A Robust LC-MS Analysis of *C. albicans* - Differential Protein Expression using Adaptive Focused Acoustics® (AFA®) Ultrasonication, SP3, and TMT Labeling

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Introduction: *Candida albicans* is the most common human pathogenic yeast and a member of the human gut flora [1]. It can reside harmlessly on the skin, in the oral cavity, and/or in the urogenital and gastrointestinal tracts causing cutaneous and mucocutaneous candidiasis [2]. It is a polymorphic organism, being able to grow in yeast form, or in hyphal and pseudohyphal types, which form filamentous structures [1]. *C. albicans* can cause life-threatening systemic infections: the hyphal form (which can be induced in vitro using BSA [3]) has an important role in causing disease by invading epithelial cells and causing tissue damage [4]. This study aimed at analyzing the protein determinants differentially expressed between yeast and hyphal forms.

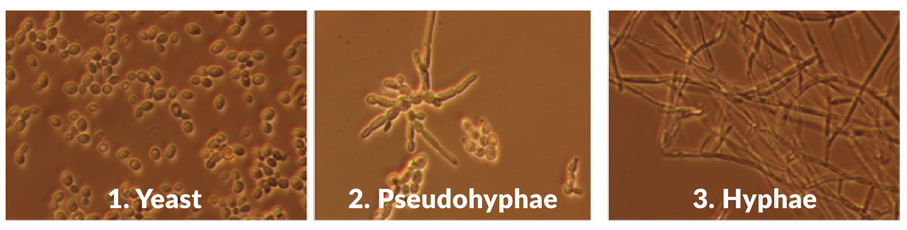

Figure 1. The three morphological forms of *Candida albicans*. Pictures from Simon Vautier. https://www.immunology.org/public-information/bitesized-immunology/pathogens-and-disease/candida-albicans

Yeast Protein Extraction Workflow from Cell Lysis to TMT Labeling

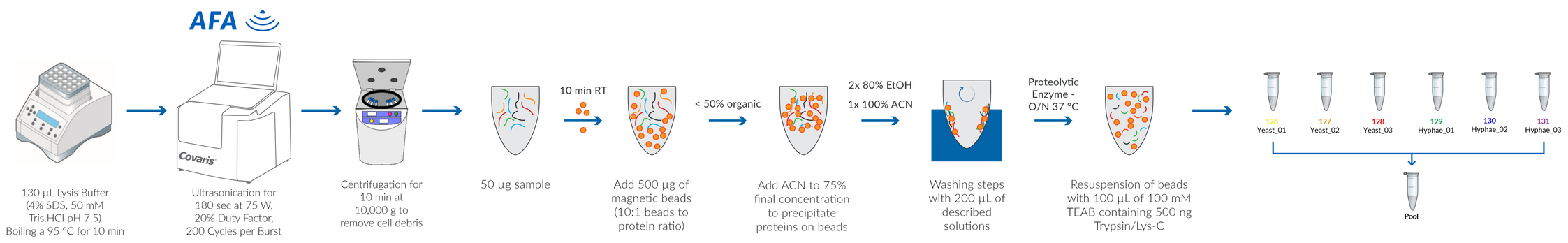

Figure 2. Yeast Protein Extraction Workflow from Cell Lysis to TMT Labeling. Six SC5314 *C. albicans* isolates were cultured in 10 mL of either YPD medium (named "Yeast") or YPD medium + 10% BSA (named "Hyphae"). Proteins were extracted with AFA. Debris was removed by centrifugation and supernatant was transferred in a tube. Beads were added to perform clean-up and digestion through SP3 [5]. Peptides were labeled via TMT and pooled. LC-MS analysis was performed using Thermo Q Exactive High Resolution Accurate Mass Spectrometer.

