E. *coli* Nissle 1917

A Unique Medical Probiotic and it's Clinical Applications

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- E. *coli's* background
- Gut Microbiome
- Pathogenic E. *coli*
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- Clinical Applications

Background

BOX 1: *E. coli* wins record number of Nobel Prizes!

The Nobel Prize is science's highest honor. Each year there are only a few Nobel prizes awarded. These go to scientists whose discoveries "have conferred the greatest benefit on mankind." Below is a list of the Nobel-worthy discoveries to which *E. coli* has contributed and the year that they received the prize.

1958: Bacterial sex, and other ways bacteria can share genes with one another

1959: DNA replication, how life copies its genetic code

1965: Gene regulation, how genes are turned on or off

1968: The genetic code, the language in which our DNA is written

1969: Virus replication, how viruses reproduce inside cells

1978: Restriction enzymes, cellular "scissors" that allow scientists to cut DNA

1980: Recombinant DNA, the creation of the first genetically engineered DNA

1989: RNA as an enzyme, additional roles for RNA discovered

1997: ATP generation, how cells make ATP, the energy molecule that powers life

1999: Signal sequences on proteins, one way that cells organize themselves

2008: Green fluorescent protein, a tag scientists use to track cell components

Commensal in the human gut

- Consistent with lower gut physiology

Discovered by Theodore Escherich in 1885

- German Paediatrician
- Coli meaning 'from the gut'

Most well studied and scientifically used bacteria

- Strain K12 model organism for scientific study

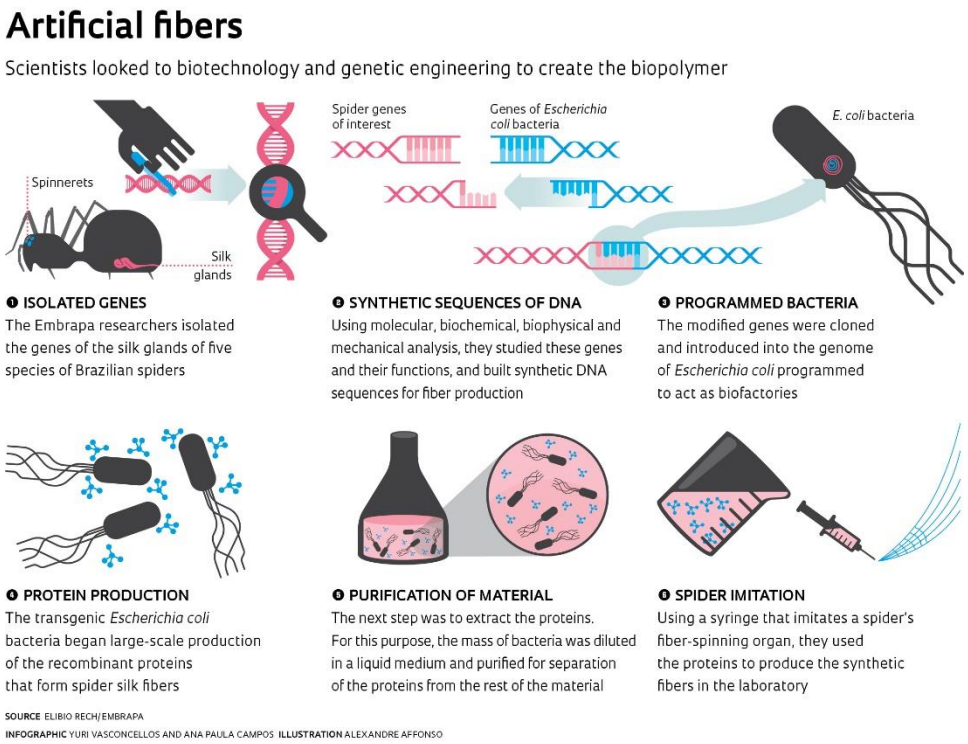
Jumpstarted the Biotechnology Industry

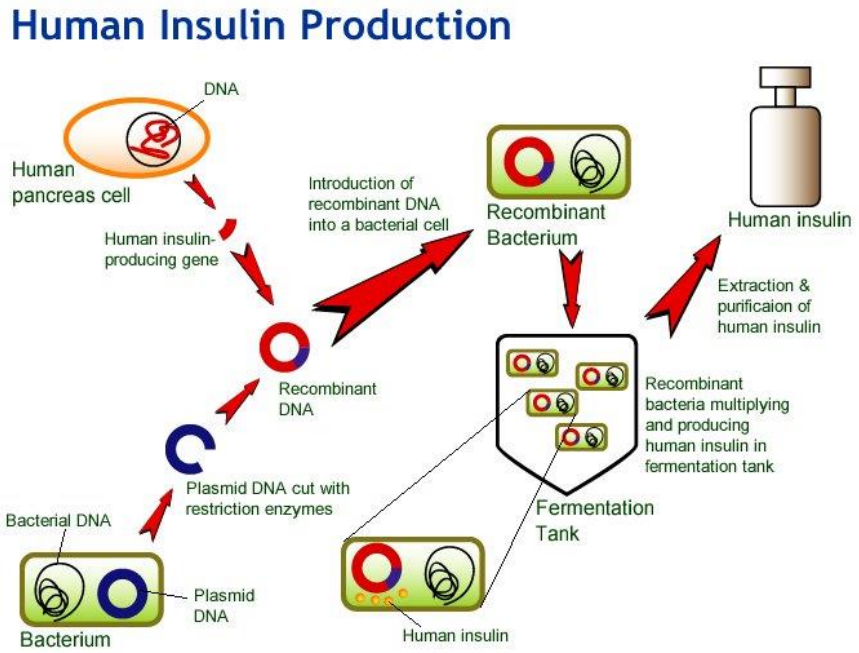
- Production of pharmaceuticals and foods
  - » Insulin, HGH, IFN-γ, peptides
  - » Rennin
  - » Biofuels

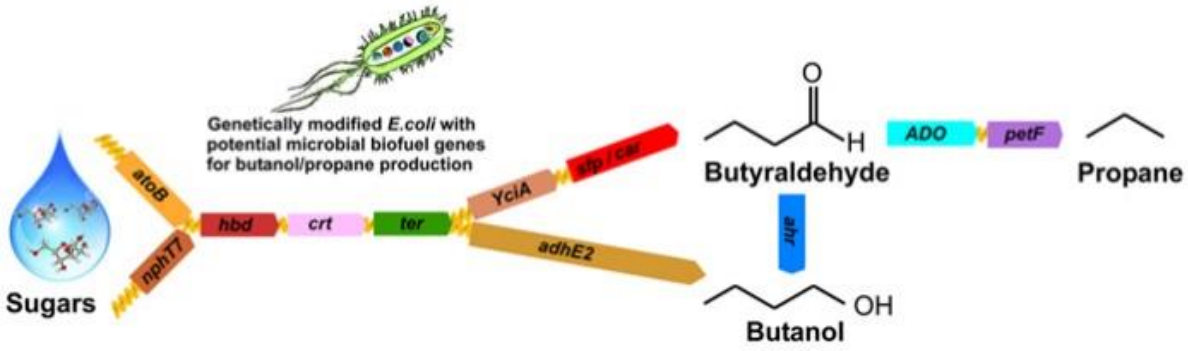
Serotype descriptions (O,H,K)

- Species and strains

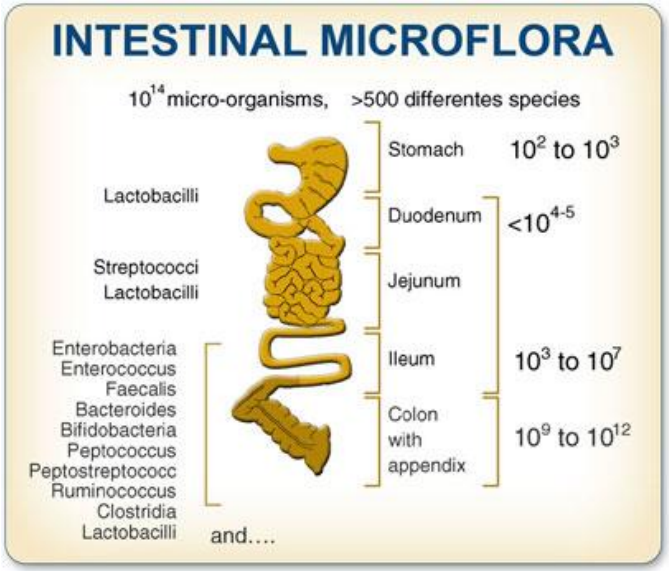
E. *coli* in Biotechnology

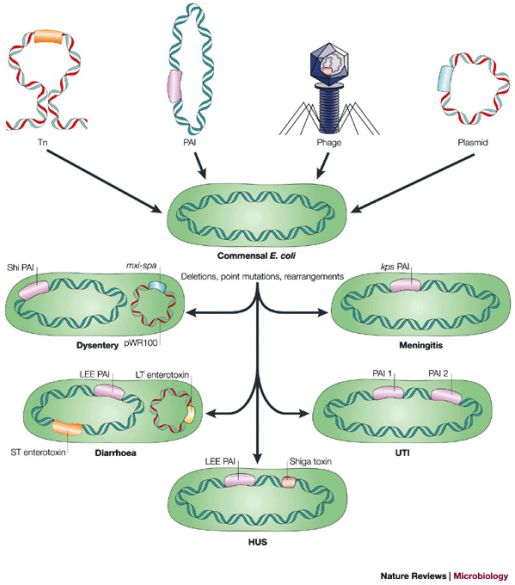






E. *coli* in the Human Gut





- Gut **commensal** E. *coli* (Average 5)
  - Most abundantly in the colon
  - Harmless and often beneficial
- E. *coli* ferments complex sugars and facilitates colonic motility
- E. *coli* produces important short chain fatty acids like acetate, lactate and succinate that stimulate colonocyte activity
- E. *coli* produces and modulates neurotransmitters like norepinephrine, serotonin and dopamine
- Commensal E. *coli* can become **harmful** by sharing or taking in pathogenic DNA via plasmids, from exposure to bad E. coli and other bacterial, yeast or viral pathogens

