MSC THESIS

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CHARACTERIZATION AND ANTAGONISTIC ACTIVITY OF YEAST AND BACTERIA ISOLATED FROM FRUITS

MASTER'S THESIS (MSC)

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SUMMARY

The research specifically aimed to select, characterize and identify antagonistic yeast and bacteria obtained from specific fruits by examining their morphological and biochemical traits, as well as their biocontrol efficacy against pathogens through contact method and finally identification by MALDI test and DNA sequencing. In this study, 23 strains isolated from specific fruits were screened and selected for their antagonistic activity against 7 spoilage yeast strains and 6 and 14 strains out of 23 isolated strains(60.8%) showed positive antagonistic activity against the tested spoilage microorganisms and were selected for further characterization and identification.

Initial cell and colony morphology characterization was done and the selected antagonists grouped into two types: yeast and bacteria, from this grouping we had 8 bacteria strains and 6 yeast strains. Yeast cells, characterized by their larger size, eukaryotic nature, and typically round to oval shape, contrasted with bacterial cells, which were smaller, prokaryotic, and exhibited various shapes such as bacilli or rod shape. Additionally, the appearance of yeast colonies on solid media differed from bacterial colonies in terms of texture, color, and overall morphology. Utilizing these evaluation techniques ensured accurate differentiation of yeast and bacteria, thereby facilitating various proceeding microbiological test and analysis for accurate differentiation and analysis.

Biochemical characterization of the antagonistic strains was carried out. For yeast strains, fermentation test, urease test and carbon assimilation test were carried out. All the six yeast strains showed no significant color change for the urease test thus showing negative results. Yeast typically do not test positive for the urease test. For fermentation 4 strains were fast fermenting while 2 strains slow fermenting. For carbon assimilation test, all the six strains showed positive reaction with glucose while only strain 4(2.Ha.1.1.w) and strain 12(Bc65.2.2) showed positive reaction with sucrose. All the strain showed negative reaction with lactose and raffinose. For the bacterial antagonistic strains, Gram staining, KOH test, Catalase test, Oxidase test and Urease test were carried out only strain 1(2MG.1.2) was gram. All the strains were catalase positive. Only strains 1(2MG.1.2) and 2(VC.1.2) showed positive reaction for oxidase test. Only strain 3 (VC.1.1) showed positive results for urease test.

Both MALDI test and DNA sequencing methods showed same identity for strain 1 (2MG.1.2) as *Pseudomonas japonica* , strain 11 (La.1.2) as *Bacillus mojavensis* with a high percentage identity of above 99%. Strain 12(Bc65.2.2) was identified as *Metschnikowia pulcherrima* and strain

13(Bc65.2.1) as *Hanseniaspora uvarum* by both methods however rDNA sequencing results showed low percentage identity of 96.60% and 95.96% respectively. Comparing and relating the identification results and the inhibition results, the study findings showed that strain 1 (2MG.1.2) identified as *Pseudomonas japonica* inhibited the growth of Y1 (*Zygosaccharomyces rouxii* 19), Y6 (*Candida parapsilosis* Y1011), Y7 (*Saccharomyces cerevisiae* CBS 1171), B5(*Escherichia coli* ATCC 8739) and B6 (*Listeria innocua* CCM 4030). Strain 11(La.1.2) as *Bacillus mojavensis* inhibited the growth of Y1 (*Zygosaccharomyces rouxii* 19), Y7 (*Saccharomyces cerevisiae* CBS 1171), B3 (*Staphylococcus aureus* 1755) and B6 (*Listeria innocua* CCM 4030). Strain 12 (Bc65.2.2) was identified as *Metschnikowia pulcherrima* inhibited the growth of B3 (*Staphylococcus aureus* 1755), B5(*Escherichia coli* ATCC 8739) and B6(*Listeria innocua* CCM 4030). Strain 13 (Bc65.2.1) as *Hanseniaspora uvarum* showed inhibition against B3 (*Staphylococcus aureus* 1755) and B4(*Pseudomonas aeruginosa* ATC 9027).

MALDI-TOF-MS is a good strategy for various identifications: bacteria, and yeast cultures. It is to date the fastest method, providing excellent results without prior extraction however as a limitation can only provide a result between two possible identifications. DNA sequencing was more effective as it gave elaborate identity of the sample strains giving all the matches within the same genera but different species. Thus, although DNA sequencing is an expensive approach, it's a more robust, accurate and elaborate method of species identification.