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Original article

Assessment of efficacy of a potential probiotic strain and its antiuremic and antioxidative activities

Arpita Mandal [a], Shreya Mandal [a], Suchismita Roy [a], Arpita Patra [a], Shrabani Pradhan [a], Koushik Das [a], Tanmay Paul [b], Keshab Ch. Mondal [b], Dilip Kumar Nandi [a,*]

[a] Department of Microbiology, Nutrition, and Human Physiology, Raja N L Khans Women's College, Midnapore 721102, West Bengal, India
[b] Department of Microbiology, Vidyasagar University, Midnapore 721102, West Bengal, India

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SUMMARY

Background & aims: Kidney disease requires dialysis or kidney transplantation. No generally applicable therapies to slow progression of renal disease are available. The aim of this study was to characterize *Sporosarcina pasteurii* (MTCC 1761, Type strain) as an effective probiotic strain for acetaminophen induced uremic patent.

Methods: Antibacterial and antioxidative activities, bile salt and pH tolerance, starch and protein digestibility, hydrophobicity, opsonophagocytic assay and antibiotics sensitivity of the strain was performed to investigate its probiotic potentialities. Blood uremic profiles, DNA fragmentation assay of kidney tissue and kidney histological studies were investigated on acetaminophen-induced nephrotoxic rats (Wister strain albino male).

Results and discussion: The cell free extract of *S. pasteurii* showed high *in vitro* antioxidative property and potential antibacterial activity (average diameter of 6 mm) against some reference enteropathogenes. The strain can survive at highly acidic environment (pH 3.0) and showed bile resistance upto 0.8% (w/v) along with 8% (w/v) salt and 0.8% (w/v) phenol. The strain able to digest starch and milk protein and show medium hydrophobic attachment with non-polar solvent. Bacterial strain completely destroyed in the presence of blood components and sensitive to all tested 20 antibiotics. After oral administration of the strain significantly lowered the level of blood urea, creatinine, and uric acid level and minimized the glomerular necrosis, DNA damage of uremic rats.

Conclusion: Therefore, the strain *S. pasteurii* may be exploited as a potent probiotic organism and oral ingestion of bacteria decrease uremic syndrome.

1. Introduction

Renal insufficiency leads to uremia and each year, the number of patients with chronic kidney failure increases by an astounding 11%.[1] Globally, kidney transplantation is opted by a very few kidney failure patients for effective treatment, due to shortage of donor because of high cost and high probability of organ rejection. Therefore, there is a great necessity for an unconventional, affordable therapy for patients who cannot afford expensive dialysis or kidney transplant to keep them alive.

Acetaminophen is the major metabolite of phenacetin and may contribute to kidney injury through a specific mechanism different from other kidney failure due to other type of analgetics. Acetaminophen (APAP) overdose can result in nephro-toxicity. APAP also interact with cytochrome P450 (CYP)-and produce the toxic electrophile N-acetylp-benzoquinone imine (NAPQI) both in liver and kidney.[2] APAP overdose produces extremely large amount of NAPQI and only a part of it can form GSH conjugate with subsequent depletion of cellular GSH. The remaining part of NAPQI binds to cellular proteins and induces oxidative stress, leading to renal injury.

"Enteric dialysis" is an adaptive physiological process for removal of solutes from the body. High concentration gradients can facilitate diffusion of solutes from plasma to intestinal lumen. The intestinal barrier functions as a semipermeable membrane. Concentration gradient makes solutes scatter from plasma into the lumen. When amount of uremic toxins become larger in plasma than lumen, a large amount of uremic solutes become distributed

* Corresponding author. Tel.: +91 9434229882; fax: +91 3222275426, +91 3222297865.
E-mail address: dilipnandi2004@yahoo.co.in (D.K. Nandi).

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throughout the intestine. Recent studies indicated that uremic toxins like urea, creatinine etc. can excrete through enteric dialysis.[3] "Enteric dialysis" thus become an alternative therapy for solute removal in uremia. The live bacteria which degrade uremic toxins within the gut have been acceptable therapy of today.[4] Probiotics by the generally accepted definition, are "live microorganisms which when administered in adequate amounts confer a health benefit on the host". To shift the microecological balance in the intestines in favor of probiotic bacteria prebiotics, such as dietary oligosaccharides, are used as nutritional supplements. Combination nutritional products comprised of probiotics and prebiotics named synbiotics are also available. Probiotics and prebiotics evaluate the impact on solute concentration in serum or on their fecal or urinary excretion.[4] Semipermeable microcapsules containing genetically engineered live cells of *Escherichia coli*DH5 lower the high plasma urea level to normal in uremic rats when orally administred.[5] Oral administration of *Bifidobacterium longum* to hemodialysis patients is effective in reducing the serum toxin levels of by correcting the intestinal microflora.[6]

Sporosarcina pasteurii (Sp) formerly is known as *Bacillus pasteurii* from older taxonomies and is a non-pathogenic spore-forming ureolytic gram positive bacterium and it has been isolated from human feces.[7] It can use urea as sole nitrogen source, and according to literature, needs urease only to generate a nitrogen source, ammonia,[7] which prompted us to initiate the present study. Our previous study demonstrated that feeding with *S. pasteurii* attenuates blood urea-nitrogen levels (BUN) improves in the life span of uremic animals.[8] In this present study we want to establish *S. pasteurii* as a potent probiotic organism and antiuremic agent in uremia.

2. Materials and methods

2.1. Microorganisms

Sporosarcina pasteurii (MTCC 1761) was collected from IMTECH Chandigarh, India. Some pathogenic reference strains like *Escherichia coli* (ATCC 8739), *Bacillus subtilis* (ATCC6633), *Staphylococcus aureus* (ATCC 25093), *Shigella dysentery*, *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumonia* (ATCC 10145), were also collected from Department of Microbiology, Vidyasagar University, West Bengal, India.

2.2. Probiotic characteristic of S. pasteurii

Composition of basal media: Basal media containing several components such as Yeast extract-20.0 g, $(NH_4)_2SO_4$-10.0 g. All ingredients were dissolved in 1000 mL of 0.13 M Tris buffer (pH 9.0).

