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Ability to Synthesize Extracellular Metabolites of Rice Endophytic Bacteria Before and After *gfp* Gene Transformation

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Abstract. The purpose of this research was to investigate the ability of rice endophytic bacteria to synthesize extracellular metabolites before and after *gfp* (Green Fluorescent Protein) gene transformation. These bacteria were grown in liquid media M63+2mM Tryptophan for 3 days and the supernatants were extracted with absolute methanol 1:1 (v/v). The extracts were then analysed with Gas Chromatography-Mass Spectrophotometry (GC-MS). The GC-MS result showed that all isolates that were tested be it the control (without transformation) and the mutants (transformed) were able to synthesize growth hormones with different composition patterns, namely Indole 3 Butyric Acid (IBA), Indole Acetic Acid (IAA), Kinetin, Cit. Zeatin, Zeatin, Benzylaminopurine, Gibberellic Acid (GA3), GAs) as well as Zeatin Riboside. Furthermore, the isolates also synthesised osmoprotectants, namely Glycine, Proline, Arginine, Thiamine, and Trehalose with different composition pattern as well.

Keywords—Endophytic Bacteria, Extracellular, Fluorescent, Metabolic

1. INTRODUCTION

Endophytic bacteria are bacteria that live in the plant tissue and form a symbiotic relationship with the host plants [1]. Malanova [2] stated that endophytic bacteria that colonized the plant tissue obtain their host plant's nutrition and in return give phytohormone to the host plants. Some endophytic bacteria are able to synthesize compounds that are beneficial to the plants such as antimicrobial compounds, degradation enzyme for cell wall and growth regulators auxin, cytokinin and ethylene [3]. Hallman, and Berg [4] stated that the endophytic bacteria live in the plant tissue that is healthy and play a role in stimulating plant growth by producing growth regulatory compounds such as IAA (*Indole Acetic Acid*), GA3 (*Gibberellic Acid*), dan Cytokinin. Moreover, the endophytic bacteria are able to maintain and improve the soil fertility by providing P and fixing N [5].

Secondary metabolites that are produced by the microbes are beneficial for the growth and development of the plants. Growth regulators that are produced can increase the root length, number of root branches and root hairs, dry mass of root as well as the length of the stem in some plants. The fast root growth is beneficial for young plants to absorb water and nutrients from the environment. This is advantageous especially for plants that grow in dry area where scarcity of water is the main growth inhibitor. Antimicrobials and degradation enzyme for cell wall that are produced are able to

help resist pathogen attack that may cause diseases in plants [4].

Rice endophytic bacteria isolates that are able to synthesize phytohormone and osmoprotectants are obtained from previous experiment result. These bacteria are also already genetically marked with gene *gfp* (green fluorescent protein) to examine their distribution in the plant tissue. The previous experiment did not study the effect of the transformation with gene *gfp* to the change in the bacteria's ability to synthesize growth hormones and osmoprotectants extracellularly. Therefore, this experiment aims to study further the bacteria's ability to synthesize growth hormone and osmoprotectants before and after transformation of gene *gfp*.

II. METHODS

A. Date and Place of Research
This research was conducted in Biotechnology Laboratory of Universitas Muhammadiyah Malang from August 3, to October 29, 2017.

B. Materials
The materials that were used comprised untransformed endophytic bacteria IM-3 (control) and transformed IM-3 with *gfp* (mutant IM-3 + *gfp*) and mineral media M63 + 2 mM Tryptophan + 0.5 M NaCl. The composition of the M63 media was KH₂PO₄ 100 mM, KOH 75 mM, (NH₄)₂SO₄ 15 mM, MgSO₄ 0,16 mM, FeSO₄ 3,9 µM and D-glukosa 10 mM [6].

C. Growth Analysis
Isolates IM-3 (control) and mutant IM-3 + *gfp* were grown on the media M63+ 2 mM tryptophan + 0,5 M NaCl at a temperature of 36 °C and shake of 125 rpm overnight. The bacteria growth was observed every 2 hours by spectrophotometry, with the wavelength 420 nm.

D. Extracellular Metabolite Preparation
Cell culture was harvested, and the supernatant obtained was extracted with absolute ethanol 1:1 (v/v), and concentrated with cold ethanol to 50 times. The extracellular metabolite extract was then analysed with Gas Chromatography Mass Spectrometer (GC-MS) [7].

E. Extracellular Metabolite Analysis
The metabolite was analyzed using GC-MS type Shimadzu QP 2010 SE, with semi-polar column RX1-5MS,

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Artikel 1

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E. Extracellular Metabolite Analysis

The metabolyte was analyzed using GC-MS type Shimadzu QP 2010 SE, with semi-polar column RXi-5MS,

length of 30 m and inner diameter (ID) 0.25 mm, helium-carrying gas with the flow rate of 0.5ml/min, and pressure of 27.4 kPa. The initial temperature of the GC-MS was 120 °C and 5 minute after injection, it was raised at a rate of 5 °C/min until it reached 320 °C and the sample was inserted using a microinjector with a volume of 1-2 ul [8].

III. RESULTS AND DISCUSSION

A. Analysis of Bacteria Growth on Media M63 + 2mM Tryptophan + 0.5M NaCl

The result of the analysis of the endophytic bacteria on media M63 + 2 mM Tryptophan + 0.5 M NaCl shows that there was a difference in the growth pattern between the control (untransformed) and the mutant. The initial bacteria growth of the control was faster than the mutant bacteria, but the mutant bacteria was able to achieve higher growth. According to Allison and Sattenstall [9], insertion of foreign genes to the host cell would increase the size of the DNA genome of the cell which affects the metabolism and the cell growth.

