Original Research Article

A Biosensing Technique through a Coadhesion Study between Saccharomyces cerevisiae and Lactobacillus plantarum

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A B S T R A C T

Primary and secondary microbial adhesion onto solid surfaces has been the onset of development of a mature biofilm in aqueous environments that is predominantly the mode of bacterial contamination and spread of diseases. Adhesion and co-adhesion assays are therefore useful in understanding the adhesive interactions between microorganism and its surfaces. Various factors influence cell adhesion and biofilm formation, depending on aqueous medium and the type of microorganism in place. Similarly, factors such as ionic strength, pH and temperature are vital factors that influence cell growth and surface attachment. Different methods are available for testing adhesion and coadhesion assays such as macroscopic methods, microscopic methods, steady state and kinetic turbidometric methods, mathematical methods and slide based. However, out of these, parallel plate flow chambers (PPFC) are reportedly convenient and easy to use.

Introduction

Earlier studies on microbial adhesion were aimed at understanding the adhesion phenomenon of microbial cells onto solid surfaces such as a glass slide. Cell adhesion phenomenon is influenced by hydrodynamics and is also dependent on the shear strength of the cells to withstand high fluid shear force i.e. cell retention to surfaces (Busscher et al., 2001). Hydrodynamic shear assay techniques are used to investigate the adhesion phenomenon of cells on solid surfaces that react invariably different under the influence of turbulent or laminar flows (Bakker et al., 2002). From a clinical context, microbial adhesion on surgical instruments and implants becomes a menace in hospitals and microbiological laboratories where aseptic conditions are mandatory (Vo-Dinh and Cullum, 2000). Such microbial presence may aid in the transmission of pathogens (Katsikogianni and Missirlis, 2004) and distribution of harmful bacteria. Coadhesion is a phenomenon where two different microorganism pair up and one aids in the adhesion and attachment of the other microorganism. An adhesive interaction
between yeast and bacterial strain is one such combination for studying coadhesion behaviour. Flow chamber experiments (Sharma et al., 2005) that provide information about microbial surface behaviour have been a vital source of information. This has been useful in the field of medicine, where knowledge of microbial adhesion aids in limiting pathogenic infections (Dunne 2002).

Busscher et al (1997), studied the adhesive interaction between yeast and bacteria on silicone rubber within a PPFC their study investigated coadhesion and interaction of different bacterial strains with yeast (Busscher and Mei, 1997). The study showed that mostly bacterial adhesion was not favouring coadhesion with yeasts. However, a few strains stimulated adhesion of yeasts when a suitable medium of interaction was in place such as change in ionic strength or change in pH of solution. This was observed in this research work too which will be discussed later in the results.

Materials and Methods

Cell culture

(1) *S.cerevisiae* WT Flo11, *S.cerevisiae* SSN6, and *L.plantarum* ATCC 11974: were cultured using a MYGP medium which contained 3g/l of Yeast extract (Sigma-Aldrich, UK), 5g/l of Mycological Peptone (Lab M, International diagnostic group plc idg), 3g/l of Malt extract (Lab M, International diagnostic group plc idg), 10g/l of Glucose (Sigma-Aldrich, UK) for liquid broth and for solid media 20g/l of agar (London Analytical and Bacterial Media Ltd., UK) was added with the MYGP medium (I. Campbell and J. H. Duffus, 1988).

Flow setup

A rectangular parallel plate flow chamber (Figure 1) was fabricated in-house and flow cell experiments were carried out with suitable hydrodynamic conditions (Busscher and Van Der Mei 2006). Flow of cell suspension into the rectangular PPFC was regulated using a peristaltic pump (Ismatec, Germany) at different flow rates, 0.05, 0.5, 1, 2, 3, 5, 8, 11, 13, 15 and 18 ml/min respectively.

