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Research paper

Study of the *Lactobacillus rhamnosus* Lcr35® properties after compression and proposition of a model to predict tablet stability

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ABSTRACT

The beneficial effects of probiotic bacteria on human health are now widely acknowledged, and this has prompted growing interest in research and development in the pharmaceutical field. However, to be viable when they reach their target, the bacteria must be able to survive during the manufacturing process and the biological pathway. Tablet form best meets the requirements for protecting acid labile drugs, but the tableting process could be an additional stress for the bacteria.

This study evaluated the initial effect of compression pressure on the Lcr35® strain in a vaginal (*Lcr regenerans*®) and an intestinal (*Lcr restituo*®) formulation. A stability study was also performed on the tablets and revealed a beneficial effect of this form. The obtained destruction rates (k) demonstrated that the bacterial stability was greater in tablets than in powders ($k_{powders} > k_{tablets}$). A new mathematical model was developed combining compression and temperature parameters to predict the bacterial viability at any pressure and time.

Moreover, the genetic profile of Lcr35® (Rep-PCR, microarrays), its resistance to acidity and its ability to inhibit *Candida albicans* growth, after compression, were determined to evaluate the target product profile (TPP) in a Quality by Design (QbD) approach.

The Rep-PCR analysis validated the strain identity and the microarrays demonstrated the genetic stability of Lcr35® strain after compaction. Additionally, ability to inhibit the *C. albicans* growth was maintained and the resistance to gastric conditions of Lcr35® was even improved by tableting. As a dosage form, tablets containing probiotic can guarantee that an adequate amount of bacteria reaches the therapeutic target (intestinal or vaginal) and that the product remains stable until the time of consumption.

1. Introduction

Probiotics are defined by the World Health Organization (WHO) as viable live microorganisms, which, when administered in adequate amounts, confer a health benefit on the host [1]. Probiotic bacteria are currently of great interest for the pharmaceutical development because of their widespread effects on human health. They have numerous therapeutic applications including gastric disorders, vaginal infections, food allergies or serum cholesterol reduction [2–6]. The beneficial effects depend on the strain, and the most commonly used, for both food and pharmaceutical applications, belong to the *Lactobacillus* and *Bifidobacterium* bacterial genus [4,7,8].

To be defined as a probiotic strain, the microorganisms must have biological attributes such as human origin, acid and bile stability, adherence to human epithelial cells, competition with pathogens for mucosal binding sites and growing capacity at the site of action [2,5,9]. A probiotic formulation must also possess properties such as safety, genetic stability, suitability to large-scale production, product stability (viability), and acceptable flavor or taste [7,9–11]. One of the most important properties needed to

Abbreviations: TPP, target product profile; QbD, Quality by Design; Rep-PCR, Repetitive Polymerase Chain Reaction; WHO, World Health Organization; API, active pharmaceutical ingredient; CPP, critical process parameter; ICH, International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use; RH, relative humidity; MPa, MegaPascals; DNA, deoxyribonucleic acid; NCBI, National Center of Biotechnology Information; RNA, ribonucleic acid; MRS, Man, Rogosa, Sharp; ATCC, American Type Culture Collection; KEGG, Kyoto Encyclopedia of Genes and Genomes; CQA, critical quality attribute; CMA, critical material attribute.

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confer a health benefit is the maintenance of viability during the product life cycle up to the time of consumption [9,12–14]. Depending on the biological target and on whether they are incorporated in food or pharmaceutical products, the number of viable bacteria necessary to produce the health effect can vary [3,11,15]. However, as previously demonstrated, product stability and hence bacterial viability, can be modified by the growth medium formulation, the manufacturing process and the storage conditions [12,13,15–19]. Generally, probiotic powders are obtained from freeze-dried culture media because the lyophilization process is widely used to obtain biological products with a long shelf-life [18,19]. Nevertheless, several studies showed that, probably owing to the manufacturing process, the number of viable bacteria in some marketed food probiotic products was lower than the announced levels [7].

The gastrointestinal tract, because of its low pH environment, is a critical step for the passage of acid labile drugs including probiotic bacteria. The strain's ability to survive under these harsh conditions can be improved depending on the active pharmaceutical ingredient (API), on the formulation and on the intrinsic resistance [14,20–23]. To ensure the survival of the beneficial microorganisms throughout the biological pathway, the development of new probiotic products should take into consideration the impact of the environment encountered up to the therapeutic target.

