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Proteus penneri encapsulation with maltodextrin and sodium alginate using a spray-drying method

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Abstract

Proteus penneri has been isolated from the digestive tract and used for coffee fermentation. As its viability is compromised rapidly by unfavorable environmental conditions, encapsulation technique is used to protect it. The purpose of this study is to develop a bacterial encapsulation method that can preserve viability of *Proteus penneri*. The *Proteus penneri* were isolated from civet feces. Bacterial encapsulation was performed using a spray-drying method, and maltodextrin and sodium alginate were used as coating materials. We tested 15%, 20%, and 30% (w/v) concentrations of maltodextrin and 0.50%, 0.75%, and 1% (w/v) concentrations of sodium alginate. The encapsulated bacteria were stored for two weeks to study the stability of enzyme activity. We found that the spray-drying technology using maltodextrin as coating material resulted in a stable encapsulated bacterium. However, *Proteus penneri* did not survive well during the process. Loss of viability is caused by the membrane cell wall damage. After two weeks storage, bacterial viability decreased significantly. The enzymatic activity of the encapsulated cellulolytic bacteria was influenced by concentration of the bacteria, however the activity appeared to be stable after two weeks of storage.

Keywords: *Proteus penneri*; encapsulation; maltodextrin; sodium alginate; spray drying.

Practical Application: Bacterial encapsulation for producing artifical civet coffee.

1 Introduction

Proteus penneri, formerly known as *Proteus vulgaris,* a facultatively anaerobic bacterium, is an invasive pathogen and a cause of nosocomial infections of the urinary tract or open wounds. Recently this bacterium has been found and isolated from the digestive tract of civet by (Dewi, 2012) and has been produced as a dry isolate for the manufacture of artificial civet coffee by (Setyowati, 2016). This bacterium has an important role in civet digestion.

Civet coffee is known as the most expensive coffee in the world. It derives from coffee fruit eaten by the mongoose (*Paradoxurus hermaphroditus*). The supply of the natural product is insufficient to meet the increasing demand for civet coffee, so producers manufacture the coffee artificially through bacterial fermentation. The process is problematic. The bacteria used in it are unstable and must be re-isolated and multiplied before beginning production. A practical, effective, and easily controlled technique is necessary to improve the bacterial fermentation process.

One method for maintaining bacterial viability is bacterial encapsulation. Bacterial encapsulation is the process of wrapping (coating) bacteria with materials that help to maintain their viability and protect them from unfavorable environmental factors such as heat and chemicals (Raise et al., 2020; Ayama et al., 2014; Mahmoud et al., 2020). Bacterial viability is the ability of bacterial cells to grow normally under optimal conditions (Both et al., 2018). Besides increasing the duration of bacterial viability, bacterial encapsulation creates a product that is easier to use and less prone to damage: a fine powder with low water content (Afzaal et al., 2020) and (Patil et al., 2019).

Several studies using encapsulation techniques have been undertaken on probiotic bacteria (Samedi & Charles, 2019), Lactobacilli (Pourjafar et al., 2020), proteolytic bacteria (Sundari et al., 2020; Silva et al., 2018), and bacteriophages (González-Menéndez et al., 2018). Encapsulation methods and materials and the concentration of the coating material affect bacterial cell viability. Common methods used in the encapsulation process are spray drying, freeze drying, and emulsion techniques. Capsules can be derived from gum, carbohydrates, and proteins such as skim milk, lactose, sucrose, alginate, gum Arabic (Vahidmoghadam et al., 2019), starch, maltodextrin (Dewi et al., 2017), agar, gelatin, carrageenan, albumin, and casein (Dewi et al., 2018). The concentration of the material used for the coating can be varied to provide the most effective protection for the material to be encapsulated.

An ideal encapsulation technique for the bacteria *Proteus penneri* would improve its shelf life without affecting its viability. The method used in this study was spray drying. Bacterial cell damage from spray drying can minimized by adding coatings before conducting the process. The purpose of this study is to develop a bacterial encapsulation method for *Proteus penneri* that maintains bacterial viability.

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2 Materials and methods

2.1 Materials

The *Proteus penneri* isolates used in this research were cellulolytic bacterium isolates from civet feces, obtained using the method described by (Dewi, 2012) and (Rohman, 2013). The growth media were Carboxy Methyl Cellulose (CMC) and Plate Count Agar (PCA). The coating materials employed for encapsulation were maltodextrin (Foodchem) and sodium alginate (Foodchem). Other materials used were DNS solution, concentrated H_2SO_4 and 5% phenol to test cellulase enzyme activity based on glucose formation, 0.85% NaCl, distilled water, 70% alcohol, cotton, heat-resistant plastic, and aluminum foil.

2.2 Methods

The stages of the research are illustrated in Figure 1.

