Available online at https://ejournal.unisayogya.ac.id/ejournal/index.php/ijhst

International Journal of Health Science and Technology, 3 (2), 2021, 155-163

The effect of adding casein hydrolysate as a protein source in the culture of the bacteria *Paenibacillus polymyxa* at wilker office of food and horticultural plants protection (PTPH) Bojonegoro

Nanda Putri Insan Khasana[1*], Miwing Nurul W[2], Annisa Khumaira[3]

[1,3]Faculty of Science and Technology, Aisyiyah University Yogyakarta

[2]Protection of Food Crops and Horticulture in Bojonegoro

[1]nandaputri074@gmail.com*; [2]miwing87@gmail.com; [3]annisakhumaira@unisayogya.ac.id

Submission date: 18 September 2021, Receipt date: 2 Oktober 2021, Publication date: 1 November 2021

Abstract

Agencia (Biological Control Agents) are currently widely used to control pests and diseases or plant-disturbing organisms. One of the biological agents that has many benefits for controlling several types of diseases in both food crops and horticulture is Paenibacillus polymyxa. These bacteria are beneficial in nitrogen fixation, promotion of plant growth, solubilization of soil phosphorus, and production of exopolysaccharides, hydrolytic enzymes, antibiotics, and cytokinins. Paenibacillus polymyxa also produces polymyxin antibiotics and it is known that these bacteria contain the hormone gibberellins. Casein hydrolyzate is one of the growth media that can be used for the growth of microorganisms. The media is a complex mixture of 18 amino acids, vitamins, calcium, phosphate, and several microelements which results in their high price. Casein hydrolyzate is known to be more effective for plant tissue culture. Therefore, the purpose of this study was to determine whether the amino acid content in casein hydrolyzate could affect the growth of bacteria Paenibacillus polymyxa. The simple medium used in this study was soybean boiled water. Soybean as an alternative medium for protein sources to substitute beef extract, beef extract, and bacto peptone for the growth of bacteria Paenibacillus polymyxa. Based on TPC (Total Plate Count) calculations with graded dilution in Paenibacillus polymyxa, it was found that there was no difference in the number of bacteria to the addition of casein. This shows that bacteria can grow optimally even though casein hydrolyzate is not given during the growth process.

Keywords*: paenibacillus polymyxa; biological agents; casein hydrolyzate; soya bean; total plate count*

INTRODUCTION

Agencia Controller Conservation *(Biological Control* Agents) which is any organism that includes subspecies, species, varieties, all types of protozoa, insects, bacteria, fungi, viruses, and other organisms are in the stage of development can be used to control pests and diseases or pests plants in the production process and the management of other agricultural products. *Paenibacillus polymyxa* has various benefits, including nitrogen fixation, promotion of plant growth, solubilization of soil phosphorus, and production of *exopolysaccharides*, hydrolytic enzymes, antibiotics,

Doi: 10.31101/ijhst.v3i1.2244

cytokinins (Tantiani & Fauzi, 2020). Based on (Kantikowati *et al*., 2018), the bacterium *Paenibacillus polymyxa* is a Gram-positive antagonist bacteria that can be morphologically recognized from the shape of a convex elevation with a cloudy white color. These bacteria can be used to control several types of diseases in both food crops and horticulture.

Paenibacillus polymyxa produces polymyxin antibiotics. The resulting antibiotic is more effective in controlling plant pathogenic bacteria. In agriculture, *Paenibacillus polymyxa* can be found in soil and plants. Biofilms of these bacteria show the production of exopolysaccharides in plant roots that can protect against pathogens. The results of the Biogen BB Test (Center for Research and Development of Biotechnology and Agricultural Genetic Resources), this bacterium also contains the hormone gibberellins (Syamsiah, 2013).

