Annals of Microbiology, **59 (3)** 419-424 (2009)

Yeasts colonizing the leaves of fruit trees

Elena SLÁVIKOVÁ[1]*, Renata VADKERTIOVÁ[1], Dana VRÁNOVÁ[2]

[1]Culture Collection of Yeasts, Institute of Chemistry Slovak Academy of Sciences, Dúbravská cesta 9, 845 38 Bratislava, Slovakia; [2]Institute of Food Science and Technology, Faculty of Chemistry, BUT, Purkyňova 118, 612 00 Brno, Czech Republic

Received 7 April 2009 / Accepted 10 July 2009

Abstract - Yeasts were isolated from leaf surfaces of five species of fruit trees located in southwest Slovakia. One hundred and fifty five yeast strains belonging to 11 genera were isolated from 300 samples of leaves. 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. We found only few differences in the yeast community isolated from leaves of different tree species although dominant species occurred regularly on the majority of leaves. Furthermore, yeast species varied throughout years. In spite of the fact that the yeast community occupying the leaves of the fruit trees was studied in the samples harvested in three localities, which are distanced from each other some kilometers, the qualitative representation of the most isolated yeasts was identical. The differences were only in the frequency of the incidence of individual species in the samples.

Key words: yeasts; leaves of fruit trees; colonization; isolation.

INTRODUCTION

All aerial plant surfaces are inhabited by diverse assemblages of microorganisms that have profound effects on plant health and impact on ecosystem and agricultural functions. This environment is usually named phylloplane or phyllosphere. Many works on phyllosphere microbiology have focused on leaves, a more dominant aerial plant structures (Lindow and Brandl, 2003).

Yeasts are important members in many ecosystems (Fleet, 1998) and also form a major component of the population on leaves (Nakase, 2000; Inácio *et al*., 2002). Little is known about the ecological role of the phylloplane yeasts. The leaf surface characteristics may affect, both qualitatively and quantitatively, the immigration of yeasts to the phylloplane (Blakeman, 1973).

Yeasts are active as competitors for nutrients, antagonists or symbiotic associates or as victims of the behaviour of their neighbours (Do Carmo-Sousa, 1969). Leaf surfaces are colonized by members of several genera of saprophitic yeasts that provide a natural barrier against plant pathogens (Fokkema *et al*., 1979). Leaves are exposed to rapid fluctuation of temperature and relative humidity values, which may have an impact on the yeast population. Large fluxes of UV radiation are also one of the most prominent features of the leaf surface environment to which micoorganisms have presumably had to adapt (Lindow and Brandl, 2003). Many plants contain a number of compounds whose adaptive significance may be a defense against invertebrates and microorganisms (Robinson, 1974). These compounds also act, in some cases, as selective agents which shape the yeast community composition (Lachance, 1990).

The external surface of the leaf as a habitat for yeasts has been recognized more recently as an interior of flowers or fruits of higher plants. Several years ago, the extensive isolation studies of yeasts from plant materials were carried out (Middelhoven, 1997; Nakase, 2000; Inácio *et al*., 2002) and yeast species were isolated and described from these habitats.

Yeasts were also isolated from the leaves of apple and mango trees (Jager *et al*., 2001; Fiss *et al*., 2003; Camatti-Sartori *et al*., 2005). Some of them have a potential use as antagonists and can serve as a biological control against post-harvest decay of apples and pears (Ippolito and Nigro, 2000; Seibold *et al*., 2004).

No information on yeasts associated with the phyllosphere on the territory of Slovakia and neighbouring countries is available. Only the yeasts associated with fruits and grapes were studied in Slovakia. In our previous investigations, we studied the yeast population colonizing the surface of the leaves and needles of various wood plants from a forest park (Sláviková *et al*., 2007).

The purpose of this work was to study the yeast community colonizing the leaf surface of various fruit trees during two consecutive years in order to find out which yeast species are predominant and what is their frequency.

* Corresponding Author. Phone: + 421-2-59410262; Fax: + 421-2-59410222; E-mail: Elena.Slavikova@savba.sk Slavikova Slavikova mailto:.Slavikova@savba.sk

MATERIALS AND METHODS

Sampling. Yeasts were isolated from leaf surfaces of five species of fruit trees: apple, cherry, apricot, peach, and plum. The leaves of these fruit trees were harvested in three localities: peripheral part of Bratislava, Malé Leváre (approximately 50 km from Bratislava), and Malé Zálužie (approximately 100 km from Bratislava). All three sites are located in southwest Slovakia.

