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Chapter

Potential of *Escherichia coli* Probiotics for Improved Health and Disease Management

Nareshkumar Gattupalli and Archana Gattupalli

Abstract

Although natural gut microbiota contains *Escherichia coli* as a commensal, this bacterium, along with other members of the *Enterobacteriaceae* family, are usually known for their pathogenic potential. Interestingly, *E. coli* colonizes first and remains all through life, and in fact, some strains possess beneficial properties such as antibacterial colicin secretion. Among the beneficial strains, *E. coli* Nissle, isolated in 1917, has been the most extensively explored strain. Adaptability to survive under diverse conditions coupled with facile genetic manipulations enabled the design of *E. coli* strains with properties to deliver antioxidant, anti-inflammatory, and antitumor molecules. Moreover, genetically modified *E. coli* strains secreting enzymes for converting sucrose and fructose into insulin and mannitol, respectively, were very effective in preventing the onset of metabolic disease by acting as synbiotics. Thus, *E. coli* is emerging as a very potent probiotic platform for developing strains with the potential of controlling many metabolic and multifactorial diseases, including cancer.

Keywords: *E. coli* Nissle, probiotic, prebiotic

1. Introduction

Escherichia coli resides in the gastrointestinal (GI) tract of animals along with a few hundreds and thousands of different microbiota. In humans, *E. coli* is present at less than 1% of gut microbiota and it is not among the 25 most prevalent bacteria [1, 2]. But *E. coli* is the predominant *Enterobacteriaceae* species in humans [3]. Interestingly, *E. coli* is the first to colonize the intestines and persists all through life in humans [4]. Mucin layers do not allow any direct interaction of gut microbiota with the enterocytes. However, the diversity of gut microbiota is known to influence the intestinal permeability involving LPS, peptidoglycan, lipoproteins, deoxynucleic acid (DNA), and ribonucleic acid (RNA). Most *E. coli* strains are nonpathogenic and exist as commensals, but some pathogenic strains are associated with severe diseases [5]. Additionally, some *E. coli* strains known as pathobionts do not cause any disease in healthy individuals but exacerbate chronic inflammatory diseases [1]. The *E. coli* population in the GI tract is dynamic with a turnover in months to years [3, 6]. Humans contain five different strains of *E. coli* [7]. Oxygen diffusion from intestinal epithelium is favorable for *Enterobacteriaceae* members including *E. coli* to be present in close proximity to the mucus layer [8]. *E. coli* is known to play an important role in the maintenance by decreasing oxygen content,

E. coli Probiotic	Properties	References
Mutaflor, EcN 1917	Serotype O6:K5:H1, Motile, flagella, present, Microcin M and H47	[4, 15, 16]
Symbioflor 2	20% strain G1/2 (DSM16441), 20% G3/10 (DSM16443), 20% G4/9 (DSM 16444), 10% G5 (DSM 16445), 20% G6/7 (DSM 16446), and 10% G8 (DSM 16448). Microcin S, Non-motile	[19, 35]
Colinfant (A0 34/86)	O83:K24:H3;	[16, 20, 21]
CFR16	Colicin E1/Ia1b	[9, 22]

Table 1.
Characteristics of E. coli *probiotic strains.*

by facilitating the colonization of anaerobes and vitamin K production, and protects against colonization of pathogens. *E. coli* produces microcins and colicins, which prevents the colonization of pathogens. In contrast to antibiotics, colicins act on bacteria related to the bacteria producing these antibacterial proteins. Many *E. coli* isolates from rat fecal matter were found to produce E1-, 1a/1b-, and B/D-type colicins with antimicrobial properties against enteropathogens [9].

2. *E. coli* as a probiotic

International Scientific Association for Probiotics and Prebiotics (ISAPP) defines probiotics as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" [10]. Although fermented foods with 10^5–10^8 microorganisms per gram can be constituted probiotics, recent oral supplementations contain $10^{10–12}$ live cells per single dose [11, 12]. *Lactobacillus* sp., *Bifidobacterium* sp., *Streptococcus* sp., *Enterococcus* sp., and *Saccharomyces boulardii* are common probiotics [13]. Some *Bacillus*, *E. coli,* and *Clostridium* sp. were found to be probiotics.