2.3 Isolate characterization

Cellulolytic ability test

The characterization of cellulolytic bacterial isolates was carried out at 30ºC for 54 hours on CMC media. The isolate characterization was done by determining the clear zone, growth curve, enzyme activity, total sugar content, and reducing sugar. Clear zone measurements were determined by growing the isolates on 1% CMC solid media incubated at room temperature for 48 hours. The media were then soaked with Congo red solution for 15 minutes and rinsed three times with NaCl for 20 seconds. A clear zone formed on the media indicates cellulase activity. If a clear zone was not visible, the bacterial isolates were re-incubated for 24 hours at 4 °C.

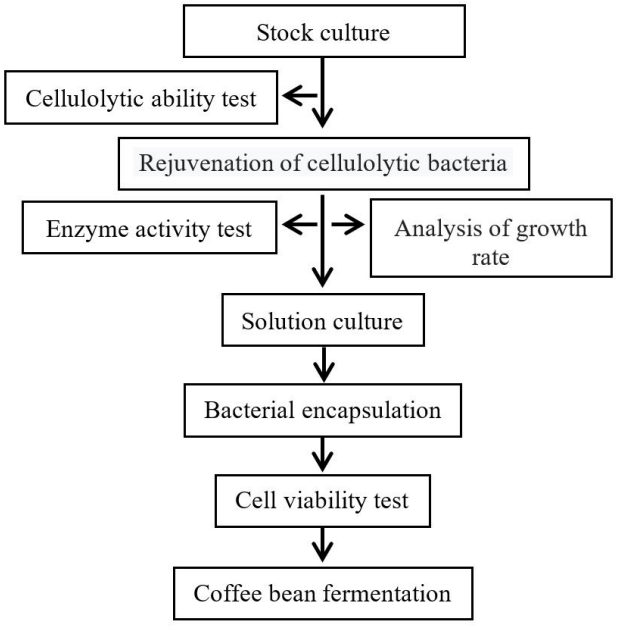

Figure 1. Flow chart of the research methods.

Rejuvenation of cellulolytic bacteria

Cellulolytic bacteria were rejuvenated in CMC media. As much as 1 g of CMC was dissolved and mixed in 100 ml of distilled water and sterilized using an autoclave at 121 °C for 15 minutes. Sterile media was poured into a petri dish and test tube and allowed to stand until solidified. Cellulolytic bacteria were rejuvenated by inoculating 1 loop in a slanted media and 1 ml in a petri dish media. The media were incubated for 24 hours at 37 °C.

Growth curve

A total of 1 loop of rejuvenated cellulolytic bacteria was inoculated into 10 mL of sterile liquid media composed of 1 g CMC, 0.10 g glucose, 0.20 g yeast extract, and 100 mL distilled water. The bacteria were then incubated in a shaker for 24 hours at 37 °C and 100 rpm. Samples were taken to measure the absorbance of the culture using spectrophotometry at a wavelength of 600 nm until it reached an absorbance of approximately 0.80. A total of 10 mL (10^6 cells/mL) of culture was inoculated into 100 mL of liquid media and incubated in a shaker at 100 rpm at 37 °C. The culture was assessed by the turbidimetry method with a spectrophotometer at a wavelength of 620 nm every 6 hours for 54 hours. The absorbance value obtained was then calculated using the standard growth curve equation. The number of cellulolytic isolate cells was calculated using the Total Plate Count (TPC) method on dilutions of 10^{-4} and 10^{-5}.

Bacterial encapsulation

Homogenization was employed to encapsulate the bacteria, using solutions with maltodextrin concentrations of 15%, 20% and 30% and sodium alginate concentrations of 0.50%, 0.75% and 1%. The bacterial cell was mixed with maltodextrin or sodium alginate at the designated concentration using a magnetic stirrer at 500 rpm for 5 minutes. A total of 1 mL of each sample was stored in Eppendorf for viability analysis before encapsulation. The remaining samples were encapsulated with a spray dryer at 125 °C until a powdery mass was obtained. This result was stored in heat-resistant plastic sealed with a hot zipper to ensure a sterile environment.

Viability analysis of the encapsulated bacteria

Viability analysis before encapsulation was carried out by taking 0.1 mL of the sample and diluting it to 10^{-7} with a physiological NaCl solution of 0.85%. The bacterium was than planted into a solid medium. Calculation of the colonies were performed after 24 hours of incubation at 30 °C. The viability analysis of the encapsulated bacteria was carried out immediately after the encapsulation process was completed and after two weeks of storage at 4º C and 37° C. The TPC method was performed by diluting 0.1 g of the powdery bacterial sample with 0.9 ml physiological NaCl 0.85% continuously until a dilution of 10^{-7} was reached. Colonies were counted after 24 hours of incubation at 30 °C. The viability of the colonies were calculated using the Equation 1.

$$Viability\ (\%) = \frac{total\ of\ encapsulated\ cellulolytic\ bacteria}{total\ of\ unencapsulated\ cellulolytic\ bacteria} x\ 100\% \quad (1)$$

Coffee bean fermentation

Coffee bean fermentation was performed using the powdered preparation of encapsulated cellulolytic bacteria in a solution with a water content of 60%. The skin of the bean was peeled off and cut into small pieces. A total of 5 g of coffee skin was mixed with 10 g of coffee beans (the ratio of beans and coffee skin was 2 to 1), put in a bottle, and sterilized at 121 °C for 15 minutes. The sterile substrate was stirred to distribute the particles evenly, aseptically inoculated with 10% powdery preparation, and incubated for 3 days at 30 °C.

