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BIOMASS PRODUCTION OF *TRICHODERMA HARZIANUM* (RIFAI) IN PALM OIL MILL EFFLUENTS (POME)

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ABSTRACT

Trichoderma harzianum strain FA 1132 has shown potential as a biological control agent of *Ganoderma boninense*, the causal pathogen of basal stem rot (BSR) of oil palms based on previous nursery trials. This study investigates the suitability of the agrowaste slurry palm oil mill effluents (pome) as a feedstock for the possible mass production of strain FA 1132, with mycelial yield taken as the indicator of the biomass production. Results showed that the best growth of FA 1132 in pome was at 1:3 parts dilution to water, which gave a yield that was statistically comparable to its growth in the laboratory media Potato Dextrose Broth (PDB) and Richard's Solution. Production of the mycelial biomass increased by 141.25% when 1:3 pome was supplemented with sucrose. When supplemented with glucose, the best biomass yield was obtained from the 1:1 diluted pome, which gave a 217.99% yield increase. Thus, pome offers a potential as a feedstock for the biomass production of FA 1132, with the mycelial yield being significantly enhanced when the media was supplemented with sucrose or glucose as a carbon source.

Keywords : *Trichoderma harzianum*, biomass production, palm oil mill effluents, feedstock, biocontrol agent.

INTRODUCTION

The genus *Trichoderma* is a relatively common fungus found in most soil types (Roiger *et al.*, 1991). *Trichoderma* is saprophytic in nature, but the fungus can turn hyperparasitic, particularly on plant pathogenic fungi. In his review, Samuels (1996) reported that the genus was first identified in 1794 by Persoon, but it was not until 138 years later that the first report on its hyperparasitism was documented by Weindling (1932). Progress on the screening of *Trichoderma* as potential biocontrol agents of fungal pathogens was rapid from then onwards. By the 1990's, several *Trichoderma*-based formulations were already available in the market. For example, Trichodex ™ 25P is available for the control of grey mould on grape vines, caused by *Botrytis cinerea* (O'neill *et al.*, 1996) and Gliogard ™ is for the biocontrol of *Rhizoctonia solani* and *Pythium ultimum* on ornamental plants (Lumsden and Locke, 1989). Soytong *et al.* (1999) prepared *Chaetomium* and *Trichoderma* pellets for the control of *Phytophthora parasitica*, the causal pathogen of root rot of sweet orange, *Citrus reticulata* Blanco.

Oil palms are grown on a large scale commercial basis in Malaysia. One important disease that infects this crop is basal stem rot (BSR), caused by *Ganoderma boninense* (Ho and Nawawi, 1985). *In vitro* studies showed that several strains of *Trichoderma* inhibited the growth of *G. boninense* by hyperparasitism (Abdullah and Ilias, 1999a) and by production of unidentified diffusible (Abdullah and Ilias, 1999b) and volatile compounds (Ilias, 2000). Previous studies by Abdullah *et al.* (1999) showed that the mycelia, its abiotic extracts, as well as the conidial suspension of *T. harzianum* (strain FA 1132) each had the capacity to curb *Ganoderma* disease development at various levels, using artificially-infected oil palm seedlings as disease models. The treatment using a conidial soil drench supplemented with a *Trichoderma* surface mulch was the most successful, where up to 90% protection against the disease can be conferred. These results are enough justification for investigations into the possible large-scale production of FA 1132, using a cheap source of material as fungal feedstock.

This study investigates the fungal biomass production of FA 1132 when cultured in palm oil mill effluents or 'pome', a viscous by-product of milling processes during distillation of the cooking oil. The first objective of this study was to determine the dilution factor of pome that would give the best yield and whether the biomass production is higher in shake or in static flask cultures. The second was to determine the performance of pome as a culture media of FA 1132 compared to other types of laboratory liquid media. The third was to determine the effect of carbon nutrient supplements in pome on mycelial biomass of the fungus, using non-laboratory grade sucrose and glucose respectively.

MATERIALS AND METHODS

Source of pome

Fresh pome was collected from Seri Ulu Langat Palm Oil Mill Sdn. Bhd. in Dengkil, Selangor. They were collected in plastic containers with lids and immediately brought back to the laboratory where they were stored at 10°C ± 1°C until required.

