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Isolation, Characterization and Screening of Functional Properties Probiotic Candidates for Lactic Acid Bacteria Bali Traditional Drink Wong Tea in Vitro

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Abstract

One of the functional foods in Bali, especially in Gianyar Regency, is teh wong (mushroom), which has a sweet-sour taste. The purpose of the study was to determine the characteristics of probiotic candidates isolated from the fermented tea wong drink and to determine the LAB of Teh Wong which could potentially be probiotic candidates. The samples used were 20 lactic acid bacteria (LAB) isolates from wong tea. The process of isolating the LAB candidate used the spread plate method from the fermented liquid of Teh Wong for 7 days. Isolation was carried out after the plates were incubated for 24 hours at 37°C. Colonies suspected of being LAB were then streaked on new De Man, Rogosa and Sharpe (MRS) Agar media to obtain a single colony. The total LAB produced from LAB isolates ranged from 3.0 x 105 cfu/ml to 7.0 x 108 cfu/ml. Of the 20 isolates from gram staining, it was found that the LAB Teh Wong isolate was dominated by rods (bacilli) and only 4 isolates were spherical (coccus). The results of the catalase test on 20 isolates growing on mannitol salt agar (MSA) showed that all isolates (bacteria) showed a negative reaction and those that formed gas were classified as the heterofermentative type and those that did not form gas were classified as the homofermentative type. The LAB isolates of wong tea were able to survive at pH 2 which had a BAL viability of 45.5-62.5. The highest antibacterial activity for E. coli was isolate TW28 with an inhibition zone of 9.6 mm (medium category) and the highest antibacterial activity for S. aureus was isolate TW28 with an inhibition zone of 12 mm (strong category). All BAL isolates from Teh Wong have very strong antioxidant activity, except for sample code TW29.

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Contents

1 Introduction

Wong tea is one of the traditional Balinese drinks which has a distinctive taste and aroma, namely sweet and sour taste. Wong tea is made from sugar water (sweet tea water) which is fermented using a starter from the fungus (wong) tuak. This tuak fungus (wong) lives in water containing sugar to sustain its life and is usually reared in sweet tea water (Scherf et al., 2011; Sarı et al., 2020). This fermented water is called wong tea which can be consumed directly. Fermentation will turn the sugar into alcohol and then the bacteria will turn the alcohol into acid, then the sweet tea water turns into a slightly sweet sour. This mushroom tea is usually consumed by the people of Guwang Sukawati as a drink. Research Antarini et al. (2022), that the total LAB ranges from 5.8 x 103 to 5.1 x 104 cfu/g. Based on this, the researchers intended to explore, isolate and identify the functional properties of probiotic candidates from lactic acid bacteria from wong tea in vitro.

2 Materials and Methods

The samples used were 20 LAB isolates from Wong tea which were stored in glycerol stock at -20°C. The isolation process for LAB candidates used the spread plate method from the fermented liquid of Teh Wong which had been fermented for 7 days. Isolation was carried out after the spread plates were incubated for 24 hours at 37°C. Colonies suspected to be LAB were then streaked on new MRS Agar media to obtain single colonies.

Total LAB analysis with spread plate

Total bacteria was determined by surface method (Fardiaz, 1992). A total of 100 µl of sample was put into a microtube containing 900 µl of physiological saline solution (0.85% NaCl), so that a 10-1 dilution was obtained, then shaken until homogeneous, then 100 µl pipetted and put into a microtube which already contained 900 µl physiological saline solution, so that a 10-2 dilution is obtained, and so on to get a larger dilution.

From the desired dilution, 100 µl was pipetted into a petri dish containing the bacteria, then grown into the MRS agar medium which had been previously prepared, 60 ppm bromcresol purple (BCP) was added as an acidity indicator. Then, spread over the entire surface of the media (spread plate method) with a bent glass rod. The planted petri dishes were then placed upside down in the incubator and incubated at 37°C for 24 hours (Hur et al., 2011; Witte et al., 2006).

LAB colonies will appear as colonies surrounded by a yellow zone, then isolated and scratched on MRS media in order to obtain a single colony. The single isolate was then stored in a glycerol solution with a final concentration of 15% and stored as culture stock at -20°C for working cultures made in the form of broth/agar (Sujaya et al., 2008; Suardana et al., 2007; Nuryadi et al., 2013; Nur et al., 2015).

