Mutaflor®

The probiotic drug for life!

Inflammatory Bowel Disease
and
Chronic Functional Bowel Diseases

Effective in the colon
A Bacterial Strain Becomes a Medicine

The Origin

The Mutaflor® strain was isolated by Professor Alfred Nissle in 1917 in the First World War from the feces of a soldier who, in contrast to all of his comrades, did not develop enterocolitis in the region of Dobrudsha on the Balkan peninsula, highly contaminated by enteropathogens at that time.

For that reason, the strain is known by the name Escherichia coli strain Nissle 1917 until today.

Professor Nissle cultured the strain and filled it into capsules, each containing the yield of 1 to 3 agar plates.

Nowadays, the bacteria are grown in fermenters and subsequently lyophilized. They are filled into hard gelatine capsules which are enteric coated in order to open up not before the terminal ileum is reached. After this procedure, they have a shelf-life of several months when kept under refrigeration.

In summary, Mutaflor® is a probiotic drug that contains viable, nonpathogenic bacteria and it is on the market for almost a century.

Mutaflor® – the probiotic drug containing E. coli strain Nissle 1917 as active ingredient.
Significance of *E. coli* for Intestinal Microbial Ecology

The intestinal microflora consists of about $10^{14}$ microorganisms, representing a complex ecological entity with diverse metabolic activities. These microorganisms exceed in numbers the roughly $10^{13}$ cells of our body by a factor of ten. With regard to weight as well as to metabolic activity the intestinal microflora can be compared e.g. to the liver. These purely numerical reflections already point to the significance of the indigenous microflora for physiological as well as pathophysiological processes in the gut.

*E. coli* is the most prominent early colonizer of the sterile gut of the neonate. In this context, it is left to chance (hygienic conditions in the hospital, microbial colonization of the mother) whether the newborn is colonized by commensal or by pathogenic microorganisms.

The metabolic activities of early aerobic colonizers like *E. coli* prepare the intestinal milieu for subsequent colonization by anaerobes. Commensal *E. coli* predominantly colonize in the vicinity of the colonic mucosa (mucus layer). Here they help to establish a microbial barrier against pathogenic and potentially pathogenic invaders. Due to their distinct immunogenicity, they significantly contribute to development and ‘training’ of the gut-associated lymphoid tissue (GALT). In addition, their metabolic products support the energy supply of the colonic mucosa. Furthermore, short-chain fatty acids (SCFA) of microbial origin stimulate water re-absorption, motility and blood circulation within the colonic mucosa.

"Life without gut bacteria would be extremely unpleasant, if not impossible.”

(Gibson and Williams, Brit. J. Nutr., 1999)
The *E. coli* strain Nissle 1917 as contained in Mutaflor® has several specific properties which are important for survival, persistence within the gut ecosystem, and therapeutic effects.

Because of these characteristics the strain is
- readily mobile and able to colonize the gut
- antagonistically active against pathogens
- vital and strong (‘biologically fit’)
- able to modulate the immune system.

The *E. coli* strain Nissle 1917 colonizes the gut by non-pathogenic adhesion to epithelial cells and mucus. For this reason, it expresses so-called type-1 and F1C fimbriae. The strain also expresses two microcins and has an antagonistic effect on other microorganisms. To enhance its growth, multiplying and success, i.e. its vitality or fitness, it possesses different systems for iron uptake, like aerobactin, colibactin, enterobactin, and yersiniabactin, as well as a haemin and a citrate-dependent iron uptake system. It expresses type-H1 flagellae and therefore, is extremely mobile.

The genome of *E. coli* strain Nissle 1917 has been largely sequenced. All gene loci of functionally important structures that are currently known, have already been determined. As an example, a point mutation in the gene for O-antigen polymerase was detected, leading to only one ‘repeating’ unit in the side chain of the lipopolysaccharide (LPS) sugar.

This unusual or rather unique LPS structure enables the Mutaflor® strain to show immunomodulating effects without being immunotoxic. It also confers sensitivity to the antimicrobial activity of blood serum, an important biosafety aspect.

The total of its biological characteristics forms the basis for the specific effects of *E. coli* strain Nissle 1917.