3 Result and discussion

3.1 Isolates characterization

The bacterial isolate was able to produce clear zones on cellulolytic specific media (Figure 2). This indicates that the bacteria were able to produce cellulase enzymes. Bacterial isolates which have high cellulase enzyme activity can hydrolyze cellulose to glucose and produce a large clear zone around the colony. This is due to breakdown of fibrous cellulose into glucose. CMC media which are hydrolyzed by cellulase enzymes will not be colored if inundated with Congo red coloring. The interaction takes place non-covalently. Congo red is used as an indicator of β-D-glucan degradation in agar media (Hartanti, 2013).

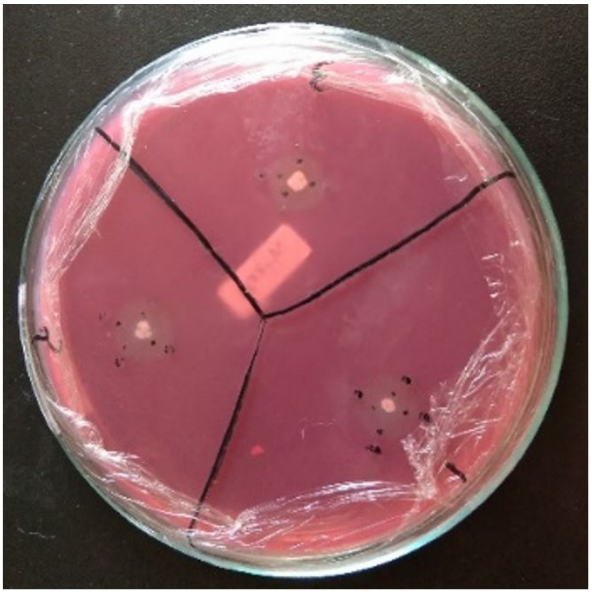

Figure 2. The formation of a clear zone around the bacterial colony.

Bacterial growth was determined by TPC and optical density (OD) was calculated. To determine bacterial growth by TPC, the number of visible bacterial cells are counted. The determination of bacterial growth based on OD values evaluates the density of the bacteria in a turbidity medium. An increase in the number of cells in a liquid culture increases its turbidity, which is measured using a spectrophotometer that assesses the intensity of the absorbed light. Enzyme activity is expressed as the amount of reducing sugars released per unit of time.

Bacterial growth determined by TPC and OD is presented on Figure 3. The results showed that the pattern of bacterial growth was sigmoidal, with an apparent slow phase followed by a more rapid increase in numbers to reached its maximum, and followed by the phase where the number of bacterial cells begins to decline as the nutrients become exhausted until the end of incubation process. This finding was in line with other research results (Russell et al., 2009; Dubey et al., 2014). At the same time OD values continued to increase until reaching a stationary phase, however OD value tended to decrease at the end of the incubation process. At that point the available nutrients began to decrease, while the cells continued to divide.

The pattern of total sugar produced by bacteria during incubation process (Figure 4) was closely related to the bacterial growth curve (Figure 3). The breakdown of polysaccharides into simple sugars by enzymatic activity produced an increase in total sugar. The bacteria break down polysaccharide to get their energy for their lives and produces sugar. As the number of bacterial cells decreased was followed by the decrease of total sugar produced.

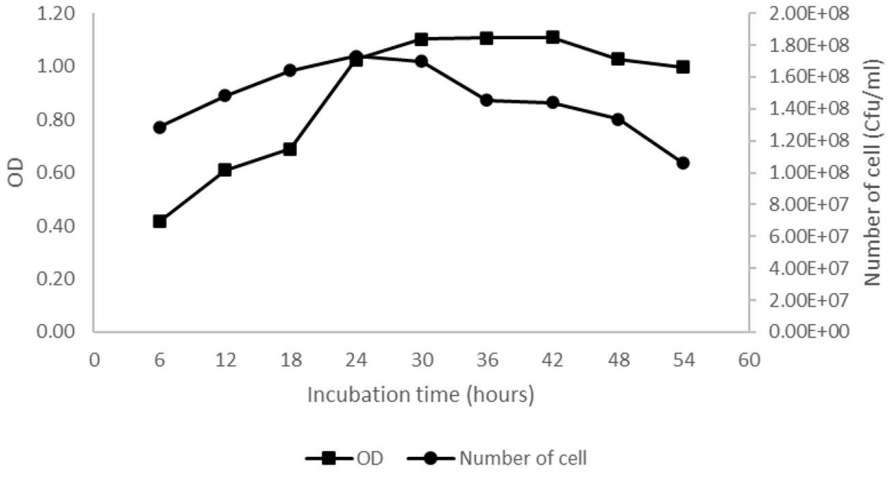

Figure 3. Growth of bacterial cells and optical density during incubation.

