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Review

Paraprobiotics: Evidences on their ability to modify biological responses, inactivation methods and perspectives on their application in foods

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ABSTRACT

Background: The classical definition of probiotics indicates “they are live microorganisms which, when ingested in adequate amounts can provide health benefits to the host”. These benefits are provided due to interactions between the probiotics and the gastrointestinal microbiota and immunological system. On the other hand, non-viable probiotics have been known as “ghost probiotics”, “postbiotics” and “inactivated probiotics”, but recently the term “paraprobiotics” has been coined.
Scope and approach: In this study, the main methods used to inactivate probiotics to produce paraprobiotics, their role as modifiers of biological responses as well as their potential application in foods are discussed.
Key findings and conclusions: A number of biological effects have been associated with paraprobiotics, highlighting that they could constitute an excellent option to improve health status and wellness. Although health benefits have been associated to paraprobiotics, most data in literature show these effects are linked to their direct consumption. Therefore, the use of foods as carriers for paraprobiotics seems to constitute a field to be explored with several opportunities and challenges. Among them, are of special importance the selection of probiotic species and strains to be used for paraprobiotics production, the use of appropriate methods for inactivation and delivery, the evaluation of their stability and activity in foods during shelf life, and the use of adequate methods to assess their biological effects.

1. Introduction

The human gastrointestinal tract (GIT) contains a rich, complex microbiota, which composition and activity play an important role in nutrition, immunology and in specific diseases (de Almada, De Almada, Martinez, & de Souza Sant'Ana, 2015; Dong, Rowland, Thomas, & Yaqoob, 2013). There is evidence to support the idea that the balance of the intestinal microbiota confers important benefits on the health of the host. Under normal conditions, the composition of the intestinal microbiota is stable, but can be altered due to factors such as changes in the diet, medications and stressful situations (Generoso et al., 2011). One of the most effective contemporary strategies to maintain a healthy equilibrium of the intestinal microbiota is the administration of probiotic supplements or the consumption of probiotic foods (Adams, 2010; Biswas et al., 2013; Dantas et al., 2016; Zhang et al., 2014).

The most widely accepted definition of probiotic microorganisms is “*live microorganisms that when administered in adequate amounts confer health benefits on the host*” (FAO/WHO, 2001). It has been reported that health benefits are provided due to interactions between the gastrointestinal microbiota and the immune system (Adams, 2010). In general, the mechanisms related to the beneficial effects of probiotics are unknown, but are believed to be multifactorial. For example, some mechanisms related to the antagonistic effects of probiotics against various microorganisms include the secretion of antimicrobial substances, competitive adherence to the mucous membrane and epithelium, strengthening of the intestinal epithelial barrier and modulation of the immune system (Bermudez-Brito, Plaza-Díaz, Muñoz-Quezada, Gómez-Llorente, & Gil, 2012). In addition, there is evidence to suggest that the mechanism of action of probiotics is strain dependent, and thus each strain will present specific health benefits. As such, the beneficial properties of one probiotic strain must not be

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extrapolated to others. It is also known that the health benefits of a probiotic strain depend on the dose administered and on the purpose of application of the probiotic strain, apart from the means and frequency of consumption (Oelschlaeger, 2010).

In general, the beneficial health effects provided by probiotics can be classified into three levels according to their site of action (Rijkers et al., 2010): *i)* direct interaction with the intestinal microbiota or by enzyme activity within the GIT; *ii)* direct interaction with the epithelium and the intestinal mucous layer, influencing the intestinal barrier function and the mucous immune system; *iii)* action outside the GIT, in the immune system and other organs such as the liver and brain.

In general the probiotics have been used to alleviate diarrhea (Sudha, Bhonagiri, & Kumar, 2013), the symptoms of lactose intolerance (Li et al., 2012), the irritable bowel syndrome (Ducrotté, Sawant, & Jayanthi, 2012), against certain types of cancer (Rajpal & Kansal, 2008; Shmuely, Domniz, & Cohen, 2012), insulin resistance (Hsieh et al., 2013), to control cholesterol (Bordoni et al., 2013), blood pressure levels (Chiang & Pan, 2012), and obesity (Arora, Singh, & Sharma, 2013).

Various types of microorganisms, mainly bacteria and yeasts, have been recognized as presenting probiotic properties, and these can be used in foods and clinical treatments (Amara & Shilb, 2013). Strains belonging to the following species/genera are the most commonly studied probiotics/potential probiotics worldwide: *Lactobacillus acidophilus, Lactobacillus sporogenes, Lactobacillus plantarum, Lactobacillus rhamnosus, Lactobacillus delbrueckii, Lactobacillus reuteri, Lactobacillus fermentum, Lactobacillus lactis, Lactobacillus cellobiosus, Lactobacillus brevis, Lactobacillus casei, Lactobacillus farciminis, Lactobacillus paracasei, Lactobacillus gasseri, Lactobacillus crispatus, Bifidobacterium bifidum, Bifdobacterium infantis, Bifidobacterium adolescentis, Bifidobacterium longum, Bifidobacterium thermophilum, Bifidobacterium breve, Bifidobacterium lactis, Bifidobacterium animalis, Streptococcus lactis, Streptococcus salivarius, Streptococcus intermedius, Streptococcus thermophilus, Streptococcus diacetylactis, Leuconostoc mesenteroides, Pediococcus, Propionibacterium, Bacillus clausii, Bacillus coagulans, Bacillus subtilis, Bacillus licheniformis, Bacillus cereus, Enterococcus mundtii, Enterococcus faecium, Saccharomyces cerevisiae, Saccharomyces boulardii*, and *Candida pintolopesii* (Amara & Shilb, 2013; Cutting, 2011).

