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A modern view of pro-/eukaryote interactions in the human body as the basis for development of next-generation probiotics

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Multicellular organisms and the saprophytic flora form complex, highly integrated chimeric systems (associative symbioses, metaorganisms) characterized by interplay between pro- and eukaryotic components. To be able to interact symbiotically microorganisms (MO) need a whole body.

When grown on artificial media for a long time, symbiotic MO have to adapt to the artificial environment and gradually, though reversibly, lose their ability of associative interaction with the human body, thus causing a decrease in the therapeutic efficacy of MO-derived probiotic products. To increase the therapeutic activity of probiotic MO, they must be functionally rehabilitated.

A pathological process induces development of a secondary metabolic dysbiosis; as a result, changes in the regulatory processes of an individual interfere with the restoration of the normal microflora. Therefore, functional rehabilitation of probiotic MO must take place during cultivation, while the cultivation process must replicate the whole-body conditions.

Keywords: *probiotics; regulatory peptides; protein hydrolysate; dysbiosis; correction.*

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Современные представления о про-/эукариотических взаимодействиях организма человека — основа создания нового поколения пробиотических препаратов

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Многоклеточные организмы в совокупности с сапрофитной флорой формируют сложные, глубоко интегрированные химерные системы (ассоциативные симбиозы, метаорганизмы) с выраженным взаимным влиянием про- и эукариотического компонентов. Способность микроорганизмов (МО) к симбиотическому взаимодействию формируется только в условиях целостного организма.

При длительном выращивании на искусственных средах симбиотические МО, адаптируясь к существованию в искусственных условиях, постепенно и обратимо утрачивают способность к ассоциативному взаимодействию с организмом человека, что приводит к снижению терапевтической эффективности пробиотических препаратов, приготовленных на их основе. Для повышения терапевтической активности пробиотических МО необходима их функциональная реабилитация.

При развитии патологического процесса складываются предпосылки формирования вторичного обменного дисбиоза, вследствие этого изменения регуляторных процессов самого индивида становятся препятствием для восстановления нормальной микрофлоры. Поэтому функциональная реабилитация пробиотических МО должна проводиться на этапе культивирования, а процесс культивирования должен в необходимой степени воспроизводить условия целостного организма.

Ключевые слова: *пробиотики; регуляторные пептиды; белковый гидролизат; дисбиоз; коррекция.*

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Introduction

A human is a multicellular eukaryote, which evolved in a world of bacteria existing long before its emergence. Phylogenetically, the evolving immune system came across bacterial signals for many millions of years and learned how to respond to them; therefore, studying of development and correction of the symbiotic microbiota structure has received increasing attention in the recent years.

Despite the multitude of scientific literature on development and correction of the symbiotic microbiota structure, studies in this area lack consistency. On the one hand, this fact suggests that most of the researchers still rely on the primary data and their assessment within the narrow bounds of traditional (infection-focused, antagonistic) concepts. On the other hand, the diversity of "dysbiosis-associated" pathological conditions speaks about the overall significance of symbiotic microbiota in development of diseases in humans and about the role of dysbiosis correction in comprehensive therapy.

However, the mere recognition of the important role, which dysbiosis plays in development of pathological conditions in humans, cannot solve the problems. In our opinion, the current controversy stems from the oversimplified (distorted) view of the relationship between the host body and its microbiota, when the integrated, whole-body pro-/eukaryotic system is split into two individualized, frequently antagonistic subjects.

Development of adequate understanding of pro-/eukaryote interactions is an important practical task, which will help identify the role of microbiota among the causes and mechanisms of development of a pathological condition and will help outline potential therapeutic approaches, including the required properties of pharmaceutical products, their production methods and uses.

Impact of host regulatory processes on the structural and functional condition of the symbiotic microflora

The first step towards the holistic view of the pro-/eukaryote interactions in the human body was made by academician O.V. Bukharin et al. [1] who offered the concept of "associative symbiosis" (AS). "AS is a multi-component integrated system including the host acting as a macro-partner, the stable dominant micro-symbiont and associated micro-symbionts characterized by multidirectional impacts affecting the development, existence stability and productivity of the symbiosis" [1]. The AS concept postulates a strong relationship between micro- and macro- symbionts, though giving very few details, thus making its full-scale application difficult.