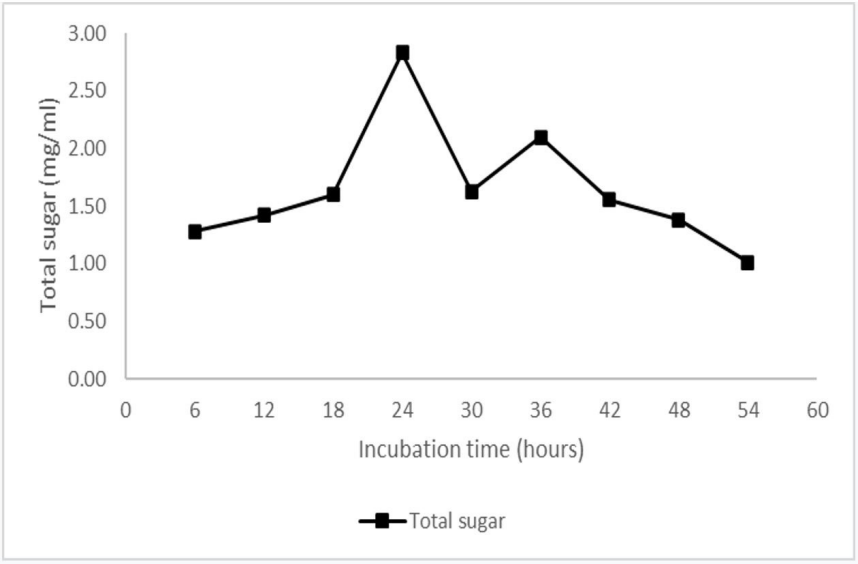

Figure 4. Total sugar produced by bacteria during incubation.

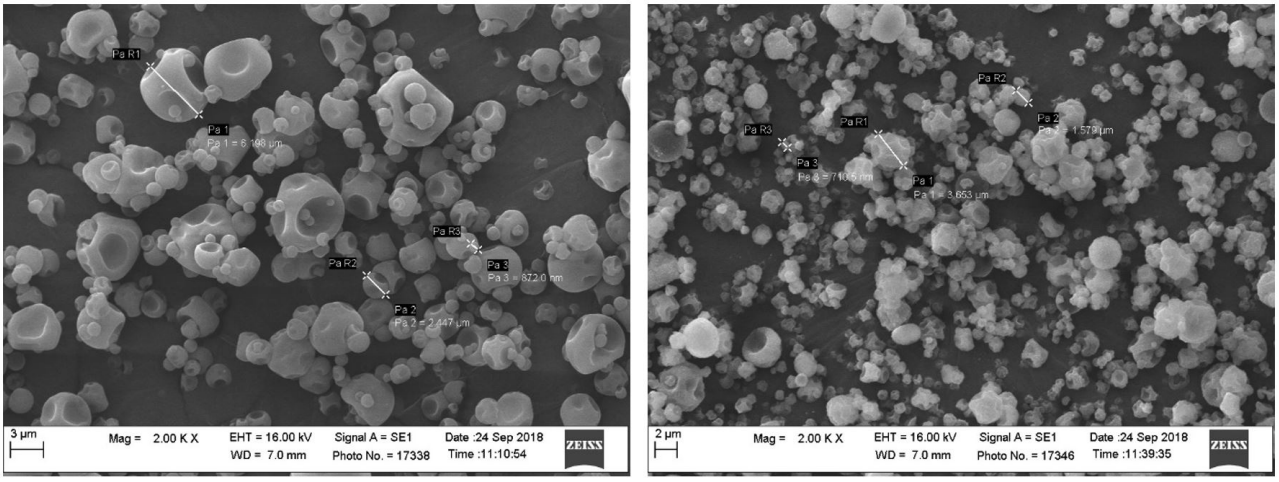

Figure 5. SEM encapsulation of maltodextrin (left) and sodium-alginate (right).

3.2 Bacterial encapsulation

Encapsulation is a process to entrap active agents within a carrier material in order to maintain viability of the living cells and extend their storage life. In this study, encapsulation was carried out using a spray-drying method with maltodextrin and sodium alginate as coating materials. These materials have been widely used in encapsulation and have been shown to extend the viability of bacteria during storage (Vahidmoghadam et al., 2019; Akdeniz et al., 2017; Călinoiu et al., 2019).