In addition to their use as supplements, probiotic microorganisms can be intentionally added to traditional foods and beverages such as fermented milks, salted fish, yogurts, various types of cheeses and a great variety of other products (Amara, 2012; Batista et al., 2015; Fernandes et al., 2013; Jesus et al., 2016). There is currently a wide range of foods containing probiotic microorganisms, but despite this, the survival of these microorganisms in certain foods or their addition during food processing still represent considerable technological challenges. Such challenges are associated with *i)* the need to guarantee the survival of probiotic microorganisms during the shelf life of foods that are in fact stressful substrates and *ii)* the need to add probiotics to food formulations after thermal processing, given their low thermal resistance. The need to ensure a minimal concentration of probiotic microorganisms in foods has posed limitations in terms of shelf life of certain probiotic foods. Although yogurt is an adequate matrix for probiotic delivery (Ranadheera, Baines, & Adams, 2010), the extension of probiotic yogurt shelf life is limited by the occurrence of oxidative stress suffered by probiotic microorganisms (Cruz et al., 2013). In addition, some food matrixes *per se*, for example, orange juice and dehydrated foods represent stressful substrates for the survival of probiotic microorganisms due to their low pH and water activity (Anekella & Orsat, 2013; Betoret et al., 2003; Krasaekoopt & Watcharapoka, 2014; Marhamatizadeh, Rezazadeh, Kazemeini, & Kazemi, 2012). The production of probiotic foods can also be challenging if it is required to add probiotics after a thermal processing, which increases the chances of microbiological recontamination. The application of encapsulation methods has been proposed as an alternative to reduce the deleterious effects of thermal processing over probiotic microorganisms (Rokka & Rantamäki, 2010). Nonetheless, it is known that the effectiveness of this approach is limited (Manojlović, Nedovic, & Kailasapathy, 2010). Hurdles of technological nature, such as impossibility to add the probiotic microorganisms at post-thermal processing steps (after cooking of meat products, for instance), among others, are limiting factors for the application of probiotics in a wide variety of foods.

Even though the classical definition of probiotics indicate that they should be alive in order to provide health benefits to the hosts, recent studies have proved that inactivated probiotic microorganisms (herein referred as "paraprobiotics") can also provide such benefits (Imaoka et al., 2008; Rampengan, Manoppo, & Warouw, 2010; Villena, Barbieri, Salva, Herrera, & Alvarez, 2009).

Current knowledge allows stating that paraprobiotics provide health benefits to the hosts through several pathways. For instance, paraprobiotics are known to modulate the immune system (compounds of the cell wall might boost the immunological system) (Fujiki, Hirose, Yamamoto, & Murosaki, 2012; Ou, Lin, Tsai, & Lin, 2011) and to have increased adhesion to intestinal cells which further result in inhibition of pathogens (Grzeskowiak, Collado, Beasley, & Salminen, 2014). Also, paraprobiotics can provide health benefits to hosts through secretion of metabolites by the dead cells (Shin et al., 2010). In spite of these findings, it is known that the mechanisms of action of paraprobiotics are not completely elucidated and more research to understand their interaction with gastrointestinal, respiratory and other systems is required.

Studies have shown that the paraprobiotics can be obtained through different methods, such as heat, high pressure, sonication, irradiation and ultra-violet rays, amongst others (Ananta & Knorr, 2009; Awad et al., 2010; Kamiya et al., 2006; Shin et al., 2010). The application of paraprobiotics in foods could offer certain advantages in relation to probiotics, such as: *i)* less or no interaction with other components of the food products, which could directly reflect in extended shelf life; *ii)* greater food processing facility, as paraprobiotics could be added before thermal processes so as their activity remain to the level required for the intended health benefits; *iii)* storage and transport simplicity, which could result in longer shelf life and greater convenience for their administration also as supplements to immune-compromised individuals (Chuang et al., 2007; Ishikawa et al., 2010). .

Given the above, it seems feasible that the use of paraprobiotics could constitute an excellent strategy to obtain beneficial health effects through processes and foods normally considered deleterious to probiotics. Herein, the main methods used to obtain paraprobiotics and their role as modifiers of biological responses and potential for the application in foods are reviewed.

2. The concept of paraprobiotics

The definition given by the FAO/WHO (2001) for probiotics is restricted to live microorganisms. Nonetheless, non-viable (dead), therefore non-culturable, and possibly immunologically active microbial cells have been reported to provide health benefits to hosts (Adams, 2010; Ananta & Knorr, 2009). Thus, the term "paraprobiotics" has been coined to "*indicate the use of inactivated microbial cells (non-viable) or cell fractions to confer a health benefit to the consumer*" (Taverniti & Guglielmetti, 2011). Paraprobiotics have been previously referred in the literature as "inactivated probiotics" and "ghost probiotics" (Tsilingiri & Rescigno, 2013; Tsilingiri et al.,