

Lyophilisation of Probiotic Bacteria for Inclusion in Poultry Feed

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Abstract

Probiotics are extensively used in animal nutrition to improve feed conversion parameters, to boost the immune response and ultimately to improve animal health and welfare. The aim of our study was to obtain a lyophilised product for use as supplement in poultry feeding and to determine the optimal concentration required for inclusion in feed. In our experiment we have used the following human isolated probiotic bacteria: *Lactococcus lactis* CMGB 31, *Lactobacillus paracasei* CMGB 18, *Lactococcus lactis* CMGB 33, *Lactobacillus rhamnosus* CMGB 34 and *Lactococcus lactis* CMGB 32. The strains were preserved by freezing at - 80°C. After lyophilisation, using skimmed milk and MRS mixture as preservative and MRS broth as growth media the recorded concentration was of 1·10⁹ CFU/g lyophilized product. This product can now be used as probiotic supplement in poultry feed at a concentration of 1 g/kg feed.

Keywords: lactic acid bacteria, lyophilisation, poultry, viability

1. Introduction

According to their primary definition, probiotics are known as live nutritional supplements with a beneficial effect on host's intestinal microbial balance [1].

Their role as digestive bioregulators or direct-fed microbials (DFMs) is limited to products that consist of one or several strains of microorganisms defined [2].

The use of probiotics in livestock feeding has increased considerably in the last 25 years. Once ingested, probiotics can change the microbiota composition and functionality with a direct impact in the intestinal homeostasis. It has been suggested that in some cases, factors such as dietary and management constraints can modify the structure

and the metabolic role of intestinal microbial communities, leading to health and growth deficiencies in animals [3].

Lactic acid bacteria must satisfy the existing safety, functional and technological regulations prior to their classification as probiotics. From a technological point of view, it is vital that the new probiotic strains are able to grow to high cell density in the fermentation medium, to survive and remain viable during storage, and to withstand the harsh gastrointestinal environment [4].

Freeze-drying represents the preferred method for long-term storage of microorganisms for the food and pharmaceutical industry where large amounts of bacteria are needed. Even though lyophilisation is widely used technique, there are still huge variations due to a plethora of different desiccation techniques related to individual microorganisms [5].

Freeze-drying is also known as a process of stabilization in during which first the substances

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are frozen followed by two drying steps in order to reduce the water content to a level not able to support the growth and enzymatic activity [6,7,8]. The existing literature, related to methodologies involved in producing probiotics for animal feeding is still unclear. Our study aimed to obtain a lyophilized product, of five new probiotic strains, suitable to be applied in poultry feed.

2. Materials and methods

The biological material: we have used the following human isolated lactic acid bacteria *Lactococcus lactis* CMGB 31, *Lactobacillus paracasei* CMGB 18, *Lactococcus lactis* CMGB 33, *Lactobacillus rhamnosus* CMGB 34, *Lactococcus lactis* CMGB 32. The growth conditions were: MRS-broth (CM 0359, OXOID LTD, Basingstoke, Hampshire, England), 37°C, in aerobic conditions.

The obtaining of the lyophilized product: After thawing the bacteria stains were grown for 24 hours in MRS broth, then the cultures were centrifuged (6000 rpm for 5 min). The pellet was then suspended in the lyophilisation mixture. The lyophilisation mixture contained MRS broth and 20% skimmed milk solution (1:1, vol:vol). This mixture was then frozen at -80°C and lyophilized using II Shin Europe equipment. After lyophilisation (for 40 hours) the relative humidity of the products was determined using a thermo balance (Radwag WPS 210S) and all samples had a recorded humidity of less than 1%. After lyophilisation the product was stored at room

temperature, in dark and in sealed containers. After 7 days of storage the quality of the product was evaluated as CFU/g.

Quality evaluation of the lyophilized probiotic: In order to evaluate the efficiency of the lyophilisation, CFU determination was performed before and after lyophilisation. CFU was determined from the lyophilisation mixture and from the product resulted after lyophilisation using the serial dilution method, insemination on MRS agar (CM 0361 OXOID LTD., Basingstoke, Hampshire, England) and colonies were enumerated after 24-48 hours of growth.