Leaves were collected in the springtime, in the middle of June, and in autumn, in the late September, during two consecutive years (June 2006 to September 2007). Seventy five samples were collected during each sampling. In total, this resulted in 300 samples from which yeasts and yeast-like organisms were isolated. Leaves were carefully ripped out of twigs and put into sterile plastic bags, transported to the laboratory, and processed within 2 h after the harvesting.

Yeast isolation. Each sample (5 g) was cut up and placed in the 250-ml flask containing 50 ml of sterile distilled water and shaken on a rotary shaker at 25 °C for 2 h. Leaf washing solutions were serially diluted and 0.1 ml of each dilution was spread on malt agar (MEA; Oxoid) containing 80 $\mu g \cdot ml^{-1}$ of streptomycin. The plates were incubated at 25 °C. After 3, 5 and 10 days different colonies were picked and streaked pure on the malt agar plates. Cultures were maintained on the malt agar slants.

Yeast identification by conventional methods. The morphological and physiological characteristics of isolates were examined by the methods described by Yarrow (1998). Strains were identified according to Kurtzman and Fell (1998) and Barnett *et al.* (2000).

Yeast identification by molecular methods. The identification of the strains belonging to the species *Cryptococcus laurentii*, *Rhodotorula glutinis* and *Saccharomyces cerevisiae* was also confirmed by the sequencing analysis of the rRNA gene internal transcribed spacer (ITS) regions according to Esteve-Zarzoso *et al.* (1999) and Leaw *et al.* (2006).

DNA extraction. Cells were collected from a fresh yeast colony. The DNA was extracted by using the Ultra Clean Microbial DNA Isolation kit (MOBIO Laboratories, USA) in accordance with the manufactures instructions. The extracted DNA was stored at –20 °C.

PCR reaction and DNA digestion. To amplify the ITS region two primers: ITS1 (5′ TCCGTAGGTGAACCTGCGG 3′) and ITS4 (5′ TCCTCCGCTTATTGATATGC 3′) were used. The PCR was performed in a total reaction volume of 50 µl consisting of Taq polymerase buffer (Apligene), 0.02 mM dNTPs, 0.02 µM of each primers, 1 unit of Taq polymerase (Apligene), 3-10 ng DNA in 1-2 µl of TE buffer PCR amplification was carried out in PTC-100 Programmable Thermal Controller (MJ Research, Inc., USA). After an initial denaturation at 94 °C for 4 min, 25 cycles of amplification were conducted as follows: denaturation at 94 °C for 1 min, annealing at 48 °C for 30 s, and extension at 72 °C for 1 min. The final extension was at 72 °C for 10 min. A negative control was performed for each run by replacing the template DNA with sterile water in the PCR mixture. The amplification products were precipitated by ethanol and diluted in an appropriate buffer. Amplicons were digested with *Hae*III*, Taq*I*, Tru*I*, Hinf*I*, Hha*I and *Eco*88I restriction endonucleases (Promega). The digests were analyzed by 2% agarose gel electrophoresis in TBE buffer (10X stock solution/l: 108 g Tris base, 55 g boric acid, 40 ml 0.5 M EDTA pH 8.0; working solution was 1X TBE). Gels were stained with ethidium bromide and visualised under UV light (Ultra. Lum, Inc.). The amplification products of the unknown strains were compared with the amplification products of reference strains (Zarzoso *et al.,* 1999; Leaw *et al.,* 2006).

Reference strains. *Cryptococcus laurentii* CCY 17-3-2 (Type), *Rhodotorula glutinis* CCY 20-2-34 (Type), and *Saccharomyces cerevisiae* CCY 21-4-96 (Type) are maintained in the Culture Collection of Yeasts, Institute of Chemistry, Slovak Academy of Sciences, Bratislava.

