Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 10, Issue, 05, pp.69349-69357, May, 2018

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

INFLUENCE OF *LACTOBACILLUS* STRAINS ON GASTRIC CANCER INDUCED BY *HELICOBACTER PYLORI*

[1*]Kinjal Bhadresha, [2]Manthan Trivedi, [1]Bhaumik Vaghela, [1]Rakesh Rawal and [1]Nayan Jain

[1]Department of Life Science, School of Science, Gujarat University, Ahmadabad, Gujarat, India
[2]Head of Research and Development Department, Lincoln Pharmaceuticals ltd, Ahmadabad, Gujarat, India

ARTICLE INFO

Article History:
Received 17th February, 2018
Received in revised form
21st March, 2018
Accepted 03rd April, 2018
Published online 30th May, 2018

Key words:

Helicobacter Pylori,
lactobacillus acidophilus,
lactobacillus Rahmnosus.

ABSTRACT

Helicobacter pylori, a gastric pathogen that colonizes approximately 50% of the world's population. Infection with *H.pylori* is the strongest known risk factor for some GI disorders including gastric cancer. Probiotics have considerable potential for preventive or therapeutic applications in various gastrointestinal disorders. So, the aim of this study was *in vitro* study of the potential inhibitory effect of *Lactobacillus* strains on *H.pylori* Growth. The aim of the potential inhibitory effect of *Lactobacillus* strains on *H.pylori* by using a standard antimicrobial plate well diffusion assay and liquid media assay. Among the two, *L. acidophilus* showed maximum zone of inhibition of a diameter of 22-18 mm against *H.pylori*. When the supernatant of the culture media of *L. acidophilus* was diluted with PBS, the zone of inhibition was decreased. On the other hand, *L. rhamnosus* showed maximum zone of inhibition of 23 mm in diameter against *H.pylori*. Unlike *L. acidophilus*, supernatant of the culture media of *L. rhamnosus* did not show any zone of inhibition upon dilution with PBS. Bacteriocins from *Lactobacillus* strains were found to be heat-stable (121^0C for 15 min) and active over a wide pH range of 5.0-9.0. Addition of surfactants (EDTA, SDS, CTAB) up to 1% to crude supernatant containing Bacteriocins showed increase in antibacterial activity; whereas NaCl concentration of 2% increased the antibacterial activity. Study concluded that the growth of the cancer bacteria *H.pylori* can be controlled by *Lactobacillus* strain but still requires optimization.

****Corresponding author:***

Citation: Kinjal Bhadresha, Manthan Trivedi, Bhaumik Vaghela, Rakesh Rawal and Nayan Jain. 2018. "Influence of *Lactobacillus* strains on Gastric cancer induced by *Helicobacter pylori"*, *International Journal of Current Research*, 10, (05), 69349-69357.

INTRODUCTION

Helicobacter pylori are a gram negative, micro-aerophilic bacterium which can inhabit various areas of the stomach, particularly the antrum. *Helicobacter pylori* are a causative agent of a range of human disease and it causes a chronic low-level inflammation of the stomach lining and is strongly linked to the development of duodenal and gastric ulcers and stomach cancer. Over 80% of individuals infected with the bacteria are asymptomatic (Oscariz, 2001). *Helicobacter pylori* were designated a class-I (definite) carcinogen for stomach cancer in 1994 after the epidemiological investigation by the International Agency for Research on Cancer (IARC), a subordinate organization of the World Health Organization[30]. Furthermore, the association of primary malignant gastric lymphoma with *Helicobacter pylori* has been reported in a large-scale cohort study. However, the role of *Helicobacter pylori* as an obligate pathogen has been questioned, and it has been proposed that *Helicobacter* strains could be part of the indigenous microbiota of the human stomach and that *Helicobacter pylori* could have both pathogenic and symbiotic features (Pande *et al*., 2009). The treatment of *Helicobacter pylori* remains a challenging clinical problem despite extensive research over the last 25 years. Increasing antimicrobial resistance and falling eradication rates are the results of the widespread use of antibiotics. The third Maastricht Consensus Report agreed that effective treatment for *H. pylori* should achieve an intention-to-treat (ITT) eradication rate of over 80% (Garza-González *et al*., 2014). Fermented dairy products and vegetables have been used for thousands of years. As early as 1907, Nobelist Elie Metchnikoff attributed the longevity of Bulgarian peasants to their consumption of fermented milk products (Sadaf, 2009). He suggested that regular consumption of dairy yogurt may suppress "putrefactive" bacteria in the colon. Since then, several definitions have been used to describe these probiotics, such as substances that are produced by one microorganism and stimulate the growth of other microorganisms (Bulent *et al*., 2010), live microbial feed supplements that beneficially affect the host animal by improving its intestinal microbial balance, and more recently,

live microorganisms that, when administered in adequate amounts, confer a health benefit on the host (Pantoflickova, 2013). The most commonly used organisms in probiotic products belong to *Lactobacillus* sp., and *Bifidobacterium* sp.. Other organisms have also been used including *Bacillus* sp., and yeast such as *Saccharomyces boulardii* (Soccol *et al*., 2010)-. Results were found by Thirabunyanon *et al*. (2011) concluded that the probiotics, *Enterococcus faecium* RM11 and *Lactobacillus fermentum* RM28, isolated from some fermented dairy products could inhibit the growth of pathogenic *H. pylori* in human. Therefore, in this study, we investigated how *lactobacilli* strains can affect the *H. pylori* growth by *In Vitro*. The finding opens for research the characterization of the *Lactobacillus* strains are effectors molecule that reduces *H. pylori* Growth and further investigation of its mode of action.

MATERIALS AND METHODS

Bacterial strains

- The Pathogenic Bacterial strain *Helicobacter pylori* (ATCC 26695) were gifted from Dr. Asish K. Mukhopadhyay, National Institute of Cholera and Enteric Diseases, Kolkata, India.
- A strain of *Lactobacillus acidophilus* (MTCC 10307) and *Lactobacillus rhamnosus* (MTCC 8712) was received from Microbial Type Culture Collection and GenBank, IMTECH, Chandigarh, India.

Media and growth conditions

- *H. Pylori* strain was cultivated in Brain heart infusion (BHI) agar with 5-7% sterile horse serum and rehydrated contents of 1.5 ml of Campylobacter supplement-I (Blaser-wang) under micro-aerophilic atmosphere (5% O_2, 10% CO_2 and 85% N_2) for 3 to 6 days (Kovvuri *et al*., 2012) .
- *Lactobacillus acidophilus* and *Lactobacillus rhamnosus* strains were cultivated in MRS agar (Himedia) under anaerobic conditions. Before use, the bacteria were propagated in proper medium (MRS) by incubated overnight at optimal temperature.

