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Germination Studies of *Ganoderma boninense* Spores from Oil Palms in Malaysia

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ABSTRAK

Spora Ganoderma boninense *yang terdapat daripada sporofor yang mensporulasi, bercambah dengan mudahnya, tetapi spora yang dipicitkan daripada sporofor tidak. Spora tidak boleh bercambah dalam eraman 24 jam pertama, tetapi selepas 30 jam, 31.5 – 64.0% spora bercambah pada berbagai media. Media yang terbaik untuk percambahan spora ialah lima bean agar dan diikuti oleh corn meal agar dan rice dextrose agar. Percambahan spora pada Czapeks agar kurang baik. Suhu 25 – 31 °C lebih sesuai jika dibandingkan dengan suhu 21 – 23 °C.*

ABSTRACT

Spores of Ganoderma boninense *obtained from sporulating sporophores attached to infected oil palms or infected cut stumps, germinated readily but spores which were squeezed out from detached sporophores did not. None of the spores germinated during the first 24 hrs of incubation but by 30 hrs, 31.5 – 64% of the spores germinated on various media. The most suitable medium for germination was lima bean agar followed by corn meal agar and rice dextrose agar. Spore germination was poor on Czapeks agar. Warmer temperatures of 25 – 31 °C were more favourable to germination than cooler temperatures of 21 – 23 °C.*

INTRODUCTION

Spore germination implies a change from the dormant to an actively growing state. Factors favourable to spore germination may not necessarily be favourable to mycelial growth. Growth of mycelium can sometimes continue under conditions that are inhibitory to germination. Likewise, spore germination can occur under conditions adverse to mycelial growth.

The optimum temperature for mycelial growth of *Ganoderma boninense* Pat. isolated from oil palms in Malaysia was found to be 27 – 29°C and the best medium to be lima bean agar (Ho and Nawawi, 1986a). Factors affecting spore germination are not known and some of these will be investigated here.

MATERIALS AND METHODS

Effect of Media on Germination

Preliminary studies in this investigation showed that spores squeezed out from detached sporophores usually remained ungerminated. The squeezed-out spores were probably immature. The simplest method of obtaining mature spores of *G. boninense* was by exposing sterile petri dishes under sporulating sporo-

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phores, preferably at night when spore discharge was maximum (Ho and Nawawi, 1986b). Spores collected in the petri dishes were made into a suspension (approximately 2×10^3 spores/ml) with an appropriate amount of sterile distilled water. One ml of the spore suspension was then plated on to petri dishes with various media.

Media used were 2% and 3% malt agar (2% MA, 3% MA) carrot dextrose agar (CDA), Difco lima bean agar (LBA), rice dextrose agar (RDA), Difco potato dextrose agar (PDA), Difco corn meal agar (CMA), Difco prune agar (PA), Difco Czapek agar (CA) and modified Elliott's medium (EM). Preparation of CDA, RDA and EM was the same as that given by Ho and Nawawi, 1986a. Six replicates were made for each medium. The plates deposited with spores were incubated at 27 ± 1°C and examined after 12, 24, 30 and 48 hrs. Five hundred spores were counted at random for each plate and their percentage germination recorded. The length and number of branches of the germ tube at 30 hrs were recorded.

Effect of Temperature on Germination

The medium used was LBA and the spores were obtained by the same method described above. The plates were incubated at 21°C, 23°C, 25°C, 27°C, 29°C and 31°C for 48 hrs after which they were examined and 500 spores counted at random for each plate. The percentage germination, number and lengths of germ tubes were recorded. Six replicates were made for each temperature.

RESULTS AND DISCUSSION

Effect of Media

None of the spores germinated during the first 24 hrs of incubation, but by 30 hrs 31.5 – 64% of the spores germinated on the various media (Table 1). Prior to germination, the spores were observed to swell slightly. Each spore then produced a single germ tube from the truncated end *(Fig. 1a)*.

Of the various media tested, LBA was found to be the most suitable medium for germination. At 48 hrs germination on LBA was 97.5%. Mean length of the germ tubes at 30 hrs was 50 ± 4.1 μm and branching of the germ tube was not very common. However, at 48 hrs most germ tubes produced more than 3 branches *(Fig. 1b)* and by 72 hrs the branching was so profuse that a small colony of mycelium was formed around the germinated spores *(Fig. 1c)*.

Although percentage germination of spores was also high on CMA and RDA (94% and 92.3% respectively), growth of the germ tube was much slower on these 2 media when compared to that on LBA (Table 1).

Germination was poor on CA and the length of the germ tube at 30 hrs was only 9 ± 1.0 μm.

Lima bean agar which was the best medium for germination was also found to be the most suitable medium for mycelial growth (Ho and Nawawi, 1986a). The next best media for germination, RDA and CMA, were also suitable for mycelial growth. Czapek agar which was a poor medium for germination was also reported to be a poor medium for mycelial growth (Ho and Nawawi, 1986a). These results indicate that germination like mycelial growth is more successful on natural rather than synthetic media such as Czapek agar.

