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RESEARCH NOTE

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Effects of Food Components on the Antibacterial Activity of Chitosan against *Escherichia coli*

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Abstract The antibacterial activity of chitosan against *Escherichia coli* was investigated in the presence of NaCl, sucrose, and ethanol to assess the potential use of chitosan as a biopreservative in food products containing these components. The inhibitory activity of chitosan decreased slightly upon the addition of NaCl and sucrose, respectively to culture broth containing 100 ppm of chitosan (Mw 3,000), while the addition of ethanol enhanced the inhibitory activity of chitosan on growing cells. The addition of these components to non-growing cells prior to chitosan treatment demonstrated that NaCl protected the cells from the inhibitory activity of chitosan, while sucrose had no effect. Ethanol addition to non-growing cells increased cell death by chitosan treatment. Finally, binding of fluorescein isothiocyanate (FITC)-labeled chitosan to *E. coli* was measured in the presence of the food components. The FITC-labeled chitosan binding to cells decreased upon NaCl addition, was not affected by sucrose, and increased following treatment with ethanol.

Keywords: antibacterial activity, chitosan, fluorescein isothiocyanate-chitosan, food component effect, *Escherichia coli*

Introduction

Chitosan, the deacetylated derivative of chitin, is widely used in pharmaceuticals, agriculture, and the food industry, and the antimicrobial activity of chitosan against food-borne pathogens has been actively studied over the past 15 year's attention (1-4). Recently, the consumer concerns regarding food safety are leading to increased demand for foods that do not contain chemical preservatives. Chitosan, a nontoxic, biodegradable polysaccharide, has the potential to replace chemical preservatives in food (5-9). However, not enough is known about the factors that affect the antimicrobial activity of chitosan in foods. Therefore, the effects of the common food components NaCl, sucrose, and ethanol on the inhibitory activity of low molecular weight chitosan against *Escherichia coli* were investigated.

Materials and Methods

Chitosan preparation Low molecular weight chitosan (Mw 3,000) was obtained from Amicogen Inc. (Jinju, Korea). The chitosan was minimally deacetylated at 95%. Stock solution containing 5,000 ppm of chitosan in 0.5% acetic acid were sterilized by membrane filtration.

Strains and culture conditions *Escherichia coli* KCTC 1039 was purchased from the Korea Collection for Type Cultures (Daejon, Korea) and subcultured twice in tryptic soy (TS) broth (pH 6.0) for 16 hr at 37°C for all experiments.

Growth inhibition assay TS broth containing 100 ppm of chitosan was inoculated with 10^5 cells/mL. NaCl, sucrose, and ethanol were added to TS broth (pH 6.0) containing 100 ppm chitosan (Mw 3,000) to determine the effects of these components on the inhibitory activity of chitosan. Growth was measured by optical density (OD) at 600 nm.

Bactericidal activity against non-growing cells *E. coli* was grown in TS broth (pH 6.0) for 16 hr, centrifuged, and washed with 50 mM phosphate buffer (pH 6.0). NaCl, sucrose, and ethanol were added to the cell suspensions, allowed to incubate for 20 min, and then centrifuged. The cells were resuspended in phosphate buffer (pH 6.0) containing 100 ppm chitosan (Mw 3,000) for 20 min. Viable cells were counted after plating on TS agar and incubation at 37°C.

Fluorescein isothiocyanate (FITC)-chitosan binding to cells *E. coli* was grown in TS broth (pH 6.0) for 16 hr, centrifuged, and washed with 50 mM phosphate buffer (pH 6.0). Cells were resuspended in phosphate buffer (10^6 cells/mL) and incubated with NaCl, sucrose, and ethanol for 10 min. After centrifugation and washing, FITC-labeled chitosan was added to cell suspensions at 200 ppm. Bound FITC-chitosan was detected with a Quantech fluorometer (FM109530-33; Barnstead/Thermolyne Co., Dubuque, IA, USA) at intervals of 5 and 10 min. FITC-chitosan was prepared as described by Qaqish and Amiji (10).

