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Inhibition of Food Pathogenic Bacteria by Azelaic Acid

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Abstract: In the present study, the effects of concentration, pH, growth phases and heat treatment on the inhibition growth against several strains of food pathogenic bacteria by azelaic acid (Aza) was investigated to evaluate its possibility as food preservatives. The result showed that 300 ppm of Aza almost completely inhibited the growth of tested bacteria. Aza added at the beginning of the incubation had the high inhibitory effect. In the range of pH 5-9, Aza had the highest inhibitory at pH 9. The degree of antibacterial activity of Aza was higher than those of sodium benzoate and potassium sorbate and similar to those of dimethylfumarate and fumaric acid. Antibacterial activity of Aza was stable even in the heating treatment.

Key words: Azelaic acid, heating treatment, food preservatives, antibacterial activity, growth phases

INTRODUCTION

Natural derived compounds and other natural products may have applications in controlling pathogen in foods[1,2]. Some spices are known to contain essential oils that posses antimicrobial activity such as eugenol in cloves, allicin in garlic and cinnamic aldehyde in cinnamon[3-6].

Our previous studies found that the ethylacetate extract of atung seed had strong antibacterial activity against six strains of spoilage and pathogenic bacteria[7]. On the basis of the results, an experiment was continued to purify and isolate the antibacterial compound of atung seed. As a result, it was found that azelaic acid is antibacterial compound of atung seed[8].

Azelaic acid is a saturated dicarboxylic acid with 9 carbon atoms (COOH-$(CH_2)_7$-COOH) that owes its name to the fact that it was originally obtained from the oxidation of oleic acid by nitric acid (AZ= azote = nitrogen; elaoin = oil).

Azelaic acid lacks acute or chronic toxicity and is non-teratogenic and non-mutagenic[9,10]. Azelaic acid have already been successfully applied to the treatment of acne, rosacea and melasma[11-15].

The antimicrobial effects of azelaic acid are shown against aerobic microorganisms such as *Staphylococcus aureus*, *Proteus mirabilis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *candida albicans* and the anaerobic *Propionibacterium acnes*[16-18]. Azelaic acid was found as an antibacterial substance from buckwheat hull, which is effective against food pathogenic bacteria[19].

Therefore, in the present study, the effects of concentration, pH, growth phases and heat treatment on the inhibition growth against several strains of food pathogenic bacteria by azelaic acid was investigated to evaluate its possibility as food preservatives.

MATERIALS AND METHODS

Bacterial strains and culture media: *Bacillus cereus*, *Bacillus subtilis* IFO-13719, *Micrococcus luteus* IFO-12708, *Escherichia coli* IFO-3301, *Staphylococcus aureus* IFO-14462 and *Salmonella enteritidis* IFO-3313 were tested for antibacterial activity. Bacteria were maintained on the nutrient agar with the composition containing agar powder 15 g; peptone 10 g; meat extract 10 g; NaCl 5 g; distilled water 1000 mL; pH 7.2 and these were stored at 4°C.

Chemicals: Azelaic acid was provided by Wako Pure Chemical Industries Ltd, Japan. For comparison, four other compounds, namely, fumaric acid (Wako Chemical, Japan), potassium sorbate (Kanto Chemical, Japan), dimethyl fumarate (Wako, chemical, Japan) and sodium benzoate (Wako Chemical, Japan), were tested.

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Growth inhibition: The growth of tested bacteria was monitored by measuring their growth on the absorbance at 560 nm (A_{560}). Growth inhibition tests were carried out by adding appropriate amount of stock cultures into sterile media to give an initial A_{560} of about 0.1. A certain amount of preservatives was added to obtain the required concentrations after autoclaving of the broth unless otherwise specified. The cultures were incubated at 37°C and the A_{560} values of cell suspensions were determined at interval time periodically. Sterile media without addition of any preservatives were used as the control.

