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General Perspective and Assessment of the Potential of Utilizing Paraprobiotics in Food Products †

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Abstract: Paraprobiotics are non-viable microbial cells that, when administered in adequate amounts, confer some health benefits to the consumer. Paraprobiotics can be obtained by subjecting probiotics to physical or chemical treatments, and inactivation of the microorganism would lead to the release of some compounds, such as exopolysaccharides, peptidoglycans, surface proteins, and lipoteichoic acids, all of which have a variety of positive health effects. Paraprobiotics also have numerous technological advantages. Therefore, paraprobiotics are promising components and have great potential for producing functional food products. However, there are limited studies, most of which concentrate on using paraprobiotics in clinical research and using them directly. The objective of this study is to summarize the ways to obtain paraprobiotics, their health benefits, technological advantages, and their potential for utilization in food products.

Keywords: paraprobiotic; probiotic; functional food; non-thermal technologies; health benefit

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1. Introduction

Functional foods refer to the food conferring health advantages beyond their nutritional value, and so the consumers' interest is ever increasing. One of the most important features of functional foods is maintaining the total balance of the intestinal system [1]. Probiotics, live microorganisms that, when administered in adequate amounts confer a health benefit on the host. Probiotics have often been used to produce functional foods, based on the beneficial effects like antioxidant, antidiabetic, anti-inflammatory, and hypercholesterolemia [2,3]. These beneficial effects of probiotics are ensured by interactions between probiotics and gastrointestinal microbiota, as well as the immunological system. Probiotics have different properties and effects depending on the strain and administered dose, so each strain will exert different health benefits [4]. Different types of microorganisms, mainly bacteria, are acknowledged as probiotics and the probiotic species that appear in the literature; the most frequent are *Lactobacillus*, *Bifidobacterium*, *Streptococcus*, *Bacillus*, and *Enterococcus* [5]. Probiotics are used to relieve the symptoms of high blood pressure, lactose intolerance, diarrhea, irritable bowel syndrome, and obesity [6].

There are various foods that contain probiotics, primarily dairy products, which are a good food matrix for the growth of probiotics. However, there are still many challenges associated with adding probiotics during food processing, including the survival of microorganisms, shelf-life stability, and proper delivery to the gut microbiota [7]. The probiotics will be affected by the food's composition, water activity, antibiotic content, processing conditions of temperature, time, pH, storage conditions such as oxygen content, and packaging materials [8]. The term "paraprobiotic" has come into prominence to overcome these problems, and in light of recent research showing that inactivated probiotic microorganisms may also provide such benefits [9]. There are limited studies, most of which concentrate on using paraprobiotics in clinical research and using them directly. More research is necessary because the paraprobiotics' interactions and mechanisms are not completely understood. Herein, the way of obtaining paraprobiotics, their health benefits, technological advantages, and their potential for utilization in food products are reviewed.

2. Paraprobiotics

Until recent years, the idea and consensus were that probiotics should be alive and present in the final product at a minimum concentration of 106–107 CFU/g or CFU/mL until reaching the gut microbiota to confer health benefits [10]. However, this admission begins to change in light of the findings concerning non-viable microorganisms and their health effects [11]. Paraprobiotics are non-viable microbial cells that, when administered in adequate amounts, also confer some health benefits for consumers. Paraprobiotics are also referred to as "inactivated probiotics" and "ghost probiotics" [12].

Microorganism viability is lost when microbial cell structures are altered by mechanical damage to the cell envelope, DNA filament breaks, and cell membrane disruption. Furthermore, enzyme inactivation and changing membrane selectivity are other factors affecting microorganism viability [13].

Paraprobiotics are acknowledged as non-culturable and immunologically active. There are several methods for paraprobiotic assessment, including plating and flow cytometry (FC). Even though the plating is easy to implement, there are some drawbacks, such as the lack of information about metabolic activity and cell integrity of paraprobiotics. FC, on the other hand, is a more advanced method for comprehensive paraprobiotic determination. In this regard, flow cytometry can characterize both cell structure and function in real-time [14]. The best inactivation method can be chosen more precisely and easily based on data collected by FC. Beyond that, FC helps to understand the mechanisms by which the health effects of paraprobiotics come out. Furthermore, FC can be used to track the metabolic activity of the paraprobiotics from processing to the end of the product's shelf life [11]. Additionally, there are alternative methods with high sensitivity that depend on the presence of specific nucleic acids including polymerase chain reaction (PCR) and mass spectrometry. On the other hand, a scanning electron microscope can be used for morphological change detections in inactivated cells [15].

