GENETICS AND PHYSIOLOGICAL CHARACTERIZATION OF *GANODERMA LUCIDUM* STRAINS

By

Miss Korapan Sawetsuwannakun

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree

Master of Science Program in Microbiology

Department of Microbiology

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การศึกษาคุณลักษณะทางพันธุศาสตร์และสรีรวิทยาของเห็ดหลินจือสายพันธุ์ต่างๆ

โดย

นางสาวกรพรรณ เศวตสุวรรณกุล

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต

สาขาวิชาจุลชีววิทยา

ภาควิชาจุลชีววิทยา

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The Graduate School, Silpakorn University has approved and accredited the Thesis title of Genetics and physiological characterization of *Ganoderma lucidum* strains submitted by Miss Korapan Sawetsuwannakun as a partial fulfillment of the requirements for the degree of Master of Science in Microbiology

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Nine strains of *Garnoderma lucidum* and one strain of *G. sinensis* isolated from China and Thailand were characterized based on physiology and genetics. The radial mycelia growth rate of *Ganoderma* strains were measured on potato dextrose agar (PDA) for 14 days. Except for strain KALASIN, the mycelium of all strains grew at 25ºC-30ºC, but did not grow at 35ºC-40ºC. The strain KALASIN grew at 25ºC-37ºC. Yeast extract, malt extract and ammonium chloride could enhance growth rate of some strains. The internal transcribe spacers (ITS) were amplified and sequenced, and revealed that the ITS regions of *Ganoderma* strains were 636-658 bp. By comparison of ITS sequences, *Ganoderma* species could be classified into 3 groups. Group 1 consisted of *G. lucidum* strains isolated from China, group 2 composed of *G. lucidum* isolate from Thailand and group 3 was made up by *G. sinensis*. The similarity between Chinease and Thai strains were 95-96%. The similarity between *G. lucidum* strains and *G. sinensis* were 89-92%. The random amplified polymorphism DNA analysis could distinguish *Ganoderma* species into 2 groups. Group 1 composed of *G. lucidum* strains and group 2 consisted of a single strain of *G. sinensis*. Group 1 could be divided into 2 subgroups, i.e. group of Chainease strains and group of Thai strain. The similarity between *G. lucidum* and *G. sinensis* were 39-47%. The similarity between *G. lucidum* strains isolated from China and Thailand were 50 -61%. The numerical taxonomy could divide the mushrooms into 2 groups, i.e. group of *G. lucidum* strains and group of *G. sinensis*. The crude polysaccharides of *G. lucidum* strains were 0.68-2.86% and *G. sinnesis* were 7.93%. The total carbohydrate, reducing sugar, protein content and phenol content of *Ganoderma* species were 316.54-923.46 mg/g, 62-120.11 mg/g, 3.35-17.74 mg/g, and 4.11-16.56 mg/g of crude polysaccharide, respectively.

Department of Microbiology Graduate School, Silpakorn University Academic Year 2011
Student's signature .....
Thesis Advisor's signature .....

๕๑๓๑๓๒๐๑ : สาขาวิชาจุลชีววิทยา

คำสำคัญ : *Garnoderma. lucidum* สารสกัดโพลีแซคคาไรด์ Internal transcribe spacer (ITS)
Random amplified polymorphism DNA (RAPD)

กรพรรณ เศวตสุวรรณกุล : การศึกษาคุณลักษณะทางพันธุศาสตร์และสรีรวิทยาของเห็ดหลินจือสายพันธุ์ต่างๆ. อาจารย์ที่ปรึกษาวิทยานิพนธ์ : ผศ. ดร. เอกพันธ์ บางยี่ขัน.๑๒๑ หน้า.

เห็ดหลินจือแดง *Ganoderma lucidum* ๙ สายพันธุ์ และเห็ดหลินจือม่วง *G. sinensis* ๑ สายพันธุ์ ที่แยกจากประเทศจีนและประเทศไทย ถูกนำมาศึกษาเพื่อเปรียบเทียบคุณลักษณะทางพันธุศาสตร์และสรีรวิทยา นำเส้นใยของเห็ดทุกสายพันธุ์มาเลี้ยงบนอาหาร potato dextrose agar ที่อุณหภูมิต่างๆ เป็นเวลา ๑๔ วัน พบว่าเส้นใยสามารถเจริญได้ที่อุณหภูมิ ๒๕-๓๐ องศาเซลเซียส แต่ไม่สามารถเจริญได้ที่อุณหภูมิ ๓๕-๓๗ องศาเซลเซียส ยกเว้น เห็ดหลินจือแดงสายพันธุ์ KALASIN สามารถเจริญได้ที่อุณหภูมิ ๒๕-๓๗ องศาเซลเซียส เห็ดบางสายพันธุ์มีการเจริญได้ดีขึ้นเมื่อมีการเติม yeast extract, malt extract และ ammonium chloride ลงในอาหาร ลำดับเบสบริเวณ Internal transcribed spacer (ITS) มีขนาด ๖๓๖-๖๕๘ เบสแพร์และเมื่อเปรียบเทียบความเหมือนของลำดับเบสบริเวณ ITS สามารถแบ่งเห็ดออกเป็น ๓ กลุ่มคือ กลุ่มที่ ๑ เห็ดหลินจือแดงสายพันธุ์จากประเทศจีน กลุ่มที่ ๒ เห็ดหลินจือแดงสายพันธุ์จากประเทศไทย กลุ่มที่ ๓ เห็ดหลินจือม่วง กลุ่มที่ ๑ และ ๒ มีความเหมือนกันร้อยละ ๙๕-๙๖ กลุ่มที่ ๑ และ ๒ เหมือนกับกลุ่มที่ ๓ ร้อยละ ๘๙-๙๒ การศึกษาความหลากหลายของรูปแบบสารพันธุกรรมด้วย Random Amplified polymorphism DNA พบว่า สามารถแยกเห็ดเป็น ๒ กลุ่มคือกลุ่มของเห็ดหลินจือแดง และกลุ่มของเห็ดหลินจือม่วง กลุ่มของเห็ดหลินจือแดงสามารถแยกออกเป็น ๒ กลุ่มย่อยคือ เห็ดหลินจือแดงจากไทยและเห็ดหลินจือแดงจากจีน ในกลุ่มของเห็ดหลินจือแดงมีความเหมือนกันร้อยละ ๓๙-๔๗ สายพันธุ์จากประเทศไทยมีความแตกต่างจากสายพันธุ์ของประเทศจีนร้อยละ ๕๐-๖๑ การศึกษาอนุกรมวิธานเชิงตัวเลขพบว่าสามารถแยกเห็ดเป็น ๒ กลุ่มคือ กลุ่มของเห็ดหลินจือแดงและเห็ดหลืนจือม่วง ปริมาณสารสกัดหยาบในเห็ดหลินจือแดงมีร้อยละ ๐.๖๘-๒.๘๖ และในเห็ดหลินจือม่วงมีร้อยละ ๗.๕๓ มีปริมาณสารสกัดคาร์โบไฮเดรต น้ำตาลรีดิวซ์ โปรตีน และฟีนอล ในเห็ดหลินจือ ๓๑๖.๕๔-๕๒๓.๔๖ มิลลิกรัมต่อกรัม ๖๒-๑๒๐.๑๑ มิลลิกรัมต่อกรัม ๓.๓๕-๑๗.๗๔ มิลลิกรัมต่อกรัม ๔.๑๑-๑๖.๕๖ มิลลิกรัมต่อกรัมของสารสกัดหยาบตามลำดับ

ภาควิชาจุลชีววิทยา บัณฑิตวิทยาลัย มหาวิทยาลัยศิลปากร ปีการศึกษา ๒๕๕๔

ลายมือชื่อนักศึกษา.....