Pathogenic E. *coli*

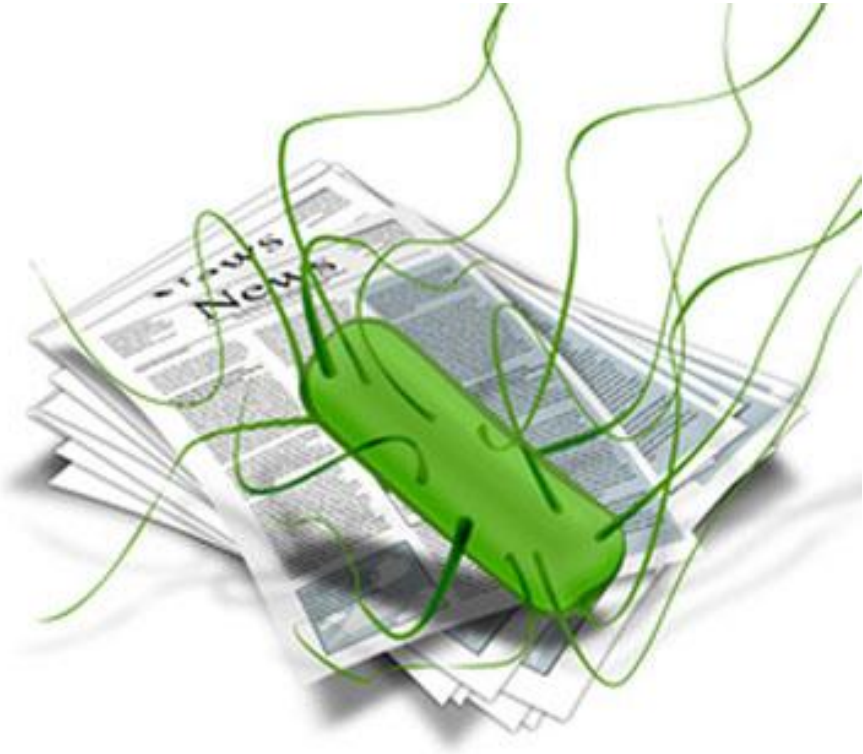
- Diarrhoeagenic E. *coli*
  - Most common in foodborne outbreaks
    - » Enterohaemorrhagic E. *coli* (EHEC)
    - » Shiga toxin-producing E. *coli* (STEC)
    - » Verocytotoxin-producing E. *coli* (VTEC)
  - Enterotoxigenic E. *coli* (ETEC)
  - Enteropathogenic E. *coli* (EPEC)
  - Enteroaggregative E. *coli* (EAEC)
  - Enteroinvasive E. *coli* (EIEC)
  - Diffusely adherent E. *coli* (DAEC)

- Extraintestinal Pathogenic E. *coli* (ExPEC)
  - Uropathogenic E. *coli* (UPEC)
  - Meningitis-associated E. *coli* (MNEC)
  - Septicemic E. *coli* (SPEC)

Food-based Outbreaks

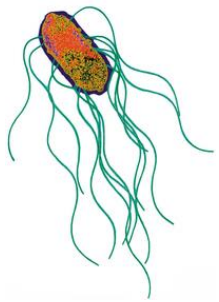
- **0157:H7**
- O104:H4
- O121
- O145
- O26

- In Asia, clinical outbreaks common (ETEC) and often associated with traveller's diarrhoea - nicknamed after the countries of first association
  - 'Delhi' Belly condition associated with travellers to India
  - 'Bali' Belly condition associated with travellers to Indonesia



Significant Other Gut Pathogens

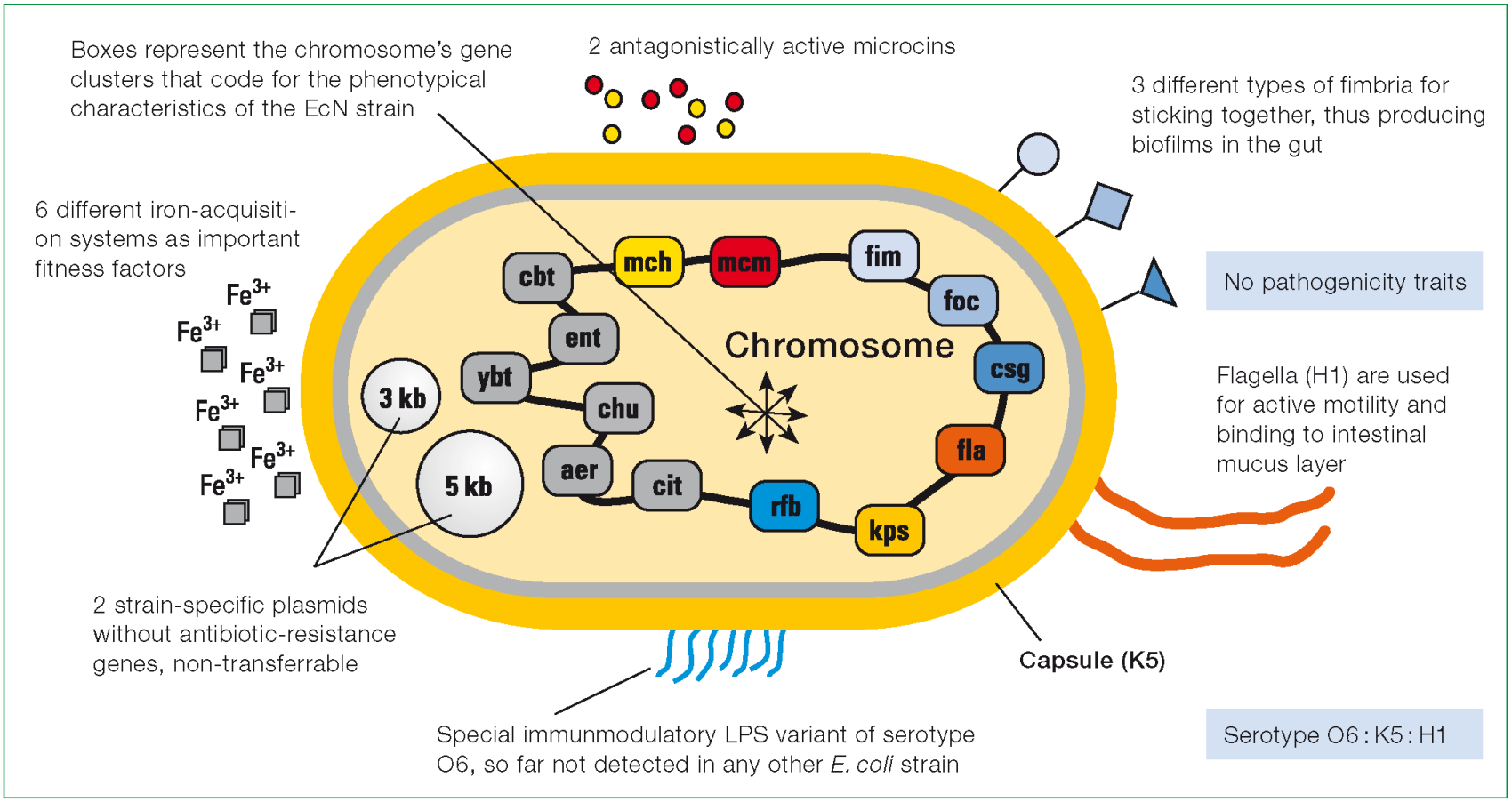
- Vibrio *cholerae*
- Salmonella *typhimurium*
- Listeria *monocytogenes*
- Klebsiella *pneumoniae*
- Candida *albicans*



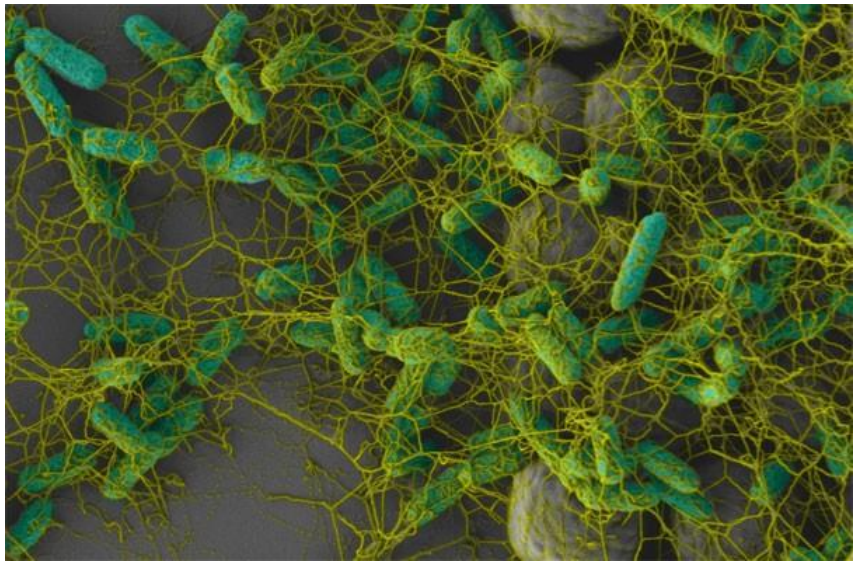
Good E. *coli*

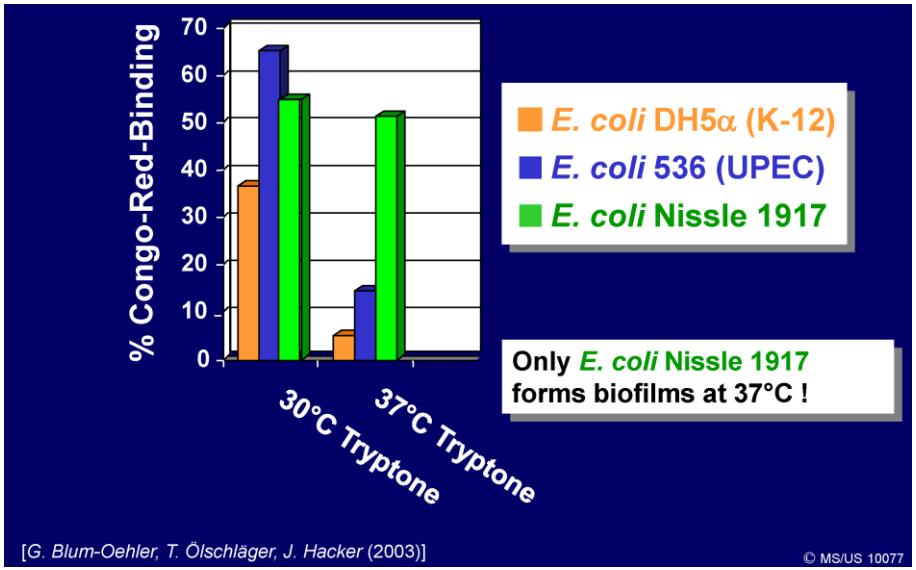
- **Nissle 1917** strain
  - Discovered by German military surgeon Alfred Nissle during World War I, from the stools of a soldier who did not develop any form of severe diarrhoea despite being in a high cholera and dysentery environment despite his fellow soldiers becoming severely ill
  - After the war, Prof Nissle's work on the strain continued to yield substantial results that showed it effective and safe in protecting humans and livestock from a variety of gut-related conditions
  - Today, the strain is produced and branded **Mutaflor®** by Ardeypharma, based in Herdecke, Germany

