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Wild *Saccharomyces cerevisiae* strains display biofilm-like morphology in contact with polyphenols from grapes and wine

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ABSTRACT
Polyphenols are a major component of wine grapes, and contribute to color and flavor, but their influence upon yeast growth forms has not been investigated. In this work we have studied the effect of polyphenols on the ability of natural isolates of wine-related *Saccharomyces cerevisiae* strains to form biofilms attaching to plastic surfaces, to grow as mat colonies, to invade media, and to display filamentous growth. The use of carbon- and nitrogen- rich or deficient media simulated grape juice fermentation conditions. Addition of wine polyphenols to these media affected biofilm formation, and cells exhibited a wide variety of invasiveness and mat formation ability with associated different growth and footprint patterns. Microscopic observation revealed that some strains switched to filamentous phenotypes which were able to invade media. The wide range of phenotypic expression observed could have a role in selection of strains suitable for inoculated wine fermentations and may explain the persistence of yeast strains in vineyard and winery environments.
Keywords: Wine *Saccharomyces cerevisiae*, Biofilm/mat formation, Filamentous growth, Invasive growth, Polyphenols.

1. Introduction

Natural isolates of *Saccharomyces cerevisiae* have a wide range of genetic diversity, which reflects the phenotypic diversity required to inhabit different ecological niches. *S. cerevisiae* shows great genomic diversity between strains from different origins (Legras et al., 2007) and between domestic and wild yeasts (Liti et al., 2009; Schacherer et al., 2009). Genomic differences discovered between wine yeasts are likely to favour growth in the wine environment (Tofalo et al., 2013).
S. cerevisiae can undergo different modes of invasive or adhesive growth, where single cells give filamentous forms, or undergo mat formation (Cullen and Sprague, 2012; Lo and Dranginis, 1998). Yeast mat requires growth on semisolid media (Reynolds and Fink, 2001) and it is controlled by both glucose and pH gradients (Reynolds et al., 2008) and by genetic factors (Martineau et al., 2010). The ability of *S. cerevisiae* to adhere to surfaces was described in strain Σ1278 (Gimeno and Fink, 1994) and it continues to be studied in this well-described laboratory strain. Proteins named flocculins including Flo1, 5, 9, 10, and 11, confer flocculence, adherence to agar, solid surfaces and other yeast cells to give rise to these phenotypes (Guoet al., 2000; Granek and Magwene, 2010; Purevdorj-Gage et al., 2007; Reynolds and Fink, 2001).The genetic variability of the Flo adhesion family is large and expression is dependent on the environment in which the yeasts inhabit (Honigberg, 2011).
The ability to adhere and invade is present in yeasts isolated from different environments (Zupan and Raspor, 2008), and indeed Flo-dependent phenotypes in yeasts enable strains to colonise and infect grapevines (Gognies et al., 2006) and to persist as biofilms on winery surfaces (Joseph et al., 2007). Much phenotypic variability exists between strains of flor *S. cerevisiae* used in the production of sherry wine with respect to biofilm and mat forming ability (Zara et al., 2002; 2005; 2010). Through concerted population behavior using fusel alcohols as signalling molecules (Ceccato-Antonini and Silva, 2002; Lorenz et al., 2000; Martínez-Anaya et al., 2003) yeasts may undergo this mode of growth to search for nutrients. The presence of this growth state may also confers abiotic resistance to the yeast cells in difficult environmental situations (Honigberg, 2011).
The winemaking environment, whether in the vineyard or winery, contains many environmental stresses to impact upon the ability of *S. cerevisiae* to persist and metabolise sugars into ethanol to make wine (Pretorius, 2000). Interestingly, many of these environmental stresses on the yeasts have also been implicated in the production of mat colonies, filamentous growth and biofilms. For example, nitrogen limitation can induce air-liquid biofilm formation (Zara et al., 2011), nitrogen and carbon limitation can induce biofilm formation (Cullen and Sprague 2000, 2002; Gagiano et al., 2002; Gimeno 1992; Klis et al., 2002; Kuchin et al., 2002), via induction of signalling networks that regulate the response (Granek and Magwene, 2010).
Secondary plant compounds important for wine quality such as polyphenols could be other environmental stresses that induce biofilm-like morphology in yeasts. Polyphenols act as strong antioxidants and interact with proteins involved in cell proliferation (Middleton et al., 2000; van der Woude et al., 2005) and their concentration varies in grapes according to the cultivar, climate, and cultivation practices. There is ample interest for studying the polyphenolic content of grape cultivars and musts (Giuffrè, 2013a; Guerrero et al., 2009; Krammer et al., 2004; Singh Brar et al., 2008) particularly as their antioxidant function may have a health effect (Giuffrè, 2013b) and will impact of the colour of wine. Polyphenols are found to interact with *S. cerevisiae* strains during winemaking and affect the final color of wine (Caridi et al., 2007; Caridi, 2013; Sidari et al., 2007), but little is known about how this important class of compounds interacts with, and affects yeast morphology and metabolism during industrial fermentation.

