

Probiotics and Antimicrobial Peptides: The Creatures' and Substances' Future in the Twenty-First Century: An Opinion Letter

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While the number of scientific publications related to health-promoting bacteria and antimicrobials of proteinaceous nature is growing, there is an obvious need for a better comprehension of what these studies actually provide the community and which directions these studies should be taking. With no intention of neglecting fundamental science (in which true knowledge helps separate fact from fiction, and eventually finds its practicality), I would still like to step on a dangerous path and speculate on the near-future progress in the aforementioned field of science with emphasis on possible beneficial outcomes.

The number of marketed food products and dietary supplements labeled as “probiotics” or “containing probiotic bacteria” is virtually incalculable. In 2010, the global probiotic market was estimated at \$22.6 billion and is projected to reach \$28.8 billion by 2015 [1]. Very few microorganisms have been subjected to thorough in vitro studies confirming their specific health-promoting activity, and even fewer were subsequently subjected to and passed the appropriate human trials. A few studies of interest include those which investigated the strains *Lactobacillus plantarum* 299v, *Lactobacillus casei* Shirota, *Lactobacillus rhamnosus* GG should probably be mentioned—yet even the latter was reported on several occasions as associated with bacterial sepsis (for review see: [2]). Additionally, probiotics can be truly dangerous, posing as a wolf in the sheep's clothing: They have been linked to an increase in mortality rate if administered to severely immunocompromised patients [3]. Those interested in “legal” probiotics may consider looking at the list of so-called “Commercial

Strains Sold As Probiotics” [4] where the phrase “sold as” is perhaps the most telling in indicating the less than firm certainty about these strains being large-scale human trial-proven as delivering measurable benefits. Still, there is WHO guidance on probiotics [5] and a brief overview of the claims discussed as required and sufficient for placing a microorganism into a group of “good fellows” [6]. Finally, NIH Grant 5R01HG005171-02 resulted in a document entitled “Federal Regulation of Probiotics: An Analysis of the Existing Regulatory Framework and Recommendations for Alternative Frameworks” [7] which only confirms the murky reality of less than stringent and frequently disconnected regulations currently being observed.

As of December 14, 2012, the European Food Safety Authority (EFSA) posed serious and soundly justified [8] restrictions on labeling food products containing probiotics—the matter was strongly opposed by the major world manufacturers of microorganism-fermented/containing food products and by the large-scale manufacturers of microbial cultures [9]. Currently, there are two (European and North American) different statuses resulting in two different approaches addressing the real problem: how to separate the truly beneficial microorganisms from the crowd of overall harmless but “indifferent” bugs broadly used to make some products attractive to the consumers [10].

All of these leave a few questions and hopefully some answers. The diversity and multiplicity of the human microbiota cannot be ignored. While broad generalizations can be made concerning the frequency of certain species in our body's ecological niches, they are genetically variable [11]. Certainly, the common strains associated with healthy human subjects should do no harm when ingested with a food product or administered otherwise. The additional health benefits are somewhat doubtful; why should they deliver anything in addition to what they already provide?

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Also, if a product claims to contain live, active and health-promoting bacteria but does not deliver the expected effect to some (few? many?) consumers, should this claim be withdrawn or carefully revised (e.g., [12])? Clearly, probiotics showing statistically significant and reproducible positive effects in clinical [13] or other large-scale trials [14, 15] should keep their well-deserved and earned name. Furthermore, while the society's primary concern is focused at the use of probiotics for various human benefits, the issue is similarly important when it concerns animals of agricultural importance [16], aquacultures [17], and poultry [18].

Therefore, it is only logical to expect an increase in the number of studies which focus on understanding the mechanisms of action of health-promoting bacteria which show positive and reproducible results in clinical and other, specific large-scale environments when used under the supervision of medical or other appropriate for the study/application professionals. In addition, with already numerous statistically sound and highly reproducible results, the science of probiotics for the improvement in animal husbandry will continue blooming. As for the species *Homo sapiens*, we still have to find how diverse our individual and group (ethnic, social, age, gender, etc.) microbiomes are (e.g., see: [19]).