Nissle 1917 Characteristics



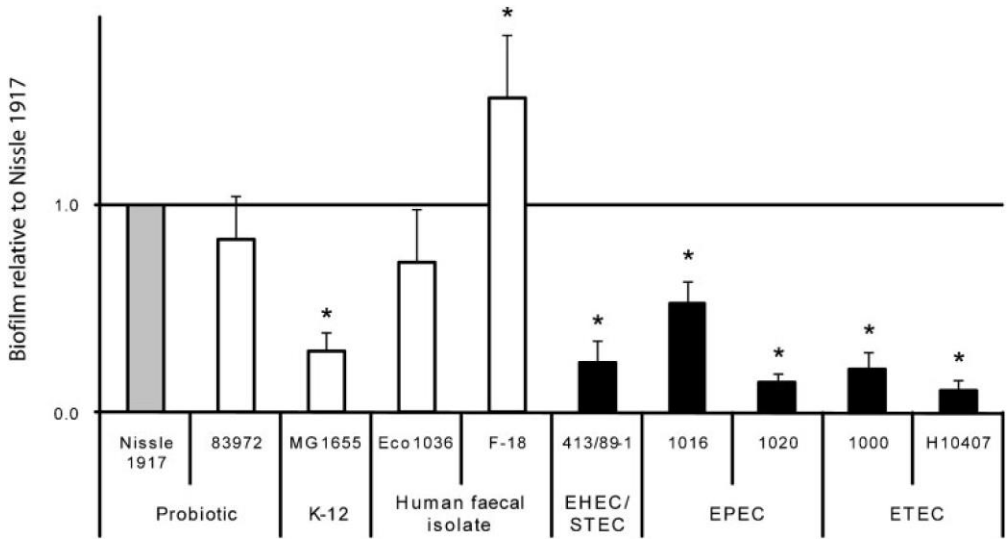
Nissle 1917 Biofilms





- **ECN** is the best natural E. *coli* strain colonizer in the lower gut, through optimal biofilm formation at 37 degC

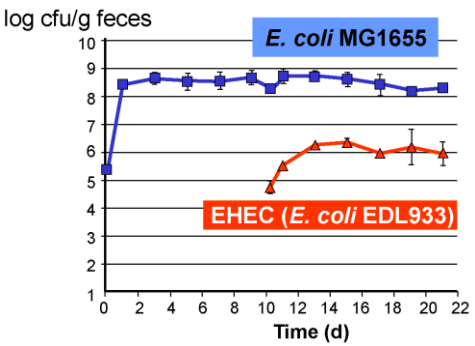
- **ECN** biofilms effectively
  - protect colonocyte mucus layers
  - improve tight junctions integrity
  - starve off pathogens

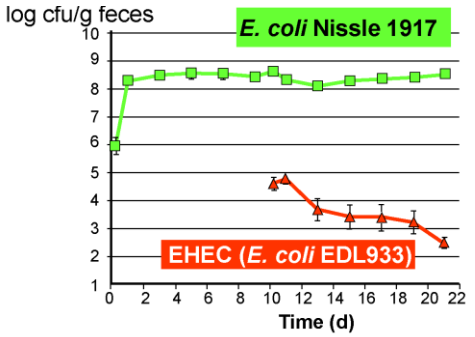


Nissle 1917 Outcompetes Pathogens

Approved antagonism of the *E. coli* strain Nissle 1917 against:

- Adherent invasive *E. coli* LF 82
- Enterohemorrhagic *E. coli* O157:H7
- Enteroinvasive *E. coli* O143:H-
- Enteropathogenic *E. coli* O112 ab
- Uropathogenic *E. coli* O6:K15:H31
- *Shigella flexneri* strain M90T
- *Shigella dysenteriae*
- *Salmonella enterica* (two strains: Serovar Typhimurium C17 and SL1344)
- *Salmonella enteritidis*
- Yersinia enterocolitica (two strains: WA314 and WA-C)
- *Proteus vulgaris*
- *Vibrio cholerae*
- *Listeria monocytogenes* strain EGD
- *Legionella pneumophila* strain Corby
- *Candida albicans*





Method:
Mice were pre-treated with Streptomycin in drinking water 24 hours before the application of bacteria. Streptomycin was continued for the whole course of the experiment. On day 0, either the *E. coli* K-12 strain MG1655 or *E. coli* strain Nissle 1917 were administered p.o. On day 10, mice were infected orally with the EHEC reference strain EDL933. Cell counts of viable bacteria were determined in fecal pellets as colony-forming units (cfu).

Nissle 1917 Limits Pathogen Growth

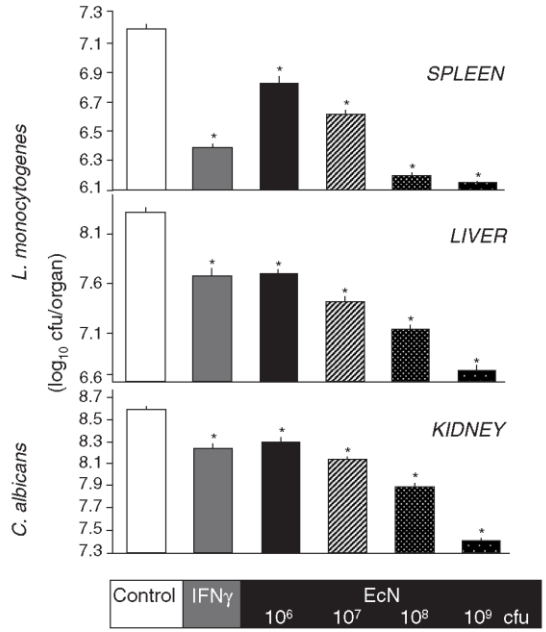


Figure 14. Augmentation of host defense against systemic bacterial and fungal infections in mice by oral pretreatment with *E. coli* Nissle 1917 (EcN) (132). Four groups of mice were pretreated once with 10^6, 10^7, 10^8, or 10^9 viable cells of EcN by oral administration, before they were challenged 24 h later by intravenous infection with *Listeria monocytogenes* (6×10^3 cfu) or *Candida albicans* (5×10^5 cfu). For each infection model, further groups of mice served as controls. These were either pretreated with placebo (negative controls, open bars) or with murine IFN-γ (positive controls, gray bars). Three days after infection with *L. monocytogenes* and 1 day after infection with *C. albicans*, mice were sacrificed and the parasite burden of the respective main target organs was determined. Compared with placebo, the pretreatment with IFN-γ resulted in a significant decrease of parasite load in spleen and liver of mice infected with *L. monocytogenes*, and a significant decrease of *C. albicans* counts in the kidneys. Likewise, pretreatment with EcN via the oral route significantly and dose-dependently reduced the parasite burden in spleen, liver, and kidneys. $^*p < 0.05$ vs negative controls.

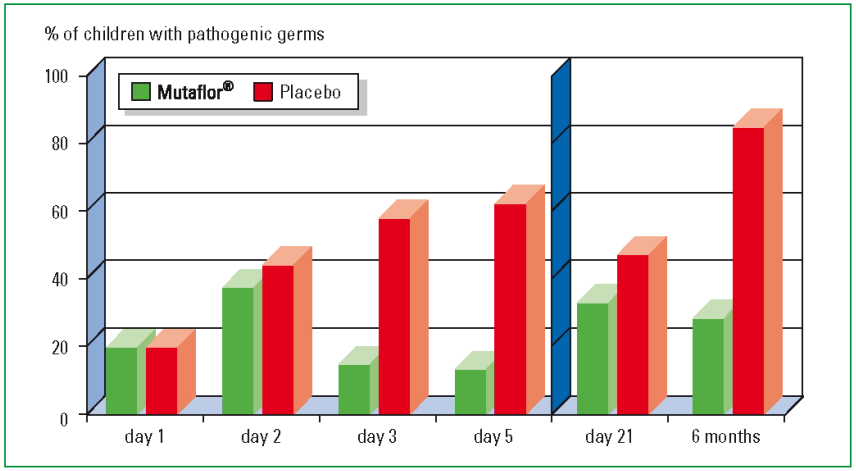


Fig. 2: Colonization of the infants gut with pathogenic or potentially pathogenic bacteria in the Mutaflor® and in the placebo group (Lodinová-Zádníková & Sonnenborn, 1997).