Observation and count population (total bacteria)

Calculation of the total number of bacteria was obtained by means of the number of colonies per cup times 1/dilution factor. Confirmation of LAB isolates, catalase test and Gram stain were performed. One drop of the culture above is dripped over a 10% H2O2 solution. Positive catalase is indicated by the formation of air bubbles (Kozaki et al., 1992 in Sujaya et al., 2008). Gram staining is done to see the shape of the cells and the nature of the Gram. Formation of gas from glucose is carried out by a hot loop which is characterized by the formation of foam (Sperber & Swan, 1976 in Sujaya et al., 2008).

Identification of microbes by Gram Stain Test

One ose of pure isolate was grown on MRS Broth medium and then incubated at 37oC for 24 hours. Then a Gram stain test was carried out, such as drops with crystal violet, Lugol, alcohol and safranin. After the staining process with the reagent is complete, the preparate glass is then heated using a Bunsen until the preparate layer becomes dry. After drying, look at the microscope (Corry et al., 1995; Eloff, 1998).

Test turbidity with a spectrophotometer

One ose of pure isolate was grown on MRS Broth medium and then incubated at 37oC for 24 hours. Pipette 2 ml into a cuvette for analysis on a UV-Vis spectrophotometer. Then measured OD (Optical Density) with a spectrophotometer (absorbance = 660 nm)

Catalase test

The catalase test was carried out by making a smear of LAB isolate on the surface of the object glass, adding 2 drops of 10% H2O2 and observing the gas bubbles formed on the preparation. Positive results are indicated by the formation of oxygen gas bubbles by the catalase enzyme (Agestiawan et al., 2014).

Gas production test results of glucose metabolism

The hot loop needle is inserted into the BAL isolate suspension. Positive results are indicated by the formation of carbon dioxide gas as a result of glucose metabolism. Homofermentative LAB gave negative results in this test, while heterofermentative LAB showed positive results in this test (Sujaya et al., 2008).

Antibacterial activity testing

All pure bacterial isolates were tested for their anti-bacterial activity by disc diffusion method. The pathogenic bacteria tested were E. coli and S. aureus (concentration 105 – 106 CFU/ml). The test uses Nutrient Agar (NA) media as a non-selective medium, the pathogenic bacteria are first spread on the agar surface until evenly distributed, then let stand for 10 minutes.

Disc diffusion paper that had been incubated in MRSB media for 24 hours containing probiotic candidates, was then placed on agar media along with negative control treatment. Then it was incubated for 24 hours at 37ºC. The diameter of the clear zone formed around the well was measured as the zone of inhibition of lactic acid bacteria against pathogenic bacteria (Uriot et al., 2017; Behbahani et al., 2019).

Testing of LAB Resistance to Low pH

Prepare 5 ml of MRS Broth media in a screw tube whose pH has been adjusted by adding HCl (0.1 N and 0.5 N) until pH 2, 3 and 4 are obtained using a pH meter. The media whose pH has been adjusted are then sterilized using an autoclave, after sterilization is complete and the media has cooled, LAB isolate is added with a volume of 50 µl. Furthermore, the media that had been suspended with LAB were incubated for 24 hours at 37oC. The results were observed based on the turbidity formed in the media, measured using a UV-Vis spectrophotometer with an absorbance value of 600 nm (Nocianitri, 2011; Agestiawan, 2014).

Testing the Antioxidant Activity of LAB tea wong

The probiotic strain was grown in MRS broth and incubated at 37oC for 24 hours. Probiotic cells were harvested by centrifugation at 5000 g for 10 minutes at 4oC and washed with 20 mM sodium phosphate buffer (PBS; 0.85% NaCl, 2.86 mM KCl, 10 mM Na2HPO4, and 1.76 mM KH2PO4, pH 7) 2 times. The probiotic cell pellet was added with 20 mM sodium phosphate buffer pH 7, the probiotic cell was then destroyed with an ultrasonic cell disrupter in an ice bath. Cell debris was separated by centrifugation at 10000 x g for 10 min at 4oC. and filtered (0.45 m, Millipore) (Kim et al., 2006b). The protein concentration for each extract (1 mg/ml) was determined by the Bradford method.