Images of the encapsulated bacteria obtained using Scanning Electronic Microscopy (SEM) are presented in Figure 5. This figure showed that the size of the maltodextrin particles is larger than the particle of sodium alginate. Images of SEM also showed that the size of the *Proteus penneri* corresponds to the size of this bacteria as listed in the Bergey's *Manual of Determinative Bacteriology.* Maltodextrin (left) was better able to encapsulate bacteria than sodium alginate (right). This is may be due to the larger particle and cavity size of maltodextrin, compared with sodium alginate. These results are in line with (Akdeniz et al., 2017), in which encapsulation was more efficient when maltodextrin alone was used as a coating material rather than used in conjunction with gum Arabic. Encapsulation efficiency is defined as the capacity of the coating materials to trap and maintain within their structure the material to be encapsulated (Piñón-Balderrama et al., 2020).

3.3 Viability test of encapsulated bacterium

Number of bacterial cells were found fewer after encapsulation than before, indicating that the cells could not withstand the heat of the encapsulation process (Table 1). *Proteus pinneri* is a mesophilic bacterium that has a protein that is not resistant to high temperatures. *Proteus* strains are able to grow in temperatures

ranging from 10 to 43 °C, but the optimal temperature is 25 °C (Wang & Pan, 2014). This bacterium is generally sensitive to both moist heat (121 °C for at least 15 minutes) and dry heat (160 to 170 °C for at least 1 hour) (Piñón-Balderrama et al., 2020). This experiment also showed that the storage temperature had no significant effect on the number of colonies after 2 weeks of storage. The coating materials had a greater effect on the number of colonies, where the decrease of the colonies number slightly higher when the bacteria were encapsulated with maltodextrin than when sodium alginate was used. This finding was similar to some other research results. The effectiveness of encapsulation is also affected by capsule materials (Dianawati et al., 2016), while the encapsulation efficiency is determined by the nature of hydrogel materials (Afzaal et al., 2020).

This experiment also shows that after the spray-drying process cell viability of the encapsulated bacteria declined, probably due to osmotic stress and heat (Table 2). The spray-drying process was conducted at 125 °C, which may cause thermal or dehydration inactivation of microbial cells (Tan et al., 2018). Inactivation caused by heat stress is the dominant factor in cell death when drying is conducted at high temperatures.

The viability of encapsulated bacteria affected by concentration of coating materials, where bacterial viability increased as the concentration of coating materials was increased (Table 2). The bacterial viability, however, tended to decrease after 2 weeks storing. This probably due to the temperature of incubation storage. Some literatures stated that bacterial cell viability depends on storage temperature (Cota & Alvim, 2018; Costa et al., 2002). (Arepally et al., 2020) also found that the colder the storage temperature, the longer the culture will retain viable cells.

3.4 The use of encapsulated bacteria for coffee fermentation

The encapsulated bacteria were used to ferment coffee fruits to obtain a bean quality equivalent to that of natural civet coffee. Enzymatic activity was observed to assess the effect of concentration of the coating materials on bacterial activity.

Figure 6 presents the enzyme activity of the encapsulated cellulolytic bacteria, showing that it decreased with increasing concentration of the coating materials. The reduction of enzyme activity when 30% maltodextrin was employed most likely due to lower bacterial concentration in the solution (Table 1). This result was in line with (Sırt Çıplak & Akoğlu, 2020), where the lower enzymatic activity was observed in the samples when lower bacterial counts were observed. This is similar to other research results, which stated that in most cases of encapsulation have shown no effect enzyme activities. However, encapsulation slightly reduced activity of yeast cytosine deaminase (Inoue et al., 2008) and reduced the activity of peptidase E (Fiedler et al., 2010).

This experiment also proves that the enzyme activity of the encapsulated cellulolytic bacteria stabilized during storage (Figure 7). This result was in agreement with the studies of (Natalia et al., 2014) who observed the activity of immobilized K80 lipase of *Proteus vulgaris* was found more stable. Similarly, (Almulaiky et al., 2021) reported immobilized α-amylase preparations maintained and enhanced biochemical properties of the free enzyme.

Table 1. Number of bacterial colonies observed using different concentrations of coating materials.

Coating material	Colony (Cfu/ml)			
	Unencapsulated bacteria	Encapsulated bacteria	After 2 weeks' storage	
			30 °C	4 °C
Maltodextrin 15%	3.79×10^8	1.93×10^8	1.60×10^8	1.58×10^8
Maltodextrin 20%	3.14×10^8	1.87×10^8	1.27×10^8	1.34×10^8
Maltodextrin 30%	5.12×10^8	2.47×10^8	8.22×10^7	8.31×10^7
Sodium alginate 0.50%	1.68×10^8	1.57×10^8	1.11×10^8	8.98×10^7
Sodium alginate 0.75%	3.90×10^8	1.40×10^8	9.55×10^7	8.39×10^7
Sodium alginate 1%	5.91×10^8	1.19×10^8	6.47×10^7	8.44×10^7

Table 2. Viability test of encapsulated cellulolytic bacteria.