Biochemical Test

Production of *helicobacter pylori* supernatant: *Helicobacter Pylori* species were cultured in 200 ml BHI broth (pH 7.0) with aseptically added 5-7% sterile horse serum and rehydrated contents of 2 ml of Campylobacter supplement-I (Blaser-wang). The Broth was then incubated at 37°C in a micro-aerophilic atmosphere for 3 to 6 days. For extraction of Supernatant, a cell-free solution was obtained by centrifuged 5000 rpm for 20 minutes at 4˚C the culture and was adjusted to pH 7.0 (10). Then CFS was again centrifuged in 10K Macrosep advance Centrifugal Device (Pall Corporation). After centrifugation collected the less than 10 kDa protein Supernatant and greater than 10 kDa proteins Supernatant and then sterilized by filter membrane (0.25 µm, Nylone filter membrane).

Purification of supernatant: Soluble proteins present in the culture supernatant were precipitated by mixing with a 0.1 volume of Trichloroacetic acid (TCA) and kept on ice for 15 min. Subsequently, the mixture was centrifuged 10 000 g at 4 °C for 20 minutes and the pellets were washed with cold acetone to remove residual TCA. After this procedure was repeated, the pellets were air-dried and used for analysis of extra cellular released proteins from *H. pylori* (Tsuzuki, 2002).

Production of crude bacteriocins samples: *Lactobacillus* two species were cultured in 200 ml MRS broth (pH 7.0) The broth were then incubated at 37°C for 48 hr in a under anaerobic conditions. For extraction of bacteriocins, a cell-free solution was obtained by centrifuged 6000 rpm for 20 minutes at 4˚C the culture and was adjusted to pH 7.0 (Mahrous *et al*., 2013). Then supernatant was Lyophilized and Dissolved in PBS (1gm/1ml). Then CFS was again centrifuged 6000 rpm for 20 minute at 4˚C in 10K Macrosep advance Centrifugal Device (Pall Corporation). After centrifugation collect the less than 10kDa protein Supernatant and adjusted to pH 7 by 0.1 N NaOH (to exclude the effect of organic acids) and then sterilized by filter (0.25 µm, Nylone filter membrane).

Determination of protein concentration: Protein concentration of the supernatant/ ml was determined by the method of Lowry *et al,* (1951), using bovine serum albumin as the standard.

Screening of LAB strains for antibacterial activities: The inhibitory activity was screened by agar well diffusion assay (Soumya, 2012). First Prepared 150 ml hard base agar and 50 ml soft base agar with 7.5 ml sterile horse serum and 1.5 ml rehydrated contents of Campylobacter supplement-I (Blaser-wang) seeded with 10 ml of freshly grown indicator strain (about 10^5 cfu/ml), *Helicobacter Pylori*. Then wells of 5 mm in diameter were cut into the agar plate by using a cork borer. Then the wells were filled with the different concentration of Bacteriocin solution (125 µl). Then plate were put into anaerobic jar and passed out the gases (5% O_2, 10% CO_2 and 85% N_2). The plates were then incubated at 37°C in a micro-aerophilic atmosphere for 3 to 6 days. After 6 days incubation at proper temperature and anaerobic condition, inhibition zone was observed.

Minimum inhibitory concentration (MIC) determination: The Minimum inhibitory concentration activity was determined by Broth Dilution Method. Broth dilution method was done following the standard method of NCCLS (Ravi *et al*., 2010). The tubes were then incubated at 37°C in a micro-aerophilic atmosphere for 3 to 6 days. After 6 days incubation at proper temperature and anaerobic condition, turbidity was measured by OD at 600 nm.

Characterization of bacteriocin: The curd Bacteriocin samples were characterized with respect to Heat and pH stability, Surfactants, NaCl Concentration (Ogunbanwo *et al*., 2003). Agar well diffusion assay was performed to detect the Characterization of Bacteriocin.

Effect of heat treatment on bacteriocin activity: Culture supernatant was both *lactobacillus* strains heated for 10, 20 and 30 min at 40 ºC, 50 ºC, 60 ºC, 70 ºC, 80 ºC, 90 ºC, 100 ºC and agar well diffusion assay was performed to detect residual activity (Maria *et al*., 2012).

Effect of pH treatment on bacteriocin activity: The pH of the culture supernatant was both *lactobacillus* strains adjusted to 3, 4, 5, 6, 7, 8, 9 and 10.0 (from approximately 4.8 to 4.0, 3.0, and 2.0 with HCI and to 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0 with

NaOH). Then crude Bacteriocin was kept at room temperature for 4 hr. Residual activity was determined by agar – well diffusion method (Zamfir *et al*., 2000).

Effect of surfactants on bacteriocin activity: Crude Bacteriocin was both *lactobacillus* strains treated with different detergents (SDS, EDTA, CTAB), concentration ranging from 0.2%, 0.4%, 0.6%, 0.8%, and 1.0 %. Then Curde Bacteriocin was incubated at 35^0C for 3 hr, and activity was determined by the well diffusion method **(**Mojgani *et al*., 2009**).**

Effect of NaCl concentration on bacteriocin activity: Different NaCl Concentration 2, 4, 6, 8, 10% was added into the culture supernatant of both *lactobacillus* strains. Then crude Bacteriocin was kept at room temperature for 1 hr and agar well diffusion assay was performed to detect residual activity (Mahrous *et al*., 2013).

RESULTS

Bacterial strain Identification of *H.pylori* and *Lactobacillus* strains

- ***Helicobacter pylori***: After 7 days of incubation at 37°C in a micro-aerophilic atmosphere, fine colonies, Small round colonies, bulging and glossy, appear in the agar plate, these cultural characteristics correspond to *Helicobacter pylori*. The colonies were spread on agar and delivery incubated to obtain a bacterial lawn growth on the agar plate. The microscopic examination of a smear prepared from suspect colonies by setting the drop of the bacterial suspension in the flame and then the smear is stained by Gram's Method has allowed observing fine cells pink proving membership the Gram negative. Strains of *H. pylori,* showed the presence of a very active urease, oxidase and Catalase biochemical test (Figure I).
- ***Lactobacillus* strains:** After 48 hr of incubation at 37°C in a micro-aerophilic atmosphere the *Lactobacillus* strains were characterized on the basis of their colonies morphology and other biochemical characteristics. *Lactobacillus* Colonies were circular, small and large and cream-white after incubation on MRS agar plate. *Lactobacillus* strains studied were non-motile, Gram-positive, rod-shaped bacteria. The strains, catalase and oxidase were not produced.