ลายมือชื่ออาจารย์ที่ปรึกษาวิทยานิพนธ์.....

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CHAPTER 1

INTRODUCTION

Ganoderma lucidum, also well known as Lingzhi, is a polyporous basidiomycetous mushroom with pores under cap. The young cap is yellow to yellow-brown at central and white to cream at the margin and become to dark red to red-brown with lacquered appearance. Its texture is corky or woody and the shape is circular to semi-circular to fan shape. The stalk is dark brown to black, hard and eccentric. The hymenophore composes of tiny circular pores, white and smooth surface. The spore is ovoid and double layer (Arora, 1986).

Lingzhi is a famous mushroom which has been used for a traditional medicine and health supplement in East Asia. Several studies revealed that there are several bioactive compounds in this mushroom, such as polysaccharide (β-glucan) and ganoderic acid. *G. lucidum* extract inhibited proliferation and enhanced apoptosis of cancer and tumor cells (Calvino *et al.*, 2010, Liu *et al.*, 2002, Li *et al.*, 2005, Lu *et al.*, 2004, Muller *et al.*, 2006, Sliva *et al.*, 2002, Xie *et al.*, 2006, Zhao *et al.*, 2010), activated the immune system (Bao *et al.*, 2002, Huang & Ning, 2010, Kuo *et al.*, 2006, Russell & Paterson, 2006, Zhu *et al.*, 2007), exhibited antimicrobial activities against virus, bacteria and fungi (Eo *et al.*, 1999, El-Mekkawy *et al.*, 1998, Wang & Ng, 2006) and showed antioxidant activity (Chen *et al.*, 2008, Hu *et al.*, 2009, Liu *et al.*, 2010, Mau *et al.*, 2005, Tseng *et al.*, 2008, Chen *et al.*, 2009).

The study of the variation of *G. lucidum* strains based on only morphological characteristics are a difficult task due to a slightly morphological difference presented in different strains. Presently, a multiplicity of biochemical methods, molecular genetics technique and numerical taxonomy has been used to study variation of mushroom. These techniques included Internal transcribe spacer (ITS) sequence analysis (Zheng *et al.*, 2009, Smith & Sivasithamparam, 2000, Lee *et al.*, 2006, Moncalvo *et al.*, 1995), Random amplified polymorphism DNA (RAPD; Hseu *et al.*, 1996), Polymerase chain reaction-

Restriction fragment length polymorphism (PCR-RFLP; Lee *et al.*, 2006, Gottlieb *et al.*, 2000), Amplified fragment length polymorphism (AFLP; Zheng *et al.*, 2009), and isozyme technique (Gottlieb & Wright, 1999). For example, Zheng *et al.*, 2009 applied AFLP and ITS PCR-RFLP techniques to identify *Ganoderma* species complex.

G. lucidum is commonly found in countries located in temperate zone, such as in China, Japan, Korea, and India. Recently, *G. lucidum* strain KALASIN was isolated from Kalasin province, North-Eastern of Thailand. To elucidate diversity of *G. lucidum*, 8 strains isolated from China and one strain isolated from Thailand were characterized by using growth rate on different temperatures, morphology, ITS sequence analysis, RAPD analysis and quality of crude polysaccharides, carbohydrates, reducing sugar, protein, and phenol compound.

CHAPTER 2

REVIEW LITERATURE

1. *Ganoderma lucidum*

1.1 Morphology and habitat

Ganoderma lucidum, also well known as Lingzhi, belongs to the family Ganodermataceae. Fruiting body is shelf-like with cap and stalk. Flesh is tough or woody. Spores are produced in a layer of pores. The taxonomic description of *G. lucidum* is described below (Arora, 1986).

> **Fruiting body** annual, corky and tough; often emerging as a whitish or pallid knob but soon becoming shelf-like or developing a cap and stalk. **Cap** 2-20 (35) cm broad, 4-8 cm thick, circular to semi-circular to fan-shaped or kidney-shaped in outline; surface usually with a varnished (shiny) surface crust, smooth or often concentrically zoned and grooved; color variable: dark red to reddish-brown, orange-brown, mahogany, or reddish-black, but often ochre or yellowish toward the margin (which is often white when actively growing); surface sometime covered with brownish spore powder. Flesh ochraceous-brown to dark brown, or pallid near the cap and brownish near the tubes; soft-corky or punky when fresh, tough when dry or old. **Pores** minute (4-7 per mm), whitish or yellowish-white when fresh, usually bruising or aging brown; tube 2-20 mm long, one layer only (rarely two). **Stalk** sometimes absent, but often present; usually attached laterally, but often vertical and well-developed, 3-14 cm long, 0.5-3 (4) cm thick; often gnarled or twisted, equal or enlarge below; dark red to reddish-black and appearing vanished like the cap. **Spore print** brown; spore 7-13x 5-8 microns, elliptical, double walled, appearing minutely rougheded

.

G. lucidum is a parasite on live hardwoods and a saprophyte on deadwood or stump. The mushroom is fruitfulness during mushroom season but often persisting round year. The distribution of *G. lucidum* is in both of temperate zone, such as China, Japan, Korea, India, and Argentina and tropical zone, such as Australia and Thailand (Smith & Sivasithamparam, 2000).

1.2 Life cycle of *Ganoderma lucidum*

The *G. lucidum* life cycle is similar to that of other mushrooms. The spores germinate and develop to primary mycelium, called monokaryon (Fig. 1 a). Then, two different mating types of monokaryon are fused to form the secondary mycelium or dikaryon (Fig. 1 b). At this process, only mycelia are fused but the nucleus does not, i.e. plasmogamy. The dikaryotic cells can grow for a long period and become primodium and fruiting body, respectively (Fig. 1 c). All cells in fruiting body are dikaryon and only certain dikaryotic cells, basidia, will produce basidiospores. The basidia are generated in a definite layer, hymenium, in pores. In basidia, the karyogamy and miosis take place and give rise to 4 haploid nuclei. In the meantime, 4 small outgrowths termed sterigmata push out at the top of the basidium and their tips enlarge, eventually forming the basidiospore initials. The nuclei are pushed from basidium into the basidiospore initials by the action of vacuole to form the basidiospores (Fig. 1 d). Then, the spores are released into the air to start another cycle. The life cycle of *G. lucidum* showed in Fig. 1.

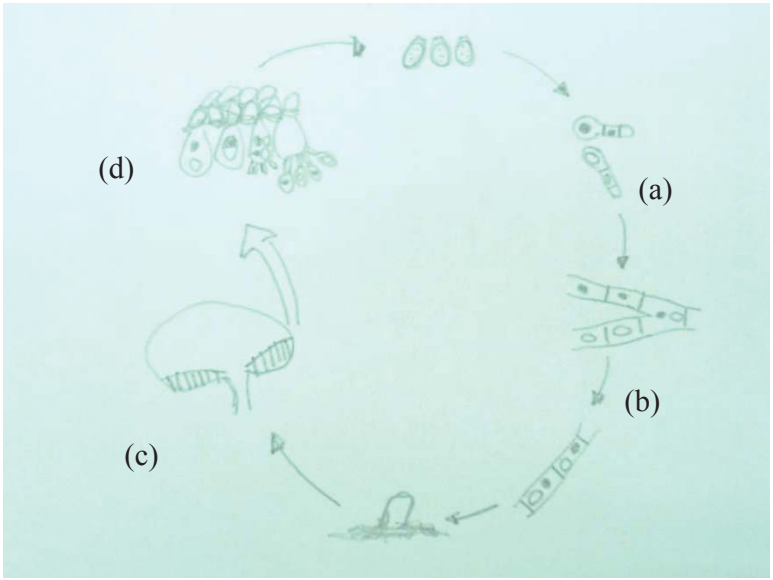


Figure 1 The life cycle of *Ganoderma lucidum*.