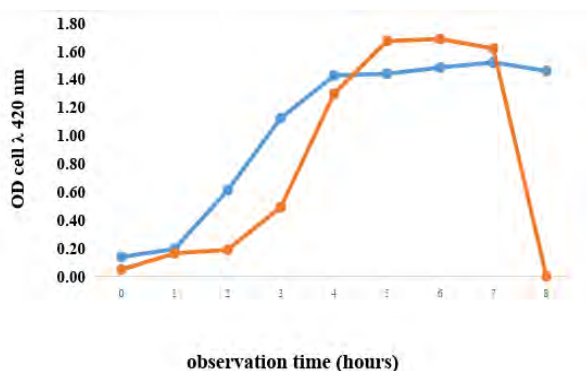


Figure 1. The growth of isolates: — IM-3 control, and — mutant IM-3 + gfp on the media M63 + 2mM Tryptophan + 0.5 M NaCl

B. Analysis of Extracellular Metabolites: Growth Hormone

The result of the analysis of extracellular metabolites using GC-MS is obtained from 9 metabolites that lead to growth hormone (Figure 2). The GC-MS analysis result also shows there was an increase in the synthesis of the extracellular growth hormone metabolites in the mutant bacteria compared to the control (untransformed). This indicates that the gene gfp that was inserted into the genome increased the metabolism of the cell in the synthesis of growth hormone and the cell has the potential to be a organic fertilizer agent [10]. According to Cardinale et al [11], foreign genetic insertion into the genome of an organism (bacteria) affects the metabolic system of the cell by rearranging the metabolism, increasing the synthesis of some metabolites and decreasing the synthesis of other metabolites¹⁰. Furthermore, the result of the analysis shows that Giberilic Acid-3 (GA3) was synthesized with the highest percentage and Benzylaminopurine with the lowest percentage. This shows that GA3 is a growth hormone that was most efficiently synthesized in the osmotic pressure NaCl condition [12].

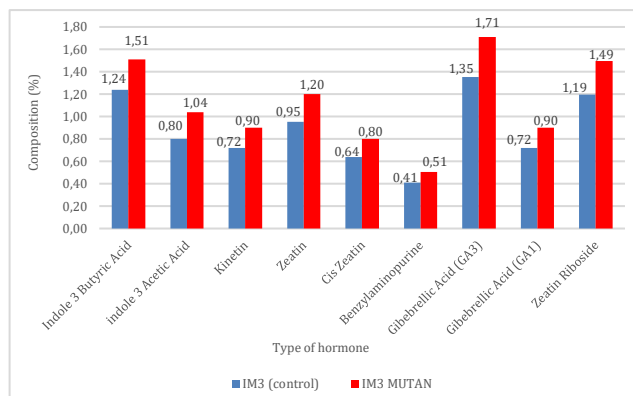


Figure 2: Results of GC-MS analysis of the composition of extracellular growth hormone: ■ IM3 control and ■ mutants-IM3 + gfp

C. Analysis of Extracellular Metabolites: Osmoprotectant

The result of the analysis of metabolites using GC-MS is obtained from five extracellular osmoprotectant compounds with different composition between the IM-3 control and the mutant IM-3 + gfp that were grown in osmotic pressure condition M63 + 0.5M NaCl (Figure 3). There were an increase in some osmoprotectants in the mutant IM-3 + gfp such as Glycine, Proline and Thiamine, but there were some that decrease such as Arabinose and Trehalose, as compared to the IM-3 control. This indicates that the insertion of gene gfp affects the synthesis of osmoprotectants to reach a new equilibrium of cell metabolism [12]. According to Serge et al. [13], Insertion of foreign gene to the cell affects the synthesis of osmoprotectant in the osmotic pressure condition. Moreover, the result of the GC-MS analysis shows that Trehalose was at the highest percentage in both IM-3 control and mutant IM-3 + gfp, while the lowest percentage is Glycine in the mutant isolate IM-3 + gfp and IM-3 control. This shows that the synthesis pathway of Trehalose is the most effective in osmotic pressure [14].

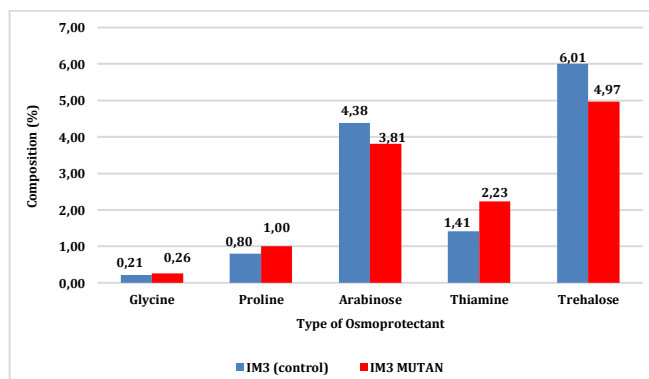


Figure 3: Results of GC-MS analysis of extracellular osmoprotectant composition: ■ IM3 control, and ■ mutants-IM3 + gfp

IV. CONCLUSION

The growth pattern of IM-3 control and mutant IM-3 + gfp differ with respect to their response to osmotic pressure of NaCl. The difference in the growth pattern is also reflected in

the pattern of synthesis of extracellular metabolite be it in growth hormone and osmoprotectant. In the synthesis of growth hormone *IBA*, *IAA*, *Kinentin*, *Cis Zeatin*, *Zeatin*, *Benzylaminopurine*, *GA3*, *GAI* and *Zeatin Riboside* there was an increase in the mutant IM-3 + *gfp* as compared to the control. On the other hand, the synthesis of osmoprotectant compounds such as *Glycine*, *Proline*, and *Thiamine* increased in the mutant and decrease in the synthesis of *Arabinose*, *Coumestrol* dan *Trehalose*.

ACKNOWLEDGEMENTS

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