Results and Discussion

Effects of NaCl, sucrose, and ethanol on chitosan-mediated cell growth inhibition Chitosan inhibition of *E. coli* growth was measured in the presence of NaCl, sucrose, and ethanol. The inhibitory activity of chitosan decreased considerably when NaCl was added to chitosan containing TS broth (Fig. 1A). Similarly, the addition of sucrose inhibited the activity of chitosan (Fig. 1B). However, the addition of ethanol slightly increased the

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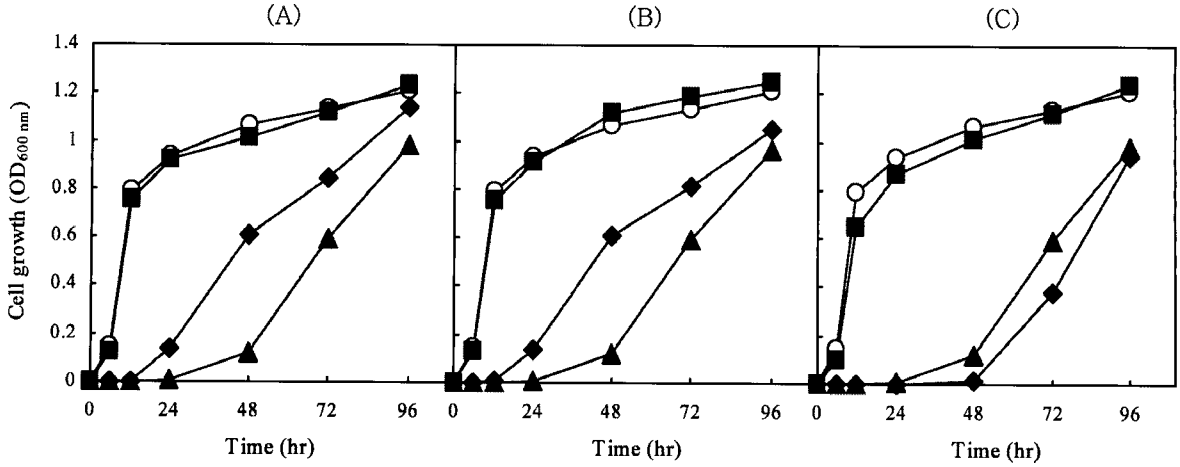

Fig. 1. Effect of 2%(w/v) of (A) NaCl, (B) sucrose, and (C) ethanol on the inhibitory activity of chitosan (Mw 3,000) on the growth of *E.coli* at a concentration of 100 ppm. Cells were cultured in TS broth at 37°C in the presence of (▲) chitosan only; (◆) chitosan with NaCl (A), sucrose (B), and ethanol (C); (■) NaCl (A), sucrose (B), and ethanol (C). Open circles indicate the control without chitosan.

