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学位論文全文に代わる要約
Extended Summary in Lieu of Dissertation

氏名：
Name Nina Artanti

学位論文題目：
Title of Dissertation

Studies on α-Glucosidase Inhibitors from *Colletotrichum* sp. TSC13, Endophytic Fungus isolated from *Taxus sumatrana* (Miq.) de Laub.
スマトライチイ(*Taxus sumatrana* (Miq.) de Laub.) から単離された *Colletotrichum* sp. TSC13 菌のα-グルコシダーゼ阻害物質に関する研究

学位論文要約：
Dissertation Summary

Since ancient time, human always depend on natural product for their health maintenance and medicine. Many current important drugs were derived from natural product. Although current modern medicine seems to prefer synthetic drugs, according to analysis on 1073 new chemical entities used for new drugs development from 1981 to 2010 (30 years) only 36% can be categorized as truly synthetic, whereas others have their origin as mimic or chromopore from natural products. Therefore, even in current situation natural product still plays important roles in drug discovery to find treatment for many serious diseases in the word.

Diabetes mellitus (DM) is one of the serious non-communicable diseases affecting numerous people in the world. It is a chronic metabolic disease that occurs either when not enough insulin (hormone that regulate blood sugar) produced by the pancreas or when the insulin produces cannot effectively use by the body. Therefore it is important to continue study for finding medicines for DM treatments. The most common DM is the Type 2 DM (non-insuline dependent DM) which accounts 85-95% of total people with diabetes. Inhibitor of α-glucosidase is one of the oral treatments to control postprandial hyperglycemia for people suffered with type 2 DM.

Besides natural products from plant origin, since the discovery of penicillin, microbial source also attract interest for finding lead compounds that can be used for drugs discovery for various

diseases including diabetes. Endophytic microbes are microbes that have symbiotic live in plant. The most frequently isolated endophytes are the fungi. This group of microbes was considered as important source of natural products for leads in drug discovery.

The objective of research reported in this Dissertation was to search for α-glucosidase inhibitors as antidiabetes from endophytic fungi isolated from *Taxus sumatrana* (Miq.) de Laub. The selected fungus having the best α-glucosidase inhibitory activity from screening result was then studies further on the isolation characterization of the α-glucosidase inhibitor compounds. Afterward, studies on the effect of media compositions and culture conditions on growth and α-glucosidase inhibitors content of this fungus were conducted.

An *in vitro* screening of 14 endophytic fungi isolated from *T. sumatrana* having α-glucosidase inhibitory activities have been conducted. Table 1. shows the screening results of antidiabetic activities of mycelium methanol extracts and medium ethyl acetate extracts of all the endophytic fungi tested. Under the same culture conditions, α-glucosidase inhibitory activity of the endophytic fungi only detected in the methanol extracts of 8 fungal mycelia (range from 2.4 to 89.5% inhibiton) and 2 ethyl acetate extracts of fungal media (18.2 and 18.6% inhibition). The screening results showed that from methanol extract fungal mycelia TSC13 had the best α-glucosidase inhibitory activity.

Study then focus on isolation characterization of α-glucosidase inhibitors from mycelium methanol extract of TSC 13 which identified as *Colletotrichum* sp. (Figure 1.). The *n*-hexane fraction of the *Colletotrichum* sp. TSC13 mycelium methanol extract had the best α-glucosidase inhibitory activity was then separated using silica gel column chromatography to give 8 fractions (F1-8). From these fractions, Fraction 3 (F3) which showed 71.4% inhibitions for α-glucosidase activity was analysed further. Analysis using GC-MS after methylation of F3 and comparison to spectra databases and confirmation using authentic sample standards showed that F3 had two saturated fatty acid methyl

esters, palmitic acid and stearic acid methyl esters, and three unsaturated fatty acid methyl esters, oleic acid, linoleic acid and linolenic acid methyl esters (Figure 2.).

Table 1. The results of antidiabetic activities of endophytic fungi from *T. sumatrana*.

