Phenotype switching affects biofilm formation by Candida parapsilosis

Sean F. Laffey and Geraldine Butler

Department of Biochemistry, Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Belfield, Dublin 4, Ireland

Generation of biofilms by the pathogenic yeast Candida parapsilosis is correlated closely with disease. The phenomenon of phenotype switching in 20 isolates of C. parapsilosis was examined and the relationship with biofilm development was investigated. Four stable and heritable phenotypes were identified – crepe, concentric, smooth and crater. Cells from crepe and concentric phenotypes are almost entirely pseudohyphal, whilst cells from smooth and crater phenotypes are mostly yeast-like. The pseudohyphae from concentric phenotypes are approximately 45% wider than those from crepe cells. The cell size of the smooth phenotype is smaller than those of the other three phenotypes.

On polystyrene surfaces, the concentric phenotype generates up to twofold more biofilm than the crepe and crater phenotypes. Smooth phenotypes generate the least biofilm. Concentric phenotypes also invade agar surfaces more than the crepe and crater phenotypes, whilst smooth phenotypes do not invade at all. The smooth phenotype, however, grows significantly faster than the others. The quorum-sensing molecule farnesol inhibits formation of biofilms by the crepe, concentric and crater phenotypes.

INTRODUCTION

The yeast Candida parapsilosis is found frequently as a commensal organism on epithelial and mucosal tissues, but it is also an increasing cause of hospital-acquired infection (Weems, 1992). Although C. parapsilosis is responsible for approximately 16% of general Candida infections, it is a particular problem in critically ill neonates (Hajjeh et al., 2001; 2004; Hawser & Diekema, 2004; Roilides et al., 2004). Fungaemia caused by C. parapsilosis is associated with the presence of catheters and the use of parenteral nutrition (Shin et al., 2002). This is probably due to the ability of the yeast to grow as biofilms, especially in high-glucose environments (Branchini et al., 1994). Biofilms are inherently resistant to treatment with antifungal drugs and the infected device must usually be removed.

Formation of biofilms by organisms growing in association with a surface is a very common phenomenon among yeast and bacterial species (Hall-Stoodley et al., 2004; Jabra-Rizk et al., 2004). In yeast, the most detailed descriptions of biofilm structure come from studies on Candida albicans (Baillie & Douglas, 1999; Chandra et al., 2001; Hawser & Douglas, 1994; Kumamoto, 2002; Shin et al., 2002). In general, a layer of cells in the yeast form is found attached to the surface, with a layer of filamentous cells above, surrounded by an exopolymeric matrix (Kumamoto, 2002). Cells in biofilms are associated with a specific gene-expression pattern, including the overexpression of amino acid biosynthetic genes, particularly those for amino acids containing sulfur (Garcia-Sanchez et al., 2004). Biofilm growth requires activation of the filamentation pathway and mutants defective in regulators of filamentation, such as efg1 and cph1, are unable to form biofilms (Ramage et al., 2002).

C. parapsilosis is not capable of forming true filaments and biofilms are often composed of clumped blastospores (Kuhn et al., 2002). Whilst it has been reported that C. parapsilosis biofilms are not as large as those generated by C. albicans (Kuhn et al., 2002), this may be related to the growth conditions used; high-glucose media, in particular, greatly increase biofilm development by C. parapsilosis (Branchini et al., 1994).

Bacteria form biofilms with somewhat similar structures to fungi. Cell attachment is also associated with changes in gene expression (Kuchma & O'Toole, 2000; Ren et al., 2004; Schembri et al., 2003; Schoolnik et al., 2001). In some species, biofilm development is particularly associated with specific colony variants. In Pseudomonas aeruginosa, for example, a small-colony variant forms significantly more biofilm than other phenotypes (Haufler, 2004). These variants consist of highly adhesive cells that are hyper-piloted (Dzieli et al., 2001). The switch between phenotypic variants is regulated by a phase-variation mechanism involving the two-component response regulator PvrR (Drenkard & Ausubel, 2001). In Vibrio cholerae, switching from a smooth to a rugose phenotype is also associated with increased biofilm formation and upregulation of genes involved in polysaccharide biosynthesis (Rashid et al., 2003; 2004; Yildiz et al., 2001).