The flow rate was regulated using an external peristaltic pump (Ismatec, Germany) at a rate of 0.1-30 ml/min through a tubing (Ismaprene tubes) with a diameter of 2.06 mm (inner diameter). The suspended cells in the buffer solution were carried into the flow chamber through the inlet and outlet tubings connected to the PPFC, a real time monitoring system was created by mounting an inverted microscope (Leitz Wetzlar, Germany) on top of the flow chamber. A charge coupled device (ccd) camera was attached to the microscope that captured images with a 10x objective over an area of 0.43 x 0.58 mm, which were then recorded and processed using a image software (Pinnacle studio) in a computer.

Hydrodynamic shear assay

Yeast cells were suspended (70 x 10^6 cells/ml) in the buffer solution in the flask, using a peristaltic pump, cell suspension was allowed to flow to the flow chamber. *L.plantarum* cells were used (1.3 x 10^8 cells/ml) along with the yeast strain types.

The flow rate was controlled using the peristaltic pump and it was turned on/off using a switch on the pump. The real time images of the PPFC were recorded using the camera as mentioned in the previous section in the flow set up. All the images were recorded and processed using Image J software for data analysis. Cell suspension from outlet of PPFC was collected in a measuring jar which was used to measure the rise of fluid with increase in fluid flow rate.
Coadhesion study with *L. plantarum* and yeast cells using “In liquid behaviour” method

The experiments were repeated similarly as above but here combination of two cells was used. Firstly *L. plantarum* ATCC 11974 and *S. cerevisiae* WT Flo11 was used as combination I and secondly *L. plantarum* ATCC 11974 and *S. cerevisiae* SSN6 was used as combination II for cell suspension in buffer at different pH values of 5, 7 and 9 at 0.1M NaCl buffer solution. Similarly, as above procedure, cell solution was allowed to spread in flow chamber PPFC and later flow rate was increased gradually to increase fluid shear (flow rates, 0.05, 0.5, 1, 2, 3, 5, 8, 11, 13, 15 and 18 ml/min respectively). This was repeated with both combinations of cells. After completing the experiments glass slides were removed from the PPFC and kept in petri dishes for Cryo SEM imaging.

Coadhesion study with *L. plantarum* and yeast cells using “On surface behaviour” method

A novel style of experimental procedure was attempted for understanding surface behaviour of bacteria and yeast cells in flow chamber. Initially, *L. plantarum* ATCC 11974 was soaked on a glass slide surface for 1 hour with high concentration (1.3 x 10^8 cells/ml) of cells. After soaking for 1 hour *L. plantarum* ATCC 11974 in glass slide was placed in the flow chamber (PPFC).

Initially yeast *S. cerevisiae* WT Flo11 (combination I: *L. plantarum* + *S. cerevisiae* WT Flo11) was suspended in NaCl solution at 0.1M concentration at pH 5 was allowed to flow inside the PPFC with flow rate of 2.0 ml/min and cells were allowed to adhere to the glass surface of the chamber for 2 minutes. Later flow rate was increased to the following flow rates 1.5, 7.5, 18 and 30 ml/min respectively, with the time interval of 2 minutes each. Similarly, the same procedures were repeated with pH 7 and 9 for the cell suspension combination I (*L. plantarum* + *S. cerevisiae* WT Flo11). Exactly same procedure was repeated for the cell suspension, combination II (*L. plantarum* + *S. cerevisiae* SSN6). After completing each experiment, glass slides were retrieved from the PPFC and kept on petri dishes for Cryo SEM imaging. At the end of each reading cells were counted and images were taken for the above set of experiments. The recorded images were used for plotting graph and analysing results.

