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ANTI – *SHIGELLA DYSENTERIAE* ACTIVITY BY PROBIOTIC LACTIC ACID BACTERIA (*PEDIOCOCCUS PENTOSACEUS*); AN *IN VITRO* STUDY

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ABSTRACT

Shigellosis caused by *Shigella* is prevalent throughout the world with approximately 164.7 million cases, of which 163.2 million are in developing countries as per the World Health Organization report. In the current study the effect of a known Probiotic Lactic acid Bacteria (PLB) *Pediococcus pentosaceus*, a previously reported strain of PLB from our laboratory on gastroenteric pathogen – *Shigella dysenteriae* was studied and its mode of action was established. In agar diffusion tests PLB lysate showed larger inhibition zones of *S. dysenteriae* than a known *Shigella* susceptible antibiotic ampicillin which shows a better potentiality of PLB lysate over standard antibiotic. Further the effect of PLB lysate on *Shigella dysenteriae* lysis was confirmed by electrophoretic and microscopic study. PLB lysate at 250 µg/mL protein concentration inhibited ~70% of *Shigella dysenteriae* growth in *vitro*. A significant protection was observed against the cellular damage caused by *Shigella dysenteriae* lysate. Red blood cells and buccal cells protection against the lysis induced by *Shigella dysenteriae* lysate substantiated the cytoprotective role of PLB, thus PLB can be an effective natural agent against *Shigella* mediated infection.

Keywords: Shigellosis, anti-*Shigella* activity, Probiotic Lactic acid Bacteria (PLB), Cellular damage and protection

INTRODUCTION

Shigellosis is an intestinal disease and a serious health problem in north-eastern parts of India and other developing countries in world **(Swapan, 2005; Reema *et al*., 2013)**. It is caused by *Shigella*, being epidemic it attained multi drug resistance. *Shigella dysenteriae* is one of the prominent infectious strains out of *Shigella* species. A report states *Shigella dysenteriae* showed resistance to Chloramphenicol (80%), Tetracycline (100%), Co-trimoxazole (100%), Nalidixic acid (100%) and Ciprofloxacin (100%) **(Datta *et al*., 2003; Deen *et al*., 2004; Alam and Shrish, 2006)**. *S. dysenteriae* causes Shigellosis along with inflammatory diarrhea and dysentery. The symptoms of Shigellosis include sepsis, dehydration, encephalopathy, intestinal perforation, toxic megacolon, and pneumonia. In few cases it may leads to hemolytic uremic syndrome which is a life threating systemic disease characterized by thrombocytopenia and kidney failure **(Laure *et al*., 2013)**. Therefore there is a need for the new potential sources of antibiotic or concurrent use of probiotic bacteria. Another group reported that bacteriocins produced by lactic acid bacteria can treat the multi-drug resistant strains **(Diekema *et al*., 2001; Paluszak *et al*., 2006; Hickson, 2011)**. *Pediococcus pentosaceus* is a Gram-positive probiotic lactic acid bacteria known to produce bacteriocins and specific isolate which we have isolated from cheese – MTCC 5151 was producing a novel bacteriocin of molecular mass of 23 kDa. Present study focuses on effect of this Lactic acid Bacteria *Pediococcus* on *Shigella dysenteriae* and reports the anti-*Shigella dysenteriae* activity followed by the inhibition of mammalian cellular damages caused by *S. dysenteriae.*

MATERIAL AND METHODS

Bacterial strains

Shigella dysenteriae obtained from JSS Medical College in Mysore, India was selected as a test organism. The culture was sub cultured once in 2 weeks in Brain Heart Infusion medium of pH 7.4, and stored in the same media at 4 °C. A cheese isolate, Probiotic Lactic acid Bacteria (PLB) *Pediococcus pentosaceus* was maintained in the laboratory as per the protocol standardized earlier in the laboratory. The culture was maintained by sub culturing in de Man Rogosa Sharpe (MRS) broth of pH 6.4, once in 2 weeks. The cultures were confirmed by gram staining. PLB being Gram-positive stains violet while; *Shigella* being Gram-negative, stains pink with the crystal violet and saffranine reagents.

