Isolation of air-borne yeast yeast fungi

Five replicate plates of 9 cm diameter of each of two media (DYM and DRBC) were exposed for five minutes at a height of 60 cm above the ground level during the same hours of the day (10 am - 2 pm) at each of the six sites. The plates were then sealed and brought back to the laboratory and incubated at 25 ºC for 7-15 days, during which, the developing colonies were counted, isolated and identified.

The meteorological data during the period of study were as follows: the maximum temperatures varied from 25 ºC to 46 ºC, the relative humidity from 36-86%. Since the concentration of fungal spores generally differed from location to location and even fluctuated with time in a given location, the same hours of the day (10 am - 2 pm) were chosen. The exposure plate method and two isolation media: yeast extract malt extract agar supplemented with dichloran (DYM) and dichloran rose Bengal chloramphenicol agar (DRBC) were used in this study. A total of 36 exposures (3 farms of each of citrus and grapevine) were carried out bimonthly, beginning from April 2008 to February 2009.

Figure 6. Exposure of DRBC and DYM (5 plates each) at a height of 60 cm above the ground level.

4. Isolation of soil yeast fungi

A. Determination of soil moisture content (MC)

The moisture content of soil was determined by drying replicates of freshly collected samples in an oven at 105°C till constant weight. The loss of weight was determined, and then the percentage of moisture content was calculated.

B. Determination of soil pH

To determine the pH in soil samples, sample extract was prepared first as follows: a known weight of the sample was shaken in a known volume of distilled water in a ratio 1: 5 (w/v) for about 30 min and the mixture was left overnight to settle. The extract was then filtered, centrifuged at 4000 rpm for 15 min. A pH meter (Orior Research Model GOHL Digital Ionalyzer) was used for the determination of the pH of soil samples. The electrode was immersed directly in the soil suspension with a ratio 1: 5 (w /v) to avoid the error through higher dilutions (Jackson 1958).

C. Isolation of soil yeast fungi

The dilution-plate method was used for enumeration of different yeast species as described by Johnson and Curl (1972) and employed in this laboratory by Moubasher and his collaborators as follows:

1. Ten g of soil sample (based on dry weight basis) were placed in a sterile 250 ml Erlenmeyer flask containing 90 ml of sterile distilled water. The flask containing the suspension was shaken on a mechanical orbital shaker for 30 minutes.
2. Ten ml of the suspension were immediately drawn (while in motion) using sterile 1 ml Menzies' dipper (1957) and transferred immediately into Erlenmeyer flasks containing 90 ml of sterile water and the dilution process was repeated until the desired final dilution was (1:3000) reached which supports a total of about 25- 40 colonies per plate. Each suspension was shaken by hand for few minutes, and was in motion while being drawn into the dipper.

3. One ml of the desired dilution using Menzies' dipper was transferred aseptically into each of several Petri-dishes and ~20 ml / plate of an appropriate agar medium cooled to just above solidifying temperature were added. The dishes were rotated by hand in a broad swirling motion, so that the dilution soil was dispersed in the agar.
4. The plates (5 plates for each type of medium) were incubated at 28 ºC for 1-2 weeks during which the developing yeast colonies were counted and isolated for further identification and the number of colony forming units (CFUs) was calculated per g dry sample. Isolates of different yeasts were maintained on YM and stored at 5°C till confirming the identification.

5. Isolation of phyllosphere yeast fungi

Small pieces of leaves (approximately 1 cm^2) were made using sterile scissors and 10 g of each sample were placed in 250 ml sterile Erlenmeyer flask containing 90 ml sterile distilled water. Flasks were shaken on orbital shaker for 15 minutes. Ten ml aliquots of the suspension were transferred into sterile Erlenmeyer flasks containing each 90 ml sterile distilled water, then were shaken for 5 minutes. The appropriate dilution which gave reasonable number of yeast colonies depends on the states of the leaves whether they were dusty or not was selected. One ml of the appropriate dilution was transferred into each sterile Petri-dish which was then poured with melted but cooled agar medium. Ten replicate plates were used for each sample (5 for each medium type).

6. Isolation of phylloplane yeast fungi

The cut pieces of leaves after thoroughly shaken in a series of sterile distilled water were removed and thoroughly dried using sterilized filter paper. Four pieces were inserted on the surface of each agar plate. Five replicate plates were used for each isolation medium and for each plant type.

7. Isolation of carposphere yeast fungi

Fruit samples were collected from citrus and grapevine trees bimonthly. Samples of fruits were collected at random, put in plastic bags and transferred to the laboratory. In case of citrus, the fruits were peeled with a sterilized blade. A known weight of the peel was placed in 250 ml sterile Erlenmeyer flask containing 100 ml sterile distilled water. Flasks were shaken on an orbital shaker for 15 minutes. Ten ml aliquots of the suspension were transferred into sterile Erlenmeyer flasks containing each 90 ml sterile distilled water, then shaken for 5 minutes. In case of grapes a known weight of the fruits was mixed thoroughly as in the citrus fruits. The appropriate dilution which gave reasonable number of fungal colonies depends on the state of the fruits whether they were dusty or not was selected. One ml of the appropriate dilution was transferred into each sterile Petri-dish which was then poured with melted but cooled agar medium. Ten replicate plates were used for each sample (5 for each medium type).

8. Isolation of carpoplane yeast fungi

In case of citrus fruit, the peel after thorough washing with sterile distilled water and thorough drying was cut into small pieces of approximately 1 cm^2 and 4 pieces were thereafter placed on the surface of each agar plate.

In case of grapes, the whole fruits after thorough washing with sterile distilled water and drying were either inserted on the agar surface as a whole fruit when young or cut into two halves when mature. Four parts were used in each of 5 replicate plates. Five replicate-plates were used for each isolation medium and for each plant type.

9. Isolation of juice yeast fungi

Fruits were surface washed by placing the whole fruits in a beaker containing sterilized water several times. The oranges were then sliced by sterilized cutter under sterile conditions and squeezed by hand into sterile universal tubes. In case of grapes, the berries after washing were squeezed by sterile lemon squeezer, the juice was collected into sterile universal tubes under aseptic conditions. One ml of the juice was transferred into each sterile

Petri- dish which was then poured with melted but cooled agar medium. Ten replicate plates were used for each sample (5 for each medium type).

10. Media used for isolation of yeast fungi from different sources

Two media were selected after screening four other media:

A. Dichloran yeast extract malt extract agar (DYM)

Yeast extract malt extract agar (Wickerham 1951), of the following composition was employed: (g/liter) yeast extract 3.0, malt extract 3.0, peptone 5.0, glucose 10.0, agar 20.0; chloramphenicol (250 µg/ml) was used as a bacteriostatic agent. The yeast extract malt extract agar medium was modified in the present work after preliminary survey by addition of 1 ml/l of 2 mg of dichloran dissolved in 10 ml ethanol which restricts the mucoraceous growth without affecting the other species (published by Moubasher *et al.* 2016).

B. Dichloran Rose Bengal chloramphenicol agar (DRBC) (King *et al.* 1979)

Dichloran Rose Bengal chloramphenicol agar, of the following composition: (g/liter) peptone 5.0, potassium dihydrogen phosphate 1.0, magnesium sulphate 0.5, glucose 10.0, agar 15.0, to which rose bengal (25 µg/ml) and chloramphenicol (100 µg/ml) were used as bacteriostatic agents (Smith and Dawson 1944, Al- Doory 1980) and dichloran (20 µg/ml).

11. Identification of yeasts

A. Morphological characters

1. Formation of pseudomycelium and true mycelium

The term pseudomycelium indicates the formation of a filamentous structure consisting of cells, which arise exclusively by budding, whilst true mycelium proliferates by continuous growth of the hyphal tip, followed by the formation of septa. Septation lags behind the growth of the hyphal tip to such a degree that the terminal cell measured from the

tip to the first septum is often longer than the subterminal cell, which is measured from the first to the second septum (Wickerham 1951). Formation of mycelia by the isolated yeast strains was performed using slide culture procedure. A Petri- dish, containing a U- shaped glass-rod support on which two glass slides were placed, was sterilized by dry heat at 160-180°C for 2 hours. Potato glucose agar was melted and poured into a second Petri-dish. The glass slides were removed from the glass rod with a flame - sterilized pair of tweezers, and were dipped into the agar after which they were replaced on the glass - rod support. After solidification of the agar on the slides, the yeast was very lightly inoculated in three lines along each slide and a sterile cover slip was placed over part of the lines. Some sterile water was poured into the Petri-dish to prevent the agar of drying out. The culture was then incubated at 25°C for 4-5 days. For microscopic examination, the slides were taken out of the Petri-dish and agar was wiped off the back of the slide. The areas of the inoculation lines under the cover slip were examined.

2. Ascospore formation

In testing for the ability to form ascospores, three sporulation media were used. These include corn meal agar, potato glucose agar and yeast extract – malt extract agar (YMA). The culture to be studied was first brought to a state of active growth and optimal nutrition by subculturing on YMA medium for 1–2 days at 25°C. The sporulation media were then inoculated with the culture, and incubated at 25°C for 3 days before being examined microscopically for the first time. Yeast strains that had not sporulated was then maintained at room temperature and examined at weekly intervals for at least 4–6 weeks. Yeast strain may only be regarded as anascogenous when it has failed to yield ascospores on a wide variety of media (Barnett *et al.* 2000).

B. Physiological characters

1. Fermentation of sugars

The ability or inability to ferment carbohydrates to ethanol and carbon dioxide depends on the presence or absence of transport system(s) to mediate the uptake of sugar at low oxygen concentrations and the presence of the relevant enzyme systems which will bring about its hydrolysis and/or mediate its anaerobic glycolytic break down to ethanol and carbon dioxide.

A basal medium, consisting of peptone (7.5 g/l) and yeast extract (4.5 g/l), was prepared and sufficient amount of bromothymol blue was added to give a sufficiently dense green color. Five ml aliquots were placed in test tubes carrying inserted tubes. The test tubes were sterilized by autoclaving. On cooling, 1 ml concentrated, filter sterilized sugar solution was added aseptically to the test tubes. Six percent aqueous solutions of different sugars (Table 2) were prepared except in the case of raffinose, which was made up in a 12 % solution. Media in the test tubes were inoculated with about 100 µl of a suspension of yeast cells made by suspending the growth of a 24 to 48-hour yeast extract malt extract agar culture in 2 ml sterile water. Test tubes were incubated at 25°C, regularly shaken and observed for the presence of gas in the inserted tubes and for change in color of indicator from green to yellow (Fig. 8) over a period of 24 days (Barnett *et al.* 2000).

2. Oxidative utilization of carbon compounds

Barnett and Kornberg (1960) and Macquillan and Halvorson (1963) showed that the ability or inability of a yeast strain to utilize a compound oxidatively depends on permeability factors and on the presence of specific enzyme systems that mediate its degradation. Before proceeding with the carbon assimilation tests, the yeast strain to be tested must first be brought to a state of active growth. This is affected by transferring the strain once or twice on YMA medium at 25°C at 2-3-day-intervals depending on its growth rate. A tenfold concentrated medium was prepared by dissolving 6.7 g of bacto-yeast nitrogen base (DIFCO) and the appropriate amount of the carbon compound equivalent to glucose (containing the same amount of carbon as 5 g glucose) in 100 ml distilled water. When raffinose was the carbon source it was used at twice this concentration. The media were filter-sterilized and 0.5 ml of tenfold concentrated solution of the various carbon compounds (Table 8) was transferred to 4.5 ml amount of sterile distilled water in cotton–plugged tubes. Media in the test tubes were inoculated with about 100 µl of a suspension of yeast cells made by suspending the growth of 24 to 48 hours in yeast extract malt extract agar (YMA) culture in 2 ml sterile water. A tube containing the nitrogen base without any carbon source served as control. Test tubes were incubated at 25°C, and the growth on the various carbon sources was regularly compared with the growth in the control tube over a period of 3 weeks (Fig. 8).

3. Assimilation of nitrogen compounds

A known weight of each nitrogen source was dissolved in separate bottles (potassium nitrate, 0.15 g; sodium nitrite, 0.21 g; ethylamine-HCl, 0.13 g; L-lysine-HCl, 0.33 g; creatine,

0.20 g; creatinine, 0.17 g; D-glucosamine, 0.33 g; imidazole, 0.10 g; or D-tryptophan,0.32 g) with 250 ml of 2X yeast carbon broth. pH of the medium was adjusted to 5.5-6.5. Ten g of agar and 250 ml of distilled water were added in each solution. The medium was then autoclaved at 120°C for 15-20 minutes and poured into Petri plates (9 cm). A pre-culture was prepared on YMA for 2-4 days. Light (not containing too much cells) suspension was prepared in yeast carbon base starvation broth. The cultures were incubated at 25°C for 2-3 days to consume the nitrogen compounds carried from the pre-culture medium. A drop of suspension was inoculated onto agar plates (multi-point inoculation) using sterilized plastic dropping pipettes. Each plate could hold 4 isolates. The plates were allowed to dry before sealing by parafilm and moving them to the incubator. The plates were incubated at 25°C for up to 3 weeks and then examined for growth (Suh *et al.* 2008) (Fig. 9).

4. Test for hydrolysis of urea

Difco Urea broth was suspended into tubes, in aliquots of 0.5 ml. A loopful of cells from 1-2-old-day culture was suspended in the broth and incubated at 37°C. Check every 30 min was performed for up to 4 hours for a change of the color to bright pink or red, which indicates urease activity (Fig. 10).

5. Growth at high osmotic pressure

Growth media were prepared of the following composition: 1 % yeast extract, 2 % agar, containing 50 % and 60 % (w/v) of D-glucose, and 10 % and 16 % (w/v) of NaCl (Table 2). The plates were inoculated lightly by streaking, incubated at 25°C and examined for growth for up to four weeks (Fig. 10). To prevent drying of the medium the plates were sealed with parafilm.

6. Growth at different temperatures

Taxonomically, it was of interest whether or not yeasts are capable of growth at different temperatures (30°, 37°, 42°, 45°C) (Table 2). The yeast strain under test was grown on YMA for 2-4 days.

7. Growth in the presence of cycloheximide

This test was done in liquid yeast nitrogen base medium with D- glucose for assessing aerobic utilization of D-glucose, but with filter-sterilized cycoloheximide added to give a final concentration of 0.1 % or 0.01 % (w/v) (Table 2, Fig. 10).

Table 2. Biochemical and morphological characteristics (as indicated in Barnett 2000) used for identification of yeast isolates investigated during the current study.

Semi-anaerobic fermentation tests			
F1, D-glucose	F2, D-galactose	F3, Maltose	F4, Me-α-D glucoside
F5, Sucrose	F6, α, α Trehalose	F7, Melibiose	F8, Lactose
F9, Cellobiose	F10, Melezitose	F11, Raffinose	F12, Inulin
F14, D- xylose	F13, Starch		
Aerobic carbon compounds utilization tests			
C1, D-glucose	C2, D-galactose	C3, L-sorbose	C4, D- Glucosamine
C5, D-ribose	C6, D-xylose	C7, L-arabinose	C9, L-rhamnose
C10, Sucrose	C11, Maltose	C12, α, α Trehalose	C13, Me α-D glucoside
C14, Cellobiose	C15, Salicin	C16, Melibiose	C18, Lactose
C19, Raffinose	C20, Melezitose	C21, Inulin	C22, Starch
C23, Glycerol	C24, Erythritol	C25, Ribitol	C26, Xylitol
C28, D-glucitol	C29, D-mannitol	C30, Galactitol	C31, myo-Inositol
C32, D-glucono-1,5 lacton	C33, 2-Keto-D-gluconate	C35, D-Gluconate	C36, D-glucuronate
C37, D-galacturonic acid	C38, DL-lactate	C39, Succinate	C40, Citrate
C41, Methanol	C42, Ethanol	C43, Propane 1,2 diol	C44, Butane 2,3 diol
C45, Quinic acid			
Nitrogen compounds utilization tests			
N1, Nitrate	N2, Nitrite	N3, Ethylamine	N4, L-lysine
N6, Creatine	N7, Creatinine	N8, Glucosamine	N9, Imidazole
N10, D-tryptophane			
Miscellaneous tests			
M1, Starch-like compound formation	M3, Urea hydrolysis	M4, Diazonium Blue B reaction	O1, 0.01 % cyclohexamide
O2, 0.1 % cyclohexamide	O4, 50 % D-glucose	O5, 60 % D-glucose	O6, 10 % Na Cl
O7, 16 % Na Cl	T2, At 30 °C	T4, At 37 °C	T6, At 42 °C
T7, At 45 °C			
Microscopic characterisations			
EI, Pink colonies	**E2**, Budding cells	**E3,** Lemon-shaped cells	**E4,** Budding on stalks

E5, Splitting cells	**E6**, Filamentous	**E7,** Pseudohyphae	**E8,** Septate hyphae
E9, Arthroconidia	**E10,** Ballistoconidia	**A1,** Ascosporogenous	**A2,** Round, oval ascosporse
A3, cap, hat shaped ascosporse			

8. Diazonium blue B (DBB) test

The yeast strain was cultured on YM agar plate for 5-7 days, and then incubated at 55-60°C for 16 hours. The plates were cooled down to room temperature before testing. DBB reagent was prepared [Diazonium Blue B salt (Fast Blue salt B) 15 mg in 15 ml of chilled 0.25 M Tris buffer, pH 7.0] in the amount needed every time, kept in ice bath or refrigerator, and was not used before it turned dark yellow (within about 30 min.). One or two drops of chilled DBB reagent were dropped onto the surface of each colony. If the culture turned dark red within 2 min., the result was recorded as positive. A positive response is characteristic of basidiomyceteous yeasts (Fig. 10).

9. Production of extracellular starch-like compounds

A culture was prepared in a medium containing 1 % glucose [or the 3 week-old-culture for glucose assimilation test (C1) was used]. One or two drops of Lugol's iodine solution (iodine 1g, potassium iodide 2g in 300 ml distilled water) were added into the culture and mixed thoroughly. A positive reaction is indicated by changing the color to the range of green to dark blue. This test helps to identify certain species, especially those of the genera *Cystofilobasidium* and *Leucosporidium*, as well as most *Cryptococcus* species, which characteristically form extracellular starch-like polysaccharides (Fig. 10).

Identification keys of Barnett *et al.* (2000) were followed to assign each isolate to its species level. Confirmations of these identifications were carried out using the molecular technique. Aliquots of killed cells by boiling in distilled water of the yeast isolates were prepared and sent to SolGent Company, South Korea, for PCR and rDNA sequencing.

Figure 7. Representative biochemical tests used for identification of yeast strains.

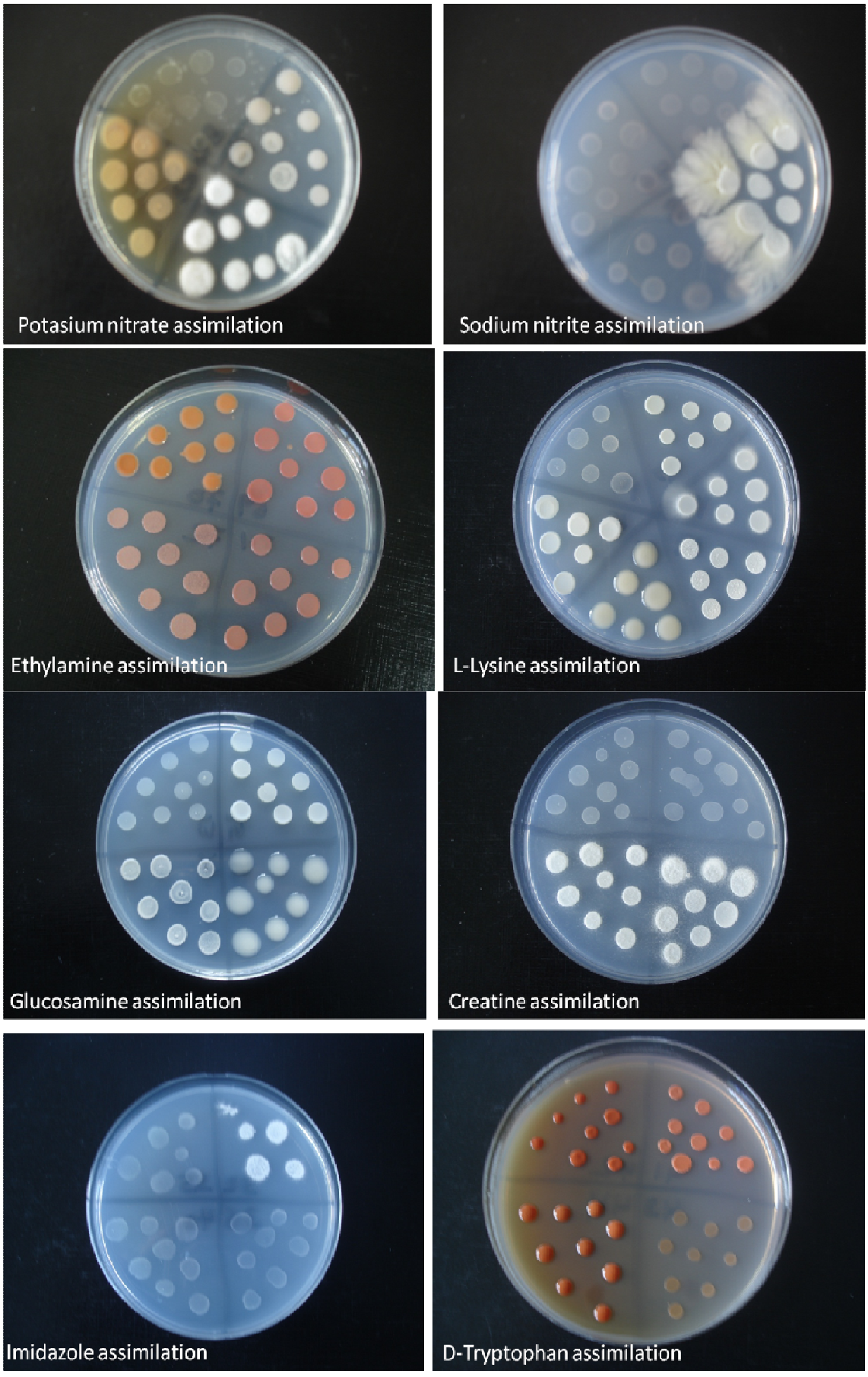

Figure 8. Representative biochemical tests used for identification of yeast strains.

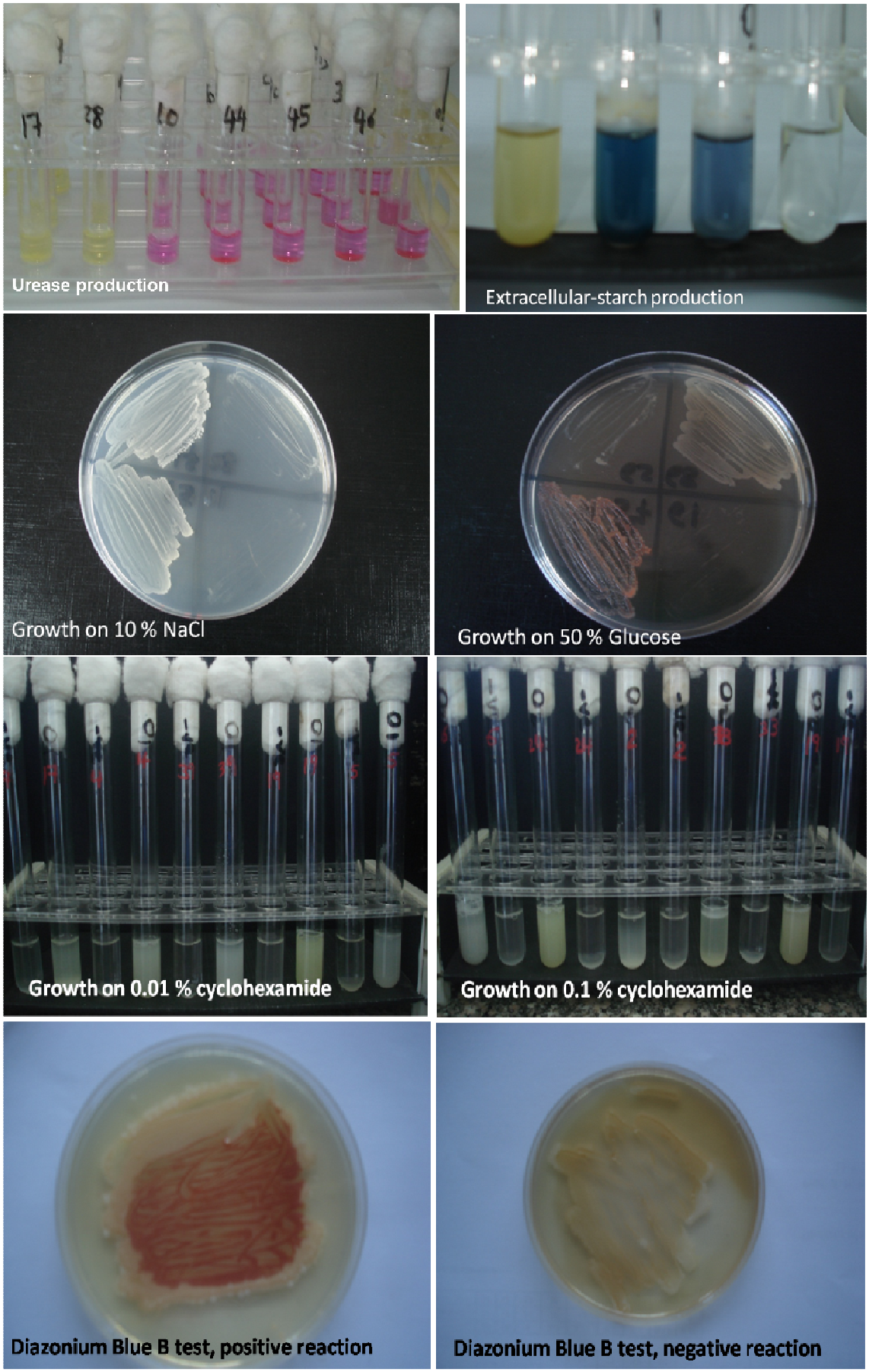

Figure 9. Representative biochemical tests used for identification of yeast strains.

10. Molecular methods

Growth of yeasts and DNA extraction

The yeast isolates were grown on YMA plates and incubated at 25° C for 2 days. A small amount of yeast growth was scraped and suspended in 100 µl of distilled water and boiled at 100° C for 15 minutes and stored at -70° C.

Yeast DNA was extracted and isolated using SolGent purification bead in SolGent Company (Daejeon, South Korea). Internal transcribed spacer (ITS) sequences of nuclear ribosomal DNA were amplified using universal primers ITS 1 (5' - TCC GTA GGT GAA CCT GCG G - 3'), and ITS 4 (5'- TCC TCC GCT TAT TGA TAT GC -3'). Then ampilification was performed using the polymerase chain reaction (PCR) (ABI, 9700). The PCR reaction mixtures were prepared using Solgent EF-Taq as follows: 10X EF-Taq buffer 2.5 µl, 10 mM dNTP (T) 0.5 µl, primer (F-10p) 1.0 µl, primer (R-10p) 1.0 µl, EF-Taq (2.5U) 0.25µl, template1.0 µl, DW to 25 µl. Then the amplification was carried out using the following PCR reaction conditions: one round of amplification consisting of denaturation at 95 °C for 15 min followed by 30 cycles of denaturation at 95 °C for 20 sec, annealing at 50 °C for 40 sec and extension at 72 °C for 1 min, with a final extension step of 72 °C for 5 min.

The PCR products were then purified with the SolGent PCR Purification Kit-Ultra prior to sequencing. Then the purified PCR products were reconfirmed (using size marker) by electrophoreses of the PCR products on 1 % agarose gel. Then these bands were eluted and sequenced. Each sample was sequenced in the sense and antisense direction.

Phylogenetic analysis

Contigs were created from the sequence data using CLCBio Main Workbench program. The sequence obtained from each isolate was further analyzed using BLAST from the National Center of Biotechnology Information (NCBI) website. Sequences obtained were subjected to Clustal W analysis using MegAlign (DNAStar) software version 5.05 for the phylogenetic analysis of ITS region along with those retrieved from GenBank database. Sequence data were deposited in GenBank and accession numbers are given for them.

COMPARISON BETWEEN YEAST BIOTA RECOVERED FROM DIFFERENT SOURCES OF CITRUS AND GRAPEVINE PLANTATIONS

1. List of identified yeast species

A total of 37 species, in addition to 5 unidentified species, related to 20 genera of yeasts were gathered from different sources of citrus and grapevine plantations (Table 3). 22 species of these yeasts are new records to Egypt. Distribution of these species and plates (1-37) of detailed structures are also included.

Identification was performed using the morphological and microscopical characteristics (Plates 1-37) in addition to the biochemical tests (Tables 12, 13, 15-18). In suspected isolates, molecular techniques [Internal transcribed spacer (ITS) sequences of nuclear ribosomal DNA were amplified using primers ITS1, ITS4] (refer to Tables 14, 19 & Figures 17, 18-20).

Table 3. Alphabetical list of yeast species recovered from different sources of citrus and grapevine plantations during the present investigation.

Ambrosiozyma *platypodis* (J. M. Baker& Kreger-Van Rji) van der Walt
Aureobasidium sp.
Candida Berkhout
C. catenulata Diddens & Lodder
C. parapsilosis (Ashford) Langeron & Talice
**C. prunicola* Kurtzman
Cryptococcus Vuillemin
**C. albidosimilis* Vishniac & Kurtzman
C. albidus (Saito) C. E. Skinner
**C. carnescens* (Verona & Luchetti) Takashima, Sugita, Shinoda & Nakase (Currently *Vishniacozyma carnescens* (Verona & Luchetti) X.Z. Liu, F.Y. Bai, M. Groenew. & Boekhout)

**C. flavescens* (Saito) C. E. Skinner (Currently *Papiliotrema flavescens* (Saito) X.Z. Liu, F.Y. Bai, M. Groenew. & Boekhout)
C. laurentii (Kufferath) C. E. Skinner Currently *Papiliotrema laurentii* (Kuff.) X.Z. Liu, F.Y. Bai, M. Groenew. & Boekhout
**C. luteolus* (Saito) C. E. Skinner
**C. magnus* (Lodder & Kreger-van Rij) Baptist & Kurtzman (Currently *Filobasidium magnum* (Lodder & Kreger-van Rij) X.Z. Liu, F.Y. Bai, M. Groenew. & Boekhout)
Debaryomyces Lodder & Kreger-van Rij
D. hansenii (Zopf) Lodder & Kreger-van Rij (anamorph: *Candida famata*)
**D. pseudopolymorphus* (C. Ramirez & Boidin) C. W. Price & Phaff (Currently *Schwanniomyces pseudopolymorphus)*
*****Filobasidium*** *floriforme* L. S. Olive
Geotrichum Link (Teleomorph: *Galactomyces*)
G. candidum Link (Teleomorph: *Galactomyces candidus* de Hoog & Smith)
**G. citri-aurantii* (Ferrairis) E. E. Butler (Teleomorph: *Galactomyces citri-aurantii* E. E. Butler)
Geotrichum sp.
Hanseniaspora *occidentalis* M. T. Smith
Issatchenkia *orientalis* Kudryavtsev (Teleomoph: *Picia kudriavzevii* Boidin, Pignal & Besson) (anamorph: *Candida krusei* (Castellani) Berkhout
Klyuveromyces *marxianus* (E. C. Hansen) van der Walt
Kodamaea *ohmeri* (Etchells & Bell) Y. Yamada *et al*.
*****Melanopsichium*** *pennsylvanicum* Hirschhorn.
Pichia E. C. Hansen
Pichia caribbica Vaughan-Mart., Kurtzman, S.A. Mey. & E.B. O'Neill (currently *Meyerozyma caribbica* (Vaughan-Mart., Kurtzman, S.A. Mey. & E.B. O'Neill) Kurtzman & M. Suzuki) (anamorph: *Candida fermentati* (Saito) Bai)
P. fermentans Lodder (anamorph: *Candida lambica* (Lindner & Genoud) van Uden & Buckley)
**P. farinosa* (Lindner) E. C. Hansen (currently *Millerozyma farinose*)

Pichea guilliermondii Wickerham (currently *Meyerozyma guilliermondii*) (anamorph:*Candida guilliermondii*)
Pseudozyma Bandoni
**P. aphidis* (Henninger & Windisch) Boekhout
**P. hubeinsis* Wang *et al.*
**P. rugulosa* (Traquair, L. A. Shaw & Jarvis) Boekhout & Traquair
Pseudozyma sp.
Rhodosporidium Banno
**R. diobovatum* S. W. Newell & I. L. Hunter
**R. paludigenum* Fell & Statzell Tallman
Rhodotorula F. C. Harrison
R. aurantiaca F. C. Harrison
R. glutinis (Fresenius) F. C. Harrison
R. mucilaginosa (A. Jorgensen) F. C. Harrison
Rhodotorula sp.
Sporidiobolus Nyland
S. pararoseus Fell & Tallman
**S. ruineniae* Holzschu *et al.*
*****Sporobolomyces*** *roseus* Kluyver & van Niel (currently *Sporidiobolus metaroseus* Sampaio & Valerio)
Trichsporon Behrend
**T. asahii* Akagi ex Sugita *et al.*
**T. japonicum* Sugita &Nakase
Black yeast sp.

* Yeasts marked with asterisks are new records to Egypt.

2. Air of citrus and grapevine

Yeasts gave rise to 3.92 % and 1.33 % of total amount of CFU of all fungi from citrus air and 5.97 % and 1.51 % of total amount of CFU from grapevine air on DYM and DRBC respectively.

A total of 14 genera and 24 species of yeast were caught from the air of citrus and grapevine plantations on DYM and DRBC agar media. From these, 8 yeast species were

isolated from the air of citrus only, while 6 were isolated only from the air of grapevine (Table 4).

Table 4. Collective data of counts, percentage counts calculated to total fungi and frequency of occurrence of fungi recovered from the air of citrus and grapevine plantations on DYM and DRBC agar media bimonthly during the period from April 2008- February 2009 (counts of CFU calculated per 5 minutes exposures in each sample, collectively in 18 samples in each plantation).

Taxa	Citrus						Grapevine					
	DYM			DRBC			DYM			DRBC		
	CFU	%CFU	F&O	CFU	%CFU	F&O	CFU	%CFU	F&O	CFU	%CFU	F&O
Filamentous fungi	10140	96.08	18 H	9219	98.67	18 H	8947	94.03	18 H	4574	98.49	18 H
Yeasts	414	3.92	16H	125	1.34	14H	557	5.86	15H	70	1.51	7M
Ambrosiozyma platypodis				1	0.01	1R						
Candida	2	0.02	2 R	5	0.05	3 L						
C. catenulata	2	0.02	2 R									
C. parapsilosis				5	0.05	3 L						
Cryptococcus	66	0.63	9 H	47	0.50	7 M	58	0.62	10 H	37	0.79	6 M
C. albidus	66	0.63	9 H	47	0.50	7 M	33	0.35	6 M	20	0.43	4 L
C. caranscence							2	0.02	1 R			
C. flavescens							6	0.06	1 R			
C. laurentii							17	0.18	4 L	17	0.37	6 M
Debaryomyces	20	0.19	4 L	10	0.11	5 M	7	0.07	4 L	2	0.04	1 R
D. hansenii	15	0.14	3 L	8	0.09	4 L	3	0.03	2 R	2	0.04	1 R
D. pseudopolymorphus	5	0.05	2 R	2	0.02	1 R	4	0.04	2 R			
Geotrichum	2	0.02	1 R	2	0.02	2 R						
G. candidum				1	0.01	1 R						
G. citri-aurantii	2	0.02	1 R	1	0.02	1 R						
Hanseniaspora occidentalis	24	0.23	2 R	2	0.02	1 R						
Issatchenkia orientalis	4	0.04	1 R	3	0.03	2 R	1	0.01	1 R			
Melanopsichium pennsylvanicum	2	0.02	1 R	1	0.01	1 R						
Pichia	18	0.17	2 R	7	0.07	1 R	53	0.57	3 L	8	0.17	3 L
P. farinose	1	0.01	1 R									
P.guilliermondii	17	0.16	1 R	7	0.07	1 R	53	0.57	3 L	8	0.17	3 L

Taxa	Citrus						Grapevine					
	DYM			DRBC			DYM			DRBC		
	CFU	%CFU	F&O	CFU	%CFU	F&O	CFU	%CFU	F&O	CFU	%CFU	F&O
Pseudozyma	2	0.02	1 R	9	0.09	1 R	5	0.05	1 R			
P. hubeinsis	2	0.02	1 R	9	0.09	1 R						
Pseudozyma sp.							5	0.05	1 R			
Rhodosporidium paludigenum	93	0.88	6 M	12	0.13	4 L						
Rhodotorula	63	0.59	10 H	18	0.19	5 M	428	4.58	9 H	18	0.39	3 L
R. aurantiaca	2	0.02	2 R									
R. glutinis	12	0.11	4 L	2	0.02	1 R	378	4.05	6 M	16	0.34	3 L
R. mucilaginosa	49	0.46	5 M	16	0.17	4 L	50	0.54	4 L	2	0.04	1 R
Sporidiobolus ruineniae	119	1.14	5 M	5	0.05	3 L	4	0.04	2 R	2	0.04	1 R
Sporobolomyces roseus							1	0.01	1 R	3	0.06	1 R
Trichsporon asahii							1	0.01	1 R			
Total CFUs	10554	100	18H	9343	100	18H	9505	100	18H	4644	100	18H
No. of genera	59			58			46			44		
No. of species	139+2			136+2			110+2			91+1		

*F = Frequency of occurrence out of 18 exposures * OR = Occurrence remarks: H = high, 9 - 18; M = moderate, 5 - 8; L = Low, 3 - 4; R = rare, 1 - 2 exposures.

Yeasts showed their peak of total propagules caught from the air of citrus plantations in December on both media and from grapevine plantations in October and April on DYM and DRBC respectively, while their trough occurred in April on both media in the air of citrus plantations and in June and December on DYM and DRBC respectively in grapevine plantations.

Two genera of yeasts were encountered in high frequency on one medium and moderate or low frequency on the other medium in the air of both citrus and grapevine plantations and these were *Cryptoccocus* (4 species) and *Rhodotorula* (3 species). On the other hand, two genera were recovered in moderate or low frequency in the air of citrus plantations and low or rare frequency in grapevine plantations and these were *Debaryomyces* (2 species) and *Sporidiobolus* (*S. ruineniae*). Some yeast genera were recovered in the air of citrus plantations only (*Ambrosiozyma*, *Candida*, *Geotrichum*, *Hanseniaspora*,

Rhodosporidium, and *Melanopsichium* while *Sporobolomyces* and *Trichosporon* in grapevine plantations only (Table 4).

3. Soil of citrus and grapevine plantations

Moisture content and pH of soil in citrus and grapevine

pH values of the citrus soil samples investigated lied in the alkaline side ranging between 7.22-7.95 and the moisture content ranged between 14.44-22.94 % at the time of sampling (Table 5). Also, pH values of the grapevine soil samples lied in the alkaline side ranging between 7.46-8.14 and their moisture content ranged between 20.18-30.12 % (Table 5).

Table 5. Mean moisture content (MC) and pH values of soil samples collected from citrus and grapevine plantations.

