Chapter 6

Evaluating the efficacy of probiotic *Escherichia coli* Nissle 1917 strain containing As(III) S-adenosylmethio--nine (SAM) methlyltransferase (*arsM*) and pyrroloquinoline quinone (*pqq)* gene cluster in amelioration of arsenic induced toxicity in rats.

6.1. Introduction

In Chapter1, section 1.7 and 1.10 dealt in detail about the toxicity of Arsenic (As) and the role of antioxidants and chelating agents in preventing the toxic effects. Additionally, bacterial methylation of arsenite have been found to be very effective against arsenite toxicity. Chelating agents such as sodium 2,3-dimercaptopropane-1-propanesulfonic acid (DMPS) and meso-2,3 dimercaptosuccinic acid (DMSA) are ineffective against As toxicity with several side effects associated with use of synthetic agents (Flora, 2002). However, citric and 2-ketogluconic acids are poor chelating agents due to negative charge of arsenate and arsenite. Alternately, detoxification of As involves the biotransformation of As to methylated As species by microorganisms using *arsM* genes encodes for As(III) S-adenosylmethionine (SAM) methlyltransferases (ArsM), eventually forming volatile nontoxic trimethylarsine gas as end product (Chen et al., 2013). Hence, the objective of this study is to genetically modify probiotic *Ec*N-20 producing PQQ to synthesize As(III) S-adenosylmethionine (SAM) methlyltransferases (ArsM) and to determine its potential in the detoxification of the hazardous As metal in rat diet. The current hypothesis summarized in **Fig. 6.1**.

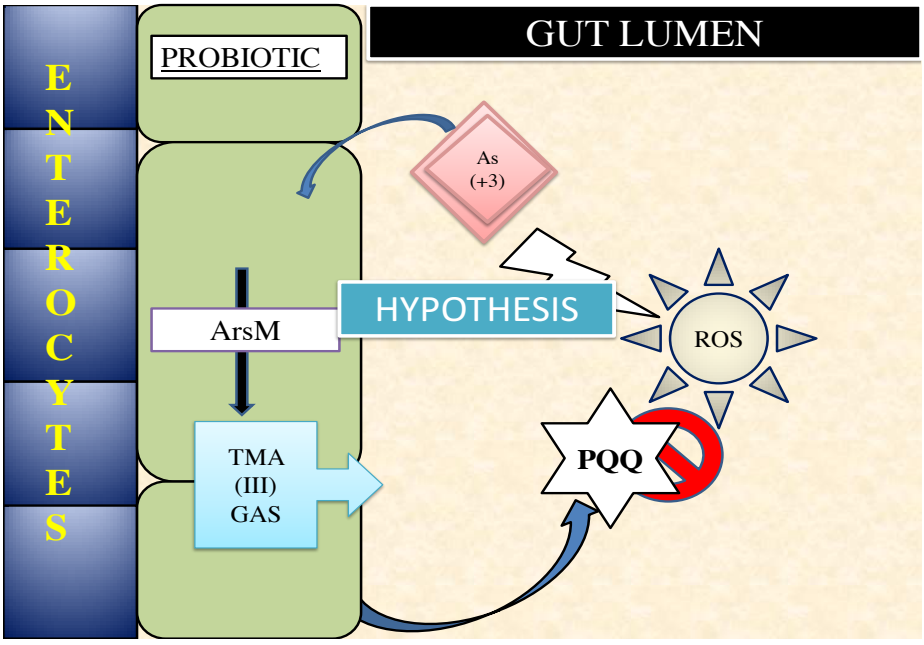


Fig. 6.1. Proposed mechanism for the detoxification of As using *Ec*N-20 and *Ec*N-24

6.2. Methods and materials

6.2.1. Animals

Free access to food and water was given to male adult Charles foster albino rats (weight 250–300g) and were maintained at photoperiod cycle (12h light: 12h dark), relative humidity (45.5%), controlled temperature (25±1ºC), according to the Committee for the purpose of control and supervision of experiments on animals (CPCSEA) guidelines of Animal Ethical Committee (M. S. University of Baroda, India, **Reg. No. 938/A/06/CPCSEA).**

6.2.2. Cloning

Forward primer F1 and reverse primer R1 were used to amplify constitutive *tac* promoter (p*tac*) from pMALp2 (**Table 6.1 and 6.2**). Forward primer F2 and reverse primer R2 were used to amplify 3.3kb *pqq* gene cluster from the genome of *Gluconobacter oxydans*. p*tac* and *pqq* gene cluster amplicons with a common overlapping regions of 24 bps were amplified using recombinant PCR with F1 and R2 primers to generate p*tac-pqq* amplicon of 3.4 kb. pRN1 was generated by cloning p*tac-pqq* amplicon into pJET. *arsM* gene of 852bp was amplified from genome of *Rhodopseudomonas palustris* using forward primer F4 and reverse primer R4. Forward primer F3 and reverse primer R3 were used to amplify constitutive p*tac* from pMALp2. Amplicons of *ptac* and *arsM* with overlapping regions of 25 bps were amplified using recombinant PCR with forward primer F3 and reverse primer R4 to obtain p*tac-arsM* amplicons of 952bp, which were then cloned in pJET to get pRN4 plasmid (**Fig. 6.2**). p*tac-arsM* was amplified from pRN4 plasmid using F3 and R4 primers, after polynucleotide kinase treatment, it was ligated with XbaI digested linear pRN1 plasmid to get pRN5 plasmid (**Fig. 6.3**). *Ec*N-20 was obtained by transforming pRN1 into *Ec*N-2 while *Ec*N-24 by transforming pRN5 into *Ec*N-2. *Ec*N was obtained as a generous gift from Dr. rer. nat. Ulrich Sonnenborn, Ardeypharm GmbH, Loerfeldstrabe 20, Herdecke (Germany). *Ec*N-2 was obtained by genomic integration of *vgb* and *gfp* genes in *Ec*N (Singh et al., 2014).

