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KAJIAN KERAGAMAN *Ganoderma* PADA TANAMAN KELAPA SAWIT DENGAN TEKNIK *ELISA*[1]

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ABSTRAK

Ekstrak protein larut dari Ganoderma *dan campuran dari sembilan isolat* Ganoderma *digunakan untuk menghasilkan antibodi poliklonal pada kelinci. Dalam teknik* ELISA *tak langsung, PAb-campuran lebih peka dibandingkan dengan PAb-3 dalam identifikasi isolat* Ganoderma *dari kebun-kebun kelapa sawit. Meskipun kedua antibodi tersebut berbeda dalam hal kepekaan, keduanya menunjukkan pola yang hampir sama dalam identifikasi adanya keragaman* Ganoderma. *Reaksi homolog antigen pada isolat* Ganoderma *bervariasi antara 105-284% untuk PAb-campuran dan 100-280% untuk PAb-3.*

Kata kunci : *ELISA, Elaeis guineensis, Ganoderma*

PENDAHULUAN

Di Asia Tenggara, penyakit yang paling serius menyerang tanaman kelapa sawit adalah penyakit busuk pangkal batang (BPB) yang disebabkan oleh jamur klas *Basidiomycetes* yang dikenal sebagai *Ganoderma sp*. Penyakit BPB dilaporkan pertama kalinya terjadi pada 1930 di Malaysia dan patogen penyebabnya adalah *G. lucidum* W. Curt (25). Setelah itu, Steyaert (23) berhasil mengidentifikasi enam spesies *Ganoderma* lainnya pada pertanaman kelapa sawit, yaitu : *G. boninense* Pat., *G. miniatocinctum* Steyaert sp. nov., *G. chalceum* (Cooke) Steyaert comb. nov., *G. tornatum* (Pers.) Bers., *G. zonatum* Murill dan *G. xylonoides* Steyaert. Turner (26) mencatat 15 spesies *Ganoderma* yang berhubungan dengan terjadinya penyakit BPB di perkebunan kelapa sawit. Di Afrika Barat, empat spesies *Ganoderma* telah diidentifikasi dari kebun-kebun kelapa sawit, yaitu *G. zonatum* Murill, *G. colossus*, *G. lucidum* W. curt dan *G. applanatum* Pers. ex S.F. Gray (19). Berbeda dengan hal di atas, kajian terakhir di Indonesia dan Malaysia menyatakan bahwa penyakit BPB disebabkan oleh hanya satu spesies, yaitu *G. boninense* Pat. (1,10). Kajian-kajian yang dilakukan tersebut didasarkan pada identifikasi karakteristik morfologi *basidioma* dan bukti-buktinya dikumpulkan dari contoh-contoh yang berasal dari daerah terbatas di Sumatera Utara (Indonesia) dan Semenanjung Malaysia. Dengan demikian, spesies tunggal sebagai penyebab timbulnya penyakit BPB pada kelapa sawit memiliki dasar yang meragukan.

Penggunaan teknik ELISA dalam deteksi dan identifikasi penyakit tanaman telah banyak dikembangkan untuk peng-

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ujian-pengujian rutin, namun kesulitan-kesulitan masih juga dilaporkan, khususnya dalam mendapatkan antiserum yang sangat spesifik (3, 15, 23). Antigen yang digunakan untuk menghasilkan antibodi dapat berasal dari cucian kultur miselia (16), bubuk miselia (5), ekstrak miselia (27), mikotoksin (28) maupun enzim ekstraseluler (12).

Identifikasi *Ganoderma* berdasarkan karakter morfologis dari *basidioma* sering menimbulkan keraguan, karena keadaan yang muncul berubah-ubah bergantung kepada kondisi lingkungan (20,24). Untuk menghindari kesulitan identifikasi *Ganoderma* pada tanaman kelapa sawit, para peneliti telah mengembangkan metode yang berbeda dalam mempelajari keragaman *Ganoderma*, antara lain melalui tes reaksi kompatibilitas vegetatif (8), isozim pektinase ekstraseluler (18), *random amplified polymorphic DNA* (RAPD) (2), dan metode serologi (6).

Tujuan penelitian ini adalah untuk menghasilkan antibodi poliklonal yang didapatkan dari protein miselia yang diencerkan untuk menentukan keragaman *Ganoderma* yang dilakukan secara serologis.

