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# Biodiversity of Yeasts in the Gastrointestinal Ecosystem with Emphasis on Its Importance for the Host

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## 1. Introduction

Thinking of the diversity of the microbial world most readers will focus their attention to the bacteria and archaea. However, among most of the ecosystems present on Earth, such as soil or intestine of animals, another microbial group has established: the yeasts. Their biodiversity has been hardly investigated although they possess probably as much adaptation potential as bacteria, considering the enormous differences between the habitats and the challenges the different ecosystems must face.

In the chapter the authors would like to provide to the reader the state of the art in the field of intestinal yeast research, with focus on the diversity of the yeasts in the gastrointestinal tract of animals - insects and mammals. Up to date there are about 1,500 yeast species known, belonging to two phyla *Ascomycota* (Suh et al., 2006a) and *Basidiomycota* (Fell et al., 2000; Scorzetti et al., 2002) of the Dikarya subkingdom (Kurtzman & Fell, 2006). These unicellular fungi are considered as ubiquitous microorganisms, which can be found in a vast variety of different ecological systems associated with terrestrial and underwater flora and fauna (Rosa & Peter, 2006). Nevertheless, based on the currently researches it could be suggested that only 1% of the diversity of yeast species has been described yet (Kurtzman & Fell, 2006).

The gastrointestinal tract (GIT) of animals remains a largely unexplored habitat. Most of the yeasts were isolated from the GIT of beetles and other insects. The current knowledge about yeasts' diversity in the digestive tract of vertebrates, especially of farm animals, is still based generally on the findings from 50's and 70's of the XX<sup>th</sup> Century. Furthermore, the taxonomy of yeasts undergoes continuous revision, e.g., variety of yeasts has double names or even many synonyms. This came off due to the fact that sometimes the same yeasts have been described by different scientists (Kurtzman & Fell, 1998) or several yeasts were invalidly classified, e.g., species assigned to genus *Torulopsis* were reclassified to the genus *Candida* (Yarrow & Meyer, 1978). Moreover, it transpires frequently when yeast species previously described based on its phenotypic characteristics has been later phylogenetically analysed and on that basis reclassified into another genus, consequently obtained a new name (Kurtzman & Fell, 2006). Therefore, few yeast species will be mentioned in the further sections with a double name.

Furthermore, in this review we will provide some consideration to the importance of the yeasts for the host. Advantages and disadvantages of the contemporary methods used for diversity studies will also be pointed.

## 2. Biodiversity of yeasts in the gastrointestinal ecosystem

Microorganisms live in the diverse habitats of the world. In course of evolution, some microbes adapted to the extreme environment prevailing in the gastrointestinal ecosystem of human and animals. Gastrointestinal tracts of mammals (Hooper & Gordon, 2001; Bauer et al., 2006; Ley et al., 2008) and insects (Dillon & Dillon, 2004; Hongoh, 2010; Grunwald et al., 2010) harbour vast bacterial communities which undoubtedly play an important role for the maturation and proper function of mucosal and systemic immune systems, nutrient metabolism and host health. In contrast, the knowledge of yeasts which naturally occur in intestine and thereby belong to the intestinal microbiota still remains deficient.

### 2.1 Yeasts' diversity in the alimentary tract of insects

Insects are among the most diverse group of animals that has been found worldwide (Chapman, 2009) and they unavoidably come into contact with yeasts widespread in various habitats like soil (Botha, 2006), plants (Fonseca & Inácio, 2006) and fresh and marine water (Nagahama, 2006). This has been confirmed by the fact that yeasts can be found on body surface as well as in the entrails of insects: beetles, bees, flies, lacewings, termites, and mosquitoes; and their larvae. Table 1 summarises the yeast species that were recently discovered in the GIT of insects and that have particularly been identified using molecular methods.

#### 2.1.1 Yeasts associated with flowers and gut of bees

In general, insects can be considered as either as a vector carrying yeasts on the body surface or the consumers of the yeasts (for review see Phaff & Starmer, 1987; Ganter, 2006). For instance, since yeasts regularly occur in flowers they are considered as autochthonous for this environment, and so they are closely associated with flower-visiting insects (Lachance et al., 2001; Brysch-Herzberg, 2004). In floristic nectar, ascomycetous yeasts belonging to the genera *Metschnikowia*, *Kodamaea*, *Wickerhamiella* have been found in higher abundance, whereas basidiomycetous yeasts (e.g. *Cryptococcus* spp., *Rhodotorula* spp., *Pseudozyma* spp.) were rarely isolated (Lachance et al., 2001; Brysch-Herzberg, 2004). Brysch-Herzberg (2004) counted an astonishing number of yeast cells (up to 16,000 cells/ $\mu$ l nectar) from the nectar samples of *Digitalis purpurea*. In this study, yeasts were isolated from nectar, plant materials, honey and from body of bumblebees, but unfortunately not from the GIT. In another study (Batra et al., 1973), the same yeasts (species of *Candida*, *Endomycopsis*, *Oidium*, *Hansenula*, *Rhodotorula*, *Saccharomyces*, *Schizosaccharomyces*, *Pichia*, and *Zygosaccharomyces*) were found in nectar and in the crops of bees, however, in the last niche yeasts were determined to be in higher, about 10 to 100 fold, concentration. Gilliam et al. (1974) summarized the data of yeasts isolated from the digestive tract of honey bees and also reported about their own investigations on yeasts in intestines of 388 adult worker honey bees.

Seven species were observed in the study, but three: *Candida (Torulopsis) magnoliae*, *Candida parapsilosis*, and *Candida (Torulopsis) glabrata* were found most frequently providing evidence for their dominance in this environment.

#### 2.1.2 Yeasts associated with digestive tract of ants and termites

Ants, belonging like bees to the order *Hymenoptera*, have been also associated closely with variety of yeasts harbouring their nests (Carreiro et al., 1997; Rodrigues et al., 2009; Pagnocca et al., 2010). Some yeasts can pass into the infrabuccal pocket, a pouch in the ants'

Species	Host organism	References
<i>Ambrosiozyma monospora</i>	owlfly ( <i>Ascalaphidae</i> )	(Nguyen et al., 2007)
<i>Aureobasidium pullulans</i>	leaf beetles ( <i>Chrysomelidae</i> )	(Molnar et al., 2008)
<i>Candida aglyptinia</i> sp. nov.	round fungus beetle ( <i>Leiodidae</i> )	(Suh et al., 2006b)
<i>Candida alai</i> sp. nov.	click beetle ( <i>Elateridae</i> )	(Suh et al., 2008)
<i>Candida ambrosiae</i>	pleasing fungus beetle ( <i>Erotylidae</i> ); darkling beetle ( <i>Tenebrionidae</i> ); sap-feeding beetle ( <i>Nitidulidae</i> )	(Suh et al., 2004b)
<i>Candida amphixiae</i> sp. nov.	handsome fungus beetle ( <i>Endomychidae</i> )	(Suh et al., 2005b)
<i>Candida anneliseae</i> sp. nov.	pleasing fungus beetles ( <i>Erotylidae</i> ); darkling beetles ( <i>Tenebrionidae</i> ); rove beetle ( <i>Staphylinidae</i> ); false darkling beetles ( <i>Melandryidae</i> ); clown beetle ( <i>Histeridae</i> ); minute tree-fungus beetle ( <i>Ciidae</i> )	(Suh et al., 2004b)
<i>Candida arabinofermentans</i>	bark beetles ( <i>Scolytinae</i> )	(Rivera et al., 2009)
<i>Candida ascalaphidarum</i> sp. nov.	owlfly ( <i>Ascalaphidae</i> ); largus bug ( <i>Largidae</i> ); fungus weevil ( <i>Anthribidae</i> )	(Nguyen et al., 2007)
<i>Candida atakaporum</i> sp. nov.	pleasing fungus beetle ( <i>Erotylidae</i> )	(Suh et al., 2004b)
<i>Candida atbi</i> sp. nov.	sap-feeding beetles ( <i>Nitidulidae</i> )	(Suh et al., 2006b)
<i>Candida athensensis</i> sp. nov.	beetles: cucujoid, curculionid, <i>Megalodacne fasciata</i> ( <i>Erotylidae</i> )	(Suh & Blackwell, 2004)
<i>Candida barrocoloradensis</i> sp. nov.	sap-feeding beetles ( <i>Nitidulidae</i> )	(Suh et al., 2006b)
<i>Candida blattae</i> sp. nov.	cockroach ( <i>Blattidae</i> ); dobsonfly ( <i>Corydalidae</i> )	(Nguyen et al., 2007)
<i>Candida blattariae</i> sp. nov.	cockroach ( <i>Blattaria</i> )	(Suh et al., 2005b)
<i>Candida bohienensis</i> sp. nov.	click beetles ( <i>Elateridae</i> ); leaf beetle ( <i>Chrysomelidae</i> )	(Suh et al., 2008)
<i>Candida bokatorum</i> sp. nov.	pleasing fungus beetles ( <i>Erotylidae</i> ); ground beetle ( <i>Carabidae</i> ); sap-feeding beetle ( <i>Nitidulidae</i> ); false darkling beetle ( <i>Melandryidae</i> ); darkling beetle ( <i>Tenebrionidae</i> )	(Suh et al., 2004b)
<i>Candida bolitotheri</i> sp. nov.	darkling beetles ( <i>Tenebrionidae</i> ); pleasing fungus beetle ( <i>Erotylidae</i> )	(Suh et al., 2004b)

