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Evaluation of *Pediococcus acidilactici* from Dadiah Bukitinggi (dairy food) as an insulin promotor by bioinformatics

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ABSTRACT

Dadiah is one of traditional food in West Sumatera. It is made from naturally fermented buffalo milk in bamboo tubes by lactic acid bacteria found naturally in the buffalo milk and bamboo tubes themselves. Lactic acid bacteria strains are the main probiotics in the food and pharmaceutical markets. Probiotics are live microorganisms or components of a microbial cell that give good effect to other organisms. Probiotics have an important role in the health of the gastrointestinal tract in general. *Pediococcus acidilactici* is one of the lactic acid bacteria that acts as a probiotic isolated from Dadiah. Insulin is a hormone produced by pancreatic beta cells, which plays an important role in regulating blood glucose levels, so that they remain in a normal state, and its promoter regulates the rate of transcription in response to physiological regulators. In human pancreatic cells, the insulin promoter interacts with loci associated with diabetes risk and controls genes involved in insulin metabolism. Insulin promoters are tissue-specific and typically only activate in islet beta cells in adult tissues to stimulate insulin production. This study aimsto determine the secondary metabolites of *P. acidilactici* to insulin promotor expression by looking at the canonical and isomeric SMILE (simplified molecular-input line-entry system) structures in the PubChem database. After conducting bioinformatics analysis to determine the secondary metabolites of *P. acidilactici*, its compound has potential as a diabetes treatment, because the QSAR results show potential as an insulin promoter (0.75).

Keywords: Probiotics, Dadiah, Insulin Promotor, *Pediococcus acidilactici.*
Article type: Research Article.

INTRODUCTION

In Indonesia, there are around 2.5 million heads of buffalo living in the wild. On the other hand, the number of buffalo used for livestock in Indonesia has been dropped. The population of buffalo on Sumatra Island increased slightly from 1.1 to 1.2 million heads in the same year, or experiencing a population growth of 9%. However, the population of tigers on Sumatra Island decreased dramatically from 3.3 million in 1985 to only 2.4 million in 2001, or experienced a population decline of 26% (Purwati *et al.* 2016). According to the research on milk production, the daily production of buffalo milk can reach a maximum of 4,100 liters. According to Purwati (2008), buffaloes in West Sumatra produce between 1.5 and 2 liters milk per day. If the protein content of buffalo milk is 5.26%, then the consumption of buffalo milk each day would result in 216 kg available protein. In West Sumatra, the districts of Sijunjung, Agam, and Limapuluh Kota are where the majority of buffalo development is concentrated.This demonstrates that the natural and socio-cultural conditions of the people who live on the island of Sumatra provide an appropriate environment for the growth of buffalo as a producer of dadiah (Purwati *et al.* 2016). Dadiah is a traditional food that is popular in several West Sumatra districts, such

as Bukittinggi, Padang Panjang, Solok, Lima Puluh Kota, and Tanah Datar district. According to Surono (2015), dadiah is a source of probiotics, which are known to improve human health. A Minangkabau yogurt is one possible name for this product. Although it has been consumed by the people of Minangkabau for a considerable amount of time, its consumption has decreased in recent years due to the fact that most people, especially younger people, are unaware of the nutritional benefits it provides. For this reason, it is essential to reintroduce dadiah to the community by making use of a variety of different approaches, such as packaging it in a bamboo cup (Helmizar & Surono 2019). According to the prior research, it has been established that the dadiah contains highly effective probiotics in the form of lactic acid bacteria. *Lactobacillus* and *Bifidobacterium* are the two types of probiotic bacteria that can be found in the curd (Ramakrishnan 2010). Lactic acid bacteria and the products derived from them have the ability to prevent the development of a number of diseases, including colorectal cancer, diarrhea caused by antibiotics, viruses, and bacteria, and diarrhea, as well as spurring the health and workings of the heart, good food to increase stamina and endurance, preventing colorectal cancer, improving intestinal microflora, repairing the intestinal condition affected by antibiotic treatment, treating diarrhea caused by antibiotics, and producing digestive enzymes (Rodrigues *et al.* 2007).