RESULTS AND DISCUSSION

One hundred and fifty five yeast strains belonging to 11 genera and 17 species were isolated from 300 samples of leaves. Table 1 provides a list of isolated species. *Aureobasidium pullulans*,

TABLE 1 - Occurrence of yeasts and yeast-like species isolated from the leaves of trees

Species	Apple	Plum	Cherry	Apricot	Peach
Aureobasidium pullulans	35*	20	28	33	30
Candida tropicalis	2				3
Cryptococcus albidus	10	3	5		2
Cryptococcus laurentii	28	22	25	27	23
Geotrichum candidum		7			3
Hanseniaspora uvarum	5	2	3	7	18
Metschnikowia pulcherrima	30	32	13	32	33
Pichia anomala	4	5	2		5
Pichia guilliermondii	3	3			3
Pichia membranifaciens	3	2	2	3	2
Pseudozyma aphidis	2		2	4	
Pseudozyma fusiformata				2	2
Rhodotorula glutinis	4	2	3	3	2
Rhodotorula minuta					2
Rhodotorula mucilaginosa	3	2			
Saccharomyces cerevisiae	2	10		8	10
Yarrowia lipolytica			2		

* The number gives % of positive samples.

Cryptococcus laurentii, and *Metschnikowia pulcherrima* were the most frequently isolated species and occurred on leaves of all five kinds of fruit trees.

The basidiomycetous yeasts *A. pullulans* was present in 20-35% of the samples and *C. laurentii* in 22-28% of samples (Table 1). The species *A. pullulans* and *C. laurentii* have been often associated with the surface of plants. They were isolated from all leaves regardless of plant or location (Andrews *et al*., 2002; Inácio *et al*., 2002; Pereira *et al*., 2002). *Cryptococcus laurentii* has been reported to be heterogenous based on DNA G + C content, whole-cell protein electrophoretic patterns, and the sequences of the D_1/D_2 region of 26S rDNA and ITS regions (Sugita *et al*., 2000; Takashima *et al*., 2003). We have not found such heterogenity among the studied strains (Fig. 1). *Cryptococcus albidus* occurred less frequently, but it was present in 10% of samples of apple leaves.

A marked dominance of basidiomycetous yeasts on phylloplane was reported by Inácio *et al*. (2002), but Middelhoven (1997) reported only about one third of the strains frequently found on plants growing in an arid climate as basidiomycetous species. We found out that the ascomycetous and basidiomycetous species were present in the leaf samples in approximately equal frequency.

Table 1 shows low incidence of *Rhodotorula glutinis* on leaves and together with another two carotenoids producing species *Rhodotorula mucilaginosa* and *Rhodotorula minuta* occurring only in 3-7% of the samples. *Rhodotorula glutinis* has a world-wide distribution and some studies have suggested that this species might encompass more than one species (Gadanho and Sampaio, 2002). Figure 1 shows that the strains isolated from the fruit trees exhibited similar profiles as the type strain *R. glutinis* (Fig. 1). The red yeast species *Sporobolomyces* also belongs to the yeasts frequently occurring on leaf surfaces (Phaff and Starmer, 1987; Nakase, 2000). A surprising result was the absence of ballistoconidia-forming yeasts in the samples of fruit tree leaves. A low incidence of this group of yeasts was also found on leaves and needles of wood trees in the forest park (Sláviková *et al*., 2007) and on the phylloplane of plants in the Arrábida Natural Park in Portugal (Inácio *et al*., 2002). On the other hand, *Sporobolomyces roseus* was the dominant species of the fish-pond water samples collected in the locality Železná Studnička in autumn, when the water contained many fallen leaves (Sláviková and Vadkertiová, 1995) and probably became more easily released from the decomposed leaves.

The ascomycetous species *Metschnikowia pulcherrima* was the third most frequently isolated yeast species. It was isolated from 13-33% of the samples and dominated in spring samples (Table 1, Fig. 2). *Metschnikowia pulcherrima* has been considered to be a typical constituent of flowers and fruits (Phaff and Starmer, 1987; Lachance *et al*., 2001) although Kvasnikov *et al.* (1975) reported this species typical of phyllosphere, both wild and cultural plants. The apiculate yeast *Hanseniaspora 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). It belongs to the most common fermentative spoilage yeasts. Similar to *M. pulcherrima, H. uvarum* ferments only glucose. Flowers, especially their nectars, are considered as an ideal habitat for yeasts because they contain sugar. These yeasts can be distributed between different plants or between flowers and leaves by pollinating insects. *Hanseniaspora uvarum* was present in samples of all kinds of fruit trees in both seasons and all localities (Table 1, Figs. 2 and 3).