3. Inactivation Methods to Produce Paraprobiotics

Paraprobiotics can be obtained by subjecting probiotics to physical or chemical treatments. Physical treatments can also be classified as thermal or non-thermal treatments, such as high pressure, sonication, ultraviolet rays, ionizing radiation, irradiation, pulsed electrical field, and supercritical CO_2 [14]. The following changes have taken place: destroying the viability of microorganisms by rupturing and/or damaging cell walls, cell membranes, and DNA, as well as inactivating enzymes, lowering intercellular pH, and denaturing or altering the structure of nucleic acids, proteins, and ribosomes [13].

Probiotics have a strain-specific mechanism of action and efficiency, and various approaches have different effects on biological activities and components. The most convenient inactivation technique should be chosen based on the desired health benefits and the targeted microorganism. The chosen approach should inactivate probiotics while maintaining the beneficial effects of microorganisms. Furthermore, the method has the potential to influence the immunomodulatory activity of paraprobiotics [6,12]. Nonetheless, the thermal process has been used primarily for cost-effectiveness and provides a wide range of time-temperature combinations. Additionally, a variety of processes may be used in combination to carry out the inactivation [13]. The mechanisms of inactivation methods are listed below for each:

i. Thermal treatments. Cell membrane damage, inactivation of enzymes, protein coagulation, ribosome aggregation, nutrient and ion loss, RNA filaments break;
ii. High pressure. Cell membrane damage, inactivation of enzymes, protein denaturation, changes in ribosome and nucleoids, solute loss, reduction in pH;

iii. Sonication. Cell membrane damage and rupture, DNA damage;
iv. Ultraviolet rays. Protein denaturation, formation of DNA photoproducts;
v. Ionizing radiation. Nucleic acid damage;
vi. Pulsed electrical field. Cell membrane disrupture;
vii. Supercritical CO_2. Cell membrane damage, inactivation of enzymes, removal of cell and cell membrane vital constituents, disorder in the intracellular balance of electrolytes, direct effects of CO_2 in the metabolism, reduction in pH;
viii. Ohmic heating. Cell membrane damage, inactivation of enzymes, protein coagulation, ribosome aggregation, nutrient and ion loss, DNA filaments break, electroporation;
ix. Dehydration. Cell membrane damage, changes in the structure of proteins, nucleic acids, and ribosomes;
x. pH. Cell membrane damage, chemical changes in fundamental components [6].

4. Health Benefits of Paraprobiotics

The results of studies demonstrated that when a microorganism is inactivated, it releases compounds such as exopolysaccharides, peptidoglycans, surface proteins, and lipoteichoic acids, all of which have a variety of positive health effects. Even if the cells are dead, the metabolites continue secretion and provide health benefits [16]. Thus, paraprobiotics can provide some health benefits to consumers, such as modulating the immune system and inhibiting pathogens through adhesion to intestinal cells [17]. Moreover, paraprobiotics can help the recovery of intestinal injuries; decrease bacterial translocation and maintain the intestinal barrier; alleviate the symptoms of diarrhea, inflammation, lactose intolerance, respiratory diseases, and liver diseases, especially alcohol-induced; lower cholesterol; treat of dental caries, atopic dermatitis, colitis, intestinal lesions, and visceral pain; prevent the onset of aging manifestations; and reduce stress and anxiety [6]. Pharmaceutical companies have already begun using paraprobiotics to create pharmaceutical products. Two examples are Nyaditum resae® and LacteolTM which are produced by heat-inactivated strains of *Mycobacterium manresensis* and *Lactobacillus*, respectively. Furthermore, CytoFlora® is produced as an immunomodulatory supplement, by utilizing the cell walls of different paraprobiotic strains including *Lactobacillus*, *Bifidobacterium,* and *Streptococcus* [11].

5. Technological Advantages and the Potential of Utilizing Paraprobiotics in Food Products

Paraprobiotics have numerous technological advantages, such as stability, over a wide pH and temperature range, and no interaction with other components in the food matrix, which facilitate easy food processing, industrial usage, commercialization, and extending the shelf life of food [18]. The use of paraprobiotics can be promising, especially when the processing and shelf-life conditions deleterious for probiotics. Moreover, antibacterial and antifungal agents have no effect on paraprobiotic bioactivity, so they can be used concurrently [12]. On the other hand, even if the precautionary conditions are met, some of the probiotic bacteria become inactive during processing and this loss ever increases along with shelf-life. Herewith, foods with probiotic bacteria contain both live and dead probiotics, of which the exact numbers of live and dead bacteria are unknown [19]. According to some reports, some commercially produced probiotic foods do not actually contain the levels of probiotics that are stated [13]. For this reason, the health benefits of probiotic foods are highly likely to provide not only live bacteria but also inactivated microorganisms and metabolic by-products [12,19].