Genes		
***tac* promoter**	**F1 (forward)**	CCCTCGAGGGTTGACAATTAATCATCGGCTCGTATAATGGATCG AAT TGT GAG
	R1(reverse) (overlapping region of pqq gene cluster)	CCA GGCCAT AAT CTA TGG TCC TTG TTG GTG AAG TG
	F3(forward)	TTGACAATTA ATC ATC GGC TCGTATAATG GATCG AAT TGT GAG
	R3 (reverse) (overlapping region of arsM)	CAGTGGGCAT AAT CTA TGG TCC TTG TTG GTG AAG TG
***pqq* (pyrroloquinoline quinone)**	**F2 (forward)**	CAAGGA CCATAGATT ATG GCC TGG AAC ACA CCG A
	R2 (reverse)	CC CTCGAG GG TTA CGT ATA ACG CCT GTA GAA CAA CGT GC
***arsM* (As(III)S-adenosylmethionine methlyltransferases)**	**F4 (forward)**	CAA GGA CCA TAG ATT ATG CCC ACT G AC ATG CAA GAC GTG AA
	R4(reverse)	TCA CCC GCA GCA GCG CG

Table 6.1. Primers

Plasmids/ Strain	Characteristics	References
pMALp2	P*tac*, *malE*, Amp^r	NEB
pJET	pJET1.2/blunt is a linearized cloning vector, Recircularized pJET expresses a lethal restriction enzyme after transformation and is not propagated, Amp^r	Thermo Scientific CloneJET PCR Cloning Kit #K1231, #K1232
pRN1	pJET harboring *Gluconobacter oxydens pqq* gene cluster (3.3 Kb) under *tac* promoter, Amp^r	This study
pRN4	pJET harboring *arsM* gene from *Rhodopseudomonas palustris* under *tac* promoter, Amp^r	This study
pRN5	pJET harboring Gluconobacter oxydens *pqq* gene cluster under *tac* promoter and *arsM* gene from *Rhodopseudomonas palustris* under *tac* promoter	This study
***Ec*N**	*Escherichia coli* Nissle 1917 (*Ec*N) (Probiotic strain)	Sonnenborn et al., 2009
***Ec*N-2**	*Ec*N strain with genomic integration of *vgb* and *gfp* genes	Singh et al., 2014
***Ec*N-20**	*Ec*N-2 harbouring pJET-*tac-pqq* producing PQQ	This study
***Ec*N-24**	*Ec*N-2 harbouring pJET-*tac-pqq-tac-arsM* producing PQQ and As (III)S-adenosylmethionine methlyltransferase	This study

Table 6. 2. Plasmids and bacterial strains

6.2.3. Characterization of *Ec*N transformants

*Ec*N-20 and *Ec*N-24 transformants were confirmed by activity of PQQ dependent glucose dehydrogenase resulted into appearance of red colour due to gluconic acid secretion indicated pH drop by growing in Tris-buffered medium with methyl orange as pH indicator (Gyaneshwar et al., 1999). PQQ extraction from *Ec*N transformants was performed according to Suzuki et al. (1990) and quantified using Hitachi fluorescence spectrophotometer (Hitachi High-Technologies Corporation, Tokyo, Japan) with excitation 375 nm and emission 465 nm. 20% liver tissue homogenate and 10% colonic content homogenate in phosphate buffer saline was used for PQQ extraction and quantification. ArsM activity of *Ec*N-24 was confirmed with increased tolerance to As as compared to *Ec*N-2 by growing in luria broth.

6.2.4. Bacterial strains and culture conditions

Probiotic strains including *Ec*N-2, *Ec*N-20, *Ec*N-24 were grown in luria broth overnight at 37°C. Then reinoculated in fresh medium to achieve colony forming unit (CFU) of 10^9 cells/ml culture. One ml of the culture was pellet down, washed twice with saline, redissolved into saline and eventually tube fed to rats.

6.2.5. Experimental design

To determine the effect of *Ec*N-20 and *Ec*N-24 on As exposure, rats were divided into 5 groups (6 rats in each group): Control, As, As+*Ec*N-2, As+*Ec*N-20, As+*Ec*N-24. Probiotic treatment was given for 3 consecutive days following streptomycin wash, thereafter, colonization was confirmed after 7 days of treatment by fecal count. Afterwards, 25 ppm As was given in drinking water for one month followed by further probiotic treatments which were given once per week for one month.

6.2.6. Preparation of tissue homogenates

Similar as described in section 2.2.6 of chapter 2.

6.2.7. Biochemical assays

Catalase activity was determined by protocol of Beers and Sizer (1952). Superoxide dismutase activity was determined by the method of Marklund and Marklund (1974). Reduced GSH and GPx activity was determined by the method of Beutler et al. (1963). Lipid peroxidation was measured according to method described by Buege and Aust (1978). NO levels were determined by method of Green et al. (1982). ROS estimation was done by method of Socci et al. (1999). Blood free fatty acids (FFAs) were determined by the method of Lauwerys (1969).

6.2.8. ALT, AST, ALP, urea, creatinine and blood lipid estimaton

ALT (alanine aminotransferase), AST (aspartate aminotransferase), ALP (alkaline phosphatase), urea, creatinine as well as triglycerides (TG), HDL cholesterol, total cholesterol (TC) in serum were measured using kit as per manufacturer protocol (Beacon Diagnostics Pvt. Ltd., Navsari, Gujarat, India).

6.2.9. Histopathological changes

Similar as described in section 2.2.9 of chapter 2.

6.2.10. Metal determination

Estimation of As was done from colonic contents by Atomic Absorption Spectroscopy in accordance with the protocol described in Salinska et al., 2012. The content of As was expressed in mg/g of rat faeces.

6.2.11. Statistical analysis

Similar as described in section 2.2.11 of chapter 2.

6.3. Results

6.3.1. Cloning and characterization of *Ec*N-2 transformants

pRN4 and pRN5 were confirmed by PCR of *arsM* using primers F4 and R4 to give amplicons of 852bp (**Fig. 6.2; 6.3**). *Ec*N-20 and *Ec*N-24 transformants produced 5.6±0.12 μg and 5.2±0.24 μg PQQ/ml of culture respectively after 24 h in M9 minimal medium at 37°C with glucose as carbon source. Both transformants acidified Tris-buffered medium with methyl red as indicator which is due to secretion of gluconic acid by PQQ dependent glucose dehydrogenase. As compared to *Ec*N-2 tolerance towards As was increased to 2-folds for *Ec*N-24.

6.3.2. Effect of *Ec*N-2 transformants against As induced liver and kidney damage in rats

As treatment for one month decreased the body weight, Hb, GSH, albumin levels, Catalase, SOD, GPx activities while increased the lipid peroxidation, ROS, NO levels significantly in liver and kidney compared to control (**Table 6.3, Fig. 6.4 and 6.5**). AST, ALT, ALP activities, urea and creatinine levels were also significantly increased in serum compared to control while *Ec*N-2 treatment had no effect. However, *Ec*N-24 was more effective against As exposure, in increasing the body weight, Hb, GSH, albumin levels, Catalase, SOD, GPx activities; in decreasing the lipid peroxidation, ROS, NO levels in liver and kidney; and in decreasing AST, ALT, ALP activities, creatinine, urea levels in serum compared to *Ec*N-20.

