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SHORT COMMUNICATION

Influence of whey on viability of *Lactobacillus gasseri* during freeze-drying process

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ABSTRACT

Probiotic bacteria have positive impacts on a numerous of physiological functions including immunomodulation and also on prevention of various intestinal diseases. However, acidic environment of the stomach and heavy conditions of freeze-drying process decrease survival of these bacteria. There are necessary researches on cheap raw materials that increase cell protection. The aim of the present study was to determine the impact of whey and other compounds on survival *Lactobacillus gasseri* during the freeze-drying process and storage. There were prepared culture media for cell protection during lyophilisation, which contained a variety of mixture whey, skim milk, sucrose, starch, maltodextrin and whey protein. Lyophilisate was rubbed in aerobic and anaerobic condition with lower humidity. Survival tests were investigated directly after freeze-drying process and then periodically during storage under refrigeration. Within 6 months of storage bacterial viability was similar in the samples containing liquid whey except for media containing starch. The results of viability did not deviate than those obtained with using the reference medium, which contains skim milk and sucrose. This shows that the liquid whey may be successfully used to protect the lactic acid

bacteria during lyophilisation. Moreover, the use of whey can reduce the amount of waste produced in the dairy industry.

***Keywords*:** freeze-drying, lactic acid bacteria (LAB), whey

1. INTRODUCTION

Freeze-drying is one of the most common methods for the production at an industrial scale of concentrated microbiological cultures. This method is mainly used for food biotechnology (fermentation and probiotics food) to the preservation and long period storage of lactic acid bacteria (LAB). The freeze-drying process causes high environmental stress for bacteria, that its survival depends on many factors, such as type of cryoprotectants, freezing rate and temperature, bacterial species, initial cell concentration and the physiological status of the bacteria [1,2].

A special group *Lactobacilli* are often have beneficial effects on human health and they are called probiotics. The main function of Lactobacilli is to limits the growth of pathogenic microorganisms through to keep for them a adverse environment. The interfering growth of pathogens is due to by different mechanisms, such as high adhesion and to colonize to the intestine mucosa (competitive for pathogens), production of antimicrobial compounds, competition for nutrients and stimulation of the immune system [1-15]. The one of most interesting *Lactobacilli* is *Lactobacillus gasseri* which is an anaerobic and gram-positive. These bacteria have resistance to gastric acids and have good adhesion properties that allow biofilm formation. Moreover *L. gasseri* 57℃ has a unique ability to produce hydrogen peroxide, which is especially responsible for the inhibition of growth anaerobic bacterial [1-9,12-14].

The microencapsulation processes and addition of cryoprotectants are used to improve the survival of lactic acid cultures due to better stabilise the cells, enhance the viability and stability during the production and storage. As a result of encapsulation, the medium containing proteins which surround the bacterial cells and protect them the outside environment. Furthermore addition of cryoprotecting compounds helps in reducing the osmotic difference between inside and outside of cells [1-9].

Whey is the liquid residue obtained from the coagulation of milk for the manufacture of cheese. It contains lactose and soluble proteins (20% proteins of milk), which make it a potential substance to enhance survival of bacteria. Some part of the whey is used as a food additive but the rest of it is considered as very problematic waste [7,8]. Therefore, the use of whey in freeze-drying as a raw material can reduce its negative impact on the environment.

2. MATERIALS AND METHODS

2. 1. Materials and methods

Production of lyophilisate

Lactobacillus gasseri strain was obtained from the IBSS BIOMED S.A. (Kraków, Poland) collection. Cultures were grown 24 h in modified MRS-broth at 37 °C under

anaerobic conditions to stationary phase by using AnaeroGen™ (Oxoid Limited, UK). The modified MRS mediums were prepared according to IBSS BIOMED S.A. protocol. The cells were harvested by centrifugation (5000 x *g*, 10 min, 4 °C), washed with sterile phosphate buffered saline and were centrifuged second time. The obtained bacterial biomass was suspended in various freeze-drying media. The reference medium contained 80 g/L skim milk and 60 g/L sucrose and the other media used in this study was its modifications where the skim milk and/or sucrose was replaced by different compounds. Tested mixtures of lyophilisates: whey, skim milk and sucrose; whey and sucrose; whey and starch; whey and maltodextrin; whey and whey protein; whey protein and sucrose. The used whey was post-production liquid by-product obtain from regional Dairy Plant (STARCO, Poland). It was the acid whey so before using its pH was adjusted to 6.

The samples were freeze-dried for 48h in freeze-dryer CHRIST® BETA. After freeze-drying process samples were closed in this apparatus under vacuum condition. Samples were transferred to anaerobic cabinet with low humidity (<10%), than was obtained lyophilised powder by using a sieve of size 0.5 mm.

Storage experiment

The freeze-dried powdered samples were stored at 4 °C in a glass vials with a rubber stopper.

Survival test

The survival rates of bacteria in freeze-dried powder were expressed as log CFU/g. The survival rates were evaluated after 1, 3 and 6 month of storage for each the suspension media used for freeze-drying. After 1, 3 and 6 months samples of 0.1 g lyophilisate were added to 100 ml of previously prepared sterile saline solution. Then serial decimal dilutions were made and 100 µL of the selected dilutions were transferred into triple sets of Petri dishes with modified MRS-agar. The samples were incubated under anaerobic atmosphere at 37 °C for 48h. The colonies were counted and expressed as CFU g^{-1}.