1.3 Techniques for study of diversity of *Ganoderma lucidum*

A variety of laboratory-based techniques have been used to study diversity in *Ganoderma*, such as numerical taxonomy, random amplified polymorphism DNA (RAPD) (Hseu *et al.*, 1996), ITS sequence analysis (Zheng *et al.*, 2009, Moncalvo *et al.*, 1995, Manassila *et al.*, 2005, Matsumoto *et al.*, 2005), amplified fragment length polymorphism (AFLP; Zheng *et al.*, 2009, Gottlieb & Wright, 1999) and PCR-restriction fragment length polymorphism (PCR-RFLP; Zheng *et al.*, 2009).

1.3.1 Numerical taxonomy

Numerical taxonomy is a classification system in biological systematic. This technique can generate closely related groups by numerical methods of taxonomic units based on their character states. Numerical taxonomy is used as a tool for analyses of mushroom. To study diversity of *G. lucidum* complex, Moncalvo *et al* (1995) used character of growth rate at low, medium and high temperature, and number and shape of chlamydospore to characterize 29 strains of *Ganoderma* complex. The results showed that these mushrooms could be classified according to geographical origins, i. e. Asia, America, and Europe. Gottlieb & Wright (1999) applied 26 characters to study relationship of 45 specimens of *Ganoderma* complex from Southern South America. This work indicated that mushroom could be grouped into 9 taxa by only spore character but other characters remained doubtful.

1.4.2 Internal transcribed spacer sequence analysis

The ITS refer to a piece of non-functional region situated between structural ribosomal RNAs (rRNA) on a common precursor transcript (Fig. 2). From 5' to 3', the precursor transcript contains 5' external transcribed sequence (5' ETS), 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA and 3'ETS. The non transcribed spacers (NTS) are between pieces of rDNA tandem. The ITS regions in fungi are highly variable. Hence, these regions are probably important for molecular systematic in order to distinguish the species or strains of fungi (Moncalvo *et al.*, 1995). The ITS sequence analysis is performed by polymerase chain reaction (PCR) of DNA, sequencing and sequence comparison. There have been many reports published on the analysis of the ITS regions to establish taxonomic relationship within *Ganoderma* species (Smith & Sivasithamparam, 2000, Moncalvo *et al.*, 1995, Gottlieb *et al.*, 2000). For examples, Güzeldağ & Çolak (2007) used 5.8S and ITS sequence for identification of *G. lucidum* from Turkey. The results showed that the 5.8S sequences of the mushroom samples were

absolutely identical (100%) to *G. lucidum*. Smith & Sivasithamparam (2000) revealed that ITS sequence analysis could be classified Australian *Ganoderma* species into 5 clades. Hseu *et al*. (1996) analyzed ITS sequences of 36 strains of *G. lucidum* complex and the results showed that the mushrooms could be clearly differentiated into 6 groups.

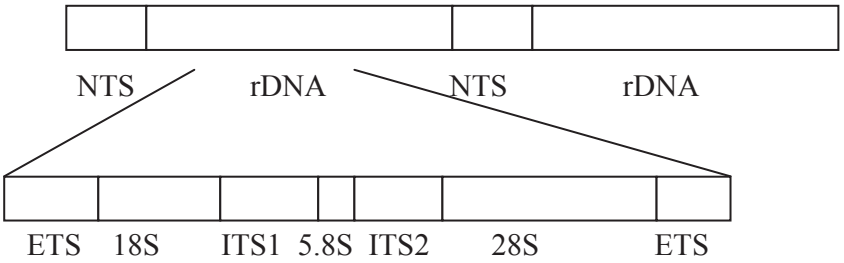


Figure 2 The rDNA consists of a tandem repeated of a unit segment. The gene segment composes of 5' ETS, 18S, ITS 1, 5.8S, ITS1, 28S, and 3'ETS.

1.4.3 Random amplified polymorphism DNA (RAPD)

RAPD is a PCR based technique that employed several arbitrary and short primers to create polymorphism DNA. RAPD is not a specific PCR amplification. The primers randomly prime on complementary DNA template. RAPD is sensitive and efficient method, which is currently available for distinguish between different strains of a species. For examples, Rolim *et al.* (2011) used 48 random primers for RAPD analysis to distinguish on Brazilian and Chinese *Ganoderma lucidum* strains. Hseu *et al.* (1996) used 5 primers to differentiate and group the *G. lucidum* complex to 9 groups.

All techniques can be used for identification, differentiation, and grouping of organisms but there are advantages and disadvantages in each methods. Numerical taxonomy is a technique based on morphology, but not on genetics. In some cases, numerical taxonomy could not distinguish strains of organisms, but genetics characterization could. This method is cheap and simple. The ITS sequence analysis can identify variability of intraspeies and has been extensively used to study the taxonomy and phylogenetic (Zheng *et al.*, 2009). The advantage of RAPD techniques is cheap and quick, but has to be proven because of its sensitivity to experimental conditions (Zheng *et al.*, 2009). The AFLP and RFLP are used as the tools for studying of genetic diversity, but these techniques are expensive, difficult and several steps for work. The several studies applied several techniques to investigate the diversity of mushroom to ensure the accuracy (Moncalvo *et al.*, 1995, Hseu *et al.*, 1996, Gottlieb *et al.*, 2000). Manassila *et al.*

(2005) used ITS sequence analysis and PCR-RFLP to study the relationship of *Russula* species in North East Thailand and found that the mushroom could be divided into three groups.

2. Bioactive compound of *Ganoderma lucidum*

G. lucidum is a well known medicinal mushroom. There are several bioactive compounds in this mushroom, such as polysaccharides, triterpenoids, glycoproteins, sterols etc. The majority of bioactive compounds are polysaccharides and triterpenoids. These compounds are present in the fruiting body (Calvino *et al.*, 2010, Muller *et al.*, 2006, Zhao *et al.*, 2010, Pillai *et al.*, 2010), mycelium grown in submerged cultures (Kuo *et al.*, 2006, Tang *et al.*, 2006) and spores (Liu *et al.*, 2002, Lu *et al.*, 2004). The compound showed anticancer, antitumor, antivirus, antioxidant activity and could activate the immune system. It was shown that triterpenoid could be direct cytotoxicity to cell lines (Yue *et al.*, 2010). In the other hand, it was suggested that polysaccharide could not directly react to the cells, but the activity might be due to the activation of immune systems (Zhu *et al.*, 2007). A list of the important bioactive compounds and their biological functions found in *Ganoderma* species was summarized in Table 1.

Table 1 Bioactive compounds isolated from *Ganoderma* species.

