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Preliminary Identification of Inhibition Activities towards *Eschericia coli* and *Salmonella spp.* by Pickle's Indigenous Halotolerant Bacteria

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***Abstract*— Research objectives was to determine inhibition activities towards *E. coli* and *Salmonella spp.* by pickle's indigenous halotolerant bacteria. Experimental methods used in the research and data were analyzed descriptive-exploratory. In order to determine pickle's indigenous halotolerant bacteria, three isolates cultured on Nutrient Agar (NA) with addition of 5 and 10% (w/v) NaCl then the total bacteria population counted. *Escherichia coli* and *Salmonella spp.* inhibition activities determined by diffusion well method on NA. Results showed that isolate P3 shown halotolerant characteristic with total bacteria population of 5.3 x 10^5 cfu/g on 5% NaCl then decreased into 5.2 x 10^3 cfu/g on 10% NaCl with no inhibition clear zone towards *E.coli* and 7.5 mm inhibition clear zone towards *Salmonella spp.*.**

***Keywords*— Pickle, Halotolerant Bacteria, Inhibition, E.coli, Salmonella spp.**

I. INTRODUCTION

Preservation of vegetables can be done by fermentation, direct acidification, or combination of it with other process and additives [1]. Cucumber, eggplant, or even watermelon were fermented in salt solution to resulting in pickle.

Pickles could produced by two ways. First are the brine process which involving natural lactic acid bacteria fermentation, and the second are the fresh-pack processes without involving fermentation processes [2]. Commercial fermented cucumber mainly done with the addition of brine solution containing 6-8% NaCl [3].

Both methods using saline solution which can decrease the water activity where only certain bacteria with halotolerant abilities will grow [4]. Salting process in the making of pickle, can be role as inhibitor of undesired microoganisms growth [5].

However, the growth of pathogenic bacteria still found in pickling condition [4],[6],[7]. *Eschericia coli* found in apple cider and also *Salmonella spp.* in orange juice which the condition were similar to many pickle products [8]. Contamination risks of *E.coli* and *Salmonella spp.* in high salinity products like pickle still occur.

Inhibition abilities towards food borne pathogenic bacteria also found in bacteria isolated from home-made fermented vegetables products [9]. The growth of pathogenic bacteria such as *E.coli* and *Salmonella spp.* can inhibited by the bacteria that involve in pickle fermentation processes through their metabolites. Generally, bacteria isolated from spontaneous pickle fermentation are *Lactobacillus plantarum, L. brevis, Leuconostoc mesenteroides, Pediococcus pentosaceus* and *Enterococcus faecalis* [10],[11],[12]. The bacterias produce metabolites including organic acids, carbondioxide, hydrogen peroxide, ethanol and others which role as inhibitor for pathogenic bacteria [13].

The research aims to determine pickle's indigenous halotolerant bacteria inhibition activities towards *E.coli* and *Salmonella spp.*. Potential isolates could be used to decrease contamination of *E.coli* and *Salmonella spp*. in pickle or salted products.

II. MATERIALS AND METHODS

Commercial pickle (Figure 1) bought from market, 1g sample diluted on 9 ml of 0.85% NaCl then cultured on Nutrient Agar (NA) at 37ºC for 24h. Separate colony of three different isolates taken with osse and cultured on NA with addition of 5 and 10% (w/v) NaCl then the total bacteria population counted [14]. *Escherichia coli* and *Salmonella spp.* inhibition activities determined by diffusion

well method on NA [15]. Selected halotolerant bacteria, *E. coli* and *Salmonella spp.* each cultured into 15 mL Nutrient Broth (NB) and incubated for 24h at 37ºC. Sweep aseptically the incubated NB of *E. coli* and *Salmonella spp.* into NA plates until covered using sterile swabs. Plug NA plates aseptically, use sterile forceps or needle to carefully pick up the plug then place 0.1 mL of incubated NB of selected isolates. Incubate the NA at 37ºC for 24h then diameter of clear zones were measured.

Fig. 1. Commercial Pickle

III. Results and Discussions

The results of preliminary characterization of the isolates shown in Table 1. Three different isolates characterized. Isolates P1 was reddish cream, cocci shape and gram negatives. Isolates P2 was cream, cocci shape and gram negatives. While isolates P3 was cream, rod shape and gram negatives.

Natural succession of microbial population in cucumber fermentations begin with high levels of aerobic bacteria, sometimes including pathogenic and spoilage organisms [16]. *Lactobacillus* and *Leuconostoc* were dominating the early stage fermentation, and *Pediococcus* dominated the late stage of pickle fermentation [1]. Bacteria such as *L. plantarum* and *Leuconostoc mesenteroides* was the main microorgaisms that responsible for the natural fermentation of vegetables, but *L.paracasei*, *L. delbrueckii* and *L.brevis* also reported as well [13]. Three *bacillus* strain grow well at pickling condition such as wide range of pH (3.5-10.0), temperature (15-50ºC) and 4-7% NaCl [17].

