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# EVALUATION OF ADHESIVE PROPERTIES OF *LACTOBACILLUS* SPECIES IN TERM OF HYDROPHOBICITY, AUTO-AGGREGATION AND CO-AGGREGATION ISOLATED FROM CHICKENS

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## ABSTRACT

This study was conducted to evaluate the probiotic potential of *lactobacillus* species in terms of hydrophobicity, auto-aggregation and co-aggregation which provide preliminary criteria for selecting strains with high adhesion ability. The *lactobacillus* species were isolated from various regions of gastrointestinal tract of chickens. Among the 220 samples 100 were identified as *lactobacillus* species upon preliminary screening which were further confirmed by using gene specific primer amplification and sequencing. Twenty one (21) out of 100 were confirmed as *Lactobacillus* species, upon sequencing 16 of them were identified as *L. paracasei* (6), *L. jhonsonii* (3), *L. salivarius* (3), *L. fermentum* (1), *L, agilis* (1), *L. sakei* (1) and *L. curvatus* (1).The adhesion attributes of these strains were assessed through hydrophobicity with chloroform and xylene which ranges between 23.7 – 75.7% and 71.1 – 88.8%, respectively. Among 16 strains, 8 showed > 80% hydrophobicity with xylene. A significant ($p < 0.05$) and comparatively higher values (> 90%) for auto-aggregation were observed for *lactobacillus* (n = 7) strains after 24 h, rendered a great potential of probiotic bacteria. A significant ($p < 0.05$) higher values of percent co-aggregation were found 52.3% (ZA64Cl) and 33.6% (ZA62Cl) with *S. aureus* and *S. typhimurium*, respectively. A non-significant ($p > 0.05$) but higher value of percent co-aggregation 39.3% (ZA68Cl) was noted with *E. coli*. These strains also showed antagonistic effect with enteropathogenic bacteria which prevent the intestinal epithelium by forming a barrier.

**Key words:** Probiotics, *lactobacillus*, chickens, hydrophobicity, auto-aggregation, co-aggregation

## INTRODUCTION

The researchers are making their untiring efforts to explore new approaches to tackle bacterial infections without using antibiotics due to emerging resistance. So, probiotics provides safe and better alternate in this context which can be isolated and used to get maximum benefits of newly developed strains and the functions associated with them (Fung *et al*., 2011). Lactic acid bacteria belong to the genus *Lactobacillus* and collectively define as Gram positive, catalase-negative bacterial species capable to produce lactic acid on fermentation of glucose as an end-product (Felis and Dellaglio, 2007). These lactic acid bacteria harbored in gastrointestinal tract of chickens especially in small intestine and caeca. They are colonized after seven days seven days of hatching eggs (Mead, 1997).

Many of the species of genus *Lactobacillus* found in meat and dairy products, sewage, plants, and animal's gastrointestinal tract and now being isolated (Kandler, 1986). Recently, it has been known that there are more than 130 species belong to genus *Lactobacillus* (Neville and O'Toole, 2010). Out of many species some species including *Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus casei, Lactobacillus paracasei, Lactobacillus johnsonii, Lactobacillus reuteri,* and *Lactobacillus rhamnosus*, have been used as probiotics (de Vos, 2011).

Probiotic strains must have the property to cross acidic or bile barrier in the gut followed by adhesion to intestinal tract and exert its immune-modulatory and bacterial antagonism activity (Otutumi *et al*., 2012). Microbial adhesion to solvent is considered as criteria to evaluate the adhesive properties of bacteria (Kiely and Olson, 2000). Hydrophobicity or microbial adhesion to hydrocarbon (MATH), in combination with auto-aggregation is considered as an important bacterial surface characteristics and classified as low, medium and high with a hydrophobicity <33, 33%<66 and >66%, respectively (Bouchard *et al*., 2015). Both auto-aggregation and hydrophobicity causes the bacterial strain to act synergistically when attached to mucosal surface of the host (Li *et al*., 2015). Hydrophobicity may affect auto-aggregation and also alter the adhesion of bacteria to epithelial lining (Del Re *et al*., 2000).

