

Prospects of red passion fruit seeds (*Passiflora edulis* Sims.) as a source of halal probiotics

Iif Hanifa Nurrosyidah¹, Ni Made Mertaniasih², Isnaeni^{3*}

¹Faculty of Health Sciences, Universitas Anwar Medika, Sidoarjo 61263, East Java, Indonesia;

²Department of Medical Microbiology, Faculty of Medicine, Airlangga University, Mayjen Prof. Dr. Moestopo 6-8, Surabaya 60268, East Java, Indonesia

³Faculty of Health Science, Universitas Muhammadiyah Surabaya, Jl. Sutorejo No.59, Surabaya 60113, East Java, Indonesia.

Corresponding author: isnaeni@um-surabaya.ac.id

ABSTRACT

Indonesia is the most biodiversity country in the world, with a majority Muslim population, so halal issues are a priority. Natural resources are abundant, especially the tropical fruit that Allah SWT bestowed with a wide variety. Red Passion fruit (*Passiflora edulis* Sims.) is one of the tropical fruits widely consumed because of its delicious taste, rich in vitamins, minerals, and other active ingredients. The research reported that probiotics of *Bacillus* spp. Isolated from the red passion fruit seeds was proven to inhibit pathogenic microbes. Extended Strain Beta-Lactamase and Methicillin Resistant *Staphylococcus aureus*. The isolates also showed the ability to increase gamma interferon levels in the peripheral blood mononuclear cells in adult TB patients. Halal critical points for products containing probiotics can derive from raw material sources, especially those from animals. The Red passion fruit is classified as a safe, halal category. The process of isolating probiotics from red passion fruit seeds using fermentation technology in De man Rogose Sharp media, using simple materials, tools, and steps guaranteed to be halal. The compatibility of two probiotic isolates is the potential to be developed into a probiotic consortium for health supplement preparations and therapeutic complements.

Keywords: Halal Probiotics, *Passiflora edulis* Sims, Red Passion Fruit Seeds

INTRODUCTION

The world's Muslim population spread across various countries is a promising potential market. The total Muslim population is estimated at 1.6 billion of the total population in the world. The 2013 Thomson Reuters report reports that the amount of food consumed by the world's Muslim population reached around USD 1.088 billion in 2012, or about 16.6 percent of total global consumption. Global Muslim consumption is expected to grow to USD 1.626 billion in 2018, or around 17.4 percent of total world consumption (Kurniadi & Frediansyah, 2016). Its population is increasing, and the purchasing power of Muslims worldwide and a new modernization era reinforce the increasingly halal trend of Islamic lifestyle and trade.

Indonesia is one of the countries with the largest Muslim population in the world, so the halal issue is very important. Today's halal lifestyle trend in Indonesia consists of the food sector halal, Islamic finance, halal travel, halal clothes or clothing, halal media and recreation, halal medicines, halal cosmetics, and halal medical treatment (hospitals) (Adinugraha & Sartika, 2019). Based on the State of the Global Islamic report, the latest Economy is 2017/2018, which reports that halal food companies can become a halal lifestyle with opportunities to expand into the nutrition sector. The Halal lifestyle is a new pattern for the 1.6 billion Muslim population. The global market must respond to unique market needs, preferences, orientations, values, and economic trade (Ambali & Bakar, 2014).

Indonesia is a country with the most significant biodiversity in the world. One of the indigenous fruits is *Passiflora edulis* Sims. Passion fruit (*Passiflora edulis*), a member of the *Passifloraceae* family, has more than 500 species (dos Reis et al., 2018). This plant originates in Brazil and has spread to other countries in Asia, Australia, Africa, India, South America, and the Caribbean. Passion fruit has different variants that can be identified from the color of the fruit (dos Reis et al., 2018), such as yellow (*P. edulis* var. *Flavicarpa*), purple or red (*P. edulis* var. *edulis*), and orange (*P. edulis* var. *Caerulea*).

Passion fruit contains many secondary metabolites, including flavonoid glycosides (Ingale and Hivrale, 2010), luteolin-6-C-echinacoside, luteolin-6-C-fucoside, cyanogenic glycosides passiflorin,

epipassibiflorin, passicapsin, passicoriacin, epipassicoriacin, cyanogenic-b- rutoside, epitetraphilin B, amygdalin, prunasin, triterpenoid glycosides, and salicylic glycosides. Other chemical compounds such as b-carboline alkaloids harman, harmine, harmaline, harmalol, phenol, carotene, and g-lactone are also found in passion fruit (Barnes et al., 2007). Passion fruit has high nutritional value and contains many multi-minerals such as magnesium, phosphorus, various vitamins, carbohydrates, and water (Zibadi & Watson, 2004). Passion fruit has adequate nutritional content, so it is a suitable habitat for developing probiotic bacteria. Passion fruit, rich in health benefits, has yet to be further developed in the pharmaceutical field. At the same time, passion fruit can be grown as a source of halal probiotics. Therefore, in this study, the isolation of probiotics from red passion fruit flesh was carried out.