Extraction with Precision - Adaptive Focused Acoustics (AFA) in Pre-analytical Sample Prep

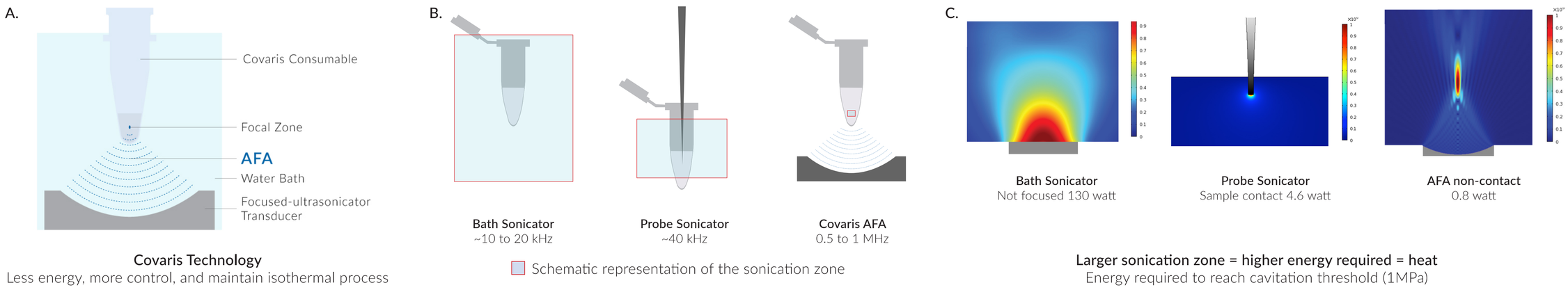

Figure 3. Characteristics of AFA technology: ***A***. Focalization of energy is permitted by high frequency acoustics dispensing. ***B.*** The resulting sonicated zone depends on the wavelength, corresponding to 1 to 3 mm in the case of AFA. ***C***. Less power is needed with AFA, resulting in a non-contact and isothermal processing of the samples which ensures preservation of proteins, unlike conventional sonication techniques.

Protein and Peptide Quantification

All raw files that used DDA LC–MS/MS were analyzed by MaxQuant v1.6.0.1 software.

a - Proteins are quantified across 6 channels with ≥ 2 unique peptides and CV < 30% for each conditions.

b - Significantly differentiated proteins are defined as those with Benjamini-Hochberg adjusted p-value < 0.01 and a log2 ratio exceeding ± 1.

Proteins[a]	Unique Peptides	Total Peptides	Sig. Dif. Proteins Up	Sig Dif. Proteins Down[b]
2,993	29,148	29,648	65	130

1D SDS PAGE Analysis

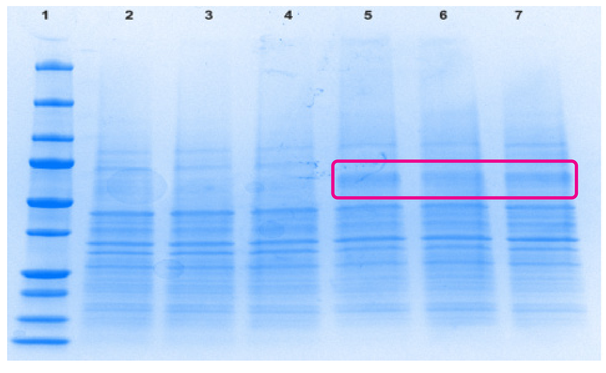

Figure 4. 1D SDS PAGE analysis. Thirty µg of protein from each sample was analyzed on a 12% acrylamide SDS-PAGE gel (Biorad) under reducing conditions followed by Coomassie Brilliant Blue staining.

- Lane 1: MW marker
- Lanes 2 to 4: Yeast samples
- Lanes 5 to 7: Hyphae samples

Several bands seemed differentially expressed between the two phenotypes. The main observation came from a 60 kDa band clearly over-expressed in the Hyphae samples.