Month	Citrus plantations		Grapevine plantations	
	Mean MC	Mean pH	Mean MC	Mean pH
April 2008	15.97	7.91	24.34	7.98
June 2008	22.94	7.95	30.12	8.14
August 2008	17.96	7.81	20.18	8,04
October 2008	21.57	7.82	26.61	7.98
December 2008	14.44	7.82	24.44	8,09
February 2009	15.53	7.22	22.55	7.46

*MC (moisture content) and pH were calculated out of three replicates and their means were calculated out of the three farms.

Yeasts contributed 0.15 % and 0.21 % of total fungi from 3 farms in the six bimonthly trips in grapevine soil on DYM and DRBC respectively, whereas they constituted 0.47 and 0.49 % respectively in citrus soil. A total of 9 genera and 13 species were recovered from soil of both citrus and grapevine plantations

Yeasts showed their peak of total propagules in soil of citrus plantations in April and in grapevine plantations in February on both media. Their trough occurred in June

and October on DYM and in August on DRBC in soil of citrus plantations and in April and December on DYM and in October and December on DRBC in grapevine plantations.

From yeasts, *Candida catenulata*, *Debaryomyces* (2 species), *Geotrichum* (3 species), *Hanseniaspora occidentalis,* and *Kluyveromyces marixianus* were encountered in low or rare frequency from soil of citrus plantations only, while *Cryptoccocus laurentii, Issachenkia orientalis,* and *Rhodotorula* sp. were encountered in rare frequency from soil of grapevine plantations only. *Pichia* (2 species) was recorded in soil of both citrus and grapevine plantations, *P. caribbica* was isolated from only soil of citrus plantations while *P. guillermondii* from only grapevine plantations (Table 6).

Table 6. Collective data of counts, percentage counts calculated to total fungi and frequency of occurrence of fungi recovered from soil of citrus and grapevine plantations on DYM and DRBC agar media bimonthly during the period from April 2008-February 2009 (counts of CFU calculated per gm soil in each sample, collectively in 18 samples in each plantation).

Taxa	**Citrus**						**Grapevine**					
	DYM			**DRBC**			**DYM**			**DRBC**		
	CFU	**%CFU**	**F&O**	**CFU**	**%CFU**	**F&O**	**CFU**	**%CFU**	**F&O**	**CFU**	**%CFU**	**F&O**
Filamentous fungi	25422	99.53	18 H	29386	99.51	18 H	16548	99.85	18 H	17513	99.79	18 H
Yeasts	120	0.47	5 M	144	0.49	6 M	24	0.15	3 L	36	0.21	4 L
Candida catenulata	6	0.02	1R	12	0.04	1 R						
Cryptococcus laurentii							12	0.07	2 R	6	0.03	1 R
Debaryomyces	96	0.05	3L	72	0.24	2 R						
D. hansenii	18	0.07	1R	48	0.16	2 R						
D. pseudopolymorphus	78	0.31	3L	24	0.08	1 R						
Geotrichum	12	0.05	2R	36	0.12	3 L						
G. candidum	6	0.02	1 R									
G. citri-aurantii				24	0.08	1 R						
Geotrichum sp.	6	0.02	1R	12	0.04	2 R						
Hansenispora occidentalis				12	0.04	1 R						
Issachenkia orientalis										6	0.03	1 R
Kluyveromyces marixianus	6	0.02	1R									

Taxa	Citrus						Grapevine					
	DYM			DRBC			DYM			DRBC		
	CFU	%CFU	F&O	CFU	%CFU	F&O	CFU	%CFU	F&O	CFU	%CFU	F&O
Pichia				12	0.04	2 R	12	0.07	1 R	12	0.07	2 R
P. carribia				12	0.04	2 R						
P. guillermondii							12	0.07	1 R	12	0.07	2 R
Rhodotorula sp.										12	0.07	2 R
Total CFUs	25542	100	18 H	29530	100	18 H	16572	100	18 H	17549	100	18 H
No. of genera	42			51			38			45		
No. of species	111			129			97			118		

*F = Frequency of occurrence out of 18 samples in case of citrus and 15 samples in grapevine

*O = Occurrence remarks for citrus: H = high, 9-18; M = moderate, 5-8; L = Low, 3-4; R = rare, 1-2 samples.

4. Phyllosphere of citrus and grapevine

Yeasts comprised minor proportions of the total fungi from citrus plantations (0.69 % on DYM and 0.42 % on DRBC). From grapevine, yeasts yielded relatively medium proportion of total fungi (21.01 % on DYM and 16.37 % on DRBC).

Yeasts were represented by 14 genera and 23 species. Eight of these were isolated only from the phyllosphere of citrus, and 5 from only the phyllosphere of grapevine (Table 7).

Yeasts showed their peak of total propagules recovered from the phyllosphere of citrus in February on both media and from grapevine plantations in December (senescent leaf) on both media, while their trough occurred in August on both media in the phyllosphere of citrus and in April (juvenile leaf) in the phyllosphere of grapevine on both media.

Two genera of yeasts were encountered in high frequency on one or both media and moderate or low on the other medium in the phyllospheres of both plants and these were *Cryptoccocus* and *Rhodotorula*.

Cryptococcus (6 species) was recovered in high frequency in the phyllosphere of grapevine contributing 9.84 and 5.43 % of total fungi on DYM and DRBC respectively while in moderate frequency and small counts in citrus. *C. albidus* was the most common

Cryptococcus species in the phyllosphere of grapevine constituting 9.79 and 5.03 % of total fungi on DYM and DRBC respectively.

Rhodotorula (2 species) was recovered in high and moderate frequencies in the phyllosphere of grapevine on DRBC and on DYM respectively, accounting for 10.48 and 10.05 % of total fungi on DYM and on DRBC respectively while in low and rare frequencies and relatively small proportions of propagules in citrus plantations. *R. mucilaginosa* was the main component of *Rhodotorula*, constituting 10.31 and 10.05 % of total fungi on DYM and on DRBC respectively in grapevine phyllosphere.

Some yeast genera were recovered only from the phyllosphere of citrus and these were *Candida* (*C. catenulata*), *Geotrichum* (*G. citri-aurantii*), *Pseudozyma* (3 species), and *Trichosporon* (*T. japonicum*), while others from grapevine only, namely *Pichia* (*P. guilliermondii*) and *Rhodosporidium* (*R. paludigenum*).

Other species were met with more frequently in one phyllosphere but less frequently or missed in the other (Table 7).

Table 7. Collective data of counts, percentage counts calculated to total fungi and frequency of occurrence of phyllosphere fungi recovered from citrus and grapevine on DYM and DRBC agar media bimonthly during the period from April 2008 - February 2009 (counts of CFU calculated per gm fresh leaf in each sample, collectively in 18 samples in case of citrus and 15 samples in grapevine).

Taxa	Citrus						Grapevine					
	DYM			DRBC			DYM			DRBC		
	CFU	%CFU	F&O	CFU	%CFU	F&O	CFU	%CFU	F&O	CFU	%CFU	F&O
Filamentous fungi	1032184	99.48	18 H	1280560	99.58	18 H	25258	78.99	15 H	231860	83.63	15 H
Yeasts	7200	0.69	13 H	5352	0.42	13 H	68160	21.01	12 H	45384	16.37	12 H
Candida catenulata	1880	0.18	4 L	1560	0.12	5 M						
Cryptococcus	3744	0.36	8 M	1736	0.14	8 M	31456	9.84	11H	15060	5.43	10H
C. albidosimilis										184	0.07	3 L
C. albidus	3592	0.35	5 M	1552	0.12	6 M	31288	9.79	10H	13936	5.03	10H
C. carnescens	16	0.002	1 R	32	0.002	1 R	4	0.001	1R	140	0.05	5 M
C. laurentii	56	0.005	2 R	96	0.01	2 R	164	0.05	3L	768	0.28	5 M
C. luteolus	80	0.008	1 R	40	0.003	1 R						
C. magnus				16	0.001	2 R				32	0.01	2 R
Filobasidium	8	0.001	1 R	120	0.01	1 R				4	0.001	1 R

Taxa	Citrus						Grapevine					
	DYM			DRBC			DYM			DRBC		
	CFU	%CFU	F&O	CFU	%CFU	F&O	CFU	%CFU	F&O	CFU	%CFU	F&O
floriforme												
Geotrichum citri-aurentii	280	0.03	1 R	760	0.06	1 R						
Issachenkia orientalis	40	0.004	1 R	240	0.02	2 R	40	0.01	1R	12	0.004	1 R
Kluyveromyces marixianus	840	0.08	1 R	320	0.02	1 R	320	0.10	2R	440	0.16	2 R
Pichia gluiliremondiil							20	0.006	1R	760	0.27	3 L
Pseudozyma	64	0.006	2 R	136	0.01	1 R						
P. aphidis	40	0.004	1 R									
P. rugulosa	24	0.002	1 R	16	0.001	1 R						
Pseudozyma sp.				120	0.01	1 R						
Rodosporidium paludigenum							820	0.26	4L	660	0.24	3 L
Rhodotorula	160	0.02	2 R	360	0.03	3 L	33492	10.48	8M	27876	10.05	9 H
R. glutinis	120	0.01	1 R	360	0.03	3 L	540	0.17	1R	8	0.003	1 R
R. muclaginosa	40	0.004	1 R				32952	10.31	8M	27868	10.05	9 H
Sporidiobolus							440	0.14	3L	444	0.16	4 L
S. pararoseus							440	0.14	3L	400	0.14	2 R
S. ruineniae										44	0.02	2 R
Sporobolomyces roseus	24	0.002	1 R				20	0.006	2R	24	0.009	2 R
Trichosporon japonicum	120	0.01	1 R									
Yeast sp. (black)	40	0.004	1 R	120	0.01	1 R						
Total CFUs	1037584	100	18 H	1285916	100	18 H	320688	100	15 H	277244	100	15 H
No. of genera	48			43			34			37		
No. of species	109+1			94+1			66			71+1		

*F = Frequency of occurrence out of 18 samples in case of citrus and 15 samples in grapevine.

*O = Occurrence remarks for citrus: H = high, 9-18; M = moderate, 5-8; L = Low, 3-4; R = rare, 1-2 samples = For grapevine: H, 8-15; M, 5-7; L, 3-4; R= 1-2 samples.

5. Phylloplane of citrus and grapevine

From citrus yeasts contributed 6.54 % and 2.71 % of total fungi on DYM and DRBC, respectively. From grapevine, yeasts shared by 5.73 % of total fungi on DYM and 5.86 % on DRBC. Yeasts were represented by 12 genera and 16 species were recovered from the phylloplane of citrus and grapevine. It is worth mentioning that 7 of yeast species were isolated from citrus phylloplane only, while 2 were isolated from grapevine phylloplane only (Table 8).

Yeasts showed their peak of total propagules recovered from citrus phylloplane in October and June on DYM and DRBC respectively, and from grapevine in August (mature leaf) on both media, while their trough occurred in April and February in the phylloplane of citrus, and in October (mature leaf) and June (young leaf) in grapevine phylloplane on DYM and DRBC respectively.

Cryptococcus (5 species) was recovered in moderate frequency from both phylloplanes, contributing 2.26 and 1.16 % of total fungi from citrus phylloplane and 1.59 and 2.36 % from grapevine on DYM and DRBC respectively.

Rhodotorula mucilaginosa was recovered in moderate frequency from grapevine phylloplane on both media, accounting for 2.54 and 1.49 % of total fungi on DYM and DRBC respectively, while in low and rare frequencies and relatively small proportions of propagules in citrus plantations.

Some yeast genera were recovered from citrus phylloplane only and these were *Candida* (*C. catenulata*), *Geotrichum* (*G. citri-aurantii*), *Issachenkia orientalis, Kluyveromyces marixianus, Pseudozyma* (*P. aphidis*), and *Trichosporon* (*T. japonicum*), while *Sporidiobolus pararoseus* and *Sporobolomyces roseus* from grapevine phylloplane only (Table 8).

Table 8. Collective data of counts, percentage counts calculated to total fungi and frequency of occurrence of phylloplane fungi recovered from citrus and grapevine plantations on DYM and DRBC agar media bimonthly during the period from April 2008 - February 2009 (counts of CFU calculated per 20 fresh leaf pieces in each sample, collectively in 18 samples in case of citrus and 15 samples in grapevine).

Taxa	Citrus phylloplane						Grapevine phylloplane					
	DYM			DRBC			DYM			DRBC		
	CFU	%CFU	F&O	CFU	%CFU	F&O	CFU	%CFU	F&O	CFU	%CFU	F&O
Filamentous fungi	2273	93.46	18 H	2766	97.29	18 H	1003	94.27	15 H	1076	94.14	15 H
Yeasts	159	6.54	13 H	77	2.71	15 H	61	5.73	10 H	67	5.86	12 H
Candida catenulata	36	1.48	5 M	19	0.67	5 M						
Cryptococcus	55	2.26	7 M	33	1.16	8 M	17	1.59	5 M	27	2.36	8 M
C. albidus	41	1.69	4 L	9	0.32	4 L	10	0.94	4 L	19	1.66	8 M
C. carnescens	2	0.08	1 R	9	0.32	1 R				1	0.09	1 R
C. laurentii	4	0.16	2 R	2	0.07	2 R	5	0.47	1 R	4	0.35	1 R
C. luteolus	8	0.33	1 R	6	0.21	1 R						
C. magnus				7	0.25	1 R	2	0.19	1 R	3	0.26	2 R
Filobasidium floriforme	2	0.08	1 R							1	0.09	1 R
Geotrichum citri-aurantii	8	0.33	2 R	5	0.18	1 R						
Issachenkia orientalis				1	0.04	1 R						
Kluyveromyces marixianus	48	1.97	1 R	10	0.35	1 R						
Pseudozyma aphidis				2	0.07	1 R						
Rhodosporidium paludigenum	2	0.08	1 R	2	0.07	1 R	13	1.22	2 R	14	1.22	2 R
Rhodotorula muclaginosa	5	0.21	2 R	6	0.21	4 L	27	2.54	7 M	17	1.49	7 M
Sporidiobolus pararoseus							3	0.28	1 R	5	0.44	1 R
Sporobolomyces roseus							1	0.09	1 R	3	0.26	2 R
Trichosporon japonicum	3	0.12	1 R									

Taxa	Citrus phylloplane						Grapevine phylloplane					
	DYM			DRBC			DYM			DRBC		
	CFU	%CFU	F&O	CFU	%CFU	F&O	CFU	%CFU	F&O	CFU	%CFU	F&O
Total CFUs	2432	100	18 H	2843	100	18 H	1064	100	15 H	1143	100	15 H
No. of genera	37			41			32			29		
No. of species	74+1			75+1			57+1			58		

*F = Frequency of occurrence out of 18 samples in case of citrus and 15 samples in grapevine. *O = Occurrence remarks for citrus: H = high, 9-18; M = moderate, 5-8; L = Low, 3-4; R = rare, 1-2 samples, = For grapevine: H, 8-15; M, 5-7; L, 3-4; R= 1-2 samples.

6. Carposphere of citrus and grape fruits

From citrus, yeasts gave rise to 37.49 and 25.69 % on DYM and DRBC of total propagules respectively which was relatively high values comparable with those of other sources (air, soil, phyllosphere and phylloplane) (Tale 9). From grape fruits, yeasts contributed relatively medium proportions of total fungi (17.95 and 19.08 % respectively) from grape carposphere (3 farms in the five trips studied bimonthly during the period from April 2008 to December 2008).

It should be mentioned that the dates of successive stages of development of fruit are as following: in citrus: primordial, in April; immature, in June and August; mature in October and December; senescent, in February, and in grape: primordial, in April; immature, in June and August; mature in October and; senescent, in December.

Yeasts were represented by 13 genera and 23 species. They showed their peak of total propagules recovered from citrus carposphere in December and from that of grapevine in October on both media, while their trough occurred in April on DYM and in February on DRBC in citrus carposphere and in August in that of grape on both media.

Yeast species were recorded in high frequency in grape carposphere while in moderate frequency in citrus although they constituted higher proportions of propagules in citrus carposphere (8460 CFUs on DYM and 7784 CFUs on DRBC respectively) than those of grape (3342 and 3682).

Rhodotorula (2 species) was encountered in moderate frequency on both media in grape carposphere constituting 0.34 % and 0.16 % of total fungi on DYM and DRBC

respectively, while was recorded in rare frequency on DYM (0.01 %) and absent on DRBC in citrus carposphere.

Issachenkia orientalis was recovered in low frequency in the carposphere of both plants contributing 26.48 and 23.01 % of total fungi on DYM and DRBC respectively in citrus carposphere while its counts retrograded sharply in grape carposphere (2.29 and 2.94 %).

Hanseniaspora occidentalis was recovered in low frequency in grape carposphere contributing 9.21 and 6.87 % of total fungi on DYM and on DRBC respectively, and in rare frequency in citrus carposphere.

Candida catenulata and *C. parapsilosis* were isolated from citrus carposphere only, while *C. prunicola* was recorded in grape carposphere only (Table 9).

Some yeast species were recovered only from citrus carposphere and these were *Geotrichum citri-aurantii*, *Kodemaea ohmeri* and *Pseudozyma* sp. while others from grape carposphere only: *Pichia guillieromondii*, *Rhodosporidium diobovatum* and *R. paludigenum*.

Table 9. Collective data of counts, percentage counts calculated to total fungi and frequency of occurrence of carposphere fungi recovered bimonthly from the citrus and grape on DYM and DRBC agar media during the period from April 2008 - February 2009 (counts of CFU calculated per gm fresh fruit rind (citrus) or fresh fruit (grape) in each sample, collectively in 17 samples in case of citrus and 14 samples in grape).

Taxa	**Citrus carposphere**						**Grape carposphere**					
	DYM			**DRBC**			**DYM**			**DRBC**		
	CFU	**%CFU**	**F&O**	**CFU**	**%CFU**	**F&O**	**CFU**	**%CFU**	**F&O**	**CFU**	**%CFU**	**F&O**
Filamentous fungi	14108	62.51	16 H	22512	74.31	17H	15274	82.05	14 H	15617	80.92	14 H
Yeasts	8460	37.49	5 M	7784	25.69	8M	3342	17.95	9 H	3682	19.08	8 H
Candida	1670	7.40	3 L	206	0.68	3 L	996	5.35	2 R	1296	6.72	3 L
C. catenulata	1670	7.40	3 L	204	0.67	2 R						
C. parapsilosis				4	0.01	1 R						
C. prunicola							996	5.35	2 R	1296	6.72	3 L
Cryprococcus	4	0.02	1 R	24	0.08	4 L	24	0.13	2 R	302	1.56	4 L
C. albidus				12	0.04	2 R	40	0.21	3 L	178	0.92	4 L
C. carnescens							4	0.02	1 R	32	0.17	3 L
C. laurentii	4	0.02	1 R	12	0.04	3 L	12	0.06	2 R	88	0.46	3 L
C. magnus										4	0.02	1 R

Taxa	Citrus carposphere						Grape carposphere					
	DYM			DRBC			DYM			DRBC		
	CFU	%CFU	F&O	CFU	%CFU	F&O	CFU	%CFU	F&O	CFU	%CFU	F&O
Debaryomyces	136	0.60	2 R	28	0.09	1 R	4	0.02	1 R			
D. hansenii	136	0.60	2 R	16	0.05	1 R						
D. pseudopolymorphus				12	0.04	1 R	4	0.02	1 R			
Geotrichum citri-aurantii	20	0.09	1 R	12	0.04	2 R						
Hanseniaspora occidentalis	526	2.33	2 R	358	1.18	2 R	1714	9.21	4 L	1326	6.87	3 L
Issachenkia orientalis	5976	26.48	3 L	6970	23.01	3 L	428	2.29	4 L	568	2.94	3 L
Kluyveromyces marixianus							12	0.06	2 R	2	0.01	1 R
Kodemaea ohmeri	4	0.02	1 R	4	0.01	1 R						
Pichia	120	0.53	3 L	174	0.57	2 R	10	0.05	2 R	76	0.39	3 L
P. caribbica	2	0.01	1 R	4	0.01	1 R						
P. fermentans	118	0.52	3 L	170	0.56	2 R	2	0.01	1 R			
P. guillieromondii							8	0.04	1 R	76	0.39	3 L
Pseudozyma sp.	4	0.02	1 R									
Rhodosporidium							22	0.12	2 R	22	0.11	3 L
R. diobovatum							20	0.11	1 R	12	0.06	1 R
R. paludigenum							2	0.01	1 R	10	0.05	2 R
Rhodotorula				4	0.01	1 R	64	0.34	5 M	30	0.16	5 M
R. glutinis							12	0.06	2 R	4	0.02	1 R
R. muclaginosa				4	0.01	1 R	52	0.28	4 L	26	0.13	4 L
Sporobolomyces roseus							36	0.19	3 L	60	0.31	3 L
Total CFUs	22568	100	17 H	30296	100	17H	18616	100	14 H	19299	100	14 H
No. of genera	34			32			33			31		
No. of species	67+1			64+1			58			59		

*F = Frequency of occurrence out of 17 samples of citrus fruits or 14 of grapevine fruits.

*OR = Occurrence remarks: for citrus samples; H = high, 9-17; M = moderate, 5-8; L = Low, 3-4; R = rare, 1 or 2 samples, and for grapevine: H, 7-14; M, 5-6; L, 3-4; R = 1-2 samples.

7. Carpoplane of citrus and grape fruits

From citrus fruits, yeasts gave rise to moderate proportions of the total fungi (30.71 % and 35.22 % on DYM and DRBC respectively). From grape fruits, yeasts yielded 20.56 % and 23.08 % on DYM and DRBC respectively from grape carpoplane (3 farms in the six trips studied bimonthly during the period from April 2008 to February 2009).

Yeast fungi were represented by 12 genera and 14 species. It is worthy to mention that 6 of yeast species were isolated from the carpoplane of citrus only, while 6 were isolated from grape carpoplane only (Table 10).

Yeasts showed their peak of total propagules in the carpoplane of citrus in December and in grape carpoplane in October on both media, while their trough occurred in April on DYM and in April and June on DRBC in citrus carpoplane and in December on DYM and in August on DRBC in grape carpoplane.

Issachenkia orientalis was recovered in low frequency in citrus carpoplane and in low and rare frequencies in grapevine constituting 9.49 % and 9.42 % of total fungi on DYM and DRBC respectively in citrus carpoplane and 10.95 % and 12.23 % in grapevine.

Debaryomyces (*D. hansenii* and *D. pseudopolymorphus*) was isolated in low frequency in citrus carpoplane while it was missed in grape.

Candida (2 species) contributed medium proportion of propagules despite its record in rare frequency in the carpoplane of both plants on both media. It was represented by *C. catenulata* in citrus carpoplane (10.30 % and 11.68 % of total fungi on DYM and DRBC respectively) only and by *C. prunicola* in grape carpoplane (6.51 % and 7.97 %) only.

Hanseniaspora occidentalis was recovered in rare frequency in both plants contributing 3.64 % and 3.39 % of total fungi in grape carpoplane on DYM and on DRBC respectively, and 2.07 % and 1.10 % in citrus carpoplane.

Some yeast genera were recovered from the carpoplane of citrus only and these were *Geotrichum* (*G. citri-aurantii*), *Kodemaea* (*K. ohmeri*), and *Pichia* (*P. fermentans*), while others from grape carpoplane only, namely *Cryptococcus* (*C. laurentii*), *Rhodosporidium* (*R. paludigenum*), *Rhodotorula* (*R. mucilaginosa*), *Sporobolomyces roseus* and yeast sp. (black) (Table 10).

Table 10. Collective data of counts, percentage counts calculated to total fungi and frequency of occurrence of carpoplane fungi recovered from citrus and grape on DYM and DRBC agar media bimonthly during the period from April 2008 - February 2009 (counts of CFU calculated per 20 fresh fruit rind pieces (citrus) or fresh fruit pieces (grape) in each sample, collectively in 17 samples in case of citrus and 14 samples in grape).

Taxa	Citrus						Grape					
	DYM			DRBC			DYM			DRBC		
	CFU	%CFU	F&O	CFU	%CFU	F&O	CFU	%CFU	F&O	CFU	%CFU	F&O
Filamentous fungi	343	69.29	17 H	344	64.78	17 H	537	79.44	14 H	560	76.92	13 H
Yeasts	152	30.71	5 M	187	35.22	6 M	139	20.56	4 L	168	23.08	7 H
Candida	51	10.30	2 R	62	11.68	2 R	44	6.51	1 R	58	7.97	2 R
C. catenulata	51	10.30	2 R	62	11.68	2 R						
C. prunicola							44	6.51	1 R	58	7.97	2 R
Cryptococcus laurentii										1	0.14	1 R
Debaryomyces	23	4.65	3 L	26	4.89	3 L						
D. hansenii	3	0.61	2 R	2	0.38	2 R						
D. pseudopolymorphus	20	4.04	3 L	24	4.52	1 R						
Geotrichum citri-aurantii	3	0.61	1 R	5	0.94	2 R						
Hanseniaspora occidentalis	18	3.64	1 R	18	3.39	1 R	14	2.07	1 R	8	1.10	2 R
Issachenkia orientalis	47	9.49	3 L	50	9.42	3 L	74	10.95	2 R	89	12.23	3 L
Kodemaea ohmeri	1	0.20	1 R	2	0.38	1 R						
Pichia fermentans	9	1.82	1 R	24	4.52	1 R						
Rhodosporidium paludigenum							2	0.29	1 R			
Rhodotorula muclaginosa										5	0.69	2 R
Sporobolomyces roseus							2	0.29	1 R	5	0.69	2 R
Yeast sp. (black)							3	0.44	1 R	2	0.27	1 R
Total CFUs	495	100	17 H	531	100	17 H	676	100	14 H	728	100	14 H
No. of genera	27			28			26			27		
No. of species	55			51+1			39			37		

*F = Frequency of occurrence out of 17 samples of citrus and 14 of grapevine.

*O = Occurrence remarks for citrus: H = high, 9-17; M = moderate, 5-8; L = Low, 3-4; R = rare, 1-2 samples = For grapevine: H, 7-14; M, 5-6; L, 3-4; R = 1-2 samples.

8. Juice of citrus and grape fruits

Yeasts comprised the extreme majority of total fungi (95.42 % and 91.60 % on DYM and DRBC respectively from citrus juice and 99.39 % and 99.14 % on DYM and DRBC respectively from juice of grape berries collected from the three farms in August 2008 and October 2008 respectively. However, extremely smaller numbers of propagules were recovered from citrus juice (908 CFUs/1 ml fresh citrus juice in 8 samples on both isolation media) compared with those from grape (72215 CFUs/1m fresh grape juice in 6 samples).

Yeasts were represented by 11 genera and 16 species, 7 genera and 7 species from citrus juice and 9 genera and 11 species from grape juice. From these, 4 species were isolated from citrus juice only, while 8 were isolated from grape juice only (Table 11).

Yeasts regularly showed their peak of total propagules in October on both citrus and grape juices on both media, while their troughs occurred in December in citrus juice and in August in grape juice on both media.

Issachenkia orientalis was recovered in moderate frequency in citrus juice on both media and in high and moderate frequencies in grape constituting 30.75 and 26.60 % of total fungi on DYM and DRBC respectively in citrus juice and 18.91 and 27.21 % in grape juice.

Candida (2 species) was recorded in moderate frequency in citrus juice on both media while it was recorded in high frequency on DYM and in moderate frequency on DRBC, contributing in grape juice 80.22 % and 71.41 % of total fungi on DYM and DRBC respectively and in citrus juice (3.44 % and 4.16 %). It was represented by *C. catenulata* in citrus juice and *C. prunicola* in grape juice.

Debaryomyces (*D. hansenii* and *D. pseudopolymorphus*) was isolated in moderate frequency in citrus juice while it was recorded in low frequency on DYM and missed on DRBC in grapevine.

Hanseniaspora occidentalis was recovered in moderate frequency, while *Cryptococcus* (2 species) was recorded in low frequency in juices of both fruits.

Some yeast genera were recovered from the juice of citrus only and these were *Geotrichum* (*G. citri-aurantii*), and *Pichia* (*P. carribbica* and *P. fermentans*), while others

from grape juice only, namely *Rhodosporidium* (*R. paludigenum*), *Rhodotorula* (*R. glutinus* and *R. mucilaginosa*), *Sporidiobolus* (*S. pararoseus* and *S. ruinenniae*) and *Sporobolomyces* (*S. roseus*) (Table 11).

Table 11. Collective data of counts, percentage counts calculated to total fungi and frequency of occurrence of fungi recovered from citrus and grapevine juices on DYM and DRBC agar media bimonthly during the period from April 2008 - February 2009 (counts of CFU calculated per ml juice in each sample, collectively in 8 samples in case of citrus and 6 samples in grapevine).

Taxa	Citrus						Grapevine					
	DYM			DRBC			DYM			DRBC		
	CFU	%CFU	F&O	CFU	%CFU	F&O	CFU	%CFU	F&O	CFU	%CFU	F&O
Filamentous fungi	20.0	4.58	7 H	39.6	8.40	7 H	305.4	0.61	6 H	189.6	0.86	6 H
Yeasts	416.4	95.42	5 H	431.8	91.60	5 H	49978	99.39	6 H	21741.6	99.14	6 H
Candida	15	3.44	2 M	19.6	4.16	2 M	40338.2	80.22	3 H	15661.8	71.41	2 M
C. cateulata	15	3.44	2 M	19.6	4.16	2 M						
C. prunicola							40338.2	80.22	3 H	15661.8	71.41	2 M
Cryprococcus	0.2	0.046	1 L				0.4	0.001	1 L	0.2	0.001	1 L
C. albidus										0.2	0.001	1 L
C. laurentii	0.2	0.046	1 L				0.4	0.001	1 L			
Debaryomyces pseudopolymorphus	10	2.29	2 M	3.6	0.76	2 M	0.4	0.001	1 L			
Geotrichum citri-aurantii	2	0.46	1 L	4.4	0.93	2 M						
Hanseniaspora occidentalis	6.2	1.42	2 M	3	0.64	2 M	113.6	0.23	2 M	108.6	0.49	2 M
Issachenkia orientalis	134.2	30.75	2 M	125.4	26.60	2 M	9510.2	18.91	3 H	5967	27.21	2 M
Pichia	248.8	57.01	3 M	265.8	56.42	4 H						
P. carribbica				0.6	0.13	1 R						
P. fermentans	248.8	57.01	3 M	265.2	56.26	4 H						
Rhodosporidium paludigenum							0.2	0.0004	1 L	0.2	0.001	1 L
Rhodotorula							14	0.03	3 H	2	0.01	3 H
R. glutinis							0.4	0.001	1 L			
R. muclaginosa							13.6	0.03	3 H	2	0.01	3 H
Sporidiobolus							0.2	0.0004	1 L	0.6	0.003	2 M
S. pararoseus										0.2	0.001	1 L

Taxa	Citrus						Grapevine					
	DYM			DRBC			DYM			DRBC		
	CFU	%CFU	F&O	CFU	%CFU	F&O	CFU	%CFU	F&O	CFU	%CFU	F&O
S. ruineniae							0.2	0.0004	1 L	0.4	0.002	1 L
Sporobolomyces roseus							0.8	0.002	1 L	1.2	0.01	1 L
Total CFUs	436.4	100	8 H	471.4	100	8 H	50283.4	100	6 H	21931.2	100	6 H
No. of genera	15			17			17			14		
No. of species	23			26			29			22		

*F = Frequency of occurrence out of 8 samples for citrus juice and 6 samples for grapevine juice.

*O = Occurrence remarks for citrus juice: H = high, 4-8; M = moderate, 2-3; L = Low, 1 samples, = For grapevine juice: H, 3-6; M, 2; L = 1 sample.

9. Yeast fungi associated with different growth stages of leaf and fruit

A. In grape phyllosphere

In the early stages (juvenile leaves) in April, the basidiomyceteous yeasts, *Cryptococcus*, *Rhodotorula*, and *Sporidiobolus* contributed less than 3 % of total fungi (Fig. 11).

On mature leaves in August, yeasts constituted more than half of total fungi, in which *Rhodotorula* was the most dominant genus contributing the greatest percentage counts (47.12 % of total fungi) followed by *Rhodosporidium* and *Cryptococcus*.

On mature leaves in October, yeasts were recorded in low counts (3.74 % of total fungi), of which *Sporidiobolus* gained the highest numbers (1.33 %) followed by *Cryptococcus*, *Klyuveromyces*, and *Rhodosporidium.*

On senescent leaves in December, yeasts constituted 21.23 % of total fungi, of which *Cryptococcus* was the most dominant genus followed by *Rhodotorula* (Fig. 11).

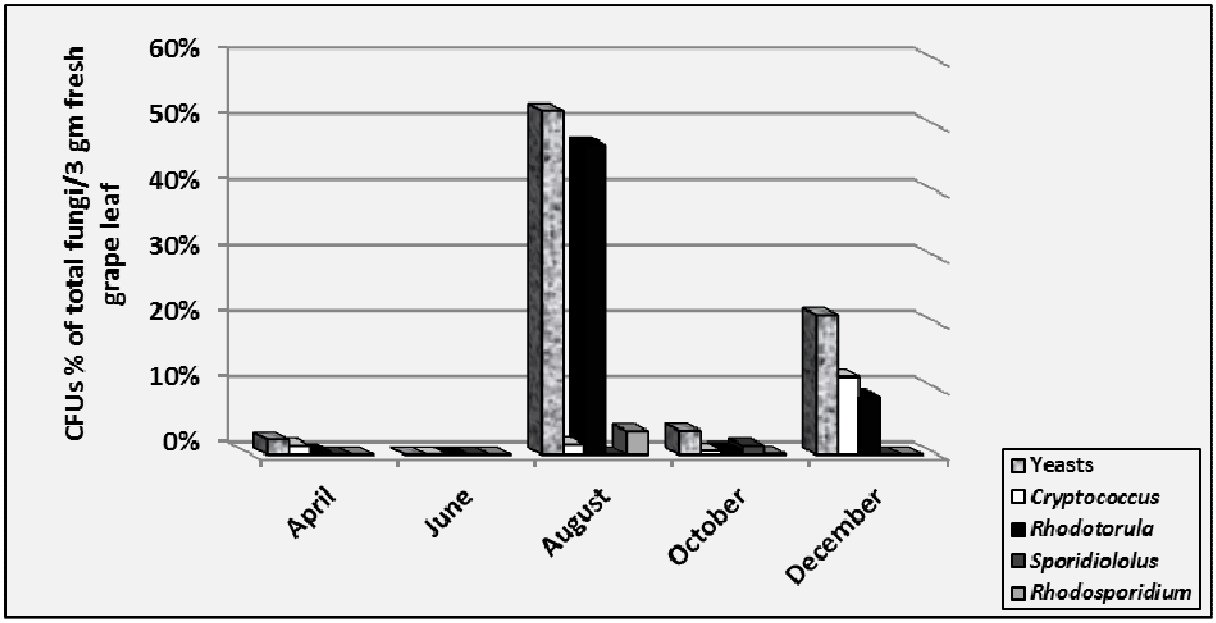

Figure 10. Percentage counts of total and common yeast fungi of grapevine leaf phyllosphere during the different stages of development: juenvile in April; immature in June; mature in August and October; senescent in December.

B. In grape phylloplane

In the early stage (on juvenile leaves) in April, the basidiomyceteous yeasts contributed 7.58 % of total fungi. *Cryptococcus* came ahead followed by *Rhodotorula*, *Sporidiobolus* and *Filobasidium* (Fig. 12).

On young leaves in June, Only *Rhodotorula* was recorded in small proportions (3.84 % of total fungi).

On mature leaves in August, yeasts constituted 12.16 % of total fungi, in which *Rhodotorula* and *Rhodosporidium* were the most prevalent genera followed by *Cryptococcus*.

On mature leaves in October, yeasts were recorded in low percentage (4.28 %), and *Sporidiobolus* came ahead of *Rhodosporidium*, *Cryptococcus* and *Rhodotorula*.

On senescent leaves in December, yeasts constituted 1.98 % of total fungi, in which *Cryptococcus* was of denser population (1.42 % of total fungi) than *Rhodotorula* (0.57 %).

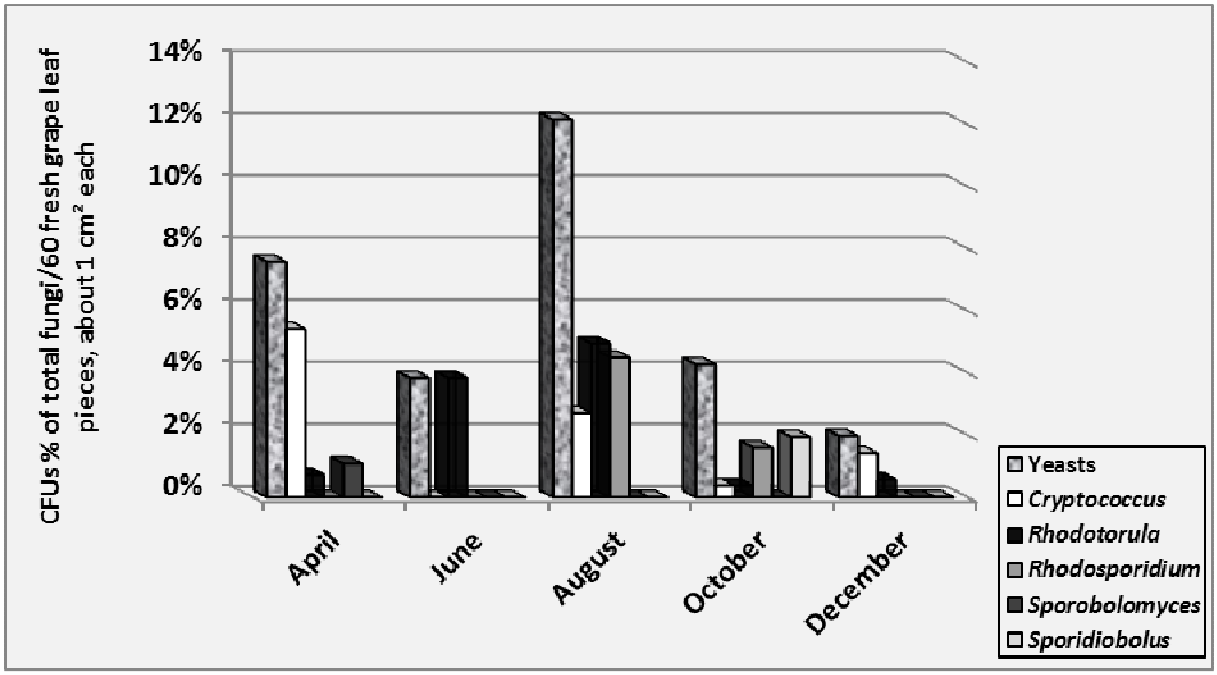

Figure 11. Percentage counts of total and common yeast fungi of grapevine leaf phylloplane during the different stages of development: juenvile in April; immature in June; mature in August and October; senescent in December.

C. In citrus carposphere

In the early stage of fruiting (primordial) in April, yeasts were encountered in minute proportions (0.29 % of total fungi), whereas on young immature fruits, yeasts were missed.

In the mature stage in October and December, the yeast accounted for 91.25 % and 95.47 % of total fungi respectively. *Candida*, *Hanseniaspora*, *Issachenkia*, and *Pichia* were the most dominant, contributing the greatest proportion of the total count (more than 90 %) (Fig. 12).

In the senescent stage in February, yeasts were missed.

