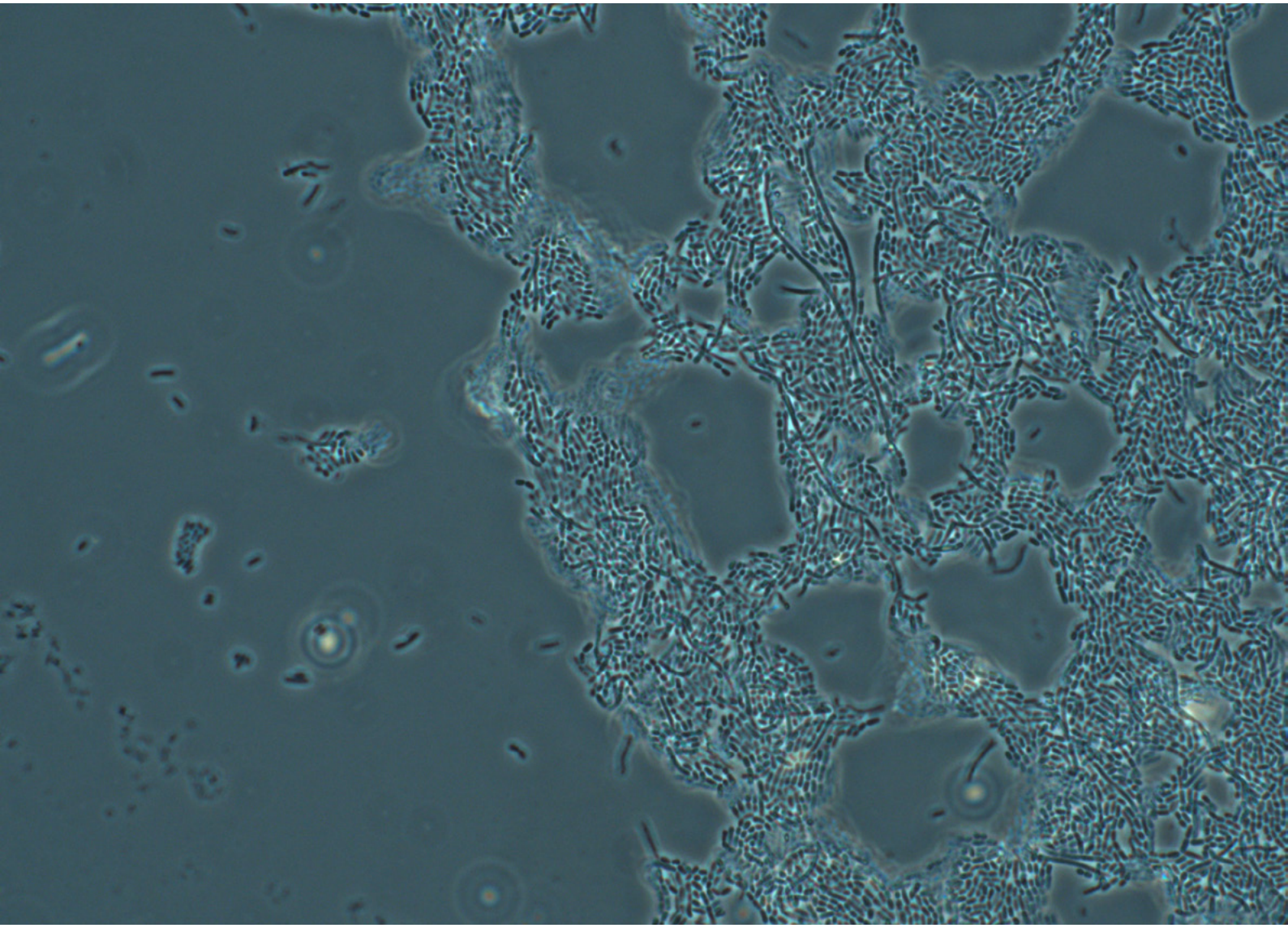
Application Note



Microscopic Analysis of Probiotic Foods

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Probiotics are among the functional food products that mark of a healthy way of life. The shelves of supermarkets and pharmacies are full of them: from yogurt drinks and bio-active compounds to starter cultures for yogurt and bread. They promise to improve health, strengthen the immune system, or aid digestion. Are these claims believable? Do these remedies really help?

Facts about probiotics

Probiotics are substances that contain viable microorganisms such as lactic acid bacteria or yeasts. They are used in foods and in medicinal products [1]. In Germany, the different types of products containing probiotics are governed by different laws and approval regulations. For example, products sold as medications at a pharmacy must be analyzed and have their safety assessed by the Federal Institute for Drugs and Medical Devices (BfArM). If a probiotic is intended to be sold as a food product, the situation is more complicated. In this case, the product is regulated by the Food and Other Commodities Act (LMBG) and its safety is only checked by the Federal Institute for Risk Assessment (BfR) in exceptional cases.