Switching between heritable colony phenotypes has been
described in C. albicans (Slutsky et al., 1985; Soll et al., 1993) and C. parapsilosis (Enger et al., 2001; Lott et al., 1993). In C. albicans, some phenotypes adhere differentially to mammalian cells (Vargas et al., 1994). We therefore tested whether there is a correlation between phenotype and biofilm formation in C. parapsilosis. We show that one phenotype (called concentric), isolated readily from clinical isolates, forms quantitatively more biofilm and invades agar more readily than others. A second phenotype (smooth) forms less biofilm and does not invade agar to any measurable degree.

**METHODS**

**Strains and media.** C. parapsilosis strains were grown routinely in YPD medium (1% yeast extract, 2% peptone, 2% glucose) at 30°C and maintained on YPD agar (2% agar). Where indicated, phloxine B was added at a concentration of 5 mg ml⁻¹ (Anderson & Soll, 1987). For biofilm development, YPD medium with a glucose concentration of 8% was used (Shin et al., 2002). The type strain (CLIB 214) was obtained from the Collection de Levures d’Intérêt Biotechnologique, Thiverval Grignon, France. All other isolates were obtained from Professor Frank Odds, Aberdeen, UK (Tavanti et al., 2005) (Table 1).

**Analysis of phenotypic switching.** To observe phenotype switching, approximately 1000 cells from each C. parapsilosis isolate were plated onto YPD medium containing 5 mg phloxine B ml⁻¹ and incubated at 30°C for 96 h. Phenotypes were observed visually. To determine the switching rate, C. parapsilosis 74/046 cells from a primary colony that were visually homogeneous for a single switch phenotype were inoculated into liquid YPD medium containing 5 mg phloxine B ml⁻¹ and grown at 30°C until they reached a concentration of approximately 5×10⁶ cells ml⁻¹. The cells were then distributed evenly on YPD agar plates containing 5 mg phloxine B ml⁻¹ at a density of 50–70 c.f.u. per plate. The plates were incubated at 30°C for 4 days and scored for the proportions of the different switch phenotypes (Brockert et al., 2003).

**Microscopy.** To visualize cellular morphology of the four phenotypes exhibited by C. parapsilosis strain 74/046, cells of each colony phenotype were grown overnight in YPD medium. Aliquots (100 µl) of cells were washed twice in PBS and then resuspended in 100 µl calcofluor white solution (1 mg ml⁻¹) and 10 µl DAPI solution (4',6-diamidino-2-phenylindole; 1 mg ml⁻¹). Aliquots (5 µl) of the samples were then mounted on glass slides and cells were visualized and images were collected by using an F-View 2 digital camera from Soft Imaging Systems (SIS). The difference in width of pseudohyphae was calculated from measurements of 100 cells.

**Biofilm formation.** Biofilms were generated essentially as described by Ramage et al. (2001). For assays using crystal violet, cells were grown to mid-exponential phase in YPD medium at 30°C, 5×10⁶ cells ml⁻¹ were added to each well of a 96-well polystyrene plate and incubated at 37°C (Kuhn et al., 2002). The cells were allowed to adhere for 2 h and wells were washed twice with PBS to remove non-adhered cells; 100 µl fresh growth medium then was added to the wells and the plates were returned to the incubator for 48 h. To determine the effect of farnesol, cells were incubated in YPD medium containing 8% glucose supplemented with 30 or 300 µM trans,trans-farnesol (Sigma). After 12–60 h, the wells were washed twice with 200 µl PBS and biofilm mass was measured by using a crystal violet assay (Djordjevic et al., 2002). Each of the washed wells was stained by adding 100 µl 0-1% aqueous crystal violet solution and incubating at 30°C for 15 min. The plate was then washed with sterile distilled water in an automated plate washer and destained with 100 µl 33% (v/v) glacial acetic acid. After 4 h destaining, 100 µl H₂O was added and 50 µl was then transferred to a new well. The amount of crystal violet stain in the destaining solution was measured.

