**NAME:** Oluwaseun Adeola **ADEWARA**

**MATRIC. No.:** 152728

**TITLE: STRESS RESPONSES OF LACTIC ACID BACTERIA AND YEASTS ISOLATED FROM SORGHUM GRUEL AND RETTED CASSAVA AND THEIR APPLICATION IN FOOD FERMENTATION**

**ABSTRACT**

Lactic Acid Bacteria (LAB) and yeasts are exposed to constant physical and chemical fluctuations in food matrix during fermentation, resulting in stress which often reduce the quality of food products. Microorganisms are known to develop adaptive responses to harsh fluctuations. However, these adaptive mechanisms are rarely investigated. This study was designed to investigate the physiological and proteomic responses of LAB and yeasts to acid and oxidative stresses as well as the application of stress-adapted LAB and yeasts in food fermentation.

Lactic acid bacteria and yeasts were isolated and identified from sorghum gruel and retted cassava using standard methods. The physiological responses of identified LAB and yeasts to acidic, hydrogen peroxide (H2O2) stresses and control were measured using turbidimetry method. Proteins (dehydrogenase enzymes and stress response proteins) extracted from the LAB and yeasts were separated using SDS-PAGE and two-dimensional gel electrophoresis. Induced and repressed proteins were identified by LC-MS. The best stress-adapted LAB and yeasts were selected as starters in single and in combination for the fermentation of sorghum gruel and retted cassava while spontaneous fermentation served as control. Chemical composition (Proximate, mineral, anti-nutritional) and sensory properties of the products were evaluated using standard procedures. Data were subjected to descriptive statistics and ANOVA at α0.05.

Sixty-four LAB and seventy-two yeasts isolated from sorghum gruel and retted cassava were identified as *Lactobacillus amylovorus* (13), *L. acidophilus* (8), *L. fermentum* (5), *Pediococcus pentasaceus* (6), *L. plantarum* (11), *L. brevis* (2), *L. paracasei* (7), *Leuconostoc pseudomesenteroids* (2), *Enterococcus faecalis* (10)*, Candida kefyr* (12), *C. glabrata* (19), *C. tropicalis ­*(12) and *Saccharomyces cerevisiae* (29). The optical-density of *Lactobacillus amylovorus*LS07 (0.235) and *C. kefyr*YS12 (0.367) from sorghum gruel adapted best to pH 1 and to 5mM H2O2 0.32 and 0.737 respectively. The optical-density of *Lactobacillus plantarum*LC03 (0.659)and *C. glabrata*YC02 (0.967) from retted cassava had best adaptation at pH 4 andto1mM H2O2 0.986 and 0.868 respectively. Bifunctional acetaldehyde CoA/alcohol dehydrogenase showed increased intensity at pH 4 and 5mM H2O2 in*L. amylovorus*LS07 while increased intensity of 6-phosphogluconate dehydrogenase was detected in *C. kefyr*YS12 at pH 4 and 1mM H2O2. *Lactobacillus plantarum*LC03 showed increased intensity of Elongation Factor Thermo unstable at pH 3 and 5mM H2O2. Increased intensity of enolase was observed in *C. glabrata*YC02atpH 4. These suggested increased microbial metabolism, which reduced stress encountered. Sorghum gruel produced with combined starters of *L. amylovorus*LS07 and *C. kefyr*YS12 had the highest crude protein (10.94%) and iron (85.50ppm). Cassava fermented with combined starters of *L. plantarum*LC03and *C. glabrata*YC02 recorded the lowest tannin (0.0007%), phytate (0.0078%), alkaloids (0.14%), cyanide (6.49ppm) and highest overall acceptability (7.92) which were significantly different from values obtained from spontaneous fermentation: 0.0018%, 0.0093%, 0.17%, 7.11ppm, 7.13, respectively. The combined starters yielded foods with improved sensory properties, mineral and reduced anti-nutrient contents.

Isolates from sorghum gruel (*Lactobacillus amylovorus*LS07 and *Candida kefyr*YS12) and retted cassava (*L. plantarum*LC03and *C. glabrata*YC02) showed increased protein production in the presence of acid and oxidative stress. Hence, the stress-adapted organisms as starters are encouraged in food fermentation.

**Keywords:** Stress adaptation, Induced and repressed enzymes, *Lactobacillus amylovorus*LS07, *Candida kefyr*YS12 and SDS-PAGE

**Word count:** 500