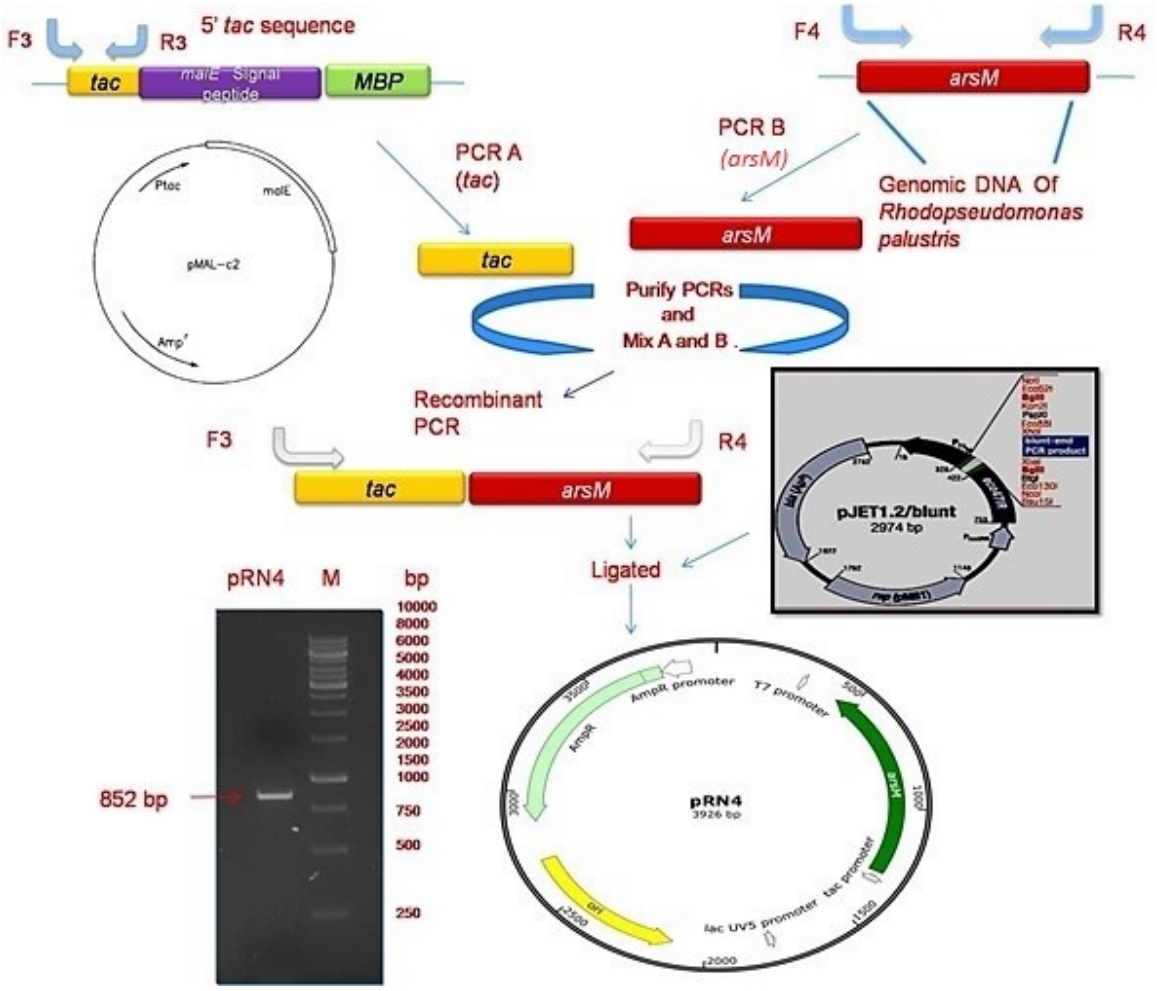


Fig. 6.2. Cloning strategy for construction of pRN4 and its confirmation by PCR.

In liver mRNA levels of Metallothionein (Mt) and iNOS were significantly increased in group treated with As compared to control. Treatment with *Ec*N-24 significantly decreased the Mt levels compared to As treated group (**Fig. 6.6**). Mt levels were also found to be increased in group treated with *Ec*N-20 as compared to control showing PQQ mediated increase in Mt levels. *Ec*N-24 treatment also significantly decreased the mRNA levels of iNOS compared to As treated group in liver.

