Mushroom Biology and Mushroom Products

Proceedings of the 7th International Conference on Mushroom Biology and Mushroom Products

4-7 October, 2011, Arcachon, Fran

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Congress organisation

This 7th International Conference of the World Society for Mushroom Biology and Mushroom Products (WSMBMP; Web: <u>http://wsmbmp.org</u>) was arranged and hosted by INRA, the French National Institute of Agronomical Research:

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Foreword

The World Society for Mushroom Biology and Mushroom Products is an international organization devoted to the enhancement and application of knowledge related to basic and applied aspects of mushroom biology and mushroom products. It was established to provide a platform for mushroom biologists from all over the world, coming from both well-established centers and emerging groups in countries with increasing interests on mushroom biology and mushroom products. A major activity of WSMBMP is its three-yearly Conference organized by the host country under the banner of the Society.

The proceedings document of the seventh conference includes both oral (in volume 1) and poster presentations (in volume 2) submitted at this event by scientists from more than 35 countries. For the first time, the proceedings are provided in electronic format on a memory stick available for the participant at the date of the conference. This saves trees and allows for keyword searching in a document of more than 720 pages. We are grateful to the Scientific Committee members for their assistance in evaluating the papers submitted. The task of error corrections in the manuscripts, technical editing, and compiling the proceedings into an electronic format, was provided by the scientific editors of the proceedings. An open access online publication of the proceedings is going to be proposed by QUAE Edition at the address http://www.symposcience.org. It needs an additive work of edition that will be done after the conference.

The purpose of the 7th International Conference on Mushroom Biology and Mushroom Products (ICMBMP7) was to bring together scientists from all subdivisions of biology with an interest in mushrooms and mushroom products, including those associated with economic, environmental and engineering issues. The main focus of ICMBMP7 is "Towards a Significant Contribution of Mushroom Biology to Sustainable Development" and the following areas are covered: (i) Genomics, Genetics, Molecular Biology and Breeding, (ii) Evolution, Biodiversity and Systematics, (iii) Biochemistry, Physiology, Nutritional and Medicinal Aspects, Innovative Products from Mushrooms, and (iv) Cultivation Technology and Bioconversions.

The development of high-yielding strains of both well-established and more recently cultivated mushroom species using strain improvement and breeding technologies was reported in the 6th International Conference (Bonn, Germany, 2008). Since 2008, the availability of the entire genome sequences of two important cultivated mushrooms, *Pleurotus ostreatus* and *Agaricus bisporus*, has been a key development. This new opportunity for advancing our knowledge and understanding of mushroom genetics and mushroom biology is a major feature of ICMBMP7. The expected progresses in breeding lead to question the ways to disseminate and protect the new strains.

Evolution, systematics, and biodiversity are important topics for the future development of new mushroom-derived products and the cultivation of new mushroom species. They are clearly identified for the first time in the WSMBMP conferences. Research output in these areas should offer a plethora of new opportunities, drive the choice of the species to be studied and influence policy makers in terms of biological resource preservation and management.

Several papers report on medicinal effects observed on cellular models and test animals, and others on mushroom as sources of nutritional and active biomolecules. If there is to be confident in the use of such mushroom extracts and supplements for the treatment of various medical conditions, then the quality and production of the products needs to be standardized and regulated. There is a major lack of such regulation at the moment, and it is essential that progress be made in this area in the future to give consumers confidence and to protect public health from substandard products. The proceedings address this question by contributing to the dissemination of scientifically validated information and proposing discussions between participants from Asia, America and Europe.

Knowledge on the biology of mushrooms (their physiology, their life cycles, their development, their nutrition) is a source of information for the development on new mushroom cultivations or the improvements on conventional cultivation techniques. The presentations on these topics show strong influence of genomics and all the approaches of the molecular biology on the mushroom biology of today.

Technological developments in the mushroom industry in general have resulted in increased production capacities, innovations in cultivation techniques, improvements in the quality of the final products, and the utilization of the natural qualities of mushrooms for environmental benefits. Papers in these Proceedings show the importance of edible and medicinal mushroom cultivation in integrated managements of agricultural wastes products. However, significant challenges remain in maintaining and further improving the current cultivars and cultivation techniques for mushroom production, in reducing mushroom diseases by integrated pest management, and in continuing to seek out new opportunities.

These Proceedings highlight many aspects of mushroom biology and mushroom products and it will our honor if they reach the objective of promoting the contribution of mushroom biology to sustainable development.

