

Department of Botany and Microbiology

**Studies on production of antimicrobial substances
and enzymes from probiotic bacteria**

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5. SUMMARY

There has been a growing interest during the last decades in developing new natural compounds safer for human use than those chemically synthesized. Moreover, a great awareness was subjected to the importance of products from Lactic acid bacteria with antimicrobial activity and to the value of using probiotics in maintaining human health and preventing many diseases.

The present work aims to study the isolation of most potent LAB from different dairy product sources from different regions in Egypt, which have the ability to produce antimicrobial substances and enzymes. In addition to screening strains that able to tolerate the human GIT harsh conditions as pH tolerance, bile salt tolerance, phenol, and other important parameters. After the determination of most potent probiotic isolates, these isolates were identified by phenotypic and genotypic methods also, examined the autoaggregation and coaggregation ability. Optimization of different parameters to obtain highest antimicrobial effect against pathogens (*S. aureus* and *C. albicans*) was also done.

The data obtained in this work can be summarized in the following points:

1. Different fermented dairy product and mother milk were collected from different regions in Egypt. The isolation LAB was done using MRS agar medium.
2. The isolated strains were purified, and characterized morphologically to select nineteen isolates
3. The nineteen selected isolates were examined for their antimicrobial activity against the pathogenic microorganisms. The cell free supernatant of this bacterium inhibited the growth of *Candida albicans*, *Staphylococcus aureus* and *pseudomonas aeruginosa* but it can't inhibit *Eshericia coli*.
4. The antagonistic ability of the seven isolates were tested and resulted in a clear inhibition zones obtained due to producer LAB strains isolated from mother milk and cottage cheese (kariesh cheese) that isolated from El-bahera, Egypt against another sensitive LAB isolated from cottage cheese (kariesh cheese)that isolated from Alexandria, Egypt.

5. Physiologically and biochemically characterization were performed for the seven isolates to find *Lactic acid* strains, were identified by phenotypic and genotypic methods. For the phenotypic characterization, morphologic examination, catalase enzyme test were applied, resistance to different temperatures and salt concentrations, gas production from glucose and determination of sugar fermentation profiles were applied. Only two of the seven isolates covered all the expected *Lactic acid* strains results, it was gram positive, catalase negative, tolerated high temperature and more than 4% NaCl tolerant. The two isolates have the ability to ferment glucose, lactose and sucrose sugar by TSI test.
6. Characterization and determination of probiotic properties of Lactic Acid Bacteria to tolerate the GIT conditions, different tests were applied such as resistance to low pH, NaCl, bile salt, phenol, and pancreatine tolerance, only two of the seven selected isolates showed tolerance to acidic pH (3.0), bile salt (3%), NaCl (4%), Pancreatine (0.5%), and phenol (0.2%), each property tested at different concentrations.
7. One of the most important probiotic characteristic is the hemolytic activity. The two tested isolates showed α -haemolytic result, were considered a non-hemolytic result.
8. The most potent two isolates were identified genotypic methods. The molecular identification, based on 16S rRNA gene was performed and 16S DNA sequencing was applied, to be identified as *L.acidophilus* LA-G80-11 and *L.plantarum* TMW 1.1623 with the accession number CP054559 and CP017383 respectively.
9. The results of susceptibility studies of the *L. acidophilus* isolate tested against different types of antibiotics showed that it was susceptible to Ampicillin, Pencillin of the β -lactam group of antibiotics, Erythromycin of the macrolide group, Ciprofloxacin of quinolones and the Tetracycline. In addition, the isolate were also resistant to a large number of antibiotics which include Vancomycin and Cefoxitin, while *L. plantarum* isolate showed a resistance results to all tested antibiotics.
10. The autoaggregation ability showed a result of 52.3, 65.32% for *L. acidophilus* and *L. plantarum* respectively. The ability was necessary for the adhesion to intestinal epithelial cells, while the ability to coaggregate with other bacteria such as pathogens may form a barrier that prevents colonization by pathogenic

microorganisms exhibited a highly significant results 73.23, 81.78% for *L. acidophilus* and *L. plantarum* respectively against *S. aureus*, also exhibit 78.54, 79.22% for *L. acidophilus* and *L. plantarum* respectively against *Candida albicans* after 24h of incubation at 37°C.

The hydrophobicity is the affinity of microorganisms to a solvent, and it was tested using ethylacetate, chloroform, xylene solvents, and the isolates showed higher hydrophobicity with ethyl acetate 88.1 and 82.8% with *L. acidophilus* and *L. plantarum* respectively. Also showed high results with chloroform 78.57 and 80% with *L. acidophilus* and *L. plantarum* respectively, and xylene solvent showed 73.81 and 75.6% with *L. acidophilus* and *L. plantarum* respectively.

2. The present study also revealed that *L. plantarum* is a potential probiotic bacteria and produced amylase and protease enzyme where the Amylolactic acid bacteria can be recommended in the development of cereal based foods, fermented foods such as bread, dumplings and non-alcoholic beverage production. The combination of starch and amylo-lactic acid bacteria is a cost effective fermentation process.

13. The *L. acidophilus* showed the ability to produce high concentration of lipase enzyme, there is various industrial applications of microbial lipases in the detergent, food, flavour industry, biocatalytic resolution of pharmaceuticals, esters and amino acid derivatives, making of fine chemicals, agrochemicals, use as biosensor, bioremediation, cosmetics and perfumery

In conclusion, the results of the present investigation collectively indicate that the *L. acidophilus* and *L. plantarum* which are probiotic strains occurring in human dairy food, and also naturally occurring in the human mother milk, have a potential and promising antimicrobial activities, therefore can indicate the possibility of using *L. acidophilus* and *L. plantarum* in therapeutic and prophylactic fields as natural and biological sources of antimicrobial substances, *L. acidophilus* and *L. plantarum* also have the ability to produce different extracellular enzymes as amylase, lipase, and protease. That enter in different industrial fields.

Finally, many studies are now in progress on the applicability of probiotic bacteria as an alternative biotherapeutic treatment for and protection against pathogenic infections.