The role of *FLO11* in *Saccharomyces cerevisiae* biofilm development in a laboratory based flow-cell system

Boloroo Purevdorj-Gage[1], Miranda E. Orr[2], Paul Stoodley[3], Kathy B. Sheehan[1] & Linda E. Hyman[1]

[1]Division of Health Sciences, Montana State University, Bozeman, MT, USA ; [2]Department of Cell Biology and Neuroscience, Montana State University, Bozeman, MT, USA; and [3]Center for Genomic Sciences, Allegheny-Singer Research Institute, Pittsburgh, PA, USA

Correspondence: Linda E. Hyman, Division of Health Sciences, WWAMI Medical Education Program, 308 Leon Johnson Hall, PO Box 173080, Montana State University, Bozeman, MT 59717 USA. Tel.: +406 994 4411; fax: +406 994 4398; e-mail: lhyman@montana.edu

Present address: Boloroo Purevdorj-Gage, Department of Microbiology and Immunology, Stanford University School of Medicine, Stanford, CA, USA.

Received 23 May 2006; revised 6 October 2006; accepted 19 October 2006.
First published online 19 January 2007.

DOI:10.1111/j.1567-1364.2006.00189.x

Editor: José Ruiz-Herrera

Keywords
biofilm adhesion; biofilm development; invasive growth; cell aggregation; *FLO11*; *Saccharomyces cerevisiae*.

Abstract

A role of the *FLO11* in *Saccharomyces cerevisiae* biofilm development in a flow cell system was examined. We carried out an ectopic *FLO11* expression in the wild type (wt) BY4741 strain that has low levels of endogenous *FLO11* transcript. In contrast to the nonadhesive wt, the *FLO11* overexpression strain (BY4741 FLO11$^+$) readily adhered to both liquid-hydrophobic and liquid-hydrophilic solid interfaces and was able to grow as a biofilm monolayer in a flow system. Cellular features associated with *FLO11* were examined and found to be consistent with the previous studies conducted in different strains of *S. cerevisiae*. When grown in suspended liquid culture, BY4741 FLO11$^+$ formed larger cellular aggregates (clumps), consisting of from five to 60 cells, and displayed an increased cell surface hydrophobicity, without changes in the cell size or growth rate, compared to wt. However, the invasive growth associated with *FLO11* expression was not observed in BY4741 FLO11$^+$. The significance of these findings is discussed in the context of clinically and industrially relevant biofilms.

Introduction

Biofilms are functionally and architecturally differentiated microbial communities that are embedded in a polysaccharide-rich matrix and are frequently attached to biotic and abiotic surfaces. The majority of microorganisms in nature and roughly 80% of all microbial infections in humans are found in biofilms (Costerton *et al.*, 1999; Stoodley *et al.*, 2002). Biofilms are inherently resistant to disinfectants and antibiotics (Costerton *et al.*, 1987), as well as to host immune responses (Nickel *et al.*, 1985). Thus, biofilms are difficult to eradicate and detrimental in many medical and industrial settings. In particular, the biofilms of pathogenic fungal species such as *Candida albicans* are frequently isolated from patients with indwelling device-related infections that frequently lead to chronic as well as life-threatening acute systemic infections (Mavor *et al.*, 2005; Mukherjee *et al.*, 2005). While a great deal of knowledge is available on the biology and pathogenesis of bacterial biofilms, more studies on fungal biofilms is needed in order to devise effective means of controlling infections and contaminations due to detrimental biofilms.

Saccharomyces cerevisiae is an ideal model organism for studies of fungal biofilms because it has well-defined and easy-to-manipulate genetic and biochemical systems. Biofilm studies in *S. cerevisiae* would provide clues to the mechanisms of biofilm development and biofilm-associated infections caused by the pathogenic yeasts that are genetically more difficult to manipulate (i.e. *C. albicans*). Biofilm studies in *S. cerevisiae* also contribute towards the advancement of the immobilized yeast cell technology currently employed in the beer production industry (Branyik *et al.*, 2005).

The ability of *S. cerevisiae* to adhere to an inanimate surface was documented in strain Σ1278 which has the ability to undergo pseudohyphal growth (Gimeno & Fink, 1994). Reynolds & Fink (2001) found, in microplate adhesion assays, that Flo11p was required for the attachment of the cells to a liquid-hydrophobic solid interface. *FLO11*, also known as *MUC1*, belongs to a gene family of adhesins that encode glycosyl-phosphatidylinositol (GPI)-linked glycoproteins with

a domain structure similar to domains found in pathogenic fungi (Lo & Dranginis, 1996). *FLO11* is expressed on the cell surface and is required for flocculation (Lo & Dranginis, 1996), nutritionally induced invasive growth in haploids and pseudohyphal development in diploids (Lo & Dranginis, 1998). Recently, *FLO11* was implicated in flor formation on the surface of wine after alcoholic fermentation (Ishigami *et al.*, 2004) and in biofilm formation at an air-liquid interface (Zara *et al.*, 2005). However, the role of *FLO11* in the progression of biofilm development in *S. cerevisiae* in the liquid flow environment is not known.