Study of *Shigella* and PLB interaction

Effect of PLB on *Shigella* was studied employing microscopic, electron microscopic and agar diffusion tests followed by protein profiling.

Scanning electron microscopic studies

Shigella and PLB cells were harvested after growing in their respective media, washed in Phosphate Buffered Saline (PBS), pH 7.4 (3X). 10^5 cells/mL of *Shigella* and PLB each were incubated at 37 °C for 30 min. Respective controls for both *Shigella* and PLB were also maintained at similar set of experimental conditions. After the incubation, cells were fixed with 2% gluteraldehyde in PBS and processed further with alcohol treatment followed by coating with gold particles for Scanning Electron Microscopic (SEM) observation. Multiple fields of visions were viewed and documented by photography at different magnifications. Results were compared between the morphological changes in PLB as well as *Shigella* in their respective untreated and treated samples.

Light microscopic observation

Cell concentration about 10^5 cells/mL of freshly harvested and washed *Shigella* and PLB were incubated at 37 °C for 30 min. Respective controls for both *Shigella* and PLB were maintained individually at similar set of experimental conditions. After allowing interaction between PLB and *Shigella*, 20 µL aliquots of each were taken on to glass slides, heat fixed and processed for gram staining. Slides were observed under the light microscope and documented by photography. Effect of PLB on *Shigella* was monitored using differential gram staining properties between PLB and *Shigella.*

Agar diffusion assay

Agar diffusion assay was performed to understand the *Shigella* inhibitory effect of PLB. PLB was grown as described above. Lysate and the incubated media

were collected to study their effects on *Shigella* growth. 10^7 PLB cells/mL were washed 3 times with PBS and washed PLBs were lysed by sonication; supernatant was collected after centrifugation at 2500g for 15 min at 4 ^{0}C and the clear supernatant obtained was estimated for total protein and designated as 'PLB-Lysate (PLB-L)'. ~2-3 µg protein equivalents of PLB-L and PLB-Media (PLB-M) were added to the wells (3 mm diameter) created on the 2% BHI agar plate with *Shigella* inoculum at 10^2 cells/mL concentration. Ampicillin at 30 µg was used as a positive control. The plate was incubated at 37 ^{0}C, overnight. *Shigella* growth inhibition was determined as the diameter of the inhibition zones around the wells. The growth inhibition diameter was an average of four measurements taken at four different directions. Efficiency of inhibition was compared with that of the known *Shigella* - susceptible ampicillin antibiotic.

Effect of PLB lysate on growth index of *Shigella*

A 10µL of 10^7 cells/mL was added to 1.0 mL of *Shigella*-specific media in triplicates, in presence and absence of 2 µg protein equivalents of PLB-Lysate/mL. Growth index and percent growth inhibition was calculated to understand the effect of PLB lysate on *Shigella* growth. Growth was measured as a turbidometric measure at A_{660} nm in a Beckman spectrophotometer. Percentage inhibition in presence of given concentration is calculated as follows:

$$\text{Growth inhibition (\%)} = \frac{100 - \text{Absorbance of } \textit{Shigella} \text{ culture tube with PLB lysate} - \text{Absorbance of PBS}}{\text{Absorbance of } \textit{Shigella} \text{ culture tube} - \text{Absorbance of PBS}}$$

Confirmation of PLB lysate induced *Shigella* lysis by electrophoresis

If *Shigella* is lysed by PLB lysate, *Shigella* proteins are expected to be released and this is studied by electrophoresis. The effect of PLB cell lysate on *Shigella* cells was studied comparing the protein profile data of PLB lysate and supernatant of *Shigella* culture with and without treatment with PLB-L. The procedure described briefly is as follows: 100 µL aliquot from 10^7 cells/mL of *Shigella* culture was washed thoroughly with sterile PBS and suspended in 100 µL of sterile PBS. 50 µL of this aliquot was treated with 50 µL of PLB cell lysate and incubated at 37 ^{0}C for 6 h. Respective controls of *Shigella* cells and PLB-lysate were also maintained under similar experimental conditions. After incubation, the supernatant from all the three tubes were subjected to SDS-PAGE. Proteins were stained by coomassie blue reagent and profiles were compared between PLB alone, *Shigella* alone and that of PLB + *Shigella.*

Interaction between mammalian cells and *Shigella* cells and its lysate

Red Blood Cells (RBCs) and Buccal Cells (BC) from humans were used during the study. The cytotoxic effects of *Shigella* were studied by taking *in vitro* models like RBC and BCs to study the protection offered by PLB against *Shigella* induced cellular toxicity.