Species	Host organism	References
<i>Candida bribrorum</i> sp. nov.	pleasing fungus beetles ( <i>Erotylidae</i> ); darkling beetles ( <i>Tenebrionidae</i> )	(Suh et al., 2004b)
<i>Candida buenavistaensis</i> sp. nov.	longhorned beetle ( <i>Cerambycidae</i> ); scarab beetle ( <i>Scarabaeidae</i> )	(Suh et al., 2008)
<i>Candida cf neerlandica</i>	owlflies ( <i>Ascalaphidae</i> ); earwig ( <i>Labiduridae</i> ); cricket ( <i>Gryllidae</i> )	(Nguyen et al., 2007; Suh et al., 2008)
<i>Candida chickasaworum</i> sp. nov.	pleasing fungus beetles ( <i>Erotylidae</i> ); minute tree-fungus beetle ( <i>Ciidae</i> )	(Suh et al., 2004b)
<i>Candida choctaworum</i> sp. nov.	darkling beetles ( <i>Tenebrionidae</i> ); minute tree-fungus beetles ( <i>Ciidae</i> ); fungus weevil ( <i>Anthribidae</i> )	(Suh et al., 2004b)
<i>Candida chrysomelidarum</i> sp. nov.	leaf beetles ( <i>Chrysomelidae</i> )	(Nguyen et al., 2006)
<i>Candida corydali</i> sp. nov.	dobsonflies and fishflies ( <i>Corydalidae</i> )	(Nguyen et al., 2007)
<i>Candida derodonti</i> sp. nov.	tooth-necked fungus beetles ( <i>Derodontidae</i> )	(Suh & Blackwell, 2005)
<i>Candida dosseyi</i> sp. nov.	dobsonflies ( <i>Corydalidae</i> )	(Nguyen et al., 2007)
<i>Candida elateridarum</i> sp. nov.	click beetle ( <i>Elateridae</i> )	(Suh & Blackwell, 2004)
<i>Candida emberorum</i> sp. nov.	pleasing fungus beetles ( <i>Erotylidae</i> ); handsome fungus beetle ( <i>Endomychidae</i> )	(Suh et al., 2004b)
<i>Candida endomychidarum</i> sp. nov.	handsome fungus beetle ( <i>Endomychidae</i> )	(Suh et al., 2005b)
<i>Candida ernobii</i>	bark beetles ( <i>Scolytinae</i> )	(Rivera et al., 2009)
<i>Candida fermentati</i>	sap-feeding beetle ( <i>Nitidulidae</i> ); scarab beetle ( <i>Scarabaeidae</i> ) fishfly ( <i>Corydalidae</i> ) mosquitoes ( <i>Culicidae</i> )	(Suh & Blackwell, 2004) (Nguyen et al., 2007) (Gusmão et al., 2010)
<i>Candida frijolesensis</i> sp. nov.	handsome fungus beetle ( <i>Endomychidae</i> ); elephant beetle ( <i>Scarabaeidae</i> )	(Suh et al., 2008)
<i>Candida gatunensis</i> sp. nov.	sap-feeding beetles ( <i>Nitidulidae</i> )	(Suh et al., 2006b)
<i>Candida gigantensis</i> sp. nov.	click beetles ( <i>Elateridae</i> )	(Suh et al., 2008)
<i>Candida guaymorum</i> sp. nov.	pleasing fungus beetles ( <i>Erotylidae</i> ); scarab beetle ( <i>Scarabaeidae</i> )	(Suh et al., 2004b)
<i>Candida intermedia</i>	mosquitoes ( <i>Culicidae</i> )	(Ricci et al., 2011a)

Species	Host organism	References
<i>Candida kruisii</i>	sap-feeding beetles ( <i>Nitidulidae</i> )	(Suh et al., 2006b)
<i>Candida kunorum</i> sp. nov.	sap-feeding beetle ( <i>Nitidulidae</i> )	(Suh et al., 2004b)
<i>Candida labiduridarum</i> sp. nov.	earwig ( <i>Labiduridae</i> ); cricket ( <i>Gryllidae</i> ); owlflies ( <i>Ascalaphidae</i> )	(Suh et al., 2008)
<i>Candida lessepsii</i> sp. nov.	unidentified beetle	(Suh et al., 2005b)
	bark beetles ( <i>Scolytinae</i> )	(Rivera et al., 2009)
<i>Candida lycoperdinae</i> sp. nov.	sap-feeding beetle ( <i>Nitidulidae</i> ); scarab beetle ( <i>Scarabaeidae</i> )	(Suh et al., 2006b)
<i>Candida maltosa</i>	click beetles ( <i>Elateridae</i> ); bess beetles ( <i>Passalidae</i> ); scarab beetle ( <i>Scarabaeidae</i> )	(Suh et al., 2008)
<i>Candida maxii</i> sp. nov.	darkling beetle ( <i>Tenebrionidae</i> )	(Suh et al., 2004b)
<i>Candida membranifaciens</i>	crane fly ( <i>Tipulidae</i> ); dobsonfly ( <i>Corydalidae</i> ); green lacewings ( <i>Chrysopidae</i> )	(Suh et al., 2005b; Nguyen et al., 2007)
<i>Candida michaelii</i> sp. nov.	handsome fungus beetle ( <i>Endomychidae</i> )	(Suh et al., 2005b)
<i>Candida nodaensis</i>	mosquitoes ( <i>Culicidae</i> )	(Gusmão et al., 2010)
<i>Candida oregonensis</i>	bark beetles ( <i>Scolytinae</i> )	(Rivera et al., 2009)
<i>Candida pallodes</i> sp. nov.	sap-feeding beetles ( <i>Nitidulidae</i> )	(Suh et al., 2006b)
<i>Candida panamensis</i> sp. nov.	sap-feeding beetles ( <i>Nitidulidae</i> ); darkling beetle ( <i>Tenebrionidae</i> )	(Suh et al., 2006b)
<i>Candida panamericana</i> sp. nov.	pleasing fungus beetle ( <i>Erotylidae</i> ); rove beetle ( <i>Staphylinidae</i> ); darkling beetle ( <i>Tenebrionidae</i> )	(Suh et al., 2004b)
<i>Candida picachoensis</i> sp. nov.	green lacewings ( <i>Chrysopidae</i> )	(Suh et al., 2004a)
	leaf beetles ( <i>Chrysomelidae</i> )	(Nguyen et al., 2006)
<i>Candida piceae</i>	bark beetles ( <i>Scolytinae</i> )	(Rivera et al., 2009)
<i>Candida pimensis</i> sp. nov.	green lacewings ( <i>Chrysopidae</i> )	(Suh et al., 2004a)
		(Nguyen et al., 2006)
<i>Candida plutei</i> sp. nov.	rove beetle ( <i>Staphylinidae</i> )	(Suh & Blackwell, 2005)
<i>Candida pseudorhagii</i>	click beetle ( <i>Elateridae</i> )	(Suh et al., 2008)
<i>Candida quercitrusa</i>	dobsonfly ( <i>Corydalidae</i> )	(Nguyen et al., 2007)
	cotton bollworm ( <i>Noctuidae</i> )	(Molnar et al., 2008)