Our main objective in this study was to determine the role of *FLO11* in progression of a flow-cell based biofilm development in *S. cerevisiae* strain BY4741. We reasoned that the complete genome sequence available for BY4741, coupled with the collections of the isogenic deletion series (Dolinsky *et al.*, 2004) and green fluorescent protein-tagged clones (Huh *et al.*, 2003), would provide a powerful tool for genetic and proteomic analyses. Previously Liu *et al.* (1996) demonstrated that S288c strain of *S. cerevisiae* lacked the *FLO11* transcripts due to naturally occurring mutations in one of its major transcriptional activators called Flo8p. We hypothesized that BY4741, as a derivative of a laboratory-selected S288c strain (Brachmann *et al.*, 1998) has reduced *FLO11* expression and thus, is unable to undergo filamentation (Gimeno *et al.*, 1992; Liu *et al.*, 1996), form cellular aggregates or initiate surface adhesion (Reynolds & Fink, 2001). Subsequently, an ectopic overexpression of *FLO11* in BY4741 would confer a biofilm forming ability to BY4741 and would demonstrate the phenotype associated with *FLO11* expression. We introduced the *FLO11* gene into a multicopy plasmid expression vector for a regulated expression in the wild type (wt) BY4741. The resulting strain, BY4741 FLO11$^+$, was tested for adhesion to glass (hydrophilic) and polystyrene (hydrophobic) surfaces in comparison to the parental strain (wt BY4741), the isogenic *FLO11* deletion strain and the parental strain with a plasmid vector lacking the *FLO11* sequence (control vector). Biofilm maturation in the strains was evaluated by examining the extent of the surface colonization and by observing the spatiotemporal behavior of *in situ* cells in a continuous flow-through system. The strains were examined for phenotypic and functional characteristics associated with *FLO11* expression, such as: cell–cell adhesion (aggregation), morphology, surface hydrophobicity, and invasive growth.

Materials and methods

Strains and growth conditions

Saccharomyces cerevisiae BY4741 haploid, MATa (*his3*Δ1 *leu2*Δ0 *met15*Δ0 *ura3*Δ0) and isogenic *FLO11* deletional mutant (Invitrogen, Carlsbad, CA) strains were used in this study. *Saccharomyces cerevisiae* Σ1278 MATa (Reynolds & Fink, 2001), which has an adhesive phenotype, was used as a positive control in flow cell biofilm studies. Strains were grown in Synthetic Complete (SC) medium as described in Guthrie & Fink (1991) with 2% (w/v) galactose and 1% (w/v) raffinose (2% agarose was added for agar plates). The strains were isolated for CFU on SC medium.

Construction of the BY4741 FLO11$^+$

A *FLO11* gene sequence from the wt BY4741 genomic DNA was PCR-amplified and cloned into pYES2.1 expression vector (Invitrogen, Carlsbad, CA) under GAL1 promoter following the manufacturer's instructions, except for incubating the reaction mix for 90 min instead of the recommended 5 min due to the large gene size. The wt BY4741 cells were transformed using lithium acetate transformation (Adams, 1997), resulting in BY4741 *FLO11*$^+$ strain. Ura$^+$ clones were selected, and the orientation of the *FLO11* insert in the clones was verified by PCR. SC medium without uracil (SC–ura) was used for the BY4741 *FLO11*$^+$ strain. Galactose in the medium (see above) induces expression of the *FLO11* within pYES2.1 vectors containing the *S. cerevisiae* GAL1 promoter.

Growth curve analysis

Overnight cultures of the yeast strains ($OD_{600\,nm}$ = 0.5–1.5) were diluted (1 : 100) into SC medium and incubated at 30 °C with constant shaking at 200 r.p.m. Cell densities were determined periodically by measuring the OD at 600 nm. The doubling time (generation time) was calculated from growth curves generated from three independent replicate experiments for each strain.

Microscopic measurements

Cells size

Cell volume was calculated from cell dimensions (width and length), assuming that cells were prolate ellipsoidal with a smooth surface (Powell *et al.*, 2003). For overall population cell size measurements, 100 cells consisting of individual budded (50 cells) and unbudded (50 cells) collected during exponential growth were examined for each strain. The overall population cell size data was calculated from three replicate samples measured three independent times for each strain.

Cell clumping

The percentage of cells connected to five or more individuals was counted in a hemacytometer using a bright field microscope (Olympus, Hamburg, Germany) in three replicate

experiments. For each replicate experiment at least 200 cells were scored from five independent microscopic views.

Biofilm assays

Ninety-six-well adherence assay

Overnight cultures of yeast strains ($OD_{600\,nm} = 0.5–1.5$) were harvested. Cells were washed once in sterile H_2O and diluted in fresh medium to an $OD_{600\,nm}$ concentration = 1.0. One hundred microliters aliquots of the cell suspensions were transferred into 96-well polystyrene plates (Becton-Dickinson Labware, Franklin Lakes, NJ) and 96-well glass bottom plates (Becton-Dickinson Labware, Franklin Lakes, NJ). The plates were incubated at 30 °C for 0, 50, 95, 160, 195 and 240 min in a rotary shaker incubator at 200 r.p.m. The wells were stained for 15 min with 100 μL of 1% crystal violet (Fisher, Hampton, NH) and washed four times with sterile H_2O. The crystal violet was dissolved in 95% ethanol and absorbance was read at OD at 595 nm. Five replicate samples were measured for each incubation time-point and for each strain.

Once-through flow cell assay

We chose the once-through flow cell system, widely used in both bacterial and fungal biofilm studies (Charaklis & Marshall, 1990; Dalton *et al.*, 1996), for continuous biofilm culturing for prolonged periods under a continuous supply of nutrients. Details of the system can be found elsewhere (Stoodley *et al.*, 1999). Briefly, the yeast biofilms were grown in 1 × 1 mm square glass capillaries (Friedrich & Dimmock, Milleville, NJ), incorporated in the flow cell system. The design of the flow-cell system allowed *in situ* tracking of biofilm development. By implementing a digital time-lapse imaging system, growth parameters such as the rate of biofilm accumulation and percentage of biofilm surface coverage over time were quantified using the Scion image software as described (Purevdorj *et al.*, 2002).

