Int.J.Curr.Microbiol.App.Sci (2016) *5*(12): 869-883

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 5 Number 12 (2016) pp. 869-883**
Journal homepage: http://www.ijcmas.com

Original Research Article http://dx.doi.org/10.20546/ijcmas.2016.512.095

Therapeutic Potential of Probiotic *Lactobacillus plantarum* MYS94 against *Campylobacter jejuni*

K. Poornachandra Rao[1], N.K. Hemanth Kumar[2] and M.Y. Sreenivasa[1]*

[1]Department of Studies in Microbiology, University of Mysore, Manasagangotri, Mysore, Karnataka, India-570006
[2]Department of Studies in Botany, University of Mysore, Manasagangotri, Mysore, Karnataka, India-570006
**Corresponding author*

Keywords

Lactobacillus plantarum MYS94, Gastrointestinal tract, *C.jejuni*, Probiotics.

Article Info

Accepted:
12 November 2016
Available Online:
10 December 2016

A B S T R A C T

Campylobacter jejuni is one of the most common intestinal pathogen causes of human gastroenterocolitis thereby leading to diarrhea and other serious post-infectious complications. Controlling the *C. jejuni* infection is critical for reducing campylobacteriosis for a healthy gut. In the current study, *Lactobacillus plantarum* MYS94 was isolated from traditional fermented wine and characterized for its probiotics properties and therapeutic potential against *C. jejuni*.The probiotic attributes revealed that, the strain could resist the gut pH, bile salt tolerance, adherence ability to chicken crop epithelial cells, sensitivity to penicillin, amoxicillin, bacitracin and erythromycin, further the strain also exhibited inhibitory potential against common food pathogens. The neutralized cell-free supernatant of *Lp* MYS94 exhibited 62.04% inhibition of *Pseudomonas aeruginosa* MTCC 7903 biofilm. The study also evaluated the cholesterol-lowering property of the *Lp* MYS94 using hen egg yolk as the cholesterol source. The cholesterol in hen egg yolk was assimilated by 62.18% and 31.42% by *Lp* MYS94and its CFS respectively. In addition, *Lp* MYS94-CFS tested against *C. jejuni* by well diffusion and time kill assay revealed a significant effect on *C. jejuni* with strong inhibitory activity. Collectively, the results suggests that *L. plantarum* MYS94 could be useful as potential probiotic strain suppressing *C. jejuni*.

Introduction

Campylobacter jejuni is one of the leading causes of the enteric bacterial infections that can interfere with normal functions of gastrointestinal tract (GIT). This pathogen causes gastroenteritis in humans characterized by fever, abdominal cramps and diarrhea (Nishiyama *et al.*, 2014). Enteric infections like human gastro-enterocolitis generally treated with antibiotic interventions and supportive therapy can lead to the development of antibiotic-resistant strains. Studies revealed that probiotic therapy is proved to be attractive control strategy for campylobacter infections (Bratz *et al.*, 2014). Dietary probiotics in the form of traditional fermented foods would

be of better choice targeting the beneficial effects. Traditional fermented foods are the rich source for probiotic lactic acid bacteria (LAB) and are involved with many therapeutic attributes (Kumar *et al.*, 2013). Among LAB, *Lactobacillus plantarum* perform an vital role in the production and preservation of traditional fermented foods. Several probiotic *Lactobacillus* strains have been documented for their probiotic properties such as tolerance to acidic pH and bile salt, resistance to gastric transit and desirable antibiogram (Hashemi *et al.*, 2014). Other probiotic attributes include, adherence to the intestinal epithelium of the host and inhibition of the pathogenic bacteria by competitive exclusion and their capability in promoting the food safety and contribute health benefits.

In addition to the nutritional values, ingestion of LAB through fermented foods has been suggested to confer a range of therapeutic effects including immune-modulation, increased resistance to infectious illness and malignancy (Soccol *et al.*, 2010). Probiotic supplementation could restore the microbial imbalance caused by antibiotic treatment in the gut. Studies revealed that *Lact. plantarum* exerts therapeutic potential with regard to important biological functions such as anti-inflammatory activity, enhancing the gut immune system, antagonistic effects against *C. jejuni* (gastro-enterocolitis) and *Salmonella enterica* (food poisoning) have been investigated (Bratz *et al.*, 2015; Pattani *et al.*, 2013). LAB strains may receive (either manufacturer's self-proclaimed or governmental agency affirmed) GRAS status regardless of their probiotic characteristics.

