SPi	Journal Code				Article ID				Dispatch: 19.08.14	CE:
	Y	E	A		3	0	3	6	No. of Pages: 10	ME:

Yeast
Yeast (2014)
Published online in Wiley Online Library
(wileyonlinelibrary.com) **DOI:** 10.1002/yea.3036

Research Article

Quantitative analysis of morphological changes in yeast colonies growing on solid medium: the eccentricity and Fourier indices

Q1 Elena Gil de Prado[1,3], Eva-María Rivas[1,3], María-Isabel de Silóniz[1,3], Belén Diezma[2,3], Pilar Barreiro[2,3] and José M. Peinado[1,3]*

[1]*Departamento de Microbiología III, Facultad de Biología, Universidad Complutense de Madrid, Spain*
[2]*LPF–TAGRALIA, Departamento de Ingeniería Rural, ETSI Agrónomos, Universidad Politécnica de Madrid, Spain*
[3]*CEI Campus Moncloa, UCM–UPM, Madrid, Spain*

**Correspondence to:*
J. M. Peinado, Departamento de Microbiología III, Facultad de Biología, Universidad Complutense de Madrid, C/José Antonio Nová 2, 28040 Madrid, Spain.
E-mail: peinado@bio.ucm.es

Abstract

The colony shape of four yeast species growing on agar medium was measured for 116 days by image analysis. Initially, all the colonies are circular, with regular edges. The loss of circularity can be quantitatively estimated by the eccentricity index, E_i, calculated as the ratio between their orthogonal vertical and horizontal diameters. E_i can increase from 1 (complete circularity) to a maximum of 1.17–1.30, depending on the species. One colony inhibits its neighbour only when it has reached a threshold area. Then, E_i of the inhibited colony increases proportionally to the area of the inhibitory colony. The initial distance between colonies affects those threshold values but not the proportionality, E_i/area; this inhibition affects the shape but not the total surface of the colony. The appearance of irregularities in the edges is associated, in all the species, not with age but with nutrient exhaustion. The edge irregularity can be quantified by the Fourier index, F_i, calculated by the minimum number of Fourier coefficients that are needed to describe the colony contour with 99% fitness. An *ad hoc* function has been developed in Matlab v. 7.0 to automate the computation of the Fourier coefficients. In young colonies, F_i has a value between 2 (circumference) and 3 (ellipse). These values are maintained in mature colonies of *Debaryomyces*, but can reach values up to 14 in *Saccharomyces*. All the species studied showed the inhibition of growth in facing colony edges, but only three species showed edge irregularities associated with substrate exhaustion. Copyright © 2014 John Wiley & Sons, Ltd.

Received: 2 December 2013
Accepted: 28 July 2014

Keywords: **yeast colony shape; inhibition among colonies; colony eccentricity; colony edge irregularity**

UNCORRECTED PROOF

Introduction

The colony shape and its edge have been morphological features used traditionally in the identification process of microbial strains, including yeasts (Kurtzman *et al*., 2011). This purely descriptive methodology has been improved lately by the incorporation of new techniques of image analysis that automatically produce quick and quantitative results (Robinson *et al*., 1998; Dorge *et al*., 2000; Yang *et al*., 2001; Hansen *et al*., 2003; Banada *et al*., 2007; Clemmensen *et al*., 2007; den Hertog *et al*., 2010; Puchkov, 2010).

Differences in colony morphology have been attributed to genotypic and phenotypic factors. Their link to the taxonomic affiliation (genus, species) implies the phylogenetic value attributed to these characters (Kocková-Kratochvilová, 1990). On the other hand, many external factors have been involved, including the size of the inoculum, and also environmental growth conditions, such as the carbon source, agar concentration, pH and growth

Copyright © 2014 John Wiley & Sons, Ltd.

temperature (Boschke and Bley, 1998; Reynolds and Fink, 2001; Vopálenská *et al*., 2005; Granek and Magwene, 2010), which can also influence the production of extracellular structures or pseudohyphae (Halme *et al*., 2004). They have also been associated with the strain origin (nature, culture collection or industry) (Šťovíček *et al*., 2010) and the age of the culture (Granek and Magwene, 2010). More recently, some cellular properties, such as cell adhesion, budding pattern and ploidy, have been associated with morphological changes in *Saccharomyces cerevisiae* (Vopálenská *et al*., 2005; Granek and Magwene, 2010; Šťovíček *et al*., 2010).

Genes associated to the morphology of the colonies, as related to biofilm-like structures, have been identified, mainly in *S. cerevisiae*, although they have also been identified in a few other species, such as *Candida glabrata*, *C. albicans* and *Kluyveromyces waltii* (Verstrepen and Klis, 2006). The gene *Flo11* of *S. cerevisiae*, which codifies an adhesin, has pleiotropic effects that include adhesion to surfaces (Lo and Dranginis, 1996; Verstrepen *et al*., 2001), effects on the colony architecture (Šťovíček *et al*., 2010; Vopálenská *et al*., 2010), the formation of mats (Reynolds and Fink, 2001) and the formation of Velcro-like interconnections between cells (Váchová *et al*., 2011).

Besides these characteristics of colony morphology linked to the interaction between genes and the environment, morphological changes can be also produced by interaction among colonies. One colony can inhibit the growth of a neighbour colony by intercolony signalling, in which the chemical messenger is a volatile compound, ammonia, a system that has been studied mainly in *S. cerevisiae* (Palková *et al*., 1997; Palková and Forstová, 2000; Váchová *et al*., 2012).