The AS concept was further extended and specified, taking into account all dynamic transformations typical of prokaryotic and eukaryotic elements of the system. As a result, the concept of pro-/eukaryotic chimerism of the human body was offered. "The mutual functional penetration of cytokine and QS mediator systems makes it possible to see the microbiocenosis as an extracorporeal, cytokine-like, non-genetically inheritable regulatory system of the host body, while the human whole body is perceived as a pro-/eukaryotic chimera, thus bridging the gap between bacterial, cytokine and cell therapies" [2].

The above concept is based on the clinical data on probiotics and cytokines, which supported similar synergistic results obtained from the therapeutic use of probiotic and cytokine products [3]. Later, the concept received confirming evidence. In their works, professor A.V. Zurochka et al. [4–7] demonstrated the ability of microorganisms (MO) to produce a wide range of cytokine-like mediators, matching the amounts of cytokines produced by human blood cells. The synthetic analogue of the granulocyte-macrophage colony-stimulating factor was added to the culture medium to stimulate production of cytokine-like MO agents [7]. The researchers use the term "cytokine-like" agents, as their structural identity with cytokines has not been verified, though identification can be done with standard test systems used for detection of cytokines.

The above results not only broaden our knowledge of the pro-/eukaryote relationship in the whole body and explain the diversity of therapeutic effects inherent in probiotics, but also, as we believe, are of profound practical importance for development of probiotic products: The functional activity of probiotic properties that must and can be modulated (see below).

From a holistic perspective, the human body can be seen as a functional chimera or, following the Western terminology, as a metaorganism comprised of two equal subsystems organized under the similar principle (auto-/paracrine network regulation at the level of intercellular and inter-bacterial interactions), having the similar language of communication, and characterized by inevitable cross-interaction resulting from the structural and functional (phylogenetic) relatedness. Changes in one subsystem generate changes in the other, thus

suggesting the presence of the single, two-directional microbiocenosis–cytokines–hormones (MCH) regulatory system.

For example, microbiota imbalance is seen as the cause or the predisposing factor of type 1 diabetes and metabolic syndrome (obesity, atherosclerosis, hypertension, type 2 diabetes) [8], while the improperly monitored hormone therapy is seen as a pathogenetic factor of secondary hormone-related dysbiosis [9].

The involvement of the MCH regulatory system is well observed in clinical practice, during critical periods in the development of an organism, during pregnancy-related hormonal changes: stress-induced dysbiosis and post-stress syndrome; dysbiosis in pregnant women developing complicated pregnancies; dysbiosis in elderly people developing age-related pathology; negative or positive puberty changes during adolescence. The process is launched by natural changes in the hormone profile of the macro-partner; however, clinical changes are caused by changes in the system of micro-symbionts. "Changes in the normal microflora composition, immune system disorders and disease symptoms should be investigated in combination,… as it is the macroorganism's immune responsiveness rather than the pathogen's virulence that plays the leading role" [10].

However, the above conditions do not develop in all people, while the negative or positive scenario is predetermined long before the clinical manifestation and is associated with development of microbiocenosis during the perinatal period (perinatal programming) when affected by the maternal microflora [11]. In our opinion, the active growth of the potentially pathogenic flora, which is associated with the manifestation of the disease and affects its development, results from neuro-cytokine-hormonal changes that are caused by stress (including infection) or by natural changes in the hormonal status (pregnancy, aging, puberty); the potentially pathogenic flora is activated as a depressive (temporally hidden) microbiocenosis component of a perinatal origin. This explains the non-genetic heritability (i.e. contact transmission from the mother) of the predisposition to a pathologic process and adulthood pathology "programmable" in infancy.

The close relationship between microbiota and metabolic processes of the host opens the way for new and unique approaches to treatment and prevention of inflammatory infections. For example, testosterone used in prostatitis treatment helps restore the urogenital microflora and suppress inflammation without antibiotics [12], or the Selank synthetic analogue of tuftsin, a natural immunomodulator and adaptogen, used in prevention of post-stress, including dysbiotic [13], disorders.