BAHAN DAN METODE

Isolat jamur. Isolat *Ganoderma* yang dimurnikan berasal dari berbagai kebun kelapa sawit, antara lain dari Sei Pancur (Dy x Dy)'76 (SP1), Sei Pancur (D x P)'90 (SP2), Sei Pancur (D x P)'76 (SP3), Sei Pancur (D x D)'90 (SP4), Aek Pancur (D x P) (AP1), Aek Pancur (Dyx P)'78 (AP2), Bukit Sentang II (D x P) (BS1), Bukit Sentang III (D x P) (BS2), Bah Jambi VII (D x P) (BJ1), Bah Jambi VIII (D x P) (BJ 2), Gunung Bayu (D x P) (GB), Adolina (D x P) (Ad), Tanjung Morawa (D x P) (TM), Pagar Merbau (D x P) (PM), Sei Bamban (D x P) (SB), Matapao (D x P) (Mt) dan Marihat (D x P) (Mr). Dua isolat *Ganoderma lucidum* dari *Deutsche Sammlung von Mikro-organismen*, Braunschweig, Jerman juga dimurnikan. Semua isolat dikulturkan pada labu Erlenmeyer 250 ml yang berisi 100 ml media campuran 1,5% ekstrak malt dan 0,5% ekstrak ragi. Inkubasi dilakukan pada suhu kamar selama 30 hari. Setelah miselia dipanen, dibilas beberapa kali dengan akuades dan ditumbuk dalam cawan porselin di atas es dengan ekstrak bufer (0,248% $Na_2S_2O_3.5H_2O$, 2,5% PVP dalam bufer karbonat 0,159% Na_2CO_3, 0,293% $NaHCO_3$, pH 9,6). Suspensi kemudian disentrifugasi pada 13.000 x g selama 10 menit pada suhu 4°C. Supernatan diambil dan disimpan pada suhu -20°C sampai dipergunakan.

Penyiapan Antigen, Imunisasi dan Teknik ELISA. Miselia segar sebanyak 0,4 gr diekstraksi dengan 6 ml bufer salin fosfat (BSF, 0,8% NaCl, 0,02% KCl, 0,29% $Na_2HPO_4.12H_2O$, 0,02% KH_2PO_4, pH 7,4) dalam cawan porselin dan ditumbuk di atas es. Dua antigen berbeda yang dipersiapkan untuk menghasilkan antibodi poliklonal, pertama adalah miselia segar dari *Ganoderma* SP1 (dilakukan di *Biologische Bundesanstalt fur Land und Forstwirtschaft*, Braunschweig dan kedua, miselia segar dari campuran sembilan isolat *Ganoderma* (SP1, AP1, BS1, BS2, Ad, PM, SB, Mt dan GB) yang dilakukan di Medan, Indonesia. Suspensi yang dihasilkan disentrifugasi pada 13.000 x g selama 10 menit pada suhu 4°C. Kelinci

kemudian diberi tiga suntikan, untuk yang pertama, 1,5 ml antigen + 1,5 ml larutan Freund lengkap, untuk yang kedua dan ketiga dengan larutan Freund tidak lengkap dengan selang waktu 10 hari. Kelinci-kelinci dibuat untuk berdarah dari telinganya 2 minggu setelah suntikan terakhir. Antiserum disentrifugasikan pada 6.000 x g selama 30 menit pada suhu 10°C. Supernatan kemudian dihisap dan ditambah 0,02 g $NaNO_3$ tiap 10 ml antiserum. Antiserum dimurnikan dengan kaolin. Teknik ELISA tak langsung ini dilakukan menurut Knapova (13) dengan sedikit modifikasi seperti terlihat pada Gambar 1.