**Table 1.** C. parapsilosis strains used in this study

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Origin</th>
<th>Phenotype(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLIB 214</td>
<td>Puerto Rico; faeces</td>
<td>Crepe, concentric</td>
</tr>
<tr>
<td>74/046</td>
<td>Leeds, UK; aortic valve</td>
<td>Crepe, concentric, smooth, crater</td>
</tr>
<tr>
<td>81/041</td>
<td>Mayo Clinic, USA; vagina</td>
<td>Crepe, concentric, smooth, crater</td>
</tr>
<tr>
<td>73/037</td>
<td>Leeds, UK; vagina</td>
<td>Crepe, concentric, smooth, crater</td>
</tr>
<tr>
<td>103</td>
<td>London, UK; anus</td>
<td>Concentric, smooth, crater</td>
</tr>
<tr>
<td>J931058</td>
<td>Belgium; nail</td>
<td>Crepe, smooth, crater</td>
</tr>
<tr>
<td>73/107</td>
<td>London, UK; mouth</td>
<td>Crepe, concentric, smooth</td>
</tr>
<tr>
<td>J950218</td>
<td>USA; unknown</td>
<td>Concentric, crater</td>
</tr>
<tr>
<td>J930631/1</td>
<td>Africa; cat hair</td>
<td>Crepe, smooth</td>
</tr>
<tr>
<td>J960578</td>
<td>Hong Kong; nail</td>
<td>Crepe, smooth</td>
</tr>
<tr>
<td>J931845</td>
<td>Japan; unknown</td>
<td>Concentric, crater</td>
</tr>
<tr>
<td>90-137</td>
<td>San Jose, USA; orbital tissue</td>
<td>Crepe, concentric</td>
</tr>
<tr>
<td>J961250</td>
<td>Lisbon, Portugal; nail</td>
<td>Crepe, crater</td>
</tr>
<tr>
<td>J930733</td>
<td>Beerse, Belgium; cat hair</td>
<td>Crepe</td>
</tr>
<tr>
<td>711701</td>
<td>Aberdeen, UK; unknown</td>
<td>Crepe</td>
</tr>
<tr>
<td>81/042</td>
<td>London, UK; ear</td>
<td>Crepe</td>
</tr>
<tr>
<td>73/114</td>
<td>Leeds, UK; anus</td>
<td>Crepe</td>
</tr>
<tr>
<td>J951066</td>
<td>Korea; nail</td>
<td>Crepe</td>
</tr>
<tr>
<td>81/253</td>
<td>London, UK; nail</td>
<td>Crepe</td>
</tr>
<tr>
<td>81/040</td>
<td>London, UK; toe</td>
<td>Crepe</td>
</tr>
</tbody>
</table>
spectrophotometrically \( (A_{570}) \) (Stepanovic et al., 2004). For comparison of isolates of \textit{C. parapsilosis}, each assay was carried out by using two biologically independent samples measured in triplicate. For determining the effect of farnesol concentrations, six independent measurements were made. For microscopy, the wells were air-dried and images were captured with a light microscope (Zeiss Axiovert 200).

For dry-weight measurements, cells were counted by using a hemocytometer and resuspended at \( 5 \times 10^7 \) cells ml\(^{-1} \) in YPD medium containing 8 % glucose. Cell suspension (20 ml) was added to untreated Petri dishes (90 mm diameter) and allowed to adhere for 2 h. The plates were washed twice with PBS to remove non-adhered cells and reincubated in 20 ml fresh growth medium for 48 h. The biofilms were then washed three times in PBS and suspended in 10 ml PBS by using a cell scraper. The biofilms were collected on pre-dried and weighed cellulose filters (0.45 \( \mu \)m pore size, 47 mm diameter) and washed three times with water (10 ml). The filters were dried at 37 \( ^\circ \)C for 24 h and the dry weight of cells per filter was calculated. Dry weights were determined by using three independent cultures measured in triplicate (Hawser & Douglas, 1994).

**Agar invasion.** Equal volumes of overnight cultures of each phenotype were spotted onto YPD agar plates and incubated at 30 \( ^\circ \)C for 96 h. The cells were photographed and those on the surface were removed by washing under running water. The cells remaining under the agar were then photographed.

**Growth-kinetics determination.** Cell number was related to \( A_{600} \) readings by plating serial dilutions of exponential-phase cultures of the four switch phenotypes onto YPD agar. Growth curves were produced by inoculating 100 ml YPD medium with cells of each of the four switch phenotypes to give an approximate density of \( 7 \times 10^4 \) cells ml\(^{-1} \). A volume of 1 ml was then taken from each growing culture every 30 min and the \( A_{600} \) was measured. The readings over 24 h were then converted to cell number and represented graphically. Each assay was carried out with three independent cultures.

