Influence of cell surface characteristics on adhesion of *Saccharomyces cerevisiae* to the biomaterial hydroxylapatite

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Abstract

The influence of the physicochemical properties of biomaterials on microbial cell adhesion is well known, with the extent of adhesion depending on hydrophobicity, surface charge, specific functional groups and acid-base properties. Regarding yeasts, the effect of cell surfaces is often overlooked, despite the fact that generalisations may not be made between closely related strains. The current investigation compared adhesion of three industrially relevant strains of *Saccharomyces cerevisiae* (M-type, NCYC 1681 and ALY, strains used in production of Scotch whisky, ale and lager, respectively) to the biomaterial hydroxylapatite (HAP). Adhesion of the whisky yeast was greatest, followed by the ale strain, while adhesion of the lager strain was approximately 10-times less. According to microbial adhesion to solvents (MATS) analysis, the ale strain was hydrophobic while the whisky and lager strains were moderately hydrophilic. This contrasted with analyses of water contact angles where all strains were characterised as hydrophilic. All yeast strains were electron donating, with low electron accepting potential, as indicated by both surface energy and MATS analysis. Overall, there was a linear correlation between adhesion to HAP and the overall surface free energy of the yeasts. This is the first time that the relationship between yeast cell surface energy and adherence to a biomaterial has been described.

Keywords: Hydroxylapatite, cell adhesion, *Saccharomyces*, surface properties, hydrophobicity

Introduction

The adhesion of yeast to abiotic surfaces has major implications for biomedical, environmental and bioprocessing industries. In the medical field, adhesion of microorgansisms to the surfaces of bioimplants and human tissues is an imperative step in the pathogenesis of infection. Pathogenic yeast such as *Candida* spp. produce biofilms on medical devices, which renders them relatively refractory to medical therapy (Kojic and Darouiche 2004). In industry, biofouling of surfaces in food processing equipment, fermenters, heat exchangers and cooling water systems is problematic. However, in certain cases adhesion of microorganisms may be desirable. Many wastewater treatment and bioremediation systems depend upon biofilm formation. In brewing and wine fermentations, cell-cell adhesion (flocculation) is a desirable property of *S. cerevisiae* strains, allowing the easy separation of cells from the fermentation media (Verstrepen et al. 2003). The adhesion of microorganisms is inherent in immobilised cell technology with immobilised yeast systems offering considerable advantages to the fermentation industry (Kourkoutas et al. 2004; Verbelen et al. 2006). Immobilised yeast systems have been used in ethanolic fermentations with successful adhesion of *S. cerevisiae* to diverse solid carriers such as polyurethane foam (Baptista et al. 2006), spent grains (Dragone et al. 2007), ceramics (Demuyakor and Ohta 1992), and porous glass (Tata et al. 1999).

The adhesion of yeast to a surface is the first step in immobilisation and results from complex physicochemical interactions between the cell, the surface and the liquid phase. It depends on physicochemical properties of the support and cells (surface charge, hydrophobicity, functional groups, electron donor-electron acceptor properties and support porosity and roughness) and environmental conditions (ionic strength,

temperature and contact time). Initial interactions between microbes and biomaterials may be explained by the extended DLVO (Derjaguin, Landau, Verwey, Overbeek) theory, which considers the four fundamental, non-covalent interactions: Lifshitz-van der Waals, electrostatic, Lewis acid-base and Brownian motion forces (van Oss et al. 1986; Bos et al. 1999). The first two are usually termed long-range forces i.e. initially involved in bringing the two surfaces together, while Lewis acid-base depends on short-range specific interactions between molecular groups. Research has mainly focused on the influence of the support surface properties on adhesion (Guillemot et al. 2006; Kang and Choi 2005; Vernhet and Bellon-Fontaine 1995), while there has been little information on the influence of yeast surface properties. This is surprising as not all yeasts bind in the same manner and generalisations concerning the hydrophobic and acid-base properties of microbial cell surfaces may not be made (Van der Mei et al. 1998). One of the main aims of this research was to compare the surface characteristics of three industrially relevant *S. cerevisiae* strains and to distinguish the influence of these properties on adhesion potential to hydroxylapatite (HAP).