Models of metabolic (hormone-related) dysbiosis clearly demonstrate the crucial role of host regulatory processes in development and maintenance of dysbiotic deviations; these processes create a vicious loop: metabolic and regulatory deviations — dysbiosis — aggravation of the metabolic and regulatory disorders and development of dysbiosis-associated pathologic conditions.

Thus, by creating the vicious loop, the host body and its regulatory processes become an obstacle for restoration of the normal microflora. In this context, any probiotic products with low metabolic activity (lyophilized bacterial products) will be inefficient. It suggests that probiotics based on archival (pharmaceutical) strains can be used successfully if preceded by a functional rehabilitation to increase their metabolic activity and improve their adaptation to the host environment (see below).

"Changes in the composition of mucosal microflora take place long before the clinical symptoms; therefore, they can be seen as antecedents of pathological processes caused by a potentially pathogenic microflora" [10]. What causes the pause between the development of intestinal dysbiosis and the manifestation of its clinical symptoms?

The processes of pro-/eukaryotic chimerism can be clearly demonstrated by the model of endogenous microbiocenosis, which captures, to the maximum extent, the relatedness between pro- and eukaryotic elements of the integrated system of the whole body as well as the interplay between the symbiotic microflora and the host body.

The discussion of human dysbiosis is generally narrowed down to assessment of qualitative and quantitative composition of the microflora in natural cavities of the body. Many authors keep supporting the thesis of sterility of a human body's interior. In the meantime, in plants, the saprophytic microflora is divided into exophytic and endophytic (exogenous and endogenous) domains. Is it different in humans? What causes formation of biofilms on endoprostheses, MO occurrence in atherogenic plaques, intracellular persistence of infectious agents, etc.? The list of MO capable of intracellular persistence is extending from year to year. The role of the endogenous flora comes to the fore when there is an apparent infectious or infection-induced inflammatory process. Does it mean that the endogenous flora does not exist, if the body develops immunological tolerance or immunological hyperactivity, thus displaying poor or zero inflammatory response? It is most unlikely.

Major success in studying of the endogenous microflora and related phenomena has been achieved in horticulture. Plants' capacity for vegetative reproduction creates temptation to replicate the most economically viable and productive species; propagation can occur not only through separate organs, but also through small tissue fragments (explants). However, the negative role of the endogenous (endophytic) microflora can be a serious obstacle for wide-scale cloning.

The relatively high tolerance of plant cells to culture conditions, their high regenerative capacity,

and multiple passaging of explants made it possible to create an informative model for identification and exploration of the endogenous microbiota, its functional characteristics, and its role in forming a phenotype of the host. No similar models based on animal and human cells have been created so far. However, scientific publications point out the relative legitimacy of the traditional division into human, animal and plant microflora [14, 15], as the same pathogenic bacteria can persist both in a human body and in plants [14], while the same probiotic MO can protect human, animal and plant organisms against pathogenic impacts (numerous products based on *Bacillus subtilis)*. From the perspective of general biology, the division of models into plant and animal models is also highly relative; therefore, the phyto-model of the endogenous microbiocenosis attracts attention of phyto-biologists as well as representatives of other biological and medical professions.

Endogenous MO exist in the uncultivated form in the internal environment of the host, thus making it difficult to use classical microbiology methods for their detection and exploration. For a long time, it has been believed that non-culturable bacteria owe their existence to factors associated with traumatic impact. The recent data demonstrate the one-sidedness of this opinion. Being elements of the integrated pro-/eukaryotic system, MO naturally obey the laws of the system, and these laws do not allow any autonomous, uncontrollable growth. While their capability of proliferation (autonomous growth) is lost reversibly, endogenous MO acquire the ability to function efficiently within the heterogeneous pro-/eukaryotic system, influencing metabolic processes in the host, participating in creating his phenotypic characteristics, governing MO's resistance to infectious diseases [16]. In the latter case, the protective effect is implemented not only through direct antagonism, but also through activation of protective mechanisms of the host [17]. In this context, the probiotic function of MO should be seen as a functional, non-constitutive condition that develops only in the whole body and is induced by regulatory signals of the host. Outside the regulatory impact of the host (the archival content outside the system), the probiotic function, apparently, declines as useless. This circumstance should be taken into consideration when developing probiotic products.