**Shear equation**

Shear rate (s^{-1}) of the microorganism adhering to the surface of substrate within the PPFC was calculated. Increase in fluid flow in the chamber, increased the shear rate, for a laminar flow profile the shear force acts parallel to the surface of the PPFC and depends on the viscosity of the liquid medium. Wall shear rate and Reynolds number (Re) calculations for rectangular PPFC were done using the following equations, here σ is wall shear rate in (s^{-1}), Q is volumetric flow rate in (m^3.s^{-1}), and ρ is fluid density in (Kg.m^{-3}), wo and ho is the width and height of the PPFC in (m) and η is absolute viscosity in (Kg.m^{-1}.s^{-1}) using the following equations (Busscher and Van Der Mei, 2006):

\[
\sigma = \frac{3 \times Q}{2 \times (\frac{ho}{2})^2 \times w_0} \quad \ldots (1)
\]

\[
Re = \frac{\rho \times Q}{(wo + ho) \times \eta} \quad \ldots (2)
\]

**Results and Discussion**

*L. plantarum* was used along with the two yeast cell types for studying coadhesion phenomenon. From the coadhesion study, it
was observed that, *L. plantarum* that was loosely adhering to glass surface due to the effect of shear force (Sharma et al., 2005), was able to adhere well in combination with yeast cells. The experiments were conducted with 0.1M NaCl buffer solution with pH 5, 7 and 9. Both the yeast cells were able to co-adhere with the bacteria and were able to stick to the surface against high fluid shear. All experiments were repeated for 3 times and the average values of the 3 repeats were used to determine the coadhesion of bacteria and yeast cells.

In this research, it was found that combination I: *L. plantarum* + *S. cerevisiae* WT Flo11 showed better coadhesion in comparison with combination II: *L. plantarum* + *S. cerevisiae* SSN6. The coadhesion data results for the total number of cells attached during the coadhesion process on the glass surface are as shown in table 1 and 2.

From table 1 of coadhesion results, we can see that *L. plantarum* had 140 cells/mm² and *S. cerevisiae* WT Flo11 had 118 cells/mm² adhered on the glass surface with cell buffer at pH5 achieved at the lowest flow rate of 0.05 ml/min. For a similar flow rate and at pH7 *L. plantarum* had 94 cells/mm² and *S. cerevisiae* WT Flo11 had 88 cells/mm²; at pH9 *L. plantarum* had 79 cells/mm² and *S. cerevisiae* WT Flo11 had 84 cells/mm². However, at the highest flow rate of 18ml/min the cell adhesion greatly reduced and from the table 1 we find that *L. plantarum* were 23 cells/mm² and *S. cerevisiae* WT Flo11 were 51 cells/mm²; at pH7 *L. plantarum* were 28 cells/mm² and *S. cerevisiae* WT Flo11 were 35 cells/mm² and at pH9, *L. plantarum* were 30 cells/mm² and *S. cerevisiae* WT Flo11 were 23 cells/mm². This meant that increase in flow rate greatly reduced the bacterial adhesion as compared to the yeast adhesion.

From table 2 of coadhesion results from combination II of *L. plantarum* + *S. cerevisiae* SSN6 shows a comparison with combination I results of table 1. Here, at pH5 the total cells adhered on the glass slide for *L. plantarum* were 138 cells/mm² and for *S. cerevisiae* SSN6 were 83 cells/mm² at the lowest flow rate of 0.05 ml/min. At a similar flow rate, at pH7, *L. plantarum* were 86 cells/mm² and *S. cerevisiae* SSN6 were 73 cells/mm²; at pH9, *L. plantarum* were 78 cells/mm² and *S. cerevisiae* SSN6 were 75 cells/mm². At the highest flow rate of 18ml/min, the number of cells significantly reduced, at pH5 there were 37 cells/mm² for *L. plantarum* and 48 cells/mm² for *S. cerevisiae* SSN6; at pH7, there were 34 cells/mm² for *L. plantarum* and 35 cells/mm² for *S. cerevisiae* SSN6 and at pH9, there were 33 cells/mm² for *L. plantarum* and 25 cells/mm² for *S. cerevisiae* SSN6.

