The Viability of *Bifidobacterium* BBP6 at Different Storage Temperatures and Periods

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Abstract. *Bifidobacterium* sp. is known as probiotic bacteria that able to inhibit the growth of pathogenic bacteria and provide health benefits to disgestive tract. The number of probiotic cells must meet a sufficient amount to give a positive effect on health. Therefore, the probiotic viability is important factor. The probiotic cell can be protected by encapsulation. This research aim to know the viability of encapsulated *Bifidobacterium* BBP6 at different storage temperature and period, and the optimum storage temperature and period to maintain the viability of encapsulated *Bifidobacterium* BBP6. The main parameter measured is the total amount of lactic acid bacteria. The supporting parameters are *Bifidobacterium*BBP6 viability, water content, and storage time. The results showed that temperatures of 4°C and 28°C can maintain the viability of *Bifidobacterium* BBP6for 4 weeks of storage, while the 37°C and 80°C cannot. The temperature 4°C was able to maintain *Bifidobacterium*BBP6 viability for 3 weeks of storage, with the highest viability percentage i.e. 102,78% at the second week.

1. Introduction

The livestock production in Indonesia is now getting lower (Wina, 2005) regarding the use of animal feed in industry. Feed quality can affect the livestock production. Therefore, it is necessary to do innovation in improving the feed quality, such as using probiotic. Probiotic use in animal fees can reduce level, trigger the animal growth, increase feed conversion, and health control of pathogen infection (Firmanyah, 2001).

Probiotic can provide beneficial effects for the body by creating a balance of intestinal microflora (Firmansyah, 2001). Probiotic from the group of lactic acid bacteria (LAB) have been widely researched and used in fermented food production (Mortazavian et al., 2007), such as *Bifidobacterium*. *Bifidobacterium* can produce bifidine that has an antibacterial activity (Gagnon et al., 2004).

The probiotic viability is a considering factor during food production. According to Hattingh and Viljoen (2001), to be able to provide health benefits, the probiotic viability in the product must range between 10^8 CFU/g. The probiotic viability can be improved by protecting or encapsulating the cell. Several studies have been conducted on probiotic encapsulation, such as using alginate-starch (Sultana et al., 2000), whey protein (Picot and Lacroix, 2004), yogurt (Adhikari et al., 2000), and alginate-calcium (Chandramouli et al., 2004).

Storage temperature and period can affect the probiotic viability (Kailasapathy, 2002). Storage temperature is an important factor on survival of bacteria. Yuan and Seppo (2009) stated that 4^0C is the ideal temperature of probiotic growth in the refrigerator, 28^0C in the room, and 37^0C in the incubator. Storage period can also affect the bacterial viability, because it causes cell death by increasing the storage time (Onayanti et al., 2015).

This research aim to know the viability of encapsulated *Bifidobacterium* BBP6 at different storage temperature and period, and the optimum storage temperature and period to maintain the viability of encapsulated *Bifidobacterium* BBP6.

2. Research Method

This research was carried out in Laboratory of Microbiology and Laboratory of Agricultural Technology, Universitas Jenderal Soedirman, Purwokerto for 5 months.

2.1. Research Design

The experiment was conducted experimentally using a factorial completely randomized design with 2 factors. The first factor is storage period (in week 0-4) i.e. K0, K1, K2, K3, K4. The second factor is storage temperature i.e. 4°C, 28°C, 37°C, 80°C (T1, T2, T3, T4). Each treatment was repeated 3 times.

The independent variable is storage temperature (4°C, 28°C, 37°C, and 80°C) and storage period (0, 1, 2, 3, and 4 weeks), while the dependent variable is the viability of *Bifidobacterium* BBP6. The main measured parameter is the total number of lactic acid bacteria. The supporting parameters are the viability of *Bifidobacterium* BBP6 and water content.

2.2. Working Procedure

2.2.1. Sterilization. Tools and materials were sterilized by following the sterilization procedure from Lestari (2006).

2.2.2. Growth medium preparation. The growth medium (MRSA and MRSB) were prepared by following the procedure from Oxoid (1998).

2.2.3. Re-culturing the bacteria as working-stock culture (Lestari, 2016). An ose of *Bifidobacterium* BBP6 was inoculated in de Man Rogosa and Sharpe Agar (MRSA) medium, then incubated at 37^0C for 24 hours in anaerobic jar.

2.2.4. Study of bacterial growth (Lestari, 2016). A 1 mL Bifidobacterium BBP6 was inoculated into 9 mL MRSB, then incubated at 37^0C. The sampling was carried out every two hours during 24 hours of incubation, followed by multilevel dillution (10^{-8}), the last two diLlutions were inoculated into MRSA medium.The growth medium was incubated incubated at 37^0C for 24 hours in anaerobic jar. Then, the growing bacteria was enumerated by using TPC to know the exact time with bacterial density i.e. 10^7-10^8 cells/mL.