Source of fungal isolate

Strain FA 1132 is a *T. harzianum* isolate obtained from the stock culture collection of the Mycology Laboratory, Department of Biology, Universiti Putra Malaysia. It was previously isolated from soils of an oil palm plantation in Batang Melaka, Negeri Sembilan, Malaysia. The strain has also shown to be a potential biological control agent based on previous studies.

Optimum dilution factor for mycelial growth in pome

To test the difference between yields from static versus shake cultures, 2 experiments at 5 replicates per set were prepared. Based on preliminary trials, a pome dilution of 3 times its volume in water (1:3) was prepared as culture media, at 250 mL per Erlehnmeyer flask and autoclaved at 121°C at 1.05 kg/cm^2 for 15 minutes. Starter cultures were made by cutting out 6mm-diameter agar discs of FA 1132 from an actively-growing colony and placed into the media at 1 disc per flask. One set of culture media was put on an orbital shaker and agitated at 100 rpm while the second was placed on a bench top as static cultures. Both sets were left in the same room under the same ambient conditions of temperature (28 ± 2 °C) and light (12 hours light, 12 hours darkness) for 14 days.

To obtain the optimum dilution, pome was diluted with water in the ratios of 1:1, 1:2, 1:3 and 1:4. From this, 500 mL of each solution was poured into 1 L culture flasks and then autoclaved in the same manner for 15 minutes. Each flask was inoculated with one disc of 6 mm diameter of FA 1132 and maintained as shake cultures at 100 rpm, 8 to 12 hours per day. After 14 days, the mycelial mass was poured out of each flask onto several layers of filter paper. They were then oven-dried at 60°C until a constant weight was achieved. Readings of mycelial dry weights from each of the treatment were recorded and subjected to statistical analysis.

Growth of FA 1132 in 4 types of liquid media

To compare the performance of pome as a liquid media, FA 1132 was cultured in 1 defined and 2 non-defined media. The former consisted of Richard's Solution (1.0 g KNO_3, 0.5 g KH_2PO_4, 0.25 g Mg $SO_4.7H_2O$, 34g glucose, trace amounts of $FeCl_3$ in 1L distilled water) while the latter consisted of a laboratory-grade Potato Dextrose Broth (PDB) and a non-laboratory media of 1:3 dilution of Guinness Malta® (GM), a commercial malt-based beverage fortified with vitamin B complex. Starter cultures were prepared as described earlier, where only one agar disc culture was placed per flask containing 250 mL sterilized media and incubated under ambient conditions as shake cultures. The experiment was run for 14 days, after which the mycelial mass were harvested and treated in the same manner as the previous experiment.

FA 1132 biomass production in supplemented pome

Table sugar and Glucolin® were purchased from grocery stores and used to represent sucrose and glucose sources respectively, for use as nutrient supplements. The same range of pome dilution as in the previous methodology (i.e. from 1:1 to 1:4) was prepared. Into each media series was added 1:10 parts weight to volume (or 1g supplement to 10mls media). No supplement was added to the control flasks. The sterilized media were then inoculated with a 6mm-diameter of FA 1132 as done in previously experiments. They were maintained as shake flask cultures for 14 days and treated as in the previous experiment. The experiment was carried out in triplicates. The calculation of percentage increase of FA 1132 biomass in supplemented to non-supplemented pome was as below:

$$\% = \frac{\text{Supplemented pome - non-supplemented pome}}{\text{Non-supplemented pome}} \times 100\ \%$$

RESULTS AND DISCUSSION

Shake flask cultures and optimum dilution factor

Shake flask cultures at 100 rpm for 12hrs/day gave a higher and statistically significant biomass yield compared to static cultures (Table 1). A preliminary study done showed no significant difference between yields of shake cultures at 12 hours/day versus 24 hours/day. Thus, biomass productions for all ensuing experiments were made as shake cultures at 100rpm for 8 to 12 hours per day. The best and most significant dilution factor of pome for growth of FA 1132 was at 1:3 (Table 2). Mycelial growths for all the other dilutions were comparable and were not significantly different from each other.