In contrast to the high number of molecular studies on the genetic basis of colony morphology and complexity, there is not a well-established and validated methodology to measure and describe quantitatively the morphology of yeast colonies. The aim of this study was the search for and validation of methods that permit a quantitative measurement of these important features of yeast colonies. They would be useful tools to relate quantitatively the effect of genetic studies (knock-out, gene dosage, etc.) on morphological changes, and also for comparison among yeast species. In this paper we describe a methodology and propose two indices that measure accurately two processes linked to colony morphology: (a) the change in morphology induced in pairs of colonies by intercolony signalling; and (b) the increase in irregularity of the colony edge with the age of the colony. This methodology has been applied to the study of colony morphology in four different yeast species: *S. cerevisiae*, *Debaryomyces fabryi*, *Rhodotorula glutinis* and *Zygosaccharomyces rouxii*.

Materials and methods

Strains

Four species, selected by their differences in their phylogeny and energy metabolism have been used in this study: *S. cerevisiae* ATCC 7754, purely fermentative; *Z. rouxii*, Bch, with higher fermentation than oxidation, a strain isolated in our laboratory from gas-spoiled chocolate bun (Wrent *et al*., 2010); *D. fabryi* PR66, fermentative only in the absence of oxygen, also isolated by us from a gas-spoiled sausage (Romero *et al*., 2005); and *R. glutinis* CECT 10145, a purely oxidative, basidiomyceteous species.

Media and growth conditions

Strains were inoculated into Erlenmeyer flasks containing 100 ml yeast morphology broth [YMB; 0.5% w/v yeast extract (Difco, Detroit, MI, USA), 0.3% w/v proteose-peptone No.3 (Difco), 0.3% w/v malt extract (Difco) and 1% w/v glucose (Panreac Quimica SA, Barcelona, Spain)] and were incubated at 28 °C overnight in an orbital incubator. Exponential samples of these cultures were diluted in saline solution to obtain inocula of ca. 10^3 colony-forming units (CFUs). Aliquots of 10 µl of these suspensions were inoculated as droplets on the surface of agar plates containing 15 ± 1 ml of yeast morphology agar (YMA; the same composition as YMB plus 2% w/v agar). Plates were inoculated to obtain: (a) only one colony, placed in the centre; (b) two colonies placed in the centre and separated by a distance of 2, 3 and 5 cm; and (c) six colonies in the same plate, separated by the same distances. The plates were covered with parafilm to avoid water evaporation and incubated for 116 days at 28 °C. Three replica plates were made for each experimental condition (one colony/plate; two colonies at three different

UNCORRECTED PROOF

Copyright © 2014 John Wiley & Sons, Ltd.

DOI: 10.1002/yea

distances; and six colonies/plate) for the four species, which amounted to 156 colonies analysed.

Measurement of colony parameters by image analysis

After 2 days of incubation, digital photos in greyscale of the colonies were captured over the course of time using a Vilber Lourmat camera, under normalized conditions, in order to obtain a constant spatial resolution. An algorithm was developed in Matlab v. 7.0 (MathWorks), based on several image analysis routines from the image-processing toolbox™ to automate measurements of the colonies. First, a segmentation of images was performed according to a grey segmentation threshold, self-adjusted for each image by application of Otsu's method. Then, the objects of the images (yeast colonies) were differentiated from the background. Finally, the colony parameters (area, major and minor diameters) were calculated in pixels. The colonies of three different plates for each growth condition and strain were analysed over the course of time. Another routine was created for the conversion of pixels to millimetres (mm) through a conversion factor obtained from photographic images made under the normalized conditions of rules scaled in mm. The eccentricity index (E_i) was calculated as the ratio of the values of the major and minor diameters of each colony. For each experimental condition, the mean of the E_i of the colonies of three different plates was calculated. The images were taken over 116 days at several time intervals, which were shorter at the beginning. A minimum of 25 images were taken at those time intervals in each colony. As a whole, almost 4000 images were analysed by this method.

Determination of Fourier index on yeast colonies

Several different boundary descriptors have been defined to characterize the external representations of an object, including Fourier descriptors (Gonzalez *et al*., 2004). The elliptic Fourier method, proposed by Kuhl and Giardina (1982), uses the periodical variation of x and y coordinates of a mass point that moves on a contour at the same speed. Elliptic Fourier harmonics descriptors, which we simplified here as Fourier coefficients, can delineate any type of shape with a closed two-dimensional (2D) contour, and have been effectively applied to the evaluation of various biological shapes in animals (Agüera and Brophy, 2011) and plants (Camargo Neto *et al*., 2006; Mebatsion *et al*., 2012). In practical approaches, the truncated Fourier series expansions are performed, calculating the x_p and y_p for n (number) elliptic Fourier harmonics. An *ad hoc* function was developed in Matlab v. 7.0 to automate the computation of the Fourier coefficients. In this study, the complexity of the edges of the colonies was evaluated through the number of Fourier coefficients needed to reproduce the x and y coordinates with a certain correlation. We have called this number the 'Fourier index' (F_i). Allowing F_i values to increase, the fitness of the geometrical figure with the actual colony edge increases but, in accordance with previous studies (Carlo *et al*., 2011), we decided to limit the maximum number of Fourier descriptors to 20. Stopping the fitting process when F_i reached a value of 20 produced correlation coefficients of 0.998 or higher for all the colonies. Figure 1 depicts the development of the correlation process with the fitting of the elliptic Fourier approximation to the edge of a colony of *S. cerevisiae*, generated with the 1st, 8th, 14th and 30th F_i values and their corresponding correlation coefficients.