In particular, variation of phenotypes in wild winemaking strains of *S. cerevisiae* in contact with polyphenols has not been investigated and it may have implications for the winemaking sector. For example, maintenance of dispersed or aggregate yeast cells, production of desirable metabolites, cell resistance to adverse conditions during fermentation (Ceccato-Antonini, 2008), and the undesirable persistence of yeast cells in wineries (Joseph et al., 2007) may all be affected by the presence of polyphenols. Attachment phenotypes are dependent on nutritional status of the growth media, which are also relevant to conditions found during winemaking.
The aim of this work was to study the effect of polyphenols on the attachment phenotypes of wild *S. cerevisiae* strains under simulated wine conditions.

2. Materials and methods

2.1. Strains

Forty-four wine *S. cerevisiae* strains, belonging to the strain collection of the Unit of Microbiology of the Department AGRARIA of Reggio Calabria and isolated from Calabrian grapes and musts during different years, were identified as *S. cerevisiae* by RFLP of the internal transcribed spacers (ITS1 and ITS2) and the 5.8S by using *Hae*III, *Cfo*I, and *Hinf*I restriction enzymes (Promega) according to Esteve-Zarzoso et al. (1999) (data not shown). These strains were screened and from these, eleven strains were chosen to carry out further trials. The *S. cerevisiae* strain Σ1278b, (MATa), which has adhesive and filamentous phenotypes (Reynolds and Fink, 2001) and *S. cerevisiae* strain BY4742, (MATα, Δflo8), which has a non-adhesive phenotype (Euroscarf collection, Frankfurt) were used as common strains tested for the phenotypes studied.