3. RESULTS AND DISCUSSION

Maintenance of bacterial viability is the most crucial in the industrial application of probiotics. However, economic aspects are also important, so reasonable is searching of cheap raw materials for freeze-drying process. The preliminary obtained results were encouraging for using liquid whey as a main component of freeze-drying medium. Generally tested samples containing whey have comparable results as this prepared in standard medium. For instance, directly after lyophilisation there was no significant differences in viability of *L.gasseri* and it was about 10^{11} CFU·g^{-1} (Fig. 1 – "0" time). After 6 months storage results were also similar between samples with whey or skim milk. For example, in the case of samples D, E, F and J there noticed decrease of bacteria viability about 0-0,5 log whether the second compound was whey or skim milk. Moreover, comparing results obtained for the reference medium to the modified medium containing only whey with sucrose the differences were only 0,35 log for the benefit of modified medium. However, the reference medium has a bit higher viability than whey with sucrose mixture. The maximum reduction of bacteria was

0,54 log for samples containing whey or skim milk (Fig. 2). The addition starch or maltodextrin has a negative impact on the viability of bacteria.

Whey contains many nutrients, among others approximately 5% lactose, 1% milk protein, 0.5% fat and numerous of mineral salts and vitamin. De Castro-Cislaghi et al. [7] reported that the whey has a positive effect on the stability of bacteria during storage under refrigeration after spray drying process. Whey has good properties for bacteria preservation because the main component is lactose. As was reported by Nag and Das [9] lactose protects bacterial cells, not only during lyophilization but also against the influence of gastric juices. It is tempting to suggest that whey is a promising to be used as a cryoprotectant media for freeze-drying. Using of whey carrier could reduce costs of freeze-drying process because it is cheaper than skim milk.

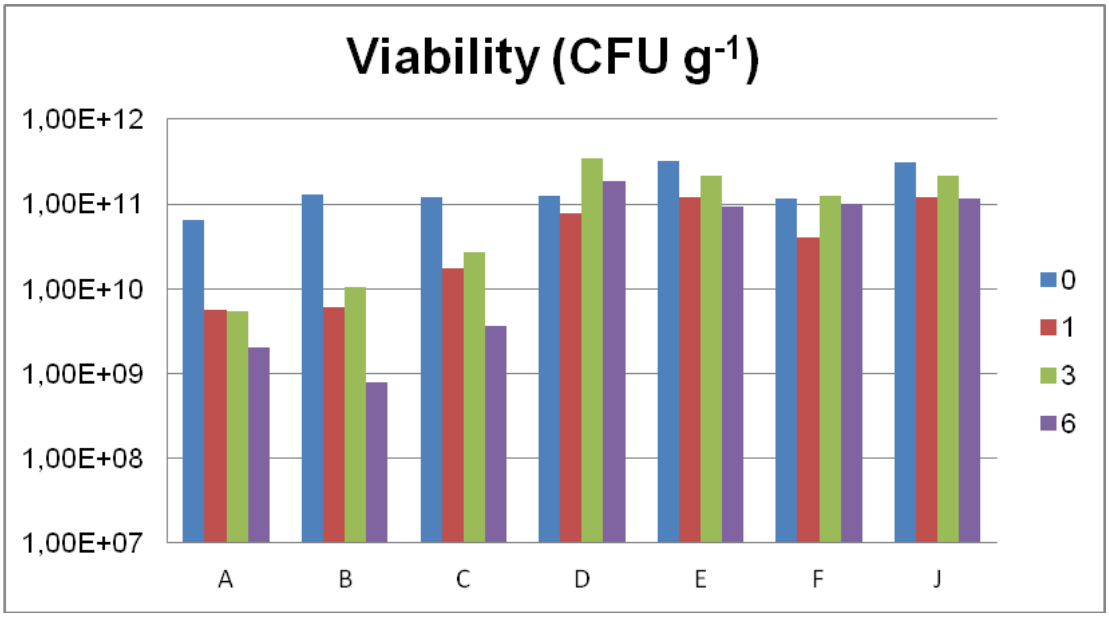

Figure 1. Viability of *L.gasseri* after storage 0, 1, 3, 6 months: A) whey + starch HS, B) whey + starch TA, C) whey + maltodextrin, D) whey + sucrose + skim milk, E) whey + whey protein, F) whey + sucrose, J) skim milk + sucrose.

A	B	C	D	E	F	J
1.51	2.21	1.51	-0.17	0.54	0.06	0.43

Figure 2. Logarithmic decrease viability of *L.gasseri* between 0 and 6 months: A) whey + starch HS B) whey + starch TA C) whey + maltodextrin D) whey + sucrose + skim milk E) whey + whey protein F) whey + sucrose J) skim milk + sucrose.

4. CONCLUSIONS

- The liquid whey could be successfully used as a cryoprotectant in freeze-drying process of LAB
- The viability of *Lactobacillus gasseri* was comparable to the reference medium in case of 3 tasted mixtures of lyophilisates with whey (whey, skim milk and sucrose; whey and sucrose; whey and whey protein).
- The mixture of lyophilisate containing only whey and starch or maltodextrin is not good enough to protect freeze-dried *L. gasseri* during long-term storage.
- The use of whey in freeze-drying process may be one of the possibilities to reduce the amount of waste from dairy industry.

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