Uses of probiotics

- Effects on treating and preventing illnesses
- Use in food products
- Combating allergies
- Combating stress and anxiety
- Weight loss
- Refreshing intestinal flora

In 2002, a commission of experts from the World Health Organisation (WHO) and the Food and Agriculture Organization of the United Nations (FAO) developed guidelines describing the current definition[1] of probiotics in food products: "Probiotics are living microorganisms which, when administered in adequate amounts, confer a health benefit on the host" [2]. Previous definitions did not account for the full variety of probiotics, including intestinal, vaginal, and topical probiotics. By contrast, the current definition requires only that they be "living microorganisms" which offer a "health benefit".

The guidelines contain a set of requirements that food products should meet in order to be called probiotic [2]:

1. The genus and species of the probiotic strain must be known.
2. *In vitro* tests must have been carried out to verify the harmlessness and suitability for use of the probiotic organisms.
3. Probiotics must meet requirements to ensure the harmlessness of the strain and to ensure that the strain is not contaminated.
4. *In vivo* tests must be carried out on animals and humans to define the health benefits of the probiotic.

1 The word prebiotic is most often heard in association with probiotics. These were originally described as "...non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and / or activity of one or a limited number of bacterial species in the colon, and thus improve host health." [4] More recent definitions refer to the International Scientific Association for Probiotics and Prebiotics (ISAPP), which defines a prebiotic as "a selectively fermented ingredient that results in specific changes in the composition and / or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health" [5]. Usually, a combination of probiotics and prebiotics is needed to achieve the desired result, and they are combined to this end.

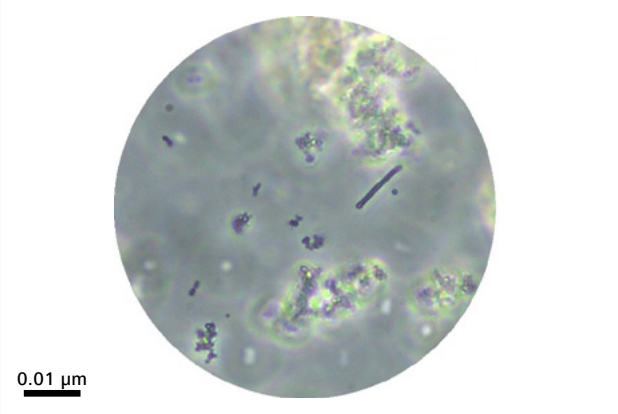

Figure 1 *Long rod-shaped bacteria in yogurt*

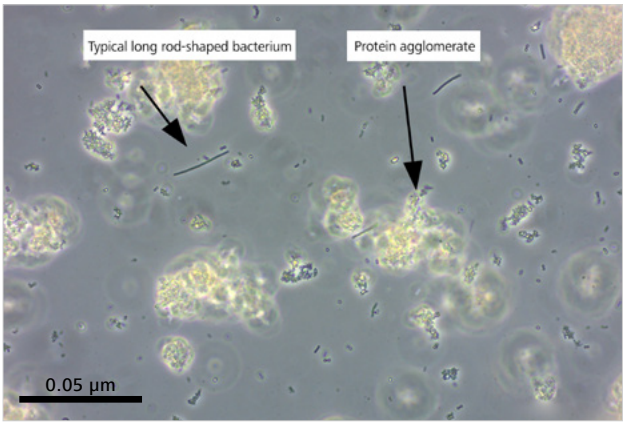

Figure 2 *Probiotic yogurt, overview*

Milestones in the history of probiotics

2001 The Food and Agriculture Organization of the United Nations and the World Health Organization (FAO / WHO) introduce the current definition of probiotics: "living microorganisms which, when administered in adequate amounts, confer a health benefit on the host"

1980s The first probiotic yogurt is made available to the European market

1965 The term "probiotic" is developed and used for the first time

1935 The first commercial product containing lactic acid bacteria is made available to the Asian market

1908 The Ukrainian-born zoologist, microbiologist, and Nobel Prize-winner Élie Metchnikoff (1845 – 1916) proposes the idea that lactic acid bacteria may promote beneficial effects, and that it may be possible to "modify the flora in our bodies and to replace the harmful microbes"[1]

1857 The French chemist and microbiologist Louis Pasteur (1822 – 1895) discovers lactic acid bacteria

Probiotic bacteria

A probiotic is defined by its genus, its species, and its strain. For example, Lactobacillus fermentum is a Gram-positive bacteria (species) of the genus Lactobacillus. The strain ME-3 is considered an antimicrobial and antioxidative probiotic. The most well-known probiotic bacteria are Bifidobacteria, Lactobacilli, and *Escherichia coli*. Probiotic lactic acid bacteria are the largest group, followed by the Bifidobacteria genus. They were discovered in the nineteenth century. The various species of the genus *Lactobacillus* were deemed safe for human consumption, and as a result were widely used in the fermentation of food products. Over 50 % of lactic acid is produced as a product of fermentation in this way. As a result of their distinct metabolic pathways, the fermentates vary in their composition and have differing effects on the resulting food safety and organoleptic properties of the final product.

