EKSPLORATION OF GIAM SIAK KECIL-BUKIT BATU BIOSFEER RESERVE FOR CARBOLYTIC FUNGI

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ABSTRACT

GiamSiak Kecil-Bukit Batu (GSKBB) Biosphere Reserve in Riau Province, Indonesia, has a unique tropical peat swamp forest with peat depth reaching up to 20 meters deep. As a tropical peat swamp forest it is a potential source for carbolytic enzyme producing microbes. In this paper we present our exploration at two different sites of the GSKBB Biosphere Reserve forest, representing primary and secondary forest, for carbolytic fungi. Although the isolation media was design for the specific isolation of *Trichoderma,* we isolated carbolytic enzyme producers from the genus *Trichoderma, Penicillium*and*Aspergillus.* From the primary forest, a total of four cellulase and two chitinase producing fungi were isolated to purity. From the secondary forest, a total of two cellulase and one chitinase producing fungi were isolated to purity. Highest specific activity of cellulase was produced by a *Penicillium* secondary forest isolate. Highest specific activity of chitinase was produced by an *Aspergillus* primary forest isolate, with a total chitinase crude extract specific activity of 0.04 U/mg at 40^{o}C, pH 5.5.
Key words: Cellulase, Chitinase, Trichoderma, PenicilliumandAspergillus

INTRODUCTION

Tropical peat swamp forest soil is a potential source of carbolytic enzyme producing microbes.The microbial community in tropical peat swamp forest soil plays an important role in the decomposition of organic matter found on the forest bed, may it be from plant, animal or insect origin (Edwards *et al*., 2008; Wiznaet *al.,* 2007). Based on the composition of peat swamp forest soil, the most probable carbolytic enzymes produced by the microbial community would be cellulases and chitinases (Wiznaet *al.,* 2007;Seidl, 2008). Although bacteria produces these enzymes, industry have favoured the used of cellulase and chitinases from fungal sources, as fungi are easy to grow at ambient temperatures, not easely contaminated,and the carbolytic enzymes they produce are non-complexed extracellular systems which are easy to produce and harvest(Hartl, Zach, & Seidl-Seiboth, 2012). . In this paper we present briefly the results of our exploration at two different sites of the GiamSiak Kecil Bukit Batu (GSKBB) Biosphere Reserve peat swamp forest, representing primary and secondary forest, for carbolytic fungi producing cellulase and chitinase.

RESEARCH METHODS

Fungi was isolated from forest soil collected at two different locations of GSKBB peat swamp core zone forrest, each representing a primary forest and secondary forest site, as determined by the canopy of vegetation. Soil was collected at several spots for each site, 5 cm from the surface, and covering a radius of one Km. Collected soil for each area was mixed, and the fungi was isolated on selective media plates containing antibiotics (neomycin sulphate, bacitracin, penicillin G, chlortetracycline, nystatin), sodium propionate to produce small fungal colonies, minerals and citric acid, and an appropriate sole carbon source for the target enzyme. For cellulase, the sole carbon source was either microcrystalline cellulose (avicel PH-101) or colloidal cellulose (Carboxy methyl cellulose = CMC). For chitinase, the sole carbon source was crab shell chitin. Single separate colonies were repeatedly purified, to obtain single colonies from single spores.Fungi was identified by morphology based on color, size and shape of spores, appearance of mycelium, and microphotographed. All purified strains were tested on their ability to produce either endoglucanases, exo-cellulases or chitinases, by growing the fungi in liquid cultures containing either CMC, avicel or chitin at room temperature, and harvesting the enzymes daily. Enzyme activity was determined, using the appropriate substrates, and based on the release of

reducing sugars when the crude enzyme was incubated with the appropriate substrate, pH 5,5 at 40^{o}C. The reducing sugars where determined using the Nelson-Somogyi method, using glucose as the reducing sugar standard. Protein from each crude enzyme extracts were determined by the Lowry method using bovine serum albumin as the standard (Alexander & Griffiths, 1993).One unit of enzyme (U) is the enzyme amount that produces 1 µmol reducing sugar equivalent per minute determined under the conditions of the assay.

