Effectiveness of Lactobacillus plantarum from Dadiah Payakumbuh yoghurt as Immunomodulator in hypertension

by Dessy Abdullah

Effectiveness of *Lactobacillus plantarum* from *Dadiah* Payakumbuh yoghurt as Immunomodulator in hypertension

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ABSTRACT

Dadiah is a traditional food for the people of West Sumatra made from fermented buffalo milk in a bamboo container. As a fermented product, dadiah contains many lactic acid bacteria that have the potential as probiotics, including *Lactobacillus plantarum* isolated from dadiah payakumbuh which has the potential as a probiotic. As a probiotic, *L. plantarum* exhibits a number of beneficial health effects in humans and animals. It is capable of enhancing the body's immune system or as an immune-modulator. Probiotics help regulate the intestinal microflora and have immunomodulatory properties. This study aims to determine the effectiveness of *L. plantarum* from dadiah payakumbuh as an immune-modulator, both bio-informatically and *in vitro*. This study looked at the activity of *L. plantarum* as an immune-modulator in bioinformatics by searching for secondary metabolites by looking at the structure of the canonical and isomeric SMILE (simplified molecular-input line-entry system) in the PubChem database, as well as analyzing IgA in jejunum tissue of Norway Rat White (*Rattus novernicus*) given hypertension treatment using KIT ELISA. It was found that there was a predictive result of SAR Way2 Drug pass server from *L. plantarum* as an immune-modulator with a value of 0.47 and the IgA value in the jejunum tissue of *R. novernicus*. Hypertension in the rats increased compared to those who were not treated. *L. plantarum* from dadiah payakumbuh is able to increase the body immunity, since it produces immunomodulatory secondary metabolites.

Keywords: Probiotics; Dadiah; Immuno-modulators; *Rattus novernicus*.
Article type: Short Communication.

INTRODUCTION

Lactic acid bacteria (LAB) are found naturally in a variety of environmental habitats, including dairy, meat, vegetable, cereal and plant environments, where fermentation can occur. Historically, the traditional roles for many LAB have been as starter cultures to drive food and dairy fermentations, leading to their widespread human consumption and generally recognized as safe (GRAS) status (Bintsi 2018). Dadiah is made from buffalo milk which is fermented in a bamboo container for more than 2-3 days in airtight conditions. It is a food that has been passed down from generation to generation so far, hence we can still find it in several areas in West Sumatra such as Bukittinggi, Payakumbuh, Sijunjung, Solok, Tanah Datar, Agam, Limapuluh Kota and Solok Selatan. This product is like yogurt with a smooth and glossy surface, even consistency, creamy colour, good aroma, and sour taste (Purwati *et al*. 2016). Dadiah also has many benefits for health, including enhancement of the body immunity, since it contains antioxidants of high nutritional value and the presence of lactic acid bacterium, so it can be considered as one of the functional foods (Arnold *et al*. 2021). Previous studies have identified species of lactic

