

Autochthonous yeasts as one of the tools to produce wines by original technologies

Eva Vontrová¹, Petra Kubizniaková¹, Jaromír Fiala¹, Jiří Sochor², Dagmar Matoulková*¹

¹Research Institute of Brewing and Malting, Lípová 15, 120 44 Praha

²Faculty of Horticulture, Mendel University in Brno, Valtická 337, 691 44 Lednice

*Corresponding author: matoulkova@beerresearch.cz

Abstract

This review focuses on autochthonous wine yeasts that occur naturally in a specific geographical area, and their role in wine production. The distribution of individual species changes during ripening and processing of grapes and fermentation of the grape must. The composition of autochthonous microflora is influenced by many factors including interspecific interactions. Autochthonous yeast populations lend to wine flavor and taste typical for a specific geographical area. The article describes the fermentation of grape must, changes of autochthonous yeast microflora during spontaneous or combined fermentation, and includes an overview of the basic species of autochthonous yeasts.

Key words: autochthonous yeast, extracellular enzymes, *Hanseniaspora uvarum*, non-*Saccharomyces*, spontaneous fermentation, wine

1. Introduction

Autochthonous wine yeasts are naturally occurring in a specific geographical area and are typical for each vineyard. They produce many sensory-active compounds and thus influence the aromatic character of the wine. Yeast other than *Saccharomyces*, the so-called non-*Saccharomyces*, produce important extracellular enzymes that influence grape yield, wine purity, etc. (Manzanares et al., 2011). Due to different composition of yeast species on grapes / land with grapevine / vineyard, each wine fermented by autochthonous yeasts receives unique aroma and flavor. On the other hand, when only commercially produced active dried yeast is used in induced fermentation, it leads to a narrow aromatic profile and uniformity of wine (Furdíková and Malík, 2008).

The predominant species on the grapes is *Hanseniaspora uvarum* (formerly classified as *Kloeckera apiculata*). Strictly aerobic and / or weakly fermentative yeasts may also be found in lesser proportions. The grape microflora can be affected by a wide variety of factors such as temperature, rainfall, altitude, use of fungicides or ripeness of the grape. The vector is especially air (wind) and insects. The microflora associated with winery equipment is largely composed of *Saccharomyces cerevisiae* that is responsible for alcoholic fermentation (Aranda et al., 2011).

2. Wine yeast

Yeast taxonomy often uses two names for a single genetic species depending on the life cycle of the cell – the teleomorph (sexual stage) and the anamorph (vegetative stage). In the vegetative form, yeasts reproduce by budding or fission, during sexual reproduction they create spores. Wine yeasts with both stages include (teleomorph / anamorph): *Dekkera* / *Brettanomyces*, *Metschnikowia*

pulcherrima / *Candida pulcherrima*, *Hanseniaspora uvarum* / *Kloeckera apiculata* and *Torulasporea delbrueckii* / *Candida colliculosa*. Winemakers use an informal name of yeast based on their morphology, function and other characteristics. *Hanseniaspora uvarum* (*Kloeckera apiculata*) is often referred to as an apiculate yeast due to its lemon-shaped morphology. *Hanseniaspora* together with *Candida*, *Cryptococcus*, *Debaryomyces*, *Hansenula*, *Issatchenkia*, *Kluyveromyces*, *Metschnikowia*, *Pichia* and *Rhodotorula* are also called “native / wild” yeasts because they originate in the wine environment. However, other yeasts also are present naturally in the wine environment (e.g. *Saccharomyces*), and so the term “native / wild” ceased to be used (Fugelsang and Edwards, 2006). The term “non-*Saccharomyces* yeasts” is now more commonly used to describe yeasts that are not of the genus *Saccharomyces*. Members of the genus *Saccharomyces* involved in the primary fermentation are sometimes called “fermentative yeasts”. *Candida*, *Hansenula* or *Pichia* can grow in unfilled containers in the presence of oxygen, growing on the surface of the young wine in the form of flor (pellicle). These yeasts are referred to as pellicle-forming yeasts or also “film yeasts” (Fugelsang and Edwards, 2006; Minárik, 2009).