Sterile SC medium was pumped through the system via a peristaltic pump at a flow rate of 1.0 mL min^{-1}. At this rate, the flow was laminar with a Reynolds number of 16 and a shear stress of 0.3 Pa along the center of the lumen. The flow cells were positioned in a polycarbonate holder, which was mounted on the stage of an Olympus BH2 upright microscope (Olympus, Hamburg, Germany) so that the biofilm could be imaged using SCION IMAGE software (Scion Corporation, Frederick, MD) *in situ* without interrupting the flow. The overnight cultures ($OD_{600\,nm} = 1.0–1.5$) were washed once in sterile H_2O and diluted in SC medium to $OD_{600\,nm}$ concentration = 2.0. One milliliter of this sample was used to inoculate the flow cell through the septum-sealed inoculation port that was positioned upstream of the flow cell. The cells were incubated for 2 h without flow to allow adherence to the surface of the glass flow cells. Once the flow was initiated the cells were grown for 3 days. Triplicate flow cell experiments were run for 3 days for each test strain. To asses the hydrophilic nature of the glass, a water contact angle measurement was performed, under conditions that mimic the flow parameters. The demonstrated angle of $21.2^\circ \pm 4.3$ (mean and SD, $n = 4$ measurements from three different samples) lies squarely below the established measure of 30° or less as a hydrophilic surface.

Quantitative reverse transcriptase real-time PCR (qRT-PCR)

Culturing and RNA isolation

For each strain, a single colony from freshly streaked agar medium were aseptically inoculated into 5 mL of nutrient broth and incubated in a roller drum overnight at 30 °C. The cultures was used to inoculate 50 mL of medium (1 : 1000 dilution) in 250-mL Erlenmeyer flasks, then incubated in rotary shaker incubator at 30 °C at 200 r.p.m. The cells were pelleted by centrifugation at 5000***g*** for 5 min during the logarithmic growth phase ($OD_{600\,nm} = 0.5–1.5$) and during the early stationary phase ($OD_{600\,nm} \sim 2.2$). The pellets were stored at −80 °C until further processing. The RNA from the yeast cell pellets was isolated via the hot phenol/glass bead method (Ausubel *et al.*, 1995). The quality of RNA was evaluated by gel electrophoresis. Absorbance at 260 nm was used to quantify the yield.

Quantitative real-time PCR (qRT-PCR)

RNA samples were treated with DNAse I (Invitrogen, Carlsbad, CA) to remove any residual DNA contamination, following the manufacturer's instructions. qRT-PCRs were performed on RNA samples from three independent replicate experiments during exponential and stationary growth phases, as described by Purevdorj-Gage *et al.* (2006). The *FLO11* expression levels in each wt BY4741, BY4741 $FLO11^+$ and wtΣ1278 strains vs. the BY4741 *FLO11* deletion strain were quantified by the $2^{-\Delta\Delta CT}$ method (Purevdorj-Gage *et al.*, 2006) and were reported as *n*-fold difference.

Agar invasion assay

Strains were patched on SC-ura and SC plates as described previously (Li & Palecek, 2003). The plates were incubated for 3, 6 and 12 days at room temperature (24 °C). Nine replicate plates were examined for each strain and time-point.

Aqueous-hydrocarbon biphasic assay

Overnight cultures ($OD_{600\,nm} \sim 1.5$) were washed once in sterile dH_2O by centrifuging cells at 1957***g*** for 5 min. The

cells were resuspended in fresh medium to an $OD_{600\,nm}$ of 1.0. After 3 h of stationary incubation at room temperature, the $OD_{600\,nm}$ for each culture was measured. In 15 mm by 100 mm borosilicate glass tubes, 1.2-mL aliquots of the cultures were overlaid with 0.6 mL of octane (Sigma, St Louis, MO). The tubes were vortexed for 2 min and incubated for 15 min to allow for the phases to separate. The $OD_{600\,nm}$ of the aqueous layer was measured. The difference between the $OD_{600\,nm}$ of the aqueous layer, before and after the addition of octane, was used to determine hydrophobicity. We determined the mean ± SD for three independent measurements for each strain. This assay was adapted from Reynolds & Fink (2001).

Statistical analysis

Statistical comparisons were made with a One-Way ANOVA test using MINITAB (version 13.3; Minitab Inc, State College, Pa.) software; the differences were reported as significant when $P \leq 0.05$.

Results and discussion

The main objective of our work was to examine the role of *FLO11* in *S. cerevisiae* biofilm development in a flow-through system. In addition, a comparative study between the *FLO11* mediated cell adhesion to glass and polystyrene were conducted. To our knowledge, these studies have not been published previously. *FLO11* in *S. cerevisiae* has sequence similarities with the functional domains of the adhesin proteins found in the pathogenic yeasts (Lo & Dranginis, 1996). For the present study, we chose the genetically well-defined laboratory strain BY4741, which we determined by qRT-PCR, had low levels of endogenous *FLO11* transcripts, and lacked an adhesive and biofilm forming phenotype. Thus, we expected that ectopic over expression of *FLO11* in BY4741 would enable the strain to adhere to solid substrates and to develop biofilms in a liquid flow environment.

