REVIVAL OF MICROBIAL THERAPEUTICS, WITH EMPHASIS ON PROBIOTIC LACTOBACILLUS (REVIEW)

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Development of probiotic concept

The idea to prevent and treat infections by the means of living microorganisms goes back to early 20th century. Health benefits of lactic acid bacteria (LAB) were first described by Elie Metchnikoff in the monograph of 1907 "The prolongation of life" [35]. He implicated that regular intake of LAB rich products, could normalise bowel health and enhance longevity [25]. The term probiotics itself was originally introduced by Werner Kollath in 1953, where he described probiotics as an inorganic and organic supplement necessary to reinstate healthy state of patients suffering from malnutrition [17]. Today the term probiotics is exclusively linked to the heath beneficial bacteria [1].

During the twentieth century, members of the *Lactobacillus* and *Bifidobacterium* genera and their derivatives were extensivly marketed as health promoting agents, often with irrelevant or false claims, leading to misunderstanding and confusion in the interpretation of probiotics and guidelines for their proper use [39]. Only in the year 2001 a clear definition of the probiotics was introduced by World Health Organization and Food and Agriculture Organization of the United Nations (WHO/FAO) as "Live microorganisms which when administered in adequate amounts confer a health benefit on the host" [19]. In the light of the rapid progress of the latest scientific and clinical developments of the field, the WHO/FAO guidelines were revised by The International Scientific Association for Probiotics and Prebiotics (ISAPP) in 2016. Recommendations concerning delivery of live, well-defined strains, with a suitable viable count and with a reasonable expectation of deliverable benefits to the host were made and the guideline was reinforced as relevant [18].

Today, the microbial composition of probiotic products ranges from a single strain to multi-strain or species compositions, while most of the strains used are members of *Lactobacillus* genera [19]. Information acquired to date shows that *Lactobacilli* have a long history of use as health beneficial microorganisms without established risk to humans and this remains to be the best proof of their safety [30].

As a future perspective, a personalized microbial therapy with selected autoprobiotic strains, where the recipient is the donor of his/her own microbial library is gaining more relevance [47]. Anticipated microbiome applications are new types of preparation such as Next-Generation Probiotics (NGPs) and Live Bio-Therapeutic Products (LBPs) [36]. NGPSs and LBPs are experimental types of probiotics, most often constituted of different genera of commensal and indigenous bacteria, such as *Bacteroides* and are targeted towards cancer, intestinal inflammation and heart disease therapy [10,15,16,48,49].

Re-evaluation of Dysbiosis

The rapid development of high-throughput sequencing and metagenomics opened a new window in the research of microbial ecosystems. This lead to rethinking of the traditional dichotomic understanding of human bacteriology and its role in maintaining immunological and metabolic balance [51]. According to the latest studies, the ratio between human somatic cells and microbial cells inhabiting external and internal surfaces, including intestinal, urogenital and respiratory tracts, is approximated to be 1:1 [45], while the „Human Microbiome Project" estimates more than eight million unique genes associated with the various microbiomes in the human body [29]. Its functions and overall role of the microbiome in human livelihood is not yet fully understood. Conformably not only the treatment, but also the understanding of this huge and fragile ecosystem is a challenge for modern medicine.

Deregulation of the normal homeostasis of microbiota i.e. dysbiosis is one of the prevalent cause that leads to an unhealthy state of human microbial ecosystems [3]. Any change to the composition of resident commensal communities relative to the communities found in healthy individuals is defined as dysbiosis [37]. But this type of definition arises questions about interpretation of the "normal" state and composition of the microbial community. Studies on this topic suggest that human microbiota are highly variable, therefore only general trends can be suggested based on typical species abundances in certain groups when sufficient data is available. For example, the diagnosis of vaginal dysbiosis is routinely performed by evaluating the pH and the presences of *Lactobacillus spp*. While the study performed by *Serrano M. G. et al.* [9] concerning the biodiversity of vaginal microbiota in healthy women revealed that *Lactobacillus (L.) iners, L. crispatus, L. gasseri,* or *L. jensenii* are dominant species in groups of European and Asian ancestry, whereas higher proportions of strictly anaerobic organisms and lower proportions of LAB and corresponding higher pH values were found in healthy women of African and Hispanic ancestry [9] [38]. This example shows that the differences between groups and individuals should be taken into account in risk assessment and diagnosis of dysbiosis.

