

Conclusion

The majority of epithelial surfaces of mammalian body, such as the skin and mucosa, are colonized by a vast number of microorganisms called normal microflora or the microbiota. Most of the commensal bacteria are symbiotic; however, under specific conditions, such as immunodeficiency, commensal bacteria could cause pathology. These bacteria are present at anatomical locations that provide suitable conditions for their growth and proliferation. Our microbiota represents a complex ecosystem with enormous microbial diversity. Microbiome produces an enormous amount and diversity of molecules and metabolites, which interact with the host; however, the role of the majority of these remains to be elucidated. The existence of enormous population of bacteria in the large intestine and their fundamental functions in nutrition and metabolism (fermentation of nondegradable oligosaccharides, metabolism of xenobiotics and activation or destruction of mutagenic metabolites) makes the colonic microbiota a large fermentative organ of the body. The different microbial communities (Bacteria, viruses and eukaryotes) interact with themselves and host, and alter the outcome of disease. Probiotics are a very important group of microorganisms and have been studied from a very long time. Incorporating desired characteristics in the probiotics using genetic engineering tools has raised newer opportunities to treat certain diseases or disorders where conventional native probiotics are ineffective.

We developed genetically modified probiotic *E. coli* strains Nissle 1917 capable of secreting novel antioxidant Pyrroloquinoline quinone (PQQ) which is derived from the peptide encoded by *pqqA* gene. PQQ synthesis requires several genes including substrate, enzymes, carriers and transporters. Interestingly, capacity to synthesize and secrete PQQ is limited to only few aerobic bacteria, in spite of its crucial and important role in plants and animals (Mammals). Thus, plants and animal (humans) get PQQ from these bacteria. Most importantly, microbes harbouring in the gut and other parts of humans and other mammals also do not synthesize PQQ. Therefore, humans depend upon plants for its fulfilment. PQQ, apart from strong water soluble antioxidant, is nutritionally essential molecule. Its deficiency in diet leads to various developmental related defects.

The present study demonstrates the beneficial effects of PQQ producing probiotic *E. coli* Nissle against ethanol induced metabolic disorder and age associated oxidative damage and lipid imbalance in rats. This study shows that PQQ secreting *E. coli* Nissle 1917 is effective in counteracting acute as well as chronic ethanol mediated oxidative damage in rats. Moreover, It also reduced the lipid accumulation in blood and liver. Accumulated PQQ in tissues prevents hepatic and systemic oxidative damage. PQQ along with SCFAs reduced hyperlipidemia, which can be correlated with changes in mRNA expression of hepatic lipid metabolizing genes. Our study suggests that endogenous generation of PQQ by EcN could be an effective strategy in preventing alcoholic liver disease.

PQQ is also shown to interact with various cell signaling pathway and induces mitochondrial biogenesis in mammalian cells. Results of chapter 3 and 4 demonstrates the role of genetically modified probiotic EcN-5 in counteracting oxidative stress and mitochondrial inefficiency in rotenone induced accelerated ageing and in naturally ageing rat models. Interestingly, PQQ given daily and EcN-5 given weekly have more or less similar effects. However, low frequency dose of EcN-5 makes it more efficient and cost effective. In conclusion, it prevents hepatic oxidative damage caused by increased mitochondrial ROS and promotes mitochondrial biogenesis in rotenone treated animals. It also, alleviates hepatic and colonic oxidative stress promoting hepatic mitochondriogenesis and metabolism in naturally aging animals. Additionally, it reduces blood and hepatic lipid accumulation in these animals. Therefore, it can be opted as natural therapeutic or nutritional supplement against age associated oxidative stress and dyslipidemia.

In all the animals experiments elevated levels of SCFAs (Butyrate, propionate and acetate) were observed in rats treated with PQQ or PQQ secreting probiotic EcN. However, exact mechanism was not known. Interestingly, all *E. coli* expresses apo form of membrane bound glucose dehydrogenase enzyme which utilizes PQQ as a cofactor and converts glucose in to gluconic acid. Gluconic acid is considered as prebiotic, and feeding sodium gluconate in animals increases the levels of SCFAs in mammals. It was hypothesized that probiotic *E. coli* and commensal *E. coli* both produces

gluconic acid in presence of PQQ and thus increase in colonic gluconic acid leads to increase in SCFAs, since it is metabolized by numerous bacteria in the gut. Thus, to test the hypothesis rats were fed starch rich diet along with PQQ secreting EcN, PQQ alone and gluconic acid. Interestingly, increased gluconic acid levels in the colon was found in rats treated with PQQ secreting EcN or PQQ only. This increase in gluconic acid correlates with elevated levels of butyrate and acetate in the colon. Thus, the probiotic bacteria capable of secreting PQQ in the gut has multifactorial benefits and could be potential therapeutic agent against various metabolic disorders in humans.

Genomic integrants of *pqqABCDE* gene cluster were found to be comparatively less effective because of lower production PQQ. Further studies aiming to generate stable and high expressing genomic integrants of *pqqABCDE* gene cluster could be a significant milestone in development of probiotic therapy.

Further, the strong antioxidant property of PQQ was utilized in order to study the significance of reactive oxygen species generated in the colon observed in various pathologies such as IBD and IBS. Surprisingly, it was observed that strong antioxidant did not provide any amelioration in DSS induced murine colitis model as well as in *C. rodentium* infection model. However, wild type probiotic EcN was found to be effective. Thus it was hypothesized that antioxidant (PQQ) is scavenging free radicals and probably also inhibiting the wound healing and other signals mediated by them. Since, these signals are very important for gut homeostasis especially during inflammation and injury, inhibiting them could lead to more severe pathology as observed in case of PQQ secreting EcN treated mice. More detailed and elaborate studies must be carried out in order to validate the hypothesis.

In conclusion, genetic modification of probiotic EcN is protective against oxidative damage caused by ethanol, rotenone and ageing. Also, it is ameliorative in reducing lipid accumulation. Genomic integrants are most suitable in order to achieve stable and safe expression. New strategies must be employed to develop genomic integrates capable of producing high amount of PQQ.