**RESULTS**

**Identification of colony phenotypes**

Switching between variant colony morphologies in \textit{C. parapsilosis} was first described by Lott et al. (1993) and extended by the Soll group (Enger et al., 2001). Enger et al. (2001) described five colony phenotypes from one isolate – crepe, concentric, snowball, rough and smooth. We examined 20 isolates [mostly from clinical sources (Tavanti et al., 2005)] on YPD medium and identified four phenotypes (Table 1, Fig. 1). The phenotypes ‘crepe’ and ‘smooth’ were similar to those described by Enger et al. (2001) and both studies describe a phenotype with a structure containing concentric circles. We therefore retained this nomenclature. We did not, however, detect any phenotypes that were readily identifiable as ‘rough’ or ‘snowball’, and instead identified a fourth phenotype with a fuzzy outline that we call ‘crater’. Our analysis was conducted by using YPD medium containing phloxine B, as this is used routinely to detect white–opaque switching in \textit{C. albicans} (Anderson & Soll, 1987). However, the observed phenotypes were identical in the absence of phloxine B (not shown). We will therefore refer to four phenotypes.

Of the 20 isolates tested, seven generated only the crepe phenotype (Table 1). Overall, the crepe phenotype was the most common and was identified in 17 out of 20 isolates. Only three of the isolates tested generated all four phenotypes, although it is possible that they also occurred in other isolates at a lower frequency. For one of these (strain 74/046), we tested the frequency of switching (Fig. 1). This occurs at a relatively high rate (from \( 10^{-1} \) to \( 10^{-3} \)), but the parental phenotype is stable enough for further study.

The cell morphology of the four colony phenotypes also varies considerably (Fig. 2). Whilst the crepe and concentric phenotypes are mostly pseudohyphal, the cells of the concentric phenotype are approximately 45 % wider. Staining with calcofluor white (Fig. 2) shows that chitin is distributed along the length of the cell, as well as at the bud neck, particularly in the concentric phenotype. Cells from the crater phenotype are elongated, but not pseudohyphal, and chitin is distributed around the cell wall and in the bud neck. Cells from the smooth phenotype are small and yeast-shaped, with chitin localized predominantly to the bud scars. These cells also have the smallest nuclei.

**Phenotype affects biofilm formation**

To determine the effect of phenotype on biofilm formation, we measured the amount of biofilm generated by 13 strains...
that formed at least two phenotypes, and one that formed only one (Table 1; Fig. 3). The strains were incubated in medium containing 8% glucose, as this maximizes biofilm development by C. parapsilosis (Branchini et al., 1994; Shin et al., 2002). Equal numbers of cells from all phenotypes from all strains were allowed to adhere to the bottom of 96-well polystyrene plates for 2 h; non-adherent cells were then removed and biofilms were allowed to develop over 48 h. The amount of biofilm generated was determined by using a crystal violet assay for all strains (Fig. 3a). To ensure that the assay was an accurate measurement of mass, we also measured the dry weight of biofilms generated by the three strains that produced all four phenotypes (C. parapsilosis 74/046, 81/041 and 73/037; Fig. 3b).

There is no significant difference between the mass of biofilm generated by the crepe and crater phenotypes, as measured by either method (Fig. 3). The concentric phenotype, however, generates approximately 1.75-fold more biofilm than either the crepe or crater phenotypes in the crystal violet assay, and twofold more biofilm as measured by dry weight (Fig. 3, P < 0.001, ANOVA). The smooth phenotype generates 20–60% less biofilm (Fig. 3, P < 0.001). The mass of biofilm generated is specific to the phenotype and independent of the strain origin. For example, isolate J930733, which produces only the crepe phenotype, generates a biofilm mass equivalent to those of other crepe phenotypes (Fig. 3a).

The structure of the biofilms, as visualized by light microscopy, also varied with the phenotype (Fig. 3c). Cells from the concentric phenotype are entirely filamentous and cover the bottom of the polystyrene well. Cells from the smooth phenotype are small and yeast-shaped, and are distributed widely on the polystyrene surface with many gaps. Cells from the crepe and crater phenotypes are more elongated and form denser structures.

**Phenotype affects invasion of agar**

Several factors required for adhesion in C. albicans are also involved in invasion of tissue and other substrates (Dieterich et al., 2002; Fu et al., 1998). The effect of phenotype on agar invasion in C. parapsilosis was determined by using isolate 74/046, which switches between all four phenotypes. Cells from each phenotype were grown in liquid culture to mid-exponential phase and equal volumes were inoculated onto solid YPD medium and incubated for 96 h. The colonies were photographed and then washed off the plate with running water (Fig. 4). The mass remaining after washing is an indication of the amount of invasion of the agar. The concentric phenotype invades the most, leaving the greatest mass of cells after washing. The crepe and crater phenotypes also invade, although not to the same extent as concentric. Cells from the centre of the colony, in particular, are removed by washing (Fig. 4). Cells from the smooth phenotype do not invade to any measurable extent. The invasion phenotypes therefore correlate well with the ability to generate biofilms.