Effect of PLB against *Shigella* induced toxicity on red blood cells (RBC)

RBCs were obtained from healthy donors after taking their consent. Heparinized blood was centrifuged at 2500g for 10 min. After removal of plasma and buffy coat, the RBCs were washed three times with Phosphate Buffered Saline (PBS - pH 7.4) at room temperature and resuspended in PBS four times its volume for subsequent analysis. 100 µL of RBC was incubated with 30 µL (0.84 µg of protein) of PLB cell lysate in presence of increasing amounts of *Shigella* cell lysate and the total volume was made up to 300 µL with PBS. It was incubated at 37 ^{0}C for 20 min and centrifuged at 2500g for 10 min at room temperature. Respective controls of *Shigella* and PLB cell lysates were maintained under similar conditions. Hemoglobin released from cells in the supernatant, due to hemolysis was diluted four times with PBS and read spectrophotometrically at 410 nm.

Effect of PLB against *Shigella* induced toxicity on buccal cells

Buccal cells (BC) were isolated from human volunteers and washed with phosphate buffered saline and resuspended in minimum amount of PBS. 30 µL of BC suspension ($1x10^4$cells/mL of PBS) were incubated for 20 min at room temperature with 30 µL of *Shigella* cell lysate (20 µg of protein) or *Shigella* incubated media respectively. In the second set 30 µL of BC suspension pre-incubated with PLB lysate or PLB incubated media was treated with *Shigella* lysate or *Shigella* incubated media were set up at similar set of experimental conditions. Treatment with either PLB cell lysate or PLB incubated media alone will serve as the control. Cells both treated and untreated were observed under the microscope upon staining with acridine orange and ethidium bromide at different magnifications. The results were documented by photography.

RESULTS

Effect of Probiotic Lactic acid Bacteria on *Shigella*

The effect of Probiotic Lactic acid Bacteria on *Shigella* was studied by performing microscopic studies, agar diffusion assay and protein profiling.

Electron microscopic observation of *Shigella* upon treatment with PLB

Studies with Scanning Electron Microscopy suggested that the incubation of *Shigella* cells (Fig. 1a) with PLB (Fig. 1b) resulted in the aggregation and condensation of cytoplasmic components of the *Shigella* cells which looks like a white condensed dot (Fig. 1c). Further, prolongation of incubation for 30 min resulted in emptying of cellular content of *Shigella* (Fig. 1d), while PLB remained unaffected (Arrow in Fig. 1d). Thus the result suggests that PLB may have cytotoxic effect against *Shigella*, a bacterial pathogen.

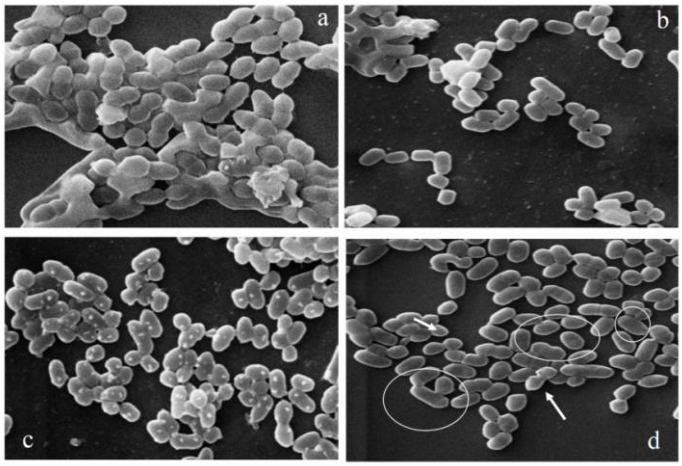

Figure 1 Photographs of scanning electron microscopic study, depicting **(a)** *Shigella* cells; (**b)** PLB cells; (**c)** *Shigella* incubated with PLB for 10 min; **(d)** *Shigella* incubated with PLB for 30 min. Elongated cells of *Shigella* with emptiness indicate the lysis shown in circles; Small PLB cells remained unaffected indicated by arrows.