Growth performance of FA 1132 in 4 types of liquid media

The highest biomass reading was obtained from 1:3 Guinness Malta® (GM) at 9.88 g/L, which was statistically significant compared to yields from all of the other media (Table 3). This was followed by pome, Potato Dextrose Broth and Richard's Solution in descending order; but all 3 were not statistically different from each other. The GM media was commercially fortified with vitamins and gave good results; however, it is percieved as not cost-effective for large scale production. On the other hand, pome gave results comparable to the laboratory-grade PDB and thus offers a promising feedstock for mass production of the fungus.

Table 1. FA 1132 biomass on static and shake cultures in 1:3 pome at 250 ml 14 days incubation.

Pome 1:3	Mean dry weight (g/L)
Shake culture	9.200 [a]
Static culture	2.828[b]

Values with the same alphabets indicate no significant difference at $p \leq 0.05$ by Tukey HSD Test.

Table 2. Biomass production of FA 1132 in 500 mL pome at 4 different dilutions, 14 days incubation.

Dilution of pome in water	Mean dry weight (g/L)
1:1	17.129 [a]
1:2	14.628 [b]
1:3	21.356 [a]
1:4	16.274 [a]

Values with the same alphabets indicate no significant difference at $p \leq 0.05$ by Tukey HSD Test.

Table 3. Biomass production of FA 1132 in 250 mL on 4 types of liquid media at 14 days incubation.

Liquid Media	Mean dry weight (g/L)
Guinness Malta (GM)	9.880[a]
Palm Oil Mill Effluent (POME)	8.316[b]
Richard's Solution (RS)	7.616[b]
Potato Dextrose Broth (PDB)	8.260[b]

Values with the same alphabets indicate no significant difference at $p \leq 0.05$ by Tukey HSD Test.

FA 1132 biomass production in supplemented pome

The best and most significant combination of diluted pome with sucrose was at 10:30:1 or 2.5% of added supplement (Table 4). At this combination, the biomass production increased by 141.25% compared to the corresponding unsupplemented media (10:30:0). The best dilution for glucose-supplemented pome was at 10:10:1 or 5% of the solution, which gave an increased percentage of 217.99 %. This combination was also the single best biomass production recorded overall for the 2 supplements.

This study showed that the use of sugar-supplemented pome offers potential as a feedstock for the large-scale production of *Trichoderma* biomass. Pome was selected as a culture media because of its ease of availability and relatively insignificant market value. Devendra *et al.* (1981) reported that among others, pome consisted of 10-16% crude protein, 11-26% crude fiber, 7-52 % nitrogen-free extract, 0.6-4% calcium, 0.002-0.2% phosphorus and 0.1-0.5 % magnesium. Pome is rich in minerals and nitrogenous sources, but the carbon content is relatively low. Thus,

the addition of sugars as a carbon supplement correctly boosted its biomass production, which in this study showed that as high as 217.99% can be achieved.

Table 4. Biomass production of FA 1132 on unsupplemented and supplemented pome.

Liquid media (pome)		Mean dry weight (g)
Pome:water	10:10:0	8.564 [d]
	10:20:0	7.314 [d]
	10:30:0	10.678 [c, d]
	10:40:0	8.137 [d]
+ Sucrose	10:10:1	24.594 [b]
	10:20:1	12.142 [c, d]
	10:30:1	25.761 [b]
	10:40:1	14.756 [c]
+ Glucose	10:10:1	33.952 [a]
	10:20:1	22.066 [b]
	10:30:1	24.019 [b]
	10:40:1	22.506 [b]

Values with the same alphabets indicate no significant difference at $p \leq 0.05$ by Tukey HSD Test

The studies also showed that yield was better as shake flask cultures and gave an approximate three-fold increase. Klein and Eveleigh (1999) reported that the formulation of *Trichoderma* biomass as pellets (consisting of mycelium and chlamydospores) into soils resulted in 1000-fold CFU (colony-forming unit) proliferation. Many industrialists prefer the use of whole fungal biomass, which consisted of a mixture of spores, mycelia and chlamydospores all in one, for purposes of upscale production. Chlamydospores and conidia appeared less sensitive to fungistasis than mycelia (Papavizas, 1985). Although strain FA 1132 was selected because of its antagonistic properties, the feedstock can still be used for the cheap production of *Trichoderma* selected for other purposes, such as producers of enzymes and antibiotics – two other properties of the fungus which are of great interest both scientifically as well as for economic reasons.

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