Coating material	Viability (%)		
	Encapsulated bacteria	After 2 weeks' storage	
		30 °C	4 °C
Maltodextrin 15%	85.07	48.32	57.93
Maltodextrin 20%	90.75	53.88	48.76
Maltodextrin 30%	91.91	66.07	55.24
Sodium alginate 0.50%	85.27	48.81	57.80
Sodium alginate 0.75%	87.28	40.57	48.31
Sodium alginate 1%	93.88	50.88	65.33

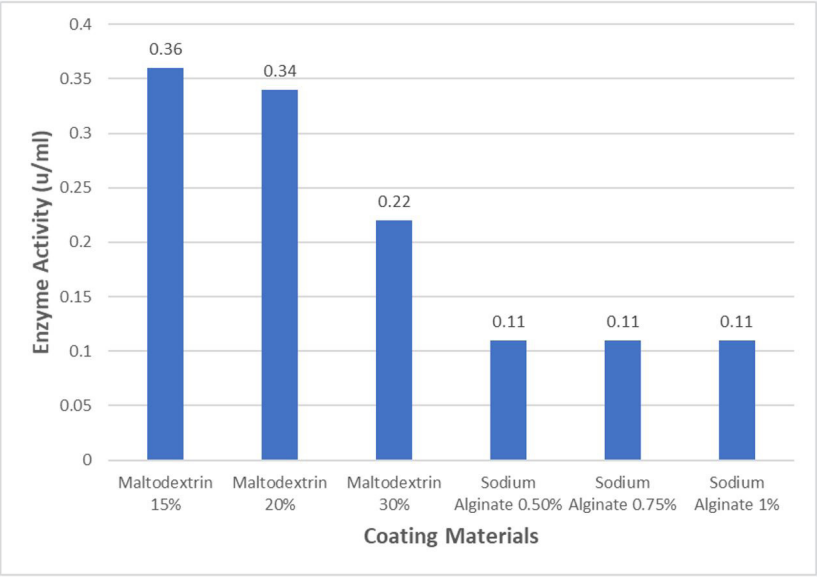

Figure 6. Enzyme activity of encapsulated cellulolytic bacteria with different coatings.

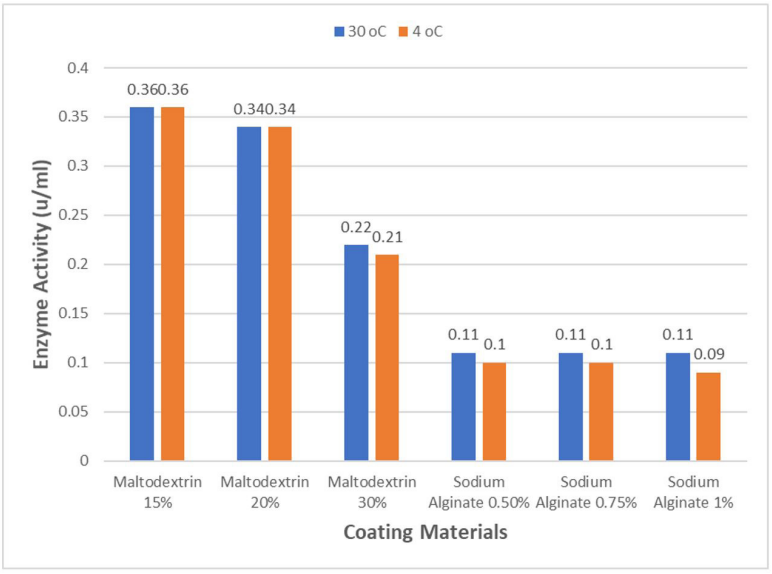

Figure 7. Enzyme activity of encapsulated cellulolytic bacteria with different coatings, after being stored for 2 weeks.

4 Conclusion

Our study showed that spray-drying technology using maltodextrin as coating material resulted in a stable encapsulated bacterium. However, *Proteus penneri* showed poor survival under the high temperature and dry environment of the spray-drying process. Two weeks of storage of the encapsulated product decreased bacterial viability. The enzyme activity of the encapsulated cellulolytic appeared to be stable after 2 weeks of storage.

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References

Afzaal, M., Khan, A. U., Saeed, F., Arshad, M. S., Khan, M. A., Saeed, M., Maan, A. A., Khan, M. K., Ismail, Z., Ahmed, A., Tufail, T., Ateeq, H., & Anjum, F. M. (2020). Survival and stability of free and encapsulated probiotic bacteria under simulated gastrointestinal conditions and in ice cream. *Food Science & Nutrition*, 8(3), 1649-1656. http://dx.doi.org/10.1002/fsn3.1451. PMid:32180972.

Akdeniz, B., Sumnu, G., & Sahin, S. (2017). The Effects of Maltodextrin and Gum Arabic on Encapsulation of Onion Skin Phenolic Compounds. *Chemical Engineering Transactions*, 57, 1891-1896.