acid bacteria in dadiah, one commonly- found is *Lactobacillus plantarum* 2DA isolated from dadiah payakumbuh (Abdullah *et al.* 2021). One of the impact factors of decreased immunity or impaired immune function is by observing the aging process, so that the body is very susceptible to infection and disease (Nikolich Žugich 2018). This can be anticipated in several ways, one of them is by increasing immunity-stimulating agents that are able to elevate immunoglobulins either naturally or using recommended chemicals, so that they do not have side effects. Therefore, using safe and natural materials that act as immune-modulators is highly recommended, especially for people with impaired immune function. Research on the effect of the immune response on the gut microbiota is very influential both directly and indirectly by enhancing the body immunity, since the gut microbiota display a dominant role in activating pathways related to the immune system (Frei *et al.* 2105; Hoseinifar *et al.* 2017; Murtadha & Hammod 2022), especially bacterium that are probiotic, because in this case probiotics help improve and maintain the immune system and are also able to prevent and reduce the impact of infection. Probiotic bacterial therapy is directly related to immunogenic and immune modulation (production of interleukin (IL)-6 and IL-10) and their tumour-reducing activity (Reid *et al.* 2003; Amdekar *et al.* 2010; Van Hoang *et al.* 2018). Immunoglobulin A (IgA) is the main immunoglobulin found in the mucosa, also known as secretory immunoglobulin A (sIgA), since it will enter and leave the lumen or the circulatory system which in turn will stimulate the formation of IgG and IgM. Probiotic bacteria produce IgA about 80% which is present in the intestinal mucosa/intestinal lamina propria (Galdeano *et al.* 2007). Immunoglobulin A (IgA) is a protein produced by B lymphocyte cells and is the main immunoglobulin found in about 80% of the digestive tract mucosa, the rest is found in the blood circulation. The content of small intestine IgA can be used as an indicator of digestive tract health. IgA production in the digestive tract plays a role in preventing the attachment of pathogenic microorganisms to intestinal epithelial cells (Wilson 2005), so that it can protect the intestines from pathogen attack. Probiotic bacteria will stimulate the activation of immunocompetent cells, both macrophages and dendritic cells. Hence, the lymphoid tissue in the lamina propria will trigger plasma cells to produce IgA which plays a role in the mucosal immune system. So, probiotic bacteria can be used as immune-modulators by producing IgA in the mucosa. Antibodies are immunoglobulins (Ig) which can react specifically with antigens that stimulate their production. Antibodies encompass 20% of plasma proteins (Brooks *et al.* 2001), formed by plasma cells originating from B cell proliferation due to contact with antigens (Baratawidjaya & Engganis 2009). This study aims to determine the secondary metabolism of *L. plantarum* 2DA isolated from dadiah payakumbuh by bioinformatics as an immune-modulator and an increase in IgA in the digestive tract in hypertensive *Rattus novernicus* Wistar strain given oral probiotic *L. plantarum*.

MATERIALS AND METHODS

Bacteria used were *Lactobacillus plantarum* 2DA (culture isolated from the payakumbuh dadiah collection from the Animal Products Technology Laboratory, Andalas University, Indonesia.

Search of secondary metabolite

Search for bacterial secondary metabolites was carried out by studying literature from published journals. Each compound that has been determined is then searched for its canonical structure and isomeric SMILE (simplified molecular-input line-entry system) in the PubChem database (https://pubchem.ncbi.nlm.nih.gov/).

Prediction of bacterial content's Structure-activity relationship (SAR)

Secondary metabolites in bacteria from the literature study were analysed for their potential using WAY2DRUG PASS prediction (http://www.pharmaexpert.ru/passonline/predict.php) as a cholesterol-lowering treatment. Previously, each compound needed to find the SMILE structure obtained from the PubChem database. Then the potency of the compound was analysed using WAY2DRUG PASS prediction to determine its potential for cholesterol reduction treatment. We recommend using a score of 0.5 as the cut-off score. The Pa value means the accuracy of the prediction function obtained, the higher the Pa value of a function, the better the accuracy level.

Analysis of immunoglobulin (IgA)

The test refers to the KIT ELISA (Bioassay Technology Laboratory) protocol. We prepared the reagents and samples at room temperature, drew map and prepared the map of the sample position; Then we entered 50 μL of standard solution into the marked well, and added 50 μL of standard diluent to the well then marked. Afterward, we entered 40 U mL^{-1} sample and 10 U mL^{-1} Anti-IgA in wells that have been marked. In standard wells and

sample wells, we added 50 U mL^{-1} streptavidin-HRP, covered with a cover plate and incubated for 1 hour in an incubator 37 ℃, then dissolved in the wells are discarded. Each well was washed with 350 μL of wash buffer and allowed to stand for 1 minute. Washing was carried out 5 times. Thereafter, we entered substrate solution A (50 μL) then substrate B (50 μL), covered with a cover plate and incubated for 1 hour in an incubator 37 ℃, followed by adding 50 μL Stop Solution on all wells, waiting for 10 minutes, then reading the OD value with ELISA Reader Multiskan Ex (Thermo Scientific) at 450 nm.

RESULTS AND DISCUSSION

Results of the SAR prediction (Table 1) with the Way2Drug Pass Server showed that the *Lactobacillus plantarum* 2DA metabolite has the ability as an immune-modulator, since it has a disease treatment prediction value of 0.47, where a value close to 0.90 is the best lift from this SAR prediction result. This ability can prove that *L. plantarum* 2DA from dadiah payakumbuh has the ability to increase immunity for humans and animals. In addition, for patients with hypertension, *L. plantarum* also has a good treatment prediction.