Traits of the wine yeast *S. cerevisiae* relate to the variety of grapes and geographical origin (Tab. 1). Wild strains of yeast produce wine with earthy and sulphurous aroma, whereas domesticated strains produce predominantly fruity and floral aroma (Hyma et al., 2011). The final taste and quality of wine are affected by both the variety of grapes, the technological process and genotype of yeast, as well as by stress factors, for example changes in the concentration of oxygen, osmotic pressure, pH, ethanol concentration, temperature stress, availability of nutrients, etc. (Belda et al., 2017; Vontrová et al., 2017).

Traditional wine-production uses autochthonous yeasts that are on the surface of grapes and in the wine

Table 1: Specific traits of *S. cerevisiae* strains from different locations (Tofalo et al., 2013; modified)

Geographical origin	Specific traits
West Africa	Poor utilization of galactose
	Hypersensitivity to high temperatures
European	High respiratory capability (ethanol growth)
	Good proliferation in wine must
	Good tolerance to copper, tartaric acid, Na ⁺ and Li ⁺
Malaysia	Utilization of melibiose and mannitol
North America	Unable to metabolize maltose
	Tolerance to oxalic acid

environment. Yeasts appear on grapes at the beginning of the ripening and are transmitted to grapes by insects or air. The amount of yeast on berries of *Vitis vinifera* ranges from 10⁴ to 10⁶/cm² of the surface of berries. The highest number of yeasts on grapes occur in a warm climate (Fleet et al., 2002).

The predominant species on the surface of the grapes is *Hanseniaspora uvarum* (*Kloeckera apiculata*) that account for more than 50% of the total yeast population. Strictly aerobic (e.g. *Rhodotorula*) and slightly fermentative yeasts with low tolerance to alcohol are found in low levels on grapes. Included are, for example: *Candida* (*C. famata*, *C. stellata*), *Cryptococcus*, *Debaryomyces*, *Hansenula* (*H. anomala*), *Issatchenkia*, *Kluyveromyces*, *Metschnikowia* (*M. pulcherrima*), *Pichia* (*P. fermentans*, *P. membranifaciens*) and *Rhodotorula* (Aranda et al., 2011; Ribereau-Gayon et al., 2006). Fermentative yeasts *S. cerevisiae* and *S. bayanus* are found on grapes only to a limited extent. Especially *S. cerevisiae* is found on the technological equipment in the winery. *Brettanomyces*, *Candida*, *Hansenula*, *Hanseniaspora*, *Pichia* and *Torulaspora* have also been isolated from the winery (Aranda et al., 2011).

3. Types of grape must fermentation

There are three types of wine fermentation: induced, spontaneous and combined. Induced fermentation uses active dry wine yeast *S. cerevisiae*, that ensures a seamless fermentation with minimal formation of by-products of anaerobic glycolysis. By-products can create unwanted side-effects of wine tones. When dry yeasts are used, yeasts that are not a natural part of the vineyards get in the must and the authenticity of the wines is thus disturbed. Induced fermentation is used by most winemakers. Recently, more and more wineries are turning to spontaneous fermentation (Furdíková and Malík, 2008).

Starter cultures are able to initiate fermentation in a short time while limiting the potential for spoilage. The starter culture of *Saccharomyces* can inhibit the population of non-*Saccharomyces* (Fugelsang and Edwards, 2006). There are some exceptions, when non-*Saccharomyces* (e.g. *Hanseniaspora uvarum* and *Candida*) develop in the presence of a *Saccharomyces* starter culture (Heard and Fleet, 1985). During must fermentation by mixed non-*Saccharomyces* and *Saccharomyces* cultures the starter *Saccharomyces* culture is affected in the level of expression of genes that are

involved in, e.g., higher alcohol production (Barbosa et al., 2015). Thus the sensoric quality of wine has improved when Chenin Blanc must was inoculated by a combined culture of *C. pulcherrima* and *S. cerevisiae* (Jolly et al., 2003). The growth of non-*Saccharomyces* yeasts can be improved by reducing fermentation temperature (10 - 15 °C) (Fugelsang and Edwards, 2006). Spontaneous / natural fermentation uses only autochthonous microorganisms. When autochthonous yeasts and bacteria are used, a typical aromatic profile is preserved. During spontaneous fermentation, different species and strains of yeast and bacteria are being promoted with different intensity. The control of the fermentation process is more difficult. Depletion of growth factors and unpredictable enzymatic effects can occur (Furdíková and Malík, 2008). Spontaneous and induced fermentation can be combined. The combined fermentation uses autochthonous yeasts isolated from the vineyard as starter culture. In the Czech Republic Znovín Znojmo, a.s., Sonberk, a.s. or Popice produce wine with autochthonous yeasts (Furdíková and Malík, 2008).