3 Results and Discussions

Total Lactic Acid Bacteria (LAB)

The isolation process for LAB candidates used the spread plate method from the fermented liquid of Teh Wong which had been fermented for 7 days. Isolation was carried out after the spread plates were incubated for 24 hours at 37oC. Colonies suspected to be LAB were then streaked on new MRS Agar media in order to obtain a single colony. (see table 1)

Table 1
Total LAB Population of Wong's Tea Drink

Sample Code	LAB population (cfu/ml)
314	10.0×10^5
324	3.0×10^5
334	7.0×10^8
344	6.0×10^8

Table 1 shows the total LAB of Wong tea ranging from 3.0 x 105 cfu/ml to 7.0 x 108 cfu/ml. Analysis of total LAB using selective media for LAB in the form of MRS Agar. From the results obtained, not all of them are LAB. From all samples analyzed, there were colonies in the form of molds and yeasts that appeared. The presence of colonies in the form of molds and yeasts was successfully observed with a microscope from several colonies that were suspected of not being bacterial colonies. Research conducted by Wijonarko et al. (2021), reported that in sap fermentation (starter for making Wong's Tea), the total colonies of molds and yeasts ranged from 55 – 65 x 102 cfu/ml.

Catalase Test and Gas Test

The catalase test is one of the stages in the morphological characterization of bacteria. This process is used to differentiate several catalase positive species which are pathogens, such as Staphylococcus sp. (Toelle, 2014). Positive catalase is indicated by the appearance of gas bubbles when the bacterial colony is dripped with H_2O_2.The results of the analysis did not find any bacterial colonies which were catalase positive, so that all LAB have the potential to become probiotic candidates. In addition, further tests in the form of gas testing using a hot loop found that the majority were heterofermentative bacteria (Vitali et al., 2012; Vinderola et al., 2008).

The results of the catalase test on 20 isolates growing on MSA in this study found that all isolates (bacteria) showed a negative reaction (Table 2). Negative catalase test results show no air bubbles, which means no oxygen gas is formed (Romadhon et al., 2012 in Febrianti et al., 2016).

Catalase is an enzyme that catalyzes the breakdown of hydrogen peroxide into H2O and O2. Hydrogen peroxide is toxic to cells because it inactivates enzymes in cells. Hydrogen peroxide is formed during aerobic metabolism, so microorganisms that grow in an aerobic environment must decompose this material (Lay, 1994).

Table 2
Catalase Test, Turbidity and Gas Test on LAB Isolates

Isolate Code	Catalase Test	Growth Media Turbidity	Gas Test
TW1	-	++	GG
TW2	-	+++	GG
TW3	-	+++	GG
TW6	-	++	GG
TW7	-	+++	GG
TW8	-	+++	GG
TW9	-	++	GG
TW10	-	+++	GG
TW14	-	++	G
TW15	-	++	-
TW16	-	+++	GG
TW17	-	+++	-
TW18	-	+	-
TW22	-	++	GG
TW23	-	+++	G
TW24	-	++	GG
TW25	-	+++	G
TW27	-	++	-
TW28	-	++	GG
TW29	-	+++	G

Note:
+: slightly cloudy media; ++: cloudy medium; +++: very cloudy media
-: no gas is generated; G: slightly raised gas; GG: lots of gas

The gas test was carried out using the hot loop method (Sperber & Swan, 1976), a hot loop needle was inserted into the MRS Broth medium containing BAL culture suspension. Positive results are indicated by the formation of CO2 gas in the form of bubbles in the culture media, this CO2 gas is the result of glucose metabolism (Suryani et al., 2012). From these results, isolates that form gas are classified as heterofermentative types and those that do not form gas are classified as homofermentative types (Apriyantono, 1989).

In the tea wong bacterial isolates, there were several isolates that did not grow on the MRS broth media which were indicated by no growth/turbidity in the media, so the isolates were not tested for further tests. The bacterial isolates that did not grow were caused because the bacteria isolated were not from the LAB group, because MRS media was a selective medium specifically for the LAB group. (see table 3)

Table 3
LAB Isolate Code of Wong Tea Strain that does not grow

Isolate Code	Turbidity in the Media
TW4	-
TW5	-
TW11	-
TW12	-
TW13	-

TW19	-
TW20	-
TW21	-
TW26	-

Note: (-): no turbidity/growth

In Tables 2 and 3, from a total of 29 isolates of Teh Wong bacteria, only 20 isolates were obtained which belonged to the Lactic Acid Bacteria group (there was turbidity/growth on the MRS media) and 9 isolates were not alive which were indicated by the absence of turbidity in the media so they were not continued for the test further probiotic potential. In the Gas Test on LAB isolates, 16 isolates produced gas (heterofermentative) and 4 isolates did not produce gas (homofermentative).

