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IDENTIFICATION OF PHYSIOLOGICAL AND BIOCHEMICAL PROPERTIES OF LACTIC ACID BACTERIAL ISOLATES FROM SNOW LOTUS (*Saussuera involucrate)* HEALTH DRINK

Identifikasi Sifat Fisik dan Biokimia Isolat Bakteri Asam Laktat dari Minuman Kesehatan Teratai Salju (Saussuera involucrate)

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ABSTRACT

Snow lotus is a 72-hour fermented health drink from a palm sugar solution with snow lotus (*Saussuera involucrate*) without using other additives. Fermentation of snow lotus into lactic enzymes and active lactic acid bacteria is to help maintain the body in prime condition, establish and strengthen the immune system and is beneficial for overall health. Based on previous research, 33 isolates of lactic acid bacteria had been isolated from snow lotus health drink. From the isolates that had been successfully isolated, potential for lactic acid bacteria has no known properties as a probiotic candidate. Therefore research is necessary to obtain lactic acid bacteria isolates from snow lotus health drinks which are potential as probiotic isolates. The purpose of this study was to identify the physiological properties of the snow lotus health drink which included gram staining, observation of the shape of the colonies and cell shapes, and catalase testing and also to identify the biochemical properties which included growth at a variety of different temperatures, growth ability at low pH, growth on media containing salt or NaCl, production of CO_2 from glucose, and production of dextran from sucrose. Based on the physiological properties, results showed that all 33 LAB isolates from the snow lotus health drink had negative catalase properties, positive gram staining in the form of single and chain rods, and also in the form of coccus, where 13 isolates were coccus and 20 were rod-shaped isolates. Based on the biochemical properties, from the 33 isolates, almost all LAB isolates were able to grow at temperatures of 10°C, 25°C, 37°C, 45°C, pH 2, 4, 6, and media containing salt concentrations of 4% and 6.5 %, 19 isolates were able to produce CO_2 from glucose, and 15 isolates were able to produce dextran from sucrose.

Keywords : Snow lotus, Isolate, Lactic acid bacteria, Physiological properties, Biochemical properties

ABSTRAK

Teratai salju merupakan minuman kesehatan hasil fermentasi selama 72 jam dari larutan gula aren dengan teratai salju (Saussuera involucrate) tanpa zat tambahan lainnya. Fermentasi teratai salju ini menjadi enzim laktat dan bakteri asam laktat aktif untuk menjaga kondisi tubuh tetap prima, membentuk dan memperkuat daya tahan tubuh serta bermanfaat bagi kesehatan tubuh secara menyeluruh. Berdasarkan penelitian sebelumnya telah berhasil diisolasi 33 isolat bakteri asam laktat dari minuman kesehatan teratai salju. Dari isolat yang berhasil diisolasi tersebut belum diketahui potensi bakteri asam laktat yang memiliki sifat-sifat sebagai kandidat probiotik. Untuk itu perlu dilakukan penelitian untuk memperoleh isolat bakteri asam laktat dari minuman kesehatan teratai salju yang berpotensi sebagai isolat probiotik. Tujuan penelitian ini adalah untuk mengidentifikasi sifat fisik dari minuman kesehatan teratai salju yang meliputi pewarnaan gram, pengamatan bentuk koloni serta bentuk sel, dan uji katalase serta mengidentifikasi sifat biokimia yang meliputi pertumbuhan pada berbagai suhu yang berbeda, produksi CO_2 dari glukosa, produksi dekstran dari sukrosa, dan pertumbuhan pada media yang mengandung garam atau NaCl. Berdasarkan sifat fisik dari minuman

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kesehatan teratai salju, hasil penelitian menunjukkan bahwa 33 isolat BAL yang diuji semua memiliki sifat katalase negatif, pewarnaan gram positif dengan bentuk sel batang baik tunggal maupun batang berantai dan kokus dimana 13 isolat berbentuk kokus dan 20 isolat berbentuk batang. Berdasarkan sifat biokimia, 33 isolat bakteri asam laktat dari minuman teratai salju hampir seluruh isolat BAL mampu tumbuh pada suhu 10°C, 25°C, 37°C, 45°C, pH 2, 4, 6, serta konsentrasi garam fisiologis 4% dan 6,5%, 19 isolat mampu memproduksi CO_2 dari glukosa, dan 15 isolat mampu memproduksi dekstran dari sukrosa.

