OPEN ACCESS

Int. Res. J. of Science & Engineering, 2018; Vol. 6 (2): 31-34 http://www.irjse.in
ISSN: 2322-0015 UGC Approved Journal No. 63628

RESEARCH ARTICLE

Adhesion inhibition of *Salmonella enterica* subspecies *typhimurium* to HT-29 cells by LAB isolates from traditionally fermented Indian foods

Walhe Rajan A[1], **Patole** Milind S[2], **Diwanay** Sham S[1*]

[1]MES Abasaheb Garware College, Pune 411004, Maharashtra, India.
[2]National Centre for Cell Science, Savitribai Phule Pune University Campus, Pune 411007, Maharashtra, India.
[*]Corresponding author: diwanay@rediffmail.com

Manuscript Details
Received : 05.03.2018
Accepted: 25.03.2018
Published: 31.03.2018

ISSN: 2322-0015

Editor: Dr. Arvind Chavhan

Cite this article as:
Walhe Rajan A, Patole Milind S, Diwanay Sham S. Adhesion inhibition of *Salmonella enterica* subspecies *typhimurium* to HT-29 cells by LAB isolates from traditionally fermented Indian foods. *Int. Res. Journal of Science & Engineering,* 2018, 6 (2): 31-34.

ABSTRACT
Fermented foods are known to contain probiotic bacteria that impart health benefits to its consumers in addition to nutrition. We obtained five lactic acid bacterial (LAB) isolates from traditionally fermented Indian foods and these were characterized for their probiotic potential. The aim of this study was to check the ability of these five LAB isolates to adhere HT-29 cells and to inhibit the adhesion of *Salmonella enterica* subspecies *typhimurium* to HT-29 cells. All five LAB isolates exhibited ability to adhere to HT-29 cells. To investigate ability of these isolates to inhibit adherence of intestinal pathogens; these were allowed to adhere to HT-29 cells and then actively growing culture of *S. enterica* subspecies *typhimurium* was added. Adherence to mucosal epithelia is an important determinant of pathogenicity. Pathogenicity of *S. enterica* subspecies *typhimurium* used in this study was established by determining its adhesion ability to HT-29 cells. Reduction in adhesion capacity of *S. enterica* subspecies *typhimurium* by 70 % on an average was seen by our LAB isolates. Thus, these five LAB isolates from traditionally fermented Indian foods have the potential to offer immunity against intestinal pathogens.

Keywords: Adhesion inhibition, HT-29, Probiotic, *Salmonella* sp., LAB isolates

INTRODUCTION

For intestinal pathogens, the adhesion to epithelium is an important step, as it allows the initiation of process that facilitates the invasion. Thus, adhesion to epithelial cells is crucial step for bacteria to survive and colonize in the gastrointestinal tract (GIT).

The epithelial cells of GIT are protected from intestinal pathogens by number of mechanisms [1]. One of these is a reduction in infection dose through competition by microbiota for adhesion sites with that of intestinal pathogens and production of antimicrobial substances.

Cell adhesion is a complex process related with contact between bacterial cell membrane and interacting surface. Caco-2 and HT-29 cells of human intestinal epithelium derived colonic adenocarcinomas are commonly used as *in vitro* models in the study of human enterocytic function; as these convey structural and functional features of human enterocyte [2, 3].

To establish infectivity and disease, intestinal pathogen must adhere to enterocytes. This primary step of adhesion in case of *Salmonella* species is mediated by bacterial fimbriae which identify certain receptors on epithelial cells [1]. Several reports in this aspect have suggested that lactic acid bacteria (LAB) could prevent the adhesion and in turn reduce pathogen colonization and thus prevent infection. LAB is known to display different types of surface determinants that are responsible for their interaction with intestinal mucosal epithelia. Secretions of LAB comprise composite glycoprotein mixture that is known to inhibit the adhesion of pathogenic bacteria [4].

In the present study, we have evaluated the ability of adhesion inhibition of *Salmonella* sp. to HT-29 cells by five LAB isolates having probiotic potential, that were obtained from traditionally fermented Indian foods.

METHODOLOGY

Bacterial cultures, culture media and growth conditions: The five LAB isolates obtained from traditionally fermented Indian foods that have exhibited promising probiotic potential were used for this study. These have been identified by morphotyping and molecular typing as: G11: *Pediococcus pentosaceus*, AP3: *Pediococcus pentosaceus*, EF: *Enterococcus faecium*, Chole1: *Enterococcus faecium*, and 7MP: *Lactobacillus pentosus* [5]. These cultures were grown in de Man Rogosa and Sharpe (MRS) medium (HiMedia) under microaerophilic conditions. *Salmonella enterica* subspecies *typhimurium* was procured from Pure Microbes, Pune and grown in Luria-Bertani (LB) medium (SRL).

Bacterial adhesion to HT-29 cells: Adhesion of LAB isolates and *Salmonella enterica* subspecies *typhimurium* was studied using HT-29 cell line.