Tolerance to inhibitory substances: The organism was grown at 37 °C in different medium pH (1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0) different NaCl concentration [1, 2, 3, 4, 5, 8, 9 and 10% (w/v)] Bile salt concentrations (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7 and 0.8 g sodium taurocholate/100 mL) Phenol level (0.1–1.0 g phenol/100 mL) were monitored at 620 nm after 24 h.[9]

Survival under conditions simulating the human GI tract: Tolerance to stomach condition and small intestine condition was tested.[10]

Determination of bacterial hydrophobicity: Hydrophobicity was determined using Bacterial Adherence to Hydrocarbons (BATH) Percentage bacterial adhesion to hydrocarbon (toluene) is calculated as shown below: Adhesion (%) = [(OD_{600} cells free of hexane) − (OD_{400} aqueous)]/(OD_{600} cells free of n − octane) × 100%.[11]

Antibiotic resistancy study: Antibiotic resistancy of the bacteria was done using Icosha Disc (HiMedia, India). The plates were incubated at 37 °C overnight, and diameters of the zone of inhibition around the discs were measured.

Antimicrobial activity and nature of antimicrobial substances: One mL cell free culture supernatant (CFCS) of *S. pasteurii* was retained as untreated filtrate. To determine the organic acid function, 1 mL CFCS was adjusted to pH 6.5. In order to test the heat sensitivity, 1 mL CFCS was incubated at 100 °C for 15 min and treated with protease-K. The antimicrobial activity of all samples was tested using the Agar-well diffusion assay.[12]

Detection of enzymatic activities: Modified nutrient agar containing skimmed milk (HiMedia, India), tributyrin and soluble starch was used for detecting the protein, lipid and starch digesting capabilities of selected strain, respectively. The digesting capability of the tested strain was classified as positive when the diameters of clear zone were more than 1 mm. Each assay was performed in triplicate.[13]

Urease assay: The urease activity was determined for *S. pasteurii*, by measuring the amount of ammonia released from urea according to the phenol-hypochlorite assay method.[14]

Antioxidative activity of bacteria strain: Cell-free culture supernatants (CFCS) were obtained by centrifugation (10,000× *g*, 4 °C, 20 min) of *S. pasteurii*. Antioxidative activity of *S. pasteurii* (MTCC 1761) was performed by the thiobarbituric acid (TBA) method via the measurement of lipid per oxidation,[15] based on the monitoring of inhibition of linoleic acid and human plasma peroxidatin by CFCS. Twenty mL of linoleic acid emulsion was made up of 0.1 mL of linoleic acid (Amersham Life Science, Cleveland, Ohio), 0.2 mL of Tween 20, and 19.7 mL of deionized water. Five tenths mL of phosphate buffer solution (0.02 M, pH = 7.4), 1 mL of linoleic acid emulsion, 0.2 mL of FeSO4 (0.01%), 0.2 mL of H_2O_2 (0.56 mM), and 0.4 mL of CFCS were mixed and incubated at 37 °C. Intracellular extract was replaced by deionized water in the control samples. After 12 h of incubation, 2 mL of the reaction solution was mixed with 0.2 mL of trichloroacetic acid (TCA; 4%), 2 mL of TBA (0.8%), and 0.2 mL of butylated hydroxytoluene (BHT; 0.4%). This mixture was incubated at 100 °C for 30 min and allowed to cool. Two mL of chloroform was then added for extraction. The extract was obtained and the absorbance was measured at 532 nm. The percentage of inhibition of linoleic acid peroxidatin was defined as follows: [1-A532 (experimental sample)/A532 (control)] × 100%. The plasma lipid peroxidation was also analyzed. Four tenths mL of plasma, 0.1 mL of $FeSO_4$ solution (50 μM), and 0.2 mL of CFCS were mixed and incubated at 37 °C in a water bath. CFCS was replaced by deionized water in the control samples. After 12 h of incubation, the reaction solution was mixed with 0.375 mL of 4% TCA and 75 μM of BHT (0.5 mM) and placed in an ice bath for 5 min. The upper phase was obtained by centrifugation at 3000× *g* for 10 min. Two tenths mL of TBA (0.6%) was then added. This mixture was incubated at 100 °C for 30 min and allowed to cool. The absorbance was then measured at 532 nm. The percentage of inhibition of plasma lipid peroxidation was also defined as [1-A532 (experimental sample)/A532 (control)] × 100%.

Quantitative determination of a,a-Diphenyl-b-Picrylhydrazyl (DPPH) radical: The DPPH radical scavenging activity was measured by the method 0f Shimada[16] with slight modification. Intact cells of 0.8 mL and CFCS were mixed separately and allowed to react for 30 min with 1 mL of freshly prepared DPPH solution (0.2 mM in methanol). Blank samples contained either PBS or deionized water. The scavenged DPPH was then monitored by measuring the decrease in absorbance at 517 nm. The scavenging ability was defined as follows: [A517 (blank) − A517 (sample)/A517 (blank)] × 100%.

2.3. Safety assays in vitro

Opsonophagocytic assay (*in vitro*): The opsonophagocytic assay has been performed as described by Huebner.[17]

2.4. Evaluation of the antiuremic effect of S. pasteurii against acetaminophen induced uremic rats

2.4.1. Selection of animals, care and grouping of animals and experimental procedure

The study was conducted on twenty four healthy, adult, male albino rats of Wister strain (Supplied from Ghosh animal, animal foods and animal cages Supplier, Kolkata-54, India) having a body weight of 100 ± 15 g. They were acclimatized to laboratory condition for 1 week prior to experimentation. Animals were housed six per cage in a temperature-controlled room (22 ± 2 °C) with 12–12 h dark–light cycle at a humidity of 50 ± 10%. They were provided with standard food (pellet diet) and water *ad libitum*. The principle of laboratory animal care National Institute of Health USA guideline was followed throughout the duration of experiment & our Institute Ethics committee approved the experimental protocol.[18] Animals were randomized and divided into four groups (NC, U, VC and UP) of six animals each. Group NC (Natural Control) and VC (Vehicle Control) served as untreated control and was injected with distilled water 1 mL/100 g body weight daily for 7 days. Groups UP (uremic + probiotic) and U (uremic group) animals were treated with 500 mg/kg body weight of the acetaminophen by intraperitoneal injection for 7 days respectively for inducing oxidative stress.