Results

Morphological evolution of yeast colonies growing on solid surfaces

Yeast colonies originated from 10^3 cells inocula begun to be visible as circles of 2 mm in diameter

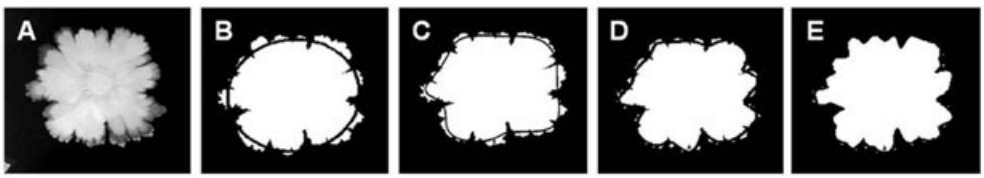

Figure 1. Elliptic Fourier shape approximations for a colony of *S. cerevisiae*: (A) original image; (B) $F_i = 1$ and correlation coefficients for x and y (CCxy) = 0.992; (C) $F_i = 8$ and CCxy = 0.996; (D) $F_i = 14$ and CCxy = 0.998; and (E) $F_i = 30$ and CCxy = 0.999

Copyright © 2014 John Wiley & Sons, Ltd.

DOI: 10.1002/yea

UNCORRECTED PROOF

enclosing separated microcolonies. In all the species, after about 2 days of incubation under our experimental conditions (nutrient-rich medium and water activity near to 1), the microcolonies were unified, forming one single colony, without apparent interaction among neighbouring colonies (column 1, F2 Figure 2). These colonies grew for 116 days, changing their appearance into more and more complex forms, as can be seen in Figure 2. At first, colonies of all the species were circular, with a smooth edge and uniform texture. Over time, the colonies showed lobed edges and lost homogeneity, although with different intensities, depending on the species. The fermentative ones (*S. cerevisiae* and *Z. rouxii*) presented more irregular and lobed edges than oxidative species (*R. glutinis* and *D. fabryi*); *D. fabryi* was the species that presented the least irregular edges, even in very old colonies. These changes were especially well observed, qualitatively, in plates with one or two colonies, producing bigger colonies.

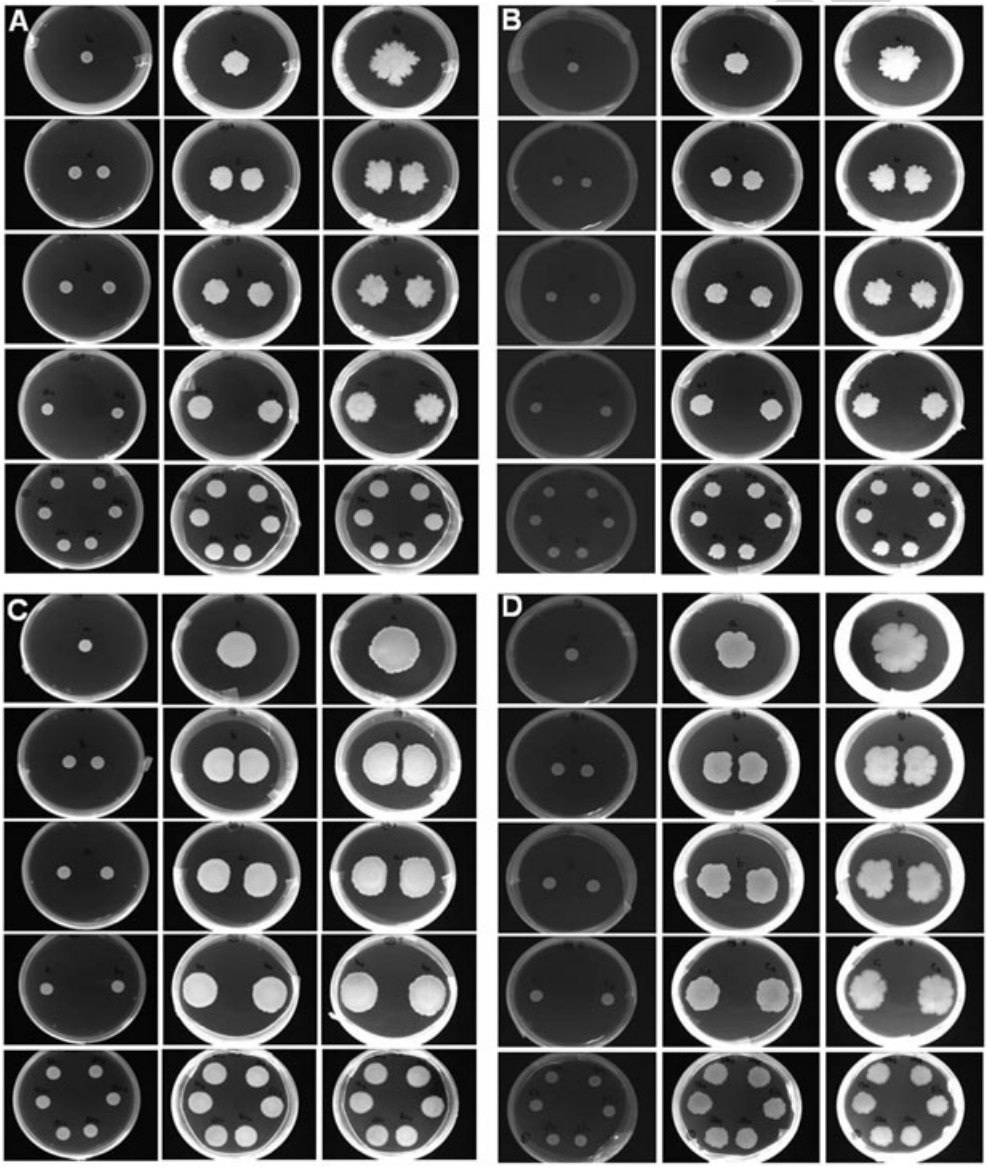

Figure 2. Morphology of yeast colonies on YMA with time: (A) *S. cerevisiae* ATCC 7754; (B) *Z. rouxii* Bch; (C) *D. fabryi* PR66; (D) *R. glutinis* CECT 10145. From left to right, 2, 21 and 116 days of incubation. From top to bottom: one colony/plate; two colonies separated by 2 cm; two colonies separated by 3 cm; two colonies separated by 5 cm; and six colonies/plate separated by 2, 3 and 5 cm

Copyright © 2014 John Wiley & Sons, Ltd.

