Evidence for Self-Medication
International Review Journal

In vitro tests allow a better understanding of the therapeutic effects of commercialized probiotic strains

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The gut microbiome is unique to each person and has a major impact on health. Orally administered probiotics are used to prevent and/or treat gastrointestinal (GI) disorders and additionally show potential in the treatment of non-GI conditions. In vitro studies lead to a better understanding of the biological properties of therapeutically used microorganisms and the positive effects they can have in vivo.

Two in vitro studies tested properties of nine established microbial strains isolated from commercial preparations (see **Tab. 1**) [1, 2]. Due to the complexity of the gastrointestinal tract, individual in vitro results cannot definitively explain the physiological effects. But they provide important information for clinical research and for the understanding of in vivo effects.

Probiotics and their stability in simulated intestinal fluid

The investigated probiotic strains exert their positive effects in the intestine, which is why the stability of the cells under simulated intestinal conditions is relevant (see **Tab. 1**). Noteworthy is the ability of the different *B. clausii* strains (*Bacillus clausii* OC, NR, SIN, T) to multiply after an initial decrease in cells count without nutrient sources in the simulated intestinal fluid (*B. clausii* SIN: decrease after 2 h compared to t_0 [$p < 0.05$], proliferation after 8 h of incubation compared to 4 h [$p < 0.05$]). After 8 hours, there was only a slight reduction of 0.240-Log compared to t_0. The tolerance of *B. clausii* and *B. coagulans* to simulated intestinal conditions is well documented considering their ability to form spores compared to non-spore forming strains usually found in commercial products [1].

Probiotics and their binding to host cells

Probiotic microorganisms can compete with pathogens for mucosal binding sites and thus, counteract infections caused by pathogenic organisms. For this effect, adhesion to the gastrointestinal mucus is necessary. The incubation of microbes on agar containing porcine mucins is an established method to study this binding behavior. The mucin-containing agar plates as well as mucin-free agar plates for negative control were inoculated with the bacterial suspension. The plates were then incubated at 37 °C under both, aerobic and anaerobic conditions and the number of cells (CFU [colony forming units]) per inoculated well was determined. In *B. clausii* strains, *B. coagulans* and *B. breve*, the CFU/well obtained after incubation of mucins under both aerobic and anaerobic conditions was significantly higher compared to the negative controls ($p < 0.05$ to $p < 0.001$). *L. reuteri* adhered to mucins only under anaerobic conditions ($p < 0.001$), *S. boulardii* only under aerobic conditions ($p < 0.01$) [1].

Probiotics for lactose intolerance

Probiotics can produce food-degrading enzymes, such as β-galactosidase, which may support digestion e.g. in people with lactose intolerance by potentially reducing digestive symptoms. All *B. clausii* strains, *B. coagulans*, *B. breve* and *L. reuteri* strains were able to produce significantly more β-galactosidase compared to the negative control ($p < 0.01$ to $p < 0.001$) [1].

Probiotics for oxidative stress

Due to the numerous metabolic processes within cells, an accumulation of reactive oxygen species (ROS) may cause toxic effects. Probiotics that produce antioxidants such as catalase (CAT) and superoxide dismutase (SOD) may be beneficial in reducing oxidative stress. All tested strains showed the ability to produce CAT and SOD [1].

Probiotics for vitamin deficiency

Probiotics have been shown to primarily produce B vitamins which could be helpful in maintaning gut eubiosis and address

Evid Self Med 2024;4:240013 | https://doi.org/10.52778/efsm.24.0013
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certain forms of deficiency. Probiotic microorganisms that are able to secrete riboflavin (vitamin B_2) could compensate a vitamin B_2 deficiency of the host. Riboflavin deficiency is frequently due to a diet lacking riboflavin-rich products and is the most common vitamin deficiency in developing countries. *B. clausii, B. coagulans* and *L. rhamnosus* were able to produce riboflavin ($p < 0.001$ compared to the negative control) [1].