Pembungkusan dan Inkubasi Antigen yang diencerkan dengan ekstrak bufer
(200 µl, 16 jam, 4°C)
↓
Pencucian dengan PBST (@ 3x3 menit)
↓
Penghambatan dengan 0,2% BSA dalam bufer karbonat
(250 µl, 0,5 jam, 37°C)
↓
Pencucian ulang
↓
Penambahan antibodi utama yang dilarutkan dalam PBST + 0,2% BSA
(200 µl, 0,5 jam, 37°C)
↓
Pencucian ulang
↓
Penambahan *Goat antirabbit IgG Alkaline Phosphatase conjugate*
yang diencerkan dengan PBST + 0,2% BSA
(200 µl, 0,5 jam, 37°C)
↓
Pencucian ulang
↓
Penambahan substrat p-nitrofenil fosfat
diencerkan dengan bufer substrat
(200 µl, 1-2 jam, suhu kamar)
↓
Pengukuran dengan fotometer pada 405 nm

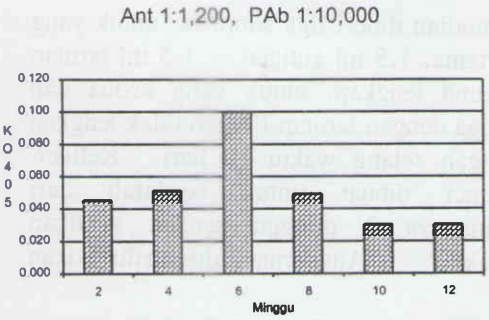
Gambar 1. Tahap-tahap pada teknik ELISA untuk mendeteksi antigen *Ganoderma*.

HASIL DAN PEMBAHASAN

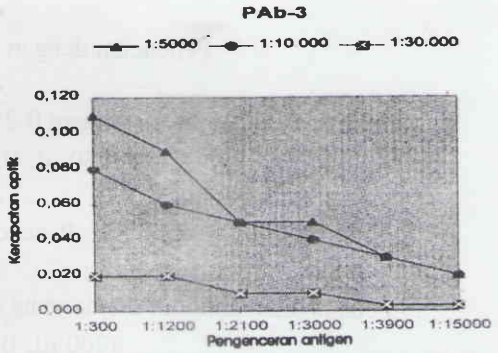
Antibodi poliklonal. Enam antiserum terhadap *Ganoderma* SP1 diambil dalam interval 2 minggu. Tingkat optimum dari produksi antibodi poliklonal ditunjukkan 6 minggu setelah suntikan terakhir atau pada pendarahan ke-3 (kode : PAb-3 dan dipergunakan dalam penelitian ini). Perkembangan akumulasi antibodi yang ditunjukkan dalam nilai kerapatan optik diperlihatkan dalam Gambar 2. Pola akumulasi yang sama juga dilaporkan terjadi dalam menghasilkan antibodi poliklonal terhadap ekstrak miselia *Phomopsis longicolla* (4) dan *Phytophthora infestans* (13). Setelah mencapai puncak akumulasi, antibodi-antibodi cenderung meningkatkan reaksi-silang dengan jamur lainnya (4). Dalam studi ini, akumulasi puncak dari antibodi-antibodi lebih lama dibandingkan dengan yang dilaporkan untuk antibodi-antibodi yang diproduksi terhadap kultur kayu *Ganoderma* dalam kuning telur (7). Untuk antibodi-antibodi poliklonal yang diambil dari campuran sembilan isolat *Ganoderma*, pengumpulan antiserum dilakukan hanya sekali (kode: Pab-camp).

Sensitivitas PAb-campuran lebih tinggi dalam mendeteksi antigen-*Ganoderma* SP1 dibandingkan dengan PAb-3. Nilai kerapatan optik teknik ELISA untuk PAb-campuran (Gambar 4) pada larutan yang lebih encer dan yang lebih pekat adalah berturut-turut 4 kali lebih tinggi dan 10 kali lebih tinggi dibandingkan dengan nilai kerapatan optik PAb-3 (Gambar 3).

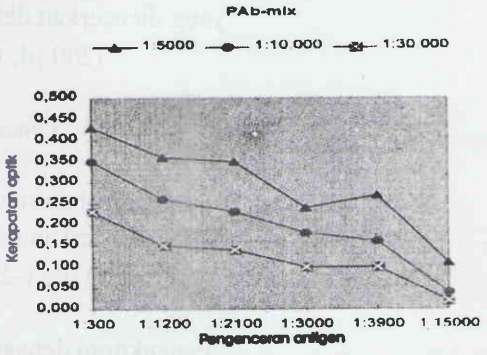
Keragaman Isolat *Ganoderma*. Meskipun penggunaannya belum meluas dalam ilmu taksonomi dan dalam pengidentifikasian fungi secara tepat, tek-



Gambar 2. Akumulasi antibodi terhadap *Ganoderma* SP1.