Gram stain

From the isolation process, there were 29 LAB isolates that showed negative results on the catalase test and Gram positive there were 20 isolates that were round and rod-shaped. (see table 4)

Table 4
Results of Gram Staining LAB from Wong's Tea Fermentation

No	Strain Code	Bacterial Morphology	Gram Staining
1.	TW1	Bacillus Panjang	Gram +
2.	TW2	Bacillus Panjang	Gram +
3.	TW3	Bacillus Panjang	Gram +
4.	TW6	Bacillus Panjang	Gram +
5.	TW7	Bacillus Pendek	Gram +
6.	TW8	Bacillus Panjang	Gram +
7.	TW9	Bacillus Pendek	Gram +
8.	TW10	Bacillus Pendek	Gram +
9.	TW14	Bacillus Panjang	Gram +
10.	TW15	Coccus (Belum Single Colony)	Gram +
11.	TW16	Bacillus Panjang	Gram +
12.	TW17	Coccus	Gram +
13.	TW18	Bacillus Panjang	Gram +
14.	TW22	Bacillus Panjang	Gram +
15.	TW23	Bacillus Pendek	Gram +
16.	TW24	Bacillus Pendek	Gram +
17.	TW25	Bacillus Panjang	Gram +
18.	TW27	Coccus	Gram +
19.	TW28	Bacillus Panjang	Gram +
20.	TW29	Bacillus Panjang	Gram +

Based on the results of gram staining, it was found that the LAB isolate Teh Wong was dominated by rods (bacilli) and only 4 isolates were spherical (coccus). In general, the genus of bacteria that has been well identified as a probiotic is the genus Lactobacillus. According to Sneath (1986), in Rahayu et al. (2000), suggested that the LAB group which was rod-shaped, gram-positive and catalase-negative were LAB from the genus Lactobacillus. From the results of the gram staining and catalase test, it can be assumed that the rod-shaped isolates can be classified from the genus Lactobacillus. However, to provide final results, further testing is needed with molecular or biochemical characterization.

LAB Resistance Test to low pH

The LAB isolates that were successfully identified/characterized were then tested for their resistance at low pH. Testing at low pH aims to see whether a LAB has the potential to become a probiotic candidate (Zinatullina et al., 2021). One of the requirements for probiotics is that LAB must be able to survive at low pH, especially at the pH of the digestive tract, such as pH 4, pH 3 and pH 2. In this study all LAB isolates showed resistance at low pH (Table 8). As shown in the table above, all LAB can be classified as resistant to low pH because the final OD value is > 0.01.

Table 5
Resistance of LAB Isolates at pH 2

pH	Code	Final OD	Early OD	Final Control OD	Early Control OD	Viability LAB %
2	TW1	0.202	0.157	0.217	0.118	45.4545
	TW2	0.175	0.152	0.186	0.136	46.0000
	TW3	0.192	0.145	0.227	0.131	48.9583
	TW6	0.187	0.144	0.224	0.136	48.8636
	TW7	0.178	0.147	0.204	0.136	45.5882
	TW8	0.159	0.142	0.164	0.132	53.1250
	TW9	0.196	0.156	0.217	0.134	48.1927
	TW10	0.185	0.142	0.222	0.134	48,8636
	TW14	0.198	0.146	0.252	0.140	46.4285
	TW15	0.185	0.142	0.238	0.153	50.5882
	TW16	0.184	0.142	0.231	0.148	50.6024
	TW17	0.142	0.125	0.186	0.149	45.9459
	TW18	0.184	0.128	0.249	0.148	55.4455
	TW22	0.187	0.147	0.231	0.145	46.5116
	TW23	0.188	0.145	0.234	0.141	46.2365
	TW24	0.159	0.124	0.200	0.144	62.5000
	TW25	0.162	0.148	0.163	0.133	46.6666
	TW27	0.146	0.132	0.154	0.130	58.3333
	TW28	0.158	0.146	0.166	0.146	60.0000
	TW29	0.154	0.147	0.147	0.134	53.8461

Table 6
Resistance of LAB Isolates at pH 3

pH	Code	Final OD	Early OD	Final Control OD	Early Control OD	Viability LAB %
3	TW1	0.193	0.138	0.217	0.118	55.5555
	TW2	0.211	0.185	0.186	0.136	52.0000
	TW3	0.187	0.143	0.227	0.131	45.8333
	TW6	0.199	0.156	0.224	0.136	48.8636
	TW7	0.184	0.145	0.204	0.136	57.3529
	TW8	0.156	0.136	0.164	0.132	62.5000
	TW9	0.177	0.136	0.217	0.134	49.3975
	TW10	0.188	0.147	0.222	0.134	46.9090
	TW14	0.191	0.139	0.252	0.140	46.4285
	TW15	0.183	0.114	0.238	0.153	81.1764
	TW16	0.189	0.147	0.231	0.148	50.6024
	TW17	0.142	0.115	0.186	0.149	72.9729
	TW18	0.179	0.109	0.249	0.148	69.3069

