Biolex MB40

Effect of brewers' yeast cell walls on pathogen binding and gut barrier integrity after infection

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Escherichia coli (E. coli) is the most important representative of the Gram-negative bacterial genus Enterobacteriaceae. It forms part of the normal flora of both the small and large intestines of warm-blooded animals and humans. But they are also able to trigger different diseases. Endo-, entero- and cytotoxins as well as adhesion factors are important in the pathogenic effects of E. coli. Newborn and young animals such as puppies, kittens and foals often suffer from diarrhoea after E. coli infection. In adult dogs, E. coli has also been reported to be involved in urinary tract infections, uterine inflammation, and septicaemia. Sources of infection can be other animals or humans as well as contaminated food and particular types of food such as raw meat, but also water. Polish scientists (WOLNY - KOLADKA, 2018) were able to detect a total of 200 different strains of E. coli from smears of air, manure and nostrils in equine stables - many of them were resistant to several commercially available antibiotics. E. coli is mainly found in fresh faeces. Through cross-contamination, multiresistant germs (ESBL - Escherichia coli producing extended spectrum beta-lactamase) can also be transmitted to humans or, conversely, from humans to animals. This is seen as an increasing risk because it not only applies to horses, also to other animals such as cats and dogs. The latter are often even closer to humans, their living spaces and frequently even their beds. ESBL-carriers can cause serious diseases. There is a higher risk of infection especially for those with a weakened immune system, e.g. kittens and puppies, elderly animals and animals weakened by illness, but also newborn babies or humans suffering from autoimmune or chronic diseases.

Specific components of brewers' yeast have already been shown to improve resistance against infections in animals. The potency of **Biolex® MB 40** to reduce pathogen binding or to protect gut barrier integrity after infection was evaluated in different in vitro and in vivo trials.

Trial I:

Highly purified beta-glucan and a brewer's yeast cell wall **(Biolex® MB40)** with a natural content of mannan oligosaccharides (MOS) and ß-gllucans were tested (NIZO 2013). Caco-2 cells were used as a model for the intestinal epithelium. The effect of yeast cell wall components on the adhesion of E. coli (ETEC strain H10407) was evaluated in decoy experiments. Caco-2 cells were exposed to a mixture of yeast cell wall components and pathogens, after which adhering pathogens were measured by CFU counts after O/N culture on agar plates. Barrier integrity was evaluated using differentiated Caco-2 cells, cultured on transwell filters. This would show the components' ability to reduce the risk of infections such diarrhoea. Transepithelial electrical resistance (TEER) was used as a measure of barrier integrity as an indicator of the potential of yeast cell wall components to protect gut barrier integrity, which is important for the resistance against pathogens.

Additionally, the area under the curve (AUC) of the different conditions was calculated, with subtraction of the AUC of the negative control (infection with ETEC alone).

The higher the AUC, the higher the protective effect on barrier integrity. As positive control, zinc oxide (ZnO) was included, which has been described as reducing pathogen binding (ROSELLI, 2003).

As negative control, the pathogen was incubated in medium without any addition, or in a medium containing 1-0.6 -0.3 mg/ml cellulose. Cellulose was included to mimic sedimentation of the yeasts but does not have any pathogen binding capacity.

Results:

Decoy experiments (Fig. 1) shows the effect of yeast cell wall components on binding of ETEC to Caco-2 cells. Purified Beta-Glucans and esp. **Biolex® MB40** reduced significant ETEC binding to Caco-2, whereas native brewers' yeast and autolysed yeast showed no effect on ETEC binding.

Figure 1: Pathogen binding as measured in an anti-adhesion assay. Cells were exposed to medium (neg. control), a positive control (zinc oxide 1 mM), and yeast components 1-5 at 4 different concentrations (3 – 1 – 0.33 – 0.11 mg/mL), mixed with pathogens (107 CFU/mL). Values are presented as mean (n=3) ± SEM. * p<0.05 compared to negative control. 1. Native brewers' yeast; 2. Yeast autolysate; 3. Beta-glucan; 4. Biolex® MB40 Batch 08/2013; 5. Biolex® MB40 Batch 07/2013.

