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Review Article

Studies on free and nano-encapsulated Lactobacillus casei under simulated gastrointestinal fluid and thermal conditions

Sabah A. AboELmaaty1, Ashraf A Ad El-Tawab2, Mohamed K. Morsy3, Hadeer A Hassan1, Ebtesam Z Gar1

1 Botany and Microbiology Department, Faculty of Science, Benha University

2 Bacteriology, Immunology and Mycology Department, Faculty of Veterinary Medicine, Benha University

3 Food Technology Department, Faculty of Agriculture, Benha University.

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ABSTRACT

The main goal of this research was to see how nanoencapsulation affected the stability and vitality of the probiotic bacteria in a simulated gastrointestinal digesting and temperature environment. The mechanical encapsulation approach was used to encapsulate probiotics using sodium alginate and pullulan. Further, scanning electron microscopy (SEM) was used to examine the interaction between the capsule matrix and probiotic bacteria in the created nano-capsules. Probiotic bacteria's survival and stability were tested in simulated gastrointestinal, intestinal, thermal, and refrigeration conditions. Nano encapsulation has a considerable impact on probiotic bacteria survival and stability. The viability of probiotic bacteria decreased at a similar rate across all treatments. In contrast, free probiotic cells were shown to have a quick decline in CFU/gram when held at 4 □C. Not only did nano-encapsulated bacteria have a lower reduction in total viable cells than free bacterial cells in an in vitro gastrointestinal assay, but the results of the viable count in the case of nano-encapsulated cells were also above the recommended level (106 CFU/gram) under the thermal and simulated GIT conditions.

1. INTRODUCTION

According to FAO/WHO (2001), probiotics are live non-pathogenic bacteria that interact with the gastrointestinal microbiota and directly with the immune system to provide health benefits to the host when consumed at the recommended level "106–107 CFU/gram" (Frakolaki et al., 2021). Probiotics may have anticancer, antimicrobial and antimutagenic properties, as well as lower cholesterol levels. Probiotic bacteria have been utilized to preserve food items because of the metabolites they create, such as antimicrobials and flavoring compounds can improve the food product stability. Probiotics are used in the production of a variety of beneficial dairy and non-dairy food items. Functional foods containing probiotic bacteria provide a variety of health advantages (Nakai et al., 2017), such as probiotic jelly sweets, which may be beneficial to individuals and children who have dairy allergies or who eat vegetarian diets (Misra et al., 2021). Probiotics also refer to a broad spectrum of microorganisms such as bacteria and yeast (Yilmaz et al., 2020), with lactic acid bacteria, Lactobacillus acidophilus, and bifidobacteria being the most investigated (Ayyash et al., 2020). All of the anticipated probiotic health effects are linked to their dietary and gastrointestinal stability and durability (Vallianou et al., 2020). To be effective, a significant number of probiotics must stay in the carrier food throughout storage and consumption (Ermis, 2021). Probiotic survival is influenced by certain parameters, including acidity in fermented goods, pH and bile salts in the gastrointestinal tract, and the temperatures during processing (Nami et al., 2020). The storage process may reduce the level of probiotics in the food product, and in this case, probiotics do not deliver the claimed benefits. Not only the storage process, but also the hostile conditions in the stomach and intestine affect probiotic survival, so it is critical to maintain the viability of probiotic bacteria under the same stressed conditions of GIT and storage (Ouis and Hariri, 2018; Muhammad et al., 2021). However, nano-encapsulation is gaining popularity as a way to guarantee that probiotic bacteria are delivered at the required dosage (Oberoi et al., 2021). The nano encapsulation technology protects probiotics against a variety of harsh circumstances (Gharibzahedi and Smith, 2021) and keeps probiotic levels in food products at therapeutic levels. The GRAS status of various encapsulating materials is used for probiotic encapsulation. Proteins, lipids, polysaccharides, and their derivatives, as well as sodium alginate and pullulan, are regarded to be efficient matrices owing to their versatility, biocompatibility, nontoxicity, economy, and ease of handling (Gorgieva and Kosev, 2018). Accordingly, the goal of this research is to see how long nano-encapsulated Lactobacillus casei can survive under simulated GIT, intestinal, and temperature environments.

