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SHORT COMMUNICATION

Selection of Carbon and Nitrogen Source for 8-Hydroxy-9, 12-Octadecadienoic Acid Production using Endophytic Fungi *Curvularia lunata* BioMCC FE-00283

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Hydroxyoctadecadienoic acid (HODE) is one of hydroxy fatty acids that has anticancer activity. HODE was previously produced by chemical synthesis or bioconversion from linoleic acid. This is the first paper reported production of HODE by *Curvularia lunata* an endophytic fungi of *Cibotium barometz.* Various carbon and hydrogen sources have been tested for their effects on the production of HODE by *C. lunata*. Glucose, lactose, maltose, xylose, and sucrose were used as carbon sources, while yeast extract, monosodium glutamate, urea, and NH_4Cl were used as nitrogen sources. Fermentation was done using 100 ml medium in 250 ml Erlenmeyer flask at 150 rpm, 28 °C for 10 days. HODE products were analyzed by high pressure liquid chromatography using C18 coloumn and eluted by gradient system of acetonitril-water from 15% to 100%. Glucose and monosodium glutamate were found to be the best carbon and nitrogen source. The optimum concentration of glucose and monosodium glutamate for the production of HODE were 10 mg L^{-1} and 12 mg L^{-1} respectively.

Key words: hydroxy octadecadienoic acid, *Curvularia lunata, Cibotium barometz*, carbon source, nitrogen source, endophytic fungi

Asam hidroksioktadekadienoat (Hydroxyoctadecadienoic acid, HODE) adalah salah satu kelompok hidroksi asam lemak yang mempunyai khasiat antikanker. Pada awalnya HODE dihasilkan dengan cara sintesis kimia atau biokonversi dari asam linoleat. Dalam tulisan ini dilaporkan untuk pertama kali produksi HODE oleh kapang endofit *Curvularia lunata* yang diisolasi dari tanaman *Cibotium barometz*. Telah dilakukan percobaan untuk melihat pengaruh beberapa jenis sumber karbon dan nitrogen terhadap produksi HODE oleh kapang *C. lunata*. Jenis sumber karbon yang digunakan adalah glukosa, laktosa, maltose, xilosa, dan sukrosa, sedangkan sumber nitrogen yang digunakan adalah ekstrak khamir, monosodium glutamat, urea, dan NH_4Cl. Fermentasi dilakukan didalam tabung Erlenmeyer 250 ml yang berisi 100 ml media pada suhu 28 °C dan kecepatan pengocokan 150 rpm selama 10 hari. HODE hasil fermentasi dianalisis dengan kromatografi cair kinerja tinggi mengunakan kolom C18 dan dielusi secara gradien menggunakan campuran asetonitril-air dari 15% sampai 100%. Glukosa dan monosodium glutamat masing-masing adalah sumber karbon dan sumber nitrogen terbaik untuk produksi HODE. Konsentrasi terbaik dari glukosa dan monosodium glutamat berturut-turut adalah 10 mgL^{-1} dan 12 mgL^{-1}.

Kata kunci: asam hidroksioktadekadienoat, *Curvularia lunata, Cibotium barometz*, sumber karbon, sumber nitrogen, kapang endofit

Hydroxyoctadecadenoic acid (HODE) is a member of hydroxyl fatty acids, well known as oxylipins, which shows bioactivity. 8-HODE produced by *Laetisiria arvalis,* a basidiomycetes, can control pathogenic fungi *Rhizoctonia solani* dan *Phoma betae* (Bowers *et al.* 1986). 13-HODE, a product of enzymatic conversion from linoleic acid was reported for its ability to inhibit tumor adhesion to endhothelium (Liu *et al.* 1991). Other report by Mundt *et al.* (2003) showed that 13-HODE produced by *Oscillatoria redekei* HUB 051 was able to inhibit gram positive bacteria *Bacillus subtillis* SBUG 14, *Micrococcus flavus* SBUG 16, and *Staphylococcus aureus* SBUG 11. HODE can be produced by macro fungi (Bowers *et al.* 1986; Wadman *et al.* 2005) or micro fungi *(Su and Oliw 1996; Wadman et al.* 2009) or cyanobacteria (Mundt 2003). HODE can also be produced by enzymatic or chemical conversion of linoleic acid (Omar *et al.* 2003; Rombi and Bordighera 2002).

During the study of endophytic fungi, we found that *C. lunata* could synthesize HODE in liquid medium. This study reports the effects of carbon and nitrogen sources on the production of HODE by *C. lunata* in liquid fermentation system.