The gut microbiota is considered as primary example of the complexity of the issue. First of all, the intestinal biodiversity and the span of functions of commensal bacteria are yet hardly comprehensible. Second, as an open ecosystem, the ecological community of the gut is dynamic. The development and fluctuation of this biota is affected by a number of factors, including, birth and infant feeding, environment, diet, medication intake, co-morbid conditions, stage of lifecycle and exposure to stress [4]. With the best estimates, the dominant phyla across the gut, were assigned to *Firmicutes, Bacteriodetes, Actinobacteria, Proteobacteria, Fusobacteria* and *Archaea* [12]. Such a huge span of commensal variety makes it very tricky to estimate the "normal" composition of the gut biota. At the same time the relationship between gut dysbiosis and chronic health conditions, such as inflammatory bowel disease, metabolic syndrome, cardiovascular disease, obesity and cancer were observed [3]. Furthermore, the link between abnormal conditions in the gut and predisposal to neurodevelopmental disorders, such as Alzheimer's disease, Autism Spectrum Disorders (ASD) and Parkinson's disease were established, underlining the immanent role of gut microbiota in healthy function of the gut-brain axis [21]. Considering these issues, treatment of gut dysbiosis requires reinstatement of natural eubiosis, with minimal disruption to the body's often unknown natural ecosystem.

Microbiome therapeutics

Today, the common reasons for dysbiosis is use of antibiotic therapy [31]. Antibiotics, the most prominent means for treatment of bacterial infections, have non-specific and general

effects. Therefore, while eliminating pathogens, they considerably damage the native microbiome, that often leads to acute or chronic antibiotic associated dysbiosis [24]. Furthermore, the recent antibiotic resistance crises leads to abate of antibiotic options and emergence of resistant infectious diseases. Therefore, non-antibiotic based methods for elimination of multi-drug resistant (MDR) pathogens and reinforcement of practises for natural restoration of eubiosis are prioritized [7]. Along with probiotics alternative means for treatment and prophylactics, microbiome therapeutics are emerging, such as microbiota transfer therapy (MTT), prebiotics, phage therapy and their combined applications with probiotic *Lactobacillus*.

In recent years MTT or Ecotherapeutics took a form of faecal transplantation, which is a medical procedure based on the replacement of the dysfunctional intestinal ecosystem with a healthy faecal microbiota of a donor [14]. This method was strongly associated with the risks of transmission of opportunistic pathogenic bacteria from donor to the patient. Unfortunately, in June 2019 two immunocompromised adults who received investigational fecal transplantations for treatment of *Clostridium difficile* infections developed invasive infections caused by extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli*. One of the individuals died. This case obliged authorities to enforce testing of donor stool and exclusion of samples that tests positive for multi-drug resistant organisms, as well as exclusion of recipient individuals at higher risk of colonization with opportunistic pathogens [13]. This unfortunate case showed the necessity of safety assessment of microbiomes of the natural complexes of microorganisms before applying NGPSs, LBPs and MTT.

Prebiotics – "a substrate that is selectively utilized by host microorganisms conferring a health benefit" [11] currently include carbohydrate-based, polyphenols and polyunsaturated fatty acid substances, for which the beneficial health effects are documented. For example fructooligosaccharides are commonly used in combination with probiotic LAB to support their viability. To this point, the health benefits of prebiotics mainly refer to the gastrointestinal tract, such as immune stimulation and inhibition of pathogens [30].

