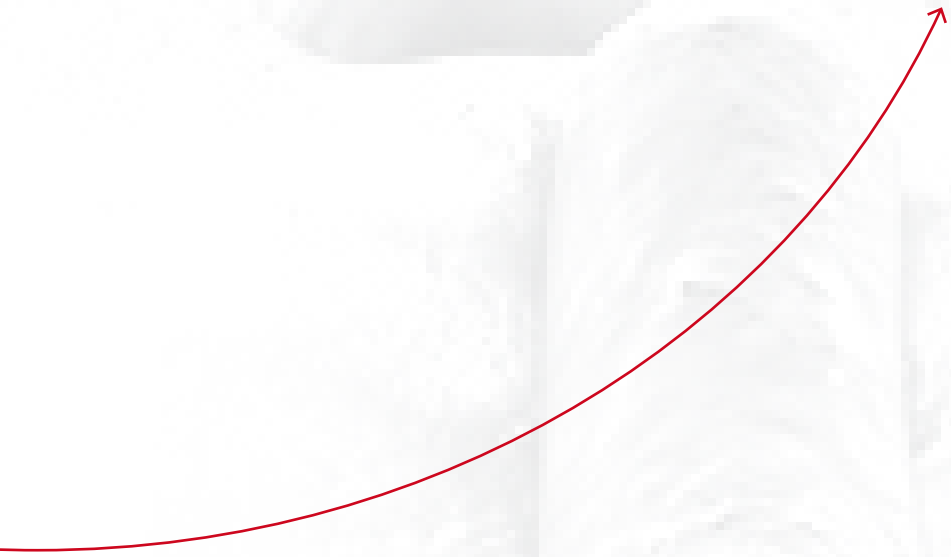
bio|vis' DIAGNOSTIK

Mikrobiom 2.0

We know by far not everything,
but we do already know a lot – an update

Mikrobiom 2.0

The **intestinal microbiome** is in full trend.
No wonder: The influence of the microbiome on human health is immense (see Fig.). In the past years, research has gained a lot of knowledge and hereto related publications have rapidly increased!