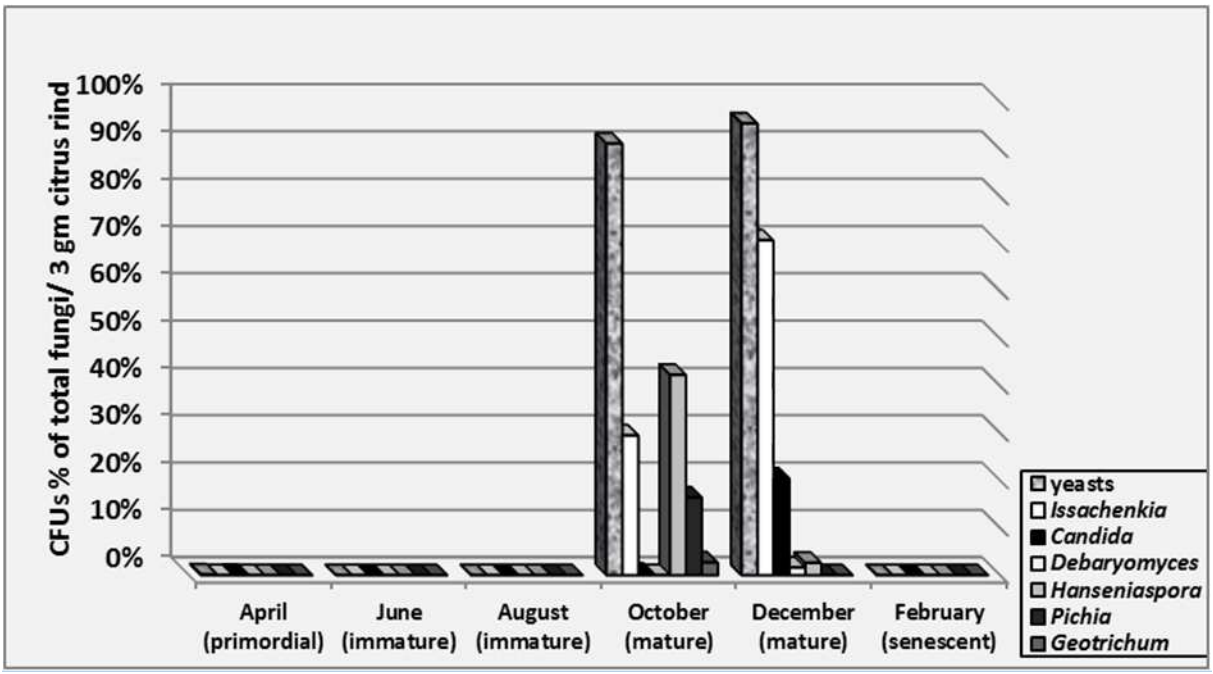

Figure 12. Percentage counts of total and common yeast fungi of citrus carposphere during the different stages of development.

D. In citrus carpoplane

The highest total count of fungi was recorded in the mature stage of fruit in December while the lowest in the immature stage in June. In the early stage of fruiting (primordial), yeasts were missed at this stage (Fig. 13). On young immature fruits, the yeast fungus *Debaryomyces* possessed 20.83 % of total fungi in June while missed in August.

In the mature stage in October and December, yeast fungi represented by *Candida*, *Debaryomyces*, *Hanseniaspora*, *Issachenkia*, and, *Pichia* were the most dominant, contributing (34.29 % of total fungi) in October and (62.79 %) in December. In the senescent stage in February, yeasts were missed.

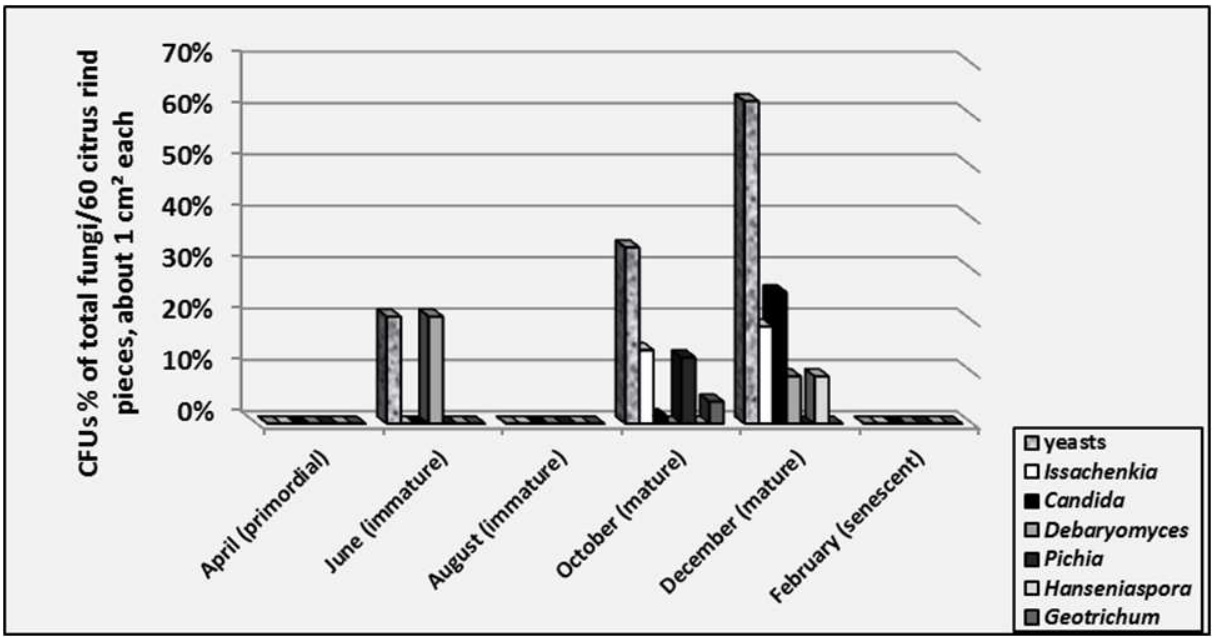

Figure 13. Percentage counts of total and common yeast fungi of citrus carpoplane during the different stages of development.

E. In grape carposphere

In the early stage of fruiting (primordial) in April, yeasts were encountered in small percentage counts (3.19 % of total fungi), in which *Cryptococcus* was the most genus followed by *Rhodosporidium* and *Rhodotorula* (Fig. 14).

On young immature fruits in June and August, yeasts were missed in June while *Rhodotorula* contributed small percentage counts in August.

In mature stages, *Candida*, *Hanseniaspora*, and *Issachenkia* were the most dominant genera, contributing the largest proportion of the total fungi (59.54 %) in October but only 7.32 % in December (Fig. 15).

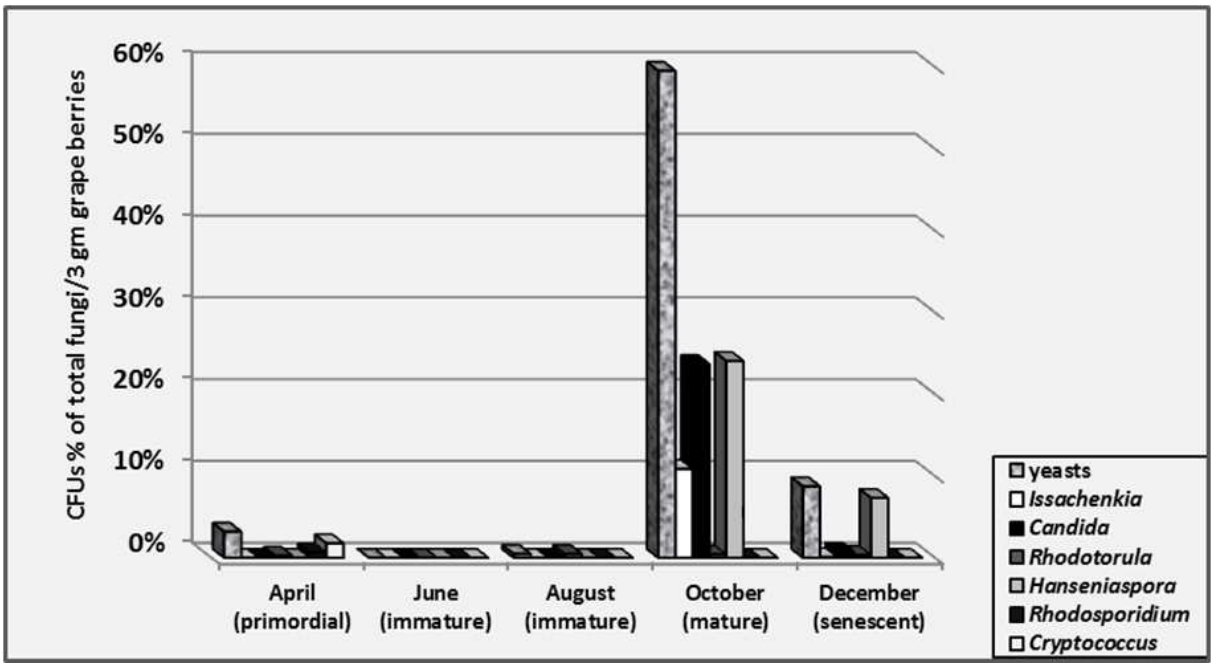

Figure 14. Percentage counts of total and common yeast fungi of grape carposphere during the different stages of development.

F. In grape carpoplane

In the primordial stage in April, *Cryptococcus* and *Sporobolomyces* constituted minute proportions.

On young immature fruits in June and August, yeasts were missed in June while *Rhodotorula* contributed small percentage count in August.

In the mature stage, *Candida* and *Issachenkia* were the most dominant genera, contributing the largest proportion of the total counts (71.43 % of total fungi) in October but disappeared in December. *Hanseniaspora* was recorded in low percentages in both months, *Rhodosporidium* and *Sporobolomyces* were recorded in December only (Fig. 15).

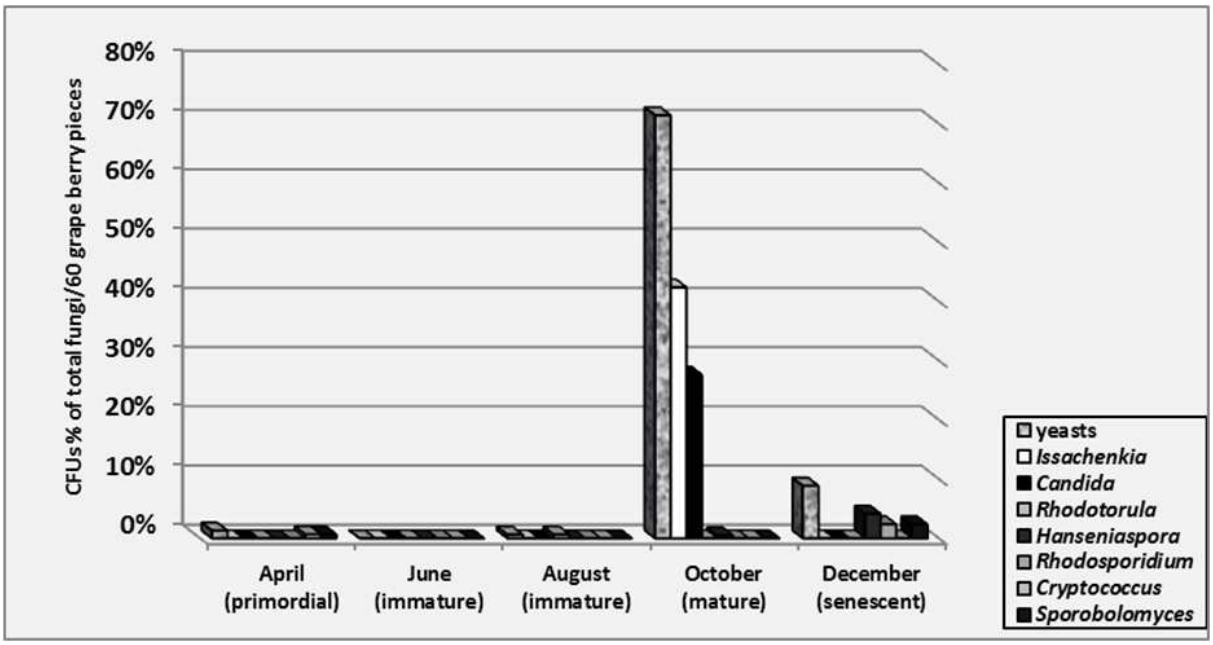

Figure 15. Percentage counts of total and common yeast fungi of grape carpoplane during the different stages of development.

DIVERSITY, BIOCHEMICAL AND MOLECULAR CHARACTERISATION OF YEAST SPECIES RECORDED FROM CITRUS AND GRAPEVINE PLANTATIONS

1. Ascomyceteous yeasts

Ambrosiozyma J.P. van der Walt

This genus was isolated only from citrus air. It was recorded in rare frequency on DRBC contributing 0.01 % of total fungi. It was represented by *A. platypodis* (Soliman 2012, Moubasher *et al.* 2016). This species was reported previously from tunnel of ambrosia beetle *Platypus cylindrus* in Turkey oak in UK, and in tunnels of other *Platypus* and insect spp. in *Ficus* and some other plant species in Tasmania and South Africa (Barnett *et al.* 2000).

Strain tested

Ambrosiozyma platypodis (J. M. Baker& Kreger-Van Rji) van der Walt AUMC 7233 (Plate 1)

Aureobasidium Viala & Boyer (as *Aureobasidium* sp.)

It was isolated from soil of citrus plantations.

Strains tested: AUMC 7757 (Plates 2 & 3)

Candida Berkhout

The genus *Candida* was recovered infrequently from different sources in both plantations while it was missed from grapevine air, soil, phyllosphere, and phylloplane. Its highest percentage count was recorded from grape juice (27.21 % - 80.22 % of total fungi). Three species were recorded from both plantations; *C. catenulata* and *C. parapsilosis* were recovered from citrus plantations only and *C. prunicola* from grapevine only.

In the air, it was recorded in cirus air in low or rare frequency, contributing minute percentage counts (0.02 % - 0.05 % of total fungi) but was missed in grapevine air. *C. catenulata* was recovered on DYM while *C. parapsilosis* was isolated on DRBC.

In the soil, it was recorded in rare frequency in citrus soil on both media represented by *C. catenulata*, contributing minute percentage counts (0.02 % - 0.04 % of total fungi). It was not recorded from grapevine soil.

In the phyllosphere, *Candida* yielded less percentage counts in citrus. It was recovered in moderate or low frequency represented by *C. catenulata*, contributing minute percentage counts (0.12 % - 0.18 % of total fungi). It was missed in grapevine phyllosphere.

In the phylloplane, it was isolated in moderate frequency from citrus phylloplane on both media represented by *C. catenulata*, contributing small percentage counts (0.67 % - 1.48 % of total fungi) exceeding their respectives in phyllosphere. It was missed in grapevine phylloplane.

In the carposphere, *Candida* was recovered in low frequency from citrus carposphere on both media while it was recovered in low or rare frequency in grape carposphere. It contributed 0.68 % - 7.40 % of total fungi in citrus carposphere and 5.35 % - 6.72 % in grape carposphere. *C. catenulata* and *C. parapsilosis* were recovered from citrus carposphere while *C. prunicola* was recorded from grape carposphere only.

In the carpoplane, it was encountered in rare frequency on both media despite its relatively high contributions, 10.30 % - 11.68 % of total fungi in citrus carpoplane and 6.51 % - 7.97 % in grape carpoplane. It was represented by *C. catenulata* in citrus carpoplane and by *C. prunicola* in grape carpoplane.

In fresh fruit juice, it was recovered in high or moderate frequency in grape juice and in moderate frequency on both media in citrus juice. It contributed 10.30 % - 11.68 % of total fungi in citrus juice and 6.51 % - 7.97 % in grape juice. It was represented by *C. catenulata* in citrus juice and by *C. prunicola* in grape juice. *C. prunicola* was first described from exuded gum of a black cherry (Prunus serotina Ehrh.) tree, growing in Peoria, IL, USA (Kurtzman 2001).

Five *Candida* species were recovered from different sites of soil in Zagazig area, Egypt (El-Sherbeny 1987). *C. parapsilosis* and *Candida* spp. were also isolated from soil in the Brazilian Amazon Basin (Mok *et al.* 1984). *Candida* sp. was isolated from the phyllosphere of *Bauhinia forficate*, *Tabebuia* sp. and *Terminala catappa*, southeastern Brazil (Valarini *et al.*, 2007). *Candida* sp. was presented in the air, soil and phyllosphere of tea plantation areas of Barak Valley, Assam, India (Dutta *et al.* 2010). *Candida* was the genus

most frequently found in different angiosperm fruits in southeastern Brazil (Prada and Pagnocca 1997), and certain varieties of grapes in southern Spain (De la Torre *et al.* 1999).

Candida has often also been reported as spoilage-causing organism in citrus juices (Hays 1951, Grawmlich *et al.* 1986, Parish and Higgins 1989, Teller and Parish 1992).

It was frequently isolated from pasteurized fruit juices in Venezuela (Mendoza *et al.* 1982). *C. parapsilosis* was the dominant species in citrus juices (Hatcher *et al.* 2000), in fresh passion juice, Uganda (Ismail 2006), and pasteurized and subsequently recontaminated single-strength orange juice, Florida (Arias *et al.* 2002).

C. parapsilosis is occasionally involved as an opportunist in systemic mycoses (de Hoog *et al.* 2000), particularly in patients with impaired natural immunity due to leukemia (Martino *et al.* 1993, Girmenia *et al.* 1996). It was also reported from olive in Italy, bladder in Denmark, udder in cow with subclinical mastitis in New Zealand, infected and healthy skin, sputum in Norway, infected nail in Austin and Italy (Barnett *et al.* 2000). *C. catenulata* was mentioned as one of the fungi occurring in cancer patients (Smolyanskaya *et al.* 1996, Radosavljevic *et al.* 1999), in onychomycosis (Crozier and Coats 1977), in faeces of man with dysentery, gut of chicken, cheese and sputum (Barnett *et al.* 2000).

Strains tested:

***Candida catenulata* Diddens & Lodder**

AUMC 7756, AUMC 7760 (Plate 4)

***Candida parapsilosis* (Ashford) Langeron & Talice**

AUMC 7750 (Plate 5)

***Candida prunicola* Kurtzman**

AUMC 7767 (Plate 6), AUMC 7768

Debaryomyces Lodder & Kreger-van Rij

The genus *Debaryomyces* was recovered infrequently from different sources on both plantations while it was missed in grapevine soil, citrus phyllosphere, the phylloplane of both plants, and grapevine carpoplane. Its highest percentage count was recorded from citrus carpoplane (4.65 % - 4.89 % of total fungi). *D. hansenii* and *D. pseudopolymorphus* were recovered from both plantations.

In the air, it was recorded in citrus air in moderate or low frequency while in low or rare frequency in grapevine air, contributing minute percentage counts (0.11 % - 0.19 % of

total fungi) in citrus air and (0.04 % - 0.07 %) in grapevine air. *D. hansenii* was recovered in low frequency on both media in citrus air and in rare frequency in grapevine air. *D. pseudopolymorphus* was recorded in rare frequency in the air of both plantations.

Debaryomyces hansenii was isolated from the air of El-Minia city, Egypt (Haridy 1992).

In the soil, it was recorded in low or rare frequency in citrus soil contributing small percentage counts (0.24 % - 0.38 % of total fungi). *D. pseudopolymorphus* was recovered in low or rare frequency and *D. hansenii* was recovered in rare frequency on both media. It was not recorded from grapevine soil.

Debaryomyces hansenii was isolated from soil of cultivated wheat field and a garden at the Karachi University campus, Pakistan (Mushtaq *et al.* 2004), soil in Zagazig area, Egypt (El-Sherbeny 1987) and soil in South Victoria Land, Antarctica (Connell *et al.* 2008).

In the phyllosphere, it was recovered in rare frequency from grapevine on DRBC only, represented by *D. hansenii* while it was missed citrus phyllosphere.

Debaryomyces hansenii has been isolated frequently from leaves in the arid climate of the Canary Islands (Middelhoven 1997), and sugarcane leaves in Rio de Janeiro, Brazil (Azeredo *et al.* 1998).

In the phylloplane, it was not encountered in both plants.

In the carposphere, it was recovered in rare frequency from citrus carposphere on both media while it was recovered in rare frequency on DYM from grape carposphere. It contributed 0.09 % - 0.60 % of total fungi in citrus carposphere and 0.02 % in grape carposphere. *D. pseudopolymorphus* was recorded in rare frequency in the carposphere of both plants while *D. hansenii* in rare frequency on both media from citrus carposphere only.

Debaryomyces polymorphus was the most common yeast species found in fruit salads including cantaloupe, citrus fruits, honeydew, pineapple, cut strawberries and mixed fruit salads, Washington (Tournas *et al.* 2006).

In the carpoplane, it was recovered in low frequency in citrus carpoplane on both media contributed 4.65 % - 4.89 % of total fungi while it was missed in grape carpoplane. *D. pseudopolymorphus* was recorded in low or rare frequency constituting 4.04 % - 4.52 % of total fungi while *D. hansenii* in rare frequency on both media.

In the fresh juice, it was recovered in moderate frequency from citrus juice on both media and in low frequency on DRBC only in grape juice, contributing 0.76 % - 2.29 % of total fungi in citrus juice and 0.001 % on DRBC in grape juice. It was represented by *D. pseudopolymorphus* in both plants.

D. hansenii was recorded in rennet in New Zeeland, skin scales from case of psoriasis, sausage, fermenting Kentucky and Maryland tobacco in Italy, infected hand in Hungary, miso in Japan, cheese in Czechoslovakia and Russia, grape juice, skin lesion, throat of angina patient, case of periostitis, salt, beaf, horse-meat, pasty, beaf and pork, sausage and horse-meat sausae in france, atmosphere, tomato puree, cheese, brine bah in cheese factory, salami, salted beans, spoiled pickled, cucumbers, nail of corpse, tobacco and refuse in the Neherlands, persistent case of furunculosis and tobacco in UK, film on pickling prines in USA, sausage in Belgium, child beaf in Australia, atmosphere, sake-moto and takuan salted pickle in Japan, gut of rainbow trout *Salmo gairnerii* in Sweeden, cherries, beaf sausage, salt pork, infected nail, muchroom (Barnett *et al.* 2000). *D. pseudopolymorphus* was previously recorded in tanning fluid prepared from park of sweet chestnut trees in France (refer to Barnett *et al.* 2000).

Strains tested:

***Debaryomyces hansenii* (Zopf) Lodder & Kreger-van Rij**

AUMC 7751 (Plate 7), AUMC 7241 (Plate 8).

***Debaryomyces pseudopolymorphus* (C. Ramirez & Boidin) C. W. Price & Phaff**

= *Schwanniomyces pseudopolymorphus* (C. Ramírez & Boidin) M. Suzuki & Kurtzman

AUMC 7752 (Plate 9).

Geotrichum Link

The genus *Geotrichum* was recovered infrequently from different sources in citrus plantations only. Its highest percentage count was recorded from citrus carpoplane (0.61 % - 0.94 % of total fungi) followed by juice (0.46 % - 0.93 %). *G. candidum*, *G. citri-aurantii* and *Geotrichum* sp. were recorded from all sources in citrus plantations.

In the air, it was recovered from citrus air in rare frequency on both media constituting minute percentage counts (0.02 % of total fungi). It was represented by *G. candidum* and *G. citri-aurantii*.

In the soil, it was encountered in citrus soil in low or rare frequency constituting 0.05 % - 0.12 % of total fungi. *G. candidum* and *Geotrichum* sp. were recorded on DYM and *G. citri-aurantii* was recoverd on DRBC.

In the phyllosphere, it was identified in citrus phyllosphere in rare frequency on both media yielding less percentage counts (0.03 % - 0.06 % of total fungi) than those in the

phylloplane (0.25 % - 0.33 %). It was represented by *G. citri-aurantii* in both phyllosphere and phylloplane.

Geotrichum candidum was isolated from plum leaves, southwest Slovakia (Slavikova *et al.* 2009).

In the carposphere, it was recorded in citrus in rare frequency carposphere on both media contributing also less percentage counts (0.04 % - 0.09 % of total fungi) than those in carpoplane (0.61 % - 0.94 %). *G. citri-aurantii* was recorded only in both carposphere and carpoplane.

In the fresh fruit juice, it was recovered in moderate or low frequency in citrus juice contributing 0.46 % - 0.93 % of total fungi. Only *G. citri-aurantii* was recorded from citrus juice.

Geotrichum spp. were present in 40 % of the grapefruit juice in Washington (Tournas *et al.* 2006). *Geotrichum citri-aurantii* was isolated from pasteurized and subsequently recontaminated single-strength orange juice, Florida (Arias *et al.* 2002). *G. candidum* was reported to cause human disorders represented by colonization of intestinal tract (Vasei and Imanieh 1999) and bronchial or pulmonary infections (Rhyan *et al.* 1990). *G. candidum* was also found in milk, cheese, plants, fruis, soil, insects, man and other mammals (Barnett *et al.* 2000).

Strains tested:

***Geotrichun candidum* Link**

***Geotrichun citri-aurantii* (Ferrairis) E. E. Butler**

AUMC 7247, AUMC 7754 (Plate 10).

***Geotrichum* sp.**

AUMC 7749 (Plate 11).

Hanseniaspora Berkh.

This genus was represented by *H. occidentalis* only. It was recorded infrequently from citrus air, soil and carposphere, and carpoplane, and juice of both plants. Its highest percentage count was recorded from grapevine carposphere (6.87 % - 9.21 % of total fungi) followed by citrus carpoplane (3.39 % - 3.64 %).

In the air, it was recovered in citrus air in rare frequency on both media constituting minute percentage counts (0.02 % - 0.23 % of total fungi). It was missed in grapevine air.

In the soil, it was identified in citrus soil in rare frequency on DRBC only constituting 0.04 % of total fungi while it was absent in grapevine soil.

In the carposphere, it was recovered in low frequency in grape carposphere on both media while it was recovered in rare frequency in citrus carposphere. It contributed 1.18 % - 2.33 % of total fungi in citrus carposphere and 6.87 % - 9.21 % in grape carposphere.

Hanseniaspora species (anamorph *Kloeckera*) are common yeast constituents on grapes (Phister *et al.* 2007), on the surface of ripe grapes (Prakitchaiwattana *et al.* 2004), on grapes and musts in Europe (Bioletti and Cruess 1912). The apiculate yeast *H. uvarum* is also often associated with plants and fruits and is the usual resident species of yeasts, regardless of the cluster sector or the ripeage (Phaff and Starmer 1987).

In the carpoplane, it was isolated in rare frequency from both fruits on both media constituting 3.39 % - 3.64 % of total fungi in citrus carpoplane and 1.10 % - 2.07 % in grape carpoplane.

Hanseniaspora was a common genus found in different angiosperm fruits in southeastern Brazil (Prada and Pagnocca 1997). *Hanseniaspora uvarum* was the main yeast species observed on the pineapple fruit skins in two different areas of both Thailand and Australia (Chanprasartsuk *et al.* 2010).

In the fresh juice, it was identified in moderate frequency from both fruits on both media contributing 0.64 % - 1.42 % of total fungi in citrus juice and 0.23 % - 0.49 % in grape juice.

Hanseniaspora was commonly found in citrus juices (Hatcher *et al.* 2000). *Hanseniaspora uvarum* was the main yeast species observed on the pineapple fresh juice in two different areas of both Thailand and Australia (Chanprasartsuk *et al.* 2010). *Hanseniaspora occidentalis* and *H. uvarum* were isolated from orange juice, Florida (Arias *et al.* 2002). *H. occidentalis* is reported from rotten persimmon *Diospyros* sp. in China, pollen carried y wild bees in Brazil, rumen contents in Germany, skin, baker's yeast in Ialy, bread, diseased caterpillar, case of chronic bronchitis, moist barley in UK, chicken feed in Guatemala, oily detritus and banana in Japan, silage in USA, sputum in the Netherlands (Barnett *et al.* 2000).

Strains tested:

***Hanseniaspora occidentalis* M. T. Smith**

AUMC 7254, AUMC 7758 (Plate 12)

Table 12. Physiological comparison of the strains tested of the Ascomyceteous genera *Ambrosiozyma, Aureobasidium, Candida, Debaryomyces* and *Geotrichum*: **1** *Ambrosiozyma platypodis* AUMC 7233, **2** *Aureobasidium* sp. AUMC 7757, **3** *Candida catenulata* AUMC 7756, **4** *C. catenulata* AUMC 7760, **5** *C. cparapsilosis* AUMC 7750, **6** *C. prunicola* AUMC 7767, **7** *C. prunicola* AUMC 7768, **8** *Debaryomyces hansenii* AUMC 7241, **9** *D. Hansenii* AUMC 7751**,** **10** *D. pseudopolymorphus* AUMC 7752, **11** *Geotrichun citri-aurantii* AUMC 7247, **12** *G. citri-aurantii* AUMC 7754, **13** *Geotrichun* sp. AUMC 7749.

Species no.	**S**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Fermentation														
D- glucose	F1	-	-	+	d	-	-	-	-	-	-	-	-	-
D-galactose	F2	-	-	+	-	-	-	-	-	-	-	-	-	-
Maltose	F3	-	-	+	-	-	-	-	-	-	-	-	-	-
Me-α-D glucoside	F4	-	-	-	-	-	-	-	-	-	-	-	-	-
Sucrose	F5	-	-	+	-	-	-	-	-	-	-	-	-	-
α-α Trehalose	F6	-	-	-	-	-	-	-	-	-	-	-	-	-
Melibiose	F7	-	-	d	-	-	-	-	-	-	-	-	-	-
Lactose	F8	-	-	+	-	-	-	-	-	-	-	-	-	-
Cellobiose	F9	-	-	d	-	-	-	-	-	-	-	-	-	-
Melezitose	F10	-	-	-	-	-	-	-	-	-	-	-	-	-
Raffinose	F11	-	-	+	-	-	-	-	-	-	-	-	-	-
Inulin	F12	-	-	-	-	-	-	-	-	-	-	-	-	-
Starch	F13	-	-	-	-	-	-	-	-	-	-	-	-	-
D-xylose	F14	-	-	-	-	-	-	-	-	-	-	-	-	-
Assimilation														
D-glucose	C1	+	+	+	+	+	+	+	+	+	+	+	+	+
D-galactose	C2	+	+	+	+	+	+	+	-	+	+	+	+	+
L-sorbose	C3	d	d	- w	-w	+	d	d	+	+	+	+	+	+
D-ribose	C5	d	d	+	d	-	d	w	d	+	+	-	-	d
D-xylose	C6	+	+	+	+	+	+	+	+	+	+	+	+	+
L-arabinose	C7	+	+	+	-	+	-	-	+	+	+	-	-	d
L-rhamnose	C9	+	+	+	-	-	-	-	+	+	+	-	-	d
Sucrose	C10	+	+	+	-	+	-	d	+	+	+	d	-	+
Maltose	C11	+	+	+	+	+	+	+	+	+	+	+	+	+
α, α-trehalose	C12	+	+	+	+	+	+	+	+	+	+	-	-	d
Methyl-α-D-glucoside	C13	+	+	-	-	+	-	-	+	+	+	-	-	+

Species no.	**S**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Cellobiose	C14	+	+	-	-	-	-	-	+	+	+	-	-	+
Salicin	C15				-	-								
Arbutin	C16				-	-								
Lactose	C18	-	d	+	+	-	-	-	-	+	+	-	-	-
Raffinose	C19	d	d	+	-	-	-	-	+	+	+	-	-	+
Melezitose	C20	+	+	-	-	+	-	-	+	+	+	-	-	+
Inulin	C21	d	-	-	+	-	-	-	+	d	d	+	-	d
Soluble starch	C22	-	+	+	-	-	-	-	-	+	+	-	-	+
Glycerol	C23	+			+	+	+				+			
Meso-erythritol	C24	+	+	-	-	d	-	-	d	+	+	-	-	+
Xylitol	C26				d	d					+			
D-glucitol	C28	+	+	+	+	+	+	+	+	+	+	+	+	+
D-mannitol	C29	+	+	+	+	+	+	+	+	+	+	+	d	+
Galactitol	C30	d	-	-	-	-	-	-	d	d	+	-	-	d
Myo-inositol	C31	d	+	-	-	-	-	-	-	-	-	-	-	-
Glucono-d-lactone	C32	+	+	+	d	+	+	+	d	+	+	d	d	+
D-glucuronate	C36	d	+	+	-	-	-	-	-	+	-	-	-	d
D-galacturonate	C37	+	d	-	-	-	w	-	w	w	-	+	+	+
Succinate	C39				+	+					+			
Citrate	C40	+	+		+	+	+	+	w	+	+	+	+	+
Methanol	C41	w	-	-	d	w	-	-	w	-	w	-	-	-
Ethanol	C42	+	d	+	+	+	+	+	+	+	+	+	+	+
Propane 1,2 diol	C43	-	d		-	-	-	-	-	-	-	-	-	-
Butane 2,3 diol	C44	-	d		+	-	-	-	d	-	-	+	+	+
Quinic acid	C45	-	+	-	-	-	-	-	+	+	+	-	-	+
Nitrogen compounds														
Nitrate	N1	+	+	-	-	-	-	-	-	-	-	-	-	-
Nitrite	N2	+	+	-	-	-	-	-	-	-	+	-	-	-
Ethylamine	N3	+	w	-	-	+	+	+	+	+	+	+	+	+
L-lysine	N4	+	-	+	+	+	+	+	+	+	+	+	+	+
Creatine	N6	-	-	-	-	-	-	-	+	+	-	-	-	+
Creatinine	N7	-	-	-	-	-	-	-	-	+	-	-	-	+
D-glucosamine	N8	+	-	-	-	-	-	-	+	-	+	-	w	-

Species no.	**S**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Imidazole	N9	+	-	-	-	-	-	-	-	-	-	-	-	-
D-tryptophane	N10	+	w	-	-	-	-	-	-	-	-	-	-	+
Miscellaneous														
0.01 % cycloheximide	O1	+	d	-	+	-	-	-	+	+	+	+	+	+
0.1 % cycloheximide	O2	+	d	-	+	-	-	-	+	+	+	+	+	+
50 % D-glucose	O4	+	-	+	+	+	+	+	+	+	+	-	-	+
60 % D-glucose	O5	-	-	-	-	+	+	+	-	+	+	-	-	+
10 % NaCl	O6	+	-	+	+	+	-	+	+	+	+	-	-	+
16 % NaCl	O7	-	-	-	-	-	-	-	-	+	-	-	-	-
Starch formation	M1	-	+	-	-	-	-	-	-	-	-	-	-	-
Urea hydrolysis	M3	-	+	-	-	-	-	-	-	-	-	-	-	-
Diazonium blue B	M4	-	-	-	-	-	-	-	-	-	-	-	-	-
Growth at 30°C	T2	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth at 37°C	T4	+	+	+	+	+	+	+	+	+	-	-	-	+
Growth at 42°C	T6	+	+	-	-	+	-	-	+	-	-	-	-	-
Growth at 45°C	T7	-	-	-	-	-	-	-	-	-	-	-	-	-
Pink colony	E1	-	-	-	-	-	-	-	-	-	-	-	-	-
Budding	E2	+	+	+	+	+	+	+	+	+	+	-	-	-
Lemon-shaped cells	E3	-	-	-	-	-	-	-	-	-	-	-	-	-
Budding on stalk	E4	-	-	-	-	-	-	-	-	-	-	-	-	-
Splitting cells	E5	-	-	-	-	-	-	-	-	-	-	+	-	+
Filamentous	E6	+	+	-	+	-	-	-	-	-	-	+	+	+
Pseudohyphae	E7	-	-	+	-	+	-	-	+	+	-	-	-	-
Septate hyphae	E8	-	-	+	-	+	-	-	-	-	-	-	+	-
Arthroconidia	E9	-	-	-	-	-	-	-	-	-	-	+	+	+
Ballistoconidia	E10	-	-	-	-	-	-	-	-	-	-	-	-	-
Ascosporogenus	A1	-	-	-	-	-	-	-	-	-	+	-	-	-
Ascospores round	A2	-	-	-	-	-	-	-	-	-	+	-	-	-

+: growth; w: weak growth; d, delayed, -: no growth, gap: not tested.

Fermentation of Me-α-D glucoside, α, α Trehalose, melezitoze, Starch and D-xylose gave negative results with all species tested and were omitted from the table.

Issatchenkia Kudriavzev

This genus was exemplified by *I. orientalis* only. It was isolated infrequently from all sources on both plantations but it was missing in citrus soil. Its highest percentage count was recorded from grape juice (18.91 % - 71.41 % of total fungi) and citrus juice (26.60 % - 30.75 %). followed by citrus carposphere (23.01 % - 26.48 %).

In the air, it was recorded in rare frequency in citrus plantations on both media and on DYM only in grapevine air, contributing 0.03 % - 0.04 % of total fungi in citrus air and 0.01 % on DYM in grapevine air.

In the soil, it was isolated in grapevine in rare frequency on DRBC contributing 0.03 % of total fungi. It was not recorded from citrus soil.

In the phyllosphere, it was recovered in rare frequency from both plantations on both media, contributing 0.004 % - 0.02 % of total fungi in citrus phyllosphere and 0.01 % - 0.16 % in grapevine phyllosphere.

In the phylloplane, it was recorded in rare frequency in citrus phylloplane on DRBC, contributing 0.04 % of total fungi. It was missed in grapevine phylloplane.

In the carposphere, it was recovered in low frequency from both plants on both media. Its percentage counts in citrus carposphere (23.01 % - 26.48 % of total fungi) noticeably exceeded those in grape carposphere (2.29 % - 2.94 %).

Issatchenkia orientalis was the most frequent species recorded in *Parahancornia amapa* fruits in the Mocambo Forest, Salvaterra (Morais *et al.* 1995).

In the carpoplane, it was recovered in low frequency in citrus carpoplane on both media and in low or rare frequency in grape carpoplane, contributing 9.42 % - 9.49 % of total fungi respectively in citrus carpoplane and 10.95 % - 12.23 % in grape carpoplane. It yielded lower percentage counts in citrus carpoplane than those in citrus carposphere while its counts in grape carpoplane surpassed those in grape carposphere.

Issachenkia orientalis was isolated from Thai fruits and vegetables, Thailand (Chanchaichaovivat *et al.* 2007).

In the fresh juice, it was recovered in high or moderate frequency in grape juice and in moderate frequency on both media in citrus juice. It contributed 26.60 % - 30.75 % of total fungi in citrus juice and 18.91 % - 71.41 % in grape juice.

Issatchenkia orientalis was isolated from pasteurized and subsequently recontaminated single-strength orange juice, Florida (Arias *et al.* 2002).

Candida krusei (anamorph of *I. orientalis*) was occasionally involved in fatal systematic candidiasis, usually in patients with impaired innate immunity (Gordon *et al.* 1980, Wingard *et al.* 1991, Iwen *et al.* 1995). *I. orientalis* was reported also from soil, cabbage refuse, domestic sewage and homare miso in Japan, yoghurt, sputum in Italy and Sri Lanka, fermentation vat in citric acid factory in Poland, ginger beer in West Africa, fermenting cacao in Ghana and West Indias, the atmosphere, film on pickles in USA, silage in UK, faeces of man in Brazil, contaminant of industrial fermentation in Hungary, fermenting extract of fruit of tamarind *Tamarindus indica*, pus from infected fingernail of woman in Argentina, baker's yeast in Finland, t-beer fungus, fruit juice, baker's yeast, beer wort (Barnett *et al.* 2000).