Gambar 3. Nilai kerapatan optik yang diperoleh dari reaksi antara PAb-3 dengan antigen pada berbagai pengenceran.



Gambar 4. Nilai kerapatan optik yang diperoleh dari reaksi antara PAb-campuran dengan antigen pada berbagai pengenceran.

nik serologi telah berhasil dalam mengidentifikasi beberapa spesies atau strain fungi patogen, seperti strain-strain *Phytophtora fragariae* (3), spesies *Fusarium* (11), spesies *Phytophthora* (17), spesies *Verticillium* (9, 29), *Botrytis cinerea* (22), dan spesies *Armillaria* (14, 21).

Keragaman dari *Ganoderma* dievaluasi dengan PAb-campuran dan PAb-3 didasarkan pada ekstrak miselia dari 19 isolat *Ganoderma*. Semua ekstrak miselia disesuaikan dengan pengenceran 1: 1.200. PAb-campuran dan PAb-3 mengenali semua isolat *Ganoderma* yang diuji dan ditemukan keragaman dari seluruh isolat. Nilai ELISA dari PAb-campuran dan PAb-3 menunjukkan pola yang relatif sama seperti pada keragaman *Ganoderma*. Persentase dari respon *Ganoderma* homolog SP1 terhadap *Ganoderma* beragam di antara 100% dan 280% untuk PAb-3, dan *Gano*-campuran yang homolog antara 105% dan 284% untuk PAb-campuran (Tabel 1 dan 2). Pada dua lokasi, Sei Pancur dan Bah Jambi, dua isolat *Ganoderma* ditemukan dengan ciri-ciri serologis yang berbeda pada tiap lokasi (SP1, SP3, dan BJ1, BJ2). Temuan yang sama juga dilaporkan oleh Darmono, *et.al* (6). Mengenai kompleksitas strain-strain *Ganoderma*, Darus *et.al* (8) mengidentifikasi 3 strain *Ganoderma* yang berbeda dalam satu pohon kelapa sawit melalui tes reaksi kompatibilitas vegetatif. Perbedaan nyata dalam nilai ELISA dari *G. lucidum* yang dibandingkan dengan semua isolat *Ganoderma* dari kelapa sawit mengindikasikan bahwa *Ganoderma* pada kelapa sawit merupakan spesies yang berbeda dengan *G. lucidum*.

Walaupun kajian keragaman *Ganoderma* dengan ELISA memberikan sedikit informasi dalam spesies atau strain *Ganoderma*, namun kajian ini mempertegas adanya variasi antigen di antara isolat *Ganoderma* yang penting untuk pengelompokan *Ganoderma*. Berdasarkan kajian ini, dianjurkan untuk menggunakan antibodi poliklonal yang diproduksi dari campuran isolat *Ganoderma* yang dipergunakan untuk pendeteksian dini adanya *Ganoderma* pada kelapa sawit.

Tabel 1. Nilai-nilai kerapatan optik (KO) dari PAb-campuran terhadap *Ganoderma* dari berbagai kebun kelapa sawit

No	Asal *Ganoderma*	KO_{405}	KO_{405} nisbi (%)*
1	BJ2	0.20	105
2	GB	0.21	111
3	SP1	0.23	121
4	BS2	0.25	132
5	SP4	0.25	132
6	AP1	0.27	142
7	SP2	0.27	142
8	Ad	0.31	163
9	AP2	0.31	163
10	TM	0.31	163
11	PM	0.35	184
12	SB	0.36	189
13	Mt	0.36	189
14	BS1	0.37	195
15	BJ1	0.42	221
16	SP3	0.47	247
17	Mr	0.54	284
18	*G.lucidum* 9621	1.58	832
19	*G.lucidum* 103	1.63	858
20	*Gano*-camp. (kontrol)	0.19	100

*Nilai kerapatan optik dihitung terhadap *Gano*-camp.