An endophytic fungi, *Curvularia lunata* BioMCC FE-00283, used in this study was obtained from BioMCC culture collection, Biotech Center, BPPT.

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It was isolated from a medicinal plant *Cibotium barometz* from East Lombok, West Nusa Tenggara using surface sterilization method developed by Tomita (2003).

Cultivation was started by inoculating the fungus, previously grown on one block of potato dextrose agar (PDA) into 250-ml flask containing 100 mL potato dextrose yeast extract (PDY). The flask was then incubated at 150 rpm, 28 °C for 2 days. Five milliliters of growing mycelia were transferred into 250 mL flask containing 100 mL fermentation medium, and then incubated at 28 °C, for 10 days with shaking at 150 rpm. Fermentation medium consisted of 20% potato extract, 4 g L^{-1} C from various carbon sources (glucose, lactose, maltose, xylose, or sucrose), 0.33 g L^{-1} N from various nitrogen sources (yeast extract, monosodium glutamate, urea, or KNO_3). The optimization of carbon and nitrogen sources concentrations were performed in the range between 0-50 g L^{-1} and 4-20 g L^{-1}, respectively.

Supernatant was obtained by filtration and HODE was extracted using ethyl acetate with volume 1:1 for 1 hour. The organic phase was separated from the water phase, the volume was measured and then concentrated using vaccuum centrifuge. These dry extracts were redisolved using methanol for HPLC at 100 fold concentration. Subsequently, these extracts were analyzed using HPLC system (Waters) over C18 coloumn (Puresil, 150 x 4.6 mm) and eluted using gradient system of acetonitril-water from 15% to 100% for 25 min. Each treatment was done in triplicate, and the concentration of 8-HODE for each treatment was statistically analyzed using 2 ways ANOVA (Montgomerry 1991)

This study showed that by using glucose as carbon source *C. lunata* produced 0.18 mg L^{-1} 8-HODE, which is much higher compared to using lactose, sucrose, xylose or maltose as carbon source (Fig 1). When different glucose concentrations were used as carbon source in the medium, the highest 8-HODE production was found at 10 gL^{-1}glucose concentration. At higher concentration the synthesis of 8-HODE by *C. lunata* was depressed significantly, suggesting that at higher concentration of glucose the growth and HODE synthesis were inhibited. It was reported that in fungi the fatty acid is synthesized in the similar manner with polyketide synthesis (Jenie *et al.* 2006). Biosynthesis of fatty acid was directly connected to carbon metabolism prior to citric acid cycle. The lower energy required to metabolize carbon source will stimulate fatty acid biosynthesis in fungi. The reported biosynthesis of hydroxy fatty acid has correlation with sporulation in genus *Aspergillus* (Wadman *et al.* 2008). The utilisation of simple sugar as a sole carbon source better stimulates the sporulation of *Aspergillus* in comparison to complex carbon source (Calvo *et al.* 2002). In this study, glucose was also shown to be the best carbon source for 8-HODE production by *C. lunata*. It was proposed that glucose also stimulated biosynthesis of 8-HODE in *C. lunata* in a biosynthesis pathway similar to the one used for the production of hydroxy fatty acid in *Aspergillus*. Increasing glucose concentration to more than 10 g L^{-1} in cultivation media, however, decreased 8-HODE production by *C. lunata* (Fig 2). Rawlings (1998) reported that pyruvate conversion to acetyl Co-A was catalysed by the action of pyruvate dehydrogenase under glucose limited condition during fatty acid production in *Saccharomyces cerevisae*. This might also be the reason why 8-HODE production decreased when glucose concentration increased. The result of this study showed that glucose at the lowest concentration