HAP [$Ca_5(PO_4)_3OH$] has numerous biomedical applications and is the most bioactive material that can bond directly to living bone. Its affinity for proteins is exploited in high performance liquid chromatography systems (Kandori et al. 2005) and its metal adsorption capacity is used for removal of metals (Baillez et al. 2007; Gómez del Río et al. 2004; Misra et al. 2007). It is used widely in medicine as bioactive ceramic materials for bone and dental implants (Gross and Berndt 2002; Ong and Chan 2000; Itoh et al. 2006). However, like all bioimplants, it is susceptible to microbial infections. It is therefore apparent that an understanding of the factors controlling

adhesion of yeasts to surfaces is important from both medical and industrial perspectives.

In this paper, the adhesion profiles of three *S. cerevisiae* strains, used in Scotch whisky, ale and lager beer production, respectively, to HAP were compared. Ale and lager yeast strains were selected, as they have known differences in surface properties leading to specific flocculation characteristics at the end of fermentation. Yeast flocs may sediment to the bottom (lager yeast) or float to the surface (ale yeast), thereby facilitating their separation from the medium. Top-fermenting strains are often described as being more hydrophobic compared to bottom-fermenting strains (Dengis and Rouxhet 1997) while there are also major differences in the polysaccharide properties of the two strain types (Alsteens et al. 2008). *S. cerevisiae* 'M-type' strain was also compared as it is central to whisky production in Scotland. The van der Waals, electron donor and acceptor characteristics of cells were assessed by two methods: surface energy characterisation by contact angle (Zhao et al. 2005) and microbial adhesion to solvents, MATS analysis (Bellon-Fontaine et al. 1996). These properties were then compared with the extent of cell adhesion to HAP.

Materials and Methods

S. cerevisiae strains and culture conditions

S. cerevisiae M-type, whisky strain (Kerry Biosciences Ltd, Menstrie, Scotland), NCYC 1681, ale strain (National Centre for Yeast Culture, Norwich, England) and ALY, lager strain (University of Abertay yeast culture collection) were routinely maintained on malt extract agar slopes (Oxoid Ltd., Hampshire, UK). Yeasts were cultured in 100 ml malt extract broth (Oxoid) in 250 ml Erlenmyer flasks at 25°C on a rotary shaker at 150

r.p.m. For experimental purposes, cells from 48 h cultures were used to inoculate 100 ml media to an initial density of 1–2 × 10^6 cells/ml. Cells were grown for 24 h and then washed three times with 10 mM MES, pH 5.0 buffer by centrifugation at 1500 *g* prior to use in adhesion or surface characterisation experiments. The concentration of yeast cells was determined by counting with a haemocytometer.

Hydroxylapatite Tablets

HAP tablets (5 mm diameter, 2–3 mm height), were supplied by the Institute of Silica Materials, Riga Technical University, Latvia and prepared by dry pressings of HAP nanopowders as described previously (Aronov et al. 2007). Tablets were pressed by a two-stage compaction, with pressures of 250 and 375 MPa for the first and second stages, respectively. After pressing, tablets were sintered with a heating rate of 5°C/min to 1100°C with annealing at this temperature for 1 hour.

Yeast adhesion to HAP

Yeast cells from 24 h cultures were washed with 10 mM MES, pH 5.0 buffer and re-suspended to a cell density of 10^8 cells/ml. For each adhesion experiment, three tablets were incubated with 3 ml yeast suspension at 25°C for 24 h with manual agitation of samples during the incubation. Replicate samples were prepared for assessing adhesion by two methods: (i) direct enumeration of cells following sonication and (ii) quantitative microscopic analysis by methylene blue-staining of cells *in situ*. After immobilisation, tablets were rinsed with MES buffer. Immobilised cells were visualised by fixing cells with 1:1 ethanol/diethyl ether, staining with 0.01% methylene blue and rinsing with water. Images of stained cells on the surface of HAP were obtained using a

light microscope (Leica DMR Fluorescent microscope, Leica Microsystems, Richmond Hill, ON, Canada) fitted with a 20× lens and with top illumination of the HAP surface with an external lamp. Images were captured with a digital camera. To quantitatively assess the degree of immobilisation, cells were detached from HAP into buffer using a sonicating water bath (ELMA Sonic S30H, Singen, Germany) for 30 s. The number of cells released per tablet was determined by the standard count plate method on malt extract agar. All qualitative and quantitative analyses were performed in triplicate.