Commensal and probiotic *E. coli* modulates innate adaptive immune responses in the intestinal epithelium by activating the secretion of defensins, cytokines, IgA, and CD4 T cells. Additionally, siderophore production and iron scavenging by probiotic *E. coli* strains prevent the survival of the pathogens [14]. Interestingly, Alfred Nissle discovered an *E. coli* strain that prevented the growth of pathogenic *Salmonella* when it was cultured with stool samples [15]. The strain was isolated from a soldier who had no diarrhea when other soldiers had suffered *Shigella* infection. Upon oral supplementation, this strain was protected from diarrhea and this strain was named as *E. coli* Nissle 1917(EcN) from the year after its use [16]. Commercially, this strain is known as Mutaflor. EcN is the most commonly used gram-negative probiotic [17]. Interestingly, EcN could get established in swine herds with variation in the colonization of individual animals [18]. Some other *E. coli* strains have also been shown to possess probiotic properties (**Table 1**).

3. Probiotic *E. coli* Nissle 1917

EcN is effective against infection by *Salmonella enterica* serovar, *Typhimurium* strain C17, *Yersinia enterocolitica*, *Shigella flexneri*, *Legionella pneumophila*, and *Listeria monocytogenes* [19]. EcN is serum sensitive, forms semi-rough colony, and

has low levels of smooth lipopolysaccharide (sLPS) [23]. *EcN* prevents the colonization of pathogens by efficient adhesion with the help of fimbriae and capsule to the epitheliumt but not activating inflammation as its lipopolysaccharide (LPS) has a short O chain and weak binding to toll-like receptor 4. EcN decreases pro-inflammatory cytokine and increases anti-inflammatory cytokine formation [24]. EcN repairs leaky gut by increasing the expression and phosphorylation of tight junction protein zonula occludens-1 (ZO-1), ZO-2, and claudin 14 [25–27]. Additionally, EcN prevents disruption of epithelial tight junctions by inhibiting NF-κB-mediated activation of the MLCK-P-MLC signaling pathway [28]. EcN mediates pathogen elimination by secretion of low molecular weight microcin H47 and microcin S. Probiotic EcN, but not commensal *E. coli* MG1655, increases serotonin (5-hydroxytryptamine) secretion by enterochromaffin cells [29].

Interestingly, bacteria are known to secrete vesicles known as membrane vesicles (MVs) [30]. Gram-negative bacterial outer membrane vesicles contain LPS and the size and complexity of O-antigen, the number, and nature of fatty acid components of lipid A determining the beneficial or toxic effects on the host cells. EcN outer membrane vesicles (OMVs) prevent the inflammation and progression of dextran sodium sulfate (DSS)-induced colitis in mice [26, 31, 32]. EcN OMVs get internalized by macrophages and activate the phagocytosis, which increases pro-inflammatory cytokine secretion and killing of pathogens [33].

4. Symbioflor-2

Symbioflor-2 is a commercial product containing six *E. coli* strains, which brings about an increase in β-defensin-2 and reduces mast cell activation [19]. Symbioflor-2 is effective in reducing symptoms of irritable bowel syndrome [34]. Microcin S is produced by Symbioflor G3/10 strain. Surprisingly, virulence genes have also been detected in Symbioflor-2 genomes suggesting that the presence of virulence genes does not imply pathogenicity [2]. Transcriptomic analysis of ileum and colon upon inoculation with Symbioflor-2 strains indicated the increase in defense responses involving dual oxidase/nitric oxide pathway mediated reactive oxygen species generation along with β-defensin-2 activity [35]. Transcription profiles were distinct with EcN and Symbioflor-2.