Kata kunci: *Teratai salju, Isolat, Bakteri asam laktat, Sifat fisik, Sifat Biokimia*

INTRODUCTION

Snow lotus health drink is one of the health drinks made from snow lotus (*saussuera involucrate*). The benefits of this health drink is to cure various diseases and strengthen body health (Darumus *et al*., 2015). Snow lotus is a health drink fermented for 72 hours from a solution of palm sugar with snow lotus without using other additives (Sulistiani and Zen, 2015). The fermentation of snow lotus into lactic enzymes and active lactic acid bacteria helps to maintain a healthy body, form and strengthen the body's resistance, and maintain body homeostatis. Consumption of snow lotus has many benefits such as curing heart disease, lower cholesterol levels, maintaining normal blood pressure, curing stomach and uric acid, lower levels of diabetes, helps in digestion process, and many other benefits (Anon., 2015).

From the results of previous studies, 33 isolates of lactic acid bacteria were successfully isolated from the snow lotus health drink (Arihantana and Sugitha, 2018). From the 33 isolates of lactic acid bacteria that were isolated, the potential of these isolates as probiotic candidates as not been known yet. Lactic acid bacterial isolates which have probiotic properties has many benefits and can be used to treat disease problems, especially in the digestive tract.

Lactic acid bacteria with probiotic characteristics, can generally be isolated from foods that is processed by fermentation, whereas snow lotus drink is a product processed through fermentation. Fermentation using lactic acid bacteria is widely used in food fermentation. Based on these findings, it is necessary to identify the physiological and biochemical properties of lactic acid bacteria isolated from snow lotus health drink so that lactic acid bacteria isolates which have properties as probiotic candidates can be obtained.

METHODS

The main materials used in this study were 33 isolates of lactic acid bacteria from snow lotus drink. This study consisted of two stages, the first stage: identifying the physiological properties of the lactic acid bacterial isolates from snow lotus health drink such as gram staining, observing colony and cell shape, and catalase test, the second stage: identifying the biochemical properties of the lactic acid bacterial isolates from snow lotus health drink such as growth at a variety of different temperatures, growth ability at low pH, growth on media containing salt or NaCl, production of CO_2 from glucose, and production of dextran from sucrose.

Gram Stainning (Harrigan and McCance, 1998; Tankeshwar, 2015)

Add one ose of sterile water on the glass slide then add one loop of isolate and spread the culture evenly with an inoculation loop. Air-dry the culture and fix it or over a gentle flame, while moving the slide in a circular fashion to avoid localized overheating. Add crystal violet stain over the fixed culture. Let stand for 60 seconds or 1 minute, pour off the stain and gently rinse the excess stain with a stream of

water from a faucet or a plastic water bottle. Add the lugol solution on the smear, enough to cover the fixed culture. Let stand for 2 minutes then pour off the lugol solution and rinse the slide with running water. Shake off the excess water from the surface. Tilt slide and decolorize with solvent (acetone-alcohol solution) until purple colour stops running. Wash immediately (within 10-20 seconds) with water and shake off excess. Add a few drops of safranine. Let stand for 1 - 2 minutes, wash briefly with water and shake off excess.

Observation of Bacterial Form (Harrigan and McCance, 1998)

Gram positive preparations obtained from Gram stainning, are then continued with observation of microbial forms. Microbes in the form of coccus, streptococcus, short rod or bacillus, long bacillus, diplobacillus and Y-shaped bacillus (or bone shape) are continued for the next test.

Catalase Test (Harrigan and McCance, 1998; Aryal, 2018)

Use a loop or sterile wooden stick to transfer one ose of bacterial colony in the surface of a clean, dry glass slide. Place two drops of 3% H_2O_2 in the glass slide and mix, observe for the evolution of oxygen bubbles. A positive result is the rapid evolution of oxygen (within 5-10 seconds) as evidenced by bubbling. A negative result is no bubbles or only a few scattered bubbles.