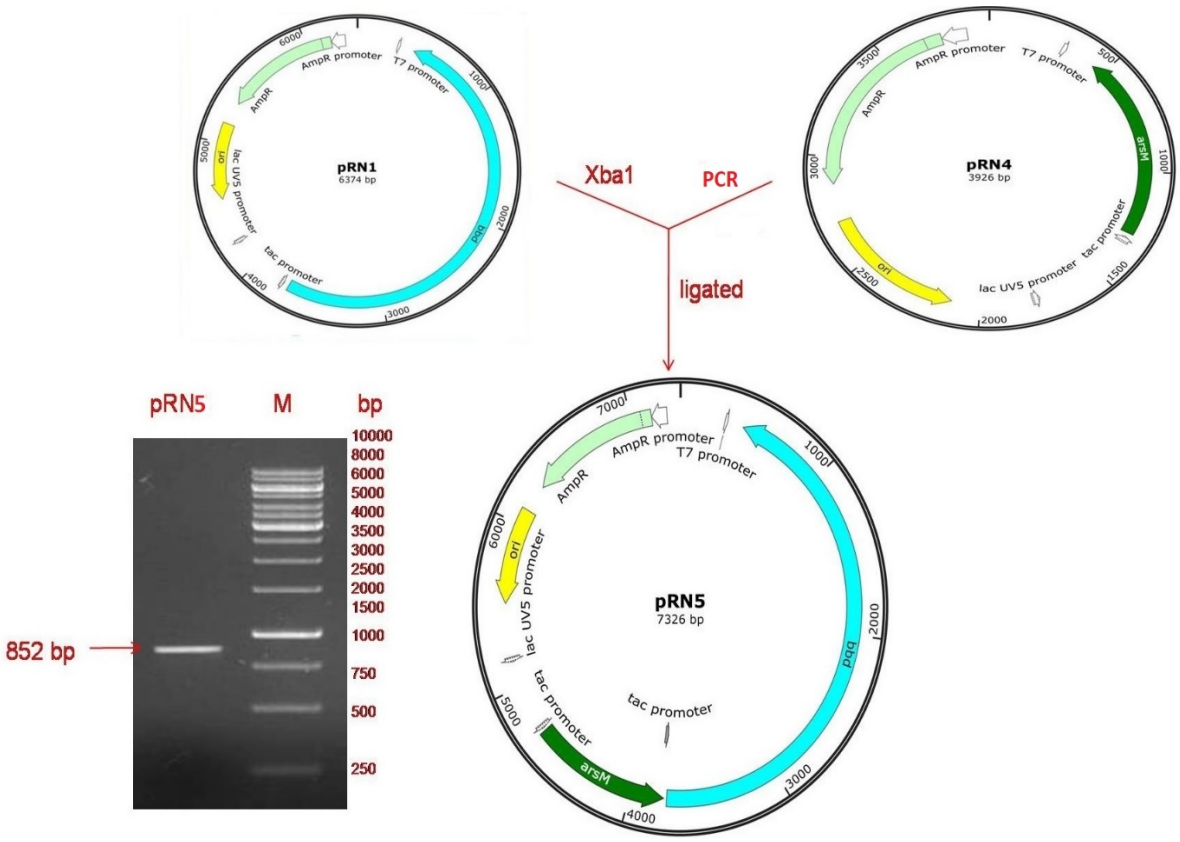


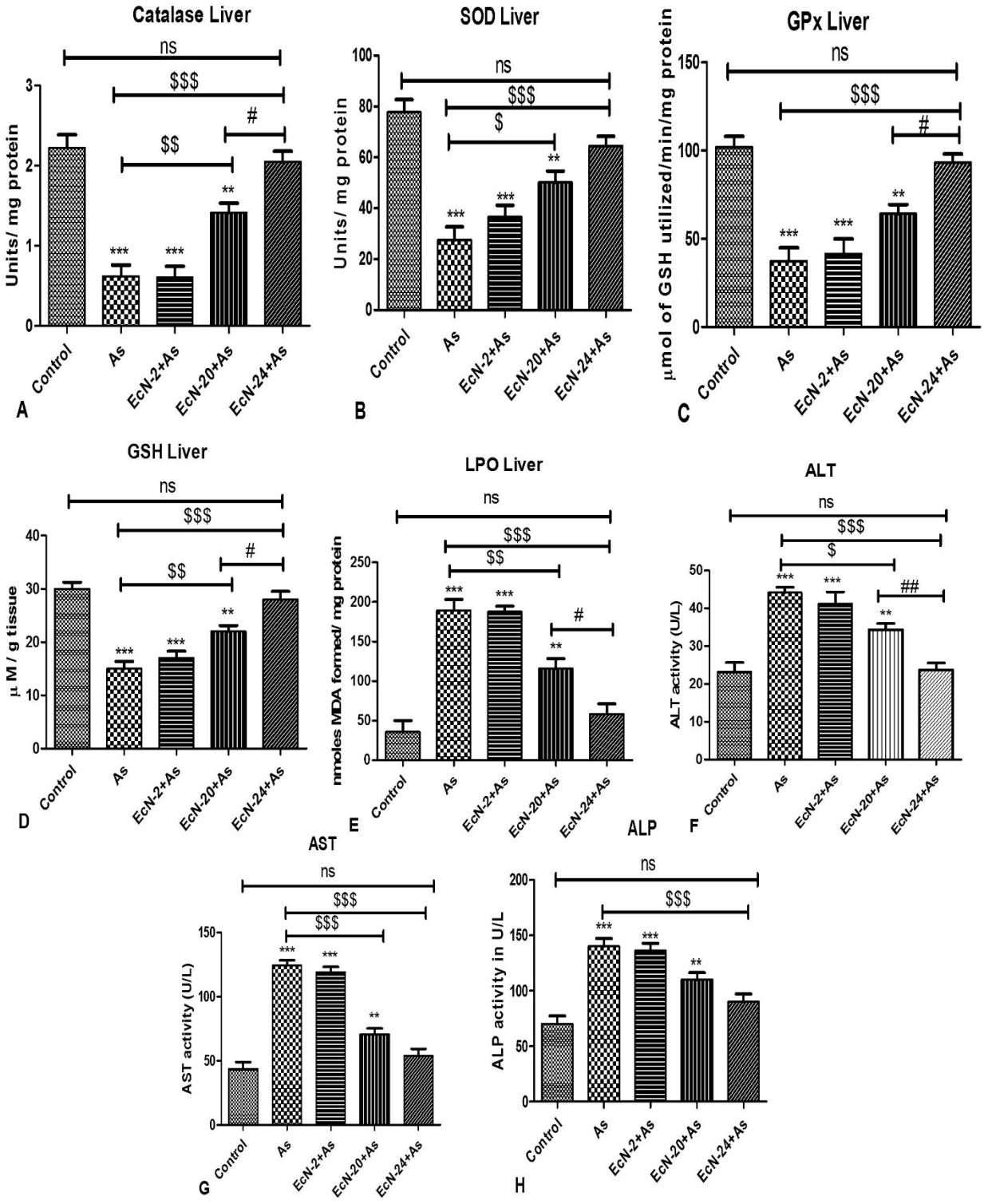
Fig. 6.3. Cloning strategy for construction of pRN5 and its confirmation by PCR.

6.3.3. Effect of *Ec*N-2 transformants against As induced dislipidemia in rats

Serum total cholesterol (TC), triglycerides (TG), free fatty acid (FFA) levels were increased and HDL-cholesterol was decreased significantly in As treated group as compared to control while *Ec*N-2 had no effect. *Ec*N-24 was found to be more effective as compared to *Ec*N-20 in bringing the levels near to control (**Table 6.3**).

6.3.4. PQQ Quantification from faeces and liver

Levels of PQQ were found to be 4.32±0.23, 4.53±0.28 nmoles/g wet wt of feces and 242.32±7.3, 255.62±6.7 picomoles/g liver tissue in groups treated with *Ec*N-20 and *Ec*N-24, respectively, which are significantly higher as compared to control (**Table 6.4**).