2.2.5. Probiotic encapsulation by extrusion method (Purwandhani et al., 2007) with modifications. Cell encapsulation used a coating method, which is a single layer method, by using tapioca flour. Fish flour (80 g), tapioca flour (40 g), bran (20 g), Bifidobacterium BBP6 (40 mL) and water (60 mL), with ratio 4: 2: 1: 2: 3. The extrusion method conducted by using syringe with drain hole 0.4-0.8 mm at the bottom, then pressurized to produce droplets of starch suspension (granules). The granules were driedin oven at 37^0C for 12 hours, then stored in 4°C, 28°C, 37°C, and 80°Cfor 4 weeks.

2.2.6. The water content of granules (AOAC, 1990). The empty dishwas weighed. A 1-2 gr of encapsulated sample (granules) was weighed in adish, then stored in oven at 105^0C for 24 hours. Then, thesample containing-dish was cooled in a desiccator and weighed (weight after dried) for 3 times.The formula for calculating water content is as follows:

$$water\ content\ (\%) = \frac{(\text{initial sample weight (g)} - \text{final sample weight (g)})\ x\ 100\%}{\text{initial sample weight (g)}}$$

2.2.7. Viability test of Bifidobacterium BBP6 (Puspawati et al., 2010) with modifications. A gram of bacterial biomass (before and after encapsulation) were put into 9 mL distilled water (10^{-1}), then multilevel dilluted up to 10^{-6}. The last two dillutions were inoculated into MRSA medium by using pourplate method, then incubated at 37^0C for 48 hours. The growing bacteria was calculated using TPC method. The bacterial viability (%) was calculated based on the ratio of the number of growing bacteria per gram before and after treatment. The formula is as follows:

$$viability\ (\%) = \frac{(\log of\ total\ probiotic\ after\ treatment)\ x\ 100\%}{\log of\ total\ probiotic\ before\ treatment}$$

2.2.8. Storage time calculation (Yulinery and Nurhidayat, 2012). To determine the cell storage time (t) in units of CFU/g/ hour and cell death rate (k) to reach encapsulated cell(10^6 CFU/g) using the equation:

$$|\ \text{Log N4} - \text{Log N}_1\ | = k\ (t)\ /\ 2{,}303$$

Note:

Log N_1: Logarithm of viability after encapsulation

Log N: Logarithm of viability at 4 weeks after encapsulation

k: Death rate per hour

t: Storage time

2.3. Data Analysis

The data was analyzed by using Analysis of Variance (ANOVA) at error rate of 5% and 1%. The significantly different results was analyzed by the Smallest Significant Difference test.

3. Result and Discussion

Sample storage for 0 week obtain the highest *Bifidobacterium* BBP6 cells at 37ºC i.e. 7.32 log CFU/g, while the lowest was at 28ºC i.e. 7.03 log CFU/g. Sample storage for 1 week obtain the highest *Bifidobacterium* BBP6 cells at 4ºC i.e. 7.34 log CFU/g, while the lowest was at 80ºC i.e. 4.6 log CFU/g.

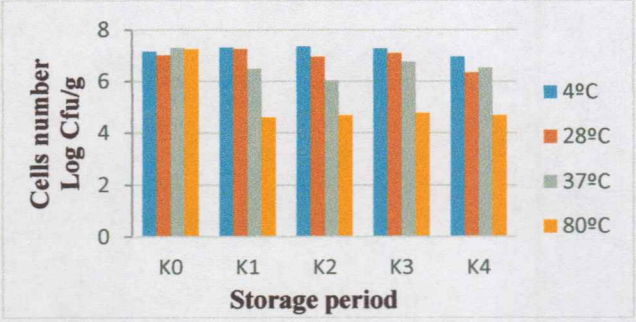

Figure 1. Bifidobacterium BBP6 cells for 4 weeks of storage in different temperatures

Sample storage for 2 weeks obtain the highest *Bifidobacterium* BBP6 cells at 4ºC i.e. 7.38 log CFU/g, while the lowest was at 80ºC i.e. 4.69 log CFU/g. Sample storage for 3 and 4 weeks obtain the highest *Bifidobacterium* BBP6 cellsat 4ºC i.e. 7.3 log CFU/g and 4.77 log CFU/g and the lowest were

at 80°C i.e.6.98 log CFU/g and 4.69 log CFU/g(Figure 1.).Decreasing number of probiotic cells is possible, because bacteria cannot survive along with increasing temperature. Winarwi (2006) stated that continuesly incresing temperature can cause the bacterial growth to stop, because of the cell component inactivation.