Almulaiky, Y. Q., Khalil, N. M., El-Shishtawy, R. M., Altalhi, T., Algamal, Y., Aldhahri, M., Al-Harbi, S. A., Allehyani, E. S., Bilal, M., & Mohammed, M. M. (2021). Hydroxyapatite-decorated ZrO2 for α-amylase immobilization: Toward the enhancement of enzyme stability and reusability. *International Journal of Biological Macromolecules*, 167, 299-308. http://dx.doi.org/10.1016/j.ijbiomac.2020.11.150. PMid:33275970.

Arepally, D., Reddy, R. S., & Goswami, T. K. (2020). Studies on survivability, storage stability of encapsulated spray dried probiotic powder. *Current Research in Food Science*, 3, 235-242. http://dx.doi.org/10.1016/j.crfs.2020.09.001. PMid:33103122.

Ayama, H., Sumpavapol, P., & Chanthachum, S. (2014). Effect of encapsulation of selected probiotic cell on survival in simulated gastrointestinal tract condition. *Songklanakarin Journal of Science and Technology*, 36(3), 291-299.

Both, E., Bodori, Z., & Albert, B. (2018). Effect of microencapsulation on viability and survival in simulated gut conditions of probiotic bacteria. *Romanian Biotechnological Letters*, 23(5), 14140-14145.

Călinoiu, L.-F., Stefănescu, B. E., Pop, I. D., Muntean, L., & Vodnar, D. C. (2019). Chitosan coating applications in probiotic microencapsulation. *Coatings*, 9(3), 194. https://doi.org/10.3390/coatings9030194.

Cota, A. L. S., & Alvim, R. G. (2018). Effect of storage temperature on Streptococcus mutans viability. *Revista de Odontologia da UNESP*, 47(2), 74-78. http://dx.doi.org/10.1590/1807-2577.08317.]

Costa, E., Usall, J., Teixido, N., Torres, R., & Vinas, I. (2002). Effect of package and storage conditions on viability and efficacy of the freeze-dried biocontrol agent Pantoea agglomerans strain CPA-2. *Journal of Applied Microbiology*, 92(5), 873-878. http://dx.doi.org/10.1046/j.1365-2672.2002.01596.x. PMid:11972691.

Dianawati, D., Mishra, V., & Shah, N. P. (2016). Survival of microencapsulated probiotic bacteria after processing and during storage: a review. *Critical Reviews in Food Science and Nutrition*, 56(10), 1685-1716. http://dx.doi.org/10.1080/10408398.2013.798779. PMid:25853290.

Dewi, E. N., Kurniasih, R. A., & Purnamayanti, L. (2018). Physical properties of spirulina phycocyanin microencapsulated with maltodextrin and carrageenan. *Philippine Journal of Science*, 147(2), 201-207.

Dewi, E. N., Purnamayati, L., & Kurniasih, R. A. (2017). Physical characteristics of phycocyanin from spirulina microcapsules using different coating materials with freeze drying method. *IOP Conference Series: Earth and Environmental Science*, 55, 012060. http://dx.doi.org/10.1088/1755-1315/55/1/012060.

Dewi, S. L. (2012). *Isolasi bakteri xilanolitik dan selulolitik dari feses luwak* [skripsi]. Java, Indonesia: Institut Pertanian Bogor.

Dubey, S. K., Meena, R. K., Sao, S., Patel, J., Thakur, S., & Shukla, P. (2014). Isolation and characterization of cellulose degrading bacteria from biogas slurry and their RAPD profiling. *Current Research in Microbiology and Biotechnology*, 2(4), 416-421.

Fiedler, J. D., Brown, S. D., Lau, J. L., & Finn, M. G. (2010). RNA-directed packaging of enzymes within virus-like particles. *Angewandte Chemie International Edition*, 49(50), 9648-9651. http://dx.doi.org/10.1002/anie.201005243. PMid:21064070.

González-Menéndez, E., Fernández, L., Gutiérrez, D., Pando, D., Martínez, B., Rodríguez, A., & García, G. P. (2018). Strategies to Encapsulate the Staphylococcus aureus Bacteriophage phiIPLA-RODI. *Viruses*, 10(9), 495. http://dx.doi.org/10.3390/v10090495. PMid:30217072.

Hartanti. (2013). *Isolasi dan seleksi bakteri selulolitik termofilik dari kawah air panas Gunung Pancar, Bogor* [skripsi]. Java, Indonesia: Institut Pertanian Bogor.

Inoue, T., Kawano, M. A., Takahashi, R. U., Tsukamoto, H., Enomoto, T., Imai, T., Kataoka, K., & Handa, H. (2008). Engineering of SV40-based nano-capsules for delivery of heterologous proteins as fusions with the minor capsid proteins VP2/3. *Journal of Biotechnology*, 134(1-2), 181-192. http://dx.doi.org/10.1016/j.jbiotec.2007.12.006. PMid:18243389.