2.2 Media

Yeast Peptone Dextrose broth (YPD - 1% yeast extract, 2% peptone, 2% glucose), Yeast Peptone Dextrose agar (YPD - 1% yeast extract, 2% peptone, 2% or 0.1% glucose, 2% agar) was used to grow yeasts. Defined media were used to modify the carbon and nitrogen content of the growth substrate. These were: Synthetic Complete Media (SCM - 0.17% Difco™ YNB without aminoacids, 0.5% ammonium sulphate, 0.13% essential aminoacids for nutritional auxotrophies (Treco and Lundblad, 1993), 2% or 0.1% glucose); Synthetic Low Ammonium Dextrose (SLAD - 0.17% Difco™ YNB without aminoacids,0.0006% ammonium sulphate, 0.13% essential aminoacids for nutritional auxotrophies (Treco and Lundblad, 1993), 2% glucose). These media allowed us to simulate nutritional condition of early fermentation (high carbon and nitrogen concentration), mid-fermentation (rate limiting nitrogen and high concentration in carbon), and 'adjusted' fermentation for nitrogen (rate limiting carbon and high concentration in nitrogen). A quantity of 100 mg/L of (+)-catechin hydrate (Fluka Analytical) was included to make SCM and SLAD supplemented with a source of a polyphenolic compound. Black grape skins were taken from the Calabrian grape cultivars *Magliocco* and *Gaglioppo* and washed with water. The skins were dried, finely ground, and added to media. In particular, the skins were used to prepare Grape Skin Agar (GraSki) according to Caridi (2013) (6% dried grape skins, 5% citric acid monohydrate, 2.5% disodium hydrogen phosphate, 2% or 0.1% glucose, 0.75% peptone from casein, 0.45% yeast extract, and 2% or 0.3% agar) and to supplement SLAD with a mix of polyphenols. The polyphenolic content of the dried grape skin water extract used to make the media was analysed by HPLC/DAD (La Torre et al., 2006) and found to be composed of gallic acid 271.40 mg/L, ruthin 12.24 mg/L, caffeic acid 9.00 mg/L, ferulic acid 2.83 mg/L, and quercetin 25.60 mg/L. The anthocyanin content measured at 520 nm was 1.156. When not otherwise specified, the strains were pre-cultured in Yeast Peptone Dextrose broth.

2.3. Biofilm formation on a liquid-solid interface

The strains were tested according to the assay described by Reynolds and Fink (2001), with slight modifications. The strains were grown overnight at 28 °C in SCM with 2% of glucose and harvested by centrifugation (5000 rpm for 10 min) at an optical density of 600 nm (OD_{600}) from 0.5 to 1.5, washed once in sterile water and re-suspended to OD_{600} of1.0 in one of two media. Early fermentation conditions were simulated by the use of SCM (containing adequate nitrogen) and 2% of glucose and supplemented with catechin. SLAD (low nitrogen) contained 2% of glucose and supplemented with catechin to simulate the environment yeast would encounter mid-fermentation. Finally, SCM (adequate nitrogen) was prepared with 0.1% of glucose and supplemented with catechin to simulate 'adjusted' fermentation conditions where nitrogen was adequate but the carbon source was lacking, as in a stuck or sluggish fermentation where nitrogen has been added.
Cell suspensions (100 µL) were transferred into 96-well microtiter polystyrene plates (Greiner Bio-One, Germany), incubated at 28 °C for 240 min in an orbital mixer incubator, and stained for 15 min at room temperature using 100 µL of 0.1% crystal violet solution. After washing with sterile water to remove unattached cells and residual dye, 100 µL of 95% ethanol was added to solubilize the dye adsorbed by biofilm. After 30 min, 100 µL of sterile water was added and mixed by pipetting. The same amount was transferred into a new 96-well microtiter plate in order to measure the absorbance at 570 nm by a Multiskan spectrum (Thermo, Finland). The forty-four wine strains were screened using the SCM with 0.1% of glucose as low glucose concentration is reported to enhance adherence to plastic (Reynolds and Fink,2001) and then thirteen strains were chosen for detailed trials using the media described.

2.4.Mat growth assay

The trials were carried out according to Reynolds and Fink (2001), with slight modifications. Overnight culture (0.5 μL) grown at 28°C were inoculated onto the centre of Petri plates containing the following media preparations. YPD with 2% of glucose supplemented with catechin and GraSki with 2% of glucose to simulate early fermentation condition. SLAD with 2% of glucose supplemented either with catechin or with dried grape skins to simulate mid-fermentation conditions, YPD with 0.1% of glucose supplemented with catechin and GraSki media with 0.1% of glucose to simulate the nitrogen 'adjusted' fermentation. These media, contained 0.3% agar, were wrapped with Parafilm and incubated for 13 days at 25°C. To monitor the growth of each yeast strain, the plates were photographed and processed using ArchiCAD 8.1 to determine the yeast area. The forty-four wine strains were screened using YPD with 2% of glucose (Casalone et al., 2005; Reynolds and Fink, 2001) supplemented with catechin while the above mentioned media were used to further test the thirteen strains chosen for detailed trials.