Table 1. Doubling time of strains. Each datum is a mean ± SD of three replicate experiments

Strain	Doubling time (h)
WT BY4741	1.4 ± 0.3
BY4741 FLO11$^+$	1.6 ± 0.1
BY4741 FLO11$^-$	1.4 ± 0.3
BY4741 control vector	1.5 ± 0.1

Genetic analysis of BY4741 FLO11$^+$

We quantified comparative levels of *FLO11* transcripts by qRT-PCR and found that in relation to the wt, there were *c.* 1100 and 78 times more *FLO11* transcripts present in BY4741 FLO11$^+$ during the exponential and stationary growth phases, respectively (Table 2).

In agreement with similar studies conducted in S288c strains (Gimeno *et al.*, 1992; Lo & Dranginis, 1998), we report very low levels of *FLO11* transcripts in BY4741, compared with the strain Σ1278 (Table 2). This could in part, as mentioned above, be due to a mutation in the *FLO11* transcriptional activator *FLO8* in S288c strains (Liu *et al.*, 1996).

Phenotypic analysis of planktonic BY4741 FLO11$^+$ strain

Growth rate, cell morphology and cell surface hydrophobicity

We determined if the *FLO11* overexpression exerts any negative effects on growth or causes changes in cell morphology. Based on a growth curve generated in planktonic culture medium, the BY4741 FLO11$^+$ had a similar doubling time (Table 1) to wt BY4741, the isogenic FLO11 deletion mutant and BY4741 with control vector ($P > 0.05$).

Microscopic examination of BY4741 FLO11$^+$ cells showed no changes in shape or size, $P > 0.05$ (data not shown). However, in contrast to the parental strain, the BY4741 FLO11$^+$ displayed an increased tendency to form clumps that consisted of from five to 60 yeast cells (Fig. 1a). These data were consistent with previous studies (Guo *et al.*, 2000; Verstrepen *et al.*, 2004; Bayly *et al.*, 2005; Verstrepen & Klis, 2006), in which a mild flocculation phenotype was identified in different strains of *S. cerevisiae* with the expression of *FLO11*. We found that during the early- and mid-exponential phases of growth in liquid culture, 55% of the cell population in BY4741 FLO11$^+$ formed aggregates (Fig. 1b). The percentage of cell clumps in the construct decreased to *c.* 35% during the stationary phase (data not

Table 2. qRT-PCR *FLO11* expression levels in different strains relative to *FLO11* deletion strain during exponential and stationary growth phases

	Level of FLO11 expression	
Strain	Exponential ($OD_{600\,nm}$ = 1.0–1.5)	Stationary ($OD_{600\,nm}$ = 2.2)
WT BY4741	1.5 ± 0.5	8.3 ± 2.1
BY4741 FLO11$^+$	1663 ± 103.1	652 ± 23.1
WTΣ1278	63 ± 5.2	3.3 ± 1.1

Each datum shows a mean ± SE of three replicate measurements.

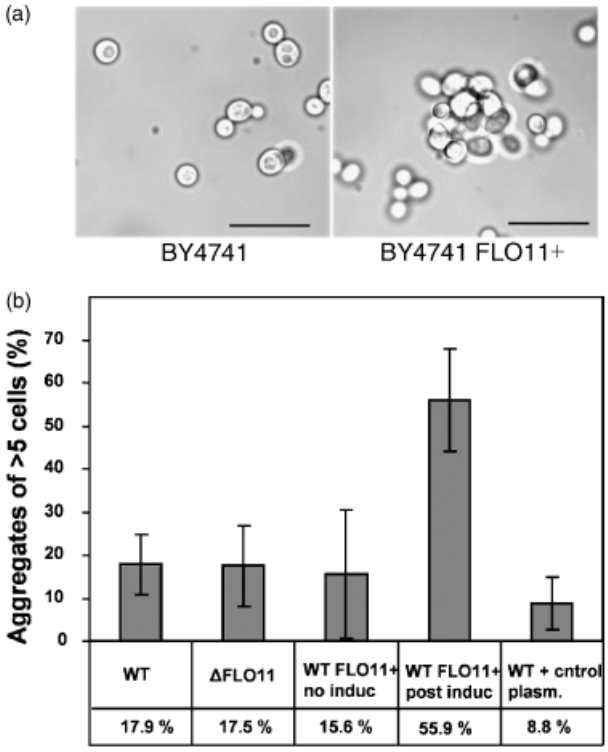

Fig. 1. BY4741 FLO11$^+$ demonstrated increased cellular clumping without changes in cell shape and size. (a) Representative micrographs of exponentially growing wt (left panel) and BY4741 FLO11$^+$ (right panel) strains. Scale bar is 20 μm. (b) The percentage of cells that are found in multiple cellular aggregates. Each data point on B is the mean ± SE from three replicate experiments. At least 200 cells were scored from five independent microscopic fields of view for each replicate experiment.