The pellicle-forming *Pichia anomala* was found in association with trees (Spencer *et al*., 1974), whereas *S. cerevisiae* predominated in the phyllosphere of wild plants (Kvasnikov *et al*., 1975). *Pichia anomala* and *Saccharomyces* sp. belonged to the most frequently found ascosporogenous yeasts isolated from the surfaces of leaves and needles in a forest park (Sláviková *et al*., 2007). Their incidence on the leaves of fruit trees was less frequent; higher incidence was detected only in autumn. The strains of *S. cerevisiae* were more common on leaves of plum, peach, and apricot and did not occur on cherry leaves. The species of the genus *Pichia: P. anomala*, *P. membranifaciens,* and *P. guilliermondii* were found together only in 4-10% of the samples (Table 1).

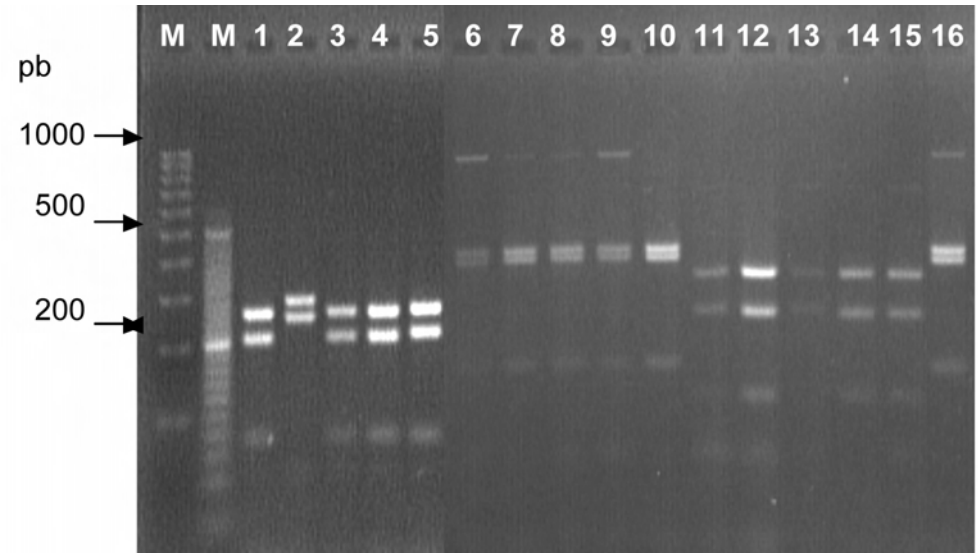

FIG. 1 - Example of PCR-RFPL analysis of the ITS region *Cryptococus laurentii, Rhodotorula glutinis* and *Saccharomyces cerevisiae* strains. Lanes 1-4: isolates from fruit tree leaves no. 31, 60_1, 116 and 251 phenotypically identified as *C. laurentii*; lane 5: *C. laurentii* CCY 17-3-2 (Type); lanes 6-10: isolates from fruit tree leaves no. 109, 235, 242, 244 and 250; lanes 11-14 isolates from fruit tree leaves no. 18, 227, 259 and 292; lane 15: *R. glutinis* CCY 20-2-34 (Type); lane 16: *S. cerevisiae* CCY 21-4-96 (Type); lanes M: DNA size marker; DNA cleaved with *Hha*I.

