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Appendix A

Colour of bee bread collected from three stingless bee species from four places in Malaysia

Sangai Merab, Kajang/ Selangor (SM)	<i>Itama</i> - Yellow Brown	
	<i>Thorasica</i> – Yellow Brown Orange	
Kedah (K)	<i>Itama</i> - Yellow	

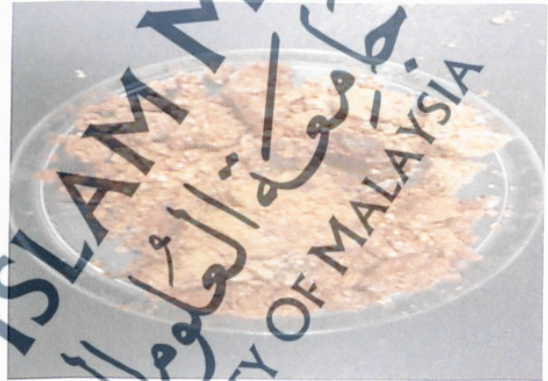
Terengganu (T)

Itama - Brown



Negeri Sembilan
(NS)

Thorasica - Yellow



Itama - Yellow



Terminata - Yellow



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Appendix B

Growth inhibition zone of target bacteria by LAB isolated from bee bread determined by dual agar overlay method

LAB isolates	Pathogenic bacteria		
	<i>S. typhimurium</i> (ATCC 13311)	<i>E. coli</i> (ATCC 25922)	<i>S. aureus</i> (ATCC 25923)
A-T-SM	24.1	19.5	19.6
B-T-SM	24.1	17.1	20.5
C-T-SM	24.0	17.0	21.5
D-T-SM	24.8	22.0	21.3
E-T-SM	29.8	20.1	25.8
F-T-SM	23.1	21.0	21.6
G-T-NS	36.6	30.6	16.3
H-T-NS	37.6	34.0	17.3
I-T-NS	35.3	28.3	18.3
J-I-NS	35.6	30.6	16.3
K-I-NS	32.3	34.3	15.3
L-I-NS	31.0	35.0	14.0
M-Te-NS	35.3	36.6	17.0
N-Te-NS	36.3	34.0	17.0
O-Te-NS	34.0	39.6	18.0

□ Diameter of growth inhibitory zone was measured in mm after 24 h incubation at 37°C.

Appendix C

ANOVA table of antimicrobial activity of LAB against pathogen by overlay method

Source	DF	SS	MS	F	P
LAB	14	15.7911	1.1279	17.83	0.000
pathogen	2	36.6253	18.3127	289.49	0.000
Interaction	28	26.1236	0.9330	14.75	0.000
Error	90	5.6933	0.0633		
Total	134	84.2333			

S = 0.2515 R-Sq = 93.24% R-Sq(adj) = 89.94%

Appendix D

D1. ANOVA table of antimicrobial activity: CFS concentration and pathogen determined by microtiter plates

Source	DF	SS	MS	F	P
concentration	8	26.1625	3.27032	423.75	0.000
pathogen	2	1.0805	0.54026	70.00	0.000
Interaction	16	0.0996	0.00622	0.81	0.680
Error	1593	12.2941	0.00772		
Total	1619	39.6367			

S = 0.08785 R-Sq = 68.98% R-Sq(adj) = 68.48%

D2. ANOVA table of antimicrobial activity of CFS concentration and time determined by microtiter plates

Source	DF	SS	MS	F	P
concentration	8	26.1625	3.27032	510.49	0.000
time	1	1.4685	1.46846	229.22	0.000
Interaction	8	1.7428	0.21785	34.01	0.000
Error	1602	10.2629	0.00641		
Total	1619	39.6367			

S = 0.08004 R-Sq = 74.11% R-Sq(adj) = 73.83

Appendix E

PH and bile acid tolerance of LAB isolated from bee bread determined by using Bio-photometer at OD_{600nm} *

