I INTEGRATIVE INTERNACIONAL CONGRESS
ON ANIMAL AND ENVIRONMENTAL HEALTH

INFLUENCE OF CRYOPROTECTANTS AND STORAGE TEMPERATURE ON THE VIABILITY OF FREEZE-DRIED ENTEROCOCCUS GALLINARUM

I Integrative International Congress on Animal and Environmental Health, 1ª edição, de 25/06/2024 a 28/06/2024
ISBN dos Anais: 978-65-5465-100-4

CRUZ; Matheus Gomes da [1], SILVA; Ana Maria Souza da [2], MEJIA; Karen Dayana Prada [3], OLIVEIRA; Cintia Mara Costa de [4], SANTOS; Kamila Oliveira dos [5], VALLADÃO; Gustavo Moraes Ramos [6]

RESUMO

Freeze-drying is a promising biotechnological technique that allows protection and stability of bacteria with probiotic potential. Here, we investigated the influence of six different cryoprotectants and storage temperatures on the cell viability of freeze-dried *Enterococcus gallianrum*. For this, a strain of *E. gallinarum* candidate for pirarucu probiotic was reactivated in tryptone soy agar and incubated for 48 h (35ºC). Posteriorly, the bacteria were inoculated in tryptone soy broth (35ºC/24h). Subsequently, the culture media containing *E. gallinarum* were centrifuged, and the bacterial cells harvested by centrifugation were washed twice and resuspended in phosphate-buffered saline (PBS) to obtain a standard cell suspension (SCS). The SCS was fractionated into equal volumes, and the cryoprotectants dextrose, fructose, skimmed milk, maltodextrin, sucrose, and trehalose were added individually. PBS was used as the control. The samples were frozen and then dried by freeze-drying (40h/-48ºC). After freeze-drying, the samples were stored at room temperature (25±1.05ºC), cooling (4±0.6ºC), and freezing (-25±1.1 ºC) for 60 days. The lyophilized strains were quantified (log CFU/g) after 60 days of storage, serially diluted in PBS, and plated in TSA medium to determine the number of viable bacterial cells after storage. The reduction in the number of viable bacterial cells after storage at three temperatures was obtained using Log N0-N1, where N0 means the count of viable cells after freeze-drying (0 days) and N1 corresponds to the number of viable cells for the time interval (60 days) and storage temperatures. After 60 days of storage, *E. gallinarum* microencapsulated with maltodextrin, trehalose, sucrose, fructose, and dextrose showed a significant decrease (3.39-9.28 log CFU/g) in their viability ($p<0.05$) at room temperature. Additionally, *E. gallinarum* lyophilized with PBS and stored at the same temperature showed a reduction of 5.99 log CFU/g. However, *E. gallinarum* lyophilized with skimmed milk remained with high viability (reduction of 1.12 log CFU/g) after storage at room temperature for 60 days. Furthermore, *E. gallinarum* lyophilized with different cryoprotectants and storage temperatures (4ºC and -25ºC) did not show significant changes ($p>0.05$) in the viable cell counts among all treatments, including the control group after 60 days. Therefore, our study highlighted that, regardless of the cryoprotectant, *E. gallinarum* stored under cooling and freezing demonstrated high viability (≥9 log CFU/g), remaining unchanged during 60 days of storage. However, we highlight the possibility of maintenance (≥8 log CFU/g) of *E. gallinarum* coated with skimmed milk at room temperature for 60 days, reducing refrigeration or

[1] Universidade do Estado do Amazonas, matheusbiogc@hotmail.com
[2] Universidade do Estado do Amazonas, juliana-lester@hotmail.com
[3] Universidade Nilton Lins, karen.pradamejia@yahoo.com
[4] Universidade Federal do Amazonas, cmaraoliveira.cmc@gmail.com
[5] Universidade Nilton Lins, kosengpesca@hotmail.com
[6] Universidade Nilton Lins, gmrv.aqua@gmail.com

freezing costs.

PALAVRAS-CHAVE: Survival;, Enterococcus gallinarum;, Shelf time

[1] Universidade do Estado do Amazonas, matheusbiogc@hotmail.com
[2] Universidade do Estado do Amazonas, juliana-lester@hotmail.com
[3] Universidade Nilton Lins, karen.pradamejia@yahoo.com
[4] Universidade Federal do Amazonas, cmaraoliveira.cmc@gmail.com
[5] Universidade Nilton Lins, kosengpesca@hotmail.com
[6] Universidade Nilton Lins, gmrv.aqua@gmail.com