Revival of frozen HT-29 cells: A vial of HT-29 cell line taken from liquid nitrogen (LN2) was thawed under running tap water and the reconstituted contents (1ml) were added to 9.0 ml of fresh Modified Eagle's medium (DMEM), mixed, and centrifuged (1000rpm,10 min.). Cell pellet was resuspended in 10 ml complete (DMEM) medium (DMEM supplemented with 10% fetal bovine serum) and the contents were transferred to 25cm^2 flask and incubated at 37 C in CO_2 incubator.

Preparation of HT-29 cells for adhesion assay: After revival, HT-29 cells were grown in DMEM complete medium. A split was given (trypsinized) and single cell suspensions were made and cell counts were determined using improved Neubauer chamber. Cells were transferred to 24 well plate containing cover slip (1.5 ml in each well) and incubated.

Cell adhesion assays:
Freshly grown LAB cultures and *Salmonella enterica* subspecies *typhimurium* cultures were pelleted and resuspended in DMEM and O.D. at 600nm was recorded. To check for adherence potential of *S. enterica* subspecies *typhimurium*, these (10 µl) were added to wells containing HT-29 and incubated for 1h.

To check for inhibition of adhesion og *S. enteritic* subspecies *typhimurium;* LAB cultures were added (10 µl) to the wells containing HT 29 cells and incubated at 37 C in CO_2 incubator for 2 h. This was followed by addition of *S. enterica* subspecies *typhimurium* (10 µl) and incubation continued for 1h. After incubation, medium was discarded and wells were washed thrice with fresh medium. Cells were fixed by chilled acetic acid-methanol (1:3) mixed and air dried. Coverslips were taken out and stained with Gram stain. Bacteria adhered to HT-29 cells were counted at 1000x using oil

immersion objective. All assays were performed in triplicates. Counts were taken from 20 different microscopic fields and adherence was expressed as bacteria adhered per 100 HT-29 cells [2, 6, 7, 8, 9,10].

RESULTS AND DISCUSSION

The antagonistic activity of LAB against pathogenic bacteria is well known [11]. This antagonistic activity has been mostly attributed to the antimicrobial substances [5] or metabolites such as organic acids, hydrogen peroxide, bacteriocins, etc. produced by the probiotic strains. This activity along with mechanism of competitive exclusion/ inhibition of adhesion of pathogen would prevent colonization of the intestinal pathogens. Thus, prevention of pathogen adherence and eventually colonization of intestinal epithelium proves to be important added benefit of probiotic organisms. We investigated adhesion of LAB cultures and of intestinal pathogen *Salmonella* sp. separately and reduction in adhesion of *Salmonella enterica* subspecies *typhimurium* by LAB cultures using HT-29 cells. We observed that *Salmonella* cells and LAB cultures adhered to the HT-29 cells (Fig.1).

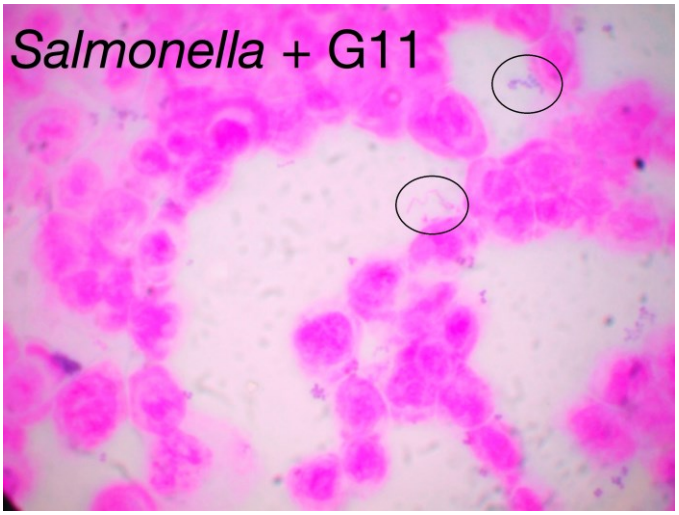

Figure 1: Adherence of *S.enterica* subspecies *typhimurium* and LAB isolate G11 to HT-29 cells (magnification 1000 X). The upper circle indicates the adhesion of G11 to HT-29 cells, while the lower circle indicates the adhesion of *S.enterica* to HT-29 cells.

All five LAB cultures were able to prevent/ inhibit adhesion of *S. enterica* subspecies *typhimurium* to HT-29 cells (Fig.2). Adherence and thus infectivity of *S. enterica* subspecies *typhimurium* was established as it adhered to HT-29 cells (53 cells per 100 HT-29 cells); while adherence of LAB cultures varied for each isolate from 125 to more than 300 cells per 100 HT-29 cells. Adherence of *Salmonella* cells to HT-29 cell decreased in presence of all LAB isolates – 63.29% (isolate G11), 65.46% (isolate AP3), 70.54% (isolate EF), 74.39% (isolate Chole 1), and 73.52% (isolate 7MP); with maximum inhibition by isolate Chole 1. This observation is indicative of our LAB isolates from traditionally fermented Indian foods have ability to provide benefit for the intestinal mucosal wellbeing.