L plantarum is a heterogeneous, indigenous species that has been isolated from diverse environmental niches such as human saliva, human intestine, human stool, milk, cheese, sauerkraut, gut of mammals, etc. (Smelt *et al.*, 2013). Food sources, including Marcha, an ethnic fermented beverage (Das and Goyal, 2014), traditional Japanese fermented food (Kanno *et al.*, 2012), traditional Chinese fermented foods (Kuda *et al.*, 2010), traditional Iranian cheeses (Hashemi, 2016) and traditional sorghum based fermented food (Rao *et al.*, 2015) have been screened for their potential probiotic *L plantarum* strains. However, there is a need on the exploration of probiotic attributes as well as therapeutic properties such as, cholesterol assimilation, antibiofilm, antimicrobial property and anti-campylobacter activity. With this background, the present study was carried out to screen traditional fermented paddy wine, collected from Coorg region of Karnataka state, India, for the presence of potential probiotic *L. plantarum* strains and studied their therapeutic potential against *C. jejuni*.

Materials and Methods

Isolation of LAB from fermented wine: Nell wine, prepared from the natural fermentation of paddy, was collected from the Coorg region of Karnataka, India. LAB were isolated by serial dilution and plating on de Man Rogosa Sharpe (MRS, Hi-Media) agar plates supplemented with 0.25 % (w/v) L-cystine (Paolillo *et al.*, 2009). The plates were incubated for 48 h at 37 ^{o}C. The well grown, individual colonies were enumerated and the subcultured isolates were stored at -20 ^{o}C in 40 % glycerol for further analysis.

Phenotypic characterization of LAB: Biochemical characteristics were studied as per the standard protocol of Cappuccino and Sherman (2004). The isolates were tested for Gram reaction, catalase activity, ammonia production from arginine, bile salt hydrolase activity, carbohydrate fermentation with different carbohydrates *viz.*, glucose, D-

fructose, L-arabinose, mannitol, maltose, raffinose, D-xylose and sorbitol (Hi-Media). The isolates were assessed for survival ability at different temperatures (10, 37 and 45^{o}C) and salinity (3, 5 and 7 % NaCl concentrations). Gram-positive and catalase negative colonies were screened as presumptive LAB.

Molecular identification: The isolate MYS94 was grown in 5 mL MRS broth supplemented with 0.25 % (w/v) L-cystine (Paolillo *et al.,* 2009) at 37 ºC overnight in a rotary shaker. The genomic DNA was extracted by conventional phenol-chloroform method and the 16S rRNA gene was amplified using the primer 27F (5'AGAGTTTGATCMTGGCTCAG 3') and 1492R (5' TACGGYTACCTTGTT ACGACTT 3') (Sahadeva *et al.,* 2011). PCR amplification reactions were carried out in a 25 µL reaction mixture. 1 µL of the DNA was amplified with 2.5 µL of 10X PCR buffer, 2.5 µL of 25 mM $MgCl_2$, 2.0 µL of 2 mM dNTPs, 1.0 µL of 20 pmol primer 27F, 1.0 µL of 20 pmol primer 1492R, 0.125 µL of LA Taq and sterile deionized water up to 14.875 µL. PCR conditions were as follows: initial denaturation at 94 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 30 sec, annealing for 57 sec at 45 °C, extension at 72 °C for 1: 30 min, followed by final extension at 72 °C for 7 min. Amplicons were confirmed by agarose gel (1 %) electrophoresis and documented using Syngene G-box gel documenting system.

Nucleotide sequence accession number: Complete sequence of the 16S rRNA gene was confirmed with NCBI BLAST database. Phylogenetic analysis of *Lp* MYS94 16S rRNA gene sequence was performed using online software MEGA 5.1 with neighbor-joining tree method. The complete 16S rRNA gene sequence of *Lp* MYS94was deposited in NCBI GenBank along with location of the isolate.

In vitro probiotic attributes of *Lact. plantarum* MYS94

Acidic pH tolerance: The pH tolerance of the strain MYS94 was determined as described by Sahadeva *et al.,* (2011). One mL of *Lact. plantarum* MYS94 (10^6 CFU/mL) was inoculated into three test tubes containing 9 mL of phosphate buffer saline (PBS) with pH 2, 3 and 6, mixed thoroughly, and incubated for 0, 90 and 180 min. After incubation, plating was carried out, and the inoculated plates were incubated anaerobically at 37 ºC for 48 h. The uninoculated MRS agar plates were served as controls. Acid tolerance of the strain MYS94 was determined by comparing the cell numbers on test MRS agar plates with control plates.

