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PHYSICAL DIFFERENCES AMONG CLASS II HYDROPHOBINS AFFECT THEIR SELF ASSEMBLY MECHANISM HENCE THEIR GUSHING POTENTIAL

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Introduction: Hydrophbins and primary gushing

Class II Hydrophobins are fungal amphipilic surface active proteins, they are produced during their vegetative growh covering spores and hyphae to make them hydrophobic and more resilent to the weather conditions(Linder, 2005). Primary gushing is a physical phenomenon caused by the interaction of hydrophobins with gaseous CO_2 producing spontaneous overfoaming out of the container without any shaking. Through trapping CO_2 into nanobubbles structures (Fig. 1) (Deckers et al, 2012) stabilizing and solubilizing it , when the bottle is opened the sudden pressure drop will explode the nanobubbles realeasing all its energy causing gushing.

How is gushing produced?

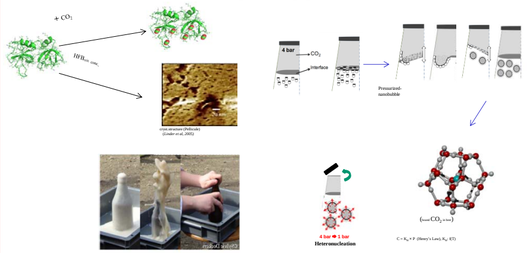


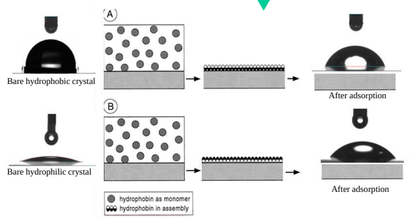
Fig 1. CO_2 Nanobubbles formation mechanism

However , it has been observed that when varies the hydrophobin involved in primary gushing the amount of overfoaming is different (Sarlin, 2012). Although all class II hydrophobins share a similar globular shape with eight conserved cysteines within their sequences and four disulfide bridges, little is known about how minor differences in sequences, protein folding and protein-protein interactions have direct effect on their interaction with other hydrophobic surfaces and hydrophobic molecules like CO_2. This research tries to elucidate how this process is achieved and how this information can be used to understand deeply primary gushing.

Materials and methods

Gushing potential determination of four different class II hydrophobins: HFBI, HFBII *(Trichoderma reesei*), HFB2-a2 (*Trichoderma harzianum*) and Fghyd5 (*Fusarium graminearum*)

Quartz crystal microbalance, adsortive properties measurements of each class II hydrophobin



Water contact angle, changes of the hydropathy of a surface after adsorption of class II hydrophobins.

Results and discussion

Hydrophobin amount added (ug/L)	Amount of sparkling water gushed (mL) (n=3)			
	HFBI	HFBII	Fghyd5	HFB2-a2
0,3	0	0	0	0
3	12	15	0	18
30	145	146	0	254
50	191	185	67	270
100	273	260	234	353
150	490	484	352	497
200	516	509	480	562
250	614	614	512	652
300	608	609	548	657

Table 1. Gushing potential determination

Gushing potential was evaluated with four different class II hydrophobins HFBI and HFBII from *Trichoderma reesei*, Fghyd5 from *Fusarium graminearum* and HFB2-a2 from *Trichoderma harzianum* (Table 1.). It was noticed that for Fghyd5 it was needed 10 times more hhydrophobin to produce the same amount of overfoaming compared with the other three. On the other hand HFB2-a2 exhibited a stronger gushing potential, reaching high overfoaming at low concentrations (30ug/L). To elucidate the reason of this behavior QCM adsorption was used to understand the behavior of this proteins with different hydropathys.

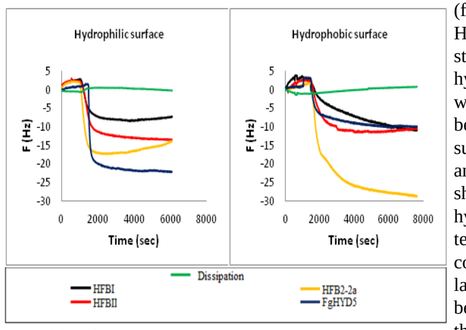


Fig 2. QCM adsorption experiments

Adsorption experiments (fig, 2) showed that HFB2-a2 exhibited a strong adsorption to hydrophobic surfaces while Fghyd5 adsorb better to hydrophilic surfaces. Water contact angle measurements showed changes in the hydropathy of the tested surfaces confirming strong and lasting interaction between the protein and the surface

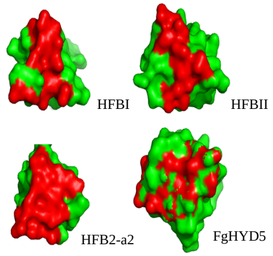


Fig 3. Molecular models of hydrophobin monomers, hydrophobic patches are colored in red

A detailed observation of the hydrophobic patches of the four tested hydrophobins showed that their size and uniformity change among them (Fig. 3). Biomolecular modelling was used using Rosetta server to understand the protein-pretein interactions when a monolayer is formed. The results showed that in the case of HFBI and Fghyd5 a glutamine residue is protruding from the hydrophobic patch augmenting the distance between the hydrophobic surface or molecule (CO_2) making it weaker. On the other hand no glutamine residue was founded in HFB2-a2 (it was buried within the strucucture)(Fig. 4). This facts can explain why this hydrophobin has a stronger gushing tendency and better adsorption for hydrophobic surfaces compared to the others

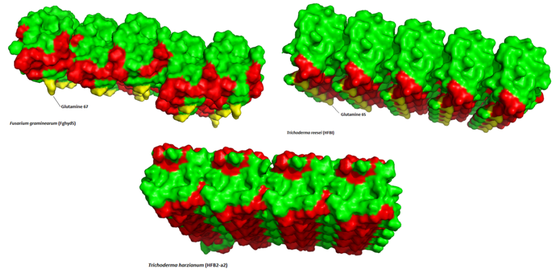


Fig 4. Hydrophobins monolayers representations hydrophobic patches colored in red

Conclusions

Self assembly mechanisms, protein-protein interactions have a definitive impact on gushing phenomenon, the presence of glutamine in the hydrophobic patch of Fghyd5, HFBI and HFBII diminish the ability to adsorption and interaction with other hydrophobic surfaces and molecules like CO_2. The uniformity and size of the hydrophobic patch is a key factor in the determination of the strenght and ability to induce gushing caused by hydrophobins.

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