* Corresponding author: sheko82004@gmail.com

2. MATERIALS AND METHODS

2.1. Maintenance of probiotic bacteria and capsule materials

Pure culture Probiotic (Lactobacillus casei BFEL 92040) was acquired from Faculty of Agriculture, Benha University. National Research Centre provided the encapsulating materials (sodium alginate; pullulan).

2.2. Nano-encapsulated probiotic preparation:

Lactobacillus casei BFEL 92040, a probiotic strain, was cultivated in 50 tubes with MRS broth and incubated at 37°C for 24 hours. The pellets were then precipitated by cooling centrifugation (High speed cooling centrifuge HSC- 310, Italy) at 35000 rpm for 10 minutes at 10°C, washed with sterilized saline solution 0.9 %, centrifuged again at the same first speed for 10 minutes at 10°C, and then stored in 0.9 %, saline solution at 4°C. Lactobacillus casei BFEL 92040 was encapsulated in two natural polymers (sodium alginate; pullulan) via mechanical nanotechnology, as described by Gazori et al. (2009), with certain changes, to provide two formulas: The initial recipe was made by creating nano-capsules of Lactobacillus casei in sodium alginate using sodium alginate. In a nutshell, 40 grams of sodium alginate were dissolved in 1000 mL deionized sterile water for 1 hour on a magnetic stirrer at 2000 rpm, then sterilized in an autoclave and stored in the refrigerator overnight. The second recipe was made by dissolving 70 grams of pullulan in 1000 mL of sterile distilled water and stirring for 2 hours at 2000 rpm with a magnetic stirrer at 50°C, then sterilizing the pullulan solution in an autoclave, and finally storing the solution in the refrigerator for 24 hours.

Ten grams of bacteria pellets were dissolved in 200 mL of 4% sodium alginate solution and swirled at 800 rpm for 15 minutes using a magnetic stirrer (PN-98, American). For breaking the particles suspended in solutions into nano particle size and emulsifications, 100 mL of the core was added to 500 mL of sterilized sodium alginate solution and the mixture was homogenized for 5 minutes in an ice bath at 10000 rpm using a High speed homogenizer (PRO- 400 PC, Germany). The obtained pre-emulsion was ultra-sonicated using a probe sonicator (VCX-750, USA) in a water bath (at 0°C) for 5 minutes to avoid recrystallization during the process, the production temperature was kept at least 5°C, and then this gel was placed in the freezer for 10 minutes to relax before receiving the capsule outer layer (pullulan), then this gel was transferred to a high-speed homogenizer at 20000 rpm for 30 minutes after (Singh et al., 2019).

2.3. Freeze and vacuum-drying technology (Freeze-drying):

This is accomplished by freezing nanoe-ncapsulated bacteria at low temperatures (Labconco freeze dryer LF-512, Germany), then vacuum dehydrating the water. After 48 hours of vacuum-drying at 0°C, the benefits of this technique include reduced energy consumption and ideal ending water content, which is one of the most essential characteristics with the best survival rate after drying and the most activation upon storage (Oberoi et al., 2021).

2.4. Nano-capsule Characterization:

A scanning electron microscope was used to examine the shape, distribution, and size of nano-capsules (QUANTA FEG 250, Japan).

2.5. The number of viable cells:

Serial dilutions from 101 to 106 were prepared weighing one gram of nano-capsule and 9 mL sterile saline solution 0.9 % were added (Etchepare et al.,2020), then transferring to MRS agar plates in triplicate, incubating at 37 °C for 72 hours and the results were recorded as CFU/gram.

2.6. Survival of L. casei, both free and nano-encapsulated, under simulated gastrointestinal circumstances:

The tolerance of free and encapsulated probiotic bacteria was tested using a modified version of Akgun et al., (2018). Pepsin (3 g/ L) was added to a sterile saline solution to create the mimicked gastric solution. 0.1 N HCL to be used to alter the pH 2 of the produced gastric juice. The gastric solution was combined with one gram of both free/unencapsulated and nano encapsulated L. casei, which was incubated at 37°C and 110 rpm. Counting the CFU/gram on MRS agar plates was used to quantify the quantity of live bacteria. The probiotic survival in free and nano encapsulated L. casei was measured over a period of time (0, 30, 60, 90, and 120 minutes).