Assume that the intra-medium and intracellular persistence of MO, while being a common biological condition for multicellular eukaryotes, is a normal, natural constituent of pro-/eukaryotic systems (metaorganisms). It is of great practical importance in medicine not only for creating phenotypical characteristics of the host (physical well-being/ill-being), but also for timing corrective measures and, though it may sound unthinkable, for establishing a methodological and, in a long run, a procedural framework for development of probiotics. In this context, the endogenous microbiocenosis is a model for identification of mechanisms responsible for regulation of functional activity of MO. The identified mechanisms will be further used for modulation of functional activity of probiotic bacteria and for creating of nature-like techniques for development of probiotic pharmaceutical products.

This poses the question: Is it important which MO are represented in the endogenous pool? It is well-known that final effects of the endogenous microflora depend on the specific affiliation of MO — for example, high or low viability of plant explants depend on the specific composition of the endogenous microflora [15].

In the event of any changes in regulatory processes of the host and in the event of dominance of the potentially pathogenic flora in microbiocenosis, both a human [10] and a plant [15] go through functional activation of the pathogenic nature of potentially pathogenic MO, while the bacteria restore their capability of cultivation (autonomous existence) [18].

In humans, MO existing in natural cavities are a natural source of the endogenous pool. For example, it is believed that endogenous bacterial infections are caused by hematogenous transmission of mucosal microflora to internal organs [19].

The aforesaid means that it is important, firstly, to divide the dysbiosis into exogenous and endogenous constituents and, secondly, to perform dysbiosis-correcting therapy, with consideration both of the cavity microflora and the endogenous constituent. Note that intestinal (exogenous) microflora is more easily accessible for corrective measures as compared to the endogenous microflora, thus explaining the delay in effects of dysbiosis-correcting therapy [9] and can be correlated with delays in clinical symptoms of intestinal dysbiosis.

The endogenous constituent is of great significance for setting timeframes for selected measures. The proper timing is important for "physiological" sanitation, i.e. natural changeover of the cell population — a reservoir of intracellular microbiological agents. More efficient methods of intracellular sanitation have not been offered so far.

Different fields of medicine demonstrate interesting correlations: Probiotic therapy performed over 6 months causes reversibility of late complications in children with type 1 diabetes (T1D) [9]; 6 months after the renal transplantation, the risk of transplant rejection decreases dramatically [20]; the age of 6 months is a critical age for children with major birth defects; if a child overcomes the threshold, his chances of survival improve significantly [21]. In the above examples, the effect is caused by the cell population changeover due to the "physiological" sanitation, which can be observed in T1D cases, or due to development of transplantation chimerism, i.e. gradual replacement of donor cells in the transplant by recipient cells, together with decreasing immunogenicity and chances for transplant rejection. The repeatable accuracy of timing is indicative of

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its functional significance and the relationship between the clinical efficacy of probiotic therapy and physiological processes of the host. In our opinion, probiotic therapy should be performed at least for 6 months, though in each case its duration depends on individual and nosological characteristics of the patient, and on properties of the administered probiotic products.

There is nothing unusual in required long-term probiotic therapy, a variety of antibacterial therapy. Long-term courses of antibiotics demonstrated their effectiveness in treatment of severe infectious diseases and inflammatory infections: tuberculosis, leprosy, rheumatism, etc. Extended antibiotic therapy and extended probiotic therapy have the same underlying principles, though probiotics have a number of advantages: Long-term treatment without complications, filling ecological niches, including prevention of endogenous reinfection, and systemic therapeutic effect.

Absence (non-maturity) of holistic ideology and methodology addressing normocenosis/dysbiosis and pro-/eukaryote interactions is the root source of problems. It impedes development of efficient treatment medications and clinical application of this approach. Probiotics should be seen as a tool that needs not only smart "tuning", but also smart usage.

Dysbiosis means disorders in the microbiological landscape of natural cavities in the human body and skin. In other words, the underlying microbiological approach completely neglects the role of the host. From the clinical perspective and in accordance with the primary principle of the healthcare system (the nosological principle), the common medical problem (microbiocenosis/dysbiosis) was divided into "spheres of influence" among gastroenterologists, dentists, dermatologists and other medical professionals. Single-discipline specialists try to solve the general pathologic and even general biological problem (common for all multicellular eukaryotes) by applying highly specialized approaches and limiting the understanding of clinical manifestations of dysbiosis by the boundaries of their professional interests. As for such specialists as hematologists, endocrinologists, etc., who "are left without natural cavities", they are not involved in solving the clinical problem of dysbiosis, unless they take initiative on their own.