3. Results and discussion

Prior to lyophilisation the CFU/ml concentration of the five probiotic stains tested was: 1.37×10^9 for *Lactococcus lactis* CMGB 31, 1.43×10^9 for *Lactobacillus paracasei* CMGB 18, 1.53×10^9 for *Lactococcus lactis* CMGB 33, 1.64×10^9 for *Lactobacillus rhamnosus* CMGB 34, and 1.89×10^9 for *Lactococcus lactis* CMGB 32. For each sample 35 ml of lyophilisation mixture was used.

After lyophilisation the resulting product was yellow in colour and water soluble. The total amount of product was 3.49 g for *Lactococcus lactis* CMGB 31, 3.51 g for *Lactobacillus paracasei* CMGB 18, 3.46 g for *Lactococcus lactis* CMGB 33, 3.59 g for *Lactobacillus rhamnosus* CMGB 34, and 3.61 g for *Lactococcus lactis* CMGB 32. Data presented in table 1 shows the efficiency of lyophilisation for the five probiotic strains studied.

Table 1. The efficiency of lyophilisation for the five probiotic strains used in the study

Probiotic	CFU/ml in lyophilized mixture	CFU/g in lyophilized product	Lyophilized product obtained from 35 ml of mixture (g)	Viability* (%)
<i>Lactococcus lactis</i> CMGB 31	1.37×10^9	1.27×10^9	3.49	16.2
<i>Lactobacillus paracasei</i> CMGB 18	1.43×10^9	1.23×10^9	3.51	15.1
<i>Lactococcus lactis</i> CMGB 33	1.53×10^9	1.37×10^9	3.46	15.5
<i>Lactobacillus rhamnosus</i> CMGB 34	1.64×10^9	1.21×10^9	3.59	13.2
<i>Lactococcus lactis</i> CMGB 32	1.89×10^9	1.25×10^9	3.61	11.9

*Viability was calculated using the following formula $V = F/I \times 100$; F- represents the total number of probiotic CFU from lyophilized product; I - represents the total number of probiotic CFU from lyophilisation mixture.

The total number of CFU in the lyophilisation mixture was calculated for each probiotic using the CFU/ml and the volume of suspension (35 ml)

methodology. We have then determined the viability (%) using as starting point the CFU/g values. We have also observed that the bacterial

viability decreases during lyophilisation as with 11.9% for *Lactococcus lactis* CMGB 32 and to 16.2% for *Lactococcus lactis* CMGB 31. Our results are similar to those reported in the literature [9].

The highest CFU/ml concentration was recorded for *Lactococcus lactis* CMGB 32, the highest CFU/g for the lyophilisate product of *Lactococcus lactis* CMGB 33 and the highest viability for *Lactococcus lactis* CMGB 31. The highest lyophilisate product was obtained from the cultures of *Lactococcus lactis* CMGB 32.

Sufficient self-life, high viability and activity are requirements for making bacterial formulations. High cell concentrations are necessary for the production of probiotic product. Also it is useful to increase the initial cell concentration as is much possible to optimize the final results. In the case of lactic acid bacteria the effect of the initial cell concentration is linked to the protective medium used [10].

Results of *in vitro* study showed that some lactic acid bacteria strains have an inhibitor effect for enteropathogenic bacteria and have the potential to be used as probiotics in poultry feeding [11].

Utilization of combined feed with included lyophilised probiotics is starting to become a current practice in farm animal feeding, especially for broilers in order to improve health, feed conversion and reduction of gut pathogens [12, 13].

Using probiotics to improve the natural defense mechanism of animals against pathogenic bacteria, *in vitro* experiments have shown that *Enterococcus faecium*, *Pediococcus acidilactici*, *Lactobacillus salivarius*, and *Lactobacillus reuteri* isolated from healthy chicken have the ability to reduce the growth of *C. jejuni* and in the *in vivo* experiments conclude that these probiotics will reduce the colonization of broilers intestinal environment [14].

4. Conclusions

Our experiments showed that human isolated probiotic bacteria could efficiently be lyophilized to be used as probiotic supplement in poultry feeding.

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