Casein is a milk protein consisting of phosphoproteins that bind to calcium to form calcium calcinate which is insoluble in water and forms a white colloid in solid media. In the presence of proteolytic enzymes from microbes, this casein will be hydrolyzed into peptides and amino acids characterized by the presence of a clear zone around the microbial colony. Casein hydrolyzate is a complex mixture of 18 amino acids, vitamins, calcium, phosphate, and several microelements. The proportion of amino acids depends on the composition of the protein source from which casein is made (Wang *et al*., 2013).

Aprevious study using the media ika kasein jaringa hydrolysates for culturing plants, with results that are more effective than the use of other media, this study used casein hydrolysates for bacterial growth media. If it is seen from the content of casein hydrolyzate which is known to be high in amino acids, it is expected that the growth of bacteria on the casein hydrolyzate media will also be high. This research needs to be done because there have not been many studies regarding the addition of casein to bacterial growth and with this research, it is expected to add references to the growth of *Paenibacillus polymyxa* bacteria on casein hydrolyzate media.

With this research as well, so it can be known reference protein sources that can be used for propagation media for bacterial culture and can be useful in controlling plant pests with the limitation of the problem, namely knowing how the effect of adding casein hydrolyzate as a protein source for the propagation of *Paenibacillus polymyxa* through measurement of Total Plate Count (TPC).

RESEARCH METHODS

2.1 Time and Place of Implementation

This Field Work Practice activity was carried out at the Food Crop Protection Technical Implementation Unit and Horticulture Bojonegoro, having its address at Jalan Diponegoro Number 77, Bojonegoro Regency. This Field Work Practice activity is carried out on January 18, 2021 to February 22, 2021.

2.2 Tools and Materials

The tools used in this research are: beaker glass, digital stirring hotplate, analytical balance, test tube, funnel, measuring pipette, pipette filler, autoclave, round loop, Bunsen, tube rack, spatula, Laminar Air Flow (LAF), steam pot, stirrer, strainer, 4 gallon, dipper, fermenter, stove, petri dish, microtip, micropipette, vortex, cotton, and plastic wrap.

The materials used in the NA media (Nutrient Agar), PCA media (Plate Count Agar), F1 broodstock of bacteria *Paenibacillus polymyxa* obtained from Brawijaya University,

ISSN 2685-8673 (online)

alcohol 70%, sterile distilled water, cotton, 2 kg sugar, 1 kg soybean, 18 L water, 120 g sweetened condensed milk, and 20 g casein hydrolyzate.

2.3 Work Activities

Here are how fieldwork activities work:

1) Preparation and Sterilization of NA and PCA

Preparation and Sterilization of NA and PCA based on the method of *(*Octavia *et al*., 2017) with modifications. Media Weighed NA media with analytical balance into a glass beaker 20 g of, and 22.5 g of PCA media with 1000 mL of distilled water added. Dissolved over hot stirring digitally by adding a magnetic stirrer. Furthermore, the NA medium was poured into the test tube as much as it was assisted by a funnel. Then, the NA tube media was covered with cotton, put in plastic, while the PCA media in the erlenmeyer was only covered with cotton and plastic was added to the mouth of the erlenmeyer and tightly tied with rubber.

NA and PCA media were sterilized in an autoclave for 30 minutes at 121^{o}C. When the sterilization was complete, the NA media was tilted on a newspaper roll and waited for it to harden. While the PCA media is stored in the refrigerator and removed when calculating the TPC.

2) Inoculation of Bacteria *Paenibacillus polymyxa* To Media NA

Inoculation of *Paenibacillus polymyxa* bacteria into medium NA carried out at the agency referred to (Pahlawi *et al*., 2019). *Paenibacillus polymyxa* F1 obtained from Brawijaya University to NA media for reproduction. Streaking was carried out in the LAF by taking 1 eye of the bacterial loop and then scraping it onto the slanted NA medium. Streaking is carried out in a zig-zag manner so that the growth of bacteria is evenly distributed on the tube media. Then, the bacteria were incubated for 1-2 days at room temperature.