Strains tested:

***Issatchenkia orientalis* Kudryavtsev (anamorph: *Candida krusei*)**

AUMC 7765, AUMC 7766, AUMC 7769 (Plate 13), AUMC 7770

Klyuveromyces Van der Walt

This genus was represented by *K. marxianus* only. It was recorded infrequently from citrus soil, the phyllosphere of both plantations, citrus phylloplane, and grape carposphere only. Its highest percentage count was gained from citrus phylloplane (0.35 % - 1.97 % of total fungi).

In the soil, it was recorded in citrus soil in rare frequency on DYM contributing 0.02 % of total fungi. It was absent from grapevine soil.

Kluyveromyces marxianus was the dominant species in soil under potato, maize, and cabbage plants in El-Minia city, Egypt (Haridy 2002).

In the phyllosphere, it was recovered in low or rare frequency in grapevine soil and in rare frequency on both media in citrus phyllosphere, contributing 0.008 % - 0.02 % of total fungi in citrus phyllosphere and 0.10 % - 0.27 % in grapevine phyllosphere.

In the phylloplane, it was recorded in rare frequency in citrus phylloplane on both media, contributing 0.35 % - 1.97 % of total fungi. It was missed in grapevine phylloplane. *Kluyveromyces* was found in elm phylloplane in California (Phaff and Starmer 1987).

In the carposphere, it was recovered in rare frequency from grape carposphere on both media constituting 0.01 % - 0.06 % of total fungi. It was not identified in citrus carposphere, and the carpoplane and juice of both plants.

Klyuveromyces marxianus was isolated from soft apples, grapes, dates, and strawberries, El-Minia city, Egypt (Haridy 1994), from olive fruits and brines during fermentation process (Hernández *et al.* 2007).

Candida kefyr (anamorph of *K. marxianus*) was occasionally involved in superficial candidiasis (Hernandez-Molina *et al.* 1994), and was described from a cardiac transplant patient with pulmonary infection (Lutwick *et al.* 1980). *K. marxianus* was reported also from buttermilk, yoghurt, pressed yeast, kefyr grain, leaking tin of apples, atmosphere, cow, sputum, brine bath in cheese factory in the Netherlands, Danish dry yeast, lungs of tuberculosis patieit, lesion on tonsils, yogurt, effluent of sugar refinery, cheese in Italy, stomach of lion cub in France, Bantu beer and soil in South Africa. pozol (fermented maize dough) in Mexico, kummis in Estonia, yeasty cream and dairy products in USA, bronchitic patient in Sri Lanka, infected nail and lung in Austria, cheese in Czechoslovakia, post-mortem material from German woman, bovine mastitis in Norway, milk of mast cow in Yugoslavia, fermenting figs, rotting sisal leaf *Agave arigida* var. *sisalana* in Tanganyika, sewage slick in Forth estuary, milk of mastitic Cow in UK (Barnett *et al.* 2000).

Strain tested

***Klyuveromyces marxianus* (E. C. Hansen) van der Walt (anamorph: *Candida kefyr*)**

AUMC 7759 (Plates 14 & 15)

Kodemaea Y. Yamada, Tom. Suzuki, M. Matsuda & Mikata

This genus was represented by *K. ohmeri* only. It was isolated in rare frequency from citrus carposphere and carpoplane. It contributed 0.01 % - 0.02 % of total fungi in citrus carposphere and 0.20 % - 0.38 % in citrus carpoplane. *K. ohmeri* was previously reported from sambal-ulak (Indonesian fermented chilli peppers), film on 5% brine and salted cucumber in USA, pleural fluid from patient in Java, torani, jooseberry jelly, figs or dates (Barnett *et al.* 2000).

Strains tested

Kodamaea ohmeri* (Etchells & Bell) Y. Yamada *et al.

AUMC 7748 (Plate 16), AUMC 7764

Pichia E. C. Hansen

The genus *Pichia* was recovered infrequently from most sources in both plantations while it was missed in citrus phyllosphere, the phylloplane of both plants, and grape carpoplane and juice. Its highest percentage count was recorded from citrus juice (56.42 % - 57.01 % of total fungi) followed by citrus carpoplane (1.82 % - 4.52 %). Three species were collected, *P. fermentans* and *P. guilliermondii* from both plantations and *P. carribbica* and *P. farinose* from citrus plantations only.

In the air, it was recovered in low frequency in grapevine air on both media and in rare frequency in citrus air. It contributed 0.07 % - 0.17 % of total fungi in citrus air and 0.17 % - 0.57 % in grapevine air. It was represented by *P. guilliermondii* in the air of both plantations and *P. farinose* from citrus plantations only. *P. farinosa* was repored earlier from beer in Poland, miso, mash of rice vinegar (koji), sake and dung of giraffe Giraffa camelopardalis in Japan, maize meal in South Africa, cow with mastitis in Switzerland, sputum in Norway, soy sauce in China, fermenting cacao in Trinidad, sorbitol solutions in South Africa and Germany (refer to Barnett *et al.* 2000).

In the soil, it was encountered in rare frequency from grapevine soil on both media, represented by *P. guilliermondii*, and in rare frequency on DRBC only represented by *P. carribbica*. It contributed 0.07 % of total fungi on each medium in grapevine soil and 0.04 % on DRBC in citrus soil.

Pichia guilliermondii (= teleomorph of *Candida guilliermondii*) was isolated from soil in the Brazilian Amazon Basin (Mok *et al.* 1984). *P. caribaea* was found in soils in China (Barnett *et al.* 1983).

In the phyllosphere, it was recovered in low or rare frequency in grapevine phyllosphere, represented by *P. guilliermondii*, contributing 0.01 % - 0.04 % of total fungi. It was missed in citrus phyllosphere and the phylloplane of both plantations.

Pichia guillermondii was isolated from the phyllosphere of *Bauhinia forficate*, *Tabebuia* sp. and *Terminala catappa* in southeastern Brazil (Valarini *et al.* 2007), apple, plum, and peach leaves in southwest Slovakia (Slavikova *et al.* 2009).

In the carposphere, it was isolated in low frequency from both fruits on one medium and rare frequency on the other. It contributed 0.53 % - 0.57 % of total fungi in citrus carposphere and 0.05 % - 0.39 % in grape carposphere. *P. fermentans* was recovered in rare frequency on both media in the carposphere of both plants while *P. carribbica* was

isolated in rare frequency from citrus carposphere and *P. guilliermondii* from grape carposphere only.

Pichia spp. were the most common yeasts found in fruit salads including cantaloupe, citrus fruits, honeydew, pineapple, cut strawberries and mixed fruit salads in Washington (Tournas *et al.* 2006), different angiosperm fruits in southeastern Brazil (Prada and Pagnocca 1997). *Pichia guilliermondii* was the most frequent species isolated from fruits of *Anacardium giganteum* at the Mocambo Forest, Salvaterra (Morais *et al.* 1995). It was isolated from soft apricot fruits, El-Minia city, Egypt (Haridy 1994). *P. caribaea* was the predominant species in Arbequina olive varieties from Castilla La Mancha region, Spain (Romo-Sánchez *et al.* 2010).

In the carpoplane, it was recovered in rare frequency from citrus fruit on both media represented by *P. fermentans* yielding more percentage counts than those in citrus carposphere (1.82 % - 4.52 % of total fungi), while it was missed in grape carpoplane.

Pichia guilliermondii was the main yeast species observed on the pineapple fruit skins in two different areas of both Thailand and Australia (Chanprasartsuk *et al.* 2010) and Thai fruits and vegetables in Thailand (Chanchaichaovivat *et al.* 2007).

In the fresh juice, it was isolated in high or moderate frequency from citrus juice contributing 56.42 % - 57.01 % of total fungi. *P. fermentans* was the extremely dominant (56.26 % - 57.01 % of total fungi) in citrus juice while *P. carribbica* was recorded in rare frequency on DRBC. It was missed in grape juice. It was previously reported from rotting, prickly-peer, cacti *Opuntia stricta* and columnar cacti *Cephalocereus royenii* in the west Indias (Barnett *et al.* 2000).

Pichia was frequently isolated from pasteurized fruit juices in Venezuela (Mendoza *et al.* 1982), from citrus juices (Hatcher *et al.* 2000). *Pichia guilliermondii* and *P. fermentans* were the most common yeast species from the fresh sugarcane juice in Brazil (El-Tabey Shehata 1960). *Pichia guilliermondii* was the main yeast species observed in the fresh pineapple juice in two different areas of both Thailand and Australia (Chanprasartsuk *et al.* 2010), and in the orange, apple, lemon, and grapefruit juices in Zagreb, Croatia (Uhitil *et al.* 2009).

Pichia fermentans was isolated from fresh-squeezed single-strength orange juice, Florida (Arias *et al.* 2002), and from orange fruit and juice in a spontaneous fermentation (Las Heras-Vazquez *et al.* 2003). It was also reported from buttermilk, potato flour in the Netherlands, lambic beer in Belgium, cheese in Italy, spoiled orange juice in USA, sputum in Norway, pharynx of goose and rectal contents of swan in France, bear dung in Ursus, arctos

yesoensis in Japan, brewer's yeast in UK, cattle feed in Denmark, kefyr grains (refer to Barnett *et al.* 2000).

Candida guilliermondii (anamorph of *Pichia guilliermondii*) was reported from disseminated cases (Dick *et al.* 1985, Vazquez *et al.* 1995), an osteomyelitis (Tietz *et al.* 1999), and occasionally from cutaneous (Ellis 1994) or subcutaneous (Graham and frost, 1973) infections. It was also reported from insect frass on elm tree, fig wasps in USA, soil in Italy butter milk, lung and canal water in Netherlands, ulcr on horce and kidney of child, grape juice soil seweag in jaban fermented maize dough in Mexico, case of cystitis, air, blood of woman with ulcerated check sputum of bronchial patient (refer to Barnett *et al.* 2000).

Strains tested

Pichia caribbaea* Phaff *et al.

AUMC 7753 (Plate 17)

***Pichia farinosa* (Lindner) E. C. Hansen**

AUMC 7236

***Pichia fermentans* Lodder**

AUMC 7755 (Plate 18)

***Pichia guilliermondii* (anamorph: *Candida guilliermondii*)**

AUMC 7771

Physiological tests

Table 13. Physiological comparison of the strains tested of the Ascomycetous genera *Hanseniaspora, Issatchenkia, Klyuveromyces, Kodemaea* and *Pichia*: **1** *Hanseniaspora occidentalis* AUMC 7254, **2** *H. occidentalis* AUMC 7758, **3** *Issatchenkia orientalis* AUMC 7765, **4** *I. orientalis* AUMC 7766, **5** *I, orientalis* AUMC 7769, **6** *I, orientalis* AUMC 7770, **7** *Klyuveromyces marxianus* AUMC 7759, **8** *Kodemaea ohmeri* AUMC 7748, **9** *K. ohmeri* AUMC 7264**, 10** *Pichia caribbica* AUMC 7753, **11** *P. farinosa* AUMC 7236, **12** *P. fermentans* AUMC 7755, **13** *P. guilliermondii.*AUMC 7771.

Species no.	**S**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Fermentation														
D- glucose	F1	+	+	+	+	+	+	+	+	-	+	w	+	d
D-galactose	F2	-	-	-	-	-	-	+	-	-	d	-	-	-
Maltose	F3	-	-	-	-	-	-	-	-	-	-	-	-	-
Me-α-D glucoside	F4	-	-	-	-	-	-	-	-	-	-	-	-	-
Sucrose	F5	-	-	-	+	-	-	+	+	-	+	-	-	d
α-α Trehalose	F6	-	-	-	d	-	-		-	-	-	-	-	-
Melibiose	F7	-	-	-	-	-	-	-	-	-	-	-	-	-
Lactose	F8	-	-	-	-	-	-	-	-	-	-	-	-	-
Cellobiose	F9	-	-	-	-	-	-	-	-	-	-	-	-	-
Melezitose	F10	-	-	-	-	-	-	-	-	-	-	-	-	-
Raffinose	F11	-	-	-	d	-	-	+	+	-	+	-	-	d
Inulin	F12	-	-	-	-	-	-	+	-	-	d	-	-	-
Starch	F13	-	-	-	-	-	-	-	-	-	-	-	-	-
D-xylose	F14	-	-	-	-	-	-	-	-	-	-	-	-	-
Assimilation														
D-glucose	C1	+	+	+	+	+	+	+	+	+	+	+	+	+
D-galactose	C2	-	-	-	+	-	-	+	+	+	+	+	-	+
L-sorbose	C3	-	-	-	+	-	-	w	+	+	d	-	-	+
D-ribose	C5	-	d	-	d	-	-	+	d	d	+	-	d	+
D-xylose	C6	w	-	d	+	d	d	+	-	-	+	d	d	+
L-arabinose	C7	-	-	-	+	-	-	+	-	-	+	-	-	+
L-rhamnose	C9	-	-	-	+	-	-	-	-	-	d	d	-	+
Sucrose	C10	d	+	d	+	d	+	+	+	+	+	-	-	+
Maltose	C11	+	+	+	+	+	+	+	+	+	+	d	+	+
α, α-trehalose	C12	-	-	-	+	-	-	d	+	+	+	d	-	+

Species no.	**S**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Methyl-α-D-glucoside	C13	-	-	-	+	-	-	-	+	+	+	d	-	+
Cellobiose	C14	+	+	-	+	-	-	d	+	+	+	-	-	+
Salicin	C15	+	+	-	+	d	-	+	+	+	+	d	-	+
Arbutin	C16	+	+	-	+	-	-	+	+	+	+	+	-	+
Lactose	C18	-	-	-	-	-	-	+	-	-	-	-	-	d
Raffinose	C19	-	d	-	+	-	-	+	+	+	+	-	-	+
Melezitose	C20	-	-	-	+	-	-	-	w	w	+	+	-	+
Inulin	C21	+	-	-	+	-	-	+	d	d	+	-	-	+
Soluble starch	C22	+	d	-	-	-	d	+	-	+	d	+	+	+
Glycerol	C23	d	d	+	+	+	+	+	+	+	+	d	+	+
Meso-erythritol	C24	-	-	-	-	-	-	-	-	-	-	+	-	d
Xylitol	C26		-	d	+			+	-		+	d	w	+
D-glucitol	C28	-	-	-	+	-	-	+	+	+	+	+	-	+
D-mannitol	C29	-	-	-	+	-	-	d	+	+	+	+	-	+
Galactitol	C30	-	-w	-	+	-	-	-	-	-	d	-	-	+
Myo-inositol	C31	-	-	-	-	-	+	-	-	-	-	-	-	d
Glucono-d-lactone	C32	+	d	-	+	+	d	w	+	+	d	+	d	d
D-glucuronate	C36	-	-	-	-	-	-	-	-	-	-	-	-	d
D-galacturonate	C37	-	-	-	-	-	-	d	-	-	-	d	-	-
Succinate	C39	-	-	+	+	+	+	d	+	+	+	+	+	+
Citrate	C40	+	-	d	+	+	d	+	+	+	+	-	+	+
Methanol	C41	-	-	-	d	-	-	w	-	-	w	-	-	w
Ethanol	C42	-	+	+	+	+	+	+	+	+	+	+	+	+
Propane 1,2 diol	C43	+	-	-	-	-	-	-	-	-	-	-	-	-
Butane 2,3 diol	C44	+	-	-	-	-	-	+	-	-	d	-	-	-
Quinic acid	C45	-		-	-	-	-	-	-	-	-	-	-	-
Nitrogen compounds														
Nitrate	N1	-	-	-	-	-	-	-	-	-	-	-	-	-
Nitrite	N2	-	-	-	-	-	-	-	-	-	-	-	-	-
Ethylamine	N3	+	w	+	+	+	+	+	+	+	+	+	+	+
L-lysine	N4	+	+	+	+	+	+	+	+	+	+	+	+	+
Creatine	N6	-	-	-	-	-	-	-	-	+	-	-	-	-
Creatinine	N7	-	-	-	-	-	-	-	-	-	-	-	-	-
D-glucosamine	N8	-	-	-	+	-	-	w	-	-	+	-	-	-

Species no.	**S**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Imidazole	N9	-	-	-	-	-	-	-	-	-	-	-	-	-
D-tryptophane	N10	-	-	-	-	-	-	-	-	-	-	-	-	-
Miscellaneous														
0.01% cycloheximide	O1	+	+	-	+	-	-	+	-	d	+	-	-	+
0.1 % cycloheximide	O2	+	+	-	+	-	-	+	-	-	+	-	-	+
50% D-glucose	O4	-	-	-	+	+	-	+	+	+	+	-	+	+
60% D-glucose	O5	-	-	-	-	-	-	-	+	+	+	-	-	-
10% NaCl	O6		-	-	+	-	-	-	-	+	+	-	-	+
16% NaCl	O7		-	-	+	-	-	-	-	-	-	-	-	-
Starch formation	M1		-	-	-	-	-	-	-	-	-	-	-	-
Urea hydrolysis	M3	-	-	-	-	-	-	-	-	-	-	-	-	-
Diazonium blue B	M4	-	-	-	-	-	-	-	-	-	-	-	-	-
Growth at 30°C	T2	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth at 37°C	T4	+	-	+	+	+	+	+	+	+	+	+	+	+
Growth at 42°C	T6	-	-	+	+	+	+	+	+	+	+	-	+	+
Growth at 45°C	T7	-	-	+	+	+	+	+	-	-	-	-	+	-
Pink colony	E1	-	-	-	-	-	-	-	-	-	-	-	-	-
Budding	E2	+	+	+	+	+	+	+	+	+	+	+	+	+
Lemon-shaped cells	E3	+	+	-	-	-	-	-	-	-	-	-	-	-
Budding on stalk	E4	-	-	-	-	-	-	-	-	-	-	-	-	-
Splitting cells	E5	-	-	-	-	-	-	-	-	-	-	-	-	-
Filamentous	E6	-	-	+	+	+	+	-	+	+	-	-	-	-
Pseudohyphae	E7	-	-	-	-	-	-	-	-	-	+	+	+	+
Septate hyphae	E8	-	-	-	-	-	-	-	-	-	-	-	+	-
Arthroconidia	E9	-	-	-	-	-	-	-	-	-	-	-	-	-
Ballistoconidia	E10	-	-	-	-	-	-	-	-	-	-	-	-	-
Ascosporogenus	A1	-	-	-	-	-	-	-	-	-	-	-	-	-
Ascospores round	A2	-	-	-	-	-	-	-	-	-	-	-	-	-

+: growth; w: weak growth; d, delayed, -: no growth, gap: not tested.

Fermentation of Me-α-D glucoside, α, α Trehalose, melezitoze, Starch and D-xylose gave negative results with all species tested and were omitted from the table.

Genotypic identification of ascomyceteous yeasts

Table 14. The Assiut University Mycological Centre accession number (AUMC) of ascomyceteous yeast strains and their isolation sources with their accession GenBank numbers given together with the closest match in the GenBank database and sequence similarity in percent to the match as inferred from Blastn searches of ITS sequences.

AUMC number	Isolation source	Accession number	Length (bp)	Closest Genbank match # ITS	Sequencing similarity (%)	Species	References
7757	Citrus soil	JQ425384	575	HM595555 GQ906942= JW40-2	93 92	*Coniochaeta* sp. M182 *Aureobasidium* sp.	
7257	Citrus leaf	JQ425344	395	GU246267 = CBS 565^{T}	100	*Candida catenulata*	Groenewald & Smith 2010
7261	Citrus soil	JQ425348	407	GU246267 = CBS 565^{T} AJ853765 = WM 6	99 100	*Candida catenulata*	Groenewald & Smith 2010
7760	Citrus leaf	JQ425389	409	GU246267 = CBS 565^{T} AJ853765 = WM 6	99 100	*Candida catenulata*	Groenewald & Smith 2010
7756	Citrus soil	JQ425361	770	GU246267= CBS 565^{T} AJ853765= WM 6	99 100	*Candida catenulata*	Groenewald & Smith 2010
7750	Air of citrus	JQ425354	503	FJ872016 = CBS 604^{T}	100	*Candida parapsilosis*	
7767	Grapevine fruit	JQ083434	432	FM178341 = WM 07.7 EU343809 = CBS 8848 ^{T}	93	*Candida prunicola*	Kurtzman 2001
7768	Grape juice	JQ425355	437	FM178341 = WM 07.7 EU343809 = CBS 8848^{T}	93	*Candida prunicola*	Kurtzman 2001
7749	Citrus soil	JQ083437	636	EF197943 = HK67-4	98	*Debaryomyces hansenii*	
7751	Citrus soil	JQ425358	632	EF643593 = LN-3 EF192227 = w-14-1	100 100	*Debaryomyces hansenii* (Anamorph:*Candida famata*)	

AUMC number	Isolation source	Accession number	Length (bp)	Closest Genbank match # ITS	Sequencing similarity (%)	Species	References
7263	Air of citrus	JQ425353	620	EF197943 = HK67-4 EF190231 = wwl-2 1	100	*Debaryomyces hansenii* (Anamorph*:Candida famata*)	
7264	Air of citrus	JQ425359	635	EF197943= HK67-4 AB220029 = IFM 54258^{T}	100	*Debaryomyces hansenii* *D. nepalensis*	Moretti *et al*. 2007
7260	Citrus soil	JQ425347	628	AJ586524= CBS 2008^{T} EF198011 = WC43-3	100	*Debaryomyces pseudopolymorphus* (=*Schwanniomyces pseudopolymorphus*)	Martorell *et al*. 2005
7752	Citrus soil	JQ425390	625	EF198011 = WC43-3 AJ586524=CBS 2008^{T}	100	*Debaryomyces pseudopolymorphus (=Schwanniomyces pseudopolymorphus)*	Martorell *et al*. 2005
7754	Citrus fruit	JQ083433	374	EU131181 = GcaCC015 AF411060	99	*Geotrichum citri-auriantii*	Arias *et al.* 2002
7758	Citrus soil	JQ425357	750	EU541358 AJ973092= CBS 6783^{T} AJ512429 = CBS 2592^{T}	100 99 97	*Hanseniaspora occidentalis* *Hanseniaspora occidentalis* var. *citrica* *Hanseniaspora occidentalis*	Cadez *et al*. 2003, 2006
7748	Citrus fruit	JQ425350	728	GU246263 = CBS 5367^{T} FJ215865	98	*Kodamaea ohmeri*	Groenewald & Smith 2010
7764	Grape juice	JQ425401	416	EF199745 = szty2w GU246263 = CBS 5367^{T}	99 98	*Kodamaea ohmeri*	Groenewald & Smith 2010
7258	Citrus leaf	JQ425345	700	HQ396523 = CHY 1612 GU256755 = ATCC 60480	100	*Kluyveromyces marxianus*	Kang *et al.* 2010
7259	Citrus soil	JQ425346	725	EF568057 = WM 39=CBS 712 GU256755 = ATCC 60480	100	*Kluyveromyces marxianus*	

AUMC number	Isolation source	Accession number	Length (bp)	Closest Genbank match # ITS	Sequencing similarity (%)	Species	References
7759	Citrus soil	JQ083435	715	HQ396523 = CHY 1612 GU256755 =ATCC 60480	100	*Kluyveromyces marxianus*	Kang *et al.* 2010
7753	Citrus soil	JQ083436	598	HQ909093 = KDLYC36-9 HQ693782 = W63245-01	99 100	*Meyerozyma caribica* (=*Pichia caribicaa)*	Kurtzman & Suzuki 2010, Jensen & Arendrup 2011
7262	Soil of grapevine	JQ425359	864	HQ909093 = KDLYC36-9 HQ693782 = W63245-01	99 100	*Meyerozyma caribbica* (=*Pichia caribbica*)	Kurtzman & Suzuki 2010, Jensen & Arendrup 2011
7771	Air of grapevine	JQ425356	590	EF197816 = EF197814 = HK53 EU568971 = CNRMA200500864	100	*Meyerozyma guilliermondii* (=*Pichia guilliermondii)* (anamorph*:Candida guilliermondii*)	Desnos-Ollivier *et al.* 2008
7765	Grapevine fruit	JQ083432	497	FM199972 = H7S6K11 FM199958 = H4S5K11	98	*Pichia kudriavzevii* (formerly *Issatchenkia orientalis*)	Daniel *et al.* 2009, Hultman *et. al.* 2008
7766	Grapevine fruit	JQ425352	516	FJ515204 = UM5 AY939808= CBS 5147^{T}	96 95	*Pichia kudriavzevii* (*Issatchenkia orientalis*)	Leinberger *et al.* 2005
7769	Grape juice	JQ425351	487	FM199972 = H7S6K11 EU798698 = NN2573	100	*Pichia kudriavzevii (=Issatchenkia orientalis)*	Daniel *et al.* 2009
7770	Grapevine soil	JQ425391	501	FM199972 = H7S6K11 FM199958 = H4S5K11 GU931323 = 5B12	100	*Pichia kudriavzevii* (= *Issatchenkia orientalis*)	Daniel *et al.* 2009

AUMC number	Isolation source	Accession number	Length (bp)	Closest Genbank match # ITS	Sequencing similarity (%)	Species	References
7755	Citrus leaf	JQ425360	518	EU315767 FM199964 =H5MandK14	79	*Issatchenkia terricola Issatchenkia orientalis*	

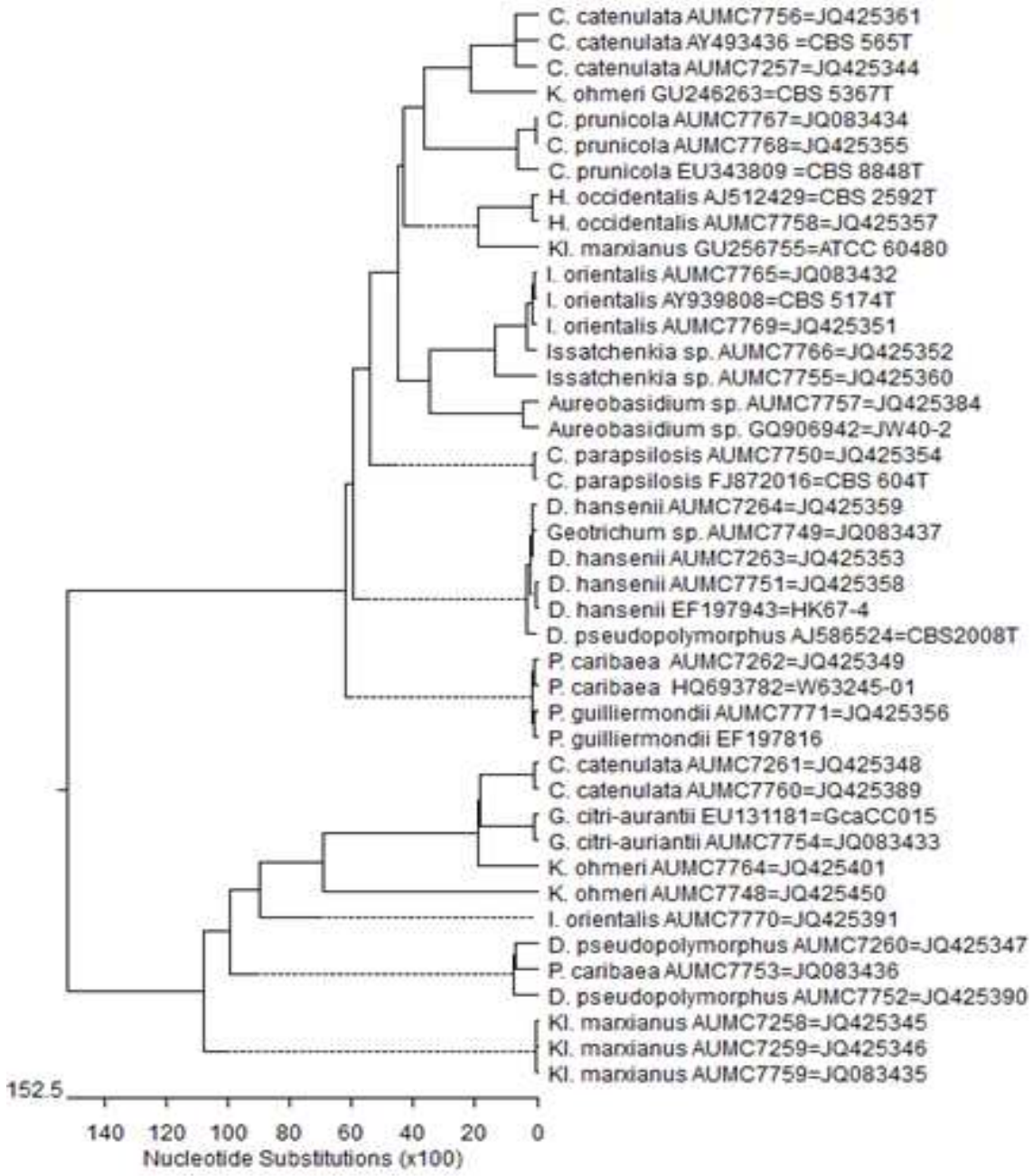

Figure 17. Phylogenetic tree for all ascomyceteous yeast strains (*C. = Candida, D. = Debaryomyces, G. = Geotrichum, H. = Hanseniaspora, I. = Issatchenkia, Kl = Kluyveromyces, K. = Kodamaea, P. = Pichia*). The scale indicates the number of nucleotide substitutions per site.

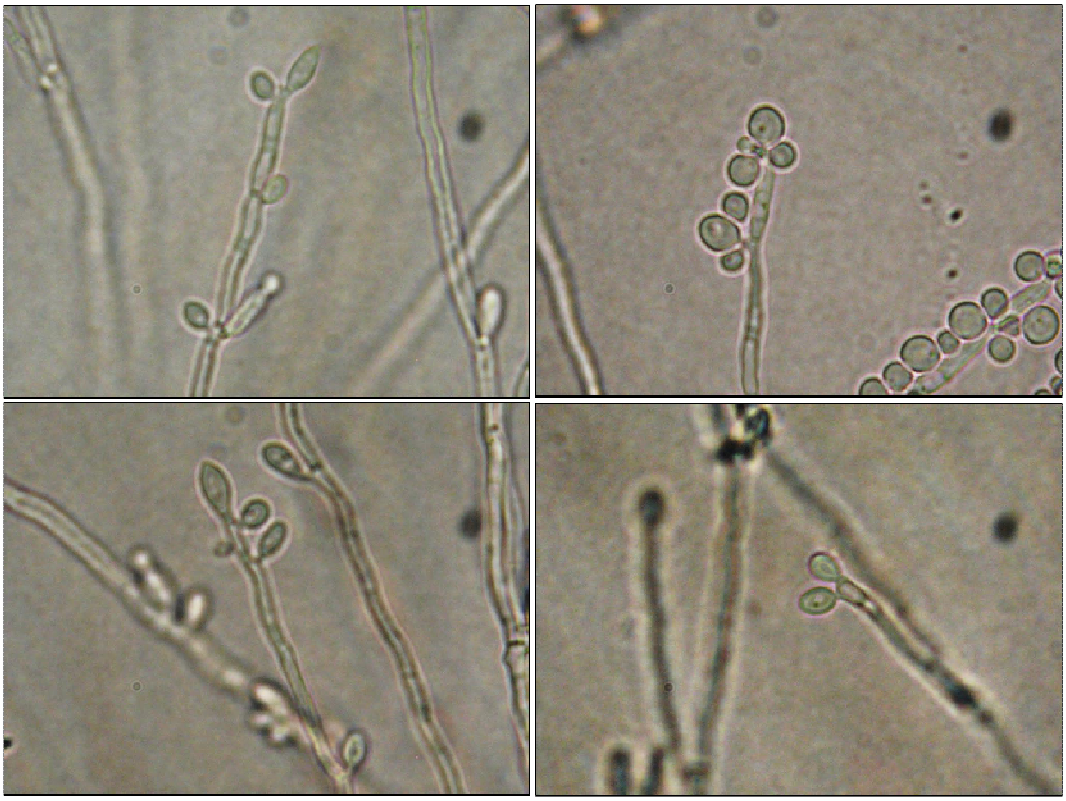

Plate 1. ***Ambrosiozyma platypodis*** AUMC 7233, true mycelium.

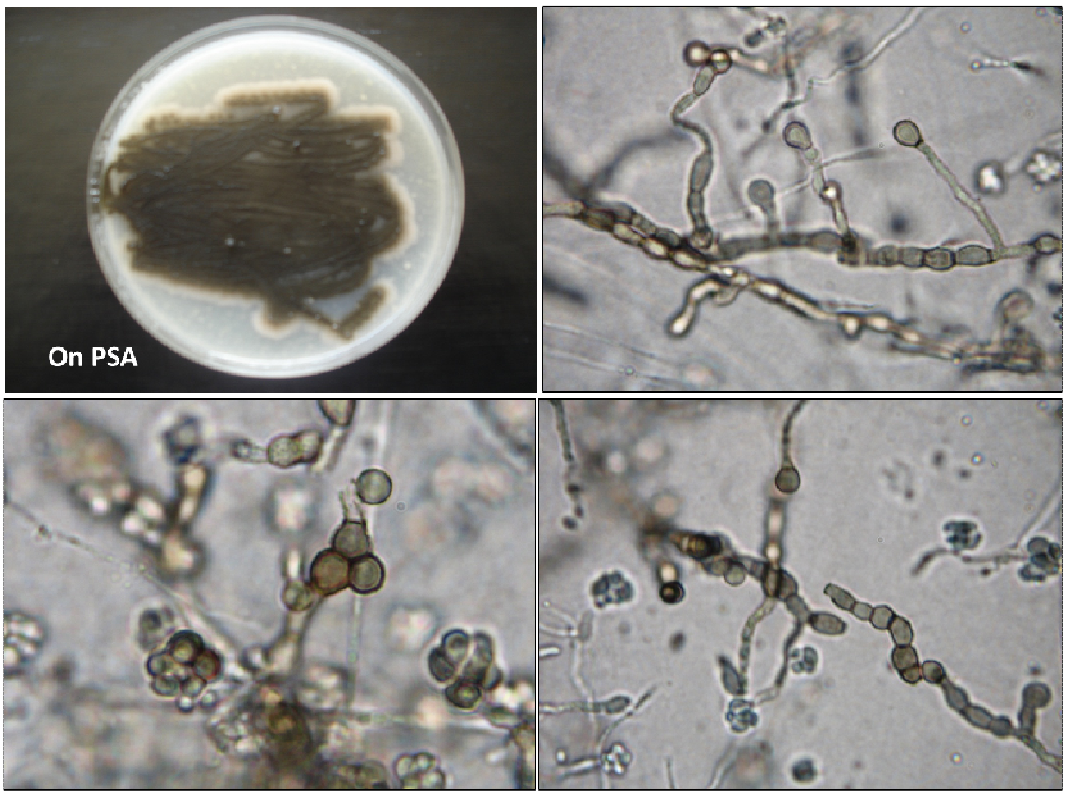

Plate 2. ***Aureobasidium*** **sp.** AUMC 7757: colony and chlamydospores (Chlamydospores dimensions 3-12 µm, Domsch *et al.* 2007).

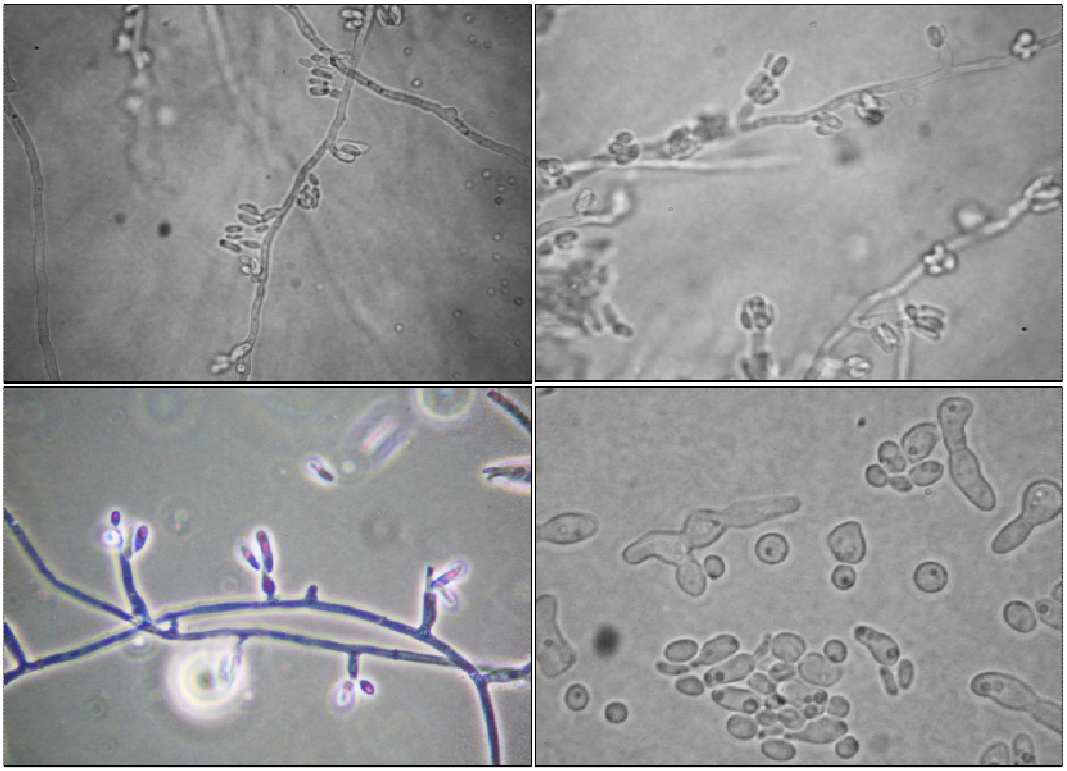

Plate 3. ***Aureobasidium*** **sp**. AUMC 7757: true mycelium and budding cells (Budding cells (7.5-)9-11(-16) x 3.5-)4-5.5(-7) µm, Domsch *et al.* 2007).