Mahmoud, M., Abdallah, N. A., El-Shafei, K., Tawfik, N. F., & El-Sayed, H. S. (2020). Survivability of alginate-microencapsulated Lactobacillus plantarum during storage, simulated food processing and gastrointestinal conditions. *Heliyon*, 6(3), e03541. http://dx.doi.org/10.1016/j.heliyon.2020.e03541. PMid:32190759.

Natalia, A., Kristiani, L., & Kim, H. K. (2014). Characterization of proteus vulgaris k80 lipase immobilized on amine-terminated magnetic microparticles. *Journal of Microbiology and Biotechnology*, 24(10), 1382-1388. http://dx.doi.org/10.4014/jmb.1404.04007. PMid:25001555.

Patil, A., Disouza, J., & Pawar, S. (2019). Shelf life stability of encapsulated lactic acid bacteria isolated from sheep milk thrived in different milk as natural media. *Small Ruminant Research*, 170, 19-25. http://dx.doi.org/10.1016/j.smallrumres.2018.09.014.

Piñón-Balderrama, C. I., Leyva-Porras, C., Terán-Figueroa, Y., Espinosa-Solís, V., Álvarez-Salas, C., & Saavedra-Leos, M. Z. (2020). Encapsulation of active ingredients in food industry by spray-drying and nano spray-drying technologies. *Processes (Basel, Switzerland)*, 8(889), 27. http://dx.doi.org/10.3390/pr8080889.

Pourjafar, H., Noori, N., Gandomi, H., Basti, A. A., & Ansari, F. (2020). Viability of microencapsulated and non-microencapsulated Lactobacilli in a commercial beverage. *Biotechnology Reports (Amsterdam, Netherlands)*, 25, e00432. http://dx.doi.org/10.1016/j.btre.2020.e00432. PMid:32099822.

Raise, A., Dupont, S., Iaconelli, C., Caliri, C., Charriau, A., Gervais, P., Chambin, O., & Beney, L. (2020). Comparison of two encapsulation processes to protect the commensal gut probiotic bacterium *Faecalibacterium prausnitzii* from the digestive tract. *Journal of Drug Delivery Science and Technology*, 56(Pt A), 101608. http://dx.doi.org/10.1016/j.jddst.2020.101608.

Rohman, H. (2013). *Produksi kopi secara enzimatis menggunakan bakteri proteolitik dan kombinasi bakteri xilanolitik dan selulolitik dari luwak* [skripsi]. Java, Indonesia: Institut Pertanian Bogor.

Russell, J. B., Muck, R. E., & Weimer, P. J. (2009). Quantitative analysis of cellulose degradation and growth of cellulolytic bacteria in the rumen. *FEMS Microbiology Ecology*, 67(2), 183-197. http://dx.doi.org/10.1111/j.1574-6941.2008.00633.x. PMid:19120465.

Samedi, L., & Charles, A. L. (2019). Viability of 4 probiotic bacteria microencapsulated with arrowroot starch in the Simulated Gastrointestinal Tract (GIT) and Yoghurt. *Foods*, 8(5), 175. http://dx.doi.org/10.3390/foods8050175. PMid:31137631.

Setyowati, N. E. (2016). *Pembuatan sediaan bakteri selulolitik dan bakteri proteolitik untuk produksi kopi luwak buatan* [skripsi]. Java, Indonesia: Institut Pertanian Bogor.

Silva, K. C. G., Cezarino, E. C., Michelon, M., & Sato, A. C. K. (2018). Symbiotic microencapsulation to enhance lactobacillus acidophilus survival. *Food Science and Technology (Campinas)*, 89, 503-509.

Sırt Çıplak, E., & Akoğlu, K. G. (2020). Enzymatic activity as a measure of total microbial acrivity on historical stone. *Hertage*, 3(3), 671-681. http://dx.doi.org/10.3390/heritage3030038.

Sundari, U. Y., Noor, E., Haditjaroko, L., Permatasari, I. D., & Rohadian, A. R. (2020). Utilization of various encapsulation methods in proteolytic bacteria to maintain biomass cells during storage. *IOP Conference Series: Earth and Environmental Science*. 460, 012022. http://dx.doi.org/10.1088/1755-1315/460/1/012022.

Tan, D. T., Poh, P. E., & Chin, S. K. (2018). Microorganism preservation by convective air-drying — a review. *Drying Technology*, 36(7), 764-779. http://dx.doi.org/10.1080/07373937.2017.1354876.

Vahidmoghadam, F., Pourahmad, R., Mortazavi, A., Davoodi, D., & Azizinezhad, R. (2019). Characteristics of freeze-dried nanoencapsulated fish oil with whey protein concentrate and gum arabic as wall materials. *Food Science and Technology (Campinas)*, 39(2, suppl 2), 475-481. http://dx.doi.org/10.1590/fst.22618.

Wang, Y., & Pan, X. (2014). Bacteria: proteus. In Y. Motarjemi (Ed.), *Encyclopedia of food safety* (Vol. 1, pp. 486-489). USA: Elsevier.