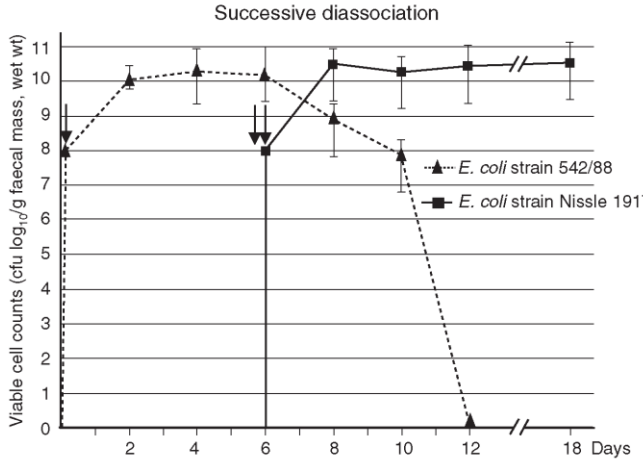
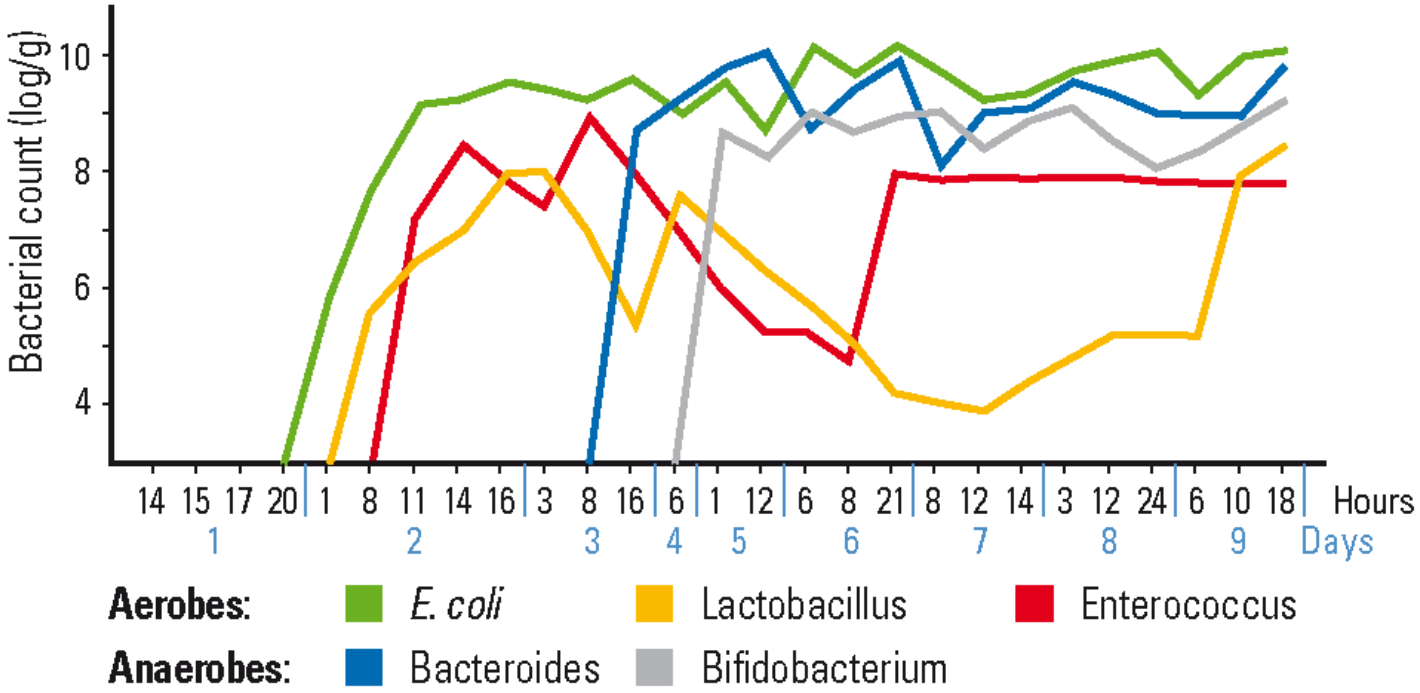


Figure 6. Antagonistic action of *E. coli* Nissle 1917 (EcN) against enteropathogenic *E. coli* 542/88 *in vivo* in gnotobiotic piglets (94). Four 7-day-old germ-free piglets were infected orally with 10^8 cfu of *E. coli* 542/88 (arrow). The enteropathogenic *E. coli* strain quickly colonized the gut (dotted black line) and reached stable bacterial counts (about 10^{10} cfu/g contents). Shortly after infection the animals showed signs of diarrheal illness. At day 6 of the experiment, when the piglets were visibly ill, they received an EcN suspension p.o. (2×10^8 cfu) (double arrow). Although the pathogenic *E. coli* strain had already settled in the gut, EcN also colonized the intestine fairly well (straight black curve). Six days later, the pathogen was completely expelled from the gut, whereas EcN continuously showed a high population level, and the piglets recovered.

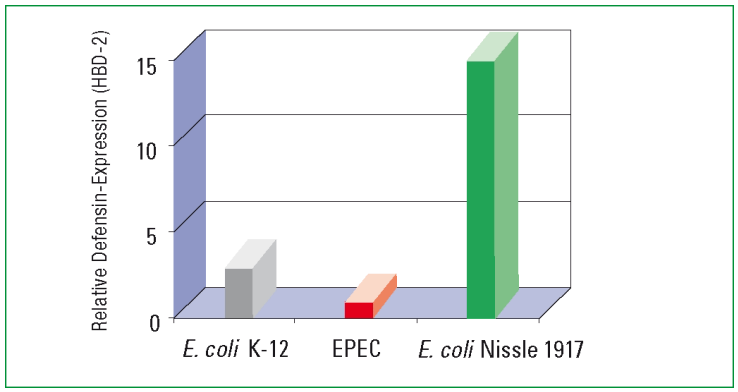
E. *coli* in Early Life

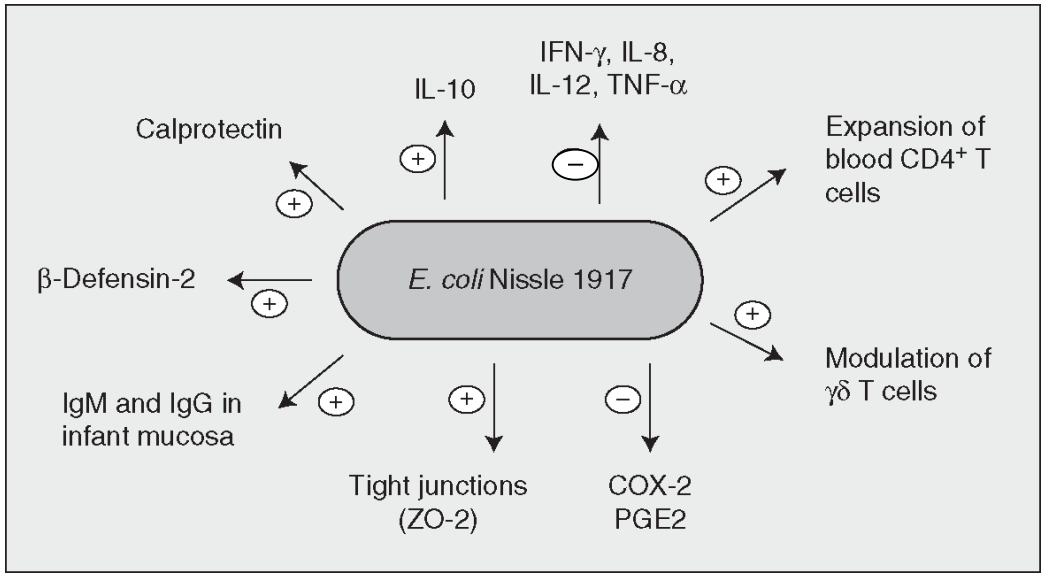


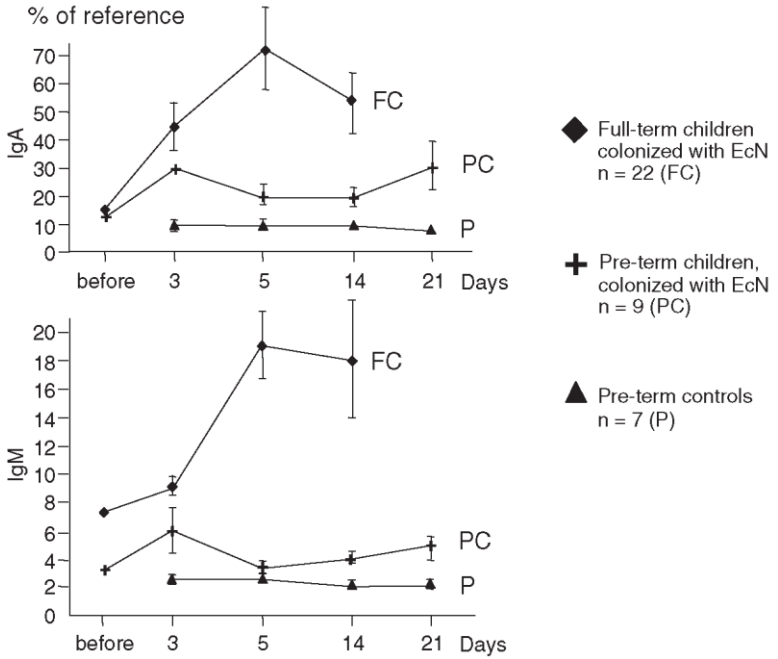
Results for the colonization of *E. coli* strain Nissle 1917 in the infant's gut

- Given directly after birth, EcN can already be detected in stool samples from the 2nd to 3rd day of life
- EcN permanently colonizes the infant's gut

Nissle 1917 Protective Effects



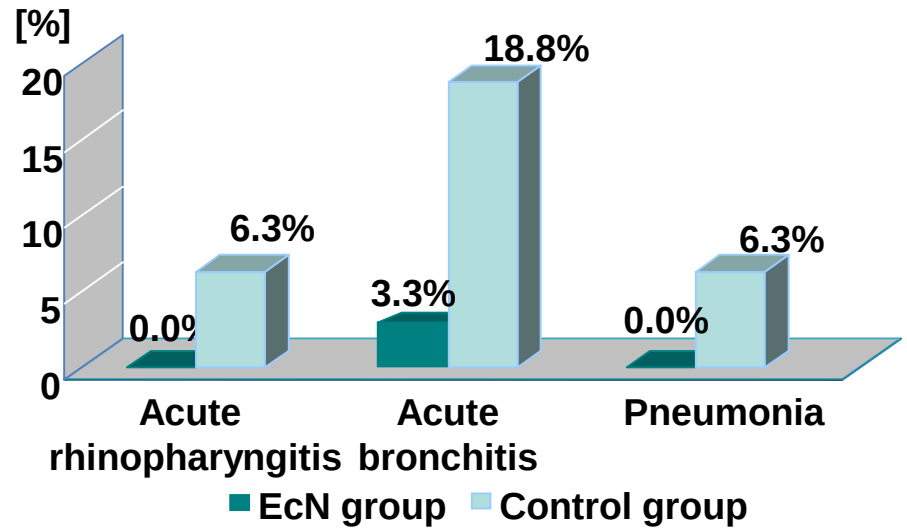




◆ Full-term children colonized with EcN n = 22 (FC)

