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Evaluation cell surface hydrophobicity of four potential probiotics Lactic acid bacteria's isolated from local dairy products

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Abstract

The purpose of this study was to investigate the cell surface hydrophobicity of four Lactic acid bacteria (LAB) strains isolated from various traditional and industry fermented yogurt and cheese sources based on the biochemical tests. The determining cell surface hydrophobicity is a critical step in selecting LAB strains with probiotic qualities and is one of the most essential elements regulating LAB's particular adherence to abiotic surfaces, as well as one of the most critical factors controlling LAB's absorption and destruction of hydrophobic organic matter. In current study, the four LAB strains had high cell surface hydrophobicity to the four separate hydrocarbon solvents xylene, hexadecane, chloroform, ethyl acetate determined via spectrophotometer at 450 nm. The highest hydrophobicity score was determined for LB3 for chloroform, n- hexadecane, ethyl acetate and xylene at 66.51, 38.23, 33.67 and 24.18% respectively. LB3 demonstrated promising cell surface characteristics, suggesting that it could be used as an indigenous probiotic.

***Keywords*:** Probiotic, adherence. xylene, hexadecane, chloroform, ethyl acetate.

1. Introduction

Historically, the knowledge and discovery of probiotics are associated with commonly consumed fermented foods, and it was approved that probiotic strains have been transferred to us since the first food raw materials were subjected to fermentation process (Ołdak et al., 2020). Cultured dairy products were probably the first functional food supplemented with probiotics. They are the best carrier of probiotic strains in the production of dairy products (Khojah et al., 2022). Lactic acid bacteria (LAB) species are gram-positive, anaerobic, catalase-negative, and motile bacteria (Sharma et al., 2021). They are well known as probiotics, and they play a key role in biotechnological products such as cheese, yogurt, and bread (Yerlikaya, 2019). LAB bacteria also play a significant role in food preservation by producing antimicrobial compounds such as lactic acid, diacetyl, hydrogen peroxide, and bacteriocins, which have been found in dairy starter cultures and could be used as food preservatives (Samet and Icen, 2022).

Furthermore, probiotic bacteria provide essential health benefits, such as enhancing gut microbiota balance and fighting pathogenic bacteria, stimulating the immune system, lowering blood cholesterol levels, producing vitamins (particularly vitamin B group) and antibacterial action. Numerous studies showed that LAB isolated from different kinds of food can effectively inhibit the growth of *S. aureus*. Anti-bacterial activity of LAB is mainly connected with the pH lowering and organic acid production and also with the possibility of bacteriocin synthesis and other antimicrobial agents such as hydrogen peroxide, reuterin or reutericyclin, and peptidoglycan hydrolases (Ołdak et al., 2020).

They are also employed as therapeutic bacteria due to limitations in traditional cancer therapies and other disorders (Sedighi et al., 2019). The most interesting applications of modern Lactococcus lactis (*L. lactis*) is as an antigen factory, allowing the bacteria to behave as live vaccines. In the last two decades *L. lactis* has emerged as a good alternative expression system to *E. coli* (Frelet-Barrand, 2022). The use of LAB as vaccine carriers is tempting because they can generate mucosal and systemic immune responses. When it comes to vaccine development, *L. lactis'* ability to surface display antigens makes it the preferable host with higher immunogenicity than intracellularly produced counterparts (Song et al., 2017).

Lactococcus lactis subsp. is commonly found in naturally fermented dairy products (Bandyopadhyay et al., 2022). This subspecies is of high economic value due to its wide application in dairy industry. However, the genetic background and evolutionary history of *L. lactis* subsp. lactis are still poorly understood (Liu et al., 2022). Lactococcus lactis, a Gram-positive bacterium, emerged at the beginning of the twenty-first century as a good alternative to the functional expression of prokaryotic and eukaryotic MPs.