2.4.2. Formulation preparation

Food balls were prepared by the casein-based diet with *S. pasteurii*, sterile 10% honey and milk mixture.[7] The formulation was stored in a −20 °C freezer in aseptic conditions. While no microbial additives were given to rats of group I and group III, rats of group II were administered with 1×10^9 cfu of *S. pasteurii* for 21 days. For formulation, composition, and dosage specifications, see the following Table.

Composition of microbial additive, fed to different groups serial no.	Groups	Microbial additive	No. of rats	Cfu/Dose*/Day
1	NC (No acetaminophen)	No microbe(s)	6	–
3	UP (acetaminophen)	*Sporosarcina pasteurii*	6	1×10^9 cfu
2	U (acetaminophen)	No microbe(s)	6	–
4	VC (No acetaminophen)	*Sporosarcina pasteurii*	6	1×10^9 cfu

After 21 days, animals were sacrificed by diethyl ether anesthesia. Blood samples were collected by hepatic artery punche, using 21 gauge (21 G) needles mounted on a 5 mL syringe (Hindustan syringes and medical devices ltd, Faridabad, India.) Liver and kidney were collected.

2.4.3. Blood uremia profile

Biochemical estimation of Blood Urea: The collected blood was centrifuged and plasma fraction was separated. Urea level of plasma was measured by commercially available standard blood urea kit (Merck, Japan) by semiautoanalyser by standard protocol for photometric determination of urea according to the Urease GLDH method.[19] Biochemical estimation of Blood Creatinine: Creatinine level of plasma was measured by commercially available standard blood creatinine kit (Merck, Japan) by semiautoanalyser (Merck, Japan) by standard protocol for photometric determination of creatinine based on Jaffe kinetic method without deproteinization.[20] Biochemical estimation of Blood Uric Acid Levels: Enzymatic determination of uric acid occurred. End product of the reaction is quinoneimine and measured colorimetrically at 546 nm[21] using the diagnostic reagent kit manufactured by Merck Japan.

DNA fragmentation assay: The extent of DNA fragmentation in the kidney tissue was determined by the method as described by Lin.[22] Briefly, kidney tissue homogenates were treated with 100 mM Tris buffer, pH 8.0, 1 mM EDTA and 0.5% triton X-100 followed by centrifuged. The supernatant was transferred carefully in a tube and 1 mL of 25% TCA was added to it, the mixture was vortexed vigorously and incubated overnight at 4 °C.

2.4.4. Histological studies

Kidneys from the normal and experimental mice were fixed in 10% buffered Bruins reagent and were processed for paraffin sectioning. Sections of about 5 μm thickness were stained with hematoxylin and eosin to evaluate under light microscope. Histologic scoring system was used to quantitate renal pathology. The scoring system was as follows: 0, absent of deformed glomerulus; 1, present deformed glomerulus and 2, marked deformed glomerulus. The parameters assessed were tubular necrosis score and total histological score.[23]

2.4.5. Hematological study

Hematological parameters like total RBC by haemocytometer and hemoglobin (Hb) by standard kit method (Merck, Japan).[24]

Limited analysis of fecal: Analysis of fecal ammonia, & urea by using standard kit method (Merck., Japan) were done. Consistency of sample stool also examined.

2.5. Statistical analysis

Analysis of variance (ANOVA) followed by a multiple two-tail test with Bonferroni modification was used for statistical analysis of the collected data. Differences were considered significant when $P < 0.05$.

3. Results

3.1. Bacteria strain characterization as well as probiotic characterization

3.1.1. Tolerance to inhibitory substances

It has been found that the studied bacterium can survive upto 0.3% (W/V) bile salt (Fig. 1a). *S. pasteurii* tolerate relatively lesser amount of phenol about 0.8% (W/V) shown in Fig. 1c. *S. pasteurii* could tolerate up to 8% NaCl as shown in Fig. 1d. This bacterium is able to tolerate pH 2.0 and its significant growth was obtained from pH 5.0 to 9.0 (Fig. 1b).

3.1.2. Survival under conditions simulating the human GI tract

In simulating gastric juice, the viability of the *S. pasteurii* would remain same with the time, incubation for 1–3 h as shown in Table 1. The strain examined in this study could survive well in pancreatin solution at pH 8.0 as even after 4 h exposure, retained their viability. Our findings was on the viability of the *S. pasteurii* in the presence of pepsin at pH 2.

3.1.3. Hydrophobicity test

S. pasteurii showed hydrophobicity values 33% when toluene is used as hydrocarbon.

3.1.4. Antimicrobial activity

S. pasteurii produced clear zone (mm) against all tested pathogens. *S. pasteurii* showed antimicrobial activity against potential human pathogens. The zone of inhibition is tabulated in Table 2.