**Strain-specific characteristics of *Escherichia coli* strain Nissle 1917**

**Chromosome**

- mch
- mcm
- fim
- foc
- csg
- fla
- kps
- ybt
- chb
- ent
- aer
- cit
- rfb
- cha
- aer
- cit
- ybt
- ent

**Fitness factors**

- 6 iron-acquisition systems

- Fe⁴⁺
- Fe³⁺
- Fe³⁺
- Fe³⁺
- Fe³⁺

**Antagonistic activity**

- 2 microcins

**Adhesion and formation of biofilms**

- 3 different types of fimbriae

**Known gene clusters of the chromosome, coding for phenotypic characteristics and detected by molecular genetic analyses**

- **Fe³⁺**
- **Fe³⁺**
- **Fe³⁺**

**Special LPS of the O₆ type, not detected in any other *E. coli* so far**

**Capsule (K₅)**

**No pathogenicity factors**

**Flagsellae for active movement (H₁ type)**

The effects of Mutaflor® are strain-specific (*Escherichia coli* strain Nissle 1917).
Modes of Action of *E. coli* strain Nissle 1917

*E. coli* strain Nissle 1917 communicates with the gut epithelium as well as with constituents of the gut microflora (so-called ‘crosstalk’). Some of its interactions are regulated by bacterial ‘quorum sensing’. First DNA expression experiments revealed regulation of about 300 different enterocyte genes being triggered by this bacterial-epithelial communication.

Moreover, there is evidence for *E. coli* strain Nissle 1917 inhibiting the formation and excretion of pro-inflammatory cytokines from the gut epithelium, and stimulating the non-specific immune defense by upregulation of defensins.

Mutaflor® fights multiplication of pathogenic microorganisms and thus prevents invasion by gut pathogens.

**Communication strategies of *E. coli* strain Nissle 1917**

**Relative expression of defensins (HBD-2):** Upon co-cultivation of CaCo2 cells and *E. coli* strains only *E. coli* strain Nissle 1917 highly induces HBD-2 expression.

Antagonism of *E. coli* strain Nissle 1917, co-cultivated with:

1. *Salmonella enteritidis*
2. *Shigella dysenteriae*
3. *E. coli* O112 ab (EPEC)
4. *E. coli* O6:K15:H31 (UPEC)
5. *Proteus vulgaris*
6. *Candida albicans*

Mutaflor® research is greatly contributing to our understanding of the modes of action of probiotic drugs.
When using viable bacteria for therapy, their clearly defined identity as well as guaranteed purity are prerequisite. Necessarily, those bacteria have to be devoid of the following traits:

- Formation of enterotoxins and cytotoxins,
- enteroinvasiveness,
- formation of hemolysins,
- serum resistance, as well as uropathogenicity.

Mutaflor® meets all of today’s safety requirements and may therefore be introduced into the gut of even newborn and prematurely born children, using a high count of live bacteria. Non-pathogenicity of the Mutaflor® strain has been proven to the genetical level by using methods of molecular biology. The *Escherichia coli* strain Nissle 1917 – designated DSM 6601 in the German Collection for Microorganisms in Braunschweig – is one of the best-examined therapeutically relevant bacterial strains worldwide.

### Precise characterization of *E. coli* strain Nissle 1917

<table>
<thead>
<tr>
<th>Typing Method</th>
<th>Specific characteristics, e.g.:</th>
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<tbody>
<tr>
<td>Serological</td>
<td>No production of enterotoxins (Shiga toxins, heat-stable and heat-labile toxins)</td>
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<tr>
<td>Biochemical/ microbiological</td>
<td>No production of cytotoxins (CNF)</td>
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<td></td>
<td>No enteroinvasiveness</td>
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<td></td>
<td>No pathogenic adhesion factors (e.g. no CFA I/II, P, M and S fimbriae)</td>
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<tr>
<td></td>
<td>No hemolysins</td>
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<td></td>
<td>No serum resistance</td>
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<td>No uropathogenicity</td>
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<td>No antibiotic-resistance genes</td>
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**Mutaflor® fulfills all biosafety requirements for a modern probiotic drug.**
Evidence for Efficacy of Mutaflor®

Efficacy of Mutaflor® has been shown over more than 80 years of experience and was confirmed in numerous clinical trials.