Bile salt hydrolase assay: Bile salt hydrolysis ability of the *Lact. plantarum* MYS94 was determined by direct plate assay (Kumar *et al.,* 2012) with minor modifications. Briefly, overnight culture of *Lact. plantarum* MYS94 was added onto sterile discs (Hi-Media, Mumbai) placed on the MRS agar plates supplemented with 0.5 % (w/v) taurodeoxycholic acid (TDC) and 0.037 % of $CaCl_2$ and incubated at 37 ^{o}C for 72 h. MRS agar plates without supplementation of TDC were served as control. The presence of precipitated bile acid around the culture discs was considered positive for bile salt hydrolysis.

Antibiotic susceptibility test: Antibiotic susceptibility of *Lact. plantarum* MYS94 was tested against 8 selected antibiotics using antibiotic discs (Hi-Media, Mumbai) containing vancomycin (VA; 30 mcg), methicillin (MET; 5 mcg), norfloxacin (NX; 10 mcg), bacitracin (B; 10 mcg), kanamycin (K; 30 mcg), amoxicillin (AMC; 30 mcg), penicillin G (P; 10 mcg) and erythromycin (ERY; 15 mcg) using Kirby-Bauer disc

diffusion method (Bauer *et al.,* 1966). Twenty-four-hour old culture of *L plantarum* MYS94 was swabbed onto MRS agar plates using sterile cotton swabs. Antibiotic discs were placed on the inoculated plates, after incubation at 37 ^{o}C for 24 h, inhibition zones around the discs were measured and susceptibility was compared to the reference chart of zone size interpretative for antibiotics as per CLSI (2011).

Hemolytic activity: *Lp* MYS94 was tested for its hemolytic activity as per Argyri *et al.,* (2013). *Lact. plantarum* MYS94 was streaked onto blood agar plates containing 5 % (w/v) human blood and incubated for 48 h at 37 ^{o}C. The plates were examined for β-hemolysis, α-hemolysis, or gamma hemolysis (no zones around colonies).

Adherence of *L. plantarum* MYS94 to epithelial cells

The adhering and colonizing ability of *L. plantarum* MYS94 to epithelial cells (chicken crop) was performed by tissue adherence assay of Jakava-Viljanen and Palva (2007) with minor modifications. To clear the surface mucus, the chicken crop tissue was maintained in PBS at 4 $^{\circ}$C for 30 min followed by washing thrice with potassium phosphate buffer (pH 7.4 ± 2). After microscopy examination, the recovered epithelial cells from the tissue were diluted to get 5×10^{6} cells/mL. The live bacterial culture (10^{6} CFU) and epithelial cells were mixed in 1:4 ratio and incubated at 37 $^{\circ}$C for 30 min in a water bath. The incubated mixture was centrifuged (Eppendorf) (3000 rpm for 3 min) and washed with PBS to remove the unbound bacteria. The resulting pellet was resuspended in PBS and observed the bacterial adhesion to epithelial cells under Fluorescent microscopy (Carl Zeiss, Germany), the assay was considered positive if a minimum of 10 bacteria adhered to each epithelial cell.

Anti-Biofilm activity of *Lp* MYS94

Inhibition of Biofilm formation was carried out in a sterile 96 well microtitre plate. *Pseudomonas aeruginosa* (MTCC 7903) was grown in Tryptic Soy Broth media (Hi-Media) and allowed for the biofilm formation. After incubation the media was decanted out from the wells and washed with sterile PBS buffer. CFS of MYS94 (200 µL) was added to each well following the incubation for 24 h. Crystal violet staining was used for assessing the biofilm formation. Culture with 200 µL of sterile distilled water was served as control. The results were expressed as percentage of biofilm inhibition (Rao *et al.,* 2015).

$\% I = OD_{Control} - OD_{Sample} / OD_{Control} \times 100$

Where I (%): Percent Biofilm inhibition, OD control: Optical Density in Control, OD sample: Optical Density in sample with CFS of MYS94.

