Selection and Characteristics of Fermented Salted Seafood (jeotgal)-Originated Strains with Excellent S-adenosyl-L-methionine (SAM) Production and Probiotics Efficacy

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Abstract

This study is executed to develop probiotics which produce S-adenosyl-L-methionine (SAM), a methyl group donor of the 5-methyltetrahydrofolate methylation reaction within the animal cell. SAM is an essential substance for the synthesis, activation, and metabolism of hormones, neurotransmitters, nucleic acids, phospholipids, and cell membranes of animals. The SAM is also known as a nutritional supplement to improve brain functions of the human. In this study, the SAM-producing strains are identified in 18 types of salted fish, and then, the strains with excellent SAM productions are being identified, with 1 strain in the Enterococcus genus and 9 strains in the Bacillus genus. Strains with a large amount of SAM production include the lactic acid bacteria such as En. faecium and En. durans, En. sanguinicola, as well as various strains in the Bacillus genus. The SAM-overproducing strains show antibacterial activities with certain harmful microbes in addition to the weak acid resistances and strong bile resistances, indicating characteristics of probiotics. It is possible that the jeotgal-originated beneficial strains with overproducing SAM can be commercially utilized in order to manufacture SAM enriched foods.

Key words: S-adenosyl-L-methionine, fermented salted seafood probiotics, jeotgal

Introduction

S-adenosyl-L-methionine (SAM), first discovered by Cantoni in 1952, is a substance primarily present in tissues and body fluids of an organism, and it plays an important role in the 5-methyltetrahydrofolate (5-MTHF) methylation reaction as a methyl group donor (Shelly, 2000). SAM is an important bio-regulator made from essential amino acids, L-methionine, and ATP by methionine adenosyltransferase (Wang et al., 2001). It is mostly synthesized in the liver at a rate of about 8 g a day and is involved in many biochemical metabolism processes (Horikawa et al., 1990).

Through many studies, it has been confirmed that SAM content varies depending on the content of L-methionine in foods (Kim et al., 2008). Its functionality has been recognized in western societies including Europe and the USA, and SAM has been reported as an essential substance in processes including in polyamine synthesis in cells and the synthesis, activation, and metabolism of hormones, neurotransmitters, nucleic acids, phospholipids, and cell membranes (Santi et al., 1983). Also, it is required in the production of important brain compounds such as neurotransmitters and phospholipids, including phosphatidylcholine and phosphatidylserine, and is known to be effective in improving brain functions (Mato et al., 1990).

The metabolic reactions of SAM in the body include transmethylation, transsulfuration, and polyamine (Mato et al., 1999). In the methylation reaction, the methyl group of SAM is donated to various acceptor substrates such as DNA, phospholipids, and proteins; in the transsulfuration reaction, SAM is converted to taurine, a major antioxidant in cells, and cysteine, a precursor of glutathione, through several enzymatic reactions (Porter et al., 1986). Finally, SAM is used in the synthesis of polyamines that are essential to the growth of normal cells (Cooney, 1993). It also donates the methyl group to the propolyamine group in the synthesis of spermine and spermidine (Lee et al., 2006). It has been reported that SAM has pharmacological effects in the restoration of hepatic functions in alcoholic liver disease, in reducing muscle fatigue and
rigidity in patients with fibromyalgia syndrome, and in improvement in patients with depression with about a 66% clinical improvement (Koning, 1987; Lieber, 1999).

Up to now, studies on SAM reported its effects in the treatment of depression, and in arthritis and hepatic cirrhosis, and it has been recognized as a health-functional food with a recommended daily intake of 400 mg (Lee et al., 2008). A food containing SAM includes Jeotgal (fermented salted seafood), one of our traditional fermented foods. Thus, this study was performed to isolate jeotgal-originated strains that produce physiologically functional material, SAM, select and identify strains that produce large amounts of SAM in foods, and find strains producing SAM with probiotics through antibacterial activity to function against harmful microbes such as food poisoning bacteria.