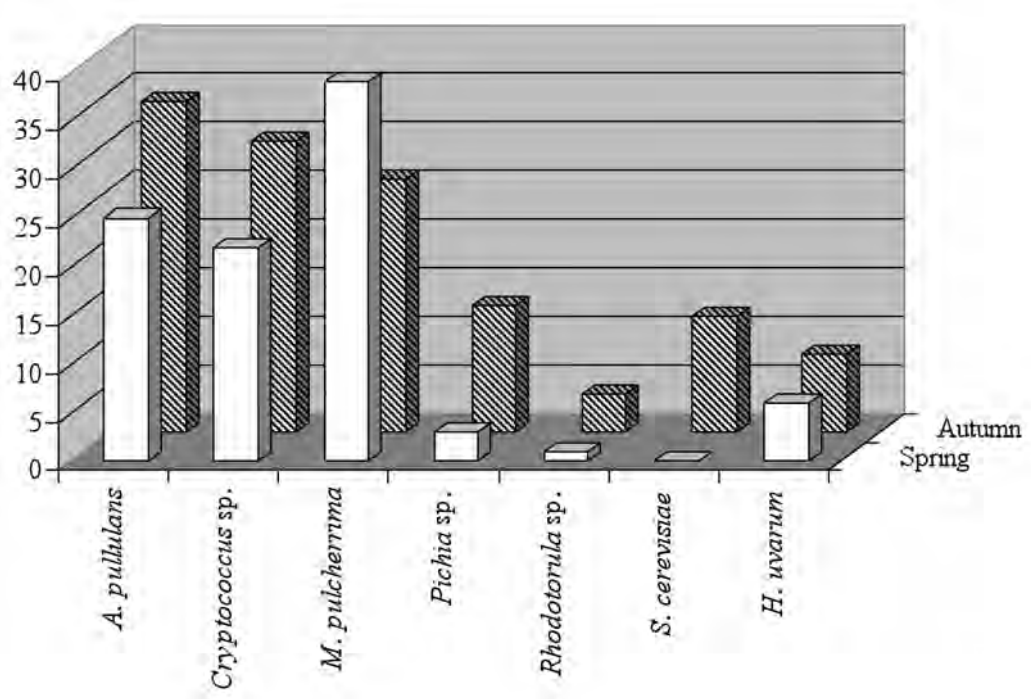

FIG. 2 - Yeast species most frequently isolated from the leaves harvested during the spring and autumn sampling (the number gives % of positive samples and is an average of two sampling).

The remaining five species: *Candida tropicalis*, *Geotrichum candidum, Pseudozyma aphidis, Pseudozyma fusiformata,* and *Yarrowia lipolytica* were isolated from the leaves less frequently.

The samples of leaves were harvested from five various fruit trees, in two seasons of year during two consecutive years in three localities distant from each other from 50 to 100 km. We found out that although the leaves as habitats for the microbial colonization came from various fruit-tree species, only few differences in the yeast community were observed. The dominant species occurred regularly on the majority of leaves. Leaves of peach trees were occupied by the most heterogenous yeast flora (Table 1). The marked differences in the frequency of individual species isolated in spring and autumn are evident (Fig. 2). The higher frequency of the yeast flora in autumn could be explained by the changes in the leaf surface, when probably due to erosion processes the leaf became more accessible for colonizing

