
UNL

International Journal of **Dairy Technology** SDT SOCIETY OF DAIRY TECHNOLOGY

doi: 10.1111/1471-0307.12141

ORIGINAL RESEARCH

Influence of dairy practices on the capacity of probiotic bacteria to overcome simulated gastric digestion

PATRICIA BURNS,[†] LISANDRO LAFFERRIERE,[†] GABRIEL VINDEROLA* and JORGE REINHEIMER

Instituto de Lactología Industrial (INLAIN, UNL-CONICET), Facultad de Ingeniería Química, Universidad Nacional del Litoral, Santiago del Estero 2829, Santa Fe 3000, Argentina

The aim of this study was to evaluate the influence of microbiological and technological factors on the viability and functionality of probiotic Lactobacillus *strains. In particular, the influence of harvesting time, food matrix, refrigerated storage, time of inoculation and refrigerated storage in fermented milk on the resistance to simulated gastric digestion (RSGD) was evaluated. Moreover, strain resistance to simulated gastric digestion was compared to human gastric fluid. Results showed that the variables studied affected, in a different way and in a strain-dependent manner, the RSGD. No direct relation was observed between cell viability and RSGD.*

Keywords *Lactobacillus*, Probiotic, Storage, Resistance to gastric digestion, Functionality.

INTRODUCTION

Probiotics are ‘live microorganisms which when administered in adequate amounts confer a health benefit on the host’ (FAO/WHO 2002). Many micro-organisms are currently used as human probiotics, the strains most widely used in fermented dairy products belonging to the genus *Bifidobacterium* or to *Lactobacillus casei* and *Lactobacillus acidophilus* groups (Vinderola *et al.* 2011a). With regard to their viability and functionality of probiotic bacteria in fermented dairy products, this can be modified by many microbiological and technological factors, which should therefore be taken into account. In fact, the factors involved during biomass production of a strain (medium pH, available sugars and their concentrations, growth phase at harvesting, etc.), technological processing and the food matrix into which micro-organisms are added may significantly affect both their resistance to biological barriers (gastric acidity and bile salts) and their capacity to interact with immune cells, thus conditioning its functionality (Vinderola *et al.* 2011b). Viability can be considered as the amount of viable cells displayed by a culture under a given condition, whereas functionality is a more sophisticated concept comprising a group of nonexhaustive features, including viability, which confer on a probiotic strain the properties responsible for health improvement. However, as viability does not always mean full functionality, it is important to establish the difference between both parameters. Under certain conditions, bacteria can survive, although some of their functional characteristics such as resistance to low pH, adhesion to the intestinal epithelium or immunomodulating capacity may not be fully displayed, which could at least partially impair its capacity to exert a beneficial effect on health (Reilly and Gilliland 1999; Vinderola *et al.* 2011a). Saarela *et al.* (2006), for example, studied the stability of freeze-dried bifidobacteria in fruit juice and low-fat milk and found no changes in cell viability but lower acid and bile tolerance of cells during storage. Freeze-dried cells of *B. animalis* subsp. *lactis* INL1 showed higher tolerance to low pH (pH 2.0) when grown at pH 5.0 than when grown at pH 6.5 (Vinderola *et al.* 2012). Similar results had been reported by Saarela *et al.* (2009) for *L. rhamnosus* VTT E-97800. The appropriate choice of a probiotic micro-organism involves, as an essential feature, its ability to reach, survive and persist in the environment in which it is intended to act. As the large intestine is the site of action for most probiotic bacteria, for these bacteria to be effective they must arrive viable in sufficient

*Author for correspondence. E-mail: gvinde@fiq.unl.edu.ar.
[†]These authors contributed equally to this work.

frontiers in
MICROBIOLOGY

REVIEW ARTICLE
published: 03 May 2011
doi: 10.3389/fmicb.2011.00070

Cell viability and functionality of probiotic bacteria in dairy products

Gabriel Vinderola*, Ana Binetti, Patricia Burns and Jorge Reinheimer

Instituto de Lactología Industrial, Facultad de Ingeniería Química, Universidad Nacional del Litoral, Santa Fe, Argentina

Edited by:
Paula Teixeira, Catholic University, Portugal

Reviewed by:
Kiiyukia Matthews Ciira, Jomo Kenyatta University of Agriculture and Technology, Kenya
Paul Anthony Gibbs, Catholic University, Portugal

****Correspondence:***
Gabriel Vinderola, Instituto de Lactología Industrial, Facultad de Ingeniería Química, Universidad Nacional del Litoral, 1° de Mayo 3250, Santa Fe 3000, Argentina.
e-mail: gvinde@fiq.unl.edu.ar