2.5. Invasive growth plate-washing assay

The invasiveness of the thirteen strains chosen for the detailed trials were assessed in Petri plates with the media used for the mat assay containing 2% agar. The trials were carried out according to Bester et al. (2006) modified as follows. Overnight cultures (20 μL) were spotted onto the different plates and incubated at 28°C for 6 days. Then, each strain biomass was removed from the surface with water gently rubbing with a finger in order to reveal cells remaining attached to or infiltrated into the media, reported in the following as footprints. Photographs were taken before and after the washing.

2.6. Morphological assessment of yeasts in contact with polyphenols

The strain biomass grown in the media used for mat and invasive growth assays was microscopically observed (Zeiss Standard 20 optical microscope) to assess the influence of the experimental conditions on cell morphology. The yeast cell morphology was also observed after growth in grape must. Here, 600μL of exponential phase culture were inoculated in12 ml tubes containing must of the cultivar *Cabernet*, obtained from Malaspina winery (Melito Porto Salvo, RC, Italy). The must was heated to remove appreciable growth of wild microorganisms. The micro-fermentations were statically incubated at 25°C and observed under the microscope after 3, 8, 25, and 40 days. At each stage, the cells showing unusual morphology such as short chains of cells and/or cells arranged in clusters were counted and expressed as a percentage of the total cells with singular elliptic morphology.

2.7. Statistical analyses

Biofilm trials were carried out in quadruplicate while invasiveness and mat growth assays were carried out in duplicate. The data were statistically analyzed by StatGraphics Centurion XVI for Windows XP from StatPoint.

3. Results and discussion

3.1. Strain identification

The PCR-amplified fragment was 880 bp for all of the forty-four strains investigated in this study. The restriction enzyme digest of the 880bp fragment showed four bands with sizes 320, 220, 180, and 150 bp for digestion with *Hae*III, three bands with sizes 380, 360, and 150 bp for digestion with *Cfo*I, and two bands with sizes 365 and 155 bp for *Hinf*I (data not shown) (Esteve-Zarzoso et al., 1999). Thus, the forty-four strains are identified as *S. cerevisiae*.

3.2. Screening of the forty-four strains for biofilm and mat formation

As a preliminary phenotypic screen, biofilm formation was induced by growth in SCM with 0.1% of glucose and the forty-four strains exhibited a wide range of variability with low, medium, and high aptitude to form a biofilm being observed. The two strains Σ1278b and BY4742 showed high and low ability to form a biofilm respectively (Sidari, Howell, Caridi, manuscript in preparation). Mat formation was also screened in the forty-four strains, by growth on YPD with 2% of glucose supplemented with catechin, and also exhibited a wide variability in the mat formation assays. The mat areas for the wild strains ranged from 3.06 to 25.69 cm^2 respectively for strains Sc2363 and Sc1741 while BY4742 and Σ1278b had values of 2.68 and 9.89 respectively (data not shown). Interestingly, the supplementation with catechin changed the strain behavior towards mat formation. Some strains (Sc329, Sc456, Sc786, Sc1674, Sc1871, and Sc2319) formed larger mat colonies on the medium without supplementation with catechin than in its presence. Some other strains (Sc76, Sc157, Sc396, Sc1526, Sc1721, Sc1741, Sc1798, Sc1803, Sc1864, and Sc2485) showed the reverse behavior (data not shown). On the basis of these preliminary screens, thirteen strains were chosen to be characterized in media with varied carbon, nitrogen and polyphenol content (Table 1).