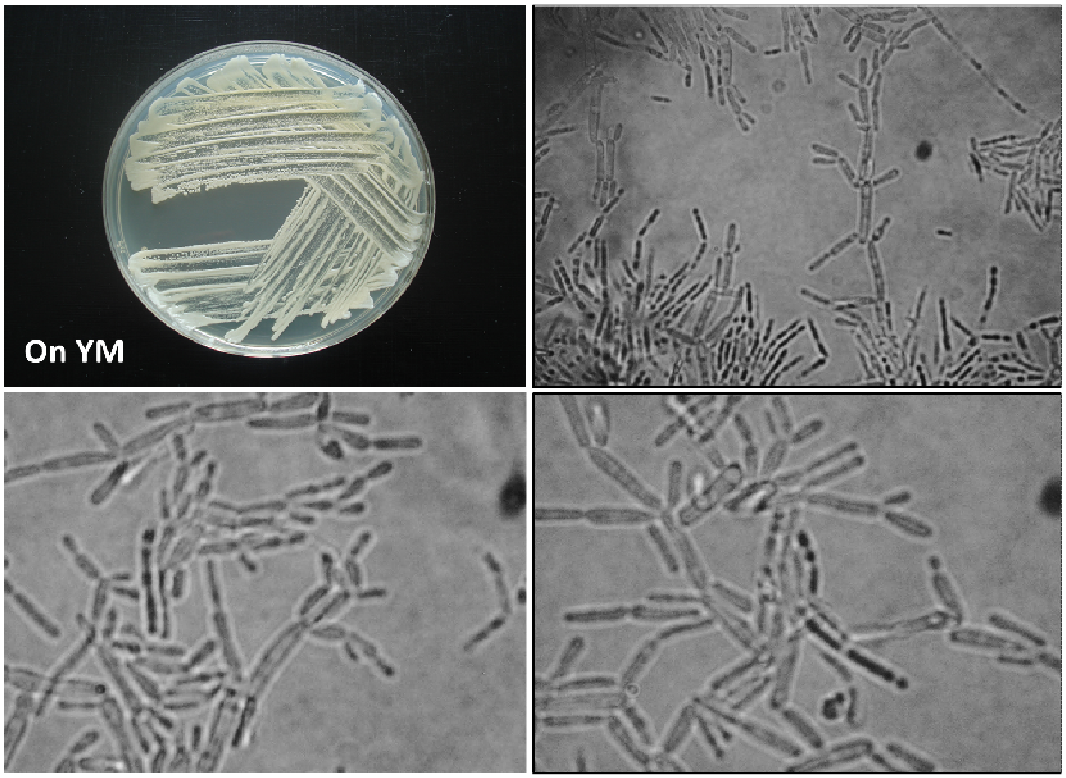

Plate 4. ***Candida catenulata*** AUMC 7760: pseudomycelium and budding cells (Budding cells 1.5-4.5 x 4-12 µm, Kurtzman & Fell 1998).

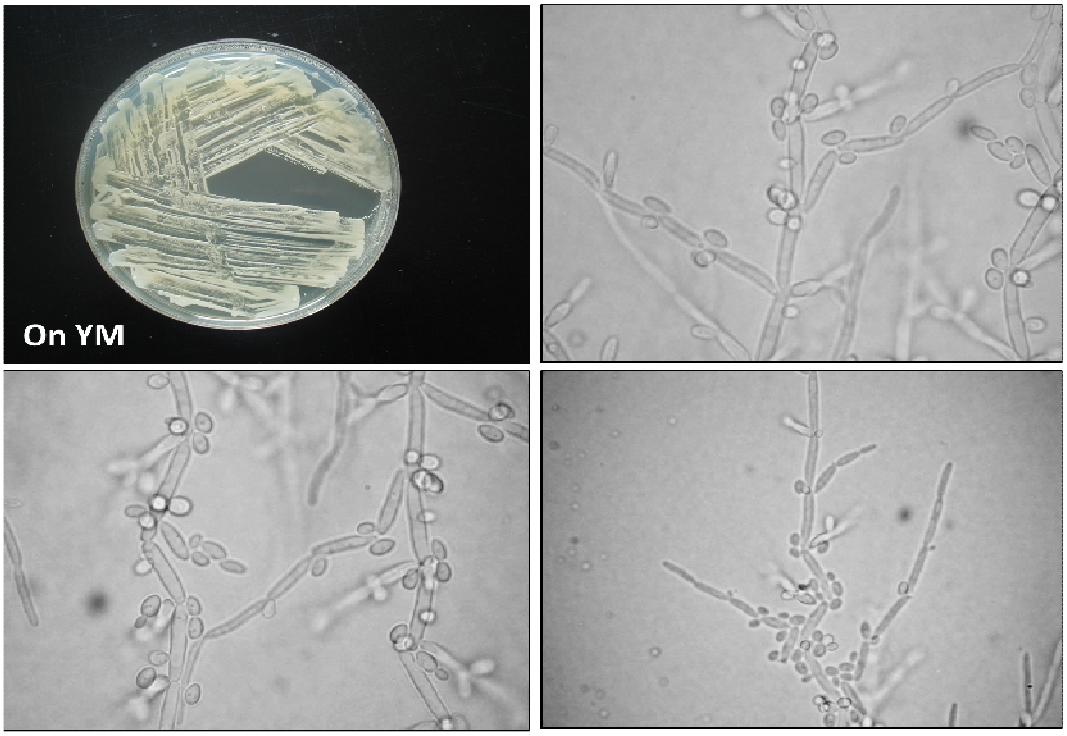

Plate 5. ***Candida parapsilosis*** AUMC 7750, pseudohyphae and budding cells (Budding cells ovoidal, 3-4 x 5-8 µm, cylindrical upto 20 µm, Kurtzman & Fell 1998).

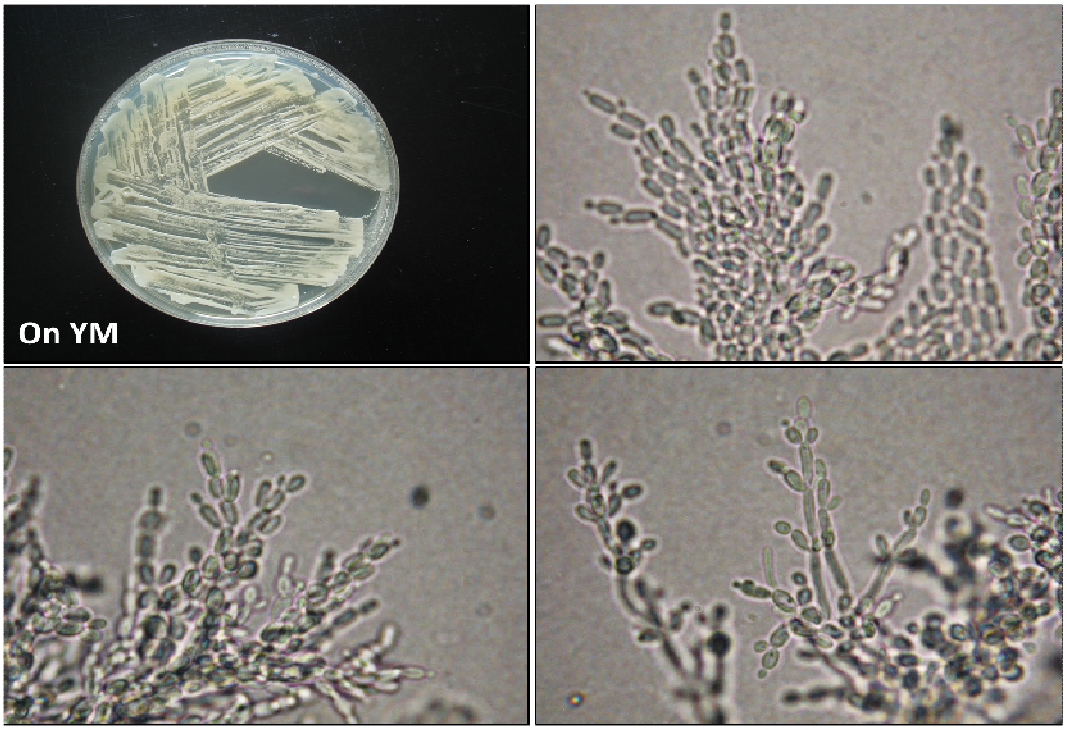

Plate 6. ***Candida prunicola*** AUMC 7767, pseudohyphae and budding cells (yeast cells are spherical (2.1-4.0 µm) to ellipsoidal (1.4-3.5x2.0-7.5 µm) to elongate (2.0-2.5x6.0-17.0 µm), and single, in pairs or occasionally in small clusters. Budding is multilateral with 1-3 buds per cell, Kurtzman 2001).

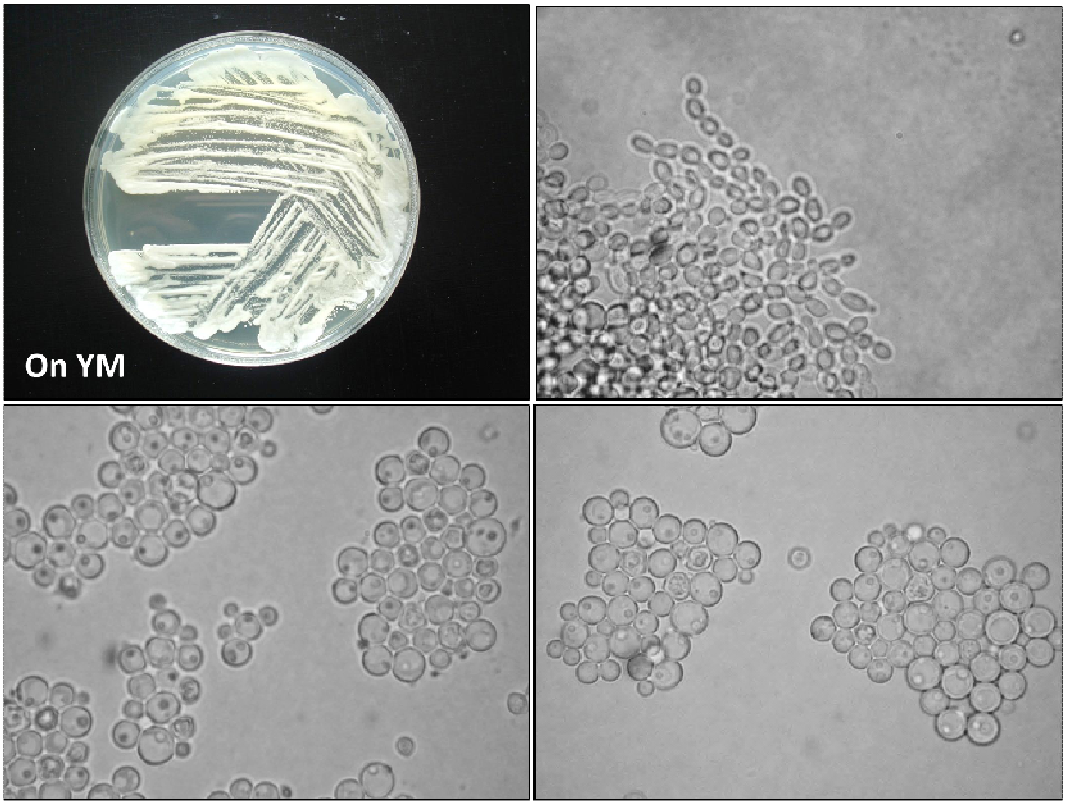

Plate 7. ***Debaryomyces hansenii*** AUMC 7751, pseudomycelium and budding cells (Budding cells 2-7.2 x 2.2-8.6 µm, Kurtzman & Fell 1998).

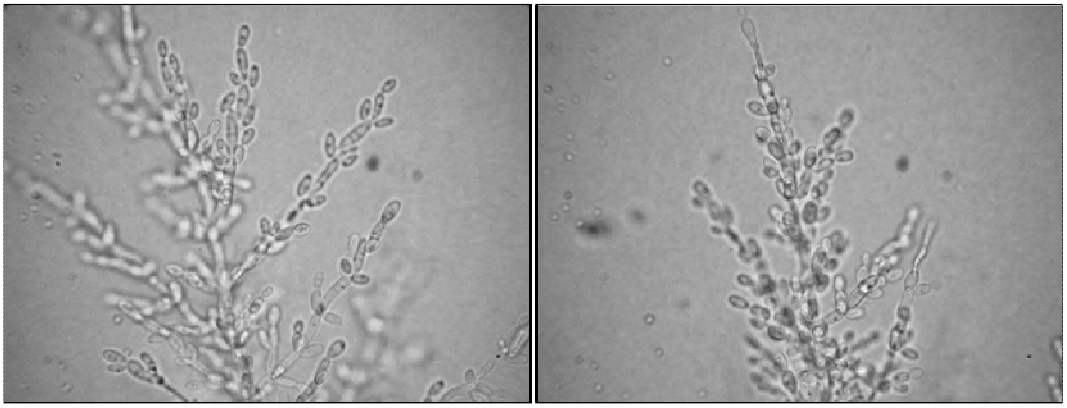

Plate 8. ***Debaryomyces hansenii*** AUMC 7241: pseudomycelium and budding cells (Budding cells 2-7.2 x 2.2-8.6 µm, Kurtzman & Fell 1998).

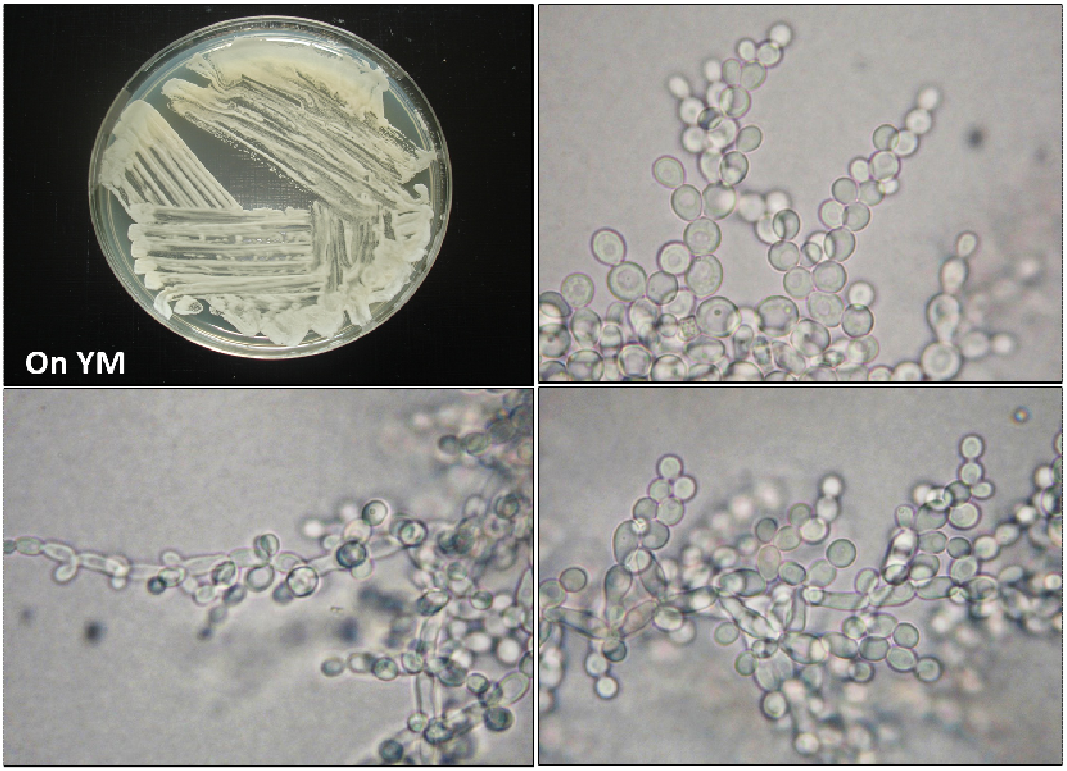

Plate 9. ***Debaryomyces pseudopolymorphus*** AUMC 7752, pseudomycelium and budding cells (Budding cells 3-6.5 x 4.5-16 µm, Kurtzman & Fell 1998).

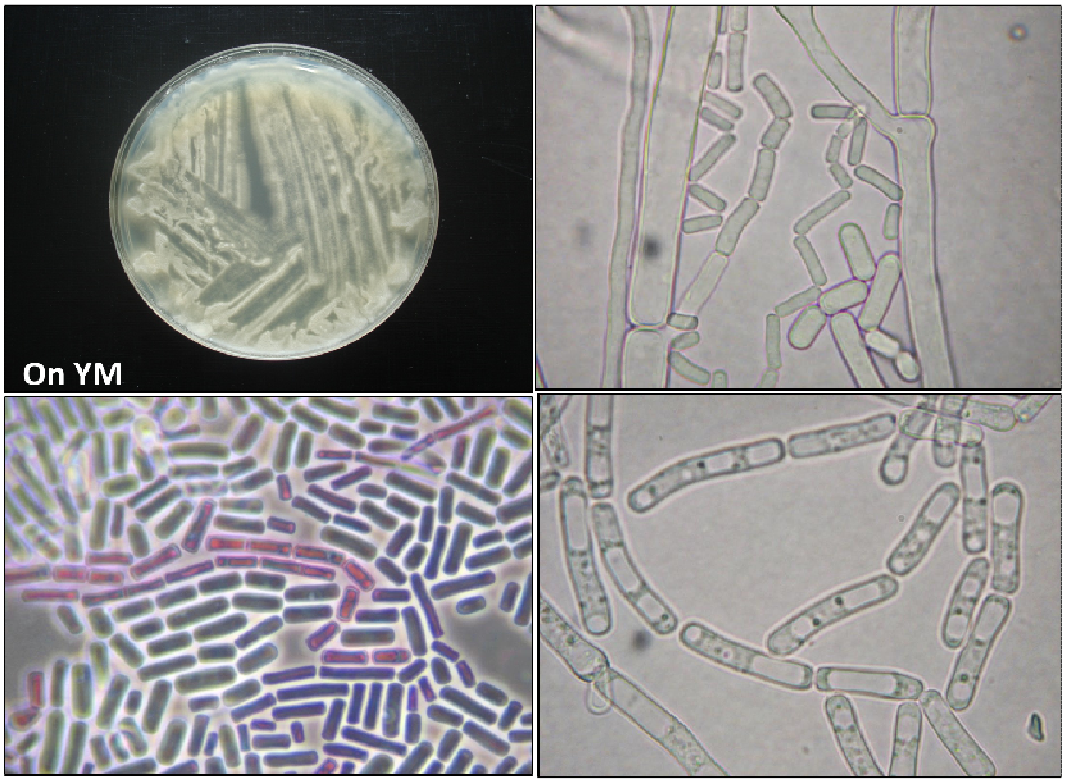

Plate 10. ***Geotrichum citri-aurantii*** AUMC 7754, true mycelium and arthrospores (arthrospores 4-6 x 5-17 µm, Kurtzman & Fell 1998).

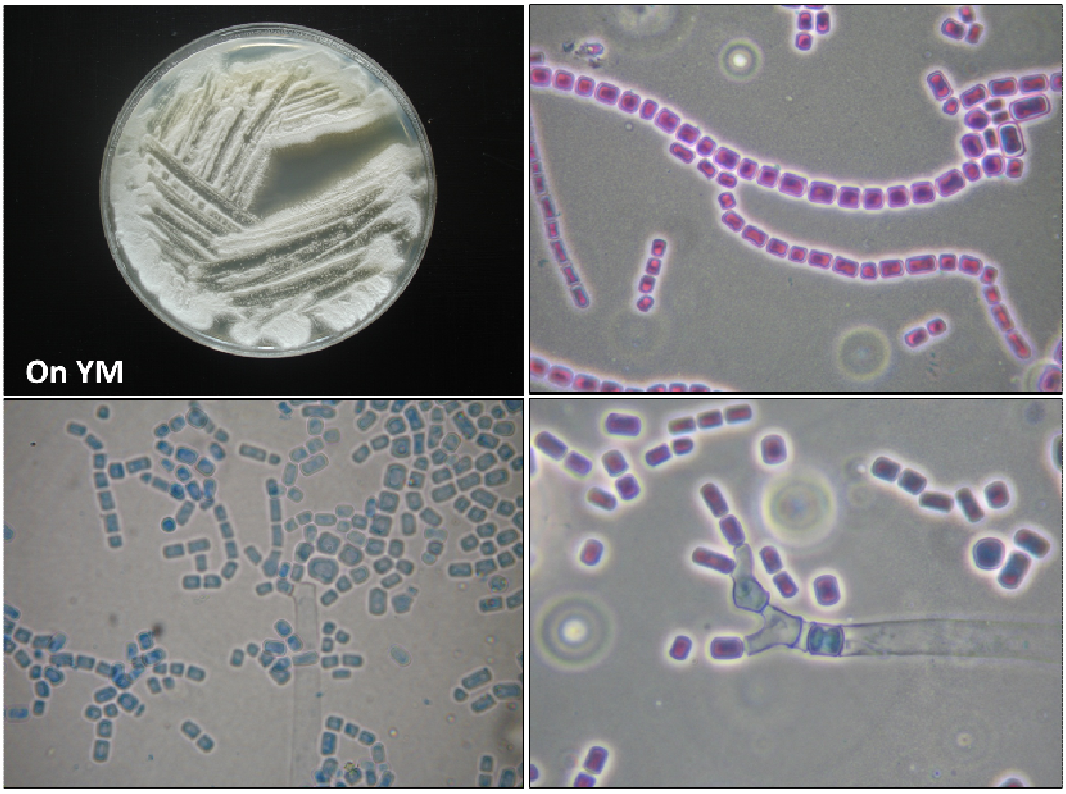

Plate 11. ***Geotrichum*** sp. AUMC 7749, true mycelium and arthroconidia.

Plate 12. ***Hanseniaspora occidentalis*** AUMC 7758: apiculate lemon-shaped cells (cells 1.8-6.2 x 3-11 µm, Kurtzman & Fell 1998).

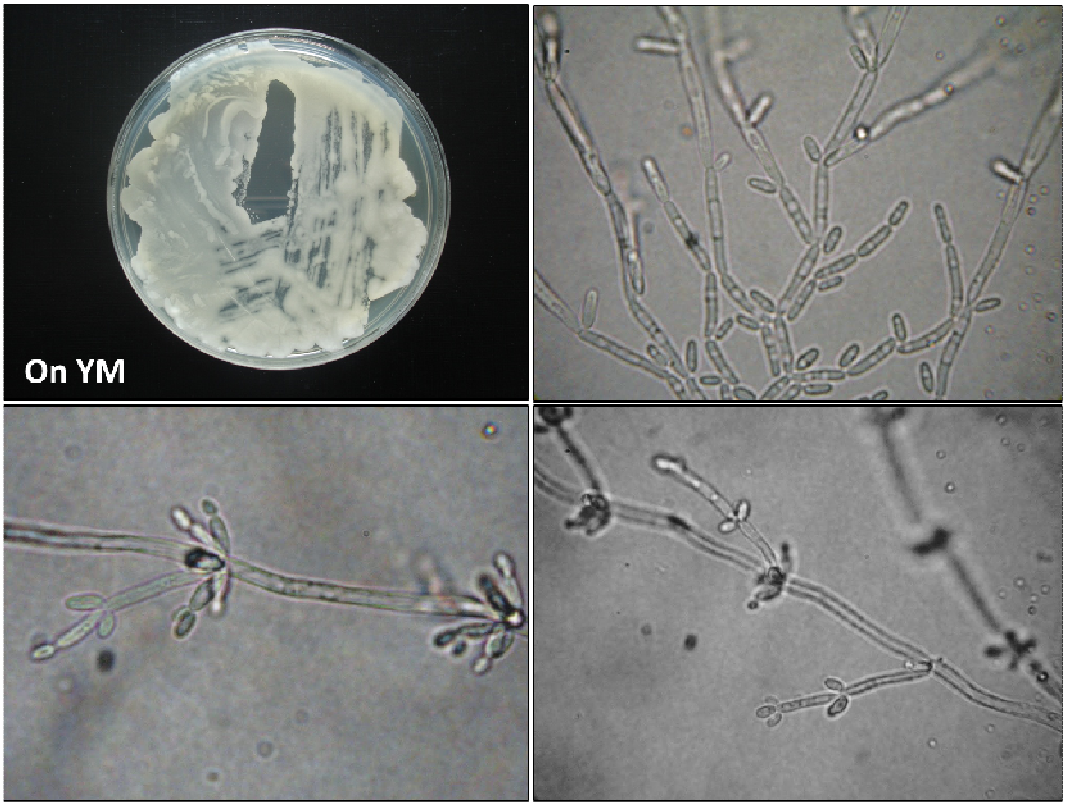

Plate 13. ***Issachenkia orientalis*** AUMC 7769: pseudomycelium and budding cells. (budding cells 1.3-6 x 3.3-14, Kurtzman & Fell 1998).

Plate 14. ***Kluyveromyces marxianus*** AUMC 7759, pseudohyphae and budding cells (budding cells 2-6 x 3-11µm, Kurtzman & Fell 1998).

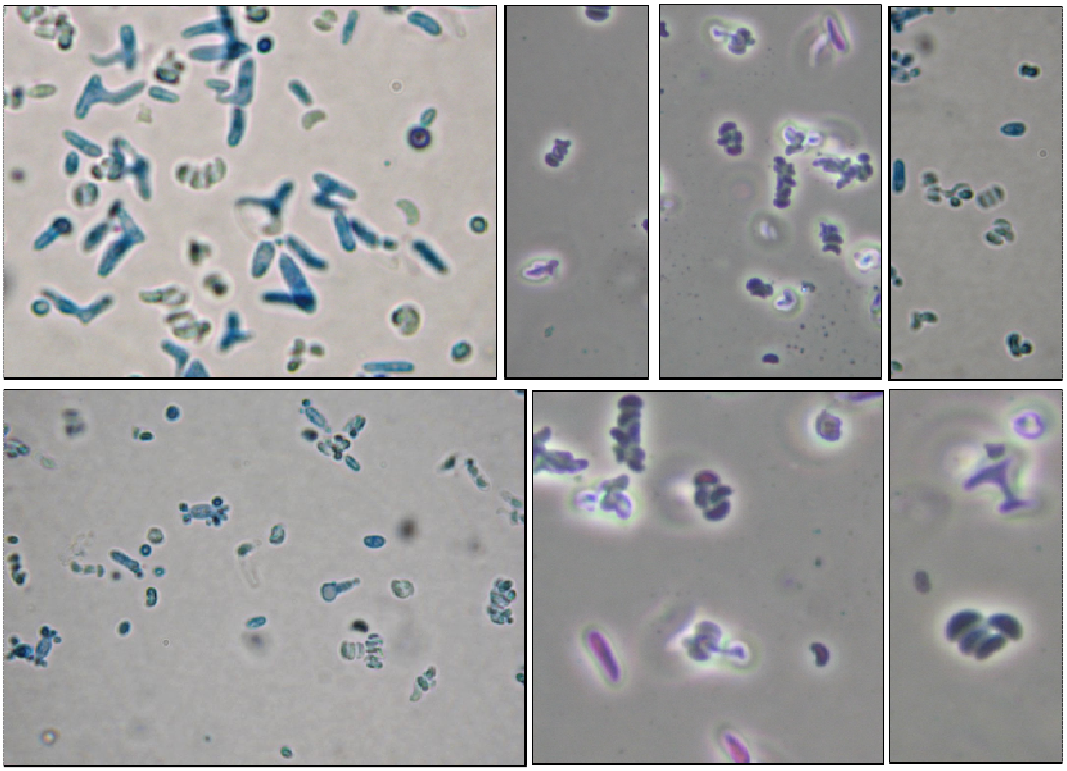

Plate 15. ***Kluyveromyces marxianus*** AUMC 7759: on YM, pseudohyphae and budding cells (top left), asci and reniform ascospores (middle top right and bottom).

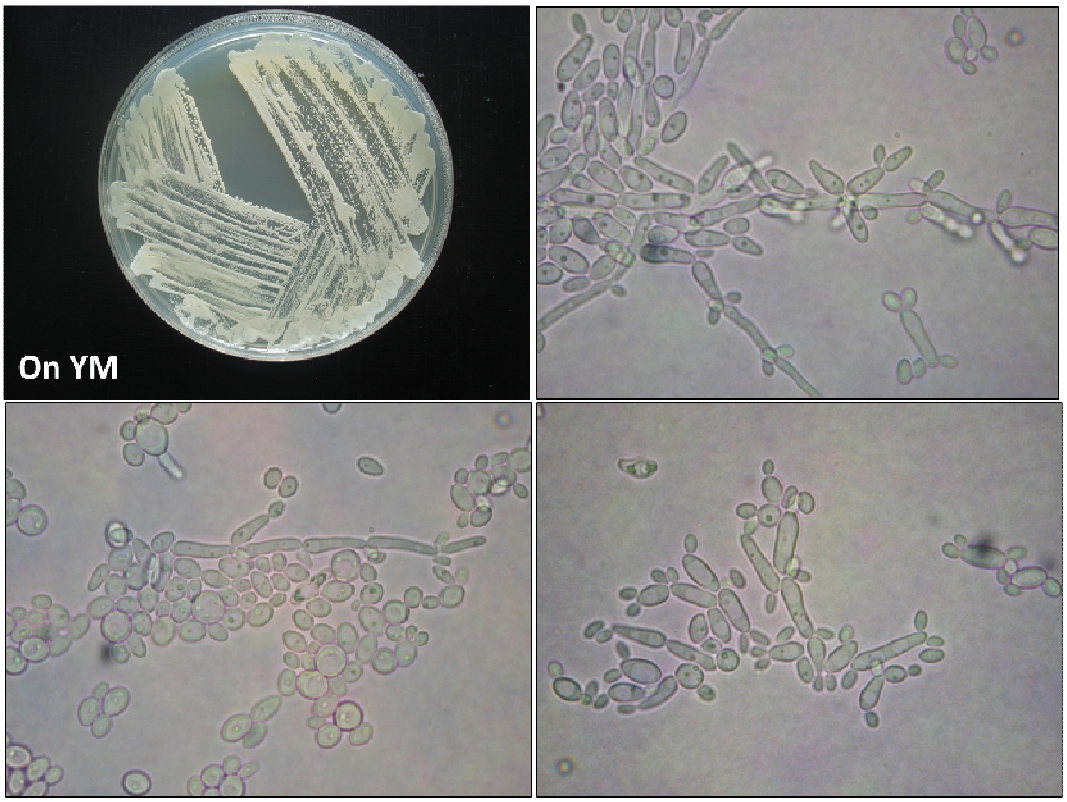

Plate 16. ***Kodamaea ohmeri*** AUMC 7748: pseudomycelium and budding cells, (budding cells 1.7-6.5 x 2.5-25 µm, Kurtzman & Fell 1998).

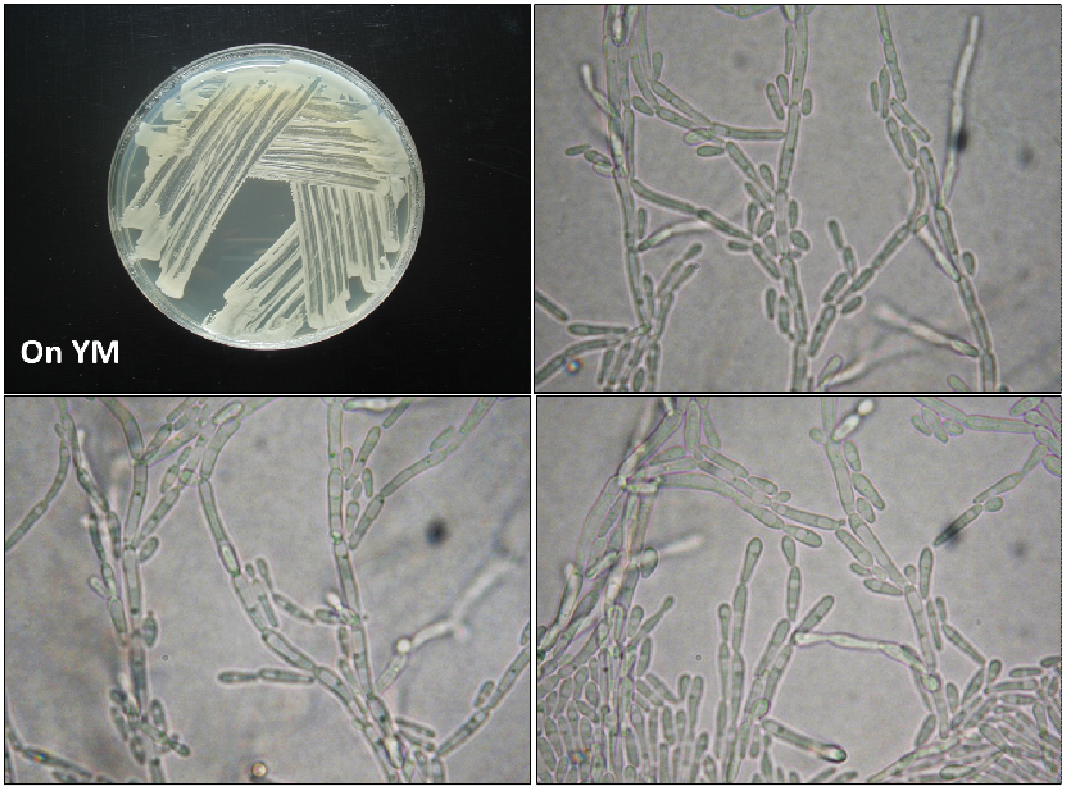

Plate 17. ***Pichia caribbica*** AUMC 7753: pseudomycelium and budding cells. (budding cells 1.8-4 x 3-10.2 µm, Kurtzman & Fell 1998).

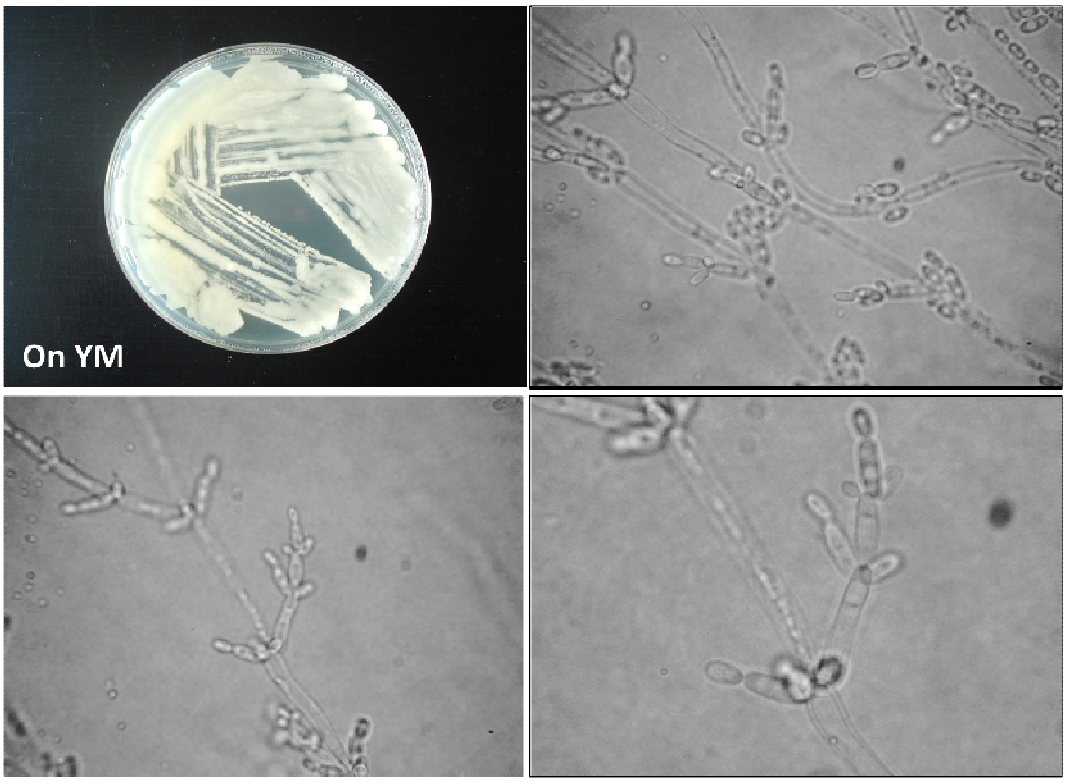

Plate 18. ***Pichia fermentans*** AUMC 7755: pseudomycelium and budding cells, (budding cells 1.9-6.5 x 4-14.4 µm, Kurtzman & Fell 1998).

2. Basidiomyceteous yeasts

Cryptococcus Vuillemin

The genus *Cryptococccus* was isolated in high frequency in grapevine phyllosphere on both media, the air of both plantations on DYM, and grapevine phylloplane on DRBC. It was recovered infrequently from the remaining sources in both plantations while it was missed in citrus soil and carpoplane on both media. Its highest percentage counts were recorded from grapevine phyllosphere (5.43 % - 9.84 % of total fungi). Seven species were recorded from both plantations (five species from citrus plantations and six species from grapevine). *C. luteolus* was recovered from citrus plantations only and *C. albidosimilis* and *C. flavescens* from grapevine only. The highest species number (5 species) was recorded in the phyllosphere of both plants and citrus phylloplane.

In the air, it was recorded in high or moderate frequency in both plantations. It contributed 0.50 % - 0.63 % of total fungi in citrus air and 0.62 % - 0.79 % in grapevine air. *C. albidus* was recovered in high or moderate frequency while it was recorded in moderate or low frequency in grapevine air. *C. carnescence, C. flavescens,* and *C. laurentii* were isolated from grapevine air only.

Dominance of *Cryptococcus albidus* in the air was reported by Di Menna (1955), Voros-Felkai (1966 1967), Al- Doory (1967) and Haridy (1992).

In the soil, it was recorded in grapevine in rare frequency soil on both media represented by *C. laurentii*, contributing minute percentage counts (0.03 % - 0.07 % of total fungi). It was missed from citrus soil.

Four *Cryptococcus* species were recovered from different sites of soil in Zagazig area, Egypt (El-Sherbeny 1987). *Cryptococcus* species accounted for 67% of the yeast species identified in the Dry Valley soil and 72% in soils surrounding the historic huts from Ross Sea region of Antarctica (Arenz *et al.* 2006), and (33%) from soil in South Victoria Land, Antarctica (Connell *et al.* 2008). *Cryptococcus albidus* and *C. laurentii* were prevalent in soil (Capriotti 1958, 1967, Monib *et al.*, 1982, Haridy 2002). *Cryptococcus albidus* was isolated from soil of garden at the Karachi University campus, Pakistan (Mushtaq *et al.* 2004).

In the phyllosphere, *Cryptococcus* yielded more percentage counts in grapevine phyllosphere than those recorded in citrus phyllosphere. It was recovered in high frequency on both media in grapevine phyllosphere and in moderate frequency in citrus phyllosphere. It

contributed 0.14 % - 0.36 % of total fungi in citrus phyllosphere and 5.43 % - 9.84 % in grapevine phyllosphere. *C. albidus* was recovered in high frequency on both media in grapevine phyllosphere and in moderate frequency in citrus phyllosphere, contributing the greatest component of the genus counts (0.12 % - 0.35 % and 5.03 % - 9.79 % of total fungi in citrus and grapevine phyllospheres respectively. *C. laurentii* was recorded in moderate or low frequency in grapevine phyllosphere while it was recorded in rare frequency in citrus phyllosphere. *C. luteolus* was recovered from citrus phyllosphere and *C. albidosimilis* from grapevine only.

Cryptococcus was prevalent in pineapple leaves, Rio de Janeiro, Brazil (Robbs *et al.* 1989). *C. laurentii* and *C. albidus* were the prevalent species isolated from sugarcane leaves, Rio de Janeiro, Brazil (Azeredo *et al.* 1998), apple, plum, and cherry leaves, southwest Slovakia (Slavikova *et al.* 2009). *C. albidus* was isolated from the phyllosphere of *Bauhinia forficate, Tabebuia* sp. and *Terminala catappa,* southeastern Brazil (Valarini *et al.* 2007).

In the phylloplane, it was recorded in high or moderate frequency in grapevine phylloplane and in moderate frequency on both media in citrus phylloplane. It contributed 1.16 % - 2.26 % of total fungi in citrus phylloplane and 1.59 % - 2.36 % in grapevine phyllosphere. *C. albidus* was recovered in high or low frequency in grapevine phylloplane and in low frequency on both media in citrus phylloplane, contributing 0.94 % - 1.66 % of total fungi in grapevine phylloplane and 0.32 % - 1.69 % in citrus phylloplane. *C. luteolus* was recovered from citrus phylloplane.