Determination of inhibitory spectrum of Bacteriocin produced by isolates

***In vitro* screening for *H. pylori* inhibition:** With the well diffusion assay, the activity of L. acidophilus and L.rhamnosus strain against H. pylori (26695) was assessed. Cultures of *H.pylori* strain were tested against supernatant *of lactobacillus sp*. which shows anti *H.pylori* activity by measuring diameter of zone on the plate. All different concentration supernatant of lactobacillus tested (natural pH) inhibit the growth of *H.pylori*. It shown that in result when concentration of supernatant was decrease the zone of inhibition was not observed. But culture supernatant of *L.acidophilus* gives clear zone of inhibition against *H.pylori* (average diameter of the zone 20 to 25 mm). However, inhibitory effect was lost when supernatant pH adjust 5, 4, 9 and 10 so that is indicating that it is mediated by organic acid. When direct culture supernatant of *L.rhamnosus* displayed only minor inhibitory effect against *H.pylori*. *L.rhamnosus* inhibitory effect was increase when supernatant was adjusted with pH and NaCl concentration (Figure II).

Minimum inhibitory concentration (MIC) determination: To determine the MIC values, the selected *H.pylori* were grown on the rising concentrations of both *Lactobacillus* supernatant (Bacteriocin). Concentrations of Bacteriocin used for MIC evaluation were selected by previously determined the resistance to Bacteriocin of selected *H.pylori* by agar-well diffusion assay. Therefore *H.pylori* was grown in the BHI broth containing Bacteriocin in the range of 1:1 to 1:8. The strain *H.pylori* was not able to grow in the presence of Bacteriocin. After 6 days incubation at proper temperature and anaerobic condition, turbidity was measured by OD at 600 nm MIC values for Bacteriocin were determined. Result showed that *L. acidophilus* and *L. rhamnosus* supernatant was inhibiting the growth of *H.pylori.* When raising the concentration of Bacteriocin decrease the growth of *H.pylori* (Ravi, 2010).

Characterization of Bacteriocin

Effect of Heat treatment on Bacteriocin activity: Effect of heat treatment on the Bacteriocin activity was tested by keeping the crude extract (final pH after the fermentation was 4.8) at 40^0, 50^0, 60^0, 70^0, 80^0, 90^0 and 100^0C for various periods. As seen from Figure IV, V and VI, crude Bacteriocin was very stable to heat with respect to the all the temperatures. The stability of bacteriocin preparations has often been shown to decrease significantly with increased purification. *Lactobacillus acidophilus* was able to grow and produce bacteriocin up to 40^0 to 70^0 of heat treatment. *Lactobacillus acidophilus* was showed antimicrobial activity against *Helicobacter pylori.* This showed the largest of growth inhibitor % around 59.56% in temp. 60°C/30 min but that showed the smallest 18.08% in temp 100°C/30 min. *Lactobacillus rhamnosus* was able to grow and produce bacteriocin up to 40^0 to 70^0 of heat treatment. *Lactobacillus rhamnosus* was showed antimicrobial activity against *Helicobacter pylori.* This showed the largest of growth inhibitor % around 54.56% in temp. 50°C/30 min but that showed the smallest 15.08% in temp. 100°C/30 min. Heat resistance is a major characteristic of many bacteriocins and bacteriocin-like substance produced by lactic acid bacteria. They can vary dramatically ranging from 60^0C or 100^0C for more than 30 min (e.g. lactocin 27, lactocin S, carnobacteriocins A and B) to autoclaving at 121^0C for 15-20 min (e.g. lactacin B, lactacin F, nisin etc.) (De Vuyst, 1994).

Effect of pH treatment on Bacteriocin activity: Bacteriocins differ greatly with respect to their sensitivity to inactivation by changes in pH and temperature. Many of the bacteriocins and bacteriocin-like compound produced by lactic acid bacteria are only stable at acid and neutral pH (De Vuyst, 1994) and are inactivated even at a pH above 8.0 (e.g. nisin, lactostrepcins, pediocin AcH, leucocin A-UAL 187). This can be attributed to the solubility of the bacteriocins of LAB (lactic acid bacteria); the isoelectric points of the bacteriocins produced by *Lactobacillus* bacteria are around 8.0-9.0 and the solubility of the bacteriocins decreases with increasing their pH. The effects of different pH such as 3, 4, 5, 6, 7, 8, 9, and 10 on crude of bacteriocin were studied. In pH 6, the largest activity of bacteriocin from *Lactobacillus acidophilus* against *Helicobacter pylori* was shown 9.8 mm.

