Studies on novel food functions of microbial metabolites and constituents

Laboratory of Marine Bioproducts of Technology
Neng Tanty Sofyana

Introduction

Microorganisms transform the chemical constituents of raw materials of plant/animal sources thereby enhancing the bioavailability of nutrients, producing antioxidant and antimicrobial compounds, stimulating the probiotic functions, and fortifying with some health-promoting bioactive compounds. Among bacteria associated with foods and alcoholic beverages, lactic acid bacteria are widely present. A gut lactic acid bacteria, *Lactobacillus plantarum* produces novel fatty acids, derived from PUFAs such as linoleic acid (LA) and α-linolenic acid (ALA) through multi-enzymatic reactions in saturation metabolism. Recent study also demonstrated the effect of gut microbial PUFA metabolites to develop functional foods for prevention of metabolic disorder diseases such as obesity and T2DM. This data suggest that fatty acid metabolites produced by bacterial flora in the gastrointestinal tract may contribute to human health.

Microalgae is also promising as functional foods resources. Fresh water microalgae, euglena, contains abundant of nutrients therefore its used as a supplement and various applied researches such as fuel, livestock feed, and use for water quality improvement. It has been reported that glucose tolerance is improved by long-term administration of euglena drugs to OLETF rats, which are type 2 diabetes model rats, however, the functional components are still unknown. This present study aim to examine the new functional properties and bioactivities of microbial metabolites and constituents in high-fat diet induced NASH mice, human hepatic (HepG2) and intestinal (Caco2) cell lines, high-fat diet induced obesity mice

1. Effects of microbial fatty acid metabolites (KetoA and KetoC) on lipid metabolism in anonalcoholic steatohepatitis mouse model

Nonalcoholic steatohepatitis (NASH) is a common liver disease that occurs in both alcoholics and non-alcoholics. Oxidative stress is a possible causative factor for liver diseases including NASH. NASH is not a simple type of obesity that affects the liver. It can lead to cirrhosis, which permanently damages the liver, requiring a liver transplant. Therefore, therapies for NASH must be developed. Oxidative stress is a possible causative factor for liver diseases including NASH. Gut microorganisms, especially lactic acid bacteria, can produce unique fatty acids, including hydroxy, oxo, conjugated, and partially saturated fatty acids. Previous study demonstrated the oxo fatty acid 10-oxo-11(*E*)-octadecenoic acid (KetoC) provides potent cytoprotective effects against oxidative stress through activation of Nrf2-ARE pathway. The aim of this study is to explore the preventive and therapeutic effects of gut microbial fatty acid metabolites in a NASH mouse model. The mice were divided into 3 experimental groups and fed as follows: 1) high-fat diet (HFD) 2) HFD mixed with 0.1% KetoA (10-oxo-12(*Z*)-octadecenoic acid) and 3) HFD mixed with 0.1% KetoC. After 3 weeks of feeding, plasma parameters, liver histology, and mRNA expression of multiple genes were assessed. There was hardly any difference in fat accumulation in the histological study; however, no ballooning occurred in 2/5 mice of KetoC group. Bridging fibrosis was not observed in the KetoA group, although KetoA administration did not significantly suppress fibrosis score (P=0.10). In addition, KetoC increased the expression level of HDL related genes and HDL cholesterol levels in the plasma. These results indicated that KetoA and KetoC may partly affects the progression of NASH in mice models.

2. Effects of fatty acid metabolites (KetoA and KetoC) on human hepatic (HepG2) and intestinal (Caco2) cell lines

Preliminary investigation using NASH STAM mouse as a model showed that KetoC had increase the level of HDL cholesterol in plasma samples. Furthermore, the gene expression level of HDL-related genes also showed the significant increased by

Keto C treatment. However the mechanism on reverse cholesterol transport (RCT) by modified fatty acid has not yet well studied. That's why this study aims to evaluate the effect of modified fatty acid (KetoA and KetoC) in cell culture (HepG2 and Caco2) and to understand the mechanism happened in this study. Human *in vitro* ELISA of Apolipoprotein A-1 (ApoA1) was used to measure the ApoA1 content of the HepG2 cell culture. KetoA and KetoC treatment resulted in a dose-dependent increase of ApoA1 concentration in the cell culture media of HepG2. HepG2 cells and Caco2 cells were cultured in DMEM medium and treated with 30 μM and 60 μM of KetoA and KetoC both respectively for 24 h. The evaluation of HDL related genes expression levels in HepG2 and Caco2 cells were conducted with RT-PCR. In agreement with ELISA protein result, the expression level of *APOA1* gene in HepG2 cells were also significantly increased by the treatment of 60 μM of KetoC. However, there were no significant differences of gene expression levels in Caco2 cells. The effects of EPA, ARA, and hidroxy form of PUFA (12-OH EPA, 15-OH ARA, 11-OH DHA, 14-OH DHA) were also investigated. However, these fatty acids showed no effect on the expression level of genes related to RCT metabolism.

3. Effects of euglena-derived carotenoid and extract on high-fat diet fed-mice

Obesity is an abnormal condition with an inflammatory process in adipose tissue and liver. Chronic inflammation is considered to be important for the development of insulin resistance. Euglena is unicellular green algae, which contained several nutrients. Euglena extract was reported to have an ability to inhibit the early stage of adipocyte-differentiation. Thus, euglena is a promising candidate for the development of a new therapeutic treatment for obesity. Diatoxanthin (Dtx) is one of major characteristic carotenoids contained in euglena, however, there are only a few reports on its functionality. Therefore, this study aims to identify the effects of euglena-derived carotenoid and extract on high-fat diet-fed mice, especially for the purpose of finding new functionalities. Mice were preliminary raised for ten days and randomly divided into 4 groups: Normal Diet (ND) group, High Fat Diet (HD) group, HD+diatoxanthin fraction (DF) group, HD+hot water extract of euglena (HWE) group. DF was found to suppress

the increase in blood glucose level by high fat diet. Dietary DF reduced the expression of lipogenesis-related genes and enhanced the expression of β-oxidation related genes in the liver. We also successfully detect the accumulation of DF in the tissues. These results suggested that functional ingredient were present in the DF fraction.

Conclusion

Fatty acid metabolites (KetoA and KetoC) produced by gut lactic acid bacteria may affect the progression of NASH in model mice through the suppression of inflammation, fibrosis, ballooning, and oxidative stress. Together with a significant increased of plasma HDL cholesterol (*in vivo*) and ApoA1 (HDL-Chol main component) concentration (*in vitro*) also the significant increased of HDL related genes both *in vivo* and *in vitro*, our results suggest that KetoC would improve RCT ability. Euglena extract and its carotenoid (diatoxanthin) are promising candidates for obesity treatment through the suppression of blood glucose level and their effects on lipid metabolism related genes in high-fat diet induced mice. In conclusion, microbial metabolites and its constituent from gut microbiota and microalgae may be beneficial as a foodstuff for the prevention of life-style related diseases.