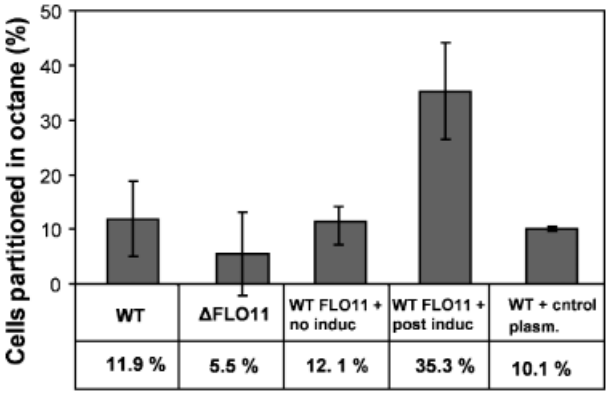

Fig. 2. BY4741 FLO11$^+$ cells are more hydrophobic. Aqueous-hydrocarbon biphasic assay was used to measure the percentage of cells partitioned in octane. Each bar on the graph shows the mean+SE of three replicate measurements performed in exponentially growing cells ($OD_{600\,nm}$ = 0.5–1.5).

shown) which coincided with lower levels of *FLO11* transcripts at this stage (Table 2).

Zara *et al.* (2005) recently reported that *FLO11* expression increased cell surface hydrophobicity in *S. cerevisiae* sherry strains. We measured cell surface hydrophobicity and found a significant proportion of BY4741 FLO11$^+$ cells partitioned in the organic phase compared to the parental strain (Fig. 2), which suggests that *FLO11* confers increased surface hydrophobicity to BY4741. Taken together, these results demonstrate that *FLO11* confers distinct phenotypic and morphological changes in the BY4741 background and are consistent with the previous characterizations of the *FLO11* in the yeast (Guo *et al.*, 2000; Bayly *et al.*, 2005; Verstrepen & Klis, 2006).

Haploid invasive growth

Upon nutrient limitations, haploid *S. cerevisiae* undergoes a dimorphic transition from the yeast form of growth on agar surfaces to an invasive form of growth (under similar conditions, the diploids under pseudohyphael growth), in which chains of cells penetrate the solid agar (Gancedo, 2001). The invasive growth is believed to be an important survival mechanism in yeast that allows for foraging for optimal growth substrates in nutrient-limited environments and for tissue invasion during pathogenesis (Gancedo, 2001). Previous work showed that an increase in *FLO11* expression alone was sufficient to induce invasive growth in Σ1278 haploid cells (Lo & Dranginis, 1998; Li & Palecek, 2003). We tested BY4741 FLO11$^+$, the parental strain for invasive growth, and found the strains were not able to penetrate the agar substratum or form filaments (data not shown). We also tested Σ1278 as a control for invasive growth and found positive results, as demonstrated previously (Lo & Dranginis, 1998). The inability of BY4741 FLO11$^+$ cells to undergo agar invasion implies that in addition to a known mutation in *FLO8*, there could be other functional defect(s) in the factors, controlling morphogenesis in BY4741 (Gancedo, 2001).

Adhesive property of the FLO11 overexpressing strain

The attachment process in microbial cells is an important initial step towards biofilm formation (Stoodley *et al.*, 2002) which dictates *in vivo* progression of biofilm-associated infections (Costerton *et al.*, 1995) and biofouling processes in industry (Wong, 1998; Costerton & Stewart, 2000). Thus, we examined the role of *FLO11* in the adhesive properties of BY4741. We tested the adhesion of the BY4741 FLO11$^+$ strain in parallel to the parental, isogenic *FLO11* deletion strain, and in the control vector strain by a method adapted from Reynolds & Fink (2001). In order to compare the extent of cell attachment for hydrophilic and hydrophobic surfaces, we utilized glass-bottom and polystyrene microplates. The results, illustrated in Fig. 3, show that the BY4741

FLO11$^+$ strain demonstrated enhanced adherence to both polystyrene (Fig. 3a) and glass (Fig. 3b) surfaces compared with controls. The level of adhesion in BY4741 FLO11$^+$ was comparable to strain Σ1278 (Reynolds & Fink, 2001). Flo11p is a highly hydrophobic protein, and when expressed in cells, enhances cell–cell aggregation (Zara *et al.*, 2005) and adhesion of cells to hydrophobic substrata, such as polystyrene (Reynolds & Fink, 2001). In order to confirm the hydrophilic nature of the glass substrate, a water contact angle measurement was performed and it established that the adhesion was indeed onto a true hydrophilic surface. This is, to our knowledge, the first report showing the role of *FLO11* in adhesion to glass, a preferred substrate in industry for yeast cell immobilization (Branyik *et al.*, 2005). The fact that the cells are able to adhere to hydrophilic surfaces such as glass implies that the function of *FLO11* in biofilms extends beyond its currently described role in mediating biofilm formation due to hydrophobicity-based cell-surface interactions (Reynolds & Fink, 2001).

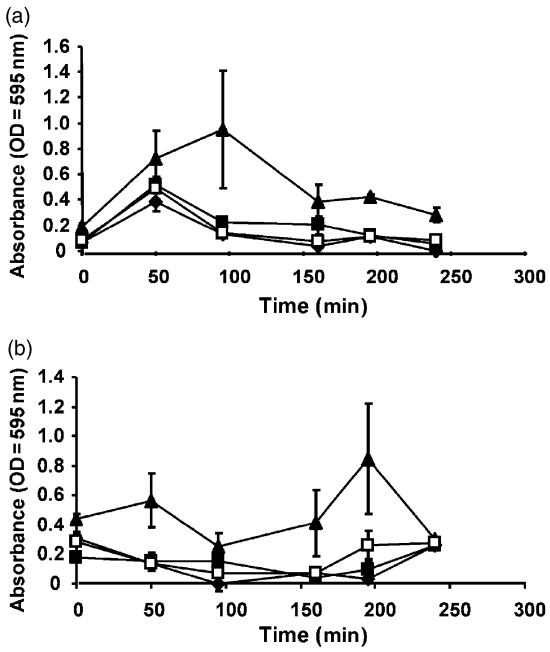

Fig. 3. BY4741 FLO11$^+$ cells demonstrate increased adhesion to both plastic (a) and glass-(b) liquid interfaces. Flow cell adhesion quantified in WT BY4741 (♦), isogenic *FLO11* deletion strain (◇), *FLO11* overexpressing construct BY4741 FLO11$^+$ (▲), BY4741+control plasmid (△), with plasmid lacking *FLO11* mean+SE of five replicate measurements.