✚ Pre-term children, colonized with EcN n = 9 (PC)

▲ Pre-term controls n = 7 (P)



Nissle 1917 Anti-Inflammatory

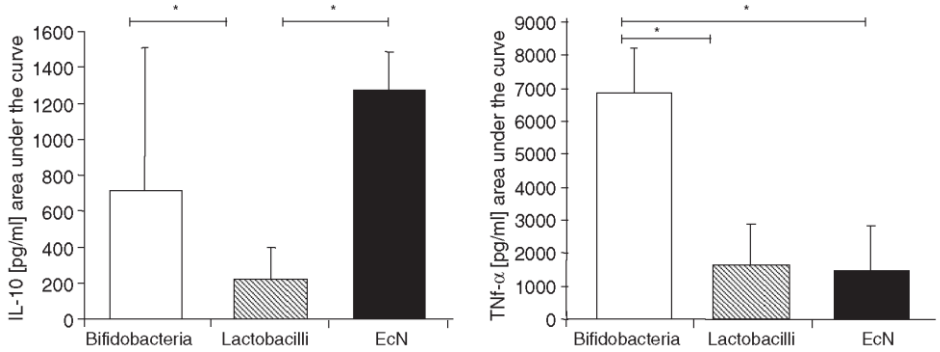
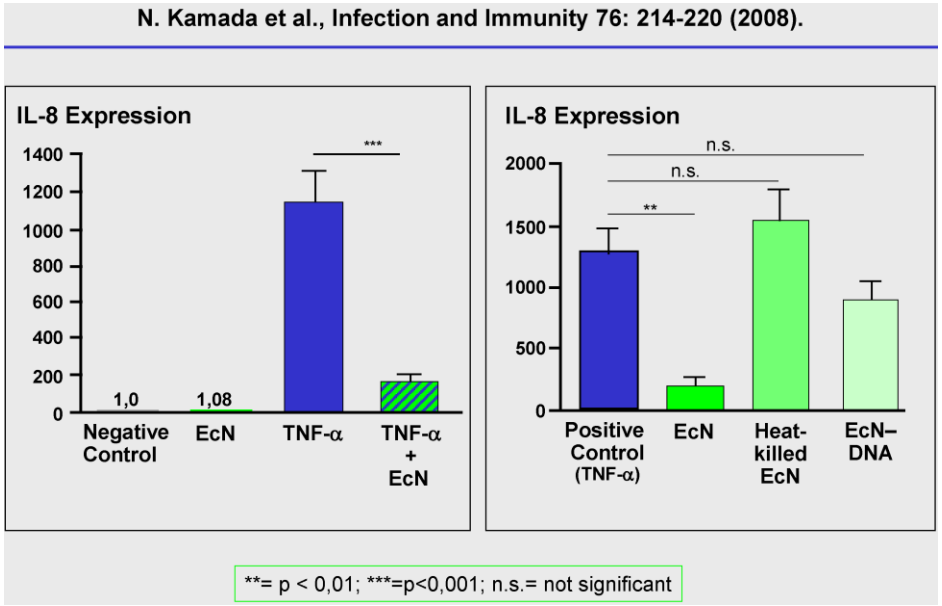


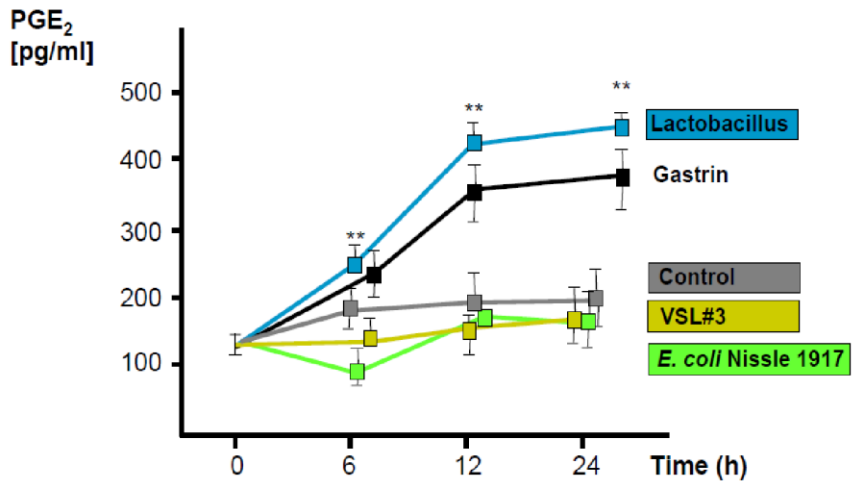
Figure 11. Immunomodulatory activities of gram-positive (bifidobacteria, lactobacilli) and gram-negative (*E. coli* Nissle 1917, EcN) bacteria on human peripheral blood mononuclear cells (PBMNCs) *in vitro* (107). Supernatant concentrations of the cytokines IL-10 and TNF-α after co-incubation of PBMNCs with cell debris of bacteria from different genera and species are shown. With regard to bifidobacteria (open bars), results are pooled from the data obtained by testing *B. breve*, *B. infantis*, and *B. longum*. With regard to lactobacilli (hatched bars), results are pooled from the data obtained by testing *L. acidophilus*, *L. bulgaricus*, *L. casei*, *L. plantarum*, and *L. rhamnosus* strain GG (LGG). Data from experiments with *E. coli* Nissle 1917 (EcN) are presented by the black bars. Concentrations of IL-10 and TNF-α are shown in pg/ml as area under the curve (AUC, mean ± SE). *Significantly different, $p < 0.05$.



Nissle 1917 may protect against Colon Cancer

***E. coli* Nissle 1917 inhibits Gastrin-induced Prostaglandin Synthesis (PGE_2) in Gut Epithelial Cells**

J.-M. Otte et al., Nutrition and Cancer 61: 103-113 (2009)



** $p < 0,01$

Reduction of COX-2/PGE-2 activity

- COX-2/PGE-2 up-regulation is known to stimulate colorectal carcinogenesis
- Celebrex® (COX-2 inhibitor) approved by US FDA for treatment of familial adenomatous polyposis

Reduction of IL-8 levels

- IL-8 - recognized autocrine growth factor for colon carcinoma cell lines

Improvements in Microsatellite Instability (MSI) compared to mesalazine in 1-year UC remission biopsies

- MSI - one of 3 main mutational mechanisms that can lead to Colitis-Associated Cancer (CAC) subset of colorectal cancer

Nissle 1917 Repairs Leaky Gut

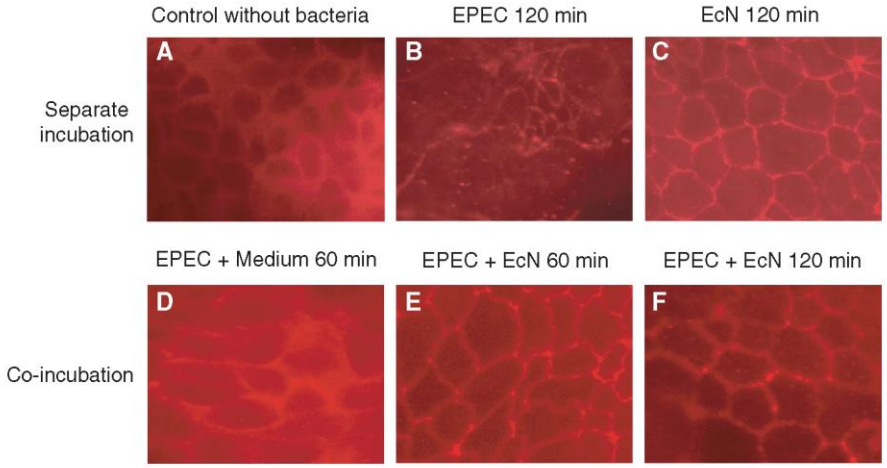


Figure 15. Effects of the non-pathogenic *E. coli* strain Nissle 1917 (EcN) and the enteropathogenic *E. coli* (EPEC) strain E2348/69 on the distribution of the tight junction protein zonula occludens-2 (ZO-2) in T_{84} epithelial cells (115). T_{84} monolayers were incubated with bacteria for different periods of time and stained for ZO-2 using a fluorescent anti-ZO-2 antibody. (A) Control, T_{84} epithelial cells without bacteria. (B) T_{84} cells incubated with EPEC for 120 min. (C) T_{84} cells incubated with EcN for 120 min. (D) T_{84} cells incubated with EPEC for 60 min, then washed and further incubated with regular medium for another 60 min. (E) T_{84} cells incubated with EPEC for 60 min, then washed and further incubated with EcN for another 60 min. (F) T_{84} cells co-incubated with EcN plus EPEC for 120 min. Bacteria were added in a 1:1 ratio. The EcN strain had no negative influence on the distribution of the tight junction protein ZO-2, but abolished the negative impact of EPEC on ZO-2 distribution.