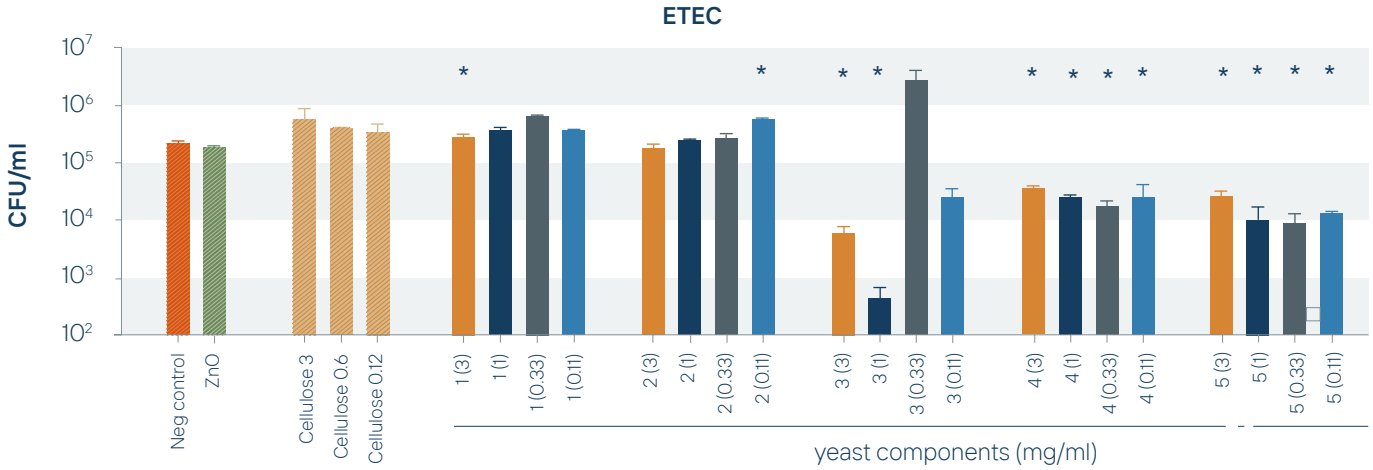
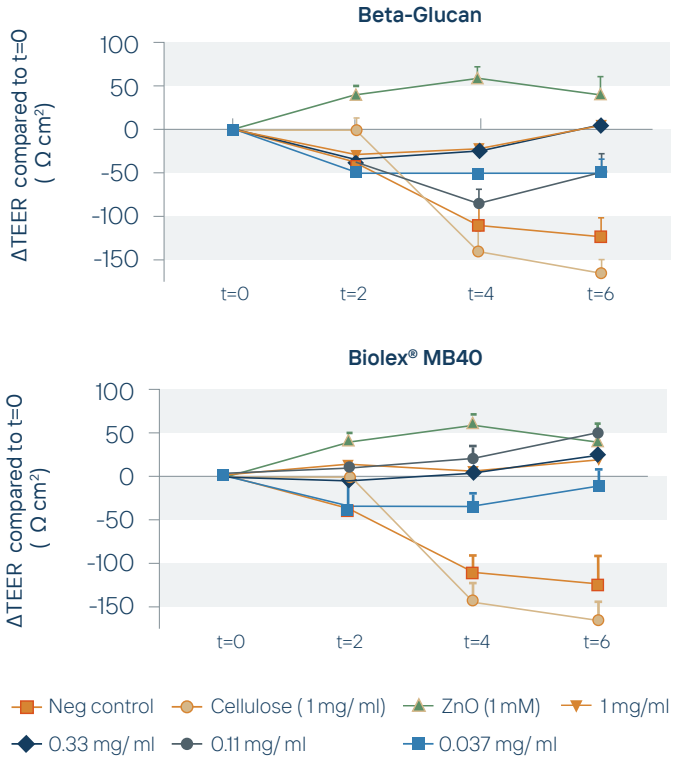


Figure 2 shows the results of the change in TEER upon exposure to the pathogens. Results of every well were related to their own TEER value at t=0, and expressed as ΔTEER (Ω.cm²). Upon infection, TEER continuously decreased in wells infected with ETEC alone (negative control) and remained unchanged in wells incubated with ZnO (positive control).