Flow cell biofilm analysis

While the microplate assay demonstrates the adhesive properties of an organism, it does not predict the ability of organisms to differentiate into a complex multicellular community. During the past decade, the studies shown that biofilm structural development is associated with virulence and resistance of many microbial pathogens (Costerton *et al.*, 1995; Stoodley *et al.*, 2002; Parsek & Singh, 2003; Purevdorj-Gage & Stoodley, 2004; Branda *et al.*, 2005). The process of biofilm structural differentiation is the subject of intense scrutiny due to medical and industrial implications. Therefore, we investigated the role of *FLO11* in biofilm structural development under liquid shear.

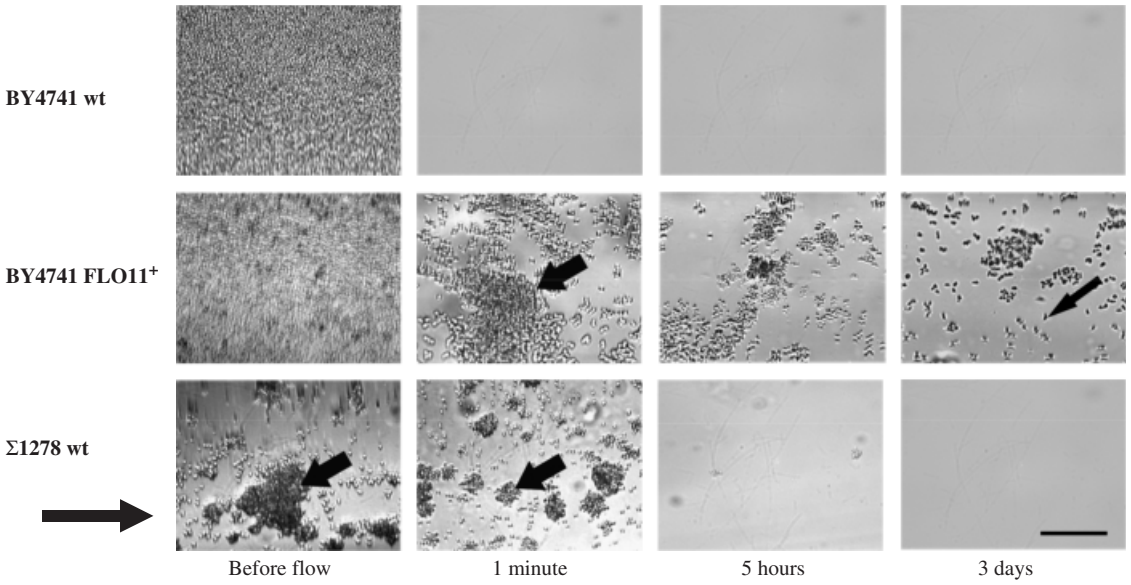

Fig. 4. BY4741 FLO11$^+$ cells form biofilms in a flowing system. Transmitted images of *in situ* biofilm cells over time are illustrated. The black arrow in the left-hand corner indicates the flow direction, the black arrows on the panels indicate yeast biofilm aggregates and a thin black arrow indicates individual cells. Scale bar is 100 μm.

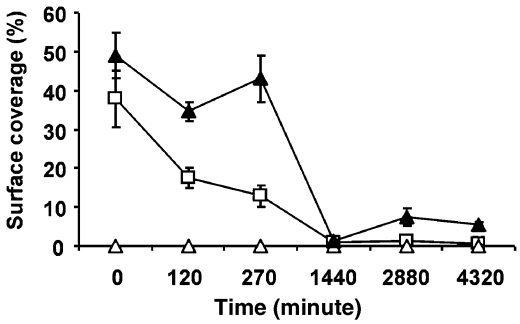

Fig. 5. Quantification of biofilm surface coverage in WT (Δ), *FLO11* overexpressing strain (▲) and Σ1278 (□). Each point on the graph is a mean+ SE of five measurements from each of the three independent replicate experiments.