Heat treatment: In order to examine the effect of heat treatment on the antibacterial activity of azelaic acid, culture media with 200 ppm azelaic acid were subjected to the heat treatment such as autoclaving at 121°C for 20 min or boiling for 20 min. Sterile distilled water was added after the heat treatment to compensate for evaporation.

pH adjustment: To consider the effect of pH, the culture media were adjusted to the desired pH from pH 5.0 to 9.0, as opposed to pH 6.5-6.6 for all the other tests. After the stock cultures of tested bacteria were added, the pH values were adjusted by adding 1N NaOH or 1 N HCl. Azelaic acid was then added to obtain a concentration of 200 ppm.

RESULTS AND DISCUSSION

Comparison of different compounds: Not all the tested compounds inhibited the growth of tested strains. However, two compounds, potassium sorbate and sodium benzoate showed little inhibition. This result indicated that these two compounds were less effective in inhibiting the tested bacteria (Fig. 1). Nguyen *et al.*[20] also found that Kumazasa extract showed stronger antibacterial activity than potassium sorbate and sodium benzoate at concentrations of 0.2-1.0%. While in this experiment, dimethyl fumarate (DMF), fumaric acid and azelaic acid were more effective in comparison to sodium benzoate and potassium sorbate. These results were in agreement with previous finding by Wang *et al.*[21], who found that antibacterial activity of potassium sorbate and sodium benzoate was less effective than dimethyl fumarate and fumaric acid.

Factor influencing antibacterial activity of azelaic acid Concentration: The different concentration (100, 200 and 300 ppm) of Aza affected the growth of all tested bacteria. However, compared with the control (no added Aza), the performance of 100 ppm of Aza showed close to that of the control. On the other hand, the inhibition of their growth was gradually increased as the concentration of

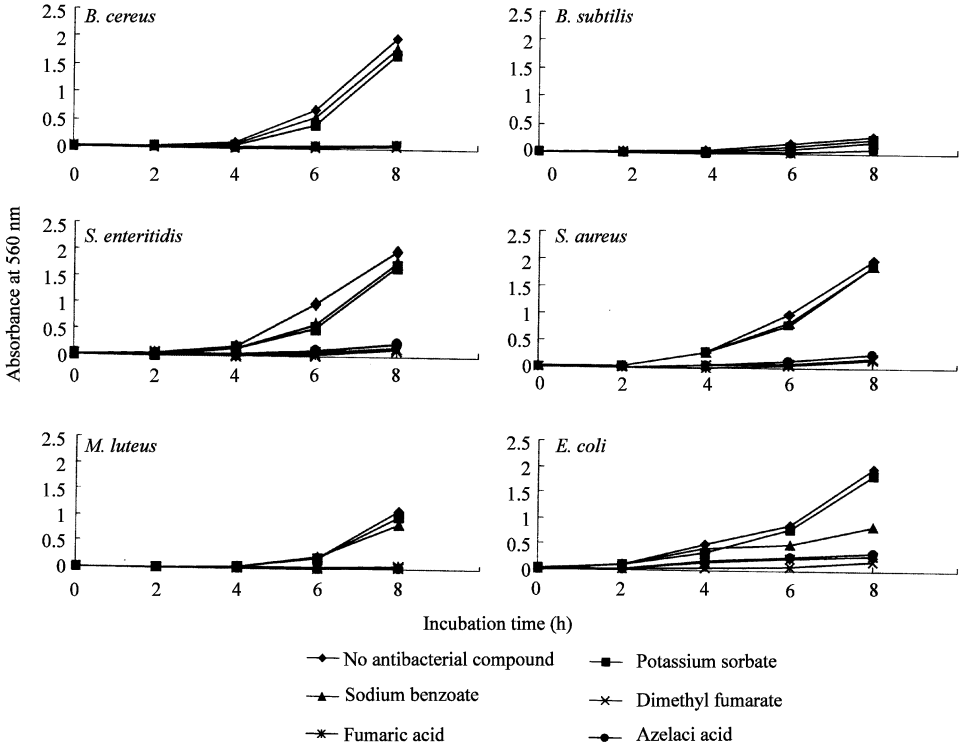

Fig. 1: Growth inhibition of food pathogenic and spoilage bacteria by antimicrobial compound (200 ppm)