Figure 2: Change in TEER upon exposure to ETEC for 2 h, 4 h and 6 h. ΔTEER is shown as mean (n=3) ± SEM.



After 6 h of incubation, barrier integrity of the negative control significantly decreased by 123 Ω.cm² compared to baseline values (t=0). Barrier integrity of the positive control stayed unchanged.

Most of the individual TEER values of the wells incubated with beta Glucan or **Biolex® MB 40** fell between the values of the negative and positive control.

Biolex® MB40 was able to attenuate a decrease in TEER. The concentrations of 1, 0.33 and 0.11 mg/ml were able to keep TEER values around baseline. Only at the lowest concentration did TEER values slightly decrease over time.

When comparing this effect to ZnO, used as positive control, the ΔTEER values may suggest that ZnO is more potent in protecting against barrier disruption than **Biolex® MB40**.

However, this is mainly due to the fact that ZnO itself caused a TEER drop during the first hour of incubation, from which the Caco-2 cells recovered during the subsequent incubation time. In contrast, **Biolex® MB40** showed a stable TEER value over time (see Fig. 3).

Figure 3: Change in absolute TEER values upon exposure to yeast components alone (t=-1 to =0) and yeast components in the presence of ETEC t= 0 to=2 h, 4 h and 6h.

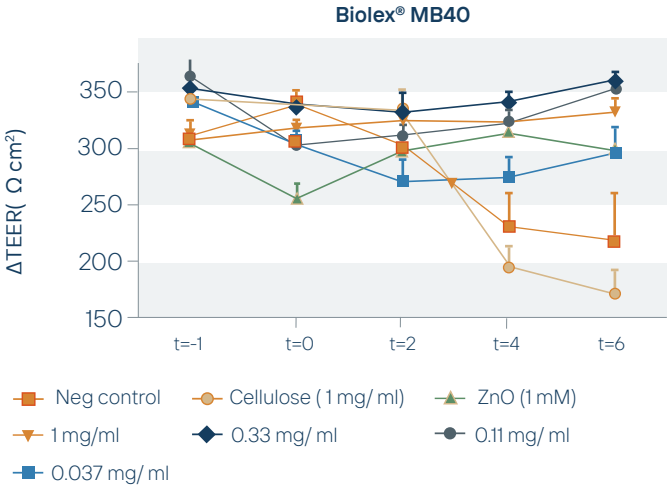
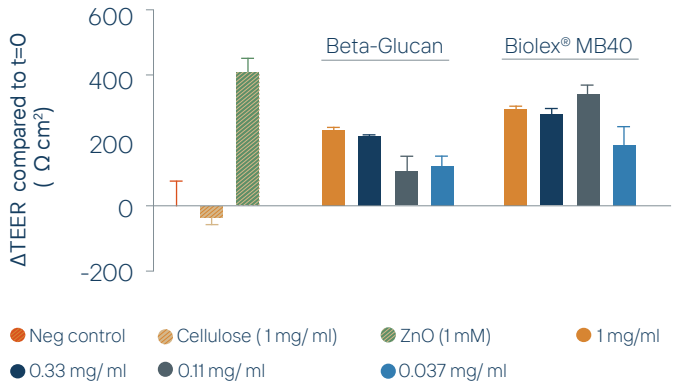


Figure 4 shows the area under the curve (AUC) over time for the yeast components compared to the negative control, providing an impression of the overall effect of the yeast cell wall components over time. It was observed that the total response over time (AUC) of ZnO was significantly different to the control condition, which is likely due to its effect on pathogen binding to Caco-2 cells. Also, a difference could be observed for wells incubated with beta-glucan and **Biolex® MB40**. Although the effects of these yeast components were not significant, they showed a trend towards protection against a decreased barrier function after infection, with the strongest and most consistent effect observed for **Biolex® MB40.**

Figure 4: AUC of the effects of the yeast components on TEER. AUC, calculated by subtraction of the AUC of the negative control (infection with ETEC alone). Values are presented as mean (n=3) ± SEM. Significant differences between the negative control and the cells incubated with yeast components are identified with an asterisk.



