

12. Screening of Starter Strains for Sailfin Sandfish Sauce Made Using Deep Sea Water Salt

Han Dae-won* · Cho Sun-yeong** · Park Ju-yong, Lee Hyeong-gil, Kim Gyeong-su ***

*Industry-University Cooperation Group, Gangneung Wonju National University **Department of Food Processing and Distribution, Gangneung Wonju National University,***Ociad Company Limited

1. Introduction

Currently, fish sauces are produced by fermentation of fish with salt, but the methods are not uniform so that the tastes, aging periods, and aging strains are not consistent and the fish sauces are produced unsanarily. In order to solve these problems, in this study, excellent strains generated in the sailfin sandfish sauce fermented with deep sea water were explored and their antibacterial activity against food poisoning bacteria, glycolytic ability, and stability were analyzed to finally isolate and identify an excellent strain. The finally isolated strain was identified as *Bacillus subtilis*.

2. Methods

To isolate starter strains, colonies that occurred in the fermentation of sailfin sandfish sauce made first hand, at 35 °C and 45 °C in the PCA medium and MRS agar were randomly harvested on the 10th, 20th, and 30th days of aging and 300 candidate starter strains were isolated. To check the morphological and physiological characteristics of the isolated strains, catalase tests and gram staining were carried out. In the catalase tests, the 300 isolated strains cultured in a liquid medium for 24 hours and the culture fluids was taken and put into 3% H₂O₂ for reaction to see if bubbles are formed. In the results, no bubble was formed in any of the culture fluid indicating that all the strains are negative. In addition, when the strains were observed with a microscope after gram staining, most of the strains were identified as gram positive diplococci or micrococci. To check the glycolytic ability and gas production ability of sailfin sandfish sauce candidate strains, 24 strains that showed strong characteristics in the isolation culture were selected and their glycolytic ability and gas production ability were reviewed. Gas production ability and glycolytic ability were identified in all 24 strains. To check the growth rates and pH production ability of the isolated strains according to temperatures, the growth rates and pH production ability of 24 strains were checked at 35 °C and 45 °C according to the fermentation conditions of fish sauces. The O.D values of the medium as a control were shown to be about 0.3217 and 0.3917 at 35 °C and 45 °C, respectively, and the pH values were shown to be similar at 6.14 and 6.27, respectively. Most of the strains cultured at 35 °C showed increased O.D values and gradually decreasing pH values compared to the control. In particular, strains no. 13 and 24 showed the highest optical densities with O.D. values of 1.7286 and 1.3489, respectively, and showed low pH values of 4.32 and 4.58, respectively. Of the strains cultured at 45°C, about half showed O.D values not much higher compared to the strains cultured at 35°C, and the remaining half showed O.D values similar to or higher than those of the strains cultured at 35°C. Strains no. 13, 14 and 24, which showed large O.D. values at 45 °C are expected to be thermophilic bacteria. As for the growth rates and pH-production abilities of the isolated strains according to salinity, strains cultured at 10% salinity and 35 °C were found to have generally much low

er growth rates compared to the strains in the above mentioned experiment conducted without the addition of salt. In addition, when the salinity increased to 20%, the bacterial productivity and pH production ability were decreased drastically than when the salinity was 10%. In addition, at the high temperature of 45 °C, the bacterial productivity of all strains except for several strains expected to be thermophilic bacteria such as strains no. 13, 14, and 24 were shown to have drastically lower bacterial productivity at a salinity of 10% and it could be identified that at a salinity of 20%, which is a worse condition, all strains had no or drastically reduced bacterial productivity and pH production ability. The antibacterial activity of the 24 selected isolated strains against pathogenic strains was checked using the paper disc method. No strain that showed antibacterial activity against *Listeria monocytogenes* or *Vibrio parahaemolyticus* could be found. Strain no. 4 showed antibacterial activity of about 4.3 mm against SA, strain no. 14 showed antibacterial activity of about 4.8 mm against EO, and strain no. 17 showed antibacterial activity of about 8.1 mm against *E. coli* and 12.4 mm against *Salmonella*. Strain No. 24 showed antibacterial activity of about 5.7 mm against *E. coli* and 8.4 mm against *Staphylococcus aureus*. Four starter candidate strains isolated from sailfin sandfish sauce were identified through 16S rRNA sequence analysis.

3. Conclusion

In order to search for excellent sailfin sandfish sauce starter strains, at least 300 strains were randomly selected and optimal candidate strains were selected first by measuring glycolytic ability, gas production ability, and growth rate according to temperatures and salinity. Thereafter, the antibacterial activity of the selected candidate strains against food poisoning bacteria was tested to finally select and identify strains with excellent antibacterial activity. As a result, *Bacillus subtilis*, which is known to be a thermophilic bacterium, was selected as the final candidate strain for shortening of the fermentation period of fish sauce. The deep sea water salt used for the manufacturing of fish sauce is a salt with cleanliness and high mineral contents that can be said to be a good raw material for production of clean food. Hereafter, studies to review the growth fitness and physiological and chemical properties of starter candidates in vitro and in situ as well as studies using other table salts will be conducted.

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References

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