### Indications
- **Ulcerative colitis**
- **Crohn’s disease**
- **Pouchitis**
- **Collagenous colitis**
- **Irritable bowel syndrome**
- **Chronic constipation**
- **Chronic diarrhea**
- **Antibiotic-associated/pseudomembranous colitis**
- **Diverticular disease of the colon**
- **Prophylaxis against colonization of pathogens and enhanced immunity of newborn and prematurely born infants**
- **Intestinally caused halitosis**
- **Polymorphous light eruption**

### Authors
- Schütz 1989
- Rembacken et al. 1999
- Kruis et al. 2004
- Malchow 1997
- Kuzela et al. 2001
- Tromm et al. 2004
- Schütz 1989
- Bruckschen et al. 1994
- Möllenbrink et al. 1994
- Schütz 1989
- Goerg et al. 1998
- Frič et al. 2003
- Schröder 1992
- Cukrowska et al. 2002
- Henker et al. 2001
- Wurzel 1999

### Ulcerative colitis

For maintenance of remission of ulcerative colitis with Mutaflor®, there are 3 controlled, randomized, double-blind (double-dummy) clinical studies which have proven equivalence of Mutaflor® and mesalazine.

<table>
<thead>
<tr>
<th>Patient numbers</th>
<th>Design/trial duration</th>
<th>Author/year</th>
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<tbody>
<tr>
<td>53 mesalazine</td>
<td></td>
<td></td>
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<tr>
<td>57 Mutaflor®</td>
<td>randomized, double-blind, 1 year</td>
<td>Rembacken et al. 1999</td>
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<tr>
<td>59 mesalazine</td>
<td></td>
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<tr>
<td>162 Mutaflor®</td>
<td>randomized, double-blind, multicentric, 1 year</td>
<td>Kruis et al. 2004</td>
</tr>
<tr>
<td>165 mesalazine</td>
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</table>

Randomized double-blind trials with Mutaflor® for remission maintenance in patients with ulcerative colitis, proving equivalence of Mutaflor® and mesalazine.

The first of these studies was carried out in multiple centers over a period of 12 weeks on a total of 103 patients. The treatment consisted of either 2 capsules Mutaflor® once daily (day 1–4: one capsule once daily) or mesalazine 500 mg 3 times daily. There were no significant differences between the two groups with respect to the following criteria:
- Clinical activity index (CAI)
- Relapse rate: 16.0 % for Mutaflor® versus 11.3 % for mesalazine
- Time to relapse: $106 \pm 5$ days for Mutaflor® versus $103 \pm 4$ days for mesalazine.

In a second study, 116 ulcerative colitis patients were included during the acute episode and treated with gentamycin 80 mg 3 times daily (day 1–7) and prednisolone at a gradually reduced dosage. As the trial medication, patients received either 2 capsules Mutaflor® twice daily or mesalazine 800 mg 3 times daily. After remission had been achieved, a maintenance treatment with either 2 capsules Mutaflor® once daily or mesalazine 400 mg 3 times daily was carried out. Patients who did not show remission within 12 weeks were excluded from the study; all others were treated and observed for 12 months. The median duration of remission was 185 days for Mutaflor® and 175 days for mesalazine. During the course of one year, 67 % of the Mutaflor® patients and 73 % of the mesalazine suffered a relapse. There was no statistically significant difference between these two groups.
On the one hand, these strikingly high relapse rates were due to the fact that patients were included during an acute episode. This per se results in a higher relapse rate than with including patients after a long-lasting period of remission. On the other hand, the authors used a very stringent definition of relapse according to which, unlike the international standard, even slight changes in the clinical and endoscopic picture were regarded as relapses of disease.