RESULTS AND DISCUSION

A total of six (6) fungal strains were isolated from the primary forest site, and a total of 3 (three) fungal strains were isolated from the secondary forest site to purity. From both sites, fungal strains each isolated based on their ability to produce either chitinase, endoglucanase or exo-glucanase could be isolated (table 1). Chitinase producing fungi was isolated using crab shell chitin as the sole carbon source, endoglucanase was isolated using CMC as the sole carbon source, and exoglucanase was isolated using avicel PH-101 as the sole carbon source. Although the selective media was adapted from a media designed to selectively isolate *Trichoderma sp.* (Papvizas, 1982), only two *Trichoderma sp.* strains were isolated. The majority of the strains isolated were from the genus *Penicillium.* A larger diversity was observed from the primary forest, with members of three genus were isolated (*Penicillium, Trichoderma*and*Aspergillus*), when compared to only two genus from the secondary forest (*Penicillium*and*Trichoderma*). One explanation is that a more diversified canopy of the forest in the primary area, provides a better environment for diversification of fungal species.

Table 1. Fungal carbolytic enzyme producers from GSK-BB isolated to purity.

Location	Type of enzyme	Number of isolates	Identified Genus
GSK-BB primary forest	Chitinase	2	*Aspergillus sp.* and *Penicillium sp.*
	Endoglucanase	2	*Penicillium sp.*
	Exoglucanase	2	*Penicillium sp.* and *Trichoderma sp.*
GSK-BB secondary forest	Chitinase	1	*Trichoderma sp.*
	Endoglucanase	1	*Penicillium sp.*
	Exoglucanase	1	*Penicillium sp.*

Quantification of the enzyme activities showed that the highest specific activity of chitinase from the GSK-BB isolates was produced by the *Aspergillus* primary forest isolate, with a total chitinase crude extract specific activity of 0.04 U/mg at 40^{o}C, pH 5.5 (Table 2). This value is significantly lower (p<0.05) than the crude chitinase specific activity of two biocontrol*T. asperellum*strains (T.N.J63 and T. N.C52) isolated from cultivated plantation soil in Riau (Nugroho*et al.,* 2003, Sawitri, 2010) (see table 2). Since chitinases are important for mycoparasitism and protection of plants by biocontrol fungi, the *Aspergillus sp.* isolated from GSK-BB primary forest may not be an effective biocontrol agent. The same conclusion can be deducted for the other chitinase producing fungal strains that were isolated from GSK-BB biosphere reverse.

Highest specific activity of cellulase was produced by the *Penicillium* secondary forest isolate. This activity per mg crude extract protein was significantly higher (p<0.05) than that of a commercial cellulase preparation from *Trichodermareesei*(data to be published elsewhere). Therefore this *Penicillium sp.* isolate is a potential candidate for the high production of efficient exoglucanase for use in industry.

Table 2. Specific activity of chitinase crude enzyme from fungal isolates of GSK-BB peat swamp forest compared to chitinases of *Trichodermaasperellum sp.*

Fungal isolate	Chitinase activity (U/mL)	Chitinase specific activity (U/mg protein)*)	Soil source	Refference
Trichoderma sp. LBKURCC22	0.0023±0.0007	0.03±0.015[b]	GSK-BB secondary forest	This study
Aspergillus sp. LBKURCC33	0.0046±0.0022	0.04±0.02[b]	GSK-BB primary forest	This study
Penicillium sp. LBKURCC34	0.0019±0.0001	0.004±0.0002[a]	GSK-BB primary forest	This study
*T. asperellum*T.N.C52	0.0149±0.0007	6.48±0.29[c]	Cocoa plantation soil	Sawitri, 2010
*T. asperellum*T.N.J63	0.0165±0.0014	10.65±0.9[d]	Citrus plantation soil	Sawitri, 2010

*)Average of three separate experiments. A different superscript letter denotes a statistically significant difference ($p<0.05$) as determined by the Duncan Multiple Range Test.

CONCLUSION

A total of 9 (nine) fungal strains, producers of the carbolytic enzymes chitinase and cellulase (endo and exo-glucanase) were successfully isolated to purity from the GSK-BB core zone peat swamp forest. A higher diversity of the fungal strains was isolated from the primary forest, compared to the secondary forest. Highest specific activity of chitinase was produced by an*Aspergillus* primary forest isolate, with a total chitinase crude extract specific activity of 0.04 U/mg at 40^{o}C, pH 5.5. Highest specific activity of cellulase was produced by a *Penicillium* secondary forest isolate, with an exoglucanase crude extract specific activity of 3.7 U/mg at 40^{o}C, pH 5.5.

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