International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 5 Sep-Oct 2021, pp: 136-138 www.ijprajournal.com ISSN: 2249-7781

Antimicrobial effect of Lactobacillus acidophilus on Salmonella Typhimurium

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Date of Submission: 30-08-2021 Date of Acceptance: 08-09-2021

ABSTRACT
Salmonella is one of the most important causes of foodborne illnesses and deaths. Overuse and misuse of antibiotics in current scenario had led to the emergence of multidrug resistant strains of Salmonella. Hence alternative approaches, other than antibiotics are need of today for prevention control and treatment of Salmonella infections. Probiotics are one of important alternatives which are considered as safe for human consumption and cause health benefits when administered in adequate amount. The aim of present study was to evaluate the antimicrobial effect of Lactobacillus acidophilus against Salmonella Typhimurium. In vitro antimicrobial effect of Lactobacillus acidophilus was evaluated using broth dilution method. Minimum inhibitory concentration (MIC) was calculated by co-culturing Lactobacillus and Salmonella Typhimurium. Selective media XLT-4 was used for enumeration of Salmonella Typhimurium. The results of our study conclude that tested Lactobacillus acidophilus strain can be used as potential candidate for further in vivo studies and its applications in control and treatment of salmonellosis.

Keywords : Salmonella, antimicrobial, Lactobacillus, in vitro

I. INTRODUCTION

Foodborne diseases cause a severe public health problem and affect people's wellbeing and leads to serious socioeconomic losses. There are many factors that influence foodborne diseases. Hence their prevention, control and treatment require multiple approaches (**Sivapalasingam et al., 2004)**. Among the major foodborne pathogens, Salmonella enterica is one of the major causes of foodborne illness ranging from acute gastroenteritis to systemic infections. The nature and severity of the infection developed depends on many factors. It includes the serovar involved, the virulence of the strain, the infective dose, the age and immune status of the host. According to some reports, Salmonella species cause 93.8 million gastroenteritis infections worldwide and resulted in 155 000 deaths each year (**Majowicz et al., 2010**). Antibiotics are the common clinical treatments for Salmonella infection. It has caused the emergence of multi drug resistant Salmonella species (**Berendonk et al., 2015**). Also, the prolonged use of antibiotics causes the imbalance in the intestinal microflora (**Ramirez et al., 2020).** Due to the emergence of multidrug resistant strains, alternative strategies to combat Salmonella infections are urgently needed. One of the promising alternative therapeutic approaches is the use of probiotics against various pathogens, including Salmonella spp (**Das et al., 2013**).

Probiotics are the live microorganisms which confer health benefit to the host when administered in adequate amount. Lactobacillus and Bifidobacterium species are most commonly studied probiotics for their antimicrobial effect against Salmonella infections (**Tsai et al., 2005)**. The mechanism of antimicrobial activity includes production of antimicrobial elements such as bacteriocins, bacteriocin like substance, volatile fatty acids and hydrogen peroxide, decreasing the pH of the environment, competition for adhesion sites on the intestinal epithelium and modulation of immune system and alterations in enzyme activity (**Parada et al., 2007)**. A great number of in vivo and in vitro studies have been conducted to evaluate the effect of probiotics to prevent and treat gastrointestinal infections caused by Salmonella species.

Because of few studies conducted on the effect of Lactobacillus acidophilus on gram-negative bacteria especially Salmonella Typhimurium, we aimed to study the antimicrobial effect of Lactobacillus acidophilus on Salmonella Typhimurium

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II. MATERIALS AND METHOD

2.1 Chemicals and re-agents

de-Man Rogosa and Sharpe (MRS), Brain–Heart Infusion (BHI) broth, agar base and Xylose lysine tergitol 4 (XLT4) media were procured from Hi-Media labs, Mumbai, India.

2.2 Bacterial strains

Lactobacillus acidophilus was procured from National Collection of Dairy Cultures (NCDC), ICAR-NDRI, Karnal, India. Salmonella Typhimurium isolates were procured from the Department of Veterinary Public Health and Epidemiology, College of Veterinary and Animal Sciences, GBPUA&T, Pantnagar. All the bacterial cultures were preserved as glycerol stocks at -80°C.