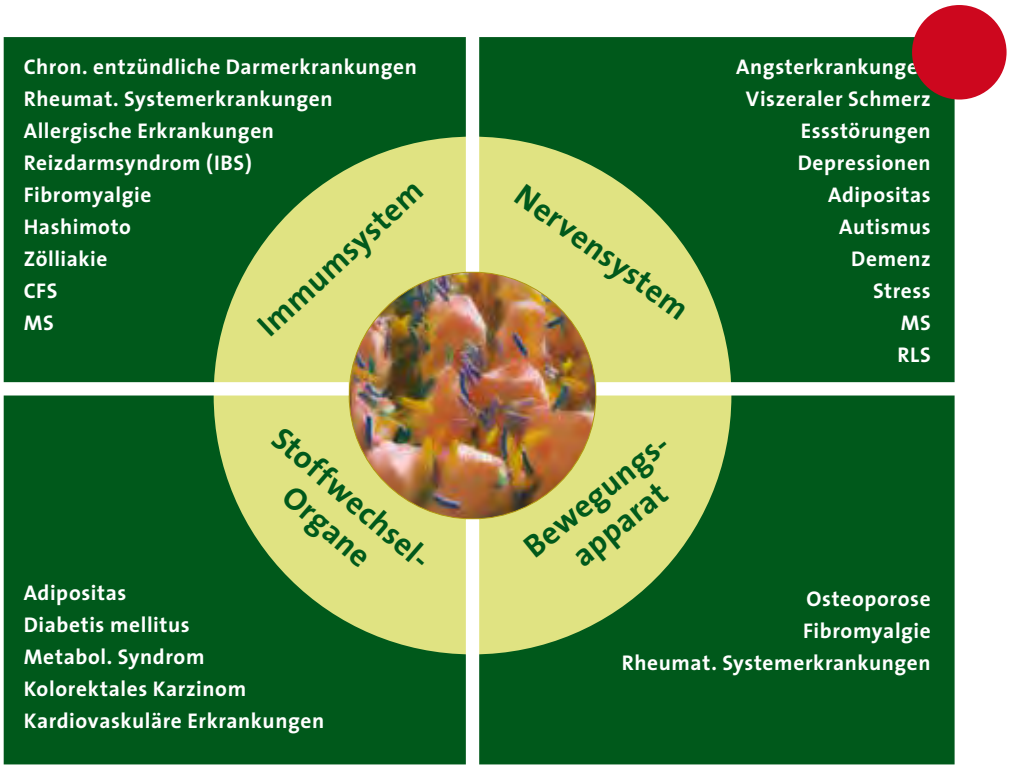


Abb: 1

Whilst in 1995 nobody was interested in this research object, in 2005 there were already 350 publications and already over 5000 publications ten years later! This vast amount of new information has the effect of making some recent studies already considered obsolete today. Thus, from today's perspective the first enthusiasm, viewed soberly, was precipitate when correlations were found between certain diseases and specific bacterial species in the microbiome.

Associating individual marker germs with a risk of certain diseases is far too simple a perception. The nature of the microbiome and its human is clearly more complex. This makes us thoughtful and cautious and this is a good thing.

It is simply impossible to fundamentally investigate the totality of organisms in the intestines with more than 1000 species within a few years and at the same time make them broadly available for diagnostic and therapeutic purposes. To solve this problem in full at least several decades are required if not a century. Here, we are confronted with an object of research that is extremely diverse, flexible and influenceable that internally presents a diverse interaction and that is in complex relation with its host and its environment. It would be an error, however, to remain sceptical and wait-and-see towards latest knowledge. There is already so much assured knowledge that a good diagnosis and following targeted and successful therapy is possible. However, both must be absolutely permanently updated based on new knowledge.

On the following pages we will therefore introduce to you interesting news of the human microbiome in the intestines – so to speak we will give you an update on microbiome 2.0.

Three enterotypes? Functional groups!

One important findings on human intestinal microbiome seemed to be the detection of the enterotypes of humans. The three enterotypes that are meanwhile repeatedly confirmed are classified after the species that colonise the most in the individual microbiome: *Bacteroides-type, Prevotella-type* and der rare *Rumninococcus-type* [1], who was corrected into *Rumninococcaceae-type* (taxonomically extended to family level) in 2016 [2]. In 2014, it was possible to prove that these enterotypes are very stable in the individual [3]. However, what is the consequence from these enterotypes for medical practice? It gives information about the specific eating habits and leads to the conclusion that changes can only be achieved by a long lasting change of eating habits with high share of specified dietary fibres and prebiotics. For daily practice, the taxonomical classification of the microbiome bacteria is of minor importance. Of major interest to both, doctor and patient, however, is the metabolism certain intestinal bacteria perform, how many of them exist in the microbiome and if they are potentially rather health-supporting or health-damaging. The quantitative analysis of so-called "functional groups" is thus more helpful. Such groups are for example butyrate builders (e.g. Faecalibacterium prausnitzii, Eubacterium rectale and Ruminococcus bromii), mucin dreceasing bacteria (e. g. *Akkermansia muciniphila*), lactic acid-producing bacteria (e. g. *Lactobacillus, Bifidobacteria*), sulfat reducers (e.g. *Desulfovibrio piger, Desilfonomonas pigro*) and others. The quantitative determination of the presence of these groups in the individual microbiome gives information about the physiological or altered conditions in the intestines and indicates deficiencies, compensatory protection possibilities or the risk impairment to the mucous membrane.

Further considerable is the functional group of the equol producing bacteria (e. g. *Adlercreutzia spp., Eggerthella spp., Slackia spp.*). They are capable of producing non-steroidal estrogene equol from isoflavone daidzein (also see from soy). Equol can bind Era and ERß to the estrogene receptors and promotes the production of the sex hormone-binding globulin (SHBG). Additionally, it possesses antioxidant, immune stimulant and anti-inflammatory properties. It supports the protection against osteoporosis, heart diseases, peripheral circulatory disturbances, strengthens the cognitive abilities and reduces the risk of mamma or prostate carcinoma [4] . The effectiveness of isoflavones, commonly being used for therapeutic purposes in menopausal women, largely depends on the conversion of daidzein to equol in the intestines by the equol-producers. Unfortunately, only 20 to 30 percent of the Western population carry equol-

Diverse Bakterienansammlungen auf der Darmoberfläche
3-D-Illustration,

producers in the intestines, whereas there is a 50 to 60 percent [5] frequency of equol-producers in adults from Asian countries in which soy foods are commonly consumed at any age. An equol-producer test can quickly show if a treatment with isoflavones is appropriate or if the microbiome cannot sufficiently realise the necessary conversion.