At present, the dysbiosis condition was split unintentionally into microbiological and clinical conditions. Microbiologists deal with diagnosis and recommendations for correction of dysbiosis as a microbiological condition, while specialist physicians handle its clinical forms. It means that the former do not see the results of the microbiocenosis impact on the host, are not aware of specifics of the particular disease, and neglect the role of the host in dysbiosis development, while the latter, due to their filed-related specialized training, are not able to assess the systemic nature of cause-and-effect processes, which led to development of the specific clinical condition. As a result, both groups of specialists tend to underestimate the importance of the problem. Only specialists who can overcome this gap are able to solve the dysbiosis problem successfully.

In the clinical context, the AS condition is changeable and is composed of current microbiological characteristics, a positive and negative impact of microbiocenosis on the host, and the host's existing ability to respond negative and positive impacts of micro-symbionts. Rehabilitation measures cannot be efficient without restored metabolic processes and without restored energy supply. Means of normalization of metabolic and regulatory processes in the host are an integral component of treatment of dysbiosis as a clinical condition. Therefore, in clinical practice, the human body should be seen as an integral pro-/eukaryotic system, and not only microbiocenosis, but also the entire AS system should be corrected to achieve successful results. Such comprehensive approach is used in real-life practice; however, the adequate methodology could contribute to more knowledgeable and efficient application of available tools and to their diversity.

Generally speaking, the clinical (not to be confused with microbiological) condition of dysbiosis is, first of all, a disruption of metabolic and regulatory processes of the host (the dysfunction of the cytokine-like regulatory system), initiation of tissue hypoxia. The process acquires a nosological form when affected by additional factors: The initial microbiological spectrum in combination with the external microbiological impact ("microsymbiocenosis" according to O.V. Bukharin [22]), individual immunoreactivity (tolerance), genetic characteristics, daily habits, etc. Specific clinical manifestations represent an immediate AS condition (the condition of pro-/eukaryotic chimerism) of a particular individual, i.e. clinical manifestations of dysbiosis are caused by multiple variables, the microbiological variable being one of them. It results in complexity of the diagnosis (prognosis) of clinical manifestations by using only microbiological data; the process cannot be modelled or rather, the currently existing conceptual microbiological tools cannot be used for modelling and correction of processes causing AS.

The concept of the MCH regulatory system implies that the current condition of the individual must be perceived as an integral reflection of the functional condition of the AS system, thus offering a two-directional, synergistic approach (i.e. simultaneous impact on prokaryotic and eukaryotic elements) to correction of the system condition.

The above theses make sense only when a physician has efficient tools for correction of microbiocenosis.

The postulated equality of the subsystems in AS enhances the biological significance of the microflora in life processes of the host, implies the leading role of dysbiosis in development of acquired and congeni-

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tal pathology, expands the range of therapeutic approaches, emphasizes the significance of development of efficient probiotic products and forms the methodological basis for solution of the problem associated with their development.

Use of nature-like technologies in development of therapeutic probiotics

The model of endogenous microbiocenosis suggests the possible existence of two forms of symbiont microorganisms: the form of free-living (independent of the host) bacteria and bacterial associations, and the form of close relationship with the host body. Both modes of existence are established under the influence of current external conditions; they are interchangeable and accompanied by functional, physiological, genetic, and sometimes morphological changes in MO [23]. MO existing in the human intestine are important for cambial cell population in replenishment of endogenous microbiota and, apparently, remain transient, as they are still connected with regulatory processes of the host, while being able to grow freely.

Initially, many probiotic MO were isolated from the human intestine; however, long-term cultivation of MO in laboratory conditions triggers bacteria's transition to the condition typical of free-living forms, thus contributing to lower probiotic activity. In development of probiotic products, it should be remembered that free-living forms are not capable of functional reversion in the patient's body due to intrinsic blocking impacts. This explains insufficient clinical efficacy of the existing probiotics; therefore, the functional rehabilitation (reversion) should be performed during cultivation of commercial strains.