Probiotics are live microorganisms (most bacteria) that, if consumed, can provide health benefits by maintaining and improving the balance of microorganisms in the gut (Zendeboodi et al., 2020). Probiotics can produce various compounds that inhibit the growth of pathogenic bacteria, including organic acids (lactic acid and acetic acid), bacteriocins, and re-uterine. Organic acids lower pH, which can affect the growth of pathogenic bacteria and can also be toxic to pathogenic microbes (Pundir et al., 2013). Oral administration of probiotics enhances the immune system through interaction with intestinal epithelial cells (IECs) or immune cells associated with the lamina propria via Toll-like receptors (TLRs) and induces the production of various cytokines or chemokines. Macrophage chemoattractant protein 1, produced by IECs, sends signals to other immune cells leading to mucosal immune system (MIS) activation, characterized by increased immunoglobulin A+ cells in the intestine, bronchi, and breast. Glands, and T cell activation. Specifically, probiotics activate regulatory T cells that release IL-10. Probiotics strengthen the intestinal barrier by increasing mucin and Paneth cells, thereby modulating the good microbiota in the gut by suppressing the growth of potentially pathogenic bacteria in the gut. Therefore, the interaction of probiotics with IEC, macrophages, and dendritic cells (DC) plays an essential role in this immune response without causing an inflammatory pattern (Maldonado Galdeano et al., 2019).

Natural probiotics, including passion fruit, can be found in the digestive tract of humans and animals, vegetables, and fruits (Halim & Zubaidah, 2013). Probiotics can also be isolated from the digestive tract of animals and human and animal feces. The source of probiotics still needs to be evaluated again for the halal aspect.

RESEARCH METHOD

Materials

The materials used were red passion fruit pulp (*Passiflora edulis* Sims.) obtained from the Krembung area (Sidoarjo-East Java), which had been identified by Herbarium Malangensis (University of Malang), media de Men Rogosa Sharpe Broth (MRS-B), de Men Rogosa Sharpe Agar (MRS-Agar), triple sugar iron agar (TSA) media, Sulfide Indole Motility (SIM) media, Methyl-Red Voges-Proskauer (MR-VP) media, Simmons citrate media, Kovac's reagent, methyl red indicator, gram stain test material (crystal violet, Lugol's iodine, safranin, 95% alcohol, and distilled water), H₂O₂, and indole reagent.

Methods

Isolation of probiotic candidate bacteria from red passion fruit (*Passiflora edulis* Sims.) seeds by preparing fresh passion fruit. Passion fruit was split into two parts, and the contents (seeds) were weighed as much as 5 grams, then suspended in 45 mL of sterile 0.9% NaCl, and shaken using a shaker for 24 hours at room temperature. Passion fruit suspension that has been shaken with a shaker for 24 hours, pipette 1 mL suspended in 9 mL of 0.9% NaCl (10⁻¹ dilution obtained). Dilutions were made up to 10⁻¹⁰. Each dilution series (10⁻¹ to 10⁻¹⁰) was pipetted 1 mL, grown on MRS-Agar and 1% CaCO₃ media, and then incubated at 37⁰C for 24 – 48 hours. Colonies with morphological forms, such as lactic acid bacteria, are characterized by a clear zone around the colony. The probiotic candidate bacteria were purified using the quadrant method after obtaining pure bacterial isolates inoculated on slanted MRS-Agar media. The culture is ready for further testing (biochemistry, Genotype identification, probiotic character testing, and compatibility test). Analysis of halal parameters regarding probiotic sources, isolates obtained, equipment, and substrates used.

RESULT AND DISCUSSION

Table 1. Identification results of 16S rRNA probiotic isolates from red passion fruit (Nurrosyidah et al., 2020).

Sample	Homolog (% Identity)	Identity
MM1	97,44	<i>Bacillus subtilis</i> strain IAM 12118
MM2	85,80%	<i>Bacillus wiedmannii</i> strain FSL W8-0169
MM3	100	<i>Bacillus cereus</i> ATCC 14579

Based on previous studies, it has been proven that red passion fruit contains *Bacillus spp* probiotics which have activity against resistant pathogenic bacteria such as Extended Strain Beta-Lactamase and Methicillin Resistant *Staphylococcus aureus* (Nurrosyidah et al., 2022) and also showed the ability to increase gamma interferon level in the peripheral blood mononuclear cells in adult TB patients, have compatibility with each of these probiotic isolates, as well as being susceptible to erythromycin and vancomycin (Nurrosyidah et al., 2020).

The next halal critical point is the place or equipment to isolate the probiotics. In this study, the process of isolating probiotics from red passion fruit seeds using fermentation technology in de man rogosa sharpe media, using simple materials, tools, and steps guaranteed to be halal. All equipment and places used are clean and sterile to ensure that all processes and places or equipment used are halal.

The substrate used was de man rogosa sharpe media. De man rogosa sharpe media is a bacterial growth medium selective for the lactic acid bacteria (LAB) group. LAB are a group of Gram-positive bacteria capable of producing organic acids. Some of its member species are the microbiota of the human digestive tract, and several species act as probiotics. The composition of the media is peptone, yeast extract, lablamco powder, glucose, sorbitan mono-oleate, Dipotassium hydrogen phosphate, triammonium citrate, magnesium sulfate 7H₂O, magnesium sulfate 4H₂O, agar, distilled water. The substrate used doesn't contain animals that are forbidden or have blood, so it can be categorized as halal.

CONCLUSION

Halal critical points, which include sources of probiotics isolates obtained, substrates, and places or equipment used, are known to be halal. In this study, the metabolites produced by probiotics have not been identified. For further research, it is suggested to analyze the metabolites of probiotics, and the probiotics have the potential to be developed into relevant probiotic preparations.

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