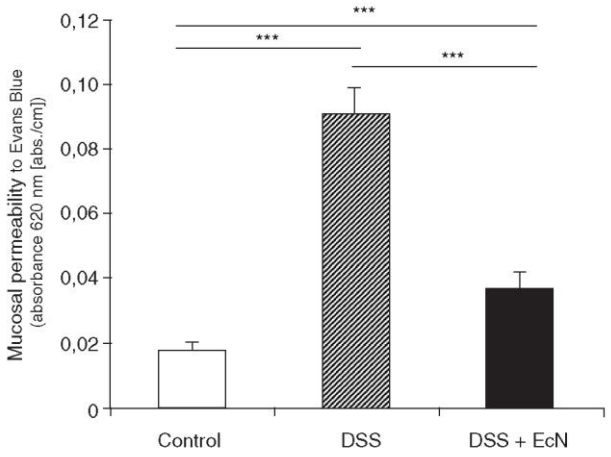
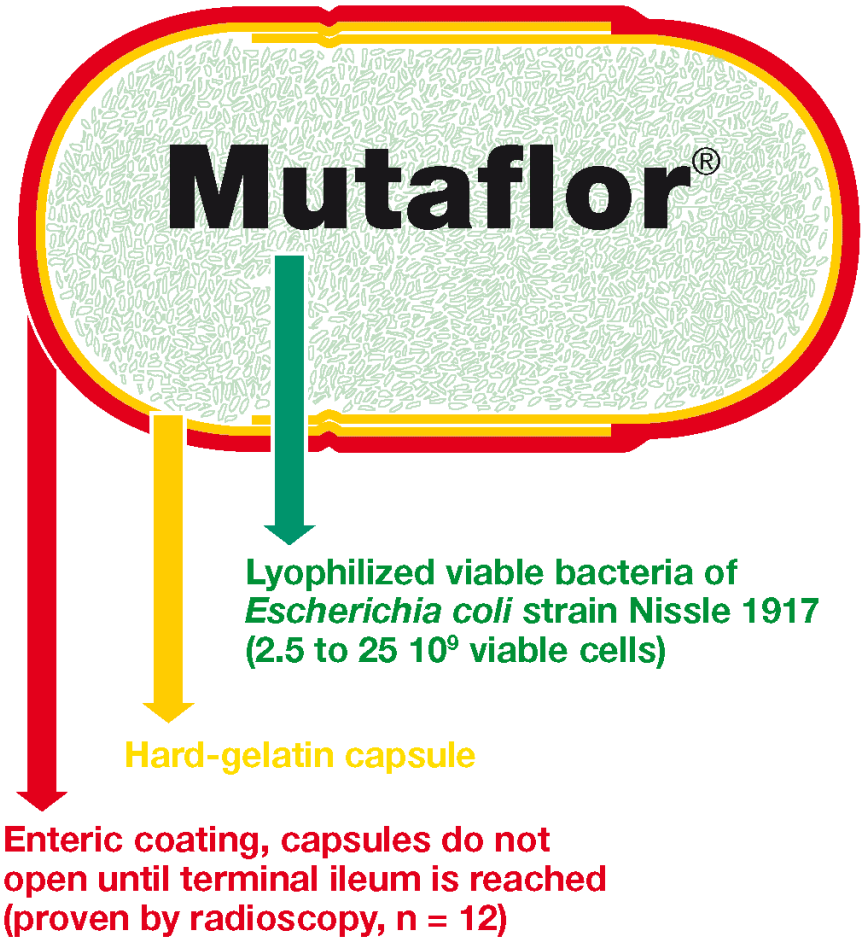


Figure 17. Inhibition of 'leaky gut' phenomena by oral administration of *E. coli* Nissle 1917 (EcN) to mice with dextran sodium sulfate (DSS)-induced colitis (130). Intestinal permeability to Evans Blue was determined in healthy mice (control, open bar), mice with DSS-induced colitis (hatched bar), and mice treated with DSS plus EcN (black bar). Compared with healthy control mice, a significant increase in the uptake of Evans Blue by the colonic mucosa of DSS-treated mice was observed. This increase was strongly reduced in the group of mice treated with DSS plus EcN to almost normal values. $^{***}p < 0.001$.

Nissle 1917 in a Quality Product

The *E. coli* strain Nissle 1917 is

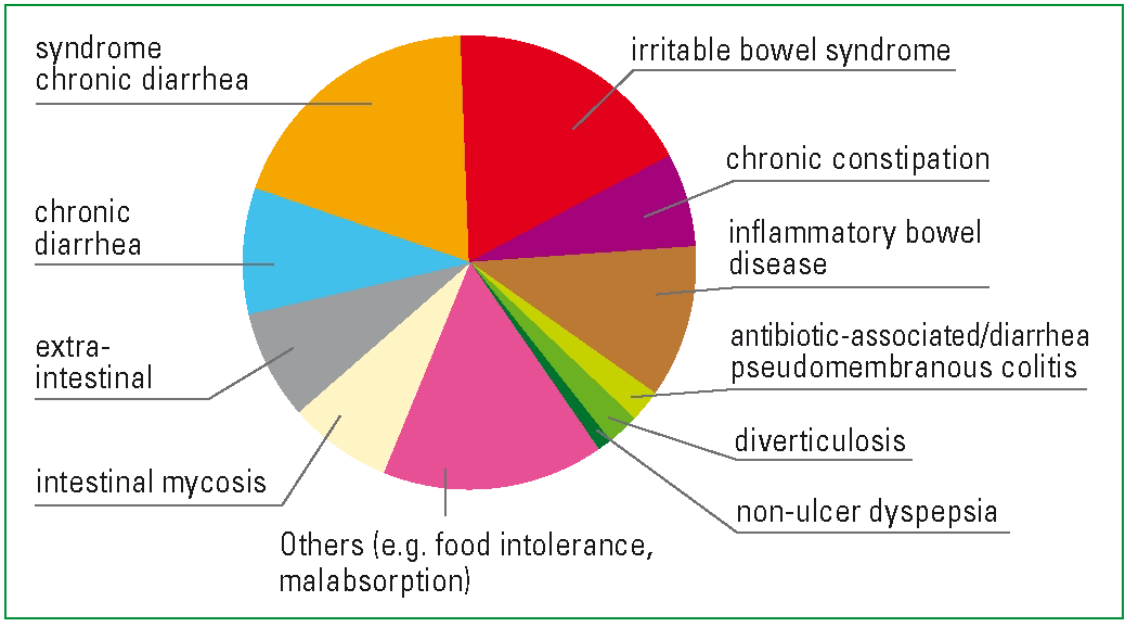
- vital and assertive ("biologically fit")
- antagonistically active against pathogens
- immune-modulatory
- anti-inflammatory and
- stabilizes the intestinal barrier (preventing leaky gut-syndrome)



Clinical Experience

Overview of Mutaflor® studies

Diarrhea	Henker et al. 2007, 2008
Irritable bowel syndrome	Krammer et al. 2006, Plassmann 2007, Keller et al. 2010 Kruis et al. 2012
Antibiotic-associated-/ pseudomembranous colitis	Goerg u. Schlörer 1998, Goerg et al. 2008
Ulcerative colitis	Kruis et al. 1997, Rembacken et al.1999, Kruis et al. 2004, Henker et al. 2008, Matthes et al. 2010
Crohn's disease	Malchow 1997
Pouchitis	Kuzela et al. 2001
Collagenous colitis	Tromm et al. 2004
Chronic constipation	Bruckschen et al. 1994, Möllenbrink et al. 1994
Diverticulitis of the colon	Frič et al. 2003
Colonization prophylaxis and augmentation of immuno-competence in full-term and pre-term infants	Lodinová-Žádníková et al. 1992, 1997, Schröder 1992, Cukrowska et al. 2002
Halitosis caused by intestinal problems	Henker et al. 2001
Polymorphous light dermatosis (sun allergy)	Wurzel 1999



Spectrum of therapeutic experiences for *E. coli* strain Nissle 1917 according to the study results (Post marketing surveillance) of Krammer et al., 2006.

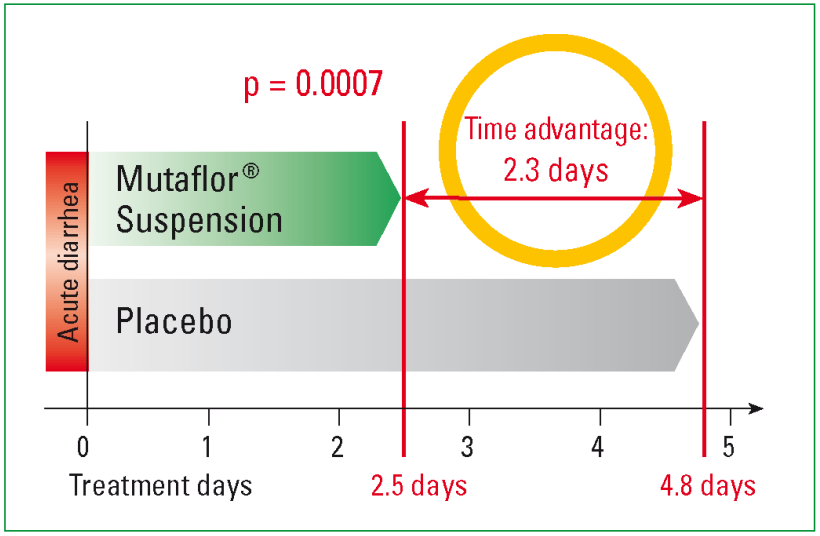
Diarrhoea (Infants/Toddlers)

Acute Diarrhoea

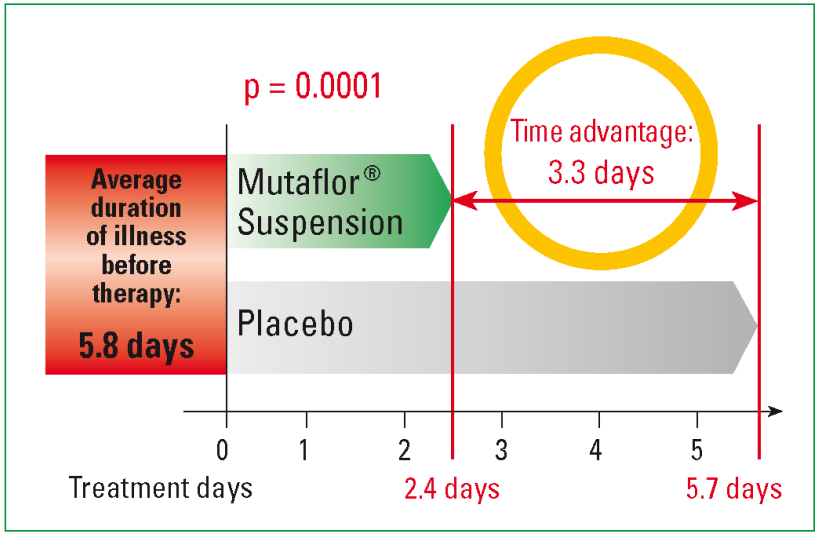
- 113 children (aged 2 – 47 months)
- >3 water/loose stools in 24h
- **ECN** superior to placebo, safe & well-tolerated