2.3 In vitro antimicrobial effects of Lactobacillus acidophilus on Salmonella Typhimurium

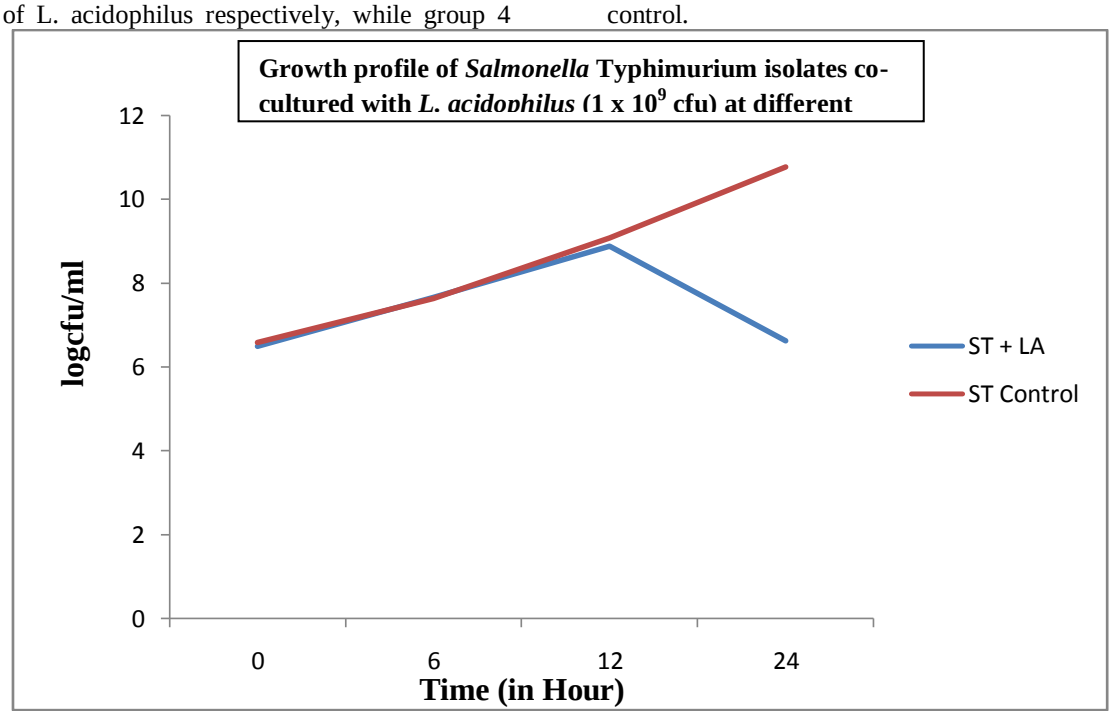
A single colony of Salmonella Typhimurium isolate and L. acidophilus were separately inoculated in Brain–Heart Infusion broth and incubated at 37°C for 4 h to attain log phase cultures. The log phase cultures were pelleted and suspended in sterile Normal Saline Solution (NSS) individually to match with 0.5 MacFarland standard (equivalent to 1.5 x 10^8 cfu/ml). After adjusting the count, the cultures of Salmonella Typhimurium and L. acidophilus were suspended in 5 ml BHI Broth in 4 different groups. Group 1, 2 and 3 consist of 1 x 10^7 cfu, 1 x 10^8 cfu and 1 x 10^9 cfu of L. acidophilus respectively, while group 4 was Salmonella control. Each group contains 1 x 10^6 cfu of Salmonella Typhimurium. The mixed co-cultures of respective groups along with appropriate controls were incubated at 37°C upto 24 h. To enumerate antibacterial effect of L. acidophilus strain on Salmonella Typhimurium isolate, an aliquot of 100 µL from all the four groups were drawn at 0 h, 6 h, 12 h and 24 h. At each time point, aliquot i.e. 100 µL of each respective group was serially diluted 10 fold in NSS and 10 µL from last three dilutions were placed on Xylose lysine tergitol 4 (XLT4) agar plates. The individual bacterial colonies were counted on XLT4 agar plates and expressed as Salmonella Typhimurium log cfu/ml.

2.4 Statistical analysis

In vitro experiments were performed three times independently and the results were expressed as the mean ± standard deviation. The level of significance between groups and controls was analyzed by one-way analysis of variance (ANOVA) followed by Duncan's test using IBM SPSS Statistics.

III. RESULTS

Dose dependent antimicrobial effects of L. acidophilus were analyzed. Number of colonies at different time interval were presented as logcfu/ml. Significant ($p < 0.05$) antimicrobial effect of L. acidophilus (10^9 cfu) was observed at 24 h, whereas bacterial growth was observed in the control.



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IV. DISCUSSION

Recently, there has been a great interest in products with natural origin. In the same line, wide range of lactobacillus strains that were isolated from dairy products were evaluated as a valuable source of novel therapeutic tools. The results of the current study revealed that L. acidophilus exhibited significant antimicrobial activity against Salmonella Typhimurium. Antimicrobial activity of lactobacilli may be due to production of organic acids, hydrogen peroxide, bacteriocins, and bacteriocin-like substances **(Balamurugan et al., 2014** Significant reduction in the growth of Salmonella Typhimurium in our indicates that L. acidophilus exhibit antimicrobial activity. **)**. Several similar studies have explained the effect of probiotics on different gastrointestinal disturbances such as irritable bowel syndrome caused by Helicobacter **(Ducrotte et al., 2012)**, C. difficile infection **(McFarland, 2015).**

V. CONCLUSION

The results of our study conclude that L. acidophilus exhibited antibacterial activity against Salmonella Typhimurium isolates. More intensive in-vitro and in-vivo model studies need to be carried out revealing the phenotypic and genotypic characteristics of probiotics, in order to explicate their role in human health.

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