Although induced fermentation is predominant in wine industry, spontaneous fermentation is becoming more and more used by winemakers. Autochthonous yeasts produce many sensory-active compounds and extracellular enzymes that affect the final quality of wine. Wine produced by spontaneous fermentation has a characteristic aroma and flavour which distinguish it from the wine on the market (Fig. 1).

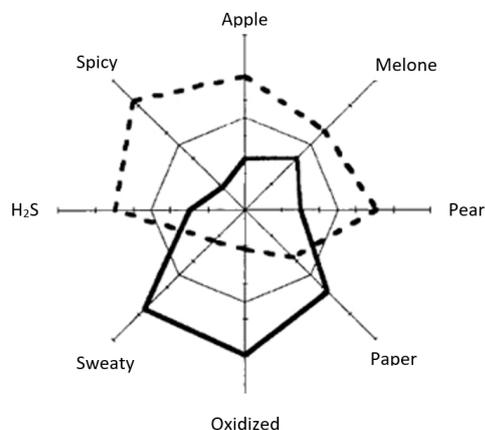


Figure 1: The effect of spontaneous (dashed line) and induced (thick line) fermentation on the sensory characteristics of Riesling wine (thin line) (Jackson 2008; modified).

Non-*Saccharomyces* yeasts, inter alia, differ from saccharomycetes by producing extracellular enzymes that significantly affect some phases of the production process (grape maceration, filtration, clarification) and at the same time increase grape yield and extraction of colors (Charoenchai et al., 1997). The grapes contain various structural substances (pectins, cellulose, glycans, lignin, etc.), aromatic precursors, phenols and enzymes. Under normal vinification conditions, the grapes produce only small quantities of enzymes with very limited activity which is not sufficient for effective degradation of structural substances that are contained in the grapes. The controlled use of non-*Saccharomyces* yeasts increases yield and color extractions and positively improves particular phases of the process (must clarification or wine filtration) (Aranda et al., 2011). Non-*Saccharomyces* yeasts produce esterases, glycosidases, lipases, β -glucosidases, proteases, cellulases, etc. The production of individual enzymes is described below within the taxon characteristics.]

4. Changes in yeast populations during fermentation

The composition of yeasts is changing during spontaneous fermentation of grape must (Fig. 2). During the first few hours after filling the fermentation vessel, yeasts occurring in the must are identical to the grape microflora. Most common non-*Saccharomyces* yeasts (A) are *Hanseniaspora uvarum* (predominant) and *Candida stellate* (Jackson, 2008). *Saccharomyces* that control alcoholic fermentation appear in higher numbers after 20 hours and are predominant after 3 - 4 days. After the primary fermentation is finished, malolactic fermentation (C) may be induced by lactic acid bacteria (e.g. *Oenococcus*). Spoilage yeasts and bacteria are also present during the production of wine and their concentration is highest during the aging of wine (D).

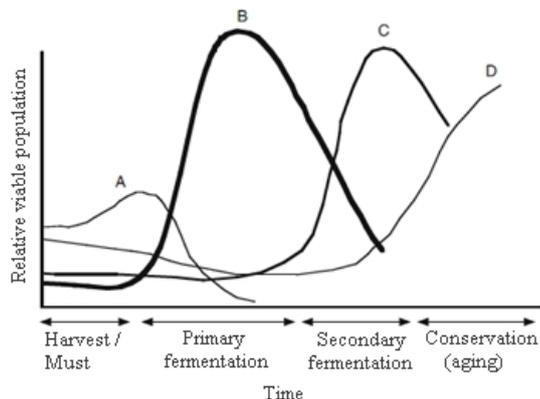


Figure 2: The composition of yeasts during fermentation of grape must; A, non-*Saccharomyces* yeasts; B, *Saccharomyces* yeasts; C, *Oenococcus oeni*; D, spoilage yeast and/or bacteria (Fugelsang and Edwards, 2006; modified).

