

## Effect of Chaff Vinegar on the Growth of Food-Borne Pathogenic Bacteria

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### Abstract

The growth inhibitory effect of chaff vinegar against various food-borne pathogenic bacteria was evaluated. Bacterial growth was evaluated in chaff vinegar at concentrations of 15, 30, 50, 65, 80, and 100% using the paper disc diffusion method and 0.5, 1.0, 1.5, 1.8, 2.0, 2.2 and 2.5% in broth. In the paper disc diffusion assay, chaff vinegar showed a clear zone on both the Gram-positive bacteria; *Listeria monocytogenes*, *Staphylococcus aureus* and Gram-negative bacteria; *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Vibrio parahaemolyticus*. Chaff vinegar exhibited the greatest growth inhibition for *V. parahaemolyticus*. The bactericidal effect of chaff vinegar on the *E. coli* O157:H7 was tested at concentrations ranging from 0.5 to 2.5% (v/v) in the LB broth media. Chaff vinegar retarded the lag phase time of the growth curve in proportion in a concentration-dependent manner. Chaff vinegar at 2.5% completely inhibited the growth of *E. coli* O157:H7.

**Key words:** growth inhibition, chaff vinegar, food-borne pathogenic bacteria

### INTRODUCTION

Food-borne pathogens such as *Escherichia coli* O157:H7, *Salmonella enterica* serovar Typhimurium, and *Listeria monocytogenes* are found in a wide variety of foods. *E. coli* O157:H7 has a low infective dose (1,2) and causes hemorrhagic colitis, which is occasionally complicated by hemolytic uremic syndrome (3-6). *E. coli* O157:H7 has been implicated in over 73,500 cases of illness each year in the United States (7). Although most outbreaks have been associated with undercooked ground beef and raw milk (1), a variety of acidic foods which were traditionally considered of low risk have also been implicated in outbreaks; including unpasteurized apple juice, salami, yogurt, and mayonnaise (8-11).

In South Korea from 1993~1997, various sources of food poisoning occurred from *Salmonella* sp. (46.5%), *Vibrio* sp. (21%), *Staphylococcus* sp. (19.2%), natural poisoning (2.4%) and etc. (11.9%) (12). It is important to rapidly, efficiently and inexpensively decontaminate foods from bacterial pathogens to protect humans from food-borne infectious diseases. The sources of food poisoning by microbial pathogens can be controlled by various means. One method is to use synthetic antimicrobial agents (13), however, consumers have recently become interested in natural antimicrobial materials including grapefruit seed extract (14), organic acids (15), and green tea extracts (16) as safer alternatives to syn-

thetic antimicrobial chemicals.

Currently, agricultural and industrial residues are becoming attractive sources of natural anti-microbial agents (17). More than one million tons of chaff (rice hulls) are produced annually in South Korea during rice processing. However, rice hulls are wasted or destined to undervalued uses. Chaff vinegar is manufactured during production of chaff charcoal. The rice chaff is roasted at high temperature inside a furnace, the smoke produced is cooled, and the supernatant of this coolant is chaff vinegar. Chaff vinegar has a pH of 2.8~3.3, and contains more than 200 types of minerals. Chaff vinegar is composed of 10~20% organic compounds and 80~90% water (17). The term, chaff vinegar, came from its acetic acid content. Vinegar is advertised as a deodorizing agent and for use as a soil conditioner due to its ability to decompose nitrate nitrogen and oxidize heavy metal by its powerful oxidative capacity (17,18). In this study, we evaluated the inhibitory activity of chaff vinegar on the growth of food-borne pathogens.