Tabel 2. Nilai-nilai kerapatan optik dari PAb-3 terhadap *Ganoderma* dari berbagai kebun kelapa sawit

No.	Asal *Ganoderma*	KO_{405}	KO_{405} nisbi (%)*
1	BJ2	0.05	100
2	BS2	0.06	120
3	GB	0.06	120
4	SP4	0.06	120
5	AP1	0.07	140
6	TM	0.07	140
7	SP2	0.07	140
8	PM	0.08	160
9	Ad	0.08	160
10	SB	0.08	160
11	Mt	0.08	160
12	AP2	0.08	160
13	BS1	0.09	180
14	BJ1	0.12	240
15	SP3	0.13	260
16	Mr	0.14	280
17	*G.lucidum* 9621	0.84	1680
18	*G.lucidum* 103	0.95	1900
19	SP1 (kontrol)	**0.05**	**100**

*Nilai kerapatan optik dihitung terhadap SP1

KESIMPULAN

Baik Pab-campuran maupun PAb-3 mampu mendeteksi dan menegaskan terdapatnya keragaman dari isolat *Ganoderma* pada tanaman kelapa sawit secara serologis. Berdasarkan isolat yang dianalisa dengan antibodi-antibodi tersebut, hanya sedikit saja informasi yang diberikan untuk menunjukkan perbedaan-perbedaan pada spesies *Ganoderma*, tetapi memberikan kegunaan yang berarti dalam penge-lompokan isolat-isolat *Ganoderma*. Untuk tujuan-tujuan identifikasi yang lebih baik, diperlukan lebih banyak antibodi-antibodi yang spesifik. Antibodi-antibodi mono-klonal dapat memenuhi kebutuhan ini dengan memberikan perbedaan secara kualitatif dari spesies atau strain yang menunjukkan antibodi-antibodi mono-klonal spesifik.

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Study on the Variability of *Ganoderma* in Oil Palm by ELISA Technique[1]

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Abstract

Soluble protein extracts of single Ganoderma *and the mixture of nine* Ganoderma *isolates were used to raise polyclonal antibodies in rabbits. In the indirect ELISA technique, PAb-mix was more sensitive than PAb-3 in the determination of* Ganoderma *isolated from various oil palm estates. Although both antibodies differ in the sensitivity, but they showed similar pattern in recognizing of the variability of* Ganoderma. *Homologous reactions of antigens to* Ganoderma *isolates varied from 105-284 % for PAb-mix and 100-280 % for PAb-3.*

Keywords: ELISA, *Elaeis guineensis, Ganoderma*

Introduction

In South-East Asia, the most serious disease attacking oil palm is the basal stem rot (BSR) caused by various basidiomycete fungi known as *Ganoderma* sp. BSR was first reported in 1930 in Malaysia and the pathogen was identified as *G. lucidum* W. Curt (25). Later, Steyaert (23) identified six *Ganoderma* species from oil palm field, *i.e.*, *G. boninense* Pat., *G. miniato-cinctum* Steyaert sp. nov., *G chalceum* (Cooke) Steyaert comb.nov., *G. tornatum* (Pers.) Bers., *G. zonatum* Murill and *G. xylonoides* Steyaert. Turner (26) has listed 15 *Ganoderma* species which are associated with BSR in oil palm. In West Africa, four *Ganoderma* species have been identified from oil palm field namely, *G. zonatum* Murill, *G. colossus*, *G. lucidum* W.curt and *G. applanatum* Pers.ex S.F Gray (19). In contrast, recent studies in Indonesia and Malaysia revealed that BSR is caused only by a single species, *G. boninense* Pat. (1, 10). The studies were based on the identification of morpho-logical characteristics of basidioma and the evidence was drawn from few

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specimens collected from limited region in North Sumatra (Indonesia) and Malaysia Peninsular. Therefore, single species as causal agent of BSR in oil palm has a rather dubious basis.

The use of ELISA technique in detection and identification in plant pathology has been developed for routine assay, even so difficulties are still reported, especially in obtaining highly specific antisera (3, 15, 23). Antigens for eliciting antibodies can be raised against mycelial surface washing (16), powdered mycelia (5), mycelial extract (27), mycotoxin (28) and extracellular enzymes (12).

Identification of *Ganoderma* based on morphological characters of basidioma may often produce ambiguities since such features can vary depending on environmental conditions (20, 24). To avoid confusion surrounding the identification of *Ganoderma* in oil palm, some researchers have developed different methods to study the variability of *Ganoderma*, such as through vegetative compatibility reaction tests (8), extracellular pectinase isozymes (18), random amplified polymorphic DNA (RAPD)(2) and serological method (6).

The aim of this research was to produce polyclonal antibodies raised against soluble mycelial proteins in order to evaluate the variability of *Ganoderma* serologically.