The spontaneous gathering of cells to precipitate in the medium in which they are suspended is term as aggregation (Gobin, 2011). Auto-aggregation is the gathering of bacterial cells belongs to same strains while co-

aggregation takes place between two different bacterial strains (Janković *et al*., 2012). Auto-aggregation plays a vital role for probiotic bacteria to attach with the epithelial lining while co-aggregation is important to prevent the colonization of pathogenic bacteria with the epithelial lining of intestine by forming a barrier (Schellenberg *et al*., 2006).

On adherence to intestinal epithelium of the host the LAB probiotics mainly *Lactobacillus, Pediococcus* and *Enterococcus* competitively invade the disease causing organism in the intestine (Chen *et al*., 2013) and due to this ability they stimulate the immune system and intestinal barrier of the hosts (Chen *et al*., 2012; Ganguly *et al*., 2011)

Lactic acid bacteria reside in gut symbiotically and they struggle to capture the nutrient and adhesive sites on the epithelial lining of the intestine through a general mechanism (Grajek *et al*., 2016). *Lactobacillus* enhances the immune response by producing bacteriocins (Fong *et al*., 2015) which are associated with antagonism (Wen *et al*., 2016).

Probiotics play its effective role against pathogenic bacteria by secreting metabolites such as bacteriocins and short chain fatty acids (SCFAs). A decrease *coliform* count has been found due to short chain fatty acids (SCFAs) which are secreted by probiotic *bacillus subtilis* (Ranilla and Carro, 2013). The bacteriocins characterized as antimicrobial substances which is capable to reduce the growth of pathogenic bacteria such as bacteriocins secreted by *lactobacillus salivarius* and *E. coli* greatly decline the gram positive bacteria, *Campylobacter jejuni* (Pilasombut *et al*., 2006) and *salmonella* contamination in chickens (Stern *et al*., 2006), respectively.

## MATERIALS AND METHODS

### Collection of samples and growth conditions

A total 220 samples were collected from various region of gastrointestinal tract (GIT) of chickens including intestinal, caecum and cloacal regions. A selective media MRS broth (Oxoid Ltd. UK) which is specific for *lactobacillus* was used to enrich the culture, incubated at 37 °C for 18 – 24 h and streaked on MRS agar (Oxoid Ltd. UK) for 24 hrs. After incubation of 24 h the well isolated and prominent colonies were picked with sterile loop and incubated in MRS broth for 24 h and stored in -80 °C after addition of 50% glycerol solution.

### Identification of *lactobacillus* species

Out of 220 samples 100 *Lactobacillus* species were isolated from various regions of chicken intestine. In our previous study, they were further characterized on the basis of their morphology, staining and catalase test. Species level identification was made by amplifying the *lactobacillus* specific 16s rRNA gene; out of 100 isolates 21 were selected for sequencing on the basis of band intensity and better amplification. Among 21 sequences 16 were identified as *L. paracasei* (n = 6), *L. salivarius* (n = 3), *L. jhonsonii* (n = 3), and *L. agilis, L. fermentum, L. sakei,* and *L. curvatus* (n = 1 each).

### Hydrophobicity Assay

Cell surface hydrophobicity was assessed by microbial adherence to hydrocarbon (MATH) a procedure described by Rosenberg *et al*. (1980). The bacterial cells were grown in MRS broth grown in MRS broth for 24 h at 37 °C. The cells were centrifuged at 7000× g for 15 minutes and washed twice with the PBS (pH 7.2). The optical density of bacteria was measured and set to 1.0 at 540 nm. Later, 1 mL of bacterial cell suspension was mixed with 1 ml of each hydrocarbon i.e. xylene and chloroform and vortexed vigorously. Optical density of aqueous phase was again measured at 540 nm after phase separation of 30 min and hydrophobicity was calculated by using the following formula.

$$\textbf{Hydrophobicity (\%)} = (A_i - A_f) / A_i \times 100$$

Where, $A_i$ and $A_f$ represent the initial and final (after phase separation) absorbance.