In the third study on remission maintenance of ulcerative colitis with Mutaflor®, 327 patients (54.7% male) were enrolled. The study was carried out in multiple centers (60 centers in 10 European countries) over a period of one year. The treatment consisted of either 2 capsules of Mutaflor® and 3 tablets of mesalazine placebo or 2 capsules of Mutaflor® placebo and 3 tablets of mesalazine 500 mg daily. Relapse was defined by a CAI $\geq 6$ or an increase of the CAI by at least 3 points with simultaneously exceeding a CAI of 4 and by an endoscopically and histologically ascertained inflammation.

Of the total of 327 patients, 222 were included in the per-protocol analysis (Mutaflor® n = 110, mesalazine n = 112). Of these patients, 36.4% of the Mutaflor® group and 33.9% of the mesalazine group suffered a relapse.

Both in the per-protocol analysis and in the intention-to-treat analysis, the efficacy of the two drugs was equivalent ($p < 0.01$ and $p < 0.02$, respectively, one-sided test of equivalence). Both therapeutic strategies proved to be well tolerated.

Crohn’s disease

There are numerous experience reports from clinical practice and the results of a pilot study on the treatment of Crohn’s disease with Mutaflor®. In patients with Crohn’s disease of the large intestine, there were far fewer relapses in the group treated with Mutaflor® than in the control group (33.3% versus 63.6%).

The treatment was carried out for one year, and the adjuvant administration of Mutaflor® also reduced steroid use. The Mutaflor® treatment clearly showed a lower side-effect potential than the steroid treatment. Considering the long period for which a remission-maintenance treatment may be required, this aspect becomes even more important.
Chronic functional bowel diseases

Mutaflor® is effective in the therapy of chronic functional bowel diseases, as was shown in various clinical studies. E.g., in a drug application survey on patients with chronic recurrent diarrhea (n = 269) and patients with irritable bowel syndrome (n = 228), a “very good” or “good” therapeutic result was established for Mutaflor® in 85% and 76% of patients, respectively (Schütz 1989).

There are two randomized studies in patients with chronic constipation treated with Mutaflor®. In a placebo-controlled, double-blind, cross-over trial, the mean number of bowel movements per week was significantly increased under Mutaflor® compared to a placebo already in the fourth week of treatment, and the frequency increased further in the Mutaflor® group until the end of the study (after 8 weeks).

In a second randomized study in patients with chronic constipation, Mutaflor® proved to be at least as efficacious as lactulose.

Diverticular disease of the colon

A first pilot study resulted in improvement of the symptoms of diverticular disease of the colon by therapy with Mutaflor®.

Symptomatic diverticular disease of the colon, improvement of complaints under Mutaflor®, Frič et al. 2003

The clinical efficacy of Mutaflor® was proven by clinical trials conducted according to GCP (good clinical practice).
Storage precautions
Mutaflor® should be stored under refrigeration (2–8°C)! A short interruption of refrigeration does not do any harm to the product. In cases of prolonged storage at room temperature or even higher temperatures, bacteria will lose their viability but not their safety profile.

Combination with antibiotics
If treatment with antibiotics is necessary, in principle these can also be combined with Mutaflor®. If the antibiotic is directed against Gram-negative bacteria, however, loss of efficacy of Mutaflor® is to be expected.

With antibiotics that are mainly directed against Gram-positive bacteria (such as Clindamycin, Erythromycin, Metronidazol, Penicillin G, Quinupristin/Dalfopristin, Rifampin, Teicoplanin, Vancomycin, Cefsulodin), no loss of efficacy of Mutaflor® occurs.

Dosing of Mutaflor® capsules
Adults and teenagers should take 1 capsule Mutaflor® daily from day 1 to day 4, then the standard dose of 2 capsules Mutaflor® daily. In case of persistent constipation it may be useful to increase the dose up to 4 capsules Mutaflor® per day.

The standard dose should be taken with a meal, if possible with breakfast, and an appropriate amount of fluid. Avoid to chew the capsules.

In order to avoid initial flatulence due to the high metabolic activity of E. coli strain Nissle 1917, the dose may be slowly increased by first taking Mutaflor® mite. With onset of flatulence or with a daily dose higher than the standard dose, the daily dose may be split and administered with meals spread evenly throughout the day.

In case of ulcerative colitis, experience from controlled trials exists for a duration of administration of 12 months. For relapse prevention in ulcerative colitis, Mutaflor® should be taken continuously.