TABLE I
Characteristics of Isolates

Isolate	Morphology		Microscopic	
	Colour	Shape	Shape	Gram
P1	reddish cream	round	cocci	-
P2	cream	round	cocci	-
P3	cream	round	rod	-

Table 2 shown the results of halotolerant bacteria identification. Only isolates P1 that survived at 5-10% salinity with the average population of 5.3 x 10^5 cfu/g on 5% NaCl then decreased into 5.2 x 10^3 cfu/g on 10% NaCl. Other isolates did not show their ability in tolerate salinity concentration which shown by less than 30 colonies on every plates so that can not be counted.

Halotolerant bacteria categorized based on their abilities to tolerate different salinity concentrations [14]. Bacteria with halotolerant characteristics survived in 1-5% salinity, moderately halotolerant bacteria survived in 5-20% salinity [7]. Based on the results can conclude that isolates P1 was moderately halotolerant bacteria.

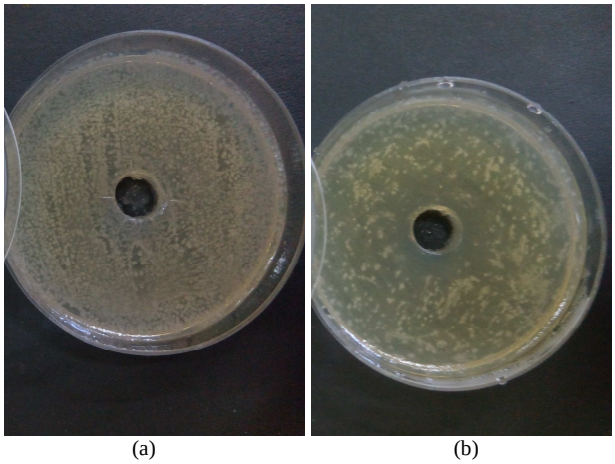
TABLE II
Identification of Isolates Halotolerant Characteristics

Isolate	5% NaCl (x10^4cfu/g)			10% NaCl (x10^2cfu/g)		
	R1	R2	R3	R1	R2	R3
P1	53	55	52	51	50	55
P2	<30	<30	<30	<30	<30	<30
P3	<30	<30	<30	<30	<30	<30

The results of inhibition abilities towards *E.coli* and *Salmonella spp.* shown on Table 3. Table 3 showed that isolates P1 did not have inhibition abilities towards *E.coli*, but shown 7.5 mm average clear zone that indicating inhibition abilities towards *Salmonella spp.* (Figure 2). Two strain of *L. plantarum* and *P. Pentosaceus* isolated from pickle shown their abilities in inhibiting *Salmonella* invasion in animal metabolism [18].

TABLE III
Inhibition Abilities Towards Pathogens

Isolate	*E. coli* (mm)			*Salmonella spp.* (mm)		
	R1	R2	R3	R1	R2	R3
P1	-	-	-	5	10	7.5



(a) (b)

Fig. 2. Inhibition abilities (a) Isolates P1-*E.coli*, (b) Isolates P1 - *Salmonella spp*.

Pathogenic gram negatives bacteria such as *E.coli* and *Salmonella spp.* could inhibited by the production of organic acid and hydrogen proxide [19]. Lactic acid bacteria as main microflora that involves in pickle fermentation, has abilities to produce antimicrobial compound such as formic acid, benzoic acid, hydrogen peroxide, diacetyl, acetoin and bacteriocin such as nicin [20]. However, *E.coli* were more

resistant to the effects of acid than *Salmonella spp.* under typical pickling condition [21].

Bacterias isolated from pickles such as *L.plantarum*, *B. acidicola*, *B. amyloliquifaciens* and *B. mycoides*, could inhibited various food borne pathogens through production of organic acids and bacteriocins, also survived in 4-11% salinity [17]. Bacteria with halotolerant characteristics and pathogen inhibition has wide opportunities in food production. Application in salted or fermented products could decrease the contamination risk of pathogenic bacteria especially *E.coli* and *Salmonella spp.*.

IV. CONCLUSIONS

Isolate P3 shown halotolerant characteristic with total bacteria population of 5.3 x 10^5 cfu/g on 5% NaCl and 5.2 x 10^3 cfu/g on 10% NaCl with no inhibition clear zone towards *E.coli* and 7.5 mm inhibition clear zone towards *Salmonella spp.*

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