Materials and Methods

Fungal isolates. *Ganoderma* was isolated from various oil palm estates, i.e. Sei Pancur (Dy x Dy)`76 (SP1), Sei Pancur (D x P)`90 (SP2), Sei Pancur (D x P)`76 (SP3), Sei Pancur (D x D) 90 (SP4), Aek Pancur (D x P) (AP1), Aek Pancur (Dy x P)`78 (AP2), Bukit Sentang II (D x P) (BS1), Bukit Sentang III (D x P) (BS2), Bah Jambi VII (D x P) (BJ1), Bah Jambi VIII (D x P) (BJ2), Gunung Bayu (D x P) (GB), Adolina (D x P) (Ad), Tanjung Morawa (D x P) (TM), Pagar Merbau (D x P) (PM), Sei Bamban (D x P) (SB), Matapao (D x P) (Mt) and Marihat (D x P) (Mr). Two isolates of *Ganoderma lucidum* from Deutsche Sammlung von Mikroorganismen, Braunschweig, Germany was also isolated. All isolates were cultured in 250 ml Erlenmeyer containing 100 ml 1,5 % malt extract + 0.5 % yeast extract. Incubation was done at room temperature for 30 days. The mycelia were harvested, washed several times with distilled water and macerated using a mortar and pestle on ice with extraction buffer (0.248 % $Na_2S_2O_3.5H_2O$, 2.5 % PVP in carbonate buffer 0.159 % Na_2CO_3, 0.293 % $NaHCO_3$, pH 9.6). The suspension was centrifuged at 13,000 x g for 10 min at 4 °C. The supernatant was taken and stored at - 20 °C until used.

Antigen Preparation, Immunization and ELISA technique. Fresh mycelia of 0.4 g was extracted by 6 ml Phosphate buffer saline (PBS, 0.8 % NaCl, 0.02 % KCl, 0.29 % $Na_2HPO_4.12\ H_2O$, 0.02 % KH_2PO_4, pH 7.4) in a mortar and pestle on ice. Two different antigens were prepared to produce polyclonal antibodies, first, fresh mycelia of *Ganoderma* SP1 (conducted in *Biologische Bundesanstalt für Land und Forstwirtschaft*, Braun-

schweig) and second, fresh mycelia of a mixture of nine *Ganoderma* isolates (SP1, AP1, BS1, BS2, Ad, PM, SB, Mt and GB) conducted in Medan, Indonesia. The suspension was centrifuged at 13,000xg for 10 min at 4 °C. Rabbits were given three injections, for the first, 1,5 ml antigen + 1.5 ml Freund`s complete adjuvant, for the second and third with Freund`s incomplete adjuvant with 10 days interval. Rabbits were made to bleed from the ear 2 weeks after the last injection. Antisera were centrifuged at 6,000xg for 30 min at 10 °C. The supernatant was pipetted and added with 0.02 g $NaNO_3$ per 10 ml antiserum. Antisera were purified by kaolin. Indirect ELISA technique was adopted from Knapova (13) with minor modification as described in Figure 1.