The viability of immobilised cells was confirmed by comparing tablets stained with either Cell Tracker Orange CMRA or bis(1,3 diethylthiobarbituric acid) trimethine oxonol, $DiSBAC(2)_3$ (both from Molecular Probes, Invitrogen Corp, Paisley, UK). Stock solutions, 0.1 mg ml^{-1} CMRA and 1 mg ml^{-1} $DiSBAC(2)_3$, were prepared in dimethyl sulfoxide. Immobilised tablets were incubated with either 10 µl CMRA or 0.5 µl $DiSBAC(2)_3$ for 20 min in 10 mM PIPES, 0.9% NaCl, pH 7.0 buffer. Fluorescent cells were examined using a Leica DM R fluorescent microscope fitted with Filter Cube I3 (blue excitation filter, bandpass 450-490 nm; green dichromatic mirror 510 nm). It was previously confirmed with planktonic yeast cells that CMRA stained all the cell population while $DiSBAC(2)_3$, which is negatively charged, could only enter dead cells (results not shown).

MATS testing of yeast

The hydrophobic and Lewis acid-base (electron donor/acceptor) characteristics of yeast cell surfaces were determined using the MATS test as first described by Bellon-Fontaine et al. (1996). The MATS test compares affinity of microbial cells for pairs of monopolar/apolar solvents of similar Lifshitz-Van der Waals surface tension

components. Affinity to the following pairs of solvents was compared: (i) the apolar solvent hexadecane and the acidic monopolar solvent, chloroform and (ii) the apolar solvent decane and the strongly basic, monopolar solvent ethyl acetate. As these solvents have different surface tension properties, differences between the affinity of chloroform and hexadecane and between ethyl acetate and decane are indicative of the electron-donor or electron-acceptor properties of the yeast surfaces, respectively. The hydrophobic character is reflected by affinity for the apolar solvents.

The MATS test was adapted for use with yeast cells (Mortensen et al. 2005). Briefly, yeast cells from 24 h cultures were washed with 10 mM MES, 0.9% NaCl, pH 5.0 buffer and re-suspended to an optical density (OD) of 0.8 at 400 nm (A_0). NaCl was included to prevent any electrostatic repulsion between similarly charged solvent droplets and yeast cells. To start the test, 0.4 ml solvent was added to 2.4 ml cell suspension and vortex-mixed for 1 min. The mixture was allowed to stand for 15 min to ensure complete separation of the two phases before a 1 ml sample was carefully removed from the aqueous phase and OD measured at 400 nm (A). The percentage of bound cells was subsequently calculated by

$$\%\,\text{affinity} = \left(1 - \frac{A}{A_0}\right) x100 \qquad (1)$$

where A_0 is the OD measured at 600 nm of the cell suspension before mixing and A is the absorbance after mixing.

Alcian blue dye retention assay

Adsorption of alcian blue by yeast cells was used as an indicator of cell surface charge according to the method of Powell et al. (2003) with some slight modifications. Yeast

cells from 24 h cultures were centrifuged at 1500 *g* for 3 min and cells resuspended in 10 mM MES buffer, pH 5.0 to a concentration of 5×10^6 cells/ml. Aliquots of 10 ml each were washed a further two times and cells suspended in the same buffer containing alcian blue (15 mg/l). The suspension was incubated for 5 min on an orbital shaker at 100 r.p.m. Samples were centrifuged (1500 *g*, 3 min) and the dye remaining in the supernatant determined by OD at 607 nm. The concentration of alcian blue was determined by reference to an alcian blue standard curve prepared from the original dye/buffer solution. Alcian blue retention by yeast was expressed as mg alcian blue per 10^6 cells. All yeast strains were tested in triplicate.

Contact angle measurements and surface energy calculations

Contact angles were obtained using the sessile drop technique with a Dataphysics OCA-20 contact angle analyser as described by Liu and Zhao (2005). The contact angles of three test liquids were used for surface free energy calculations: distilled water, diidomethane (Sigma, Dorset, UK) and ethylene glycol (Sigma, Dorset, UK). The data for surface tension components is provided in Table 1. All measurements were made at 25°C. Due to the small size of the tablets, only one liquid contact angle could be determined per tablet. At least 9 HAP tablets were examined for each test liquid with mean values ± standard deviation reported.

