Microbial biomass and phospholipid fatty acid (PLFA) profile changes in bioconversion of wheat straw

I Nurika
Department of Agroindustrial Technology, Faculty of Agricultural Technology, Universitas Brawijaya, Malang, Indonesia
Email: irnia@ub.ac.id

Abstract. *Serpula lacrymans* was shown to grow rapidly on wheat straw solid state fermentation (SSF). This study aimed to determine the pattern of ergosterol and the relative composition of phospholipid fatty acid (PLFA) produced during fungal growth. The PLFA analysis was carried out on the upper layer of the solvent extraction and was performed on the incubation from 0 up to 35 days. Relative abundant (%) of each fatty acid methyl ester (FAME) produced during the fungal growth was measured by comparing the amount of an individual FAME with the total FAME detected. The dominant PLFA released by the fungi were: 18:2n6c (linoleic acid) 24:1n9 (nervonic acid), 18:1n9c (oleic acid), and 16:0 (palmitic acid). The highest amount of fatty acid produced during the fungal growth is linoleic acid ranged from 186.92-345.78 μg g⁻¹.

1. Introduction
In the temperate region, wheat straw, which has a complex structure of lignocellulose is abundant and cheap. Lignocellulose is a recalcitrant compound consists of cellulose (16-21%), hemicellulose (26-31%) and lignin (29-35%) which is not easy to be degraded [1]. Therefore, the cost beneficial of wheat straw bioconversion is necessary, especially for the development of the biorefinery concept. Lignocellulose is highly resistant to decay by microorganisms [2], which is mostly depolymerized by white and brown rot fungi [3,4]. Brown rot fungi play a significant role in wood degradation, especially in the sub-tropical region. However, the brown rot fungi do not typically produce ligninolytic enzymes such as lignin peroxidase, manganese peroxidase and laccase. The generating of hydroxyl radical under Fenton reaction which plays a role in depolymerisation of lignocellulose was known as the key for the brown rot fungi to degrade lignocellulose [5]. *Serpula lacrymans* is one of the most Basidiomycetes, which has the ability to grow rapidly and destroy the lignocellulosic substrate.

Ergosterol is known as the main sterol in fungi cell membranes [6]. It is specifically a major component of spores, mycelia and vegetative cell [7]. Ergosterol is mostly used as a method of estimating fungal biomass since there is a good relationship between ergosterol content and hyphae length [8], which also recognized as a biomarker for quantifying fungal biomass [9]. The conversion factor was used to quantify the fungal biomass in wheat straw solid state fermentation (SSF) [9,10]. Most of microorganisms contain phospholipids in their membranes and the different microbial
environment differs in type of fatty acids [11]. Number of studies on fatty acid profiling of Basidiomycetes in liquid culture have recently been reported [12–14]. However, data from solid substrate and information on sequential production of fatty acids over the duration of fungal culture have until now been limited. This study reveals the production of phospholipid fatty acid (PLFA) and their dynamics following culturing in wheat straw. The objective of this study was to determine the relationship between the growths of S. lacrymans indicated by the amount of ergosterol (fungal biomass) released and PLFA profile in S. lacrymans, which was incubated in wheat straw solid state fermentation (SSF) for 35 days. Since the quantification method of fungal biomass is not an easy task and both ergosterol measurement and specific PLFA identification are often used to estimate the amount of fungal in certain media, therefore this study will contribute on giving the alternative method for particular fungal species.

2. Materials and Methods

2.1. Microorganism
The S. lacrymans culture was grown on malt extract agar (MEA) media at 20 ºC for a week and inoculated to rye grain afterward to produce grain spawn (inocula). The grain spawn was then used as inoculum on wheat straw SSF.

2.2. Solid state fermentation (SSF)
10 g of wheat straw was added with 13 ml of water before undergoing sterilization of 121 ºC for 1 hour. The sterilized straw was then inoculated with 1 g of grain spawn and incubated for 35 days at 20 ± 2 ºC. Four replicates of each sample were applied.

2.3. Fungal biomass (ergosterol assay)
S. lacrymans was grown on malt extract agar (MEA) media and five plugs agar inocula were added to 100 ml of liquid media (1% w/v Malt extract) and incubated for 35 days. The mycelium produced was collected and freeze dried. The prepared dried mycelium then was used for preparing a standard curve for the ergosterol assay, which followed the protocol from Gong et al. [15].