5. Colinfant

Colinfant is an *E. coli* (A0 34/86) strain that is used as prophylactic in infants for allergy, nosocomial infection, and diarrhea [20, 21]. Additionally, it is effective in later years in preventing infections and developing allergies. Some strains of *Klebsiella oxytoca* are implicated in antibiotic-associated diarrhea, which could be reduced by the administration of Colinfant in infants. Colinfant also prevents infection of pathogenic *E. coli*.

6. Genetic modifications of probiotic *E. coli*

Probiotic *E. coli* strains have been modified to improve colonization, to secrete metabolites, proteins, and enzymes exploiting a variety of genetic manipulations (**Table 2**). EcN was tagged with a green fluorescent protein (Gfp), which facilitated monitoring the colonization and survival in stomach, ileum, colon, and Peyer's patches [36]. EcN was detected in the fecal matter at 45 days after oral inoculation.

***E. coli* strain**	**Nature of modification**	**Properties**	**References**
EcN	pUC-gfp	48-h residence in stomach, cecum and rectum; the presence in Peyer's patches; detected in feces up to 45 days after oral administration in rats	[36]
Ec16 (*vgb*)	*Green Fluorescent protein-Vitreoscilla* hemoglobin (*Vgb*) gene	Improves survival and ameliorates carbon tetrachloride toxicity	[37]
Ec16::*vgb-gfp* operon	Plasmid containing *Pseudomonas fluorescens* Bf1 *pqqABCDE* gene cluster	Ameliorates dimethylhydrazine-induced colon and liver damage; improved the neurotransmitter status	[38, 39]
Ec16::*vgb-gfp (inuJ)*	pMAL-p2ΔlaclQ Inulosucrase (*inuJ*) gene	Extracellular secretion of inulosucrase	[22]
EcN::*vgb-gfp* (*pqqABCDE*)	Plasmid containing *Pseudomonas fluorescens* Bf1 *pqqABCDE* gene cluster	Preventing chronic alcohol-induced oxidative damage	[40]
EcN::*vgb-gfp* (*pqqABCDE)*	Plasmid containing *Gluconobacter suboxydans* 621 *pqqABCDE* gene cluster	Prevents rotenone-induced mitochondrial oxidative stress and improves mitochondrial biogenesis	[41]
EcN::*vgb-gfp* (*pqqABCDE)*	Plasmid containing *pqq pqqABCDE* gene cluster of *Gluconobacter oxydans*	EcN strain secretes PQQ, gluconic acid with citric acid supplementation decreases the Cd- and Hg-induced liver toxicity effects in rats	[42]
EcN::*vgb-gfp* (*pqqABCDE-gad)*	Plasmid containing *pqqABCDE* gene cluster of *A. calcoaceticus* and gluconate dehydrogenase (*gad*) operon of *P. putida* KT 2440	EcN strain secretes PQQ, gluconic and 2-ketogluconic acids decrease the Cd, Hg, and Pb toxic effects in rats	[43]
EcN(*pqqABCDE-arsM)*	P*tac* G. oxydens pqqABCDE—Rhodopseudomonas palustris arsM* gene	EcN strain converts arsenite gets converted to non-toxic trimethyl arsenite and reduces arsenite toxicity	[44]
EcN::*vgb-gfp* (*pqqABCDE-glf-mtlK)*	*Ptac*-pqqABCDE* gene cluster of *G. suboxydans*-glucose facilitator (*glf)* of *Zymomonas mobilis*-mannitol dehydrogenase *(mtl*K)	EcN strain secrets PQQ and produces Glf protein and MtlK enzyme that coverts dietary fructose into mannitol	[45]
EcN::*vgb-gfp* (*pqqABCDE-fdh)*	*Ptac*-pqq* gene cluster of *G. suboxydans—Fructose dehydrogenase (fdh)* from *Gluconobacter frauteuri*IFO3260	EcN strain secrets PQQ and produces Fdh enzyme that converts dietary fructose to 5-keto-D-fructose	[45]
EcN::*vgb-gfp (pqqABCDE-inuJ)*	Genomic integration of *vgb*, *gfp, pqqABCDE,* and *inuJ* genes-	High dietary sucrose-induced oxidative damage and hyperlipidemia were decreased	[46]