Light microscopic observation of *Shigella* upon treatment with PLB

Gram staining followed by observation of untreated *Shigella* (Fig. 2b) and PLB (Fig. 2a) treated *Shigella* (Fig. 2c and Fig. 2d) in light microscopy revealed aggregation of *Shigella* pathogen (pink colored gram negative bacteria) with uniformly distributed PLB suggesting the attack of PLB on *Shigella* rather than the vice versa. Aggregation of pink *Shigella* cells (Fig. 2c) observed after 10 min of incubation, was lysed during prolonged incubation of 30 min (Fig. 2d). Data thus suggests the ability of PLB to kill *Shigella.*

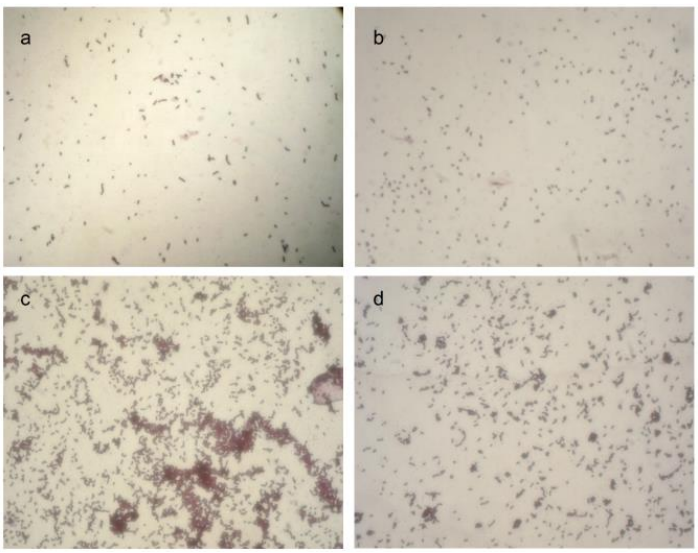

Figure 2 Photograph of Gram staining showing **(a)** Probiotic Lactic acid Bacteria; **(b)** *Shigella*; **(c)** *Shigella* interaction with PLB for 10 min at 37 ^{0}C; **(d)** *Shigella* interaction with PLB for 30 min at 37 ^{0}C.

Agar diffusion assay

Agar diffusion assay showed clear growth inhibition zone of 14 mm diameter around the well containing PLB cell lysate at 3.5 µg protein concentration (Fig. 3b) as opposed to Ampicillin (34 mm) antibiotic at a concentration of 30 µg (Fig. 3a) suggesting the lysing ability of PLB against *Shigella.* PLB incubating media (Fig. 3c) also showed inhibitory zone, but to lesser extent which could be due to the dilution effect. Similar growth inhibition zones of *Shigella* by PLB was reported **(Smita and Vaijayanti, 2014)**.

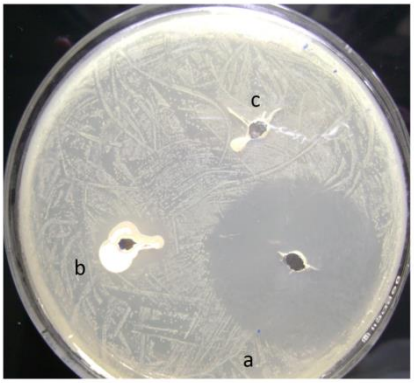

Figure 3 Agar plate showing growth inhibition zones of *Shigella*: **(a)** Ampicillin; **(b)** PLB cell lysate; **(c)** PLB incubated media. Inhibition was more with Ampicillin (34 mm) than PLB-L (14mm); than PLB-M (<6mm).

