SCREENING LACTIC ACID BACTERIA FOR ANTIMICROBIAL COMPOUND PRODUCTION

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Lactic Acid Bacteria was known as potential probiotic used in food industries and dairy products and probable to produce antimicrobial compound that inhibit variety of microorganisms. The objectives of the research are to determine the optimum condition and glucose utilization in relation to antimicrobial compound production. Two species of Lactic Acid Bacteria namely *Lactococcus* and *Lactobacillus* were used as probiotic. The Lactic Acid Bacteria were fermentated in different medium, initial substrate pH and incubation temperature for the production of antimicrobial compound. The test organisms such as *E.coli* and *Salmonella* were selected as test organisms. Amongst the two species of Lactic Acid Bacteria, *Lactococcus* produced the highest amount of antimicrobial compound than *Lactobacillus*.

Keywords: Antimicrobial compound, *Lactococcus*, *Lactobacillus*

INTRODUCTION

Lactic acid bacteria have been used for centuries in the foods and beverages industries, and are nowadays used in numerous fermentation processes. As well as silage manufacture, they are used in the manufacture of fermented dairy products, in the

production and preservation of sausages and meat, in the production of wine and in baking.

They can be characterized as Gram-positive, catalase negative, non-sporulating, non-pigmented mesophils. The tolerated temperature range is generally between 5° C and 50° C, with the optimum for most strains being about 30° C. Shape is variable, from cocci through to elongated rods. Although metabolically similar, there is a lack of DNA homology between them. The most important genera in terms of silage microbiology are the lactobacilli, which are non-motile, Gram-positive, obligate saccharolytic fermenters. They lack the pathways for nitrate reduction, for the production of catalase, and for the production of cytochromes and other pigments. They also have a complex and variable nutritional requirement, differing according to species.

MATERIALS AND METHOD

Lactic Acid Bacteria

The lactic acid bacteria consisted of two species, a gram positive chain rod and a gram positive chain coccus.

3.1.2 Fermentation Media

The fermentation media employed were BM and GYP (Fujitoshi Yanogida et al., 2005) media respectively. The compositions of both media are shown in Table 1.1 and 1.2

Table 1.1: BM medium.

INGRIDIENTS	g/L
Polypeptone	22.0
Tryptose	3.0
Bacto-liver extract	25.0

Yeast extract	4.0
Tween 80	0.11
Glucose	11.0
Fructose	3.0
DL-malic acid	1.0
MnCl2.4H2O	0.08
Filtered tomato juice	300
Deionized water	1 litre

Table 1.2: GYP medium.

INGREDIENTS	g/L
Glucose	20.0
Yeast extract	15.0
Peptone	15.0
Sodium acetate	15.0
Fructose	3.0
Salt solution	5.0
Deionized water	1 litre

3.1.3 Test Organisms

The antimicrobial activities of the extracts were tested against two bacterial species (*Escherichia coli* and *Salmonella).*

3.1.4 Microbiological Media and Chemicals

The Nutrients Agar (NA) and Niacin (SIGMA, U.S.A) was obtained from the Department of Microbiology, Faculty of Applied Sciences, UiTM, Shah Alam. All chemicals used in this study were of ANALAR grade.

METHODS

3.2.1 Sampling

All media and glassware were sterilized at 121°C for 15 minutes in a floor standing vertical autoclave. The initial substrate pH of 5.5 and 7.0 were adjusted for both media using NaOH or HCl. A total of 8 fermentation media was prepared for the experiment. All fermentation media were contained in 250ml Schott bottles and were incubated under anaerobic condition. All bottles were placed inside an incubator at 35°C and 37°C for 1-4 days respectively. 5ml sample were aseptically taken at 2 day intervals and commenced from day 5, 7, 9, 11, 13 and 15. The pH changes of samples were determined using a pH meter (Mettler Toledo). All samples were clarified by centrifugation at 5000rpm for 5 minutes using an Eppendorf centrifuge (Heraeus, Germany)

