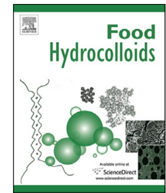
Food Hydrocolloids 41 (2014) 60–70

Contents lists available at ScienceDirect

Food Hydrocolloids

journal homepage: www.elsevier.com/locate/foodhyd



Significance of bacterial surface molecules interactions with milk proteins to enhance microencapsulation of *Lactobacillus rhamnosus* GG

Jennifer Burgain [a], Joel Scher [a], Sarah Lebeer [b,c], Jos Vanderleyden [b], Catherine Cailliez-Grimal [a], Magda Corgneau [a], Gregory Francius [d], Claire Gaiani [a,*]

[a] Université de Lorraine, LIBio, Laboratoire d'Ingénierie des Biomolécules, 2 av de la Forêt de Haye, TSA 40602, 54518 Vandoeuvre lès Nancy, France
[b] KU Leuven, Centre of Microbial and Plant Genetics, Kasteelpark Arenberg 20, Box 2460, B-3001 Leuven, Belgium
[c] University of Antwerp, Department of Bioscience Engineering, Groenenborgerlaan 171, B-2020 Antwerp, Belgium
[d] Université de Lorraine, LCPME, Laboratoire de Chimie Physique et Microbiologie pour l'Environnement, UMR 7564, 54600 Villers-lès-Nancy, France

ARTICLE INFO

Article history:
Received 4 February 2014
Accepted 21 March 2014
Available online 3 April 2014

Keywords:
Probiotic
Encapsulation
Interactions
Milk proteins
Adhesion

ABSTRACT

Probiotic bacteria are being increasingly encapsulated to enhance their delivery in an active state at their preferred site of action. In this study, an encapsulation process based on emulsification and requiring only food grade components was used to protect wild-type *Lactobacillus rhamnosus* GG (LGG) and three of its surface mutants into dairy matrices. The mechanism of microencapsulation was studied at the molecular level by comparing the encapsulation efficiency of LGG wild type and three of its surface mutants with Atomic Force Microscopy. A significant decrease in the encapsulation efficiency was observed when the bacteria were depleted for pili, while the pilus also appeared to be crucial for location of LGG inside the microparticle. Hereto, the *spaCBA* mutant lacking pili), the *welE* mutant lacking long exopolysaccharides) and the *dltD* mutant having modified lipoteichoic acids were used. Atomic Force Microscopy enabled the confirmation of specific interactions between bacteria and whey proteins, in contrast to the observed nonspecific interactions with micellar casein. The role of the pili, i.e. multimeric appendages of several micrometers, was also modeled using WLC (Worm-Like Chain) or FJC (Freely Jointed Chain) models. This revealed that understanding molecular mechanisms of microencapsulation of probiotic bacteria should ultimately benefit their targeted application.

© 2014 Elsevier Ltd. All rights reserved.

1. Introduction

The concept that probiotic yoghurt and other fermented milk products can improve digestion, boost immunity and provide other health benefits are long claimed by food companies and modern consumers increasingly adhere to this trend (Burgain, Gaiani, Linder, & Scher, 2011). Probiotic microorganisms used in functional foods are generally members of the genera *Lactobacillus* and *Bifidobacterium*. Probiotic bacteria are defined as "live microorganisms, which when administrated in adequate amounts can provide a health benefit on the host" (FAO/WHO, 2002). However, to exert these beneficial effects, probiotic bacteria must maintain their viability first during storage and then during their passage through the upper gastro-intestinal tract in order to arrive in the intestine in a viable state (Burgain et al., 2011). Functional foods containing probiotics are also increasingly subject to regulation by regulatory authorities, such as the European Food Safety Authority (EFSA), to ascertain that the probiotic health claims are based on solid scientific evidence, both with respect to the characterization of the probiotic micro-organism and human intervention studies to validate health benefits. In this sense, the design of biopolymer based microparticles to encapsulate, protect and release probiotic bacteria at the required site of action is now gaining interest (Matalanis, Jones, & McClements, 2011). Milk proteins can be used as an encapsulating carrier in order to produce this kind of delivery system (Doherty et al., 2011; Heidebach, Först, & Kulozik, 2009; Livney, 2010). In most cases, microencapsulation with dairy proteins led to an increase in bacterial survival during digestion. However, even if in some cases the bacterial location into the microparticle was observed, the mechanisms behind the interaction between the probiotic bacteria and the matrix were never studied.