This change in the yeast population is linked to the increasing concentration of ethanol, the anaerobic conditions, the use of sulphites (Fig. 3), higher temperature and the change of sugar content (Aranda et al., 2011). Low temperatures of fermentation (10-15 °C) can cause

increased tolerance of non-*Saccharomyces* yeasts to ethanol (Gao and Fleet, 1988).

The initial concentration of yeast ranges from 10^3 to 10^6 cells / ml. Non-*Saccharomyces* yeasts (predominantly *Hanseniaspora uvarum*) dominate. Most strains present at the start of fermentation soon pass into decline. This is often caused by the increasing concentration of ethanol produced by *Saccharomyces* yeasts or the addition of sulfur dioxide (Jackson, 2008).

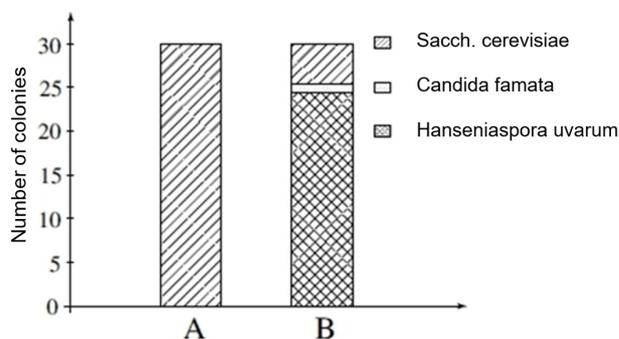


Figure 3: Comparison of yeast species present at the start of alcoholic fermentation. Yeast in a tank of sulfited red grapes in Bordeaux (A). Yeast in a tank of unsulfited white must in Cognac (B) (Ribereau-Gayon et al., 2006; modified).

Recent studies indicate that interspecies / interstrain effects may play a role such as the production of acetaldehyde by hybrid strain of *S. cerevisiae* and *S. uvarum* (Cherait et al., 2005) or a cell-cell contact (Jackson, 2008). The content of acetaldehyde in wine may range from 30 to 300 mg/l. The concentration of acetaldehyde in white wine is usually higher (80 mg/l) than that in red wine (30 mg/l). The presence of acetaldehyde can be masked by the addition of SO_2 (Vontrobová et al., 2017).

During the initial stages of fermentation, non-*Saccharomyces* yeasts produce different chemical compounds such as acetic acid, glycerol and esters. This can modify the final wine's aroma. Some strains of *Hanseniaspora uvarum* produce up to 25 times the amounts of acetic acid typically produced by *H. uvarum*. These yeasts may inhibit some *S. cerevisiae* strains, possibly due to the production of acetic, caprylic and capric acids (Jackson, 2008). In favorable conditions, characterized by a complete exhaustion of sugars at the end of fermentation, the number of yeasts decreases. What remains is almost exclusively *S. cerevisiae*. The viable populations of *S. cerevisiae* also drop rapidly, falling below a few hundred cells/ml. In some cases, spoilage yeasts (e.g. *Brettanomyces intermedius*) can contaminate the aging wine. Some yeasts such as *Pichia*, *Candida*, *Hansenula* and certain strains of *S. cerevisiae* form a veil on the surface of the wine. Strains of *S. cerevisiae* that form a veil are used in the production of specialty wines. Refermentation yeasts can develop significantly in sweet or botrytized wine during aging or storage, the major species found are *Saccharomycodes ludwigii*, *Zygosaccharomyces bailii* and also some *S. cerevisiae* strains that are particularly resistant to ethanol and sulfur dioxide (Ribereau-Gayon et al., 2006).