Catalase Liver
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EcN-20+As
EcN-24+As
SOD Liver
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GPx Liver
µmol of GSH utilized/min/mg protein
C
GSH Liver
µ M / g tissue
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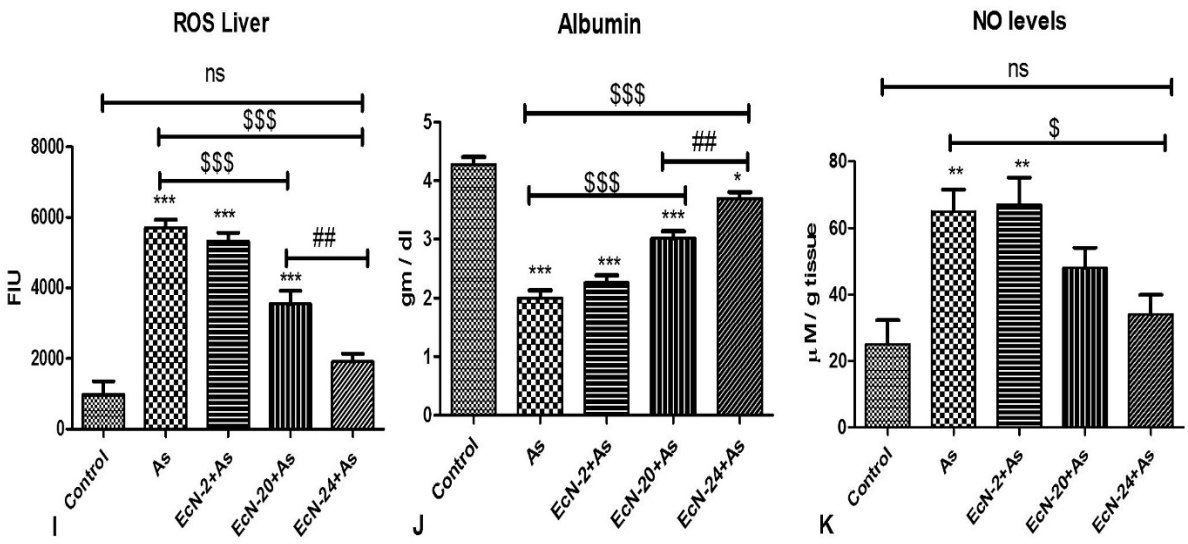
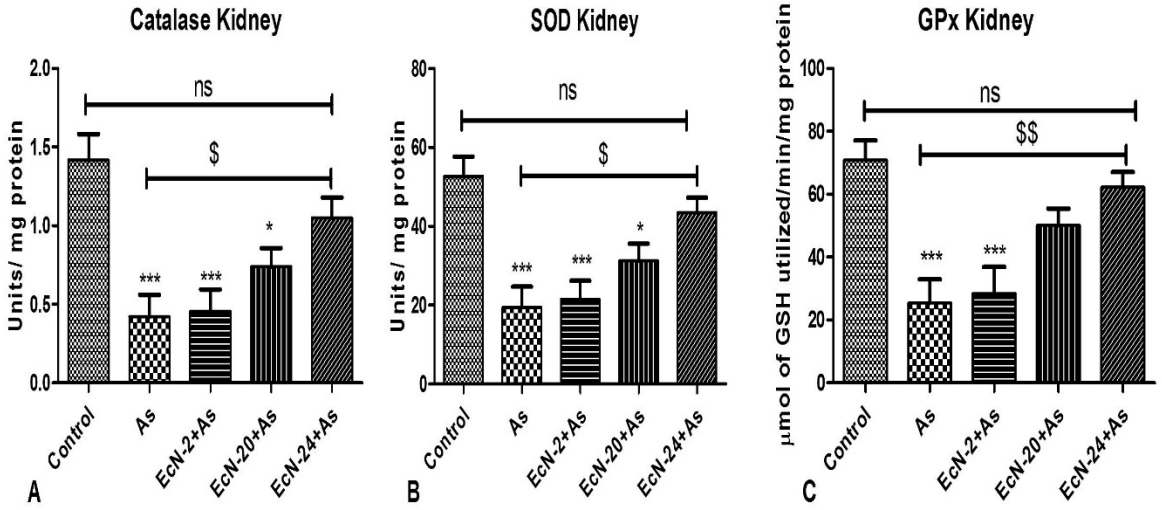


Fig. 6.4. Effect of genetically engineered probiotic *E. coli* Nissle 1917 on As exposure in liver: (A) Catalase, (B) SOD, (C) GPx activity, (D) GSH levels, (E) Lipid peroxidation, (F) ALT, (G) AST, (H) ALP activity in serum, (I) ROS levels in liver, (J) Albumin levels in serum, (K) NO levels in liver. Values are expressed as mean ± SEM (n=6 each group). * P ≤ 0.05, ** P ≤ 0.01,*P ≤ 0.001 compared to control. $^{\$}$P ≤ 0.01, $^{\$\$}$P ≤ 0.05 $^{\$\$\$}$P ≤ 0.001 compared to As treated group. $^{\#}$P ≤ 0.01, $^{\#\#}$P ≤ 0.05 $^{\#\#\#}$P ≤ 0.001 compared to *Ec*N-20+As group.**