The results from table 1 and 2 showed similar coadhesion data in terms of adhesion for *L. plantarum* cells but comparing the two yeast types we find that *S. cerevisiae* WT Flo11 had a better surface adhesion to glass than *S. cerevisiae* SSN6 at pH5 (from low to high flow rate). However, the coadhesion results for *S. cerevisiae* WT Flo11 and *S. cerevisiae* SSN6 at pH7 and pH9 were quite similar (from low to high flow rate). This lead to the optimization of the experiment using the on surface behaviour methods as described in methods section and the flow rates were changed to 1.5, 7.5, 18 and 30ml/min respectively. Figure 2 shows the cell attachment between combination I: *L. plantarum* + *S. cerevisiae* WT Flo11 and combination II: *L. plantarum* + *S. cerevisiae* SSN6 for the “in liquid” behavior of *L. plantarum* with yeast cells. Table 3 and 4 shows the coadhesion results for the on surface behaviour method for combination I and combination II of cells. Coadhesion experiments with “on surface behavior” produced results that were similar in comparison to that of “in liquid behavior” method experiments; at pH5, *L. plantarum* had 384 cells/mm² and WT Flo11 had 191
cells/mm$^2$ (Table 3) at the lowest flow rate of 1.5ml/min; whereas for similar conditions $L$. $plantarum$ and $S$. $cerevisiae$ SSN6, resulted in 382 cells/mm$^2$ and 164 cells/mm$^2$ (table 4) at the lowest flow rate of 1.5ml/min. This behavior was observed at the highest flow rate of 30ml/min as well for both combinations I and combination II of cells (table 3-4) and was similar for both the pH7 and pH9. In both type of experiments (table 1-4) it was clear that $S$. $cerevisiae$ WT Flo11 adhered more in numbers with $L$. $plantarum$ than $S$. $cerevisiae$ SSN6. This is an important result suggesting that $S$. $cerevisiae$ Flo11 shows selective adhesion to the glass surface (Guillemot et al., 2006) when compared to the $S$. $cerevisiae$ SSN6 strain. For the first time, $S$. $cerevisiae$ WT Flo11 and $L$. $plantarum$ were investigated for coadhesion study so a suitable comparison has been made with previous research works based on similar cell characteristics. Figure 3 shows the cell attachment between combination I: $L$. $plantarum$ + $S$. $cerevisiae$ WT Flo11 and combination II: $L$. $plantarum$ + $S$. $cerevisiae$ SSN6 for the on surface behavior of $L$. $plantarum$ with yeast cells.

The influence of pH (Mozes et al., 1987) significantly contributed towards the coadhesion of $L$. $plantarum$ ATCC 11974 and $S$. $cerevisiae$ yeasts, where pH5 was most suitable. Further, it was concluded that van der Waals interaction described by DLVO and the interaction between the outer cell surface macromolecules and the sample substrate, were important factors that described the microbial adhesion with respect to ionic strength and pH (Skvarla, 1993, Bos et al., 1999, Rijnaarts et al., 1999). For the same reason 0.1 M NaCl at pH5 were found appropriate for coadhesion of $L$. $plantarum$ ATCC 11974 with $S$. $cerevisiae$ yeast strains SSN6/ WT Flo11 showing similar response for ‘‘in liquid’’ and ‘‘on surface’’ methods.

Millsap et al (2000) developed a dot assay technique for determining the adhesive interactions between yeast and bacteria under controlled hydrodynamic conditions using a parallel plate flow chamber. Four different bacterial strains ($Streptococcus$ gordonii NCTC 7869, $Streptococcus$ sanguis PK 1889, $Actinomyces$ naeslundii T14V-J1 and $Staphylococcus$ aureus GB 2/1) at two different concentrations were used along with $Candida$ albicans ATCC 10261. Polymethylmethacrylate (PMMA) was used in the PPFC as a substratum surface and the microorganism were suspended in a TNMC buffer (In one liter: 1 mM Tris-HCl (pH 8.0), 0.15 M NaCl, 1 mM MgCl$_2$, 1 mM CaCl$_2$). It was found that on an acrylic surface, the presence of adhering bacteria suppressed adhesion of $C$. $albicans$ ATCC 10261 to various degrees, depending on the bacterial strain involved. Suppression of $C$. $albicans$ ATCC 10261 adhesion was strongest by $A$. naeslundii T14V-J1, while adhering $S$. gordonii NCTC 7869 caused the weakest suppression of yeast adhesion.