**Farnesol inhibits biofilm formation**

It has been reported previously that farnesol, a quorum-sensing molecule, inhibits filamentation and biofilm formation by C. albicans (Ramage et al., 2002a). We therefore tested the effect of farnesol on biofilm formation by C. parapsilosis. Farnesol was added either immediately after the cells were placed in the 96-well plates, or following 2 h.
adhesion. As shown in Fig. 5(a), the addition of 300 μM farnesol before allowing cells to adhere resulted in a reduction in biofilm formation by the crepe, concentric and crater phenotypes of C. parapsilosis 74/046 (P < 0.001, ANOVA). The addition of farnesol had little or no effect on biofilm formation by the smooth phenotype. There was no effect on biofilm formation by any phenotype when farnesol was added after the cells were allowed to adhere for 2 h. Farnesol affects only biofilm mass, and has no effect on cell phenotype (data not shown). Fig. 5(b) shows that the addition of farnesol to concentric cells reduces biofilm mass developed over 24–60 h. Biofilm development by crepe and crater cells is reduced in a similar manner (data not shown).

Smooth phenotype grows rapidly

Whilst preparing cells for biofilm analysis, we observed that the smooth phenotype in all isolates grew more rapidly. We confirmed this observation by comparing the growth rate of the four phenotypes in C. parapsilosis isolate 74/046 (Fig. 6). The cultures were analysed in triplicate. There is no consistent, detectable difference in the growth curves between the four phenotypes over the period of observation.

**Fig. 3.** Phenotype affects biofilm formation. (a) Cells from different phenotypes from 14 C. parapsilosis isolates were allowed to adhere to polystyrene plates as described in Methods and the amount of biofilm formed after 48 h was determined by using a crystal violet assay. The mean ± SD was determined from two independent samples measured in triplicate. Gaps represent phenotypes not present in individual strains. (b) Cells from three C. parapsilosis isolates (74/046, filled bars; 81/041, shaded bars; 73/037, empty bars) that form all four phenotypes were allowed to adhere to the bottom of Petri dishes and the dry weight of biofilm formed after 48 h was determined. The mean ± SD was determined from three independent samples measured in triplicate. (c) Adherent cells from each phenotype from strain 74/046 were photographed in situ on the base of a 96-well polystyrene plate after 48 h.

**Fig. 4.** Phenotype affects agar invasion. Equal volumes and cell numbers from stationary-phase cultures of the four phenotypes of strain 74/046 were spotted onto YPD agar plates and incubated at 30 °C for 4 days. Colonies were photographed before and after removing cells on the surface by washing under running water.
of the crepe, concentric or crater phenotypes. However, the cell number of the smooth phenotype is significantly higher for all time points from 4-5 h onwards ($P<0.001$, ANOVA).

**DISCUSSION**

The generation of biofilms by *Candida* species begins with adherence of the cells to plastic or other surfaces, resulting in a change in morphology and behaviour. Phenotypic switching has been associated with adherence of *C. albicans* to mammalian cells. In the well-studied white–opaque transition, cells expressing the white phenotype are more adhesive to buccal epithelia than cells expressing the opaque phenotype (Kennedy et al., 1988). Vargas et al. (1994) showed that, in a different *C. albicans* switching system with four phenotypes, there is a hierarchy of adhesion of the yeast to buccal epithelia and stratum corneum from pigs. The phenotype o-smooth is the most adhesive, followed by irregular wrinkle, revertant smooth and star. However, no association has been shown between phenotype switching and adhesion to plastics, or subsequent biofilm formation.

We have extended the observations of Lott et al. (1993) and Enger et al. (2001) that phenotypic switching occurs in *C. parapsilosis*. We identify four core phenotypes, three of which are similar to those described by Enger et al. (2001). The fourth phenotype (crater) has not been described. Switching between all four phenotypes occurs at a high rate in three out of 20 *C. parapsilosis* isolates that were tested. The majority of isolates (13) switched between at least two different morphologies (Table 1). We used a crystal violet assay to compare the ability of 14 isolates to generate biofilms and found that the concentric phenotype isolated from nine different strains formed the greatest amount of biofilm (Fig. 3), whilst the smooth phenotype from eight strains formed the least biofilm. We also measured the dry weight of biofilm formed by the four phenotypes from three *C. parapsilosis* isolates. Both methods show that the concentric phenotype forms the most biofilm (from 1.75- to twofold higher), whilst the smooth phenotype forms the least.