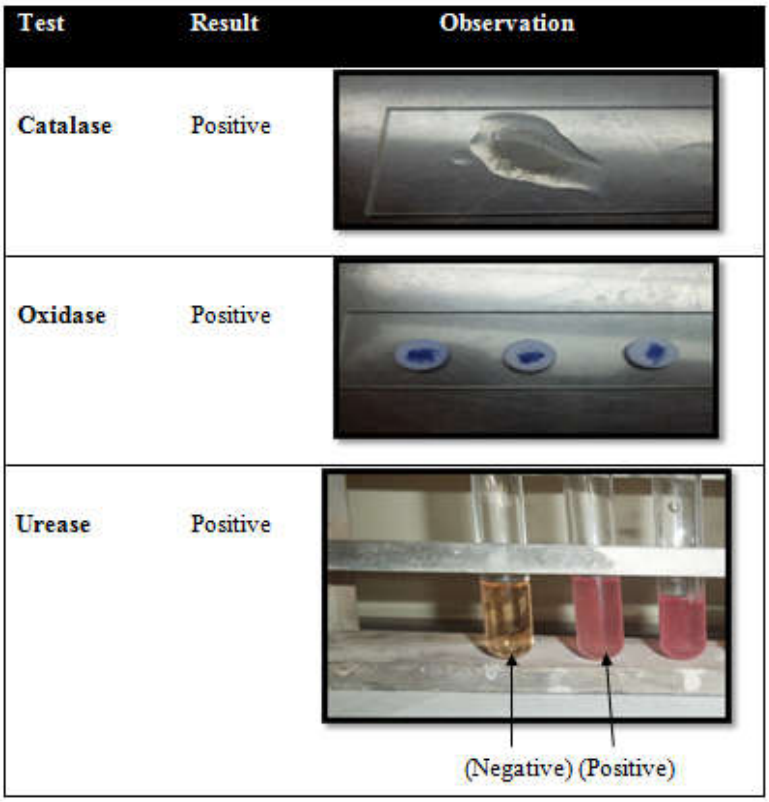

Figure 1. Helicobacter pylori Biochemical characteristics

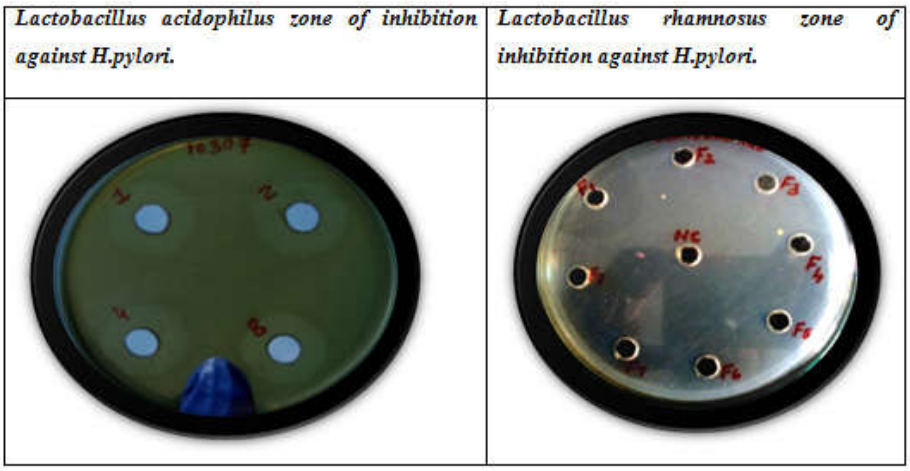

Figure 2. Zone of inhibition against h pylori

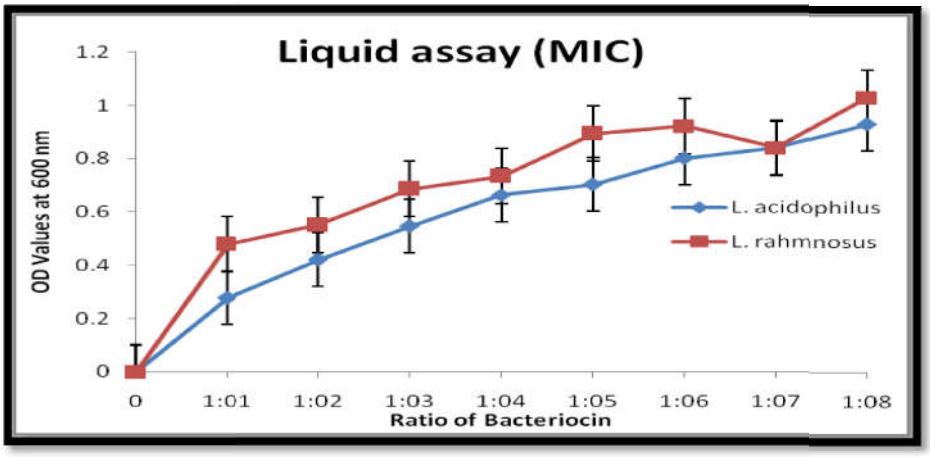

Figure 3. MIC values for *Bacteriocin* by OD at 600 nm

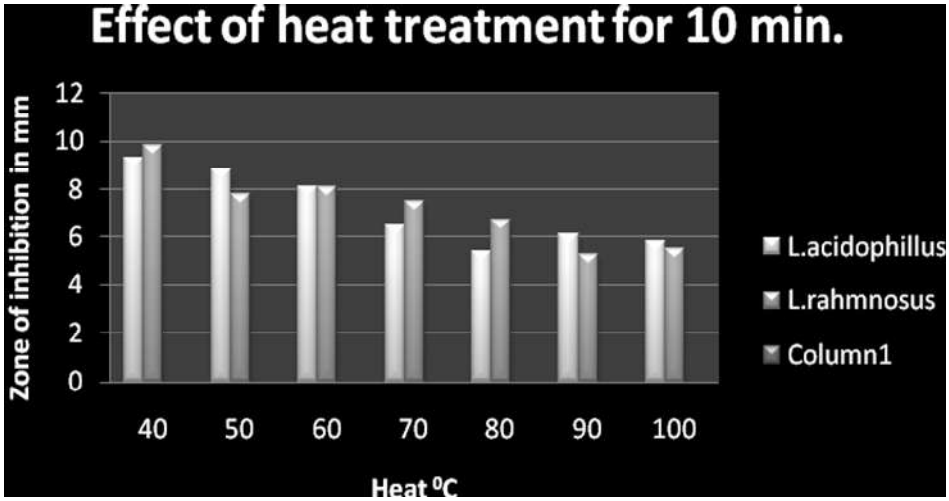

Figure 5. Effect of Heat treatment on *Bacteriocin* activity for 20 minute

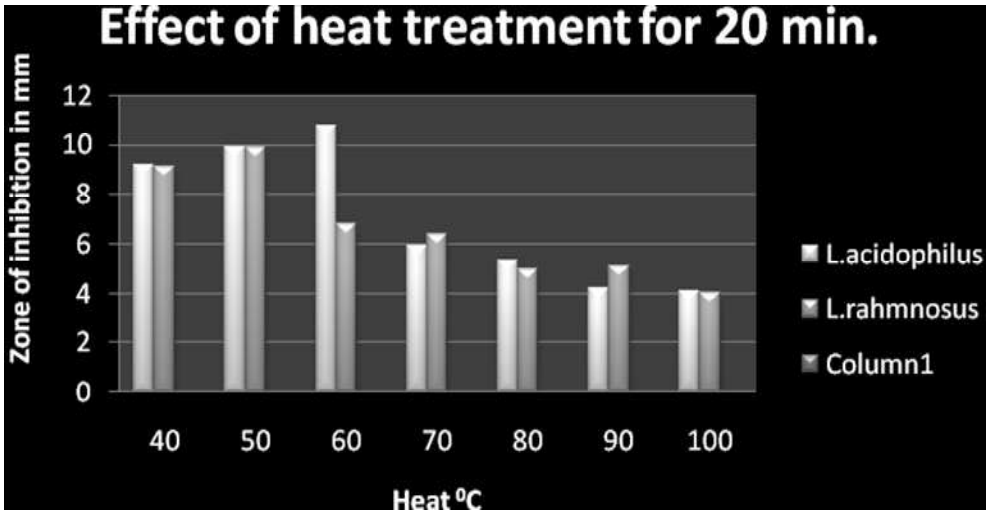

Figure 6. Effect of Heat treatment on *Bacteriocin* activity for 30 minute

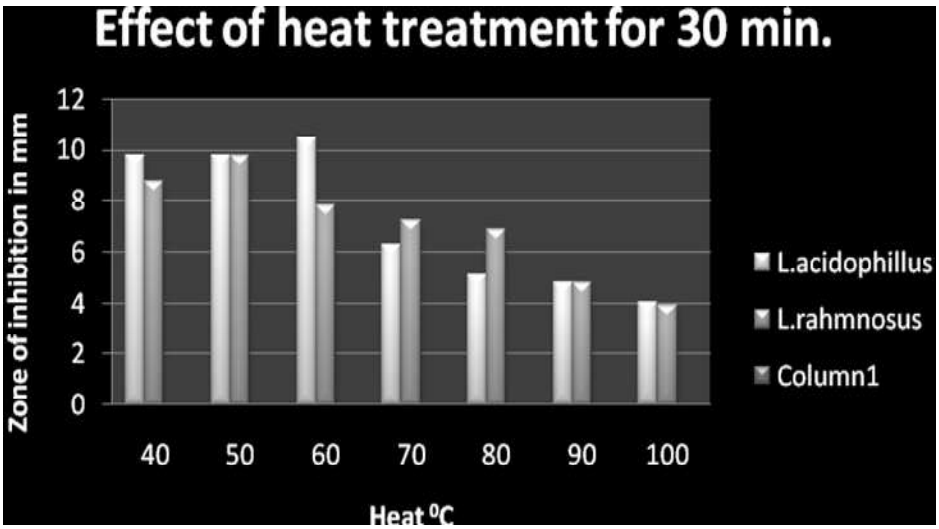

Figure 7. Effect of Different pH on *Bacteriocin* activity

Figure 8. Effect of Different Surfactant on *Bacteriocin* Activity by *L.acidophilus*

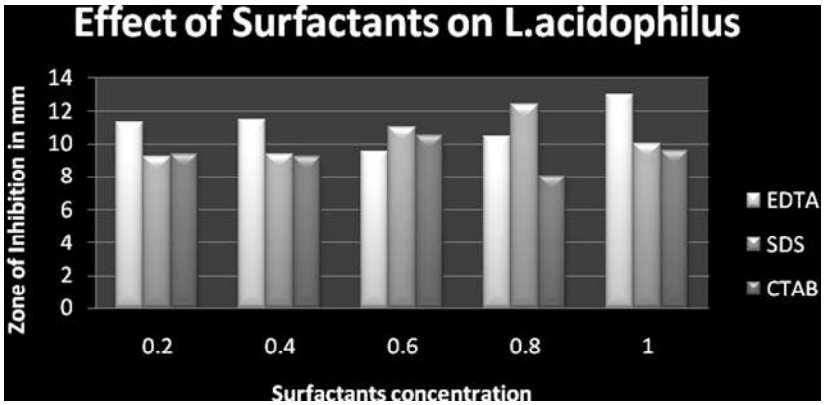