Cryptococcus species were the most common species in phylloplane communities (Hislop and Cox 1969, McBride and Hayes 1977, Fokkema *et al.* 1979, McCormack *et al.* 1994a). *C. albidus* (Fonseca *et al.* 2000, Sugita *et al.* 2001) and *C. laurentii* (Sugita *et al.* 2000, Takashima *et al.* 2003) were deemed to be ubiquitous phylloplane colonists regardless of plant type or geography (Inacio *et al.* 2002, Maksimova and Chernov 2004).

In the carposphere, it was recovered in low frequency in grape carposphere on both media while it was recovered in low or rare frequency in citrus carposphere. It contributed 0.02 % - 0.08 % of total fungi in citrus carposphere and 0.30 % - 1.56 % in grape carposphere. *C. albidus* was recovered in low frequency on both media in grape carposphere while in rare frequency on DRBC only in citrus carposphere. *C. carnescence* and *C. magnus* were recorded from grape carposphere only.

Cryptococcus was prevalent in pineapple fruit in Rio de Janeiro, Brazil (Robbs *et al.* 1989), isolated from different angiosperm fruits, southeastern Brazil (Prada and Pagnocca 1997), and from olive fruits and brines during fermentation process (Hernández *et al.* 2007).

C. albidus and *C. laurentii* were isolated from soft grapes and peach, El-Minia city, Egypt (Haridy 1994).

In the carpoplane, it was recovered in grape carpoplane in rare frequency on DRBC only represented by *C. laurentii* contributing 0.14 % of total fungi, while it was missed in citrus carpoplane.

Cryptococcus albidus was part of the natural microbiota of certain varieties of grapes in southern Spain (De la Torre *et al.* 1999).

In the fresh juice, it was recovered in low frequency in grape juice contributing 0.001 % of total fungi on both media and in low frequency on DYM only from citrus juice constituting 0.05 % of total fungi. *C. laurentii* was isolated from both juices while *C. albidus* was recorded from grape juice.

C. albidus was reported from cases of meningitis (Cunha and Lusins 1973, Yasin *et al.* 1988). *C. laurentii* was reported from a pulmonary abscess (Lynch *et al.* 1981).

Strain tested

***Cryptococcus albidosimilis* Vishniac & Kurtzman**

AUMC 7784 (Plate 19).

***Cryptococcus albidus* (Saito) C. E. Skinner**

AUMC 7234, AUMC 7242, AUMC 7244, AUMC 7246, AUMC 7761, AUMC 7775

***Cryptococcus carnescens* (Verona & Luchetti) Takashima, Sugita, Shinoda & Nakase**

AUMC 7790.

***Cryptococcus flavescens* (Saito) C. E. Skinner**

AUMC 7794.

***Cryptococcus laurentii* (Kufferath) C. E. Skinner**

AUMC 7237, AUMC 7239, AUMC 7255, AUMC 7763, AUMC 7799 (Plate 20), AUMC 7798 (Plate 21).

***Cryptococcus luteolus* (Saito) C. E. Skinner**

AUMC 7291 (Plate 22), AUMC 7792.

***Cryptococcus magnus* (Lodder & Kreger-van Rij) Baptist & Kurtzman**

AUMC 7772 (Plate 23), AUMC 7793.

Table 15. Physiological comparison of the strains tested of *Cryptococcus* species (Basidiomycete species): **1** *Cryptococcus albidosimilis* AUMC 7784**, 2** *C. albidus* AUMC 7234, **3** *C. albidus* AUMC 7242, **4** *C. albidus* AUMC 7244**, 5** *C. albidus* AUMC 7246**, 6** *C. albidus* AUMC 7761, **7** *C. albidus* AUMC 7775**, 8** *C. carnescens* AUMC 7790**, 9** *C. flavescence* AUMC 7794**, 10** *C. laurentii* AUMC 7237, **11** *C. laurentii* AUMC 7239, **12** *C. laurentii* AUMC 7255, **13** *C. laurentii* AUMC 7763, **14** *C. laurentii* AUMC 7798, **15** *C. laurentii* AUMC 7799, **16** *C. luteolus* AUMC 7792, **17** *C. magnus* AUMC 7772 and **18** *C. magnus* AUMC 7793**.**

Species no.	**S**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
Fermentation																			
D- glucose	F1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Assimilation																			
D-glucose	C1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-galactose	C2	+	d	d	d	d	+	+w	+	+	+	+	w	+	+	+	+	+w	+
L-sorbose	C3	+w	w	d	-	+	w	-	w	-	-	w	-	-	-	-	-	d	d
D-ribose	C5	+	d	d	d	+	w	d	+	d	d	+	-	+	+	+	+	d	d
D-xylose	C6	+	d	d	+	+	+	+	d	+	+	+	+	+	+	+	+	+	+
L-arabinose	C7	+	+	d	+	+	+	+	d	+	+	d	d	+	+	+	+	+	+
L-rhamnose	C9	+	d	+	-	+	d	d	+	+	+	+	d	+	d	+	+	+	+
Sucrose	C10	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Maltose	C11	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
α, α-trehalose	C12	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Methyl-α-D-glucoside	C13	+	+	+	w	+	+	+	+w	+	+	+	w	d	+	+	+	+	+
Cellobiose	C14	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salicin	C15	-	-	-	-	-	-	-	-	-	-	-	d	-	-	-	-	-	d
Arbutin	C16	-	-	-	-	-	-	-	-	-	-	-	d	-	-	-	-	-	d
Lactose	C18	d	+	+	+	+	+	+	d	+	+	+	-	+	+	+	+	+	+
Raffinose	C19	+	+	+	+	+	+	+	+	+	+	+	d	+	+	+	+	+	+
Melezitose	C20	+	+	+	+w	+	+	+	+	+	+	+	-	+	+	+	+	+	+
Inulin	C21	+	+	d	d	+w	+	+w	d	+	+	+	d	d	+	+	+	d	+
Soluble starch	C22	d	+	+	+	+	+	+	d	+	d	+	+	+	+	+	+	+	+
Glycerol	C23																		d
Meso-erythritol	C24	+	-	-	-	-	w	-	+w	w	+	+	-	d	+	+	d	-	-
Xylitol	C26												d						d
D-glucitol	C28	+	d	-	+	+	+	d	d	d	+	+	d	+	-	d	+	w	d
D-mannitol	C29	+	+	+	+	+	+	+	+	+	+	+	d	+	+	+	+	+	+

Species no.	**S**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
Galactitol	C30	+	d	+	w	+	w	d	d	d	+	+	-	d	+	+	+	-	d
Myo-inositol	C31	d	+	d	d	+	+	+	d	+	+	d	-	+	+	+	+	+	+
Glucono-d-lactone	C32	+	-	d	-	d	-	d	d	d	+	+	-	+	+	+	+	d	-
D-glucuronate	C36	d	+	+	d	d	+	d	d	+	+	+	-	d	+	+	+	+	+
D-galacturonate	C37	d	d	-	-	w	d	d	+w	+	+	+	-	+	d	+	+	d	d
Succinate	C39												d						d
Citrate	C40	+	+	d	d	+	+	d		+	+	+	d	+	+	+	+	+	+
Methanol	C41	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	w	-	-
Ethanol	C42	+	d	-	d	-	w	d	-	+	+	-	d	+	+	+	+	-	-
Propane 1,2 diol	C43	-	-	-	d	-	-	-	d	-	-	+	d	-	-	-	-	-	-
Butane 2,3 diol	C44	d	-	-	-	-	-	-	-	-	-	d	d	-	-	-	-	d	-
Quinic acid	C45	w	w	-	-	-	-	-	-	-		-	-	-	-	-	-	+	d
Nitrogen compounds																			
Nitrate	N1	+	+	+	+	+	+	+	-	-	-	-	w	-	-	-	-	+	+
Nitrite	N2	+	+	+	+	+	+	+	w	+	-	+	w	+	-	-	+	+	+
Ethylamine	N3	+	w	-	-	-	-	-	-	+	+	-	+	+	+	+	+	+w	-
L-lysine	N4	+	-	-	w	-	-	-	-	+	+	w	+	+	+	+	+	d	-
Creatine	N6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Creatinine	N7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D-glucosamine	N8	+	-	-	-	-	-	d	-	+	+	+	-	-	+	+	+	w	w
Imidazole	N9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	d	-
D-tryptophane	N10	-	-	-	-	-	-	-	-	+	+	+	d	+	-	+	+	w	-
Miscellaneous																			
0.01% cycloheximide	O1	+	-	-	+	-	-	+	+	d	-	-	-	-	d	d	+	-	+
0.1 % cycloheximide	O2	+	-	-	-	-	-	d	w	w	-	-	-	-	d	d	+	-	-
50% D-glucose	O4	-	-	-	-	-	-	-	+	-	-	-	-	+	-	+	-	-	-
60% D-glucose	O5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
10% NaCl	O6	-	+	+	+	+	+	-	+		+	+		+			+	-	+
16% NaCl	O7	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	+
Starch	M1	-	-	-	-	-	-	+	-	+	-	-	-	+	+	+	+	+	-

Species no.	S	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
formation																			
Urea hydrolysis	M3	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Diazonium blue B	M4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth at 30°C	T2	+	-	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+
Growth at 37°C	T4	+	-	-	-	-	-	-	-	+	+	-	-	-	+	+	-	-	-
Growth at 42°C	T6	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Growth at 45°C	T7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pink colony	E1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Budding	E2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lemon-shaped cells	E3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Budding on stalk	E4	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
Splitting cells	E5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Filamentous	E6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pseudohyphae	E7	+	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-
Septate hyphae	E8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Arthroconidia	E9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ballistoconidia	E10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ascosporogenus	A1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ascospores round	A2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

+: growth; w: weak growth; d, delayed, -: no growth, gap: not tested.

Fermentation of Me-α-D glucoside, α, α Trehalose, melezitoze, Starch and D-xylose gave negative results with all species tested and were omitted from the table.

Filobasidium L. S. Olive

This genus (represented by *F. floriforme*) was isolated only from the phyllosphere and phylloplane of both plants. It was recorded in rare frequency on both media in citrus phyllosphere, on DRBC in grapevine phyllosphere and phylloplane, and on DYM in

citrus phylloplane. *F. floriforme* was reported earlier from dead florets of plume grass *Erianthus giganteus* in South Carolina, USA (Barnett *et al.* 2000).

***Filobasidium floriforme* L. S. Olive**

Strains tested: AUMC 7238, AUMC 7243, AUMC 7245.

Melanopsichium Beck

This genus was isolated only from citrus air. It was recorded in rare frequency on both media contributing 0.01 % - 0.02 % of total fungi. It was represented by *M. pennsylvanicum*.

***Melanopsichium pennsylvanicum* Hirschhorn**

Strain tested

AUMC 7785 (Plate 24).

Pseudozyma Bandoni

It was recorded infrequently from the air of both plantations, and from citrus phyllosphere, phylloplane, and carposphere only. Its highest percentage count was recorded from citrus air (0.02 % - 0.09 % of total fungi). Three species and one unidentified were recovered from both plantations. *P. aphidis*, *P. hubeinsis*, and *P. rugulosa* were recorded from citrus plantations only.

In the air, it was recoverd in rare frequency from both plantations on both media constituting 0.02 % - 0.09 % of total fungi. *P. hubeinsis* was recorded from citrus air and *Pseudozyma* sp. from grapevine air.

In the soil, it was missed in both plantations.

In the phyllosphere, it was encountered in rare frequency in citrus phyllosphere on both media contributing 0.006 % - 0.01 % of total fungi. *P. aphidis*, *P. rugulosa*, and *Pseudozyma* sp. were recorded from citrus phyllosphere. It was missed in grapevine phyllosphere. *P. rugulosa* was repored earlier from leaf of maize (*Zea mays*) in Canada (Barnett *et al.* 2000).

In the phylloplane, represented by *P. aphidis* only, was recovered in rare frequency from citrus phylloplane on DRBC only contributing 0.07 % of total fungi. It was missed in grapevine phylloplane.

Pseudozyma aphidis was isolated from apple, cherry, and apricot leaves, southwest Slovakia (Slavikova *et al.* 2009).

In the carposphere, it was recorded in rare frequency from citrus carposphere on DYM contributing 0.02 % of total fungi. It was absent in grape carposphere and the carpoplane and juice of both plants.

Strains tested

***Pseudozyma aphidis* (Henninger & Windisch) Boekhout**

AUMC 7787 (Plate 25).

Pseudozyma hubeinsis* Wang *et al.

AUMC 7786 (Plate 26).

***Pseudozyma rugulosa* (Traquair, L. A. Shaw & Jarvis) Boekhout & Traquair**

AUMC 7240 (Plate 27).

***Pseudozyma* sp.**

AUMC 7235, AUMC 7256 (Plate 28).

Table 16. Physiological comparison of the strains tested of the basidiomyceteous genera *Filobasidium*, *Melanopsichium* and *Pseudozyma*: **1** *Filobasidium floriforme* AUMC 7238**, 2** *F. floriforme* AUMC 7243, **3** *F. floriforme* AUMC 7245, **4** *Melanopsichium pennsylvanicum* AUMC 7285**, 5** *Pseudozyma aphidis* AUMC 7787, **6** *Pseudozyma hubeinsis* AUMC 7786, **7** *Pseudozyma rugulosa* AUMC 7240**, 8** *Pseudozyma* sp. AUMC 7235**, 9** *Pseudozyma* sp. AUMC 7256**.**

Species no.	**S**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Fermentation										
D- glucose	F1	-	-	-	-	-	-	-	-	-
Assimilation										
D-glucose	C1	+	+	+	+	+	+	+	+	+
D-galactose	C2	d	+w	d	+	+	+	w	+	+
L-sorbose	C3	+	d	+	d	d	w	-	-	-
D-ribose	C5	d	d	d	+	d	d	-	+	d
D-xylose	C6	+	+	+	+	+	+	+	+	+
L-arabinose	C7	+	+	+	+	+	+	+	d	+
L-rhamnose	C9	+	+	+	+	+	-	-	d	-
Sucrose	C10	+	+	+	+	+	+	+	+	+
Maltose	C11	+	+	+	+	+	+	+	+	+

Species no.	**S**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
α, α-trehalose	C12	+	+	d	+	+	+	+	+	+
Methyl-α-D-glucoside	C13	+	+	+	+	+	+	+	d	+
Cellobiose	C14	+	+	+	d	+	+		d	+
Lactose	C18	+	+	+	d	d	d	-	-	-
Raffinose	C19	d	+	+	+	+	+	d	+	+
Melezitose	C20	+	+	+	+w	+	+	+	+	+
Inulin	C21	+w	-	d	+	+	+	d	d	+
Soluble starch	C22	d	d	+	+	+	+	+	-	+
Meso-erythritol	C24	-	-	-	d	+	+	w	d	d
D-glucitol	C28	+	d	+	d	+	+	+	d	d
D-mannitol	C29	+	+	d	+	+	+	+	+	+
Galactitol	C30	+	d	d	-	d	-	-	-	-
Myo-inositol	C31	+	+	+	d	+	d	d	-	-
Glucono-d-lactone	C32	d	-	-	+	+	d	d	d	+
D-glucuronate	C36	+	+	d	d	+	+	+	-	-
D-galacturonate	C37	+	-	-	+	+	w	-	-	-
Citrate	C40	-	d	+	d	d	+	-	+	-
Methanol	C41	-	-	-	-	-	w	-	-	-
Ethanol	C42	-	-	d	+	+	+	-	d	d
Propane 1,2 diol	C43	w	-	-	-	-	-	-	-	+
Butane 2,3 diol	C44	w	-	d	d	-	-	-	-	-
Quinic acid	C45	-	-	-	+	+	+	d	+	+
Nitrogen compounds										
Nitrate	N1	+	+	+	+	+	+	+	+	+
Nitrite	N2	+	+	+	+	+	+	+	+	+
Ethylamine	N3	-	w	-	+	+	+	d	+	+
L-lysine	N4	-	w	-	+	+	+	+	-	-
Creatine	N6	-	-	-	-	-	-	-	-	-
Creatinine	N7	-	-	-	-	-	-	-	-	-
D-glucosamine	N8	-	+	+	+	+	+	+	-	-
Imidazole	N9	-	-	-	-	-	-	+	w	-
D-tryptophane	N10	-	w	w	-	w	d	-	-	-
Miscellaneous										
0.01% cycloheximide	O1	-	-	-	+	+	d	+	-	-

Species no.	S	1	2	3	4	5	6	7	8	9
0.1 % cycloheximide	O2	-	-	-	+	+	d	+	-	-
50% D-glucose	O4	-	-	-	-	-	-	-	+	-
60% D-glucose	O5	-	-	-	-	-	-	-	-	-
10% NaCl	O6	-	-	+	-	-	-	-	+	+
16% NaCl	O7	-	-	-	-	-	-	-	-	
Starch formation	M1	-	+	-	-	-	+	-	-	
Urea hydrolysis	M3	+	+	+	+	+	+	+	+	+
Diazonium blue B	M4	+	+	+	+	+	+	+	-	
Growth at 30°C	T2	-	-	+	+	+	+	+	+	+
Growth at 37°C	T4	-	-	-	-	+	-	-	-	-
Growth at 42°C	T6	-	-	-	-	-	-	-	-	-
Growth at 45°C	T7	-	-	-	-	-	-	-	-	-
Pink colony	E1	-	-	-	-	-	-	-	-	-
Budding	E2	+	+	+	+	+	+	+	+	+
Lemon-shaped cells	E3	-	-	-	-	-	-	-	-	-
Budding on stalk	E4	-	-	-	-	-	-	-	-	+
Splitting cells	E5	-	-	-	-	-	-	-	-	-
Filamentous	E6	-	-	-	+	-	-	+	+	+
Pseudohyphae	E7	+	+	+	-	+	-	-	-	-
Septate hyphae	E8	-	-	-	-	-	-	-	-	-
Arthroconidia	E9	-	-	-	-	-	-	-	-	-
Ballistoconidia	E10	-	-	-	-	-	-	-	-	-
Ascosporogenus	A1	-	-	-	-	-	-	-	-	-
Ascospores round	A2	-	-	-	-	-	-	-	-	-

+: growth; w: weak growth; d, delayed, -: no growth, gap: not tested.

Rhodotorula F. C. Harrison

It was isolated infrequently from all sources in both plantations except citrus soil, carpoplane, and juice. Its highest percentage count was recorded from grapevine phyllosphere (10.05 % - 10.48 % of total fungi) and grapevine air (0.39 % - 4.58 %) followed by grapevine phylloplane (1.49 % - 2.54 %). It was more common in grapevine than citrus plantations. Two species were recorded from both plantations, *R. glutinis* and *R. mucilaginosa* while

R. aurantiaca from citrus plantations only and *Rhodotorula* sp. from grapevine plantations only.

In the air, it was recorded in high or moderate frequency in citrus air, contributing 0.19 % - 0.59 % of total fungi. In grapevine air, it was isolated in high or low frequency constituting 0.39 % - 4.58 % of total fungi. *R. mucilaginosa* was more common in citrus air than *R. glutinis* while the reverse occurred in grapevine air. *R. aurantiaca* was recorded in rare frequency from citrus plantations only. *R. aurantiaca* was reported earlier from atmosphere in Japan, soil and Bantu beer in South Africa, leaf of bottle-brush plant *Callistemon viminalis* in Australia, bark beetle *Dendroctonus jeffreyi* in *Pimis jejfreyi* in USA, brine bath in cheese factory in the Netherlands (Barnett *e al.* 2000).

Rhodotorula mucilaginosa was dominant species in the air (Di Menna 1955, Voros-Felkai 1966, 1967, Al- Doory 1967, Haridy 1992).

In the soil, it was isolated in rare frequency from grapevine soil on DRBC contributing 0.07 % of total fungi. It was not recorded from citrus soil.

Rhodotorula mucilaginosa was isolated from soil, south Victoria Land, Antarctica (Connell *et al.* 2008). *Rhodotorula glutinis* and *R. mucilaginosa* were prevelant in soil (Capriotti 1958, 1967, Monib *et al.* 1982, El-Sherbeny 1987, Haridy 2002).

In the phyllosphere, *Rhodotorula* yielded more percentage counts in grapevine phyllosphere than those recorded in citrus phyllosphere and the same situation occurred in the phylloplane of the two plants. It was recovered in moderate and high frequencies respectively constituting 10.05 % - 10.48 % of total fungi. In citrus phyllosphere, it was recovered in low or rare frequency contributing minute percentage counts (0.02 % - 0.03 % of total fungi). *R. mucilaginosa* was recorded in high or moderate frequency in grapevine phyllosphere while in rare frequency on DYM in citrus phyllosphere. *R. glutinis* was recorded in low or rare frequency in citrus phyllosphere while in rare frequency on both media in grapevine phyllosphere.

Rhodotorula was prevalent in pineapple leaves, Rio de Janeiro, Brazil (Robbs *et al.* 1989), the leaf surfaces of *Banksia collina* and *Callistemon viminalis* (Shivas and Brown 1986). *Rhodotorula mucilaginosa* was prevalent in the sugarcane leaves, Rio de Janeiro, Brazil (Azeredo *et al.* 1998). *Rhodotorula glutinis* and *Rhodotorula mucilaginosa* were isolated from apple and plum leaves southwest Slovakia (Slavikova *et al.* 2009).

In the phylloplane, it was encountered in moderate frequency from grapevine phylloplane on both media and in low or rare frequency in citrus phylloplane, contributing

more percentage counts (1.49 % - 2.54 % of total fungi) in the former habitat than those in the latter (0.21 % on each medium). *R. mucilaginosa* was isolated from boh phylloplanes.

Phylloplane communities usually comprise deeply pigmented species belonging to the genera *Rhodotorula* and *Sporobolomyces* (Hislop and Cox 1969, McBride and Hayes 1977, Fokkema *et al.* 1979, McCormack *et al.* 1994b). *R. glutinis* and *R. mucilaginosa* appear to be prevalent regardless of plant type or geography (Inacio *et al.* 2002, Maksimova and Chernov 2004).

In the carposphere, it was recovered in moderate frequency from grape carposphere on both media while it was recovered in rare frequency on DRBC in citrus carposphere. It contributed 0.16 % - 0.34 % of total fungi in grape carposphere and 0.01 % in citrus carposphere. *R. mucilaginosa* was isolated in low frequency on both media in grape carposphere and in rare frequency on DRBC only in citrus carposphere. *R. glutinis* was recorded from grape carposphere only.

Rhodotorula spp. were the most common yeasts found in fruit salads including cantaloupe, citrus fruits, honeydew, pineapple, cut strawberries and mixed fruit salads, Washington (Tournas *et al.* 2006), and pineapple fruit of in Rio de Janeiro, Brazil (Robbs *et al.* 1989).

In the carpoplane, it was isolated in rare frequency from grape carpoplane on DRBC only represented by *R. mucilaginosa* contributing, 0.69 % of total fungi. It was missed in citrus carpoplane.

In the fresh juice, it was identified in high frequency from grape juice on both media contributing 0.01 % - 0.03 % of total fungi. *R. mucilaginosa* was isolated in high frequency on both media while *R. glutinis* in low frequency on DRBC only. It was missed in citrus juice.

Rhodotorula was frequently isolated from citrus juices (Hatcher *et al.* 2000) and pasteurized fruit juices in Venezuela (Mendoza *et al.* 1982). *Rhodotorula mucilaginosa* was isolated from pasteurized and subsequently recontaminated single-strength grapefruit juice, Florida (Arias *et al.* 2002), and orange fruit and juice in a spontaneous fermentation (Las Heras-Vazquez *et al.* 2003).

R. glutinis caused fungemia in patient with compromised innate immunity (Fanci *et al.* 1997). *R. mucilaginosa* was reported from a chronic dacryocystitis (Muralidhar and Sulthana 1995).

Strains tested

***Rhodotorula aurantiaca* F. C. Harrison**

AUMC 7250, AUMC 7253 (Plate 29).

***Rhodotorula glutinis* (Fresenius) F. C. Harrison**

AUMC 7249, AUMC 7251, AUMC 7774 (Plate 30), AUMC 7776.

***Rhodotorula mucilaginosa* (A. Jorgensen) F. C. Harrison**

AUMC 7248, AUMC 7777, AUMC 7778 (Plate 31), AUMC 7780, AUMC 7782, AUMC 7795, AUMC 7796.

***Rhodotorula* sp.**

Table 17. Physiological comparison of the strains tested of the basidiomyceteous genus *Rhodotorula*: **1** *Rhodotorula aurantiaca* AUMC 7250, **2** *R. aurantiaca* AUMC 7753, **3** *R. glutinis* AUMC 7249, **4** *R. glutinis* AUMC 7251, **5** *R. glutinis* AUMC 7774, **6** *R. glutinis* AUMC 7776, **7** *R. mucilaginosa* AUMC 7248, **8** *R. mucilaginosa* AUMC 7777, **9** *R. mucilaginosa* AUMC 7778**, 10** *R. mucilaginosa* AUMC 7780, **11** *R. mucilaginosa* AUMC 7782, **12** *R. mucilaginosa* AUMC 7795, **13** *R. mucilaginosa* AUMC 7796.

Species no.	**S**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Fermentation														
D- glucose	F1	-	-	-	-	-	-	-	-	-	-	-	-	-
Assimilation														
D-glucose	C1	+	+	+	+	+	+	+	+	+	+	+	+	+
D-galactose	C2	+	+	d	+	d	d	+	+	+	+	+	+	d
L-sorbose	C3	d	+	d	d	w	d	+	d	-	d	-	d	-
D-ribose	C5	+	d	+	+	d	+	+	+	+	+	+	+	+
D-xylose	C6	+	d	+	+	+	+	+	+	d	+	+	+	d
L-rhamnose	C9	-	-	-	+	-	-	+	+	+	-	+	-	+
Sucrose	C10	+	+	+	+	+	+	+	+	+	+	+	+	+
Maltose	C11	+	+	+	d	+	d	+	+	+	+	d	+	+
α, α-trehalose	C12	+	+	+	+	+	+	+	+	+	+	+w	+	+
Methyl-α-D-glucoside	C13	-	+	-	w	d	-	-	-	-	d	-	-	-
Lactose	C18	-	-	-	-	-	-	-	-	-	-	-	-	-
Melezitose	C20	+	+	+	-	+	+	+	+	+	+	+	+	+
Inulin	C21		d			+	+		+	+	+	d	+	+
Soluble starch	C22		+			+	+		+	d	-	-	-	-
Meso-erythritol	C24	-	-	-	-	-	-	-	-	-	-	-	-	+
D-glucitol	C28	d	d	d	+	d	+	+	+	+	d	d	w	d

Species no.	**S**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
D-mannitol	C29	+	+	+	+	+	+	+	+	+	+	+	d	+
Galactitol	C30	-	-	d	+	-	-	-	-	-	-	+w	-	-
Myo-inositol	C31	-	-	-	-	-	-	d	-	-	-	-	-	-
Glucono-d-lactone	C32	+	-	+	+	d	d	+	+	+	+	+	+	+
D-glucuronate	C36	-	d	-	d	-	-	-	-	-	-	-	-	-
D-galacturonate	C37	d	-	w	+	w	+	-	+	d	d	d	d	+
Citrate	C40	d	+	+	+	+	+	d	+	+	d	d	d	d
Methanol	C41	-	d	-	-	-	-	-	w	w	-	-	-	-
Ethanol	C42	+	d	+	+	+	d	+	+	+	+	+	d	d
Propane 1,2 diol	C43	-	-	-	-	-	-	-	-	-	-	-	d	-
Butane 2,3 diol	C44	d	-	d	-	-	-	d	-	-	-	-	w	d
Quinic acid	C45	+	w	-	+	+	+	+	+	+	+w	+w	+	+
Nitrogen compounds														
Nitrate	N1	-	+	+	+	+	+	-	-	-	-	-	-	-
Nitrite	N2	-	+	+	+	+	+	-	-	-	-	-	-	-
Ethylamine	N3	+	+	+	+	+	+	+	+	+	+	+	+	+
L-lysine	N4	w	d	+	w	+w	+	w	+	w	w	-	-	+
Creatine	N6	-	-	-	-	-	-	-	-	-	-	-	-	-
Creatinine	N7	-	-	-	-	-	-	-	-	-	-	-	-	-
D-glucosamine	N8	-	-	-	-	-	-	-	-	-	-	-	-	-
Imidazole	N9	-	-	-	-	-	-	-	-	-	-	-	-	-
D-tryptophane	N10	W	W	W	-	D	D	-	+	+	-	+	-	+
Miscellaneous														
0.01% cycloheximide	O1	+	d	+	+	+	+	+	+	+	+	+	+	+
0.1 % cycloheximide	O2	+	d	+	+	+	+	+	+	d	+	+	+	+
50% D-glucose	O4	-	-	-	-	-	-	-	-	-	-	-	-	+
60% D-glucose	O5	-	-	-	-	-	-	-	-	-	-	-	-	+
10% NaCl	O6	+		+	+	-	-	-	-	-	-	-	-	-
16% NaCl	O7	-		-	-	-	-	-	-	-	-	-	-	-
Starch formation	M1	-	-	-	-	-	-	-	-	-	-	-	-	-
Urea hydrolysis	M3	+	+	+	+	+	+	+	+	+	+	+	+	+
Diazonium blue B	M4	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth at 30°C	T2	+	-	+	+	+	+	+	+	+	+	+	+	+
Growth at 37°C	T4	+	-	-	-	-	-	+	+	+	+	+	+	+

Species no.	S	1	2	3	4	5	6	7	8	9	10	11	12	13
Growth at 42°C	T6	-	-	-	-	-	-	-	-	-	-	-	-	-
Growth at 45°C	T7	-	-	-	-	-	-	-	-	-	-	-	-	-
Pink colony	E1	+	+	+	+	+	+	+	+	+	+	+	+	+
Budding	E2	+	+	+	+	+	+	+	+	+	+	+	+	+
Lemon-shaped cells	E3	-	-	-	-	-	-	-	-	-	-	-	-	-
Budding on stalk	E4	-	-	-	-	-	-	-	-	-	-	-	-	-
Splitting cells	E5	-	-	-	-	-	-	-	-	-	-	-	-	-
Filamentous	E6	-	-	-	-	-	-	-	-	-	-	-	-	-
Pseudohyphae	E7	-	+	-	-	+	-	+	+	+	+	+	+	+
Septate hyphae	E8	-	-	-	-	-	-	-	-	-	-	-	-	-
Arthroconidia	E9	-	-	-	-	-	-	-	-	-	-	-	-	-
Ballistoconidia	E10	-	-	-	-	-	-	-	-	-	-	-	-	-
Ascosporogenus	A1	-	-	-	-	-	-	-	-	-	-	-	-	-
Ascospores round	A2	-	-	-	-	-	-	-	-	-	-	-	-	-

+: growth; w: weak growth; d, delayed, -: no growth, gap: not tested.

Fermentation of Me-α-D glucoside, α, α Trehalose, melezitoze, Starch and D-xylose gave negative results with all species tested and were omitted from the table.

Rhodosporidium Banno

This genus was isolated infrequently from citrus air, grapevine phyllosphere, the phylloplane of both plantations, and grape carposphere, carpoplane, and juice only. Its highest percentage count was recorded from grapevine phylloplane (1.22 % of total fungi on each medium). *R. paludigenum* was recovered from both plantations while *R. diobovatum* from grapevine plantations only.

In the air, it was recorded in moderate or low frequency in citrus air contributing 0.13 % - 0.88 % of total fungi. It was missed in grapevine air.

In the soil, it was absent in both plantations.

In the phyllosphere, it was recovered in low frequency from grapevine phyllosphere on both media, contributing 0.24 % - 0.26 % of total fungi while it was missed in citrus phyllosphere.

In the phylloplane, it was encountered in rare frequency from both plantations on both media. It contributed lower percentage counts in citrus phylloplane (0.07 % - 0.08 % of total fungi) than those in grapevine phylloplane (1.22 % on each medium).

In the carposphere, it was identified in low or rare frequency in both fruits contributing (0.11 % - 0.12 % of total fungi). *R. paludigenum* and *R. diobovatum* were recovered from grape carposphere only.

In the carpoplane, it was recovered in rare frequency from grape carpoplane on DYM only, contributing 0.29 % of total fungi. It was not recorded in citrus carpoplane.

In the juice, it was isolated from grape juice in low frequency on both media, contributing less than 0.01 % of total fungi on. It was missed in citrus juice. *R. paludigenum* was repored earlier from sea-water, mangrove swamp and black-rush marsh in Florida, USA, and *R. diobvatum* from little Shark River, sea-water and clover *Trifolium repens* in USA, soil in Italy, cherry blossom in France (refer to Barnett *et al.* 2000).

Strains tested

***Rhodosporidium diobovatum* S. W. Newell & I. L. Hunter**

AUMC 7252 (Plate 32).

***Rhodosporidium paludigenum* Fell & Statzell Tallman**

AUMC 7783, AUMC 7789 (Plate 33).

Sporidiobolus Nyland

This genus was recorded infrequently from the air of both plantations, and grapevine phyllosphere, phylloplane, and fruit juice only. Its highest percentage count was recorded from citrus air (0.05 % - 1.14 % of total fungi) and grapevine phylloplane (0.28 % - 0.44 %). *S. ruineniae* was recovered from both plantations while *S. pararoseus* from grapevine plantations only.

In the air, it was recovered in moderate or low frequency from citrus air while in rare frequency on both media in grapevine air, contributing more percentage counts (0.05 % - 1.14 % of total fungi) in citrus air than those in grapevine air (0.04 % on each medium). It was represented by *S. ruineniae* on both plantations.

In the soil, it was missed in both plantations.

In the phyllosphere, it was isolated in low frequency from grapevine phyllosphere on both media, contributing 0.14 % - 0.16 % of total fungi. It was missed in citrus phyllosphere.

S. pararoseus was recorded in low or rare frequency while *S. ruineniae* was recorded in rare frequency on DRBC only. It was not recorded from citrus phyllosphere.

In the phylloplane, it was recorded in rare frequency from grapevine phylloplane on both media, contributing more percentage counts (0.28 % - 0.44 % of total fungi) than those in grapevine phyllosphere. *S. ruineniae* and *S. pararoseus* were isolated in rare frequency. It was missed in citrus phylloplane and carposphere and carpoplane of both plants.

In the fresh juice, it was encountered in moderate or low frequency from grape juice contributing 0.002 % - 0.003 % of total fungi. *S. ruineniae* and *S. pararoseus* were isolated in low frequency from grape juice. It was misssed in citrus juice. *S. ruineniae* was reported earlier from herbaceous culm in Jamaica, dung of goat in Pakistan, leaves of *Malphigia coccigera* in Indonesia, and *S. pararoseus* from soil in Russia, oil brine in Yabase oil field, *Fragaria* sp. and soil in Japan, sea water from Atlantic Ocean off Florida and atmosphere in USA, barley (refer to Barnett *et al.* 2000).

Strains tested

***Sporidiobolus pararoseus* Fell & Tallman**

AUMC 7791.

Sporidiobolus ruineniae* Holzschu *et al.

AUMC 7773 (Plates 34 & 35), AUMC 7781.

Sporobolomyces roseus Kluyver & van Niel

This genus was represented by *S. roseus* only. It was recorded infrequently from citrus phyllosphere only and all sources in grapevine plantations except soil. Its highest percentage count was recorded from grapevine carpoplane (0.29 % - 0.69 % of total fungi) and grapevine carposphere (0.19 % - 0.31 %).

In the air, it was recorded in rare frequency from grapevine air on both media contributing 0.01 % - 0.06 % of total fungi. It was missed in citrus air and the soil of both plantations.

Sporobolomyces was isolated from the aerospora of Hong Kong (Turner 1966), and aerospora in Jamaican banana plantations (Meredith 1962)

In the phyllosphere, it was recovered in rare frequency from grapevine phyllosphere on both media and in rare frequency on DYM only in citrus phyllosphere, contributing less

than 0.01 % of total fungi on both media in grapevine phyllosphere and 0.002 % of total fungi in citrus phyllosphere.

Sporobolomyces roseus was isolated from the phylosphere of *Bauhinia forficate*, *Tabebuia* sp. and *Terminalia catappa*, southeastern Brazil (Valarini *et al.* 2007).

In the phylloplane, it was recorded in rare frequency from grapevine phylloplane on both media, but was absent in citrus phylloplane.

Sporobolomyces roseus appeared to be prevalent in the phylloplane regardless of plant type or geography (Bai *et al.* 2002, Fell *et al.* 2002, Inacio *et al.* 2002, Maksimova and Chernov 2004).

In the carposphere, it was recovered in low frequency from grape carpoplane on both media constituting lower percentage counts (0.19 % - 0.31 % of total fungi) than those in grapevine carpoplane. It was missed in citrus carposphere.

Sporobolomyces was isolated from different angiosperm fruits, southeastern Brazil (Prada and Pagnocca 1997).

In the carpoplane, it was isolated from grape carpoplane in rare frequency on both media, contributing 0.29 % - 0.69 % of total fungi. It was not recorded in citrus carpoplane.

Sporobolomyces roseus was a part of the natural microbiota of certain varieties of grapes in southern Spain (De la Torre *et al.* 1999).

In the fresh juice, it was recovered in low frequency on both media, contributing 0.01 % - 0.002 % of total fungi. It was absent in citrus juice.

***Sporobolomyces roseus* Kluyver & van Niel**

Strain tested

AUMC 7788 (Plate 36).

Trichosporon Behrend

This genus was isolated only from the air of both plantations, and citrus phyllosphere, and phylloplane. *T. japonicum* was isolated from the air of citrus plantations while *T. asahii* from grapevine only.

In the air, it was recorded in rare frequency from grapevine air on DYM only contributing 0.01 % of total fungi, represented by *T. asahii*. It was missed in citrus air and the soil of both plantations.

In the phyllosphere, it was identified in rare frequency from citrus phyllosphere on both media, represented by *T. japonicum*, donating 0.01 % of total fungi on each medium. It was missed in grapevine phyllosphere.

In the phylloplane, it was recovered in rare frequency from citrus phylloplane on DYM only, represented by *T. japonicum*, contributing 0.12 % of total fungi. It was missed in grapevine phylloplane.

Trichosporon was one of the predominant yeasts found on sugarcane leaves in Rio de Janeiro, Brazil (Azeredo *et al.* 1998), plant surfaces (Phaff and Starmer 1987, Babjéva and Chernov 1995, Santos *et al.*, 1996),

T. asahii caused hematogenous dissemination in patients with impaired innate immunity (Gueho *et al.* 1994, Sugita *et al.* 1995, Itoh *et al.* 1996). It has also been reported from skin lesions (Hoog *et al.* 2000).

Strains tested

Trichosporon asahii* Akagi ex Sugita *et al.

AUMC 7779 (Plate 37).

***Trichosporon japonicum* Sugita &Nakase**

AUMC 7797, AUMC 7800.

Table 18. Physiological comparison of the strains tested of the basidiomycetous genus *Rhodosporidium, Sporidiobolus, Sporobolomyces* and *Trichosporon*: **1** *R. diobovatum* AUMC 7252, **2** *R. paludigenum* AUMC 7783, **3** *R. paludigenum* AUMC 7789, **4** *Sporidiobolus pararoseus* AUMC 7791, **5** *S. ruineniae* AUMC 7773, **6** *S. ruineniae* AUMC 7781, **7** *Sporobolomyces roseus* AUMC 7788, **8** *T. asahii* AUMC 7779, **9** *T. japonicum* AUMC 7797**, 10** *T. japonicum* AUMC 7800.