### MATERIALS AND METHODS

#### Bacterial strains and chemicals

*Escherichia coli* O157:H7 ATCC 43894 (responsible for human stool hemorrhagic colitis outbreak) was purchased from American Type Culture Collection (Manassas, VA). *Staphylococcus aureus*, *Listeria monocytogenes*,

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*Salmonella* Typhimurium, and *Vibrio parahaemolyticus* were purchased from KTCT (Biological Resource Center, Daejeon, Korea). Each bacterium was maintained on Luria-Bertain (LB) agar slants at 4°C and subcultured monthly, with the exception of *V. parahaemolyticus* which was cultured in LB medium. In all experiments, *V. parahaemolyticus* was cultured in LB medium containing 3% sodium chloride under the same conditions. Chaff vinegar was kindly supplied from Garak-Nonghyup, Pusan, Korea, and sterilized by passing through a 0.2 µm-micromilipore filter (Advantec, Tokyo, Japan) before use. Paper discs were also purchased from Advantec (Tokyo, Japan), and autoclaved before determining antibacterial activity. The pH of chaff vinegar was measured with an Orion 420A pH meter (ATI Orion; Boston, MA, USA). Media for microbial growth were purchased from DIFCO (Becton Dickinson Co., Franklin Lakes, NJ, USA).

#### Paper disc agar diffusion assay

Autoclaved LB agar medium of 200 mL was maintained at 50°C in a water bath and inoculated with 2 mL of approximately 10<sup>8</sup> colony forming units (CFU)/mL for each microorganism. Each agar plate was drawn with 10 mL of the LB agar medium. Various concentrations of 60 µL of chaff vinegar were loaded on the sterilized paper discs. After drying at 60°C in a drying oven for 1 hr, the discs were placed on the LB agar plates and were incubated at 37°C for 24 hr. The antimicrobial

activity was evaluated by measuring the diameter of the inhibition zone against the test microorganisms. Each assay in this experiment was performed in triplicate and the results were reported as the mean of the diameter of the inhibition zones.

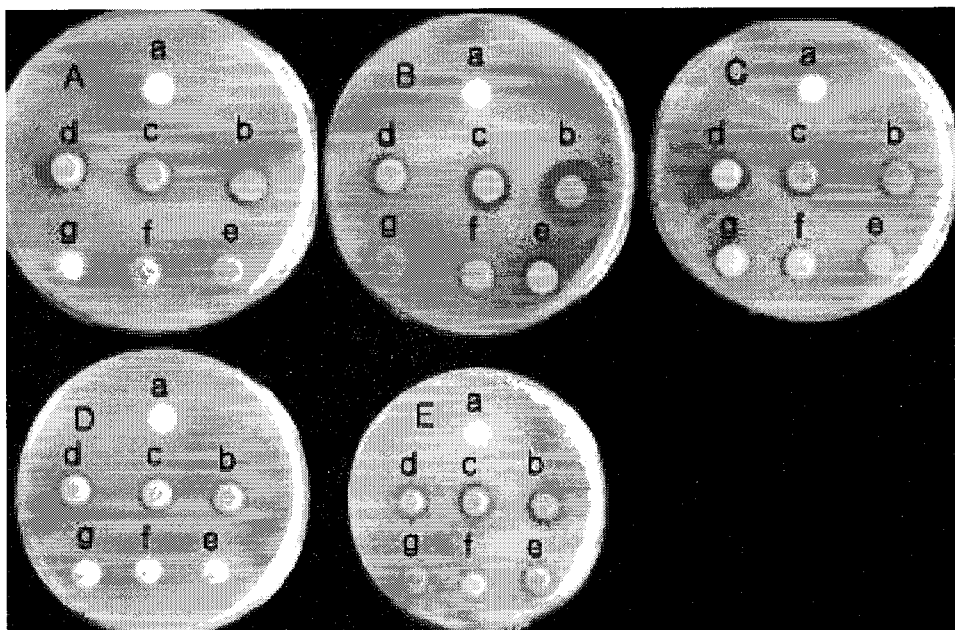
#### Growth inhibition effect of chaff vinegar on broth medium

Antibacterial activity of chaff vinegar was determined on broth medium with *E. coli* O157:H7. *E. coli* O157:H7 was cultured in LB medium overnight at 37°C with two consecutive transfers before use, and final transfer was made into fresh LB medium containing chaff vinegar and incubated for 24 hr at 37°C. The culture was aseptically withdrawn every 2 hr for 24 hr and then immediately cooled on ice to measure optical density at 600 nm using a spectrophotometer (UV-1602, Simadzu Co., Kyoto, Japan). Culture without the addition of chaff vinegar was used as a control. The experiments were carried out in triplicate. Symbols and error bars in the figure express the mean ± standard deviation.