Figure 9. Effect of Different Surfactant on *Bacteriocin* activity by *L.rhamnosus*

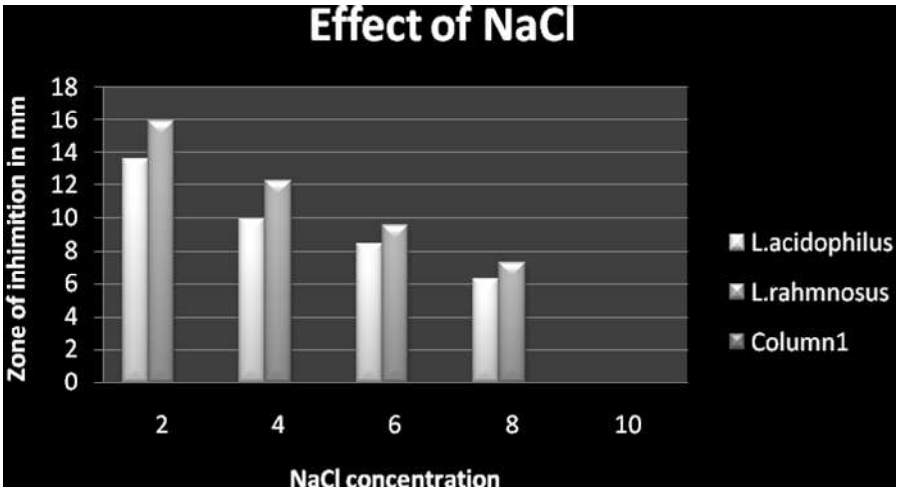

Figure 10. Effect of NaCl Concentration on *bacteriocin* activity

Lactobacillus rhamnosus was showed antimicrobial activity against *Helicobacter pylori* 9.3 mm in pH 7. But in pH 10 shows no activity of bacteriocin from *Lactobacillus* spp. against *Helicobacter pylori.* The higher activity was obtained at high pH range of 8-10 and at low pH to 3 and 10 (Figure VII).

Effect of surfactants on Bacteriocin activity: Figure clearly highlights of effect of different minerals such as SDS, ETDA and CTAB on crud bacteriocin from two LAB such as *L. acidophilus* and *L. rhamnosus.* To detect the hydrophobic nature of the Bacteriocin was treated with a different group of detergents.