Probiotic bacteria, according to the definition adopted by the World Health Organization in 2002, are live microorganisms, which when administered in adequate amounts confer a health benefit to the host. Recent studies show that the same probiotic strain produced and/or preserved under different storage conditions, may present different responses regarding their susceptibility to the adverse conditions of the gastrointestinal tract, its capacity to adhere to the intestinal epithelium, or its immunomodulating capacity, the functionality being affected without changes in cell viability. This could imply that the control of cell viability is not always enough to guarantee the functionality (probiotic capacity) of a strain. Therefore, a new challenge arises for food technologists and microbiologists when it comes to designing and monitoring probiotic food: to be able to monitor the functionality of a probiotic microorganism throughout all the stages the strain goes through from the moment it is produced and included in the food vehicle, until the moment of consumption. Conventional methodological tools or others still to be developed must be used. The application of cell membrane functionality markers, the use of tests of resistance to intestinal barriers, the study of surface properties and the application of *in vivo* models come together as complementary tools to assess the actual capacity of a probiotic organism in a specific food, to exert functional effects regardless of the number of viable cells present at the moment of consumption.

Keywords: probiotics, viability, functionality

PROBIOTIC MICROORGANISMS USED IN FOOD

The intestinal microbiota comprises about 95% of the total cells in the human body, and it contains approximately 10^{11}–10^{12} CFU/g of intestinal content. This diffuse "organ" which is not encoded in our DNA, is rapidly acquired after birth and is carried with us all throughout our lives, experiencing changes in its composition and its activities which respond not only to endogenous factors (e.g., age, stress), but also to exogenous factors (e.g., diet, medical treatments). The presence of the intestinal microbiota is of essential importance for the development of the mucosal immune system and the maintenance of its activity, and also for the numerous barrier and biochemical activities that it performs. As for the species and strains comprising the intestinal microbiota, there is a wide variability among individuals, depending on their age, diet, immunological status, stress factors, and intrinsic characteristics of the individual not yet totally known (Isolauri et al., 2004). The main genera found in the intestinal microbiota include *Bacteroides, Eubacterium, Ruminococcus, Clostridium,* and *Bifidobacterium*, and as the subdominant microbiota, *Escherichia, Veillonella, Staphylococcus, Proteus, Streptococcus,* and *Lactobacillus* have been reported (Tannock, 2003).

It is possible to classify the components of the intestinal microbiota according to the effects they exert on the host's health: bacteria with potentially pathogenic effects, bacteria presenting a mixture of pathogenic and beneficial activities, or bacteria with strictly beneficial effects (Gibson et al., 2003). *Bifidobacterium* and *Lactobacillus* belong to this last group, and are the genera most frequently chosen for the isolation and characterization of probiotic bacteria.

Among the numerous definitions of probiotics, the one with the prevailing international scientific consensus is the one adopted in 2002 by the Joint Committee of the World Health Organization (WHO) and the Food and Agriculture Organization (FAO). It was established that probiotics are "live microorganisms which when administered in adequate amounts confer a health benefit on the host." (WHO/FAO, 2002). The majority of the probiotic strains available were isolated and characterized from the following species: *Lactobacillus casei, L. paracasei, L. rhamnosus, L. acidophilus, L. gasseri, L. johnsonii, L. plantarum, L. reuteri, L. crispatus, L. fermentum, Bifidobacterium bifidum, B. adolescentis, B. lactis, B. breve, B. infantis, B. longum, Saccharomyces boulardii, S. cerevisiae,* and *Enterococcus faecium.* Certain species of the genera *Bifidobacterium* and *Lactobacillus*, common inhabitants of the intestinal microbiota, are traditionally regarded as microorganisms beneficial to health, due to the fact they exclusively promote healthy effects in their natural niche. Furthermore, some of these strains show an adequate tolerance to gastrointestinal barriers and to technological parameters applied in the production of food. These facts determine that the main commercial strains used for the manufacture of functional foods belong to these genera.

MICROBIOLOGICAL CONTROL OF PROBIOTICS IN FOOD

The microbiological enumeration of probiotic bacteria in fermented foods represents a real challenge to the industry, due to the simultaneous presence of probiotics and the acidifying starter bacteria used for the fermentation of the food. Even though there is an increasingly marked tendency to apply advanced techniques such as FISH, flow cytometry (Ben Amor et al., 2007), and real-time quantitative