Species	Host organism	References
<i>Candida sake</i>	crambid snout moths ( <i>Crambidae</i> ); cotton bollworm ( <i>Noctuidae</i> )	(Molnar et al., 2008)
<i>Candida sinolaborantium</i>	handsome fungus beetle ( <i>Endomychidae</i> ); cerambycid larvae ( <i>Cerambycidae</i> )	(Suh et al., 2005b)
<i>Candida smithsonii</i> sp. nov.	endomychid larva ( <i>Endomychidae</i> ); Iphiclus beetle ( <i>Erotylidae</i> )	(Suh & Blackwell, 2004)
<i>Candida stri</i> sp. nov.	sap-feeding beetles ( <i>Nitidulidae</i> )	(Suh et al., 2006b)
<i>Candida taliae</i> sp. nov.	darkling beetle ( <i>Tenebrionidae</i> )	(Suh et al., 2004b)
<i>Candida temnochilae</i> sp. nov.	bark-gnawing beetle ( <i>Trogossitidae</i> ); bess beetle ( <i>Passalidae</i> )	(Suh et al., 2005b)
<i>Candida tenuis</i>	minute tree-fungus beetle ( <i>Ciidae</i> )	(Suh et al., 2005b)
<i>Candida terraborum</i> sp. nov.	pleasing fungus beetle ( <i>Erotylidae</i> )	(Suh et al., 2004b)
<i>Candida tetrigidarum</i> sp. nov.	elephant beetle ( <i>Scarabaeidae</i> ); pygmy grasshopper ( <i>Tetrigidae</i> )	(Suh et al., 2008)
<i>Candida tritomae</i> sp. nov.	pleasing fungus beetles ( <i>Erotylidae</i> ); scarab beetle ( <i>Scarabaeidae</i> )	(Suh et al., 2006b)
<i>Candida tropicalis</i>	owlfly ( <i>Ascalaphidae</i> ); bess beetles ( <i>Passalidae</i> ); ichneumon wasps ( <i>Ichneumonidae</i> ); dobsonfly and fishfly ( <i>Corydalidae</i> ); roach ( <i>Blattidae</i> )	(Nguyen et al., 2007; Suh et al., 2008)
<i>Candida wounanorum</i> sp. nov.	pleasing fungus beetle ( <i>Erotylidae</i> )	(Suh et al., 2004b)
<i>Candida xestobii</i>	crambid snout moths ( <i>Crambidae</i> ); leaf beetle ( <i>Chrysomelidae</i> )	(Molnar et al., 2008)
<i>Candida yuchorum</i> sp. nov.	pleasing fungus beetle ( <i>Erotylidae</i> )	(Suh et al., 2004b)
<i>Clavispora lustansiae</i>	blister beetle ( <i>Meloidae</i> )	(Rao et al., 2007)
<i>Cryptococcus flavescens</i>	crambid snout moth ( <i>Crambidae</i> ); cotton bollworm ( <i>Noctuidae</i> ); leaf beetles ( <i>Chrysomelidae</i> )	(Molnar et al., 2008)
<i>Cryptococcus luteolus</i>	crambid snout moths ( <i>Crambidae</i> ); leaf beetles ( <i>Chrysomelidae</i> )	(Molnar et al., 2008)
	green lacewings ( <i>Chrysopidae</i> )	(Woolfolk & Inglis, 2004)
<i>Cryptococcus oeirensis</i>	leaf beetle ( <i>Chrysomelidae</i> )	(Molnar et al., 2008)
<i>Cryptococcus victoriae</i>	crambid snout moth ( <i>Crambidae</i> )	(Molnar et al., 2008)
	green lacewings ( <i>Chrysopidae</i> )	(Woolfolk & Inglis, 2004)

Species	Host organism	References
<i>Cryptococcus zeae</i>	crambid snout moths ( <i>Crambidae</i> ); leaf beetle ( <i>Chrysomelidae</i> )	(Molnar et al., 2008)
<i>Geotrichum carabidarum</i> sp. nov.	ground beetle ( <i>Carabidae</i> ); geometrid larva ( <i>Geometridae</i> ); pleasing fungus beetle ( <i>Erotylidae</i> )	(Suh & Blackwell, 2006)
<i>Geotrichum cucujoidarum</i> sp. nov.	minute tree-fungus beetle ( <i>Ciidae</i> ); cucujoid beetle ( <i>Cucujoidae</i> ); hining fungus beetle ( <i>Scaphidiinae</i> )	(Suh & Blackwell, 2006)
<i>Geotrichum histeridarum</i> sp. nov.	clown beetles ( <i>Histeridae</i> ); pleasing fungus beetle ( <i>Erotylidae</i> ); geometrid larvae ( <i>Geometridae</i> ); tiger moths ( <i>Arctiidae</i> )	(Suh & Blackwell, 2006)
<i>Hanseniaspora uvarum</i>	crambid snout moths ( <i>Crambidae</i> ) mosquitoes ( <i>Culicidae</i> )	(Molnar et al., 2008) (Ricci et al., 2011a)
<i>Hanseniaspora vineae</i>	dobsonflies and fishflies ( <i>Corydalidae</i> )	(Nguyen et al., 2007)
<i>Issatchenkia orientalis</i>	scarab beetle ( <i>Scarabaeidae</i> )	(Rao et al., 2007)
<i>Kodamaea laetipori</i> sp. nov.	darkling beetles ( <i>Tenebrionidae</i> ); scarab beetles ( <i>Scarabaeidae</i> )	(Suh & Blackwell, 2005)
<i>Kodamaea ohmeri</i>	dobsonfly ( <i>Corydalidae</i> ); sap-feeding beetle ( <i>Nitidulidae</i> ); pleasing fungus beetle ( <i>Erotylidae</i> ) mosquitoes ( <i>Culicidae</i> )	(Suh & Blackwell, 2005; Nguyen et al., 2007) (Gusmão et al., 2010)
<i>Kuraishia capsulata</i>	bark beetles ( <i>Scolytinae</i> )	(Rivera et al., 2009)
<i>Kuraishia cf. molischiana</i>	bark beetles ( <i>Scolytinae</i> )	(Rivera et al., 2009)
<i>Lachancea fermentati</i>	dobsonflies and fishflies ( <i>Corydalidae</i> )	(Nguyen et al., 2007)
<i>Lachancea thermotolerans</i>	dobsonflies and fishflies ( <i>Corydalidae</i> )	(Nguyen et al., 2007)
<i>Lodderomyces elongisporus</i>	bark and ambrosia beetle ( <i>Scolytinae</i> )	(Suh et al., 2008)
<i>Metschnikowia andauensis</i> sp. nov.	cotton bollworm ( <i>Noctuidae</i> )	(Molnar & Prillinger, 2005)
<i>Metschnikowia chrysoperlae</i> sp. nov.	green lacewings ( <i>Chrysopidae</i> ) dobsonfly ( <i>Corydalidae</i> )	(Suh et al., 2004a) (Nguyen et al., 2007)
<i>Metschnikowia corniflorae</i> sp. nov.	soldier beetles ( <i>Cantharidae</i> )	(Nguyen et al., 2006)
<i>Metschnikowia fructicola</i>	crambid snout moths ( <i>Crambidae</i> ); cotton bollworms ( <i>Noctuidae</i> )	(Molnar & Prillinger, 2005)
<i>Metschnikowia noctiluminum</i> sp. nov.	green lacewings ( <i>Chrysopidae</i> )	(Nguyen et al., 2006)
<i>Metschnikowia pulcherrima</i>	dobsonfly ( <i>Corydalidae</i> ) green lacewings ( <i>Chrysopidae</i> )	(Nguyen et al., 2007) (Woolfolk & Inglis, 2004)



