Summary

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Human gut is habitat for trillions of gut microbiota where they live in close association with the host. These gut microbiota are known to play many beneficial physiological role including modulation of immune response, SCFA production, vitamin synthesis, lipid and cholesterol metabolism, influence of energy supply, gut hormone, satiety and energy expenditure. Any imbalance in the microbiota composition may be attributed to many physiological conditions like obesity and metabolic syndrome, cancer progression, inflammatory bowel disease (IBD) and cardiovascular diseases. Among gut flora there are probiotic organism which when consumed in adequate amount confers health benefit. Diverse role of gut microbiota in health and disease has been of significant interest from past 2-3 decades and they are considered to be natural therapeutic agent.

Present study was focused on development of probiotic *E. coli* for alleviating dietary sugar induced metabolic syndrome. To address the problem two alternate strategies was implied, first; enhancing the antioxidant potential and second; incorporation of transforming enzyme to decrease intestinal absorption. Initial work was carried out by using our laboratory strain E. coli CFR 16 followed by well characterized and marketed human isolate *E. coli* Nissle 1917 (Mutaflor).

Tagging of probiotic *E. coli* with GFP served as powerful tool for tracking its colonization in gastrointestinal tract and incorporation of *vgb* gene significantly enhanced its colonization potential of both the strains in gastrointestinal tract. Most of the probiotics have excellent probiotic potential but major limitation is there colonization ability. As human intestine has micro-aerophillic environment incorporation of *vgb* gene in *E. coli* enhanced the colonization potential.

As mentioned in the previous chapters, PQQ is co-factor as well as heat stable and water soluble antioxidant molecule (20,000 oxidation-reduction cycles). Protective effect of PQQ as potential antioxidant was investigated in chemically induced model of 1,2-dimethylhydrazine (DMH) as against vitamin-C, a standard antioxidant. Although 1000 times less dose of PQQ was used in comparison to vitamin-C, similar beneficial effects were found in both cases. Additionally, PQQ was

able to modulate brain neurotransmitter status in contrast to vitamin-C implying additional neuro-modulatory property of PQQ.

E. coli genome encodes for glucose dehydrogenase apoprotein but lacks the ability to synthesize PQQ co-factor. Engineering EcN to produce PQQ confers dual advantage: first, as a good source for humans and decrease in the dependency on the plant sources for the supply of PQQ. *E. coli* producing PQQ has remarkable benefit on PQQ being continuously synthesized in gut; secondly, PQQ serves as co-factor for glucose dehydrogenase enabling EcN to convert glucose to gluconic acid. Gluconic acid is a prebiotic molecule which can be digested by gut microflora resident in lower part of gastrointestinal tract resulting in the production of short chain fatty acids (SCFAs).

In case of fructose induced mediated disorder in rats, administration of PQQ producing probiotic exhibited antioxidant status both in blood and liver to almost normal levels. However, lipid profile both in serum and liver exhibited 30-40 % decrease in comparison to fructose control. Apart from effect exerted by antioxidant and co-factor property of PQQ, *E. coli* Nissle alone facilitates reassembly of tight junction proteins like Occludin, ZO-1. In addition, it can also induce human β defensin-2 production via NFkβ. Delaying of metabolic syndrome in fructose fed rats seems to be combined effect of EcN and PQQ.

Sucrose is disaccharide made of glucose and fructose unit. Previous literature has demonstrated, fructose moiety of sucrose is responsible for the adverse effects associated with sucrose consumption. To address this problem, we modified EcN to synthesize Inulosucrase (sucrose transforming enzyme). Inulosucrase so produced converts sucrose to FOS (fructo-oligosaccharide), a prebiotic molecule. In comparison to EcN producing PQQ, EcN producing PQQ-InuJ was capable of producing two prebiotic molecules, Gluconic acid and FOS. In addition, it also decreases intestinal absorption of sugar by direct conversion to FOS. Rats fed with EcN producing both PQQ and InuJ was found to exhibit almost normal lipid profile and antioxidant status in both liver and blood.

Plasmid based expression system was found to alleviate these effects to significant extent. However, plasmid based system have few disadvantages, first, it increases plasmid load, second; there are chances of plasmid loss and thirdly; there is

risk of horizontal gene transfer i.e. antibiotic resistance. To circumvent these challenges all genes (*vgb-gfp-pqq-inuJ*) were integrated in the genome of *E. coli*. pGRG36 vector system was used for integration with couple of advantages, first; this results in marker free integration, second; stable expression. Tn7 mediated integration was confirmed by loss of ampicillin resistance and PCR. Animal experimentation to test these genomic integrants exhibited significant protection. However, these effects were found to be significantly lower than seen with plasmid based expression system. This observation is supported by the fact that genomic integrants express only one copy of gene in contrast plasmid based expression system express multiple copies.

In conclusion, genetic modifications have been effective in alleviating sucrose induced metabolic perturbations. Genomic integrants as of EcN are most suitable for this purpose. Since the present genomic integrants do not express genes at a level corresponding to the effective plasmid counterparts, new strategies with strong promoters needs to be designed in order to achieve similar reduction in metabolic affects.