	TW22	0.198	0.135	0.231	0.145	73.2558
	TW23	0.224	0.176	0.234	0.141	51.6129
	TW24	0.158	0.124	0.200	0.144	60.7142
	TW25	0.148	0.131	0.163	0.133	56.6667
	TW27	0.131	0.118	0.154	0.130	54.1667
	TW28	0.182	0.169	0.166	0.146	65.0000
	TW29	0.148	0.140	0.147	0.134	61.5384

Table 7
Resistance of LAB isolates at pH 4

pH	Code	Final OD	Early OD	Final Control OD	Early Control OD	Viability LAB %
5	TW1	0.182	0.127	0.217	0.118	55.5555
	TW2	0.194	0.165	0.186	0.136	58.0000
	TW3	0.166	0.119	0.227	0.131	48.9583
	TW6	0.168	0.126	0.224	0.136	47.7272
	TW7	0.185	0.152	0.204	0.136	48.5294
	TW8	0.138	0.117	0.164	0.132	65.6250
	TW9	0.172	0.129	0.217	0.134	51.8072
	TW10	0.176	0.121	0.222	0.134	62.5000
	TW14	0.189	0.128	0.252	0.140	54.4642
	TW15	0.161	0.118	0.238	0.153	50.5882
	TW16	0.177	0.124	0.231	0.148	63.8554
	TW17	0.148	0.114	0.186	0.149	91.8918
	TW18	0.165	0.106	0.249	0.148	58.4158
	TW22	0.192	0.150	0.231	0.145	48.8372
	TW23	0.181	0.126	0.234	0.141	59.1397
	TW24	0.168	0.124	0.200	0.144	78.5714
	TW25	0.148	0.131	0.163	0.133	56.6667
	TW27	0.132	0.118	0.154	0.130	58.3333
	TW28	0.168	0.158	0.166	0.146	50.0000
	TW29	0.137	0.128	0.147	0.134	69.2307

According to Hutkins & Nannen (1993), an organism can survive in a low pH environment by maintaining its internal pH condition relatively higher than the pH of its environment. This mechanism is carried out by activating the ATPase enzyme, resulting in energy that can be used to translocate protons from inside the cell to outside the cell, resulting in an increase in pH in the cell cytoplasm (Chou & Weimer, 1999). The results shown in table 5, 6 and 7, that all LAB isolates from Teh Wong have the potential to be developed as probiotic candidates, because they meet one of the requirements that probiotic candidates must have resistance at low pH.

Antibacterial Activity of Wong Tea Isolate against E. coli and S. aureus

Antibacterial testing on LAB is one of the requirements that must be met to become a probiotic candidate. The results shown in Table 8 stated that all of the Teh Wong BAL isolates had fairly good antibacterial activity against pathogenic bacteria (E. coli and S. aureus). Antibacterial activity in probiotic candidates occurs in various ways, such as total acid accumulation, bacteriocin production and various antibacterial compounds produced. The accumulation of these compounds in cells occurs because lactic acid bacteria do not produce the catalase enzyme (Salminen & Wright, 1993).

Table 8
Antibacterial activity of LAB against E. coli and S. Aureus

Code	The average diameter of the zone	
	E. coli	*S. aureus*
TW1	9.33	9.66
TW2	8.33	8.00
TW3	8.66	9.33
TW6	8.66	9.66
TW7	8.66	9.66
TW8	8.33	9.00
TW9	8.33	9.00
TW10	8.33	8.66
TW14	8.00	8.33
TW15	8.66	9.00
TW16	8.33	9.00
TW17	8.00	8.33
TW18	9.33	9.66
TW22	9.00	9.33
TW23	8.33	8.66
TW24	8.66	9.33
TW25	8.33	9.33
TW27	8.00	10.00
TW28	9.66	12.00
TW29	8.00	8.33

Note: The average diameter of the inhibition zone is 10 – 20 mm which means very strong, 5 – 10 mm means medium and < 5 mm means weak

Table 8 shows LAB isolates of Teh Wong generally have moderate category of antibacterial activity. The highest antibacterial activity for E. coli was isolate TW28 with an inhibition zone of 9.6 mm (moderate category) and the highest antibacterial activity for S. aureus was isolate TW28 with an inhibition zone of 12 mm (strong category). Bacterial isolate 28 had the strongest antibacterial activity against E. coli and S. aureus compared to the other isolates. The results of this antibacterial activity test, it was found that all LAB isolates from Teh Wong had the potential to be developed as probiotic candidates, because they met the requirements that probiotic candidates must have antibacterial activity to suppress pathogenic bacteria in the host gastrointestinal tract.