Growth at Different Temperatures (Nuraida, 1988)

This test aims to determine the ability to grow lactic acid bacteria at different temperatures. One drop of lactic acid bacterial culture was inoculated into a tube containing MRSB medium. Lactic acid bacterial culture inoculation was made in 4 series of tubes, then each tube series was incubated for 7-14 days at 10^{o}C, 15^{o}C, 30^{o}C and 45^{o}C (4 days). The control treatment was incubated at 37^{o}C. The presence of growth is characterized by turbidity in the tube.

Growth Ability at Low Ph (Modified from Chou and Weimer, 1999; Zavaglia *et al.*, 1998)

Probiotic bacteria must be able to live and survive in the intestines in order to continue to function for health. The upper gastrointestinal tract has a low acidity (pH) condition, which varies between 2 and 4. The food consumed will be in the stomach for about 90 minutes to 2 hours and will reach the intestines in the range of 7 to 9 hours. Based on this, the lactic acid bacteria which have the potential as probiotics will be tested by growing isolates on media that have been regulated for their acidity conditions according to the acidity conditions in the human digestive tract.

Growth on Media Containing Salt or NaCl (Nuraida, 1988)

This test aims to determine the ability of LAB growth on media containing high salt content. Salt tolerance (4% and 6.5% NaCl) is commonly used to differentiate between enterococci or vagococci, and streptococci. MRS broth medium added with 6.5% NaCl was inoculated with 1 ose inoculum. The culture was then incubated at 37°C for 5 days. Positive results are indicated by the formation of turbidity and sedimentation.

Production of CO_2 From Glucose (Modified from Nuraida, 1988; Harrigan and McCance, 1998)

The media was put in a 10 ml test tube and sterilized. Before the media is used, the temperature is lowered to 45°C. Add approximately 0.5 ml of lactic acid bacterial isolate that had been grown in MRSB for 24 hours and then pour the liquid agar over it about 2-3 cm to create anaerobic conditions. Incubation was carried out at 37°C for 2-5 days.

Production of Dextran From Sucrose (Nuraida, 1988)

This test is conducted to differentiate species from the Leuconostoc genus. Sucrose agar (SA) medium for 1 liter is prepared by mixing 10 g trypton; 5 g yeast extract; 5 g K_2HPO4; 5 g triammonium citrate; 50 g

sucrose; and 15 g bacto. The medium is then sterilized at 121°C for 15 minutes, then distributed in sterile plates.

RESULT AND DISCUSSION

The data from the observed parameters which include Gram stainning, observation of cell shape, and catalase test are shown in Table 1.

Gram Stainning

Lactobacillus is the largest genus of the family *Lactobacillaceae.* According to Widyastuti and Sofarinawati (1999), lactic acid bacteria (LAB) in general are Gram positive and catalase negative. According to Hutkins (2006) LAB is classified into *Lactobacillus, Lactococcus, Leuconostoc, Oenococcus, Pediococcus, Streptococcus, Tetragenococcus, Weisella, Carnobacterium, Aerococcus, Enterococcus,* and *Vagococcus.*

All LAB isolates used were Gram positive bacteria. Based on the composition of the cell walls, bacteria are divided into 2 groups, Gram positive and Gram negative bacteria. For Gram positive bacteria, 90% of the cell wall consists of a peptidoglycan layer, while the other thin layer is teichoic acid. For Gram negative bacteria, only 5-20% of their cell wall consists of a peptidoglycan layer, while the other layers are composed of proteins, lipopolysaccharides, and lipoproteins.

Cell Shape and Morphology

The morphology of the LAB isolates of snow lotus are of single rod or bacillus and in forms of chains and coccus. Of the 33 isolates, 13 isolates were coccus, such as RTS 1, RTS 2, RTS 5, RTS 13, RTS 14, RTS 15, MTS 4, MTS 6, MTS 8, MTS 9, TS 1, TS 2, and TS 5 while 20 isolates were bacillus, such as RTS 3, RTS 4, RTS 6, RTS 7, RTS 8, RTS 9, RTS 10, RTS 11, RTS 12, MTS 1, MTS 2, MTS 3, MTS 5, MTS 7, TS 3, TS 4, TS 6, TS 7, TS 8, and TS 9. The isolates in the form of coccus can be seen in Figure 1 (Isolate RTS 1) while the isolate in the form of bacillus can be seen in Figure 2 (Isolate MTS 2).