### Auto-aggregation

Auto-aggregation of various strains of *lactobacillus* was assessed by the procedure described by Janković *et al*. (2012) with few minor modifications. The *lactobacillus* culture was grown in MRS broth for 24 h at 37 °C. The bacterial cell centrifuged at 7000× g for 10 min and washed twice with the PBS (pH 7.2). The optical density bacterial cell suspension was adjusted to 1 at 600 nm. In order to measure the auto-aggregation 3 ml of cell suspension was equally transferred to three glass tubes and vortexed. After a time interval of 2, 5 and 24 h absorbance was again recorded at 600 nm and auto-aggregation was calculated by using the following formula.

$$\textbf{Auto-aggregation (\%)} = 1 - A_t / A_0 \times 100$$

Where, $A_t$ and $A_0$ represent the absorbance at different time interval (2, 5 and 24 h) and initial absorbance, respectively.

**Co-aggregation**

Co-aggregation of *lactobacillus* strains were assessed by the method described by the Basson *et al*. (2008) with minor modifications. The *lactobacillus* strains and pathogenic strains were grown in MRS broth and BHI broth for 24 h at 37 °C, respectively. The bacterial cell centrifuged at 5000× g for 15 min and washed twice with the PBS (pH 7.2). The optical density bacterial cell suspension was adjusted to 0.5 at 660 nm. A suspension of 2 mL of each *lactobacillus* strains mixed with equal volume (2 mL) of each of the three pathogenic strains including *S. aureus* (NCTC-6571), *E. coli* (ATCC-25922) and *S. typhimurium* (ATCC-14028). Absorbance was again recorded immediately after mixing, 1 and 3 h at 660 nm and co-aggregation calculated by the following formula.

$$\textbf{Co-aggregation (\%)} = A_{(mixing)} - A_t / A_0 \times 100$$

Where,
$A_{(mixing)}$ is the absorbance taken immediately after mixing
$A_t$ is the absorbance taken at interval of 1 and 3 h
$A_0$ is the initially adjusted absorbance of 0.5 at 660 nm.

**Statistical analysis**

One way analysis of variance (ANOVA) was performed in order to evaluate the probiotic properties of *lactobacillus* using SPSS (Statistics 22, IBM). GraphPad Prism (version 7.0) was used for graphical representations.

## RESULTS

**Cell surface hydrophobicity**

In this study two hydrocarbons xylene and chloroform were used to assess the cell surface hydrophobicity of various *lactobacillus* strains. The hydrophobicity of various *lactobacillus* strains ranges between 23.7 ± 2.15 – 75.7 ± 2.21 and 71.1 ± 2.15 – 88.8 ± 1.78 with chloroform and xylene, respectively (Table 1 and Fig. 1).

A comparatively higher value of percent hydrophobicity (>80%) was found in 8 different strains of *lactobacillus* treated with xylene. Statistical analysis revealed a significant ($p< 0.05$) difference as determined by one way ANOVA. There was no significant ($p > 0.05$) difference was found with strain ZA15SI ($p = 0.169$), ZA80C ($p = 0.053$) and ZA81Cl ($p = 0.089$) (Table 1).

**Auto-aggregation**

Auto-aggregation is an important characteristic in the preliminary screening of probiotic bacteria to be use as potential probiotic. In this study auto-aggregation of various identified strains of *lactobacillus* (n=16) was measured at different time intervals.

The percent auto-aggregation of various strains of *lactobacillus* ranges between 24.6 ± 1.86 – 73.7 ± 1.89, 48.5 ± 2.05 – 80.2 ± 1.36 and 69.6 ± 1.85 – 94.8 ± 1.83 for the period of 2, 5 and 24 h, respectively (Table 2 and Fig. 2). The value of percent auto-aggregation increases with time and comparatively higher values (>90%) with significant ($p < 0.05$) difference was found with all strains of *lactobacillus* as determined by one way ANOVA (Table 2).

**Co-aggregation**

In the preliminary screening of probiotic bacteria the co-aggregation play its role in recognition of pathogenic bacteria and establish a barrier that prevent the colonization of pathogenic bacteria to the epithelial lining. In this study different strains of *lactobacillus* were evaluated in their ability to co-aggregate with three enteric pathogens include *S. aureus* (NCTC - 6571), *S. typhimurium* (ATCC - 14028) and *E. coli* (ATCC - 25922).