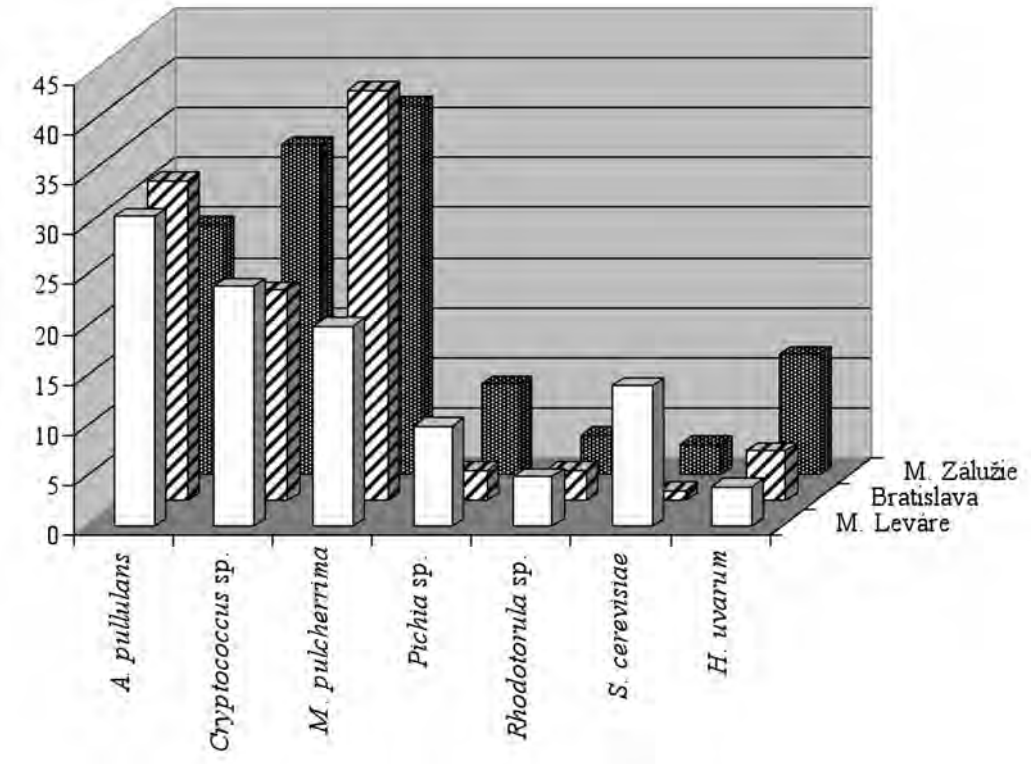

FIG. 3 - Yeast species most frequently isolated from the leaves of fruit trees harvested in three localities (the number gives % of positive samples).

by epiphillic microorganisms (Neinhuis and Barthlott, 1998). Regardless of distance of individual localities, the qualitative representation of the most isolated yeasts was identical. The differences were in the frequency of the incidence of individual species in samples (Fig. 3).

The representation of species colonizing the leaves of fruit and wood trees (Sláviková *et al*., 2007) seems to be very similar, but the frequency of individual species is distinct. Considerable differences were found in the incidence of *M. pulcherrim*a and *H. uvarum*, which occurred more frequently on leaves of fruit trees, whereas *P. anomala*, *S. cerevisiae* and *A. pullulans* were found in the higher incidence on wood trees. The abundance of *C. laurentii* and *R. glutinis* was almost similar in both types of trees.

The occurence of yeasts on fruit trees could be also influenced by chemical compounds, mainly fungicides which are used in the production system. The fungicides could substantially reduce yeast population, but some have no effect on yeasts. Comitini and Ciani (2008) noticed a drastic reduction in the yeast population caused by fungicides. *Saccharomyces cerevisiae* exhibited the highest sensitivity to some fungicides (Ribeiro *et al.,* 2000). Our results show lower incidence of this species on leaves of fruit trees in comparison to wood trees, which could be affected by the chemical spraying of fruit trees. On the other hand, *C. laurentii* and *R. glutinis* performed low sensitivity towards several fungicides commonly applied on fruits and vegetables (Lima *et al*., 1998). The incidence of these species on the leaves of wood and fruit trees was almost similar, in spite of chemical treatment of fruit trees. The occurrence of yeasts after the chemical spraying depends also on their ability to repopulate the surface of the leaves (Andrews and Harris, 2000).

Middelhoven (1997) reported that phylloplane yeasts exhibit wide biodegradative activities. From his study, it is clear that phyllosophere yeasts are able to attact and to assimilate many high-molecular and low-molecular plant constituents and that they may benefit from many compounds leaking out of the plant. By succesfully competing for nutrients, yeasts may protect the plant against phytopathogenic fungi.

Some isolates of *A. pullulans*, *P. anomala*, *M. pulcherrima*, and *C. laurentii* showed antagonistic activities against a number of pathogenic fungi (Fredlund *et al*., 2002; Allen *et al*., 2004; Seibold *et al*., 2004). The production of antibacterial compounds by phylloplane isolates of *C. laurentii* and *R. glutinis* towards bacteria were reported by McCormack *et al.* (1994). Pimenta *et al*. (2009) pointed out that the changes in the native microbiota could negatively affect the antagonistic action against pathogens.

The monitoring of the yeast community is actually fundamental to the better underestanding of their functions in natural environments. It is possible that some of our isolated strains may have the biological control potential against foliar and post harvest diseases, but it remains the aim of further studies to investigate this capability.

Acknowledgement

This work was supported by a grant from the VEGA for biological and ecological sciences No. 2/7031/27 and a grant from the Ministry of Education (FRVŠ) No. 2774/F4a.

REFERENCES

Allen T.W., Burpee L.L., Buck J.W. (2004). *In vitro* attachment of phylloplane yeasts to *Botrytis cinerea*, *Rhizoctonia solani*, and *Sclerotinia homoeocarpa*. Can. J. Microbiol., 50: 1041-1048.