3.3. Phenotypic variability of the selected strains

3.3.1. Biofilm formation on a liquid-solid interface

Biofilm formation at the liquid-solid interface was tested in conditions simulating different phases of fermentation (Table 2). The addition of catechin reduced the biofilm formed in the majority of the strains. Progress of fermentation reduced biofilm formation in eight of the thirteen strains studied, while two strains were not affected. Three strains demonstrated an increase in biofilm formation by mid-fermentation. The addition of catechin modified the strain behavior so that seven strains

out of thirteen increased biofilm formation by mid-fermentation. Media containing low carbon and adequate nitrogen allowed strains to form good biofilms compared to the other media tested. However, addition of catechin decreased biofilm formation for most strains (excepting strains Sc1128, Sc1741, and Sc2621).

3.3.2. Mat growth assay

Figure 1 shows means and standard deviations of the area of the mat colonies in media that simulate early fermentation, mid-fermentation, and nitrogen 'adjusted' fermentation. The values ranged from 2.49 to 7.22 $cm^2$and from 2.91 to 23.99 cm^2 in YPD and in YPD added with catechin, respectively. The addition of catechin caused an increase in colony area and mat formation for most strains. Among these, the strain Sc2621 exhibited the highest value. Growing in contact with a natural mix of polyphenols gave a reduction in the area of the mat colonies with values ranging from 0.94 to 3.37 cm^2 (Fig. 1a).

Mat areas increased in media supplemented with catechin (ranged from 1.45 to 2.94 cm^2 and increased to 1.59 to 4.09 cm^2 in SLAD supplemented with catechin).This trend was also observed when YPD with 2% of glucose was used. Strain Sc2621 exhibited the highest values in all cases. Presence of a natural mix of polyphenols increased the colony area for four out thirteen strains (Sc1240, Sc1321, Sc1591, BY4742) than when grown with catechin alone, where values ranged from 1.65 cm^2 to 3.11 cm^2 (Fig. 1b).

Mat areas were reduced when yeasts were grown in YPD containing 0.1% glucose compared with 2% glucose. in YPD with 0.1% glucose, and this result agrees with Reynolds and Fink (2001). Addition of catechin increased colony area for eight of the thirteen strains, and again Sc2621 exhibited the highest value. When a natural mix of polyphenols was present, three strains (Sc384, Sc1321, BY4742) increased the area colony compared to the previous conditions (Fig. 1c).

The effect of the natural mix of polyphenols and catechin on the mat formation was not consistent for all the strains tested, as some increased mat formation with polyphenol mix and reduced with catechin. Additionally, not all the strains tested in all the nutrition conditions in presence of the mix of polyphenols exhibited a decrease in the area colony, as expected considering the polyphenolic composition of the dried grape skin water extract used to prepare media. It may be that the strain differences can be due to various polyphenolic compounds acting synergistically or antagonistically.

3.3.3. Invasive growth plate-washing assay

Figure 2 shows the invasiveness of five representative strains observed after growth on agar media with various supplements and washing the colony under a stream of water. Under simulated fermentation conditions, the strains exhibited different invasive behavior, from no invasiveness (e.g. Sc384) to various invasiveness degree modulated also by either the presence of catechin or natural mix of polyphenols (e.g. Sc1321, Sc2621).

The strain differences between catechin and natural mix of polyphenols may be due to the various polyphenolic compounds acting together rather than to the lack of the catechin in the dried grape skin water extract used to prepare media. As an example, strain Sc384 in the early fermentation conditions does not invade media both in presence of catechin and mix of polyphenols, strains Sc1321 and BY4742 invade only the media with mix of polyphenols while strains Sc2621 and Σ1278b invade both the media. It is possible to observe similar trends in the other nutritional conditions.

Comparing the three simulated fermentation conditions, each strain maintained the same behavior but exhibiting different strength and pattern of the footprint attachment and infiltration into the media. This is consistent with Štòvíčeket et al. (2010) who showed specific attachment footprint for natural and domesticated yeast strains. However, our results highlight that the strain footprint and pattern is modified by the nutrients and the polyphenols present in the media.