DOI: 10.1002/yea

Quantitative analysis of growth of the colony area

After 2 days of incubation, when the first measurement of the colonies area was taken, all the species showed a similar colony size of about 70 mm^2 except *Z. rouxii*, whose colonies were about 25% smaller (Figure 3). At the beginning there was a period where, independently of the number of colonies/plate, the rate of area growth was very similar. This time period was slightly longer in the fermentative species (ca. 11 days) than in the oxidative ones (8.3 days) (Figure 3).

The maximum area reached by the colonies depended on the species and the number of colonies/plate, as can be observed in Figure 3. In YMA with 1% glucose (150 mg glucose/plate), our experimental conditions, the oxidative species reached a maximal area close to 1200 mm^2 and the fermentative ones did not exceed 800 mm^2 (much lower in the case of *Z. rouxii*). These maximal values were reached only with a single colony/plate, and decreased with the number of colonies (see maximal area for the different number of colonies/plate in Figure 3). The decrease was proportional to the number of colonies, independent of their position in the plate, indicating a constant value for the yield coefficient (cell biomass produced/carbon source consumed). It must be also remarked that these features were common for the four species (see Figures 2 and 3), although to different extents.

Quantitative analysis of the circularity of colonies over the course of time: the eccentricity index

We have defined the eccentricity index (E_i) as the ratio between their orthogonal vertical and horizontal diameters, to quantify the circularity of the colonies. The loss of circularity ($E_i > 1$) was only observed when there were at least two colonies/plate (Figure 4). With only one colony/plate the eccentricity index was maintained close to 1, and this equality between the vertical and horizontal diameters was maintained over time in all the species (results not shown, although they can be concluded from the data depicted in Figure 4 relating E_i and area). It could be concluded that the increase in eccentricity was not a consequence of the age of the colonies but of their interactions.

Visual analysis suggested that the distance between colonies was an important factor in the loss of circularity. When the initial distance was 2 cm, a clear effect could soon be observed, especially in *D. fabryi*. Increases in this distance to 3 and 5 cm retarded the effect or even made it disappear in the fermentative species (Figure 2). The images in Figure 2 also suggest that the inhibition in a pair of colonies was symmetrical, so that any of them could be considered at the same time inhibitory and inhibited. This hypothesis was tested quantitatively by analysing the effect of the growth in size of the right colony on the

CORRECTED PROOF

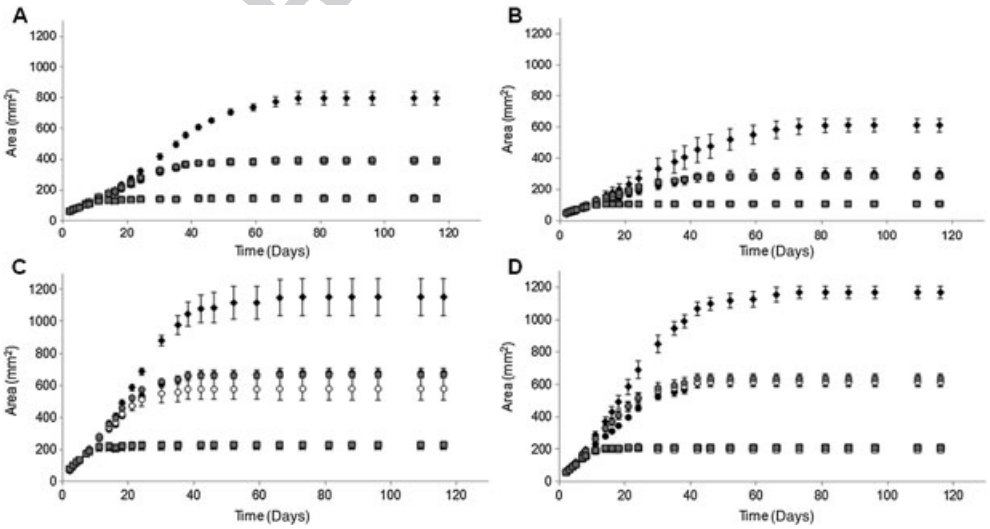

Figure 3. Increase of the area (mm^2) of the colonies with time: (A) *S. cerevisiae* ATCC 7754; (B) *Z. rouxii* Bch; (C) *D. fabryi* PR66; (D) *R. glutinis* CECT 10145; diamonds, one colony; circles, two colonies; squares, six colonies. Distances between neighbouring colonies: black, 2 cm; white, 3 cm; grey, 5 cm. Each point is calculated as the mean of at least three colonies from different plates

Copyright © 2014 John Wiley & Sons, Ltd.