Table 1. Hydrophobicity of various *Lactobacillus* strains.

| S.No. | Strain | Strain ID | Hydrophobicity (%) Mean ± SD | | F | p – value |
|---|---|---|---|---|---|---|
| | | | Chloroform | Xylene | | |
| 1 | *L. paracasei* | ZA15SI | 71.7 ± 2.27 | 74.9 ± 2.36 | 2.85 | 0.169 |
| 2 | *L. paracasei* | ZA16C | 57.4 ± 2.06 | 78.2 ± 2.76 | 109.7 | 0.000 |
| 3 | *L. paracasei* | ZA27SI | 32.6 ± 1.67 | 84.5 ± 2.73 | 789.9 | 0.000 |
| 4 | *L. paracasei* | ZA30SI | 54.8 ± 2.75 | 72.1 ± 2.81 | 58.1 | 0.002 |
| 5 | *L. paracasei* | ZA32Cl | 56.0 ± 2.70 | 75.3 ± 2.58 | 80.13 | 0.001 |
| 6 | *L. paracasei* | ZA78Cl | 32.2 ± 2.85 | 82.7 ± 2.85 | 470.7 | 0.000 |
| 7 | *L. salivarius* | ZA67C | 68.4 ± 1.90 | 81.8 ± 2.95 | 44.21 | 0.003 |
| 8 | *L. salivarius* | ZA68Cl | 48.5 ± 2.21 | 88.8 ± 1.78 | 606.8 | 0.000 |
| 9 | *L. salivarius* | ZA74Cl | 49.5 ± 2.58 | 71.1 ± 2.15 | 123.5 | 0.000 |
| 10 | *L. jhonsonii* | ZA61C | 69.5 ± 2.05 | 83.3 ± 2.21 | 62.66 | 0.001 |
| 11 | *L. jhonsonii* | ZA64Cl | 75.6 ± 2.35 | 80.7 ± 1.86 | 8.81 | 0.041 |
| 12 | *L. jhonsonii* | ZA79Cl | 56.6 ± 1.95 | 81.3 ± 2.35 | 195.6 | 0.000 |
| 13 | *L. agilis* | ZA62Cl | 31.6 ± 2.05 | 83.5 ± 2.11 | 932.4 | 0.000 |
| 14 | *L. fermentum* | ZA66Cl | 23.7 ± 2.15 | 86.9 ± 1.46 | 1767.2 | 0.000 |
| 15 | *L. sakei* | ZA80C | 69.4 ± 1.70 | 73.9 ± 2.25 | 7.41 | 0.053 |
| 16 | *L. curvatus* | ZA81Cl | 75.7 ± 2.21 | 71.5 ± 2.42 | 5.0 | 0.089 |

Data represented as Mean ± SD, each in triplicate. All parameters were calculated using one-way ANOVA.
*P* value < 0.05 taken as significant

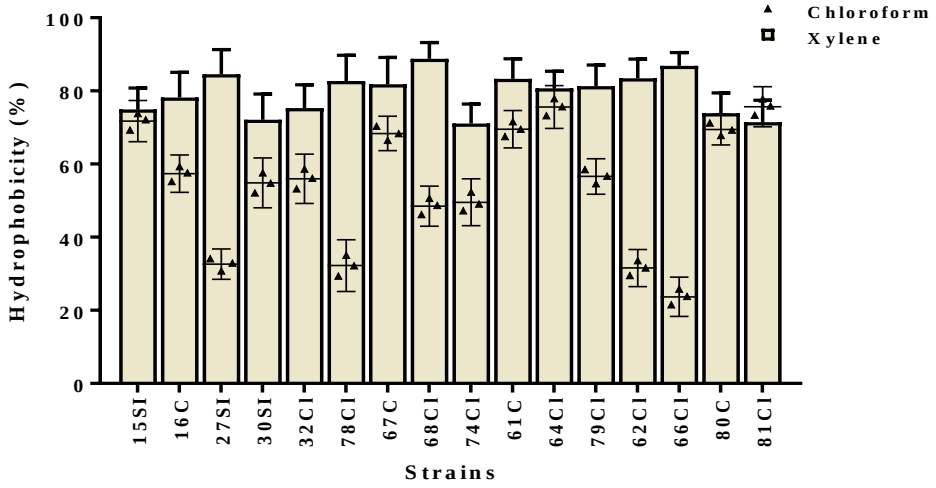


Fig. 1.The hydrophobicity of various strains of *lactobacillus* with chloroform and xylene.