In vitro Cholesterol assimilation using egg yolk

Four-percent commercial fresh hen egg yolk as the cholesterol source was supplemented with MRS broth. *Lp* MYS94(100µL) of 18 h culture was inoculated and incubated at 37 ^{o}C for 24 h. After incubation the cells were centrifuged at 5400g for 15min at 4 ^{o}C. The amount of cholesterol in the supernatant was determined by the modified colorimetric method of Rudel and Morris (1973). The percentage of cholesterol removal was estimated using the formula:

Cholesterol removal (%) = [(amount of cholesterol of the control - amount of cholesterol of the inoculated group) /amount of cholesterol of the control)] x 100.

Anti-*C. jejuni* activity

Well diffusion assay

The inhibitory activity of *L. plantarum* MYS94 was evaluated by well diffusion assay according to Santini *et al.*, (2010) with minor modifications. Briefly, overnight culture of *L. plantarum* MYS94 was centrifuged (15min at 12000xg at 4 °C), and adjusted to pH 6.5± 0.2 with 1N NaOH to obtain neutralized cell-free supernatant. Subsequently the supernatant was filter sterilized (0.22µm filter).

MH plates were overlaid with 20 mL of molten MH soft agar (0.75%) inoculated with 200 µL of *C. jejuni* culture standardized to an OD_{600} of 1.0 in MH broth. Wells of 5 mm diameter were cut into agar plates and 50 µL *Lp* MYS94-CFS was added to each well. After 24 h incubation under microaerophilic conditions zone of inhibition diameter around each well was measured for anti-*C. jejuni* activity. Uninoculated MRS broth served as control.

Time-kill assay with *Lp* MYS94-CFS on *C. jejuni*

The assay was performed by co-culturing *C. jejuni* with *Lp* MYS94-CFS according to Zhang *et al.*, (2010). One mL of *C. jejuni* suspension (10^8 CFU mL^{-1}) was added into 50 mL of *Lp* MYS94-CFS and MRS broth respectively, and incubated at 37 °C. Optical Density (O.D) was measured at initial and predetermined intervals to determine the surviving cells of *C. jejuni*.

Statistical Analysis: Statistical analysis was executed by one-way ANNOVA of mean, standard deviation and graphical representation was performed using Graph pad prism, version 5.03 software and Microsoft office excel 2007.

Results and Discussion

Screening and identification of LAB: Out of twenty-four isolated presumptive lactic acid bacteria, the strain *Lp* MYS94 showed maximum inhibitory potential against *C. jejuni* and was considered for further studies. *Lp* MYS94 was found to be Gram positive and catalase negative. Phenotypic characterization and molecular identification revealed that the isolate was homo-fermentative and had the ability to ferment maltose, glucose, mannitol, lactose and arabinose. *Lp* MYS94 was found to be negative for arginine hydrolysis, and positive for bile salt hydrolysis and tolerant to 7 % NaCl (Table 1). Sequence analysis of the amplicon confirmed that the isolate was 100 % identical to *Lactobacillus plantarum*. The assigned accession number for the complete nucleotide sequence is KM488574. The 16S rRNA sequence profiling is one of the important molecular tools for the identification of microbial communities' up to species level (Rao *et al.*, 2015). More than 98% identity to the consensus sequence of the 16S rRNA gene is essential for the successful inclusion in the species (Kunene *et al.*, 2000). The phylogenetic tree of the strain in the present study exhibited high similarity to strain *Lactobacillus plantarum* strain LBP1 and the short evolutionary distance with several *L. plantarum* strains (Fig 1).

Probiotic attributes of *Lp* MYS94: The strain was acid tolerant (pH 2) and in the same pH, the viability of the strain MYS94 decreased from 7.2×10^6 to 3.6×10^6 CFU mL^{-1} after 0 and 3h of incubation, respectively. However, there was slight reduction, yet statistically significant in the viable cell number from 7.4×10^6 to 5.2×10^6 CFU mL^{-1}, as compared to the control (pH 6) which was 7.0×10^6 to 6.8×10^6 CFU mL^{-1} after 0 to 3h of incubation at pH 3, indicating the tolerance