The contact angle of yeast strains was measured on yeast lawns (harvested after 24 h growth and washed by centrifugation as described above) deposited on sterile cellulose nitrate membranes with pore size 0.45 μm. Prior to contact angle measurement, the yeast lawns were dried in the air to a certain state, indicated by stable water contact angles over time. Usually this state lasted 30–60 min and indicated that

only bound water was present on the surface. Three independently grown samples of each strain were assessed with five replicate measurements made for each test liquid.

The free surface energy (γ_i^{TOT}) and surface energy components (Lifshitz-van der Waals, γ_i^{LW}; electron acceptor, γ_i^{+}; and electron donor, γ_i^{-}) of the yeast surfaces and HAP tablets were calculated according to the method of Liu and Zhao (2005) as based on the approach developed by van Oss et al. (1986 and 1988). The surface free energy (γ_i^{TOT}) was expressed as the sum of a Lifshitz-van der Waals apolar component (γ_i^{LW}) and a Lewis acid-base polar component (γ_i^{AB}):

$$\gamma_i^{TOT} = \gamma_i^{LW} + \gamma_i^{AB} \qquad (2)$$

where

$$\gamma_i^{AB} = 2\sqrt{\gamma_i^{+}\gamma_i^{-}} \qquad (3)$$

Hydrophobicity, ΔG_{iwi} was calculated as the free energy of interaction between two similar solute molecules immersed in water as defined by van Oss (1995):

$$\Delta G_{iWi} = -2\left(\sqrt{\gamma_i^{LW}} - \sqrt{\gamma_W^{LW}}\right)^2 - 4\left(\sqrt{\gamma_i^{+}\gamma_i^{-}} + \sqrt{\gamma_W^{+}\gamma_W^{-}} - \sqrt{\gamma_i^{+}\gamma_W^{-}} - \sqrt{\gamma_i^{-}\gamma_i^{+}}\right) \qquad (4)$$

where, the subscripts *i* and *w* correspond to the properties of the molecules and water, respectively.

Molecules or surfaces with $\Delta G_{iwi} \leq -84$ mJ/m^2 may be classified as completely hydrophobic, partly hydrophobic for ΔG_{iwi} between –84 and 0 and hydrophilic above 0 (van Oss 1995).

Results and Discussion

Adhesion of yeast strains to HAP

Adhesion of yeast to HAP was strain-dependent with the whisky strain being adhered to the greatest extent, the ale strain being half that, while the lager strain was 10-fold

lower. The number of cells immobilised per tablet was 4.3×10^5, 2.2×10^5 and 3.1×10^4 for M-type strain, NCYC 1681 and ALY, respectively (Table 2). For enumeration, cells were recovered from tablets by sonication. This method has been described previously for recovery of yeast cells from non-porous supports (Vernhet and Bellon-Fontaine 1995) and cell recovery was confirmed by the absence of methylene-blue stained cells on sonicated tablets. Similar methods have been used to recover cells from HAP tablets including sonication for staphylococci (Kinnari et al. 2009) and vortexing used to remove Candida sp. (Pereira-Cenci et al. 2008) with enumeration by plate counts. When comparing adhesion of different strains such direct methods are required, as assays such as those based on assessing metabolic activity are strain-specific and would not be applicable (Pereira-Cenci et al. 2008). Typical images of immobilised cells stained with methylene blue are provided in Fig 1. This clearly shows the difference in adhesion levels of the three yeast strains. Viability of immobilised cells was confirmed by staining with fluorescent probes. Two fluorescence probes were selected for use with HAP *in situ*: one for staining total cells (CMRA) and the other for staining dead cells only ($DiSBAC(2)_3$). Typical images of immobilised M-type strain stained with either CMRA or $DiSBAC(2)_3$ is provided in Fig 2. It is clear that the cells adhered to tablets are viable as the number of $DiSBAC(2)_3$-fluorescent cells was minimal. This confirms the validity of using plate counts to determine the number of cells recovered by sonication.