3) Preparation and Inoculation of Bacterial Isolates into Soybean Media

Inoculation of bacterial isolates into soybean media refers to the reference from (Winarsi, 2018) with modifications, which were carried out into 4 gallons. Inoculation using 2 kinds of treatment, each with two replications. Gallons 1 and 2 are control, gallons 3 and 4 with the addition of casein hydrolyzate. Soybean media was made by washing 1 kg of soybeans until clean, dissolving 2 kg of sugar, preparing 120 g of sweetened condensed milk (3 sachets), and 20 g of casein hydrolyzate media.

The media was made by boiling soybeans 3 times (Figure 1). The first stew contains a mixture of soybeans and water. Then put evenly into 4 gallons. The second stew contains soybeans, water, sweetened condensed milk, and sugar water. After that put the soybean liquid media into 4 gallons equally. The third stew containing soybeans, water, and casein hydrolyzate was added to gallons of casein in replicates I and II. Wait for the gallon soybean to cool and then isolate *Paenibacillus polymyxa.* Incubation was carried out for 5 days using a fermenter that was lit for 24 hours non-stop.

ISSN 2685-8673 (online)

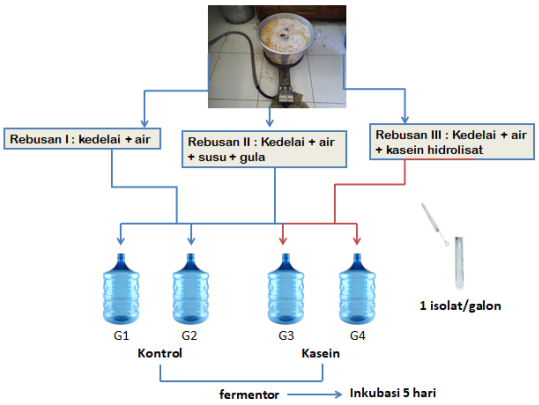

Figure 1. Illustration of making soybean liquid media

4) Multilevel Dilution and Inoculation

Multilevel dilution carried out in the agency refers to (Palawe *et al*., 2016) with modifications. Bacterial inoculation was done by pour plate method and dilution technique. Dilution is done to reduce the number of microbes in the sample so that the number of microorganisms can be observed and counted specifically. The dilutions carried out were graded dilutions of 10^{-9}, with odd dilutions, namely in tubes 10^{-3}, 10^{-5}, 10^{-7}, and 10^{-9}.

After obtaining an odd dilution, the PCA media was poured under sterile conditions. The inoculated petri dish is wrapped in plastic wrap to avoid contamination. Incubated for 3 x 24 hours.

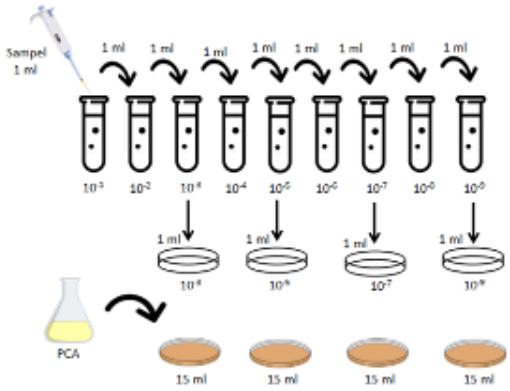

Figure 2. Illustration of the stages of dilution 10^{-9} and pouring PCA medium into a petri dish

5) Calculation of Total Plate Count (TPC)

The TPC method is a method used to calculate the number of microbes contained in one sample or preparation. The formula for calculating the number of colonies based on (Palawe *et al*., 2016) is:

> Colonies per mL or per g = number of colonies in plates x 1/dilution factor

ISSN 2685-8673 (online)

RESULTS AND DISCUSSION

According to (Mubarak *et al*., 2017) inoculation is the work of moving microbes from the old medium to the new medium with a very high level of accuracy. The process of inoculation of *Paeniacillus polymyxa* was carried out by means of an ose needle fixed on a bunsen then attached to the pure isolate and scratched in a zigzag manner on an oblique order then the test tube was closed with cotton and wrapped in plastic wrap which aims to minimize contamination from outside. Then incubated for 24 hours.

