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Metabiotics: novel idea or natural development of probiotic conception

Boris A. Shenderov*

Laboratory of Biology of Bifidobacteria, Gabrichevsky Research Institute of Epidemiology and Microbiology, Moscow, Russia

Traditionally, probiotics are live microorganisms that are considered to be both beneficial and safe. Unfortunately, their effects may be short-lived, inconsequential, or ambiguous. Some symbiotic (probiotic) microorganisms with known health benefits may cause opportunistic infections, increase incidence of allergic sensitization and autoimmune disorders, produce microecological imbalance, modify gene expression, transfer genes that are virulent and resistant to antibiotics, cause disorders in epigenome and genome integrity, induce chromosomal DNA damage, and activate signaling pathways associated with cancer and other chronic diseases. As of now, the commercially available probiotics serve as a first-generation means of correcting microecological disorders. Further development will include the selection of natural metabiotics and/or formulation of synthetic (or semi-synthetic) metabiotics that will be analogies or improvised versions of natural bioactives, produced by symbiotic (probiotic) microorganisms. Metabiotics are structural components of probiotic microorganisms and/or formulation of and/or signaling molecules with a determined (known) chemical structure that can optimize host-specific physiological functions and regulate metabolic and/or behavior reactions connected with the activity of host indigenous microbiota. Metabiotics are advantageous because of their chemical structure, dosage, safety, and long shelf-life. Thus, metabiotics should not be considered a myth; they are the result of the natural evolution of probiotic conception.

Keywords: *probiotics*; *metabolome*; *microbial bioactive molecules*

*Correspondence to: Boris A. Shenderov, Moscow Research Institute of Epidemiology and Microbiology after G.N. Gabrichevsky, Moscow, Russia, Fax: +495-452-1830, Email: shenderof@yandex.ru

Neither microbial genes nor microbial names cause any harm to the body, but microbial products may do. The presence of very active microbial biological compounds in the gut may have physiological and pathophysiological consequences for the host. *Tore Midtvedt* (2008)

According to modern scientific doctrine, the human being is a 'superorganism' – a consortium of numerous *Eukarya, Bacteria, Archaea*, and *Viruses*. Various indigenous host microorganisms should be considered as essential complex extracorporal physiological systems playing a fundamental role in human health and disease. Host microbiota and other functional and metabolic systems connected with host eukaryotic cells are working together profitably for the whole organism and for its separate components. Unfortunately, various unfavorable biotic or abiotic factors and stress agents (diet, age, sex, pharma-

ceutical interventions, surgical interventions, etc.) can produce microecological disorders, resulting in tissue, organ, and regulatory system disturbances that can lead to enhanced risk of disease development (1–5). This means that the modulation of microbe–microbe and microbe–host interactions is an extremely important, fundamental, and concrete problem in modern biology and medicine. To date, to maintain and restore the gut microbial community, three therapeutic approaches have been used: probiotics, prebiotics, and synbiotics (4, 6–10). As defined by FAO/WHO, probiotics are non-pathogenic live microorganisms which, when administered in adequate quantities, offer health benefits to the host (6). Many therapeutics, dietary foods, and additives containing probiotic microorganisms have been introduced in practice for human health support; for acute, chronic, localized, and systemic disease prophylaxis; and for treating acute or chronic diarrhea,

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1

intestinal inflammation leading to allergy, atherosclerosis, and cancer (5, 11). In the past decade, transplantation of the distal gut microbiota (fecal bacteriotherapy) has become popular in the treatment of some inflammatory intestinal diseases (12, 13). The introduction of genetically engineered probiotics based on recombinant live microorganisms in medical practice has also been actively discussed (4).