If probiotics are able to survive well in environmental conditions such as those found in the stomach, which have a low pH environment, and if they are able to avoid being damaged by enzymes produced by the stomach, then they will be effective. One of the most common types of lactic acid bacteria that are found in the gastrointestinal tract is called *Lactobacillus* (Ljungh & Wadström 2001). The fermentation of lactic acid typically takes place with the participation of lactic acid bacteria and sometimes some *Bacillus*. There are two types of fermentation that produce lactic acid: homolactic- and heterolactic- fermentation. In homolactic fermentation, all of the pyruvic acid is converted to lactic acid, whereas in heterolactic one, in addition to lactic acid, additional products such as ethanol and carbon dioxide are created (Nicklin, Graeme-Cook *et al.* 1999). Included in this category are homolactic bacteria, such as *Lactobacillus acidophilus*, *L. bulgaris*, *L. casei*, *L. lactis*, *Streptococcus thermophillus*, *Lactococcus lactis*, and *Pediococcus acidilactici*. Heterolactic bacteria, on the other hand, are carried out by hetero-fermentative bacteria, such as *Lactobacillus bervis*, *L. fermentum*, *Leuconostoc lactis* and *Weissella confusa* (Rhadhiyah 2008). *P. acidilactici* (PA), which is also a LAB member, was said to control animals' gut microbiota, reduce inflammation, release bacteriocin, and stop pathogenic bacteria from growing (Wang *et al.* 2019; Tan *et al.* 2020; Ashouri *et al.* 2020). Low-grade inflammation is prevalent in type 2 diabetic patients (T2DM). As a result of inflammation, heart disease, metabolic syndrome, and T2DM all share a higher concentration of circulatory cytokines (Calle & Fernandez 2012). Inflammatory cytokines are created by a variety of cell types and then secreted into the circulation, where they govern a variety of tissues by acting locally, centrally, and peripherally (Bruun *et al.* 2003). The pathogenesis of T2DM is complex and largely understood. T2DM is considered as a chronic inflammatory condition (Herder *et al.* 2009).

Insulin resistance (IR), which is brought on by inflammation, is a trait that is present in virtually all people who have type 2 diabetes (Tao *et al.* 2020). T2DM is believed to be caused by a number of risk factors, including genetic susceptibility, age, being over- weight or obese, and living an unhealthy lifestyle. Recent evidence suggests that the risk of acquiring T2DM may also involve gut microbiota-related variables. Patients with type 2 diabetes mellitus exhibited a moderate degree of gut microbial dysbiosis, and it was found in their stool samples that there was a drop in the amount of intestinal *Roseburia* and *Faecalibacterium prausnitzii* (Wang *et al.* 2012). In addition to taking into consideration the gastrointestinal symptoms of T2DM, the gut microbiota is suspected of playing a role in the pathogenesis of T2DM (Tao *et al.* 2020). Probiotics are defined as live microorganisms that, when provided to a host in sufficient quantities, are capable of bestowing a health benefit on the host. The findings of experiments conducted on animals suggested that the beneficial effects of probiotics can have an influence on glucose metabolism andcan increase insulin sensitivity (Kobyliak *et al.* 2016). However, there is a lack of consensus on the effects of probiotics on human T2DM. Patients with T2DM who were given probiotics exhibited significantly lower levels of HbA1c, fasting blood glucose, or insulin resistance after receiving medication (Firouzi *et al.* 2017; Khalili *et al.* 2019), whereas other research did not discover a significant difference between patients who were given probiotics and those who were given a placebo (Mobini *et al.* 2017; Razmpoosh *et al.* 2019). We performed a meta-analysis to evaluate the effects of probiotics on three indicators of T2DM, including HbA1c, FBG, and homeostasis model assessment of IR.