Table 2. Auto-aggregation of various *Lactobacillus* strains.

| S.No. | Strain | Strain Id | Auto-aggregation (%) Mean ± SD | | | F | p - value |
|---|---|---|---|---|---|---|---|
| | | | 2 H | 5 H | 24 H | | |
| 1 | *L. paracasei* | ZA15SI | 24.6 ± 1.86 | 63.5 ± 1.70 | 69.6 ± 1.85 | 551.1 | 0.000 |
| 2 | *L. paracasei* | ZA16C | 55.1 ± 1.67 | 56.5 ± 1.81 | 94.6 ± 2.22 | 410.7 | 0.000 |
| 3 | *L. paracasei* | ZA27SI | 45.7 ± 2.50 | 62.0 ± 1.85 | 82.3 ± 1.86 | 230.4 | 0.000 |
| 4 | *L. paracasei* | ZA30SI | 36.7 ± 1.42 | 65.9 ± 1.65 | 84.3 ± 1.84 | 637.6 | 0.000 |
| 5 | *L. paracasei* | ZA32Cl | 73.7 ± 1.89 | 80.2 ± 1.36 | 84.2 ± 1.53 | 32.6 | 0.001 |
| 6 | *L. paracasei* | ZA78Cl | 44.1 ± 1.50 | 48.5 ± 2.05 | 94.8 ± 1.83 | 823.6 | 0.000 |
| 7 | *L. salivarius* | ZA67C | 70.5 ± 1.70 | 73.3 ± 1.85 | 84.1 ± 1.87 | 274.3 | 0.000 |
| 8 | *L. salivarius* | ZA68Cl | 33.3 ± 2.20 | 71.5 ± 1.92 | 85.2 ± 1.50 | 630.5 | 0.000 |
| 9 | *L. salivarius* | ZA74Cl | 45.2 ± 1.40 | 51.3 ± 0.92 | 94.3 ± 1.45 | 699.9 | 0.000 |
| 10 | *L. jhonsonii* | ZA61C | 36.2 ± 1.27 | 65.4 ± 0.81 | 88.3 ± 1.61 | 1282.4 | 0.000 |
| 11 | *L. jhonsonii* | ZA64Cl | 43.6 ± 1.79 | 50.7 ± 1.75 | 82.4 ± 1.79 | 480.0 | 0.000 |
| 12 | *L. jhonsonii* | ZA79Cl | 65.4 ± 2.31 | 71.6 ± 1.85 | 85.8 ± 2.11 | 218.0 | 0.000 |
| 13 | *L. agilis* | ZA62Cl | 61.9 ± 1.52 | 70.2 ± 2.11 | 92.3 ± 1.86 | 236.2 | 0.000 |
| 14 | *L. fermentum* | ZA66Cl | 43.6 ± 1.20 | 50.5 ± 2.12 | 92.1 ± 1.76 | 592.5 | 0.000 |
| 15 | *L. sakei* | ZA80C | 42.1 ± 1.60 | 58.6 ± 2.36 | 90.5 ± 2.21 | 503.8 | 0.000 |
| 16 | *L. curvatus* | ZA81Cl | 46.8 ± 1.50 | 52.0 ± 1.85 | 94.1 ± 1.65 | 966.5 | 0.000 |

Data represented as Mean ± SD, each in triplicate. All parameters were calculated using one-way ANOVA.
*P* value < 0.05 taken as significant