Undesired properties and adverse affects of live probiotic microorganisms

Although, more than 50 years' use of probiotics has shown them to be safe and beneficial, we are yet to define the optimal amount of bacteria for probiotic effects; there is no single mechanism of action for all probiotics. Moreover, the beneficial effects of probiotics may be short-lived, inconsequential, or ambiguous (3, 7, 14). The latter may be explained by the low concentration of biologically active probiotic substances (bioactives) found in target places during the traditional application of live probiotic microorganisms. The number of these microbial bioactives produced may be inadequate to receive desirable specific effects under *in vivo* conditions (4, 7). Besides, various molecules produced in volume by live probiotic cells may interact with different receptors of indigenous microbes and host cells, simultaneously resulting in both beneficial and detrimental effects (15). Modern 'omic'-technologies have revealed substantial diversity of the gut microbiome between individuals. Only a few microbial phylotypes (species) are common to all individuals about 80% of human intestinal microorganisms in the adult gut are individual at the strain level (7, 10, 16–19). Experimental and clinical studies published in recent years have demonstrated that it is very difficult, if not impossible, to produce industrially adequate probiotics for supporting indigenous microflora at the optimal level by the simple mechanical selection of a separate or combined set of probiotic microorganism strains. Furthermore, limited knowledge about the affect mechanisms of such biotherapeutics on the host gut microbiota, physiology, and metabolism hampers the design of effective probiotics. However, little is known about the molecular mechanisms of probiotic effects (10, 15, 20).

In recent years, our knowledge on the safety of probiotics has increased. It is necessary to bear in mind that any detrimental and harmful consequences may become apparent only after extended periods of probiotic use. In reality, some data now show that not all probiotic bacteria are safe, even if they belong to the *Lactobacillus* or *Bifidobacterium* species with no traditional genes of pathogenicity (21, 22). From medical reports it can be inferred that lactic acid bacteria and even bifidobacteria used as microbial food cultures or probiotics are rarely associated with human opportunistic infections (infective endocarditis, sepsis, bacteraemia, pneumonia,

abdominal abscesses, peritonitis, meningitis, urological infections, rheumatic vascular diseases), especially in patients receiving antibiotic treatment or those who are severely immune compromised (21–28). There are increased incidences of these bacteria being responsible for allergic sensitization and autoimmune disorders (23, 29–32). Symbiotic microorganisms (including probiotic strains) sometimes can increase platelet aggregation aggravating hemolytic uremic syndrome (21, 26); some of them may be a source of toxic metabolites (e.g. biogenic amines) (22). Probiotics found on living microbes when introduced into gastro-intestinal or vaginal tracts may cause unintended harm by gaining a competitive advantage and causing ecological imbalance by the microbe biodiversity and metabolic pathways. The vast majority of probiotic strains introduced in practice have been selected on the basis of their strong antagonistic activities against disease causative microorganisms, it seems that many probiotics can suppress the growth and development of human gut and vaginal lactobacilli as well as other different indigenous microbiota (33, 34). They may also alter intestinal metabolism due to their microbial enzymatic activities (35). It is necessary also to remember that some intestinal microbial strains can participate in the transformation of some drugs, modifying their activity and/or converting the prodrug to the active product (36–38). Unfortunately, nothing is known about interactions of live probiotic microorganisms with drug function *in vitro* and *in vivo*. The situation becomes more complicated when new strains belonging to *Enterococcus, Streptococcus, Escherichia, Bacillus, Bacteroides,* or other microbial genera are suggested as potential probiotics, including probiotics that consist of multistrains or are constructed based on genetically modified microorganisms. There are data that probiotics (*Lactobacillus GG*) inside the intestinal tract can induce the expression of more than 400 new genes involved in immune response and inflammation, cell growth and differentiation, apoptosis, cell-to-cell signaling, and cell adhesion, resulting in a wider impact on the host's gene expression than thought of before the era of macroarray technologies (39).

Oral introduction of *Enterococcus faecalis* modulates activities of 42 genes in the gut epithelial cells; these genes are involved in the regulation of the cell cycle, cell death, and signaling (40). It has also been shown that, during the passage through the intestinal tract, some silent genes of probiotic bacteria may also be induced by host cell signals; newly formed bacterial products are poorly characterized, both chemically and functionally (39, 41, 42). For example, the induction of 72 probiotic *L. plantarum* WSFS1 genes was demonstrated in these conditions: nine genes were responsible for sugar transport and the production of different enzymes involved in their fermentation; nine genes were responsible for the

synthesis of amino acids, nucleotides, cofactors, and vitamins; four genes were responsible for outer cell proteins controlling resistance to specific host factors; and 46 genes were responsible for the production of non-identified proteins (42). It means that the probiotic cells inside the gastrointestinal tract may be involved in the interaction with various host-specific networks, for example, participation in the degradation and production of different nutrients by different metabolic pathways in the small intestine and colon (18, 43), or in the modification of immune response, cell differentiation, cell signaling, adhesion (18, 44), cell proliferation, tissue development, water and ion metabolism, balance of Th1/Th2 cells, and so on (15, 45). Thus, it can be expected that the expression of known or silent microbial and eukaryotic genes may lead to undesirable effects on human health.