Materials and Methods

Sample preparation

Naturally existing strains in 18 kinds of jeotgal were isolated and their colony was analyzed to select strains that improve SAM production. Samples used in the study included 18 types of fermented salted seafood (toha shrimp, whitesaddled reeffish, baby octopus, herring roe, scallop, clam, pen-shell, hairtail, gizzard shad, hairtail guts, sand lance sauce, large-eyed herring, branchia, shrimp, anchovy, squid, small octopus, pollack roe) purchased in Gangyeong, Chungcheongnam-do (April 18, 2012).

Characteristics of selected strains

Samples were diluted 10 times (0.85% NaCl 225 mL + sample 25 g) under a sterilized environment and then homogenized using a stomacher (speed level 5, 1 min). Then, the homogenate was smeared on each selective medium and incubated to separate total microbes, lactic acid bacteria, anaerobic bacteria, yeast and mold. Microbial separation and collection were performed depending on the colony types of microbes produced. Microbes grown in different selective media were identified. The separation of microbes used tryptic soy agar (TSA) for analysis of the number of total microbes. In addition, for the analysis of total lactic acid bacteria, MRS agar with adjusted pH 5.5 was used. For a detailed analysis of the lactic acid bacteria, m-LBS (Lactobacillus species), KF-Streptococcus (Enterococcus, Pediococcus species) and PES (Leuconostoc species) were used. Also, potato dextrose agar (PDA) with an adjusted pH using tartaric acid was used for the analysis of the yeast and mold (Table 1).

Table 1. Selective medium type and isolated colony

<table>
<thead>
<tr>
<th>Medium type</th>
<th>Target bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSA (Merck)</td>
<td>Total microbes</td>
</tr>
<tr>
<td>MRS (adjust pH 5.5)</td>
<td>Total LAB</td>
</tr>
<tr>
<td>KF-Streptococcus m-LBS</td>
<td>Enterococcus/Pediococcus</td>
</tr>
<tr>
<td>lactic acid bacteria m-LBS</td>
<td>Lactobacillus</td>
</tr>
<tr>
<td>PES</td>
<td>Leuconostoc</td>
</tr>
<tr>
<td>PDA (Difco, adjust pH using tartaric acid solution)</td>
<td>Yeast and mold</td>
</tr>
</tbody>
</table>

1)TSA: Tryptic soy agar
2)MRS: Lactobacilli MRS agar
3)KF: KF-Streptococcus
4)m-LBS: modified Lactobacillus selection agar
5)PES: phenylethyl alcohol with 2% sucrose agar
6)PDA: Potato dextrose agar

Culture of strains isolated from jeotgal

To examine SAM production ability, strains were inoculated onto a nutrient medium TSB, incubated at 35°C for 24-48 h to maintain 10^8-10^9 CFU/mL, and centrifuged (4,000 rpm, 4°C, 10 min) to eliminate microbial cells and collect the supernatant for analysis.

SAM production

The production of SAM was analyzed using high-performance liquid chromatography (HPLC) under the conditions listed in the table (Guattari, 1991; Katie et al., 2006). Prepared samples were filtered using a 0.45 μm syringe filter and stored at -20°C for analysis. The standard for SAM was purchased from Sigma-Aldrich, and the ammonium acetate and methanol used in the analysis and all other reagents were purchased for HPLC use.

Analysis of sugar fermentation by isolated strains

Among the isolated strains, the Enterococcus genus strains used an API 20 Strept system kit (bioMereux, France) and the Bacillus genus strains used an API 50 CHB system kit (bioMereux, France) for the analysis of the sugar fermentation of the strains. According to the API kit manual, diluted strain samples were transferred to each selective medium and incubated at 37°C for 24-48 h to identify the presence/absence of various sugar fermentation.

Acid resistance and bile resistance of strains

For the resistance to pH, strains were activated in TSB (10^8-10^9 CFU/mL), washed with PBS or 0.85% NaCl, and then centrifuged for sample use (centrifuge use). After TSB medium preparation with pH levels of 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, and 5.5 (control pH 7.0 - medium pH), samples were spotted on TSA and changes of the colony