Casein hydrolyzate is a complex mixture of 18 amino acids, vitamins, calcium, phosphate, and several microelements (Wang *et al*., 2013). Casein hydrolyzate is a medium that is usually used to grow bacteria. This study used *Paenibacillus polymyxa* which was inoculated into soybean media and used TPC (Total plate Count) calculations.

Based on the reference from (Napitupulu *et al*., 2019), the media used to calculate the total number of bacteria (all types of bacteria) contained in each sample such as food, dairy products, wastewater, and other samples is PCA (Plate Count Agar) and bacterial counts using the TPC method. Research using casein hydrolyzate as a protein-rich medium for the growth of *Paenibacillus polymyxa* obtained the following results:

A. Observations of *Paenibacillus polymyxa* on growth media

Figure 3. Macroscopic form ofcolonies *Paenibacillus polymyxa* on NA media and PCA media in a petri dish (Source: personal document)

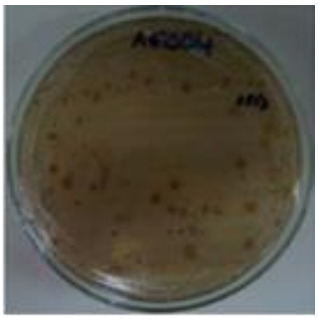

Figure 4. Macroscopic observations ofcolonies *Paenibacillus polymyxa* based on Frediansyah & Sudiana (2017)

Macroscopic observations of *Paenibacillus polymyxa* on NA media are in accordance with (Frediansyah & Sudiana, 2017), namely macroscopically (Figures 3

ISSN 2685-8673 (online)

and 4) *Paenibacillus polymyxa* has an irregular round shape and cream colored. While microscopically, seen from the Gram stain, *Paenibacillus polymyxa* is rod-shaped with Gram positive. This is also reinforced by the research of (Kim *et al*., 2015) which isolated *Paenibacillus polymyxa* from rotten ginseng roots, the study said that macroscopically *Paenibacillus polymyxa* was cream, smooth colonies, convex, irregular colonies.

B. TPC calculation

The dilution method was carried out before calculating the Total Plate Count (TPC). According to (Luna-Guevara *et al*., 2019) the aim is to minimize or reduce the number of microbes suspended in the liquid. The dilution method was carried out before calculating the Total Plate Count (TPC). This makes it easier to calculate the number of microbes. Determination of the amount or number of dilution levels depends on the estimated number of microbes in the sample.

Method used for calculating the number of bacterial colonies in this study is the TPC method. Based on (Yunita et al., 2015) the principle of the plate count method or TPC is to grow living microbial cells on agar media, so that the microbes will multiply and form colonies that can be seen directly and counted with the naked eye without using a microscope. The TPC method is distinguished in two ways, namely thepour platemethod and the method surface/spread plate. The TPC method used in this study is the method, pour plate where PCA medium is poured into a petri dish that has previously been carried out with odd dilutions in a test tube (10^{-3}, 10^{-5}, 10^{-7}, and10^{-9})

Table 1. Direct calculation results at 24 hours

Sample	Dilution				Amount (CFU/mL)
	10^{-3}	10^{-5}	10^{-7}	10^{-9}	
Without Casein I	207	142	235	69	1.8×10^{10}
Without Casein II	246	125	106	51	1.3×10^{10}
Casein I	109	58	126	97	2.4×10^{10}
Casein II	96	94	21	101	2.5×10^{10}

