Letter to the Editor

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Metabolomics analysis of medicinal insect *Protaetia brevitarsis* after *Bacillus subtilis* fermentation

Bacillus subtilis fermantasyonundan sonra tıbbi böcek *Protaetia brevitarsis*'in metabolomik analizi

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To the Editor,

Insects are attracting attention as future food substitutes around the world, and about 1.3 million species of insects can be used for resources. It is obvious that these insects are the largest unused resources on Earth and are an important research target. The use of insects for medical purposes has a long history. Wasps are effective in promoting blood coagulation, strengthening of the heart, lowering blood pressure, diuresis, etc., and are known to be effective in anti-inflammatory and toothache [1, 2]. *Protaetia brevitarsis* is an insect with a length of about 17 to 24 mm. The larvae inhabit a deciduous soil such as oak sawdust fermented by microorganisms and enter the winter after the metamorphosis until late October [3]. *P. brevitarsis* has shown physiological activity mainly in hepatic disease, thrush, tetanus, and diuretic effect [4].

Microorganisms can be used to convert substances with significantly improved functionality. As the microbes grow, they also produce nutrients that are good for human health such as vitamins and organic acids. Recently, Cheonggukjang, a traditional Korean fermented food made by fermenting soybeans with *Bacillus subtilis*, contains not only antioxidants contained in soybeans, but also peptides, free amino acids, and aglycones of isoflavones [5]. These substances are attracting attention as a new dietary supplement with various physiological activities such as anticancer activity, immune enhancing effect, blood cholesterol lowering, and heart disease prevention [5].

The aim of this study is to find and apply useful substances enhanced through microbial fermentation of medicinal insects such as *P. brevitarsis*. Air-dried *P. brevitarsis* larvae were ground into a powder and autoclaved at 121 °C for 15 min. *B. subtilis* pellet (OD at 660 nm = 1.7–1.8) was resuspended in distilled water after centrifugation. The pasteurized powder was fermented with *B. subtilis* at a ratio of 1:1 (w/v) at 30 °C. Metabolomics analysis was performed using an ultra-performance liquid chromatography (UPLC) system. Centroid MS^E mode was used to collect the mass spectrometry data. The UPLC-MS/MS method was described in supplementary section. Figure 1 shows representative UPLC/MS chromatograms before and after fermentation of *B. subtilis* with *P. brevitarsis* larvae, measured by UPLC-Q-TOF/MS. In comparison with the extract before fermentation, it was confirmed that 51 metabolites are up-regulated in the extract after fermentation (Figure 1 and Supplementary Table 1). Of the 51 metabolites identified, several biomedical efficacies are known for surfactin, notoginsenoside B, homodihyfrocapsacin, and muricatenol, but they should be further analyzed through more detailed efficacy studies. Edible insect resources have excellence in productive and nutritional terms. It is well known that the world population will reach 9 billion by 2050. Surviving a large population will require several

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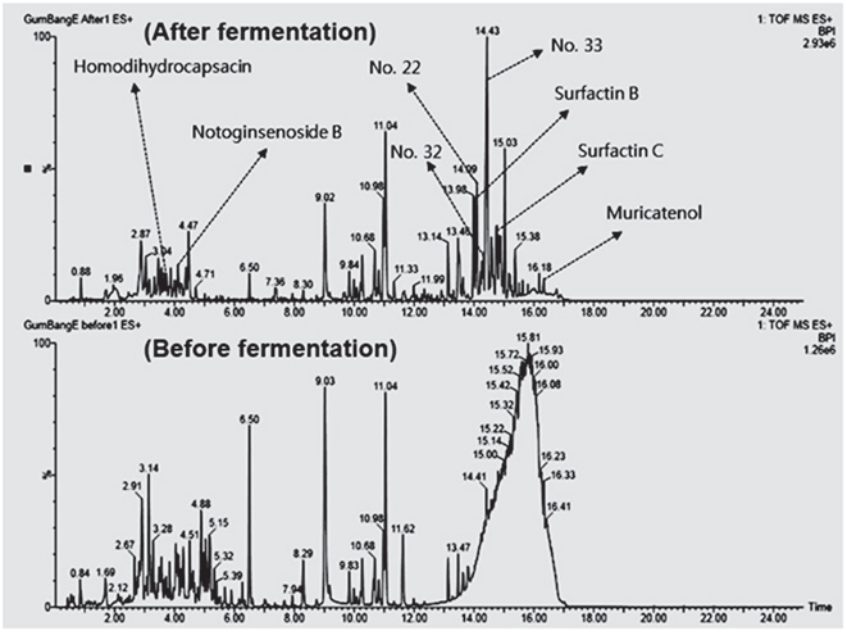

Figure 1: UPLC-Q-TOF/MS chromatograms before and after fermentation of *B. subtilis* with *P. brevitarsis* larvae.

times as much food as now. Research is also underway to develop pharmaceutical materials by separating high-functional substances from insects. Insects have been used as a medicine for a long time all over the world, and researches on developing new substances for the treatment of antibiotic-resistant diseases have been actively conducted. In summary, *P. brevitarsis*-derived metabolites that are specifically increased after fermentation are expected to show useful biomedical activity to humans, and their functions need to be revealed through further detailed studies.

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