Species	Host organism	References
<i>Pichia americana</i>	bark beetles ( <i>Scolytinae</i> )	(Rivera et al., 2009)
<i>Pichia canadensis</i>	bark beetles ( <i>Scolytinae</i> )	(Rivera et al., 2009)
<i>Pichia caribbica</i>	pleasing fungus beetle ( <i>Erotylinae</i> )	(Rao et al., 2007)
	mosquitoes ( <i>Culicidae</i> )	(Gusmão et al., 2007)
<i>Pichia glucozyma</i>	bark beetles ( <i>Scolytinae</i> )	(Rivera et al., 2009)
<i>Pichia guilliermondii</i>	fishfly and dobsonfly ( <i>Corydalidae</i> ); owlfly ( <i>Ascalaphidae</i> )	(Nguyen et al., 2007)
	bark beetle ( <i>Scolytinae</i> )	(Rivera et al., 2009)
	crambid snout moths ( <i>Crambidae</i> ); leaf beetle ( <i>Chrysomelidae</i> )	(Molnar et al., 2008)
	mosquitoes ( <i>Culicidae</i> )	(Gusmão et al., 2010)
<i>Pichia mexicana</i>	bark beetles ( <i>Scolytinae</i> )	(Rivera et al., 2009)
<i>Pichia nakazawae</i> var. <i>akitaensis</i>	handsome fungus beetle ( <i>Endomychidae</i> )	(Suh et al., 2005b)
<i>Pichia scolyti</i>	bark beetles ( <i>Scolytinae</i> )	(Rivera et al., 2009)
<i>Pseudozyma apsidi</i>	cotton bollworms ( <i>Noctuidae</i> ); leaf beetles ( <i>Chrysomelidae</i> )	(Molnar et al., 2008)
<i>Pseudozyma flocculosa</i>	cotton bollworms ( <i>Noctuidae</i> )	(Molnar et al., 2008)
<i>Pseudozyma prolifica</i>	cotton bollworms ( <i>Noctuidae</i> )	(Molnar et al., 2008)
<i>Rhodotorula aurantiaca</i>	leaf beetle ( <i>Chrysomelidae</i> )	(Molnar et al., 2008)
<i>Rhodotorula glutinis</i>	crambid snout moths ( <i>Crambidae</i> ); leaf beetle ( <i>Chrysomelidae</i> )	(Molnar et al., 2008)
<i>Saccharomyces cariocanus</i>	dobsonflies ( <i>Corydalidae</i> )	(Nguyen et al., 2007)
<i>Saccharomyces cerevisiae</i>	dobsonflies and fishflies ( <i>Corydalidae</i> )	(Nguyen et al., 2007)
<i>Saccharomyces fermentans</i>	fishfly ( <i>Corydalidae</i> )	(Nguyen et al., 2007)
<i>Saprochete gigas</i>	dobsonfly and fishfly ( <i>Corydalidae</i> )	(Nguyen et al., 2007)
<i>Sporobolomyces coprosmae</i>	leaf beetles ( <i>Chrysomelidae</i> )	(Molnar et al., 2008)
<i>Tilletiopsis washingtonensis</i>	leaf beetle ( <i>Chrysomelidae</i> )	(Molnar et al., 2008)
<i>Torulaspora delbrueckii</i>	fishfly ( <i>Corydalidae</i> )	(Nguyen et al., 2007)
<i>Trichosporon insectorum</i> sp. nov.	bess beetles ( <i>Passalidae</i> ); scarab beetles ( <i>Scarabaeidae</i> )	(Fuentefria et al., 2008)
<i>Trichosporon mycotoxinivorans</i> sp. nov.	lower termite ( <i>Mastotermitidae</i> )	(Molnar et al., 2004)
<i>Trichosporon xylopinii</i> sp. nov.	darkling beetles ( <i>Tenebrionidae</i> ); bess beetles ( <i>Passalidae</i> )	(Gujjari et al., 2010)
<i>Wickerhamomyces anomalous</i> ( <i>Pichia anomala</i> )	mosquitoes ( <i>Culicidae</i> )	(Ricci et al., 2011a)

Table 1. Yeasts detected in the alimentary tract of insects

oral cavity (Hansen & Klotz, 2005). Based on physiological characteristics using a microbial identification system BIOLOG, Mankowski & Morrell (2004) identified 19 species from 155 yeast isolates collected from nest, surrounding soil and frass as well as from exoskeleton and infrabuccal pockets of carpenter ants. From 17 isolates found in the infrabuccal pockets, ten were identified as *Debaryomyces polymorphus* and other species (*Pichia guilliermondii*, *Candida ergatensis*, *Candida edax*, *Bulleromyces* spp. and *Cryptococcus laurentii*) occurred only once or twice. Besides of soil samples, *D. polymorphus* was the most often isolated yeast from the all analysed materials. Further social insects, such as termites may harbour high yeasts' numbers ( $10^7$ - $10^8$  cells/ml) in their gut (Schäfer et al., 1996). Schäfer et al. (1996) cultured 35 yeast isolates from the intestinal contents of termite species, *Zootermopsis nevadensis* and *Neotermes castaneus*, but the authors reported presence of only 15 yeast strains, as their enzymatic activity were significant to the study. These phenotypes were related to the genera *Candida*, *Sporothrix*, *Pichia* and *Debaryomyces*. In another study, *Debaryomyces hansenii* and *Sporothrix albicans* as well as species of *Trichosporon* and *Rhodospordium* could also be found in the hindgut of the termites from families: *Mastotermitidae*, *Hodotermitidae*, *Kalotermitidae* and *Rhinotermitidae* and roaches (Prillinger et al., 1996).

### 2.1.3 Yeasts associated with the gut of some pests

In the gut of some maize' pests (*Diabrotica virgifera*, *Helicoverpa armigera* and *Ostrinia nubilalis*), Molnar et al. (2008) isolated 97 yeast strains; furthermore they detected yeasts as well as other fungi of the genera: *Acremonium*, *Aspergillus*, *Cladosporium* and *Fusarium* by means of cloning and denaturing gradient gel electrophoresis (DGGE). The occurrence of clones was given in percents. All methods revealed that *Metschnikowia* spp., closely related to *Metschnikowia pulcherrima*, *Cryptococcus* spp. (*Cr. luteolus*, *Cr. zaeae* and *Cr. flavescens*) as well as *Candida* spp., bearing close similarity to *C. xestobii* or *C. sake*, and *Pseudozyma* spp. were the most frequently identified yeasts. *Pichia guilliermondii* and *Rhodotorula* species were less common. Some of occasionally found yeasts e.g. *Aureobasidium pullulans*, *Candida quercitrusa*, *Hanseniaspora uvarum*, *Sporobolomyces coprosmae*, *Tilletiopsis washingtonensis* were detected however only via culturing. There are some publications reporting presence of the yeasts in the gut of mosquitos (*Diptera: Culicidae*), which are known to be vectors of many diseases in humans. Gusmão et al. (2007; 2010) identified *Pichia caribbica*, *Pichia guilliermondii*, *Pichia* (syn. *Kodamaea*) *ohmeri*, *Candida fermentati* and *Candida nodaensis* in the diverticulum of *Aedes aegypti*. Ricci et al. (2011a) investigated yeasts in the gut of *Anopheles stephensi* using molecular and cultivation-dependent methods. Forty six clones that expressed fragments of the 18S rRNA gene retrieved from the gut samples of 6 adults were sequenced. Eleven clones were identified as *Wickerhamomyces anomalus*, known also as *Pichia anomala*, while others could be assigned either to genus *Candida* or *Pichia* or to unidentified fungus. Moreover, 100 colonies were cultured from 10 insect specimens, classified based on their morphology and identified as *Candida intermedia*, *Hanseniaspora uvarum* and *W. anomalus* (77%, 15% and 8% respectively) by sequencing analysis of 18S and 26S rRNA genes and ITS fragments. *W. anomalus* was detectable using both approaches. Furthermore, Ricci et al. (2011a; 2011b) observed the presence of *W. anomalus* in the midgut of different mosquitos species *Anopheles stephensi*, *Anopheles gambiae*, *Aedes albopictus* and *Aedes aegypti* of both sexes as well as on larvae, pupae and gonads, thereby supposed close relationship between this yeast species and mosquitos.

