EFFECT OF GALTO OLIGO SACCHARIDES ON ENCAPSULATED LAB AND ITS PROBIOTIC EFFICACY

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Abstract

Lactic acid bacteria are the predominant microbial group in fermented products, which play an important role in fermentation processes. The LAB used in commercial starter cultures possesses numerous metabolic characteristics such as production of organic acids, aroma compounds, bacteriocins and exopolysaccharides. Our reports show high values of hydrophobicity, coaggregation as well as a strong biofilm production of *Lactobacillus* sp was recorded . Various immobilization/encapsulation techniques have been proposed and tested for application in functional food production. In the present research, the use of Cissus quadrangularis as prebiotic with probiotic microorganisms was found to be beneficial and LAB become more active. . Subsequently, immobilization/encapsulation applications in the food industry aiming at the prolongation of cell viability are described together with an evaluation of their potential future impact, which is also highlighted and assessed. The total Polysaccharide was estimates Cissus quadrangularis acted as prebiotic and stimulate or suatain the viability of cells. High values and the strongest biofilm production was also recored in chitosan immboilzed cells. The *Lactobacillus* strains and pathogenic strains tested could all auto aggregate. Auto aggregation increased as a function of time and was highest at the 24 h time point.A moderate coaggregation and hydrophobicity was registerd at non immobilized cells and starch based polymers. The auto aggregative percentages of all pathogenic isolates ranged between 10 and 16 %, which is 9- fold below the range for the *Lactobacillus* isolates (between 99 %) after 24 h in the same conditions. These results demonstrate that there is relationship between auto aggregation and adhesiveness ability of *L. acidophilus* also showed strain-specific co aggregation abilities with the tested indicator strains. It has been suggested that coaggregate with pathogens, may constitute an important host defiance mechanism against infection by inhibitor producing LAB, which. Biofilm formation by secretion of exopolysaccharide (EPS) was observed.

Key: Lactobacillus, Biofilm, Immobilization

INTRODUCTION

Bacteria to survive in human gastrointestinal tract to delivered in food system and must be acid and bile tolerant. Lactic acid bacteria (LAB) are the most commonly used probiotic microorganisms due to their beneficial effects on the gastrointestinal tract.They are recognized as very potential bacteria and are also they remove the harmful bacteria from the intestine

Therapeutic benefits have led to an increase in the incorporation of probiotic bacteria such as lactobacilli and bifidobacteria in dairy products, especially yogurts.Probiotic survival in products is affected by the factor includes post-acidification during products fermentation, a range of factors including pH and, hydrogen peroxide production and storage temperatures (Krasaekoopt et al., 2014)INn an approach currently receiving considerable interest of probiotic living cells with a physical barrier against adverse conditions (Favaro-Trindade et al.,2011). Many lactic acid bacteria they produce insufficient exo-polysaccharides to be able to encapsulate themselves fully even synthesiseexo-polysaccharides, but they produce insufficient exo-polysaccharides to be able to encapsulate themselves fully (Shah, 2000).

MATERIAL AND METHODS

Sample collection

The curd sample was collected from the local market for thr production of lactic acid bacteria and regular sub culturing. After isolation the culture was strained with grams staining and the biochemical test such as Indole test,Methyl red, Voges-Proskauer test, Catalase test, Oxidase test was done for identification of *lactobacillus* bacteria.

Isolation of polysaccharides

20 g of dried sample *Cissus quadrangularis*dissolve 20 ml distilled water andheated at 50^{o} C for 30 MIN. The aqueous phase filtered and mixed with 20 ml ethanol and kept under refrigeration overnight. The precipitate was collected by centrifugation and sugar was estimated.

Estimation of reducing sugar by DNS method

Seven test tubes are taken and marked as blank , S1, S2, S3, S4, S5 and T1 respectively . blank and T1 test tubes contains 1ml of water and 0.4ml of polysaccharide respectively. Glucose solution of 0.2ml, 0.4ml, 0.6ml, 0.8ml, 1ml is added into S1, S2, S3, S4, S5 test tubes. And the glucose solution in the test tubes is maked up to 1ml by adding water. Then 2ml of DNS reagent is added into the each test tubes . the test tubes are subjected to heating process in the heating mantle at 75^{0}c for 10 minutes. After heating, 1ml of sodium potassium

tartarate solution is added each tubes observed is for the colour changes and the OD value is taken.