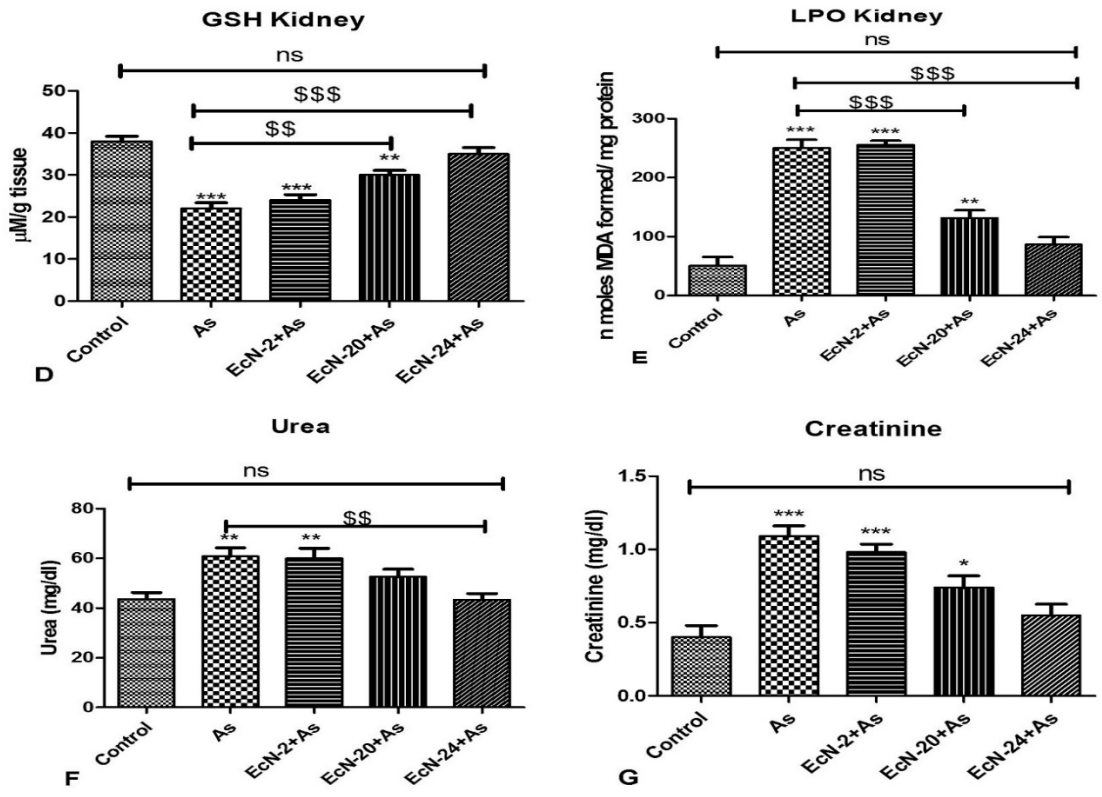


Fig. 6. 5. Effect of genetically engineered probiotic *E. coli* Nissle 1917 on As exposure in kidney: (A) Catalase, (B) SOD, (C) GPx activity, (D) GSH levels, (E) Lipid peroxidation; (F) Urea, (G) Creatinine levels in serum. Values are expressed as mean ± SEM (n=6 each group). * P ≤ 0.05, ** P ≤ 0.01,*P ≤ 0.001 compared to control. $^{\$}$P ≤ 0.01, $^{\$\$}$P ≤ 0.05 $^{\$\$\$}$P ≤ 0.001 compared to As treated group.**

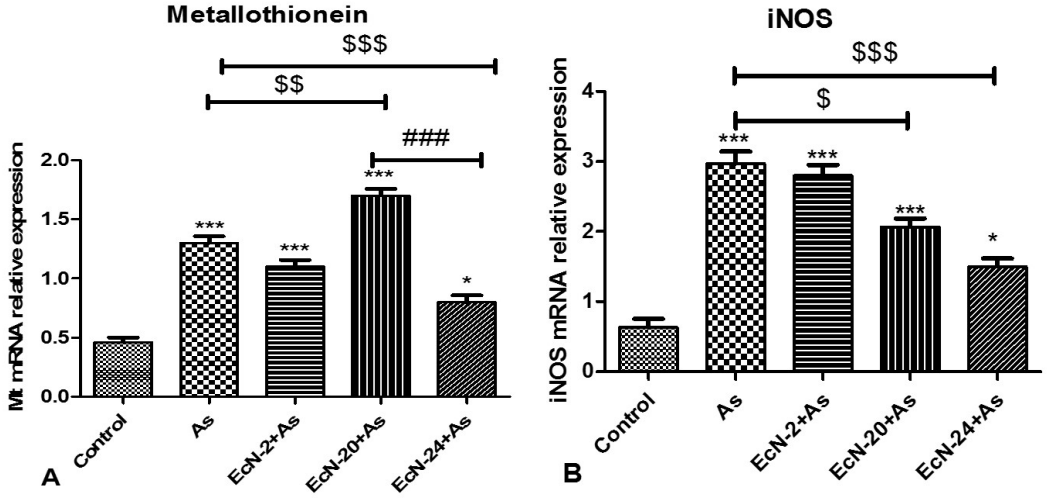


Fig. 6.6. Effect of genetically engineered probiotic *E. coli* Nissle 1917 on mRNA expression levels of: (A) Metallothionein, (B) iNOS in liver on As exposure. Values are expressed as mean ± SEM (n=6 each group). * P ≤ 0.05,*P ≤ 0.001 compared to control. $^{\$}$P ≤ 0.01, $^{\$\$}$P ≤ 0.05, $^{\$\$\$}$P ≤ 0.001 compared to As treated group. $^{\#\#\#}$P ≤ 0.001 compared to *Ec*N-20+As group.**

Groups	Control	As	*Ec*N-2	*Ec*N-20	*Ec*N-24
Total cholestrol (mg/dl)	43.21±3.17a***	65.42±2.63	63.61±2.72	57.42±2.31	51.87±2.73 a*
Triglycerides (mg/dl)	30.45±2.48a***	55.26±1.45	53.96±3.17	44.52±1.88a*	36.33±2.53a***
HDL (mg/dl)	31.48±1.11a***	20.91±1.38	21.46±1.42	24.97±1.93	27.48±1.85
Free fatty acids (mg/dl)	19.42±1.76a**	30.88±2.16	29.62±2.24	24.78±1.83	21.58±1.61a*
Body weight change (g)	64.32±4.04	32.34±5.31 a**	35.17±4.32 a**	42.29 ±6.21a*	53.25 ±4.63
Hb (g/dl)	15.17±0.33	12.37±0.21	12.15±0.36	13.64±0.38	14.53±0.24b*
Liver (μg/mg)	nd	5.2±0.25	5.0±0.29	4.8 ±.31	3.5 ±0.21c**

a*$p \leq 0.05$, a$p \leq 0.01$, a***$p \leq 0.001$** compared to As

a*$p \leq 0.01$, a$p \leq 0.05$** compared to Control , **b*$p \leq 0.01$** compared to As; **c**$p \leq 0.05$** compared to As, *Ec*N-2, *Ec*N-20

Table 6.3. Serum lipid profile, body weight change, haemoglobin (Hb) levels and As concentration in liver.

Groups	Control	As	*Ec*N-2+As	*Ec*N-20+As	*Ec*N-24+As
Fecal (n moles/ g fecal wet weight)	0.724±0.15	0.711±0.17	0.732±0.19	4.32±0.23***	4.53±0.28***
Liver (picomoles/ g tissue)	27.43±8.3	26.12±5.2	29.64±4.7	242.32±7.3***	255.62±6.7***
Acetate	100.37±3.5	74.48±2.7 a***	78.62 ±3.8 a***	91.53 ±2.4b**	103.41±3.4b***
Propionate	18.31±1.8	11.66±2.1	12.53±1.6	24.17±2.2b***	30.68±1.4a***b***
Butyrate	11.04±1.2	6.17±1.4a*	7.19±0.8	15.87±0.9a*b***	22.51±1.1a***b***

***$p \leq 0.05$** compared to Group Control, As, *Ec*N-2+As

a*$p \leq 0.05$, a*$p \leq 0.001$** compared to Control. **b*$p \leq 0.05$, b**$p \leq 0.01$, b***$p \leq 0.001$** compared to As**.** Values are expressed as μmoles/g colonic content. Values are mean±SEM (6 rats each group).

Table 6.4. PQQ concentration in fecal matter and liver homogenate; Short chain fatty acids (SCFAs) concentration in colonic matter.

6.3.5. SCHAs quantification from faeces

Short chain fatty acids (SCFAs) were significantly decreased in As treated groups as compared to control while the *Ec*N-20 and *Ec*N-24 treatment increased the levels of acetate, propionate and butyrate near to normal. *Ec*N-24 was more effective as compared to *Ec*N-20 in increasing SCFAs levels, however, *Ec*N-2 had no effect (**Table 6.4**).

