

ISSN-0976-0245 (Print) • ISSN-0976-5506 (Electronic)

Volume 10 / Number 09 / September 2019

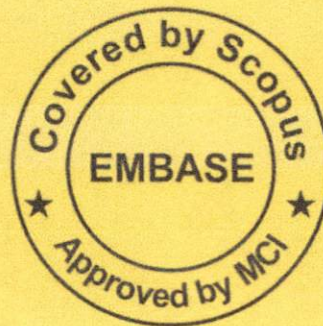


Indian Journal of Public Health Research & Development

An International Journal

SCOPUS IJPHRD CITATION SCORE

Indian Journal of Public Health Research and Development
Scopus coverage years: from 2010 to till date. Publisher:
R.K. Sharma, Institute of Medico-Legal Publications
ISSN:0976-0245E-ISSN: 0976-5506 Subject area: Medicine:
Public Health, Environmental and Occupational Health
CiteScore 2017-0.03
SJR 2017 - 0.108
SNIP 2017-0.047



Website:

www.ijphrd.com



Indian Journal of Public Health Research & Development

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Volume 10, Number 9

September 2019

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Utilization of *Lactobacillus Acidophilus* FNCC-0051 Microencapsulation: Potential Benefit of Giving Combination of Sodium Alginate and Gelatin to Attributes and Role of Probiotic Against *Staphylococcus Aureus*

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ABSTRACT

Background: The use of *Lactobacillus acidophilus* as an antimicrobial is still not optimal, even though this type of probiotic has benefits for skin health. One of bacteria causes the turnaround cause effects of the skin is *Staphylococcus aureus*.

Objective: To evaluate the effect combination of sodium alginate and gelatin matrix 2.25%: 0.75% to the characteristics, viability, and antibacterial activity of probiotic microparticles.

Method: The experimental study used the ratio of sodium alginate and gelatin 2.25%: 0.75% (FI), 3% sodium alginate (FII) and 3% gelatin (FIII). Tests were carried out to assess viability (TPC method), making microparticles by extrusion method and antibacterial activity (agar diffusion method). The data obtained was analyzed by statistical tests.

Results: Particle size distribution was obtained, including 8.85 μm (FI), 9.69 μm (FII) and 5.40 μm (FIII). The viability of probiotics after being made microparticles is still in the range of probiotic minimum requirements despite a decrease. The lowest decrease in viability was $1.32\% \pm 0.06$ (FI) and the highest decrease in viability was $14.77\% \pm 1.21$ (FIII) which was significantly different ($p < 0.05$). The lowest antibacterial activity test 9.27 ± 0.19 mm (FIII) and the highest antibacterial activity 10.83 ± 0.51 mm (FI) which was significantly different from FIII.

Conclusion: The combination of sodium alginate and gelatin 2.25%: 0.75% can increase the role of *Lactobacillus acidophilus* probiotic as antimicrobial against *Staphylococcus aureus* and can be an alternative in preventing infection.

Keywords : *Lactobacillus acidophilus* FNCC-0051, role, attributes, sodium alginat, gelatin

Introduction

Studies of probiotics and their use in various products have developed in recent years.¹ Probiotics provide health benefits if given in sufficient quantities.² *Lactobacillus* and *Bifidobacteria* are common microbes used as probiotics.³ Probiotics are widely used in the form of nutrients and orally.⁴ Probiotics can also provide health benefits such as antimicrobial activity, immunomodulators, and antidiarrheal.⁵ Probiotics is used in the fields of dermatology, cosmetics and preventive or therapeutic benefits, especially *Lactobacillus acidophilus* as an antimicrobial.⁶

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The probiotic suspension has more optimal antimicrobial activity compared to probiotic supernatants.⁷ In this study *Lactobacillus acidophilus* was used in the form of probiotic suspensions that being cultured on MRS media.⁸ In topical use, it is expected that the active ingredient's stability must be maintained, that last long enough in the skin, and released gradually by means of encapsulation in the form of microparticles.⁹

The matrix that commonly used in microencapsulation is alginate, carrageenan, Chitosan, and protein.³ Gelatin is a matrix in the form of proteins that are often used in making microparticles.¹⁰ Gelatin is biocompatible, biodegradable, non toxic, inexpensive, swelling index good and easy to experience cross linking.¹¹ Sodium alginate is also common, used as a microencapsulation matrix of probiotic bacteria.¹² The advantage of microencapsulation using sodium alginate is easy, safe and cheap.¹³ Microencapsulation can be done by several methods, namely, emulsification, coacervation, spray drying, and extrusion.¹⁴

Sodium alginate evidently has a disadvantage, namely the particles produced were too porous to protect probiotics from environmental factors¹⁵ So that an alternative is needed through a combination with gelatin.^{16,17} The commonly used gelatin concentration is between 5-15%¹⁸, the concentration of sodium alginate is 0.5-4% and calcium chloride is 0.05- 1.5 M.¹² In this effort a Crosslink is needed. Crosslinking commonly used in gelatin and sodium alginate is calcium chloride.¹¹

The effectiveness of *Lactobacillus acidophilus* needs be improved as an effort to prevent skin infections in health services. This study aimed to determine the effect of sodium alginate and gelatin combination with a ratio of 2.25%: 0.75% on particle shape and size, viability, and antibacterial activity of *Lactobacillus acidophilus* microparticles, by extrusion method, and CaCl₂ 1.5M cross linker.