Numerous studies have recently shown that paraprobiotics have positive health effects when consumed directly. In this regard, the use of foods as carriers is a novel concept that requires further investigation. In this instance:

- Probiotic species and strains should be selected carefully due to each species and strain exerting different health benefits;

- Even though various inactivation techniques have been applied there are not approved or optimized standard methodologies, the most adequate inactivation method should be determined for relevant species;
- The biological effects of paraprobiotics should be carefully evaluated;
- The stability and activity of the paraprobiotics in the food matrix should assess throughout shelf-life [6].

Although clinical studies on paraprobiotics are the main focus of research, recent years have seen an increase in the use of foods to deliver paraprobiotics, as shown in Table 1.

Table 1. Applications of paraprobiotics in foods.

Probiotic Strain	Food Matrix	Inactivation Method	Results	References
L. acidophilus and *B. lactis*	Yogurt	Heat treatment (121 °C, 15 min)	Viscosity ↑, WHC ↑, Syneresis ↓, Storage modulus ↓, Loss modulus ↓, Stress crossover point ↓, Loss tangent ↓, Sensory properties ↑, *L** ↔, *a** ↔, *b** ↔, pH ↓, Acidity ↑, Redox potential ↑	[20–22]
L. casei subsp. *paracasei* 01	Whey-grape juice	Ohmic heating (8 V/cm, 95 °C/ 7 min, 60 Hz)	Glucose rate ↑, Maximum glucose value ↔, Glucose incremental percentage ↔, Peak blood glucose time ↔, Glycemic responses (AUC, AIg, PGV, HP, GB) ↔, Glucose postprandial level ↓	[23]

↑ indicates an increment that is statistically different; ↓ indicates a decrease that is statistically different; ↔ indicates an increment or decrease that is not statistically different.

Paraprobiotic yogurt produced with *Lactobacillus acidophilus* ATCC SD 5221 and *Bifidobacterium lactis* BB-12 was compared to probiotic yogurt in terms of physicochemical, microstructural, biochemical, rheological, microbiological, and sensory properties during 28 days of storage. The results demonstrated that paraprobiotics increased water holding capacity and apparent viscosity while decreasing syneresis due to producing of exopolysaccharides by heat-killed cells. Another crucial point is that the viability of starter cultures is enhanced by providing nutrients released from the paraprobiotics. Moreover, paraprobiotics affected the rheological characteristics and sensory properties of yogurt. To sum up, the incorporation of paraprobiotics into a food product is a promising alternative to probiotics with some technological and health advantages, such as no interaction with other ingredients in the food matrix, enhanced shelf-life, resistance to environmental changes, and ease of processing and commercialization. Nevertheless, more research is required to validate the findings using different paraprobiotics and food products [20–22].

Lacticaseibacillus casei subsp. *casei* 01 was inactivated by using ohmic heating and produced a paraprobiotic whey-grape juice drink. According to the results, the paraprobiotic drink displayed reduced glucose postprandial levels like the control probiotic drink. Even though more research is needed, it can be claimed that paraprobiotics can be effective in lowering postprandial glycemia [23].

6. Conclusions

To summarize, the paraprobiotic term has been evolving and gaining attention in recent years. Clinical studies demonstrated that paraprobiotics have health benefits for the consumers like probiotics. Therefore, paraprobiotics can be an alternative to probiotics for people with a sensitive immune system or who are immunocompromised/immunodeficient and avoid probiotic consumption. Moreover, paraprobiotics can be used when the use of probiotics is a technological challenge. In the case of processing and shelf-life conditions, not convenient probiotics survive. However, there is not enough information and research in the literature about paraprobiotics just yet. The mechanism of action of paraprobiotics is not fully understood and requires further investigation. In this regard, the following studies should focus on determining valid conditions for emerging inactivation methods, the biological activities and stability of paraprobiotics in vitro and in vivo, and the terms for wide application and easy commercialization of paraprobiotics. Furthermore, it is critical to establish a precise definition by subject-matter experts and prevent the misuse of paraprobiotics.

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