Bioactive compound	**Ganoderma species**	**Stage of mushroom**	**function**
Polysaccharide	*G. lucidum*	Fruiting body	Anticancer (Lu *et al.*, 2004, Sliva *et al.*, 2002) Antitumor (Zhao *et al.*, 2010) Immunological effector cells (Zhu *et al.*, 2007) Antiherpetic activity (Eo *et al.*, 1999, Lin *et al.*, 1995) Antioxidant activity (Fan *et al.*, 2012) Enhancement of repair of DNA strand (Pillai *et al.*, 2010)
		Mycelia Fermentation filtrate	Immunomodulatory (Kuo *et al.*, 2006)
		Spore	Anticancer (Lu *et al.*, 2004, Sliva *et al.*, 2002) Antitumor (Liu *et al.*, 2002)

Table 1 Bioactive compounds isolated from *Ganoderma* species. (continuous)

Bioactive compound	Ganoderma species	Stage of mushroom	function
Polysaccharide	*G. tsugae*	Mycelia	Antioxidant (Tseng *et al.*, 2008)
	G. formosanum *G. neo-japonicum*	Fruiting body	Radical scavenger and Antihepatotoxic activity (Lin *et al.*, 1995)
Triterpenoid	*G. lucidum*	Fruiting body	Anticancer (Lu *et al.*, 2004) Antitumor (Calvino *et al.*, 2010) Anti-HIV (El-Mekkawy *et al.*, 1998) Cytotoxicity (Yue *et al.*, 2010) Antiinflammatory (Ko *et al.*, 2008)
		Mycelia	Antitumor (Tang *et al.*, 2006)
		Spore	Anticancer (Lu *et al.*, 2004)
	G. tsugae	Fruiting body	Antiinflammatory (Ko *et al.*, 2008)
	G. atrum	Fruiting body	Antimicrobial, antioxidant activity (Li *et al.*, 2012)
	G. amboinense	Fruiting body	Anticancer (Chang *et al.*, 2006a)
Essential oil	*G. japonicum*	Mycelia	Antimicrobial activity (Liu *et al.*, 2009)

Polysaccharides

Polysaccharides are the biological macromolecules with diverse of structures and physiochemical properties. The major bioactive polysaccharides in *Ganoderma* species are β-1-3 and β-1-6-glucans (Rahar *et al.*, 2011; Fig. 3). Bioactive polysaccharides have been isolated from the fruiting body (Lu *et al.*, 2004, Sliva *et al.*, 2002, Zhao *et al.*, 2010, Zhu *et al.*, 2007, Eo *et al.*, 1999, Pillai *et al.*, 2010, Lin *et al.*, 1995, Fan *et al.*, 2012), mycelium (Kuo *et al.*, 2006), and spore (Liu *et al.*, 2002, Lu *et al.*, 2004, Sliva *et al.*, 2002).

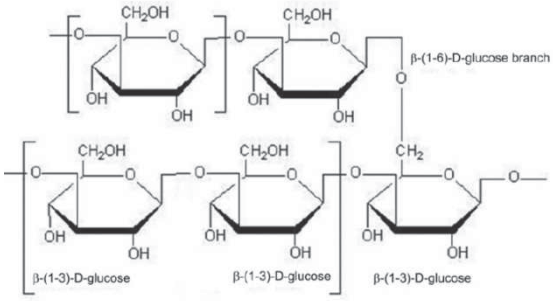


Figure 3 Structure of β-glucan (Rahar *et al.*, 2011).

Several studies revealed the crude polysaccharides isolated from *G. lucidum* exhibited anticancer activity. For examples, the extraction of fruiting body inhibited the growth of HUC-PC cells, MTC-11 cells and breast cancer cells (Lu *et al.*, 2004, Wu *et al.*, 2006) and the extraction of sporoderm-broken germinate spores could significantly prevent growth of mouse hepatoma, sarcoma S-180, reticulocyte sarcoma L-II cells and malignant human breast carcinoma cells by80–90% (Liu *et al.*, 2002, Xie *et al.*, 2006). Most of the anticancer glucans were reported to contain a branched glucan core with an average molecular weight of 1,050 kDa (Bao *et al.*, 2002). Recently, Zhao *et al.* (2010) extracted polysaccharides from fruiting body and purified using filtration, DEAE cellulose-52 chromatography and sephadex G-100 size-exclusion, and found that the fraction GP-1 with 1.926 kDa and GP-2 with 1086 kDa showed inhibition effect to the human breast cancer cell (MDA-MB-231).

The polysaccharides of *G. lucidum* also showed antiviral activity. The crude polysaccharides (Eo *et al.*, 1999) and protein bound polysaccharide (APBP) from fruiting body reduced plaque formation of HSV-1 and HSV-2 to Vero cells (Pillai *et al.*, 2010).

The polysaccharides extracted from fruiting body also exhibited an antioxidant activity. Chen *et al* (2009) found that crude polysaccharides showed superoxide anion radical-scavenging activity and significantly enhanced the antioxidant enzyme (SOD, CAT and GPx) activities. Tseng *et al.* (2008) reported that hot water extracted polysaccharides from fruiting body, primodium, mycelia and fermentation filtrate of *G. tsugae* showed antioxidant activity by 78-88%, while the hot alkaline extracted of those exhibited only 55%

Several researchers reported the polysaccharides of *Ganoderma* species could activate immune system. For instances, polysaccharides could activate natural killer cell (Huang & Ning, 2010), enhance immunostimulating activities (Zhu *et al.*, 2007), enhance activity of T and B cell (Manassila *et al.*, 2005), stimulate TNF-α and IL-6 production, activated NF-κB (Kuo *et al.*, 2006) and increase antihepatotoxic acitivity (Manassila *et al.*, 2005). The majority of notable polysaccharide for their ability to activate immune system is glucan and derivative of glucan (Lu *et al.*, 2004, Bao *et al.*, 2002). For examples, Bao *et al.* (2002) discovered that two heteroglycan (PL-1 and PL-4) and glucan from fruiting bodies and six derivative of the (1, 3)-α-D-glucan from spore of *G. lucidum* are able to enhance the proliferation of T and B lymphocytes *in vitro*. Moreover, PL-1 exhibited an immune-stimulating activity in mice. The soluble glycoprotein fraction (F3)

purified from the water-soluble extracts of *G. lucidum* could enhanced CD56+ NK-cell cytotoxicity in cord blood (Chien *et al.*, 2004).

The biological function of polysaccharides in anticancer activity might be a result of enhancement of host mediated immune system rather than a direct toxicity to the cancer cells (Lu *et al.*, 2004, Sliva *et al.*, 2002).

Triterpenoids and sterols

Triterpenoids are unsaturated hydrocarbon. Their chemical structure is based on the ground structure of lanosterol, which is an important intermediate in the biosynthetic pathway for steroids and triterpenes in microorganisms and animals. Triterpenoids found in *Ganoderma* mushroom named Ganoderic acids. There are more than 100 types of Ganoderic acid found in *Ganoderma* species. For examples, Ganoderic acid Me, Ganoderic acid T, ganoderic acid X, etc. The type of ganoderic acid was classified based on functional groups at position R1, R2 and R3. Ganoderic acid could be isolated from fruiting body, mycelium and spore (Calvino *et al.*, 2010, Lu *et al.*, 2004, El-Mekkawy *et al.*, 1998, Tang *et al.*, 2006, Yue *et al.*, 2010, Ko *et al.*, 2008). The structure and types of ganoderic acid were shown in the Fig. 4 and Table 2.