Septembre, 2011

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THE POTENCY OF OIL PALM PLANTATION WASTES FOR MUSHROOM PRODUCTION

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ABSTRACT

Indonesia has become the world's largest palm oil producer with total area of oil palm plantation being 7.3 million ha in 2009 wich produced a huge quantity of biomass by-product such as empty fruit bunches (EFB) estimated at 43 million tons, as well as produced liquid and solid wastes from palm oil mill effluent (POME). This research was aimed to study the potency of EFB and POME on growth of white oyster mushroom (F isolate of *Pleurotus* sp.) and GKSA isolate of *Ganoderma boninense*. F isolate was grown on EFB, *Paraserianthes falcataria* sawdust and mixture of both substrates with proportion 1:1 respectively. The results showed that EFB could be used as a substrate of *Pleurotus* fruit body production with biological efficiency that reached 152%, and even the growth went faster than the other substrates. As well as GKSA isolate could used the EFB as a substrate of fruit body production and it could decrease C/N ratio up to 84% at vegetative phase and lignin concentration up to 66% at reproductive phase. In addition the POME have induced significant increasing of the GKSA isolate growth up to 62% at 20% of liquid POME concentration in malt media and reached 64% of growth at 10% of solid POME in the same media. Based on these results the abundant wastes from the oil palm plantation were considered suitable for mushroom production and spent mushroom substrates can be used as soil

Keywords: Oil palm, wastes, cultivation, Pleurotus sp., Ganoderma boninense

fertilizers at oil palm plantation as well as for animal feed.

INTRODUCTION

Oil palm tree has become a plantation icon in Indonesia and neighboring Malaysia. Both countries have now become the world's biggest producers of palm oil. Data from Indonesia Crude Palm Oil Council showed that total area of oil palm plantation in Indonesia was 7.3 million ha in 2009 which produced a huge quantity of by-product biomass such as empty fruit bunches (EFB) estimated at 43 million tons, as well as produced liquid and solid wastes from palm oil mill effluent (POME). In a palm oil mill with modern technology, every ton of fresh fruit bunch (FFB) produced 0.23 ton of EFB, 0.13 ton of mesocarp fiber and 0.55 ton of kernel shells as well as 0.2 m³ of POME and 0.6-1.2 m³ of waste water. Annually, 27,600 tons of EFB and 96,000 m³ of POME are produced by a 30 tons/hour capacity mill with an input of 120,000 tons FFB. On average processing of 1 million ton FFB in palm oil mills generates 230,000 tons

of EFB and 650,000 tons of POME as residues [10]. The palm oil industry also produces fronds and trunks pruned when harvesting fruit bunches and felled during the replantation. For most mills, EFB and POME are still considered as an unwanted wastes because of their storage, distribution, and treatment costs. Concerning the environmental problem especially high air pollution, the EFB incineration is prohibited therefore palm oil mills have started to bring EFB back to the plantation and just dump them.

Millions of tons of agriculture industrial wastes are discarded, burned and neglected. They mainly content the lignocellulose compounds. These useless by-products can be recycled to produce value-added mushrooms. With the exploitation of these wastes as a raw materials for cultivation of mushrooms, the wastes can be curbed and the nutritional quality of the diet of people in plantation region improved.

The use of EFB as a substrate for the cultivation of *Pleurotus* spp. and *Volvariella* sp. is a viable alternative for the management of the solid waste and nutritional security in the oil palm plantation region. *Volvariella* sp. grows naturally on EFB. Only several reports about oyster mushrooms cultivated on EFB were published [5, 1, 10, 8]. Bioconversion of two formulations of substrates containing only 10% and 20% oil palm fiber respectively using *Pleurotus ostreatus* and *P. eous* was reported by [12]. The results showed that both species displayed a higher rate colonization and yielded on corncob-based formulations than on cocoa and rice husk-based media containing oil palm fiber. Lower biological efficiency (BE) of 50-70% was achived by *Pleurotus* spp. grown on coffee pulp. Then BE were improved with ensiled coffee pulp, reaching 82% in the cultivation of *P.sajor-caju* and 73% with *P. ostreatus* [9]. The BE of *P. ostreatus* grown on a mixture of 70% *Digitaria decumbens* grass and 30% coffee pulp varied between 59.79% and 93% [4]. High yield of *P. ostreatus* was obtained by waste paper amended with 20% husk rice [2] with BE of 140%. For *P. sajor-caju*, 200% BE has been recorded [3].

This research was aimed to study the potency of empty fruit bunches on growth of white oyster mushroom and *Ganoderma boninense* which is one of important pathogen of oil palm. We also studied the influence of liquid and solid wastes from palm oil mill effluent on growth of *G. boninense*.

MATERIALS AND METHODS

Isolates. F isolate of *Pleurotus* sp.was obtained from mating between two monocaryotic isolates of white oyster mushrooms (BNK 2 and BBR 14 isolates). Both of those dicaryotic isolates were isolated from fruit body bought from the markets in Bangkok, Thailand and Madiun, Indonesia respectively. GKSA isolate of *Ganoderma boninense* was isolated from fruiting body grown on standing oil palm in Adolina, The Fourth State-Owned Estate, North Sumatera, Indonesia.