5. Representatives of autochthonous yeasts

Candida

The genus *Candida* represents a very broad group of species found in wines. The teleomorphic forms are represented by a number of yeasts: *Issatchenkia*, *Kluyveromyces*, *Pichia*, *Metschnikowia*, *Saccharomyces*, *Torulasporea* and *Zygosaccharomyces*. Various sugars may be fermented, and nitrate may be assimilated depending on the species. *C. stellata* assimilates and ferments glucose, sucrose, and raffinose. Other species (e.g. *C. pulcherrima*) ferment only glucose but assimilate glucose, galactose, sucrose, cellobiose, ethanol, glycerol, succinate and other compounds (Fugelsang and Edwards, 2006). *Candida vini* form a veil on the surface of wine and synthesize sensorially active compounds such as ethyl acetate or acetoin (Clemente-Jimenez et al., 2004). Some *Candida* species produce extracellular enzymes pectinases, enzymes cleaving heteropolysaccharides, structural compounds of grapes and proteolytic enzymes that are involved in the removal of protein turbidity of wine (Aranda et al., 2011; Lagace and Bisson, 2000).

Dekkera/Brettanomyces

Dekkera (teleomorph) / *Brettanomyces* (anamorph) is considered to be a cause of wine spoilage due to the production of volatile phenols (4-vinylphenol, 4-vinylguaiacol, 4-ethylphenol and 4-ethylguaiacol) and nitrogenous compounds (2-acetyl-3,4,5,6-tetrahydropyridine, 2-acetyl-1,2,5,6-tetrahydropyridine and 2-ethyl-3,4,5,6-tetrahydropyridine). In the case of special wines, the contamination by *Dekkera* may be desirable (Schiffedercker et al., 2014). All species ferment glucose, and other carbohydrates may be fermented depending on strain. Wine strains of *Brettanomyces* can be separated on the basis of lactose fermentation and succinate assimilation. The species *B. anomalous* (ferments lactose, assimilates succinate) and *B. bruxellensis* (does not ferment lactose, does not assimilate succinate) can be distinguished. Both yeasts can assimilate nitrate. Some members use ethanol as an only carbon and energy source (Silva et al., 2004). Species of *Dekkera* produce a large amount of acetic acid when grown on glucose (Freer, 2002).

Brettanomyces are not primarily found on grapes. They are transmitted by infected wine, equipment or fruit flies (Fugelsang and Edwards, 2006). *Brettanomyces* were found in air samples (Connell et al., 2002) and are generally observed in barrel-aging red wine, although they have also been noted in sparkling wines or white wine (Chardonnay and Sauvignon Blanc) (Ciani and Ferraro, 1997). The presence of the yeast is related to the disaccharide cellobiose which results from the toasting process of wooden barrels (Blondin et al., 1982). *Brettanomyces* yeasts grow very slowly. Their growth reaches a maximum population density 5 to 7 months after vinification (Fugelsang and Zoeklein, 2003). The low content (0.275 g/l) of residual sugar supports the growth of *Brettanomyces*; they are therefore more often isolated from dry wine (Chatonnet et al., 1995).

Hanseniaspora

Hanseniaspora forms ovoid/spherical (young cultures) or apiculate (older cultures) cells (Fugelsang and Edwards, 2006). The species is most commonly found in grapes.

H. uvarum (anamorph *Kloeckera apiculata*) ferments only glucose and assimilates glucose, cellobiose, 2-keto-D-gluconate and salicin. *Hanseniaspora* is often fructophilic and prefers fructose over glucose (Ciani and Fatichenti, 1999). Some *Hanseniaspora* species are the highest producers of proteolytic activity and affect the protein profile (and thus turbidity) of the finished wines (Dizy and Bisson, 2000; Lagace and Bisson, 2000). *Hanseniaspora* yeasts can produce significant quantities of some wine compounds; *H. guilliermondii* produces 2-phenylethyl acetate and *H. uvarum* produces isoamyl acetate (Moreira et al., 2008).

Issatchenkia

Issatchenkia terricola and *I. orientalis* (anamorph *Candida krusei*) are found in wine. *I. orientalis* forms ovoid cells, assimilates glucose, N-acetyl-D-glucosamine, ethanol, glycerol, D-lactate and succinate (Fugelsang and Edwards, 2006). *I. terricola* appears as ovoidal to elongated cells, assimilates glucose, ethanol, glycerol and succinate (Kurtzman et al., 2011). Malic acid and tartaric acid are the major organic acids in the grapes. *Issatchenkia orientalis* degrades malic acid, which can have a negative impact on wine quality. Among the yeasts degrading malic acid are *Schizosaccharomyces pombe*, *Schizosaccharomyces malidevorans* and *Zygosaccharomyces bailii* (Kim et al., 2008). *Issatchenkia terricola* shows β -glucosidase activity. β -Glucosidases can promote the liberation of aromatic compounds from monoterpene glycoside precursors present in young wine (González-Pombo et al., 2011).