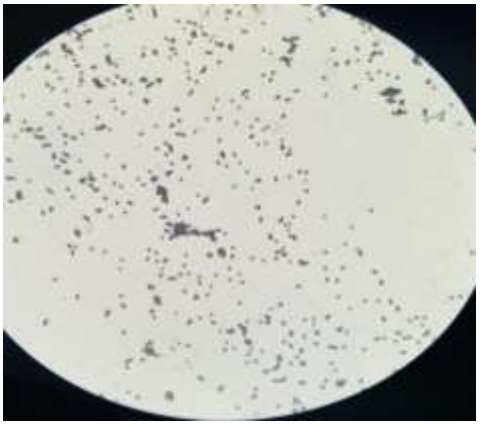

Figure 1. Coccus shape LAB

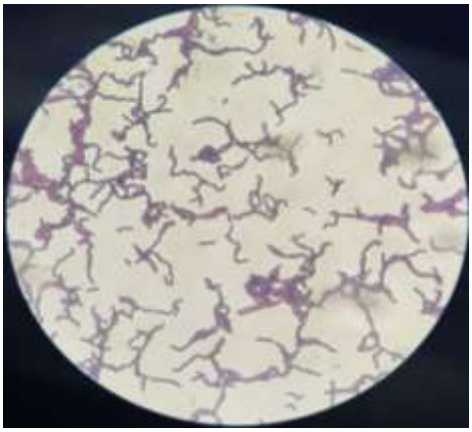

Figure 2. Bacillus shape LAB

Catalase Test

Based on Table 1, the results of the catalase test showed that 33 isolates isolated from the snow lotus health drink were catalase negative bacteria. This can be seen when the isolate was added with H_2O_2 solution, there were no bubbles formed. This showed that the isolates from these bacteria did not produce the catalase enzyme which would convert H_2O_2 into water and oxygen.

The data from the observed parameters which include growth at a variety of different temperatures, growth ability at low pH, growth on media containing salt or NaCl, production of

Table 1. Gram Stainning, Observation of Cell Shape, and Catalase Test From Snow Lotus Isolates

No.	ISOLAT CODE	GRAM STAIN	CELL SHAPE	CATALASE TEST
1	RTS 1	Positive (+)	Coccus	Negative (-)
2	RTS 2	Positive (+)	Coccus	Negative (-)
3	RTS 3	Positive (+)	Bacillus	Negative (-)
4	RTS 4	Positive (+)	Bacillus	Negative (-)
5	RTS 5	Positive (+)	Coccus	Negative (-)
6	RTS 6	Positive (+)	Bacillus	Negative (-)
7	RTS 7	Positive (+)	Bacillus	Negative (-)
8	RTS 8	Positive (+)	Bacillus	Negative (-)
9	RTS 9	Positive (+)	Bacillus	Negative (-)
10	RTS 10	Positive (+)	Bacillus	Negative (-)
11	RTS 11	Positive (+)	Bacillus	Negative (-)
12	RTS 12	Positive (+)	Bacillus	Negative (-)
13	RTS 13	Positive (+)	Coccus	Negative (-)
14	RTS 14	Positive (+)	Coccus	Negative (-)
15	RTS 15	Positive (+)	Coccus	Negative (-)
16	MTS 1	Positive (+)	Bacillus	Negative (-)
17	MTS 2	Positive (+)	Bacillus	Negative (-)
18	MTS 3	Positive (+)	Bacillus	Negative (-)
19	MTS 4	Positive (+)	Coccus	Negative (-)
20	MTS 5	Positive (+)	Bacillus	Negative (-)
21	MTS 6	Positive (+)	Coccus	Negative (-)
22	MTS 7	Positive (+)	Bacillus	Negative (-)
23	MTS 8	Positive (+)	Coccus	Negative (-)
24	MTS 9	Positive (+)	Coccus	Negative (-)
25	TS 1	Positive (+)	Coccus	Negative (-)
26	TS 2	Positive (+)	Coccus	Negative (-)
27	TS 3	Positive (+)	Bacillus	Negative (-)
28	TS 4	Positive (+)	Bacillus	Negative (-)
29	TS 5	Positive (+)	Coccus	Negative (-)
30	TS 6	Positive (+)	Bacillus	Negative (-)
31	TS 7	Positive (+)	Bacillus	Negative (-)
32	TS 8	Positive (+)	Bacillus	Negative (-)
33	TS 9	Positive (+)	Bacillus	Negative (-)