2.7. Lactobacillus casei survival in simulated intestinal juice, both free and nano encapsulated:

The approach developed by Singh et al. (2019) was used to determine the survival of free and nano-encapsulated probiotic bacteria. In a nutshell, bile salt (3 g/ L) was dissolved in a phosphate buffer. NaOH was used to alter the pH of the intestinal fluid (7.5). One gram of free and nano-encapsulated were added to prepared simulated solutions separately and incubated at 37°C and 110 rpm. On MRS agar plates with encoded intervals of time, the viability of surviving free and nano encapsulated was determined (0, 30, 60, 90 and 120 minutes).

2.8. Free and encapsulated probiotics' thermal stability:

Heat treatments were applied to both free and nano-encapsulated L. casei according to Ouis and Hariri's approach (2018). At 65°C for 0, 15, 30, and 45 minutes, the viability of free and nano-encapsulated probiotics was tested. In test tubes containing 9 mL of sterile saline solution 0.9 %, one gram of both types of probiotic cells were introduced. The test tubes were incubated in a water bath at 65°C for various amounts of time. All of the samples were chilled to bring them down to room temperature. On MRS agar plates, the number of free and nano- encapsulated bacteria that survived was counted in triplicate.

2.9. Free and encapsulated cell viability under cold conditions:

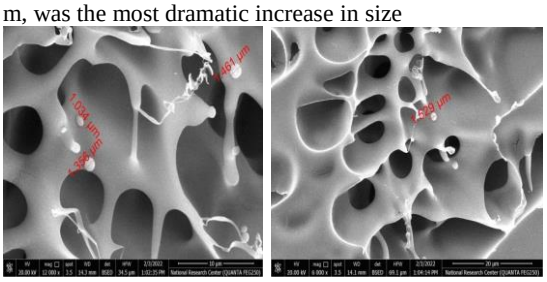
The viability of free and encapsulated probiotics was determined by subjecting them to cold temperatures using the technique of (Hariri et al., 2018). Briefly, both types of bacteria (cell free/nano encapsulated) were kept at 4°C and vitality was counted at intervals of 30 minutes (0, 5, 10, and 15 days).

3. RESULTS:

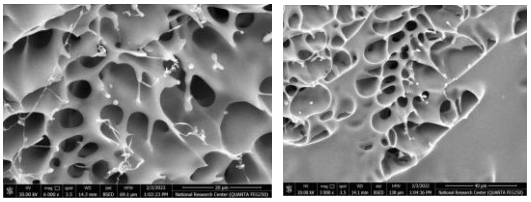
3.1. Nano-capsule Characterization

SEM images (Fig. 1) revealed that nano-encapsulated L. casei exhibit a spherical shape morphology, with typical particle sizes ranging from 1.034 m to 1.529 m. Lactobacillus casei was evenly distributed within the nano-capsule. The presence of alginate and L. casei was confirmed throughout the nano-capsule's interior, indicating that the

active material (probiotic bacteria) is not only in the core, but also throughout the capsule and even on the surface. The capsules shrank throughout the lyophilization process, resulting in uneven forms. The creation of the second alginate layer, which increased the capsule diameter to 1.529 m, was the most dramatic increase in size



(b) (a)

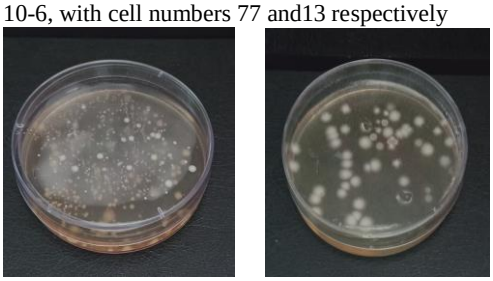


(c) (d)

Fig (1): Scanning electron microscope images.