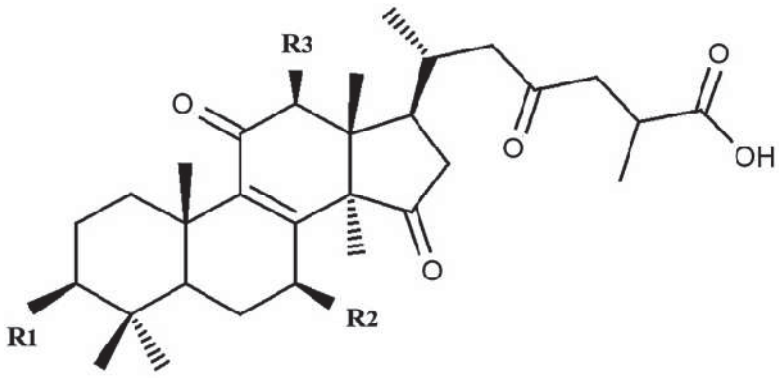


Figure 4 The structure of ganoderic acid (Yue *et al.*, 2010).

Table 2 The type of ganoderic acid.

Type of ganoderic acid	Position R1	Position R2	Position R3	Refference
B	OH	OH	H	Yue *et al.*, 2010
D	O	OH	H	Yue *et al.*, 2010
F	O	O	OAc	Yue *et al.*, 2010
K	OH	OH	OAc	Yue *et al.*, 2010
Me	OAc	OAc	H	Liu *et al.*, 2009
T	OAc	OAc	OAc	Tang *et al.*, 2006
X	OH	H	H	Li *et al.*, 2005

Triterpenenoids are considered to be potential anticancer agents. They act a direct cytotoxicity against tumor cells (Russell & Paterson, 2006). Ganoderic acid DM could induce DNA damage, G1 cell cycle arrest and apoptosis in human breast cancer cells (Wu *et al.*, 2012). Ganoderic Mf and ganoderic S isolated from mycelia of *G. lucidum* could induce apoptosis in HeLa cells and stimulate cysteine proteases (caspase-3, caspace-9) indicating there essential roles in apoptosis, necrosis, and inflammation (Liu & Zhong, 2011). Calvino *et al.*, 2010 observed that the semi-purified fraction obtained from *G. lucidum* fruiting body exhibited toxic effect to and induced apoptosis on NB4 leukemia cells. The extraction of fruiting bodies of *G. lucidum* caused apoptosis of leukemia, lymphoma and multiple myeloma cells (Muller *et al.*, 2006).

Steroidal compounds isolated from *Ganoderma* species exhibited antimicrobial effects. An essential oil extracted from the mycelium of *G. japonicum* revealed a broad spectrum bactericidal activity (Liu *et al.*, 2009). Ganomycins A and B extracted from *G. pfeifferi* showed antibacterial activity against both Gram-negative and Gram-positive bacteria (Mothana *et al.*, 2000).

The triterpenenoids and sterol compounds obtained from *Ganoderma* species exhibited antiviral effects. Ganoderiol F and ganodermanontriol isolated from fruiting

body of *G. lucidum* acted as the anti-HIV-1 agents with an inhibitory concentration of 7.8 μg ml^{-1} (Chang *et al.*, 2006b). The ganoderic acid B, ganoderiol B, ganoderic acid C1, 3β-5α-dihydroxy-6β-methoxy-ergosta-7,22-diene, ganoderic acid α, ganoderic acid H and ganoderiol A moderately inhibited HIV-1 PR (El-Mekkawy *et al.*, 1998). Ganodermadiol, lucidadiol and applanoxidic acid G isolated from *G. pfeifferi* showed antiviral activity against influenza virus type A and HSV type 1 (Mothana *et al.*, 2000).

CHAPTER 3

MATERIALS AND METHODS

1. Source of mushroom strains

Ganoderma lucidum strain A1, G2, DOA, G45, G5A, G5Z, HHK, and SUN, and *G. sinensis* strain A2 were isolated from China. *G. lucidum* strain KALASIN was isolated from Kalasin province, North-East of Thailand. All strains were kindly provided by Dr. Suraporn Rukpratom. The mushroom mycelia were maintained on potato dextrose agar (PDA) medium and sorghum as mushroom spawn at 4°C.

2. Effect of temperature and nitrogen source on growth rate of *Ganoderma lucidum*

The mycelia of *G. lucidum* strains were cultured on PDA or PDA supplement with 4g/l of either ammonium chloride, yeast extract or malt extract at 30ºC for 10 days. Then, 6 mm. diameter inocula were transferred to new medium and incubated at 25ºC, 30ºC, 35ºC and 40ºC for 14 days. Colonial diameters were measured daily. Growth rates were calculated from increase in the diameter per day. The experiments were set up with three replicates.

3. DNA extraction

The mycelia were cultured in potato dextrose broth (PDB) at 30ºC with shaking for 10 days. Then, it was collected by filtration and rinsed three times with steriled water. The mycelia in liquid nitrogen were ground with ceramic mortar and pestle. 10-20 mg of mycelia powders were transferred to new eppendorf tube. 400 µl of lysis buffer was added and mixed by vortex mixer. After that, the samples were incubated at 65ºC for 1 hour. 1.5 µl of 20 mg/ml Protinase K was added to samples and incubated at 55ºC for 1 hour. 1.5 µl of 20 mg/ml RNase A was added to tubes and incubated at 37ºC for 30 minutes. 400 µl of Chlorofrom:TE-saturated phenol (1:1 v/v) was added to tubes and mixed by vortex mixer. Then, the samples were centrifuged at 13000 rpm for 5 minute. 350 µl of upper layer was taken to new 1.5 ml tubes. 10 µl of 3M sodium acetate and 200 µl of isopopanol were added and inverted gently for 2-3 times. The tubes were centrifuged at 13000 rpm for 5 minutes. The supernatant was discarded. 1 ml of 70%

ethanol was added to the DNA pellet and inverted gently for 1 minute. The tubes were centrifuged at room temperature for 5 minutes. The supernatant was drained and the DNApellet was air dried for 15 minutes. The DNA was dissolved with 50 µl of TE buffer and incubated at 95ºC for 10 minutes. The DNA concentration and purity was measured absorbance at 260 and 280 nm in spectrophotometer. Then, DNA was stored at -20ºC.

4. PCR amplification of the internal transcribed spacer (ITS) regions

The PCR reaction was carried out in 50 µl volumes containing 100 ng/µl of DNA, 10 mM Tris-HCl (pH 8.8), 50 mM KCl, 2 mM of $MgCl_2$, 0.2 mM of each dNTP (Fermentas, UK), 36.5 µl of water, 2.5 units of taq polymerase (Fermentas, UK) , 0.2 µM primer. The primer sequences were 5'TCCGTAGGTGAACCTGCGG3'for ITS1 and 5'TCCTCCGCTTATTGATATGC3' for ITS2. Amplifications were performed in Esco GeneAmp PCR system programmed for 1 cycle of initial denaturation at 94ºC for 2 minutes followed by 40 cycles consisting of denaturation at 94ºC for 30 second, annealing at 55ºC for 30 second, extension at 72ºC for 30 second and the final extension at 72ºC for 20 minutes. The PCR products were then stored at 4ºC. 10 µl of PCR products were separated on 1% agarose gel electropholysis, dyed with ethyldiumbromide and visualized under UV transmittion. 100 bp DNA ladder was used as the molecular weight marker.