Metschnikowia

Metschnikowia is found in the grapes, musts or wines. *M. pulcherrima* (anamorph *Candida pulcherrima*) ferments glucose and assimilates a number of compounds (e.g. galactose, maltose, cellobiose, ethanol, glycerol, D-manitol) but not nitrate (Fugelsang and Edwards, 2006). As nitrogen sources it assimilates cadaverine, L-lysine and ethylamine. The yeast tolerates 10 mg/l cycloheximide but is completely inhibited by it at 100 mg/l. Some species produce pulcherrimin – a brown/red pigment (Pallmann et al., 2001). *M. pulcherrima* has antimicrobial activity against the non-*Saccharomyces* yeasts *Brettanomyces* / *Dekkera*, *Hanseniaspora* and *Pichia*, but does not have any influence on the growth of *S. cerevisiae* and can co-ferment with it. The antimicrobial activity is not based on protein compounds but is related to the production of pulcherriminic acid (precursor of pulcherrimin pigment) which immobilizes the iron in the growth medium (Oro et al., 2014). Growth of *S. cerevisiae* is not inhibited by *M. pulcherrima* and these species can thus co-ferment, resulting in elevated production of ethyloctanoate (pear aroma) by *M. pulcherrima* (Sadoudi et al., 2012). Some strains of *M. pulcherrima* are able to produce higher concentration of 3-mercaptohexanol that is important for white wine aroma (Belda et al., 2017). *M. pulcherrima* produces aspartic protease which can increase the concentration of assimilable nitrogen sources, impact aromatic profile and reduce wine protein haze (Reid et al., 2012).

Meyerozyma

The species *Meyerozyma guilliermondii*, which was previously classified as *Pichia guilliermondii*, occurs in must and on

winery equipment (Dias et al., 2003; Kurtzman et al., 2011). Asexual vegetative stage (anamorph) is referred to as *Candida guilliermondii* (Romi et al., 2014). *Meyerozyma* forms ovoid/elongated cells, ferments sugars and assimilates hexoses, disaccharides, polyols and organic acids (Kurtzman et al., 2011).

Pichia

The genus *Pichia* has previously included more than 100 types of yeast; most of them were reclassified into other genera; at present the genus *Pichia* includes about 20 species. The only fermented sugar is glucose (Kurtzman et al., 2011). Three species are linked to viticulture: "*P. anomala*" (now *Wickerhamomyces anomalus*), *P. membranifaciens* and "*P. guilliermondii*" (now *Meyerozyma guilliermondii*) which are found in grape must, wine or on winery equipment (Dias et al., 2003).

P. membranifaciens is found in wine, slightly ferments glucose and assimilates only a limited number of compounds (glucose, N-acetyl-d-glucosamine, ethanol). Some species of the *Pichia* genus (*P. anomala*, *P. kluyveri*, *P. membranifaciens*, *Pichia guilliermondii* - now *Meyerozyma guilliermondii*) produce pectinases which positively affect the wine aroma (Aranda et al., 2011). Elevated production of 3-mercaptohexanol was observed during co-fermentation of *P. kluyveri* with *S. cerevisiae* (Anfang et al., 2009). Yeasts of the genus *Pichia* utilize acids and thus increase pH (Fugelsang and Edwards, 2006). They produce proteins to prevent the growth of spoilage microorganisms (Błaszczuk et al., 2015)