3.2. Total viable cells:

The results showed that a huge number of cells were entrapped in nano-encapsule Lactobacillus casei in dilution 10-4 with cell numbers 360 as compared to dilution 10-5 and 10-6, with cell numbers 77 and13 respectively



(a) (b)

(c)

Fig (3): Total viable cell to nano-encapsulated bacteria from dilutions (a: 10-4, b: 10-5, c: 10-6)

3.3 Stability of the nano-encapsulated probiotic bacteria in gastric juice:

The results showed reduction in the CFU/gram of free cells in contrast to the cells encapsulated with sodium alginate and pullulan (Fig. 4). The cell vitality of free cells decreased from 7.8 CFU/gram to 2.2 CFU/gram after 120 minutes. While in case of nano-encapsulation with sodium alginate and pullulan the viability decreased from 8.8 CFU/gram to 5.8 CFU/gram.

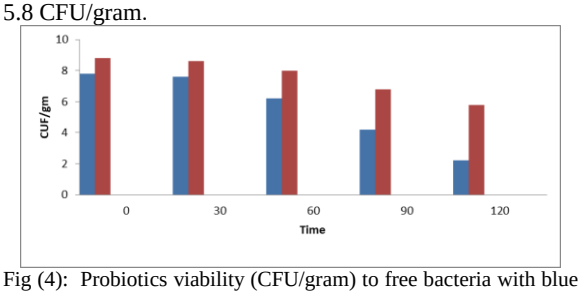


Fig (4): Probiotics viability (CFU/gram) to free bacteria with blue column and nano-encapsulated with red column in simulated gastric conditions .

There is something here not correct. May be the count is with Log10 CFU/gm not CFU/gm. Pleases check it. Also in Fig. 5 and 6

3.4. Stability of the nano-encapsulated probiotic in simulated intestinal Juice:

A reduction in the viability of free cells was observed as compared to the nano- encapsulated probiotic bacteria in simulated intestinal fluid (Fig. 5). The nano-encapsulation had significant effect on the survival of the cell. The vitalityof free cells decreased from 8.7 to 2.7 CFU/gram after 120 minutes. While in nano encapsulation decreased from 8.8 to 4.2 CFU/gram .

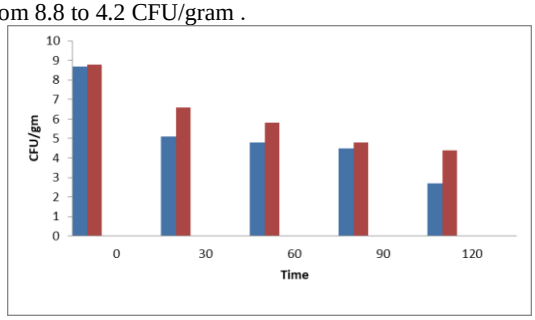


Fig (5): Viability offree probiotic (CFU/gram) with blue column and nano encapsulated bacteria with red column in simulated intestinal conditions.

3.5. Stability of free and nano encapsulated bacteria under thermal conditions:

The results of the present study indicated that high temperature affected the survival of free probiotic cells (Fig. 6). On the other hand, nano encapsulated probiotic bacteria cells showed better survival during exposure to temperature 65°C. The cell viability in case of free cells when exposed to 65°C decreased from 8.6 to 1.8 CFU/gram over an exposure of 120 minutes. While in case of nano encapsulation decreased from 8.8 to 6.2 CFU/gram .

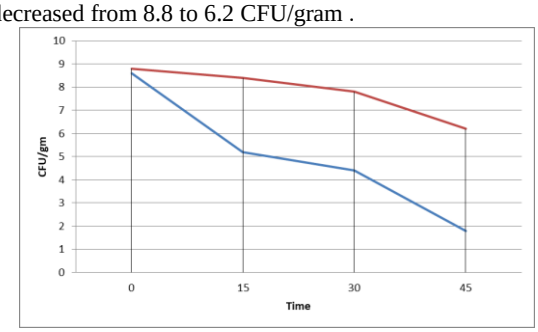
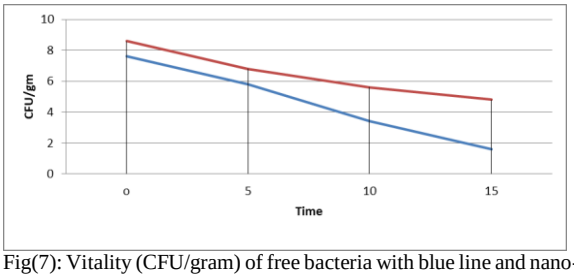


Fig (6):Free bacteriaviability (CFU/gram) with blue line and nano encapsulated bacteria with red line under thermal conditions.