6.3.6. As levels

As was estimated from liver by Atomic Absorption Spectroscopy. As level was undetected in control while it is significantly decreased in group treated with *Ec*N-24 compared to other As treated groups (**Table 6.3**).

6.3.7. Histological analysis

Histological analysis showed that the control group had normal architecture while As treatment has induced the pathological changes in liver and kidney (**Fig. 6.7 and 6.8**). As exposure caused necrosis, inflammatory cell infiltration, derangement of hepatic cords while in kidney, it leads to necrotic renal tubules, distention of Bowmen's cavity, shrinkage and reducing size of glomeruli. *Ec*N-24 treatment resulted in the recovery of histopathological damage in liver and kidney.

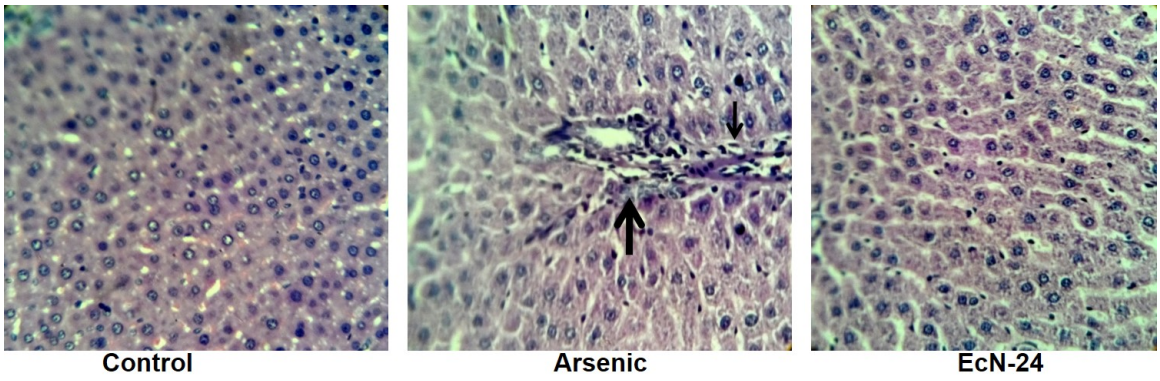


Fig. 6.7. Photomicrograph of liver stained with HE (Magnification=40x). Control showing normal liver architecture, Arsenic treated group showing inflammatory cell infiltration (black arrow), necrosis (bold arrow), *Ec*N-24 treatment showing near to normal appearance of liver.

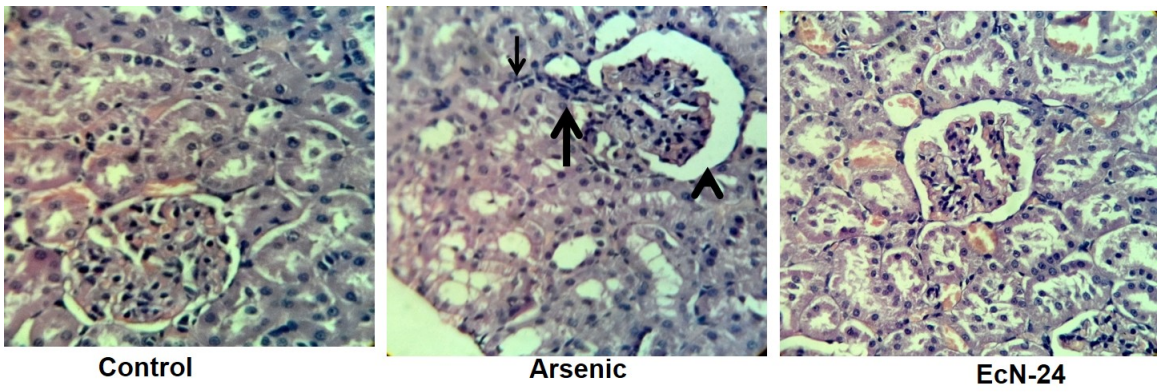


Fig. 6.8. Photomicrograph of kidney stained with HE (Magnification=40x). Control showing normal kidney architecture, Arsenic treated group showing inflammatory cell infiltration (black arrow), necrosis (bold arrow), shrinkage of glomeruli and distention of bowman's capsule (arrow head), *Ec*N-24 treatment showing near to normal appearance of kidney.

6.4. Discussion and conclusion

Mitochondrial membrane potential destabilized by As and can also induce the morphological changes in mitochondrial integrity thus initiating cascade of reactions for free radical formation (Jomova et al., 2011). H_2O_2 generated during oxidation of As(III) to As(V) can generate highly reactive hydroxyl radicals by Fenton reaction. Another mechanism for generating oxidative stress by As is by reducing the levels of antioxidants like GSH, possibly through utilizing it as a donor for reducing pentavalent forms of As to trivalent As forms or by oxidizing GSH or either due to high affinity of As towards GSH (Flora et al., 2008). In the present study, significantly higher levels of ROS, lipid peroxidation, and decrease in levels of GSH were observed on As treatment.

As inhibits more than 200 enzymes by forming complexes and has higher affinity for thiol groups in proteins (Roy and Saha, 2002). Activity of antioxidant enzymes such as SOD, CAT, GPx and GR in liver and kidney was reported to be decreased in previous studies (Mershiba et al., 2013; Gupta and Flora, 2005). Additionally, As by forming complexes with the sulphydryl groups, decreases the blood haemoglobin (Hb) (Lu et al., 2004). In present study also, decrease in levels of antioxidant enzymes as well as in Hb levels were observed. Furthermore, along with reactive oxygen species (ROS) induction, As can also produce reactive nitrogen species (RNS). In rat liver, As treatment causes the

increase in NO levels might be due to activation of endogenous NO synthase, hence increase in NO levels and iNOS expression were seen in rats.

The increased levels of AST, ALT and ALP enzymes are the biomarkers for the hepatic injury (Rahman et al., 2000). In serum, As mediated increase in these marker enzymes is due to impairment of functional integrity of hepatic membrane (Renugadevi and Prabu, 2010; Albores et al., 1989). In current study, hepatic damage is illustrated by increase in levels of AST, ALT, ALP in serum on As treatment.

In kidney, serum urea and creatinine levels were increased by As due to tubular degeneration, changes in glomerular and interstitial infiltration (Sankar et al., 2016; Sener et al., 2016). Therefore, in As treated groups, increased levels of urea and creatinine were observed. Liver tissues degeneration and alterations in renal tubules caused by As are corroborated with previous studies of liver and kidney histopathological damage (Waalkes et al., 2000).