Principal Component Analysis of the Two Different Phenotypes

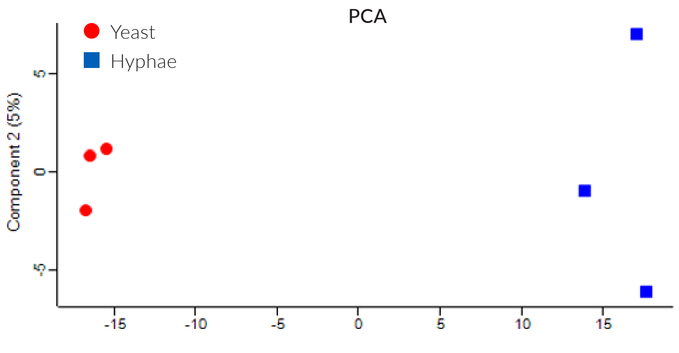

Figure 5. Principal Component Analysis of the two different phenotypes. The PCA based on the first and the second component, colored by strains shows a discrimination between Yeast and Hyphae on the first component with a variance of 84.3%. It also highlights the reproducibility of extraction, along with a clear separation between the two phenotypes.

Differential Expression Between Yeast and Hyphae Subtypes

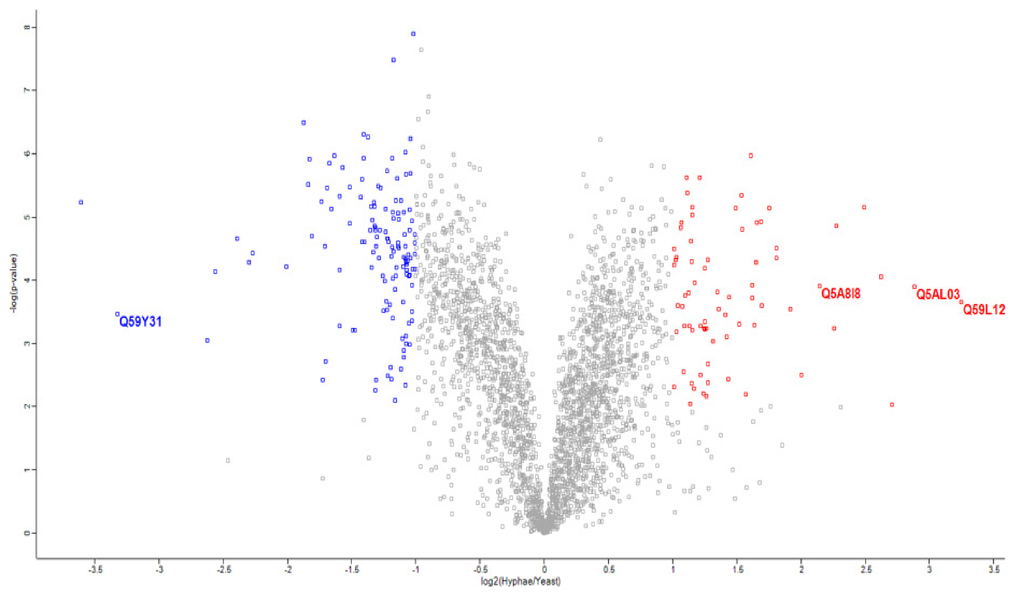

Figure 6. Differential expression between Yeast and Hyphae subtypes. Significantly differentiated proteins are defined as those with a Benjamini Hochberg adjusted p-value < 0.01 and a log2 ratio exceeding ± 1. In Hyphae, proteins in ***red*** are up-regulated and proteins in ***blue*** are down-regulated. Sixty five proteins are upregulated and 130 are down regulated in the Hyphae type. Those results are in line with previous observations. For example, Agglutinin-like protein 3 (ALS3, Q59L12), Hyphally regulated cell wall protein 1 (Q5AL03), and Induced during Hyphae Development Protein 1 (Q5A8I8) are known to be induced during Hyphae formation. Of particular interest, ALS3 is a key protein in the adherence of *C. albicans* hyphae to epithelial cells [6]. On the other hand, Yeast-form wall Protein 1 (Q59Y31) undergoes a downregulation under filamentation [7].

Conclusion: This workflow optimally combines the most advanced preparation techniques for proteomics, namely the precise energy and thermal control of Covaris AFA with bead-based clean up (SP3) and TMT labeling. It can be easily adapted for automated, higher-throughput processing as already demonstrated for mammalian cells and tissues in 8-well strips [8] and 96-well plates [9].

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