#### 2.1.4 Yeasts in the digestive tract of lacewings

Lacewings (*Neuroptera: Chrysopidae*) are one of the predators admitted as biological control agents of pests. During the scanning and transmission electron microscopical studies, a large numbers of yeast cells were observed within lacewings' alimentary tract (Woolfolk et al., 2004; Woolfolk & Inglis, 2004; Chen et al., 2006). Woolfolk & Inglis (2004) investigated yeasts in the different parts: diverticulum, foregut, midgut, and hindgut of digestive tract of 24 lacewing adults (*Chrysoperla rufilabris*) collected at two field locations in Mississippi. With the exception of 7 insects that were yeasts-free, lacewings harboured a high concentration ( $\approx 10^3$  colony forming units; CFU) of yeasts distributed in the all analysed gut sections; however the highest ( $5.4 \times 10^5$  CFU/g) density was in diverticulum. In total 752 yeasts were isolated in the study and arranged in five groups based on their phenotypic properties; some specimens were randomly chosen from each group for further genotyping analysis. Interestingly, 89% of the isolates were identified as *Metschnikowia pulcherrima* and the remaining 11% involved either *Cryptococcus victoriae* or *Cryptococcus luteolus* or strains that could not be assigned by the authors to any known species. Sometimes, closely related yeast species could be separated only according to the genotypic characterization, while they were showing similar physiological properties (Kurtzman & Fell, 2006) as it was the case in the study of Suh et al. (2004a). These authors isolated 14 yeasts from digestive tract of *Chrysoperla* spp. which were closely related to *M. pulcherrima*, however sufficiently variable in the D1&D2 domains of the 26S rRNA gene of the large subunit (LSU) to represent three new species: *Metschnikowia chrysoperlae*, *Candida picachoensis* and *Candida pimensis*. Recently, several new yeasts of *Metschnikowia* and *Candida* (see table 1) were discovered in the gut of other members of the *Neuroptera*, too (Nguyen et al., 2006; 2007).

#### 2.1.5 Yeasts in the digestive tract of beetles

At the present time, the most yeasts were isolated from the digestive tract of beetles (*Coleoptera*). Shifrine & Phaff (1956) collected bark beetles (*Dendroctonus* and *Ips*) and their larvae from the various coniferus trees in Northern California. After sterilization of the outer surface, the beetles were dissected and yeasts were isolated from internal parts of the insects. Total of 169 yeast strains could be assigned to 13 species. *Candida silvicola* (41.4%; teleomorph *Hansenula holstii*), *Hansenula capsulata* (21.3%), *Pichia pinus* (18.9%) and *Candida curvata* (8.9%) were frequently found; other species (e.g. *C. parapsilosis*, *C. mycoderma*, *C. rugosa*, *Cryptococcus diffiuens*) were rarely (from 0.6 to 1.8%) isolated. Some yeasts as *Candida* (*Torulopsis*) *nitratophila*, *C. (T.) melibiosum*, *Rhodotorula crocea* and *C. silvicola* were described by these authors as new species. Recently, Rivera et al. (2009) provided an account of yeasts associated with alimentary tract of *Dendroctonus* beetles. Yeasts (403 strains) were isolated from different parts of intestine, the midgut (anterior & posterior) and the hindgut, as well as from the ovaries, eggs and frass of the beetles collected from pine trees at 34 locations in Mexico, Cuatemala and the USA. Based on the sequence analysis of several DNA regions (18S, 26S rRNA genes and ITS1) and phenotypical characteristics, the yeasts were reletated to three genera: *Candida* spp. (*C. ernobii*, *C. piceae*, *C. membranifaciens*, *C. lessepsii*, *C. arabinofermentans* and *C. oregonensis*), *Pichia* spp. (*P. americana*, *P. guilliermondii*, *P. scolyti*, *P. mexicana*, *P. glucozyma* and *P. canadensis*) and *Kurashia* spp. (*K. capsulata* and *K. cf. molischiana*). The exact numbers of the yeast strains isolated from the different gut sections have not been provided by the autors, however, they indicated that yeasts were present in eggs, ovaries and frass to much lesser extent than in the guts. For instance, *P. americana*, *C.*

*ernobii* and the strains related to the one *Candida* sp. were prevalent in all parts of gut and frass and *P. guilliermondii* and *C. ernobii* were cultured most frequently from the posterior midgut.

In relation to high number of the yeast isolates (richness) described above, comparatively low yeast diversity was found in the assemblage of *Dendroctonus* beetles. It thereby underlined the impact of the host and/or environmental factors on the yeasts diversity. Nevertheless, examination of yeasts harbouring the GIT of beetles from 27 families revealed a huge variety of yeasts (Suh et al., 2005a).

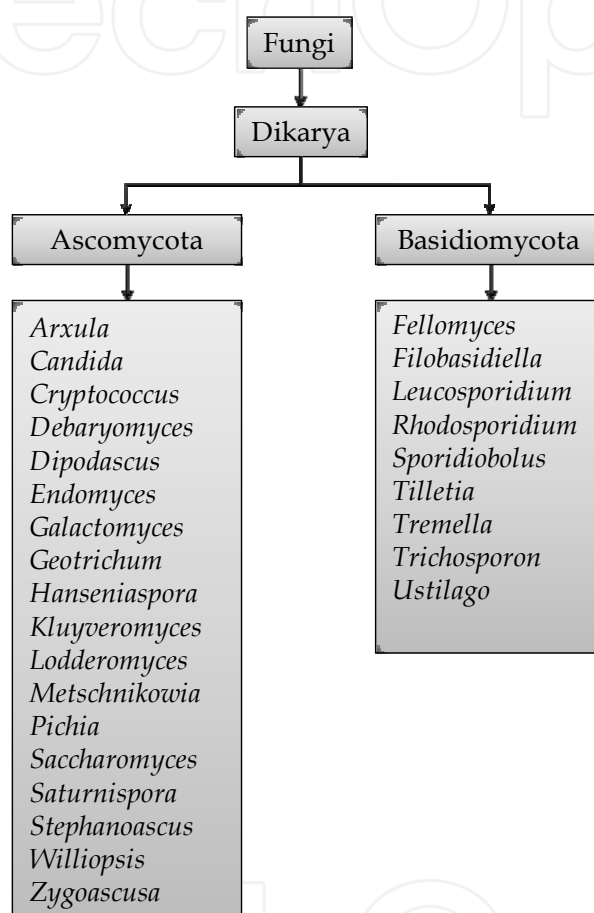


Fig. 1. Schematic representation of the different yeast taxa belonging to the two phyla *Ascomycota* and *Basidiomycota* of the Dikarya subkingdom isolated from the intestinal tract of insects during the study of Suh et al. (2005a).

During three-years-period, Suh et al. (2005a) isolated about 650 yeasts from the gut of diverse beetles collected from the south-eastern USA and Panama. Sequence analyses of the D1&D2 domains of LSU rRNA gene revealed 290 single species belonging to at least 27 taxa (Fig. 1.); the great majority of which were ascomycetous and some basidiomycetous yeasts. It is noteworthy that nearly 200 yeasts determined throughout the study were considered by the authors to represent new, not yet described species. In the meantime, some of them (table 1) were characterized by Suh, Nguyen, Blackwell and their co-workers. Based on their observation Suh et al. (2005a) suggested that almost each beetle species may be a host for at least one unknown yeast species. In the last decades, describing of many novel species of yeasts isolated from the gut of insects corroborates this supposition.

Nowadays, there are over one million of accepted insect species; however, their number has yearly increased and is still largely undiscovered (Chapman, 2009). Thus, it can be supposed that the number of yeasts would tremendously rise, even if only the intestinal tracts of the currently known insects were explored.

## 2.2 Yeasts' diversity in the GIT of vertebrates with focus on farm animals

The more intensive investigations of the yeast population present in the GIT of vertebrates, based on various cultivation procedures, began in the fifties of the XX<sup>th</sup> Century. Van Uden et al. (1958) and Van Uden & Carmo Sousa (1957b) examined yeasts in the caecal samples of large number of animals: 252 cattle, 252 horses, 503 sheep, 250 goats and 250 pigs. Yeasts were also studied by Parle (1957) in the digestive tract of cows, rabbits, sheep, guinea pigs, opossums, monkeys, cats, dogs, hedgehogs, mice, pigs and rats. Lund (1974) explored yeasts and moulds in the bovine rumen. Lately, yeasts were also described in the intestinal tract of reptiles (Kostka et al., 1997), birds (Cafarchia et al., 2006; 2008; Brillhante et al., 2010; Costa et al., 2010), mice (Scupham et al., 2006), dogs (Brito et al., 2009) and fish (Gatesoupe, 2007). In these studies, the scientists have detected various ascomycetous and basidiomycetous yeasts chiefly representing the genera *Candida*, *Trichosporon*, *Pichia*, *Rhodotorula*, *Debaryomyces*, *Kluyveromyces* and *Saccharomyces*.