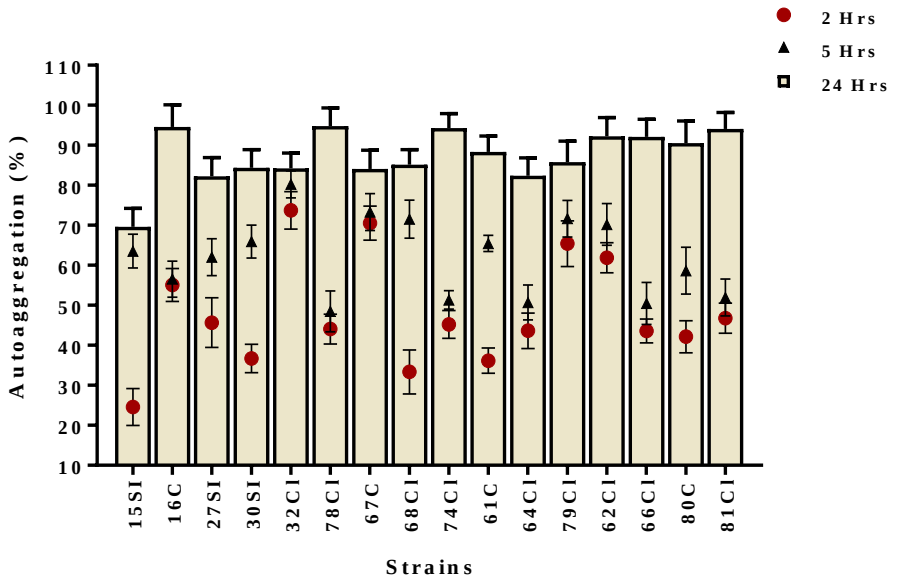


Fig. 2. A comparison of percent auto-aggregation of various strains of *lactobacillus* after 2, 5 and 24 h.

All strains of *lactobacillus* (n = 16) co-aggregated with enteric pathogens with the variable degree of co-aggregation. A relatively, higher values of percent co-aggregation were obtained after 3 h as compared to their co-aggregation after 1 h (Table 3).

Table 3. Co-aggregation of various *Lactobacillus* strains with pathogenic strains.

| | | Co-aggregation (%), Mean ± SD | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | *S. aureus* | | | *S. typhimurium* | | | *E. coli* | | |
| **S.No.** | **Strain ID** | **1 Hr** | **3 Hrs** | **p-value** | **1 Hr** | **3 Hrs** | **p-value** | **1 Hr** | **3 Hrs** | **p-value** |
| 1 | ZA15SI | 42.5 ± 1.47 | 48.7 ± 1.30 | **0.005** | 25.3 ± 1.81 | 32.3 ± 2.01 | **0.011** | 19.9 ± 1.72 | 24.1 ± 2.40 | **0.070** |
| 2 | ZA16C | 25.5 ± 1.53 | 32.2 ± 1.83 | **0.008** | 8.6 ± 2.11 | 8.3 ± 1.86 | **0.847** | 20.1 ± 2.00 | 23.0 ± 2.40 | **0.187** |
| 3 | ZA27SI | 25.3 ± 1.81 | 31.1 ± 2.00 | **0.021** | 28.9 ± 1.80 | 30.7 ± 2.30 | **0.362** | 19.9 ± 2.00 | 22.1 ± 1.40 | **0.194** |
| 4 | ZA30SI | 21.9 ± 2.05 | 35.4 ± 1.83 | **0.001** | 16.5 ± 2.20 | 29.9 ± 1.17 | **0.001** | 11.3 ± 3.78 | 18.7 ± 1.72 | **0.037** |
| 5 | ZA32CL | 12.1 ± 1.30 | 32.1 ± 1.72 | **0.000** | 7.5 ± 2.05 | 13.5 ± 2.05 | **0.023** | 24.1 ± 2.05 | 26.2 ± 0.87 | **0.184** |
| 6 | ZA78Cl | 18.0 ± 1.51 | 42.2 ± 1.71 | **0.034** | 12.7 ± 1.92 | 26.9 ± 2.00 | **0.001** | 14.8 ± 1.64 | 18.7 ± 1.10 | **0.026** |
| 7 | ZA67C | 13.7 ± 1.36 | 18.1 ± 1.92 | **0.030** | 7.7 ± 2.21 | 18.3 ± 1.70 | **0.003** | 20.3 ± 2.10 | 23.4 ± 1.31 | **0.099** |
| 8 | ZA68Cl | 29.7 ± 1.40 | 33.5 ± 1.42 | **0.030** | 12.3 ± 1.62 | 16.5 ± 1.60 | **0.034** | 36.1 ± 1.80 | 39.3 ± 1.01 | **0.055** |
| 9 | ZA74Cl | 14.9 ± 2.20 | 34.7 ± 1.62 | **0.000** | 20.5 ± 2.12 | 28.1 ± 1.90 | **0.010** | 19.5 ± 2.30 | 22.1 ± 0.95 | **0.137** |
| 10 | ZA61C | 22.3 ± 2.00 | 34.1 ± 1.22 | **0.001** | 9.8 ± 2.00 | 14.3 ± 1.80 | **0.043** | 25.6 ± 2.23 | 37.3 ± 1.81 | **0.002** |
| 11 | ZA64Cl | 37.0 ± 1.31 | 52.3 ± 1.42 | **0.000** | 3.5 ± 0.70 | 14.1 ± 2.00 | **0.001** | 25.8 ± 0.80 | 17.1 ± 1.94 | **0.002** |
| 12 | ZA79Cl | 12.3 ± 1.67 | 29.3 ± 1.60 | **0.000** | 13.9 ± 2.00 | 21.6 ± 2.42 | **0.013** | 17.1 ± 0.92 | 20.5 ± 2.52 | **0.093** |
| 13 | ZA62Cl | 18.6 ± 1.40 | 34.3 ± 1.67 | **0.000** | 15.7 ± 2.01 | 33.6 ± 2.20 | **0.000** | 16.2 ± 0.92 | 21.7 ± 0.64 | **0.001** |
| 14 | ZA66Cl | 38.5 ± 1.40 | 42.7 ± 1.10 | **0.017** | 9.1 ± 1.60 | 21.3 ± 1.81 | **0.001** | 22.3 ± 1.90 | 28.2 ± 1.83 | **0.018** |
| 15 | ZA80C | 7.6 ± 1.31 | 32.8 ± 1.60 | **0.000** | 5.7 ± 2.25 | 24.2 ± 1.60 | **0.000** | 12.7 ± 1.53 | 15.0 ± 0.92 | **0.092** |
| 16 | ZA81Cl | 15.2 ± 1.40 | 30.8 ± 2.03 | **0.000** | 5.9 ± 2.01 | 21.3 ± 2.00 | **0.001** | 17.5 ± 1.70 | 18.6 ± 1.06 | **0.383** |