Immobiliztion

Freshly grown lab was centrifuged and the cell pellete was diluted in pbs to obtain an od 1 at 600 nm. about 1 ml of lab cell suspension was mixed with polymers for entrapment. the entrspment of cells were done Group 1:0.3% alginates in cacl2,Group 2:05 % chitosan in 0.5 m NaOH,Group 3: alginate+ starch,Group 4:Alginate+ OGP ,Group 5:Chitosan+Starch,Group 6: Chitosan+ OGP.

All the entrapped cells maintained in phosphate buffer for 72 h and cells were recovered and allowed to grown on MRS. The non immobilized cells and immobilized LAB probiotic potential was studied by Biofilm and co aggregation test

Biofilm Assay

The quantification of biofilm production for immobilized and naïve cells were performed as described previously by Borges et al. (2012) with some modifications. The wells of a sterile 12-well polystyrene microtiter plate were filled with 2 ml of MRS broth, absorbance (A600 nm) of bacterial suspensions in MRS was adjusted to 0.25 ± 0.05 in order to standardize the number of bacteria (107–108 CFU/ml) and 200 µl of overnight was added to each well. The plates were incubated aerobically for 48 h at 30°C. To quantify the biofilm formation, the wells were gently washed three times with 2 ml of sterile distilled water. The attached bacteria were fixed with 2 ml of methanol for 15 min, and then, microplates were emptied and dried at room temperature. Subsequently, 2 ml of a 2% (v/v) crystal violet solution was added to each well and held at ambient temperature for 5 min. Excess stain was then removed by placing the plate under gently running tap water. Stain was released from adherent cells with 2 ml of 33% (v/v) glacial acetic acid. The optical density (OD) of each well was measured at 595 nm using a plate reader

Aggregation and co aggregation

Five test tubes were taken and labelled as T1, T2, T3, T4, T5 respectively. 2ml of *pseudomonas* and *lactobacillus* suspension was added into the T1. The bacterial suspension of *klebsiella* and *lactobacillus* was added into T2. *E.coli* and *Klebsiella* sp alone was taken in T3 and T4. *Lactobacillus* alone was taken in T5. All the the cultures were previously adjusted to OD 1 at 600 nm to get an uniform cell suspension. The tubes were allowed to kept

undisturbed for 24 hrs and were observed for aggregation and the OD values of aqueous are taken for 5 and 24 hours.

Surface hydrophobicity

The test was determined using the microbial adhesion to solvents (MATS) method of Bellon-Fontaine. Briefly, overnight cultures of *Lactobacillus* strains were harvested by centrifugation at 5,000 × *g* for 10 min, washed twice, and resuspended to an OD_{600} of 1 (A_0) in PBS. Five milliliters of bacterial suspension was mixed by 2 min of vortexing with 2 ml petroleum ether (the solvent) in a 10-ml glass tube. The tubes were incubated statically for 15 min at room temperature to allow phase separation of the mixture. The aqueous phase was collected, and its OD_{600} was measured (A_1). The cell surface hydrophobicity (CSH) is presented as the percentage of microbial cells retained in the solvent and calculated as

$$[1 - (A_1/A_0)] \times 100.$$

RESULT AND DISCUSSION

From the curd sample **(plate 1)** pure bacterial colonies were isolated. Bacteria in plate 1b were observed by compound microscope followed by gram and capsule stain. It is clear that the bacteria was gram positive, non capsulated, rod shaped coccobacilli **(plate 2),** occurring singly or in chains. The results indicated that the isolated bacteria could be identified as *lactobacillus acidophilus through* the gram staining From the fresh raw milk, commercial liquid milk and yoghurt samples. LAB were enumerated the total viable aerobic cured samples showed higher LAB at aerobic conditions. Aerobic bacterial count was 7.2×10^6 CFU. For example ,The cow's, goat's and sheep's milk samples in Western Algeria that shows lower than total LAB count in milk and yoghurt .Traditional koopeh cheese in Iran, camel's milk,yoghurt, and goat's yoghurt reported for the Similar LAB count under aerobic condition were also reported by Kacem (2011).

Cissus quadrangularis:

Plate 3 shows *Cissus quadrangularis* processing and its sugar was estimated as 28 mg/ml. the presence of sugar was observed by reduction of DNS reagent (**plate 4**). Cells were immobilized successfully with alginate and chitosan (**plate 5**) Growth rate of isolated LAB was found to be moderate at static and hihgh at 150 rpm with an OD 0.2 and 1.5 at 600 nm

(**Plate 3**). Further immobilized group 6 immobilized cells in chitosan along with extracted polysaccharide showed enhanced growth and maximum growth of 2.7 was observed under 150 rpm with in 20 h. addition of starch to alginates and chitosan showed a significant chabges in growth of LAB.