In general, the diversity of the yeast population depended on the host; but many species occurred at diverse, also not intestinal ecosystems; and several exhibited direct relationship to the individual animal. It should be noticed, however, that yeasts could not be always isolated from the investigated GIT and often they were present in small numbers. Nevertheless, taking into consideration the scarce information existing on yeasts in the gastrointestinal ecosystems of vertebrates, it is well known that relatively high variety as well as quantity of yeasts can be found in the GIT of pigs.

Here, the yeasts diversity in the GIT of farm animals representing diverse nutritional types: omnivores (pig), monogastric herbivores (horse) and ruminants will stay in focus and will be compared.

### 2.2.1 Yeasts in the GIT of pigs

Comparatively to all animals investigated in the study of Van Uden et al. (1958), the most frequent occurrence (88.8%) of yeasts was detected in the caecum of pigs (horses 52.4%, cattle 46.8%, sheep 6.8%, and goats 6.4%). The yeasts *Candida slooffiae*, *Candida krusei*, *Saccharomyces telluris*, *Candida albicans*, *Candida (Torulopsis) glabrata*, were commonly found in the porcine gut. However, *C. slooffiae* was isolated most frequently (48.4%). A few other yeasts such as *Saccharomyces* spp., *Pichia membranifaciens*, *Pichia farinose* and *Candida mycoderma* could also be identified. Roughly the same situation has been confirmed in the following studies (Van Uden & Carmo-Sousa, 1962; Mehnert & Koch, 1963), where the scientists isolated almost the same variety of yeasts from the different parts of porcine GIT. After investigation of digesta samples collected from stomach, three parts of small intestine as well as caecum and rectum of healthy 57 pigs, Van Uden & Carmo Sousa (1962) reported high animal-individual qualitative and quantitative variability of the yeast occupation. In total 15 yeast species were identified; while *C. slooffiae* was present in 27 pigs, many other species mentioned above occurred only sporadically. Moreover, *C. slooffiae* was highly abundant, from  $10^2$  to  $10^3$  CFU/g of chyme in the stomach and up to  $10^6$  CFU/g intestine contents in the rectum. A still higher occurrence of yeasts in the gut of pigs was detected by Mehnert & Koch (1963), up to  $10^7$  CFU/g in rectum. They isolated 292 yeasts from 200

digesta samples collected from stomach and rectum from 98 (of 100 examined) pigs. Apart from the *C. slooffiae* which was detectable in 75% of pigs, yeast species such as *C. krusei*, *S. telluris*, *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. parapsilosis* and *C. pintolopesii* (60%, 26%, 9%, 4%, 3%, 3% and 2% respectively) were isolated, too. Also in this study the appearance of yeasts was variable within a part of the GIT and among examined animals. Thus, stomach was generally colonized by yeasts at lesser intense than rectum. In most animals, *C. slooffiae* and *C. krusei* were detected both in stomach and rectum, while just in a few cases the yeasts could be found only in a single part of the GIT. *C. slooffiae* and its closely related species: *S. telluris* and *C. pintolopesii* have been newly molecularly investigated and based on multigene sequence analyses they were assigned to the teleomorphic genus *Kazachstania* (Kurtzman et al., 2005).

Recently, Urubschurov et al. (2008) described yeasts' diversity in the gut of piglets around weaning which were reared at two facilities: at experimental farm (EF) with improved husbandry conditions than at commercial farm (CF). Most piglets, 33 at CF and 35 at EF, were weaned at 28 days (d) of age and fed with the same diet until 39 d in both farms. A number of piglets, namely 18 at CF and 9 at EF, were left by the sows without additional feeding. All piglets were sacrificed at 39 d of age and digesta samples from GIT were collected. D1&D2 domains of 26S rRNA gene from 173 yeast isolates obtained from 95 piglets were sequenced. The alignment to known sequences revealed close relationship to 17 species, of which the most dominated are presented in figure 2. Urubschurov et al. (2008) observed distinction of yeasts variety between both facilities that were proven by calculation of different similarity and diversity indices. In piglets from CF *Galactomyces geotrichum*, *Kazachstania slooffiae* and *Candida catenulata* were the most abundant ones and the other were present only at low abundances. Unlike at CF, at EF two species, namely *K. slooffiae* and *C. glabrata* were found to be the most dominating ones and the others were rarely isolated. Some of the other species could be found in piglets either only at the EF (*P. fermentans*, *C. tropicalis*, *C. oleophila*, *C. parapsilosis*, *P. guilliermondii*, *Rh. mucillaginosa*, *T. montevidense*) or at the CF (*C. silvae* and *P. farinose*). This study provided evidence for association of *K. slooffiae* with the porcine GIT. *K. (C.) slooffiae* was found for the first time in 6 of 252 examined horses (Van Uden & Carmo-Sousa, 1957a), however, due to frequent occurrence and high concentration in the porcine digestive tract it can be considered to be specific for pigs.

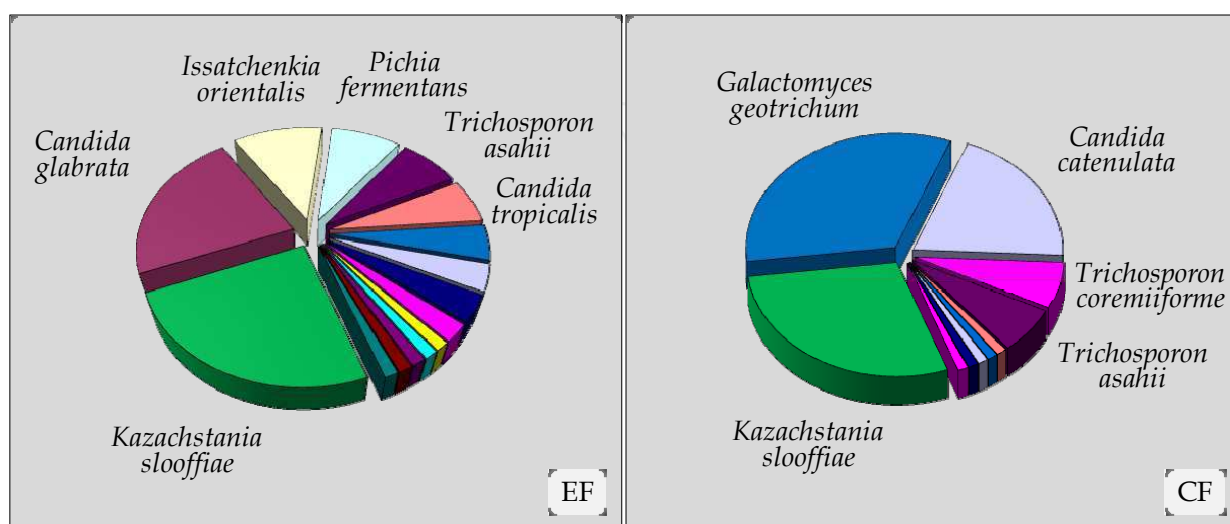


Fig. 2. Dominated yeasts isolated from the gut of 39 d old piglets, kept at experimental (EF) and commercial farm (CF), in the study of Urubschurov et al. (2008).

Furthermore, compared to other yeasts occurring in the porcine GIT, which can survive also in other ecological niches outside the animals, *K. (C.) slooffiae* seems to be well adapted to the porcine gastrointestinal habitat, as this species is one of those that need high temperature to grow, comparable to the temperature of animal body, being characterized as thermophilic or psychrophobic (Travassos & Cury, 1971).

