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RESEARCH ARTICLE

The Effect of *Moringa oleifera* Extract as Candidate Prebiotic to Increase the Growth of *Lactobacillus acidophilus* and *Lactobacillus plantarum* Probiotics

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Abstract

Objectives: The potential of probiotics can be increased by adding prebiotics, which are substances that cannot be digested, but can be used by probiotics to increase their growth. Prebiotics generally consist of oligosaccharides. Moringa oleifera has potential as a prebiotic candidate. This study aims to prove the use of *Moringa oleifera* (*M. oleifera*) extract as a prebiotic candidate to increase the growth of *Lactobacillus acidophilus* (*L. acidophilus*) and *Lactobacillus plantarum* (*L. plantarum*) probiotics. Methods: This study consisted of 4 treatments and each treatment was repeated 5 times. The treatments consisted of: P0: 0% *Moringa oleifera* extract, P1: 0.1% *Moringa oleifera* extract (v/v), P2: 0.2% *M. oleifera* extract (v/v), P3: 0.3% *M. oleifera* extracts (v/v). The probiotic concentration is $1.5x10^8$CFU/ml. Results: The results showed that there was a significant difference ($p<0.05$) between treatments on the growth of L. *plantarum* and *L. acidophilus* probiotics. The use of 0.3% *M. oleifera* extract produced the highest number of colonies on the growth of L.plantarum, which was not different from the use of 0.2% *M. oleifera* extract, while the use of 0.3% *M. oleifera* extract resulted in the highest number of colonies on the growth of *L. acidophilus* which was different with all treatments. Conclusion: Based on the research results, it can be concluded that the use of 0.1%, 0.2% and 0.3% *M. oleifera* extract can increase the growth of *L. acidophilus* and *L. plantarum* probiotics, so that *M. oleifera* extract can be used as a prebiotic candidate.

Keywords: *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Moringa oleifera* extract, Synbiotic.

Introduction

Probiotic culture is live microbial and non-pathogenic, if consumed in a certain amount by host it can improve the balance of microbes in the gut so that it can improve host health, increase growth performance, feed efficiency and livestock productivity [1-3]. Several studies have shown that the use of the probiotic *Lactobacillus casei* (*L.casei*), *Lactobacillus acidophilus* (*L.acidophilus*), *Bifidobacterium spp* can increase the growth performance of poultry [4, 5].

The characteristics of probiotic bacteria are that they must be resistant to low pH, survive in acidity conditions in the gastric, tolerate bile salt and are able to attach to intestinal mucosal cells. Another characteristic of probiotics is the ability to ferment oligosaccharides. [6-10].Lactobacillus *plantarum* is classified as lactic acid bacteria which have an important role in the fermented food industry, and they are important for probiotic properties [11, 12].

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Some strains of the *L. plantarum* have high tolerance to acid, alkaline and osmotic stress (parente). Probiotic *L. acidophilus* La-5 had a higher survival in the in vitro gastric environment. *Lactobacillus acidophilus* strains were resistant to low pH and to the presence of different gastrointestinal enzymes [13, 14]. Prebiotics are substrates that can be used to increase probiotic growth in the gastrointestinal tract [15].The probiotic Bifidobacteria and Lactobacilli can ferment oligosaccharides, but they are specific strains [7, 9].

M. oleifera leaves are rich in nutrients such as protein, methionine, cystine, tryptophan and lysine, ß-carotene and ascorbic acid, calcium, potassium, iron and phytochemicals which have activity as antioxidants [16, 17].

An evaluation of the use of polysaccharide from *M. oleifera* (MOs-2-a) in mice has been evaluated which shows a beneficial effect on the growth of probiotics on the gut micro ecology [18].The use of *M. oleifera* shows that it has potential property as a prebiotic and contains caffeic acid and chlorogenic acid, flavonoid aglycon compounds such as quercetin and kaempferol. The best solvents for the extraction of antioxidant compounds from *M. oleifera* leaves are 80%methanol and 70% ethano [19].

Research on the use of *M. oleifera* extract as a prebiotic candidate for probiotic growth is still very limited, so this study aims to prove the potential of *M. oleifera* extract on the probiotic growth of *L.acidophilus* and *L.plantarum*.