Commercialized probiotic products are available in diverse dosage forms such as capsules, sachets and tablets [2]. Bacterial protection against environmental stress can vary according to the formulation and the dosage form and promising results have been obtained with tablets [20,24–27]. In addition, the tablet dosage form has the advantage of good patient acceptance, ease of administration, accurate API dosage and good stability during manufacturing and storage conditions [26]. For these reasons, tablets are one of the most widely used dosage forms in the development of pharmaceutical drugs to protect the API against environmental conditions.

In the case of probiotic products, tablets could preserve the bacterial strain's integrity until it reaches its target. Indeed, because of its low water activity, bacterial survival during storage can be improved. Previous studies added polymers to the bacteria to control the release of the probiotic strain from the tablet and to improve bacterial viability during the biological pathway [28–30]. However, tableting of freeze-dried probiotic powders could lead to a loss of viability [12,27,31].

Hence, the new challenge in the development of probiotic products is to determine the ideal tableting conditions that would limit the loss of viability. The compression parameters should thus be considered as a critical process parameter (CPP) in a QbD approach.

This study investigates the impact of tableting on bacterial viability after compression and during stability studies of the *Lactobacillus rhamnosus* Lcr35® strain which is used to restore the intestinal and the vaginal flora after disruption by factors such as antibiotic treatment or stress [32,33]. Its physical and biological properties including stability during manufacturing and storage are well documented [23,32,34,35] but no studies have been performed to estimate the loss of viability during tableting and the sensitivity to the compression stress (biological strain properties and genetic stability).

All assays were performed according to ICH recommendations [36] and the bacterial properties were measured by microbiological and genetic analysis.

2. Materials and methods

2.1. Probiotic strain and products

Two different industrial probiotic powders, provided by Probionov (Aurillac, France), were used for this study: Gynophilus® (capsule), which has a vaginal application, and Bacilor® (capsule), which has an intestinal application. These formulations are close in their composition but differ especially by their manufacturing process.

The industrial products and their corresponding APIs are shown in Table 1. Henceforth in this article, the powders will be called with their respective API names, *Lcr regenerans*® and *Lcr restituo*®.

The apparent particle densities of these two products were determined with a helium pycnometer (Acupyc 1330, Micromeritics, USA). The corresponding values are 1.607 ± 0.003 g/cm^3 for *Lcr regenerans*® and 1.600 ± 0.003 g/cm^3 for *Lcr restituo*®.

2.2. Tableting and tablets testing

The two probiotic industrial products were compacted with a Stylcam 200R compaction simulator (Medelpharm, Bourg-en-Bresse, France) associated with a data acquisition software (Analis, Medelpharm, Bourg-en-Bresse, France). The running of this single station press and the experimental conditions were described in detail elsewhere [37,38]. In our experiments, no pre-compression pressure was used and a direct cam profile was chosen with a compaction speed of 10 tablets per minute. The punches used had a diameter of 11.28 mm (Euro B round flat-face) and the die height was adjusted at 10 mm. During compaction, the pressure level was controlled through the thickness of the tablet in the die. The thickness was adjusted for each product to obtain tablets under seven compression pressure levels (50, 100, 150, 200, 250, 300 and 400 MPa) in constant environmental conditions (RH < 30% and temperature around 20 °C). The mean tablet weight obtained was 669 mg ± 11 mg.

A sufficient number of tablets were produced to perform all the tests described later (viability, stability, porosity...). Immediately after compaction, all the tablets were stored at 4 °C in a glass container with a hermetic plug until testing.

48 h after compaction, the tablet porosity was calculated from the dimensions, the weight of each tablet and the apparent particle density of the powder. At the same time, a micropress (CEGITAB, France) was used to measure the diametral breaking force (F_r) of the tablets. The experimental conditions were described in [37]. The tensile strength (σ_r) was calculated from this breaking force using the following equation [39]:

$$\sigma_r = \frac{2F_r}{\pi dt} \quad (1)$$

F_r: diametral breaking force (N).
d: tablet diameter (mm).
t: tablet thickness (mm).

2.3. Genome analysis of the Lcr35® strain within the tablets after compaction

The genome analysis of *Lactobacillus rhamnosus* Lcr35® after compaction was performed on tablets obtained at 100, 200, 300 and 400 MPa for the Rep-PCR and at 100, 200 and 400 MPa for the microarrays method in comparison with the powder.

Table 1
Industrial API powders provided by Probionov (Aurillac, France) and their respective commercial name.

Commercial name	API name	Packaging	Application
Gynophilus®	*Lcr regenerans*®	Capsule	Vaginal
Bacilor®	*Lcr restituo*®	Capsule	Intestinal