In recent years, evidence has appeared that horizontal gene transfer may take place between lactic acid bacteria and enteric bacteria as well as between different lactic acid bacteria. Natural gene transfer usually occurs via the uptake of naked DNA (transformation), viruses (trans-duction), or plasmids (conjugation). It is well-known that the spread of antibiotic resistance is a major global health issue (46). Probiotic bacteria can possess acquired anti-biotic resistance genes associated with mobile genetic elements (plasmids, transposons) that permit these or-ganisms to transfer this genetic information to strains of the same species, different species, or even different genera, including both commensals and pathogens. Such recombination events might not only result in the distribution of undesired genes among intestinal micro-organisms but can also produce rearrangements in microbial genomes and change gene expression patterns in recipient bacteria. The gene transfer and recombina-tion events associated with probiotics may have long-term environmental and health consequences (21, 22, 26, 46), including chromosome rearrangement and death of recipient cells (47), alteration of the genomic and epi-genomic regulation of gene expression, post-translation modification of gene products, and host/microbial cross talk resulting in the change of human metabolic and behavior reactions (20, 48). Recently, it has been shown (on the *E. coli* model) that the DNA methyltransferases represent potential threats to the epigenomic integrity of cell genomes. When the methylation system enters the cell and begins to methylate the host genome, the methylated DNA-specific microbial DNAse senses the epigenetic changes, causing cell death via chromosomal cleavage (49). Traditional virulence traits should not be present in microorganisms used in food fermentation and probiotics (21, 22).

Investigations made during the last decade have shown that some human symbiotic microorganisms can produce substances possessing genotoxic affects in intestinal epithelial cell DNA. Therefore, some commensals (including probiotic strain *E. coli* Nissle 1917) possess a set of genes (*pks* island) that are responsible for the production of double-strand breaks in host cell DNA. Bacteria containing the *pks* genes induce in eukaryotic cells a process called megalocytosis, in which the cell body and nucleus become enlarged and mitosis stops. Sometimes, 4 hours is enough for the *pks* island carrying bacteria with eukaryotic cells to prompt an increase in the DNA double-strand break level. A total of 34% of *E. coli* strains isolated from healthy human intestine content had such *pks* islands. A small number of bacterial cells caused minimal DNA damage in eukaryotic cells; in contrast, exposure to 100 or more bacteria per cell has broken down the most nuclear DNA. Thus, the breaks in host cell DNA caused by peptide-polyketide genotoxins of some indigenous (probiotic) bacteria could trigger the various cell disorders, including intestinal cancer devel-opment (50, 51).

Live cells of *E. faecalis* being inside the intestinal tract release substantial extracellular superoxide, hydroxyl radical, and H_2O_2 during carbohydrate fermentation and via autoxidation of membrane demethylmenaqui-none. These oxidants can damage DNA and facilitate development of sporadic adenomatous polyps and color-ectal cancer (40, 52). Ethanol and its first metabolite acetaldehyde have been recently classified as a class I carcinogen. The acetaldehyde concentration required for a mutagenic DNA effect increases from 100 to 500 μM. Many microbes (including lactic acid bacteria) used as microbial food cultures in fermented food products and in the manufacture of probiotics can convert ethanol and/ or glucose to carcinogenic acetaldehyde. The acetalde-hyde levels formed may exceed the aforementioned levels markedly. Furthermore, because probiotic bacteria can colonize the intestinal tract for a long period of time and produce carcinogenic acetaldehyde locally, they can potentially be more dangerous than traditional dairy lactic acid bacteria because of increased total exposure to this bacterial metabolite (53, 54).