Antioxidant Activity of BAL Isolates

Testing of antioxidant activity using the DPPH method with an assessment indicator in the form of IC50 value. The IC50 value is the effective concentration of BAL isolate needed to suppress 50% of DPPH (free radicals) (Tristantini et al., 2016). The IC50 value of the LAB isolate from Teh Wong can be seen in table 9.

Table 9
IC50 value of BAL isolates using the DPPH method

Sample Code	Sample Weight	Absorbance	IC_{50} (mg/ml)
TW1	0.1049	0.485	207.926
TW2	0.1040	0.470	25.1064
TW3	0.1055	0.508	14.4258
TW6	0.1047	0.480	22.2010
TW7	0.1052	0.437	33.8108
TW8	0.1043	0.459	28.0570

TW9	0.1036	0.457	28.7998
TW10	0.1057	0.473	23.8891
TW14	0.1064	0.473	23.7319
TW15	0.1030	0.489	20.0631
TW16	0.1057	0.475	23.3468
TW17	0.1058	0.491	18.9903
TW18	0.1043	0.480	22.2862
TW22	0.1053	0.484	20.9858
TW23	0.1037	0.460	27.9429
TW24	0.1055	0.480	22.0327
TW25	0.1035	0.503	16.0892
TW27	0.1045	0.492	18.9523
TW28	0.1047	0.475	23.5698
TW29	0.1058	0.353	56.3749

Note: IC50 value < 50 indicates strong antioxidant activity

The free radical that is commonly used in measuring the capture power of free radicals is 1,1-diphenyl-2-pikrihidazyl (DPPH). Free radicals are very unstable and very reactive, that is, they tend to react with other molecules to achieve stability. These free radicals with high reactivity can start a chain reaction at a time when they are formed, causing abnormal compounds and starting chain reactions that can damage important cells in the body (Badarianth et al., 2010). Free radicals can be overcome by using antioxidants (Mandal et al., 2009).

One potential source of antioxidants in today's era apart from the use of natural ingredients, can also be obtained from probiotics. One of the requirements for BAL isolates to be said as probiotic candidates is that they must have good functional properties for the host. Antioxidants are one of the functional properties that can provide good benefits for the host, especially in suppressing the rate of free radicals. Based on the results shown in Table 9, all BAL isolates from Teh Wong had very strong antioxidant activity, except for the isolate with the code TW29. The BAL isolate code TW3 has the highest IC50 value. In the antioxidant analysis with the IC50 indicator, the smaller the value of the number of samples needed to suppress 50% of free radical activity, the better. So from the results of the antioxidant test, as many as 19 LAB isolates have the potential to be developed into probiotic candidates.

4 Conclusion

From the results of the gram staining and catalase test, it can be assumed that the rod-shaped isolates can be classified from the genus Lactobacillus. And 20 LAB isolates from met the requirements that probiotic candidates must have resistance at low pH. All LAB isolates from Teh Wong have the potential to be developed as probiotic candidates, because they meet the requirements that probiotic candidates must have antibacterial activity to suppress pathogenic bacteria in the host gastrointestinal tract.

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References

Agestiawan, I. G. A. M., Swastini, D. A., & Ramona, Y. (2014). Uji Ketahanan Bakteri Asam Laktat yang Diisolasi dari Kimchi Terhadap pH Rendah. *Jurnal Farmasi Udayana*, *3*(2), 279858.

Antarini, A. A. N., Agustini, N. P., & Mataram, I. K. A. (2002). The Effect of Using Various Tea Types on the Characteristics of Wong Tea Balinese Tradisional Beverage. *International Research Journal of Engineering, IT and Scientific Research*, *8*(2), 36-48.

Antarini,N., N.P. Agustini., S. Puryana, K. Wiardani dan A. Malongi. 2018. Identification of microbes, chemical and organoleptic characteristic toward The Wong during Fermentation. Indian Journal oh Public Health Research and Development 9 (5) 378 – 382.

Apriyantono, A., Fardiaz, D., Puspitasari, N. L., & Sedarnawati, S. B. (1989). Analisis Pangan. PAU Pangan dan Gizi.