The purpose of this study was to provide a theoretical basis for the extensive clinical application of probiotics in the treatment of T2DM, as well as to provide a comprehensive evaluation of the role that probiotics play in the

treatment of T2DM patients (HOMA-IR; Tao *et al.* 2020). In spite of the fact that probiotics were able to lower blood sugar levels, a growing body of studies (Firouzi *et al.* 2017; Tonucci *et al.* 2017) indicate that probiotics do not help reduceinflammation. Insulin resistance is associated with obesity in patients with T2DM; yet, surprisingly, probiotics decreased insulin resistance without affecting body mass index (BMI). Animal studies showed that a change in the microbiota may contribute to the development of IR and T2DM in several ways. Short-chain fatty acids (SCFA) like butyrate, propionate, and acetate are mostly made by gut microbiota by proximal digestion of carbohydrates and fermentation of indigestible oligosaccharides. At the moment, when people talk about the role of SCFA, they mostly talk about how it helps endocrine cells in the colon mucosa release glucagon-like peptide-1 (GLP-1) and peptide YY gastrin regulator. These hormones display an effect on the GI tract. For example, they stop the production of gastric juice and GI peristalsis, slow down the emptying of the stomach, and stimulate the hypothalamus in the central nervous system, which makes you feel full and increases your appetite. So, T2DM-related indexes like fasting blood glucose level, weight, and others can be lowered (Tao *et al.* 2020).

This study aims to determine the secondary metabolites of *P. acidilactici* to insulin promotor expression by looking at the canonical and isomeric SMILE (simplified molecular- input line-entry system) structures in the PubChem database. After conducting bioinformatics analyses to determine the secondary metabolites of *P. acidilactici*, its compound has potential as a diabetes treatment, since the QSAR results show potential as an insulinpromoter (0.75).

MATERIAL AND METHODS

Search for secondary metabolites

The 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 quantitative structure-activity relationship (QSAR) bacterial content as a DM treatment

The potential secondary metabolites in bacteria from the literature study were analyzed using WAY2DRUG PASS prediction (http://www.pharmaexpert.ru/passonline/predict.php) as a diabetes treatment. Previously, each compound needed to find the SMILE structure obtained from the PubChem database. Then the potential of the compound was analyzed using WAY2DRUG PASS prediction to determine its potential for diabetes treatment. The value of Pa (Probability to be active) is a value that describes the potential of a compound being tested. Determination of this value is done by comparing the structure of the input compound with a compound that has been proven as a particular treatment. If the Pa value is more than 0.7, it indicates that the compound is predicted to have high potential as an anti-inflammatory, e.g., due to the high similarity with the compound in the database that has been proven as a 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 (Filimonov *et al.* 2014).

Protein-Protein Interaction

STITCH DB 5.0 (http://stitch.embl.de/) was used to predict target proteins. The input used is the minimum required interaction score (high confidence 0.7) in the *Homo sapiens* model. The prediction results are then used as the basis for protein - protein interaction (PPI) with the STRING DB version 11.5 webserver (https://string-db.org/) with a confidence score of 0.4 on the *Homo sapiens* model. Afterward, the analysis was carried out using Cytoscape version 3.8.2. to determine the role of proteins in the pathway.

The GOlorize plug-in and Network Analysis on CytoScape are used in this study. GOlorize is used to determine the role of genes / proteins in the pathway using the BinGO (Gene Ontology) approach and visualized with a certain color. For BinGO-GOlorize, a significant value of 0.05 is used, while the statistical test used is the Hypergeometric Test with Multiple Testing Correction Benjamini-Hochberg False Discovery Rate (FDR) correction with the ontology file Biological Process on *Homo sapiens*. FDR describes the statistical approach used in multiple hypothesis testing to correct for multiple comparisons.

RESULTS AND DISCUSSION

Prediction of Structure-activity Relationship (SAR) of bacterial content as a DM treatment