Method

This study was an experimental study. Microbiological examination was carried out at the Health Laboratory Center in Surabaya and *Lactobacillus acidophilus* material came from the Center for Food and Nutrition Studies at Universitas Gajahmada. This study consisted of 3 formulas namely formula I (Probiotics

+ sodium alginate and gelatin combination with a ratio of 2.25%: 0.75%), formula II (Probiotics + 3% sodium alginate) and formula III (Probiotics + 3% gelatin). Sodium alginate and single gelatin were used as a comparison, compared with a combination of sodium alginate and gelatin.

The procedures in this study were carried out in several stages. The first stage was the examination of the materials, including probiotics, gelatin, sodium alginate and CaCl₂. The second stage was carried out for the preparation of probiotic *Lactobacillus acidophilus* starter, optimization of growth at 0, 6, 12, 14, 18, 24 and 48 hours. In this phase the viability test was carried out by the TPC (Total Plate Count) method. Probiotics were mixed into the matrix (sodium alginate - gelatin) then viability tests are carried out using the TPC method. The next step was the formation of formula I (Probiotics + sodium alginate and gelatin combination with a ratio of 2.25%: 0.75%) , formula II (Probiotics + sodium alginate 3%) and formula III (Probiotics + gelatin 3%). Making microparticles with an extrusion method with cross linker CaCl₂ 1.5 M, pH checked at pH 8.5. Evaluation of the characteristics of the microparticle including the shape examination, viability and particle size distribution using an optical microscope. Activity test for *Staphylococcus aureus* bacteria with the agar well diffusion method set after preparation of *S. aureus* bacteria. Antibacterial activity tests were carried out before and after microencapsulation by measuring the diameter of the inhibition zone (mm) against *Staphylococcus aureus*. Next, to find out the equivalence of probiotic concentrations in microparticles with antibiotics, then comparing probiotic inhibitory zones that have been encapsulated with antibiotic inhibition zones (gentamicin) at various concentrations.

Qualitative examination carried out for the *Lactobacillus acidophilus* bacteria is by gram staining. Qualitative examination was also carried out in the sodium alginate and calcium chloride matrix including organoleptic examination, FTIR and DTA, and also in a gelatin matrix including organoleptic examination and FTIR. The data obtained were then analyzed through statistical tests with the One-Way ANOVA variance analysis method, then analyzed by Tukey's Honesty Significant Difference (HSD) test with a confidence level of 0.95 ($\alpha = 0.05$).

Results

Identification results of *Lactobacillus acidophilus* FNCC-0051 showed that gram-positive bacteria were formed. Sodium Alginate, Type B Gelatin and Calcium Chloride used in this study have met the requirements as listed in the literature monograph. The results of pH showed in the range $5,22 \pm 0,08$ in 48 hours and $6,49 \pm 0,07$ in early hours. The minimum TPC is $1,14 \times 10^8 \pm 0,36 \times 10^8$ cfu/ml while the maximum is $3,82 \times 10^7 \pm 3,05 \times 10^7$. The highest log TPC of *Lactobacillus acidophilus* is $8,46 \pm 0,17$ log cfu/ml while the lowest is $7,09 \pm 0,13$ log cfu/ml.

Interactions between polymers and Crosslink CaCl_2 solutions are characterized by the wave number of C = O either shifted or disappears from alginate and gelatin. Sodium alginate-gelatin combination (2.25%: 0.75%) has the wave number of 3432.0 for the Sodium Alginate hydroxyl group and 1633.6 for C = O Stretching in Gelatin. The measurement results of a single raw material were found that IR spectrum (cm^{-1}) of the sodium alginate Hydroxyl group was 3466.38 and gelatin C = O is stretching was 1651.63. The results obtained from an optical microscope of 400x magnification are presented in Figure 1.