Materials and Methods

This research is an experimental study by conducting an experiment giving *M. oleifera* extract as a prebiotic candidate to increase the growth of *L.acidophilus* and *L.plantarum* probiotic. The experimental design used in this study was a completely randomized design consisting of four treatment groups, namely P0, P1, P2, and P3 with five replications.

The treatment in this study was the administration of *M. oleifera* extract in different doses on each growth medium for the bacteria *L.acidophilus* and *L.plantarum* probiotic. Measurement of the number of bacterial cells was carried out to determine the number of bacterial cells isolates before being given *M. oleifera* extract, a comparison of the bacterial suspension was carried out using the Mc Farland standard method of 0.5 scales with a cell concentration of $1.5x10^8$CFU / ml. The preparation of the bacterial suspension was done by mixing 10 ml aquadest and the colony of bacteria taken using sterile ose until it got the same turbidity as the Mc Farland standard scale of 0.5. After obtaining the same turbidity with the Mc Farland standard scale of 0.5, a gradual dilution was carried out until the sixth dilution, so that the bacterial concentration was obtained 10^2. In this study, the bacterial concentration of 10^1 was used.

Treatment

The sterile test tube was filled with 9 ml of MRS Broth media and added 1 ml of bacteria with a concentration of 10^2. After the inoculant was cultured in MRS Broth media then *M. oleifera* extract was added with the concentration according to the treatment.

The dosage of *M. oleifera* extract is taken based on research, as follows:

P0: 0% *M. oleifera* extract

P1: 0.1% *M. oleifera* extract (v/v)

P2: 0.2% *M. oleifera* extract (v/v)

P3: 0.3% *M. oleifera* extract (v/v)

0.1% *M. oleifera* extract was obtained from 0.1 g *M. oleifera* extract and dissolved with 100 ml aquadest (v/v). Furthermore, 0.1 ml of *M. oleifera* extract was taken and mixed into a sterile tube containing 10 ml of isolate in MRS Broth media (v/v) and then incubated for 24 hours, 37°C. After the incubation period was complete, 0.1 ml of bacterial culture suspension was taken and then poured on the MRS Agar media, then shaken gently so that it is evenly distributed and incubated for 12-24 hours, 37°C. Calculation of bacterial colonies was carried out using the Total Plate Count (TPC) method.

Statistical analysis

All the data obtained were evaluated by adopting the analysis of variance (ANOVA) method using the Statistical Product and Service Solution (SPSS, IBM Corporation, USA) for Windows 22.0 program, if difference was detected ($p<0.05$), another test was carried out using the Duncan's Multiple

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Range Test method to determine the most effective treatment.

Result and Discussion

The results of research using *M. oleifera* extract in different doses on the growth of *L.plantarum* probiotics are listed in Table 1.

Table 1: Total plate count of *Lactobacillus plantarum* added by *M. oleifera* extract

Treatment	Total plate count of *Lactobacillus plantarum*	
P0=0% *M. oleifera* extract	86.00 [a] ± 26.08	8.6 x 10^1 ± 26.08
P1=0.1% *M. oleifera* extract	156.00 [b] ± 27.93	1.56 x 10^2 ± 27.93
P2=0.2% *M. oleifera* extract	198.00 [bc] ± 50.70	1.98 x 10^2 ± 50.70
P3=0.3% *M. oleifera* extract	210.00 [c] ±31.62	2.10 x 10^2 ±31.62

[a,b,c] means within the same column having significantly different ($p< 0.05$)

The lowest average growth of *L. plantarum* was found in treatment P0 (8.6 x 10^1CFU/ml) and followed by P1 treatment (1.56 x 10^2 CFU/ml). Treatment P1 showed that there was no difference with P2 (1.98 x 10^2 CFU/ml). The highest number of colony growth was found in P3 treatment of 2.10 x 10^2 CFU/ml, where P3 was not different from treatment P2. This indicates that the addition of *M. oleifera* extract at doses of 0.2% and 0.3% showed no different growth to the probiotic *L. plantarum*. The results of research using *M. oleifera* extract at different doses on the growth of *L. acidophillus* probiotics are listed in Table 2.