Effect of PLB lysate on growth index of *Shigella*

Shigella cell density decreased with increase in PLB lysates protein concentration (Table 1). Quantitative study results, the percentage of *Shigella* inhibition by PLB lysate was depicted (Fig. 4). PLB lysate inhibited *Shigella* growth in the culture broth confirming the ability of PLB lysate on *Shigella* lysis.

Table 1 Percentage of *Shigella* growth inhibition by PLB lysate at varied protein concentration.

Protein concentration of PLB Lysate (µg/mL)	***Shigella* growth (%)**
0	100
20	95
40	83
60	81
80	79

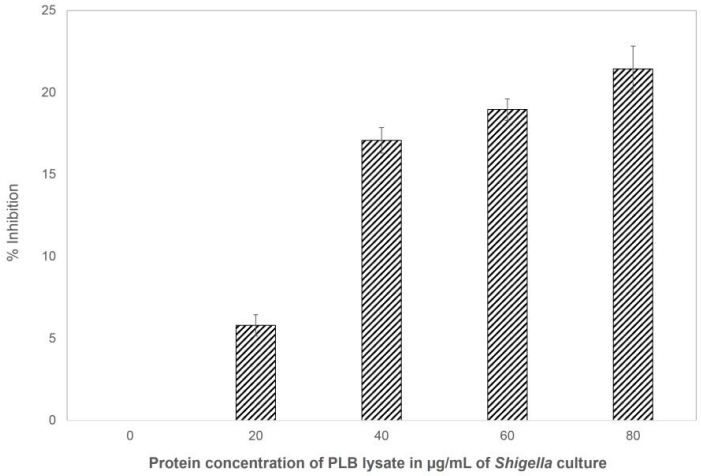

Figure 4 PLB lysate protein concentration dependent growth inhibition of *Shigella.*

Confirmation of PLB lysate induced *Shigella* lysis by electrophoresis

Results of electrophoresis (Fig. 5) showed different band pattern of proteins. The band pattern in Lane 1 represents PLB lysate, Lane 2 represents *Shigella* cell supernatant and Lane 3 represents the proteins in PLB lysate along with proteins released from *Shigella* when treated with PLB lysate which causes *Shigella* lysis. Differential band patterns between PLB lysate protein profile in Lane 1 and *Shigella* + PLB lysates protein profile in Lane 3 is due to additional proteins from *Shigella* lysis which confirms the capacity of PLB lysate on *Shigella* lysis.

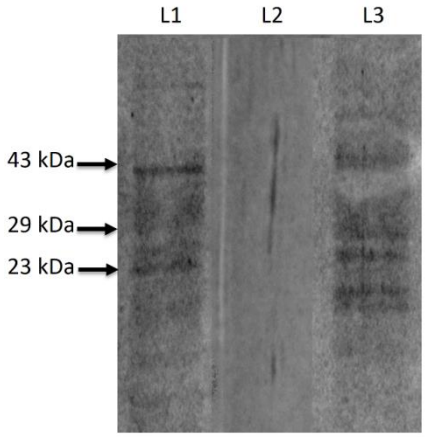

Figure 5 Gel photograph of SDS – PAGE showing protein profiles.
L1- PLB lysate, L2-Supernatant of *Shigella* cells and L3- Supernatant of *Shigella* cells treated with PLB lysate.

Interaction of mammalian cells with PLB and *Shigella*

Determination of cytoprotectivity of PLB against the cytotoxic effects of Shigella

From the above results it is proven that *Shigella*, a bacterial pathogen can be degraded by PLB or PLB lysate. Current experiment addresses the effect of *Shigella* on mammalian cells; and its effect in presence of PLB. In course the effect of *Shigella* lysate on human red blood cells and buccal cells were studied.

Effect of PLB against Shigella induced toxicity on red blood cells (RBC)

Data (Fig. 6) revealed that *Shigella* lysate lysed RBCs in a dose dependent manner. At 1.0 µg protein concentration of *Shigella*, 100% damage was observed. However 0.8 µg protein concentration of PLB protected RBCs up to 46% suggesting that PLB may offer protection against *Shigella* induced cellular damage.