Information:
RTS : LAB isolate Rogosa Teratai Salju
MTS : LAB isolate deMann Rogosa Sharpe Agar Teratai Salju
TS : LAB isolate Teratai Salju

CO_2 from glucose, and production of dextran from sucrose are shown in Table 2.

Growth at a Variety of Different Temperatures and Growth on Media Containing Salt or NaCl

The temperatures used to determine the growth ability of LAB in snow lotus health drinks were 10ºC, 25ºC, 37ºC, and 45ºC, while the physiological salt concentrations used were 4% and 6.5%. In Table 2, it is shown that from 33 isolates of lactic acid bacteria obtained, the growth ability at various temperature levels were quite high. Almost all LAB isolates (31 isolates) were able to grow at 10°C, 32 isolates were able to grow at 45°C, while all snow lotus health drink isolates were able to grow at 25ºC and 37ºC. The growth temperature range of lactic acid bacteria were wide starting from 10°C to 45°C. Based on the results, the isolates

Table 2. Growth at a Variety of Different Temperatures, Growth Ability at Low pH, Growth on Media Containing Salt or NaCl, Production of CO_2 From Glucose, and Production of Dextran From Sucrose.

No.	Isolate Code	Temperature				pH			NaCl		CO_2	Dextran
		10°C	25°C	37°C	45°C	2	4	6	4%	6,5%		
1	RTS 1	+	+++	++++	-	++	+++	++++	++	+	++	+
2	RTS 2	+	++	++	+	+	++	++	+	+	-	+
3	RTS 3	+	++	+++	++	+	+	+++	+	+	+++	+
4	MTS 1	+	++	++	+	+	++	++	+	+	+	-
5	MTS 2	++	+++	+++	+	++	++	+++	++	+	-	-
6	RTS 4	+	++++	++++	++	+++	++++	++++	+++	++	+++	-
7	RTS 5	+	++++	++++	+	++	++++	++++	++++	++	-	-
8	MTS 3	+	++	++	+	+	++	++	++	+	+++	-
9	RTS 6	++	++++	++++	++	++	++++	++++	+++	++++	-	+
10	RTS 7	+	++++	++++	++	++	+++	++++	+++	++++	+++	-
11	RTS 8	++	++++	++++	++	++	++++	++++	+++	++++	++	-
12	RTS 9	+	++++	+++	++	+	++++	+++	++	++	++	-
13	MTS 4	++	++++	++++	+++	++	++++	++++	++++	++++	+	+
14	MTS 5	++	++++	+++	++	++	++++	+++	++++	+++	-	+
15	MTS 6	+	++++	++++	+++	+	++++	++++	++	++	-	-
16	RTS 10	+	+++	+++	++	++	+++	+++	+	++	++	+
17	RTS 11	++	++++	++++	++	++	++++	++++	+++	++	-	+
18	MTS 7	+	++++	++++	+++	++	+++	++++	+++	+++	-	+
19	RTS 12	-	+++	+++	+	-	++++	+++	+++	++	++++	+
20	MTS 8	++	++++	++++	++	+	++	++++	++	++	-	-
21	RTS 13	+	++++	+++	++	+	++	+++	+++	+++	-	-
22	RTS 14	+	++++	+++	++	++	++++	+++	++	++	-	-
23	MTS 9	++	++++	++++	+++	++	++++	+++	++	++	+	-
24	TS 1	+	++	++	++	+	++	++	++	++	++++	-
25	TS 2	+	++	++	++	+	+	++	++	++	+++	+
26	TS 3	+	+++	++	++	++	+++	++	++	++	++++	-
27	TS 4	+	++++	++++	+++	++	+++	++++	++++	++	-	+
28	TS 5	+	++++	++	++	++	+	++	++	++	++	+
29	TS 6	+	++++	++++	+++	++	++++	++++	+++	++	-	+
30	TS 7	+	++++	+++	+++	++	++++	+++	+++	+++	+++	+
31	RTS 15	+	++++	+++	+++	++	+	+++	+++	++	++++	-
32	TS 8	+	++++	++++	+++	++	++++	++++	+++	+++	-	-
33	TS 9	-	++	++	++	-	++	++	++	++	++++	-