3.6. Stability of free and nano encapsulated bacteria under refrigeration conditions:

The results indicated that nano-encapsulation is able to increase the viability of the probiotic bacteria at 4°C (Fig. 7) and the cell vitality in case of free cells when exposed to 4°C decreased from 7.6 to 1.6 CFU/gram after two weeks. While nano encapsulation decreased from 8.6 to 4.8 CFU/gram after 2 weeks .



Fig(7): Vitality (CFU/gram) of free bacteria with blue line and nano-encapsulated with red line under cold conditions with different times (0, 5, 10, and 15 days).

4. DISCUSION

Due to the lyophilization process, which caused water loss during the dehydration process, the bacteria spread homogeneously throughout the particle of nano capsule with spherical or irregular shape, the bacteria spread homogeneously throughout the particle of nano capsule with spherical or irregular shape (Chen et al., 2017). Particle sizes ranged from 1.034 m to 1.529 m on average. The most significant increase in size occurred with the formation of the second layer, resulting in a capsule diameter of 1.529 m, as observed by Agudelo-Chaparro et al. (2021), who found that when particles with two layers of alginate interacted electrostatically with the last layer, size became larger than the parent particle.

In dilutions (104) and (105), a huge number of cells were entrapped in sodium alginate and pullulan nano-encapsulate to Lactobacillus casei, respectively. These findings are consistent with Moghanjougi et al.(2021) .

Over the course of a 120-minute exposure, the vitality of free cells dropped. In the case of nano encapsulation, the viability was reduced somewhat, according to Singh et al. (2019). In order to get the intended results, probiotics must be viable in stomach and intestinal conditions. The findings of this research correlate with those of Afzaal et al. (2019), who found that using a polymer like sodium alginate for nano-encapsulation of probiotics protects and maintains bacterial viability at low pH.

In comparison to the nano-encapsulated probiotics, a fast drop in free cell viability was found. The nano-encapsulation of the cells had a major impact on the cell's ability to survive. The survival of cells in stomach and intestinal settings is critical for getting the intended advantages of probiotics, according to Li et al. (2019). The encapsulated cells release is influenced by the intestinal solution. When sodium alginate is exposed to an acidic pH, it begins to disintegrate, resulting in the fast release of cells from the nano-capsule (Agudelo-Chaparro et al. 2021).

Bacteria that are encapsulated have a better chance of surviving at extreme temperatures according to Misra et al. (2021). The current research found that high temperatures had an impact on the survival of free probiotic cells. Encapsulated cells, on the other hand, fared better at 65°C, since encapsulation of bacteria resulted in less heat transmission from the surrounding environment. The present findings are consistent with those of Li et al. (2019), who investigated the lifespan of free and encapsulated probiotics under various heat treatments.

Low temperature has an effect on the survivability of free probiotic bacteria since it reduces their enzymatic activity and makes them unable to absorb nutrients, according to Ermis.(2021)

According to Muhammad et al. (2021) who said that encapsulation ensures the stability and vitality of functional food during storage at 4°C, the findings showed that nano encapsulation may boost the stability of probiotic bacteria at 4°C. In another investigation, probiotics (L. acidophilus) encapsulated with pullulanmaterial exhibited negligible decline during 4 weeks of storage at 4°C when compared to free cells. The findings of this research correspond with those of Oberoi et al. (2021), who investigated the storage stability of probiotics and found that encapsulation may improve probiotic longevity.

5. CONCLUSION:

Under GIT, intestinal, thermal, andrefrigeratedtemperature simulations, the synthesis of nano-capsuled L. casei had a beneficial influence on the survivability of the probiotic bacteria. The nanoencapsule particles shield probiotic bacteria from stress while also allowing them to deliver their benefits. In terms of storage time, nanoe-ncapsules were shown to sustain probiotic viability and preserve for a longer amount of time. When compared to free cells, the coating materials put on L. casei may preserve cell structure and enhance probiotic vitality and stability.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest for current data

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