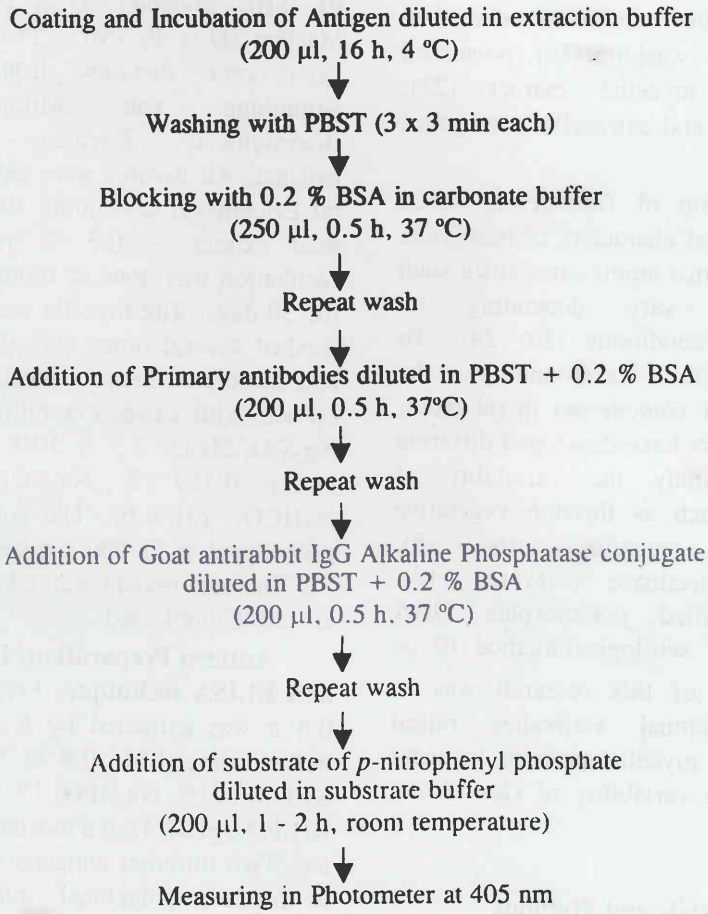

Figure 1. Protocol of ELISA technique for the detection of *Ganoderma* antigen.

Results and Discussion

Polyclonal antibodies. Six antisera raised against *Ganoderma* SP1 were taken with an interval of 2 weeks. The optimum level of polyclonal antibodies production was exhibited 6 weeks after the last injection or at the third bleeding (code: PAb-3 and used in this research). The development of antibody accumulation reflected as optical density values is shown in Figure 2. The same pattern of accumulation was also reported in producing polyclonal antibodies raised against mycelial extracts of *Phomopsis longicolla* (4) and *Phytophthora infestans* (13). After the peak of accumulation, antibodies tend to increase cross-reactivity with other fungi (4). In this study, the peak accumulation of antibodies was longer than that reported for antibodies produced against exudation of wood culture of *Ganoderma* in egg yolk (7). For polyclonal antibodies raised against a mixture of nine *Ganoderma* isolates, the antisera collection was carried out only once (code: PAb-mix)

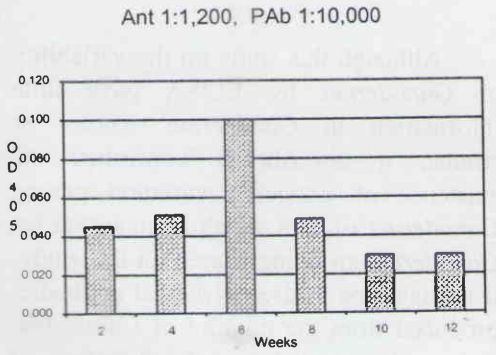

Figure 2. Accumulation of antibodies raised against *Ganoderma* SP1.

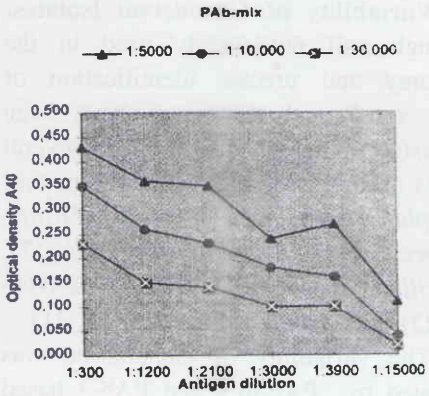

Figure 3. Optical density (OD) values obtained from reaction of the diluted PAb-3 and antigens.

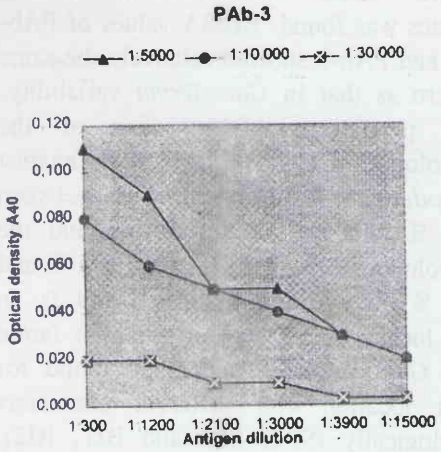

Figure 4. OD values obtained from reaction of the diluted PAb-mix and antigens

Sensitivity of PAb-mix was higher in detection antigen-*Ganoderma* SP1 than in PAb-3. The optical density values of ELISA of PAb-mix (Fig. 4) at lower dilution and at higher dilution were about four times higher, and ten times higher than those of PAb-3 (Fig. 3), respectively.