Effect of Temperature

The optimum temperature for germination was 27°C *(Fig. 2)*. At this temperature, germination exceeded 90% and the germ tube measured up to 260 μm with many branches. Germination was also high at 25°C, 29°C and 31°C – around 80%. However at 23°C and 21°C, germination was significantly less. The germ tubes were very short (mean length was 10 ± 1.8 μm) with no branching and some of them produced abnormal swollen protuberances at the tips. Thus temperatures above 25°C were more conducive while temperatures below that level seemed to have a critical adverse effect on

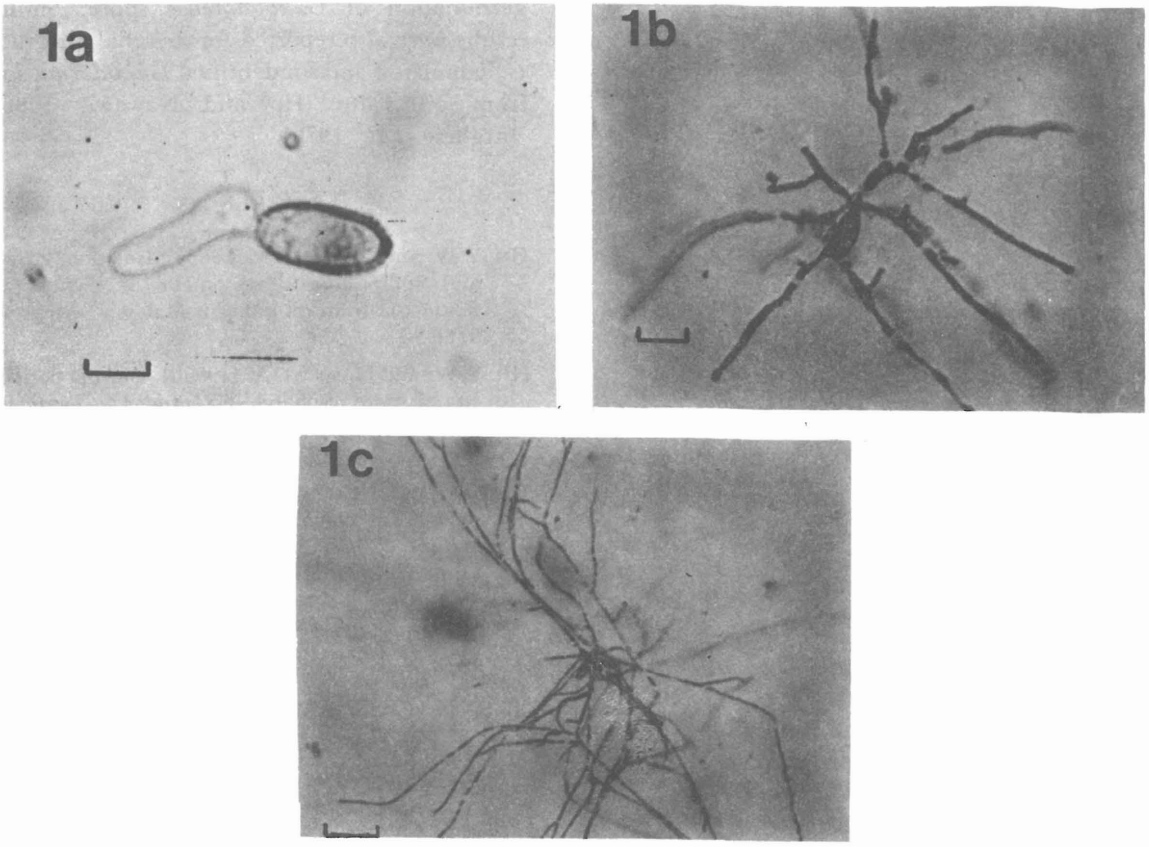

Fig. 1: Germinated spores of G. boninense, *(a) after 30 hrs (bar = 5 μm); (b) after 48 hrs (bar = 10 μm); (c) after 72 hrs (bar = 25 μm).*

TABLE 1
Percentage of germination and lengths of germ tubes of *G. boninense* spores

Media	Germination of basidiospore (in %)			Mean lengths of germ tubes (in μm)	
	24 hrs	30 hrs	48 hrs	24 hrs	30 hrs
2% MA	0	48.5	66.3	0	16.3 ± 1.9
3% MA	0	37.0	66.5	0	10 ± 1.2
PDA	0	46.8	60.4	0	16 ± 1.5
CDA	0	52.0	85.0	0	11.2 ± 2.1
PA	0	50.1	86.8	0	15 ± 2.2
RDA	0	57.4	92.3	0	20 ± 1.8
CMA	0	58.9	94.0	0	25 ± 1.4
LBA	0	64.0	97.5	0	50 ± 4.1
Mod. Elliott's med.	0	53.3	82.0	0	13 ± 1.2
Czapek sol. agar	0	31.5	52.5	0	9 ± 1.0

± — standard error.

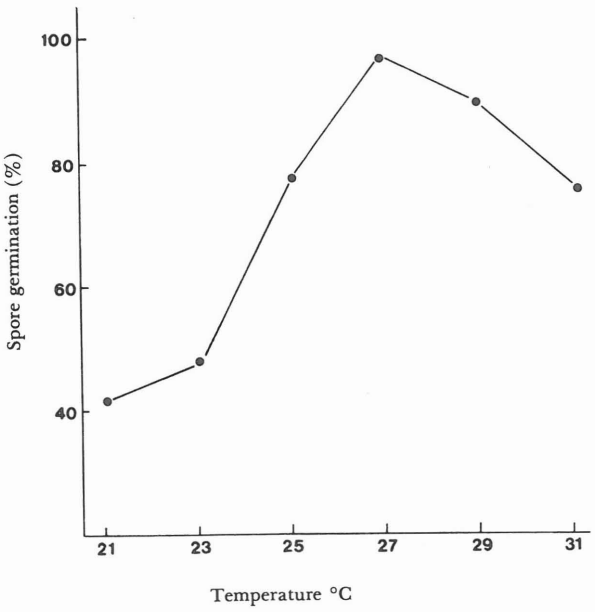

Fig. 2: Effect of temperature on germination of G. boninense *spores.*

germination of *G. boninense* spore. Similar results were also reported for mycelial growth of *G. boninense* and unidentified *Ganoderma* spp. from oil palm (Ho and Nawawi, 1986a; Varghese *et al.*, 1976).

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