

Effect of initial inoculum on growth and fatty acid content as an α -glucosidase inhibitor in *Colletotrichum* sp. TSC13 mycelium that cultures under shake and static conditions

Nina Artanti^{1*}, Leonardus B.S. Kardono¹, Sanro Tachibana²

¹Research Center for Chemistry, Indonesian Institute of Sciences (LIPI), Kawasan Puspiptek Serpong, Tangerang Selatan 15314, INDONESIA

²Faculty of Agriculture Ehime University, 3-5-7 Tarumi, Matsuyama, Ehime, 790-8566, Japan

*Corresponding author: ninaartanti@yahoo.com

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Abstract

Colletotrichum species were reported producing various bioactive compounds that may potentially be used in the development of anti-microbial, antioxidant, anticancer and anti-diabetes drugs. Our previous studies showed that the methanol extracts of mycelia from *Colletotrichum* sp. TSC13, an endophytic fungus isolated from *Taxus sumatrana* (Miq.) de Laub., were found to have α -glucosidase inhibitory activities. The aim of present study was to examine the effects of initial inoculum on growth and the content of fatty acids as an α -glucosidase inhibitor in *Colletotrichum* sp. TSC13 that grew under shake and static condition. The results showed that shake and static conditions had more effect compared to the initial inoculum on growth and glucose consumption in growth and glucose consumption. However in terms of fatty acids contents and α -glucosidase inhibitor activities the initial inoculum showed more effect than shake and static conditions.

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1. INTRODUCTION

In many plant species, *Colletotrichum* is a genus of fungi that commonly known as the pathogens causing the anthracnose disease [1-6]. However, this fungal species also showed potential bioactivities by producing bioactive compounds such taxol, phillyrin, oleic acid etc., that may potentially be used in the development of anti-microbial, antioxidant, anticancer and anti-diabetes drugs [7-11]. The methanol extracts of mycelia from *Colletotrichum* sp. TSC13, an endophytic fungus isolated from *Taxus sumatrana* (Miq.) de Laub., were found to have α -glucosidase inhibitory activities [10]. α -Glucosidase is an enzyme that is responsible for breaking down carbohydrates into glucose in the human intestine, and inhibitors of this enzyme may be used in the management of postprandial hyperglycemia in patients with type II diabetes [13]. The bioactive compounds of the α -glucosidase

inhibitor detected in *Colletotrichum* sp. TSC13 were identified as the unsaturated fatty acids, oleic acid, linoleic acid, and linolenic acid [12]. Several published reports suggested that media compositions and culture conditions might have affected mycelial growth and the content of bioactive compounds, including fatty acids in fungal cultures [14-16]. The effects of the carbon source, nitrogen source, metal ions, and desaturase and fatty acid synthase inhibitors on fungal growth and the content of fatty acids in *Colletotrichum* sp. TSC13 have already been reported [17]. The aim of present study was to examine the effects of initial inoculum on growth and the content of fatty acids as an α -glucosidase inhibitor in *Colletotrichum* sp. TSC13 that grow under shake and static condition.

2. EXPERIMENTAL SECTION

2.1. Materials

2.1.1. Fungal culture

Colletotrichum sp. TSC13 is an endophytic fungus isolated from *Taxus sumatrana* (Miq.) de Laub that was kindly supplied by Ms. Harmastini Sukiman (Research Center for Biotechnology, Indonesian Institute of Sciences) and used in the present study.

2.2. Methods

2.2.1. Fungal culture maintenance and preparation

The fungus was transferred to a Potato Dextrose Agar (PDA) plate. It was cultured for 7 days (Fig.1.) prior to use for inoculums of mycelial plugs (1 cm diameter) in the experiments using liquid media Potato Dextrose Broth (PDB).



Fig. 1. *Colletotrichum* sp. TSC13 on PDA medium (7 days)

2.2.2. Effect of initial inoculum

Fungal cultures with different initial inoculums (1, 2 and 5 mycelial plugs) were conducted in 60 mL PDB media in 200 mL flask at 25 °C for 7 days under the shake (75 rpm) or static condition (Fig. 2). Fungal growth was measured as mycelium dry weight (mg) and glucose consumption which was determined as the percentage of the glucose concentration consumed at harvesting time relative to that in the fresh media.

2.2.3. Extract preparation.

Dried mycelia were extracted with methanol. The methanol extracts were evaporated to dryness under a reduced pressure and then used to determine the

content of fatty acids and α -glucosidase inhibitory activity.

2.2.4. Fatty acid analysis

Analysis of fatty acids content was conducted using GC-MS, Shimadzu-QP-2010. The column used was a SPB-50 column (30 m x 0.25 mm I.D., 0.25 μ m film thickness).

2.2.5. α -Glucosidase inhibitory assay.

Sample (10 μ g/ml) was added to a test tube containing 3 mM pNPG (*p*-Nitrophenyl α -D-glucopyranoside) in phosphate buffer at pH 7.0, then incubated for 5 min at 37 °C. The reaction was initiated by addition of enzyme solution followed with 15 min incubation at 37 °C as previously described [10]. The absorbance of *p*-nitrophenol released from pNPG at 400 nm was measured with a spectrophotometer.