Samples	α-glucosidase % inhibition	
	FB	M
TSC1	nd	nd
TSC2	nd	nd
TSC3	2.7	nd
TSC7	nd	nd
TSC8	nd	nd
TSC12	55.3	nd
TSC13	79.5	18.2
TSC14	45.3	nd
TSC17	68.4	nd
TSC22	nd	nd
TSC23	66.3	18.6
TSC24	4.7	nd
TSC26	nd	nd
TSC28	2.4	nd

Notes: Antidiabetic activity showed α-glucosidaseinhibitory activity. Value is shown as average of duplicate measurements. (FB=fungal mycelium MeOH extract; M= fungal medium EtOAc extract; nd= not detected)

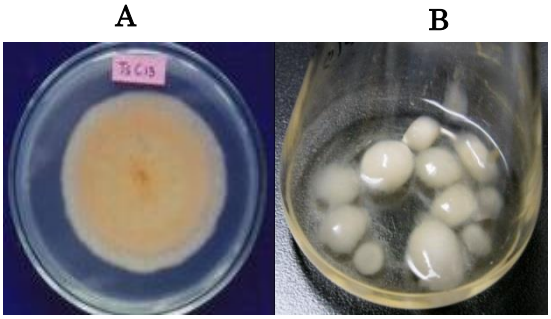


Figure 1. Endophytic fungus TSC13 identified as *Colletotrichum* sp. on PDA plate (A) and PDB liquid medium (B)

Further analysis using an ethyl acetate extract of fungal mycelia confirmed that most of the fatty acids were present in the form of free acids (Figure 3 and Table 2 and 3). α-Glucosidase inhibitory activity of *Colletotrichum* sp. TSC13 were conducted and found that the α-glucosidase

inhibitor compounds in *Colletotrichum* sp. TSC13 were three unsaturated fatty acids. The highest α-glucosidase inhibitory activity in the three unsaturated fatty acids was oleic acid (IC_{50}: 2.2μg/mL), next was linoleic acid (IC_{50}: 2.9μg/mL) and linolenic acid (IC_{50}: 4.4μg/mL). The *Colletotrichum* sp. TSC13 isolated from *T. sumatrana* was found to have α-glucosidase inhibitory activity for the first time.

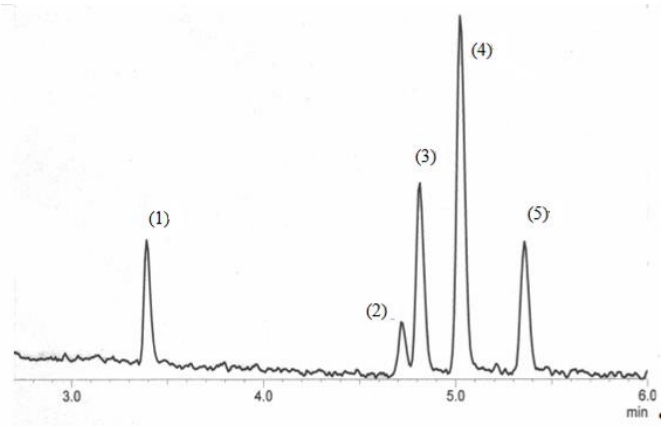


Figure 2. Total ion chromatogram (TIC) of F3 (the active fraction obtained from column chromatography of *Colletotrichum* sp. TSC13 *n*-hexane fraction). Palmitic acid methyl ester (1); stearic acid methyl ester (2); oleic acid methyl ester (3); linoleic acid methyl ester (4); and linolenic acid methyl ester (5).