5. Ligation and transformation

The ligation and transformation was performed by using pCR^R8/GW/TOPO TA cloning kit (invitrogen, USA) according to manufacture instruction. Briefly, 4 µl of DNA sequence was taken to the 1.5 ml eppendorf tube. 0.5 µl of salt solution and 0.5 µl of TOPO vector were added and mixed gently and incubated at room temperature for 5 minutes. 25 µl of competent cells were added to the ligation reaction, gently mixed and incubated on ice for 30 minutes. Then, the tube was incubated at 42ºC for 30 second and swifted on ice immediately. 125 µl of SOC medium was added to the tube, incubated at 37ºC with shaking for 1 hour. The transformants were selected on LB agar medium containing 100 µg/ml spectinomycin. The colonies on LB agar plate were picked and transferred to the new LB medium and incubated the sample at 37ºC with shaking for 24 hours. The inserted DNA was checked ITS fragment by PCR. The positive colonies were further extracted.

6. Extraction of plasmid

The extraction of plasmid was performed by using GeneJET^{TM}Plasmid Miniprep Kit (Fermentas, UK) according to manufacture instruction. Briefly, 3 ml of the culture was taken to a 1.5 ml eppendorf tube and centrifuged at 13,000 rpm/minute at room temperature. The supernatant was removed, 250 µl of resuspension solution and 250 µl of lysis solution was added and mixed by inverting 4 to 6 times. Then, 350 µl of neutralization solution was added and mixed by inverting 4 to 6 times. The sample was centrifuged at 13000 rpm/minute for 5 minutes. The suspension was taken to a column and centrifuged at 1 minute. The flow through solution was discarded. 500 µl of wash solution was added to the column and centrifuged for 30 to 60 second. After that, the suspension was discarded and washed the column again. The column was placed to the new 1.5 ml tube. Then, the elution buffer (50 µl) was added to the column that was incubated and centrifuged at room temperature for 2 minutes. The plasmid was taken to the new tube. The plasmids were measured absorbance at 260 and 280 nm in spectrophotometer. The plasmids were sent to the First Base Laboratory, Malaysia for sequencing.

7. DNA alignment and phylogenetic tree construction

DNA sequences were analyzed by chromaslite 201, and CAP3 sequence assembly program. DNA alignment was perform using multiple alignment program ClustalW. Phylogenetic tree was constructed using Mega 4 software.

8. Random Amplified Polymorphism DNA (RAPD)

The RAPD reactions were carried out in 50 µl volumes containing 100 ng/µl of DNA, 10 mM Tris-HCl (pH 8.8), 50 mM KCl, 2 mM of $MgCl_2$, 0.2 mM of each dNTP, 0.2 µM primer (the primers; described below), 37.5 µl of water and 2.5 units of taq polymerase. The following primers were employed A01 (5'-CAGGCCCTTC-3'), A02 (5'TGCCGAGCTG-3'), A03 (5'-AGTCAGCCAC-3'), A04 (5'-AATCGGGTCG-3'), A05 (5'AGGGGTCTTG-3'), A06 (5'- GGTCCCTGAC-3'), A07 (5'-GAAACGGGTG-3'), A08 (5'-GTGACGTAGG-3'), A09 (5'-GGGTAACGCC-3'), A10 (5'-GTGATCGCAG-3'), A11 (5'- CAATCGCCGT-3'), A12 (5'-TCGGCGATAG-3'), A13 (5'-CAGCACCCAC-3'), A14 (5'-TCTGTGCTGG-3'), A15 (5'-TTCCGAACCC-3'), A16 (5'- AGCCAGCGAA-3'), A17 (5'-GACCGCTTGT-3'), A18 (5'-AGGTGACCGT-

3'), A19 (5'-CAAACGTCGG-3'), A20 (5'-GTTGCGATCC-3'). Amplifications were performed in Esco GeneAmp PCR system programmed for 1 cycle of initial denaturation at 94ºC for 2 minutes, 30 cycles of denaturation at 94ºC for 30 second, annealing at 37ºC for 30 second, extension at 72ºC for 1 minute and a final extension at 72ºC for 7 minutes. The PCR products were then stored at 4ºC. 10 µl of PCR products were separated on 1% agarose gel. The 100 bp DNA ladder and λDNA/hindIII were used the molecular weight marker. Gel were stained with ethidium bromide, visualized by UV illumination and photographed with Gel document (Amersham Pharmacia Biotech).

The RAPD-PCR was performed at least three replication, and only repro ducibly amplified markers were scored as present (1) or absent (0). Similarity coefficients (F) between 2 isolates were calculated according to the formula of Nei & Li (1979) as followed;

$$F = 2Nxy/Nx+Ny$$

When Nxy is the number of common fragments between 2 isolates, and Nx and Ny are the number of fragments in isolated X and Y, respectively.

Pooled data from primers gave polymorphism pattern were used for calculation. Similarity coefficients values close to 1.0 indicate high genetic similarity among the strains. The dendrogram was constructed based on similarity matrix and generates by UPGMA (unweighted pair-group method with arithmetic average) with Mega 4 software.

9. Mushroom cultivation

Mycelium was cultured on PDA at 30ºC for 10 days, cut by using cock borer (diameter 0.6 cm) and inoculated to new bottle of sorghum. The bottle was incubated at 30ºC for 10 days. After the sorghum was fully covered by mycelia, a full spatula of sorghum was transferred to sawdust bag and incubated at room temperature. After completion of mycelium running on sawdust, the fruiting bodies were induced by removed of plastic bag, put into basket and covered with soil. The baskets were watered everyday. Fruiting bodies were collected after 60 days.

10. Morphology and Numerical taxonomy of fruiting body of *Ganoderma lucidum* strains

10.1 Morphology

Descriptions of basidiomes were made according to their macroscopic and microscopic features of cultured specimens. Dermic elements were carefully examined and measured in thin sections perpendicular to the pileus surface and stained with Melzer's reagent. Spores were studied using light microscope.

10.2 Numerical analysis

Characters and state assignments were selected from those typically used in taxonomic keys. As a consequence, 20 characters were recorded and codified (Table 3). The similarity matrix was obtained by applying Gower's similarity coefficient. A dendrogram based on this matrix was constructed clustering method using UPGMA (unweighted pair-group method using arithmetic averages) cluster analysis to visualize relationship.

Table 3 Morphological characters and their character-state descriptions

1	Pileus surface : dull-semidull-laccate
2	Pileus colour : orange to reddish- Bordeaux to almost black
3	Pileus consistency : soft, brittle, easily broken with the nail-intermediate-totally hard
4	Pileus width : up to 0.5 mm-0.5-0.9 mm-more than 0.9 mm
5	Basidiome margin : sharp-blunt/rounded
6	Cutis of hymeniodermis vera type : absent-present
7	Cutis of hymeniodermis with capitates elements : absent-present
8	Cutis of hymeniodermis with diverticulate club-shaped elements : absent-present
9	Amyloid reaction of dermic elements : absent-present
10	Context colour : cream-wholly light brown to dark brown-dark brown partially or totally discoloured

11	Width of older context layer : up to 5 mm-5-10 mm-more than 10 mm
12	Height of older tube layer : up to 5 mm -5-10 mm-more than 10 mm
13	Tube colour : cream-wholly light brown to dark brown-dark brown partially or totally discoloured
14	Number of pores per mm : up to 4-4 to 6-more than 6
15	Pore surface colour : whitish, pinkish, grayish-cream to light brown-white
16	Mean basidiospore length * : quantitative
17	Basidiospore shape (SI) **: quantitative
18	Basidiospore of the smooth type (at 400x) : absent-present
19	Basidiospore of the semirugose type (at 400x) : absent-present
20	Basidiospore of the rugose type (at 400x) : absent-present

* The spore length was calculated on the mean of 20 measurements.