The result of water content test of granules (encapsulated cells) after dried was 4.62%. This result is proper with the Indonesian National Standard (2006), the water content of feed is should be ≤14%. The results of viability of *Bifidobacterium* BBP6 during 4 weeks storage at different temperatures showed that sample storage at 4^0C was able to maintain bacterial viability up to 3 weeks(K3) i.e. 101.67%, and 102.78% in 2 weeks of storage (K2) (Table 1.).

The bacterial viability in the 4th week of storage at 4^0C was decreased.This is presumably due to the accumulation of metabolic results which have a less favorable effect on *Bifidobacterium* BBP6. According to Dodd and Gasson (1994), bacterial viability can decrease, due to the anti-microbial effects of diacetyl acid, acetic acid, and lactic acid, which appear in the product and sometimes due to bacteriocin.

Table 1. Viability of *Bifidobacterium* BBP6 for 4 weeks of storage in different temperatures

Treatments	K1	K2	K3	K4
T1	102,22%	102,78%	101,67%	97,21%
T2	103,55%	99,14%	101,28%	90,18%
T3	88,79%	82,51%	92,48%	89,34%
T4	63,36%	64,60%	65,70%	64,60%

Table 2. Result of Smallest Significant Difference test

T1	T2	T3	T4
1.0008E2[a]	97,8333[a]	84,8333[b]	53,667[c]

Note: significantly difference treatment is followed by different alphabet

Bifidobacterium BBP6 thatstored at 28^0C was only able to survive for one week (K1) i.e. 103.55%, and fluctuative at the 2nd, 3rd, and 4th weeks. While, *Bifidobacterium* BBP6 that stored at 37^0C cannot survive for 4 weeks of storage (Table 1.). Significant decrease of bacterial viability can be caused by oxygen expossion during encapsulation process that may affect the bacterial growth. Salminen and Wright (1998) explained that *Bifidobacterium bifidum* is an anaerobic lactic acid bacterium but also micro-aero tolerant. Viability of *Bifidobacterium* BBP6 at 80^0C also can not survive for 4 weeks of storage with viability value i.e. ≤100% (Table 1.). Salminen and Wright (1998) explained that *Bifidobacterium bifidum* has an optimal growth temperature at 36^0C-38^0C and will die at above 60^0C.

The results of variance analysis of *Bifidobacterium* BBP6 viability on different storage temperatures and periods showed that the treatment of temperature can affect (P> 0.05)the viability of *Bifidobacterium* BBP6, while the storage periodcannot (P <0.05). There is no statistical interaction between temperature and storage period in determining the viability of *Bifidobacterium* BBP6 (P <0.05). The storage time of *Bifidobacterium* BBP6 and its viabilityare not affected by the storage temperature.

Advanced data analysis showed that temperature 4^0C (T1) and 28^0C (T2) expressed relatively similar influence in the viability of *Bifidobacterium* BBP6. While, temperature 37^0C (T3) and 80^0C (T4) expressed different effect in the viability of *Bifidobacterium* BBP6 (Table 2.).This showed that the higher temperature, the smaller viability percentage. The highest viability was at 4^0C (T1) and 28^0C (T2). The optimum temperature is chosen to be 28^0C, because it does not require any cooling device during its application.

The average storing time of encapsulated *Bifidobacterium* BBP6 at 4^0C was 47.54 days and at 28^0C was 17.33 days. Encapsulated *Bifidobacterium* BBP6 storage that over the preset time will not give

any beneficial effect, because its viability is low. According to Yulinery and Triana (2015), if the sample is stored beyond its shelf time limit, it will cause the probiotic cells to be much decreased. Therefore, it will be less than the minimum standard ofconsumed probiotic.

4. Conclussions

- The temperature 4^0C and 28^0C can maintain the viability of *Bifidobacterium* BBP6 for 4 weeks of storage, while 37^0C and 80^0C cannot.
- The optimum temperature to maintain the encapsulated *Bifidobacterium* BBP6 for 3 weeks is at 4^0C with viability i.e. 102.78% at 2^{nd}week.

References

[1] Adhikari, K, Mustapha, A, Grun, U, and Fernando, L 2000 Viability of Microencapsulated Bifidobacteria in Set Yougurt During Refridgerated Storage, *Journal Dairy Science*, 83, pp194-1951

[2] AOAC 1990 *Official Method of Analysis of The Association of Official Analytical Chemists*. AOAC Inc. Washington D.C

[3] Chandramouli, V, Kailasapathy, K, Peiris, P, and Jones, M 2000 An Improved Method of Microencapsulation and Its Evaluation to Protect Lactobacillus in Simulated Gastric Condition, *Journal of Microbial Methods*, 56, pp 27-35

[4] Dodd, H M and Gasson, M J 1994 *Bacteriocins of Lactic Acid Bacteria*. Blakie Acad, Profess Public. London

[5] Firmansyah, A 2001 Terapi Probiotik dan Prebiotik pada Penyakit Saluran Cerna Anak, *Sari Pediatri*, 2, pp 210-214