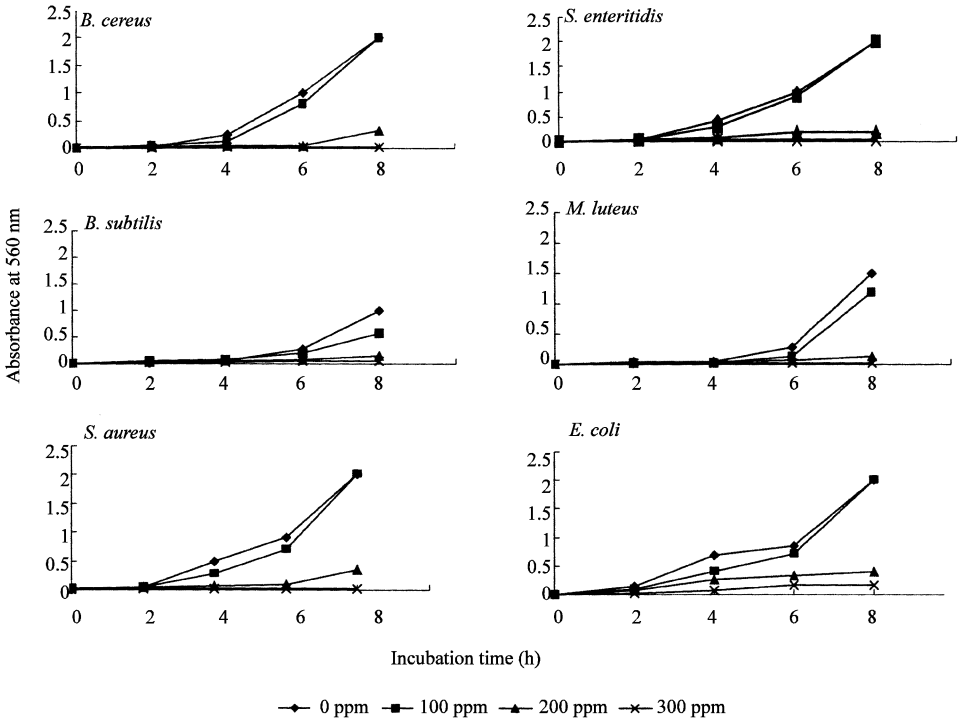

Fig. 2: Effect of concentration of azelaic acid on the growth inhibition against food pathogenic and spoilage bacteria