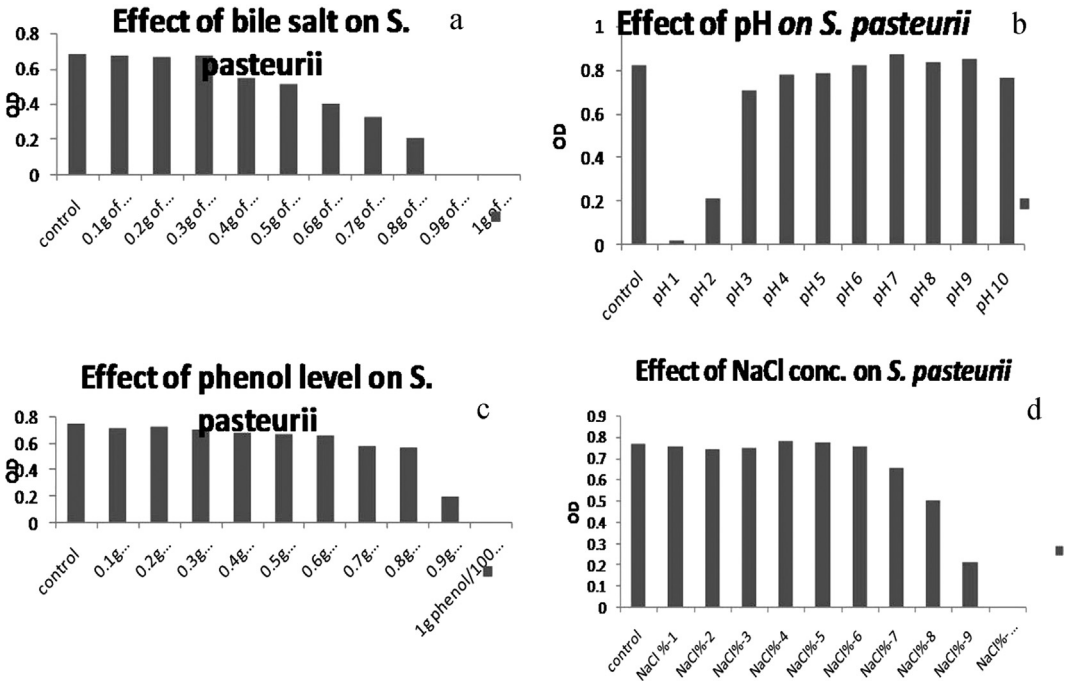

Fig. 1. Probiotic charecterization of *S. pasteurii*, survival of *S. pasteurii* at different bile salt concentration, pH values, phenol level and NaCl concentration (OD at 620 nm).

3.1.5. Starch, protein, and lipid digesting capabilities

S. pasteurii exhibited starch and protein digestibility on modified nutrient agar plates containing starch and milk protein.

3.1.6. Antibiotic resistance

This work reports showed the susceptibility patterns of *S. pasteurii* against all of twenty antibiotics in Icosha disc (Hi-media, India). *S. pasteurii* is susceptible to all of the antibiotics tested (Table 3).

3.1.7. Urease assay

In our present study *S. pasteurii* produce a large amount of urease, about 288 unit urease activities (U/ml) in basal media with additional urea in media.

3.1.8. Antioxidative activity

Culture free supernatant of tested bacteria shows the inhibitory lipid peroxidetion of linoleic acid, about 53% and 59% in case of human plasma.

3.1.9. DPPH free radical scavenging ability

The intact cells and cell-free extracts of *S. pasteurii* exhibited the ability of reduction of DPPH radical by 61.50 ± 6.12% and 53.99 ± 6.09%, respectively. Data are means ± SD of triplicate experiments.

3.2. Safety assays in vitro

3.2.1. Opsonophagocytic assays

Tested strain was susceptible to killing, when complement with polymorphonuclear neutrophils, used in the oponophagocytic assay (Fig. 2).

Table 1
S. pasteurii survival under conditions simulating the human GI tract.

Strain	Pepsin at pH 2 (log CFU/ml)			3% Oxgall (log CFU/ml)	
S. pasteurii	1 h 9.34 ± .52	2 h 9.04 ± .6	3 h 8.74 ± .23	1 h 9.04 ± .12	4 h 8.64 ± .56

3.3. Evaluation of the antiuremic effect of S. pasteurii against acetaminophen induced uremic rats

3.3.1. Uremic profiles

APAP exposure significantly increased plasma level of BUN (Fig. 3a), creatinine (Fig. 3b), and uric acid level (Fig. 3c), However, *S. pasteurii* treatment to APAP administration reduced the plasma level of blood urea nitrogen (BUN), creatinine, uric acid, compared to APAP-exposed animals (group U).

3.3.2. Assessment of DNA fragmentation

Fig. 4 represents the results of DNA fragmentation analysis. A smear on agarose gel has been observed in the DNA isolated from APAP-exposed animals. *S. pasteurii* post-treatment was found to be effective to prevent this APAP-induced smear formation (group UP).

3.3.3. Histological assessment

Histological assessments of different renal segments of control and experimental animals have been presented in Fig. 5. Sections

Table 2
The antimicrobial activity of CFCS of *S. pasteurii* strain by several treatments.

Indicator strains	Inhibition zone (mm)a			
	Non treatment of CFCS	Proteinase -K	CFCS adjust pH 6.5	Heat (100 °C, 15 min)
E. coli (ATCC 25922)	+	+	−	+
Shigella dysenteriae (MTCC 1457)	++	++	−	++
Klebisella pneumoniae (ATCC 15380),	++	++	−	++
Staphylococcus aureus (ATCC 6538)	++	++	−	++
Pseudomonas aeruginosa (ATCC 15442).	++	++	−	++
Bacillus subtilis (ATCC6633)	++	++	−	++

Symbols refer to the size of the inhibition zone diameter observed with growing cells: +, 5 mm; ++, 10 mm; −, absence of an inhibitory zone.

Table 3
Antibiotic sensitivity profile of *S. pasteurii*.

Sl. No	Antibiotic	Concentration	Susceptible/registrant
1.	Cephalothin	30 mcg	Susceptible
2.	Clindamycin	2 mcg	Susceptible
3.	Co − Trimoxazole	25 mcg	Susceptible
4.	Erythromycin	15 mcg	Susceptible
5.	Gentamycin	10 mcg	Susceptible
6.	Ofloxacin	5 mcg	Susceptible
7.	Penicillin	10 units	Susceptible
8.	Vancomycin	30 mcg	Susceptible
9.	Ampicillin	10 mcg	Susceptible
10.	Chloramphenicol	30 mcg	Susceptible
11.	Oxacillin	1 mcg	Susceptible
12.	Linezolid	30 mcg	Susceptible
13.	Azithromycin	15 mcg	Susceptible
14.	Amikacin	30 mcg	Susceptible
15.	Clarithromycin	15 mcg	Susceptible
16.	Teicoplanin	10 mcg	Susceptible
7.	Methicillin	5 mcg	Susceptible
18.	Amoxyclave	30 mcg	Susceptible
19.	Novobiocin	5 mcg	Susceptible
20.	Tetracycline	30 mcg	Susceptible

from APAP unexposed animals (NC) showed normal histology shown in Fig. 5a. On the other hand, renal sections from APAP-exposed animals (U) showed extensive tubular damage by swollen and necrotic epithelial cells shown in Fig. 5b. Treatment with *S. pasteurii*, (UP) however, reduced these alterations (Fig. 5c). In Fig. 5d histological score of respective groups prove the fact.

3.3.4. Levels of hemoglobin and RBC

In the present study, receiving acetaminophen interperitonially for 7 days significantly decreased the Hb levels and also lowered the total RBC count in group U (the acetaminophen treated control group), compared to group NC. But in group UP significantly higher levels of hemoglobin and RBC are observed, compared to group U and the values resettled to the control group. In group VC, levels of hemoglobin has increased significantly after receiving *S. pasteurii* for 3 weeks (Table 4).