Phage therapy utilizes viruses of bacteria called lytic bacteriophages (phages). This type of viruses specifically kill only a target bacterium, without damaging human somatic cells or disturbing surrounding microbiota. Phages are proven to be effective against multi-drug resistant bacterial cells as well [34]. The potential of bacteriophages to selectively reduce target organisms without global disruption of the gut community has been demonstrated in a double-blinded, placebo-controlled crossover trial [8]. Therefore, therapeutic use of phages have greater potential for treatment of infectious disease and dysbiosis [33]. Even more, recent trends suggests usage of bacteriophages as prebiotics to promote the growth of beneficial bacteria (symbionts and probiotic) by decreasing harmful bacterial populations and releasing nutrients into the environment [5,36]. For example *Escherichia coli* specific phages (LH01 - Myoviridae; LL5 - Siphorviridae; T4D - Myoviridae; LL12 – Myoviridae) are marketed as prebiotics and available in combinations with *L. acidophilus, L. rhamnosus, L. paracasei, L. casei,* as pre-probiotic blends. This products are sold as health supplements under the brand names: Florassist GI, bVital, Probiophage DF60, Prephage Probiotic, EcoPhage and FloraPhage in the United States. Considering that, the host's virome may be an important factor that determines the efficacy of some probiotic formulations, development of phage-probiotic complexes can be the novel approach in promoting heathy microbiotas.

Mechanisms of action

Probiotic LAB can be of human, animal or plant origins and can be utilized in a variety of different forms. However, it is the specificity of the action and not the source of the microorganism that matters. It is estimated that a majority of health benefits of probiotics are the result of interactions with the host biome and the creation of favourable microbiota, via promoting native microbial homeostasis, rather than replacing its composition [43]. This is the main advantage of probiotic *Lactobacillus* when applied for reinstatement of microbiota without known "normal" microbial composition.

The translation of probiotic mechanisms depends on the environmental context to which the

LAB strains are exposed during application [26]. Even more so, they may have distance effects by transfer of the organisms away from the initial administration site or by producing molecules that are adsorbed and transferred thought the host organism. Considering this ambiguities, the underling mechanisms of action can be principally divided in the following groups:

- *Microbiota targeted mechanisms*, where probiotic effects are achieved through broad antimicrobial activity and modulation of composition of the indigenous microbiota [32].
- *Interaction with the epithelial barrier*, including epithelial permeability decrease, through promoting tight junction functionality and enhance cell proliferation [22].
- *Immune system modulation*, via interaction with innate and adaptive immunity, mainly thought monocytes, macrophage, M-cells in the gut mucosa and dendritic cells [27].
- *Modulation of systemic metabolic responses*, can be induced by bile salt hydrolase activity, impacting on leptin and endocrine modulation [2].
- *Signalling via the central nervous system*, with direct and indirect mechanisms, such as tryptophan-derived products, g-amino-butyric acid or oxytocin production [20].

Probiotics are selected based on the traits, that enables activation of their mechanisms of action. In nature the frequency of the traits carried by the *Lactobacillus* vary significantly.

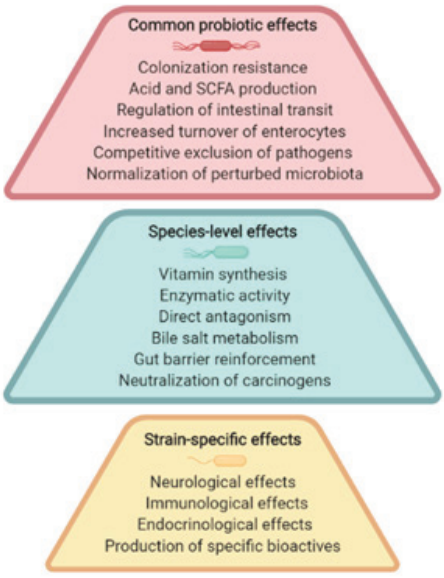

Fig. 1. Different shared and specific effects among probiotics related to their health beneficial and therapeutic action. Classification is based on ISAAP consensus statement [18].