***E. coli* strain**	**Nature of modification**	**Properties**	**References**
EcN	Curing of Mut1 and Mut2 plasmids	Growth is similar to the wild type in Luria broth	[47]
SYNB1618	Phenylalanine ammonia lyase (*stlA*) gene	Phenyl alanine conversion to *trans*-cinnamate	[48]
	L-amino acid deaminase (*pma*) gene	Phenyl alanine conversion to phenylpyruvate	
EcN (Δ*frdA*, Δ*ldhA*, Δ*adhE*, Δ*pta*)	P_L-*atoDAEB* operon; gsA::PL-*LacO-hbd-crt-ter*	Butyric acid secretion	[49]
EcN	Trefoil factor	Curly fiber matrix restitutes intestinal epithelium effective against DSS-induced colitis	[50]
EcN	*Staphylococcus aureus* α-hemolysin	Tumor regression by forming pores	[51]
EcN	Hemolysin E (*hylE*) under *araBAD* promoter	EcN strain had regressed tumors in mice by pore formation	[52]

Gad—gluconate dehydrogenase, Gfp—green fluorescent protein, DSS—dextran sodium sulfate, Ptac—constitutive tac promoter.*

Table 2.
Characteristics of genetically modified probiotic E. coli *strains.*

EcN contains two cryptic plasmids MUT1 and MUT2, and these plasmids were cured using CRISP-Cas9-assisted double-strand breaks [47]. EcN strain cured of these plasmids had similar growth under Luria broth conditions despite differences in the DNA content. Effects of colonization and survival of the plasmid-cured strain with decreased DNA content as compared to the wild-type strain need to be investigated to determine the impact of metabolic load. Alternatively, both the cryptic plasmids of EcN have been engineered for stable maintenance and expression of recombinant proteins [53].

Vitreoscilla hemoglobin (VHb) with a high affinity for oxygen facilitates the survival and functionality of bacteria under microaerobic conditions [54] promoted colonization of genetically modified *E. coli* in the gut. *E. coli* 16 double transformants of *gfp* and *Vitreoscilla* hemoglobin (*vgb*) genes at 10^8 cfu/g were present in the rat fecal matter after 70 days of oral administration, while Ec16 *gfp* was not found after 48 days [37]. Additionally, catalase activity of VHb scavenges the reactive oxygen species, which decreased the carbon tetrachloride-induced hepatotoxicity in rats.

Pyrroloquinoline quinone (PQQ) is a water-soluble antioxidant with the highest redox cycles of 20,000, promotes mitochondrial biogenesis and cellular signaling, and provides health benefits [55]. *E. coli* 16 strain tagged with *gfp-vgb* genes and transformed with *pqqABCDE* operon from *Pseudomonas fluorescens* Bf1 prevented colon and liver damage by dimethylhydrazine (DMH) due to the combined beneficial effects of effective colonization and antioxidant properties of Vhb and PQQ, respectively [38]. DMH had systemic oxidative damage, and decreased brain serotonin and norepinephrine levels, but epinephrine levels were increased [39]. In addition to decreasing the oxidative damage, *E. coli* 16 *vgb-pqq* strain had near-normal levels of neurotransmitters in rats. These beneficial effects

were not similar with treatments of Ec16, vitamin C, or PQQ alone suggesting other than its additional ability to confer antioxidant properties, probiotic *E. coli* 16 had synergistic effects related to the continuous secretion of PQQ in the gastrointestinal tract. These beneficial effects were also seen in EcN strain that was modified in a similar manner to that of Ec16 strain [40]. EcN *vgb-pqq* recombinant strain effects were monitored in rats for alcohol toxicity in chronic and acute exposure. Chronic alcohol caused extensive oxidative damage and induced hyperlipidemia and the EcN::*vgb-gfp*(*pqq*) probiotic strain prevented the deleterious effects, while EcN, PQQ, and vitamin C alone had no significant effects. These effects were also correlated with increased short-chain fatty acids (SCFA) in the colon. However, oral PQQ had better effects than recombinant EcN strain in acute alcohol damage. These studies further supported the significance of endogenous PQQ biosynthesis by probiotic *E. coli*.