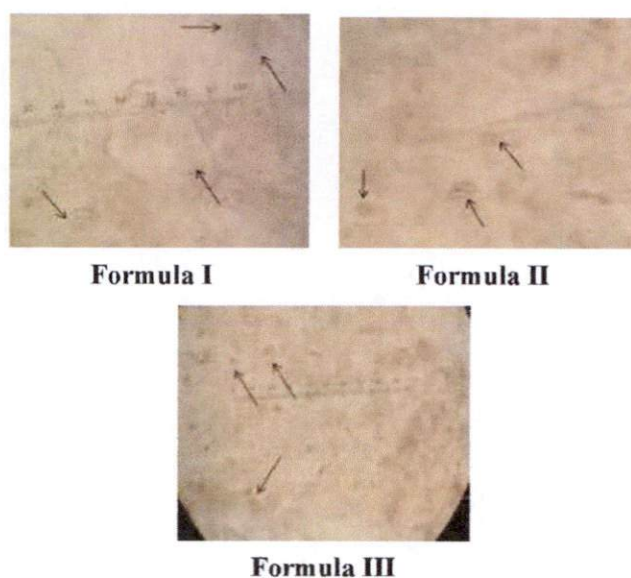


Figure 1: Microparticles form of probiotic Formula I, II, and III with 400x magnification optical microscope

The mean size of probiotic microparticles in each formula, as follow; formula I ($8.85 \mu\text{m}$), formula II ($9.69 \mu\text{m}$) and formula III ($5.40 \mu\text{m}$). Based on the particle size, percentage calculation of microparticles between

formulas, FIII (3% gelatin) has the smallest particle size and FII (3% sodium alginate) has the largest particle size, while the combination between the two polymers causes smaller size than just single sodium alginate and larger from gelatin. The particle size obtained from the microparticles of each formula has an unequal distribution, the smallest particle size is $3.06 \mu\text{m}$ found in formula I, II, III while the largest particle size is $18.38 \mu\text{m}$ at FI, $24.5 \mu\text{m}$ at FII, and $15, 31 \mu\text{m}$ on FIII.

The microparticle viability test results were obtained from viability percentage, which compared the TPC and Log TPC values of *Lactobacillus acidophilus* at Formula I, Formula II, and Formula III before microparticles and after microparticles. The smallest TPC value after manufacturing process of dry microparticles found in Formula III, but it still covered the required viability requirements, namely 106 - 107 cfu/ml or log results which was 6-7 cfu/ml.

The viability decrease of *Lactobacillus acidophilus* in probiotic microparticles on the manufacturing process of microparticles (extrusion and oven). The highest decrease in viability was obtained in Formula III at 14.77% and the lowest decrease in viability was obtained in Formula I at 1.32%. The entrapment efficiency of *Lactobacillus acidophilus* in probiotic microparticles on the manufacturing process of microparticles (extrusion and oven) was decreasing compared to viability before microparticles manufacturing process. The highest entrapment efficiency obtained in Formula III at 87.94% and the lowest entrapment efficiency was obtained in Formula I at 76.35%. Viability and entrapment efficiency of *Lactobacillus acidophilus* against the microparticles manufacturing process were presented in table 1.

Table 1: Viability of *Lactobacillus acidophilus*

Group	Viability decrease	Entrapment Efficiency
Formula I	$1,32 \pm 0,06$	$76,35 \pm 0,22$
Formula II	$7,27 \pm 0,19$	$84,20 \pm 0,16$
Formula III	$14,77 \pm 1,21$	$87,94 \pm 0,43$

The results of the One-Way ANOVA test with Tukey test, it can be seen that Formula III has the highest reduction in viability against the manufacturing process of microparticles and has a significant difference with Formula I and Formula II. From the results of the One Way ANOVA test with post hoc Tukey, it can be seen

that Formula I produces the highest inhibitory activity compared to other Formulas and has a significant difference with Formula III. Table 2 presents the Tukey test results of *Lactobacillus acidophilus* viability against the manufacturing process of microparticles (extrusion and oven) and One Way ANOVA test with post hoc Tukey on inhibition zone diameter for each formula against *Staphylococcus aureus*.

Table 2: Tukey's test results of *Lactobacillus acidophilus* viability on the microparticles manufacturing process (extrusion and oven) and One Way ANOVA test with post hoc Tukey of inhibition zone diameter in each formula against *Staphylococcus aureus*

Group	Formula I	Formula II	Formula III
One-Way ANOVA with Tukey's test			
Formula I		+	+
Formula II	+		+
Formula III	+	+	
One Way ANOVA with post hoc Tukey			
Formula I		-	+
Formula II	-		-
Formula III	+	-	

Description: (+) = there is significant differences (-) = there is no significant differences

The highest inhibition zone produced by Formula I with value of 10.83 ± 0.51 mm compared to other formulas. The lowest inhibition zone produced by Formula III with value of 9.27 ± 0.19 mm. The results of the inhibitory activity test for each probiotic microparticles formula against *Staphylococcus aureus* are presented in Table 3.