The important criteria used in the selection of probiotic strains, include hydrophobicity, tolerance to gastrointestinal conditions (acid and bile), aggregation, antimicrobial activity against pathogenic bacteria, sensitivity to antibiotics and lack of pathogenicity (Bhushan et al., 2021). Bacterial cell surface hydrophobicity is defined as adhesion to a non-polar solvent (xylene). The affinities to a basic (ethyl acetate) and an acidic (chloroform) solvent reveal the electron acceptor and electron donor properties of bacteria's cell surfaces, respectively. Lewis' acid-base and carboxylic group interactions resulted in this outcome (Kos et al., 2003; Khojah et al., 2022). Because the usage of probiotic bacteria has been more interested with their beneficial effects in the gastrointestinal tract, it is important to investigate basic factors that influence on physicochemical cell surface and adhesive features of selected

probiotic strains. Bacterial hydrophobicity was determined to evaluate the attachment properties of microorganisms to the hydrocarbon surface which is a measure of adhesion to epithelium cells in gut (Yadav et al., 2016). The hydrophobic character depends on the strain and organism specify and is affected by different factors such as aging, chemical structure of the surface, even composition of culture medium and experimental method (Samet and Icen, 2022; Marin et al.,1997).

The aim of this study was to determine one of most important characteristics used in the selection of probiotic bacterial strains which is cell surface hydrophobicity bacterial strains. Cell surface hydrophobicity of local isolated bacteria was evaluated by measure the adherence to four separate hydrocarbon solvents xylene, hexadecane, chloroform, ethyl acetate.

2. Material and methods

2.1. Bacterial isolation and identification

This studied was carried out in the Industrial Microbiology Dept-Directorate of Agricultural Research from May 2021 to March 2022. Samples were collected from different traditional and industry fermented yogurt and cheese sources purchased from Al -Krada local market in Baghdad. Traditional samples as laben arab and cheese arab whereas industry samples as Activa yogurt, canon yogurt and Almzrha cheese. 10% diluted samples were plated on MRS agar plates containing 3% $CaCO_3$ (wt/vol). The plates were incubated at 37 ºC for 24–48h. The colonies with distinct morphology were picked and purified by further sub-culturing morphological and physiological assessment to identify potential LAB strains (Sharma et al., 2021). The morphological characterization for the strains was performed through Gram staining kit (Hi-Media, India). The cultures were examined under a bright field microscope (Olympus, Japan). The strains were then investigated for the presence of catalase enzyme using 3% H_2O_2 (Reiner, 2010).

2.2. Cell surface hydrophobicity

The cell surface hydrophobicity was determined using xylene, ethyl acetate and chloroform according to the method described by Kumari et al. (2022). Bacteria were cultured overnight and 3 ml of each culture was divided to different sterile falcon tubes. Falcon tubes were centrifuged at 4000 rpm at 4°C for 10 minutes. Washed 3 times with 5 mL phosphate buffer solution (pH=6.5). Initial cell absorbance value was determined using spectrophotometer at 450 nm. approximately 0.4. Then 0.6 ml of n-Hexane, chloroform, ethyl acetate and Xylene were added on the bacterial suspension slowly. The mixed solution was put into the water bath at 37 °C for 15 minutes within vortexing per 2 minutes. Then keep at room temperature for 25 minutes without agitation to split into two layers and separate the aqueous and organic phases. The absorbance value of aqueous phase was determined via spectrophotometer at 450 nm. Their results were recorded and percent hydrophobicity was calculated by the using following formula:

Hydrophobicity % = ((OD N0-OD N1) / ODN0) x100

where OD N1= is the absorbance value for late bacteria concentration after applying chemicals and ODN0= is the absorbance value for initial bacteria concentration before applying chemicals.

2.3. Data statistics

The data statistical analysis was performed by using SPSS 19.0 software. The comparisons of differences between the means of the treatments were tested by one-way ANOVA tests at a significance level of $p<0.05$.