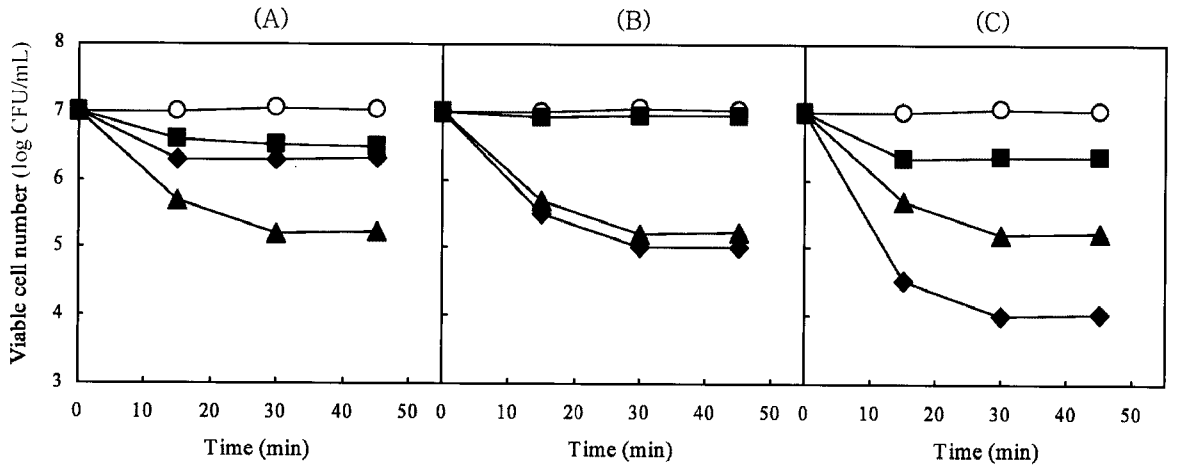

Fig. 2. Survival of non-growing cells of *E.coli* at a concentration of 100 ppm chitosan after the addition of 2%(w/v) of (A) NaCl, (B) sucrose, and (C) ethanol. (▲) Only chitosan added; (◆) chitosan treatment after the addition of NaCl (A), sucrose (B), and ethanol (C); (■) NaCl (A), sucrose (B), and ethanol (C) added. Open circles indicate the control without chitosan.

inhibitory activity of chitosan (Fig. 1C).

Several reports have demonstrated that chitosan, a non-toxic natural biopolymer, can be used as a food product preservative (6,9). Practically, it is important to determine the influence of food components on the activity of chitosan before it is used as a biopreservative. It is likely that food components interfere with chitosan activity by two possible modes. First, these substances could act on the cell surface and alter the sensitivity of the cells to chitosan (hypothesis 1). Alternatively, the food components could directly interact with chitosan (hypothesis 2). To determine the cause of food component effects, the interactions of these components with the non-growing cells were investigated.

Effects of NaCl, sucrose, and ethanol on the bactericidal activity of chitosan on non-growing cells The viability of non-growing cells after chitosan treatment in the presence of various food additives is shown in Fig. 2. The viable cell count was nearly 10-fold higher upon NaCl addition to the cell suspension prior to chitosan treatment. Considering the inhibitory activity of NaCl on the cells alone, the actual effect is estimated to be much higher. The results clearly demonstrate that NaCl represses the inhibitory activity of chitosan by acting directly on the cells, supporting hypothesis 1. While sucrose had a similar effect on growing *E. coli*, it did not repress the inhibitory activity of chitosan when sucrose was added to the non-growing cells prior to chitosan treatment. In contrast, the viable cell count was minimally affected by sucrose addition (Fig. 2B). Therefore, the attenuation of chitosan activity by sucrose addition to the culture broth could be explained by the interaction of sucrose with chitosan and is consistent with hypothesis 2. Ethanol addition to the cells prior to chitosan treatment decreased cell viability (Fig. 2C). The results indicate that ethanol interaction with the cell envelope sensitizes the cells to chitosan.

FITC-chitosan adherence to *E. coli* The effect of NaCl, sucrose, and ethanol on the activity of chitosan was further investigated using FITC-labeled chitosan to measure binding of chitosan to *E. coli*. As shown in Fig. 3, NaCl