Andrews J.H., Harris R.F. (2000). The ecology and biogeography of microorganisms on plant surfaces. Annu. Rev. Phytopathol., 38: 1-36.

Andrews J.H., Spear R.N., Nordheim E.V. (2002). Population biology of *Aureobasidium pullulans* on apple leaf surfaces. Can. J. Microbiol., 48: 500-513.

Barnett J.A., Payne R.W., Yarrow D. (2000). Yeasts: Characteristics and Identification. Third Edition, Cambridge University Press.

Blakeman J.P. (1973). The chemical environment of leaf surfaces with special reference to spore germination of pathogenic fungi. Pestic. Sci., 4: 575-588.

Camatti-Sartori V., da Silva-Ribeiro R.T., Valdebenito-Sanhueza R.M., Pagnocca F. C., Echeverrigaray S., Azevedo J.L. (2005). Endophytic yeasts and filamentous fungi associated with southern Brazilian apple (*Malus domestica*) orchards subjected to conventional, integrated or organic cultivation. J. Basic Microbiol., 45: 397-402.

Comitini F., Ciani M. (2008). Influence of fungicide treatments on the occurrence of yeast flora associated with wine grapes. Ann. Microbiol., 58: 489-493.

Do Carmo-Sousa L. (1969). Distribution of yeasts in nature. In: Rose A.H., Harrison J.S., Eds, The Yeasts, Vol. I, Academic Press, London, pp. 79-105.

Esteve-Zarzoso B., Belloch C., Uruburu F., Qerol A. (1999). Identification of yeasts by RFLP analysis of the 5.8S rRNA gene and the two ribosomal internal transcribed spacers. Int. J. Syst. Bacteriol., 49: 329-337.

Fiss M., Barckhausen O., Gherbawy Y., Kollar A., Hamamoto M., Auling G. (2003). Characterization of epiphytic yeasts of apple as potential biocontrol agents against apple scab (*Venturia inaequalis*). J. Plant Dis. Protect., 110: 513-523.

Fleet G.H. (1998). Yeasts in natural habitats. Food Technol. Biotechnol., 36: 285-289.

Fokkema N.J., den Houter J.G., Kosterman Y.J.C., Nelis A.L. (1979). Manipulation of yeasts on field-grown wheat leaves and their antagonistic effect on *Cochliobolus sativus* and *Septoria nodorum*. Trans. Br. Mycol. Soc., 72: 19-29.

Fredlund E., Druvefors U., Boysen M.E., Lingstein K.-J., Schnürer J. (2002). Physiological characteristics of the biocontrol yeast *Pichia anomala* J121. FEMS Yeast Res., 2: 395-402.

Gadanho M., Sampaio J.P. (2002). Polyphasic taxonomy of the basidiomycetous yeast genus *Rhodotorula*: *Rh. glutinis* sensu stricto and *Rh. dairensis* comb. nov. FEMS Yeast Res., 2: 47-58.

Inácio J., Pereira P., De Carvalho M., Fonseca Á., Amaral-Collaco M.T., Spencer-Martins I. (2002). Estimation and diversity of phylloplane mycobiota on selected plants in a Mediterranean-type ecosystem in Portugal. Microb. Ecol., 44: 344-353.

Ippolito A., Nigro F. (2000). Impact of preharvest application of biological control agents on postharvest diseases of fresh fruits and vegetables. Crop Protection, 19: 715-723.

Jager E., Wehner F., Korsten L. (2001). Microbial ecology of the mango phylloplane. Microb. Ecol., 42: 201-207.

Kurtzmann C.P., Fell J.W., Eds (1998). The Yeasts, A Taxonomic Study, 4th edn., Elsevier Science B.V., Amsterdam.

Kvasnikov E.I., Nagornaia S.S., Shchelokova I.F. (1975). Yeast flora of plant rhizosphere and phyllosphere. Mikrobiologiia, 44: 339-346.

Lachance M.A. (1990). Yeast selection in nature. In: Panchal Ch.J., Ed., Yeast Strain Selection, Vol. 8, M. Dekker, New York, pp. 2-41.

Lachance M.A., Starmer W.T., Rosa C.A., Bowles J.M., Barker J.S., Janzen D.H. (2001). Biogeography of the yeasts of ephemeral flowers and their insects. FEMS Yeast Res, 1: 1-8.