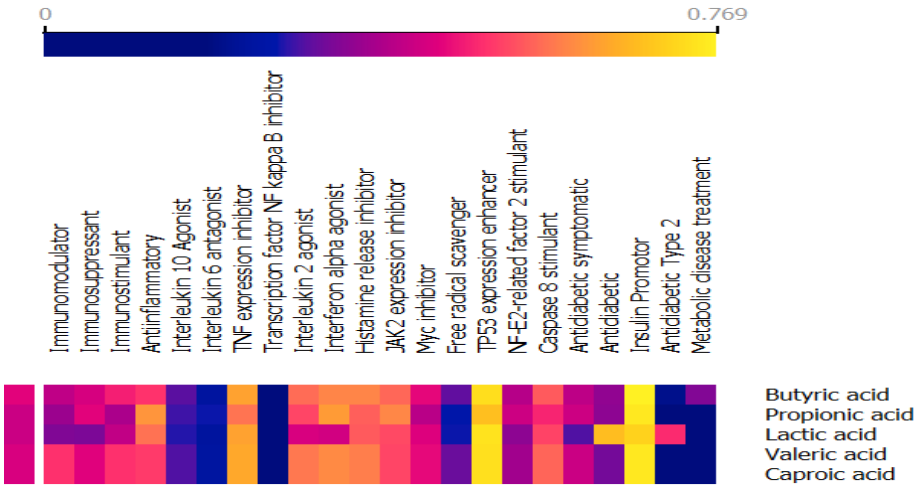

Fig. 1. Heat Map Potensi QSAR Metabolite *Pediococcus* (the closer to yellow the higher the value).

Based on the QSAR analysis, the probiotic content of dadiah has potential for immunity, stress scavenger and diabetes treatment. The potential of dadiah as a diabetes treatment is supported by the potential of dadiah compounds as insulin promoters (0.75). In addition, this compound has a great potential as a TP53 expression enhancer (0.73). P53 is a key molecular node in stress responses including inflammation. It is involved in inflammation, cell cycle arrest, apoptosis, DNA repair, and cellular senescence which are essential for normal cells to maintain normal cellular homeostasis and genome integrity (Hussain *et al.* 2006). The pathogenesis of type 2 diabetes mellitus (T2DM) and the development of insulin resistance is one of the causes of reactive oxygen species (ROS), epigenetic factors, and activation of pro-inflammatory cytokines. *Pediococcus* metabolites have a high potential as an anti-inflammatory (0.55). This score is supported by the potency of dadiah as a TNF expression inhibitor (0.63), Histamine Release Inhibitor (0.58), and JAK2 expression inhibitor (0.55). TNF (Tumor Necrosis Factor) is one of the pro-inflammatory cytokines that can trigger inflammation. TNF plays a crucial role in the regulation of innate immune responses. TNF triggers the secretion of inflammatory mediators and is involved in the activation and function of innate immune cells. TNF is also involved in the proliferation, differentiation and survival of macrophages (Schral 2015). TNF-alpha is one of the pro-inflammatory mediators involved in the pathogenesis of T2DM (Akhas *et al.* 2018). High histamine levels have been reported to be associated with diabetic long-term complications (Pini *et al.* 2016). JAK-signaling hyperactivity is a hallmark of immune diseases and is involved in inflammation (Perner *et al.* 2019). Many cytokines can increase receptor affinity for Janus kinases (JAKs). Activated JAK binds to signal transducers and activators of transcription, insulin receptor substrates (IRSs), and collagen protein (SHC). Insulin itself can activate JAK2. The additionof siRNA against JAK2 (siJAK2) which can reduce JAK2 protein expression by 75%, proves that when JAK2 is decreased, it is significantly correlated with a decrease in insulin-mediated cell proliferation (Thirone *et al.* 2006). Nuclear Factor-Kappa B (NF-κB) is a family of dimeric transcription factors consisting of 5 proteins, namely p65 or RelA, RelB, c-Rel, p50 and p52 (Collins *et al.* 2016). NF-B activation can be stimulated by TNFα molecules. TNF will bind with TNF receptors. Then signal transduction occurs when the TNF receptor has been activated. Due to the TNF-TNFR interaction then IκB undergoes IκB phosphorylation. Phosphorylation of IκB leads to degradation. When IκB is degraded, NF-B dimers (e.g., p65/p50 or p50/p50 subunit combinations are possible) and translocate to the nucleus, where it binds by consensus to the DNA sequence of the target DNA and activates gene expression such as genes, involved in pro-inflammation including TNFα (Albensi 2019; Fig. 3). Based on the prediction results, SCFA can target the g-protein coupled free fatty acid receptor, namely FFAR2. This prediction result is obtained from the interaction results of STITCH DB 5.0 (http://stitch.embl.de/) with a minimum required interaction score (high confidence 0.7) on the *Homo sapiens* model. The prediction results are then used as the basis for protein – protein interaction (PPI) with the STRING DB version 11.5 webserver (https://string-db.org/).