## RESULTS AND DISCUSSION

#### Growth inhibition effect of chaff vinegar in the paper disc agar diffusion assay

Chaff vinegar inhibited the growths of selected food-borne pathogenic bacteria (Fig. 1). It can be seen that



**Fig. 1.** Typical plates showing growth inhibition effects of chaff vinegar against food-borne pathogenic bacteria by the paper disc diffusion assay.

The plates were inoculated with A, *Staphylococcus aureus*; B, *Vibrio parahaemolyticus*; C, *Salmonella* Typhimurium; D, *Escherichia coli* O157:H7; and E, *Listeria monocytogenes*. In all plates, a, without chaff vinegar; b, 100% chaff vinegar; c, 80% chaff vinegar; d, 65% chaff vinegar; e, 50% chaff vinegar; f, 30% chaff vinegar; and g, 15% chaff vinegar.

**Table 1.** Antibacterial activity of chaff vinegar against food-borne pathogens by the disc diffusion assay (mm)

Strains	Concentration (%)							
	0	15	30	50	65	80	100	
Gram (+)	<i>Listeria monocytogenes</i>	8.00±0.00 <sup>1)</sup>	8.00±0.00	8.00±0.00	8.67±0.16	9.67±0.20	12.33±0.15	13.00±0.19
	<i>Staphylococcus aureus</i>	8.00±0.00	8.00±0.00	8.00±0.00	8.00±0.00	8.33±0.08	9.33±0.10	9.83±0.10
Gram (-)	<i>Escherichia coli</i> O157:H7	8.00±0.00	8.00±0.00	8.00±0.00	8.00±0.00	8.83±0.13	9.67±0.16	10.33±0.16
	<i>Salmonella</i> Typhimurium	8.00±0.00	8.00±0.00	8.67±0.10	8.83±0.13	9.67±0.19	10.33±0.16	12.00±0.14
	<i>Vibrio parahaemolyticus</i>	8.00±0.00	8.00±0.00	8.00±0.00	9.67±0.20	12.33±0.05	13.17±0.10	13.50±0.12

<sup>1)</sup>Means of three replicates±standard deviations of the diameters of the clear zones.

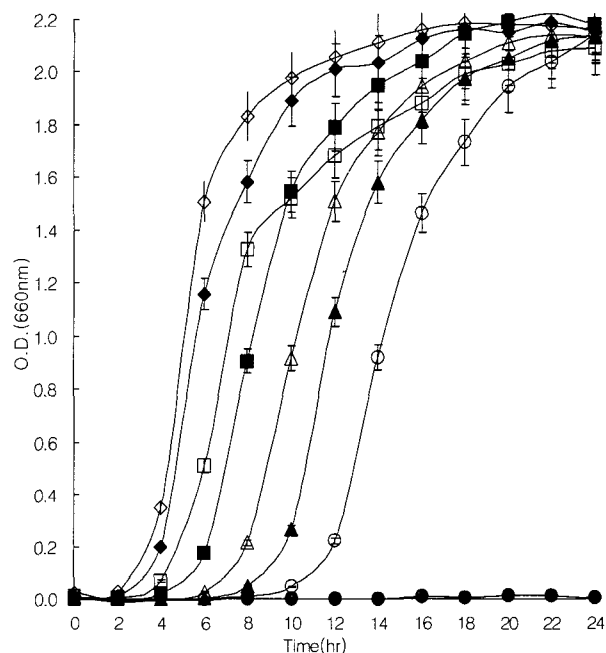
the size of inhibition zone increased with increasing concentrations of chaff vinegar on the paper discs, and the growths of all tested food pathogens were inhibited at or above the 65% concentration of chaff vinegar. It has been reported that the antimicrobial activity of vinegar is mainly dependent on the acetic acid concentration (18). Chaff vinegar showed the most significant inhibition against growth of *V. parahaemolyticus*, and at concentrations above 65%, chaff vinegar inhibited the growth of all test bacteria (Table 1).