To overcome any interference of ions with potential binding sites on the HAP surface, an ion-free buffer was chosen for adhesion studies (the ionic strength of the biological buffer MES is essentially zero). It is known that sodium and potassium ions adsorb to HAP (Henriques et al. 2004) and HAP undergoes surface solubility in

aqueous solution (Bertazzo et al. 2010). Ion-free buffer and static incubation were chosen to minimise variation in incubation conditions and HAP dissolution. In this case, differences in adhesion between the strains could be attributed to the characteristics of the strains rather than variation in HAP surface properties. The different adhesive interactions of the three strains presented here highlights the fact that the three strains of *S. cerevisiae* do not interact with HAP in the same manner.

Yeast cell surface properties

To elucidate the reasons for different adhesion levels, the physicochemical properties of the yeast strains were assessed. The alcian blue retention assay was used as an indication of the overall negative charge of the yeast cell surface (Table 2). The strains adsorbed similar levels of dye, with greater than 86% of the dye removed in all cases. Adsorption of positively charged alcian blue to cells is an electrostatic interaction. This indicated that the overall cell wall electrostatic charge was not a principal determinant in cell adhesion.

The Lifshitz-van der Waals, electron donor and electron acceptor characteristics of the cells were assessed by two methods: MATS analysis and contact angle measurements using the approach of van Oss (van Oss 1986 and 1988). MATS is a qualitative method with the Lewis acid and base parameters being deduced by the difference in cell affinity for apolar and polar solvent pairs that have similar van der Waals properties, but differ in their electron donating (in the case of hexadecane and chloroform) or electron accepting (decane and ethyl acetate) properties (Bellon-Fontaine et al. 1996). Affinity of the yeasts for these solvents is presented in Fig 3. According to MATS, the ale strain was the most hydrophobic with 76 and 87% affinity

for hexadecane and decane, respectively. The whisky and lager strains had similar affinity for the apolar solvents, varying between 31 and 43%. Based on this, these strains would be described as moderately hydrophilic. The three *S. cerevisiae* strains were strongly electron donating as indicated by M-type strain, NCYC 1681 and ALY having 75, 98 and 89% affinity for chloroform, respectively. Yeast cells are predominantly negatively charged due to the presence of carboxyl, phosphoryl and hydroxyl groups. As the strains tested removed greater than 86% of the positively charged alcian blue from solution, this confirmed their overall electron donating character. All strains had minimal electron accepting capacity with affinities for decane greater than ethyl acetate in all cases.

Cell hydrophobicity properties as determined by contact angle measurements did not concur with MATS analysis. According to MATS, the ale strain was hydrophobic with a strong affinity for the apolar solvents, hexadecane and decane. In contrast, the water contact angle for all yeast was less than 20° (Table 3) and ΔG_{iwi} was positive (Table 4), indicating that all yeast were hydrophilic. It is argued that microbial adhesion to solvents does not provide an accurate account of cell surface properties with only contact angles providing a real estimate of cell surface hydrophobicity (van der Mei et al. 1998). The water contact angles are within the range of those reported for other *S. cerevisiae* strains. The water contact angle of wild type strain was 20.7° compared to 32.8° for a hydrophobic strain (Nakari-Setälä et al. 2002), the water contact angle for baker's yeast was 24.5° (Kang and Choi 2005) and between 7.2-9.5° for *S. uvarum* (*carlsbergensis*) (Brányik et al. 2004).

The total surface energy (γ_i^{TOT}), Lewis acid-base polar components (γ_i^+ and γ_i^-) and Lifshitz-van der Waal parameter (γ_i^{LW}) were calculated from the contact angles of

three solvents (apolar diidomethane and two polar solvents, water and ethylene glycol) (Table 4). The acid-base parameters indicate that all strains were electron donating with low electron accepting capacity. All yeast strains had low electron acceptor potential with γ_i^+ less than 1. This is in line with other reports (Nakari-Setälä et al. 2002; Kang and Choi 2005). Most biological surfaces have a γ_i^+ value which is exceedingly low (i.e. of the order of 0.1 mJ/m^2 or less) and when hydrophilic, a high γ_i^- (more than 28.5 mJ/m^2) and may be designated as essentially 'monopolar' (van Oss 1995).