### 2.2.2 Yeasts in the equine GIT

Several investigators focused on the effect of yeast, *Saccharomyces cerevisiae*, on intestinal microbiota of horses and on the digestibility of different diets (e.g. Medina et al., 2002; Jouany et al., 2008; 2009). But little is known about the yeasts naturally occurring in the equine gut. Van Uden et al., (1958) studied yeasts in the caecal contents of 252 horses, and these authors revealed presence of yeasts in over half (52.4%) of the investigated animals. With occurrence of 21.8%, *Trichosporon cutaneum* was the most frequently isolated yeast, whereas in porcine intestine this species was found only one time. However, other yeasts: *C. krusei* (8.3%) as well as *C. tropicalis* (4.4%), *C. albicans* (4.4%), *C. parapsilosis* (3.6%), *C. slooffiae* (2.4%), *C. glabrata* (1.2%) and *S. telluris* (1.2%) detected in the GIT of horses were also commonly recorded in pigs.

### 2.2.3 Yeasts in the GIT of ruminants

As reported by Lund (1974), a different number of yeasts has been observed in the ruminal contents from cows and sheep depending on the culture conditions and incubation temperature. After at 39°C incubation of the rumen contents collected at different times from five cows, Clarke & Menna (1961) quantified yeast colonies rated from 80 to 13,000 per gram of samples; 134 colonies were isolated for further characterization. Yeasts from *Candida* spp. (*C. krusei*, *C. albicans*, *C. tropicalis* and *C. rugosa*), *Trichosporon* spp. (*T. cutaneum* and *T. sericeum*) and *Rhodotorula* spp. (*Rh. rubra/mucilaginoso*, *Rh. glutinis* and *Rh. macerans*) were identified; however, the *Rhodotorula* spp. could be cultured only at the temperature of 19°C. Lund (1974) examined fungal microbiota in rumen liquid of 10 fistulated and 2 non fistulated cows fed different diets. Forty nine collected samples were plated and incubated simultaneously at 25°C and at 39°C. A considerably larger number of yeast colonies, up to 1000 fold and about 20 fold on average, were observed after incubation at 25°C, while sometimes even none could be obtain after incubation at 39°C. Nevertheless, only 67 yeast isolates growing at 39°C, as it is the temperature proper to the rumen environment, were used for further identification. The largest share (77.6%) of them was identified as *C. krusei*, *T. cutaneum* and *T. capitatum* and the rest were *C. valida*, *C. ingens*, *C. pintolepesii*, *Klyveromyces bulgaricus*, *Saccharomycopsis lipolytica* and *Hansenula fabianii*. Other fungi (molds) belonging to the order *Mucorales* have been also found in the study. Additionally, Lund (1974) observed two yeast species *C. krusei* and *T. capitatum* in faeces of the cattle. However, their counts were lower than in the rumen samples of corresponding host. Later, Lund (1980) conducted a similar study where the researcher investigated yeasts microbiota in 16 rumen samples of musk oxen. Only 6 strains of one species, *C. parapsilosis* were identified after incubation at 37°C, while 41 yeast strains belonging to *Candida* spp., *Cryptococcus* spp., *Trichosporon* spp., *Rhodotorula* spp., *Torulopsis* spp. and *Pichia* spp. were characterized after growing at 25°C. But, the authors indicated that the rumen contents were kept frozen for a long period (more than 7 weeks), what could have had considerable effects on yeast colonization. As mentioned above, Van Uden et al. (1958) cultivated caecal samples

from a much higher number of cattle (252) as well as sheep (503) and goats (250). Among the investigated animals, cattle showed the highest (46.8%) occurrence of yeasts, whereas just a few yeasts could be found in sheep and goats, 6.8% and 6.4% of the animals, respectively. The most frequently isolated yeasts were *C. tropicalis* and *C. krusei* in cattle, and *C. albicans* in sheep. These species were also isolated from the goats, but just two times each; and *C. glabrata* four times. A few other yeasts identified as members of *Saccharomyces* spp., *Candida* spp. and *Pichia* spp. have been found only occasionally.

Quite similar results regarding yeast colonization have been obtained in the cultivation dependent studies (Clarke & Menna 1961; Lund 1974; 1980; Van Uden et al. 1958) from different geographical regions. Shin et al. (2004) explored different rumen samples (fluid, solid and epithelium) from one cow, examined for yeasts population using molecular approaches. Shin et al. (2004) have succeeded to obtain 97 clones containing 26S rRNA gene fragments from the three types of samples and to assign them to the different phylogenetic groups. Compared to 4 phylotypes from the rumen epithelium showing the closest relatedness to *Geotrichum silvicola*, *Acremonium alternatum*, *Pseudozyma rugulosa* (up to 99%) and *Galactomyces* sp. (97%), and 2 phylotypes (*Geotrichum silvicola*, 99% and *Galactomyces* sp., 97%) from the rumen solid, the highest yeast' diversity was observed in the samples of rumen fluid revealing presence of 15 various phylotypes. Only 5 (*Setosphaeria monoceras*, *Raciborskiomyces longisetosum*, *Magnaporthe grisea*, *Ustilago affinis* and *Pseudozyma rugulosa*) of the 15 phylotypes showed 99% identity with the sequences deposited at the NCBI GenBank. The identification rate of the others belonging also to the classes *Pezizomycotina*, *Urediniomycetes*, *Saccharomycotina* and *Hymenomycetes* ranged from 91 to 98%. These phylotypes could represent new species, because in yeasts more than 1% of the nucleotide divergence in D1&D2 domains of the 26S rRNA gene may represent a separate species (Kurtzman & Fell, 2006). In spite of the lack of inter-individual comparison, this study showed a potential existence of the other yeasts that have not been discovered yet.

### 3. Methods for investigating biodiversity of the yeasts from GIT

From the cited references it is obvious, the biodiversity studies depend very much on the applied method. However, this is beyond the scope of this chapter to provide very detailed description of all possible methods that could be used for studies on yeasts' diversity. Nor calculation of the different biodiversity indices is in the focus of the paragraph. This paragraph is meant to provide short discussion on the existing possibilities, their limitations and advantages, and provide the reader with some input for consideration which methods he or she would choose for his/her studies.

Any application of either method mentioned below requires correct sampling of the material. Studying the biodiversity of the yeasts harbouring the GIT the dominating yeasts are in focus of most studies, as well as their abundance and changes of the abundance in time and in relation to the diet. For these purposes faecal or digesta samples have been collected from large animals (Urubschurov et al., 2008; 2011) or whole intestines from e.g. insects have been dissected (Suh et al., 2004b; 2005a; Nguyen et al., 2007). Whereas rain worms, termites or other small animals can provide the whole GIT for the studies, only part of contents of wall of the GIT can be studied in large animals. Therefore the choice of sampling is the first bottle neck in the studies on yeasts biodiversity in the GIT. Following proceedings such as homogenization, concentration or dilution of the samples must be hereby additionally considered.



Among the methods applied for investigating the biodiversity of yeasts harbouring the GIT of animals, cultivation and morphological and/or biochemical identification have been the most often used for more than 150 years. However, these methods bear limitations such as the choice of the right cultivation medium, pH, temperature and moisture. Furthermore the yeast species that need more time for growth and are at lower abundance in the community cannot be identified in this way. It has been accepted that every ecosystem consists, next to cultivable organisms, also of viable but non-cultivable (VBNC) microorganisms, that temporarily cannot be cultivated in laboratory because of nutrient limitation or lack of optimal living conditions (Edwards, 2000). This is why only approximately 1% of yeast species could be described so far (Kurtzman & Fell, 2006). Sabouraud agar is the medium most commonly used for cultivation of yeasts from clinical or ecological samples (Odds, 1991), however many others have been used for industrial purposes, providing alternatives for cultivation of more demanding species (King et al., 1986; Jarvis & Williams, 1987; Fleet, 1990; Deak, 1991). It is to remember, that various species can give similar colonies, and the same species can grow in a different way under different conditions. Cultivated species can be however observed under microscope, what helps for identification of the isolates. Spectrophotometric methods such as MALDI-TOF could also provide fast and good tool for identification of the isolates. Molecular methods can be also applied for identification of isolates, e.g. pyrosequencing of target genes (Borman et al., 2009; 2010). Further development of modified media and combinations of temperature, pH, aerobic/anaerobic conditions and moisture would probably increase the number of isolated yeasts, it is however laborious and very time consuming.