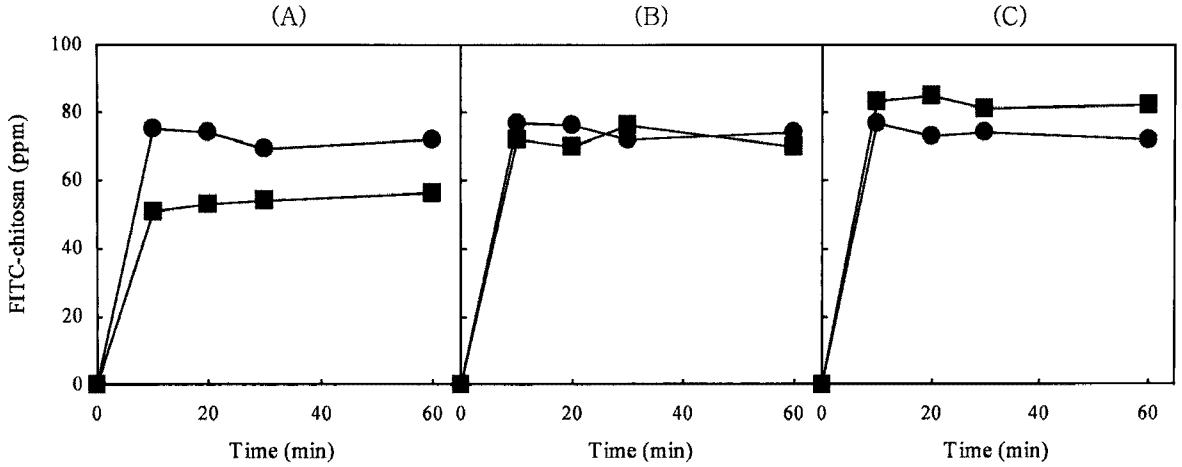

Fig. 3. Effect of 2%(w/v) of (A) NaCl, (B) sucrose, and (C) ethanol on the FITC-chitosan binding to the non-growing cells of *E.coli*. (●) FITC-chitosan added after NaCl (A), sucrose (B), and ethanol (C) treatment; (■) FITC-chitosan added.

treatment decreased the amount of FITC-chitosan binding, presumably due to Na^+ binding to the negatively charged cell surface components. The results further demonstrate the mechanism of NaCl on the inhibitory activity of chitosan during cell culture growth.

In contrast, sucrose treatment did not affect the binding of FITC-chitosan to cells. The results confirm the results on non-growing cells indicating that sucrose does not interact with the cells. Taken together, these results indicate that the attenuating effect of sucrose on the inhibitory activity of chitosan could be due to a direct interaction of sucrose with chitosan.

As seen in Fig. 3C, ethanol treatment of cells increases the binding of FITC-chitosan, suggesting that ethanol interaction with components of the cell surface increases chitosan binding to cells. These results confirm the synergistic effect of ethanol on the inhibitory activity of chitosan.

In conclusion, the results of this study elucidate the effects of NaCl, sucrose, and ethanol on the inhibitory activity of low molecular weight chitosan against *E. coli*. In addition, indirect evidence is provided that partly determines the mode of action on chitosan activity. Though further works with other foodborne pathogens are needed, these results will be of important practical interest when determining the optimal amount of chitosan needed for use as a biopreservative in food products containing these components.

References

1. Sudarshan NR, Hoover DG, Knorr D. Antibacterial action of chitosan. Food Biotechnol. 6: 257-272 (1992)
2. Tsai GJ, Su WH. Antibacterial activity of shrimp chitosan against *Escherichia coli*. J. Food Protect. 62: 239-243 (1999)
3. OH SW, Hong SP, Kim HJ, Choi YJ. Antimicrobial effects of chitosans on *Escherichia coli* 0157:H7, *Staphylococcus aureus*, and *Candida albicans*. Korean J. Food Sci. Technol. 32: 218-224 (2000)
4. Myoung HS, Choi MH, Park YH. Binding of low molecular weight chitosans on the bacterial cells. J. Chitin Chitosan 7: 18-22 (2002)
5. Liu H, Du YM, Wang XH, Sun LP. Chitosan kills bacteria through cell membrane damage. J. Food Microbiol. 95: 147-155 (2004)
6. Chung YC, Wang HL, Chen YM, Li SL. Effect of abiotic factors on the antibacterial activity of chitosan against water borne pathogens. Bioresource Technol. 88: 179-184 (2003)
7. Devlieghere F, Vermeulen A, Debevere J. Chitosan: Antimicrobial activity, interactions with food components and applicability as a coating on fruit and vegetables. J. Food Microbiol. 21: 703-714 (2004)
8. Park YH, Tumenjargal D. Control of yeast isolated from Korean soybean paste with low molecular weight chitosans. J. Chitin Chitosan 9: 180-183 (2004)
9. Rhoades J, Roller S. Antimicrobial actions of degraded and native chitosan against spoilage organisms in laboratory media and foods. Appl. Environ. Microb. 66: 80-86 (2000)
10. Qaqish RB, Amiji MM. Synthesis of a fluorescent chitosan derivative and its application for the study of chitosan-mucin interactions. Carbohyd. Polym. 38: 99-107 (1999)