Probiotics to support physiological balance through short-chain fatty acids (SCFA)

During microbial fermentation of complex carbohydrates in the human intestine SCFA are produced. The connection between SCFA deficiency and the occurrence of various diseases is confirmed, as well as the curative effects of probiotic microbacteria, which can counteract SCFA deficiency.

Acetic acid: Regulation of lipid metabolism and body weight. All nine probiotic strains tested were able to secrete acetic acid [2].

Propionic acid: Improvement of barrier function as well as intestinal integrity, glucose, and lipid homeostasis.
The four *B. clausii* strains, as well as *S. boulardii*, secreted propionic acid. *B. coagulans, B. breve, L. reuteri* and *L. rhamnosus* did not secrete propionic acid [2].

Butyric acid: Improvement of barrier function as well as intestinal integrity, source of energy for intestinal epithelial cells.
The four *B. clausii* strains showed comparable secretion, which was higher than that of *L. reuteri* and S. *boulardii* [2].

Tab. 1. Overview of the in vitro properties of each microbial strain

Bacterial strain	Survival in intestinal fluid	Binding to mucins (aerobic)	Binding to mucins (anaerobic)	Production of β-galactosidase	Production of catalase and superoxide dismutase	Production of riboflavin	Production SCFA: Acetic acid	Production SCFA: Propionic acid	Production SCFA: Butyric acid
Bacillus clausii NR	+[1]	+	+	+	+[4]	+	+	+[6]	+
Bacillus clausii OC	+[1]	+	+	+	+[4]	+	+	+[6]	+
Bacillus clausii SIN	+[1]	+	+	+	+	+	+	+[6]	+
Bacillus clausii T	+[1]	+	+	+	+[4]	+	++[5]	++[6]	+
Bacillus coagulans ATCC 7050	+[1]	+	+	+	+	+	+	–	–
Bifidobacterium breve DSM 16604	–[1]	+	+	+	+	–	+	–	–
Limosilactobacillus reuteri DSM 17938	+[1]	–	+	+	+	–	++[5]	–	+
Lacticaseibacillus rhamnosus ATCC 53103	+[1]	–[2]	–[2]	–[3]	+	+	+	–	–
Saccharomyces boulardii CNCM I-745	+[1]	+	–	–[3]	+	–	+	+[6]	+

[1] The bacterial strains *B. clausii* NR, OC, SIN and T, as well as *B. coagulans*, *L. reuteri*, *L. rhamnosus* and *S. cerevisiae* survived under simulated intestinal conditions for up to 480 minutes, while in B. breve no living cells were detectable after 6 hours.
[2] *L. rhamnosus* was unable to bind to mucins under both aerobic and anaerobic conditions ($p < 0.01$ and $p < 0.001$, respectively).
[3] *L. rhamnosus* and *S. boulardii* did not produce β-galactosidase.
[4] *B. clausii* OC showed higher SOD activity compared to NR and T ($p < 0.01$ and $p < 0.05$, respectively).
[5] *B. clausii* T and *L. reuteri* were the strongest producers of acetic acid.
[6] *B. clausii* T produced the highest concentrations of propionic acid, which differed significantly from *B. clausii* NR ($p = 0.0374$), *B. clausii* SIN ($p = 0.0112$) and *S. boulardii* ($p = 0.0007$).

Summary

A deeper understanding of probiotic mechanisms may allow a more selective application of microbiota-based treatments to patients. Future studies based on this may clarify which potential further therapeutic areas can be exploited in benefit of patients.

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Conflict of interest: P. Pellegrino, M. C. Uboldi, and M. III Perez are employees of Sanofi. D. Marquez was a Sanofi employee by the moment of submission, and by the moment of acceptance and publication Boehringer Ingelheim employee.

Disclosures: Medical Writing and publication funded by Sanofi.

Information regarding manuscript:
Submitted on: 21.09.2023
Accepted on: 09.02.2024
Published on: 05.03.2024