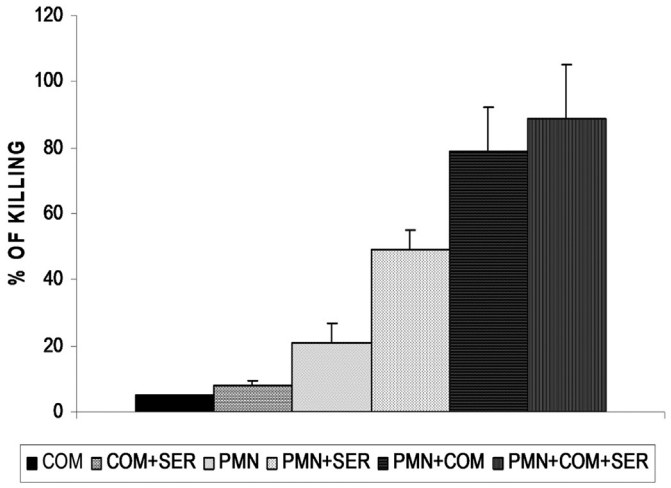

Fig. 2. Safety assessment *in vitro* comparison of % of killing of *S. pasteurii* using complement alone (Com); complement plus normal rabbit serum (Com + Ser); PMNs alone (PMN); PMNs plus normal rabbit serum (PMN + Ser); PMNs plus complement (PMN + Com); and PMNs plus complement plus normal rabbit serum (PMN + Com + Ser). Error bars represent the standard error of the mean.

3.3.5. Limited fecal analysis

Ammonia present in stool samples collected from group VC and UP from every 7 days of interval is increased slightly than APAP treated group and control group. Formation of any diarrheal condition is not occurring in experimental condition after feeding of *S. pasteurii* (Table 5).

4. Discussion

In the present study, *S. pasteurii* (MTCC 1761) is evaluated as potential probiotic for uremia in acetaminophen induced rat model. Before evaluating as therapeutic agent for uremic patents, important probiotic characteristics of the bacteria has been studied in this experiment. Therefore, it is necessary to evaluate the resistance

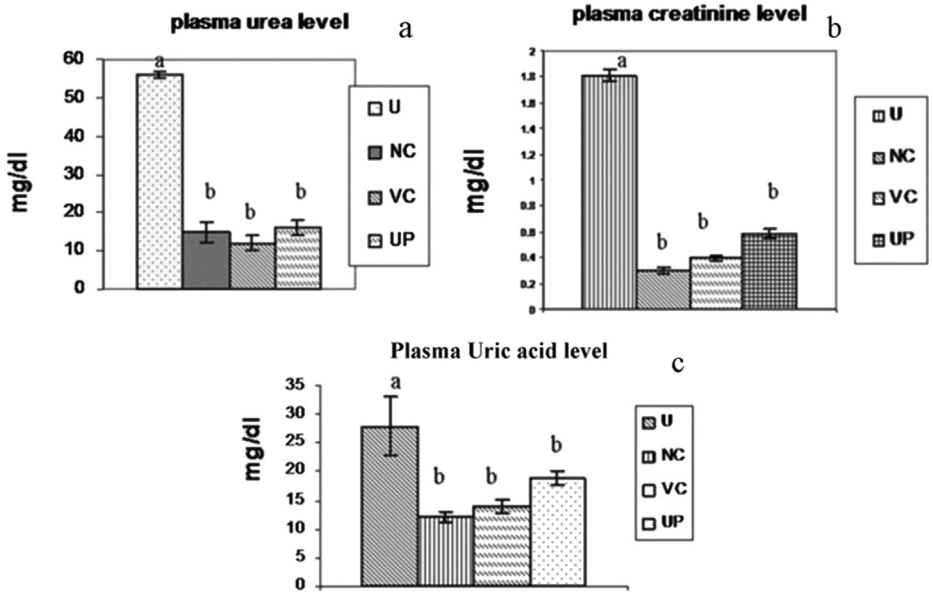

Fig. 3. Effect of acetaminophan and after then *S. pasteurii* treatment on plasma urea (a). Effect of acetaminophan and after then *S. pasteurii* treatment on plasma creatinine. (b) Effect of acetaminophan and after then *S. pasteurii* treatment on uric acid in plasma (c). In all cases data are expressed as Mean ± SE ($n = 6$). ANOVA followed by multiple two tail *t*-test. Bars for SE and a, and b specific data differ from each other significantly ($P < 0.05$).

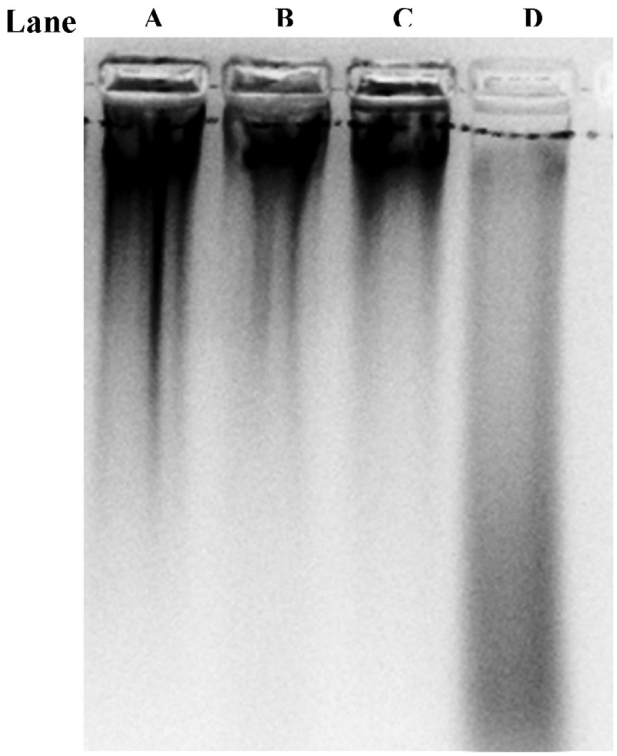

Fig. 4. DNA fragmentation pattern of the experimental rats kidney. DNA isolated from experimental rat kidney tissues was loaded onto 1% (w/v) agarose gels. Lane A: DNA isolated from UP (*S. pasteurii* treated on acetaminophen induced rats) kidney samples; Lane B: DNA isolated from VC (*S. pasteurii* treated on rats) kidney; Lane C: DNA isolated from NC (control) rats kidney and Lane D: DNA isolated from U (APAP exposed) kidney.

ability of bacteria to gastrointestinal stress before their use as probiotics. This bacterium tested for resistance to bile salt, acidic pH, phenol and salt.