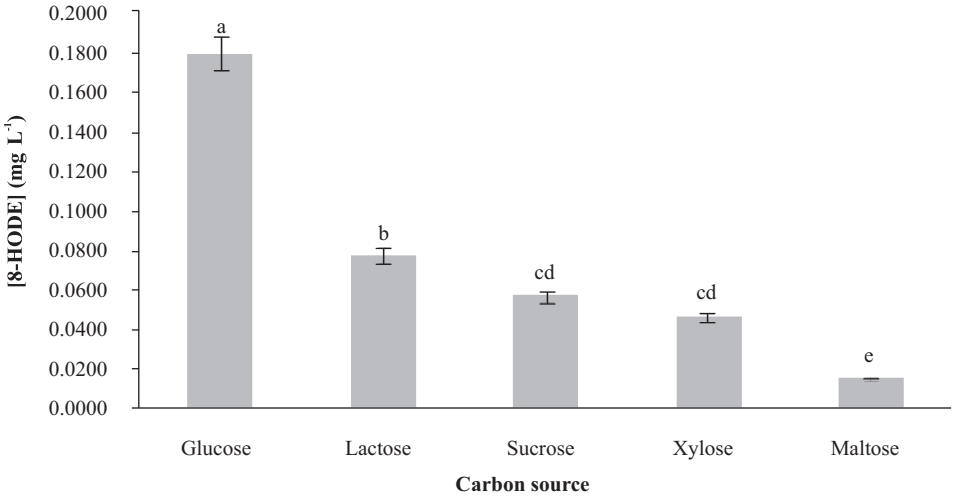

Fig 1 Effect of carbon source on 8-HODE production by *Curvularia lunata*. The concentration of various carbon sources was 4 g L^{-1}. Different of alphabet symbol shows significant different of the treatment.

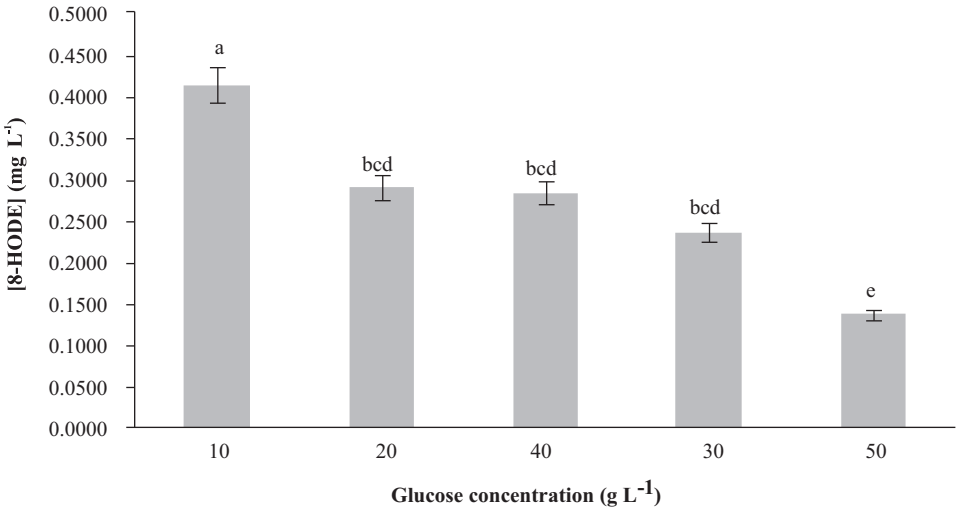

Fig 2 Effect of glucose concentration on 8-HODE production by *Curvularia lunata*. Different of alphabet symbol shows significant different of the treatment.

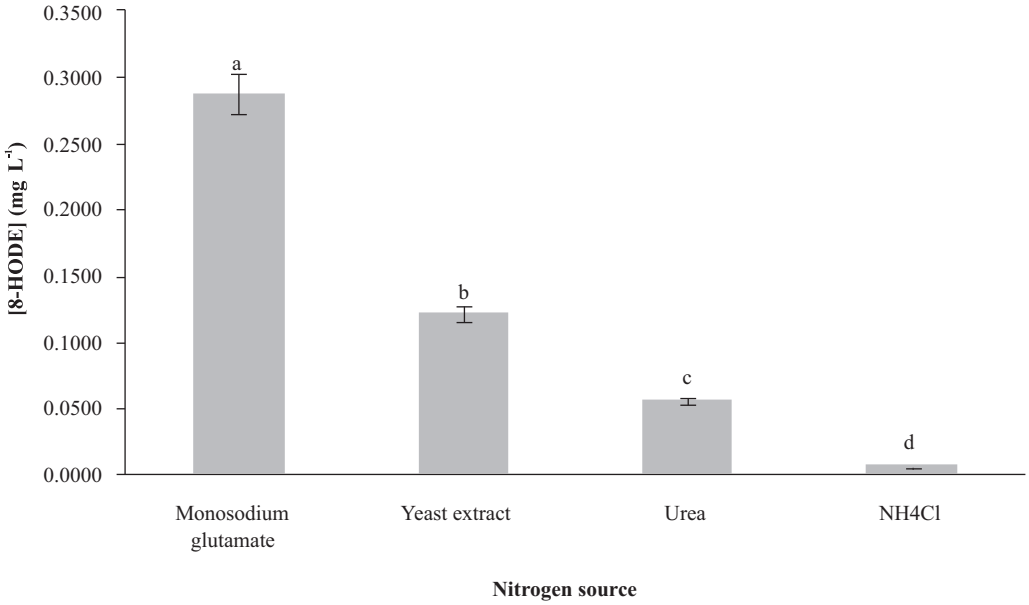

Fig 3 Effect of nitrogen source on 8-HODE production by *Curvularia lunata*. The concentration of various nitrogen sources was 0.33 g L^{-1}. Different of alphabet symbol shows significant different of the treatment.