The growth and survival of probiotic *E. coli* CFR16 in GI tract was enhanced by *vgb* gene encoding for *Vitreoscilla* haemoglobin (VHb) and protected against carbon tetrachloride induced oxidative damage due to its peroxidase activity (Kumar et al., 2014). In the present study, *Ec*N-2 producing VHb did not exhibit protection against As toxicity due to the lower copies of *vgb* gene as it is present in the genome while this gene was present in multicopy plasmid form in *E. coli* CFR16.

*Ec*N producing PQQ was more effective against rotenone, alcohol, dimethyl hydrazine (DMH) induced oxidative damage and the altered neurotransmitter levels induced by DMH as compared to orally given PQQ (Singh et al., 2014; Singh et al., 2015; Pandey et al., 2015). Likewise, beneficial effects of PQQ were found in the present study with *Ec*N-20 and *Ec*N-24 against As induced oxidative damage as compared to *Ec*N-2.

Glucose converted to gluconic acid by *Ec*N producing PQQ mediated by activity of glucose dehydrogense utilizing PQQ as a cofactor. Gluconic acid known to be utilized by beneficial gut microbiota resulting into formation of SCFAs (Kameue et al., 2004), which are known for their anti-inflammatory effects (Cox et al., 2009), therefore could exhibit

protection against pro-inflammatory effects of As. This is also supported by remarkably higher levels of SCFAs such as butyric, propionic and acetic acid in the feacal matter of the rats treated with *Ec*N producing PQQ (Singh et al., 2014). Similar effect of increased SCFAs was observed in *Ec*N-20 and *Ec*N-24 treated rats.

As intoxication also leads to dislipidemia which includes increase in plasma triglycerides, total cholesterol, free fatty acids levels and decrease in HDL cholesterol levels, food intake and body weight gain (Afolabi et al., 2015; Miltonprabu and Sumedha, 2015). In As exposed animals enhanced cholesterogenesis was reported due to increase in activity of 3-hydroxy-3-methylglutaryl Coenzyme A (HMG CoA) reductase which is the rate limiting enzyme in cholesterol synthesis leading to excessive accumulation of cholesterol. As induction also caused inhibition in the activity of cholesterol-7α-hydroxylase which is the cytochrome P450 enzyme present in the endoplasmic reticulum which functions to prevent the biosynthesis of bile acids, thereby, limits the exclusion of cholesterol from the body (Gesquiere et al., 1999; Kojima et al., 2004; Sawada et al., 2004).

As also induce hypertriglyceridemia by reducing activity of LPL (Lipoprotein lipase) involved in the catabolism of TGs releasing FFAs from VLDL and chylomicrons, thus regulating the TGs levels in circulation (Muthumani and Miltonprabu, 2015). Reduction in LPL activity causes organization of LDL, which can be aggregated by As induced LDL receptor defects in liver. Lipotoxic effects of As also involve inhibition of β-oxidation due to decrease in mitochondrial function causing increase in serum free fatty acid levels. Surplus cholesterol can be removed by HDL in presence of LCAT, where its activity was reported to reduce by As causing decrease in HDL levels. Thus, in agreement with the previous reports, increase in levels of TC, TG, FFA and decrease in HDL levels in serum were observed in As treated rats.

In mice, PQQ deficiency leads to hyperlipidemia due to direct influence of PQQ on lipid metabolism (Bauerly et al., 2011). Along with the PQQ, SCFAs are also involved in influencing the lipid metabolism such as acetate upregulates lipid oxidizing enzymes in liver (Kondo et al., 2009); fatty acid levels in liver and plasma were decreased by propionate (Sa'ad et al., 2010); butyrate regulates the cholesterol synthesis, stimulates ß-

oxidation of fatty acids and proliferation of peroxisome (Canani et al., 2011). Hence, the recovery in disturbed lipid profile was observed in groups treated with PQQ producing *Ec*N-20 and *Ec*N-24.

Heavy metal exposure induced the expression of metallothionein (Mt) which is a cysteine rich protein and detoxifies the heavy metals by binding them (Ganger et al., 2016). As mediated induction of Mt is due to its binding with the C-terminal cysteine cluster of metal regulatory transcription factor-1 (MTF-1). These findings are consistent with present study where in As treated groups significantly higher levels of Mt were measured. Mt can also be induced by antioxidant treatment (Kumar, 2012). Therefore, in *Ec*N-20 treated group higher levels of Mt was observed due to PQQ secretion. However, decrease in Mt levels were observed in *Ec*N-24 treated groups due to decrease in total bulk of As getting absorbed in gut and reaching organs.

On As intake, it gets absorbed in blood and metabolized primarily in liver, where it undergoes several reductions and oxidative methylations by Cyt19 or As3MT (in humans and rats) to form organic pentavalent As species monomethylarsonic acid (MMAV) and dimethylarsinic acid (DMAV), they are more readily excreted in urine compared to inorganic As (Gebel, 2002; Qin et al., 2006). Thus, toxic effects of inorganic As decreased by methylation by facilitating excretion. The intracellular metabolism of inorganic As in humans is conducted by As3MT which involves extensive metabolism to DMAv while till TMAO in rats (Cohen et al., 2002; Vahter, 1981). Hence, compared to the other metabolites of As biomethylation significantly higher levels of DMAv was found in human urine and of TMAO in rats. However, no significant conversion to TMA gas was observed.

For reducing the content of As in contaminated soil and water, arsM gene of bacterial origin was expressed in *P. putida* and *E. coli* to facilitate the conversion of As into methylated forms and eventually into volatile gas TMA, thus decreasing the As load in the environment (Chen et al., 2013; Qin et al., 2006). Hence, to enhance the conversion into volatile gas and excretable pentavalent methylated forms, *arsM* was constitutively expressed in probiotic *Ec*N, thereby reducing As accumulation in body.

During biomethylation of As originally which was considered as detoxification mechanism, had also known to produce reactive intermediates with cytotoxic effects like monomethylarsonous acid MMAIII and dimethylarsinous acid DMAIII (Aposhian et al., 2000; Le et al., 2000). The cytotoxicity of trivalent As species in rat bladder urothelial cells was inhibited by Vitamin C and *N*-Acetylcysteine, suggesting oxidative damage as the cytotoxic mechanism involved (Wei et al., 2004). Hence, PQQ secreted by *Ec*N-24 and *Ec*N-20 protects against oxidative stress mediated by As.

In conclusion, *Ec*N-24 producing ArsM and PQQ found to be more effective against As induced oxidative stress and dislipidemia as compared to *Ec*N-20 producing PQQ while *Ec*N-2 had no effect for protection. Since the present study is of short duration (30 days), it will be interesting to monitor the effects for longer period.