The parental BY4741 strain was not able to adhere in a flow cell (Fig. 4). One hundred percent of the cell population detached shortly after the flow was started (Fig. 4). In contrast, BY4741 FLO11$^+$ cells grew in biofilms throughout the experimental time-period, covering as much as 50% of the surface area (Figs 4 and 5). During this time, a monolayer of individual budded cells, as well as larger aggregates of cells, was present (Fig. 4). However, structural differentiation, typical of biofilms formed by pathogenic microbial species such as *C. albicans* (Kumamoto & Vinces, 2005) or *Pseudomonas aeruginosa* (Purevdorj-Gage *et al.*, 2005) was not evident. Interestingly, in the flow cell system, both the Σ1278 and BY4741 FLO11$^+$ strains were entirely in the yeast-form with no evidence of filamentation or cell elongations (Fig. 4), despite the fact that the pathway for filamentous growth is present in the Σ1278 strain (Stanhill *et al.*, 1999). In contrast, *C. albicans* is known to undergo morphogenesis in a little as 20 min upon surface contact (Suci & Tyler, 2002). Morphogenesis to the filamentous form in *C. albicans* is necessary for biofilm development and virulence progression during the course of infection (Kumamoto & Vinces, 2005) and is activated by various environmental signals and conditions including physical contact with semisolid matrixes (Kumamoto, 2005). The inability of *S. cerevisiae* to undergo morphogenesis to a filamentous type in response to surface association could be one of the underlying reasons for inability of *S. cerevisiae* to form structurally differentiated biofilms (Li & Palecek, 2003). While *S. cerevisiae* has been extensively used as a model organism for studies of morphogenesis and biofilm formation in *C. albicans*, we do not rule out important differences in their genome organization and regulatory features that may contribute to differences in the overall ability of these two organisms to form complex biofilm architectures. Further experiments are needed in order to unravel mechanisms responsible for the unique surface behavior found in *S. cerevisiae*.

In summary, the results clearly indicate the importance of *FLO11* in yeast biofilm initiation and development. This is to our knowledge a first report demonstrating the role of *FLO11* in *S. cerevisiae* adhesion to a hydrophilic surface (i.e. glass) and biofilm proliferation at the liquid-hydrophilic solid interface under continuous liquid shear. Our results are in agreement with those from both freshwater (Rickard *et al.*, 2003) and dental (Palmer *et al.*, 2003) studies that describe a positive correlation between the ability to coaggregate and biofilm formation. The findings from this study extend the functional role of *FLO11* in diverse biofilm systems and may be useful in the advancement of immobilized cell technology involving *S. cerevisiae* and related organisms.

Acknowledgements

This work was supported by NASA grant (NAG9-1559) (LEH) and NIH GMS RO1 GM60052 (PSt). We thank the laboratory of Dr Gerald Fink for his kind gift of the Σ1278 strain. We thank Sara Altenberg for her assistance in experimental executions and Dr Curtis W. Frank, Director, Center on Polymer Interfaces for advice on the water contact angle measurements.

References

Adams A (1997) *Methods in Yeast Genetics: A Cold Spring Harbor Laboratory Course Manual, 1997 ed*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Ausubel FM, Brent E, Kingston RE, Moore DD, Seidman JG, Smith JA & Struhl K (1995) *Current Protocols in Molecular Biology*. Wiley, New York.

Bayly JC, Douglas LM, Pretorius IS, Bauer FF & Dranginis AM (2005) Characteristics of Flo11-dependent flocculation in *Saccharomyces cerevisiae*. *FEMS Yeast Res* **5**: 1151–1156.

Brachmann CB, Davies A, Cost GJ, Caputo E, Li J, Hieter P & Boeke JD (1998) Designer deletion strain derived from *Saccharomyces cerevisiae* S288C: a useful set of strains and plasmids for PCR-mediated gene disruption and other applications. *Yeast* **14**: 115–132.

Branda SS, Vik S, Friedman L & Kolter R (2005) Biofilms: the matrix revisited. *Trends Microbiol* **13**: 20–26.

Branyik T, Vicente AA, Dostalek P & Teixeira JA (2005) Continuous beer fermentation using immobilized yeast cell bioreactor systems. *Biotecnol Prog* **21**: 653–663.

Charaklis WG & Marshall KC (1990) *Biofilms*. Wiley, New York.

Costerton JW & Stewart PS (2000) Biofilms and device-related infections. *Persistent Bacterial Infections* (Nataro JP, Blaser MJ & Cunningham-Rundles S, eds), pp. 423–439. ASM Press, Washington, DC.

Costerton JW, Cheng KJ, Geesey GG, Ladd TI, Nickel JC, Dasgupta M & Marrie TJ (1987) Bacterial biofilms in nature and disease. *Annu Rev Microbiol* **41**: 435–464.

Costerton JW, Lewandowski ZD, Caldwell E, Korber DR & Lappin-Scott HM (1995) Microbial biofilms. *Annu Rev Microbiol* **49**: 711–745.

Costerton JW, Stewart PS & Greenberg EP (1999) Bacterial biofilms: a common cause of persistent infections. *Science* **284**: 1318–1322.

Dalton HM, Goodman AE & Marshall KC (1996) Diversity in surface colonization behavior in marine bacteria. *J Ind Microbiol* **17**: 228–234.

Dolinsky TJ, Burgers PM, Karplus K & Baker NA (2004) SPrCY: comparison of structural predictions in the *Saccharomyces cerevisiae* genome. *Bioinformatics* **20**: 2312–2314.

Gancedo JM (2001) Control of pseudohyphae formation in *Saccharomyces cerevisiae*. *FEMS Microbiol Rev* **25**: 107–123.

Gimeno CJ & Fink GR (1994) Induction of pseudohyphal growth by overexpression of PHD1, a *Saccharomyces cerevisiae* gene related to transcriptional regulators of fungal development. *Mol Cell Biol* **14**: 2100–2112.

Gimeno CJ, Ljungdahl PO, Styles CA & Fink GR (1992) Unipolar cell divisions in the yeast *S. cerevisiae* lead to filamentous growth: regulation by starvation and RAS. *Cell* **68**: 1077–1090.

Guo B, Styles CA, Feng Q & Fink GR (2000) A Saccharomyces gene family involved in invasive growth, cell-cell adhesion, and mating. *Proc Natl Acad Sci USA* **97**: 12158–12163.