Saccharomyces

Saccharomyces yeasts feature ovoid- or ellipsoid-shaped cells. *S. cerevisiae* and *S. bayanus* are mainly found in wine. They ferment glucose, sucrose, raffinose and ethanol and do not utilize five-carbon sugars (pentoses). Over the years, there have been numerous changes to the taxonomy of the genus *Saccharomyces* (Fugelsang and Edwards, 2006). Formerly, *Saccharomyces* yeasts were divided into three groups: *sensu stricto*, *sensu lato* and *S. kluyveri*. The *sensu stricto* complex included *S. cerevisiae*, *S. pastorianus*, *S. bayanus*, which are typical for fermentation industry (Fernández-Espinar et al., 2003), and *S. kudriavzevii*, *S. cariocanus* and *S. mikatae* (Naumov et al., 2000). The *sensu lato* complex included *S. exiguus*, *S. castellii*, *S. unisporus*, *S. dairenesis* and *S. servazzii* (Petersen et al., 1999). *S. kluyveri* is now officially described as *Lachancea kluyveri* (Agier et al., 2013). Yeasts from the *sensu lato* complex were reclassified to form a separate group: *Kazachstania*, *Naumovozyma* and *Lachancea* (Kurtzman et al., 2011). Based on PCR analysis, *S. cariocanus* is considered an American variant of *S. paradoxus* (Muir et al., 2011). At present, 9 valid species belong to the *Saccharomyces* genus: *S. cerevisiae*, *S. arboricolus*, *S. paradoxus*, *S. mikatae*, *S. kudriavzevii*, *S. eubayanus*, *S. uvarum* and two species with hybrid genome *S. bayanus* and *S. pastorianus* (Fig. 4).

Interspecific crossings can arise between *S. cerevisiae* and closely related species or genera. In hybridization of closely related yeast, hybrid yeasts feature higher viability (Marinoni et al., 1999). The hybridization affects vitality and phenotypic values of the offspring. Characteristics of the hybrid genome affect the taste and aroma of alcoholic

beverages. Numerous interspecific hybrids between *S. cerevisiae*, *S. uvarum* and *S. kudriavzevii* have been isolated in the environment (da Silva et al., 2015). The increasing competition in the wine sector gives rise to yeast breeding which affects the production of metabolites and thus taste and aroma of the beverages.

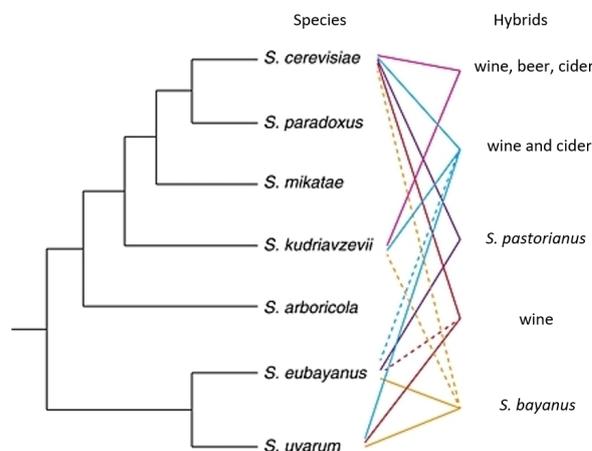


Figure 4. Phylogenetic relationships among *Saccharomyces* species. Dashed lines represent introgressions from a third or fourth species into a hybrid (Boynton and Greig 2014; modified).

Based on restriction analysis and sequencing of the mitochondrial *COX2* gene, hybrids were identified in wine industry (González et al., 2006); in particular, hybrids of *S. cerevisiae*/*S. kudriavzevii*, *S. cerevisiae*/*S. kudriavzevii*/*S. uvarum* and *S. cerevisiae*/*S. uvarum* (Sipiczki, 2008).

The *S. cerevisiae*/*S. kudriavzevii* hybrid has been found to be mainly associated with winemaking and is best suited for winemaking under the typical fermentation conditions of central Europe, low temperatures and low sugar content in grapes of *Vitis vinifera* (González et al., 2006). A VIN7 allotriploid hybrid genome is the result of crossing *S. cerevisiae* with *S. kudriavzevii*. The VIN7 hybrid has the ability to grow at low temperature and the ability to release double amounts of the fruity thiol 4-mercapto-4-methylpentan-2-one (4MMP) which affects the wine aroma (Borneman et al., 2012).