Diversity counts

The diversity of bacteria species in the individual microbiome remains an important quantity allowing current and prognostic statements regarding the health of the human. The higher the diversity the higher the possible protective function of the microbiome and the higher also the support for health and supply situation of the intestinal epithelium. A high diversity of species simply contributes to a physiological intestinal environment. Comparative international microbiome studies among test persons from the Western world and close-to-nature groups (Burkina Faso or hunter-gatherers from Peru) indicated that the later carry a significantly higher diversity, a stronger production of short-chain fatty acids (SCFA) along with a reduced (potential) presence of pathogenic germs in the intestines [6,7]

Importance of short-chain fatty acids (SCFA) in the intestines [8]

- Stabilisation intestinal environment (pH-value)
- Energy supply colonic epithelium
- Promotion mucin production
- Promotion mucosa circulation
- Reduction of inflammation
- Reduction of cellular proliferation
- Promotion apoptosis
- Promotion differentiation Ca-cells
- Gene regulation (deacetylase inhibition)
- Strengthening of mucosal barrier (reduction of Claudin-2 expression)
- Promotion of regulatory T cells

The role SCFA plays for the pathogenesis of diseases is subject of current studies of many research groups.

Unbalanced diet or frequent administration of antibiotics have negative impact on the diversity. They persistently reduce the diversity of species in the intestinal microbiome. Individuals with overweight, type 1 or type 2 diabetes, Alzheimer's disease, chronic inflammatory intestinal disease, colorectal carcinoma and irritable bowel syndrome often carry a reduced diversity.

In addition, patients with myalgic encephalomyelitis and chronic fatigue syndrome show reduced diversity compared to healthy controls [9].
In 2016 two studies with middle-aged and especially old persons (95 to 112 years old) in Italy and China indicated a correlation between healthy ageing and a high diversity in the microbiome and seems to be related to a high proportion of butyrate producers and *Akkermansia muciniphila* (see box) [10,11] .

Akkermansia muciniphila (Am)

Am is a strictly anaerobic growing, gram-negative rod and the only species of Phylum Verrucornicrobia. ***Am*** is mucin-degrading and thus it stimulates the intestinal mucosa to produce new mucus. ***biovis*** own study in 2015 showed that ***Am*** can preferably be found in the mucus layer and far less in the intestinal lumen. The intestinal mucosa and the mucus layered upon play a decisive role in the case of chronic diseases of the digestive system. When the mucus production is reduced for lack of ***Am***, pathogens, harmful substances or allergens can enter the intestinal mucosa more easily and promote local inflammatory reactions.
After cognition of these intestinal protecting functions of ***Am*** the bacteria was considered a positive marker germ for a healthy intestinal mucosa and has been used as such in many studies ever since. In 2016, however, this assessment needed to be put in a relative perspective: a study successfully showed that a low-fibre diet leads to a deficiency situation in which microbiota (primarily ***Am***) excessively degrade the mucus layer [12]. The colonic mucus production of the host was not able to compensate adequately. As expected, the consequences were devastating: an eroded intestinal barrier, Leaky gut, inflammations and an increased risk of endogenous infections. The conclusion can thus only be that neither ***Am*** nor any other germ can generally be classified as positive or negative for the intestine. What matters most is the conditions in which the respective germ thrives. Thus again, the key factors are the diversity and the actual metabolism of the microbiome. The sole analysis of particular individual germs (***Am*** or others) is counterproductive to enable conclusions to be drawn on the condition of an individual microbiota.