The electron donating-electron accepting properties as determined by contact angle MATS analysis is in agreement with contact angle measurements. For the three *S. cerevisiae* strains tested, affinity for ethyl acetate was minimal and less than that for decane, confirming the low electron acceptor capacity. ALY lager yeast and M-type whisky yeast strains had similar affinities for hexadecane but the affinity of the lager strain for chloroform was greater than M-type strain (89 compared to 75%), indicating that the lager strain was more electron donating. This corresponds to the γ_i^- parameter, where the surface free energy component of ALY was greater than the M-type strain. The HAP tablets were slightly hydrophobic with water contact angle of 53.7° and low ΔG_{iwi} value of 3.62 mJ/m^2. The tablets were electron donating with negligible electron accepting capacity. In this case the Lewis acid-base energy parameter was zero and the overall surface energy (40.65 mJ/m^2) was directly dependent on the Lifshitz-van der Waals component.

Relationship between yeast adhesion to HAP and surface properties

To elucidate a physicochemical basis for the variation in adhesion, the surface properties of the yeasts were compared. Alcian blue retention was similar for all strains,

indicating that electrostatic forces involved in dye adsorption were not involved in HAP adhesion. There was no direct relationship between affinity to the solvents used in MATS and yeast adhesion. It is not surprising that the yeast strains have different surface characteristics. Difference in cell wall composition of ale and lager strains has been reported and is related to their flocculation behaviour at the end of brewing fermentations. Ale strains are reported as being more hydrophobic than lager strains, which is related to the higher protein content of the top-fermenting strain, compared to the higher phosphate concentration of the bottom-fermenting strain (Dengis and Rouxhet, 1997). This contributes to their settling character at the end of the fermentation; it is generally accepted that the more hydrophobic flocs of the top-fermenting ale strains associate with the CO_2 bubbles and rise to the top of the fermenter while the less hydrophobic lager flocs settle at the bottom. According to MATS analysis of the three strains presented here, the ale strain could be characterised as hydrophobic with the other strains being moderately hydrophilic.

The overall surface energy of the yeast strains correlated directly with adhesion to HAP (Fig 4). It is known that adhesion of bacteria to biomaterials is dependent on the surface energy of the biomaterial (Liu and Zhao 2005). However, we have demonstrated that the surface energies of the microbial surfaces are also key factors in adhesion. This is significant as it indicates that the overall properties of the yeast cell surface may be used to predict the potential for adhesion to biomaterials. The differences in adhesion and surface energy of the strains may be related to the nanomechanical properties of the cell surface polymers. Differences in the cell wall elasticity and polysaccharide properties of top and bottom brewing yeast strains as probed by atomic force microscopy (AFM) have been reported (Alsteens et al. 2008). Polypeptides are more

exposed, or available for interaction on the *S. cerevisiae* surface (top-fermenting strain) compared to the *S. carlsbergensis* surface (bottom-fermenting strain). This is comparable to the work presented here, with ALY, an example of a bottom-fermenting lager yeast, showing the least level of interaction with HAP when compared to the ale and whisky strains.

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List of Tables

Table 1 Test liquids and their surface tension components used for contact angle analysis (Liu and Zhao 2005).

Surface tension data (mJ/m^2)	γ_i^{TOT}	γ_i^{LW}	γ_i^{AB}	γ_i^{+}	γ_i^{-}
Water	72.8	21.8	51.0	25.5	25.5
Diidomethane	50.8	50.8	0	0	0
Ehtylene glycol	48.0	29.0	19.0	1.92	47.0

Table 2 Yeast adhesion to HAP and alcian blue retention.

Strain	**Adhesion to HAP (cells × 10^5)**	**Alcian Blue retention [mg dye/10^6 cells]**
M-type	4.32 ± 1.02	0.254 ± 0.003
NCYC 1681	2.20 ± 1.05	0.213 ± 0.001
ALY	0.31 ± 0.19	0.251 ± 0.003

Table 3 Contact angles of water, diiodomethane and ethylene glycol on HAP tablets and yeast lawns.