2.4. PLFA analysis
The upper layer solvent extracted SSF of wheat straw was evaporated using a rotary evaporator (70 ºC; 125 rpm). The evaporated solution was then dried overnight in room temperature until the dry precipitate remained. 0.5mg of the extract powder was added with 10 µL 15:0 TAG (triacylglycerol) as internal standard (Sigma p/n T4257) and mixed with 500 µL 1 N HCl/MeOH. Subsequently, samples were homogenized and dried at 80 ºC for 10 hours. To obtain the fatty acids, 250 µL 0.9% KCl was added followed by the addition of 800 µL hexane and vortex mixed. The resulting layers were allowed to separate for 10 minutes. 500 µL of the upper hexane layer was used for fatty acid methyl ester measurement using gas chromatography (GC) machine (Agilent technology 6850 network GC system). The PLFA profile of the wheat straw SSF incubated by S. lacrymans was determined by fatty acid methyl ester (FAME) analysis. Relative abundance (%) of each FAME was measured by comparing the amount of an individual FAME with the total FAME detected. The amount of fatty acids pools was assessed by measurement of the summed area of peaks in the chromatogram.

3. Results and Discussion

3.1. Fungal biomass (ergosterol)
The contents of ergosterol are known to vary between different fungal species, the period of incubation, and growth conditions [8]. In this study, S. lacrymans produced ergosterol from 7 days of
culture (53.23 µg g\(^{-1}\)) and significantly increased until 35 days (138.70 µg g\(^{-1}\)). Detail of ergosterol released during the growth of *S. lacrymans* are presented in Figure 1.

![Graph showing ergosterol production](image)

**Figure 1.** The production of ergosterol during the growth of *S. lacrymans* on wheat straw SSF for 35 days.

The calculation of ergosterol content in wheat straw SSF was determined by the quantity of mycelium weight collected during a certain time of incubation. The conversion factor was derived from the liquid culture of the fungus and used to calculate the fungal biomass. The highest ergosterol content (138.70 µg g\(^{-1}\)) was obtained from the wheat straw SSF incubated for 35 days. By using the conversion factor, the predicted fungal biomass content in wheat straw SSF incubated for 35 days was 6.13 mg g\(^{-1}\). The ergosterol content in wheat straw SSF was higher than the ergosterol previously measured in fungi growing in wood, which was 22.6 µg g\(^{-1}\) for *Phlebia radiata* and 23.0 µg g\(^{-1}\) for *Phanerochaete chrysosporium* [16].

3.2. **Fungal phospholipid fatty acid (PLFA) profiling**

Figure 2 revealed the comparison between the PLFA released from wheat straw SSF inoculated by *S. lacrymans* in the initial growth of the fungus (day 0) and 35 days incubation. The most prevalent PLFA detected in this study was 18:2n6c (linoleic acid), and the amount of linoleic acid ranged between 186.92-345.78 µg g\(^{-1}\).

In this study, some other fatty acids were also obtained for fungus *S. lacrymans*, such as 16:0 (palmitic acid), 18:1n9c (oleic acid) and 24:1n9 (nervonic acid) (Table 1). The linoleic acid (18:2n6c) reached the highest percentage at 35 days of culture from the total amount of FAME. Since the linoleic acid has dominated the release of fatty acids from wheat straw SSF, it is suggested that the fungal biomass can be indicated by the production of linoleic acid [17].
**Figure 2.** The comparison patterns of fatty acid methyl ester (FAME) on wheat straw SSF inoculated by *S. lacrymans* between control (day 0) (a) and 35 days incubation (b).
Table 1. The predominant fatty acids produced by wheat straw SSF inoculated by *S. lacrymans* for 35 days.

| Microorganism | Type of fatty acids       | (%)  |
|---------------|---------------------------|------|
| *S. lacrymans* | 16:0 (palmitic acid)      | 19-29|
|               | 18:2n6c (linoleic acid)   | 16-50|
|               | 18:1n9c (oleic acid)      | 10-13|
|               | 24:1n9 (nervonic acid)    | 6-9  |

Most studies that showed a good correlation between PLFA 18:2n6c and fungal biomass were obtained when the fungi were grown on soil medium [18]. There is still very limited information on PLFA as a fungal biomarker, especially when the fungus is grown in biomass media such as wheat straw. In this study, the release of linoleic acid was the dominant PLFA produced from the bioconversion of wheat straw using *S. lacrymans*. The use of PLFA 18:2n6c as an indicator of fungal biomass could be appropriate for other biomass feedstock even though the separation between the PLFA and solid media is not an easy task. A similar trend as the production of linoleic acid during wheat straw SSF (35 days), the highest amount of ergosterol was also obtained at 35 days culture of *S. lacrymans* [Figure 1]. The findings of this experiment have supported previous research by Klamer and Bååth [13], which reported that there was a linear correlation ($R^2 = 0.782$), between ergosterol and PLFA 18:2o6,9 among 11 species of fungi cultured in agar media.

4. Conclusions

The growth of *S. lacrymans* on wheat straw SSF during 35 days incubation showed a positive correlation with ergosterol or fungal biomass. Meanwhile, the most predominant fatty acid released during the fungal growth is linoleic acid (18:2n6c).

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