Data represented as Mean ± SD, each in triplicate. All parameters were calculated using one-way ANOVA.
$P < 0.05$ taken as significant.

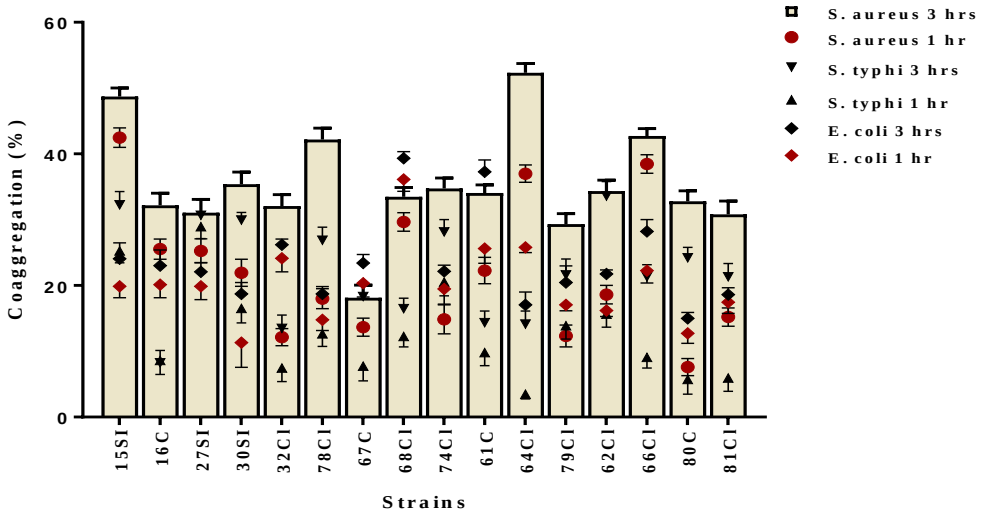


Fig. 3. A comparison of various *Lactobacillus* strains with *S. aureus*, *S. typhimurium* and *E. coli* after 1 and 3 h**.**

A significant ($p < 0.05$) higher values of percent co-aggregation were found 52.3 ± 1.42 (ZA64Cl) and 33.6 ± 2.20 (ZA62Cl) with *S. aureus* and *S. typhimurium*, respectively. A non-significant ($p > 0.05$) but higher value of percent co-aggregation 39.3 ± 1.01 (ZA68Cl) was noted with *E. coli* (Table 3).