Species no.	**S**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Fermentation											
D- glucose	F1	-	-	-	-	-	-	-	-	-	-
Assimilation											
D-glucose	C1	+	+	+	+	+	+	+	+	+	+
D-galactose	C2	+	d	+	w	d	+	w	+	+	+
L-sorbose	C3	+	d	d	d	+	+	+	d	d	-
D-ribose	C5	d	+	d	-	+	+	+	+	+	-
D-xylose	C6	-	+	+	d	+	+	d	+	+	+
L-arabinose	C7	-	-	-	-	-	-	-	+	+	+

Species no.	**S**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
L-rhamnose	C9	-	d	+	-	d	+	-	+	-	+
Sucrose	C10	+	+	+	+	+	+	+	+	+	+
Maltose	C11	+	d	+	+	d	+	+	+	+	+
α, α-trehalose	C12	+	+	+	+	+	+	+w	+	+	+
Methyl-α-D-glucoside	C13	-	d	+	+w	d	-	d	+	+	+
Cellobiose	C14	-	-	-	-	-	-	-	+	+	+
Lactose	C18	w	-	-	-	-	-	-	+	+	
Melezitose	C20	+	-	+	+	-	-	+	+	+	+
Inulin	C21	d	+	+	+	d	+	d	+w	d	+
Soluble starch	C22	+	-	-	+	-	-	d	+	+	+
Meso-erythritol	C24	-	-	-	-	-	-	+	+	+	+
D-glucitol	C28	d	+	+	d	+	+	d	+	+	d
D-mannitol	C29	+	+	+	+	+	+	-	d	d	+
Galactitol	C30	-	+	+	-	+	+	-	-	-	-
Myo-inositol	C31	-	-	-	d	-	-	-	+	d	-
Glucono-d-lactone	C32	+	+	+	d	+	+	d	+	d	-
D-glucuronate	C36	-	-	-	d	-	-	d	+	+	-
D-galacturonate	C37	w	-	-	-	+	d	-	-	-	-
Citrate	C40		+	d	d	+	d	d	+	+	+
Methanol	C41	-	-	-	-	-	-	-	-	-	d
Ethanol	C42	d	+	+	+	+	+	d	+	+	+
Propane 1,2 diol	C43	-	-	-	-	-	-	-	-	-	d
Butane 2,3 diol	C44	-	-	-	-	-	-	-	d	d	d
Quinic acid	C45	+	+	+	+	+	+	+	-	-	+
Nitrogen compounds											
Nitrate	N1	+	+	+	-	+	+	+	-	-	-
Nitrite	N2	+	+	+	-	+	+	w	+	-	-
Ethylamine	N3	+	+	+	w	+	+	w	-	-	+
L-lysine	N4	d	-	-	-	+	+	-	+	+	+
Creatine	N6	-	-	-	-	-	-	-	-	-	-
Creatinine	N7	-	-	-	-	-	-	-	-	-	-
D-glucosamine	N8	w	-	+	-	-	-	-	-	-	w
Imidazole	N9	-	-	-	-	-	-	-	-	-	-
D-tryptophane	N10	-	w	-	-	-	-	-	+	-	-

Species no.	**S**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Miscellaneous											
0.01% cycloheximide	O1	d	+	+	+	d	d	+	+	+	+
0.1 % cycloheximide	O2	d	+	+	d	d	d	w	+	+	+
50% D-glucose	O4	-	-	-	+	-	-	-	-	+	-
60% D-glucose	O5	-	-	-	-	-	-	-	-	+	-
10% NaCl	O6	-	-	+	+	-	-	-	-	+	+
16% NaCl	O7	-	-	-	-	-	-	-	-	+	+
Starch formation	M1	-	-	-	-	-	-	-	+	+	-
Urea hydrolysis	M3	+	+	+	+	+	+	+	+	+	+
Diazonium blue B	M4	+	+	+	+	+	+	+	+	+	+
Growth at 30°C	T2	-	+	+	+	+	+	-	+	+	+
Growth at 37°C	T4	-	+	-	-	-	-	-	+	+	+
Growth at 42°C	T6	-	-	-	-	-	-	-	-	-	-
Growth at 45°C	T7	-	-	-	-	-	-	-	-	-	-
Pink colony	E1	+	+	+	+	+	+	+	-	-	-
Budding	E2	+	+	+	+	+	+	+	+	+	+
Lemon-shaped cells	E3	-	-	-	-	-	-	-	-	-	-
Budding on stalk	E4	-	-	-	+	-	-	+	-	-	-
Splitting cells	E5	-	-	-	-	-	-	-	+	+	+
Filamentous	E6	-	-	-	-	-	-	-	+	+	+
Pseudohyphae	E7	+	-	-	-	-	-	-	-	-	-
Septate hyphae	E8	-	-	-	-	-	-	-	+	+	+
Arthroconidia	E9	-	-	-	-	-	-	-	+	+	+
Ballistoconidia	E10	-	-	-	-	-	-	-	-	-	-
Ascosporogenus	A1	-	-	-	-	-	-	-	-	-	-
Ascospores round	A2	-	-	-	-	-	-	-	-	-	-

+: growth; w: weak growth; d, delayed, -: no growth, gap: not tested.

Fermentation of Me-α-D glucoside, α, α Trehalose, melezitoze, Starch and D-xylose gave negative results with all species tested and were omitted from the table.

Table 19. Assiut University Mycological Centre accession number (AUMC) of basidiomyceteous yeast strains and their isolation sources with their accession GenBank numbers given together with the closest match in the GenBank database and sequence similarity in percent to the match as inferred from Blastn searches of ITS sequences.

AUMC number	Isolation source	Accession number	Length (bp)	Closest Genbank match # ITS	Sequencing similarity (%)	Species	References
7784	Grapevine leaf	JQ425387	590	AF145331 = ATCC 34633 AF145325 = CBS 7711^T	99	*Cryptococcus albidosimilis*	Scorzetti *et. al.* 2000, 2002
7790	Grapevine leaf	JQ425398	538	EU149786= CBS 10755 EU149785= CBS 10634 DQ317359 = BC43	99	*Cryptococcus carnescens Cryptococcus carnescens Cryptococcus antarcticus*	Connell *et al*. 2008, Arenz *et al*. 2006
7794	Grapevine air	JQ425400	539	FN428902 = IMUFRJ 51986 AM176643	99	*Cryptococcus flavescens*	Molnár & Prillinger 2005
7798	Grapevine soil	JQ425403	547	FN561807 = SEG-8-9 AF410468=CBS 139^T	99 98	*Cryptococcus laurentii*	Scorzetti *et al*. 2002
7799	Grapevine soil	JQ425407	665	FN561807 = SEG-8-9 AF410468=CBS 139^T	90 89	*Cryptococcus laurentii*	Scorzetti *et al*. 2002
7246	Grapevine fruit	JQ425371	661	EU871517 = S22814 AF190008= CBS 140^T	99	*Cryptococcus magnus*	Fell *et al*. 2000
7772	Citrus leaf	JQ425367	623	AF190008 = CBS 140^T EU480310 = CS11M5c59P	99 100	*Cryptococcus magnus*	Fell *et al*. 2000

AUMC number	Isolation source	Accession number	Length (bp)	Closest Genbank match # ITS	Sequencing similarity (%)	Species	References
7793	Grapevine air	JQ425369	612	AF190008 = CBS 140^{T} AF190009 = CBS 4685	89	*Cryptococcus magnus*	Fell *et al*. 2000
7777	Grape juice	JQ425364	623	AF444635 = CBS 9070 AF444541 = CBS 316 ^{T}	99 98	*Rhodotorula mucilaginosa*	Scorzetti *et al*. 2002
7780	Grapevine leaf	JQ425405	597	EU853846= ATCC 66034 AF444541= CBS 316^{T}	100 99	*Rhodotorula mucilaginosa*	Scorzetti *et al*. 2002
7796	Grape juice	JQ425366	606	AF444635 = CBS 9070 AF444541 = CBS 316 ^{T}	99	*Rhodotorula mucilaginosa*)	Scorzetti *et al*. 2002
7785	Citrus air	JQ425368	777	AY740040	96	*Melanopsichium pennsylvanicum*	Stoll *et al*. 2005
7787	Citrus leaf	JQ425372	758	HQ848933 = HX6610 AF294699 = CBS 517.83^{T} AF294697 = CBS 170.88	99	*Pseudozyma aphidis*	
7786	Citrus air	JQ425374	987	DQ008954 =CBS 10077^{T}	98	*Pseudozyma hubeiensis*	Wang *et. al*. 2006
7774	Grapevine air	JQ425397	618	HQ670677 EF194846 = MCCC2E00215	99	*Rhodotorula glutinis*	Yang *et al*. 2011
7776	Citrus air	JQ425370	618	HQ670677	99	*Rhodotorula glutinis*	Yang *et al*. 2011
7248	Citrus fruit	JQ425393	628	HQ909092 = KDLYC24-1	99	*Rhodotorula mucilaginosa*	Scorzetti *et al*. 2002

AUMC number	Isolation source	Accession number	Length (bp)	Closest Genbank match # ITS	Sequencing similarity (%)	Species	References
				AF444635 = CBS 9070 AF444541= CBS 316^{T}			
7778	Citrus air	JQ425392	629	HQ909092 = KDLYC24-1 AF444541= CBS 316^{T}	99	*Rhodotorula mucilaginosa*	Scorzetti *et al*. 2002
7795	Grapevine leaf	JQ425396	626	HQ702343 = UOA/HCPF 10538 AF444541 = CBS 316 ^{T}	99	*Rhodotorula mucilaginosa*	Scorzetti *et al*. 2002
7782	Grapevine air	JQ425399	633	HQ909092 = KDLYC24-1 AF444541 = CBS 316 ^{T}	99 98	*Rhodotorula mucilaginosa*	Scorzetti *et al*. 2002
7783	Citrus air	JQ425395	616	AF444493= CBS 6567 AF444492= CBS 6566^{T}	99 99	*Rhodosporidium paludigenum* (Anamorph: *Rhodotorula graminis*)	Scorzetti *et al*. 2002
7789	Grapevine leaf	JQ425404	614	HQ670676 AF444493 = CBS 6567 AF444492 = CBS 6566^{T}	99	*Rhodosporidium paludigenum* (Anamorph: *Rhodotorula graminis*)	Scorzetti *et al*. 2002
7791	Grapevine leaf	JQ425362	603	AF417115 = CBS 484 AY015429 = CBS 491^{T}	99	*Sporidiobolus pararoseus*	Fell *et al*. 2002
7788	Citrus air	JQ425365	582	AY015435 =	90	*Sporidiobolus*	Valerio *et*

AUMC number	Isolation source	Accession number	Length (bp)	Closest Genbank match # ITS	Sequencing similarity (%)	Species	References
				CBS 5541 EU003482 =CBS 7683^{T}	89	*metaroseus (*anamorph: *Sporobolomyces roseus*)	*al*. 2008
No 15 (dead)	Grapevine air	JQ425363	608	AY070006 = AS 2.2108 EU003482 = CBS 7683^{T}	100 99	*Sporidiobolus metaroseus* (anamorph: *Sporobolomyces roseus*)	Valerio *et al*. 2008
7773	Citrus air	JQ425373	613	AY015433 = CBS 5001^{T} AF444491 = CBS 5811	99	*Sporidiobolus ruineniae* (anamorph: *Sporobolomyces coprophilous*)	Fell *et al*. 2002
7781	Citrus air	JQ425394	610	AY015433 = CBS 5001^{T} AF444491= CBS 5811	99 99	*Sporidiobolus ruineniae* (anamorph: *Sporobolomyces coprophilous*)	Fell *et al*. 2002
7779	Grapevine air	JQ425402	553	AM900369 = YS124 FJ943429 = CBS 2479^{T}	99	*Trichosporon asahii*	
7797	Citrus leaf	JQ083438	520	AF444473 = CBS 8641^{T} EU863543 = PUMCHBY27	100	*Trichosporon japonicum*	Scorzetti *et al*. 2002
7800	Citrus leaf	JQ425388	549	AF444473 = CBS 8641^{T} EU863543 = PUMCHBY27	99	*Trichosporon japonicum*	Scorzetti *et al*. 2002

Black yeasts

They were isolated in rare frequency on DYM from citrus phyllosphere contributing 0.004 % of total fungi and from grape carpoplane on both media constituting 0.27 % - 0.44 % of total fungi. However, black yeast isolates were prevalent in pineapple fruit and leaves in Rio de Janeiro, Brazil (Robbs *et al.* 1989).

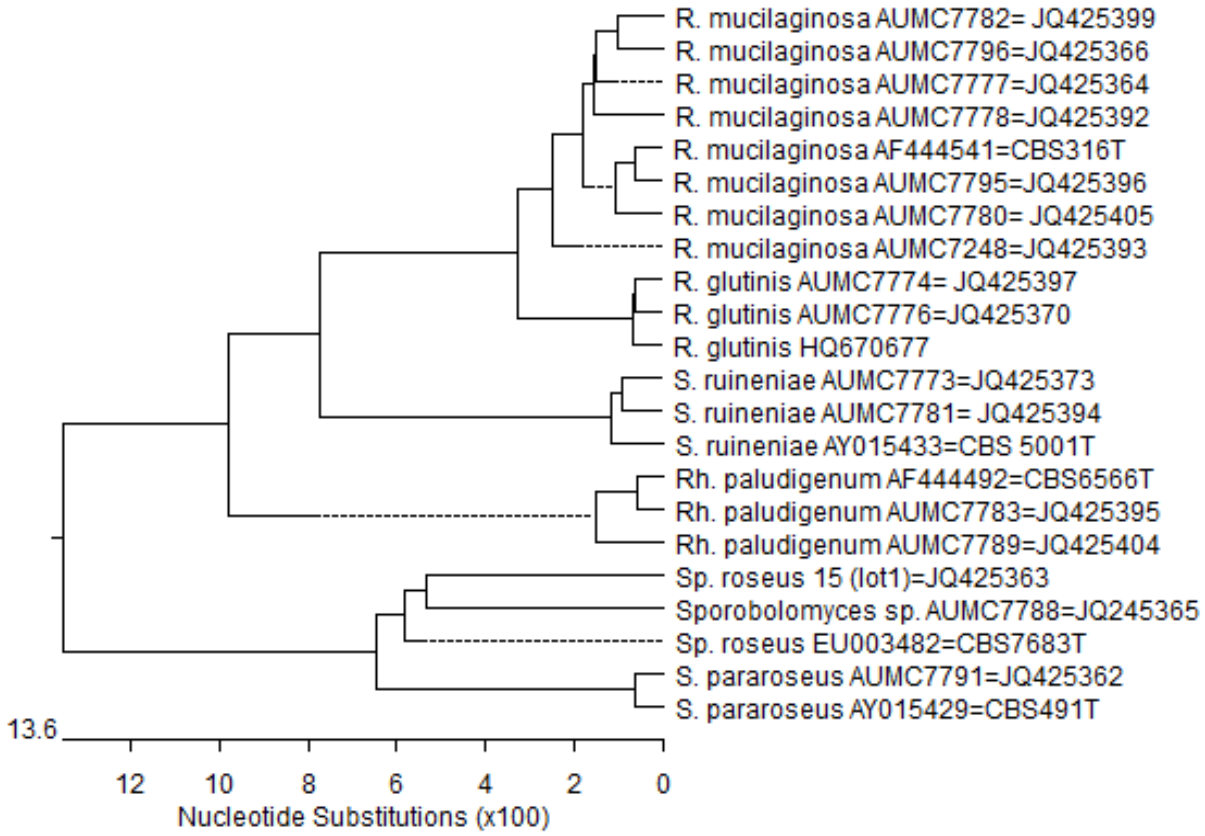

Figure 18. Phylogenetic tree for red basidiomyceteous yeast strains (*R.* = *Rhodotorula*, *Rh.* = *Rhodosporidium*, *S.* = *Sporidiobolus*, *Sp.* = *Sporobolomyces*). The scale indicates the number of nucleotide substitutions per site.

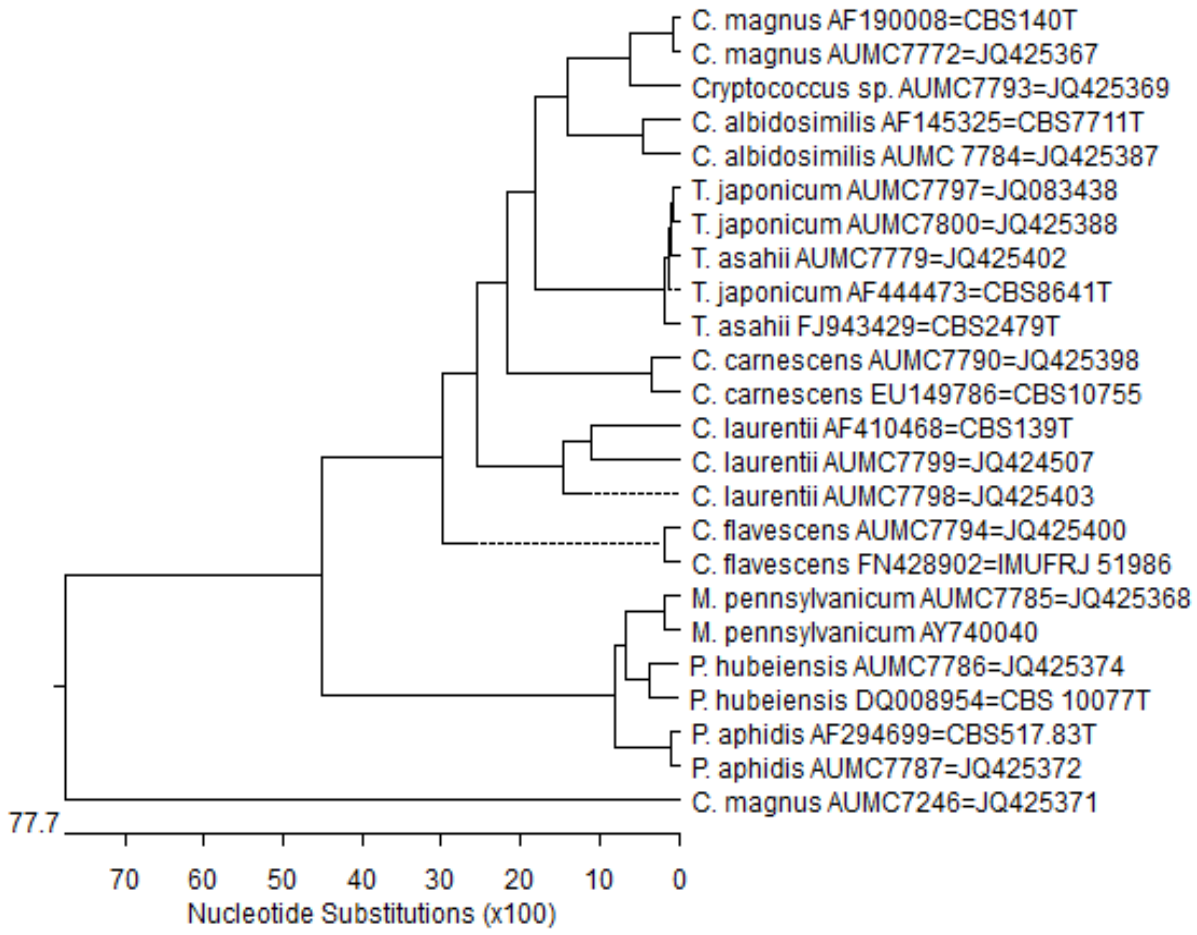

Figure 19. Phylogenetic tree for white basidiomyceteous yeast strains (*P.* = *Pseudozyma*, *M.* = *Melanopsichium*, *C.* = *Cryptococcus*, *T.* = *Trichosporon*). The scale indicates the number of nucleotide substitutions per site.

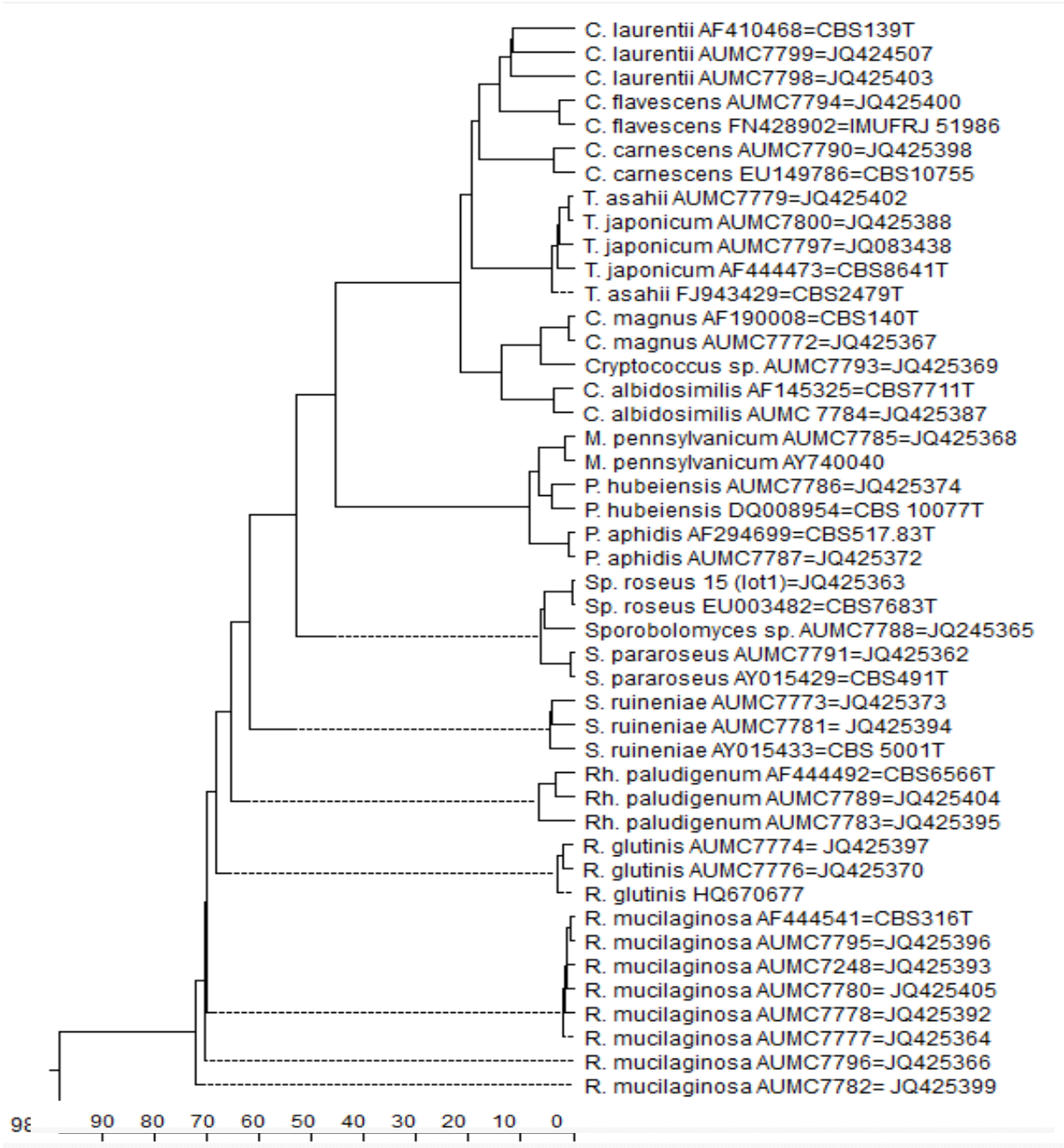

Figure 20. Phylogenetic tree for basidiomyceteous yeast strains (C = *Cryptococcus*, *T.* = *Trichosporon*, *M.* = *Melanopsichium*, *P.* = *Pseudozyma*, *S.* = *Sporidiobolus*, *Sp.* = *Sporobolomyces*, *R.* = *Rhodotorula*, *Rh.* = *Rhodosporidium*). The scale indicates the number of nucleotide substitutions per site.

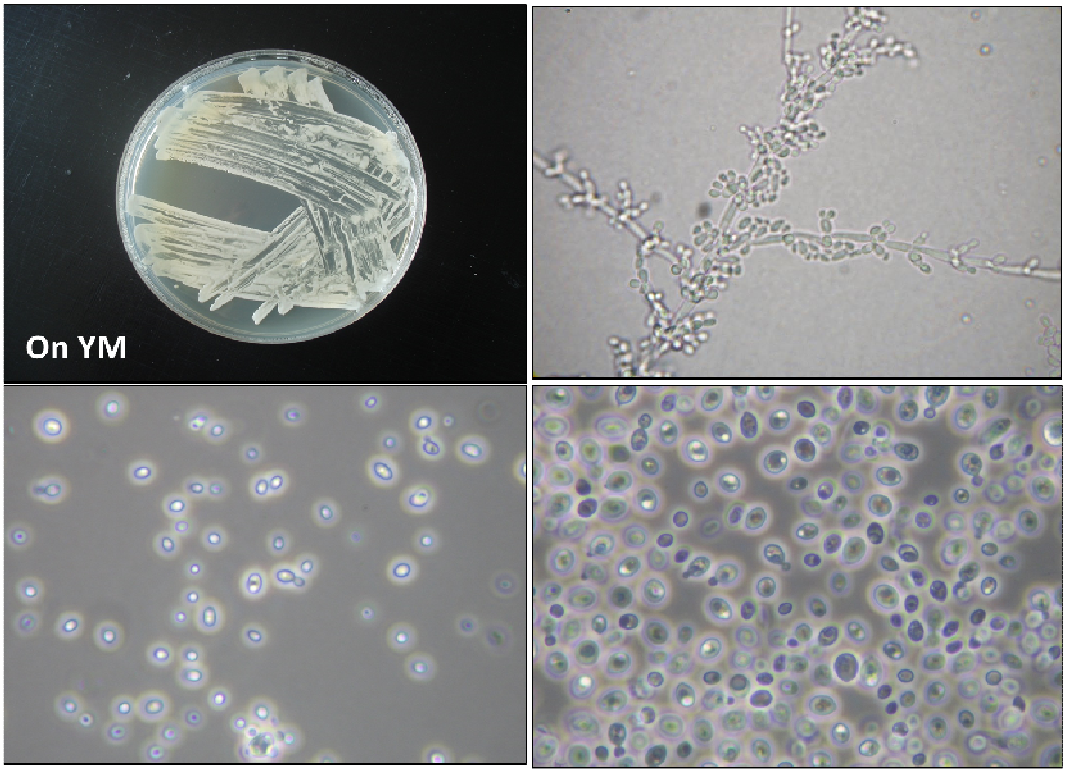

Plate 19. ***Cryptococcus albidosimilis*** AUMC 7784, pseudohyphae (top right) and budding cells, phase constrat (bottom), (budding cells 6.6 x 4.9 µm, Kurtzman & Fell 1999).

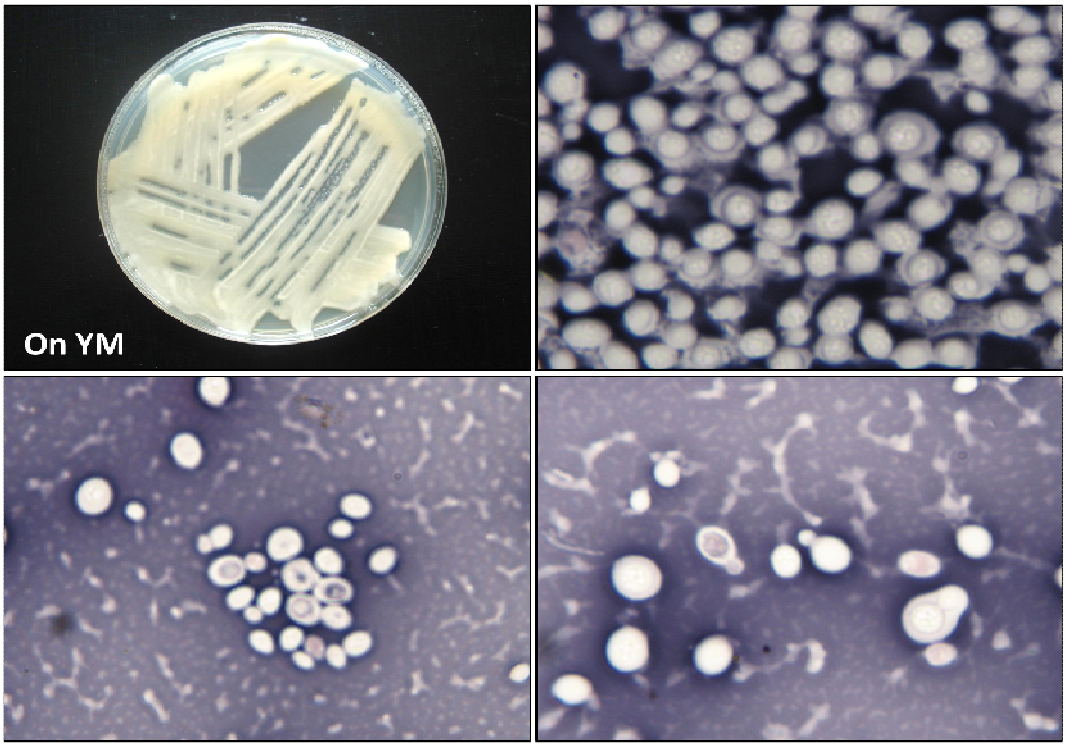

Plate 20. ***Cryptococcus laurentii*** AUMC 7799, capsules around the budding cells (PH= Phase contrast, top right and bottom), (budding cells 2-5.5 x 3-7 µm, Kurtzman & Fell 1998).

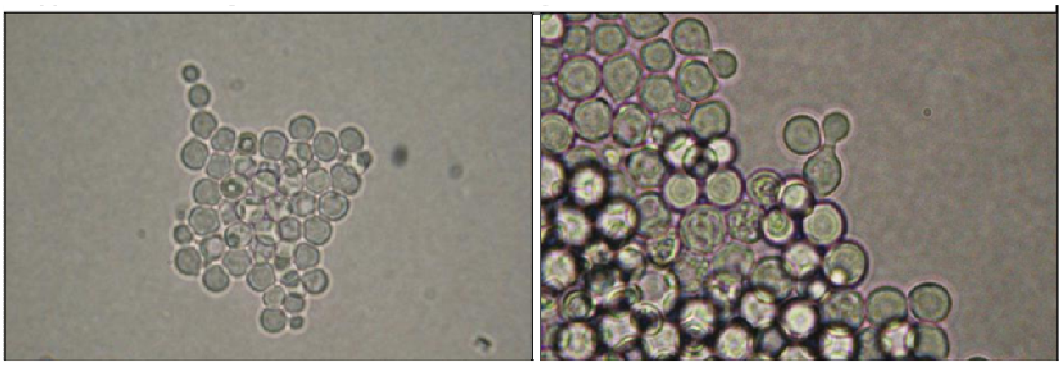

Plate 21. ***Cryptococcus laurentii*** AUMC 7798, budding cells, (budding cells 2-5.5 x 3-7 µm, Kurtzman & Fell 1998).

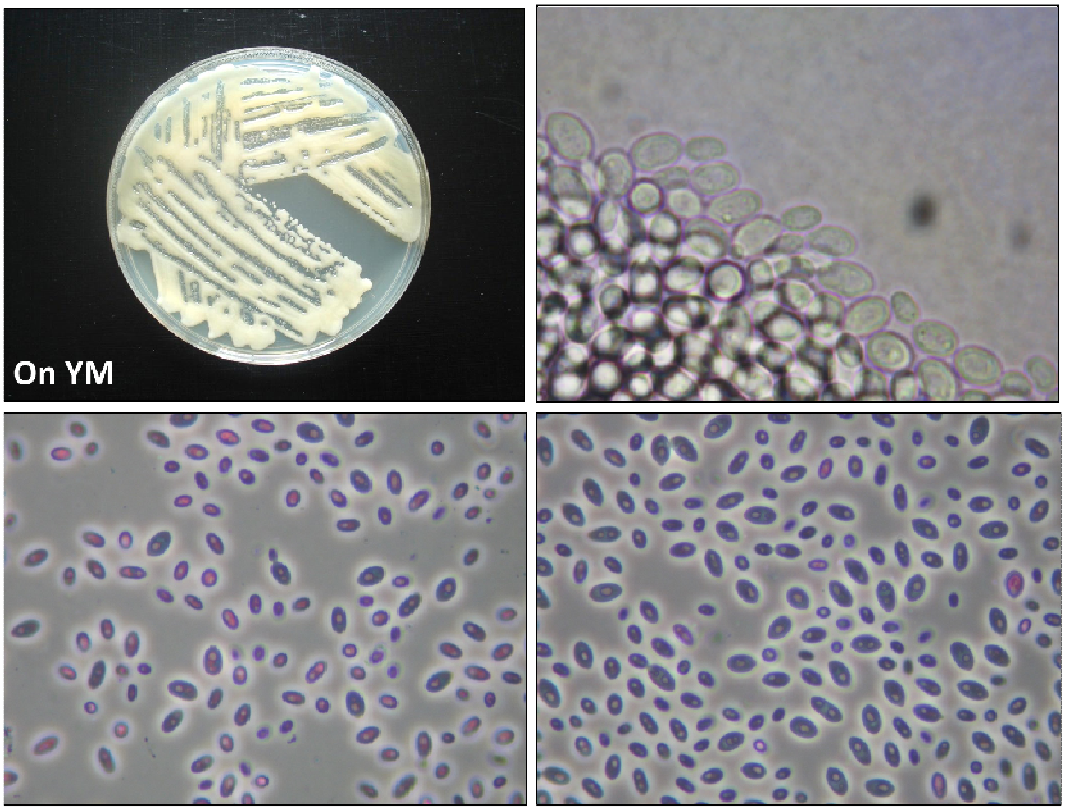

Plate 22. ***Cryptococcus luteolus*** AUMC 7291, budding cells (top right, PH= Phase contrast, bottom), (budding cells 3.1-6 x 5.5-9 µm, Kurtzman & Fell 1998).

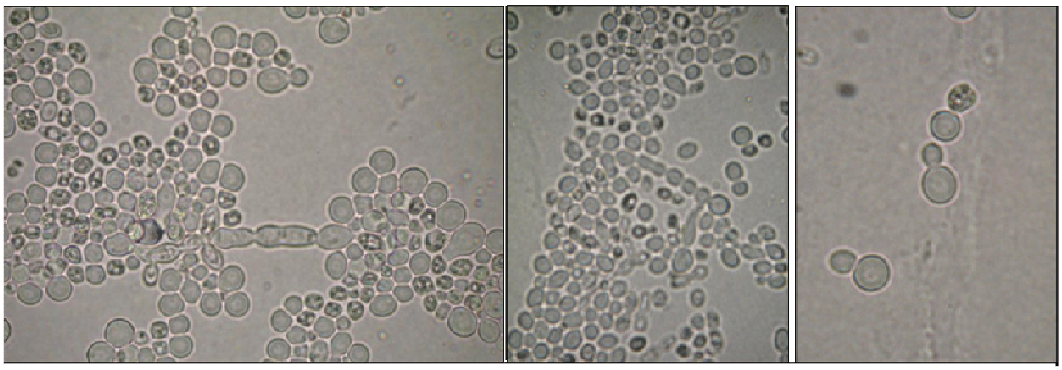

Plate 23. ***Cryptococcus magnus*** AUMC 7772, budding cells, (budding cells 3.5-15 x 4.5-45 µm, Kurtzman & Fell 1998).

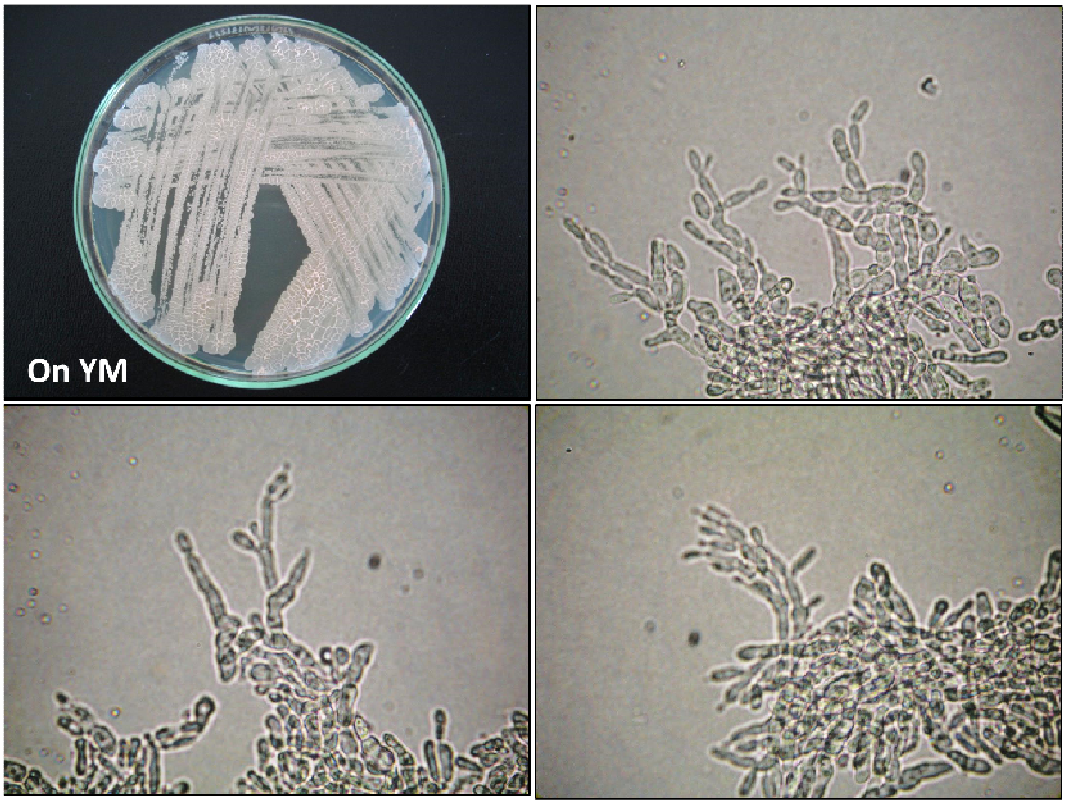

Plate 24. ***Melanopsichium pennsylvanicum*** AUMC 7785: pseudomycelium and budding cells, (budding cells 7.5–15.0 × 6–11 µm).

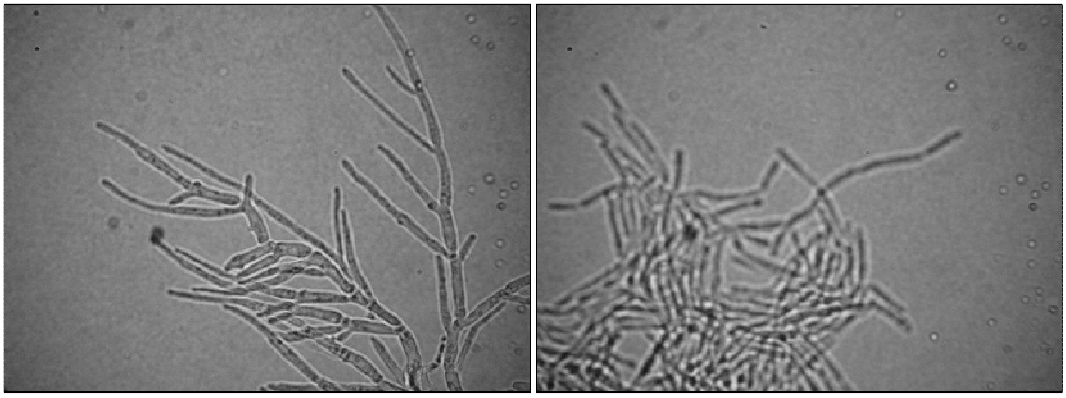

Plate 25. ***Pseudozyma aphidis*** AUMC 7787: true mycelium, (mycelium 30-50 x 2-3 µm, Kurtzman & Fell 1998).