3.3.4. Microscopic observations

Consistent with the results reported by Karunanithi et al. (2012), the mat was composed of typical round yeast-form cells and contained some randomly budding forming clumps (Fig. 3a and 3b). Some filamentous cells were observed for strain Sc1128 in SLAD media, in YPD with 2% of glucose, and in YPD with 2% of glucose supplemented with catechin (data not shown). Figure 3c shows cells grown in the SLAD media with grape skins polyphenols. The cells were elongated with many examples of aberrant forms, such as cells with hooked ends. These morphological changes were not related to the degree of invasiveness to the media in the conditions tested in these experiments.

The presence of catechin or natural polyphenols resulted in round-shaped cells in clusters and/or filamentous form with chains of elongated cells or highly branched elongated cells in some of the other strains tested. Catechin alone does not markedly change the cells microscopically yet still modified the ability of the strains to invade the media as shown in Fig 2. Microvinifications were performed to assess the response of yeasts to polyphenols in a more 'wine-like' environment. When yeasts were in contact with grape must 3 days, the percentage of cells in aggregates or short chains ranged from 0-25% (data not shown). All yeast strains showed an increase in unusual morphologies after 8 and 25 days, but showed little difference beyond 40 days of incubation. Strain Sc2621 was strongly affected and showed unusual morphology after 3 days while strains Sc1321, Sc1340, Σ1278b, and BY4742 were also affected. Strain Sc1326 was scarcely affected by the growth conditions and displayed low rates of unusual morphologies.

3.3.5. Cluster analysis

Figure 4 shows the dendrograms obtained by the cluster analysis (*furthest neighbor* method and *Euclidean distance*) of the strains tested in the various simulated nutrition/polyphenolic supplementation conditions. Numbers of clusters were reduced according to established protocols (Fowler and Cohen, 1986).
Based upon their similarities, the strains were grouped in four clusters. The laboratory strains grouped with some wild *S. cerevisiae* strains, while the group containing Sc1240, Sc1741, 2306, Sc1321 and Sc1128 form a distinct group based on similar attachment patterns. Assessing the winemaking suitability of strain Sc2621 would be interesting as it often exhibited the highest values for the phenotypes studied.

4. Conclusion

This study contributes to the better understanding of biofilm-related behavior of wild strains of *S. cerevisiae* and to understanding the ecological and biotechnological implications of various *S. cerevisiae* lifestyles.
Our study shows there are broad natural responses of yeast strains with respect to attachment, adhesion and cellular morphologies under conditions found in wine environments. The aptitude of yeasts to organize in communities is modified in presence of polyphenols (both catechin and crude grape skin extracts) and may directly trigger the biofilm-like behavioural responses in some strains.
The observed responses to polyphenols could be a mechanism useful for the adaptation for growth in the particular grape/must conditions. This phenotype could have implications in the winemaking sector for maintenance of dispersed cells during the fermentation and thus optimal production of desired metabolites. Additionally, this community could contribute to an undesirable persistence of a particular strain of yeasts. For example, forming stable adhesive and invasive communities may increase the ability of yeasts to colonize grapes, processing equipment and within the winery. Winemakers may not have the choice of selected yeast strain they desire if the dominant yeast strain is not displaced by inoculation of a new strain. Alternatively, this mechanism may provide an explanation of 'microbial terroir', where the microbial populations are stable and peculiar to a vineyard and winery ecosystem.

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Table 1. The thirteen strains of *Saccharomyces cerevisiae* chosen to perform detailed trials. The range of values for biofilm (expressed as absorbance values at 570 nm) and growth as mat colonies (expressed as area in cm^2) refers to the forty-four strains tested.