3.2.9 Bioassay

20g of nutrient agar powder (MERCK, Germany) was suspended in 1L of deionized water and mixed thoroughly. The test organisms were inoculated into Nutrient broth (NB). The inoculated broths were incubated aerobically using an orbital shaker (INNOVA 400) for 24hours. 5 ml of the stock culture of test organism was lawned to the respective surfaces. Wells were drilled into an agar by using sterilized cork barrel. Sterile media was used as negative control. Niacin used as positive control. The 60µl of clarified samples were pipetted into the remaining agars well. All the bioassay plates were incubated at room temperature. All inhibition zones were measured in millimeter (mm).

3.2.15 Determination of Static/Cidal effect

For every single types of test organism which exhibit the biggest inhibition zone, a loop of bacteria was taken from the clear zone and streaked onto new agar plates.

RESULTS

Table 1.3: pH changes for antimicrobial production in GYP and BM

Time (hour)	Temperature (°C)	pH		
		Initial	Final Fermentation Medium	
			GYP	BM
0	35	5.5	5.5	5.5
0	35	7.0	7.0	7.0
0	37	5.5	5.5	5.5
0	37	7.0	7.0	7.0
120	35	5.0	4.19	3.19
120	35	7.0	4.51	3.41
120	37	5.5	4.35	3.16
120	37	7.0	4.56	3.11
168	35	5.5	4.21	3.31
168	35	7.0	4.39	3.48
168	37	5.5	4.17	3.21
168	37	7.0	4.34	3.26
216	35	5.5	4.07	3.31
216	35	7.0	4.41	3.47
216	37	5.5	4.17	3.32
216	37	7.0	4.35	3.23
264	35	5.5	3.98	3.18
264	35	7.0	4.31	3.43
264	37	5.5	4.08	3.16
264	37	7.0	4.36	3.06
312	35	5.5	3.95	3.25
312	35	7.0	4.33	3.47
312	37	5.5	4.07	3.14
312	37	7.0	4.23	3.02
360	35	5.5	3.97	3.25
360	35	7.0	4.29	3.43
360	37	5.5	4.09	3.18
360	37	7.0	4.32	3.06

Table 1.4: Effect of antimicrobial activity against test organism (*E. coli*)