Abbreviation: SCFA - short-chain fatty acid. Illustration created with BioRender

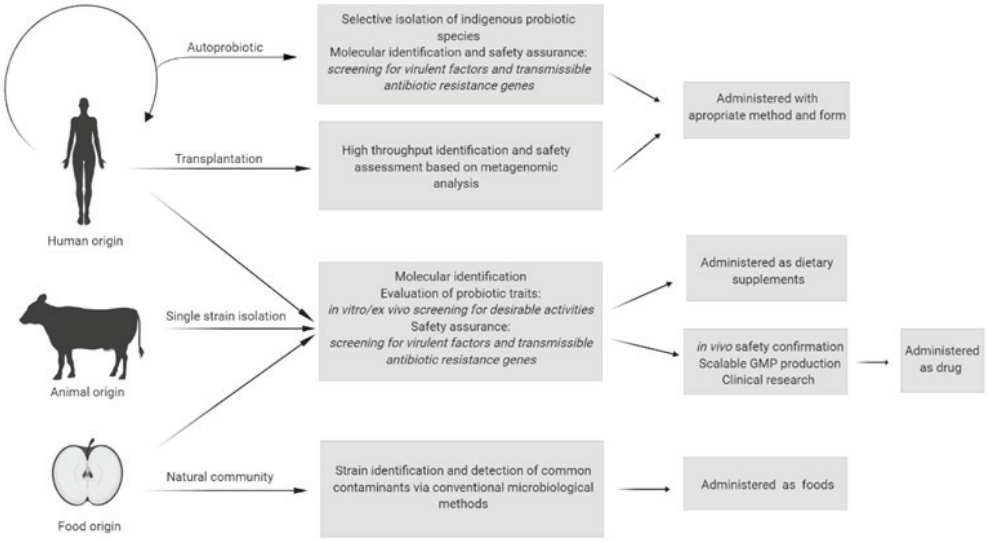

Fig. 2. Graphical representation of probiotic pipelines and requirements for their development according to administration forms. Illustration created with BioRender

According to ISAAP consensus statement some traits might be widespread among genera, thus are quite common among most LAB e. g. rapid colonisation and competitive exclusion of pathogens. While others traits can be observed only on species level, thus are less abundant, for example, bile salt metabolism or vitamin synthesis. Production of specific bioactive substances, such as Nisin, are usually strain-specific, thus harder to discover. Figure 1. shows a schematic representation of common species and strain specific effects related to health beneficial and therapeutic actions of probiotics.

As oral administration is the most prevalent delivery method for a majority of available probiotics [23,41] in addition to health beneficial characteristics, strains are required to have high survival rate and viability in gastro intestinal track (GIT) [6]. For example, the best documented probiotic microorganism *L. rhamnosus* strain GG (Gorbache-Goldin) has characteristics allowing its survival in the GIT, such as, high tolerance to low pH, resistant to bile and adhesion promoting pili, therefore is widely used in prophylactics and treatment of antibiotic associated diarrhoeas in adults and children via oral administration [46].

Regulatory requirements

In human applications the regulatory aspect of probiotics is complex. Initially, strains have to be selected on the basis of the Bradford Hill criteria [44], while the intended use of a probiotic product will determine its regulatory categorization. Probiotic strains can be delivered as food, dietary supplements or drugs [18]. The evidentiary burdens for safety assessments, medical tests, premarket and postmarket clearance requirements and types of admissible claims differ for each of these products. Furthermore different countries have particular sets of regulation for each type of preparation.

In Georgia probiotics can be assigned to the Pharmacotherapeutic groups of Anti-inflammatory and antidiarrheal microorganisms or agents, and along with bacteriophage preparations are mostly marketed as generic and re-produced pharmaceuticals [53].

In the member countries of the Eurasian Economic Union (EAEU), probiotics, as wells as bacteriophages, fall into the category of immunobiological medical products (medicinal products of biological origin meant for immunological diagnostics, prophylaxis and treatment of diseases). Authorised production strains such as *L. acidophilus* К3Ш24, *L. plantarum* 8P-A3 and *L. fermentum* 90T-C4 are deposited in official collections and can be produced as therapeutic products, in the form of pills, oral and vaginal capsules, suppositories and lubricants [42].