Some scientists consider that 'genetic engineering of the human microbiota will enable to endow its members with new desirable functions that treat diseases or promote health' (10). In spite of the scientific attractive-ness of this idea, from a prolonged point of view, the mass introduction of genetically engineered live probiotic microbes in practice may have extremely dangerous ecological and medical consequences even when using such novel platform of microbial cells design as synthetic biology approaches. Thus, the aforementioned data and discussion indicate that the present knowledge on pro-biotics is inadequate for any reliable ecological and clinical risk assessment of short- and long-term conse-quences; they could in fact often be unpredictable. Up to now, we have no sufficient scientific knowledge to

support manipulation of the human microecological, immune, and metabolic systems in an exactly predictable manner by the administration of live probiotics (including gene engineering), especially to infants and young children (26, 35). The public should have the right to participate in a discussion concerning known and potentially undesired properties of probiotics made based on living microorganisms and have information regarding the unpredictable consequences of their long-term use.

Metabiotic concept

Although the history of live probiotic use does not highlight any area of serious concern, recent well-documented events of adverse effects and uncertainty about the level of their risk require new alternative approaches in prophylaxis and treatment of pathological conditions associated with the imbalance of host microbiota. These approaches have to retain and improve the positive accumulated experience of working with live commensal microorganisms (probiotics) and increasing safety.

Investigations in the last 10–20 years have demonstrated that gut microorganisms (including probiotic strains) are capable of breaking down and metabolizing complex food nutrients and endogenous substances (saliva, gastro-intestinal juices compounds, epithelial cells, dead microbial cells, etc.), resulting in the formation of low molecular weight (LMW) bioactives that may be localized both inside and/or outside of microbial cells and found in the intestinal content or passing across the intestinal epithelial barrier determined in the various human fluids, organs, and tissues. These compounds, derived from probiotic (symbiotic) microbes, form what is called the probiotic metabolome. Interacting with corresponding prokaryotic and eukaryotic cell targets, biologically and pharmacologically active compounds may control many genetic, epigenetic, and physiological functions; biochemical and behavior reactions; and intra- and intercell exchange of information. Some commensal microbes, including probiotics, can secrete a variety of signaling molecules that can modify the inter-bacterial signaling (quorum quenching) and suppress the expression of virulence genes in pathogens or stimulate the growth of beneficial indigenous gut microorganisms (15, 18, 20, 48, 55–62). In my opinion, the probiotics that are commercially available now should be considered as a first generation of means directed for correction of microecological disorders.

Further development of traditional probiotics will include improvements to the present generation by means of production of natural metabiotics (manufactured based on current probiotic strains) and synthetic (or semi-synthetic) metabiotics that will be analogies or improvized versions of natural bioactives produced by symbiotic microorganisms. The terms 'metabiotics' (4, 20, 63, 64), 'metabolic probiotics' (65, 66), 'postbiotics' (1),

'biological drugs' (10), or 'pharmacobiotics' (15) mean small molecules; they are the structural components of probiotic (symbiotic) microorganisms and/or their metabolites and/or signaling molecules with a determined (known) chemical structure that can affect the microbiome and/or human metabolic and signaling pathways, optimizing the composition and function of indigenous microbiota and host-specific physiology, immunity, and neuro-hormonbiology, and regulating metabolic and/or behavior reactions connected with the activity of host indigenous microbiota. Different probiotic strains can become the source for hundreds (thousands) of LMW bioactives - bacteriocins and other antimicrobial molecules, short chain fatty acids, various other fatty and organic acids, biosurfactants, polysaccharides, peptidoglycans, teichoic acids, lipo- and glycoproteins, vitamins, antioxidants, nucleic acids, different proteins including enzymes and lectines, peptides with various activities, amino acids, growth and coagulation factors, defensin-like molecules or their inductors in human cells, messenger (signal) molecules, plasmalogens, various co-factors, and so on (2, 7, 10, 15, 18, 20, 48, 55, 56, 67). Various symbiotic (probiotic) strains can produce different sets of such LMW bioactive molecules, attractive candidates for metabiotic construction (Table 1).

It should be remembered that the spectrum and number of bioactives of microbial origin determined in the different human biological fluids and eukaryotic cells may also be connected with the activity of the host transmembrane transporters and/or liver and other tissue enzymes that can carry or transform various microbial substances. Metabolomic analysis of plasma extracts from germ-free and conventional mice (68) as well as other hosts and gut-microbial co-metabolomes revealed a significant interplay between gut microflora and mammalian metabolism (37).