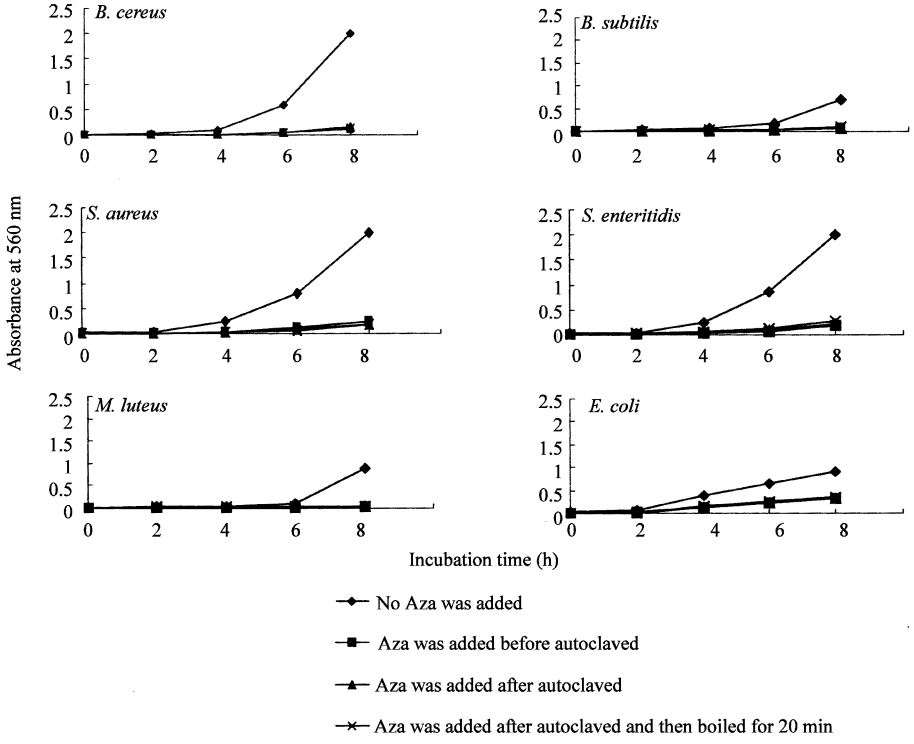

Fig. 3: Effect of heat treatment on the growth inhibition of azelaic acid against food pathogenic and spoilage bacteria (200 ppm)

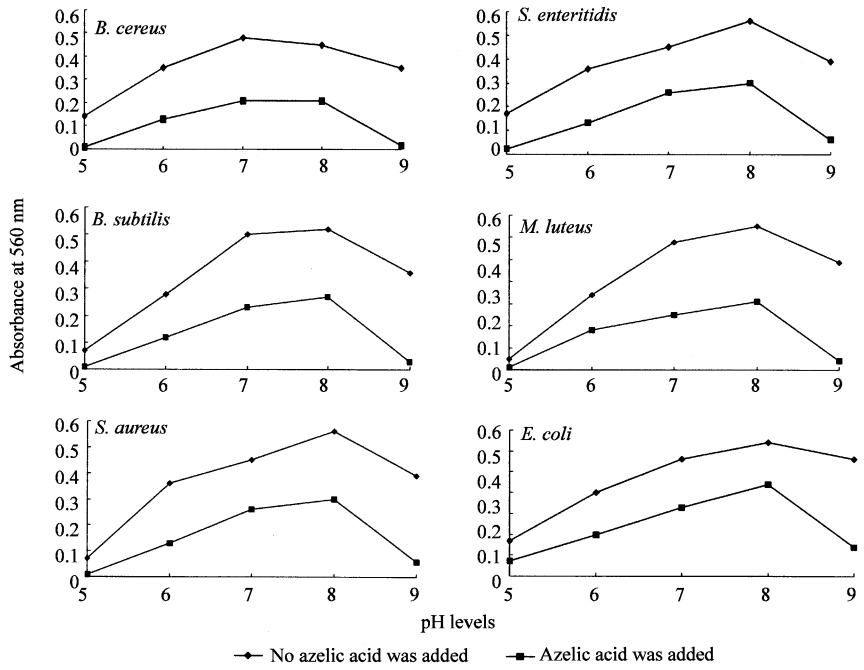

Fig. 4: Effect of pH on the growth inhibition of azelaic acid against food pathogenic and spoilage bacteria (200 ppm)