Time (hour)	Temperature (° C)	Fermentation Medium	pH (initial)	pH (final)	Inhibition zone (mm)			
					Niasin +ve control (standard)	Peptone water -ve control	Sample 1	Sample 2
120	35	GYP	5.5	4.19	**15**	**NZ**	16	14
120	35	BM	5.5	3.19	**16**	**NZ**	15	15
120	35	GYP	7.0	4.51	**15**	**NZ**	15	16
120	35	BM	7.0	3.41	**16**	**NZ**	15	20
120	37	GYP	5.5	4.35	**15**	**NZ**	18	18
120	37	BM	5.5	3.16	**16**	**NZ**	16	14
120	37	GYP	7.0	4.56	**16**	**NZ**	17	15
120	37	BM	7.0	3.11	**16**	**NZ**	13	12
168	35	GYP	5.5	4.12	**15**	**NZ**	16	17
168	35	BM	5.5	3.31	**17**	**NZ**	15	14
168	35	GYP	7.0	4.39	**17**	**NZ**	15	16
168	35	BM	7.0	3.48	**16**	**NZ**	15	15
168	37	GYP	5.5	4.17	**16**	**NZ**	16	15
168	37	BM	5.5	3.21	**15**	**NZ**	16	16
168	37	GYP	7.0	4.34	**16**	**NZ**	17	12
168	37	BM	7.0	3.26	**15**	**NZ**	14	13
216	35	GYP	5.5	4.07	**15**	**NZ**	17	18
216	35	BM	5.5	3.31	**16**	**NZ**	16	16
216	35	GYP	7.0	4.41	**17**	**NZ**	17	19
216	35	BM	7.0	3.47	**17**	**NZ**	14	15
216	37	GYP	5.5	4.17	**16**	**NZ**	19	17
216	37	BM	5.5	3.32	**17**	**NZ**	16	15
216	37	GYP	7.0	4.35	**15**	**NZ**	18	14
216	37	BM	7.0	3.23	**16**	**NZ**	13	16
264	35	GYP	5.5	3.98	**15**	**NZ**	18	19
264	35	BM	5.5	3.18	**17**	**NZ**	15	13
264	35	GYP	7.0	4.31	**17**	**NZ**	18	17
264	35	BM	7.0	3.43	**16**	**NZ**	17	19
264	37	GYP	5.5	4.08	**16**	**NZ**	16	13
264	37	BM	5.5	3.16	**16**	**NZ**	15	13
264	37	GYP	7.0	4.36	**15**	**NZ**	16	19
264	37	BM	7.0	3.06	**17**	**NZ**	17	15
312	35	GYP	5.5	3.95	**17**	**NZ**	21	21
312	35	BM	5.5	3.25	**16**	**NZ**	17	17
312	35	GYP	7.0	4.33	**17**	**NZ**	15	17
312	35	BM	7.0	3.47	**17**	**NZ**	17	14
312	37	GYP	5.5	4.07	**16**	**NZ**	16	16
312	37	BM	5.5	3.14	**17**	**NZ**	13	14
312	37	GYP	7.0	4.23	**15**	**NZ**	15	16
312	37	BM	7.0	3.02	**16**	**NZ**	14	11
360	35	GYP	5.5	3.97	**16**	**NZ**	**NZ**	**NZ**
360	35	BM	5.5	3.25	**15**	**NZ**	**NZ**	**NZ**
360	35	GYP	7.0	4.29	**15**	**NZ**	**NZ**	**NZ**
360	35	BM	7.0	3.43	**15**	**NZ**	**NZ**	**NZ**
360	37	GYP	5.5	4.09	**16**	**NZ**	**NZ**	**NZ**
360	37	BM	5.5	3.18	**17**	**NZ**	**NZ**	**NZ**
360	37	GYP	7.0	4.23	**17**	**NZ**	**NZ**	**NZ**
360	37	BM	7.0	3.06	**15**	**NZ**	**NZ**	**NZ**

NZ: No Zone

Table 1.5: Effect of antimicrobial activity against test organism (*Salmonella*)