The similar relationship between functional manifestations of symbiotic MO and living environment, through the example of potentially pathogenic microflora, is mentioned by other researchers: "Expression of genes governing virulence is not constitutive… Virulence genes are "turned off" during transition of the microorganism to the external environment and are "turned on" in the host body" [10].

The integrated pro-/eukaryotic system can be formed only if there is a common language of communication or, in other words, the language of interaction, which is understood by both parties. The system of regulatory peptides can act as this language. The ability to influence the growth and functional properties of MO is demonstrated by the tumor necrosis factor, interleikins-2 and 6, fetal serum, antibacterial peptides [24, 25]. In the latter case, it is emphasized that the antibacterial effect will occur only in high concentrations of the agent, while in physiological concentrations the effect of "antibacterial" peptides is limited to regulatory impacts [26].

The peptidic regulation is one of the mechanisms, through which the host body influences the microbiota function. Understanding and using of this mechanism by varying regulatory peptides will make it possible to create probiotic products characterized by the required functional activity. This assumption may have methodical nuances, which should be taken into account during development and production of probiotic products.

The phylogenetic relatedness in organization of pro- and eukaryotic systems is manifested in different models. It is assumed that prokaryotes use the same strategy as eukaryotes for genome amplification, differentiation and growth of cells [23]. It has been demonstrated that MO are able to produce regulatory agents similar to those produced by eukaryotes [2, 27]. The similarity of processes taking place in pro- and eukaryotic elements of the system suggests, by analogy with eukaryotes, the relationship between the functional activity of bacteria and the age of the culture: The aggregate probiotic (therapeutic) effect of the MO culture (the probiotic product), the range of agents produced by MO and, consequently, the type of the therapeutic impact will depend on the functional "maturity" of the MO culture. In eukaryotes, such relationship between the regulatory spectrum and the "maturity" of the process has been demonstrated multiple times and on different models [28], thus implying that it is typical of both pre-cellular and cellular communities.

In natural environments, regulatory agents of peptidic nature are produced during hydrolysis of large precursor-molecules, i.e. they are natural hydrolysates. This consistent pattern gives hope that the controlled hydrolysis, together with selected proteolytic agents, source substrate, required conditions, monitored and standardized regulatory activity of the produced agents, will help obtain the material with required characteristics for further use as the basis of culture media for growing probiotic cultures.

Some researchers can object: Protein hydrolysates have been used in microbiology actively and for a long time. Yes, it is true. However, being constrained by the boundaries of traditional beliefs, microbiologists used to neglect a potential regulatory role of peptide molecules of hydrolysates, which were seen as a mere source of nitrogenous nutrition, and the results were assessed from the perspective of nutritiology. Furthermore, high-temperature sterilization is traditionally used during preparation of culture media, which may cause inactivation of regulatory peptides. This fact requires further exploration. The ability of MO to perform their functions in processes occurring in the host body also needs further study.

Conclusion

The concept of AS (metaorganism) brings changes into the methodology of development of probiotic products: To initiate the probiotic function, the conditions of cultivation of probiotic MO must match the conditions

of the integral pro-/eukaryotic system. The therapeutic efficacy should be evaluated not only by antagonistic activity *in vitro* and by the ability to exert influence on the microbiological landscape *in vivo*, but also by the aggregate clinical effectiveness, with consideration of the severity of the primary condition. Clinical and experimental modelling is labor and time-consuming; therefore, it is inefficient for prompt problem-solving. The exceptionally important task is to identify the criterion that can be measured *in vitro*, but can be used for prognosis of therapeutic efficacy of a probiotic product for the whole body. This criterion can be found in the ability of MO to synthetize core mediators (cytokines and lectins), which play an important role in functioning of prokaryotic and eukaryotic systems [5, 27]. Besides, attention should be paid to the relationship between the level of production demonstrated by these mediators and the living environment, considering a sharp decrease shown by archival strains [5, 29].

The summary of the obtained data leads to the conclusion that the probiotic function of MO is not constitutive, that it is performed only in the whole-body environment, and is controlled by host regulatory processes. These key factors should be taken in consideration during development of probiotic products.

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