of *Lp* MYS94 to low pH, which is considered as an important characteristic of probiotic bacteria (Rao *et al.,* 2015). *Lp* MYS94 was bile salt hydrolysis positive and exhibited sensitivity to penicillin, amoxicillin and erythromycin and intermediate sensitivity to bacitracin and resistant to methicillin, kanamycin, vancomycin and norfloxacin. The antibiotic resistance evaluation was carried out to promote the safety assessment and for the development of potential probiotic *Lp* MYS94. Antimicrobial assay of *Lp* MYS94 showed a significant inhibitory effect against *E. coli* MTCC 7416, *B. subtilis* MTCC 121, *Enterobacter aerogenes* MTCC 7325 and *Enterococcus fecalis* MTCC 6845. The antimicrobial activity was measured as zone of inhibition and ranged from 7 to 16 mm. Hemolytic activity of the same strain showed absence of clearing zone around the growth on the blood agar plate, indicated negative for hemolysis (Table 2). The strain exhibited susceptibility to penicillin, amoxicillin and erythromycin, a macrolide antibiotic, but resistance to commonly used antibiotics, supporting the previous reports on antibiotic susceptibility (Belicova *et al.,* 2013; Rao *et al.,* 2015). The natural resistance of the strain to clinically important antibiotics provides new avenues on development of antibiotic/probiotic combination therapies for conditions like diarrhea, urogenital tract infection, and infective endocarditis (Charteris *et al.,* 1998). Probiotics are known to have the beneficial effects such as restoring the normal population levels of beneficial bacteria in the gastrointestinal tract. Hence it is one of the principal indications for its use in the treatment of diseases such as antibiotic-associated diarrhea. Despite the fact that the restoration of the gut microflora, after the antibiotic therapy, is an obvious application for probiotics, also there is scientific support for the administration of probiotics alongside antibiotic treatment. It can therefore concluded that *Lp* MYS94 has good probiotic attributes, including tolerance to acidic pH, antimicrobial activity against four enteropathogenic bacteria, antibiotic susceptibility and absence of beta-hemolysis. The results of this phase indicate that *Lact. plantarum* MYS94 may have potential probiotic value.

Biofilm inhibition ability of *Lp* MYS94

Pathogenic biofilms, a serious issue in the field of food processing and clinical fields such as nosocomial infections. The *L plantarum* has been proved to be the one of the protective weapon in controlling the pathogenic biofilms. Results of the present study support the use of CFS of *Lp* MYS94 to treat the *Klebsiella* biofilm with 52.02 % inhibition. Similar results of antibiofilm activities were reported by Slama *et al.,* (2012) and Rao *et al.,* (2015) using supernatant of *Lact. plantarum* and *Lact. pentosus* supports the present study. The CFS of MYS94 was very effective in controlling the biofilm forming pathogenic bacteria. The production of inhibitory compounds such as organic acids, bacteriocins or other associated compounds may be the possible mechanism behind the antibiofilm effects.

Cholesterol assimilation by *Lp* MYS94 and its CFS

The cholesterol in hen egg yolk was assimilated as 52.16% and 21.32% by *Lact. plantarum* MYS94 and its CFS respectively. The results of the present study clarifies that the *Lact. plantarum* MYS94 has the ability to lower the egg yolk cholesterol from the medium, which intern depicts the cholesterol-lowering ability of the host. The results indicated that the cholesterol assimilation values increased with the

bacterial growth. Cholesterol removal has decreased as the incubation time increased. Maximum cholesterol removal was seen during the lag phase of the incubation. The possibility behind the cholesterol assimilation was maybe the binding mechanism. *Lp* MYS94 was efficient over its CFS in reducing cholesterol in the media. The results of the present study were in agreement with study of Liu *et al.,* (2013) who represented similar findings.

Epithelial cell adhesion assay

Relatively higher binding ability to the epithelial cells was noticed in *L. plantarum* MYS94 (Fig 6) in regard to the control. It was stated that binding efficacy to the cells chiefly depends on the active participation of numerous adhesive proteins. Colonization improves the nutrient absorption by secreting organic acids, implies the health potential of *Lactobacilli* in the crop (Abbas Hilmi *et al.,* 2007).

Table.1 Characterization of *Lactobacillus plantarum* strain MYS 94

Tests	*Lactobacillus plantarum* strain MYS94
Morphology	Rod
Gram staining	+ve
Catalase Test	-ve
Arginine hydrolysis	-ve
Bile salt tolerance	+
Growth at different temperature	
10 ^{o}C	+
37 ^{o}C	+
45 ^{o}C	+
Growth at different NaCl concentrations	
3%	+
5%	+
7%	+
Gas production from Glucose	-ve
Carbohydrate fermentation	
Glucose	+ve
Maltose	+ve
Mannitol	-ve
Arabinose	+ve
Lactose	+ve
Xylose	-ve
Sorbitol	-ve
Raffinose	-ve
Gen Bank Accession Number	KM488573

(+ve: positive; -ve: Negative; +: presence of growth)