Sample	**Contact angle, θ [°]**		
	θ^{W}	θ^{Di}	θ^{EG}
HAP	53.7 ± 1.5	37.9 ± 1.8	39.9 ± 1.6
M-type	17.9 ± 0.6	45.4 ± 2.1	21.0 ± 1.0
NCYC 1681	18.4 ± 2.0	47.2 ± 1.3	23.9 ± 1.5
ALY	12.3 ± 1.1	50.3 ± 1.0	26.2 ± 1.7

Table 4 Surface free energy (γ^{TOT}), surface free energy components: van-der Waal (γ_i^{LW}) and Lewis acid-base (γ_i^{AB}), their acid-base parameters (γ_i^+, γ_i^-) and hydrophobicity (ΔG_{iwi}) of HAp and yeast. Calculations were based on the approach of Van Oss et al. (1988).

Sample	**Surface free energy components (mJ/m^2)**					**ΔG_{iwi}**
	γ_i^{LW}	**γ_i^+**	**γ_i^-**	**γ_i^{AB}**	**γ_i^{TOT}**	
HAP	40.65	0.00	30.44	0.00	40.65	3.62
M-type	36.80	0.14	65.45	6.05	42.85	52.96
NCYC 1681	35.82	0.12	66.66	5.66	41.48	55.14
ALY	34.11	0.11	72.57	5.65	39.76	62.73

List of figures

Fig 1: Adhesion of (a) M-type strain (b) NCYC 1681 and (c) ALY to HAP tablets. For imaging, cells were stained with methylene blue after fixing with 1:1 ethanol/diethyl ether.

Fig 2: Fluoresence of M-type strain adhered to HAP tablets. Cells were stained with either (a) CMRA, which stains all cells or (b) $DiSBAC(2)_3$ which stains dead cells only.

Fig 3: Affinity of the yeast M-type strain, NCYC 1681 and ALY for the solvents, chloroform, hexadecane, ethyl acetate and decane used for MATS analysis.

Fig 4: Correlation between the surface free energy of yeast (γ_i^{TOT}) and adhesion to HAP tablets.