Western pharmaceutical pipelines and legislation is optimised for manufacturing of defined chemicals, for which pathways of action and safety are defined in details. This leaves a limited space for biological pharmaceutical agents (bacteria, fungi, phage) of which batches are not entirely reproducible, and which effects derive from interactions with host ecosystems. Thus, the estimation of claimed effects, production methods and measurement of associated risks and benefits hardly fit in the conventional pathways. This circumstance has negative effects on probiotic legislation. Therefore, probiotic LAB are mostly marketed as dietary supplements or foods. In the United States, more than 20 strains of *Lactobacillus,* such as *L. acidophilus* NCFM, *L. rhamnosus* GG, *L. curvatus* DSM 18775, *L. plantarum* Lp-115 etc. have been accepted and are pending for the Generally Regarded as Safe (GRAS) status by the Food and Drug Administration (FDA) and can be used as foods additives [50]. Similar approach is practised in Japan, where 13 LAB strains, such as *L. acidophilus* CK60, CK92, *L. rhamnosus* GG, have been authorised as beneficial ingredients in Food for Specified Health Uses (FOSHU products). Interestingly, according to Health Canada in the Natural Health Products (NHP) category, non-strain specific probiotic claims can be made for *L. acidophilus, L. casei, L. fermentum, L. gasseri, L. johnsonii, L. paracasei, L. plantarum, L. rhamnosus* and *L. salivarius* species, when delivered in food at a level of 10^9 colony forming unit per serving.

In contrary, in the EU currently no health claims are approved for human applications with probiotics [52]. The European Food Safety Authority (EFSA) settled the probiotic claims as not sufficient and essentially banned the use of the word probiotic on labels [39]. Only several strains of *Lactobacillus* such as *L. plantarum* TENSIA® (DSM 21380) have been granted Qualified Presumption of Safety (QPS) by EFSA and are authorized as sources of food and feed additives, food enzymes and plant protection products [40].

In the case of drug development, discovery and authorisation of novel therapeutic *Lactobacillus* follows the con-

ventional drug pathway. The target application for potential probiotics can be as general as modulation of microbiome composition or function, or aiming toward particular disease through its influence on host specific pathways. As an initial step, a bank of relevant strains is screened for desirable activities *in vitro* or *ex vivo*. The next phase is safety assessment of the strains, which includes genome sequencing to screen for presumptive virulence factors, such as toxin and transmissible antibiotic-resistance genes. Following *in vivo* models are required to confirm the desired effects and safety in animal models. The pilot-scale production is defined in a manner that allows rapid Good Manufacturing Practice (GMP) scale-up. GMP standard are defined on national level and comply with the trading agreements between countries. Finally, a series of clinical research is implemented. In phase 1 often 30 to 100 human subjects are recruited to evaluates safety and dosage ranges of preparations. Phase 2 revolves around testing the drug on patients to assess efficacy and side effects. In phase 3 effectivness and safety of therapeutic doses are established on large population, followed by post marketing surveillance [36]. Unfortunately, only in rare cases preparations from Microbiota Therapeutics domain can generate sufficient data for clearance and authorities as a drug.

As the production pathway and allowable claims for dietary supplements are more flexible and can be authorised based only on preclinical studies, most probiotic *Lactobacillus* are available as supplements and claim only general health promoting properties.