Growth rate on immobilized and non immobilized cells:

Figure 1 clearly states that the LAB entered log phase within 12 h and maintained up to 20 h. Lactobacilli tested in this study showed percentage of autoaggregation around 72 % after 5 h of incubation and 97% at 12 h incubation (**Table 1**). Similarly auto aggregation of test pathogens were 20 and 10% respectively for *Pseudomonas s*p and *E.coli***(plate 6**). Probiotic organisms are considered to support the host health but with that it needs to be safe for humans. The safety of the probiotic products is appraised with the phenotypic and genotypic characteristics and the statistics of used microbes. Safety aspects of probiotic bacteria include the prime requirement that they have to be non-pathogenic and capable to co aggregate. The auto autoaggregation is an important bacterial feature in several ecological niches, especially in human and animal mucosa, where probiotics display their activities.

Immobilized LAB cells and Growth on MRS:

The ability to auto aggregate (form floccules) is a crucial factor for the maintenance of significant counts of the probiotic strain in the adverse conditions present in the oral cavity and the gastrointestinal and urogenital tracts (Nikolic et al., 2010). In general, Lactobacilli have an autoaggregation capacity ranging from low to moderate. In the present study, lactobacilli showed moderate auto aggregation close to or above those found for lactobacilli isolated from other fermented foods, including cocoa. The efficiency of auto aggregation followed by immobilized cells were moderately enhanced and 10 fold increase was observed among all tested immobilized LAB cells. The percentage of co aggregation was maximum at prebiotic supplanted (*Cissus quadrangularis)* followed by starch and less significant at starch as prebiotic. . The best co aggregation was found in chitosan immobilized cells followed by alginate under shaking condition (**Table 3**)Similarly *L. fermentum* strains isolated from fermented Chinese products presented autoaggregation ranging from 0.86 to 65.15% (Ramos et al., 2013). The extracted polysaccharide was estimated as 25mg/ml and 36mg/ml under static and shaking incubation (**plate 5**)

Biofilm formation(non and immobilized groups):

Together with autoaggregation, is considered an important bacterial surface feature and can be classified into 3 categories: low (MATH < 35%), medium (35% < 60%), or high (MATH > 60%). Hydrophobicity, also known as microbial adhesion to hydrocarbons (MATH),In this study, hydrophobicity was evaluated by the microbial adhesion to xylene (an apolar solvent) and, after 12 h of incubation results obtained and highly hydrophobic (75 %). Where as 82% of hydrophobicity was recorded at *Cissus quadrangularis* added chitosan polymer trapping. These values of hydrophobicity are much higher than those found for other lactobacilli isolated from cured (Bouchard et al., 2015). Testing a strain of *L. fermentum* and three strains of L. *plantarum*, obtained hydrophobicity values ranging from zero to 1.4% reported by Santos and coauthors. The result obtained in aggregation, co-aggregaton and hydrophobicity, tests correspond with previous works like García-Cayuela et al. (2014) a connection between hydrophobicity of cell surface and bacterial attachment, colonization, and biofilm formation. In the present study, the selected strains showed coaggregation abilities with the indicator strains tested but the percentages differed, depending on specific combinations of strains. Tested LAB isolates showed biofilm formation and was compared with a standard biofilm forming *Klebsiella pneumoniae*. The Biofilm formation was 25, 40, 72 and 76% respectively for GP 3 to GP6 at 24 h. no less than 10% biofilm activity was recorded among starch and non immobilized cells. maximum biofilm showed 95% biofilm formation which showed it as a potential probiotic (**plate 7**).Thus, biofilm formation considered a beneficial property by probiotic bacteria , because it could promote colonization and longer permanence in the mucosa of the host, avoiding colonization by pathogenic bacteriav . It has also been demonstrated that the EPS produced by some biofilm forming strains is able to inhibit the formation of biofilms by certain pathogenic bacteria (**Ramos** et al., 2012)