Bile, acidic pH tolerance is an important characteristic for the survival and growth of bacteria in the intestinal tract because gastric juice contains bile salt and HCl.[9] Bacterium can survive a good concentration of bile salt which is within the limit of intestinal content. This bacterium is able to tolerate at pH 2.0 and its significant growth was obtained from pH 5.0 to 9.0. According to Charteris[10] enteric bacteria are able to tolerate pH 2.0 for several minutes, while viable count will be affected at slightly high acidic pH. *S. pasteurii* tolerate relatively lesser amount of phenol. Tolerance to phenol is a characteristic probiotic property because phenols can be formed in the intestines by bacteria that dominate some aromatic amino acids delivered by the diet or produced by endogenous proteins.[25]

Testing the survival of bacteria in stimulated gastrointestinal tract conditions *in vitro* may have value in predicting the actual survival of a strain *in vivo* when consumed in a non-protected way. Our findings on the viability of the *S. pasteurii* in the presence of pepsin at pH 2 are in agreement with the data reported by previous literature.[10] *S. pasteurii* survive well in the stimulating small intestine environment. Most Studies so far have shown majority of the strains survived well under small intestine conditions, suggesting a potential recuperation of the initial levels during the passage of the small intestine.[10]

Regarding the percentage affinity to n-hexane, the bacteria are classified by the scale: <10% hydrophilic, 10–29% medium hydrophilic, 30–54% medium hydrophobic, >55% highly hydrophobic. Hydrophobicity of some known cultures like *Lactobacillus plantarum* ATCC8014, *L. pentosus* ATCC8041, *L. casei* NCIMB 3254, and *L. delbrueckii* NCIM2025 are found to be 5.5, 6.5, 6.2 and 3.7%, respectively, and all are very less than *S. pasteurii*. As the hydrophobicity of the cell increases, it also enhances the adhesion property. Only for hydrophobic microorganisms surface hydrophobicity is correlated to adhesions.[26]

The antimicrobial effect associated is based on the oxidative properties that results in irreversible changes in the microbial cell membrane. The production of antimicrobial compounds such as organic acids, ammonia, hydrogen peroxide and bacteriocins by intestinal microflora is probably one of the most important mechanisms responsible for the antagonistic phenomenon against pathogenic organisms[27] and therefore it is important to check this property in probiotic candidates.

The agar plate assays are used to study digesting capability of the *S. pasteurii*. In this study, sterilized skimmed milk, tributyrin and soluble starch are used for detecting protein, lipid and starch digestion capabilities, respectively. *S. pasteurii* exhibited starch and protein digestibility. The beneficial effects of enzymes in the lactic acid bacteria show the importance of enzymatic activities in bacterial strains of probiotics. Utilization of some lactic acid bacteria strains in nutrition is due to their production of enzymes such as α-amylase, phytase, lecitinase, lipase, and/or protease. Bacterial digestibility of starch, protein and lipid, showed a healthy impact to human.[28]

This reports showed the susceptibility patterns of *S. pasteurii* against all of twenty antibiotics in Icosha disc (Hi-media, India). *S. pasteurii* has susceptible to all of the antibiotics.

The tested bacteria use urea as sole nitrogen source. Urea hydrolase (urease) constitutes about 1% of the dry weight of this bacterium and is expressed constitutively, in contrast to many other ureolytic bacteria in which synthesis of urease is induced by substrate, environmental pH, or nitrogen starvation.[29] Moreover, *S. pasteurii* can use urea as a sole nitrogen source, and according to some reports, needs urease only to generate a preferable nitrogen source, ammonia.[7] Thus produced ammonia become utilized within intestine and making no adverse effect on experimental animals.

Lipid peroxidation is a key process in many pathological effects such as DNA damage, carcinogenesis, mutagenesis and aging. Therefore lipid peroxidation protection is essential. Culture free supernatant of tested bacteria shows the inhibitory lipid peroxidetion of linoleic acid, about 53% and 59% in case of human plasma. These results indicated that the culture has a good antioxidant effect on inhibition of lipid peroxidation. Intestinal lactic acid bacteria *Bifidobacterium longum* ATCC 15708 and *Lactobacillus acidophilus* ATCC 4356 are also found antioxidative. Intracellular extracts from *B. longum* ATCC 15708 and *L. acidophilus* ATCC 4356 demonstrating the inhibitory rate on linoleic acid peroxidation is 48% and 45%, respectively in previous study.[30]

DPPH scavenging ability of 1 mL of intact cells and cell free culture supernatant of *S. pasteurii* are measured and observed that it had significant ability to scavenge the DPPH (expressed in % of inhibition). The DPPH scavenging ability of intact cells is higher than that of cell-free extract of tested strain. The intact cells and cell-free extracts of *S. pasteurii* exhibited the ability of reduction of DPPH radical by $61.50 \pm 6.12\%$ and $53.99 \pm 6.09\%$, respectively. Data are means ± SD of triplicate experiments. The *in vitro* ability of probiotic bacteria to quench free radicals has been shown previously.[30] Our results confirm these findings as our data show that *S. pasteurii* had significant ability to scavenge the DPPH radicals. Intact cells had high antioxidant activity than cell-free extracts.