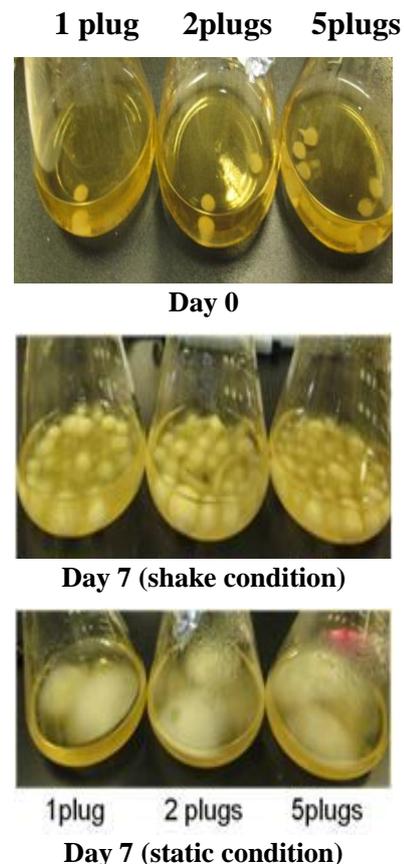


Fig. 2. *Colletotrichum* sp. TSC13 on PDB medium

3. RESULT AND DISCUSSION

The results in Fig. 3 and Table 1 showed that during 7 days of culture in 60 mL media at 25 °C, initial inoculum effected the mycelium growth of *Colletotrichum* sp. TSC13 both in shake and static conditions. Although greater effect shown in shake condition which the 5 plugs growth was almost 2 fold than 1 plug, comparing to in static condition the 5 plug growth was only 1.6 fold than 1 plug. The effect of initial inoculum in glucose consumption also had similar pattern to the effect on mycelium growth. The results clearly showed that shake and static conditions had more effect compared to the initial inoculum on growth and glucose consumption. The fold increase in growth due to initial inoculum only ranged from 1.3 to 1.9 fold whereas due to shake and static condition were ranged from 2.2 to 2.6 fold. *Mucor rouxii* and *Mucor sp. Ib.* [14], *Aspergillus niger* [18], and *Pleurotus ostreatus*, *Ganoderma lucidum*, *Auricularia auricula*, and *Lentinula edodes* [19], mycelial growth showed similar results to *Colletotrichum* sp. TSC13, that also reported to be greater under shaking than static conditions. However in terms of fatty acids contents for *Colletotrichum* sp. TSC13 the initial inoculum showed more effect than shake and static conditions. Linoleic acid is the major unsaturated fatty acid found in all treatment. The difference of linoleic acid content due to initial inoculum was ranged from 1.1 to 1.9 fold whereas similar content was obtained of linoleic acid of cultures under shake and static conditions with the same initial inoculum. Glucose consumption and the content of linoleic acid in cultures of *G. lucidum*, *A. auricular*, and *L. edodes* were significantly less under static conditions than under shaking conditions [19]. In contrary the *V. volvacea* culture, the content of linoleic acid in 8-, 12-, 14-, and 20-day cultures was always higher under the static condition than under the shaking condition, whereas no significant differences were reported in fungal growth and glucose consumption between these two conditions [20]. The effect of initial inoculum in α -glucosidase inhibitory activity of *Colletotrichum* sp. TSC13 also showed

similar pattern with the effect in fatty acids content, because the unsaturated fatty acids were the α -glucosidase inhibitor.

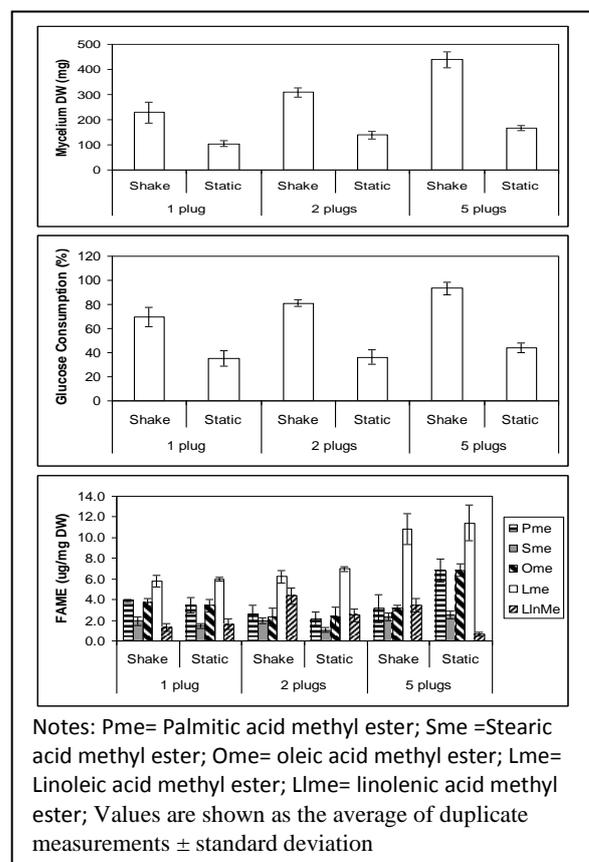


Fig. 3. Effects of initial inoculum under shake and static conditions on fungal growth (A); glucose consumption (B), and the content of fatty acids (C).

Table 1. The α -glucosidase inhibitory activities of methanol extracts from *Colletotrichum* sp. TSC13 at different initial inoculum under shake and static conditions

Initial Inoculum	Condition	Inhibition (%)
1 plug	Shake	73.2±4.1
	Static	73.8±3.3
2 plugs	Shake	71.1±4.3
	Static	75.2±5.9
5 plugs	Shake	88.6±3.2
	Static	90.8±2.5

Notes: The assay for the α -glucosidase inhibitor was conducted in 10 $\mu\text{g mL}^{-1}$ of the extracts. Values are shown as the average of duplicate measurements \pm standard deviation

4. CONCLUSIONS

The results showed that shake and static conditions had more effect compared to the

initial inoculum in growth and glucose consumption in growth and glucose consumption. However in terms of fatty acids contents and α -glucosidase inhibitor activities the initial inoculum showed more effect than shake and static conditions.

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