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Preliminary Identification of Potential Halophilic Bacteria Isolated from 'Asam Sunti' – Indonesian Traditional Herbs, in Inhibiting the Growth of *E.coli* and *Salmonella spp.*

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***Abstract*—The research aimed to determine potential halophilic bacteria isolated from Asam Sunti in inhibiting the growth of *E. coli* and *Salmonella spp.*. Research done experimentally and data analyzed by descriptive-exploratory method. Asam Sunti collected from market in Nanggroe Aceh Darussalam were cultured in Nutrient Agar (NA) with addition of 5 and 10% (w/v) NaCl and the total bacteria population was counted. Two different colonies isolated then identified by macroscopic, microscopic and gram staining. Abilities in inhibit the growth of *E.coli* and *Salmonella spp.* determined by diffusion well method on NA. Results showed that the best isolate was isolate A1 (rod shaped, gram negative bacteria) that resulting in 1.6 x 10^5 cfu/ml in 5% NaCl and 1.2 x 10^5 cfu/ml in 10% NaCl with no inhibition abilities towards *E.coli* and 11 mm of clear zone inhibition towards *Salmonella spp.*.**

Keywords*— Halophilic Bacteria, Asam Sunti, Inhibition, *E.coli*, *Salmonella spp.

I. INTRODUCTION

Asam sunti is indigenous herbs from Nanggroe Aceh Darussalam (NAD) – a province in Indonesia, which made from bilimbi (*Averrhoa bilimbi L.*). In fresh form, bilimbi also used as rust remover, cosmetics and traditional medicine [1,2]. Bilimbi has an acid flavor, unique aroma, and ability to give consistency of the food so that used as seasoning material.

Since long time ago, bilimbi were traditionally fermented into asam sunti and then utilized as seasoning material. Fermentation process of asam sunti using traditional equipment and easy methods which hereditary given by their anchestors. Asam sunti (Figure 1) is kind of fermented pickle products which processed with dry salting method, brown, soft, chewy, sour and slightly salty [3].

Fermentation method of asam sunti shown the role of bacteria as shown in other pickle products which salting processed [4]. Presence of bacteria on asam sunti were influenced by indigenous bacteria grown in bilimbi such as *Enterococcus faecalis*, *Lactococcus lactis* and *Lactobacillus plantarum* [5].

Beside the role of bacteria, salting process in the making of asam sunti will also increase the possibility of halophilic bacteria growth. When used as free cells, salinity usually limits the growth of bacteria strains [6,7]. However, the combination of salts and bacteria in Chinese traditional fermented vegetables production could improve pickle fermentation [8].

Various bacteria are well known has the ability to inhibit pathogenic bacteria such as *Eschericia coli* and *Salmonella spp.*. Lactic acid bacteria could produce bacteriocins to inhibit *Salmonella spp*. and *E. coli* [9]. Inhibition abilities towards food borne pathogenic bacteria also found in bacteria isolated from home-made fermented vegetables products [10].

Fig. 1. Asam sunti-Indonesian indigenous herbs

The research aims to determined the inhibition abilities towards *E.coli* and *Salmonella spp.* from potential halophilic bacteria that isolated from asam sunti. Selected isolates

could widely used in reducing contamination risk of *E.coli* and *Salmonella spp.* in salted products.

II. MATERIALS AND METHODS

Asam sunti bought from traditional market in NAD then diluted in 0.85% NaCl solution. Samples solution for 0.1 ml cultured in Nutrient Agar (NA) with addition of 5 and 10% (w/v) NaCl, then incubated in 37ºC for 24h and the total bacteria population was counted [11]. Two different colonies with the highest total bacteria population isolated then identified by macroscopic, microscopic and gram staining. Abilities in inhibit the growth of *E.coli* and *Salmonella spp.* determined by well diffusion method on NA [12].

III. RESULTS AND DISCUSSIONS

Based on macroscopic and microscopical characteristic, Table 1 and 2 showed that five isolates identified from asam sunti. Four bacteria with cocci shape gram positives and one bacteria with rod shape gram negatives was found. Along with previous research, five cocci shape gram positives bacteria found on bilimbi that were raw materials of asam sunti [5].