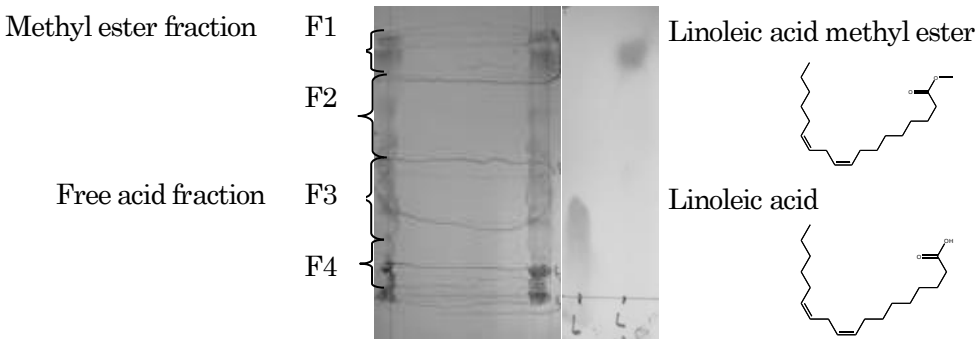


Figure 3. Preparative TLC profile of the EtOAc extract from *Colletotrichum* sp. TSC13 mycelium and TLC of linoleic acid and linoleic acid methyl ester standards.

Table 2. Fatty acid methyl esters in F1-4 of the EtOAc extract from *Colletotrichum* sp. TSC13

Fractions	Fatty acid methyl ester content (%)*				
	Pme	Sme	Ome	Lme	Llme
F1	15.0	20.9	19.8	19.3	18.5
F2	13.0	29.5	17.5	3.0	2.1
F3	67.8	46.8	58.8	76.1	77.8
F4	4.2	2.8	3.9	1.6	1.6

Notes: Pme= Palmitic acid methyl ester; Sme= Stearic acid methyl ester; Ome= Oleic acid methyl ester; Lme= Linoleic acid methyl ester; Llme= Linolenic acid methyl ester

*) Percentage of fatty acid methyl ester content in each fraction to fatty acid methyl ester content in EtOAc extract

Table 3. The α-glucosidase inhibitory activities of the EtOAc extract (EEA) and its fractions

Samples	Inhibition (%)
EEA	84.5
F1	82.5
F2	69.7
F3	90.3
F4	45.2

Notes: Values are shown as the average of duplicate measurements.

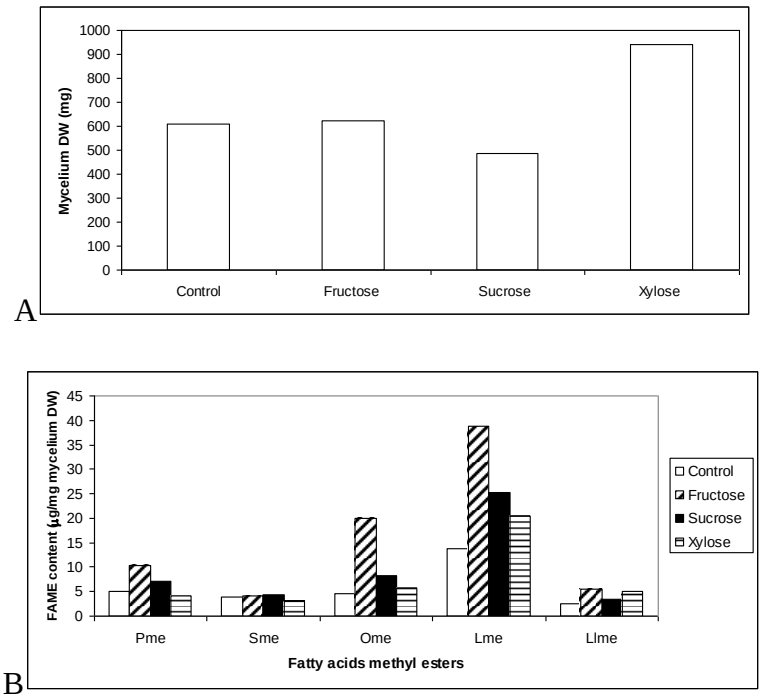
To investigate the effects of various media composition on growth (mycelium dry weight) and the unsaturated fatty acids content (μg/mg mycelium DW) of *Colletotrichum* sp. TSC13 in relation to its α-glucosidase inhibitory activity, the experiments were set up by varying the carbon and nitrogen sources, metal ions, and fatty acid synthase inhibitors in the media. *Colletotrichum* sp. TSC13 grown on potato dextrose broth (PDB) was used as control [the unsaturated fatty acid content (20.7μg/mg mycelium DW); growth (608.7mg mycelium DW)]. The α-glucosidase inhibitory activities were (range from 43.9 to 88.6%) at the concentration of 10μg/mL of the mycelium methanol extracts (Table 4). This activity seemed to correlate with the unsaturated fatty acids content of the samples. Different sugars as carbon source experiment showed that xylose gave the highest growth (938.7mg) (Figure 4A). However, the highest unsaturated fatty acids content was obtained from fructose medium which containing linoleic acid (38.8μg/mg mycelium DW) (Figure 4B). Soluble starch gave better growth (672.5mg), but very low fatty acid content (2.8μg/mg DW) was obtained. Yeast extract was