Plate 1:Bacteria isolation from crude sample

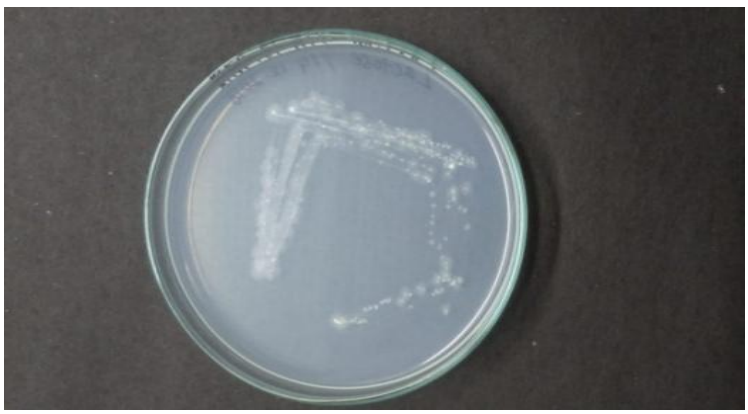


Plate:2 Grams stainiong and negative staining

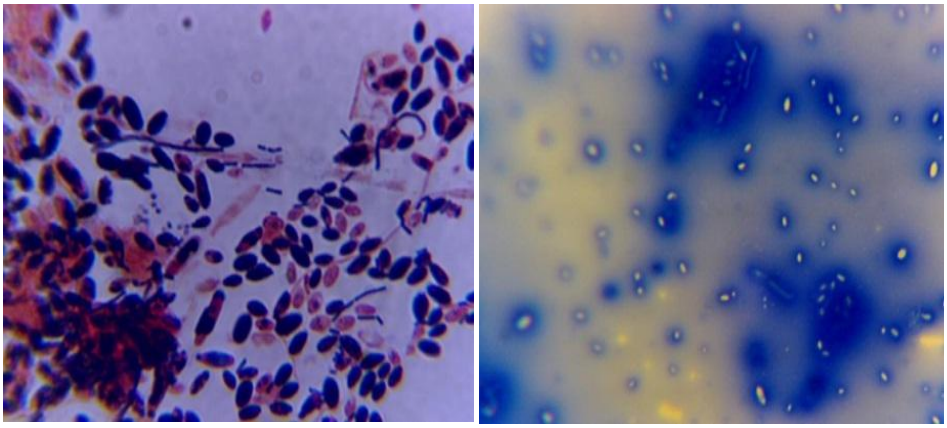


Plate : 3 *Cissus quadrangularis*

Plate:4 DNS sugar estimation

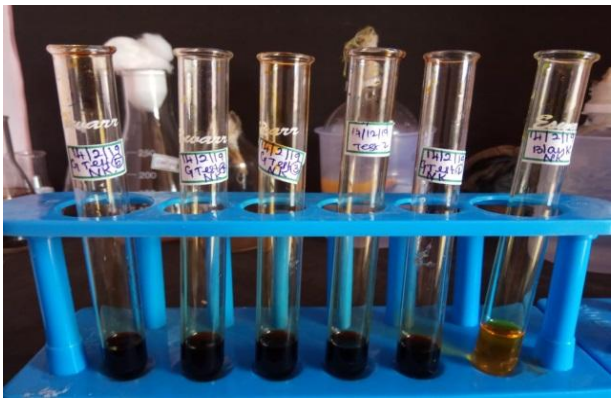


Plate :5 Immobilized LAB cells and Growth on MRS

Plate : 6 Auto aggregation and co aggregation test

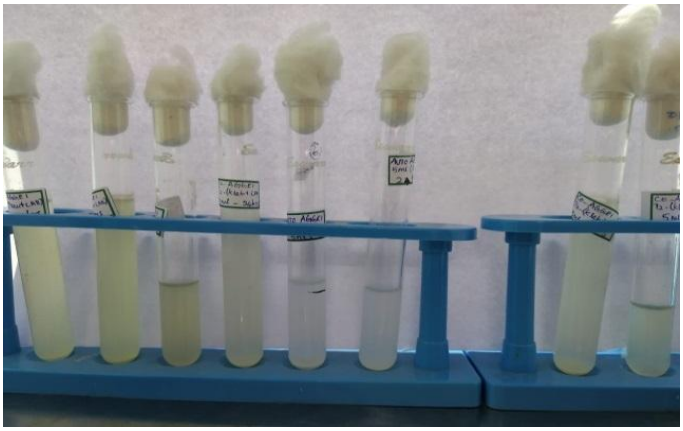


Plate : 7 Biofilm formation

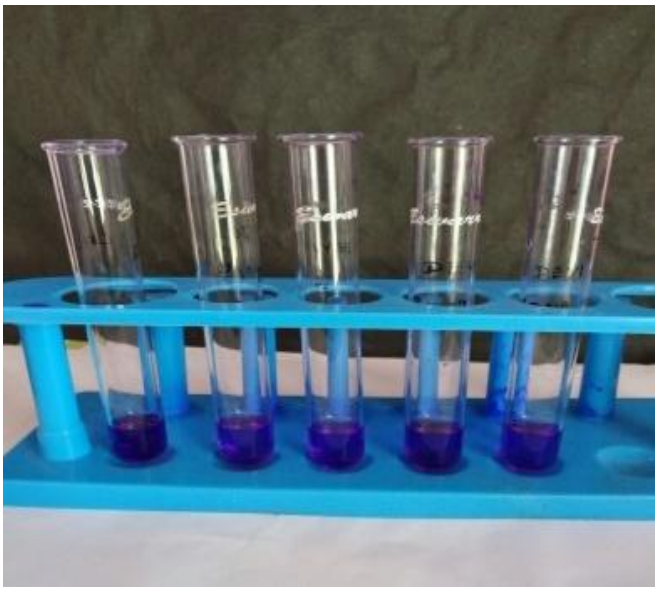


Table 1:Auto aggregation test

S.No	Organism	OD	Auto aggregation percentage