Table.2 Probiotic attributes of *Lactobacillus plantarum* MYS94

Lactobacillus plantarum MYS94			
Bile salt hydrolysis test	Positive		
	[a]log CFU ml^{-1}		
Tolerance to acidic pH	**0h**	**1h**	**3h**
pH 2	7.24 ±1.41[a]	6.04±2.12 [b]	3.62 ±2.82 [a]
pH 3	7.42 ±4.24 [c]	6.38±2.82 [c]	5.28 ±1.41 [c]
pH 6	7.34 ±1.41 [b]	7.26±2.82 [a]	6.86 ±1.41 [c]
Antibiotic susceptibility Test			
Sensitive	**Resistant**		
Penicillin G	Vancomycin		
Amoxycillin	Kanamycin		
Erythromycin	Norfloxaci		
Bacitracin			
Methicillin			
Hemolytic test	**Gamma hemolysis**		
Cell Surface Hydrophobicity	**n-Hexadecane [c]**	**Xylene[c]**	
	58.06±1.4[a]	46.90±1.2[c]	
Cholesterol Assimilation	***Lactobacillus plantarum* MYS94**	**CFS**	
	62.18%	31.42%	

n=3; [a,b,c]Mean±SD of log value of viable cell count

Fig.1 Phylogenetic relationships obtained by Dendrogram based on BLAST algorithm and Neighbour Joining Tree method between *Lactobacillus plantarum* MYS94 and other *Lactobacillus plantarum* strains

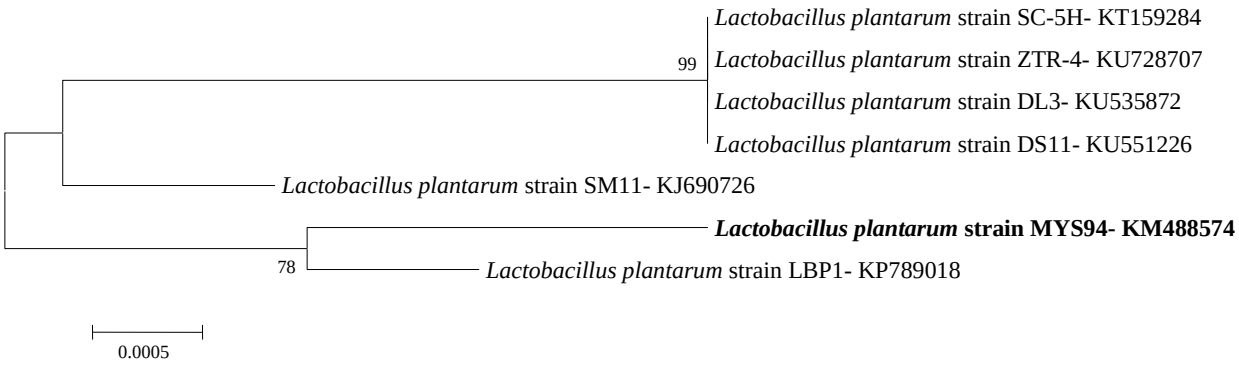


Fig.2 Inhibition of *Klebsiella* biofilm by Lp MYS94-CFS

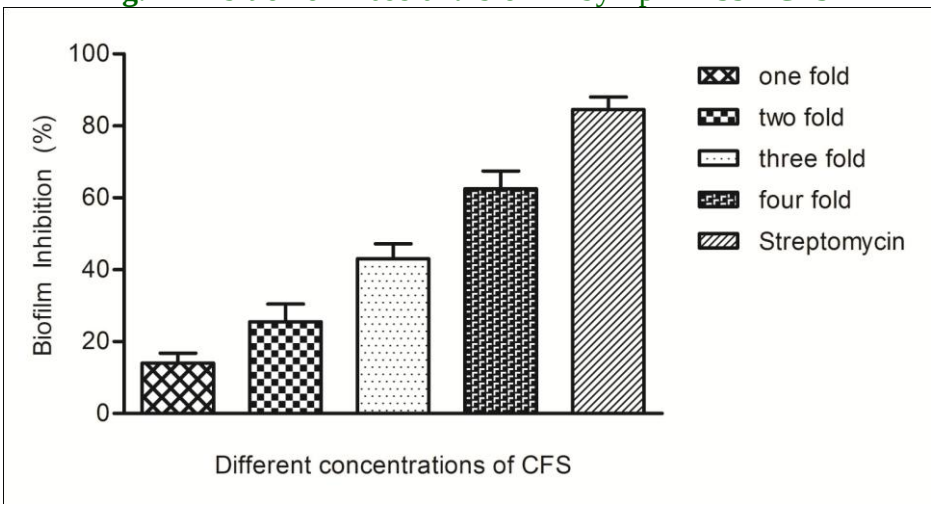


Fig.3 Adherence ability of probiotic *L. plantarum* MYS94 to chicken crop epithelial cells. A. Control- epithelial cells without bacterial adherence. B. *L. plantarum* MYS44 adhering to chicken crop epithelial cells