Tested strain is susceptible to killing when complement and polymorphonuclear neutrophils are used in the oponophagocytic assay. One possibility for the susceptibility of specific strain, in the present study, may be due to the absence of a polysaccharide

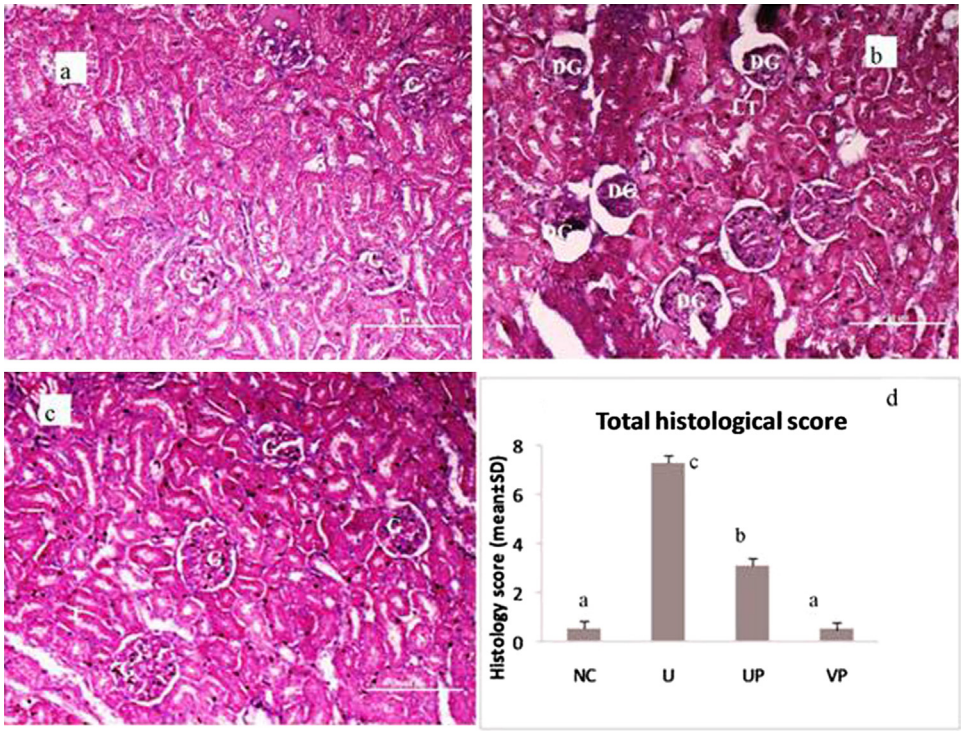

Fig. 5. Hematoxylin and eosin stained kidney section of (a) normal rats (I) (20×), showing appearance of glomeruli (marked as G) and normal tubular part (marked as T). (b) APAP exposed rats (U), showing deformed glomerulus (marked as DG) and large sweled tubular part (marked as LT) (20×), (c) kidney section of the mice post-treated with *S. pasteurii* (UP) (20×) showing almost normal appearance of glomeruli (marked as G) and normal tubular part (marked as T). (d) Histologic scoring system used to quantitate renal pathology. The scoring system was as follows: 0, absent of deformed glomerulus; 1, present of deformed glomerulus and 2, marked deformed glomerulus. Each column represents mean ± SD, $n = 6$. "a" indicates that there is no significant difference between the vehicle control (VC) and natural control group (NC) and "b" indicates the significant difference between the APAP induced uremic group (U) and other group and "c" indicates the significant difference between the *S. pasteurii* treated groups (UP) and other groups. Data of *S. pasteurii* treated group (UP) is significant lower than APAP induced uremic group (U).

capsule. However, the clinical importance of this phenomenon needs to be assessed in a comparison of pathogenic versus commensally isolates.[17] Our data, support a possible role of the opsonophagocytic assay in assessing the safety of *S. pasteurii* preparations as theraputic probiotics.

Acute large doses or chronic use of acetaminophen is commonly associated with nephrotoxicity and hepatotoxicity in humans as well as animals.[31] Thus, acetaminophen-induced nephrotoxicties are well established experimental models of drug-induced renal injury.[32] Acetaminophen nephrotoxicity occurs due to its highly reactive metabolite N-acetyl-paraamino-benzoquinoneimine, which arylates proteins in the proximal tubule, initiating cell death of renal tubular cells.[32] Drug-induced nephrotoxicities are often accompanied with marked elevations in BUN, serum creatinine and acute tubular necrosis and depletion of antioxidant enzyme.[33] In the present study, acetaminophen-induced nephrotoxicity was characterized by marked elevations in the circulating levels of BUN and serum creatinine in control (group U) rats. In this study, acetaminophen induced nephrotoxicity showed a significant ($P < 0.05$) increase in the plasma urea uric acid and creatinine concentrations in the Group U (acetaminophen induced) rats when compared to the normal group (Group NC). Moreover, oral administration of *S. pasteurii*, significantly ($P < 0.05$) decreased plasma urea and creatinine in group UP when compared to the Group U. The urease positive bacteria reduce the blood uremic toxins through enteric dialysis.[7]

A smear on agarose gel has been observed in the DNA isolated from APAP-exposed animals, indicating random DNA degradation.[23] previous observation shows that APAP induced random fragmentation of genomic DNA, with subsequent formation of a DNA smear on agarose gel without ladder formation, suggesting that APAP-induced renal cell damage occurs through necrotic pathway. All these studies show that apoptosis also takes place side by side with necrosis, although it is a less significant secondary phenomena in addition to necrosis.[22] *S. pasteurii* post-treatment was found to be effective to prevent this APAP-induced smear formation, thus stop necrosis.

APAP causes a reduction in blood flow. Because blood flowing to the kidney first reaches the renal cortex (outside) and then the renal medulla (inside), the deeper structures of the kidney are most

Table 4
Effect of acetaminophen and after then Sp treatment Hemoglobin and red blood cell count in experimental rat. Data are expressed as Mean ± SE ($n = 6$). ANOVA followed by multiple two tail *t*-test and data with different superscript (a, b, c) in specific horizontal column differ from each other significantly ($P < 0.05$).

Parameters	Group NC: control	Group U: acetaminophen receiving control	Group VC: vehicle control	Group UP: Sp treatment followed by acetaminophen receiving
Hb (mg/dl)	11.863 ± 1.29^{a}	7.861 ± 0.58^{b}	14.629 ± 1.34^{c}	11.849 ± 0.59^{a}
Total RBC count	9.03 ± 1.37^{a}	$6.537 \pm .19^{b}$	11.465 ± 0.54^{c}	11.327 ± 1.36^{c}

NC – Natural Control, VC – Vehicle Control, Groups UP – uremic + probiotic, U – uremic group.

Table 5
Result of limited fecal analysis: data are expressed as Mean ± SE ($n = 6$).