Abbreviations: AFM, Atomic Force Microscopy; CFU, Colony Forming Units; EFSA, European Food Safety Authority; EPS, Exopolysaccharides; LGG, *Lactobacillus rhamnosus* GG; LGG wt, *Lactobacillus rhamnosus* GG wild type; TEM, Transmission Electron Microscopy.

* Corresponding author. Tel.: +33 3 83 59 58 78; fax: +33 3 83 59 58 04.
E-mail address: claire.gaiani@univ-lorraine.fr (C. Gaiani).

http://dx.doi.org/10.1016/j.foodhyd.2014.03.029
0268-005X/© 2014 Elsevier Ltd. All rights reserved.

To advance the targeted microencapsulation of probiotic bacteria by milk proteins, it is of importance to investigate the specific interaction of bacterial surface molecules with the dairy matrices. It is generally known that the adhesive nature of bacteria is mainly due to cell surface structures consisting of proteins such as pili, and polysaccharides such as exopolysaccharides (EPS) and that both long range forces (steric and electrostatic interactions) and short range forces (Van der Waals, acid-base, hydrogen bonding and biospecific interactions) are involved, but – to the best of our knowledge – such adhesion studies have not yet been performed in relation to probiotic microencapsulation.

The present study aimed to investigate how changes in bacterial surface can influence the encapsulation efficiency of probiotics in dairy matrices, by combining detailed genetic methods with surface mutants and Atomic Force Microscopy (AFM). Hereto, *Lactobacillus rhamnosus* GG (LGG) was chosen as model probiotic organism, because it is one of the most intensively studied probiotic strains worldwide. It has most of the characteristics generally desired for a good probiotic bacterium including the ability to colonize the human gastrointestinal tract (Goldin et al., 1992). LGG has been shown to promote human health by reducing the risk of nosocomial rotavirus-related diarrhea in infants (Szajewska, Kotowska, Mrukowicz, Armanska, & Mikolajczyk, 2001), shortening the duration of acute diarrhea (Szajewska, Skorka, RuszczynSki, & Gieruszczak-Bialel, 2007), reducing upper respiratory tract infections among children in day care (Hojsak et al., 2010), decreasing the risk of developing atopic eczema (Kalliomäki, Salminen, Poussa, & Isolauri, 2007), alleviating the symptoms of eczema (Isolauri, Arvola, Sütas, Moilanen, & Salminen, 2000) and decreasing the risk of dental caries in children (Näse et al., 2001). Recently, various molecular genetic studies have started to unravel the molecular determinants of its peculiar probiotic characteristics (Claes et al., 2012; Lebeer, Claes, Tytgat, et al., 2012; Lebeer, Claes, Verhoeven, Vanderleyden, & De Keersmaecker, 2011; Lebeer, Vanderleyden, & De Keersmaecker, 2008; Lebeer et al., 2009) and have indicated crucial roles for surface molecules such as *spaCBA*-encoded pili, long galactose-rich EPS molecules and lipoteichoic acid (LTA) in host interactions.

2. Material and methods

2.1. Material

LGG wild-type (ATCC 53103), the pili-deficient *spaCBA* mutant (CMPG 5357) (Lebeer, Claes, Tytgat, et al., 2012), the *welE* mutant (CMPG 5351) mutated for long galactose-rich EPS (Lebeer et al., 2009, 2011) and the *dltD* mutant (CMPG 5540) (Vélez et al., 2007) having modified LTA molecules were used throughout this study. Bacterial stock cultures were stored at −80 °C in MRS (De Man, Rogosa, & Sharpe, 1960) broth containing 20% (v/v) glycerol. Preculture was initiated by inoculating 9 ml of MRS broth with 100 μl of bacterial stock. This preculture was then used to inoculate fresh MRS broth and the growth was conducted at 37 °C until the end of exponential phase when OD_{600} reached around 1.2. It was chosen to harvest the cells at this moment because pili genes are mainly expressed in exponential phase (Laakso et al., 2011). Cells were harvested by gentle centrifugation (3000*g*, 10 min, room temperature) to avoid the loss of pili (Tripathi et al., 2012). For microencapsulation, the pellet was washed with physiologic water (pH 7.4) and the cells were finally harvested by centrifugation (3000 *g*, 10 min, and room temperature). The pellet was frozen and placed on the shelves of a freeze-dryer (Christ alpha 1-2, freeze-dryer, Osterode, Germany). The microbial powders obtained after freeze-drying had a content of around 10^{11} CFU/g. For force measurements, the pellet was resuspended in 1 ml of PBS (pH 7.4).