Aging is associated with progressive loss of tissue functions mediated by reactive oxygen species-induced oxidative damage as a result of mitochondrial dysfunction [56–58]. EcN::*vgb-gfp* transformed with *pqq* gene cluster from *Gluconobacter suboxydans* 621 decreased the rotenone-induced mitochondrial oxidative damage in aging rats along with decreased lipogenesis and increased fatty acid oxidation genes correlated with increased colonic SCFA and PQQ in both feces and liver [41]. Additionally, an increase in mitochondrial biogenesis and metabolism indicates delaying of age-related tissue damage.

Heavy metal toxicity is mediated by reactive oxygen species [59]. Chelation of heavy metal ions and antioxidants is used to prevent the toxicity. EcN::*vgb-gfp* strain operon containing *pqq* gene cluster from *Gluconobacter oxydans* decreased the Cd and Hg toxicity upon oral supplementation citric acid due to the antioxidant effects of PQQ and chelation ability of citric acid [42]. Subsequently, EcN::*vgb-gfp* strain containing *pqq* gene cluster from *A. calcoaceticus* and gluconate dehydrogenase (*gad*) operon from *Pseudomonas putida* KT2440 secreted PQQ, gluconic and 2-ketogluconic acids, and this strain prevented toxicity caused by Cd, Hg and Pb without affecting the essential metal ions [43]. Thus, 2-ketogluconic acid produced by EcN recombinant strain is mimicking the chelating abilities of citric acid. Similarly, EcN strain containing As(III) S-adenosylmethionine (SAM) methlyltransferase (*arsM*) and *pqq* gene cluster prevented arsenite toxicity by scavenging arsenite-induced reactive oxygen species by secreted PQQ and converting arsenite into non-toxic trimethylarsenite in rats [44].

EcN recombinant strain containing *pqq* operon secretes 15 mM gluconic acid [43]. Gluconic acid was proposed for cancer therapy as cancer cells utilize citrate for growth and gluconic acid irreversibly inhibits citrate transporter, which is expressed on cancer cells [60]. Hence, EcN producing gluconic acid could prevent the progression of tumors, especially colorectal cancers. *Staphylococcus aureus* α-hemolysin expressing EcN recombinant strain forms pores in the tumor cells resulting in the regression of tumors in mice [51]. Similarly, tumor regression also occurred in mice xenografted with human colorectal cancer cells treated with EcN strain expressing hemolysin E (HlyE) a pore-forming protein [52].

SCFA such as acetate, propionate, and butyrate produced by gut microbiome is necessary for the survival of colonocytes, maintenance of intestinal integrity, mucus production, serotonin release by enterochromaffin cells, and secretion of gut hormone peptide YY in the intestine [61, 62]. Additionally, SCFA also regulates brain and liver functions while diminished SCFA signaling is associated with metabolic diseases [63]. Propionate and butyrate prevent the progression of these metabolic diseases [64]. In order to design EcN to secrete butyric acid, fumarate reductase (*frdA*), lactate dehydrogenase (*ldhA*), alcohol dehydrogenase (*adhE*), and phosphotransacetylase (*pta*) genes involved in the fermentation product

formation of succinic, acetic, and lactic acids were deleted to generate EcN YF005 strain [49]. The *atoDABE* operon encodes the genes for the formation of acetoacetyl CoA and butyryl CoA to the butyric acid formation, while *hbd* and *crt* from *Clostridium acetobutylicum* and *ter* from *Treponema denticola* genes convert acetoacetyl CoA into butyryl CoA. The native promoter of *atoDABE* operon was replaced with a strong, constitutive P_L promoter from phage λ, and synthetic P_L-*LacO-hpd-crt-ter* operon was integrated at methylglyoxal synthase (*msgA*) gene to generate EcN Y2023 strain. This strain produced 0.49 g/L butyric acid on glucose. It will be interesting to determine its therapeutic potential in animal studies.