Table 3: Diameter of inhibition zone against *S. aureus* (test bacteria)

Group	Inhibition Zone (mm)
Microparticles of Formula I	$10,83 \pm 0,51$
Microparticles of Formula II	$9,80 \pm 0,57$
Microparticles of Formula III	$9,27 \pm 0,19$
Positive Control	$12.15 \pm 0,05$
Negative Control	0

Description: The Data is the mean value of 3x replication \pm SD, Positive Control = Gentamicin 6 ppm, Negative Control = Microparticles without *Lactobacillus acidophilus*

Discussion

This study showed the effect of a sodium alginate and gelatin combination with concentration of 2.25%: 0.75%. The effect that occurs is the size difference of the *Lactobacillus acidophilus* FNCC-0051 microparticles and an increase in the role and effectiveness seen from the increase in viability and antibacterial activity against *Staphylococcus aureus*. This study showed that the effect of the matrix ratio (sodium alginate - gelatin) on the rate of decline percentage in the viability of *Lactobacillus acidophilus* per formula and it can be seen that FIII had the highest reduction in viability which had significant differences with FI and FII. Although there was a decrease in the viability of probiotics during the manufacturing process of microparticles from all three formulas, the number of probiotic TPCs in the three formulas still met the requirements of probiotics as antibacterial. The results of the microparticle entrapment efficiency test were obtained from percentage data on viability, the results of the viability percentage of formulas I, II, and III were 76.35%, 84.20%, and 87.94%. This can be related to the smaller particle size, the entrapment efficiency is greater because of the large surface area of the matrix.

The reason for gelatin was chosen in this study, because it has a good swelling index ability and is useful as a thermally reversible gelling agent for encapsulation.¹⁶ Besides the compact structure between alginate and gelatin will give small particle size and small particle size because of gelatin can fill these pores when cross links occur with $CaCl_2$, besides, gelatin has the ability of self-assembly, so that more compressed particles can be formed when compared to the use of a single alginate matrix that has very porous microparticle properties.¹⁵

The method used in this study was the extrusion method because it is suitable for sodium alginate and gelatin matrix. The method is simple and inexpensive, does not damage probiotic cells, produces a high probiotic viability, and can be carried out in aerobic or anaerobic conditions.³ A few factors that affect microparticles formation, including the diameter of the syringe needle and the distance between the syringe needle and the cross-linked solution can affect the shape and size of the microparticles¹², the stirring speed also affects the microparticles, the faster the stirring speed, the smaller the particles produced.

Qualitative examination of probiotic, namely gram staining on *Lactobacillus acidophilus* FNCC-0051

was obtained from gram-positive bacteria, according to the morphological characteristics of *Lactobacillus acidophilus*, which were classified as gram-positive and rod-shaped. The results of organoleptic examination of gelatin shows that it was in the form of powder, with pale yellow granules to dark yellow, odorless and tasteless¹⁹. The results of the infrared spectrum showed the same results with the literature so that it can be concluded that these results meet the characteristics of gelatin. Qualitative analysis on Calcium chloride as a cross linker also carried out, namely organoleptic and thermal analysis using DTA. The organoleptic analysis of calcium chloride showed that it was white, crystalline powder, hard, odorless and hygroscopic, whereas according to literature it was white or almost white crystalline powder, hard, odorless and hygroscopic.²⁰

Conclusion

Not only there is a difference in the size characteristics *Lactobacillus acidophilus* FNCC-0051 microparticles with a combination of sodium alginate-gelatin 2.25%: 0.75% but there is an increase in role and efficacy that observed from increased viability and antibacterial activity against *Staphylococcus aureus*.

Ethical Clearance: This research has gone through ethical tests from Faculty of Pharmacy Universitas Airlangga

Conflict of Interest: The author reports no conflict of interest of this work.

Source of Funding: This study is done with individual funding.

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Print-ISSN: 0976-0245-Electronic-ISSN: 0976-5506, Frequency: Quarterly
(Four issues per volume)

Indian Journal of Public Health Research & Development is a double blind peer reviewed international journal. It deals with all aspects of Public Health including Community Medicine, Public Health, Epidemiology, Occupational Health, Environmental Hazards, Clinical Research, and Public Health Laws and covers all medical specialties concerned with research and development for the masses. The journal strongly encourages reports of research carried out within Indian continent and South East Asia.

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Logix Office Tower, Unit No. 1704, Logix City Centre Mall,
Sector- 32, Noida - 201 301 (Uttar Pradesh)

Printed, published and owned by

Dr. R.K. Sharma
Institute of Medico-legal Publications
Logix Office Tower, Unit No. 1704, Logix City Centre Mall,
Sector- 32, Noida - 201 301 (Uttar Pradesh)

Published at

Institute of Medico-legal Publications
Logix Office Tower, Unit No. 1704, Logix City Centre Mall,
Sector- 32, Noida - 201 301 (Uttar Pradesh)