Time (hour)	Temperature (° C)	Fermentation Medium	pH (initial)	pH (final)	Inhibition zone (mm)			
					Niasin +ve control (standard)	Peptone water -ve control	Sample 1	Sample 2
120	35	GYP	5.5	4.19	**14**	**NZ**	16	17
120	35	BM	5.5	3.19	**15**	**NZ**	17	16
120	35	GYP	7.0	4.51	**16**	**NZ**	17	18
120	35	BM	7.0	3.41	**16**	**NZ**	15	15
120	37	GYP	5.5	4.35	**15**	**NZ**	12	15
120	37	BM	5.5	3.16	**16**	**NZ**	15	15
120	37	GYP	7.0	4.56	**17**	**NZ**	16	16
120	37	BM	7.0	3.11	**17**	**NZ**	16	15
168	35	GYP	5.5	4.12	**16**	**NZ**	17	17
168	35	BM	5.5	3.31	**17**	**NZ**	15	15
168	35	GYP	7.0	4.39	**15**	**NZ**	16	15
168	35	BM	7.0	3.48	**17**	**NZ**	18	17
168	37	GYP	5.5	4.17	**16**	**NZ**	16	16
168	37	BM	5.5	3.21	**14**	**NZ**	17	16
168	37	GYP	7.0	4.34	**14**	**NZ**	16	15
168	37	BM	7.0	3.26	**16**	**NZ**	16	17
216	35	GYP	5.5	4.07	**15**	**NZ**	19	19
216	35	BM	5.5	3.31	**17**	**NZ**	15	15
216	35	GYP	7.0	4.41	**15**	**NZ**	16	15
216	35	BM	7.0	3.47	**16**	**NZ**	16	16
216	37	GYP	5.5	4.17	**17**	**NZ**	16	16
216	37	BM	5.5	3.32	**17**	**NZ**	17	15
216	37	GYP	7.0	4.35	**16**	**NZ**	14	15
216	37	BM	7.0	3.23	**15**	**NZ**	20	18
264	35	GYP	5.5	3.98	**16**	**NZ**	20	20
264	35	BM	5.5	3.18	**16**	**NZ**	17	18
264	35	GYP	7.0	4.31	**16**	**NZ**	17	17
264	35	BM	7.0	3.43	**15**	**NZ**	16	15
264	37	GYP	5.5	4.08	**17**	**NZ**	17	16
264	37	BM	5.5	3.16	**15**	**NZ**	16	16
264	37	GYP	7.0	4.36	**16**	**NZ**	17	15
264	37	BM	7.0	3.06	**16**	**NZ**	17	16
312	35	GYP	5.5	3.95	**17**	**NZ**	22	20
312	35	BM	5.5	3.25	**17**	**NZ**	15	15
312	35	GYP	7.0	4.33	**16**	**NZ**	17	16
312	35	BM	7.0	3.47	**17**	**NZ**	15	15
312	37	GYP	5.5	4.07	**17**	**NZ**	19	18
312	37	BM	5.5	3.14	**16**	**NZ**	17	18
312	37	GYP	7.0	4.23	**17**	**NZ**	16	17
312	37	BM	7.0	3.02	**17**	**NZ**	15	17
360	35	GYP	5.5	3.97	**16**	**NZ**	15	17
360	35	BM	5.5	3.25	**17**	**NZ**	17	18
360	35	GYP	7.0	4.29	**17**	**NZ**	20	19
360	35	BM	7.0	3.43	**15**	**NZ**	17	15
360	37	GYP	5.5	4.09	**16**	**NZ**	20	20
360	37	BM	5.5	3.18	**16**	**NZ**	18	18
360	37	GYP	7.0	4.23	**15**	**NZ**	17	20
360	37	BM	7.0	3.06	**15**	**NZ**	17	20

NZ: No Zone

Table 1.6: Static and cidal results

Test organism	Time (hour)	Temperature (°C)	Fermentation Medium	pH		Maximum inhibition zone (mm)		Effect	
				Initial	Final			Static	Cidal
E. coli	312	35	GYP	5.5	3.95	21	21	√	
Salmonella	312	35	GYP	5.5	3.95	22	20	√	

DISCUSSION

The biggest zone exhibited by *Lactococcus* spp cultured in GYP medium indicated that this species of LAB produced diacetyl compound as reported previously by Reddy and Ranganathan (1983) who reported that this compound inhibited to a variety of gram-positive and gram-negative microorganisms. Diacetyl is known as butter aroma but it is also well recognized for its antimicrobial action. The effect of antimicrobial compound on test organisms is only bacterial static and not bacterial cidal. After the one day of incubation, the agar which was streaked with a loop of sample taken from the clear zone shows the growth of the test organisms. Centrifugal separation or membrane processes was used for harvesting cells from medium. Centrifugation is mostly used in industrial scale because the low viscosity of the medium, the properties of the cells, cell size and high temperature, which favor this technique.

CONCLUSION

The optimum condition for maximum inhibition zone production for antimicrobial activity in which produced the largest inhibition zone is 312 hours (13 days) of anaerobic incubation, temperature at 37°C, pH 5.5 and GYP as fermentation medium.

This study has shown that:

i) Amongst the two species of LAB evaluated, *Lactococcus sp* produced the highest amount of antimicrobial compound from GYP medium at an initial substrate pH of 5.5 and at an incubation temperature of 35°C.

ii) The antimicrobial compound produced by *Lactococcus* was bacterial static.

iii) Maximum antimicrobial compound production was at 312 hour of incubation.

iv) GYP medium at an initial substrate pH of 5.5 and a temperature of 35°C ascertain to be the best conditions for maximum antimicrobial compound production for *Lactococcus.*

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