** The shape of basidiospore was expressed as a spore index (SI) the ratio mean length/mean width.

11. Isolation crude polysaccharide of *Ganoderma lucidum* strains

The fruiting bodies were cut and ground to small pieces by a mill. The pieces of fruiting body were dried at 60ºC for overnight. Then, they were grounded with the mill again. The powder of fruiting body (30 g) were boiled with 600 ml of distilled water for 4 hour. Then, the sample was filtrated with mesh and filter paper (Whatman no. 4), and the supernatant was collected and made to a small volume by boiling. The supernatant was cooled down to room temperature. To extract crude polysaccharide, 4 volumes of 95% ethanol (4:1 v/v) was added and incubated at 4ºC overnight. The sediment was separated by centrifugation at 4ºC, 6000 rpm for 20 minutes. The pellet was washed with absolute ethanol, centrifuged and repeated twice. The wet crude was collected into a glass vial and weighing. The polysaccharide crude was dried by desiccators and stored at 4ºC.

12. Determination of total carbohydrates from crude polysaccharides

The 0.5 ml of 3 mg/ml crude polysaccharides was taken to glass tube. 0.5 ml of 5% phenol solution was added to sample and mixed by vortex mixer. 2.5 ml of concentrate sulphuric acid was added and incubated at room temperature for 10 minutes. The sample was mixed by mixer and incubated for 20 minutes. The absorbance of mixture was measured at 490 nm in spectrophotometer. D-glucose was used as a standard. The experiments were set up with three replicates.

13. Determination of reducing sugar from crude polysaccharides

The 1 ml of 3 mg/ml crude polysaccharides was taken to glass tube. 1 ml of DNSA solution was added in the tube and mixed by mixer. The sample was boiled in hot water for 10 minutes. Then, the sample was cooled down in cold water for 3 minutes. 10 ml of distilled water was added into the tube and mixed. The absorbance of mixture was measured at 540 nm in spectrophotometer. The D-glucose was used as a standard. The experiments were set up with three replicates.

14. Determination of protein content of crude polysaccharides

The 40 µl of 3 mg/ml crude polysaccharides were taken to the tube. Then, 2 ml of Bradford reagent was added and mixed by mixer. The absorbance of mixture was measured at 595 nm in spectrophotometer. The albumin bovine serum (BSA) was used as a standard. The experiments were set up with three replicates.

15. Determination of total phenol content

The 0.5 ml of 3 mg/ml crude polysaccharides was mixed with 0.5 ml of Folin-Ciocalteu reagent and incubated for 3 minutes. 0.5 ml of 7% (w/v) Na_2CO_3 was added to the sample and mixed by vortex mixer. The distilled water was added to the tube to adjust the total volume of 5 ml. The tube was kept in the dark for 90 minutes. The absorbance of mixture was measure at 725 nm. The gallic acid was used as a standard. The total amount of phenol contents was expressed as GAE. The experiments were set up with three replicates.

16. Statistic Analysis

The experiments were set up with three replicates. Normal distributions were analyzed by descriptive statistic and frequencies in SPSS software. The data was analyzed by Analysis of Variance (ANOVA) and Turkey's Test in SPSS software.

CHAPTER 4

RESULTS

1. Effect of temperature and nitrogen source on growth rate of *Ganoderma lucidum*

1.1 Effect of temperature on growth rate of *Ganoderma lucidum*

The mycelia of *G. lucidum* strains were inoculated on potato dextrose agar (PDA) and incubated at 25ºC, 30ºC, 35ºC, 37ºC and 40ºC for 14 days. The growth rate of mycelium was determined by measurement of colonial diameter daily. The mycelium of strain G5A was well grown at 25ºC (Fig. 5 f), the mycelia of strains A2, G2, G5Z were well grown at 25-30ºC (Fig. 5 b, d, g), the mycelia of strains A1, DOA, G45, HHK, KALASIN and SUN are well grown at 30ºC (Fig. 5 a, c, e, h, j). Except for strain KALASIN, the mycelia of all strains did not grow at temperature higher than 30ºC, The strain KALASIN grew at 35ºC and slowly grew at 37ºC (Fig. 5 i).

1.2 Effect of nitrogen source on growth rate of *Ganoderma lucidum*

Jo *et al*. (2009) studied factors for mycelium growth of *Ganoderma applanatum*. Their results showed that the nitrogen sources (malt extract and yeast extract) promoted the growth of mycelium. In this study, whether the nitrogen source could enhance the growth rate of *G. lucidum* or not was hypothesized. The growth rate of *G. lucidum* was determined on PDA compared with PDA containing ammonium chloride, yeast extract or malt extract. The media were incubated at 30ºC for 14 days. Growth rates of *G. lucidum* strains A1, DOA, G45, G5A, HHK, KALASIN and SUN on PDA containing the nitrogen sources were not statistically different from PDA (Fig. 6 a, c, e, f, h, i, j). Malt extract enhanced growth rate of strain A2 (Fig. 6 b). Yeast extract enhanced growth rate of strain G2 (Fig. 6 d). Malt and yeast extract enhanced growth rate of strain G5Z (Fig. 6 g).