The addition of anionic detergent (SDS) increased antimicrobial effect of the Bacteriocin. According to Figure VIII, the isolated *Lactobacillus acidophilus* was showed antimicrobial activity against *Helicobacter pylori.* This showed the largest of growth inhibition around 13.40 mm in SDS but that showed the smallest inhibition 8.0 mm in CTAB. Result also showed that EDTA also gives the zone of inhibition against *H.pylori.* According to Figure IX, the isolated *Lactobacillus rhamnosus* was showed antimicrobial activity against *Helicobacter pylori.* This showed the largest of growth inhibition around 12.90 mm in SDS but that showed the smallest inhibition 8.0 mm in EDTA. When CTAB was gives average zone of inhibition against *H.pylori.*

Effect of NaCl Concentration on Bacteriocin activity: The effects of different concentration of NaCl on crude of bacteriocins were studied. The supplementation with NaCl, bacteriological peptone and beef extract has resulted in reduced activity. In contrast to the present observation, growth as well as bacteriocin production in the presence of bacteriological peptone or casamino acids and NaCl was reported to be higher by previous researchers. According to Figure X, the isolated *Lactobacillus acidophilus* and *Lactobacillus rhamnosus* was showed antimicrobial activity against *Helicobacter pylori.* This showed the largest of growth inhibition around 13.60 mm and 15.90 mm in 2% NaCl Concentration. But in 10% NaCl Concentration shows no activity of bacteriocin from *Lactobacillus* spp. against *Helicobacter pylori.* So, increase the concentration of NaCl the decrease the activity of Bacteriocin. In addition, it was found that the bacteriocins activities of both isolates were diminished when it was containing more than 10% NaCl concentration (Zamfir, 1991).

DISCUSSION

Helicobacter pylori infect over 50 per cent of the world population which has been linked to the development of gastritis, gastric adeno-carcinoma and mucosa-associated lymphoid tissue lymphoma. Recently, *lactobacillus* spp. has attracted much attention due to their potential inhibitory effect on pathogens including *H. pylori*. Therefore, the effects of lactic acid bacteria on *H. pylori* were tested in the study. The use of Probiotics with bioactive components in *H. pylori*-colonized subjects with gastric inflammation is supported by many observations. Specific strains of *Lactobacillus* exert *in vitro* bactericidal effects against *H. pylori* through the release of Bacteriocins and/or inhibit its adhesion to epithelial cells. The results indicate that Probiotics generally do not eradicate *H. pylori* but decrease the density of colonization, thereby maintaining lower levels of this pathogen in the stomach; in association with antibiotic treatments, some Probiotics increased eradication rates and/or decreased adverse effects due to the antibiotics. On the other hand, the antioxidant and anti-inflammatory properties exerted by Probiotics may stabilize the gastric barrier function and decrease mucosal inflammation. These findings confirm that, as suggested by the 2000-Maastricht Consensus Conference on *H. pylori*, Probiotic micro-organisms may be used as a 'possible' tool for the management of *H. pylori* infection and its associated gastric inflammation (Naga, 2011). Recently attention had been paid to the interaction between *H.pylori* and Probiotic *lactobacilli* (Sgouras, 2005).*Lactobacillus* sp. Strains, as representative of the normal microflora of intestine, have been found to have an inhibitory effect on *H.pylori in vivo* and *in vitro*(Coconnier, 1994). Two main substances have been implicated in the inhibitory by *Lactobacillus:* Lactic acid and Bacteriocin.

In the present study *lactobacillus* strain's inhibitory effect against *H.pylori* was found *in vitro*. These strains of *lactobacillus sp* may be selected as a potential probiotic because of its safety profile in humans and its antagonistic properties *H.pylori*. The result obtains in the study suggested that there is antagonistic interaction *in vitro* between clinical *H.pylori* and *Lactobacillus* strains tested. So, the culture of *Lactobacillus* strains could inhibit strains of *H.pylori* using the agar well diffusion assay. Bhatie *et al,* (1989) were first concluding an antagonistic effect of *Lactobacillus* strain against *H.pylori*.

Since, several studies regarding the antagonistic effect of *Lactobacillus* strain such as *L.acidophilus, L.salivarius, L.plantarum, L. casei* and *L.gasseri* against *H.pylori* have also been reported are in concordance with some study. However, the inhibitory effect of *Lactobacillus* strains against *H.pylori* strain was lost after neutralization of supernatants *Lactobacillus* indicating pH mediated effect. Similarly, in the study involving MIC assay, the addition of *Lactobacillus* supernatants decreases the growth of *H.pylori* tested and dramatic decline in growth of *H.pylori* was observed when *H.pylori* culture were treated with Bacteriocin.

The finding present suggested that Bacteriocin, the major compound of *Lactobacillus* supernatants to be inhibitory for a strain of *H.pylori*. Bhatie *et al,* (1989) suggested that Bacteriocin produced by *L.acidophilus* is responsible for the inhibition of growth of *H.pylori*. Medouakh *et al.* (2006) has also reported that supernatant of *L.rhamnosus* decreases the viability of *H.pylori* strain. It has been reported earlier that *L. acidophilus* effectively reduced the attachment sites of *H. pylori* in the cell wall and has been used as a curative therapy against the infection of *H. pylori* (Mrda *et al*., 1998). While *L.rhamnosus* GG supplementation beneficially affected *H. pylori therapy*–related side effects such as bloating, diarrhea, nausea and taste disturbances (Armuzzi *et al*., 2001). Bacteriocin production was strongly dependent on pH, nutrients source and temperature various physicochemical factors seemed to affect Bacteriocin production as well as its activity. The Bacteriocin suspension of *Lactobacillus* spp. grown in MRS broth had the best inhibitory effect against wide spectrum growth of *H.pylori* bacteria. The present study demonstrated the production of the Bacteriocin by two *lactobacilli* isolates under different culture conditions. Its antimicrobial potency, pH stability, activity retention in low and high temperatures suggested its wide applicability in acidic pH conditions and in pre-processed food products (Mahrous *et al*., 2013). *Lactobacillus* is able to inhibit the growth of other microorganisms by excretion of metabolite products such as organic acids, hydrogen peroxide, diacetyl and bacteriocin (Huot *et al*., 1996). However, these results were not from acidic effect as CFS was adjusted to neutralize to get rid of the acidic effect. The Bacteriocin production of *Lactobacillus acidophilus* and *Lactobacillus rhamnosus* were found in exponential phase and the maximum of Bacteriocin activity have occurred in late exponential phase. However, the Bacteriocin activity decreased when cell entered at stationary phase. Since the production of Bacteriocin is dependent on the growth and physiological activities of the producing strains, the amount of Bacteriocin released into the medium is correlated with the quantity of biomass produced. Almost all Bacteriocin from lactic acid bacteria displays primary metabolite kinetics (Zamfir *et al*., 1999). The production of bacteriocin is growth-associated because production occurs during mid exponential phase and increase to reach a maximal level at the end of the exponential phase or the beginning of the early-stationary phase where the maximal biomass was observed. Bacteriocin production completely stopped when cell entered the stationary phase (Cheigh *et al*., 2002). The phenomenon of heat stability of bacteriocin from LAB up to 121^0C for 15min has been reported earlier in Lactocin RN 78^{17} and in *L.brevis* OGI (Ogunbanwo *et al*., 2003). Our findings are also in agreement with the above, by observing the activity of Bacteriocin in *L.acidophilus* and *L.rhamnosus* after heat treatment at 121^0C for 15min. Therefore, it could be grouped under heat stable low molecular weight Bacteriocin.