Indications according to registration
Ulcerative colitis in the phase of remission
Chronic constipation

Further indications as suggested by clinical trials
Crohn’s disease
Pouchitis
Collagenous colitis
Antibiotic-associated colitis/pseudomembranous colitis
Irritable bowel syndrome
Diverticular disease of the colon
Polymorphous light eruption

Further indications as suggested by practice reports/case histories
Non-ulcer dyspepsia
Food intolerance / malabsorptions
Halitosis
Infection sensitivity
Mycoses of the orogastrointestinal tract
Others: E.g. atopic eczema, reactive arthritis, radiation enteritis, urinary tract infections

Mutaflor® guarantees safe, effective and economical therapy!
Clinical Studies


Further evidence and preclinical studies

**Mutaflor® / Mutaflor® mite**

*Active ingredient: Escherichia coli strain Nissle 1917*

**Presentations:** 1 gastro-resistant hard capsule contains:

- **Mutaflor®:** *E. coli* strain Nissle 1917 (8.74 – 43.68 mg) with 2.5 to 25 x 10⁹ viable cells
- **Mutaflor® mite:** *E. coli* strain Nissle 1917 (3.49 – 17.41 mg) with 0.5 to 5 x 10⁹ viable cells

**List of excipients:** Maltodextrin, talc, methacrylic acid-methyl methacrylate copolymer (1:1), macrogol, dibutyl phthalat, glycerol, titanium dioxide, iron (III)-hydroxide-oxide monohydrate, gelatine, beeswax (yellow), carnauba wax, shellac, purified water.

**Therapeutic indications:** Ulcerative colitis in the phase of remission, chronic constipation.

**Contraindications:** Hypersensitivity against any ingredient of the preparation.

**Adverse drug reactions:** Frequently, initial flatulence occurs. Changes in stool consistency or stool frequency, abdominal pain, borborygm, meteorism, nausea, vomiting, cases of skin efflorescences, erythema, or skin flaking were observed very rarely. Also very rarely, headache was reported.

**Dosage, mode and duration of administration:**

- **Mutaflor®**
  - **Adults and teenagers:** *Standard dose:* From day 1 to day 4, 1 capsule Mutaflor® daily, then 2 capsules Mutaflor® daily. In case of persistent constipation it may be useful to increase the dose up to 4 capsules Mutaflor® per day.
  - The standard dose should be taken with a meal, if possible with breakfast, and an appropriate amount of fluid. Avoid to chew the capsules. In order to avoid initial flatulence the dose may be slowly increased by first taking Mutaflor® mite. With onset of flatulence or with a daily dose higher than the standard dose, the daily dose may be split and administered with meals spread evenly throughout the day.
  - In case of ulcerative colitis, experience from controlled trials exists for a duration of administration of 12 months. For relapse prevention in ulcerative colitis, Mutaflor® should be taken continuously.

- **Mutaflor® mite**
  - **Adults and teenagers:** In order to reach the standard dose of Mutaflor® for adults and teenagers individually, Mutaflor® mite should be increased according to the following scheme:
    - During the first 4 days 1 capsule Mutaflor® mite per day, for the next 2 days 2 capsules Mutaflor® mite per day and for the following days 3 capsules Mutaflor® mite per day should be given. As soon as 3 capsules Mutaflor® mite are well tolerated, the Mutaflor® standard therapy is to begin. The standard dose should be taken with a meal, if possible with breakfast, and an appropriate amount of fluid. Avoid to chew the capsules.
  - **Children:** Ulcerative colitis in the phase of remission: No dosage recommendation can be given due to lack of clinical trials in children.
  - **Constipation:** During the first 4 days 1 capsule Mutaflor® mite per day, from day 5, 2 capsules Mutaflor® mite per day. With constipation, Mutaflor® mite may be administered up to 6 weeks.

**Storage precautions:** Store under refrigeration (2 – 8 °C)!

**Package sizes:** Mutaflor® packs with 20 capsules / 50 capsules / 100 capsules
- Mutaflor® mite packs with 20 capsules

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