When adhering yeasts and bacteria were challenged with the high detachment force of a passing liquid-air interface, the majority of the yeasts detached, while $C$. $albicans$ adhering on the control on the bare PMMA surface formed aggregates. It suggested that the differences in suppression of $C$. $albicans$ ATCC 10261 adhesion shown by the bacterial strains did not appear to be dependent on bacterial size or percentage surface coverage. The largest bacterium, $A$. naeslundii T14V-J1, caused the highest bacterial surface coverage and was responsible for the strongest suppression of $C$. $albicans$ ATCC 10261 adhesion to the PMMA surface in the dot assay. However, the bacterial surface coverages for both $S$. gordonii NCTC 7869 and $S$. aureus GB 2/1 at 1x10$^9$ bacteria /ml were comparable to that of $A$. naeslundii T14V-J1 at 3x10$^8$ bacteria /ml, and both bacterial strains caused a far weaker suppression of yeast adhesion than $A$. naeslundii T14V-J1. However the yeast strains
and bacteria used in this research work were different but as the above mentioned fact it observed the same that when adhering yeast and bacteria were challenged with high shear force majority of the yeast detached compared to bacteria.

Millsap et al (1998), conducted a study on various methods of adhesive interactions between bacterial strains and yeast which ranged from simple macroscopic methods to flow chamber experiments. One of the samples study with C.albicans ATCC 10261 suspended in TNMC buffer in a parallel plate flow chamber onto glass with adhering S.gordonii NCTC 7869 showed that the presence of adhering bacteria influences the adhesion of yeast which is in comparison with this research work on L.plantarum ATCC 11974 and S.cerevisiae yeast strains WT Flo11/ SSN6.

Tallon et al., (2007) studied the agglutination test between yeast and L.plantarum which showed coadhesion behavior between yeast and L.plantarum . It observed that Mannose-containing polysaccharides (mannans) are major constituents of the cell wall of baker's yeast, S.cerevisiae. Suggested that some micro-organisms carry adhesins specific for mannose-containing receptors and, therefore, are able to agglutinate yeast cells in a mannose sensitive manner. It was found that L.plantarum strains 299V, CBE and Lp80 showed the highest titres of agglutination (32 for strain 299v and 16 for CBE and Lp80), while six other strains (529, 67G-1, BMCM12, IMG9205, T25 and CBFM19) agglutinated S.cerevisiae at lower titres (eight and two). The rest of the strains were not able to agglutinate yeast cells.

As was reported by (Adlerberth et al., 1996), L.plantarum 299v exhibited great agglutination ability in agreement with the mannose-specific adherence mechanism of these bacteria to human colonic cell line HT-29. Methyl-a-D-mannoside greatly inhibited agglutination of yeast by all strains tested. This confirmed a mannose sensitive agglutination mechanism of S. cerevisiae by L.plantarum strains. Similar results were observed in this research where L.plantarum ATCC 11974 co-adhered well with S.cerevisiae WT Flo11 than S.cerevisiae SSN6 (Table 1-4).