Specific representatives of these groups of LMW compounds isolated from symbiotic (probiotic) microorganisms or their cultural liquids may be used for manufacturing microbe-free food supplements, functional foods, and drugs for prophylaxis and treatment of chronic human diseases, as well as sport and anti-aging foods, and so on. The aforementioned approaches and

Table 1. Some groups of LMW compounds of symbiotic (probiotic) microbe origin that may become the basis for manufacture of potential metabiotics

Bacteriocins
Short-chain fatty acids, other organic acids
Proteins, peptides, amino acids
Nucleic acids, nucleotides
Polysaccharides, peptidoglycans, other cell surface molecules
Plasmalogens, vitamins, antioxidants, co-factors
Various messenger (signal) molecules

tools for the design of new types of bioactives based on LMW molecules of symbiotic microbiota origin for nutrition and medicine are already being developed in some countries (60).

Current probiotic strains may become starter strains for industrial manufacturing of such microbial bioactive molecules. The knowledge of quality and quantity of the LMW molecule profile of each industrially used or potential probiotic strain will help researchers to design novel metabiotics with increased health effectiveness, and determine the optimal frequency, dose, and mode of administration. Industrial production of metabiotics could become the novel prophylactic and therapeutic approach to address human health in the near future because of their potential ability to interfere in the processes associated with stability of the host genome and microbiome; modulation of epigenomic regulation of gene phenotypic expression in eukaryotic cells; improvement in information exchange, signaling and metabolic pathways in numerous bacteria, bacteria–host, and host systems that play an important role in the control of many genetic, epigenetic, and physiological functions, and biochemical and behavior reactions; in cell growth and host development; in supporting host health in general. We should always remember that real health effectiveness of suggested metabiotics depends on our knowledge of the physicochemical characteristics of metabiotic molecules [molecule isomerism: L or D, and α, β, or γ forms; valency and isotopic state of incoming chemical elements; substance solubility; dispersiveness, ligand binding, oxido-reduction potential of metabiotic ingredient(s), its(their) interaction with other components that can be both enhancers or inhibitors; competitive inhibitors for specific transport proteins or absorption site] as well as host physiological status, illness, constituents of diet, medication, and so on.

In biotechnology, the introduction of the concept 'Metabiotic' in practice permits inclusion of not only bifidobacteria, lactobacilli, *Escherichia*, enterococci strains but also tens and hundreds of other strains belonging to human-dominant intestinal phyla (*Bacteroides, Firmicutes, Proteobacteria, Actinobacteria*, and *Archae*) for nutrition and medical aims. There is no doubt that the future metabiotics created based on the strains belonging to the wider spectrum of dominant microorganisms may be more effective at conferring health benefits than the LMW bioactives received only based on the classical probiotic strains (bifidobacteria and lactobacilli). The known and potential microbial starter cultures of human microbiota origin that could be used as a platform for metabiotic production should first be investigated on the synthesis of structural and excreted bioactive substances participating in or regulating pathways associated with carbohydrate, fat, and cholesterol metabolism; work of immune, hormone, and nervous systems; metagenome

and epigenome stability; and gene expression regulation. Undoubtedly, among human gut microbiota there are species and strains which are potential sources of small diffusible antimicrobial agents, regulators of host energy balance, cholesterol-lowering compounds, modifiers of immune reactions, stimulants, antidepressants, cognitive enhancers, and so on. (10, 60, 63). Development of databases of microbe-derived individual compounds or groups of compounds provides key information, which could be used toward a goal-directed design of metabiotics with specific medical effects. It is also important to remember that wide rosters of LMW microbial metabolites and signal molecules produced by microorganisms and determined in the human physiological fluids can have great diagnostic value (2, 37, 69, 70). Because *in vivo* production of bioactive small molecules of human and microbial origin is often connected with prebiotic-stimulated secondary metabolism (8, 10), it would be sensible to design combined metabiotic/prebiotic foods and drugs for nutritional and medical purposes and prescription targeted to host microbiota or to indigenous microflora associated host functions, metabolic and behavior reactions.

