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Correlation of Cell Surface Properties of Industrial Yeasts to their Functional Role in Fermentations

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INTRODUCTION:

• Adhesion properties of yeasts are crucial for many essential biological processes such as sexual reproduction, tissue or substrate invasion, bio-film formation and others.

• Yeast flocculation is defined as the asexual, reversible and calcium dependent aggregation of yeast cells to form flocs containing large numbers of cells that rapidly sediment to the bottom of the liquid growth substrate (Bony et al. 1997).

• Cell surface properties play a crucial role in governing the extent of yeast flocculation NewFlo type strains are found to be suited for brewing. Flocculation occurs if wort sugars are metabolized. Flocculation properties are applicable in improving the yeast biotechnology and are supposed to be dependent directly or indirectly on characteristics of cellular surface, usually the outer layer of the cell wall.

• Exploring more about the cell wall, especially its nanoscale structure, would be helpful in gaining insights into the process of flocculation during various brewing processes.

AIMS:

- **To gain insight into the brewing yeast cell wall and try to study the phenomenon and mechanism of flocculation in these strains.**
- **The four yeast strains under study are *Saccharomyces cerevisiae* strains named according to the dominant end product yielded. Brewing Strain (lager), Wine Strain, Champagne Strain and Fuel Alcohol Strain.**
- **To understand how the Cell Surface Hydrophobicity, cell surface charge and cell wall composition of glucans and mannans could affect the flocculation ability of these strains.**
- **To understand how ultra-structure and nano-mechanical characteristics are linked to functional properties of yeast.**

RESULTS:

Flocculation Assay:

Flocculation Ability for the four industrial strains was measured by the method provided by Bony et al (1998) with some modifications. Differences in the initial absorbance (A_0) to the Final Absorbance (A) obtained by suspending the yeast cells in the Flocculation Buffer determine the percentage flocculation ability of the four industrial strains.

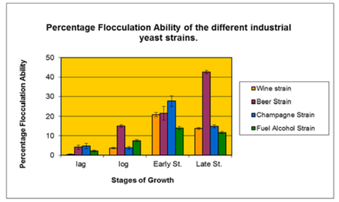


Fig 1. Flocculation ability of the industrial yeast strains at different phases of the growth curve. The Beer strain was found to be most flocculant while wine strain was least flocculant.

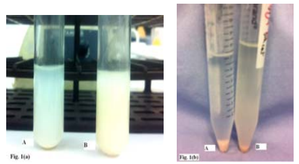


Fig 2 (a, b) Tube A:- Cells suspended in flocculation buffer containing calcium. Tube B:- Cells suspended in flocculation buffer containing EDTA.

Hydrophobicity Assay :

Cell Surface Hydrophobicity (CSH) was determined by Microsphere Latex Bead Assay. Latex Microsphere Beads were used with a bead diameter of 0.845 ± 0.001 µm .100 cells for each of the four industrial strains were counted and the percentage hydrophobicity calculated for those cells having ≥ 3 microspheres attached to it.

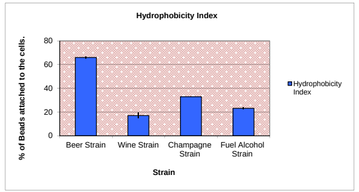


Fig 3. The Hydrophobicity Index is the direct representation of the Cell Surface Hydrophobicity (CSH). Beer Strain shows the maximum CSH levels, while the Wine Strain shows minimum CSH.

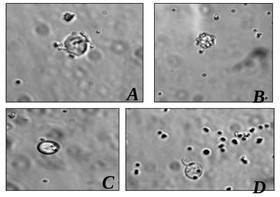


Fig 4. Latex beads attached to (A) Beer Strain, (B) Champagne Strain, (C) Fuel Alcohol Strain, (D) Wine Strain.

Atomic Force Microscopy (AFM) Measurements:

To extract information on the mechanical features of the cell wall, AFM force spectroscopy (AFM-FS) experiments were performed. These experiments yield force-distance curves. The tip interacts with the cell surface of the sample and gives insight about various forces that operate at the atomic level.