Growth of F isolate of *Pleurotus* **sp**. F isolate was grown on *Paraserianthes falcataria* sawdust, empty fruit bunches (EFB) and mixture of both substrates with proportion 1:1 respectively. Initially, EFB was chopped and shredded into smaller pieces, water soaked for one night to gain 75% moisture content. All substrates were added with 15% rice bran, 1.5% gypsum, 1.5% CaCO₃. Afterwards all substrates were placed in 30 x 20 cm sized polyethylene bags. Each bag containing of 500 gr substrate was sterilized, spawned with grain spawns and incubated in a range of 28-30°C. After completion of spawn running, the bags were unfolded at the upper parts for cropping. Five replications were used for each growing trial. Biological efficiency of each

treatment was determined as follows: BE = (fresh weight of harvested mushrooms/dry weight of substrate) x 100%.

Growth of GKSA isolate of *Ganoderma boninense* on empty fruit bunches (EFB). GKSA isolate was grown on chopped and shredded EFB supplemented with 15% rice bran, 1.5% gypsum, 1.5% CaCO₃ and added with water to gain 75% moisture content. Each of 70 gr of substrate was placed in a small bottle, then sterilized, spawned with grain spawns and incubated in a range of 28-30°C. The substrate analysis at various stages of growth i.e before inoculation, vegetative phase (after spawn run), during reproduction phase were determined. Lignin and cellulose contents were determined by Van Soest method. Organic-C content was measured by titration method and N content measured by Kjeldahl. The analyses were achieved at Laboratory of Science and Animal Feed, Faculty of Husbandry, Bogor Agricultural University and Services Laboratory, SEAMEO BIOTROP, Bogor.

Growth of GKSA isolate of *Ganoderma boninense* on liquid and solid wastes from palm oil mill effluent (POME). One hundred milliliter of 1.5% malt extract liquid medium was poured into a 250-L Erlenmeyer flask. The flasks were added with 10% (v/v), 20% (v/v) of liquid POME, 5% (w/v) or 10% (w/v) of solid POME respectively. Both POME were obtained from sixth ponds. Water or soil from oil palm plantation were used as the controls. The surface of the medium was inoculated with one disc of *G. boninense* solid medium (7-mm diameter) punched out from the edge of a 7 day-old colony grown on malt agar. The fungus was grown at 28-30°C without agitation. At the end of the incubation period the mycelium was collected, dried at 105° C, and weighed.

Data analysis. Results are expressed as means \pm standard deviation (S.D.). The analyses of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) were performed with a program of Microsoft Excel 2007 and SPSS Statistics 17.0.

RESULTS AND DISCUSSION

Growth of F isolate of *Pleurotus* **sp**. Based on this results, F isolate could grow and produce fruit body on all substrates i.e. *Paraserianthes falcataria* sawdust (PFS), empty fruit bunches (EFB), mixture of both substrates (M) with fresh weight and biological efficiencies (BE) for all substrates were not significantly different (P>0.05) in a range of 190-209 gram/bag for 8 flushes and 152-167% of BE but the highest results were obtained from PFS substrate with 209 gram/bag of fruiting body fresh weigh for 8 flushes and 167% of BE (Fig. 1). PFS is the principal substrate for oyster mushroom growing in Indonesia, although adequate production can be achieved through use of sawdust with addition of supplements that substantially increase the yield per unit weight. In addition PFS contains cellulose 48.3%, lignin 27.8%, pentosan16.2% [7] but EFB contains lower cellulose of 36%, lignin 27.3%, carbon 64.7%, N 1.1% and C/N ratio 57.8 (Sudirman, unpublished).

Vegetative phases (VP) on all substrates were observed between 25-31 days and fruit body production phase (RP) between 86-101 days and total of growth and development phase (GDP) between 111-132 days with shorter GDP on EFB and PFS substrates reaching 111 and 119 days respectively (Fig. 2).

The quantity of fruit body/bag for 8 flushes and pileus diameter of those three substrates were not significantly different (P>0.05) in a range of 31-34 pieces and 4.1-4.7 cm (Fig. 3). Number of fruit body and pileus diameter were related to the bag removal method [3] or were dependent on opened surface area for cropping. In case of larger opened surface area, the quantity of mushrooms could be larger but the diameter of pileus will be smaller.