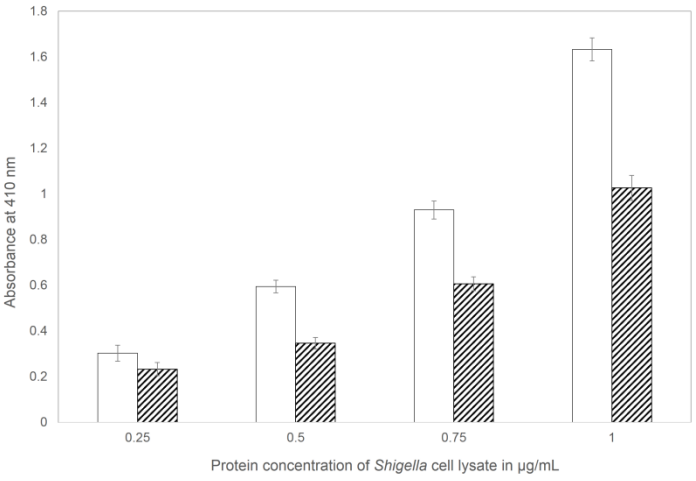

Figure 6 Effect of *Shigella* lysate on RBC at different concentrations with and without PLB lysate.

Effect of PLB on Shigella induced effect on buccal cells

Effect of *Shigella*, PLB, *Shigella* lysate, PLB lysate and in combination on buccal cells (BC) was studied. Results (Fig. 7) revealed that both *Shigella* lysate (Fig. 7c) and the *Shigella* (Fig. 7e) induced cytotoxicity of BC with disruption in cellular morphology when compared to that of untreated control (Fig. 7a). Buccal cells depicted in Fig. 7d and Fig. 7f shows the protection effect on buccal cells against *Shigella* lysate and *Shigella* respectively. Data thus suggests the presence of cytotoxic compound in the *Shigella*, which is also released into the media during the culturing as evidenced by cellular damage by the media. However, these damages could be prevented by the addition of either PLB lysate (Fig.7b) or the media (Fig. 7g) at 12 µg and 3 µg protein concentration respectively. PLB as such did not cause any toxicity to cells including the PLB lysate (Fig. 7b) and the PLB media (Fig. 7g). Data thus further suggests that PLB is potentially non – toxic to host cells; while being toxic to the pathogenic organism – *Shigella*. As well PLB has the potential to protect mammalian cells against *Shigella* induced damages. Therefore the overall data suggests that PLB may have potential to

prevent *Shigella* induced pathogenesis by either inhibiting the growth of *Shigella* or lysing them or neutralizing the toxins produced by *Shigella.*