1.	*Pseudomonas* sp	0.8	20
2.	*E.Coli*	0.92	10
3.	*Lactobacillus acidophilus*	0.03	97

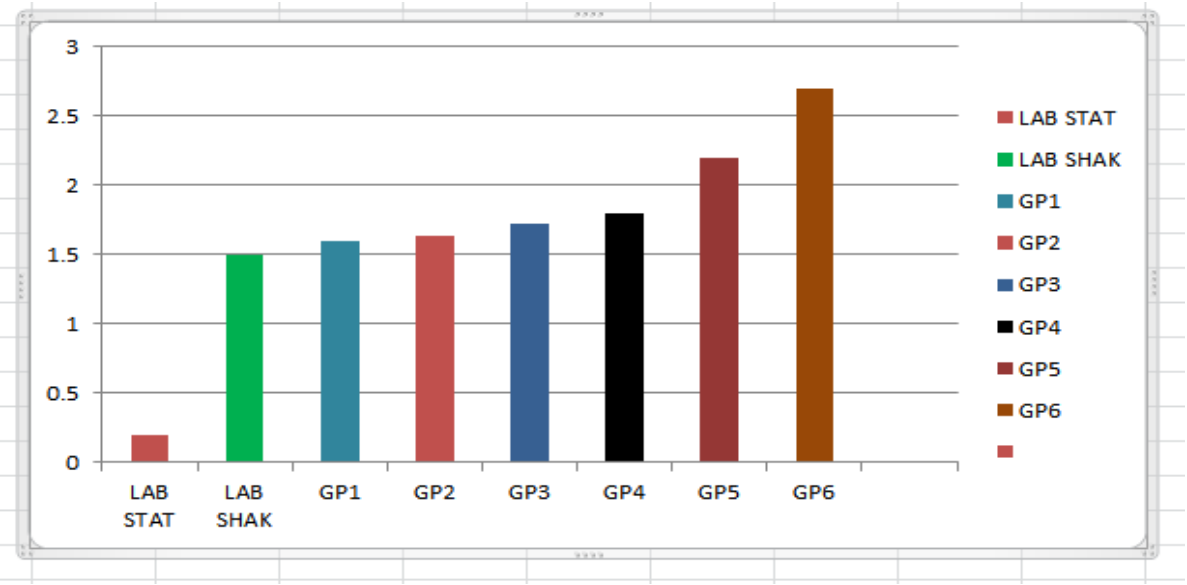
Table 2: Co-Aggrigation Test

TEST ORGANISM	% OF CO-AGGRIGATION	
	5 hrs	24hrs
E.coli +LAB	74	77.34
Klebsiella sp+LAB	74	78.33

Table 3: Immobilization effect on Co aggregation

Treatment	% OF CO AGGRIGATION
	5 hrs
Alginate immobilized	41.00
Chitosan	61.62
Alginate+starch	29.72
Alginate+OPS	78
Alginate+starch	36
Chitosan+OGP	82

Figure 1. Growth rate on immobilized and non immobilized cells



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