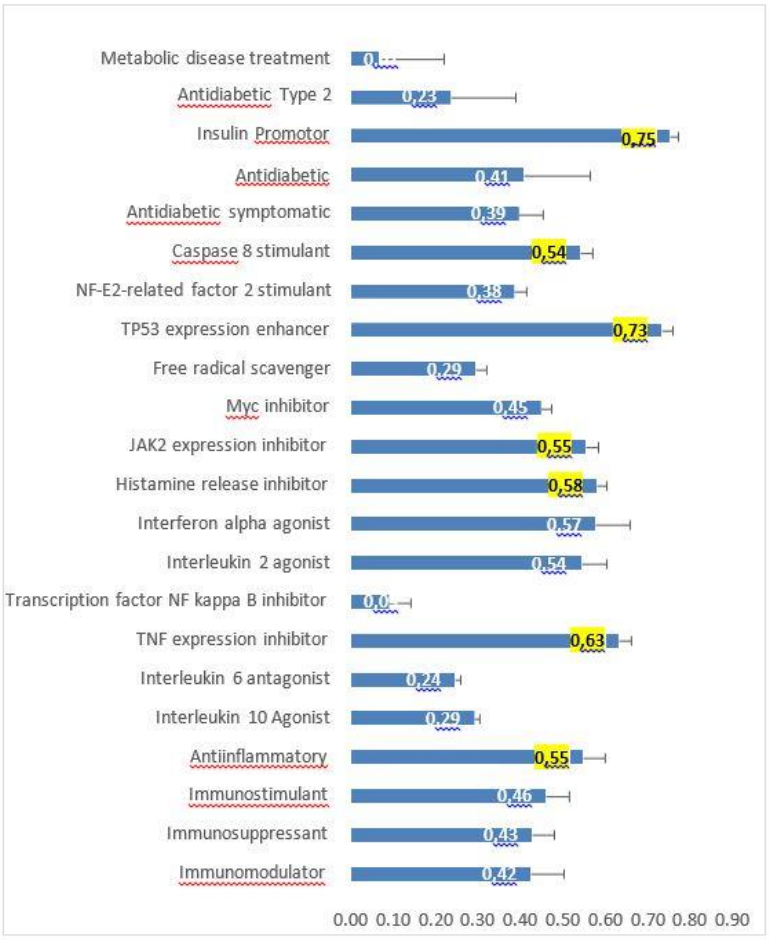

Fig. 2. Prediction Results of QSAR Way2 Drug Pass Server.

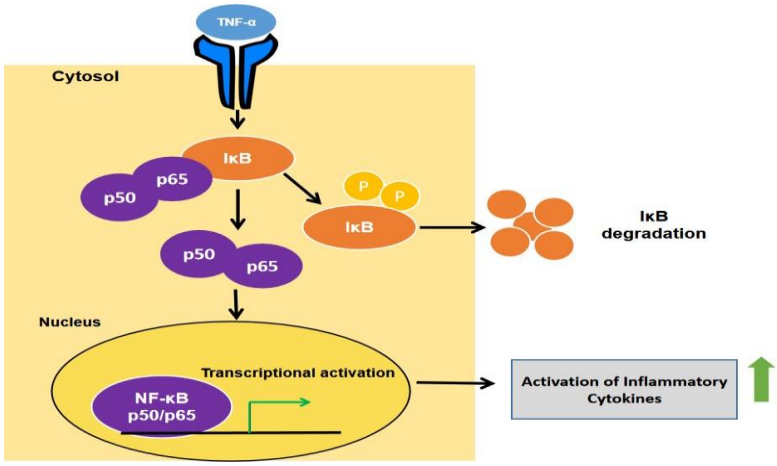

Fig. 3. Schematic of NF-Kappa B activation by TNF stimulus.

