Study of Endophytic Bacteria as Novel Source of Antioxidant Agent Based on GC-MS Analysis

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Abstract—Antioxidant plays an important role in human life. Thus, the novel source of antioxidant need to be explored. One of them is endophytic bacteria. This study aimed to evaluate the ability of BS1 isolate in order to produce antioxidant compound based on identification of chloroform extract using GC-MS. The GC-MS identification showed 15 detectable compounds. But, none of these compounds was directly addressed as antioxidant agent. Thus, the optimation of fermentation, media, and extraction process need to be conducted for better result.

Keywords— antioxidant, BS1, endophytic bacteria, GC-MS

I. Introduction

ANTIOXIDANT has become one of important issue in recent years. The term "antioxidant" refers to substance that significantly delays or prevents oxidation of the substrate [1]. In human body, the oxidation process always occurs as the consequence of cellular respiration. The cell also provide natural antioxidant which maintain the body health. But in some case, where the oxidation process are abnormal, the body produce reactive oxygen species (ROS) which harmfull for body, especially cells. This component could damage cellular systems and, in some case, could lead to health disorder. Antioxidant not only related to human health, but also in food processing. Almost all food industries face the same problem, that is oxidation process. This process could change food presentation (colors, texture, etc), taste, flavor and nutrient composition. Antioxidant could delays or prevents this process. Antioxidant also used in cosmetic industries as anti-aging formula and skin nutrition. Nowadays, antioxidant produced from synthetic materials and process, and also from plants, for example chavicol as essential oil from *Piper betle* leaf extract [2]. But, as the human population growth and the increase of healthy life awareness, today people are prefer something natural, which means come from nature. Thus, the exploration of novel source of antioxidant is unavoidable. One of the most promising source of natural bioactive compound is endophytes.

Endophytes are microorganisms that reside internal tissues of all plant species. Endophytes, including fungi and bacteria, are proven as source of novel organic natural molecules [3]. Endophytic bacteria is plant-living-inside bacteria which colonizing internal tissue of plants. This bacteria could live in one particular tissue point or spread throughout the plant [4].

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As describe earlier, some species of endophytic bacteria showed an ability to produce potential product : endophytic bacteria, *Bacillus polymixa,* which isolated from *Artemisia annua*, produce antimalarial artemisinin in the synthetic liquid medium [5]. *Streptomyces* sp. NRRL 30566 from *Grevillea pteridifolia* produce an antibiotic compound called kakadumycin [6]; *Pseudomonas viridiflava*, the plant-associated bacteria, produce unique antimycotics named ecomycins [7].

According to previous studies, endophytic bacteria could be the novel source of antioxidant. This study aimed to evaluate the ability of endophytic bacteria to produce antioxidant compound based on identification of fermentation extract using GC-MS . Bacteria used in this study was obtained from previous study, named BS1 isolate. This endophytic bacteria isolated from *Piper betle* L. in Bogor, Indonesia and has been identified as *Pseudomonas* sp. based on 16S rRNA analysis [8] and also showed antibacterial activity [9].

II. Materials And Method

A. Fermentation And Extraction

BS1 isolate was growth in 50 mL nutrient broth (NB) for 48 hours (150 rpm, 28-30°C). Culture then centrifuged in 5500 rpm for 60 minutes. Chloroform was added to supernatant for extraction. Chloroform extract was evaporated in 50ºC for 15 minutes.

B. Chloroform Extract Identification Using Gas Chromatography-Mass Spectrometry (GC-MS)

Chloroform extract was diluted in acetone then injected to GC-MS instrument. GC-MS process was conducted using capillary column type Phase Rtx-5MS, 60 meters and 0.25 mm (diameters).

III. Result And Discussion

Fermentation process conducted for 48 hours to obtain the maximum amount of metabolites from bacteria, in this study refers to BS1 isolate. BS 1 fermentation culture was centrifuged to separate bacteria cells and media that contained the metabolites. The media phase then extracted using chloroform which has non-polar characteristic. This solvent was used because NB media was already contained water, which is polar. Thus, this treatment was expected to capture wide range of bioactive compound. Water phase did not analyzed using GC-MS because of its polarity which capture the debris most, like carbohydrates, proteins and other polar molecules which usually contaminate the identification.

GC-MS identification showed that chloroform extract of BS1 culture have 15 detectable compounds (Table I). Benzotriazole substituted with acetyl carbamic acid derivates, as mentioned in earlier study, showed antioxidant activity [10]. But, carbamic acid as single compound has not been known as antioxidant. Thus, none of these compounds has been proven as antioxidant agent. Nevertheless, other studies showed different result. As reported, endophytic bacteria associated with ethnomedicinal plants in North-East India showed a good antioxidant properties [11]. Endophytic bacteria from *Myricaria laxiflora* also showed same result. The antioxidant capacity in vitro analysis showed that 10 endophytic bacteria have strong activity. Most highly active strains were from the host after blooding [12]. The endophytic bacteria *Lactobacillus sp*. has been isolated from the tissues of *Adhathoda beddomei* leaves and also showed antioxidant capacity, supported by a high total phenolic compound [13].

TABLE I
GC-MS IDENTIFICATION RESULT OF BS1 ISOLATE [8]

No	Name
1	Cyclopropane, 1,1-dibromo-2-chloro-2-fluoro- (CAS) 1,1-DIBROMO -2-CHLORO
2	Carbamic acid, monoammonium salt (CAS) Ammonium carbamate
3	Chloroform
4	Piperazine (CAS) R22
5	2,4-Imidazolidinedione, 1-methyl-
6	Phenol, 2,4-bis(1,1-dimethylethyl)- (CAS) 2,4-Di-tert-butylphenol
7	Cycloglycylalanine
8	1-Octadecene (CAS) .alpha.-Octadecene
9	3-PYRROLIDIN-2-YL-PROPIONIC ACID
10	Cyclopentanamine, N-ethyl- (CAS) N-Ethylcyclopentylamine
11	1,4-diaza-2,5-dioxobicyclo[4.3.0]nonane
12	1,4-diaza-2,5-dioxo-3-isobutyl bicyclo[4.3.0]nonane
13	5,10-DIETHOXY-2,3,7,8-TETRAHYDRO-1H,6H-DIPYRROLO [1,2-A;1',2'-D]P
14	Hexanedioic acid, dioctyl ester (CAS) Dioctyl adipate
15	3-benzyl-1,4-diaza-2,5-dioxobicyclo[4.3.0]nonane

IV. CONCLUSION

Although BS1 isolate did not showed an antioxidant properties in this study, the other experiment can be conducted. For example optimizing the condition for fermentation or using the specific media and inducer to enhance the amount of metabolites. *In vitro* test also need to be conducted.

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