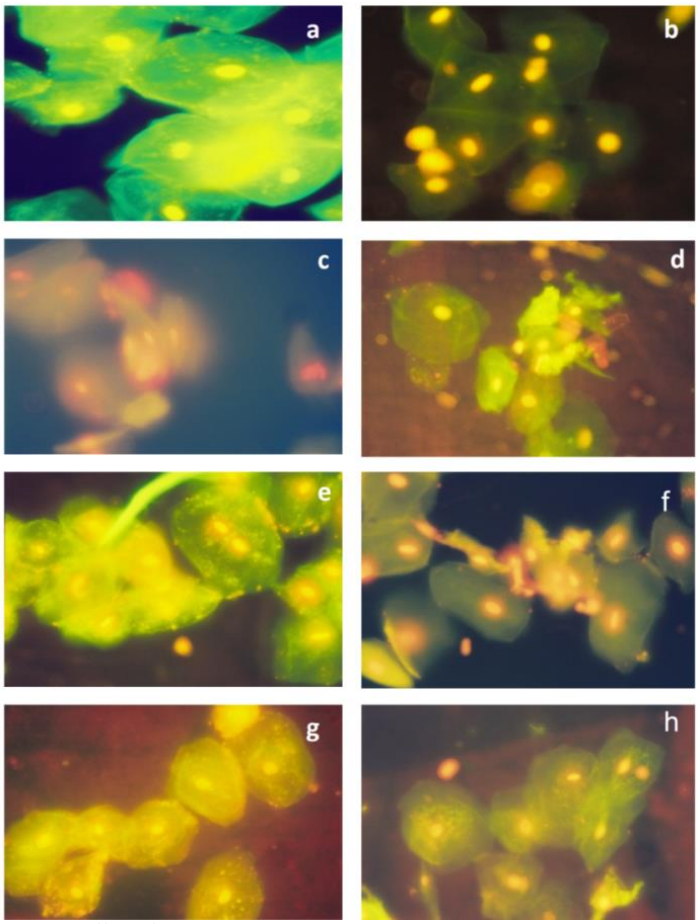

Figure 7 Effect of *Shigella* on buccal cells.
(a) untreated buccal cells; **(b)** buccal cells treated with PLB lysate; **(c)** buccal cells treated with *Shigella* lysate; **(d)** buccal cells treated with *Shigella* lysate and PLB lysate; **(e)** buccal cells treated with *Shigella* incubated media; **(f)** buccal cells treated with *Shigella* incubated media and PLB lysate; **(g)** buccal cells treated with PLB incubated media; (**h)** buccal cells treated with PLB incubated media and *Shigella* lysate.

DISCUSSION

Shigellosis is a bacterial infection that affects the digestive system. During Shigellosis, *Shigella* invades into the human intestinal mucosa, harbors there and causes dysentery, followed by recto-colitis responsible for lethal complications. Antibiotics such as azithromycin, ciprofloxacin, co-trimoxazole are often prescribed for it **(Taneja, 2007)**. Antibiotic resistance together with the loss of gut beneficial microbes indeed enhances the severity of shigellosis complications. Prolonged and frequent shigellosis may end up with development of post-infection arthritis which include joint pain, painful urination and eye irritation. Post-infection arthritis can become a chronic condition that lasts several months, years or the rest of the life.

Cultures of direct – fed microorganisms or probiotics are able to multiply in the intestinal tract to create a balance of microflora **(Biradar *et al*., 2004)**. Some lactobacillus species used in probiotic applications include *L. acidophilus*, *L. casei*, *L. reuteri*, *L. rhamnosus*, *Pediococcus pentosaceus and Bifidobacterium bidifum*. Our previous study has indicated that a cheese isolate *Pediococcus pentosaceus* Lactic acid Bacterium (PLB) which has been deposited at the Microbial Type Culture Collection centre, Chandigarh with the accession number MTCC 5151 was effective in inhibition *Shigella* growth **(Renu and Shylaja, 2012)**. In the current study we provide evidence for the anti-*Shigella* effect of PLB through microscopic and biochemical tests. Effective inhibition of *Shigella* growth by PLB was evident as per light microscopic and scanning electron microscopic studies. Formation of inhibitory zone by PLB, lysis of *Shigella* by PLB lysate as evidenced by electrophoresis further confirms the anti-*Shigella* effect of PLB and it accord with other reports **(Smita and Vaijayanti, 2014)**. Interaction of *Shigella* with mammalian cells inducing cellular damages both in the red blood cells and mammalian buccal cells; inhibition of the same by PLB, supports the fact that PLB is interacting with *Shigella*. An anti-*Shigella* activity of PLB could be due to the antimicrobial property of bacteriocin as highlighted in our earlier studies **(Renu and Shyalaja, 2012).** Hence it confirms the *Pediococcus* a Probiotic Lactic acid Bacteria as a antagonist of *Shigella dysenteriae*, similar anti-*Shigella dysenteriae* activity by few other strains of lactic acid bacteria was reported **(Devraj *et al*., 2007; Moorthy *et al*., 2010)**.

CONCLUSION

The above study indicates that the Probiotic Lactic acid Bacteria, *Pediococcus pentosaceus* has anti *Shigella* activity and it has proven that regular intake of probiotic food supplements are beneficial in enteric infections like Shigellosis.