2 E. Metchnikoff, Optimistic studies. New York: Putman's Sons, 1908, 161–183.

3 The cytoplasmic membrane of lactic acid bacteria is surrounded by a peptidoglycan layer. Peptidoglycan ("murein") is a polymer consisting of alternating N-acetylglucosamine (GlcNAc) and β-1-4-linked N-acetylmuramic acid (MurNAc) residues. Usually, this layer serves as an anchoring surface for other cell membrane molecules, such as wall techoic acid, polysaccharides, and proteins. The morphology of the Gram + lactic acid bacteria cell walls is influenced by the polysaccharides in the peptidoglycan layer, which results in varying effects on the physiology of the bacteria. For example, the N-deacetylation of GlcNAc and / or MurNAc in L. fermentum and the 6-O-acetylation of MurNAc reduces autolysis sensitivity to lysozymes in L. casei, L. acidophilus and L. fermentum. (This enzyme is found in many human bodily secretions (saliva, tears, amniotic fluid, etc.) and directly attacks the peptidoglycan cell walls of Gram-positive bacteria). Thanks to this reduction of N-acetylation, more Lactobacilli survive to reach the digestive tract [3, 6].

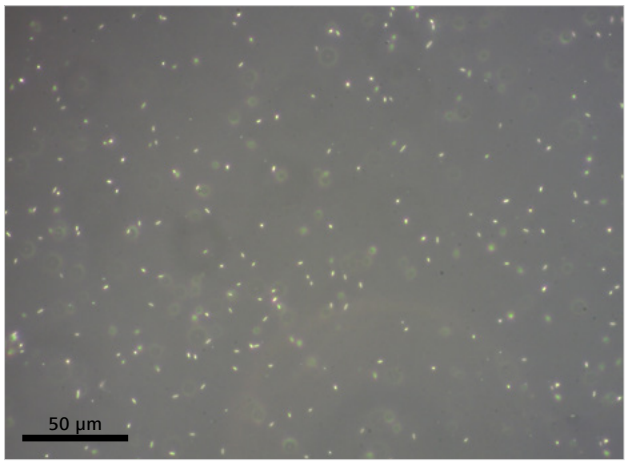

Figure 3 *Darkfield image of fermented milk beverage with lactobacilli*

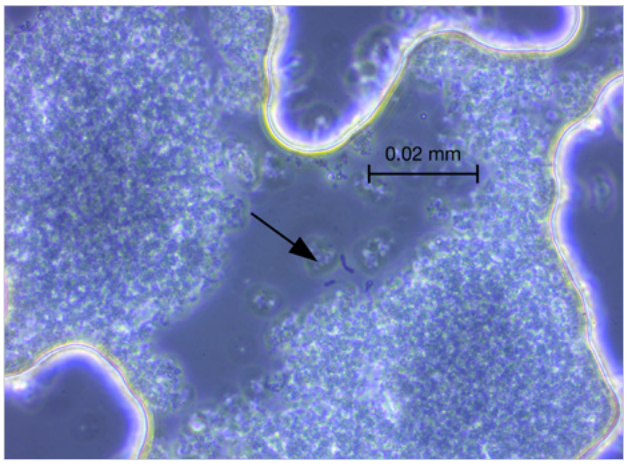

Figure 4 *Small bacteria viewed with the 100× Ph3*

The probiotic behavior[2] of the micro-organisms is defined by the way their surface membranes or excreted substances interact with the host organism. It is perhaps precisely for this reason that lactic acid bacteria are also found in the gastrointestinal tract. To date, the probiotic characteristics of only a few of the >200 species (http://www.bacterio.net/lactobacillus.html) have been tested.

For example, the strain Lactobacillus acidophilus LA-5® from the Chr. Hansen collection of dairy cultures is one of the strains of Lactobacilli which has been researched the longest. It has been used as a probiotic in food products and dietary supplements since 1979. In addition, the species received the QPS Qualified Presumption of Safety status from the European Food Safety Authority in 2007. LA-5® survives the passage through the digestive tract until it reaches the intestinal mucosa and is resistant to gastric acid, bile, and digestive enzymes. Lactobacillus acidophilus is one of the Gram-positive non-sporulating facultative or anaerobic rods. The bowel is its natural habitat. Its glucose fermentation products are lactic acid, acetic acid, and H_2O_2. These microbial metabolic products inhibit the growth of pathogens [3].