Conclusions. The idea to use living microorganisms for disease prevention and treatment was introduced more than 100 years ago, but yet the full potential and benefits of microbial therapeutics has not been entirely understood and studied. Meanwhile the recent development of high throughput sequencing and metagenomics opened a new window in the research of microbial ecosystems and lead to rethinking of traditional dichotomic understanding of human bacteriology and its role in maintaining human health. This newly obtained knowledge about human microbiota function and composition lead to a revival of microbial therapeutics and in particular probiotic LAB. As the majority of health benefits of probiotics *Lactobacillus* are the result of interactions with the host biome and the creation of favourable microbiota, rather than replacing its composition, the concept of probiotic seems to be an optimal approach in dysbiosis treatments. Even more so, the personalised therapy approach with the anticipated use of auto-probiotics is gaining more and more relevance. But as a major set-back, hurdles with authorities on approved health claims clearances, restrictions on medical research approvals and inconsistency of regulatory frameworks around the globe, put probiotic *lactobacillus* and microbial therapeutics under restriction. Nevertheless due to high frequency of dysbiosis associated chronic diseases in urban populations and rapid increase of MDR infections worldwide, Microbial Therapeutics gain more and more importance as an alternative treatment and prophylactic method. Eventually authorities will be obliged to re-evaluate the restrictive approaches and work towards more feasible solutions.

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SUMMARY

REVIVAL OF MICROBIAL THERAPEUTICS, WITH EMPHASIS ON PROBIOTIC LACTOBACILLUS (REVIEW)

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The idea to use living microorganisms for disease prevention and treatment was introduced a century ago, but yet the full potential and benefits of microbial therapeutics has not been entirely understood. In the light of developments of human microbiome studies, probiotics are gaining new momentum, where health benefit conferring by *Lactobacillus* are emerging as one of the novel approaches in the treatment and prophylactics of dysbiosis. The present review focuses on the origin and development of the probiotic's concept, mechanisms of action and anticipated use of probiotic *Lactobacillus* as well as of microbial therapeutics. The required regulatory frameworks associated with probiotic use and marketing are discussed.

Keywords: *Lactobacillus spp., Probiotics, Microbiota, Dysbiosis, Lactic Acid Bacteria (LAB).*

РЕЗЮМЕ

МИКРОБНАЯ ТЕРАПИЯ И ПРОБИОТИЧЕСКИЕ ЛАКТОБАЦИЛЛЫ (ОБЗОР)

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Идея использования живых микроорганизмов для лечения и профилактики заболеваний была представлена еще столетие назад, однако потенциал и преимущества микробной терапии по сей день до конца не изучены. В свете новых достижений в исследований микробиома человека возрастает интерес и актуальность пробиотиков, в частности *Lactobacillus,* как нового подхода к лечению и профилактике дисбактериоза. В данном обзоре рассматривается вопрос о происхождении и развитии концепции пробиотиков, обсуждаются механизмы действия и предполагаемое использование пробиотиков *Lactobacillus,* а также микробной терапии и необходимые нормативные рамки, связанные с использованием и маркетингом пробиотиков.

რეზიუმე

მიკრობული თერაპია და პრობიოტიკული რძემჟავა ბაქტერიები (მიმოხილვა)

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დაავადებათა პრევენციასა და მკურნალობაში ცოცხალი მიკროორგანიზმების გამოყენების იდეა საუკუნეზე მეტს ითვლის. თუმცა, მიკრობული თერაპიის პოტენციალი და სარგებელი დღემდე სრულად არ არის შესწავლილი. ადამიანის მიკრობიომთან დაკავშირებული ბოლოდროინდელი აღმოჩენების გათვალისწინებით, პრობიოტიკები მზარდი ინტერესის საგანი ხდება. მეტ აქტუალურობას იძენს ჯანმრთელობისთვის სასარგებლო Lactobacillus-ის გამოყენება დისბიოზის მკურნალობასა და პროფილაქტიკაში. წარმოდგენილ მიმოხილვაში განხილულია პრობიოტიკური კონცეფციის წარმოშობა და განვითარება, პრობიოტიკების მოქმედების მექანიზმები და Lactobacillus-ის მოსალოდნელი გამოყენება, აგრეთვე მიკრობული თერაპიული პრეპარატები და აუცილებელი მარეგულირებელი ჩარჩოები, რომლებიც დაკავშირებულია პრობიოტიკების გამოყენებასა და მარკეტინგთან.