### Table 1
Coadhesion results for S.cerevisiae WT Flo11 and L.plantarum ATCC 11974 at 0.1M NaCl solution at different pH values (In Liquid behaviour)

| Wall Shear rate $\sigma$ (s$^{-1}$) | Flow rate Q (m$^3$.s$^{-1}$) | pH5 Average Cell Count/mm$^2$ | pH7 Average Cell Count/mm$^2$ | pH9 Average Cell Count/mm$^2$ |
|----------------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| WT Flo11 | L.plantarum ATCC 11974 | WT Flo11 | L.plantarum ATCC 11974 | WT Flo11 | L.plantarum ATCC 11974 |
| 0.008 | 8.33E-10 | 118 | 140 | 88 | 94 | 84 | 79 |
| 0.04 | 4.17E-09 | 115 | 137 | 87 | 91 | 80 | 76 |
| 0.16 | 1.67E-08 | 110 | 134 | 85 | 87 | 77 | 71 |
| 0.32 | 3.33E-08 | 101 | 128 | 82 | 82 | 71 | 68 |
| 0.48 | 5E-08 | 95 | 111 | 78 | 76 | 68 | 64 |
| 0.88 | 9.17E-08 | 89 | 105 | 74 | 70 | 63 | 61 |
| 1.28 | 1.33E-07 | 84 | 96 | 68 | 62 | 56 | 59 |
| 1.68 | 1.75E-07 | 80 | 77 | 62 | 51 | 49 | 55 |
| 2 | 2.08E-07 | 75 | 65 | 56 | 46 | 41 | 51 |
| 2.4 | 2.5E-07 | 70 | 41 | 48 | 38 | 34 | 45 |
| 2.88 | 3E-07 | 51 | 23 | 35 | 28 | 25 | 30 |
Table 2 Coadhesion results for *S. cerevisiae* SSN6 and *L. plantarum* ATCC 11974 at 0.1M NaCl solution at different pH values (in Liquid behaviour)

| Wall Shear rate σ (s⁻¹) | Flow rate Q (m³.s⁻¹) | Average Cell Count/mm² |
|-------------------------|-----------------------|------------------------|
|                         |                       | pH5  | pH7  | pH9  |
|                         | SSN6 SSN6 | SSN6 | L. plantarum ATCC 11974 | SSN6 | L. plantarum ATCC 11974 | SSN6 | L. plantarum ATCC 11974 |
| 0.008                   | 8.33E-10 | 83   | 138  | 73   | 86     | 75   | 78     |
| 0.04                    | 4.17E-09 | 81   | 134  | 71   | 85     | 73   | 76     |
| 0.16                    | 1.67E-08 | 78   | 124  | 69   | 82     | 70   | 73     |
| 0.32                    | 3.33E-08 | 76   | 114  | 65   | 78     | 67   | 70     |
| 0.48                    | 5E-08    | 72   | 106  | 62   | 72     | 65   | 68     |
| 0.88                    | 9.17E-08 | 68   | 94   | 59   | 66     | 62   | 64     |
| 1.28                    | 1.33E-07 | 65   | 81   | 56   | 60     | 58   | 60     |
| 1.68                    | 1.75E-07 | 62   | 77   | 53   | 57     | 54   | 54     |
| 2.08E-07                | 2.08E-07 | 59   | 63   | 49   | 51     | 45   | 50     |
| 2.88                    | 3E-07    | 48   | 37   | 35   | 34     | 25   | 33     |

Table 3 Coadhesion results for *S. cerevisiae* WT Flo11 and *L. plantarum* ATCC 11974 at 0.1M NaCl solution at different pH values (on Surface behaviour)

| Wall Shear rate σ (s⁻¹) | Flow rate Q (m³.s⁻¹) | Average Cell Count/mm² |
|-------------------------|-----------------------|------------------------|
|                         |                       | pH5  | pH7  | pH9  |
|                         | WT Flo11 | L. plantarum ATCC 11974 | WT Flo11 | L. plantarum ATCC 11974 | WT Flo11 | L. plantarum ATCC 11974 |
| 0.00024                 | 2.5E-08 | 191  | 384  | 171  | 254  | 152  | 237  |
| 0.0012                  | 1.25E-07 | 124  | 344  | 108  | 210  | 86   | 198  |
| 0.0028                  | 2.92E-07 | 77   | 236  | 78   | 171  | 54   | 155  |
| 0.0048                  | 5E-07   | 28   | 166  | 25   | 133  | 23   | 110  |
Table 4 Coadhesion results for *S. cerevisiae* SSN6 and *L. plantarum* ATCC 11974 at 0.1M NaCl solution at different pH values (on Surface behaviour)

