## Application of Fatty Acid Salts for Antimicrobial Agents in Koji-Muro

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Abstract : Objectives: Aspergillus niger and Aspergillus oryzae are used as koji fungi in the spot of the brewing. Since kojimuro (room for making koji) was a low level of airtightness, microbial contamination has long been a concern to the alcoholic beverage production. Therefore, we focused on the fatty acid salt which is the main component of soap. Fatty acid salts have been reported to show some antibacterial and antifungal activity. So this study examined antimicrobial activities against Aspergillus and Bacillus spp. This study aimed to find the effectiveness of the fatty acid salt in koji-muro as antimicrobial agents. Materials & Methods: A. niger NBRC 31628, A. oryzae NBRC 5238, A. oryzae (Akita Konno store) and Bacillus subtilis NBRC 3335 were chosen as tested. Nine fatty acid salts including potassium butyrate (C4K), caproate (C6K), caprylate (C8K), caprate (C10K), laurate (C12K), myristate (C14K), oleate (C18:1K), linoleate (C18:2K) and linolenate (C18:3K) at 350 mM and pH 10.5 were used as antimicrobial activity. FASs and spore suspension were prepared in plastic tubes. The spore suspension of each fungus  $(3.0 \times 104 \text{ spores/mL})$  or the bacterial suspension  $(3.0 \times 105 \text{ CFU/mL})$  was mixed with each of the fatty acid salts (final concentration of 175 mM). The mixtures were incubated at 25 °C. Samples were counted at 0, 10, 60, and 180 min by plating (100 µL) on potato dextrose agar. Fungal and bacterial colonies were counted after incubation for 1 or 2 days at 30 °C. The MIC (minimum inhibitory concentration) is defined as the lowest concentration of drug sufficient for inhibiting visible growth of spore after 10 min of incubation. MICs against fungi and bacteria were determined using the two-fold dilution method. Each fatty acid salt was separately inoculated with 400  $\mu$ L of Aspergillus spp. or B. subtilis NBRC 3335 at 3.0  $\times$  104 spores/mL or 3.0 × 105 CFU/mL. Results: No obvious change was observed in tested fatty acid salts against A. niger and A. oryzae. However, C12K was the antibacterial effect of 5 log-unit incubated time for 10 min against B. subtilis. Thus, C12K suppressed 99.999 % of bacterial growth. Besides, C10K was the antibacterial effect of 5 log-unit incubated time for 180 min against B. subtilis. C18:1K, C18:2K and C18:3K was the antibacterial effect of 5 log-unit incubated time for 10 min against B. subtilis. However, compared to saturated fatty acid salts to unsaturated fatty acid salts, saturated fatty acid salts are lower cost. These results suggest C12K has potential in the field of koji-muro. It is necessary to evaluate the antimicrobial activity against other fungi and bacteria, in the future.

Keywords : Aspergillus, antimicrobial, fatty acid salts, koji-muro

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