The results from STRING DB are then processed using CytoScape. Each color represents a specific mechanism that has been described in Table 1 (Fig. 4). FFAR2 is a target protein of SCFA which is predicted to facilitate interaction with proteins involved in insulin regulation and immune response. GPR41/FFAR3 and GPR43/FFAR2 are activated by short-chain fatty acids. FFAR has been studied as a target for novel drugs to treat metabolic disorders, such as obesity and type 2 diabetes, since this receptor protein is involved in energy metabolism in various tissues including adipose, intestinal, and immune tissues.

Table 1. Results of GOlorize analysis with CytoScape.

Description	P-value	Corrected P- Value	Gene
Regulation of inflammatory response	0.0000197	0.0007320	JAK2 TNF IL2
Induction of apoptosis	0.0008050	0.0052500	JAK2 TP53 TNF
Regulation of response to stress	0.0008350	0.0052600	JAK2 TNF IL2
Regulation of insulin secretion	0.0000042	0.0006170	IRS1 JAK2 TNF
Regulation of glucose import	0.0001620	0.0019700	IRS1 TNF
Regulation of transport	0.0001950	0.0021500	IRS1 TNF

Note: Bold words: prediction of the target of the compound.

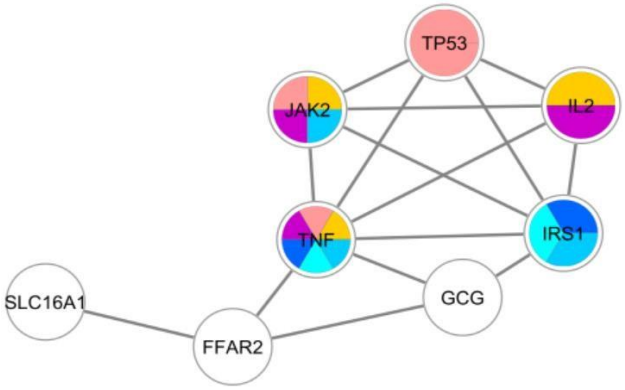

Fig. 4. Results of PPI analysis with GOlorize on CytoScape.

CONCLUSION

1. Dadiah compound has potential as a diabetes treatment, because the QSAR results show potential as an insulin promoter (0.75),

2. The pathogenesis of type 2 diabetes mellitus (T2DM) and the development of insulin resistance is one of the causes of reactive oxygen species (ROS), epigenetic factors, and activation of pro- inflammatory cytokines. QSAR results show the role of dadiah metabolites as anti-inflammatory (0.55). This score is supported by the potency of dadiah as a TNF expression inhibitor (0.63), Histamine Release Inhibitor (0.58), and JAK2 expression inhibitor (0.55). TNF-alpha is one of the pro-inflammatory mediators involved in the pathogenesis of T2DM (Akhas *et al.* 2018). High histamine levels have been reported to be associated with diabetic long-term complications (Pini *et al.* 2016). JAK-signaling hyperactivity is a hallmark of immune diseases and is involved in inflammation (Perner *et al.* 2019). Many cytokines can increase receptor affinity for Janus kinases (JAKs). Activated JAK binds to signal transducers and activators of transcription, insulin receptor substrates (IRSs), and SHC. Insulin itself can activate JAK2.

3. Dadiah compound acts as a TP53 expression enhancer (0.73). P53 is a key molecular node instress responses including inflammation. It is involved in inflammation, cell cycle arrest, apoptosis, DNA repair, and cellular senescence.

4. Dadiah affects the expression of JAK2, TNF, TP53, and IL2. JAK2, TNF and IL2 are involved in the inflammatory response, and also the stress response, while JAK2 TP53 and TNF are involved in apoptosis. TNF and JAK2 are also involved in insulin secretion. Furthermore TNF is involved in the regulation of glucose import and transport based on Gene Ontology analysis using GOlorize onCytoScape.

5. FFAR2 is a target protein of SCFA which is predicted to facilitate interaction with proteins involved in insulin

regulation and immune response. FFAR has been studied as a target for novel drugs to treat metabolic disorders, such as obesity and type 2 diabetes, since this receptor protein is involved in energy metabolism in various tissues including adipose, intestinal, and immune tissues.

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