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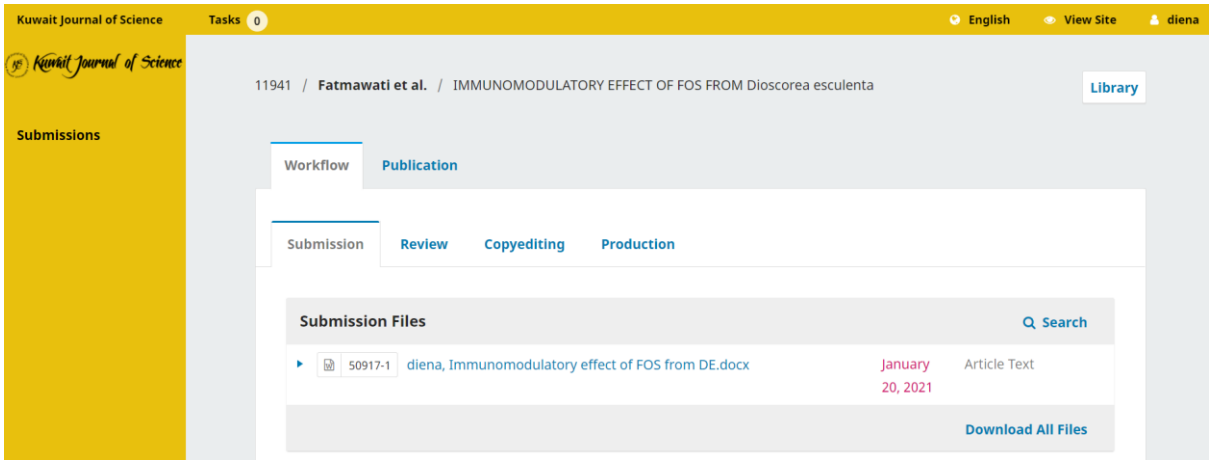

IMMUNOMODULATORY EFFECT OF FOS FROM *Dioscorea esculenta*

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ABSTRACT

Dioscorea esculenta has been shown to contain a fructooligosaccharide (FOS) and may affect immune system. However, there were few studies on the effect of FOS from *Dioscorea esculenta* on immune system. The aims of this study were to characterize the prebiotic of FOS from *Dioscorea esculenta* and determine its effect on Foxp3^{+}T cell expression, level of IL-10 and sIgA.

FOS was characterized based on its degree of polymerization. Balb/c mice were supplemented daily with FOS from *Dioscorea esculenta* by gavage for 2 weeks. Interleukin-10 (IL-10) and sIgA was investigated by ELISA. The phagocytic index was quantified by direct observation using an inverted microscope for a total of three fields per well. Independent T-test was used to determine the mean difference between FOS and control group.

The percentage of Foxp3+ T cell population was significantly increased in spleen of FOS group than that of control ($p<0.05$). In addition, FOS group had a higher level of IL-10 and sIgA compared to control group ($p<0.05$).

This study concluded that FOS from *Dioscorea esculenta* with 2 and 5 degree of polymerization can up-regulate the number of Treg cells, IL-10 level and phagocytic index in mice.

Keywords: Fructooligosaccharide, Dioscorea esculenta, sIgA level, IL-10 level

Introduction

Allergic events increase from year to year and cause problems for the world of health such as respiratory disorders (asthma, allergic rhinitis), inflammatory bowel disease (colitis), skin diseases (atopic dermatitis, urticaria) (Candra, Setiarini, & Rengganis, 2011 ; Kumar & Bhatia, 2013; Tanukusumah, Kurniati, Amelia, Department, & Children, 2015). Based on medical record in RSUP dr. Karyadi Semarang recorded 313 cases per year from 20,630 in ENT allergy polyclinic and atopic dermatitis data showed the highest rate at the age of 5 years of 62.6% (Wistiani & Notoatmojo, 2011) Allergy is a hypersensitivity reaction that begins with a specific IgE level warning against allergens and age classification of patients from children to adults. Allergic patients experience changes in the body's normal immune response such as elevated serum levels of Immunoglobulin E (IgE), local eosinophil and Th2 cell infestations and other responses caused by an imbalance between Th1 and Th2 cells resulting in polarization towards Th2 (Gourbeyre, Denery & Bodinier, 2011).

The use of probiotic Lactobacillus casei and Bifidobacterium in combination form in allergy sufferers can increase the ratio of gamma interferon (IFNɣ): interleukin-4 (IL-4) whereas the use of prebiotic inulin from *Dioscorea esculenta* in experimental animals can improve Th1 response in the form of TNF alpha (TNF-α) and IFN</s>. Several studies on probiotics and prebiotics have been carried out against allergic immune responses, however,

tend to increase Th1 responses towards inflammation. The combination of probiotics and prebiotics is a promising strategy for enhancing immune system modulation to balance the Th1 / Th2 immune response through increased CD4 + CD25 + FoxP3 + Treg expression and IL-10 levels but research on Lactobacillus casei and Bifidobacterium and FOS formulations of inulin gembili as immunomodulatory based therapy has not been performed. The aim of this research is to know the effect of FOS gibili bulb on levels of IL-10, macrophage phagocytosis power, and microbial profile in gastrointestinal tract of mouse.

Methods

Collection and determination of *Dioscorea esculentae*

The samples were obtained from farmers in Kendal area and then conducted determination in the Laboratory of Taxonomic Department of Biology, Diponegoro University. Gembili composition was analyzed using spektofotometri method.