Micellar casein powder (Promilk 872B) was obtained from Ingredia IDI (Arras, France). Whey proteins isolates powders (Prolacta 90) were purchased from Lactalis Ingredients (Bourgbarré, France).

2.2. Microencapsulation

The solutions were prepared by adding 12.5 g of protein powder into 100 g of distilled water (final concentration of 12.5%). The rehydration was done by stirring for 2 h at room temperature and then overnight at 4 °C. The denatured whey proteins were obtained by heating the native whey solution at 78 °C for 10 min then cooling it to room temperature. The chymosin enzyme (Chy-Max Plus; 199 IMCU/ml; Chr. Hansen, Hørsholm, Denmark) was prepared by diluting hundred times the initial solution with distilled water. Tween 80 was purchased from Sigma–Aldrich and sunflower oil in a local store.

The formulation consists of a mixture of 180 g of micellar casein solution and 20 g of heat treated whey protein solution. Analysis of this solution revealed that the employed technique allows the denaturation (insoluble fraction after heat treatment) of only 40% of the proteins and 60% remain native (soluble fraction after heat treatment). The bacteria were added after mixing the protein solution and before enzymatic incubation. For this, 0.5 g of freeze-dried LGG (wt or mutants) was added to 200 g of protein mixture.

Microparticles were produced by using an emulsification method employing a patented process (Burgain, Gaiani, Jeandel, Ghoul, & Scher, 2013). The enzymatic incubation was completed in a double-walled, temperature-controlled reactor, made of stainless steel. An amount of 200 g of protein mixture (protein concentration of 12.5%) was added into the first reactor (reactor for enzymatic reaction) held at 5 °C. Then, 18 ml of diluted enzyme preparation was added and mixed with the solution. The mixture was left for 30 min at 5 °C allowing the chymosin enzyme to cut the κ-casein. This solution is then pumped to a second reactor (emulsification reactor) where emulsification can take place. For this purpose, 800 ml of cooled sunflower oil containing 1% (w/v) of Tween 80, were placed under stirring at 500 RPM (Rotation Per Minute). The enzymatic phase was progressively added and cold emulsification at 5 °C was performed during 10 min. Afterwards, the temperature was raised to 40 °C for 25 min (temperature ramp: 1.4 °C/min.) under mechanical agitation.

Microparticles were removed from the reactor and separated by filtration. The harvested particles were washed with distilled water. Subsequently, the microparticles were frozen at −20 °C, then lyophilized (Christ alpha 1-2, freeze-dryer, Osterode, Germany).

2.3. Encapsulation efficiency

The enumeration of LGG (wt and mutants) living cells was done by serial dilutions. The samples (0.1 ml) were plated in duplicate on MRS agar. CFU were determined after incubation (48 h at 37 °C). The ratio between the number of bacteria added in the protein mixture and the number of bacteria in the final particles was done. The initial number of bacteria introduced in the protein mixture was obtained by determining the CFU in 1 g of solution. The result was multiplied by 200, which is the total mixture quantity used for microencapsulation. At the end of encapsulation, the microparticles total weight was measured. The final CFU in 0.2 g of microparticles was also determined and reported to the total quantity of obtained microparticles. The encapsulation efficiency (EE) was calculated by applying Eq. (1):

Download English Version:

https://daneshyari.com/en/article/605040

Download Persian Version:

https://daneshyari.com/article/605040

Daneshyari.com