Prolonged Diarrhoea

- 151 children (aged 1 – 47 months)
- >3 water/loose stools in 24h
- > 4 consecutive days, < 14 days
- **ECN** higher 7-day, 14-day, 21-day response rates, safe & well-tolerated



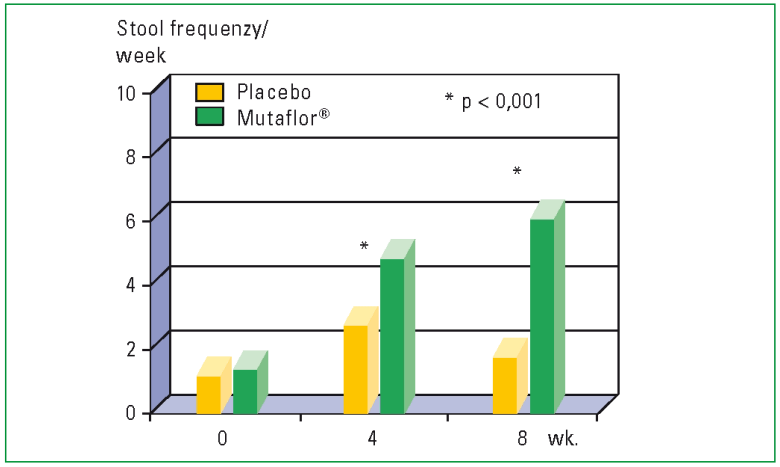
Mutaflor® Suspension reduces the duration of acute diarrhea by 2.3 days (according to Henker et al., 2007).



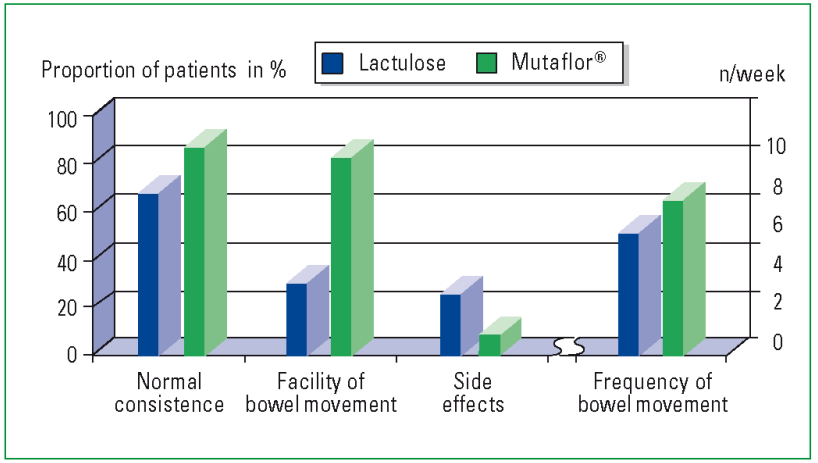
Mutaflor® Suspension reduces the duration of chronic diarrhea by 3.3 days (according to Henker et al., 2008).

Chronic Constipation (Adults)

- 2 controlled clinical studies (n = 178)
  - 8-week study showed significantly more bowel movements with **ECN** vs placebo
  - 12-week study showed comparable efficacy between **ECN** and lactulose
    - Frequency and facility of bowel movement, normal consistence and side effects all better with **ECN** than lactulose

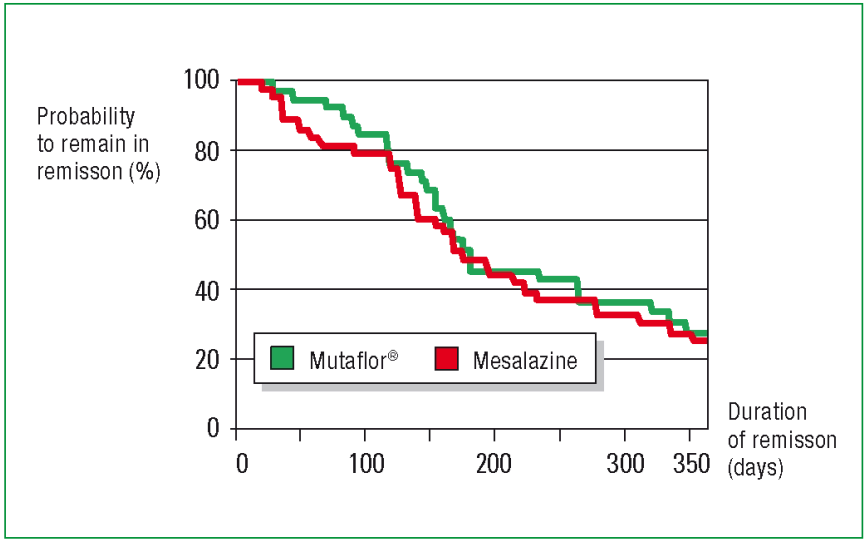


Chronic constipation, Mutaflor® vs. placebo, 8 weeks, n = 70 (Möllenbrink & Bruckschen, 1994).

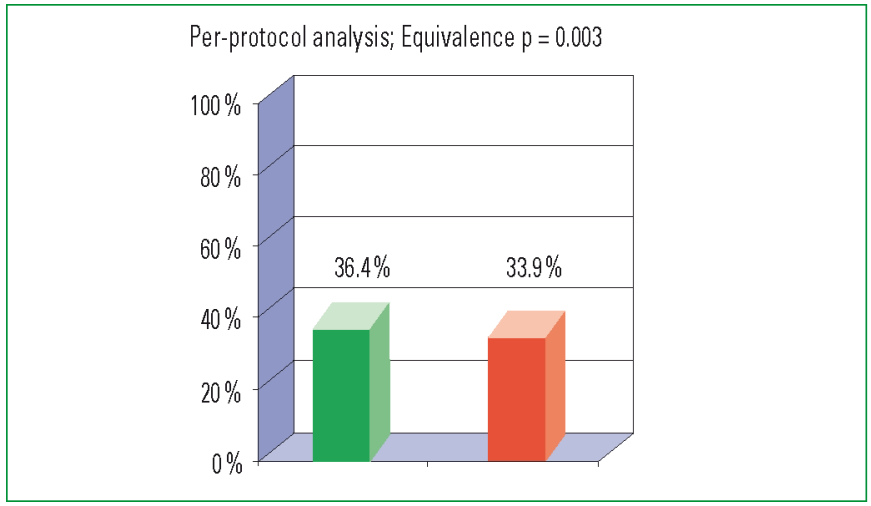


Chronic constipation, Mutaflor® vs. lactulose, 12 weeks, n = 108 (Bruckschen & Horosiewicz, 1994).

Ulcerative Colitis



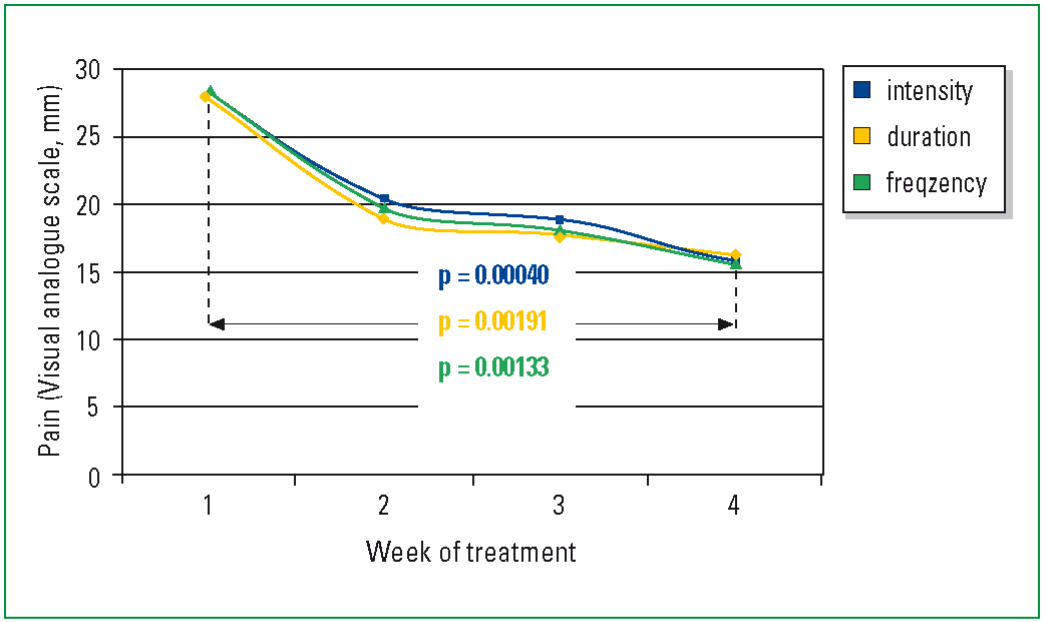
Ulcerative colitis, course of the remission within 12 months, depiction according to Kaplan-Meier (Rembacken et al., 1999).