DOI: 10.1002/yea

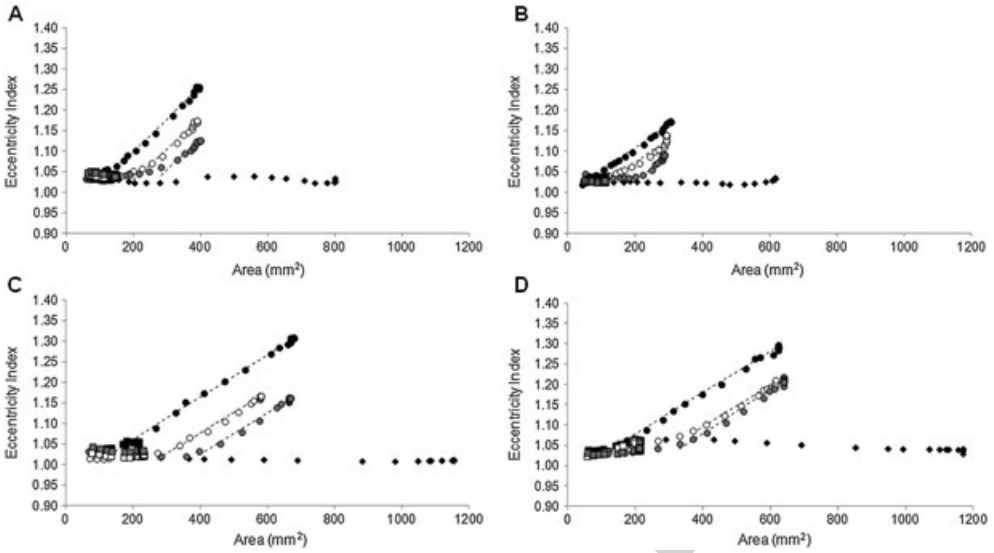

Figure 4. Relationship between the eccentricity index of a colony and its area: (A) *S. cerevisiae* ATCC 7754; (B) *Z. rouxii* Bch; (C) *D. fabryi* PR66; (D) *R. glutinis* CECT 10145. diamonds, one colony; circles, two colonies; squares, six colonies. Distances between neighbouring colonies: black, 2 cm; white, 3 cm; grey, 5 cm. Dashed lines, linear regression. Each point is calculated as the mean of at least three colonies from different plates

eccentricity of the left one, and vice versa. Plotting the eccentricity of the left colony versus the area of the right one produced overlapping results with the data of eccentricity of the right colony versus the area of the left one (results not shown). For this reason the kinetic analysis that follows, based on the data depicted in Figure 4, is based on means of eccentricities and areas of all the colonies. From analysis of the data depicted in Figure 4, two important features can be concluded: (a) a minimum size of the colonies, that depended on the distance between them, had to be reached before the inhibition began; and (b) once the inhibition had begun, the eccentricity index increased proportionally to the area of the inhibiting colonies and at a constant rate, producing straight lines in the plots, more clearly in the oxidative species (see Figure 4, Table 1). In the same plot it is also noteworthy that the straight lines, corresponding to colonies separated by different distances, are parallel, indicating identical dependence of the inhibition, once it has begun, with respect to the area of the colonies, without any further dependence of the distance between colonies. Again, these features could be observed in all the four species studied, although with relevant quantitative differences among them that are shown in Table 1, which includes results obtained with a pair of colonies/plate. All the species showed almost circular young colonies, with initial, E_i (0), values oscillating in the range 1.02–1.05 after 2 days of growth. However, the threshold area at which inhibition begun was much lower in *Z. rouxii* than in the other three species. As we have mentioned, once the inhibition had begun, the eccentricity index increased proportionally to the increase in the area of the inhibitory colony, with a ratio ($R^2 > 93\%$) very similar in three of the species but not in *S. cerevisiae*, which showed a slightly higher ratio (Table 1). The maximal E_i values depended on the number of colonies/plate, because this number limits the maximal area that can be reached. For this reason, no eccentricity could be observed when the number of colonies/plate was such that none of them was able to reach the threshold value of the area (Table 1) needed to induce eccentricity (see Figure 4).

Quantitative analysis of colony border complexity over the course of time: the Fourier index

The colony edge complexity can be measured quantitatively as the Fourier index, F_i, which we have defined as the minimum number of Fourier coefficients that are needed to describe the colony contour with at least 99.8% fitness. Figure 5 shows the evolution of the values of this index with

UNCORRECTED

Copyright © 2014 John Wiley & Sons, Ltd.

DOI: 10.1002/yea

Table 1. Eccentricity indices (E_i) and threshold values of the area to produce the effect, in pairs of colonies separated by 2, 3 and 5 cm; each value is the mean of three pairs of colonies in plates with two colonies/plate

Strain	Distance (cm)	E_i (0) ± SD	E_i (max) ± SD	Ratio (mm^2)	*A.min* (mm^2)	R^2
S. cerevisiae ATCC 7754	2	1.05 ± 0.019	1.25 ± 0.059	7.92×10^{-4}	140	99.5
	3	1.04 ± 0.019	1.17 ± 0.040	7.42×10^{-4}	212	98.4
	5	1.03 ± 0.014	1.12 ± 0.067	7.24×10^{-4}	266	96.2
Z. rouxii Bch	2	1.02 ± 0.009	1.17 ± 0.001	5.78×10^{-4}	48	99.6
	3	1.03 ± 0.020	1.13 ± 0.065	5.77×10^{-4}	122	93.4
	5	1.04 ± 0.022	1.09 ± 0.031	5.64×10^{-4}	208	93.7
D. hansenii PR66	2	1.03 ± 0.008	1.31 ± 0.032	5.19×10^{-4}	144	99.8
	3	1.01 ± 0.007	1.17 ± 0.055	4.98×10^{-4}	270	98.9
	5	1.03 ± 0.011	1.16 ± 0.014	5.06×10^{-4}	402	98.9
R. glutinis CECT 10145	2	1.02 ± 0.011	1.29 ± 0.031	5.10×10^{-4}	88	99.6
	3	1.03 ± 0.014	1.20 ± 0.040	5.12×10^{-4}	271	97.1
	5	1.03 ± 0.023	1.21 ± 0.023	5.48×10^{-4}	310	97.1

E_i (0), initial value in 48 h colonies; E_i (max), maximal value reached when the colonies have reached their maximal area; Ratio, increase in the eccentricity index/mm^2 of area growth; *A.min*, threshold value of the area of the colony below which the eccentricity index maintains a value close to E_i (0) and no inhibition is detected, estimated from the linear regression E_i/area; R^2, determination coefficient of the linear fit; SD, standard deviation.