the best nitrogen source. Production of unsaturated fatty acids (32.4μg/mg mycelium DW) was better as compared to beef extract and soytone. Various media compositions effect on unsaturated fatty acids content in *Colletotrichum* sp. TSC13 in relation to its α-glucosidase inhibitory activity was found for the first time.

Table 4. The α-glucosidase inhibitory activity of samples from media composition experiments. The assay of α-glucosidase inhibitor was conducted at concentration of 10 μg/ml mycelium methanol extracts

Samples	**Inhibition (%)**
Different Sugars	
Control	73.9
Fructose	88.6
Sucrose	80.2
Xylose	76.2
Soluble Starch	
Control	78.3
Soluble starch	56.9
Different Nitrogen Sources	
Control	75.9
Beef extract	48.9
Soytone	45.6
Yeast extract	82.7
Different Metal Ions	
Control	76.2
Cu^{2+}	43.9
Fe^{2+}	86.3
Mg^{2+}	72.9
Different Desaturase and Fatty acid Inhibitors	
Control	78.2
Kapok extract	75.6
Sesame oil	83.9
Turmeric extract	70.9
Green tea extract	72.1

Notes: Values are shown as the average of duplicate measurements

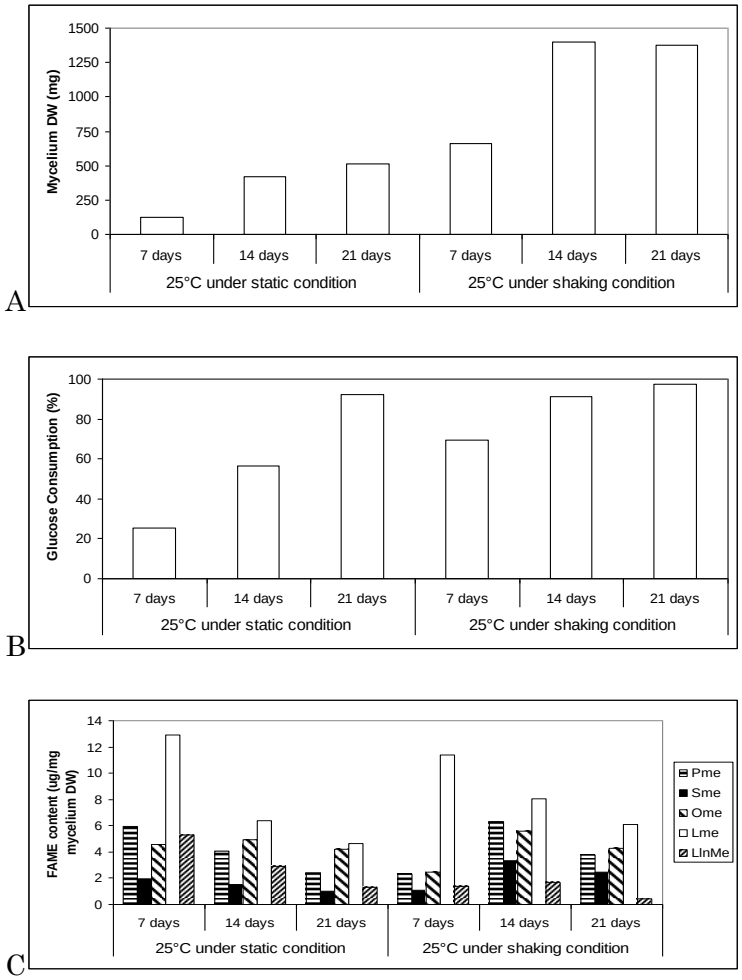


Notes: Pme= Palmitic acid methyl ester; Sme =Stearic acid methyl ester; Ome= oleic acid methyl ester; Lme= Linoleic acid methyl ester; Llme= linolenic acid methyl ester; Values are shown as the average of duplicate measurements

Figure 4. Effect of the addition of different sugars on growth (A) and fatty acid content (B).

Experiments were set up to examine the effects of culture conditions (temperature, media volume, static and shaking conditions in various culture periods, and initial pH) on *Colletotrichum* sp. TSC13 growth, glucose consumption, and the content of unsaturated fatty acids. Fungal growth and glucose consumption were greater at 25°C than at 20°C and greatest in the lowest media volume (50mL media/500mL Erlenmeyer flask) whereas the content of unsaturated fatty acids (56.9µg/mg mycelium DW) was higher at 20°C and in the highest media volume (150mL/500mL Erlenmeyer flask). Fungal growth was significantly greater under the shaking condition than under the static condition (Figure 5A), although the glucose consumption in both condition after 21 days was similar (Figure 5B). The content of unsaturated fatty acids peaked after 7 days of cultivation and declined thereafter under both the static and shaking conditions (Figure 5C). Fungal growth and glucose consumption were the greatest at an initial pH of 7.0, while the content of unsaturated fatty acids was