LAB Isolates	24 hours incubation at 37°C					
	The acidity of MRS broth (pH)			bile- salt concentration (%)		
	2 pH	3 pH	4 pH	0.1%	0.3%	0.5%
A-T-SM	0.16±0.007	0.58±0.01	4.6±0.07	7.5±0.07	3.9±0.21	3.9±0.42
B-T-SM	0.59±0.30	0.66±0.02	4.2±0.07	7.2±0.07	5.1±0.14	4.8±0.21
C-T-SM	0.20±0.007	0.67±0.02	4.4±0.07	7.2±0.07	5.1±0.14	4.5±0.42
D-T-SM	0.20±0.007	0.67±0.02	4.4±0.07	7.2±0.07	5.1±0.14	5.4±0.35
E-T-SM	0.19±0.007	0.95±0.04	4.8±0.14	7.5±0.07	5.1±0.21	4.5±0.21
F-T-SM	0.20±0.01	0.63±0.03	4.6±0.14	7.5±0.07	5.7±0.14	5.4±0.35
G-T-NS	0.20±0.02	0.21±0.007	1.8±0.07	2.7±0.07	2.1±0.07	1.7±0.21
H-T-NS	0.23±0.01	0.21±0.01	1.9±0.14	2.7±0.14	2.0±0.07	1.4±0.21
I-T-NS	0.19±0.007	0.22±0.01	1.9±0.14	2.7±0.14	2.0±0.07	1.2±0.07
J-I-NS	0.19±0.007	0.23±0.007	1.9±0.14	2.6±0.21	2.0±0.07	1.4±0.07
K-I-NS	0.19±0.01	0.21±0.01	1.8±0.14	2.8±0.14	2.0±0.07	1.3±0.07
L-I-NS	0.19±0.007	0.28±0.02	1.9±0.14	2.7±0.14	1.9±0.07	1.3±0.07
M-Te-NS	0.22±0.01	0.19±0.07	1.9±0.14	2.7±0.07	1.9±0.07	1.3±0.07
N-Te-NS	0.17±0.02	0.20±0.02	1.8±0	2.7±0.21	1.9±0.07	1.4±0.07
O-Te-NS	0.18±0.007	0.19±0	1.6±0.07	2.9±0.21	1.8±0.07	1.0±0.07

Note *Optical Density

Appendix F

F1. ANOVA table of effect of pH on survival of LAB isoaltes

Source	DF	SS	MS	F	P
LAB	14	23.843	1.7031	274.44	0.000
pH	2	133.089	66.5445	10723.38	0.000
Interaction	28	28.512	1.0183	164.09	0.000
Error	45	0.279	0.0062		
Total	89	185.723			

S = 0.07878 R-Sq = 99.85% R-Sq(adj) = 99.70%

F2. ANOVA table of survival of LAB isolates in bile-salt

Source	DF	SS	MS	F	P
LAB	14	271.131	19.3665	127.23	0.000
Bile-salt	2	61.573	30.7863	202.25	0.000
Interaction	28	19.731	0.7047	4.63	0.000
Error	45	6.850	0.1522		
Total	89	359.284			

S = 0.3902 R-Sq = 98.09% R-Sq(adj) = 96.23%

Appendix G

SPSS table of susceptibility of LAB isolates to six different antibiotics

		Sum of Squares	df	Mean Square	F	Sig.
LAB * results	(Combined)	636.532	17	37.443	2.382	.004
	Between Groups					
	Linearity	52.230	1	52.230	3.322	.071
	Deviation from Linearity	584.302	16	36.519	2.323	.006
	Within Groups	1603.468	102	15.720		
	Total	2240.000	119			

<i>Staphylococcus aureus</i> ATCC 25923	40 %	+	+	+	+	+	+	+	+	+	-	-	-	+	+	+
	30 %	+	+	+	+	+	+	+	+	+	-	-	-	+	+	+
	25 %	+	+	+	+	+	+	+	+	+	-	-	-	+	+	+
	20 %	+	+	+	+	+	+	+	+	+	-	-	-	+	+	+
	10 %	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	5 %	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Note: Growth of targeted bacteria (+); No growth of targeted bacteria (-)

Appendix I

Yahya, H. D., Z. Hassan & L. Nizam. 2016. "Antimicrobial and probiotic properties of lactic acid bacteria isolated from bee bread of Malaysian stingless bee". *Malaysian Journal of Science, Health and Technology*.