| Wall Shear rate $\sigma$ (s$^{-1}$) | Flow rate Q (m$^3$.s$^{-1}$) | Average Cell Count/mm$^2$ |
|------------------------------------|-------------------------------|---------------------------|
|                                    |                               | pH5          | pH7          | pH9          |
|                                    |                               | *S. cerevisiae* SSN6 | *L. plantarum* ATCC 11974 | *S. cerevisiae* SSN6 | *L. plantarum* ATCC 11974 |
| 0.00024                            | 2.5E-08                       | 164          | 382          | 154          | 259          | 122          | 205          |
| 0.0012                             | 1.25E-07                      | 107          | 341          | 113          | 204          | 94           | 165          |
| 0.0028                             | 2.92E-07                      | 61           | 236          | 67           | 159          | 70           | 116          |
| 0.0048                             | 5E-07                         | 32           | 168          | 28           | 111          | 42           | 85           |

Figure 1 Schematic of the flow chamber with dimensions of the glass slide along with the inlet/outlet diameter; A is top view, B is side view and C is front view

Figure 2 SEM image of *S. cerevisiae* WT Flo11 and *L. plantarum* ATCC 11974 (left); and *S. cerevisiae* SSN6 and *L. plantarum* ATCC 11974 (right), in 0.1M NaCl buffer at pH5 (in liquid behaviour)
Microbial adhesion studies are important to understand cell-cell interaction and cell-substratum behaviour, which help in medical applications. Likewise, knowledge of coadhesion behaviour of bacteria in conjunction with yeasts will contribute in developing biosensing models for inhibition and spread of bacterial contamination (Tiago et al., 2018). This research has significantly contributed through coadhesion studies to discern about the cell interaction and behaviour in parallel with another microorganism of a different species. This work is an initiative towards the development of a novel design for biosensor as microorganisms have been part of the biosensing element in the biosensors (Chang et al., 2017) and S.cerevisiae and L.plantarum have been used initially as sensing elements in biosensors. Previous studies on S.cerevisiae and L.plantarum provided the basis for this research study and for the first time, S.cerevisiae SSN6 and S.cerevisiae WT Flo11 were used in combination with L.plantarum ATCC 11974 to study their cellular interaction and surface behaviour with glass substrate. This research, therefore, successfully provided experimentation techniques for flow chamber (PPFC) assay and enhanced microscopy techniques (SEM) for qualitative and quantitative analysis that determined the adhesion and coadhesion factor and showed that pH5 and 0.1M NaCl salt concentration buffer was best suited for microbial adhesion and coadhesion on the glass surface.

References

Adlerberth, I. et al., I., Ahrne, S., Johansson, M L., Molin, G., Hanson, L. A., Johansson, Marie-louise Hanson, Lars Å, Wold, Agnes E., 1996. A mannose-specific adherence mechanism in Lactobacillus plantarum conferring binding to the human colonic cell line HT-29 . A Mannose-Specific Adherence Mechanism in Lactobacillus plantarum Conferring Binding to the Human Colonic Cell Line HT-29 , 62(7), pp.2244–2251.

Bakker, D.P., Busscher, H.J. and Van Der Mei, H.C., 2002. Bacterial deposition in a parallel plate and a stagnation point flow chamber: microbial adhesion mechanisms depend on the mass transport conditions. Microbiology, 148, pp.597–603.

Bos, R., van der Mei, H.C. and Busscher, H.J., 1999. Physico-chemistry of initial microbial adhesive interactions—it’s mechanisms and methods for study. FEMS microbiology reviews, 23(2), pp.179–230.