Table 2. Direct calculation results at 48 hours

Sample	Dilution				Amount (CFU/mL)
	10^{-3}	10^{-5}	10^{-7}	10^{-9}	
Without Casein I	254	153	289	77	2.0×10^{10}
Without Casein II	153	137	131	86	2.1×10^{10}
Casein I	146	68	147	124	3.1×10^{10}
Casein II	154	104	37	131	3.3×10^{10}

From the results obtained (Table 1), it can be seen that the number of colonies for 24 hours in the sample without casein in replicates I and II did not differ much, namely in the sample without casein in replicate I the number of colonies was 1.8×10^{10} while the sample without casein in replicate II totaling 1.3×10^{10} . The difference in the number of the two replicates was around 0.5 colonies. Then, the number of colonies in the sample with casein in replicate I was 2.4×10^{10} while the number of colonies on casein samples in replicate II was 2.5×10^{10} . And the difference between the casein samples I and II replicates is 0.1 colonies.

ISSN 2685-8673 (online)

It is known from the results of incubation for 24 hours that the number of bacteria does not meet the requirements for reporting results in the TPC calculation, that is, if the results show the same or <2 the results must be averaged. On the other hand, if the results show >2, the number of microbes used is the result of the previous dilution. And the results seen in the calculation without casein I replication is 1.8 and without casein II replication is 1.3, then these results cannot be reported. Therefore, the calculations were continued for 48 hours.

At 48 hours calculation (Table 2), the results obtained were more than the number of colonies in 24 hours. It is known that the number of colonies in the sample without casein and with casein (I and II) of all dilution factors is increasing. The results showed that the treatment without casein I was not much different from the treatment without casein II. The difference between the treatments without casein I and II was 0.1 CFU/mL, while the difference for casein I and casein II treatments was 0.2 CFU/mL. The increase in the number of colonies at 48 hours was due to the fact that at 48 hours the bacteria were known to be actively dividing. The breeding phase that occurs in bacteria for 48 hours is likely to occur in a logarithmic/exponential phase in bacteria. This phase is characterized by a period of rapid bacterial growth. Where each cell in the population divides into two cells so that at 48 hours, the bacteria grow colonies (Mardalena, 2016)

Broadly speaking, when viewed from the number of bacteria, the addition of casein and without casein has no effect on microbial growth, because the calculations both get 10^{10} (10 to the power of 10). However, when viewed from the number of bacteria obtained, the results did not show a decrease in the number of colonies from a dilution of 10^{-3} to 10^{-9}. The higher the dilution, the lower the result, because the smaller the pure sample taken by the micropipette at the dilution. In this case, the possibility of error occurs due to the wrong dilution technique, or in sampling with a micropipette of less than 1 mL so that the sample carried is also not optimal, or in pouring PCA medium that is too hot so that bacteria cannot grow properly and cause bacterial death (Siburian *et al*., 2012).

There was no difference in the number of bacteria to the addition of casein, indicating that the bacteria could grow optimally even though casein was not given during the growth process. Based on research by (Zhang *et al*., 2011) on the study of the effect of casein on lactic acid bacteria, it is known that the free amino acid and peptide collections in milk are not sufficient to guarantee optimal bacterial growth in this substrate. Many other growth factors including oligosaccharides, vitamins, and peptides have been found to increase bacterial viability. Casein as a source of protein needed by bacteria was not able to increase bacterial growth. Thus, the manufacture of biological agents with the addition of casein is not necessary. This will also provide benefits to farmers because the price of casein hydrolyzate media itself is expensive.

CONCLUSION

Based on the observation, it shows that the addition of casein has no significant effect. This can be seen from the number ofbacterial colonies *Paenibacillus polymyxa* formed in the TPC calculation. The suggestions that can be recommended for further improvement are that it is necessary to further identify the *Paenibacillus polymyxa* bacteria growing on PCA media to confirm that *Paenibacillus polymyxa* bacteria actually form colonies on the media. Furthermore, in the production of

ISSN 2685-8673 (online)

biological agents at the Bojonegoro Food Crops and Horticulture Protection Working Area, it is not necessary to add casein.