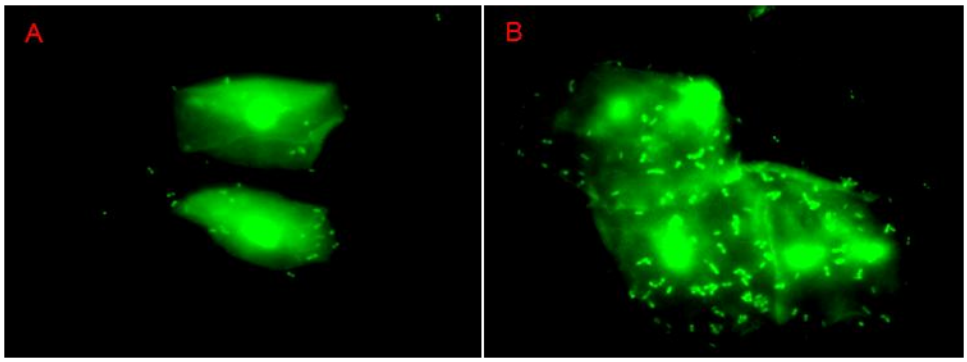


Fig.4 Evaluation of Lp MYS94 cell free supernatant kill assay on *C. jejuni* by the contact time.

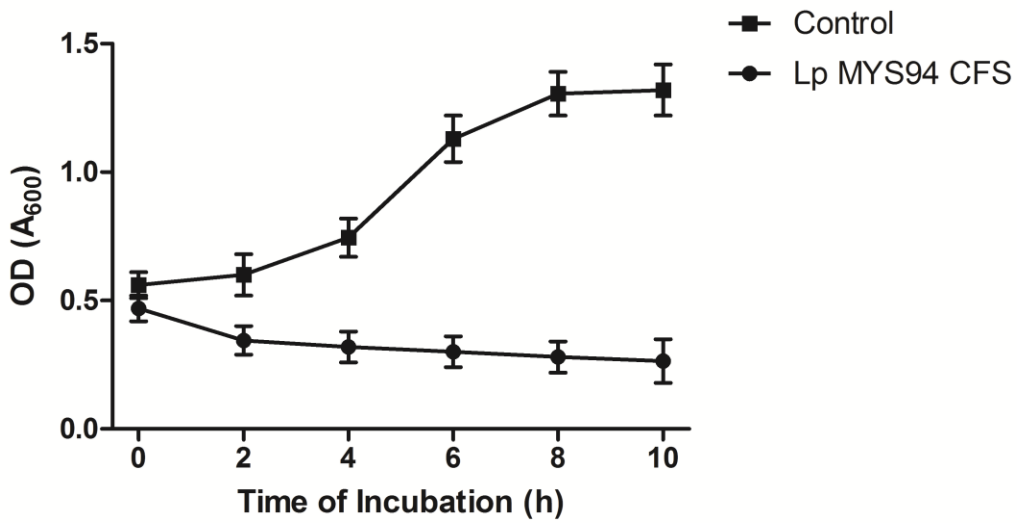
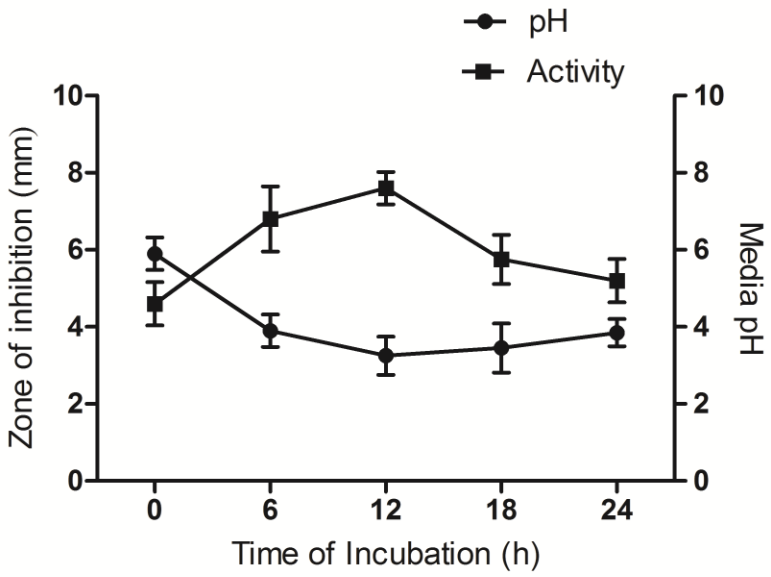