Fig 1

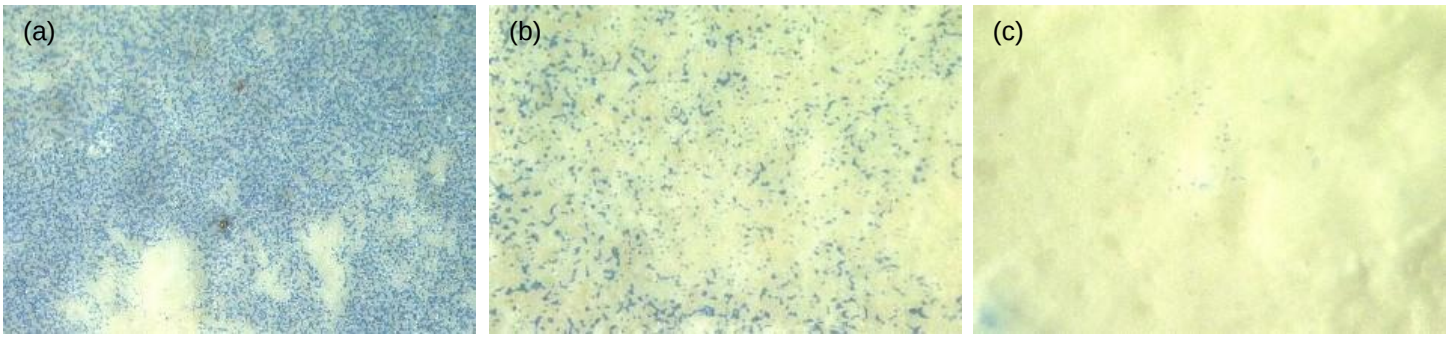


Fig 2

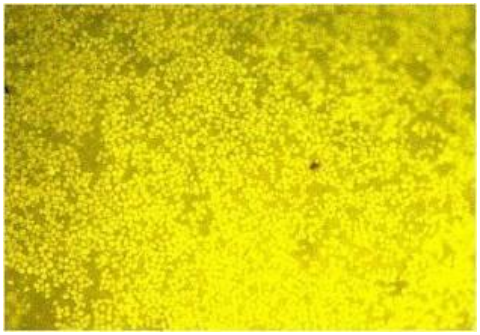
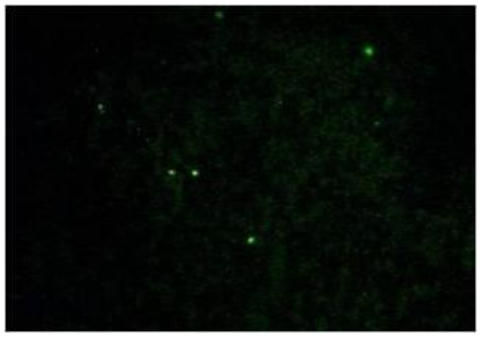
(a)  (b) 

Fig 3

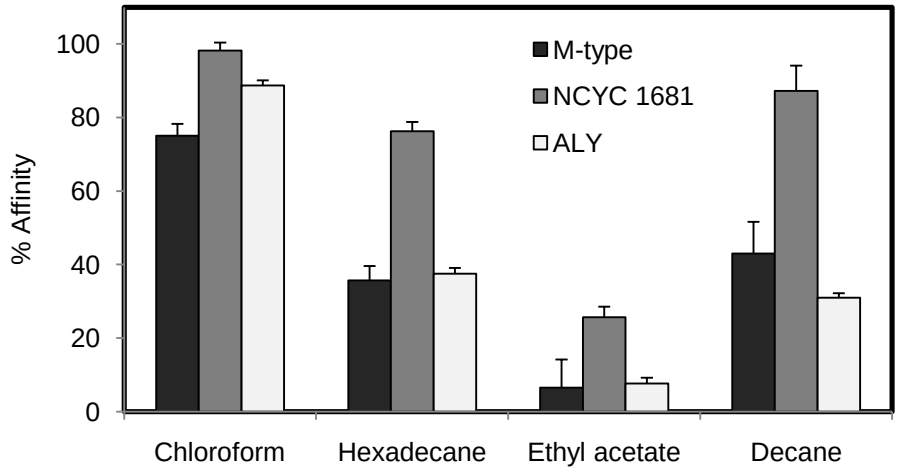
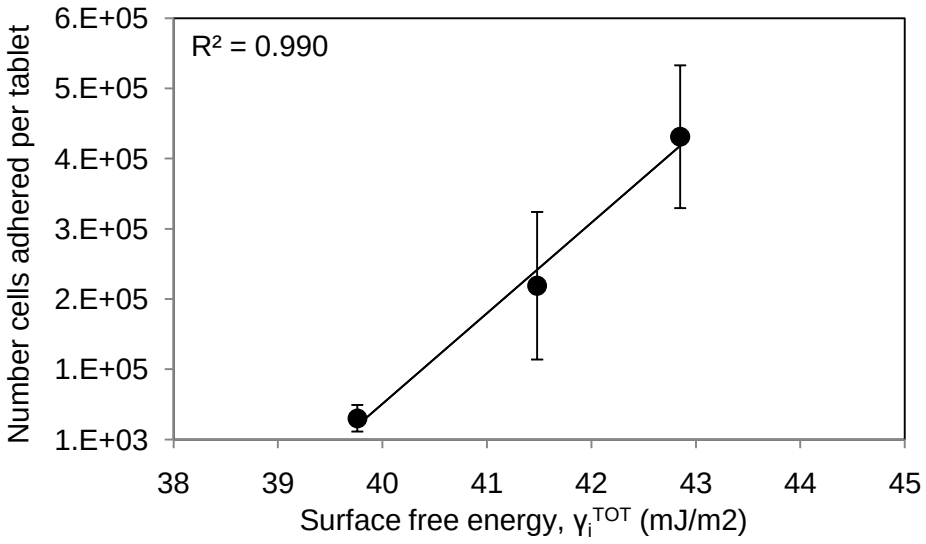

100
80
60
40
20
0
% Affinity
M-type
NCYC 1681
ALY
Chloroform
Hexadecane
Ethyl acetate
Decane

Fig 4


R² = 0.990
Number cells adhered per tablet
6.E+05
5.E+05
4.E+05
3.E+05
2.E+05
1.E+05
1.E+03
38
39
40
41
42
43
44
45
Surface free energy, γi^TOT (mJ/m2)