Immunological detection of *Fusarium* hydrophobins in barley and malt

Analytical Forum " Wort Contamination by Hydrophobins: How to detect and what to do“
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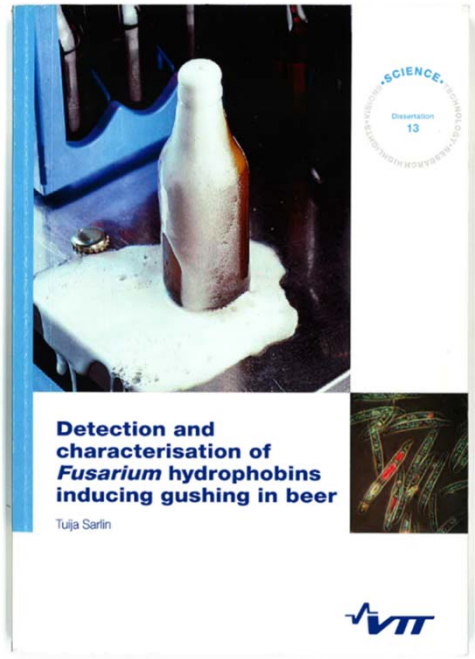
Fungal hydrophobins identified as gushing inducers

- Primary gushing commonly caused by *Fusarium* fungi
- Genes related to hydrophobin production characterized from several *Fusarium* species including
  - *F. culmorum*, *F. graminearum, F. poae, F. verticillioides*
  - Similar genes also at least in *F. oxysporum*, *F. sporotrichioides*, *F. equiseti*
- One *Fusarium* species may produce several different hydrophobins
  - 2 - 5 hydrophobin genes identified in one species
- Gushing activity of hydrophobins differs
- Only class II hydrophobins seem to be gushing active

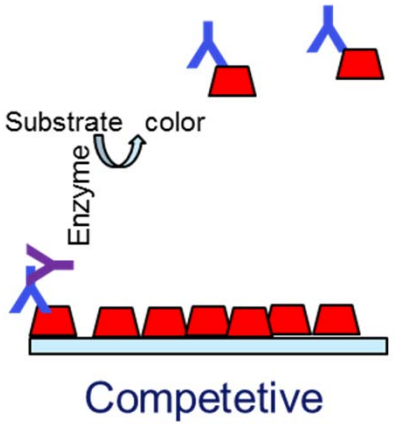


http://www.vtt.fi/publications/index.jsp

Ref. Sarlin *et al.* 2005, 2006, 2007, 2012

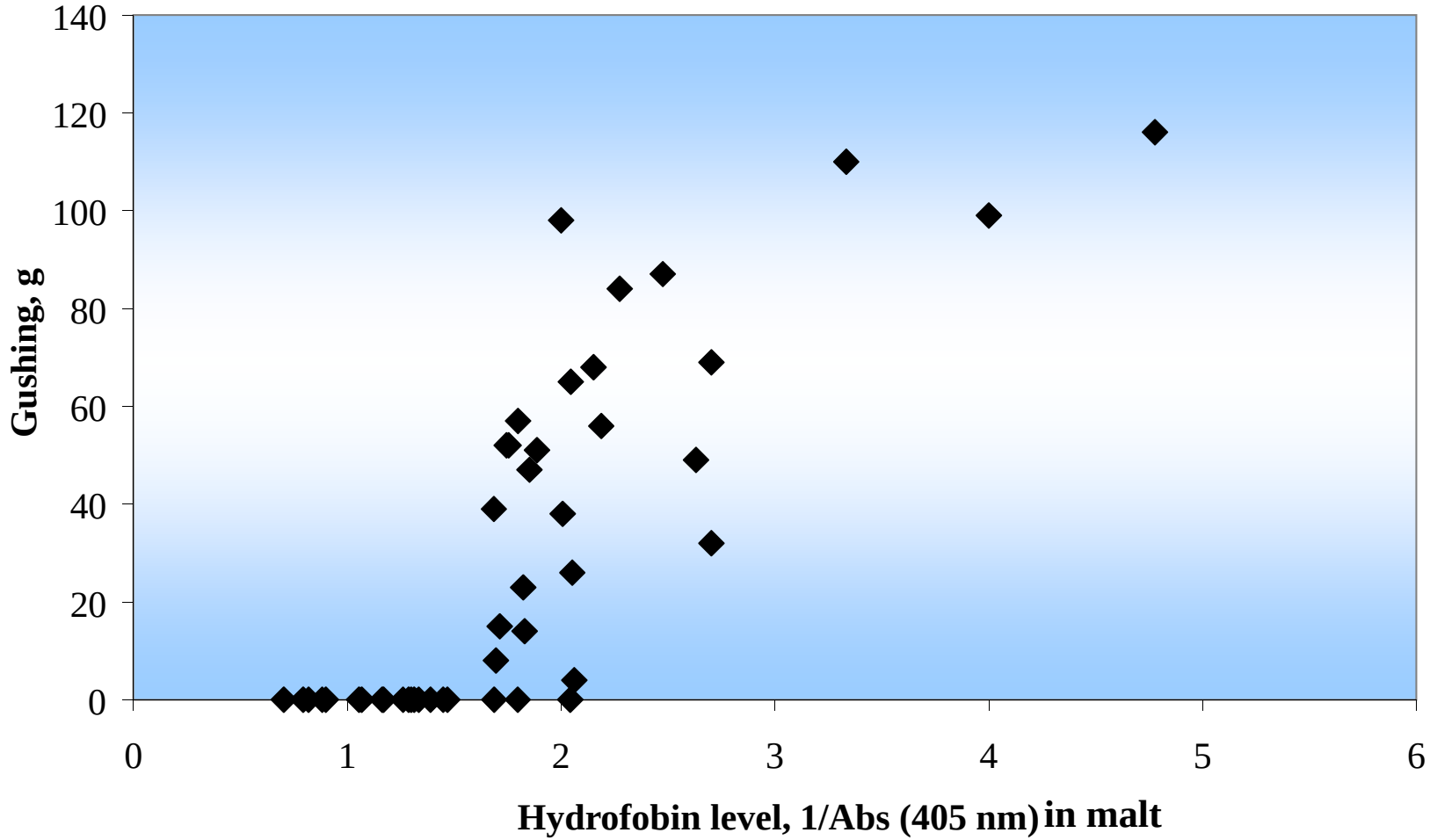
Competitive VTT ELISA test has been developed for determination of hydrophobins in barley and malt