Table 2: Total plate count of *Lactobacillus acidophillus* added by *M. oleifera* extract

Treatment	Total plate count of *L. acidophillus*	
P0=0% *M. oleifera* extract	120 [a] ± 7.07	1.20 x 10^2 ± 7.07
P1=0.1% *M. oleifera* extract	170 [b] ± 18.71	1.70 x 10^2 ± 18.71
P2=0.2% *M. oleifera* extract	240 [c] ± 39.37	2.40 x 10^2 ± 39.37
P3=0.3% *M. oleifera* extract	274 [d] ± 23.02	2.74 x 10^2 ± 23.02

[a,b,c] means within the same column having significantly different ($p< 0.05$)

The lowest average growth of *L. acidophillus* was found in treatment P0 (1.20 x 10^2 CFU/ml). The highest number of colony growth was found in the P3 treatment of 2.74 x 10^2 CFU/ml. This study indicates that the addition of *M. oleifera* extract at doses of 0.1%, 0.2% and 0.3% showed higher growth to the probiotic *L. plantarum* compared with control. The addition of *M. oleifera* extract to MRS Broth media affected the growth of *L.acidophillus* bacteria during incubation for 24 hours, 37° C.

The results showed that there were significant differences in the growth of *L.acidophillus* bacteria in 0.1%, 0.2% and 0.3% *M.oleifera* extract treatments. The lowest growth of *L.acidophillus* bacteria was control (P0), without the addition of *M.oleifera* extract. Treatment P1 showed an increase in the growth of *L.acidophillus* bacteria compared to control.

The addition of 0.2% *M.oleifera* extract showed a higher growth yield of *L.acidophillus* than P1. The addition of 0.3% *M.oleifera* extract showed the highest growth results of *L.acidophillus* bacteria compared to P2, P1 and P0 treatments.

The growth pattern of bacteria is divided into 4 phases, namely the lag phase, which is the adaptation phase of bacteria when transferred to new media. Then the log phase, which is the phase of the rapid growth of bacteria. The nutrients contained in *M.oleifera* extract are used by *L.plantarum* and *L.acidophillus* in the log phase to produce sufficient energy for their growth.

This phase also requires a number of proteins, transporters, and enzymes that are used for uptake the mannose or glucose phosphotransferase system as well as the conversion process of carbohydrates mannose, mannitol, and the amino sugar glucosamine (glucosamine-6-phosphate isomerase) into fructose-6-phosphate. All of these sugars are used as a source of carbon for cellular physiology.

After that, the stationary phase is the saturated phase of the bacteria because the media nutrition as a place for growth begins to decrease and this causes the same number of dead bacteria as living bacteria so that the number is constant. In early and late-stationary, *L. plantarum* isolates used

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glucose as a carbon source to produce energy through glycolysis as evidenced by a decrease in the amount of glucose in the stationary phase. In the late stationary phase, unfavorable conditions occur due to high acidity conditions and decreased nutrient availability [20], so that it does not support the growth of probiotic. *L. plantarum*, which is known to produce raffinose hydrolyzing α-D-galactosidase enzyme [16].

The addition of *M.oleifera* extract with a concentration of 0.1%, 0.2%, and 0.3% significantly increased the growth of *L. acidophillus* bacteria, this indicated that the increasing the addition of *M. oleifera* extract, the growth rate of *L. acidophillus* bacteria also increased. Polysaccharides or non-digestible carbohydrates (dietary fiber) are present in plants. *M. oleifera* is known to contain dietary fiber, oligosaccharides and polyphenols.

The total carbohydrate in ethanol extract was containing monosaccharides, disaccharides and oligosaccharides [21]. Oligosaccharides play an important role as a source of nutrition to increase probiotic growth. The growth rate of *L. acidophillus* bacteria in the treatment without *M. oleifera* extract (P0) showed the lowest growth compared to the growth rate of Lactobacillus bacteria added with *M. oleifera* extract. This proves that *M. oleifera* extract is a source of essential nutrients needed by *L. acidophillus* to increase and accelerate its growth. *M. oleifera* is rich in nutrients such as essential amino, vitamins, minerals and phytochemicals [22, 23].