Groups	Amount of urea mg/dl after 1st week of feeding	Amount of ammonia mg/dl after 1st week of feeding	Amount of urea mg/dl after 2nd week of feeding	Amount of ammonia mg/dl after 2nd week of feeding	Amount of urea mg/dl after 3rd week of feeding	Amount of ammonia mg/dl after 3rd week of feeding
Group NC: control	0.33 ± 0.09	1.6 ± 0.11	0.31 ± 0.11	1.8 ± 0.01	0.29 ± 0.12	1.7 ± 0.1
Group VC: vehicle control	0.43 ± 0.12	1.8 ± 0.11	0.37 ± 0.02	1.6 ± 0.5	0.39 ± 0.21	1.4 ± 0.09
Group U: acetaminophen receiving control	2.33 ± 0.3	1.6 ± 0.3	1.31 ± 0.05	1.8 ± 1.1	1.29 ± 0.3	1.7 ± 1.1
Group UP: Sp treatment followed by acetaminophen receiving	3.3 ± 1.1	2.6 ± 0.91	2.71 ± 0.6	2.8 ± 6.1	1.2 ± 0.5	2.27 ± 0.9

sensitive to decreased blood flow. Thus the innermost structures of the kidney, called the renal papillae, are especially dependent on prostaglandin synthesis to maintain adequate blood flow. Inhibition of cyclooxygenases therefore rather selectively damages the renal papillae, increasing the risk of renal papillary necrosis.[22] Most healthy kidneys contain enough physiologic reserve to compensate for this NSAID-induced decrease in blood flow. However, those subjected to additional injury from phenacetin or paracetamol may progress to analgesic nephropathy. Treatment with *S. pasteurii*, (UP) however, reduced these alterations. Different antioxidant compounds such as taurine treatment, effectively lessened APAP-induced DNA damage and tubular degeneration.[23]

In the present study, acetaminophen overdose significantly decreased the Hb levels and also lowered total RBC count in group U (the acetaminophen treated control group), compared to group NC. But in group UP, significantly higher levels of hemoglobin and RBC are observed, compared to group U and the values resettled to the control group. In group VC, levels of hemoglobin has increased significantly after receiving *S. pasteurii* for 3 weeks. Decrease in hemoglobin is supposed due to destruction on RBC and lower total RBC count due to hemolytic anemia or suppressive inflammation. Increase level of RBC and Hemoglobin also indicate that the tested bacteria remove uremia without causing toxicity and hampering physiological condition of experimental animals. However this study shows that the *S. pasteurii* could reverse the hematologic effect of acetaminophen, with ensuing improvement of hematopoiesis. The fundamental function that blood cells perform and hemoglobin together the susceptibility to xenobiotics and makes the hematopoietic system unique as a target organ.[34,35]

Ammonia present in stool samples collected from group VC and UP from every 7 days of interval has increased slightly than APAP treated group and control group. It is supposed to that *S. pasteurii* degrade more urea in intestinal tract and end product of urea such as ammonia pass through fecal matter and maximum of ammonia is utilized by bacteria as sole nitrogen source. Thus more amount of urea defused in intestinal tract and presser on kidney is minimized by passing of urea end product by gut. Blood urea level is decreased in the group UP. These results support the previous study and the concept of "enteric dialysis", an alternative strategy for solute extraction in kidney failure is based on the fact that the intestinal wall functions as a semipermeable membrane. Driven by concentration gradient, solutes with elevated concentration in circulating blood diffuse from plasma into the lumen and a large portion of uremic solutes are differentially distributed throughout the bowel.[3,4] Formation of any diarrheal condition is not occurring in experimental condition after feeding of *S. pasteurii*.

5. Conclusion

The present study demonstrates that *S. pasteurii* supplementation has both beneficial probiotic characteristic and curative role against acetaminophen-induced nephrotoxicity, probably through its antioxidant and through enteric dialysis by producing urease. *S. pasteurii* might, therefore, be considered as a potential safe therapeutic agent against renal injury caused by acetaminophen-overdose.

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Statement of authorship

Arpita Mandal (M.Sc. in Microbiology) is the INSPIRE fellow (DST, New Delhi, India) and pursuing doctoral work in the Department of Microbiology, Raja N. L Khan Women's College, India. She is the principal researcher in the field of probiotic and kidney disease. She carried out probiotic characterization, safety assessment and animal experiments. She did as well as prepared the primary manuscript.

Shreya Mandal (M.Sc in Nutrition) is the Junior Research Fellow (UGC, New Delhi, India) and pursuing doctoral work in the Department of Nutrition, Raja N. L Khan Women's College, India. She carried out the histological slide during this investigation.

Suchismita Roy (M.Sc in Nutrition) is the INSPIRE fellow (DST, New Delhi, India) and pursuing doctoral work in the Department of Nutrition, Raja N. L Khan Women's College, India. She participates in food formulation preparation and rat scarification during this investigation.

Arpita Patra (M.Sc. in Microbiology) is lecturer of Department of Microbiology, Raja N. L Khan Women's College, India. She performed the DNA fragmentation assay during this investigation.

Tanmay Paul (M.Sc. in Microbiology) is the Junior Research Fellow (UGC, New Delhi, India) and pursuing doctoral work in the Department of Microbiology, Vidyasagar University, India. He assisted during artwork preparation and taking picture of histology and DNA fragmentation.

Koushik Das (M.Sc in Physiology) is the Senior Research Fellow (UGC, New Delhi, India) and pursuing doctoral work in the Department of Nutrition, Raja N. L Khan Women's College, India. He carried out the total grammatical check of manuscript.

Keshab Chandra Mondal (M.Sc., PhD) is the Associate Professor and presently is the Head of Microbiology Department, Vidyasagar University, India. He is the expert in the field of industrial biotechnology and microbial ecology. He participates in the manuscript check.

Dilip Kumar Nandi (M.Sc., PhD) is an eminent teacher and scientist in the field of physiology and nutrition. Currently he is the Associate Professor of Department of Microbiology, Physiology and Nutrition, Raja N. L Khan Women's College, India. For more than 20

years, he is devoted with kidney disease and nutritional aspects basically probiotic and prebiotic application.

Conflict of interest statement

All the authors have no conflict of interest in publishing the paper in this journal.

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