REFERENCES

Arniah, A. (2017). Uji kadar protein total pada campuran kacang kedelai (*Glycine max l. Merr*) dan ekstrak buah nanas (*Ananas comosus*) dengan perbandingan berbeda.

Awaludin Prihanto, A., Dwi Laksono Timur, H., Abdul Jaziri, A., Nurdiani, R., & Audia Pradarameswari, K. (2018). Isolasi dan identifikasi bakteri endofit mangrove *Sonneratia alba* penghasil enzim gelatinase dari pantai sendang biru, malang, jawa timur. Indonesia Journal of Halal, 1(1), 31–42.

Frediansyah, A., & Sudiana, I. M. (2017). Potensi Paenibacillus spp. Sebagai pemacu pertumbuhan tanaman pada ekosistem gambut tropis. Widyariset, 16(2), 201–210.

Kantikowati, E., Haris, R., & Anwar, S. (2018). Aplikasi Agen Hayati (*Paenibacillus polymixa*) terhadap Penekanan Penyakit Hawar Daun Bekteri Serta Hasil dan Pertumbuhan Padi Hitam (*Oryza sativa*) Var. Lokal. Jurnal Ilmiah Pertanian Paspalum, 6(2), 134–142. https://doi.org/10.35138/paspalum.v6i2.97

Kim, Y. S., Kotnala, B., Kim, Y. H., & Jeon, Y. (2015). Biological characteristics of *Paenibacillus polymyxa* GBR-1 involved in root rot of stored Korean ginseng. Journal of Ginseng Research, 4, 1–9. https://doi.org/10.1016/j.jgr.2015.09.003

Palawe, J. F., & Antahari, J. (2018). TPC (Total Plate Count), Wac (Water Adsorbtion Capacity) Abon Ikan Selar Dan Cooking Loss Daging Ikan Selar (*Selaroides leptolesis). Jurnal Ilmiah Tindalung, 4(2), 57-60.*

Luna-Guevara, J. J., Arenas-Hernandez, M. M. P., Martínez De La Peña, C., Silva, J. L., & Luna-Guevara, M. L. (2019). The Role of Pathogenic E. coli in Fresh Vegetables: Behavior, Contamination Factors, and Preventive Measures. International Journal of Microbiology, 2019. https://doi.org/10.1155/2019/2894328

Mardalena. (2016). Fase Pertumbuhan Isolat Bakteri Asam Laktat (BAL) Tempoyak Asal Jambi yang Disimpan Pada Suhu Kamar. Jurnal Sain Peternakan Indonesia, Vol. 11 No, 58–66.

Mubarak, F., Rante, H., & Djide, N. (2017). Isolasi Dan Aktivitas Antimikroba Aktinomycetes Dari Tanah Karst Taman Wisata Bantimurung Asal Maros Sulawesi Selatan. Jurnal Ilmiah As-Syifaa, 9(1), 1–10. https://doi.org/10.33096/jifa.v9i1.226

Napitupulu, H. G., Rumengan, I. F. M., Wullur, S., Ginting, E. L., Rimper, J. R. T. S. L., & Toloh, B. H. (2019). Bacillus sp. sebagai agensia pengurai dalam pemeliharaan *Brachionus rotundiformis* yang menggunakan ikan mentah sebagai sumber nutrisi. Jurnal Ilmiah Platax, 7(1), 158–169. http://ejournal.unsrat.ac.id/index.php/platax

Octavia, A., Wantini, S., Analis, J., Politeknik, K., & Tanjungkarang, K. (2017). Perbandingan Pertumbuhan Jamur *Aspergillus flavus* Pada Media PDA (Potato Dextrose Agar) dan Media Alternatif dari Singkong (*Manihot esculenta Crantz*). Jurnal Mikologi Indonesia, 6(1).