There was no significant ($p > 0.05$) difference was found in percent co-aggregation of different strains of *lactobacillus* with *S. typhimurium* which include ZA16C ($p = 0.847$) & ZA27SI ($p = 0.362$) and with *E. coli* include ZA15SI ($p = 0.070$), ZA16C ($p = 0.187$), ZA27SI ($p = 0.194$), ZA32Cl ($p = 0.184$), ZA67C ($p = 0.099$), ZA74Cl ($p = 0.137$), ZA79Cl ($p = 0.093$), ZA80C ($p = 0.092$) and ZA81Cl ($p = 0.383$) (Table 3).

A comparison of percent co-aggregation of all *lactobacillus* strains with *S. aureus, S. typhimurium* and *E. coli* graphically represented in Fig. 3.

## DISCUSSION

The hydrophobicity of bacteria is cell surface property and used as marker to assess the non-specific adherence to epithelial lining of the intestine. A positive relationship exists between the hydrophobicity and adhesive property of many bacterial strains. According to Solieri *et al*. (2014), a bacterial strain with a value of > 70% hydrophobicity considered as hydrophobic. In this study only three strains of *lactobacillus* had > 70% hydrophobicity in chloroform but found >70% hydrophobicity for all strains when treated with xylene and these values show the adhesive property of these strains.

Many researchers have found relationship between the adhesion to epithelial cells and hydrophobicity of *lactobacillus* strains (Ehrmann *et al*., 2002; Kos *et al*., 2003). In a previous study the adhesion of 8 chicken strains of *L. salivarius, L. crispatus* and *L. jhonsonii* to xylene ranged from 78.2% to 93.2% (Heravi *et al*., 2011) , which are slightly higher to the finding of this study for the same strains of *lactobacillus*. Mota *et al*. (2006), reported approximately 80% of *lactobacillus* strains of intestinal origin were hydrophobic with a hydrophobicity > 50% which is similar to the finding of this study.

This study found a relatively higher value for auto-aggregation with *L. paracasei* after 2 h which is in accordance with the previous finding which showed 51.1% and 49.4% when *L. paracasei* was suspended in the PBS and supernatant of media after 2 h, respectively (Gudina *et al*., 2010). The presence of surface proteins are responsible to form an association between *lactobacillus* and their adhesion through auto-aggregation as found with *L. acidophilus* M92 (Kos *et al*., 2003).

The mucosa of intestinal epithelium of the intestine is protected by probiotic bacteria due to their adhesive property which makes them enable to adhere, colonizes and setup a barrier at intestinal epithelium which prevents the adherence of pathogenic bacteria (García-Cayuela *et al*., 2014). Adhesion of probiotic to intestinal epithelium not only to colonize that region but also prevent the adhesion of other disease causing bacteria (Frece *et al*., 2009)

The co-aggregation is an ability of probiotic bacteria to interact with genetically different pathogenic strain in order to keep them away from epithelium lining. Co-aggregation ability of bacteria provides to assess an interaction between the probiotic bacteria and pathogenic bacteria (Soleimani *et al*., 2010).

In present study *L. jhonsonii* (ZA64Cl), *L. agilis* (ZA62Cl) and *L. salivarius* (ZA68Cl) has comparatively higher value of percent co-aggregation than other strains of *lactobacillus* against *S. aureus*, *S. typhimurium* and *E. coli*, respectively. Valeriano *et al*. (2014), found a significant and higher value of co-aggregation with *E. coli* and some strains of *salmonella*. Messaoudi *et al*. (2012) reported a significant antagonistic activity between *lactobacillus* and *S. aureus, E. coli* and *C. jejuni* which is in accordance to finding of this study. This finding suggests these strains can be considered as potential probiotic strain subject to their efficacy in *in-vivo* trials.

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