Leaw S.N., Chang H.C., Sun H.F., Barton R., Bouchara J.P., Chang T.Ch. (2006). Identification of medically important yeast species by sequence analysis of the internal transcribed spacer regions. J. Clin. Microbiol., 44: 693-699.

Lima G., Curtis F.D., Castoria R., Cicco V.D. (1998). Activity of the yeasts *Cryptococcus laurentii* and *Rhodotorula glutinis* against post-harvest rots on different fruits. Biocontrol Sci. Technol., 8: 257-267.

Lindow S.E., Brandl M.T. (2003). Microbiology of the phyllosphere. Appl. Environ. Microbiol., 69: 1875-1883.

McCormack P., Wildman H.G., Jeffries P. (1994). Production of antibacterial compounds by phylloplane-inhabiting yeasts and yeast like fungi. Appl. Environ. Microbiol., 60: 927-931.

Middelhoven W.J. (1997). Identity and biodegradative abilities of yeasts isolated from plants growing in an arid climate. Antoine van Leeuwenhoek, 72: 81-89.

Nakase T. (2000). Expanding world of ballistosporous yeasts: distribution in the phyllosphere, systematics and phylogeny. J. Gen. Appl. Microbiol., 46: 189-216.

Neinhuis C., Barthlott W. (1998). Leaf surface contamination in beech, oak, and ginkgo in relation to micromorphology and wettability. New Phytol., 138: 91-98.

Pereira P.T., de Carvalho M.M., Girio F.M., Roseiro J.C., Amaral-Collaço M.T. (2002). Diversity of microfungi in the phylloplane of plants growing in a Mediterranean ecosystem. J. Basic Microbiol., 42: 396-407.

Phaff H.J., Starmer W.T. (1987). Yeasts associated with plants, insects and soil. In: Rose A.H., Harrison J.S., Eds, The Yeasts, 2nd edn., Vol. 1, Academic Press London, pp. 123-180.

Pimenta R.S., Morais P.B., Rosa C.A., Corrêa Jr.A. (2009). Utilization of yeasts in biological control programs. In: Satyanarayana T. and Kunze G., Eds, Yeast Biotechnology: Diversity and Applications, Springer, Netherlands, pp. 199-214.

Ribeiro I.C., Veríssimo I., Moniz L., Cardoso H., Sousa M.J., Soares A.M.V.M., Leão C. (2000). Yeasts as a model for assessing the toxicity of the fungicides Penconazol, Cymoxanil and Dichlofluanid. Chemosphere, 41: 1637-1642.

Robinson T. (1974). Metabolism and function of alcaloids in plants. Science, 184: 430-435.

Seibold A., Fried A., Kunz S., Moltmann E., Lange E., Jelkmann W. (2004). Yeasts as antagonists against fireblight. Bulletin OEPP/EPPO Bulletin, 34: 389-390.

Sláviková E., Vadkertiová R. (1995). Yeasts and yeast-like organisms isolated from fish-pond waters. Acta Microbiol. Polonica, 44: 181-189.

Sláviková E., Vadkertiová R., Vránová D. (2007). Yeasts colonizing the leaf surfaces. J. Basic Microbiol., 47: 344-350.

Spencer J.F.T., Gorin P.A.J., Gardner N.R. (1974). Yeasts occurring in the effluent disposal basins of a pulp mill in Saskatchewan. Can. J. Microbiol., 20: 993-998.

Sugita T., Takashima M., Ikeda R., Nakase T., Shinoda T. (2000). Intraspecies diversity of *Cryptococcus laurentii* as revealed by sequences of internal transcribed spacer regions and 28S rRNA gene and taxonomic position of *C. laurentii* clinical isolates. J. Clin. Microbiol., 38: 1468-1471.

Takashima M., Sugita T., Shinoda T., Nakase, T. (2003). Three new combinations from the *Cryptococcus laurentii* complex: *Cryptococcus aureus, Cryptococcus carnescens and Cryptococcus peneaus*. Int. J. Syst. Evol. Microbiol., 53: 1187-1194.

Yarrow D. (1998). Methods for isolation, maintenance and identification of yeasts. In: Kurtzman C.P., Fell J.W., Eds, The Yeasts, A Taxonomic Study, 4rd edn., Elsevier Science B.V., Amsterdam, pp. 77-100.