EcN deletion mutant of *dapA* gene coding for 4-hydroxytetra-hydropicolinate synthase was generated for incorporating phenylalanine degradation for the treatment of phenylketonuria [48]. Two different SYNB1618 strains were generated by incorporating phenylalanine ammonia-lyase and L-amino acid deaminase (*pma*) genes, which convert phenylalanine into *trans*-cinnamic acid (TCA) and phenylpyruvate, respectively. In humans, TCA is further transformed into hippuric acid in the liver and excreted in the urine. The oral load of 70 mg phenylalanine was reduced by 58% in the serum samples of individuals fed with the modified strain.

EcN was genetically modified for inflammatory bowel disease by probiotic-associated therapeutic curli hybrids (PATCH) approach using a fusion protein of amyloid domain for self-assembly (CsgA) linked to trefoil factor-3 with 6 His residues [50]. Oxidatively damaged inflamed regions are conducive for the growth of facultative anaerobes. Consequently, modified EcN strain numbers increased at the damaged regions and secreted curly fibers that facilitated the repair of damaged regions. The EcN-engineered strain could ameliorate the weight loss in DSS-induced colitis in mice.

EcN expressing a fusion protein of cholera toxin B domain and insulin growth factor-1 (CTB-IGF1) was proposed as a long-term therapeutic strategy for diabetes [65]. It was hypothesized that EcN expresses the fusion protein in the intestine that would cross the intestinal epithelium into blood circulation facilitated by CTB-specific interaction with GM1 ganglioside oligosaccharide and IGF will activate the insulin effects.

7. *E. coli* as synbiotic

The beneficial effects of probiotic *E. coli* strains are contributed by their functions in the small intestine as well as in the colon. However, prebiotics are nutrients for the survival and maintenance of the colonic microbiome, which secrete host-beneficial SCFA as fermentation products [66]. Synbiotics are a mixture of prebiotics and probiotics, which provide synergistic effects of both components [67]. Dietary fructose and sucrose are implicated in the onset and progression of metabolic diseases [68, 69]. EcN::*vgb-gfp* was modified with two different synthetic operons containing *Ptac-pqq-glf-mtlK* and *Ptac-pqq-fdh* that convert dietary fructose into mannitol and 5-keto-D-fructose that are prebiotics in the small intestine [45]. These prebiotics then serve as nutrients for colonic bacteria to produce SCFA. PQQ secreted by the synbiotic EcN will scavenge reactive oxygen species produced by fructose. Both mannitol and 5-keto-D-fructose producing strains demonstrated synbiotic activities by preventing dietary fructose-mediated metabolic disorders in rats. Fructose is known to improve iron status by its reductive ability compared to other sugars [70]. However, metabolic complications of fructose hindered its applicability. Since EcN synbiotics overcome the negative effects of fructose, these strains were found to also improve iron status [71].

High dietary sucrose also contributes to the metabolic disorders [68]. Inulosucrase catalyzes the conversion of sucrose into inulin [72]. Probiotic Ec16 transformed with inulosucrase of *Lactobacillus johnsonii* NCC 533 resulted in its secretion in the supernatant, while the enzyme was localized in the periplasm of *E. coli* BL21 suggested that extracellular enzyme in Ec16 could get transported using colicin E1/1a1b transport system [22]. EcN genomic integrant with *vgb-gfp-pqqABCDE-inuJ* gene cluster prevented high sucrose-induced metabolic disorders in rats by increased PQQ and SCFA [46].

8. Conclusion

The potential of probiotic *E. coli* is increasing over the years starting from maintaining the intestinal barrier in healthy individuals to the treatment of complex diseases such as colorectal cancer and inflammatory bowel disease. Since many commercial *E. coli* products were found to deviate by orders of magnitude in terms of claimed numbers, monitoring strain identity and numbers is imperative for exploiting their complete potential [73]. Distinct properties of *E. coli* probiotics coupled with the ease of developing strains with desired traits could greatly expand their applications.

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