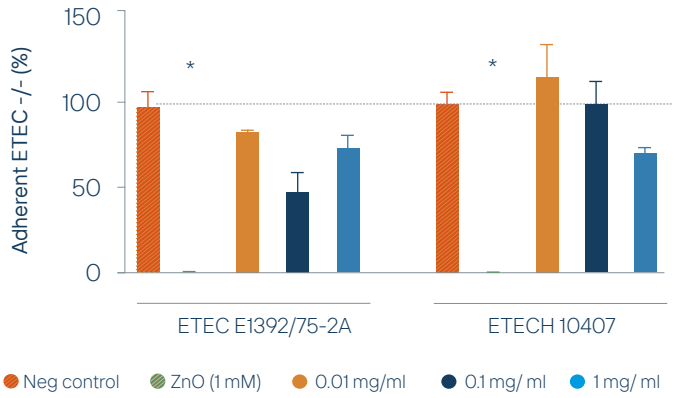
In a „Follow – Up" (NIZO 2014) the effect of **Biolex® MB40** on adhesion of ETEC H10407 (wild type E. coli strain) and ETEC E1392/75-2A (live-attenuated E. coli strain) was evaluated in decoy experiments. Figure 5 shows the results of the effect of **Biolex® MB40** on the adherence of ETEC H10407 and ETEC E1392/75-2A to Caco-2 cells. The positive control, ZnO, showed a significant reduction in binding of both ETEC strains compared to the negative control. A not significant, but dose dependent, reduction of binding of ETEC H10407 to Caco-2 cells was also observed for **Biolex® MB40**. ETEC E1392/75-2A binding to the cells was significantly reduced with 0.1 mg/ml and a trend in reduced binding was observed for the other concentrations.

An initial step of gut infection is attachment of pathogens to the gut cell surface. The ability to prevent this interaction is by binding directly to the pathogens, displacing pathogens from the gut mucosal layer or occupying the specific attachment sites on gut cells. **Biolex® MB 40** provide a large surface with a high capacity for binding and deactivating. The mannan-containing structures are thought to block pathogen binding mediated by type 1 fimbriae, which are known to be mannose-sensitive.

In addition, the beta-glucans of the yeast cell wall has been described to reduce pro-inflammatory responses in intestinal epithelial cells (SAMUELSEN et al, 2011).

Combining all results, **Biolex® MB40** can be concluded to have a beneficial effect on pathogen binding, pathogen viability, barrier integrity and barrier protection. It could thus play a beneficial role in resistance against infection.

Figure 5: Decoy results for Biolex® MB 40.
* p<0.05 compared to negative control.



Trial II:

In cooperation with the University of Ghent, we carried out a study with the aim of assessing the potential of the prebiotic activity of **Biolex® MB40** in the canine gastrointestinal tract (ABBEELE et al., 2020). An in vitro system (SCIME ™ Simulator of the Canine Intestinal Microbial Ecosystem) was used, able to reproduce the entire canine digestive tract with all microbiological processes under controlled conditions, without direct work on animals. A prebitotic effect of **Biolex® MB40** on the canine gastrointestinal digestive tract could be demonstrated:

- **significantly boosting the growth of health-promoting bacteria**
- **significantly increasing the production of health-related bacterial metabolites (SCFA- short chain fatty acids) such as propionate and butyrate**
- **decreasing intestinal pathogens such as Fusobacteria and Enterobacteriaceae (see Table 1)**

Biolex® MB40 has a positive effect on the composition and activity of microorganisms and is a promising natural agent for improving and protecting intestinal health in the canine gastrointestinal tract.

Trial III:

ESBL (Extended Spectrum Beta Lactamase) describes a protein produced in multiresistant intestinal bacteria and capable of destroying several important antibiotics. The bacteria of the Enterobacteriaceae family (e.g. E. coli) are selectively multiplied and ultimately form the new group of multiresistant ESBL germs. They are playing an increasing role in infections because of the risk of cross-contaminations between humans and animals.

Table 1: Microbial composition at the familiy level. Abundances (%) of the dominant families, evaluated by 16S Illumina sequencing, during control period (C) and treatment (TR) in luminal samples within the proximal (PC) and distal colon (DC) at three different concentrations Biolex®MB 40 (n=1). The intensity of shading indicates the absolute frequency displayed for each of the different families.