ISSN 2685-8673 (online)

Pahlawi, I. M. H., Satyantini, W. H., & Sudarno. (2019). Uji patogenitas bakteri Pseudomonas sp. pada udang vaname (*Llitopanaeus vannamei*) sebagai kandidat probiotik. Journal of Aquaculture and Fish Health, 8(2), 92–98. https://doi.org/10.20473/jafh.v8i2.13380

Palawe, J. F. P., Wod, S. I., Cayono, E., & Bahan, A. (2016). Analisis Kontaminasi Total Mikroba pada Beberapa Produk Ikan Segar Kabupaten Kepulauan Sangihe (Analysis of Total Microbial Contamination in Some Fresh Fish *in the Sangihe* Islands Regency). 2, 42–46.

Sopialena, S., Suyadi, S., Sofian, S., Tantiani, D., & Fauzi, A. N. (2020). Efektivitas Cendawan Endofit Sebagai Pengendali Penyakit Blast Pada Tanaman Padi (*Oryza sativa*). Agrifor: Jurnal Ilmu Pertanian Dan Kehutanan, 19(2), 355-366.

Siburian, E. T. P., Dewi, P., & Kariada, N. (2012). Pengaruh suhu dan waktu penyimpanan terhadap pertumbuhan bakteri dan fungi ikan bandeng. Life Science, 1(2), 101–105.

Syamsiah, M. (2013). Efektifitas aplikasi Paenibacillus polymyxa dalam pengendalian penyakit hawar daun bakteri pada tanaman padi varietas mekongga. Jurnal Agroscience, 5(1), 24–28.

Tantiani, D., & Fauzi, N. (2020). Efektivitas Cendawan Endofit Sebagai Pengendali Penyakit Blast Pada Tanaman Padi *(Oryza sativa)* Mahasiswa Progam Studi Pertanian Tropika Basah Fakultas Pertanian Universitas isolasi. XIX, 355–366.

Wang, J., Su, Y., Jia, F., & Jin, H. (2013). Characterization of casein hydrolysates derived from enzymatic hydrolysis. Chemistry Central Journal, 7(1), 1. https://doi.org/10.1186/1752-153X-7-62

Wati, R. Y. (2018). Pengaruh pemanasan media PCA berulang terhadap Uji TPC di Laboratorium Mikrobiologi Teknologi Hasil Pertanian Unand. 1(2), 44–47.

Wiranata, A., & Tantiani, D. (2021). Potential of *Paenibacillus polymyxa* bacteria and Trichoderma sp. as biological pesticides to control maize leaf blight (*Zea mays L*). In IOP Conference Series: Earth and Environmental Science (Vol. 800, No. 1, p. 012027). IOP Publishing.

Winarsi, H. (2018). Susu kecambah kedelai basah kaya protein lebih disukai sebagai minuman alternatif untuk obesitas. Jurnal Gizi Dan Pangan Soedirman, 2(1), 32–42. https://doi.org/10.20884/1.jgps.2018.2.1.896

Yunita, M., Hendrawan, Y., & Yulianingsih, R. (2015). Analisis Kuantitatif Mikrobiologi Pada Makanan Penerbangan (Aerofood ACS) Garuda Indonesia Berdasarkan TPC (Total Plate Count) Dengan Metode Pour Plate Quantitative Analysis of Food Microbiology In Flight (Aerofood ACS) Garuda Indonesia Based on the TPC (Total Plate Count) with the Pour Plate Method. 3(3), 237–248.

Zhang, Q., Ren, J., Zhao, H., Zhao, M., Xu, J., & Zhao, Q. (2011). Influence of casein hydrolysates on the growth and lactic acid production of *Lactobacillus delbrueckii* subsp. bulgaricus and *Streptococcus thermophilus*. International Journal of Food Science and Technology, 46(5), 1014–1020.

ISSN 2685-8673 (online)