MSc Thesis

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Yeasts are the most important group of microorganisms exploited for commercial purposes including fermentation, biotechnology, and medicine. However, yeasts are also known to cause spoilage of food products and beverages under certain conditions leading to their nutritional and organoleptic properties deterioration, loss of quality, and even foodborne illness. Food scientists and biotechnologists have extensively studied yeasts over recent decades and *Zygosaccharomyces* species are among the troublesome food spoilage yeast having a long history of spoilage in the food and beverage industry, especially the fermented products, and also high-salts and high-sugar containing foods.

Zygosaccharomyces species have been traditionally characterized, classified, and identified as closely related species based on morphology and physiological features. Therefore, accurate identification and isolation of these yeasts are essential for ensuring the quality and safety of food products. There is a need for new and improved media for the selective isolation of various species and/or strains capable of growing under specific environmental conditions. To isolate *Zygosaccharomyces* species from food samples, appropriate and specific culture media need to be developed that support the growth of these species while inhibiting the growth of other microorganisms commonly found in food samples. Moreover, the media should be selective enough to differentiate between different species of *Zygosaccharomyces*.

Can the growth and isolation of *Zygosaccharomyces* species be optimized by testing different culture media that vary in nutrient composition, pH, and other growth factors? This was a very fundamental research hypothesis for the chosen topic.

Therefore, the choice of the culture media was prompted by a thorough literature review of some previously studied culture media appropriate for the growth of *Zygosaccharomyces* species. Based on these data YEPD (routine cultivation), WL Nutrient agar (contains ranges of essential nutrients for their growth and metabolism), M1 (*Z. bailii* prefer fructose to glucose, M2 (fastidious xerophiles grow well in media with glycerol, M3 (*Zygosaccharomyces* genus

are lipophilic, Tween 80 solubilize and disperse the lipids), and M4 (modified TGY, for enumerating acid-resistant yeast, proved to be appropriate for this purpose.

To achieve the goal, different culture media, including (YEPD, WL Nutrient agar, M1, M2, M4, Modified M2 &M3, M2**ᵃ** & M2**ᵇ**, and Bismuth Sulfite Agar (BSA) were tested against 9 *Zygosaccharomyces* strains (five *Z. bailii*, three *Z. rouxii* strains and one strain of *Z. lentus*. To observe any macro and micromorphological differences among the investigated strains, an Olympus SZX7 Stereo microscope (for macromorphological studies) and an Olympus BX41 Fluorescence microscope (for micromorphological investigations) were used. MALDI-TOF MS was used for the identification of the yeasts.

Results of MALDI showed that most strains were not identified in the database, as the software was developed for clinical purposes, which was not appropriate for identification of food originated yeast strains. There was an observable difference in colony morphology of Z. *lentus* strains on the WL Nutrient agar. Also observed was the difference between *Z. bailii* NCAIM Y.00954^{T} and other *Z. bailii* strains on the WL Nutrient agar. Results on M1 showed no substantial differences in the colony morphology of the strains. M2 was too soft, and no clear colonies were observed. Thus, it was modified (M2) and the method of plating changed resulting in the positive growth of eight strains except for *Z. lentus* with no clear difference in colony morphology. Modified M3 resulted in poor growth of strains due to loss of moisture as a result of increased agar.

Modified M2 was further modified to M2**ᵃ** with 0.5%w/v glacial acetic acid added to lower the pH of the culture medium to make it more selective and to increase the likelihood of isolating the yeast species. However, there were no colonies formed on the plates. Modified M2 was further modified to M2**ᵇ** without using glacial acetic acid but with addition of 0.002%w/v bromophenol blue indicator to monitor the pH of the medium for the optimal growth of the strains and observe any changes based on their metabolic properties. The result revealed that *Z. bailii* strains can be differentiated from *Z. rouxii* strains. Also noted was a positive growth of *Z. lentus* while negative growth for other investigated strains on BSA. Therefore, WL Nutrient agar and BSA could be appropriate for the isolation of *Z. lentus* while M2**ᵇ** appropriate for isolating *Z. bailii* and *Z. rouxii* strains. As an important result it was possible to prove the fact of wrong identification in the case of *Z. rouxii* IV/3/7; it had been misidentified at the collection point. The results showed the applicability of WL Nutrient agar, M2^{b} medium, and BSA to be appropriate for isolation of *Zygosaccharomyces* species. I also suggested testing more yeast (mainly *Zygosaccharomyces*) isolates/strains from different origin to be able to determine the efficiency of the culture media used in this study.