Table 1. Result Prediction SAR Way2Drug Pass Server

Compound	Immuno-modulator value
Butyric Acid	0.407
Propionic Acid	0.365
Lactic Acid	0.334
Valeric Acid	0.504
Caproic Acid	0.504
Average	0.47

The prediction results using the SAR approach illustrated that *L. plantarum* metabolites have great potential as a treatment for metabolic disease, especially hypertension. This is supported by the high predictive value of *L. plantarum* bioactive as a Beta-adrenergic receptor kinase inhibitor (0.64), Vaso-protector (0.54) and Lipid metabolism regulator (0.56).
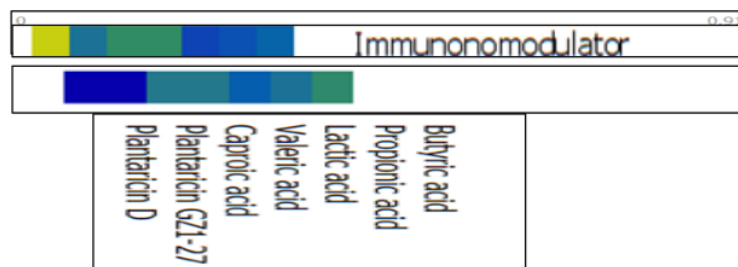

Fig. 1. Heat Map Potential QSAR Metabolite *Lactobacillus plantarum*.

On the results of the heat map, it can be observed clearly that the more the resulting image goes to yellow, the higher the value. The metabolites as immune-modulators can be seen in the middle between the blue and the middle, which means that the metabolites of *L. plantarum* are good for improving the immune system.
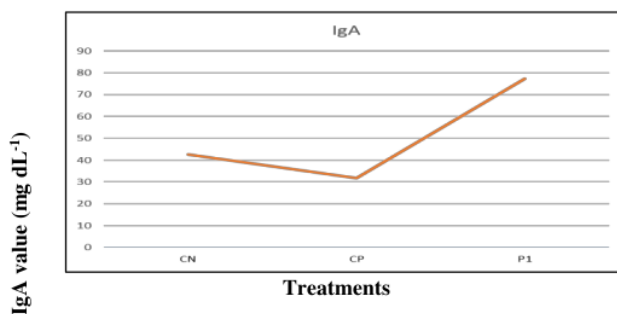

Fig. 2. Result of IgA in jejunum tissues of rats, *L. novergicus* Wistar strain.

Fig. 2 exhibits CN as control negative, CP as control positive and P1 as the group given *L. plantarum* 2DA bacterial isolates. The latter displays an elevation in IgA values. According to Fig. 2, there is an elevation in IgA from CN 42.65 with no treatment at all; CP with a value of 31.87 where rats with hypertension treatment without *L. plantarum* 2DA; and P1 hypertensive rats with acid bacteria administration lactate increases IgA. So that, it can help speed up the healing process and reduce the impact of hypertension, since *L. plantarum* 2DA contains metabolites as immune-modulators that are good for health, especially in patients with hypertension. Zoumpopoulou *et al.* (2008) reported that *L. fermentum* ACA-DC 179 *in vitro* has probiotic properties such as antimicrobial and immune-modulating activity, and *in vivo* was successfully applied to a *Salmonella*-infected mouse model, significantly reducing colitis in a TNBS-colitis mouse model. Macias-Rodriguez et al. (2009) also reported that *L. fermentum* was able to stimulate the immune system and inhibit the attachment of pathogenic microorganisms to the digestive tract of mice. The local immune response in the gut is caused by the interaction between probiotics and epithelial cells. After interaction with epithelial cells occurs, probiotics are internalized. The first cells that will interact with probiotics are antigen presenting cells (APCs), macrophages, and dendritic cells that communicate with the intestinal lamina propria, and induce the release of interleukin-6 (IL-6) and IL-10. The former stimulates the growth and differentiation of B cells into plasma cells that produce IgA, while the latter plays a role in controlling non-specific immune reactions and cellular immunity (Galdeano *et al.* 2007)

CONCLUSION

The bacteria *Lactobacillus plantarum* 2DA isolated from dadiah payakumbuh has the ability as an immune-modulator, both in terms of bioinformatics and its application to rats, *L. novergicus* Wistar strain. These rats which was given bacterial isolates, exhibited a significant effect on upraising immunoglobulin (IgA) levels in the digestive tract (small intestines) of rats suffering from hypertension.

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