The addition of *M. oleifera* extract in culture media showed a significant effect of increasing the number of *L. acidophillus* bacteria compared to bacterial growth without the addition of *M.oleifera* extract (control). The results of this study are relevant to Wang's, who's obtained a polysaccharide separated from *M. oleifera* leaf (MOs-2-a) that is composed by mannose, rhamnose, glucose, and galactose. Giving MOs-2-a showed an increase in the microbial composition of the Lactobacillaceae family [18].

Oligosaccharides, polysaccharides and galacto-oligosaccharides in plants have important functions as prebiotics to improve gut health through increased growth of beneficial bacteria [24,25].

Polysaccharide extraction from *M. oleifera* includes xylose, mannose, glucose, galactose and arabinose [22]. In this study, *M. oleifera* extract has potential as a prebiotic candidate. It is proven that the addition of 0.1%, 0.2% and 0.3% *M. oleifera* extract can increase the growth of *L. acidophilus*. Based on the results of this study, *M. oleifera* extract as a prebiotic candidate and *L. acidophilus* as a probiotic have function as a synbiotic candidate.

In this study, the optimal combination was found in the addition of 0.3% *M. oleifera* extract. The results of this study are also in line with other studies showing that *L. acidophilus* strains and *L. plantarum* strains could fermented fructo-oligosaccharides, a result consistent with that recently reported by Sghir and Kaplan [26, 27]. These results are in agreement with those obtained by El-Sayed that Moringa could stimulate the growth of *L. acidophilus* and *L. bulgaricus* [28].

Conclusion

Based on the results of the research, it can be concluded that the use of 0.1%, 0.2% and 0.3% *M. oleifera* extract can increase the growth of *L. acidophilus* and *L. plantarum* probiotics, so that *M. oleifera* extract can be used as a prebiotic candidate.

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References

1. Khanian M, Karimi-Torshizi MA, Allameh A (2019) Alleviation of aflatoxin-related oxidative damage to liver and improvement of growth performance in broiler chickens consumed *Lactobacillus plantarum* 299v for entire growth period. Toxicon., 158:57-62.
2. Yulianto AB, Lokapirnasari WP, Najwan R, Wardhani HC, Rahman NF, Huda K, Ulfah N (2020) Influence of *Lactobacillus casei* WB 315 and crude fish oil (CFO) on growth performance, EPA, DHA, HDL, LDL, cholesterol of meat broiler chickens. Iran J. Microbiol., 12(2):148.