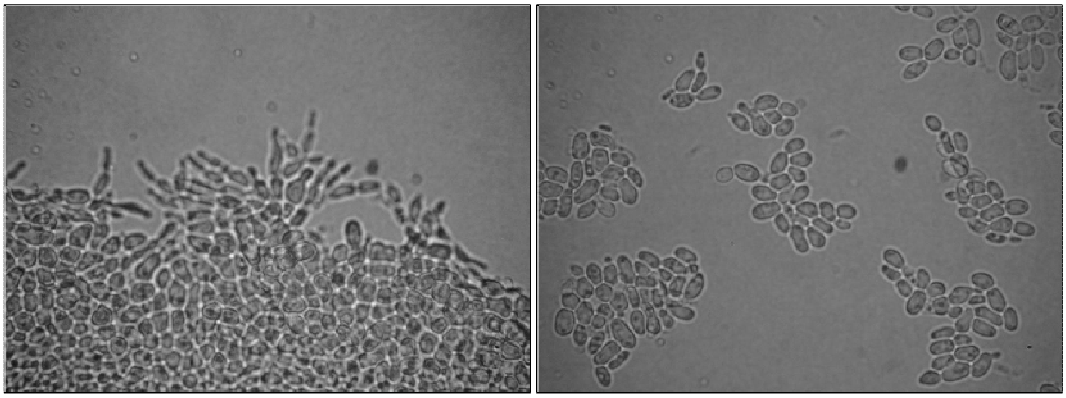

Plate 26. ***Pseudozyma hubeiensis*** AUMC 7786: pseudomycelium and budding cells, (budding cells 2.0–3.7 x 5.0–10.0 mm, Wang *et al.* 2006).

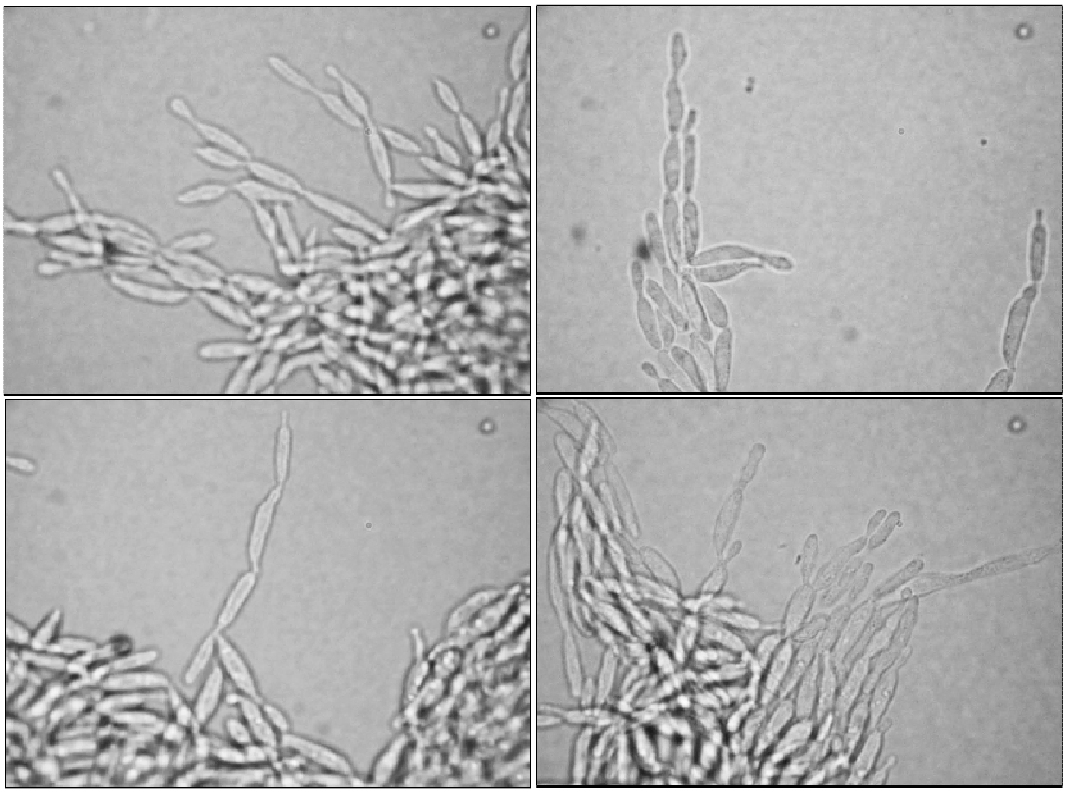

Plate 27. ***Pseudozyma rugulosa*** AUMC 7240: pseudohyphae and budding cells, (budding cells 8.0-20.0 x 2.0-2.5 µm, Kurtzman & Fell 1998).

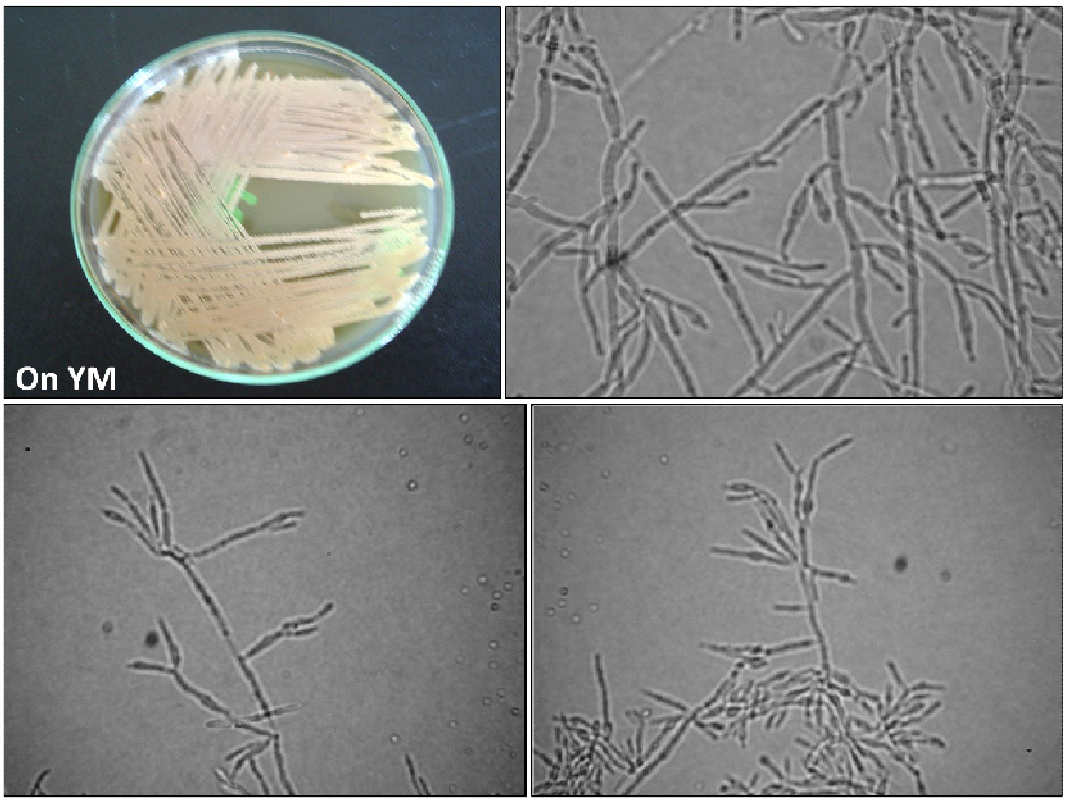

Plate 28. ***Pseudozyma* sp.** AUMC 7256: true mycelium.

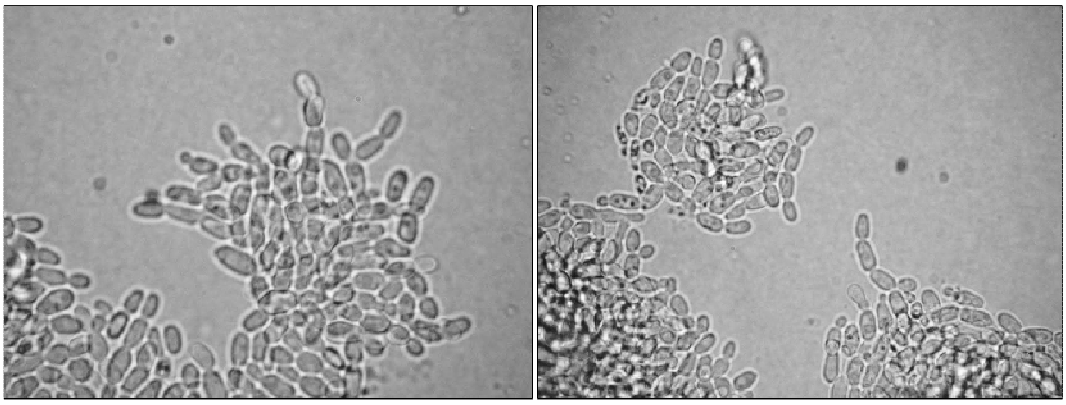

Plate 29. ***Rhodotorula aurantiaca*** AUMC 7253: pseudomycelium and budding cells, (budding cells cylindrical 3.0-.0 x 6.0-13.0 µm, Kurtzman & Fell 1998).

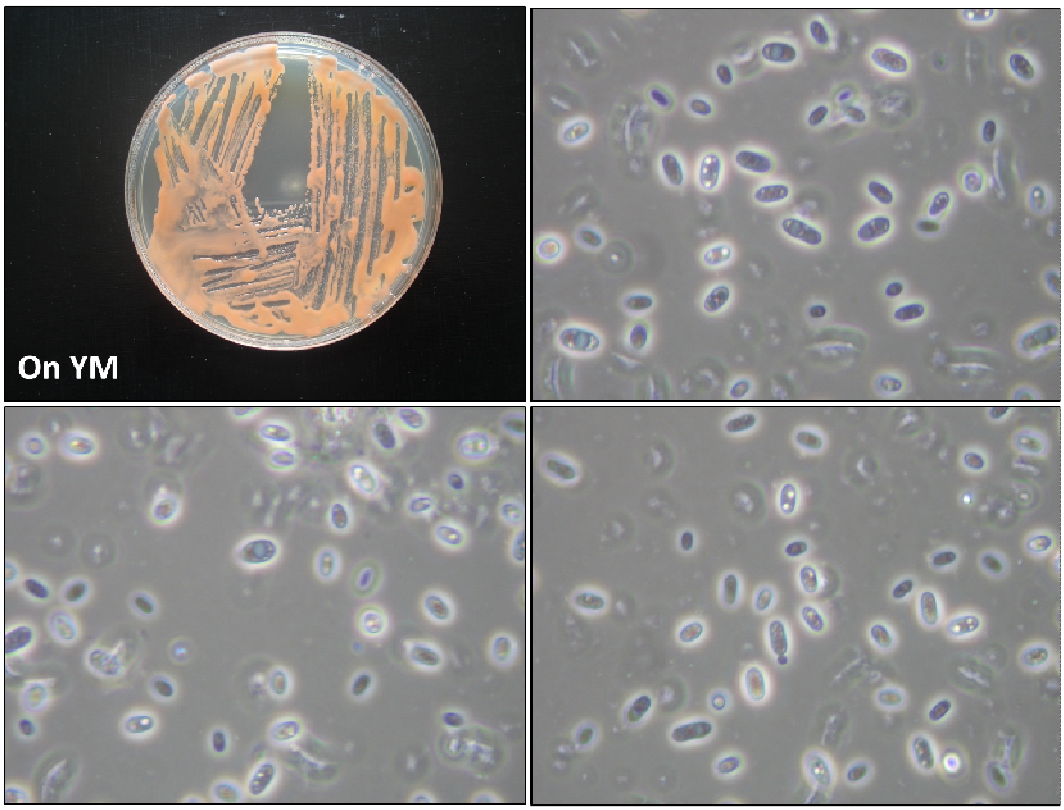

Plate 30. ***Rhodotorula glutinis*** AUMC 7774: on YM, and budding cells (Phase contrast), (budding cells ovoidal to globose 2.3-5.0 x 4.0-10.0 µm, Kurtzman & Fell 1998).

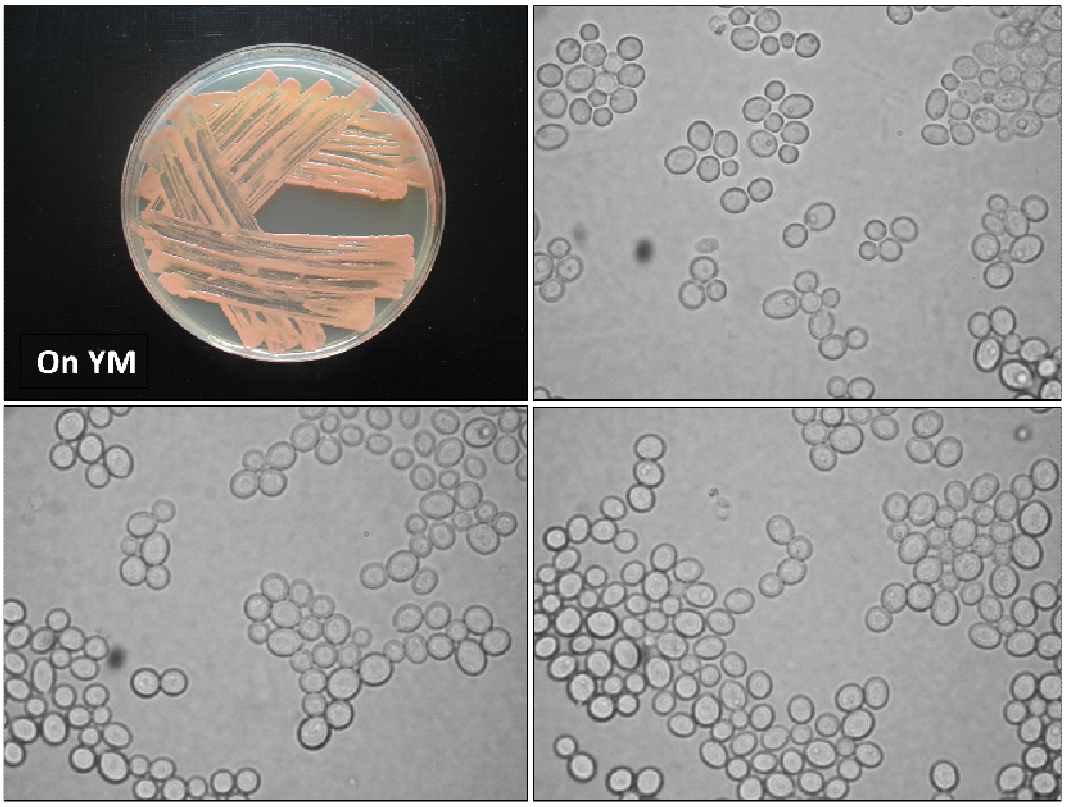

Plate 31. ***Rhodotorula mucilaginosa*** AUMC 7778: on YM, and budding cells, (budding cells ovoidal o spherical 2-8 x2-12 µm, Kurtzman & Fell 1998).

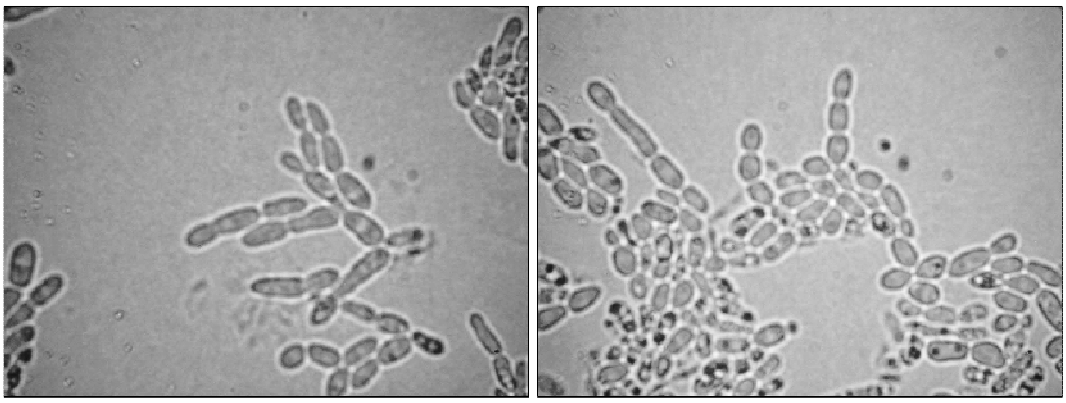

Plate 32. ***Rhodosporidium diobovatum*** AUMC 7252: Pseudomycelium and budding cells, (budding cells round to ovoid 1-6 x 2-9 µm, Kurtzman & Fell 1998).

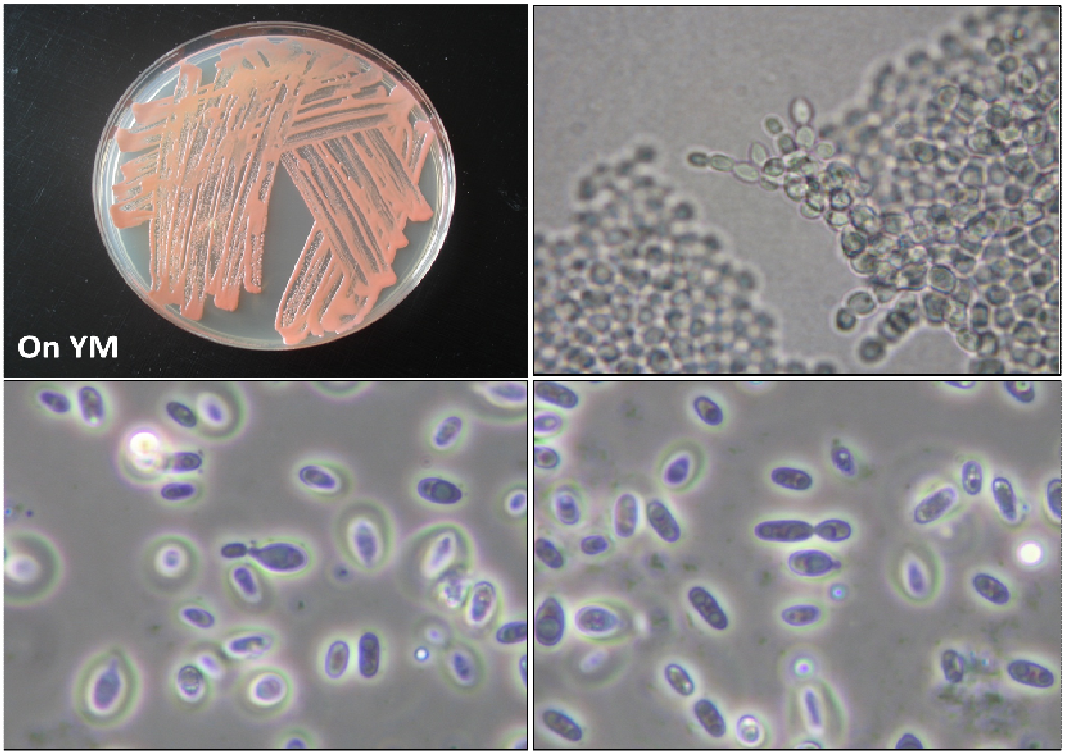

Plate 33. ***Rhodosporidium paludigenum*** AUMC 7789: budding cells (Top right and Phase contrast bottom), (budding cells ovoid to elongate 2-4 x 3-11 µm, Kurtzman & Fell 1998).

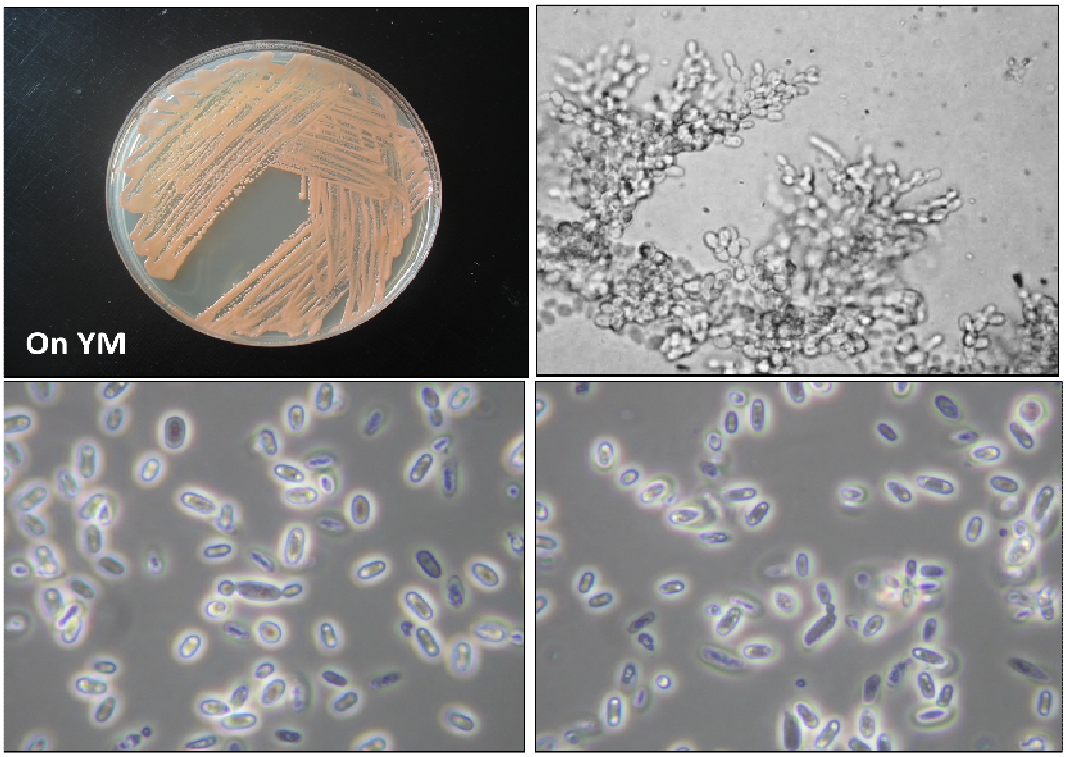

Plate 34. ***Sporidiobolus ruineniae*** AUMC 7773: on YM, and budding cells (top right and Phase contrast bottom, budding cells cylindrical or ovoidal 2-9 x 6-13 µm, Kurtzman & Fell 1998).

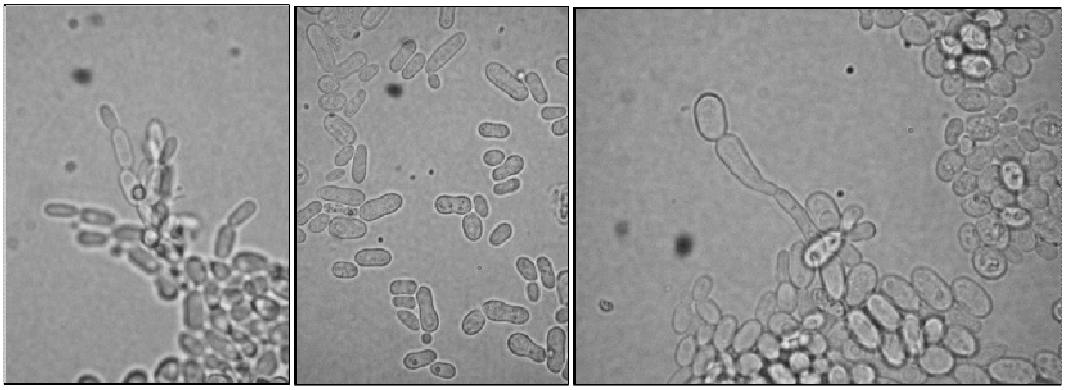

Plate 35. *Sporidiobolus ruineniae* AUMC 7773, pseudomycelium and budding cells, (budding cells cylindrical or ovoidal 2-9 x 6-13 µm, Kurtzman & Fell 1998).

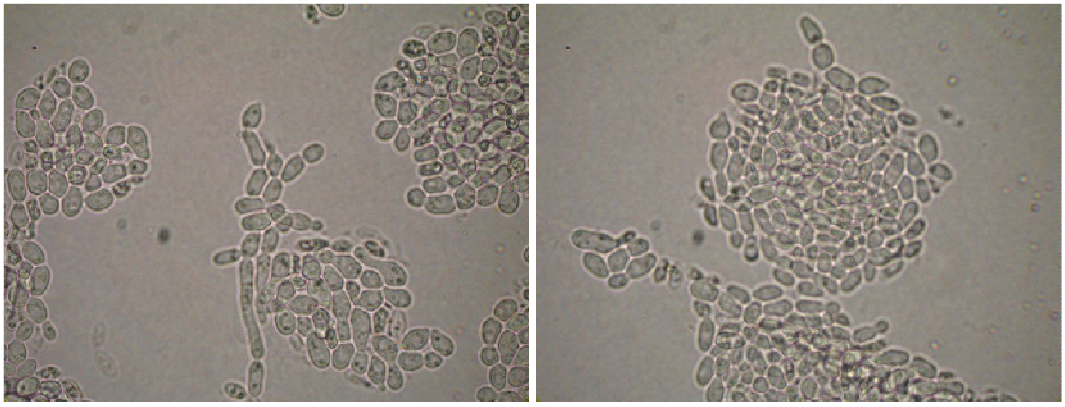

Plate 36. ***Sporobolomyces roseus*** AUMC 7788: pseudomycelium and budding cells, (budding cells ellipsoidal to cylindrical 9.0-24.0 x 2.0-3.0 µm, Kurtzman & Fell 1998).

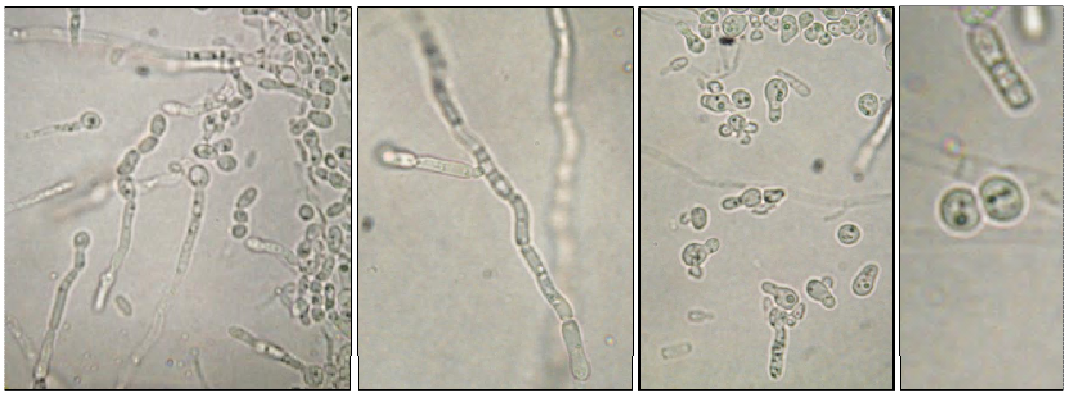

Plate 37. ***Trichosporon japonicum*** AUMC 7779, arthrospores, budding cells and splitting cells, (budding cells ovoidal, ellipsoidal, elongate, 4.5-9.6 x 5.8-9.7 µm, Sugita and Nakase 1998).

SUMMARY

The present study is an extensive survey of mycobiota from citrus and grapevine plantations in Sahel-Saleem City, Assuit Governorate, Egypt. The study was carried out during the period from April 2008 to February 2009. Identification of yeast fungi from air, soil, phyllosphere, phylloplane, carposphere, and carpoplane, in citrus and grapevine plantations, in addition to fruit juice of the two plants was conducted using morphological, biochemical characteristics and in many cases identification was confirmed using rDNA molecular sequencing. The main results were as following:

1. Total yeasts

- Yeast fungi were represented by 38 species, in addition to 4 unidentified, assigned to 20 genera. Of these, 22 species of yeasts are new records to Egypt.
- The broadest spectra of species were recorded in the following order: *Cryptococcus* (7 species), *Pichia* (4 species), *Pseudozyma* (3 species and 1 unidentified), Candida (3 species), *Rhodotorula* (3 species), and *Sporidiobolus* (3 species).
- The broadest spectra of genera and species were recorded in citrus air (12 genera and 18 species on DRBC), citrus phyllosphere (11 and 16 on DYM), and grapevine phyllosphere (10 and 16 on DRBC) and carposphere (10 and 15 on DYM), while the narrowest was recorded in grapevine soil (2 and 2 on DYM) and (4 and 4 on DRBC).
- The highest counts of yeasts were recorded from the juice of both fruits (almost more than 95 % of total fungi), followed by citrus carposphere and carpoplane where they constituted about one-third of total fungi. The lowest percentage counts (less than 1 % of total fungi) was recorded in soil of both plantations and citrus phyllosphere.

2. Yeast fungi recovered from the air of citrus and grapevine plantations

- 24 species of yeast fungi were recovered from both plantations. 10 of yeast species were isolated from the air of citrus only, while 6 from the air of grapevine only.
- Yeast fungi showed their peak in citrus plantations in December on both media and in grapevine in October and April on DYM and DRBC respectively, while their trough occurred in April on both media in citrus plantations and in June and December on DYM and DRBC respectively in grapevine.

- *Cryptoccocus* (4 species) and *Rhodotorula* (3 species) were the dominant yeast genera in both plantations while *Debaryomyces* (2 species) and *Sporidiobolus* (*S. ruineniae*) were of moderate or low frequency.
- *Ambrosiozyma*, *Candida*, *Geotrichum*, *Hanseniaspora*, *Rhodosporidium*, and *Melanopsichium* were recovered in citrus only while *Sporobolomyces* and *Trichosporon* in grapevine only.

3. Yeast fungi recovered from the soil in citrus and grapevine plantations

- 9 genera and 13 species of yeasts were recovered from both plantations. 9 species of yeast fungi were isolated from citrus only, while 4 from grapevine only.
- Yeasts comprised 0.47 % - 0.49 % of total fungi in citrus soil and 0.15 % - 0.21 % in grapevine soil. They showed their peak in soil of citrus in April and in grapevine in February on both media.
- *Candida catenulata*, *Debaryomyces* (2 species), *Geotrichum* (3 species), *Hanseniaspora occidentalis*, *Kluyveromyces marixianus*, and *Pichia caribbica* were encountered in citrus only, while *Cryptoccocus laurentii*, *Issachenkia orientalis*, *Pichia guillermondii* and *Rhodotorula* sp. in grapevine only.

4. Yeast fungi recovered from the phyllosphere of citrus and grapevine

- 14 genera and 23 species of yeast fungi were recovered from both plants. 8 species of yeast fungi were isolated from citrus only and 5 from grapevine only.
- Yeast fungi showed their peak of total propagules in citrus in February and in grapevine in December on both media, while their trough in August in citrus and April in grapevine on both media.
- *Cryptococcus* (6 species) was the most common yeast genus and possessed more percentage count in grapevine than in citrus. *C. albidus* was the most common species in of both plants.
- *Rhodotorula* (2 species) was recovered in high or moderate frequency in grapevine, while in low or rare frequency with relatively smaller count in citrus. *R. mucilaginosa* was the main component of *Rhodotorula*, in grapevine.
- *Candida* (*C. catenulata*), *Geotrichum* (*G. citri-aurantii*), *Pseudozyma* (3 species), and *Trichosporon* (*T. japonicum*) were recovered from citrus phyllosphere only, while *Pichia* (*P. guilliermondii*) and *Rhodosporidium* (*R. paludigenum*) from grapevine only.

5. Yeast fungi recovered from the phylloplane of citrus and grapevine

- 12 genera and 16 species of yeast fungi were recovered from both plants (regularly narrower spectera than in the phyllosphere). 7 yeast species were isolated from citrus phylloplane only, while only 2 from grapevine phylloplane.
- The peak of total propagules of fungi was recorded in February (permanent mature leaf) in citrus and December (senescent leaf) in grapevine on both media, while their trough in citrus in June and August on DYM and DRBC respectively, and in June (young leaf) in grapevine on both media.
- Yeast fungi contributed 2.71 % - 6.54 % of total fungi in citrus and 5.73 % - 5.86 % in grapevine. They showed their peak in citrus in October and June on DYM and DRBC respectively, and in grapevine in August on both media, while their troughs occurred in April and February in citrus, and in October and June in grapevine on DYM and DRBC respectively.
- *Cryptococcus* (5 species) was recovered in moderate frequency from both phylloplanes. *Rhodotorula mucilaginosa* was recovered in moderate frequency from grapevine and in low frequency in citrus.
- *Candida* (*C. catenulata*), *Geotrichum* (*G. citri-aurantii*), *Issachenkia orientalis*, *Kluyveromyces marixianus, Pseudozyma* (*P. aphidis*), and *Trichosporon* (*T. japonicum*) were recovered from citrus only, while *Sporidiobolus pararoseus* and *Sporobolomyces roseus* from grapevine only.

6. Yeast fungi recovered from the carposphere of citrus and grape fruits

- 13 genera and 22 species of yeast fungi were identified from both plants. 7 yeast species were isolated from citrus only, while 9 were isolated from grapevine only.
- The peak of total propagules of fungi was recorded in April (primordial fruit) in citrus and in December (senescent fruit) in grapevine on both media, while their trough was recorded in August (immature fruit) in citrus and in June (immature fruit) in grape on both media.
- Yeast fungi were recorded in high frequency in grape carposphere and in moderate frequency in citrus although they constituted higher numbers in citrus (25.69 % - 37.49 % of total fungi) than those of grape (17.95 % - 19.08 %). Their peaks were drawn in citrus in December (mature fruit) and in grape in October (mature fruit) on both media, while their trough occurred in April and February on DYM and DRBC respectively in citrus, and in August on both media in grape.

- *Rhodotorula* (2 species) was encountered in moderate frequency in grape, and in rare frequency in citrus. *Issachenkia orientalis* was recovered in low frequency in both plants contributing markedly higher number in citrus than in grape. *Hanseniaspora occidentalis* was recovered in low frequency in grape and in rare frequency in citrus.
- *Candida catenulata* and *C. parapsilosis* were isolated from citrus only, while *C. prunicola* was recorded in grape only. *Geotrichum* (*G. citri-aurantii*), *Kodemaea ohmeri*, and *Pseudozyma* sp. were recovered from citrus only while *Rhodosporidium* (*R. diobovatum* and *R. paludigenum*) from grape only.

7. Yeast fungi recovered from the carpoplane of citrus and grape fruits

- 12 genera and 14 species of yeast fungi were recovered from both plants (regularly narrower than in carposphere). 6 yeast species were isolated from citrus only and 6 also from grape only.
- The peak of total fungi was recorded in December (mature fruit) in citrus carpoplane and in October (mature fruit) in grape on both media, while their trough was regularly recorded in June (immature fruit) in the carpoplanes of both plants and media.
- Yeast fungi contributed 30.71 % - 35.22 % of total fungi in citrus and 20.56 % - 23.08 % in grape. They showed their peak of total propagules in citrus in December and in grape in October on both media, while their trough occurred in April and April, and June in citrus and in December and August in grape on DYM and DRBC respectively.
- *Issachenkia orientalis* was recovered in low frequency in both plants. *Debaryomyces* (*D. hansenii* and *D. pseudopolymorphus*) was isolated in low frequency in citrus and was missed in grape. *Candida* (2 species) contributed medium proportion of propagules despite its record in rare frequency in the carpoplane of both plants on both media. It was represented by *C. catenulata* in citrus carpoplane only and by *C. prunicola* in grape carpoplane only. *Hanseniaspora occidentalis* was recovered in rare frequency from the carpoplane of both plants.
- *Geotrichum* (*G. citri-aurantii*), *Kodemaea ohmeri*, and *Pichia* (*P. fermentans*) were recovered from citrus only, while *Cryptococcus* (*C. laurentii*), *Rhodosporidium* (*R. paludigenum*), *Rhodotorula* (*R. mucilaginosa*), *Sporobolomyces roseus* and yeast sp. (black) from grape only.

8. Yeast fungi recovered from the juice of citrus and grape fruits

- 11 genera and 16 species of yeast fungi were recovered from the fruit juice of both plants. Yeasts were represented by 7 genera and 7 species in citrus juice and 9 genera

and 11 species in grape juice. 4 yeast species were isolated from citrus juice only, while 8 were isolated from grape juice only.

- The peak of total fungi was regularly recorded in October (mature fruit) in both citrus and grape juices on both media, while their troughs were recorded in February (senecent fruit) in citrus juice and in August (immature fruit) in grape juice on both media.
- Yeast fungi were the main component of fungi accounting for 91.60 % - 95.42 % of total fungi in citrus juice and 99.14 % - 99.39 % in grape juice. They regularly showed their peak in October (mature fruit) in both juices on both media, while their troughs occurred in December (mature fruit) in citrus juice and in August (immature fruit) in grape juice on both media.
- *Issachenkia orientalis* was recovered in moderate frequency in citrus juice on both media and in high or moderate frequency in grape. *Candida* (2 species) was recorded in moderate frequency in citrus juice on both media and in high or moderate frequency in grape juice, contributing higher percentage counts in grape juice than those in citrus juice. It was represented by *C. catenulata* in citrus juice and *C. prunicola* in grape juice.
- *Debaryomyces* (*D. hansenii* and *D. pseudopolymorphus*) was isolated in moderate frequency in citrus juice and in low frequency in grape juice. *Hanseniaspora occidentalis* was recovered in moderate frequency, while *Cryptococcus* (2 species) in low frequency in both juices.
- *Geotrichum* (*G. citri-aurantii*), and *Pichia* (*P. caribbica* and *P. fermentans*) were recovered from citrus juice only, while *Rhodosporidium* (*R. paludigenum*), *Rhodotorula* (*R. glutinus and R. mucilaginosa*), *Sporidiobolus* (*S. pararoseus* and *S. ruinenniae*) and *Sporobolomyces roseus* from grape juice only.

9. Patterns of dominance of fungi

The present study reveals four patterns of correlation between dominance (counts) of certain groups of fungi and the different studied habiats:

- **Soil pattern** in which the Basidiomyceteous yeasts e.g. *Cryptococcus* and *Rhodotorula* were isolated from grapevine soil only, while Ascomyceteous yeasts were reported mainly from citrus soil but also from that of grapevine.
- **Air, phyllosphere, and phylloplane pattern** where Basidiomyceteous yeasts were dominant over ascomyceteous yeasts in these environmrnts.
- **Carposphere and carpoplane pattern** where yeast fungi were fairly dominant over filamentous fungi. Ascomyceteous yeasts were also dominant over basidiomyceteous

ones. In this pattern, sugary metabolites may leach out from the fruit surface which may be stimulatory for fair proliferation of yeast fungi and Section *Nigri* species.

- **Fruit juice pattern** where yeasts were extremely dominant over filamentous (almost over 95 % of total fungi). Ascomyceteous yeasts were dominant over basidiomyceteous ones. In this pattern, the sources are sugary and sugars are known to stimulate greatly the proliferation of yeasts leaving no much room for filamentous fungi.

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