Busscher, H.J., Gomez-Suarez, C. and Henny, C., 2001. Analysis of Bacterial Detachment from
Substratum Surfaces by the Passage of Air-Liquid Interfaces., 67(6), pp.2531–2537.
Busscher, H.J. and Mei, H.C. Van Der, 1997. Adhesion to silicone rubber of yeasts and bacteria isolated from voice prostheses: Influence of salivary conditioning films., 34, pp.201–209.
Busscher, H.J. and Van Der Mei, H.C., 2006. Microbial adhesion in flow displacement systems. Clinical Microbiology Reviews, 19(1), pp.127–141.
Chang, H., Voyvodic, P.L. and Structurale, C.D.B., 2017. Microbially derived biosensors for diagnosis, monitoring and epidemiology. Microbial biotechnology, 10(5), pp.1031–1035.
Dunne, W.M., 2002. Bacterial Adhesion: Seen Any Good Biofilms Lately? Clinical Microbiology Reviews, 15(2), pp.155–166.
Guillemot, G., Vaca-Medina, G., Martin-Yken, H., Vernhet, A., Schmitz, P., Mercier-Bonin, M., 2006. Shear-flow induced detachment of Saccharomyces cerevisiae from stainless steel: influence of yeast and solid surface properties. Colloids and surfaces. B, Biointerfaces, 49(2), pp.126–35.
I. Campbell and J. H. Duffus, E., 1988. Yeast a Practical Approach, IRL Press, Oxford.
Katsikogianni, M. and Missirlis, Y., 2004. Concise review of mechanisms of bacterial adhesion to biomaterials and of techniques used in estimating bacteria-material interactions. Eur Cell Mater, 8, pp.37–57.
Millsap, K., van der Mei, H., Bos, R.,and Busscher, H., 1998. Adhesive interactions between medically important yeast and bacteria. FEMS Microbiology Reviews, 21, pp.321–336.
Millsap, K.W., Bos, R., Van Der Mei, H. C.,and Busscher, H. J., 2000. Dot assay for determining adhesive interactions between yeasts and bacteria under controlled hydrodynamic conditions. Journal of microbiological methods, 40(3), pp.225–32.
Mozes, N., Marchal, F., Hermesse, M. P., Van Haecht, J. L., Reuliaux, L., Leonard, A. J., and Rouxhet, P. G., 1987. Immobilization of microorganisms by adhesion: Interplay of electrostatic and nonelectrostatic interactions. Biotechnology and Bioengineering, 30(3), pp.439–450.
Rijnaarts, H.H.M., Norde, W., Lyklema, J.,and Zehnder, A. J. B., 1999. DLVO and steric contributions to bacterial deposition in media of different ionic strengths. Colloids and Surfaces B: Biointerfaces, 14, pp.179–195.
Sharma, P.K. et al., Gibcus, M. J., Mei, Henny C Van Der., and Busscher, H. J., 2005. Influence of Fluid Shear and Microbubbles on Bacterial Detachment from a Surface., 71(7), pp.3668–3673.
Skvarla, J., 1993. A Physico-chemical Model of Microbial Adhesion. Journal of Chemical Society, 89(15), pp.2913–2921.
Tallon, R., Arias, S., Bressollier, P., and Urdaci, M. C., 2007. Strain- and matrix-dependent adhesion of Lactobacillus plantarum is mediated by proteinaceous bacterial compounds. Journal of Applied Microbiology, 102(2), pp.442–451.
Tiago, F.C.P., Martins, F S., Souza, E. L. S., Pimenta, P. F. P., Araujo, H. R. C., Castro, I. M., Branda, R. L., and Nicoli, Jacques R., 2018. Adhesion to the yeast cell surface as a mechanism for trapping pathogenic bacteria by Saccharomyces probiotics., (2012), pp.1194–1207.
Vo-Dinh, T. and Cullum, B., 2000. Biosensors and biochips: advances in biological and medical diagnostics. Fresenius’ journal of analytical chemistry, 366(6–7), pp.540–51.