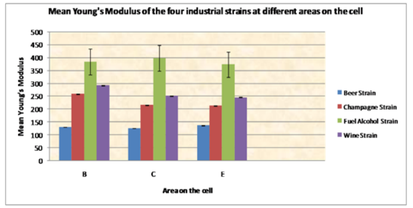


Fig 8.The mean Young's modulus for the four strains determined by AFM. It gives the measure of elasticity, i.e, lower the Mean Young's Modulus, higher the elasticity. Beer Strain shows the minimum young's modulus.

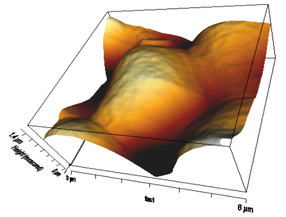
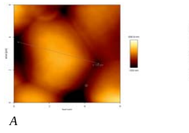
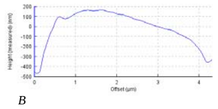


Fig 9 A 3D- rendered image of a single yeast cell (Beer Strain), produced using AFM.



A



B

Fig 10 (A) AFM image taken in contact mode for Beer Strain; (B) Height measured of a single yeast cell.

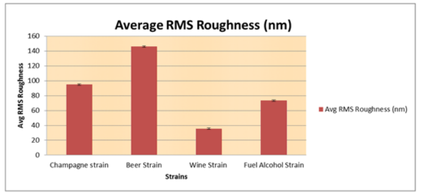


Fig 11. Higher the RMS surface roughness of the cell wall, higher are the chances that it would promote the floc formation and maintain the stability of the flocs in the liquid medium.

Cell Surface Charge (CSC) Measurement:

Alcian Blue is a phthalocyanine complex that has four charged sites in the molecule and is adsorbed by the negatively charged yeast cell surfaces. All the strains were negatively charged during their late stationary phase. The presence of carboxylic and phosphodiester groups are responsible for the negative character of yeast.

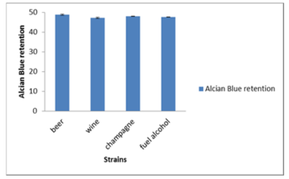


Fig 5. Alcian Blue retention (mg) /5×10^7 cells. Maximum retention was observed in the case of Brewing strain, indicating high negative charge on its cell surface compared to the other strains.

Mannan and Glucan Staining:

The mannans and glucans were identified by staining the yeast cell surface with Concanavalin A-Alexa Fluor 350 (ConA) and *Pisum sativum* (PSA)-FITC, respectively.

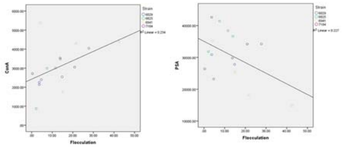


Fig 6. Correlation curve (A) ConA staining mannan subunits shows positive correlation with flocculation behaviour, (B) PSA staining glucan subunits shows a negative correlation with flocculation observed for the strains at the late stationary phase.

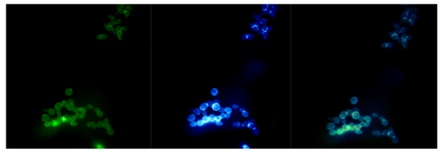


Fig 7. (from left to right) A Champagne Strain stained with PSA-FITC and then with ConA-Alexa Fluor 350, and final image showing the distribution pattern of the glucans and the mannan subunits on the cell wall.

CONCLUSION & FUTURE DIRECTIONS:

Different strains show that altering the physical parameters, such as particular phase of growth curve, initial seeding cell concentration, brings about changes in the flocculation ability.

The flocculation abilities were further correlated and supported by the Cell Surface Hydrophobicity results and AFM analysis. In particular, higher the values for CSH, CSC and RMS roughness. Higher is the flocculation ability.

Concomitantly, it was observed that the strains that have more mannan content in the cell wall were more flocculant, while a negative correlation was observed with glucan concentration in the cell wall.

Young's modulus helped us to explain the stability of flocs in the liquid environment. Higher the elasticity of the cell wall, better the stability of the yeast strain.

We aim to apply some molecular biology tools in addition to the biochemical techniques to understand the interactions between cell wall polysaccharides and lectins that lead to flocculation in the brewer's yeast strains, further investigation of the FLO genes are in the process of investigation.

REFERENCES:

• Bony, M., Barre, P. & Blondin, B. (1997) Distribution of the flocculation Protein, Flop, at the cell surface during the yeast growth. Yeast, **14** : 25-35.

ACKNOWLEDGEMENT:

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