Badarinath, A. V., Rao, K. M., Chetty, C. M. S., Ramkanth, S. T. V. S. R., Rajan, T. V. S., & Gnanaprakash, K. (2010). A review on in-vitro antioxidant methods: comparisions, correlations and considerations. *International Journal of PharmTech Research*, *2*(2), 1276-1285.

Behbahani, B. A., Noshad, M., & Falah, F. (2019). Inhibition of Escherichia coli adhesion to human intestinal Caco-2 cells by probiotic candidate Lactobacillus plantarum strain L15. *Microbial Pathogenesis*, *136*, 103677. https://doi.org/10.1016/j.micpath.2019.103677

Chou, L. S., & Weimer, B. (1999). Isolation and characterization of acid-and bile-tolerant isolates from strains of Lactobacillus acidophilus. *Journal of Dairy Science*, *82*(1), 23-31. https://doi.org/10.3168/jds.S0022-0302(99)75204-5

Corry, J. E., Post, D. E., Colin, P., & Laisney, M. J. (1995). Culture media for the isolation of campylobacters. *Progress in industrial microbiology*, *34*, 129-162. https://doi.org/10.1016/S0079-6352(05)80011-6

Eloff, J. N. (1998). Which extractant should be used for the screening and isolation of antimicrobial components from plants?. *Journal of ethnopharmacology*, *60*(1), 1-8. https://doi.org/10.1016/S0378-8741(97)00123-2

Fardiaz, S. (1992). Petunjuk Laboratorium Mikrobiologi Pengolahan Pangan. *Departemen Pendidikan dan Kebudayaan Direktorat Jenderal Pendidikan Tinggi Pusat Antar Universitas Pangan dan Gizi Institut Pertanian Bogor. Bogor*.

Febrianti, A. N., Suardana, I. W., & Suarsana, I. N. (2016). Ketahanan Bakteri Asam Laktat (BAL) Isolat 9A Hasil Isolas dari Kolon Sapi Bali terhadap pH Rendah dan Natrium Deoksikolat (NaDC). *Indonesia Medicus Veterinus*, *5*(5), 415-421.

Hur, S. J., Lim, B. O., Decker, E. A., & McClements, D. J. (2011). In vitro human digestion models for food applications. *Food chemistry*, *125*(1), 1-12. https://doi.org/10.1016/j.foodchem.2010.08.036

Hutkins, R. W., & Nannen, N. L. (1993). Ph homeostasis in lactic acid bacteria1. *Journal of Dairy Science*, *76*(8), 2354-2365.

Kim, D. H., & Austin, B. (2006). Cytokine expression in leucocytes and gut cells of rainbow trout, Oncorhynchus mykiss Walbaum, induced by probiotics. *Veterinary immunology and immunopathology*, *114*(3-4), 297-304. https://doi.org/10.1016/j.vetimm.2006.08.015

Mandal, S., Yadav, S., Yadav, S., & Nema, R. K. (2009). Antioxidants: a review. *Journal of chemical and pharmaceutical research*, *1*(1), 102-104.

Nocianitri, K. A., Permana, I. D. G. M., & Sujaya, I. N. (2011). Skrining *Lactobacillus Spp*. untuk Pengembangan Probiotik Berbasiskan Susu Kedelai. *The Exellence Research 8*: 113-120

Nur, F., Hafsan, H., & Wahdiniar, A. (2015). Isolasi bakteri asam laktat berpotensi probiotik pada dangke, makanan tradisional dari susu kerbau di Curio Kabupaten Enrekang. *Biogenesis: Jurnal Ilmiah Biologi*, *3*(1), 60-65.

Nuryadi, M. M., Istiqomah, T., Faizah, R., Uabidillah, S., & Mahmudi, Z. (2013). Isolasi dan identifikasi bakteri asam laktat asal youghurt. *Journal University of Jember*, *1*(5), 1-11.

Rahayu, E.S., Purwandhani, S.N. & Harmayani E. (2000). Isolasi Lactobacillus yang Berpotensi sebagai Kandidat Probiotik. Yogyakarta: Fakultas Teknologi Pertanian, Universitas Widya Mataram dan Universitas Gadjah Mada

Romadhon, S., & Margino, S. (2012). Isolasi dan karakterisasi bakteri asam laktat dari usus udang penghasil bakteriosin sebagai agen antibakteria pada produk-produk hasil perikanan. *Jurnal Saintek Perikanan*, *8*(1).

Salminen, S., & Wright, A. V. (1993). Future aspects in research and product development on lactic acid bacteria. *Lactic acid bacteria.*, 429-432.