TABLE I
MACROSCOPIC CHARACTERISTICS OF ISOLATES

Isolate	Colour	Shape
A1	cream	Round
A2	cream	Assymetric
A3	cream	Jagged
A4	cream	Assymetric
A5	yellow	Round

TABLE III
MICROSCOPIC CHARACTERISTICS OF ISOLATES

Isolate	Shape	Gram
A1	rod	-
A2	cocci	+
A3	cocci	+
A4	cocci	+
A5	cocci	+

Table 3 showed that only two isolates that could survive on media with the addition NaCl until 10% (w/v). Isolates A1 shown average bacteria population of 1.6 x 10^5 cfu/ml at 5% NaCl added media and 1.2 x 10^5cfu/ml population at 10% NaCl added media. While, isolates A4 shown average bacteria population of 2.1 x 10^5cfu/ml at 5% NaCl added media, however the average bacteria population decreased until 1.0 x 10^5cfu/ml at 10% NaCl added media.

Halophilic bacteria were categorized based on their tolerance towards different salt concentrations [11]. Salt lower the water activity, it is shown by decreased of water activity from fresh bilimbi (0.936) into asam sunti after dry salting (0.730) and after one month fermentation (0.704) [3]. Bacteria with halophilic characteristics grow best in 1-5% (w/v) salt, moderately halophilic bacteria tolerate 5-20% salt [13]. Based on table 3 can be conclude that isolates A1 and A4 were moderately halophilic bacteria. The growth of slightly and moderately-halophlic bacteria do not require magnesium ion, grew better at the temperature of 28-37ºC and pH of 7.0-8.0 on medium suplemented with 5-20% NaCl [14, 11].

Table 3 also shown decreased of bacteria population influenced by the increase of NaCl concentration until 10%. Between 0-6% of NaCl, bacteria reduced their growth and between 6-10% of NaCl, bacteria drastically reduced their growth [15]

TABLE IIIII
IDENTIFICATION OF ISOLATES HALOPHILIC CHARACTERISTICS

Isolate	5% NaCl (10^3) ($x10^3$cfu/g)			10% NaCl ($x10^3$cfu/g)		
	R1	R2	R3	R1	R2	R3
A1	158	174	155	114	128	119
A2	<30	<30	<30	<30	<30	<30
A3	<30	<30	<30	<30	<30	<30
A4	208	221	214	102	98	105
A5	<30	<30	<30	<30	<30	<30

*R = replication

Two isolates (A1 and A4) were tested to determine their inhibition abilities towards *E.coli* and *Salmonella spp*.. Table 4 showed that both isolates shown no inhibition abilities towards *E.coli*, however isolates A1 shown average 11 mm of clear zone diameter towards *Salmonella spp*. and 1 mm average clear zone diameter of inhibition towards *Salmonella spp.* from isolates A4 (Figure 2).

TABLE IVV
INHIBITION ABILITIES TOWARDS PATHOGENS

Isolate	*E. coli* (mm)			*Salmonella spp.* (mm)		
	R1	R2	R3	R1	R2	R3
A1	-	-	-	13	10	10
A4	-	-	-	1	0	2

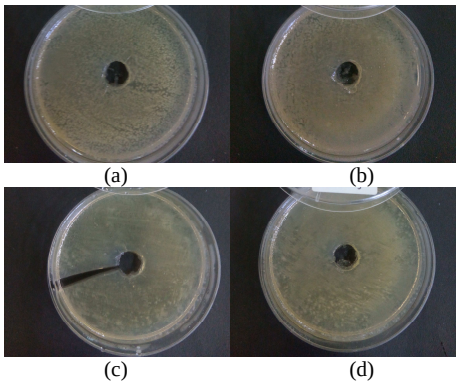

Fig. 2. Inhibition abilities (a) Isolates A1-*E.coli*, (b) Isolates A4-*E.coli*, (c) Isolates A1- *Salmonella spp.*, (d) Isolates A4-*Salmonella spp.*

Inhibition abilities occured because of the accumulation of primary metabolites such as lactic acid, acetic acid, ethanol and carbon dioxide [10]. Some of lactic acid bacteria has abilities to produce antimicrobial compound such as formic acid, benzoic acid, hydrogen peroxide, diacetyl, acetoin and bacteriocin such as nicin [6]. Inhibition activities towards gram negatives bacteria such *E.coli* and *Salmonella*

spp. could be caused by the production of organic acid and hydrogen proxide [17]. Large number of bacteria identified from variety plant sources mostly in the form of fermented and pickled products [18,19].

IV. Conclusions

Isolate A1 (rod shape, gram negative bacteria) which the best isolate shown 1.6 x 10^5 cfu/ml population in 5% NaCl and 1.2 x 10^5 cfu/ml population in 10% NaCl with no inhibition abilities towards *E.coli* and 11 mm of clear zone inhibition towards *Salmonella spp.*.

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