wew able to grow in cold and hot temperatures. From 33 LAB isolates, all isolates were able to grow at salt concentrations of 4% and 6.5%.

Growth Ability at Low pH

The resistance of isolates at low pH (2, 4, and 6) is needed to determine the ability of isolate cultures to withstand gastric conditions such as pH. The resistance of the isolates to low pH was indicated by a decrease in the growth of

the isolates after incubation in MRSB media which had been adjusted to pH 2.0; pH 4.0; and pH 6.0 at 37°C for 24 hours. The results can be seen in Table 2.

Based on Table 2, from 33 isolates it can be seen that almost all isolates were able to survive at pH 2 (31 isolates). The lower the pH value, growth of the isolates became less. Not only do most lactic acid bacteria grow more slowly at low pH, but acid damage and loss of cell viability may also occur in celss held at low pH (Hutkins and Nanned, 1993). All isolates were able to grow at pH 4 and pH 6.

Production of CO_2 From Glucose

The results from Table 2 indicates that from 33 lactic acid bacterial isolates, 19 isolates were able to produce CO_2 after incubation in media containing glucose. The ability of lactic acid bacteria to produce CO_2 indicates that lactic acid bacteria is from a heterofermentative group. Based on the production of their metabolite compounds, lactic acid bacteria are divided into 2 groups, homofermentative and heterofermentative. According to Fardiaz (1989), one of the important classification of lactic acid bacteria is the ability to ferment glucose. Glucose is frequently used as a carbon source in lactic acid fermentation. Homofermentative lactic acid bacteria can convert overall glucose into lactic acid, while heterofermentative lactic acid bacteria has the ability to ferment glucose into lactic acid, ethanol or acetic acid, and CO_2.

Production of Dextran From Sucrose

Among 33 isolates of lactic acid bacteria, 18 isolates were unable to produce dextran from sucrose media while 15 isolates were able to produce dextran. The ability of lactic acid bacteria to produce dextran indicates that these bacteria belong to the *Leuconostoc* genus.

Leuconostoc mesenteroides are round in pairs or form short chains. *Leuconostoc mesenteroides* produces an extracellular enzyme called dextransucrase which converts glucose from sucrose molecules to dextran and releases fructose into the environment. The result is therefore the formation of a glucose subunit polymer bound in the alpha 1-6 position. The glucose subunits are added to glucose at the start of the primary sucrose molecule so that each dextran molecule has one terminal sugar fructose sub-unit. Dextran is used commercially as a blood plasma extender.

Dextran is a polymer of glucose which is very important in the pharmaceutical industry as an ingredient in drug formulations as well as in the food industry as a thickening agent. In dextran fermentation, sucrose is the main carbon source which will be converted into dextran by the enzyme Dextransucrase (Raniamrhn, 2013).

CONCLUSION

From the result of the study it can be concluded that:

Based on the physiological properties, lactic acid bacteria that have been isolated are catalase negative, Gram positive, the morphology were single rod or bacillus and in forms of chains and coccus. 13 isolates were coccus while 20 isolates were bacillus.

Based on the biochemical properties, almost all LAB isolates were able to grow at 10ºC, 25ºC, 37ºC, and 45ºC, pH 2, 4, 6, and salt concentrations of 4% and 6.5%. Of 33 LAB isolates, 19 isolates were able to produce CO_2 from glucose and 15 isolates were able to produce dextran from sucrose.

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