While probiotics deliver health-promoting assistance to eukaryotic organisms in many ways (e.g., immunomodulation, cancer prevention, cholesterol reduction, etc.), the ability to produce and utilize a variety of “weapons” active against pathogens and other unwelcomed ecological niche-intruders is well documented. Healthy human lactic acid bacteria produce several potent antimicrobials such as hydrogen peroxide, bacteriocins, and of course, lactic acid. In fact, approximately 60 % of healthy vaginal lactobacilli are reported as having all three defense mechanisms [20]. Therefore, natural antimicrobial proteins go hand-in-hand with probiotics in their defense against numerous pathogens.

Natural antimicrobials of proteinaceous nature and particularly those ribosomally produced by bacteria and called bacteriocins is a young field of science. However, two of the most studied bacteriocins, namely nisin and pediocin PA-1/AcH, have already established their firm presence in line with other natural food preservatives, either claimed as an active substance in formulation or being present in a fermented active extract [21]. Bacteriocins are often inseparable from probiotics, being one of the weapons used by these healthy bacteria in their combat against pathogens [22]. The last few years have seen a trend in broadening exploration of possible use of bacteriocins in personal care and medical applications [23]. Bacteriocins from Gram-positive bacteria are increasingly reported as active against Gram-negative

microorganisms when used in combination with synergistically acting substances [24], or even alone [25]. Numerous studies published indicating increased activity and/or broader spectrum of engineered bacteriocins [26]. Recent reports demonstrate antiviral activity of some bacteriocins (e.g., [27]). All of these and particularly growing number of reports on synergy of bacteriocins with other stressors have catalyzed the exploration of bacteriocins as “helpers” with conventional antibiotics, wherein antimicrobial proteins can improve the activity of the drug and decrease the rate of the targeted microorganisms' resistance to combinational therapy (e.g., [28, 29]). The availability and convenience of using database on antimicrobial proteins is of a great assistance in these studies [30]. Since bacteriocins are biodegradable and most of them are overall positively charged, hydrophobic substances, to assure their efficiency in challenging environment of eukaryotic organisms, proper delivery systems should be considered [31]. And, while bacteriocins will probably not replace conventional antibiotics [32], they may serve as a valuable asset to already marketed drugs, especially in considering safety features of bacteriocins such as biodegradability and lack of immunogenicity [33].

Executive Summary

Nota bene: To keep this opinion letter concise, in the previous pages, some of the bullets were not elaborated on.

- Probiotics should be distinguished between food fermenting and otherwise safe, naturally occurring microorganisms.
- There is an urgent need for revisiting the definition of probiotics and for their clear classification based on functions and deliverables as confirmed in large-scale statistically sound trials.
- It is logical to expect more research focused on probiotics for following applications:
 - medicinal (cancer prevention, treatment of dysbacteriosis, immunomodulation, anti-viral prophylaxis, etc.)
 - this will include bioengineered functional probiotics;
 - personal care;
 - animals of agricultural importance;
 - poultry;
 - aquacultures.
- Novel approaches for formulation and delivery of probiotics will continue to emerge.

- Bacteriocin-producing probiotics will be gaining more attention as well as probiotics with well-characterized and fully understood functions.
- In addition to already discovered, novel bacteriocins will continue to be explored for following applications:
 - food preservations;
 - antimicrobials in personal care formulations;
 - medicinal use in combination with conventional antibiotics and/or other nature-derived synergistically acting antimicrobials;
 - anti-viral formulations;
 - spermicidal.
- To use bacteriocins in the most efficient manner in various applications, there is a growing demand for understanding of kinetics of bacteriocin–microbe interaction. This will continue being addressed at several levels:
 - conditions simulating natural environment (low number of cells and low concentration of bacteriocin);
 - bacteriocins as stressors for biofilm prevention and their activity against formed biofilms.
- A significant effort is expected to be dedicated to design and study of functional vehicles for targeted and controlled delivery of bacteriocins and synergistically acting stressors.

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