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学 位 論 文 要 旨
Dissertation Abstract

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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

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The objective of this research 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. 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. 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. 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. 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.

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. 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). However, the highest unsaturated fatty acids content was obtained from fructose medium which containing linoleic acid (38.8μg/mg mycelium DW). 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.

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. The content of unsaturated fatty acids peaked after 7 days of cultivation and declined thereafter under both the static and shaking conditions. 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.

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.