Fig.5 pH dependent anti- *C. jejuni* activity of Lp MYS94-CFS at different intervals of time



Anti *C. jejuni* assays

Time-Kill assay with CFS of *Lp* MYS94 on *C. jejuni*

The CFS of *Lp* MYS94 showed a substantial inhibitory effect against *C. jejuni*. The viability of the *C. jejuni* was decreased by 0.3 OD after 2 h treatment with CFS of *Lp* MYS94. After 6 h, the viability of the *C. jejuni* was significantly decreased by 0.4 OD. The viability of *C. jejuni* was gradualy decreased after 4 h contact with CFS of *Lp* MYS94. The CFS of *Lp* MYS94 of exhibited the strong kill effect for the growth of *C. jejuni* than that of control (Fig). Significant killing effect was observed with the CFS of a probiotic *lactobacilli* on *Shigella sonnei* (Zhang *et al.*, 2011) supporting the present study.

Well diffusion assay

The results of inhibitory activity of CFS of *Lp* MYS94 revealed the antagonistic activity against *C. jejuni*, causing a clear zone of inhibition. However, there was no inhibition effect of the CFS on neutralization and the antimicrobial activity was completly aboloshed when the pH was beyond 4.0. The organic acid production by the probiotic *Lp* MYS94 might be responsible for the antagonistic activity. Formation of low pH in the liquid media by probitoic *Lp* MYS94 can cause inhibition of *C. jejuni* (Meremae *et al.*, 2010). Furthermore, it has been reported that, probiotic LAB produce organic acids with strong inhibitory potential against several gram negative bacteria due to their permeability ability of the bacterial outer membrane and it can be considered as the major antimicrobial compound (Bartz *et al.*, 2014). The antimicrobial activity of *L casei* Shirota and *L. rhamnous* GG was largely attributed to the production of lactic acid (Makras *et al.*, 2006). Organic acids like, succinic can not only fulfil a barrier effect on bacterial pathogens, but also has a crucial role in maintaining the colon health (Cook and Sellin, 1998). The viable probiotic bacterial treatments were ineffective in controlling *C. jejuni*, while inhibitory compounds from the corresponding probiotic bacteria significantly reduced the colonization of *C. jejuni* in chickens (Stern *et al.*, 2008).

In Conclusion, *p* MYS94 exhibited good probiotic attributes such as tolerance to acidic pH, antimicrobial activity and cell surface hydrophobicity. *Lp* MYS94 was also found to be a better cholesterol assimilating and antibiofilm agent. The CFS of *Lp* MYS94 significantly inhibited the growth of *C. jejuni* and the activity was pH dependent. The production of organic acids by the probiotic *Lp* MYS94 is the main inhibitory potential to control *C. jejuni* in the study. Overall, the strain showed promising therapeutic attributes under *in vitro* conditions, however further studies are necessary to confirm its beneficial role of this probiotic strain for human health.

Acknowledgment

We acknowledge ICMR, New Delhi, India for proving funding in the form of Senior research Fellowship (ICMR-SRF Order No. 3/1/2/27/2014- (Nut) dated 18.03.2016) to the first author. We are thankful to Gangaraju, Research Fellow, Government College, Madikeri, Karnataka, India, for providing the traditional fermented wine sample for this study.

Conflict of Interest

All the authors have read and approved the manuscript and declare no conflict of Interest regarding the publication of this study.

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How to cite this article:

Poornachandra Rao, K., N.K. Hemanth Kumar and Sreenivasa, M.Y. 2016. Therapeutic Potential of Probiotic *Lactobacillus plantarum* MYS94 against *Campylobacter jejuni*. *Int.J.Curr.Microbiol.App.Sci.* 5(12): 869-883. doi: http://dx.doi.org/10.20546/ijcmas.2016.512.095