In *C. albicans*, generation of biofilms is associated closely with the dimorphic switch from yeast to hyphal growth (Baillie & Douglas, 1999). Wild-type strains that are naturally defective in hyphal growth form only a thin basal yeast layer on catheter discs, whereas strains that grow only in filamentous forms generate dense biofilm mats (Baillie & Douglas, 1999). In otherwise genetically identical strains, deleting *efg1* and *cph1*, regulators of filamentation, abolishes or greatly reduces biofilm formation (García-Sánchez et al., 2004; Lewis et al., 2002; Ramage et al., 2002b). *C. parapsilosis* strains do not form true hyphae (Odds, 1988), but they do grow in pseudohyphal or other filamentous forms (Fig. 2). The phenotype that grows only as small yeast cells on plastic (smooth) forms the least biofilm, whereas the phenotype...
that grows predominantly as a filament (concentric) forms the most biofilm. It is therefore likely that filamentation and biofilm formation in \textit{C. parapsilosis} are also correlated closely.

The cell morphology of the four phenotypes when growing on plastic is similar, but not identical, to that of the cells growing in liquid culture (Figs 2 and 3). The concentric phenotype is filamentous under both conditions and the smooth phenotype grows only as yeast cells, whether on plastic or in liquid culture. The crater cells are also similar, forming elongated yeast cells. The crepe phenotype, however, is a mixture of pseudohyphae and elongated cells in liquid culture, yet grows only as elongated yeast cells when adhered to plastic. It is likely that adherence to plastic results in a major transcriptional change, affecting genes that are also involved in cell morphology.

Factors that regulate adherence to plastic and biofilm development in \textit{C. albicans} are often also involved in cell adherence and invasion. The best characterized is \textit{Efg1}, which, as well as being required for filamentation and biofilm development (Garcia-Sanchez et al., 2004; Lewis et al., 2002; Ramage et al., 2002b), is also necessary for adherence and invasion of reconstructed human epithelia (Dieterich et al., 2002). Members of the ALS adhesin family are also required for adhesion to human cells (Fu et al., 2002; Zhao et al., 2004) and are expressed differentially in biofilms (Chandra et al., 2001). We used a very simple model to measure the ability of \textit{C. parapsilosis} phenotypes to invade solid agar. The results correlated closely with biofilm development. The phenotype that is most filamentous (concentric) generates most biofilm and invades the agar to the greatest extent. Conversely, the phenotype growing only as small yeast cells (smooth) makes the least biofilm and does not invade agar. The smooth phenotype, however, grows more rapidly. It is therefore possible that \textit{C. parapsilosis} exploits different environmental niches by undergoing phenotype switching. The smooth phenotype grows rapidly and so will be disseminated quickly. The concentric phenotype, however, is more efficient at adherence and invasion.

We do not yet understand the molecular mechanisms that regulate phenotypic switching and biofilm development in \textit{C. parapsilosis}. However, we have shown that farnesol, a quorum-sensing agent in \textit{C. albicans} (Hornby et al., 2001; Ramage et al., 2002a; Shchepin et al., 2003), also reduces biofilm formation by the crepe, concentric and crater phenotypes in \textit{C. parapsilosis}. As in \textit{C. albicans}, farnesol inhibits biofilm formation if added before allowing the cells to adhere, but has no effect following an adherence time of 2 h (Ramage et al., 2002a). It is therefore likely that farnesol exerts its effect at early stages of biofilm development. Farnesol inhibits the filamentation pathway in \textit{C. albicans} (Kruppa et al., 2004; Sato et al., 2004). \textit{C. parapsilosis} does not generate true hyphae, but it does grow in a pseudohyphal form. The amount of biofilm generated by the highly pseudohyphal phenotype (concentric) is reduced most by farnesol, when added at 30 or 300 \textmu M. However, farnesol has no effect on the morphology of the cells (data not shown). It is therefore likely that quorum sensing is also important for biofilm formation by \textit{C. parapsilosis}.

**ACKNOWLEDGEMENTS**

This work was supported by Science Foundation Ireland. We are grateful to Professor Frank Odds (University of Aberdeen, UK) for providing the strain collection and for providing access to data before publication.

**REFERENCES**