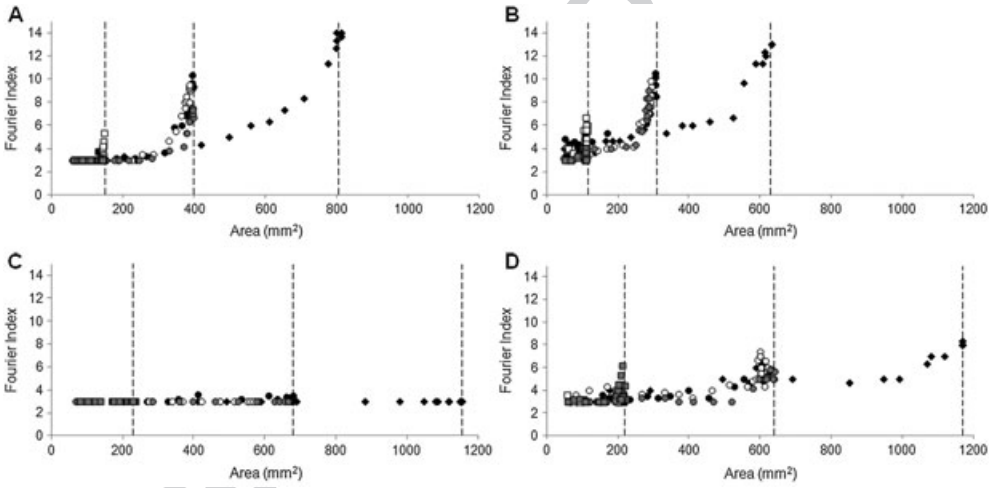

Figure 5. Relationship between the number of Fourier coefficients that are needed to describe the border complexity of a colony and its area: (A) *S. cerevisiae* ATCC 7754; (B) *Z. rouxii* Bch; (C) *D. fabryi* PR66; (D) *R. glutinis* CECT 10145. diamonds, one colony; circles, two colonies; squares, six colonies. Distances between neighbouring colonies: black, 2 cm; white, 3 cm; grey, 5 cm. Dashed lines, maximal area value. Each point is calculated as the mean of at least three colonies

colony size in the four species. It was found that this index depends strongly on the species. In young colonies (2–3 days) of all the species, the Fourier index showed a minimum value of about 3, indicating a very regular edge. This value was maintained in mature colonies, such as those of *D. fabryi*, but reached values up to 14 in the fermentative species (Figure 5). However, a common pattern could be identified in the evolution of the Fourier index with the growth of any colony: its value was maintained constant and close to 3 until the colony area approached its maximal area. Then, a sudden increase in edge complexity was observed, more abrupt as the number of colonies/plate increased (Figure 5). In all our experiments, independently of the number of colonies/plate and the distances among them, the lobed edge was a morphological change tightly linked to the final stages of colony growth, when the nutrients of the medium are supposed to be exhausted. It is noteworthy that the two fermentative species showed much higher final edge complexity than the oxidative ones.

UNCORRECTED PROOF

Copyright © 2014 John Wiley & Sons, Ltd.

DOI: 10.1002/yea

Discussion

The morphology of a yeast colony is affected by many intrinsic (genomic) and extrinsic (environmental) factors, as has been pointed out in the Introduction. Most of the genetic factors have been mainly identified and studied in *S. cerevisiae* under specific conditions. In this study we showed some common patterns of morphological change in four yeast species, differing in phylogeny (asco- and basidiomycetes) and energy metabolism. Image analyses, some of them also using Matlab software, have been described previously to characterize and analyse quantitatively the morphology of bacterial colonies (Peters, 1990; Memarian *et al.*, 2007; Mertens *et al.*, 2012). We have confirmed here the usefulness of the accurate measurements provided by image analysis and we have applied two new indices that have shown common patterns in four different yeast species. We have also confirmed the loss of circularity in pairs of colonies that is produced by the apparent inhibition of growth occurring in the facing colony edges, and we have demonstrated that the inhibition is symmetrical. The chemical messenger between colonies of nine yeast genera, growing in a complex medium with glycerol as carbon source (oxidative metabolism), has been identified as a volatile compound, ammonia (Palková *et al.*, 1997). From a teleological point of view, it is not easy to understand why, in a pair of colonies of the same age, a colony can be, at the same time, producing ammonia to inhibit the facing colony and, to the same extent, inhibited by the ammonia produced by the other colony of the pair. One possible explanation is that, once the proximity of another colony has been detected by the colonies in the pair, both produce ammonia, which accumulates between them (inhibition appears only in the intermediate space) and inhibits the growth of both to the same extent. The objective could be to maintain the individuality of the mature colonies.