Many of the Bacteriocin produced by lactic acid bacteria, particularly the ones of class I and class II, are described as small hydrophobic proteins containing little tertiary structure, which explains their heat stability. Other factors contributing to heat stability of the Bacteriocin of LAB are stable cross-linkages, a high glycine content and occurrence of strongly hydrophobic regions. Such heat stability also excludes the possibility of the inhibitory action being due to bacteriophage (De Vuyst *et al*., 1994). Bacteriocin activity of *Lactobacillus acidophilus* was stable at wide pH range 6-9 and it was heat tolerant. The stability was similar to another bacteriocin-producing *Lb. salivarius* (Cataloluk *et al*., 2003). Heat stability is another major feature of low molecular-weight bacteriocins (Oscariz, 2001). These properties would be useful for food industrial processing under pasteurization condition. However, Bacteriocin activities of *Lactobacillus rhamnosus* were found to be stable at pH range 7-9. However, higher activities were obtained at high pH range 8-9.

Exposure of the Bacteriocin sample to surfactants like anionic and cationic resulted in an increase in Bacteriocin titers. It gives indirect information about the structure of the active molecule. Ivanova *et al*, (2000) suggested that anionic detergent, SDS is known to unfold proteins by complexing to the interior hydrophobic core of their native structure thus affecting their three dimensional conformation. In our findings, addition of SDS resulted in an increased Bacteriocin effect, which might be due to the solubilization of insoluble aggregates. Results observed that the addition of detergents such as CTAB reduced the Bacteriocin activity. Activity of the Bacteriocin sample to NaCl concentration like 2% to 10% resulted in decrease the activity of Bacteriocin. Addition of salt influence changes the cell morphology to longer, thicker cells (Olfat *et al*., 2011).

Conclusion

In this work has shown that *Lactobacillus* strains are able to successfully inhibit *Helicobacter pylori in vitro*.
These strains need to be further assessed and critically evaluated by clinical research on patients colonized and infected with *Helicobacter pylori*. It may be useful to study which probiotic strain(s) combined with prebiotics are most successful in eliminating *H. pylori in vivo*. Without the use of antibiotics, which cause disturbance in the balance of the gastrointestinal microflora.

Acknowledgements

I greatly appreciate Dr. Asish K. Mukhopadhyay (National Institute of Cholera and Enteric Diseases, Kolkata) for supplying Helicobacter pylori strains for my project and provide background information about them. I warmly thank to Dr. Srinivas Murthy Dugdirala (Microbiology Department, Gujarat Vidtapeeth, Sadra) who has guided me and allowed me to use the facilities in Microbiology Lab.

REFERENCES

Armuzzi A., Cremonin F., Bartolozzi F., Candelli M., ojetti V., cammarota G., Anti M., De Lorenzo A., Pola P., Gasbarrini G. and Gasbarrini A. 2001. The effect of oral administration of *lactobacillus GG* on antibiotic-associated gastrointestinal side effects during *Helicobacter pylori* eradication therapy, *Alim. Pharm. Theap.,* 15: pp. 163-169.

Bhatia S.J., Kochar N., Abraham P., Nair P.G. and Mehta A.P. 1989. *Lactobacillus acidophilus* inhibits growth of *Campylobacter pylori in vitro*, *J.Cin. Microb*., 27: pp.2328-2330.

Bulent Y., Evren A., Hüseyin K., Burak T., Mesut S., Züleyha A., Ozcan K., Oya O. K. 2010. Efficacy of probiotics in *Helicobacter pylori* eradication Therapy, *Turk J Gastroenterol,* 21 (3): pp. 212-217.

Cataloluk O. and Gürakan C.G. 2003. Characterization of salivaricin B, A protein expressed by *Lactobacillus salivarius* M7, Turk. *J. Biol.,* 27: pp.131-136.

Cheigh C.I., Choi H.J., Park H., Kim S.H., Kook M.C., Kim T.S., Hwang J.K. and Pyun Y.R., 2002. Influence of growth conditions on the production of a nisinlike bacteriocin by *Lactococcus lactis* subsp. *lactis* A164 isolated from Kimchi, *J. Biotechnol*. 95: pp.225-235.

Coconnier M.H., Lievin V., Hemery E., Servin A.L. 1998. Antagonistic activity against *Helicobacter* infection *in vitro* and *in vivo* by the human *Lactobacillus acidophilus* strain LB. *Appl Environ Microbiol*, 64: pp.4573-4580.

De Vuyst L. and Vandamme E.J. 1994. Antimicrobial potential of lactic acid bacteria,in L. De Vuyst and Vandamme E.J. (ed), Bacteriocins of lactic acid bacteria: microbiology,genetics and applications. Blackie Academic and professional, London, United Kingdom, pp.91-142.

Garza-González E., Perez-Perez G.I, Maldonado-Garza H.J., Bosques-Padilla F.J. 2014. A review of *Helicobacter pylori* diagnosis, treatment, and methods to detect eradication, *World J Gastroenterology*, 14; 20(6): pp.1438-1449.

Huot. E. J., Meghrous C., Barrena-Gonzalez C. and Petitdemange H. 1996. Bacteriocin J 46, a new bacteriocin produced by *Lactococcus lactis* subsp. *cremoris* J46: isolation and characterization of the protein and its gene, Anaerobe 2: pp.137-145.

IARC, 1994. Schistosomes, liver flukes and *Helicobacter pylori*. In: IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Geneva: WHO Publications; 61.

Ivanova I., Kabadjova P., Pantev A., Danova S. and Dousset X., Detection, purification and partial characterization of a novel bacteriocin substance produced by *Lactococcus lactis* subsp. *lactis* B14 isolated from Boza-Bulgarian traditional cereal beverage, *Biocatalysis*, 2000, 41: pp.47-53.

Kovvuri S. and Kumar Babu P. 2012. Collection, isolation, characterization of *Helicobacter pylori* from Dental Plaque and Antibacterial Activity of Novel Drug 1-Phenyl-3-(4_-Aminophenyl)-5-(2_, 4_ Dichlorophenyl)-2-Pyrazoline, IJPCBS, 2(4), pp.552-574.