Strains	Low biofilm formation (0.13-0.38)	Medium biofilm formation (0.40-1.25)	High biofilm formation (1.41-1.78)	Low growth as mat (5.95-8.03)	Medium growth as mat (8.18-10.51)	High growth as mat (10.60-20.93)	Mat growth not negatively affected by catechin	Mat with structured architecture
Sc384	•					•	•	
Sc632		•			•		•	
Sc1128	•					•		
Sc1240	•					•		
Sc1321	•					•		
Sc1326	•				•		•	
Sc1340	•					•		
Sc1591		•			•			•
Sc1741	•			•			•	
Sc2306			•	•				
Sc2621	•					•		•
Σ1278b			•			•		
BY4742	•			•				

Table 2. Quantification of biofilm formed by the thirteen strains of *Saccharomyces cerevisiae* after incubation on polystyrene plates of cells re-suspended in media also supplemented with catechin with a) high concentration of carbon (C) and nitrogen (N) simulating early fermentation, b) high concentration of C and rate limiting N simulating mid-fermentation, and c) rate limiting C and high concentration of N an 'adjusted' fermentation with nitrogen source. Data represent means of three replicates. The arrows pointing up or down indicate the strains increase or decrease response compared to the media without catechin.

Strains	Media					
	High C - High N		High C - Rate limiting N		Rate limiting C - High N	
	SCM 2% G*	SCM 2% G + catechin	SLAD 2% G	SLAD 2% G + catechin	SCM 0.1% G	SCM 0.1% G + catechin
Sc384	0.092$^{a/a}$	0.390^{ab} ↑	0.092$^{ab/a}$	0.069^{ab} ↓	0.226^{abb}	0.067^{ab} ↓
Sc632	0.420^{bb}	0.082^{ab} ↓	0.202^{bb}	0.259^{bb} ↑	0.913^{fb}	0.503^{ab} ↓
Sc1128	0.121$^{a/a}$	0.075^{ab} ↓	0.120$^{bb/a}$	0.277^{bb} ↑	0.336$^{bb/b}$	0.513^{ab} ↑
Sc1240	0.110$^{a/a}$	0.063^{ab} ↓	0.089$^{ab/a}$	0.095$^{ab/b}$ ↑	0.332$^{bb/b}$	0.088$^{ab/b}$ ↓
Sc1321	0.359$^{b/b}$	0.061^{ab} ↓	0.073$^{a/a}$	0.271^{bb} ↑	0.572^{bb}	0.401^{bb} ↓
Sc1326	0.106$^{a/a}$	0.062^{ab} ↓	0.139^{ab}	0.094$^{ab/b}$ ↓	0.404^{ab}	0.115^{ab} ↓
Sc1340	0.127$^{a/a}$	0.066^{ab} ↓	0.105$^{ab/a}$	0.075$^{ab/ab}$ ↓	0.244$^{ab/b}$	0.083^{ab} ↓
Sc1591	0.142$^{a/a}$	0.063^{ab} ↓	0.118$^{ab/a}$	0.124^{bb} ↑	0.384^{ab}	0.111$^{ab/a}$ ↓
Sc1741	0.118$^{a/a}$	0.061^{ab} ↓	0.089$^{ab/a}$	0.202^{ab} ↑	0.192^{ab}	0.275^{ab} ↑
Sc2306	0.385$^{b/b}$	0.062^{ab} ↓	0.323$^{ab/b}$	0.184^{ab} ↓	0.567^{fb}	0.198^{bb} ↓
Sc2621	0.431$^{b/a}$	0.062^{ab} ↓	0.581^{ab}	0.831^{fb} ↑	0.432^{ab}	0.488^{ab} ↑
Σ1278b	0.432$^{b/b}$	0.087^{ab} ↓	0.437^{fb}	0.428^{ab} ↓	0.711^{ab}	0.250^{bb} ↓
BY4742	0.082$^{a/a}$	0.318^{bb} ↑	0.078$^{ab/a}$	0.075$^{ab/a}$ ↓	0.151^{ab}	0.095^{ab} ↓

*Glucose.

Superscript letters indicate the homogeneous groups (*Least Significant Difference of Fisher*, confidence level of 95%) in the same column.