Many probiotic lactic acid bacteria have been researched over the past decades. There are already a large number of probiotic products aimed at individual well-being available or in production – for example, treatments for better skin, weight loss, and stress reduction.

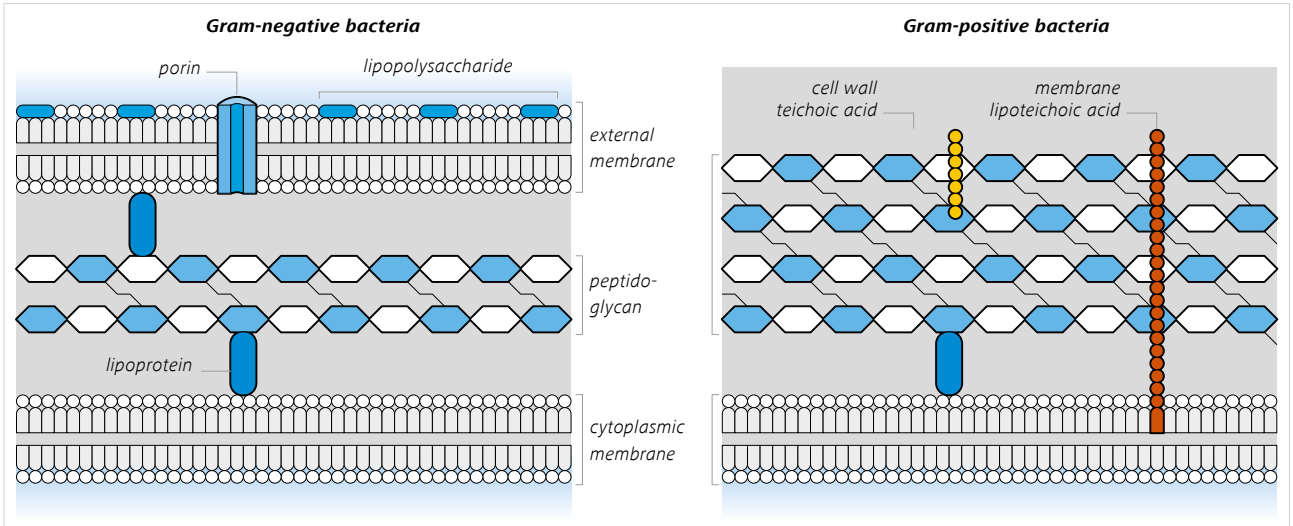

Figure 5 *Example illustration of bacterial cell walls. Various functional molecules are built into the peptidoglycan layer, which serves as an anchoring surface [7].*

Recommended microscope equipment

The commercial product (yogurt / fermented beverage) is typically diluted with physiological saline solution (e.g. 1:5). Approximately 10 µl are placed on a slide and covered with a cover slip (0.17 mm). Any excess liquid is carefully removed from the edges. Ideally, the preparation is allowed to rest for a suitable length of time.

In order to detect the bacterial variations as clearly as possible, the use of an upright, transmitted-light microscope such as ZEISS Axio Lab.A1 is recommended. The microscope should be equipped with darkfield optics and phase contrast. For example, the ZEISS achromatic-aplanatic condenser lens allows the user to record darkfield and phase contrast images in addition to brightfield images. As the process involves the observation of living organisms, the phase-contrast objective typically used for this purpose should be of high quality. Depending on the bacteria being studied, a ZEISS N-Achroplan 40×/0.65 Ph2 or ZEISS N-Achroplan 100×/1.25 Ph3 Oil objective may be used. If there is a desire to document the findings in microscopic images, a camera should be selected that has an excellent dynamic range as well as the right resolution to effectively capture images of the bacteria (e.g. ZEISS Axiocam 305 color).

References:

[1] https://de.wikipedia.org/wiki/Probiotikum.

[2] FAO/WHO, Guidelines for the Evaluation of Probiotics in Food: Joint FAO/WHO Working Group meeting. 2002: London Ontario, Canada.

[3] Lee, Y.K. and S. Salminen, Handbook of probiotics and prebiotics. second ed. 2009.

[4] Gibson, G.R. and M.B. Roberfroid, Dietary Modulation of the Human Colonic Microbiota: Introducing the Concept of Prebiotics. American Institute of Nutrition, 1995. 125(6): p. 1401–1412.

[5] Blatchford, P., et al., Prebiotic mechanisms, functions and applications – a review. International Journal of Probiotics and Prebiotics, 2013. 8(4): p. 109–132.

[6] Venema, K. and A.P. do Carmo, Probiotics and Prebiotics: Current Research and Future Trends. 2015, Norfolk, UK: Caister Academic Press.

[7] http://docplayer.org/docs-images/27/10710454/images/2-0.jpg.

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