Known and potential metabiotics

Some metabiotics based on natural (or artificial) bioactives of microbial origin have already been introduced in the last decade into medical practice and have proven their effectiveness in reducing infectious and metabolic diseases. For example, currently, in the international drug market, the most well-known metabiotic is '*Hylak Forte*' (Ratiopharm/Merckle, Germany. Code EAN: 4030096245166; N P No1497/01, 2009-05-14). This bacteria-free liquid medicine for oral use contains metabolic products of *Escherichia coli* DSM 4087 (25 g), *Streptococcus faecalis DSM 4086* (12.5 g), *Lactobacillus acidophilus DSM 4149* (12.5 g), and *L. helveticus DSM 4183* (50 g). The benefits of this sterile liquid drug are explained by the presence of SCFA, lactic acid, and some other non-identified microbial metabolites in this drug. Data prove that '*Hylak Forte*' has health benefits for adults and children by producing positive shifts in intestinal microbiota, host acid–alkaline balance, water–salt metabolism, vitamins B and K balance, and energy provision to intestinal epithelia and local immune cells (71). Other notable commercial metabiotics are 'Zakofalk' (a combination of sodium butyrate and inulin) recommended for the treatment of mild to moderately active inflammatory intestinal diseases (72); *E. coli* glycoprotein with anorexic activity (73); *Lactobacillus casei* (LEx) polysaccharide – glycopeptide with antihypertension effect (74, 75); *L. helveticus* three-peptides (Val-Pro-Pro and Ile-Pro-Pro) inhibiting angeotensin convert enzyme (ACE) and resulting in the decrease of blood pressure (76, 77); lyophilized *B. subtilis* bacteria-free culture fluid containing lysozyme, catalase, polypeptides, peptidoglycan, amino

acids, and natural sorbent ceolite with immune-modulator and gut microbiota-restoring activity ('bactistatin') (78; www.stada.ru/products/baktistatin.html); lactic acid bacteria SCFA and other organic acids ('Solcarmon', 'Frodo') (79); and Quorum sensing *Escherichia*, lactobacilli and bifidobacteria autoregulators of growth and development of indigenous intestinal microflora (65, 66). Potential metabiotics on the basis of lactobacilli, bifidobacteria, enterococci proteins, peptides, adhesions (55, 56), biosurfactants (more than 30kD) (80), lectines (81, 82), nucleic acids, and various cell wall molecules (15) connected with microbial cells or produced in cultural liquid possessing various host effects are now under development. Special attention should be paid to microbial membrane sterol-like compounds (e.g. plasmalogens) (59) and outer membrane lipoproteins (e.g. lipocalins) (83, 84) as attractive candidate molecules for the manufacture of different types of metabiotics that are capable of inhibiting lipid and energy metabolism, immune, hormone and nervous system functions.

Metabiotics, as modifiers of physiological functions, biochemical and behavior reactions, have some advantages such as chemical structure, dosage, safety, and long shelf-life. Besides, metabiotics possess better absorption, metabolism, distribution, and excretion abilities compared with classic probiotics based on live microorganisms. A detailed molecular understanding of metabiotics can turn them into significant, specific, and active contributors to the benefits derived from probiotics (4, 10, 15, 20).

If a manufacturer tries to label a metabiotic product with specific therapeutic claims (prevents or treats a specific disease or improves a specific function or metabolic reaction), such metabiotics must be regulated as a drug needing approval by individual national and international agencies controlling quality and safety of food and drugs.

Conclusion

Metabiotics are no longer a myth; they are a natural evolution of the probiotic conception. In the near future, based on probiotic LMW bioactives with proven specific beneficial effect(s), a set of semi- and/or completely synthetic metabiotics will be designed that are analogies or improved versions of the natural microbial bioactives. This is a similar route of development as in the case of traditional antibiotics which have been in production during the past 50 years. LMW bioactives of indigenous microbiota origin being chemically similar with the molecules of other origin (e.g. from food raw materials or foodstuffs) have advantages in principle. For millions of years of evolution, the human superorganism has been selecting prokaryotic and eukaryotic microorganisms from the environment that were functionally and metabolically the most optimal for human life and development.

It means that metabiotics manufactured based on the LMW bioactives of indigenous microbiota origin are the most beneficial and the safest.

The metabiotics of the future will promote further development of the probiotic concept, improve effectiveness and benefit specificity of classic probiotics, and reduce environmental and health hazards of the current micro-ecological approaches in the prevention and treatment of diseases associated with imbalance of host microbiota.

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