- Based on polyclonal antibodies raised against *F. poae* hydrophobin
- Easy to perform
  - No special skills required
  - Normal lab facilities + a microtitre plate reader
- Fast
  - ~5 h + sample milling and weighing
  - ~20 samples per plate, several plates can be run in parallel



- International evaluation of the current hydrophobin ELISA format verified the function of the assay (4 labs, 5 malt samples)
- Gives an estimation of gushing risk in malt (and in barley)

The connection between the hydrophobin level in malt and the gushing potential determined using the Carlsberg gushing test



Ref. Sarlin et al. 2005. J. Inst. Brew. 111:105-111.

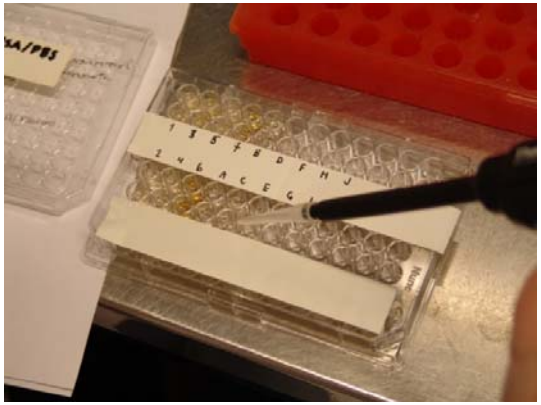
Pros and cons of the current VTT hydrophobin ELISA

Pros

- Only method available for the direct detection of gushing factors in brewing ingredients and products
- Easy and fast
- Distinguish malts with high gushing risk from low risk ones
- Detects raw materials infected with a wide spectrum of *Fusarium* species
- Has been used for research purposes and for analytical services

Cons

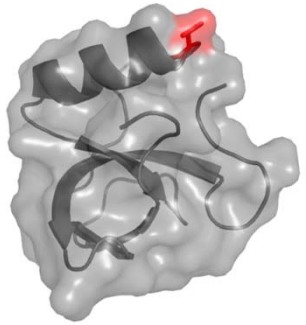
- Competitive ELISA based on polyclonal antibodies
  - Production of polyclonal antibodies suffers from batch-to-batch variation
  - Production and handling of hydrophobins used in the assay is challenging
- Not yet as a standard kit format for commercial use



Prediction of gushing risk directly from barley is limited due to the production of hydrophobins during malting. Screening of process samples and final malt is recommended.

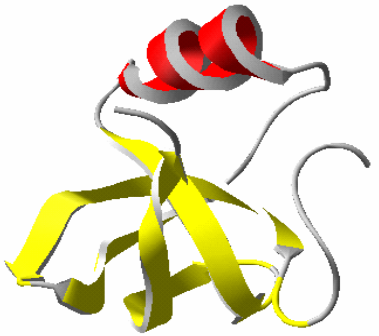
Future perspective

- Further assay development for in situ diagnostics
  - Based preferably on monoclonal antibodies
  - VTT has produced monoclonal antibodies against *F. graminearum* and *F. poae* hydrophobins
  - High specificity of the monoclonal antibodies might be a challenge
  - Commercialization partners / investors needed
- Fate of hydrophobins in industrial practice
  - Regulation of hydrophobin production in the field and during malting
  - Effects of brewing processes and preventive actions
- Identification of other factors inducing primary gushing, if any



Take home message

- Hydrophobins proved to be gushing inducers
- *Fusarium* hydrophobins can be detected with the VTT hydrophobin ELISA
  - VTT offers as analytical services
- A connection between hydrophobin level and gushing potential in malt found
- Further assay development needed for insitu diagnostics



Thank you!