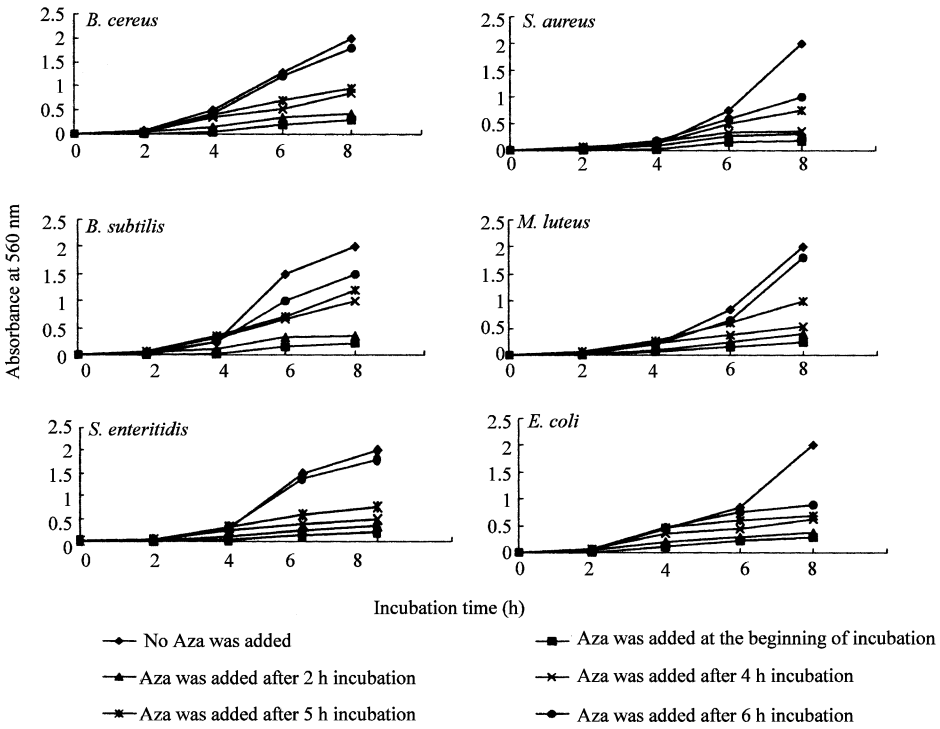

Fig. 5: Effect of growth phases on the growth inhibition of azelaic acid against food pathogenic and spoilage bacteria

Aza increased to 200 and 300 ppm (Fig. 2). However, the concentrations of 100 and 200 ppm were not sufficient to suppress the growth of tested bacteria entirely, so that they continued to grow with time. While at the concentration of 300 ppm, the growth of all tested bacteria were almost completely inhibited.

Heat treatment: Heat treatments used commonly in food processing are boiling (100°C) and autoclaving (121°C). To study the thermal stability of the azelaic acid, it was subjected to heat treatment such as autoclaving and boiling for 20 min. The Inhibitory activity of Aza was not affected by the treatment. As shown in Fig. 3, Aza had the inhibitory activity after the heat treatment. Antimicrobial activity of a mixture of the extracts corni fructus, cinnamon extract and Chinese chive and cinnamon extract was very stable to the heat treatment[22,23]. The antibacterial activity of cabbage was destroyed by the heat treatment[24,25]. Wang *et al.*[21] found that the inhibitory activity of dimethyl fumarate was affected by the heat treatment, but that dimethyl fumarate kept high inhibitory effect. While, in this study, Aza was found to be very stable to heat treatment. It is anticipated that this advantage of thermal stability will allow azelaic acid to be a potential food additive for use in food processing where the heat treatment, either autoclaving or boiling, is desired.

pH: At pH 9, the cell concentration was the lowest after 6 h incubation. This finding suggested that azelaic acid had high activity at the high pH range (Fig. 4). The antibacterial compound may penetrate into the bacterial cells more easily at higher pH than at lower pH[26,27]. Generally, the pH of processed foods usually ranges from acid to neutral, but potassium sorbate, sodium benzoate and some other commonly used food preservatives are effective only under acidic conditions. At pH 7, azelaic acid had also a high activity. On the other hand, the deterioration of foods begins when the pH of foods became basic (higher pH). Therefore, azelaic acid may be considered as alternative food preservatives.

Growth phases of tested bacteria: The growth of tested bacteria was significantly inhibited at all intervention times (Fig. 5). However, tested bacteria were able to continue growing for a short time before its growth was totally inhibited. It is interesting to note that the earlier added azelaic acid into the broth, the lower the final cell concentration of all tested bacteria were observed and hence a better inhibition effect was obtained. This finding is in agreement with Wang *et al.*[21] who found that dimethylfumarate had a best inhibitory effect against *E. coli* when it applied at the beginning of the incubation.

The result concluded that 300 ppm of azelaic acid almost completely inhibited the growth of tested bacteria. Azelaic acid added at the beginning of the incubation and at pH 9 had the high inhibitory effect. The degree of antibacterial activity of azelaic acid was higher than those of sodium benzoate and potassium sorbate and similar to those of dimethylfumarate and fumaric acid. Antibacterial activity of azelaic acid was stable even in the heating treatment. On the basis of the results, azelaic acid may be considered to be useful as food preservatives.

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