USE OF MULTIFUNCTIONAL BACTERIAL CULTURES FOR IMPROVING CHEESE QUALITY

By

AHMED MOHAMED MOAWED MABROUK

B.Sc. Agric. Sc. (Dairy Science and Technology), Cairo Univ., 2001

M.Sc. Agric. Sc. (Dairy Science and Technology), Ain Shams Univ., 2009

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This thesis for Ph.D. degree has been approved by:

Dr. Taha Abd EL-Haleem Abd EL-Aziz Nassib.....

Prof. Emeritus of Dairy Microbiology, Faculty of Agriculture, El Mansoura University

Dr. Rezk Azab Awad.....….....

Prof. of Dairy Science and Technology, Faculty of Agriculture, Ain Shams University

Dr. Zakaria Mohamed Rezk Hassan

…….....

Prof. of Dairy Science and Technology, Faculty of Agriculture, Ain Shams University

Date of Examination: 16/8/2016

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Under the Supervision of:

Dr. Mohamed Nabil Ibrahim Magdoub

Prof. Emeritus of Dairy Microbiology and Technology, Department of Food Science, Faculty of Agriculture, Ain Shams University (Principal supervisor)

Dr. Zakaria Mohamed Rezk Hassan

Prof. of Dairy Science and Technology, Department of Food Science, Faculty of Agriculture, Ain Shams University

Dr. Baher Abd EL- Khalek Effat

Researcher Prof. Emeritus of Dairy Microbiology and Technology, Department of Dairy Science, National Research Center

ABSTRACT

Ahmed Mohamed Moawed Mabrouk. Use of Multifunctional Bacterial Cultures for Improving Cheese Quality. Unpublished Ph.D. Thesis, Department of Food Science, Faculty of Agriculture, Ain Shams University, 2016.

In recent years, extensive searches have been carried out on the lactic acid bacteria (LAB) for their potential use as probiotics and biopreservatives in food and dairy industries. Nowadays, probiotic bacteria play an important role in the majority of food supplementation and extended shelf life of fermented foods. Isolation and identification of LAB from fermented dairy products gained more attention allover the world. So, total of 75 strains of LAB were isolated from local fermented dairy products. These isolates were characterized morphologically, physiologically and biochemically by using analytical profile index (API) tests. Analytical profile index (API®50 CHL) system was used for biochemical identification of *Lactobacillus* (Isolates grown on MRS agar) , while analytical profile index (API®20 CH) strep system was used for identification of *Streptococcus* (Isolates grown on M17 agar). From seventy five isolated strains only eight strains were chosen as follows: *Lactobacillus rhamnosus* (1 strain), *Lactobacillus plantarum* (1strain), *Lactobacillus pentosus* (2 strains), *Pediococcus pentosaceus* (2 strains), *Lactobacillus brevis* (1 strain) and *Lactoccocus lactis* ssp. *Lactis* (1 strain). These selected strains were proceeded to screen them in vitro for probiotic traits (tolerance to different concentrations of bile salts, low pH resistance, growth and viability % in different concentrations of NaCl, phenotypic safety assessment and antibacterial activity against five indicator pathogenic strains (*Bacillus cereus, Staphylococcus aureus, and Escherichia. coli* 0157:H7, *Listeria monocytogenes* Type I and *Pseudomonas aeruginosa*). Chosen strains were also examined for some technological properties like acidifying activity, exopolysaccharides (EPSs) production, survival in different concentrations of NaCl and milk coagulation activity. The results revealed that all chosen strains were

observed to have good antagonistic activity against tested indicator pathogens with differences in diameter of inhibition zone (mm). Meanwhile, *Pseudomonas aeruginosa* and *Listeria monocytogenes* type I were not inhibited by the extract of *Pediococcus pentosaceus* NRC AM 1 and *Pediococcus pentosaceus* NRC AM 4. According to the results of technological and probiotic properties of examined eight lactic acid strains, there were various behaviours in acidifying activity of reconstituted sterile skim milk medium and most strains had good ability to produce lactic acid and coagulate the milk. So, the good strains would be a candidate in the dairy fermentation process as starter cultures, whereas, the poor acidifier strains can be used as probiotic or adjunct cultures. The viability of selected isolated strains grown in MRS broth supplemented with different concentrations of NaCl were significantly affected ($P<0.05$) and all viabilities gradually decreased by increasing NaCl concentration. Only three strains *Lb. pentosus* NRC AM8 strain *Lb. pentosus* NRC AM5 and *Lb. plantarum* NRC AM7 were able to resist the concentration of 10 % NaCl. From the obtained results in the screening of producing exopolysaccharides (EPSs**),** many isolated strains showed ability to produce and synthesize EPS in vitro. Furthermore, all the tested strains were able to grow and survive well in MRS supplemented with different concentrations of bile salts, and the high resistant ability was observed with the strains *Pediococcus pentosaceus* NRC AM 4 and *Lactococcus lactis ssp. lactis* NRC AM3 respectively. All tested strains were significantly affected ($P<0.05$) by exposure to pH 2 for 5 hours. On the other hand, no blood haemolysis (B-hemolysis) was detected for any tested strains. From this part, only four strains *e.g. Lactobacillus brevis* NRC AM2, *Pediococcus pentosaceus* NRC AM4, *Lactobacillus rhamnosus* NRC AM6, *Lactobacillus pentosus* NRC AM8 were selected and confirmed by using 16S rRNA gene. The results confirmed that *Lactobacillus brevis* NRC AM2 was identified as *Lactobacillus casei* NRC AM2, *Pediococcus pentosaceus* NRC AM4 and *Lactobacillus rhamnosus* NRC AM6 were identified as the same and *Lactobacillus*

pentosus NRC AM8 was identified as *Pediococcus acidilactici* NRC AM8. The confirmed strains were interesting for using in dairy products. Probiotic low salt soft cheese was manufactured by using one of the selected strains *e.g. Lactobacillus casei* NRC AM2, *Pediococcus pentosaceus* NRC AM4, *Lactobacillus rhamnosus* NRC AM6, *Pediococcus acidilactici* NRC AM8. The control treatment was manufactured without adding any starter culture. The cheese treatments were manufactured from heat treated cow's milk (80^{o} C for 5 min.). The resultant cheese treatments were evaluated for chemical, bacteriological and organoleptic properties during refrigeration storage period at 7^{o} C for 28 days. Statistical analysis showed that the type of starter and/or time of storage period significantly affected the cheese analysis and sensory evaluation of all cheese treatments. The treatment containing the strain of *Lactobacillus rhamnosus* NRC AM6 had the highest score and acceptability among all probiotic low salt cheese treatments.

Key Words:

Lactic acid bacteria, multifunctional bacterial cultures, biochemical identification, antibacterial activity, indicator strains, 16S rRNA gene and low salt soft cheese.

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