Sarı, A., Hekimoğlu, G., & Tyagi, V. V. (2020). Low cost and eco-friendly wood fiber-based composite phase change material: development, characterization and lab-scale thermoregulation performance for thermal energy storage. *Energy*, *195*, 116983. https://doi.org/10.1016/j.energy.2020.116983

Scherf, A. K., Zetzl, C., Smirnova, I., Zettlitzer, M., & Vieth-Hillebrand, A. (2011). Mobilisation of organic compounds from reservoir rocks through the injection of CO2—comparison of baseline characterization and laboratory experiments. *Energy Procedia*, *4*, 4524-4531. https://doi.org/10.1016/j.egypro.2011.02.409

Sneath, P. H. A. (1986). Estimating uncertainty in evolutionary trees from Manhattan-distance triads. *Systematic zoology*, *35*(4), 470-488.

Sperber, W. H., & Swan, J. (1976). Hot-loop test for the determination of carbon dioxide production from glucose by lactic acid bacteria. *Applied and Environmental Microbiology*, *31*(6), 990-991.

Suardana, I. W., Suarsana, I. N., Sujaya, I. N., & Wiryawan, K. G. (2007). Isolasi dan identifikasi bakteri asam laktat dari cairan rumen sapi bali sebagai kandidat biopreservatif. *Jurnal veteriner*, *8*(4), 155-159.

Sujaya, N., Ramona, Y., Widarini, N. P., Suariani, N. P., Dwipayanti, N. M. U., Nocianitri, K. A., & Nursini, N. W. (2008). Isolasi dan karakterisasi bakteri asam laktat dari susu kuda Sumbawa. *Jurnal Veteriner*, *9*(2), 52-59.

Suryani, Y., Andayaningsih, P., Hernaman, I., & Muharromi, U. F. (2012). Effort of increasing production of livestock feed out of cassava waste by identifying the more suitable cellulotic degrading fungi. *Asian Journal of Agriculture and Rural Development*, *2*(3), 329-336.

Toelle, N. N. (2014). Identifikasi dan Karakteristik Staphylococcus Sp. dan Streptococcus Sp. dari Infeksi Ovarium Pada Ayam Petelur Komersial (Identification and Characteristics of Staphylococcus Sp. and Streptococcus Sp. Infection of Ovary in Commercial Layers). *Jurnal Ilmu Ternak Universitas Padjadjaran*, *14*(1).

Tristantini, D., Ismawati, A., Pradana, B. T., & Jonathan, J. G. (2016). Pengujian aktivitas antioksidan menggunakan metode DPPH pada daun tanjung (Mimusops elengi L). In *Seminar Nasional Teknik Kimia Kejuangan* (p. 1).

Uriot, O., Denis, S., Junjua, M., Roussel, Y., Dary-Mourot, A., & Blanquet-Diot, S. (2017). Streptococcus thermophilus: from yogurt starter to a new promising probiotic candidate?. *Journal of Functional Foods*, *37*, 74-89. https://doi.org/10.1016/j.jff.2017.07.038

Vinderola, G., Capellini, B., Villarreal, F., Suárez, V., Quiberoni, A., & Reinheimer, J. (2008). Usefulness of a set of simple in vitro tests for the screening and identification of probiotic candidate strains for dairy use. *LWT-Food Science and Technology*, *41*(9), 1678-1688. https://doi.org/10.1016/j.lwt.2007.10.008

Vitali, B., Minervini, G., Rizzello, C. G., Spisni, E., Maccaferri, S., Brigidi, P., ... & Di Cagno, R. (2012). Novel probiotic candidates for humans isolated from raw fruits and vegetables. *Food Microbiology*, *31*(1), 116-125. https://doi.org/10.1016/j.fm.2011.12.027

Wijonarko, G., Widjojoko, T., & Sitoresmi, I. (2021). Kajian Sifat Mikrobiologis Nira Kelapa Segar Dari Desa Gandatapa Kecamatan Sumbang. *Prosiding*, *10*(1).

Witte, F., Fischer, J., Nellesen, J., Crostack, H. A., Kaese, V., Pisch, A., ... & Windhagen, H. (2006). In vitro and in vivo corrosion measurements of magnesium alloys. *Biomaterials*, *27*(7), 1013-1018. https://doi.org/10.1016/j.biomaterials.2005.07.037

Zinatullina, A. M., Melnik, V. P., & Zimina, A. A. (2021). Personal factors of biological aging in working and non-working respondents in late maturity period. *International Journal of Health Sciences*, *5*(3), 639–648. https://doi.org/10.53730/ijhs.v5n3.2790

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