From all this can only be followed the practical consequence to preserve and promote a high diversity in the intestine. For this purpose patients should generally be advised the intake of diverse and fibre-rich foods. Administrations of antibiotics are to be reduced to the minimum and each inevitable antibiotic intervention needs to be accompanied by targeted measures to limit the damage. Despite the knowledge of the correlations between diversity and health, much research activity was done and still is in order to discover correlations between individual species of microbiome bacteria and the occurrence of diseases. This is right and proper as these correlations can possibly give information on the pathogens of diseases. However, this is of limited value for the medical practice. Informing the patient about an uncertain increased risk of a specific disease without offering concrete measures to effectively limit this risk will at best lead to the patient's uncertainty or, at its worst, to fear. Such statements are to be taken with caution but are nevertheless worth keeping an eye on. Patients carrying such „risk- bacteria", no matter what type, should

Präbiotika- Auswahl nach Wirkung									
Präbiotika	**Butyrat-bildung**	**Entzündungs-hemmer**	**Fp u/o Am**	**Bifidogene Wirkung**	**F / B-Ratio**	**LI**	**FM**	**Blähungen***	**Diversität**
RS	+	(+)	-	(+) [1]	+	ja	ja	40	+
PPb	+	+	+	+	+	ja	ja	60	+
scFOS/scGOS	+	+	+	+ +	(+)	nein	nein	100	+
FOS	+	+	+	+	(+)	ja	nein	100	+
Inulin	+	+	+	(+) [2]	(+)	ja	nein	100	+
Akazienfaser	+	+	+	+	- -	ja	ja	20	+
XOS / AXOS	+	+	-	+	?	ja	ja	50	+
Butyrat	+	+	-	-	+/-	ja	ja	10	- /+
FODMAP	-	-	- -	- -	- -	ja	ja	- -	- -
Low Carb	-	-	+/- [3]	+/- [3]	-- [3]	ja	ja	- -	- -

Erläuterungen:

* relatives Auftreten von Blähbeschwerden im Vergleich zu FOS/GOS (100%);

\+ Fördernde Wirkung;

\- keine nachweisbare oder sehr geringe Wirkung;

+/- kein Einfluss ;

- - Verminderung;

ja verträglich;

nein ggf. nicht verträglich, einschleichend dosieren (Beginn: 1 g/Tag)

1) Spaltung von RS durch B. breve u. B. adolescentis (Aliment Pharmacol Ther 2015; 42:158-179);

2) Abhängig vom Phänotyp, unvollständiger Abbau von Inulin (Appl Environ Microbiol 2009; 75:454-461);

3) Bei protein- und fettbetonten Formen der Low Carb-Kost werden abnehmende Keimzahlen von A. muciniphila (Clin Nutr Experiment 2016; 6: 39-58), F. prausnitzii- und Bifidobakterien beschrieben (Proc Nutr Soc 2015; 74: 23 – 36). Low Carb- Kostformen können zwischen 25 und 250 g Kohlenhydraten pro Tag enthalten.

RS Resistente Stärke

PPb „Pro Präbioma“ (Kombination aus mehreren präbiotischen Substanzen)

FOS/GOS Fructo-/Galactooligosaccharide: Kurzkettige Varianten (scFOS / scGOS) zeigen deutlich bessere Verträglichkeit

XOS/AXOS Xylo-, Arabinoxylooligosaccharide: Butyratbildung vorwiegend über bifidogenen Effekt („Cross-Feeding“)

FODMAP Fermentierbare Oligo-, Di-, Monosaccharide und Polyole“ (Polyole: mehrwertige Alkohole)

Fp/Am Vermehrung von Faecalibacterium prausnitzii / Akkermansia muciniphila

F/B-Ratio Firmicuten-Bacteroidetes-Ratio

LI Verträglichkeit bei Laktoseintoleranz

FM Verträglichkeit bei Fruktosemalabsorption

Diversität Diversitätsfördernde Wirkung

Nach Bekanntwerden dieser darmschützenden Eigenschaften von **Akkermansia muciniphila** galt dieses Bakterium als positiver Markerkeim für eine gesunde Schleimhaut und wird seither in vielen Studien als solcher eingesetzt. Doch 2016 musste auch diese Einschätzung relativiert werden: In einer Studie konnte gezeigt werden, dass eine nicht ausreichende Versorgung mit Ballaststoffen dazu führt, dass die Mikrobiota (und v.a. **Akkermansia muciniphila**) in dieser Mangelsituation den Mucus überstark abbaut [12]. Die Mucus-Bildung des Wirtes konnte das nicht ausreichend ausgleichen. Die Folgen waren erwartungsgemäß verheerend: eine erudierte Darmbarriere, Leaky gut, Entzündungen und eine erhöhtes Risiko für endogene Infektionen.