[6] Gagnon, M, Kheadr, E E, Blay, G L, and Fliss, I 2004 In Vitro Inhibition of *Escherichia coli* O157:H7 by *Bifidobacterium* Strain of Human Origin, *International Journal of Food Microbiology*, 92, pp 69-78

[7] Hatting, A L and Viljoen, B C 2001 Yogurt as Probiotic Carrier Food, *International Diary Journal*, 11, pp 1-17

[8] Kailasapathy, K 2002 Microencapsulation of Probiotic Bacteria: Technology and Potential Application, *Current Issues of Intestinal Microbiology,* 3, pp 39-48

[9] Lestari, W D 2016. Pengaruh Perbandingan *Lactobacillus plantarum*dan *Lactobacillus casei* Serta Lama Inkubasi Berbeda Terhadap Kualitas Produk Fermentasi Susu Sapi, *Skripsi*, Fakultas Biologi, Universitas Jenderal Soedirman, Purwokerto

[10] Mortazavian, A, Razavi, S H, Ehsani, R M and Sohrabvandi, S 2007 Principles and Methods of Microencapsulation of Probiotic Microorganisms, *Iranian Journal of Biotechnology, 5*(1), pp 1-18

[11] Onayanti, N, Budji, G, Risco, and Sartini 2015 Uji Viabilitas Probiotik Asal Saluran Pencernaan Itik Pedaging Anas domesticus Yang Dienkapsulasi Dengan Metode Cross Link, *Laporan Penelitian,* Fakultas Matematika dan Ilmu Pengetahuan Alam, Universitas Hasanuddin, Makasar

[12] Oxoid 1998 *The Oxoid Manual 8th Ed*, Complied by E. Y. Bridson (Former Technical Director of Oxoid)

[13] Picot, A and Lacroix, C 2004 Encapsulation of Bifidobacteria in Whey Protein-Based Microcapsules and Survival in Simulated Gastrointestinal Condition and in Yoghurt, *International of Dairy Journal*, 14, pp 505-515

[14] Purwandhani, S N, Suladra, M and Rahayu, E S 2007 *Stabilitas Thermal Agensia Probiotik L.acidophillus SNP 2 Terenkapsulasi Metode Ekstruksi dan Emulsi*, Seminar Nasional Teknologi 2007

[15] Puspawati, N N, Nuraida, L, and Adawiyah, D B 2010 Penggunaan Berbagai Jenis Bahan Pelindung untuk Mempertahankan Viabilitas Bakteri Asam Laktat yang di Isolasi dari Air

Susu Ibu pada Proses Pengeringan Beku, *Jurnal Teknologi dan Industri Pangan*, 21(1), pp 59-65

[16] Salminen, S and Wright, A V 1998 Lactic Acid Bacteria. Marcell Dekker Inc., New York

[17] SNI 2006 01-3929-2006 *Pakan Ayam Ras Petelur*. Badan Standardisasi Nasional, Jakarta

[18] Sultana, K, Godward, G, Reynolds, N, Arumugaswamy, R, Peiris, P, and Kailasapathy, K 2000 Encapsulation of Probiotics Bacteria with Alginate Starch and Evaluation of Survival in Simulated Gastrointestinal Condition and in Yoghurt, *International Journal of Food Microbiology*, 62, pp 47-55

[19] Wina, Elizabeth 2005 Teknologi Pemanfaatan Mikroorganisme dalam Pakan untuk Meningkatkan Produktivitas Ternak Ruminansia di Indonesia: Sebuah Review, *Wartazoa*, l5(4)

[20] Winarwi 2006 Uji Viabilitas Bakteri dan Aktivitas Enzim Bakteri Proteolitik pada Media Carrier Bekatul (Sebagai Acuan Bahan Ajar Pokok Bahasan Virus, Monera, dan Protista di SMA), *Skripsi* Universitas Sebelas Maret, Surakarta

[21] Yuan, K, Lee, and Seo, S 2009 Handbook of Probiotics and Prebiotics *Second Edition* A John Wiley and Sons Inc All Rights Reserved. Published by John Wiley and Sons, Inc., Hoboken, New Jersey, Canada

[22] Yuinery, T and Triana, E 2015 Uji Stabilitas Probiotik *Lactobacillus plantarum* Mar8 Terenkapsulasi dalam Sediaan Oralit dengan Analisis Viabilitas.*Prosiding Seminar Nasional Masyarakat Biodiversiti Indonesia,* 1(2), pp 278-282

[23] Yulinery, T and Nurhidayat, N 2012 Analisis Viabilitas Probiotik Lactobacillus Terenkapsulasi dalam Penyalut Dekstrin dan Jus Markisa *(Passiflora eduis). Jurnal Teknik Lingkungan*, 13(1), pp 109-121

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