(10 g L^{-1}) gave the highest 8-HODE production. However, a further study is needed to examine whether even lower glucose concentration will further enhance the 8-HODE production.

This study also revealed that 8-HODE production was significantly affected by nitrogen source. Monosodium glutamate was a better nitrogen source for production of 8-HODE by *C. lunata,* in comparison to urea, yeast extract or NH_4Cl. *C. lunata* even did not produce 8-HODE when NH_4Cl used as nitrogen source (Fig 3). Even though monosodium glutamate was the best nitrogen source in comparison to others, altering the concentration of monosodium glutamate did not significantly affect the 8-HODE production (Fig 4). Increasing monosodium glutamate concentration in medium affected 8-HODE production by *C. lunata* only in lower concentration (4-12 mg L^{-1}). In higher concentration (16-20 mg L^{-1}), increased monosodium glutamate concentration did not give significant effect on 8-HODE production. Even though at concentration 20 g L^{-1} monosodium glutamate gave higher production of 8-HODE, this increase was not statistically significant. Production of secondary metabolite by polyketide pathway in fungi is also influenced by nitrogen source. Hajjaj *et al.* (2001) reported that glutamic acid and histidine in cultivation media stimulated α-ketoglutarate accumulation inside fungal cell. This accumulation stimulates acetyl Co-A to enter polyketide pathway rather than citric acid cycles. Organic nitrogen source was also reported to

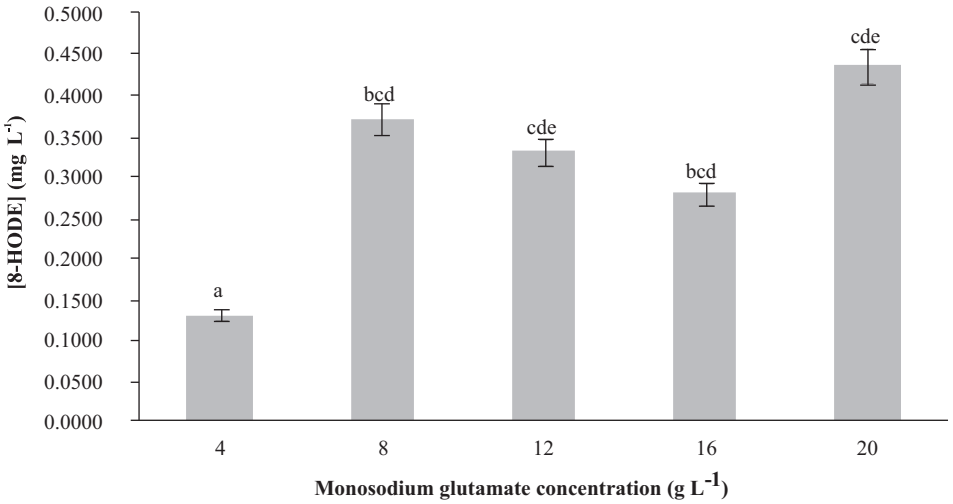

Fig 4 Effect of monosodium glutamate concentration on 8-HODE production by *Curvularia lunata*. Different of alphabet symbol shows significant different of the treatment.

better increase fatty acid production by fungi in comparison to inorganic nitrogen source (Hwang *et al.* 2005). In this study, when NH_4Cl employed as nitrogen source 8-HODE was not detected in the culture.

Limited studies has been published on the qualitative production of 8-HODE by fungi (Wadman *et al.* 2005; Bowers *et al.*1986). In quantitative production, most study discussed on the transformation of polyunsaturated fatty acid into hydoxy fatty acid. Heo *et al.* (2009) reported the production 0.4 g L^{-1} 10-hydoxy stearic acid (10-HAS) by *Flavobacterium* sp strain Ds5. They used glucose and yeast extract as carbon and nitrogen sources and olive oil as substrate. However they did not report if other carbon and nitrogen sources were used for 10-HAS production. While Arjol *et al.* (2010) reported the production oxylipins by *Pseudomonas* 42A2 using fatty acids as carbon source. They detected that after 2 h incubation the concentration of monohydroxylated compound reached 4 g L^{-1}. This study was the first report for quantitative 8-HODE production by *C. lunata*.

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