Chaff vinegar showed antibacterial activity in the order of *V. parahaemolyticus* > *L. monocytogenes* > *S. Typhimurium* > *E. coli* O157:H7 > *S. aureus*. Makino et al. (17) reported that the growth of *S. aureus* was significantly inhibited by chaff vinegar, and 1.56% chaff vinegar even showed inhibitory activity by the disc diffusion method. However, as shown in Table 1, 50% chaff vinegar in this study could not inhibit the growth of *S. aureus*. The differences of chaff vinegar, medium, origin of strain, etc., between Makino group and our study might give rise to disparity.

It is difficult to find significant differences between Gram positive and Gram negative bacteria, however, the morphology of bacteria may affect antibacterial activity of chaff vinegar. The rod shaped bacteria (*E. coli*, *S. Typhimurium*, *L. monocytogenes*, and *V. parahaemolyticus*) were more sensitive to chaff vinegar than spherical bacteria (*S. aureus*). The diffusion of chaff vinegar into bacteria across their surface membrane might be an important factor for bactericidal activity.

#### Growth inhibition effect of chaff vinegar in broth medium

The antibacterial activity of chaff vinegar in broth media was measured with *E. coli* O157:H7 (Fig. 2). Chaff vinegar gradually inhibited the growth of *E. coli* O157:H7 as the concentration of chaff vinegar increased. Concentrations of chaff vinegar of 1.8, 2.0, and 2.2%



**Fig. 2.** Effect of chaff vinegar on the growth of *Escherichia coli* O157:H7 on broth media.

Symbols indicated that  $\diamond$ , no chaff vinegar;  $\blacklozenge$ , 0.5%;  $\square$ , 1.0%;  $\blacksquare$ , 1.5%;  $\triangle$ , 1.8%;  $\blacktriangle$ , 2.0%;  $\circ$ , 2.2%; and  $\bullet$ , 2.5% of chaff vinegar.

(v/v) in LB broth resulted in lag times for of *E. coli* O157:H7 growth of 4, 6, and 8 hours, respectively. Chaff vinegar at 2.5% in the LB broth completely inhibited the growth of *E. coli* O157:H7.

The pH values of LB media containing chaff vinegar were measured (Table 2). The control LB medium had a pH value of 6.57, which decreased with the addition of chaff vinegar. Chaff vinegar contains several kinds of organic acids, mainly acetic acid (17). It has been reported that acetic acid strongly inhibited the growth of *S. Typhimurium*, *E. coli* O157:H7 and *L. monocytogenes* (19). The bactericidal activity of acetic acid was due to a change in the intracellular pH caused by diffusion across the cell membrane of the weak organic acid when

**Table 2.** The pH values of LB media depend on concentration of added chaff vinegar

(%)	0	0.5	1.0	1.5	1.8	2.0	2.2	2.5
pH	6.57±0.04 <sup>1)</sup>	6.43±0.01	6.14±0.01	5.70±0.10	5.60±0.06	5.51±0.01	5.45±0.01	5.29±0.01

<sup>1)</sup>Means of three replicates ± standard deviations.

incubated with *Bacillus subtilis* at pH values near the pK of acetic acid (20). In the present study, acetic acid of chaff vinegar might play a key role in antibacterial activity. The USDAs "Pathogen Reduction/HACCP Proposal" (21) includes recommendations for voluntary antimicrobial interventions such as organic acids, hot water, etc. Accordingly, chaff vinegar may be a candidate for use in the control of food pathogens.

In conclusion, chaff vinegar significantly inhibited the growth of food-borne pathogenic bacteria. In South Korea, most of chaffs after processing of rice are utilized with under-valuation. Chaff vinegar can be easily manufactured during production of chaff charcoal linking with Rice Processing Complex. However, further study for decolorizing and deodorizing of chaff vinegar is needed before application in the food industry.

#### ACKNOWLEDGEMENT

This work was supported by the Kyungnam University Research Fund, 2004.

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(Received April 18, 2005; Accepted June 2, 2005)