Superscript letters in bold indicate the homogeneous groups(*Least Significant Difference of Fisher*, confidence level of 95%) in the same row.

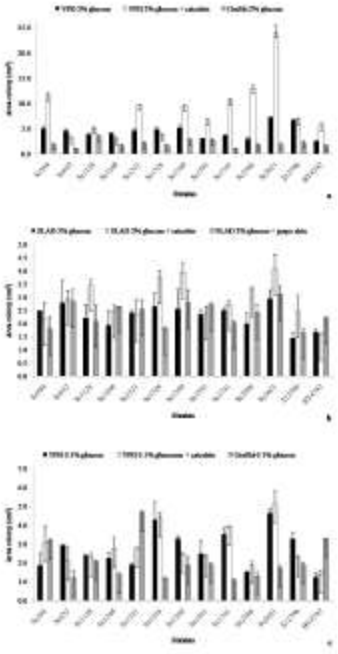

Fig. 1

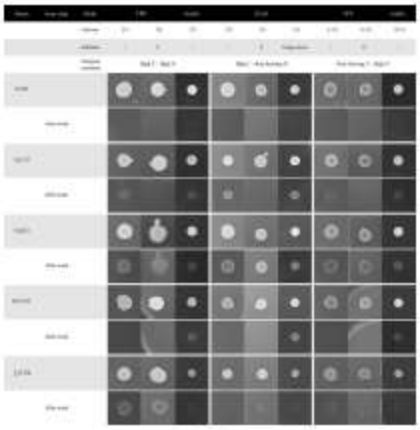

Fig. 2

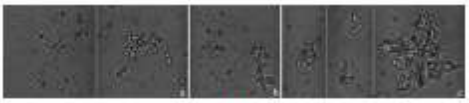

Fig. 3

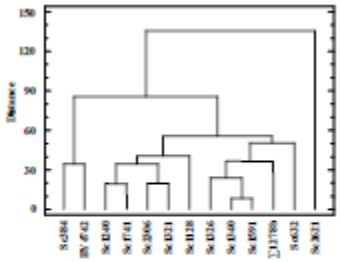
150
120
90
60
30
0
Distance
Sc284
BV4742
Sc1240
Sc1741
Sc2306
Sc1321
Sc1128
Sc1326
Sc1340
Sc1591
Y1178b
Sc632
Sc3631

Fig. 4

Figure captions

Fig. 1. Mat formation assay of the thirteen strains of *Saccharomyces cerevisiae* tested in media simulating early fermentation a), mid-fermentation b), and 'adjusted' fermentation for nitrogen c). The media was also supplemented with (+)-catechin hydrate and natural mix of polyphenols. Data are means of two experiments ± standard deviations and represent the area of the colonies expressed in cm^2 calculated by ArchiCAD.

Fig. 2. Invasive growth assay of five representative yeast strains of *Saccharomyces cerevisiae* Sc384, Sc1321, Sc2621, BY4742, and Σ1278b tested in media simulating nutritional status of the early fermentation - high carbon (C)-high nitrogen (N) - and mid-fermentation - high carbon (C)-rate limiting nitrogen (N), and of an 'adjusted' fermentation for nitrogen - rate limiting carbon (C)-high nitrogen (N) - coupled with polyphenolic supplementation. Photographs were taken before and after washing off the biomass with water from the media. Bars: 0.5 cm.

Fig. 3. Microscopic observation of invasive footprints of the strain of *Saccharomyces cerevisiae* Σ1278b grown in media with polyphenols. a) GraSki with 2% of glucose; b) GraSki with 0.1% of glucose; c) SLAD with 2% of glucose and supplemented with grape skins.

Fig. 4. Cluster diagram (furthest neighbor method and Euclidean distance) of the thirteen strains of *Saccharomyces cerevisiae* according their phenotypes in the media simulating different nutritional status coupled with polyphenolic supplementation.