3. Results and discussion

3.1.Bacterial isolation and identification

Four LAB strains were isolated from traditional and industrial fermented yogurt in Baghdad. Based on the biochemical tests, all isolates showed Gram-positive, catalase-negative characteristics (Fig.1; Table1). All isolates were studied for their morphological, physiological characteristics. Based on the temperature (37 ºC) and pH (5).

lactic acid bacteria strains were isolated: -

1-Lactobacillus LB1 showed Gram-positive, catalase-negative, cream color, round, edges colony morphology and single or paired, short chain microscopy.

2- Lactococcus LC2 showed Gram-positive, catalase-negative and cream color, round, edges clear colony morphology and spherical or ovoid cells microscopy.

3-Lactobacillus LB3 showed Gram-positive, catalase-negative, yellowish color, round, edges colony morphology and single or paired, short chain microscopy.

4- Lactobacillus LB4 showed Gram-positive, catalase-negative, slightly yellowish color, round, edges colony morphology and single or paired, short chain microscopy

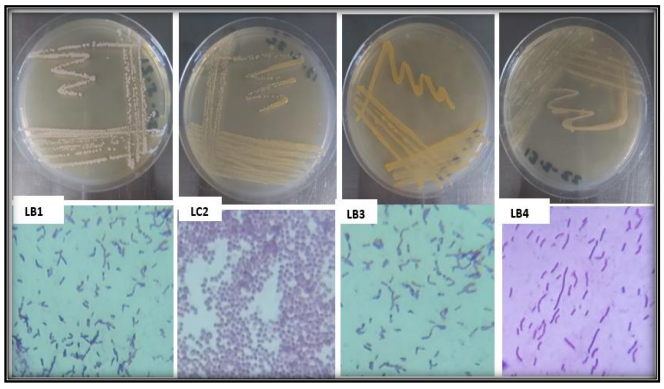

Figure 1: Examination colonies and microscopy of strains on MRS screening medium.

3.2. Cell surface hydrophobicity

Strains were tested against these hydrocarbons, and the results for cell surface hydrophobicity have been shown graphically (Fig. 2). In this research, we have found that hydrophobicity proportion differs among the tested LAB and ranged from 66.07 to 10.22 % (Fig. 2). All these strains differ significantly in their binding ability ($P < 0.05$) and showed the greatest hydrophobicity feature for Chloroform. The greatest hydrophobicity feature was observed by the LB3 for Chloroform, n-Hexane, Ethyl acetate and xylene 66.07, 38.23, 33.67 and 24.18% ($P < 0.05$) respectively. In the case of ethyl acetate LB1 and LB3 showed similar relatively affinity (33.45 and 33.67%; $P < 0.05$ respectively) as well as LB2 and LB4 showed similar relatively affinity (22.75% and 23.1%; $P < 0.05$ respectively). In case of xylene, all these strains differ significantly in their binding ability ($P < 0.05$). The greatest hydrophobicity feature was observed (Samet & Icen 2022) for n- n-hexane, n- hexadecane and xylene at 66.51%, 71.46% and 79.80% respectively. Although some researchers revealed that hydrophobicity properties for L. lactis range between 65.34% to 24.18%. for 0.8 mL xylene (Yi et al., 2019) and between 32.0 % to 54% (Prabhurajeshwar & Chandrakanth 2019).

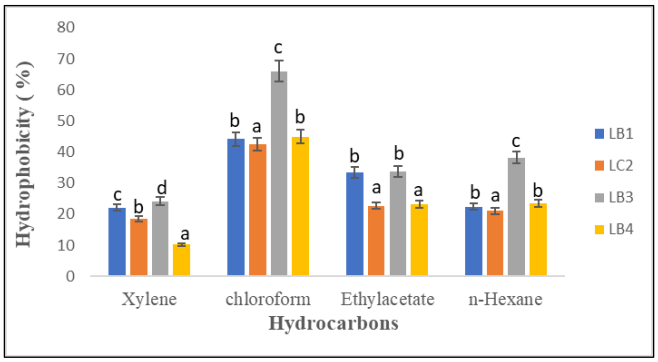

Figure 2: Cell surface hydrophobicity of LB1. LC2, LB3, and LB4 in different solvent systems (xylene, Ethyl acetate and chloroform). Values with different letters differ significantly ($p < 0.05$).