FOS production from inuline *Dioscorea esculantae*

Inulin is extracted from the bulbous bulb using hot water temperature 80-90 ° C and precipitation at -20 ° C, then the inulin deposit obtained is dried by cabinet drying method (Inulin KR) and foam mat drying (Inulin FM). The production of FOS from Inulin gembili enzymatically uses commercial endo inulase enzyme. The characteristics / properties of FOS observed include solubility, water absorption, purity, viscosity, gel strength, crystallinity, polymerization degree (DP), commercial FOS (Inulin SD) used as standard. Evaluation of prebiotic properties of inborn bulb inulin was performed in vitro using Bifidobacterium sp., *Lactobacillus casei.* dan *Escherichia coli*

Probiotic culture

Probiotic cultures used were probiotic starter consisting of *Lactobacillus casei* concentration 1 x 10^9CFU and Bifidobacterium 1 x 10^9CFU grown on growth medium. Probiotic growth observation was performed every 6 hours and plotted on a sigmoid curve to establish its log growth phase.

Formulasi sinbiotik

Probiotic formulations include a combination of both probiotics and prebiotics with different ratios and adjuvants. In this research there are 6 types of formulation which consist of:

- Formula 1 : (*Lactobacillus casei : Bifodobacterium*) 1:1 + prebiotic inulin
- Formula 2 : (*Lactobacillus casei : Bifodobacterium*) 1:2 + prebiotic inulin
- Formula 3 : (*Lactobacillus casei : Bifodobacterium*) 2:1 + prebiotic
- Formula 4 : (*Lactobacillus casei : Bifodobacterium*) 1:1+ prebiotic FOS inulin

- Formula 5 : (*Lactobacillus casei : Bifodobacterium*) 1:2 + prebiotic FOS inulin

Formula 6 : (*Lactobacillus casei : Bifodobacterium*) 2:1 + prebiotic FOS inulin

Immunodulator assay

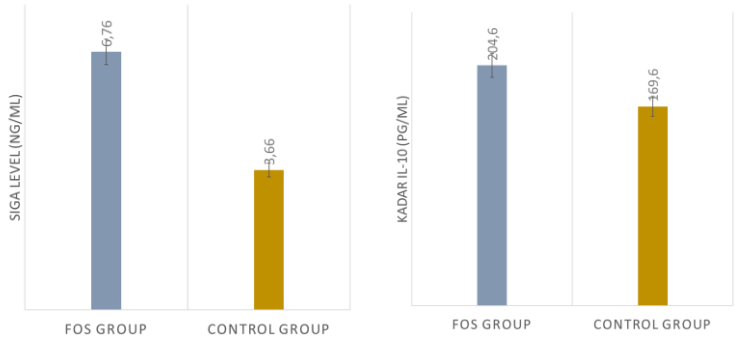
Immunomodulatory potential tests include calculations of Treg cells expressing FoxP3 + and measurements of IL-10 levels. In the immunomodulatory stage, starting with blood collection and isolation of immature dendritic cells and T lymphocyte cells using Ficoll histoplaque gradient 0.7 followed by treatment group exposure (probiotic, single probit and single adjuvant) formula in immature dendritic cell culture so that dendritic becomes matur . Mature dendritic will present antigens to T cell culture so that T cells will differentiate into effector T cells one of them Treg (CD25 +, CD4 +, FoxP3 +) and express one of the IL-10 cytokines. Calculation of FoxP3 + expression on T cell culture using immunochemical methods with the help of flowsitometry while, IL-10 levels were measured using Elisa method. Percentage analysis of T cells expressing FoxP3 + and IL-10 levels was performed using cellQuest software.

The data obtained are presented in tabular form of data and images. Data were then analyzed by using anova inferential parametric test with 5% significance level. Differences between several treatment groups will be further analyzed using the Tukey LSD test to determine the effective probiotic formulation group as immunomulators. Data that do not meet the parametric analysis rules will be tested using a cruciate wallis analysis using SPSS.20 software

Result and Discussion

The optimum result of FOS yield of gembili bulb obtained degree of polymerization ranged from 2 to 12 (table 2).

Table 2. The degree of FOS polymer based on optimization of inulinase enzyme activity



The mean rates of IL-10 and macrophage phagocytic phagocytes in the first and second groups were higher than in the third group (control) ($p < 0.05$), but the administration of FOS in both the first and second groups did not differ significantly ($p > 0.05$) .

Research conducted by Baken (2006) states that the provision of L. casei in animal try-made allergy to food is known to suppress systemic allergic reaction but, on the other hand giving L. casei can also suppress degree of autoimmune severity in diabetics and arthritis. This is due to the effects of L. casei administration on cellular immune responses such as inhibition of T cell proliferation, decreased production of IL-6, IFN-ɣ, and increased production of IL-10 and IL-12, and humoral response. A study by Smits et al. (2005) suggests that administration of L. casei causes elevated levels of IL-10 by Treg cells through modulation of dendritic cell function. Giovanni et al. (2007) Lactobacillus casei DN-1 14 001 reduced the frequency of recurrence in 64 children with allergic rhinitis. Different studies on the other hand show that administration of Lactobacillus rhamnosus GG during spring did not show any decrease in symptoms. The administration of Bifodibacterim may increase the production of Treg cells functionally as immune response regulators (Lopez et al., 2011).

Research conducted by Likotrafili et al. (2014) suggests that prebiotic administration may increase the number of probiotic species of Lactobacillus and Bifodibacterium in the distal colon of adults. Some studies suggest that the use of FOS and GOS may have an effect on bifidobacterium activity and effects associated with modulation of immune responses including decreased levels of proinflamatory cytokines and elevated anti-inflammatory cytokines IL-10 (Voreades et al., 2014).

Conclusion

FOS from *D. esculentae* can increase in the level of interleukin10 and secretory immunoglobulin A

Acknowledgment

This research was funded by Universitas Islam Sultan Agung.

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