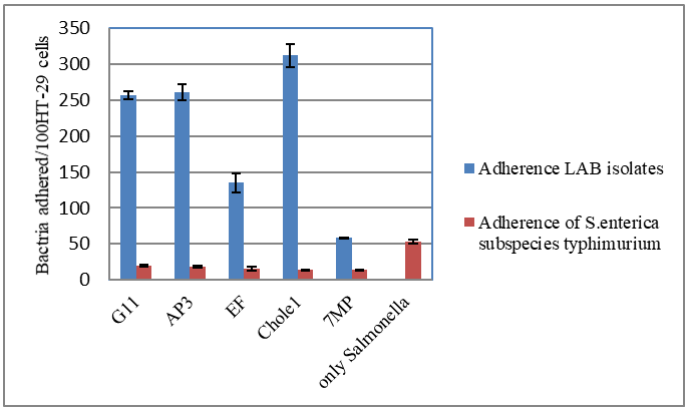

Figure 2. Adhesion of LAB isolates and *Salmonella* sp. to HT-29 cells.

Since adherence of pathogen is first requisite step in colonization and invasion for the pathogens to establish in the host; prevention of adhesion to intestinal cells will results in conferring immunity against intestinal pathogen.

CONCLUSION

Upholding and reconstitution of the normal composition of the intestinal flora by applying appropriately selected LAB may have prophylactic significance in preventing or curing intestinal colonization of *Salmonella* sp. Our study indicate the potential application of LAB isolates from traditionally fermented Indian foods as protective entities against GIT infection/colonization by pathogens like of *Salmonella* sp.

Acknowledgments:
Authors are thankful to UGC, New Delhi, India for providing financial assistance for the UGC minor research project F. No. 47-510/12 (WRO). We thank Principal, Abasaheb Garware College, Pune for

providing the laboratory facilities; Director, National Centre for Cell Sciences (NCCS), Pune for cell culture facility, Vikas Mallav and Mahendra Jamdhade for their valuable help in cell adhesion experimentation.

REFERENCES

1. Jankowska A, Laubitz D, Antushevich H, Zabielski R, Grzesiuk E. Competition of *Lactobacillus paracasei* with *Salmonella enterica* for adhesion to Caco-2 cells. Journal of Biomedicine and Biotechnology, 2008:1-6.
2. Duary RK, Rajput YS, Batish VK, Grover S. Assessing the adhesion of putative indigenous probiotic lactobacilli to human colonic epithelial cells. Indian Journal of Medical Research, 2011; 134: 664 -671.
3. Grajek W, and Olejnik A. Epithelial cell cultures *in vitro* as a model to study functional properties of food. Polish Journal of Food and Nutrition Sciences, 2004; 13(54):5-24.
4. Bermudez-Brito M, Plaza-Díaz J, Muñoz-Quezada S, Gómez-Llorente C, Gil A. Probiotic mechanisms of action. Annals of Nutrition and Metabolism, 2012; 61:160-174.
5. Walhe RA, Patole MS, Diwanay SS. Some beneficial properties of bacterial isolates from traditionally fermented Indian foods. Bionano Frontier, 2017;10 (1): 120-126.
6. Abedi D, Feizizadeh S, Akbari V, Jafarian-Dehkordi A. *In vitro* anti-bacterial and anti-adherence effects of *Lactobacillus delbrueckii* subsp *bulgaricus* on *Escherichia coli*. Research in Pharmaceutical Sciences, 2013; 8(4):260-268.
7. Ali Q S, Farid J,Kabeir B M, Sekawi Z, Mustafa S, Mohd Ghazali H, Abdul Manap Y. Adhesion properties of *Bifidobacterium pseudocatenulatum* G4 and *Bifidobacterium longum* BB536 on HT-29 human epithelium cell line at different times and pH. World Academy of Science, Engineering and Technology, 2009; 49:149-153.
8. Bianchi MA, Del Rio D, Pellegrini N, Sansebastiano G, Neviani E, Brighenti F. A fluorescence-based method for the detection of adhesive properties of lactic acid bacteria to Caco-2 cells. Letters in Applied Microbiology, 2004; 39: 301-305.
9. Shokryazdan P, Sieo CC, Kalavathy R, Liang JB, Alitheen NB, Faseleh Jahromi M, Ho YW. Probiotic potential of *Lactobacillus* strains with antimicrobial activity against some human pathogenic strains. BioMed Research International, 2014;1-16
10. Verdenelli MC, Ghelfi F, Silvi S, Orpianesi C, Cecchini C, Cresci A. Probiotic properties of *Lactobacillus rhamnosus* and *Lactobacillus paracasei* isolated from human faeces. European Journal of Nutrition, 2009; 48(6):355-363.
11. Nikolova D, Petrova M, Evstatieva Y, Danova S, Atev A. Antimicrobial activity of *Lactobacillus helveticus* strain 50P1. Trakia Journal of Sciences, 2009;7 (2):40-44.