Lowry O.H., Rosebrough N.J., Farr A.L. and Randall R.J. 1951. Protein measurement with the folin-phenol reagent, *J Biol. Chem.,*193, pp.265-275.

Mahrous H., Mohamed A., Abd El-Mongy M., El-Batal A. I., Hamza H. A. 2013. Study Bacteriocin Production and Optimization Using New Isolates of *Lactobacillus* spp. Isolated from Some Dairy Products under Different Culture Conditions, *Food and Nutrition Sciences*, 4, pp.342-356.

Mahrous H., Mohamed A., Abd El-Mongy M., El-Batal A. I., Hamza H. A. 2013. Study Bacteriocin Production and

Optimization Using New Isolates of *Lactobacillus* spp. Isolated from Some Dairy Products under Different Culture Conditions, *Food and Nutrition Sciences*, 4, pp.342-356.

Maria M. and Janakiraman S. 2012. Detection of heat stable bacteriocin from *Lactobacillus acidophilus* NCIM5426 by liquid chromatography/mass spectrometry Indian *Journal of Science and Technology,* 5 (3), pp.2325-2332.

Medouakh L., Tabak s., Bekakada A., Mahi M., Rouissat L., Yagoubi A. and Bensoltane A. 2006. Isolation and Characterization of *Helicobacter pylori* from patients suffering from gastro-duodenal ulcer disease, Egypt. *J. Appl. Sci.,* 21(3): pp.406-417.

Mojgani N., Sabiri G., Ashstiani M. and Torshizi M. 2009. Characterization of bacteriocins produced by *Lactobacillus brevis* NM 24 and *L.fermentum* NM 332 isolated from Green olives in Iran, *J. Microbiol*, 6, S2-5.

Mrda Z, Zivanovic M, Rasic J, Gajin S, Somer L, Trbojevic S, Majoros J, Petrovic Z. 1998. Therapy of *Helicobacter pylori* infection using *Lactobacillus acidophilus*. Meditsinski Pregled, 51: pp.343-345.

Naga D. C., Phani S. and Prithvi Raj, 2011. Evaluation of the various uses of Microorganisms with Emphasis on Probiotics, *J Microbial Biochem Technol.*, pp.1-7.

Ogunbanwo S.T., Sanni A.I. and Onilude A. A. 2003. Characterization of bacteriocin produced by *Lactobacillus plantarum* F1 and *Lactobacillus brevis, African Journal of Biotechnology*, 2 (8), pp. 219-227.

Ogunbanwo S.T., Sanni A.I., and Onilude A. A. 2003. Characterization of bacteriocins produced by *Lactobacillus plantarum* F1 and *Lactobacillus brevis, African Journal of Biotechnology,* 2 (8), pp. 219-227.

Olfat S. B., Ibrahim G.A., Tawfik N. F., El-kholy W. I. and Gad El-rab D.A., 2011, Identification and probiotic characteristics of *Lactobacillus* strains isolated from traditional domiati cheese, Int. *J. Microbiol. Res*., 2011, 3(1), pp.59-66.

Oscariz J.C. and Pisabarro A.G. 2001. Classification and mode of action of membrane-active bacteriocins produced by gram-positive bacteria, *Int. Microbiol.,* 4: pp.13-19.

Pande P.R., Karki B.B., Pande R., Khatri R. 2009. Evaluation of Locally made Rapid Urease Test for Diagnosis of *Helicobacter Pylori*, Postgraduate Medical Journal of NAMS, 9, (2), pp.50-53.

Pantoflickova D.L., Sy-Theulaz I.C. and Blum L.A., 2013. *Helicobacter pylori* and Probiotics, *The Journal of Nutrition*, pp.812-818.

Ravi K., Sandeep Kumar S., Arunaaloke C. 2010. Comparison of Broth Dilution and Disc Diffusion Method for the Antifungal Susceptibility Testing of *Aspergillus flavus, Am. J. Biomed. Sci.,* 2(3), pp.202-208.

Sadaf A. and Nuzhat A. 2009. Probiotic potential of *lactobacillus* strains in human Infections, *African Journal of Microbiology Research,* 3(12), pp. 851-855.

Sgouras D.N., Panayotopoulou E.G., Martinez-Gonzalaz B., Petraki K., Michopoulos S. and Mentis A. 2005. *Lactobacillus johnsonii* La1 attenuates *Helicobacter pylori* -associated gastritis and reduces levels of proinflammatory chemokines in C57BL/6 mice, *Clin Diag Lab Immunol*, 12: pp.1378-1386.

Soccol C.R., Vandenberghe L.D, Spie M.R., Medeiros A.B., Yamaguishi C.T., Lindner J.D. 2010. Pandey A. and Soccol V.T., The Potential of Probiotics, *Food Technol. Biotechnology,* 48 (4), pp.413–434.

Soumya.T.V., John R., Jose S. 2012. Characterization of Bacteriocin Produced by *Lactobacillus* SP and Optimization of Cultural Conditions, *International Journal of Scientific and Research Publications*, 2012, 2 (12) pp.1-9.

Thirabunyanon M., Biotherapy for and protection against gastrointestinal pathogenic infections via action of probiotic bacteria, *Maejo Int. J. Sci. Technology*, 2011, 5(01), pp.108-128.

Tsuzuki T., Ina K., Ohta M., Hasegawa T., Nagasaka T., Saburia N., Uedaa M., Konagaya T., Kaneko H., Imada A., T. Nishiwaki T., Nobata K., Ando T. and Kusugami, 2002. Clarithromycin increases the release of heat shock protein B from *Helicobacter pylori*, Aliment Pharmacol Ther, 16 (Suppl. 2): pp.217–228.

Wong B., Ching C., Lam S. 1999. *Helicobacter pylori* infection and gastric cancer, HKMJ, 5 (2), pp.175-179.

Zamfir M., Callewaert R., Cornea P.C. and De Vuyst L. 2000. Production kinetics of acidophilin 801. a bacteriocin produced by *Lactobacillus acidophilus* IBB 801, FEMS Microbiol. Lett., 190: pp.305-308.

Zamfir M., Callewaert R., Cornea P.C., Savu L., Vatafu I. and De Vuyst L. 1999. Purification and characterization of a bacteriocin produced by *Lactobacillus acidophilus* IBB 801, *Journal of Applied Microbiology*, 87, pp.923–931.

****