Phylum	Family	PC						DC					
		0.5g/day		1g/day		2g/day		0.5g/day		1g/day		2g/day	
		C	TR	C	TR	C	TR	C	TR	C	TR	C	TR
Fusobacteria	Fusobacteriaceae	0.0	0.0	0.0	0.0	0.0	0.0	15.7	10.8	15.9	10.6	13.0	9.4
Proteobacteria	Enterobacteriaceae	11.2	1.6	6.9	2.3	15.1	3.2	1.6	0.3	1.9	0.6	4.9	1.3

In Germany, the proportion of ESBL-producing E. coli in all E. coli from clinical examination materials (human) increased from 7 to 13% between 2008 and 2014 (Robert Koch Institute, September 18, 2016: (http: // ars .rki.de). According to BELAS (2014), up to 55% of dogs and up to 25% of cats were colonized in a range of countries. In general, ESBL are harmless to healthy people, colonization with ESBL bacteria is thus usually not treated. However, carriers can serve as a source for the transmission of the ESBL intestinal bacterium to others, e.g. in animal clinics, animal shelters, animal boarding houses or in a dog park. Patients with an underlying chronic condition, with chronic skin wounds or after major surgery are at an increased risk.

In a Turkish study (YIĞIN, 2021), the presence of ESBL genes in E. coli strains isolated from horse farms in Eastern Turkey was also detected. A total of 200 equine faecal samples were collected from 16 horse farms (70 thoroughbred and 130 Arabian horses). Out of 200 E. coli strains, 107 (53.5%) tested positive for at least one gene. ESBL-producing E. coli strains were frequently seen in the racehorses from Eastern Turkey.

Another study in Tyrol, Austria (FRANIEK et al., 2012) showed that ESBL-producing E. coli and EHEC as a possible source of human infection. Of the 228 faecal samples from dogs (n = 62) and cats (n = 134) examined, ESBL-producing E. coli could be isolated in twelve of the 228 (5.3%) faecal samples. The animals testing positive for ESBL-producing bacteria came mainly from animal welfare institutions (83%).

An in vitro test (ZENTEK, 2021) aimed to determine whether **Biolex® MB40** leads to a reduction in ESBL-positive enterobacteria in dogs. For this purpose, an ESBL-bearing Escherichia coli model strain was used, the survival of which was tested after incubation in dog faeces with yeast products.

The resulting growth curves from this incubation served as a measure of the survival of the E. coli model strain (e.g. REN et al., 2019). An E. coli strain (ESBL 10716, Phylo-type B1), was used as a model strain for ESBL-carrying enterobacteria. Only faecal samples from dogs (n = 3) without a positive result on ESBL were included in the experiment.

The most important parameter for the survival of ESBL is the LAG time, which describes both the amount of surviving bacteria and their "fitness". An increase in the LAG time compared to the control, means that the "fitness" of the bacterium is reduced by adding **Biolex® MB40.**