In hydrophobicity experiments, microbial cells were mixed by vertexing in the presence of a test liquid hydrocarbon. Following mixing, the two

phases are allowed to separate. In the case of adherence, cells from the bulk aqueous phase become bound to hydrocarbon droplets and rise with them following the mixing to form an upper 'cream' consisting of cell-coated oil droplets. When nonadherent bacteria are tested, the phases separate following the mixing procedure, with the cells remaining in the bulk aqueous suspension. The percentage of adherent cells can easily be ascertained by the decrease in absorbance of the lower aqueous phase following the assay, as compared to the absorbance of the original bacterial suspension. Cell surface hydrophobicity was used as a measurement of the ability of the probiotic strain to the host gut epithelial cells. Xylene, ethyl acetate and chloroform represent the non-polar solvent, mono-polar electron donating solvent and mono-polar electron accepting solvent respectively (Klopper and Dicks, 2018). The higher and lower affinities of strain LB3 for chloroform (polar acidic solvent) and for ethyl acetate (polar basic solvent), respectively, suggest that this strain may colonize mucus transiently, as mucus has a net negative charge. On the other hand, LB3showed a higher affinity for the solvents tested, indicating that the cells are both basic (electron-donating) and acidic (electron-accepting). Thus, LB3 will bind to negatively charged mucus, albeit lesser than cells with a net positive charge (Klopper et al., 2018). Therefore, the value of cell surface hydrophobicity can be used to select the probiotic candidates with good adhesion potential to the intestinal epithelium. The hydrophobic character depends on the strain and organism specify.

Most bacteria are classified as being either gram-positive or gram-negative, depending on whether they stain with Gram stain. This reflects fundamental differences in the structure of their cell walls and has important implications for the action of antibiotics. The cell wall of gram-positive organisms is a relatively simple structure. It is some 15–50 nm thick and comprises about 50% peptidoglycan, 40–45% acidic polymer together with 5–10% proteins and polysaccharides. The cell surface is highly polar and negatively charged and this influences the penetration of some antibiotics. The cell wall of gram-negative organisms is much more complex. From the plasma membrane outwards, it consists of the Aperiplasmic space containing enzymes and other components., Apeptidoglycan layer 2 nm in thickness, forming 5% of the cell wall mass, which is often linked to outwardly projecting lipoprotein molecules. Anouter membrane consisting of a lipid bilayer, similar in some respects to the plasma membrane, that contains protein molecules and lipoproteins linked to the peptidoglycan (Clarkand Pazdernik,2013). The hydrophilic and hydrophobic features are attributed to polysaccharides and proteins on the bacterial surface (Chauvière et al., 1992). and is affected by different factors such as aging, chemical structure of the surface, even composition of culture medium. In our study, the highest hydrophobicity score was determined for LB3 showed the highest cell surface hydrophobicity that suggested that LB3 showed the best ability to adhere to the intestinal mucosa (Carey et al., 2022).

4. Conclusion

Lactic acid bacteria (LAB) include a large number of bacterial genera among which the best known are lactobacilli, lactococci, enterococci, streptococci, leuconostoc, and pediococci. Hydrophobicity, resistance to gastrointestinal conditions (acid and bile), aggregation, antibacterial activity against pathogenic bacteria, sensitivity to antibiotics, and lack of pathogenicity are some of the main characteristics utilised in the selection of probiotic strains. Because the benefits of probiotic bacteria in the gastrointestinal system have attracted curiosity, it's critical to look into the fundamental elements that determine the physicochemical cell surface and adhesion properties of specific probiotic strains. In this study, the values of Hydrophobicity for isolates varies ranged from 66.07 to 10.22 %. also, adherence of a given strain can vary greatly depending on the growth conditions used, adherence

should be evaluated at several stages of growth, at various growth temperatures, and after growth on various media which required studies and investigation.

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