Das Fazit kann also nur lauten, weder **Akkermansia muciniphila**, noch irgendein anderer Keim sind als grundsätzlich positiv oder negativ für den Darm einzuordnen. Es kommt auf die Bedingungen an, unter denen der jeweilige Keim gedeiht. Und damit kommt es wiederum auf die Diversität und die tatsächlichen Stoffwechselleistungen des Mikrobioms an. Die alleinige Analyse von bestimmten Einzelkeimen **Akkermansia muciniphila** oder andere) ist daher nicht zielführend, um den Zustand der individuellen Mikrobiota einzuschätzen.

be monitored and are usually suitable candidates for a long-term therapy of their intestinal microbiota. Developments in microbiome research need to be followed up to put the latest findings into practice for the benefit of the affected.

Treatment via the microbiota

Everything arriving in the intestine has direct impact on the microbiome. A change of dietary fibre quantity only for ten days becomes easily and clearly evident in the microbiome [13]. As soon as this change ends, however, the “old” microbiome returns, long-term effects are minimal.

Without exemption, fibres are poly- or oligosaccharides composed of links of glucose, fructose and other sugars or sugar derivates non-fissile for humans: a specific resistant starch (RS§), fructooligosaccharides (FOS), galactooligosaccharides (GOS), xylooligosaccharides (XOS), arabinoxylooligosaccharides (AXOS), fructans (inulin) and acacia fibres as rather new but particularly well tolerated fibres types.

It has been long recognised that dietary fibres have a positive impact on microbiota and intestinal mucosa. The latter mainly occurs by the production of short-chain fatty acids (SCFA, see box above). However, the impact of dietary fibres goes far beyond the intestine. It has been known for some time that dietary fibres in overweight persons can lead to weight reduction and reduction of risk of type 2 diabetes. In 2014 a study proved the positive influence [14] on the food quantity consumed, the weight and the body fat of test persons by administration of the fibre inulin which reduced the ghrelin production (among others appetising hormone). In 2016, it has been demonstrated that the relation between intestinal dysbiosis and the development of islet cell antibodies (development of type 1 diabetes) was influenced in a positive way by administration of byrate [15]. This list of research results could be extended considerably, though. Dietary fibres are thus highly potent tools in the therapy of the microbiome. They can be used as food supplements for substantiated dysbioses. A suitable change in diet with fibre-rich foods increases the probability of success that can only be achieved with long lasting interventions.

When treating the microbiome one must remember its complexity. The treatment must not necessarily respond exactly to the one bacterium. Reflexions must be more complex. Each intervention via diet, food supplements or probiotics (see below) influences all bacteria of the microbiome. As a complex network, they work together or against each other. One example: different types of fibres are being metabolised by acetate producers (e.g. *bifid bacteria, bacteroides* etc.). Their main product, the acetic acid has importance for the total intestinal environment. First effects are the degradation or stabilisation of the pH-value in the intestine and a negative environment for certain other bacteria whose population decreases. At the same time acetate is the food resource of certain butyrates that produce the butyric acid that is of such high importance to the intestinal epithelial cells [16,17], . Without the "deviation" via the acetate, these butyrates cannot be produced and so dietary fibres indirectly promote the butyrate producers. Another example of the large number of possible interactions within the microbiome. If a diagnosed shift in the microbiome suggests the promotion of a specific functional group, an additional administration of probiotics might be advisable. This is a question of finding the right product for the individual patient. Not every bacterium mentioned on a product shows the same effect.

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There are different strains of bacteria species that strongly vary in their properties. For example, one strain may be very useful and demonstrably helpful in a dysbiosis but another strain of the same bacteria species may be not. It is thus recommendable to choose the products according to the patient's age, his symptoms and the shift of his microbiome. Furthermore, preferably products of which the effectiveness has been proven in studies should be administered. This information is available from the manufacturer. If there are no studies for the particular product, this product should always be second choice – even in the case of an apparently reasonable composition.

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