Guthrie C & Fink GR (1991) Synthetic complete media and plates and YPD plates were made as described. *Methods Enzymol* **194**: 12.

Hazen KC & Hazen BW (1987) Temperature-modulated physiological characteristics of *Candida albicans*. *J Microbiol Methods* **6**: 289.

Huh WK, Falvo JV, Gerke LC, Carroll AS, Howson RW, Weissman JS & O'Shea EK (2003) Global analysis of protein localization in budding yeast. *Nature* **425**: 686–691.

Ishigami M, Nakagawa Y, Harakawa M & Iimura Y (2004) *FLO11* is essential for flor formation caused by the C-terminal deletion of *NRG1* in *Saccharomyces cerevisiae*. *FEMS Microbiol Lett* **237**: 425–430.

Kumamoto CA (2005) A contact-activated kinase signals *Candida albicans* invasive growth and biofilm development. *Proc Natl Acad Sci USA* **102**: 5576–5581.

Kumamoto CA & Vinces MD (2005) Alternative *Candida albicans* lifestyles: growth on surfaces. *Annu Rev Microbiol* **59**: 113–133.

Li F & Palecek SP (2003) EAP1, a *Candida albicans* gene involved in binding human epithelial cells. *Eukaryot Cell* **6**: 1266–1273.

Liu H, Styles CA & Fink GR (1996) *Saccharomyces cerevisiae* S288C has a mutation in FLO8, a gene required for filamentous growth. *Genetics* **144**: 967–978.

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.

Lo WS & Dranginis AM (1998) The cell surface flocculin Flo11 is required for Pseudohyphae formation and invasion by *Saccharomyces cerevisiae*. *Mol Biol Cell* **9**: 161–171.

Mavor AL, Thewes S & Hube B (2005) Systemic fungal infections caused by *Candida* species: epidemiology, infection process and virulence attributes. *Curr Drug Targets* 863–874.

Mukherjee PK, Zhou G, Munyon R & Ghannoum MA (2005) Candida biofilm: a well-designed protected environment. *Med Mycol* **43**: 191–208.

Nickel JC, Ruseska I, Wright JB & Costerton JW (1985) *Antimicrob. Agents Chemother* **27**: 619.

Palmer RJ Jr, Gordon SM, Cisar JO & Kolenbrander PE (2003) Coaggregation-mediated interactions of *Streptococci* and *Actinomyces* detected in initial human dental plaque. *J Bacteriol* **185**: 3400–3409.

Parsek MR & Singh PK (2003) Bacterial biofilms: an emerging link to disease pathogenesis. *Annu Rev Microbiol* **57**: 677–701.

Powell CD, Quain DE & Smart KA (2003) Chitin scar breaks in aged *Saccharomyces Cerevisiae*. *Microbiology* **149**: 3129–3137.

Purevdorj B, Costerton JW & Stoodley P (2002) Influence of hydrodynamics and cell signaling on the structure and behavior of *Pseudomonas aeruginosa* biofilms. *Appl Environ Microbiol* 4457–4464.

Purevdorj-Gage B & Stoodley P (2004) Hydrodynamic considerations of biofilm structure and behavior. *Microbial Biofilms* (Ghannoum MA & O'Toole G, eds), pp. 160–173. American Society for Microbiology, Washington, DC.

Purevdorj-Gage B, Costerton WJ & Stoodley P (2005) Phenotypic differentiation and seeding dispersal in non-mucoid and mucoid *Pseudomonas aeruginosa* biofilms. *Microbiology* **151**: 1569–1576.

Purevdorj-Gage B, Sheehan KB & Hyman LE (2006) Effects of low-shear modeled microgravity on cell function, gene expression, and phenotype in *Saccharomyces cerevisiae*. *Appl Environ Microbiol* **72**: 4562–4575.

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

Rickard AH, McBain AJ, Ledder RG, Handley PS & Gilbert P (2003) Coaggregation between freshwater bacteria within biofilm and planktonic communities. *FEMS Microbiol Lett* **220**: 133–140.

Stanhill A, Schick N & Engelberg D (1999) The yeast ras/cyclic AMP pathway induces invasive growth by suppressing the cellular stress response. *Mol Cell Biol* **19**: 7529–7538.

Stoodley P, Lewandowski Z, Boyle JD & Lappin-Scott HM (1999) Structural deformation of bacterial biofilms caused by short-term fluctuations in fluid-shear: an *in situ* investigation of biofilm rheology. *Biotechnol Bioeng* **65**: 83–93.

Stoodley P, Sauer K, Davies DG & Costerton JW (2002) Biofilms as complex differentiated communities. *Annu Rev Microbiol* **56**: 187–209.

Suci PA & Tyler BJ (2002) Action of chlorhexidine digluconate against yeast and filamentous forms in an early-stage *Candida albicans* biofilm. *Antimicrob Agents Chemother* **46**: 3522–3531.

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

Verstrepen KJ, Reynolds TB & Fink GR (2004) Origins of variation in the fungal cell surface. *Nat Rev Microbiol* **2**: 533–540.

Wong AC (1998) Biofilms in food processing environments. *J Dairy Sci* **81**: 2765–2770.

Zara S, Bakalinsky AT, Zara G, Pirino G, Demontis MA & Budroni M (2005) FLO11-based model for air-liquid interfacial biofilm formation by *Saccharomyces cerevisiae*. *Appl Environ Microbiol* **71**: 2934–2939.