Patients	Design / Duration	Author / Year
50 Mutaflor® 53 Mesalazin	randomized, double blind, multicenter, 12 weeks	Kruis et al. 1997
57 Mutaflor® 59 Mesalazine	randomized, double blind, 1 year	Rembacken et al. 1999
162 Mutaflor® 165 Mesalazine	randomized, double blind, multicenter, 1 year	Kruis et al. 2004

- 3 gold-standard comparisons for maintenance of remission of adults in UC showed **ECN** equivalent to Mesalazine
- Open pilot study (Henker et al. 2008) comparing maintenance in children and adolescents with changeover from Mesalazine to **ECN** – no significant diff in recurrence rate
- **European Crohn's & Colitis Organization (ECCO)** recognizes **ECN** as the evidence-based probiotic drug for maintaining remission

Irritable Bowel Syndrome

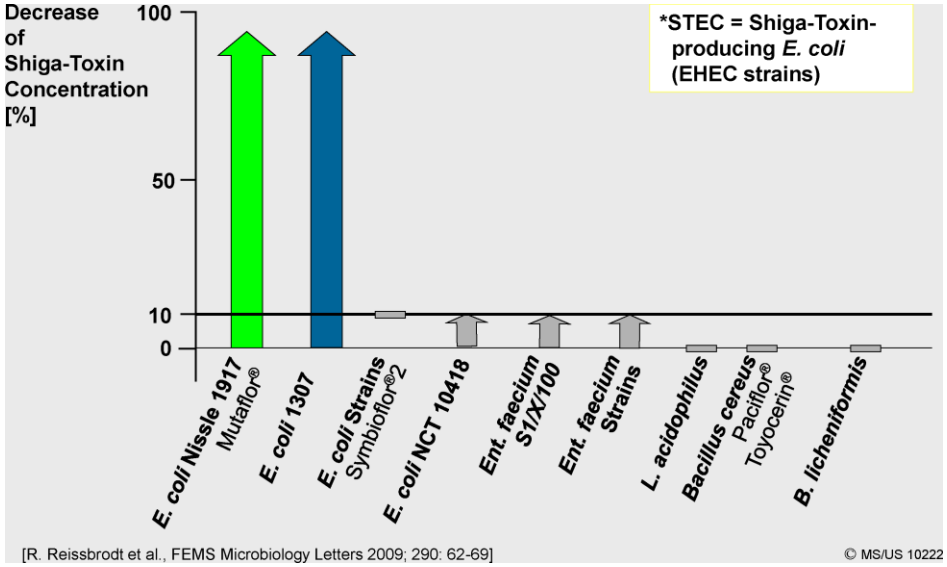


Patients' pain assessment (intensity, duration and frequency) in their diaries represented as average per week (according to Keller et al., 2010).

- Multicenter trial (Keller et al. 2010) - 41 subjects on **Mutaflor®**
  - IBS symptoms improved in most patients, particularly meteorism and pain intensity ameliorated
  - 78% stated treatment tolerance as good or very good
- IBS patients with an infection or treated previously with antibiotics improved significantly with several weeks of **Mutaflor®** therapy (Kruis et al. 2012)
- **German Association of Digestive and Metabolic Diseases (DGVS)** guideline recommends **ECN** for IBS management (Layer et al. 2011)

Clinical Support

- Traveller's diarrhoea
- Gut flora regrowth
- Antibiotic treatments
- General Gut Health



Clinical Considerations

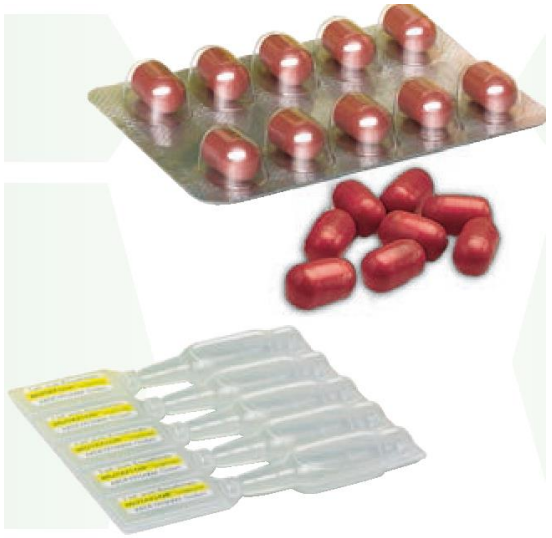
Antibiotic sensitivity of the Gram-negative *E.coli* strain Nissle 1917	
Amikacin	Gentamicin
Amoxicillin / Clavulanic acid	Imipenem
Ampicillin	Latamoxef
Azlocillin	Mezlocillin
Cefaclor	Nitrofurantoin
Cefazolin	Norfloxacin
Cefoperazone	Pipemidic acid
Cefotaxime	Piperacillin
Ceftriaxone	Tetracycline
Cephalothin	Ticarcillin
Chloramphenicol	Tobramycin
Ciprofloxacin	Trimethoprim/
Doxycycline	Sulfamethoxazole

Natural antibiotic resistance of the Gram-negative *E.coli* strain Nissle 1917	
Cefsulodin	Quinupristin / Dalfopristin
Clindamycin	Rifampin
Erythromycin	Teicoplanin
Metronidazole	Vancomycin
Penicillin G	

- If given with Gram-ve targeting antibiotics, separate doses of **ECN** and the antibiotic by 2 - 4 hours
- Natural resistance of **ECN** makes it a good combination with Triple Therapy antibiotics in H. *pylori* eradication regimes

Dosing and Side Effects

- Standard dose for Adults and Teenagers is 1-2 capsules per day
  - Best taken with water and the first meal of the day
  - Up to 4 capsules per day if supporting slow bowel movements
  - Long-term regular consumption necessary in supporting the maintenance phase of specific medical conditions



- Side effects
  - Flatulence (most common)
  - Borborygm (rare)
  - Meteorism (rare)

Mutaflor® Suspension is generally dispensed as follows:

Diarrhea:
Infants, toddlers and children:
1 – 3 x 1 ml per day

Diarrhea during tube feeding:
Infants, toddlers and children:
1 x 1 – 5 ml per day

Colonization prophylaxis:
Pre- and full-term babies:
1 x 1 ml per day

Improvement of postnatal immune competence:
1st week of life 1 x 1 ml per day;
2nd – 3rd week 3 x week 1 ml per day

Safety

- Tested and produced to **pharmaceutical standards**
  - Apathogenicity
  - Viable cell count
  - Microbiological purity
  - Genetic stability

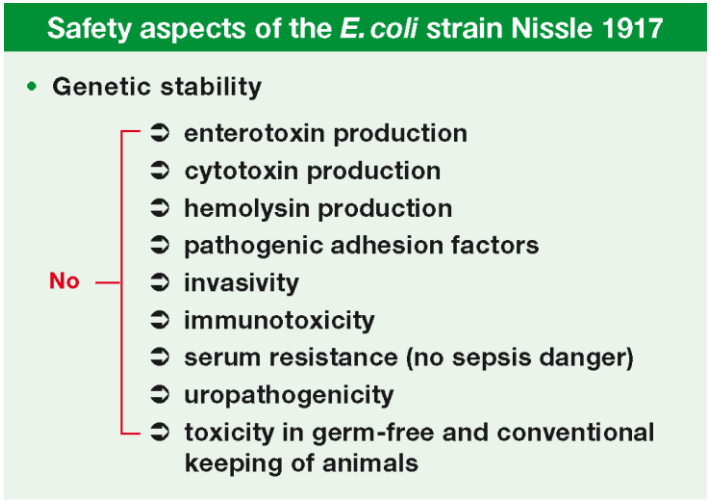
- **Best investigated**, therapeutically used E. coli strain in the world
  - Fully-sequenced genome (2014)
  - Stocked in the German Collection of Microorganisms and Cell Cultures (DSM 6601)

- History of close to **100 years** of safe, uninterrupted use in medicine
  - Continually studied and consistently validated

- **No antibiotic resistance** propagation
  - Does not share genes

- **Suitable for immune compromised**
  - LPS targeted by the complement cascade if ever it reaches the blood (serum sensitive)

- **No age restrictions**
  - Beneficial in pregnancy, full- and pre-term babies through to adults



Study examines therapeutic bacteria's ability to prevent obesity

Thursday, Jul. 17, 2014, 9:16 AM

A probiotic that prevents obesity could be on the horizon. Bacteria that produce a therapeutic compound in the gut inhibit weight gain, insulin resistance and other adverse effects of a high-fat diet in mice, Vanderbilt University investigators have discovered.

Regulatory issues must be addressed before moving to human studies, Davies said, but the findings published in the August issue of the Journal of Clinical Investigation suggest that it may be possible to manipulate the bacterial residents of the gut – the gut microbiota — to treat obesity and other chronic diseases. Davies has a long-standing interest in using probiotic bacteria — "friendly" bacteria like those in yogurt — to deliver drugs to the gut in a sustained manner, in order to eliminate the daily drug regimens associated with chronic diseases. In 2007, he received a National Institutes of Health Director's New Innovator Award to develop and test the idea. Other studies have demonstrated that the natural gut microbiota plays a role in obesity, diabetes and cardiovascular disease.

To start, the team needed a safe bacterial strain that colonizes the human gut. They selected **E. *coli* Nissle 1917**, which has been used as a probiotic treatment for diarrhoea since its discovery nearly 100 years ago. They genetically modified the E. coli Nissle strain to produce a lipid compound called NAPE, which is normally synthesized in the small intestine in response to feeding. NAPE is rapidly converted to NAE, a compound that reduces both food intake and weight gain. Some evidence suggests that NAPE production may be reduced in individuals eating a high-fat diet.

The investigators added the NAPE-producing bacteria to the drinking water of mice eating a high-fat diet for eight weeks. Mice that received the modified bacteria had dramatically lower food intake, body fat, insulin resistance and fatty liver compared to mice receiving control bacteria. They found that these protective effects persisted for at least four weeks after the NAPE-producing bacteria were removed from the drinking water. And even 12 weeks after the modified bacteria were removed, the treated mice still had much lower body weight and body fat compared to the control mice. Active bacteria no longer persisted after about six weeks.

Davies noted that the researchers also observed effects of the compounds in the liver, suggesting that it may be possible to use modified bacteria to deliver therapeutics beyond the gut. This research was supported by the **New Innovator Award (OD003137)** and by other grants from the **National Institutes of Health** (AT007830, DK059637, DK020593, RR024975, DK092993).

Recommendations

- The right probiotic involves
  - Beneficially safe strains
  - Reliable, appropriate quantities of ‘viable’ microorganisms
  - Targeted delivery mechanism

- Best strains for different aspects of gut health/age/lifestyle
  - Beneficial bacteria with good gut clinical applications are mostly specific strains of
    - » Lactobacillus
    - » Bifidobacter
  - Other key strains can include
    - » Streptococcus *thermophilus*
    - » Sacchromyces *boulardii*
  - **Nissle 1917** - natural colonizer for specific lower gut health

- **Nissle 1917** has a unique ecologically beneficial niche in the gut beyond any other commensal gut strain or probiotic