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3. Lokapirnasari WP, Sahidu AM, Maslachah L, Yulianto AB, Najwan R (2020) The effect of combination *Bifidobacterium sp* and *Lactobacillus acidophilus* probiotic on egg yolk cholesterol, HDL, and LDL. IOP Conference Series: Earth and Environmental Science, 441(1):012049.
4. Lokapirnasari WP, Pribadi TB, Al Arif A, Soeharsono S, Hidanah S, Harijani N, Najwan R, Huda K, Wardhani HC, Rahman NF, Yulianto AB (2019) Potency of probiotics *Bifidobacterium spp*. and *Lactobacillus casei* to improve growth performance and business analysis in organic laying hens. Vet world, 12(6):860.
5. Lokapirnasari WP, Sahidu AM, Maslachah L, Koestanti E (2020) Effect of *Lactobacillus casei* and *Lactobacillus acidophilus* in Laying Hens Challenged by Escherichia coli Infection. Sains Malays., 49(6):1237-44.
6. Collins MD, Gibson GR (1999) Probiotics, prebiotics, and synbiotics: approaches for modulating the microbial ecology of the gut. Am. J. Clin. Nutr., 69(5):1052s-7s.
7. Kaplan H, Hutkins RW (2000) Fermentation of fructooligosaccharides by lactic acid bacteria and bifidobacteria. Appl. Environ. Microbiol., 66(6):2682-4.
8. Gibson GR, Roberfroid MB (1995) Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J Nutr., 125(6):1401-12.
9. Macfarlane GT, Cummings JH (1999) Probiotics and prebiotics: can regulating the activities of intestinal bacteria benefit health?. Bmj., 318(7189):999-1003.
10. Roberfroid MB (1998) Prebiotics and synbiotics: concepts and nutritional properties. Br. J. Nutr., 80(S2):S197-202.
11. Tanganurat W, Quinquis B, Leelawatcharamas V, Bolotin A (2009) Genotypic and phenotypic characterization of *Lactobacillus plantarum* strains isolated from Thai fermented fruits and vegetables. J. Basic Microbiol., 49(4):377-85.
12. Ricciardi A, Parente E, Guidone A, Ianniello RG, Zotta T, Sayem SA, Varcamonti M (2012) Genotypic diversity of stress response in *Lactobacillus plantarum, Lactobacillus paraplantarum* and *Lactobacillus pentosus*. Int. J. Food Microbiol., 157(2):278-85.
13. dos Santos DX, Casazza AA, Aliakbarian B, Bedani R, Saad SM, Perego P (2019) Improved probiotic survival to in vitro gastrointestinal stress in a mousse containing *Lactobacillus acidophilus* La-5 microencapsulated with inulin by spray drying. Lwt., 99: 404-10.
14. Buriti FC, Castro IA, Saad SM (2010) Viability of *Lactobacillus acidophilus* in synbiotic guava mousses and its survival under in vitro simulated gastrointestinal conditions. Int. J. Food Microbiol., 137(2-3):121-9.
15. Hernández-Hernández O, Muthaiyan A, Moreno FJ, Montilla A, Sanz ML, Ricke SC (2012) Effect of prebiotic carbohydrates on the growth and tolerance of Lactobacillus. Food Microbiol., 30(2):355-61.
16. Vanajakshi V, Vijayendra SVN, Varadaraj MC, Venkateswaran G, Agrawal R (2015) Optimization of a probiotic beverage based on Moringa leaves and beetroot. LWT Food Sci. Technol., 63: 1268-1273.
17. Nobossé P, Fombang EN, Mbofung CM (2018) Effects of age and extraction solvent on phytochemical content and antioxidant activity of fresh *Moringa oleifera* L. leaves. Food Sci. Nutr., 6(8):2188-98.
18. Wang F, Bao YF, Si JJ, Duan Y, Weng ZB, Shen XC (2019) The Beneficial Effects of a Polysaccharide from *Moringa oleifera* Leaf on Gut Micro ecology in Mice. J. Med. Food. 22(9):907-18.
19. Siddhuraju P, Becker K (2003) Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (*Moringa oleifera* Lam.) leaves. J. Agric. Food Chem., 51(8):2144-55.
20. Cohen DP, Renes J, Bouwman FG, Zoetendal EG, Mariman E, de Vos WM, Vaughan EE (2006) Proteomic analysis of log to stationary growth phase *Lactobacillus plantarum* cells and a 2- DE database. Proteomics, 6(24):6485-93.
21. Anudeep S, Radha C (2018) Carbohydrates of *Moringa oleifera* seeds. IJRAR, 5(4):103-8.
22. Chen C, Zhang B, Huang Q, Fu X, Liu RH (2017) Microwave-assisted extraction of polysaccharides from Moringa oleifera Lam. leaves: Characterization and

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hypoglycemic activity. Ind. Crops Prod., 100: 1-1.

23. Jayawardana BC, Liyanage R, Lalantha N, Iddamalgoda S, Weththasinghe P (2015) Antioxidant and antimicrobial activity of drumstick (*Moringa oleifera*) leaves in herbal chicken sausages. LWT Food Sci. Technol., 64:1204-1208.
24. Xu X, Xu P, Ma C, Tang J, Zhang X (2013) Gut microbiota, host health, and polysaccharides. Biotechnol. Adv., 31(2):318-37.
25. Shang Q, Jiang H, Cai C, Hao J, Li G, Yu G (2018) Gut microbiota fermentation of marine polysaccharides and its effects on intestinal ecology: An overview. Carbohydrate polymers. 179: 173-85.
26. Sghir A, Chow JM, Mackie RI (1998) Continuous culture selection of bifidobacteria and lactobacilli from human faecal samples using fructooligosaccharide as selective substrate. J. Appl. Microbiol. 85(4):769-77.
27. Kaplan H, Hutkins RW (2000) Fermentation of fructooligosaccharides by lactic acid bacteria and bifidobacteria. Appl. Environ. Microbiol. 66(6):2682-4.
28. El-Sayed OF, El-Taweel HS, El-Shibiny AA, Kamal MM (2016) Using Moringa leaves powder in production of probiotic yoghurt. SINJAS. 5(2):197-208.

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