Figure 6 shows the percentage change in LAG time compared to the control incubations. **Biolex® MB40** demonstrated numerical directed changes in the LAG time with increasing concentration. A significant difference to the control was found at a concentration of 0.8 g / l (see Fig 6).
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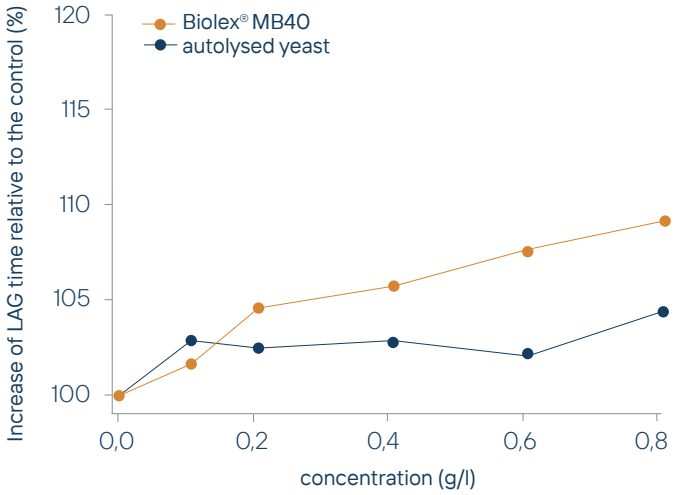
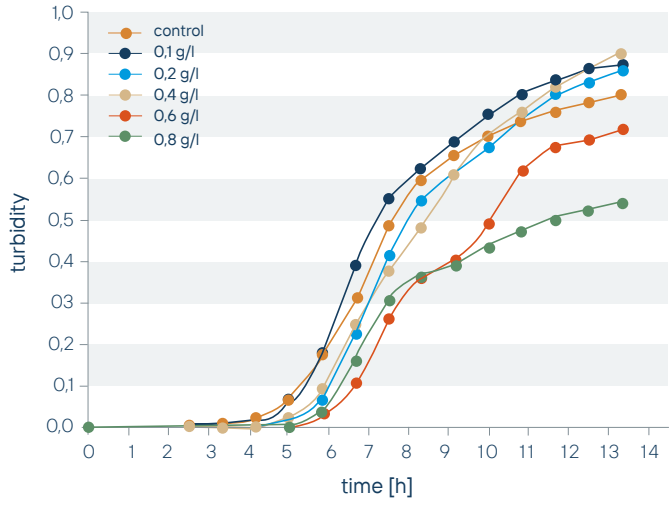
Figure 6: Changes in LAG time of E. coli model strain with increasing concentrations of Biolex ® MB 40





Growth curve of Biolex® MB 40

Biolex® MB40 showed a directed trend with regard to the inhibition of the ESBL model strain. **Biolex® MB40** seems to have a greater impact on the inhibition of pathogen growth than on direct binding. Due to its prebiotic effect, **Biolex® MB40** promotes the health-releated bacteria, while potential pathogens are displaced (see Trial II). Metabolisation of **Biolex® MB40** increases the survival pressure on the ESBL bacteria.

Take-home message for Biolex® MB40:

Mannose-binding lectins from yeast cell walls recognise carbohydrate patterns on the surface of many pathogens

| High binding strength and deactivation of pathogens and toxins in the gut lumen

- significant reduction of ETEC bindin to intestinal epithelial Caco-2 cells
- protection against gut barrier distruption induced by ETEC

| significant increase in the production of health-related bacterial metabolites (SCFA-short chain fatty acids) such as propionate and butyrate

- increasing mucin production (more goblet cells) that can limit the attachment of pathogens to the ephitel cells

| significantly boosting the growth of health-promoting bacteria

- inhibiting pathogens are inhibited from colonizing and attaching to the gut epitheliumsuch as e.g. Fusobacteria and Enterobacteriaceae
- propensity to inhibit the growth of ESBL

Biolex® MB40 could play a beneficial role in the reduction of pathogen binding or the protection of gut barrier integrity after infection.

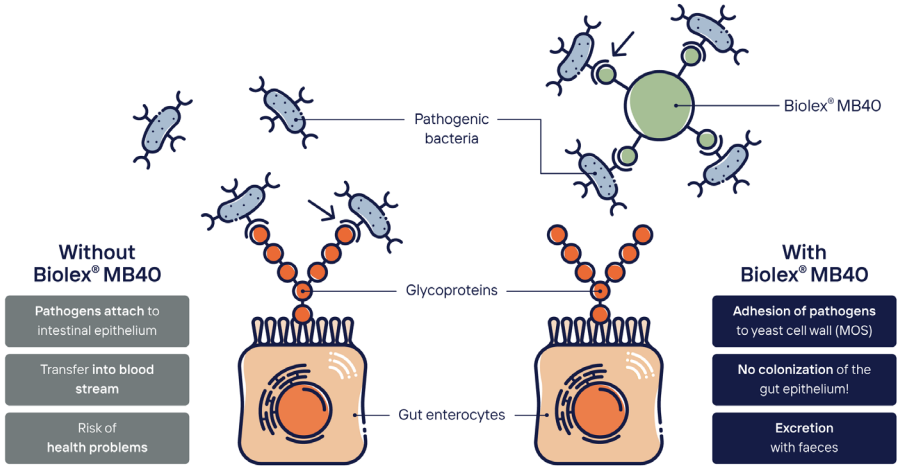


Abb.: binding effect of Biolex® MB 40

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