The inhibition of growth has sometimes been explained in terms of competition by the colonies for nutrients. Our results do not support this hypothesis. The growth data of Figure 3 show clearly that the effect of one colony on the other is not an actual inhibition of growth but a simple topological effect, inhibiting the growth only of the cells of the facing sides of the colonies, avoiding their union. Never, at any moment of their development, does one colony grow more than its facing neighbour (see the overlapping growth curves of Figure 3). All the colonies reach the same maximal area with independence of the distance among them, and this value depends only on the number of colonies/plate. In this sense, the 2% w/v agar plate should be considered a single and homogeneous environment in which the colonies, wherever they are situated, do not compete for nutrients, grow at the same rate and reach the same maximal size, which depends exclusively on their number and the limiting nutrient concentration in the plate medium (see Figure 3). Another significant result is the quantification of the threshold value that has to be reached in a colony to produce the effect, which we have called *A.min* (see Table 1); *Z. rouxii* showed the lowest threshold and *D. fabryi* the highest. These threshold values explain why, when there are six colonies/plate, even at 2 cm distance, no eccentricity effect is observed, because the maximal area that these colonies can reach is lower than the corresponding threshold value (see Figure 3, Table 1). The proportionality observed between the increase in eccentricity and the area of the colony (Figure 4) supports the hypothesis that, once the chemical messenger has reached the front colony, growth of the cells of the facing edge stops completely and the colony grows, extending its area in the non-facing edges. These results are in agreement with those of Palková and Forstová (2000), who found that growth inhibition occurred before carbon exhaustion in facing colonies of *Candida mogii* growing in glycerol agar. Similar results, indicating that morphological changes in facing colonies are due to cell growth inhibition and not to nutrient limitation, have been found in bacteria (Be'er *et al.*, 2009). No correlation has been found between the energy metabolism (from fully fermentative in *S. cerevisiae* to fully oxidative in *R. glutinis*) and the maximal eccentricity index reached by the colonies (Table 1). Although the oxidative species show slightly higher values of E_i near 1.3, the differences are not statistically significant (Table 1). Our results support the hypothesis that the intercolonial inhibition is a general property of yeast species (Palková *et al.*, 1997; Palková and Forstová, 2000). This property should be observed provided that the colonies are allowed to grow above the threshold value of the species. The eccentricity index has proved to be a good parameter to quantify the change in

UNCORRECTED PROOF

Copyright © 2014 John Wiley & Sons, Ltd.

DOI: 10.1002/yea

morphology, to follow its evolution with the growth of the colony (Figure 4) and to compare its extent among different species (Table 1). We are currently extending its application to homo- and hetero-pairs of other yeast species.

In contrast to eccentricity due to colony interaction, the formation of lobes at the edge of colonies does not seem to be a general property of yeast colonies and, in those displaying it, seems to be expressed with higher variability than the eccentricity. The evaluation of the number of elliptic Fourier harmonics, F_i, needed to reproduce the x and y coordinates of the edge points in a colony with a correlation coefficient of 0.998 or higher, has proved to be a good quantitative parameter to measure edge complexity (see Figure 5). We have called this number the 'Fourier index' and, when its value is followed over the course of time under several conditions, some clear features of the process can be defined. The number of elliptic Fourier harmonics needed for an accurate representation of the most complex colonies was 14, which is in accordance with previous studies focused on another morphometric classification. For instance, Carlo *et al*. (2011) used 20 elliptical Fourier harmonics to quantify the morphology of the outline of sclerites of eight species of gorgonian octocoral in the genus *Pseudopterogorgia*. To the authors' knowledge, the elliptic Fourier coefficients have not been applied for characterizing the morphology of yeast colonies.

Lobe formation is a consequence of nutrient depletion, such as was observed by Boschke and Bley (1998) growing 11 different yeast species in a medium with glucose as the only carbon source, at decreasing glucose concentrations. They found that lobe formation is triggered in all the cases (and in all the species) when the colony was close to its maximal area, independently of the number of colonies/plate and the distances between them. Although we have not measured the carbon source concentrations in the agar, the proportionality between the maximal areas of the colonies with the number of colonies, in plates with the same amount of carbon source available, suggests that this is the limiting nutrient. It can be concluded that when the colony is reaching its maximum the carbon source is being exhausted. In this sense, our results (see Figure 5) confirm those of Boschke and Bley (1998) in a quantitative way. The edge irregularity seems to be expressed more strongly in fermentative species than in the oxidative ones, but more species need to be studied to confirm that hypothesis. The low energy yield of fermentative metabolism would make the fermenting strains more dependent on the amount of the energy source, and this dependence could explain the different behaviour observed, more intense than in the oxidative yeasts.

The genomic basis of the colony behaviour described in this paper is poorly known and has only been explored in one of the species studied, *S. cerevisiae*. Most of the genes involved have pleiotropic effects. The two indices described here could be useful to study quantitatively the effect of genetic modifications (knock-out, gene dosage, etc.) on colony morphology.

Acknowledgements

This study was supported by UCM-SCH (Project No. G35/10-A). Research by E.G.d.P. was supported by a FPU predoctoral fellowship from the Spanish Ministry of Education, Culture and Sport; research by E.M.R.F. was supported by a PICATA predoctoral fellowship of the Moncloa Campus of International Excellence (UCM-UPM).

References

Agüera A, Brophy D. 2011. Use of sagittal otolith shape analysis to discriminate north-east Atlantic and western Mediterranean stocks of Atlantic saury, *Scomberesox saurus saurus* (Walbaum). *Fish Res* **110**: 465–471.

Be'er A, Zhang HP, Florin EL, *et al*. 2009. Deadly competition between sibling bacterial colonies. *Proc Natl Acad Sci U S A* **106**: 428–433.

Boschke E, Bley TH. 1998. Growth patterns of yeast colonies depending on nutrient supply. *Acta Biotechnol* **1**: 17–27.

Banada PP, Guo S, Bayraktar B, *et al*. 2007. Optical forward-scattering for detection of *Listeria monocytogenes* and other *Listeria* species. *Biosens Bioelectron* **22**: 1664–1671.

Camargo Neto J, Meyer EG, Jones DD, Samal AK. 2006. Plant species identification using elliptic Fourier leaf shape analysis. *Comput Electron Agr* **50**: 121–134.

Carlo JM, Barbeitos MS, Lasker HR. 2011. Quantifying complex shapes: elliptical Fourier analysis of octocoral sclerites. *Biol Bull* **220**: 224–237.