Growth of GKSA isolate of Ganoderma boninense and bioconversion on empty fruit bunches (EFB). Based on these results, GKSA isolate of G. boninense could grow and produce fruiting body, only one flush was obtained. The vegetative phase took 17 days, longer than other substrates such as oil palm leaf and root which were only 8-10 days (unpublished). Nutrition contents of substrates after vegetative and during reproduction phase are presented at figure 4. C/N ratio decreased as much as 84% with initial C/N ratio 49.1 and 7.8 after vegetative phase, but increased thereafter during reproduction phase, but not as high as before inoculation. Similar patterns were shown with cellulose and carbon contents. Cellulose content decreased as much as 41% with initial content 50.6% and 29.8% after vegetative phase, but increased thereafter during reproduction phase. This pattern was followed by decreased carbon content up to 81% with initial content 58% and 10.9% after vegetative phase, but increased during reproduction phase. Differently with lignin degradation, its content decreased up to 66% during reproduction phase with initial content 31.4%, thereafter 10.6%. The degradation pattern indicated that the rate of lignin breakdown was slow during spawn run. The most extensive degradation of lignin occured during reproduction phase, implying the release of cellulose increased during reproduction phase as well. The lignin moiety can act as a barrier to cellulose degradation [6]. Therefore, the degradation of lignin serves to increase the availability of cellulose for development of fruit body. Nitrogen content in the substrate slightly gradually increased during decomposition reaching 1.5% during reproduction phase.



Figure 1: Fresh weight of fruiting body (FW) and biological efficiency (BE) of F isolate of *Pleurotus* sp. grown on three kinds of substrate. PFS: *Paraserianthes falcataria* sawdust, EFB: empty fruit bunches, M: mixture of PFS and EFB substrates (1:1)



Figure 2: Growth phases of F isolate of *Pleurotus* sp on three kinds of substrate. PFS: *Paraserianthes falcataria* sawdust, EFB: empty fruit bunches, M: mixture of PFS and EFB substrates (1:1), VP: vegetative phase, RP: reproduction phase, GDP: growth and development phase.



Figure 3: Pileus number (PN) and diameter (PD) of F isolate of *Pleurotus* sp on three kinds of substrate. PFS: *Paraserianthes falcataria* sawdust, EFB: empty fruit bunches, M: mixture of PFS and EFB substrates (1:1).



Figure 4: Bioconversion of empty fruit bunches by GKSA isolate of *Ganoderma boninense*. BI: before inoculation, VP: vegetative phase, RP: reproduction phase.



Figure 5: The growth of GKSA isolate of *Ganoderma boninense* on malt extract media containing liquid palm oil mill effluent (LPOME). W 10% or 20%: concentration of water at 10% or 20%, LPOME 10% or 20%: concentration of LPOME at 10% or 20%, DW: dry weight, GI: growth increasing.

Growth of GKSA isolate of *Ganoderma boninense* on liquid and solid wastes from palm oil mill effluent (POME). GKSA isolate could grow on malt extract media containing liquid and solid POME respectively, even stimulated the growth of GKSA isolate with the growth increase of 33 and 62% at 10 (v/v) and 20% (v/v) of liquid POME concentration in malt media respectively (Fig. 5). Similar results were achieved for solid POME, the growth increasing reached 23 and 64% at 5 (w/v) and 10% (w/v) of solid POME concentration in malt media respectively (Fig. 6). POME as a nutrient source can speed up the process of decomposition by reducing the wide C/N ratio of the EFB [7]. POME are started to be reused in oil palm plantation (land irrigation).

Composting EFB through mushroom production could be a possible way to transform the bulky bunches into a valuable, manageable product as market product or for use in plantation. The disadvantage using EFB in mushroom production is in its preparation. Initially, EFB must be chopped and shredded into smaller pieces that need much labor but it is not needed for ready use of sawdust.



Figure 6: The growth of GKSA isolate of *Ganoderma boninense* on malt extract media containing solid palm oil mill effluent (SPOME). CS 5% or 10%: concentration of control soil at 10% or 20%, SPOME 5% or10%: concentration of SPOME at 5% or10%, DW: dry weight, GI: growth increasing.

CONCLUSION

The empty fruit bunches (EFB) could be used as alternative substrate for mushroom production. Based on this research it was suggested to build mushroom industry located near the palm oil factory where the main substrates are abundantly available. The liquid as well as solid wastes from palm oil mill effluent (POME) stimulated the growth of mushrooms. In addition spent mushroom substrates can be then used as soil fertilizers in oil palm plantation.

ACKNOWLEDGEMENTS

The authors wish to express their appreciation to Department of National Education of Indonesia, in the projects of Hibah Bersaing XVII, as well as to The Fourth State-Owned Estate (PTPN IV) for financial support of this research. Further, we are grateful to our students: Aditya Sutrisna, Puspiari, Sri Maria Ulfha, and M. Yadi Nurjayadi who were involved and helped us during research.

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