TITLE: SUPERIOR PHYSIO-TECHNOLOGICAL FITNESS OF NOVEL *SACCHAROMYCES CEREVISIAE* STRAINS FOR HIGH-GRAVITY BREWING

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ABSTRACT:

With the greater valuing of yeast role in beer brewing and product diversity, the search for novel yeast starters with superior and desirable traits arouses interest for product innovation. High-gravity brewing (HGB) allows the production of more alcoholic beers with increased sensorial complexity. However, in this condition yeasts are challenged by various stress factors, which may impact on fermentation performance and product quality. In this study, *S. cerevisiae* strains isolated from different bioprocesses were screened for employment as starters in HGB. In this initial screening, five strains (ST1-ST5) previously picked out for growth capacities in HG beer wort were evaluated for physio-technological brewing traits: foam production, hydrogen sulfide (H_2S) formation, flocculation ability, ethanol tolerance, and cell viability. S-33 commercial strain was used as control. Foam production (mm) was determined along fermentation of HG beer wort (18°P, 6 x 10^6 cells/mL, 22°C, 96h). H_2S production was estimated by differential colony culture in agar LA medium (incubation at 30°C, 7 days). Flocculation capacity (%) was determined by optical density ($OD_{600ηm}$) using Helm´s modified test. Ethanol tolerance was estimated by growth in YPD plates containing 12%, 15% and 18% (v/v) ethanol at 30°C. Cell viability was estimated by differential staining with erythrosine dye after HG beer wort fermentation. All strains showed similar (15 mm) or lower foam formation compared to S-33, but ST3 and ST4 exhibited cell flotation behavior, which is undesirable to the process due to incrustation problems. It was observed none, low and medium H_2S production for ST1 and ST2, ST3 and ST4, and ST5, respectively, while S-33 showed high production. Once H_2S has an undesirable aroma, strains with high production are not desired. Regarding flocculation percentages, ST2, ST3 and ST4 had similar ability than S-33 (~80%), while ST1 and ST5 showed medium capacity (~60-65%). All strains showed high ethanol tolerance, being able to grow under 12-15% ethanol, similar to control, while ST1, ST2 and ST3 could grow even at 18% ethanol. After fermentation, the three best strains (ST1, ST2 and ST5) were able to sustain higher cell viability rates (>94%) than control (88%), showing ability to lead with stressful conditions of HGB. Such strains exhibited superior physio-technological fitness, being potential candidates to be applied as starters in HGB to obtain high quality and differentiated beers.

Keywords: *Saccharomyces cerevisiae*, high-gravity brewing, cell viability, stress, starters.

Development Agency: grant #2020/05927-9, São Paulo Research Foundation (FAPESP).