Clemmensen LH, Hansen ME, Frisvad JC, *et al*. 2007. A method for comparison of growth media in objective identification of *Penicillium* based on multi-spectral imaging. *J Microbiol Methods* **69**: 249–255.

den Hertog AL, Visser DW, Ingham CJ, *et al*. 2010. Simplified automated image analysis for detection and phenotyping of *Mycobacterium tuberculosis* on porous supports by monitoring growing microcolonies. *PLoS One* **5**: e11008.

UNCORRECTED PROOF

Copyright © 2014 John Wiley & Sons, Ltd.

Dorge T, Carstensen JM, Frisvad JC. 2000. Direct identification of pure *Penicillium* species using image analysis. *J Microbiol Methods* **41**: 121–133.

Gonzalez RC, Woods RE, Eddins SL. 2004. Digital Image Processing Using Matlab. Pearson Prentice Hall: Upper Saddle River, NJ.

Granek JA, Magwene PM. 2010. Environmental and genetic determinants of colony morphology in yeast. *PLoS Genet* **6**: e1000823.

Halme A, Bumgarner S, Styles C, Fink GR. 2004. Genetic and epigenetic regulation of the *FLO* gene family generates cell-surface variation in yeast. *Cell* **116**: 405–415.

Hansen ME, Lund F, Carstensen JM. 2003. Visual clone identification of *Penicillium* commune isolates. *J Microbiol Methods* **52**: 221–229.

Kocková-Kratochvilová A. 1990. Yeasts and Yeast-like Organisms, 2nd edn. Wiley-VCH: Weinheim.

Kuhl FP, Giardina CR. 1982. Elliptic Fourier features of a closed contour. *Comput Graphics Image Proc* **18**: 236–258.

Kurtzman CP, Fell JW, Boekhout T (eds). 2011. The Yeasts. A Taxonomic Study, 5th edn. Elsevier: San Diego, CA.

Lo WS, Dranginis AM. 1996. *FLO11*, a yeast gene related to the *STA* genes, encodes a novel cell surface flocculin. *J Bacteriol* **178**: 7144–7151.

Mebatsion HK, Paliwal J, Jayas DS. 2012. Evaluation of variations in the shape of grain types using principal components analysis of the elliptic Fourier descriptors. *Comput Electron Agr* **80**: 63–70.

Memarian N, Jessulat M, Alirezaie J, *et al*. 2007. Colony size measurement of the yeast gene deletion strains for functional genomics. *BMC Bioinformat* **8**: 117–128.

Mertens L, Van Derlinden E, Van Impe JF. 2012. A novel method for high-throughput data collection in predictive microbiology: optical density monitoring of colony growth as a function of time. *Food Microbiol* **32**: 196–201.

Palková Z, Janderová B, Gabriel J, *et al*. 1997. Ammonia mediates communication between yeast colonies. *Nature* **390**: 532–536.

Palková Z, Forstová J. 2000. Yeast colonies synchronise their growth and development. *J Cell Sci* **113**: 1923–1928.

Peters AC. 1990. Use of image analysis to map bacterial growth on solid media. *Binary* **2**: 73–75.

Puchkov EO. 2010. Computer image analysis of microbial colonies. *Microbiology* **79**: 141–146.

Reynolds TB, Fink GR. 2001. Bakers' yeast, a model for fungal biofilm formation. *Science* **291**: 878–881.

Robinson A, Sadr-Kazemi N, Dickason G, Harrison STL. 1998. Morphological characterisation of yeast colony growth on solid media using image processing. *Biotechnol Tech* **12**: 763–767.

Romero P, Patiño B, Quirós M, *et al*. 2005. Differential detection of *Debaryomyces hansenii* isolated from intermediate-moisture foods by PCR–RFLP of the IGS region of rDNA. *FEMS Yeast Res* **5**: 455–461.

Šťovíček V, Váchová L, Kuthan M, Palková Z. 2010. General factors important for the formation of structured biofilm-like yeast colonies. *Fungal Genet Biol* **47**: 1012–1022.

Váchová L, Cáp M, Palková Z. 2012. Yeast colonies: a model for studies of aging, environmental adaptation, and longevity. *Oxid Med Cell Longev* •••: •••–•••; DOI: 10.1155/2012/601836

Váchová L, Stovícek V, Hlavácek O, *et al*. 2011. Flo11p, drug efflux pumps, and the extracellular matrix cooperate to form biofilm yeast colonies. *J Cell Biol* **194**: 679–687.

Verstrepen KJ, Derdelinckx G, Delvaux FR, *et al*. 2001. Late fermentation expression of *FLO1* in *Saccharomyces cerevisiae*. *J Am Soc Brew Chem* **59**: 69–76.

Verstrepen KJ, Klis F. 2006. Flocculation, adhesion and biofilm formation in yeasts. *Mol Microbiol* **60**: 5–15.

Vopálenská I, Hulková M, Janderová B, Palková Z. 2005. The morphology of *Saccharomyces cerevisiae* colonies is affected by cell adhesion and the budding pattern. *Res Microbiol* **156**: 921–931.

Vopálenská I, Šťovíček V, Janderová B, *et al*. 2010. Role of distinct dimorphic transitions in territory colonizing and formation of yeast colony architecture. *Environ Microbiol* **12**: 264–277.

Wrent P, Rivas EM, Peinado JM, de Silóniz MI. 2010. Strain typing of *Zygosaccharomyces* yeast species using a single molecular method based on polymorphism of the intergenic spacer region (IGS). *Int J Food Microbiol* **142**: 89–96.

Yang K, Wang J, Li X, *et al*. 2001. Strain selection of *Metarhizium anisopliae* by image analysis of colony morphology for consistency of steroid biotransformation. *Biotechnol Bioeng* **75**: 53–62.

UNCORRECTED PROOF

Copyright © 2014 John Wiley & Sons, Ltd.