Variability of *Ganoderma* Isolates. Although still not widely used in the taxonomy and precise identification of fungi, serological techniques have been successful in the identification of several species or strains of pathogenic fungi; e.g. *Phytophthora fragariae* strains (3), *Fusarium* species (11),*Phytophthora* species (17), *Verticillium* species (9, 29), *Botrytis cinerea* (22), and *Armillaria* species (14, 21).

The variability of *Ganoderma* was evaluated by PAb-mix and PAb-3 based on mycelial extracts of 19 *Ganoderma* isolates. All mycelial extracts were adjusted to dilution of 1:1,200. PAb mix and PAb3 recognized all the *Ganoderma* isolates tested and variability among isolates was found. ELISA values of PAb-mix and PAb-3 showed relatively the same pattern as that in *Ganoderma* variability. The percentage of response of the homologous *Ganoderma* SP1 against *Ganoderma* of oil palm varied between 100 % and 280 % for PAb-3 and the homologous *Gano*-mix between 105 % and 284 % for PAb-mix (Tables 1 and 2). In two locations, Sei Pancur and Bah Jambi two *Ganoderma* isolates were found for each location with different characters serologically (SP1, SP3 and BJ1, BJ2). The same findings were also reported by Darmono, *et.al.* (6). Concerning the complexity of *Ganoderma* strains, Darus, *et.al.* (8) identified three different *Ganoderma* strains within one oil palm through vegetative compatibility reaction tests. Significant differences in ELISA values of *G. lucidum* as compared to all *Ganoderma* isolates from oil palm indicated that *Ganoderma* in oil palm was a different species with *G. lucidum*.

Table 1. OD values of PAb-mix raised against *Ganoderma* from various oil palm estates

No	*Ganoderma* origin	OD_{405}	Rel. OD_{405} (%) *
1	BJ2	0.20	105
2	GB	0.21	111
3	SP1	0.23	121
4	BS2	0.25	132
5	SP4	0.25	132
6	AP1	0.27	142
7	SP2	0.27	142
8	Ad	0.31	163
9	AP2	0.31	163
10	TM	0.31	163
11	PM	0.35	184
12	SB	0.36	189
13	Mt	0.36	189
14	BS1	0.37	195
15	BJ1	0.42	221
16	SP3	0.47	247
17	Mr	0.54	284
18	*G.lucidum* 9621	1.58	832
19	*G.lucidum* 103	1.63	858
20	*Gano*-mix (control)	0.19	100

*Relative OD values calculated to *Gano*-mix

Although this study on the variability of *Ganoderma* by ELISA gave little information in *Ganoderma* species or strains, it nevertheless confirmed the existence of antigenic variation among *Ganoderma* isolates which is important for *Ganoderma* grouping. Based on this study, it is suggested to use polyclonal antibodies produced from the mixture of *Ganoderma* isolates be used for early detection of *Ganoderma* in oil palm.

Table 2. OD values of PAb-3 raised against *Ganoderma* from various oil palm estates

No.	*Ganoderma* origin	OD_{405}	Rel. OD_{405} (%)*
1	BJ2	0.05	100
2	BS2	0.06	120
3	GB	0.06	120
4	SP4	0.06	120
5	AP1	0.07	140
6	TM	0.07	140
7	SP2	0.07	140
8	PM	0.08	160
9	Ad	0.08	160
10	SB	0.08	160
11	Mt	0.08	160
12	AP2	0.08	160
13	BS1	0.09	180
14	BJ1	0.12	240
15	SP3	0.13	260
16	Mr	0.14	280
17	*G.lucidum* 9621	0.84	1680
18	*G.lucidum* 103	0.95	1900
19	SP1 (control)	**0.05**	**100**

* Rrelative OD values calculated to SP1

Conclusions

Both PAb-mix and PAb-3 were able to detect and confirm the variability of *Ganoderma* isolated from oil palm serologically. Based on the isolates analyzed by these antibodies, only little information was provided for indicating the differences of *Ganoderma* species, but provided a useful means of grouping of isolates of *Ganoderma*. For better identification purposes, more specific antibodies were needed. Monoclonal antibodies could fulfill the requirements by giving qualitative differences from obtaining species or strains-specific monoclonal antibodies.

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