Saccharomyces

This genus is represented by a single species, *Saccharomyces ludwigii*. Fermented sugars include glucose, sucrose and raffinose; assimilated compounds are glucose, sucrose, raffinose, glycerol, cadaverin and ethylamine but not nitrate (Fugelsang and Edwards, 2006). *Saccharomyces ludwigii* is highly resistant to ethanol (12-14%) and SO_2 (up to 200 mg/l) (Ivit et al., 2017). The resistance of this yeast could be due to its ability to produce acetaldehyde, which binds SO_2 (Ciani and Maccarelli, 1998). Formerly, this yeast was considered spoilage yeast. The studies have lately been focusing on its positive impact. The cells produce a β -glucosidase enzyme involved in the release of aromatic compounds from non-aromatic precursors in the grapes and *Saccharomyces* might thus positively affect the sensory profile of wine (Ivit et al., 2017).

Schizosaccharomyces

The genus *Schizosaccharomyces* was initially considered as a spoilage yeast because of the production of undesirable metabolites (acetic acid, hydrogen sulfide, acetaldehyde). Currently, *Schizosaccharomyces* species have a great potential to produce less acidic wines with a lower histamine content, a higher anthocyanin content and a higher color intensity. *Schizosaccharomyces* reduces the concentration of malic acid, leading to malolactic fermentation in must which leads to a reduction of undesirable amines.

S. pombe can be an invaluable tool for wines with a high concentration of malic acid, especially for winemakers in northern wine regions (cold climates). *S. pombe* reduces the content of gluconic acid, reduces 4-ethylphenol (Mylona et al., 2016) and has urease activity, i.e. it lowers the content of urea (precursor of toxic ethyl carbamate) during the fermentation process (Benito et al., 2013).

Wickerhamomyces

W. anomalus (formerly *P. anomala*) has a limited fermentation ability (it ferments glucose and sucrose) and can grow in oxygen as a film on the surface of wine. If *W. anomalus* grows fermentatively, it produces 0.2 to 4.5% ethanol together with a large amount of acetic acid, ethyl acetate and isoamyl acetate (Shimazu and Watanabe, 1981). The CBS 1982 protein produced by *W. anomalus* is characterized by the highest antifungal activity against *Candida pulcherrima* and *Hanseniaspora guilliermondii* (Błaszczuk et al., 2015).

Zygosaccharomyces

Species isolated from wine mainly include *Z. bailii*, *Z. bisporus*, *Z. rouxii* and *Z. florentinus*. *Saccharomyces rouxii* and *Z. barkeri* are synonyms for *Z. rouxii*. Various sugars are fermented depending on species, nitrates are not assimilated. *Z. rouxii* ferments glucose and maltose, assimilates glucose, trehalose, glycerol, D-mannitol and D-glucitol.

Zygosaccharomyces produces succinic acid, acetic acid and lactic acid, acetaldehyde and glycerol. It has tolerance to preservatives, a high content of sugar and ethanol and low temperature (2,5 °C) (Fugelsang and Edwards, 2006; Thomas and Davenport, 1985). *Zygosaccharomyces* depreciates wine; the spoilage effects include gas production, sediments and film formation in bottled wines (Loureiro and Malfeito-Ferreira, 2003).

Conclusion

The spontaneous fermentation of grape must is based on autochthonous yeasts which are present naturally in the vineyard. The composition of the yeast population changes during grape processing and fermentation of grape must. The microflora composition is influenced by a number of factors: concentration of sugar and ethanol, oxygen availability, sulfur compounds or temperature. Interspecies interactions also play an important role. The predominant species on the grapes is *Hanseniaspora uvarum* (formerly classified as *Kloeckera apiculata*). The primary fermentation is predominantly controlled by *Saccharomyces*. Autochthonous yeast populations give a typical flavor and taste to wine depending on the geographical area. On the market, the wine produced in this way has the advantage of uniqueness. The disadvantage of spontaneous fermentation is a difficult control of the fermentation process, depletion of growth factors or unpredictable enzymatic effects. Spoilage yeasts (e.g. *Dekkera*) can contaminate the aging wine and disturb the quality of wine.

Acknowledgement

The article was elaborated with the support of the Ministry of Culture of the Czech Republic - project DG16P02R017 "Viticulture and enology for maintaining and restoring cultural identity wine regions in Moravia".

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