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REFERENCES

ALAM, S., SHRISH, B. 2006. Current Status of Anti-Diarrheal and Anti-Secretory Drugs in the Management of Acute Childhood Diarrhea. *Indian J Pediatr*, 73, 693-696. http://dx.doi.org/10.1007/bf02898447

BIRADAR, S., BHAGAVATI, S., SHENGUNSHI, B. 2004. Probiotics and Antibiotics: A brief Overview. *The Internet Journal of Nutrition and wellness*, 2, 1-6. http://dx.doi.org/10.5580/fc0

DATTA, S., CHATTOPADHYAY, S., NAIR, G.B., ASISH, K.M., JABARANJAN, H., BERG, D.E., SAHA, D.R., KHAN, A., AMAL, S., BHATTACHARYA, S.K. AND ABHIJIT, C. 2003. Virulence Genes and Neutral DNA Markers of Helicobacter pylori Isolates from Different Ethnic Communities of West Bengal, India. *Journal of Clinical Microbiology*, 3737-3743. http://dx.doi.org/10.1128/jcm.41.8.3737-3743.2003.

DEEN, J., SUR, D., RAMAMURTHY, T. 2004. Shigellosis: challenges and management issues. *Ind J Med Res*, 120, 454-462.

DEVARAJ, S.N., MOORTHY, G., MURALI, M.R. 2007. Protective role of lactobacilli in *Shigella dysenteriae* induced diarrhea in rats. *Nutrition*, 23, 424-433. http://dx.doi.org/10.1016/j.nut.2007.03.003

DIEKEMA, D.J., PFALLER, M.A., SCHMITZ, F.J. 2001. Survey of infections due to *Staphylococcus* sp. Frequency of occurrence and antimicrobial susceptibility of isolates collected in the United states, Canada, Latin America, Europe and the Western pacific region for the sentry antimicrobial surveillance program. *Clin Infect Disease*, 32, 114-132. http://dx.doi.org/10.1097/00019048-200106000-00032

HICKSON, M. 2011. Probiotics in the prevention of antibiotic-associated diarrhea and Clostridium difficile infection. *Therapeutic Advances in Gastroenterology*, 4, 185-197. http://dx.doi.org/10.1177/1756283x11399115

LAURE, B.M.K., JOSEPH, L.T., JACQUES, K. 2013. The anti-shigellosis activity of the methanol extract of *Picralima nitida* on *Shigella dysenteriae* type I induced diarrhea in rats. *BMC Complementary and Alternative Medicine*, 13, 211-222.
http://dx.doi.org/10.1186/1472-6882-13-211

MOORTHY, G., MURALI, M.R., DEVARAJ, S.N. 2010. Lactobacilli inhibit *Shigella dysenteriae*-1 induced pro-inflammatory response and cytotoxicity in host cells via impediment of *Shigella*–host interactions. *Digestive and Liver Disease*, 42, 33-39. http://dx.doi.org/10.1016/j.dld.2009.04.021

PALUSZAK, Z., JUSTYNA, B.K., BEATA, S. 2006. Inhibitory effect of Lactic acid bacteria of genus Lactobacillus on the survival of *Proteus* and *Shigella* rods in mixed cultures. *Bull Vet Inst Pulaway*, 50, 335-340.

REEMA, N., LAHARI, S., GARGI, C., DAISY, S. 2013. Drug resistant *Shigella flexneri* in & around Dibrugarh, north-east India. *Indian J Med Res*, 137, 183-186.

RENU, A., SHYLAJA, D. 2012. An anti-*Shigella dysenteriae* bacteriocin from *Pediococcus pentosaceus* MTCC 5151 cheese isolate. *Turk J Biol*, 36, 177-185.

SMITA, N., VAIJAYANTI, S. 2014. Evaluation of antimicrobial activity of probiotic microflora from neonatal origin. *Asian Journal of Multidisciplinary Studies*, 2, 182-283.

SWAPAN, K.N. 2005. Shigellosis. *The Journal of Microbiology*, 43, 133-143.

TANEJA, N. 2007. Changing epidemiology of shigellosis and emergence of ciprofloxacin resistant *Shigellae* in India. *J Clin Microbiol*, 45, 678-679. http://dx.doi.org/10.1128/jcm.02247-06