the highest at an initial pH of 4.5 (the production of the acids was 1.5 higher than that of pH 7.0). Alpha-glucosidase inhibitory activity in all culture condition experiments ranged from 72.7 to 85.7% at the concentration of 10μg/mL of the mycelium methanol extract (Table 5).



Notes: Pme = Palmitic acid methyl ester; Sme =Stearic acid methyl ester; Ome = oleic acid methyl ester; Lme = Linoleic acid methyl ester; Llme= linolenic acid methyl ester; Values are shown as the average of duplicate measurements

Figure 5. Effects of static and shaking conditions at different culture periods on fungal growth (A); glucose consumption (B), and the content of fatty acids (C).

Table 5. The α-glucosidase inhibitory activities of mycelium methanol extracts from *Colletotrichum* sp. TSC13 at various culture condition.

Samples	Inhibition (%)
Different temperatures	
20℃	85.7
25℃	79.1
Different media volumes	
50 mL	83.6
100 mL	83.0
150 mL	84.2
Different pH	
4.5	79.6
5.7	77.8
7.0	77.1
Static Condition	
7 days	81.8
14 days	76.3
21 days	72.7
Shaking Condition	
7 days	80.5
14 days	77.2
21 days	76.9

Notes: The assay for the α-glucosidase inhibitor was conducted at the concentration of 10 μg/mL of the mycelium methanol extracts. Values are shown as the average of duplicate measurements.

The results showed that both media compositions and culture conditions affected the growth and the content of unsaturated fatty acids in *Colletotrichum* sp. TSC13 mycelium. However the content of unsaturated fatty acids as the α-glucosidase inhibitors more affected by the media compositions than the culture condition which expressed in yields obtained and the range of α-glucosidase inhibitory activities. This information will be useful in future studies for conducting scale up fermentation to produce the α-glucosidase inhibitors from mycelium methanol extract of *Colletotrichum* sp. TSC13. Results of these studies revealed the potential of *Colletotrichum* sp. TSC13, an endophytic fungus isolated from *T. sumatrana* to produce the unsaturated fatty acids as α-glucosidase inhibitors for the treatment of diabetes.

(注) 要約の文量は，学位論文の文量の約１０分の１として下さい。図表や写真を含めても構いません。
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