Cultivation-independent methods which have been used for the last two decades provided the researches with fast and specific tools for the biodiversity studies. Polymerase chain reaction (PCR), DNA-DNA hybridization or fluorescence in situ hybridization (FISH) applying probes targeting the RNA allow in theory detection of 1 single colony present in a sample population. Further separation of the specific DNA fragments performing denaturing or temperature gradient gel electrophoresis (DGGE / TGGE) allows studying the diversity of the complex community (Cocolin et al., 2002; Prakitchaiwattana et al., 2004; Molnar et al., 2008). Other molecular methods could be also applied for identification of members of a community, e.g. terminal restriction fragment length polymorphism (T-RFLP), amplified fragment length polymorphism (AFLP), multiple-locus variable number tandem repeat analysis (MLVA) (e.g. Tiedje et al., 1999; Gemmer et al., 2002). These methods are very specific, allowing targeting of specified species and thus quantification of the yeasts and calculating the biodiversity. The largest limitation for methods based on PCR is the low sensitivity, as the practice shows only 1-2% of the community can be detected in this way (Macnaughton et al., 1999). Furthermore, fingerprint methods have the bias combined to the fact that amplicons from different species with sequences of similar energetic profile may migrate to the same positions; multiple gene copies with slight sequence differences may give multiple bands for one strain or species; finally some species are phylogenetically very similar (Lachance et al., 2003; Janczyk et al., 2006; Borman et al., 2010). The design of probes for direct targeting needs knowledge on the sequence of the target gene and differences between species.

Pyrosequencing and other high-throughput methods provide a fast and very efficient tool for identification of the members of the complex populations. Metagenome analyses targeting the D1/D2 domain of the 26S rRNA gene or the internal transcribed regions (ITSs) allow distinction of the yeasts (Kurtzman & Fell, 2006) and seem to be very suitable methods

for studying the yeast biodiversity in the GIT of animals. Pyrosequencing is a rapid method providing up to several thousands of sequences per sample in just few days. Followed by bioinformatics processing, alignment to known species is performed resulting not only in a phylogenetic tree but also in description of the species diversity. Unknown species can be also detected in this way. The high cost provides the limitation for the wide application of this method; however it is to expect that in the near future the high-throughput sequencing will be as expensive as the other commonly used molecular tools.

A microarray has been recently developed allowing characterization of pig GIT bacterial community, targeting over 800 phylotypes (Pérez Gutiérrez, 2010). Microarrays for yeasts would need to be developed to provide further molecular tool for studying the biodiversity and its changes caused by different extrinsic factors.

#### 4. Role of yeasts in GIT

Studying diversity of yeasts harbouring the GIT of animals would be incomplete without consideration of the role that these microorganisms play for the host. For years the yeasts harbouring the GIT of animals and humans have been considered rather as harmful to the host's health. Indeed, there are some species belonging to *Candida*, *Cryptococcus*, *Malassezia*, *Trichosporon* and *Geotrichum* that could be pathogenic to members of the Animal kingdom (Fidel et al., 1999; Girmenia et al., 2005; Cabañes, 2010). Furthermore, many researchers have evaluated yeasts in association with various diseases and if they found representatives of this group they acted against them applying medical treatment (Schulze & Sonnenborn, 2009). However, there is just as little known about yeasts harbouring the GIT of healthy animals to understand their importance there, and growing evidences appear for their role in the proper function and survival of the host. In fact, the current knowledge about yeasts in the digestive tract of vertebrates is still based on the findings from 50's and 70's of the XX<sup>th</sup> Century; therefore there is a great demand for the scientific evaluation in this field. As enough reports exist concerning pathogenic yeasts, in this paragraph a possible positive impact of yeasts on the gut ecology and host health will be discussed.

There are nice reviews (e.g. Phaff & Starmer, 1987; Ganter, 2006) pronouncing a yeast-insect relationship. Gatesoupe (2007) gave an insight into the ecology of yeasts naturally occurring in the intestinal tract of fish, and thereby emphasized a possible importance of yeasts to the host.

Similarly to the probiotic strains of *Saccharomyces cerevisiae* (Buts, 2009), the cells of some intestinal yeasts could have a trophic effects since they provide a source of B vitamins, proteins, trace minerals and essential amino acids. Besides, the major portion (> 90%) of the yeast cell walls comprise of polysaccharides such as  $\beta$ -glucans, mannans and chitin, which composition and structure are specific for individual yeast (Latgé, 2007). In many human and animals studies,  $\beta$ -glucans and mannans have been comprehensively investigated; they may play important diverse roles for the host immune system and exhibit antimicrobial activity against bacteria thereby influencing the establishment of the intestinal microbiota and promising to promote host's health. Therefore, several studies concentrated on use of the live or dead yeast cells in human and animal nutrition as supplements or as a remedy for acute diarrhoea in humans (Bekatorou et al., 2006; Buts & De Keyser, 2006; Fleet, 2007; Buts, 2009). Furthermore, due to production of several enzymes, some yeast species, e.g. found in the gut of termites (Schäfer et al., 1996; Molnar et al., 2004) and beetles (Suh et al., 2003), are able to degrade hemicelluloses that are being the main carbohydrates of

herbivorous diet, and also detoxify toxins that can appear in the feed. The possibility cannot be excluded that some yeasts harbouring GIT of herbivorous animals may produce extracellular enzymes (e.g. exohemicellulases, exocellulases) or show endocellulolytic activity, and thereby contribute to their digestion by breaking down complex, indigestible fibre into simple carbohydrates.

It is still a prevalent opinion, that yeasts harbouring the digestive tract of animals have only minor importance for the host. The main scientific argument up to date is the negligible quantity of yeasts. Nevertheless, yeasts may be of physiological relevance, even though they are present to a much lesser extent than bacteria. In fact, yeasts could provide a relevant biomass, as they have a cell volume 30- to 100-fold higher than bacteria (Gatesoupe, 2007). Commensal yeasts may interact with intestinal bacteria and due to this interplay affect microbial diversity and host organism. An example of such yeasts-bacteria interrelationship provides the study of Urubschurov et al. (2011) who examined changes of yeasts and major bacterial groups (lactobacilli, enterobacteria and enterococci) in the faeces of piglets after weaning. They observed that the increase of yeasts number, where the dominating species was *Kazachstania slooffiae*, significantly correlated with the increase of lactobacilli and decrease of enterobacteria numbers. Other studies hypothesized that specific yeasts frequently occurred in high quantity at the digestive tract of lacewings (Woolfolk & Inglis, 2004; Woolfolk et al., 2004) and mosquitoes (Ricci et al., 2011a; 2011b) and were symbiotically related to the host.

These first indications need further confirmation but they already show that the yeasts cannot be considered negligible any more.

## 5. Conclusions

Yeasts belong to gastrointestinal microbiota even though they are not as frequent as the bacteria or archaea. However, it does not disclude their importance for the host and for the members of the complex microbial community. Despite long time of research, whereas our knowledge on bacterial intestinal communities has increased dramatically during last decade, still only little is known on the intestinal yeasts. This review provides an overview on what has been done in the field of intestinal yeast research up till now, and the reader surely agrees that much more work needs to be done. Not only the diversity of the intestinal yeasts and its changes depending on different conditions shall be further uncovered. The importance of yeasts for the host and the interplay between yeasts and other members of the intestinal milieu is also waiting to be explored. New cultivation techniques; cultivation combined with molecular techniques will need to be further developed to overcome the existing limitations.

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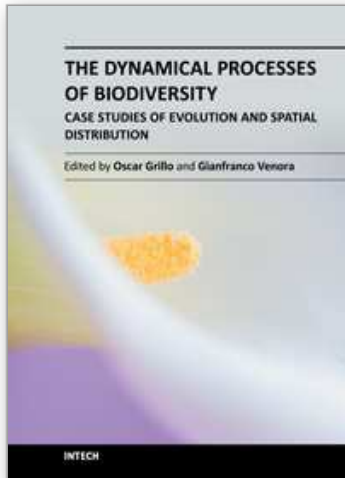


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