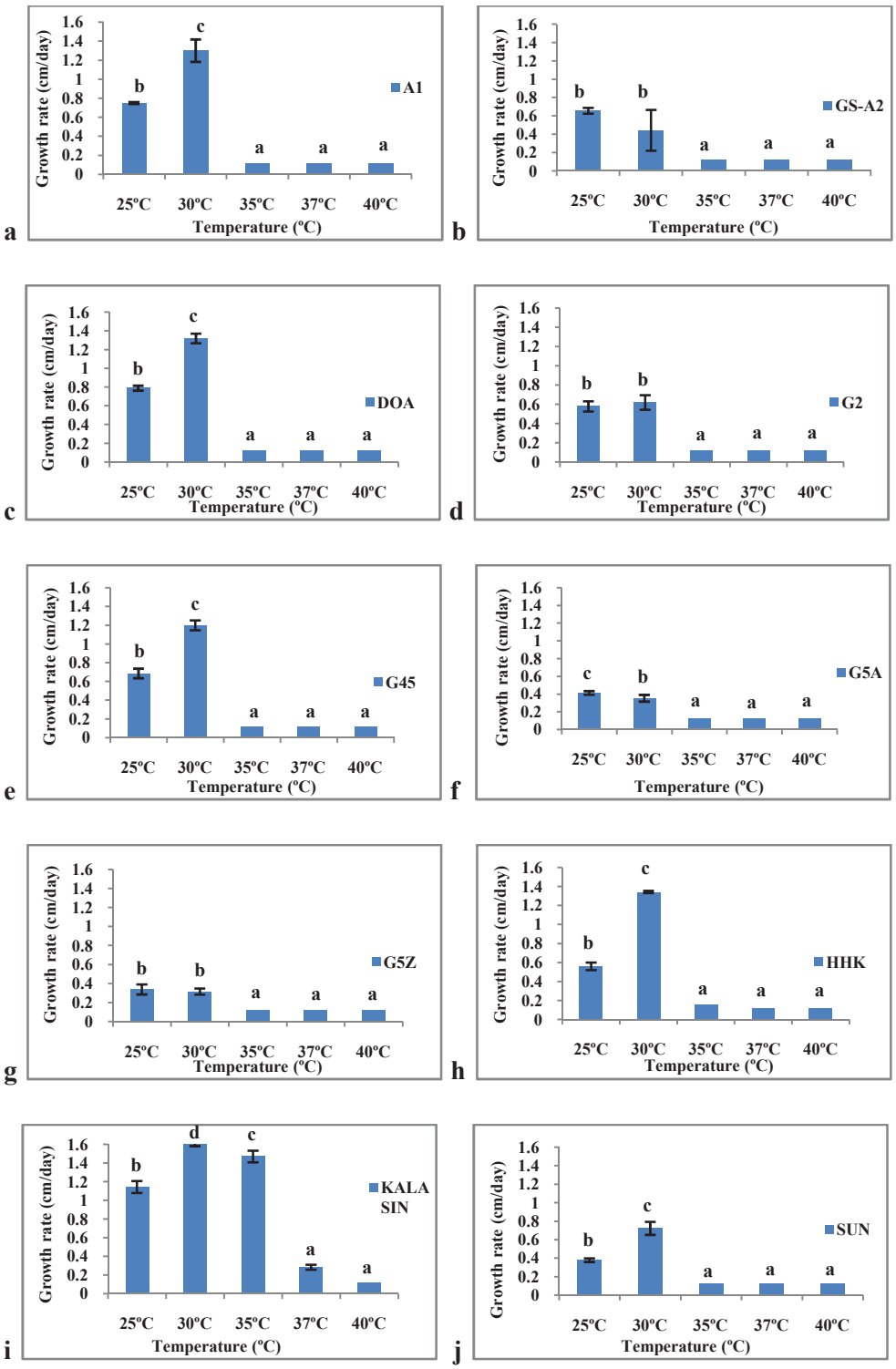


Figure 5 Growth rates of *Ganoderma lucidum* strains A1 (a), A2 (b), DOA (c), G2 (d), G45 (e), G5A (f), G5Z (g), HHK (h), KALASIN (i), SUN (j) on PDA at 25ºC-40ºC for 5 days. The values represent means of triplicate cultures. Error bars showed SD. The alphabet a, b, c and d indicate the statistical difference.

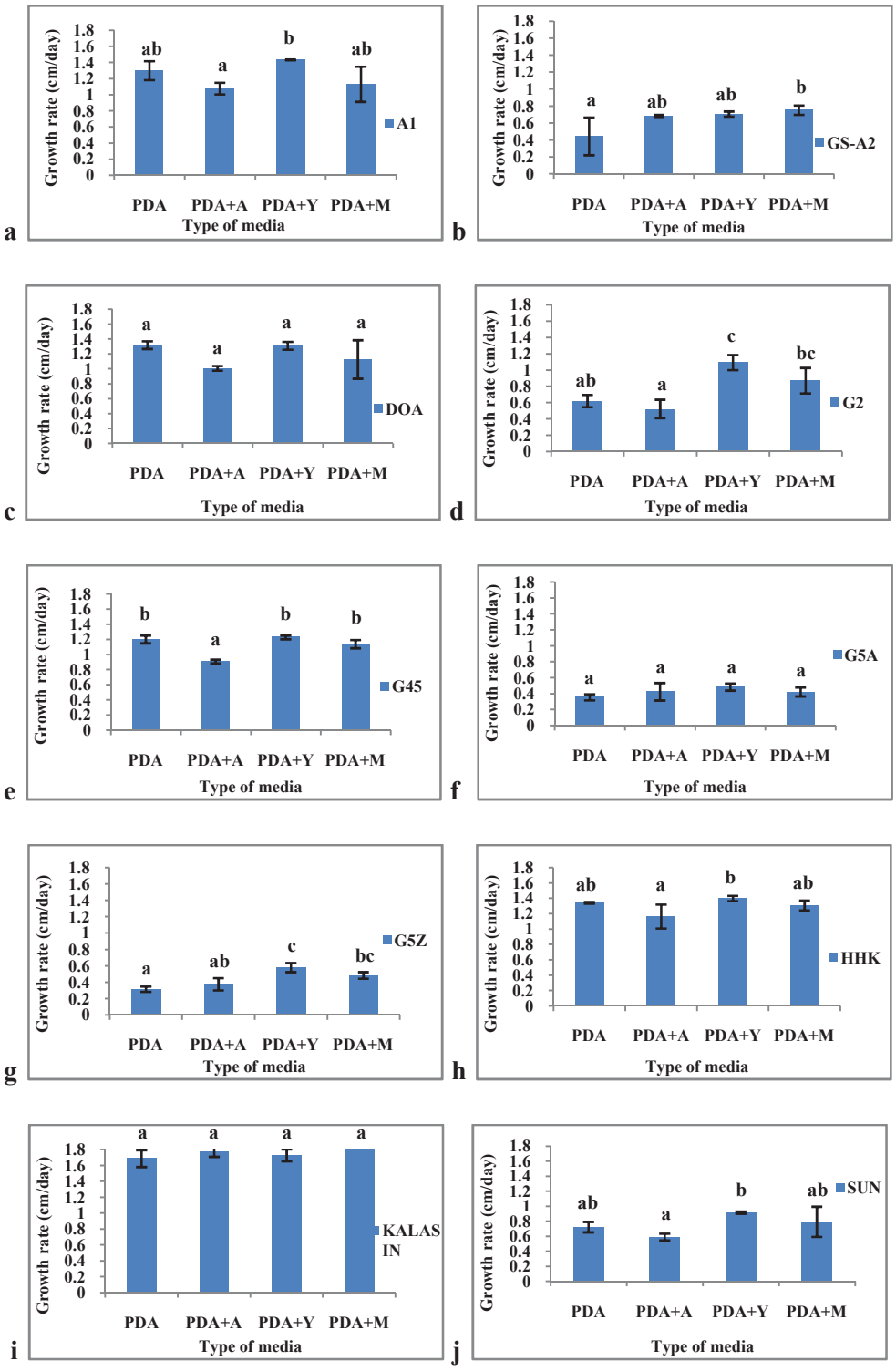


Figure 6 Growth rates of *Ganoderma lucidum* strains A1 (a), A2 (b), DOA (c), G2 (d), G45 (e), G5A (f), G5Z (g), HHK (h), KALASIN (i), SUN (j) on PDA, PDA with nitrogen source (ammonium chloride, yeast extract and malt extract) at 30ºC for 5 days. The values represent means of triplicate cultures. Error bars showed SD. The alphabet a, ab, b, bc and c indicate the statistical difference.

At 35ºC, the growth rate of *G. lucidum* is very low. To test that nitrogen source could enhance growth of the mushroom at 35ºC, the ammonium chloride, yeast extract and malt extract were added to PDA and the fungi were culture at 35ºC for 14 days (Fig. 7).Yeast extract enhanced the growth rate of strain G2 and G5A (Fig. 7 d, f). Malt extract and yeast extract enhanced growth rate of strain G5Z (Fig. 7 g). All nitrogen sources enhanced growth rate of strain HHK (Fig. 7 h). Growth rates of strains A1, A2, DOA, G45, KALASIN and SUN on PDA contained with nitrogen sources were not statistically different from PDA (Fig. 7 a, b, c, e, i, j). The nitrogen sources could enhance the growth rate of mycelia of *G. lucidum* strains G2, G45, G5A, G5Z and HHK at 35ºC that compared with PDA and yeast extract and malt extract are suitable for mycelium growth rate of *G. lucidum*. However, at 37ºC and 40ºC, the nitrogen sources could not enhance the growth rate of mycelia of *G. lucidum* (data not shown).

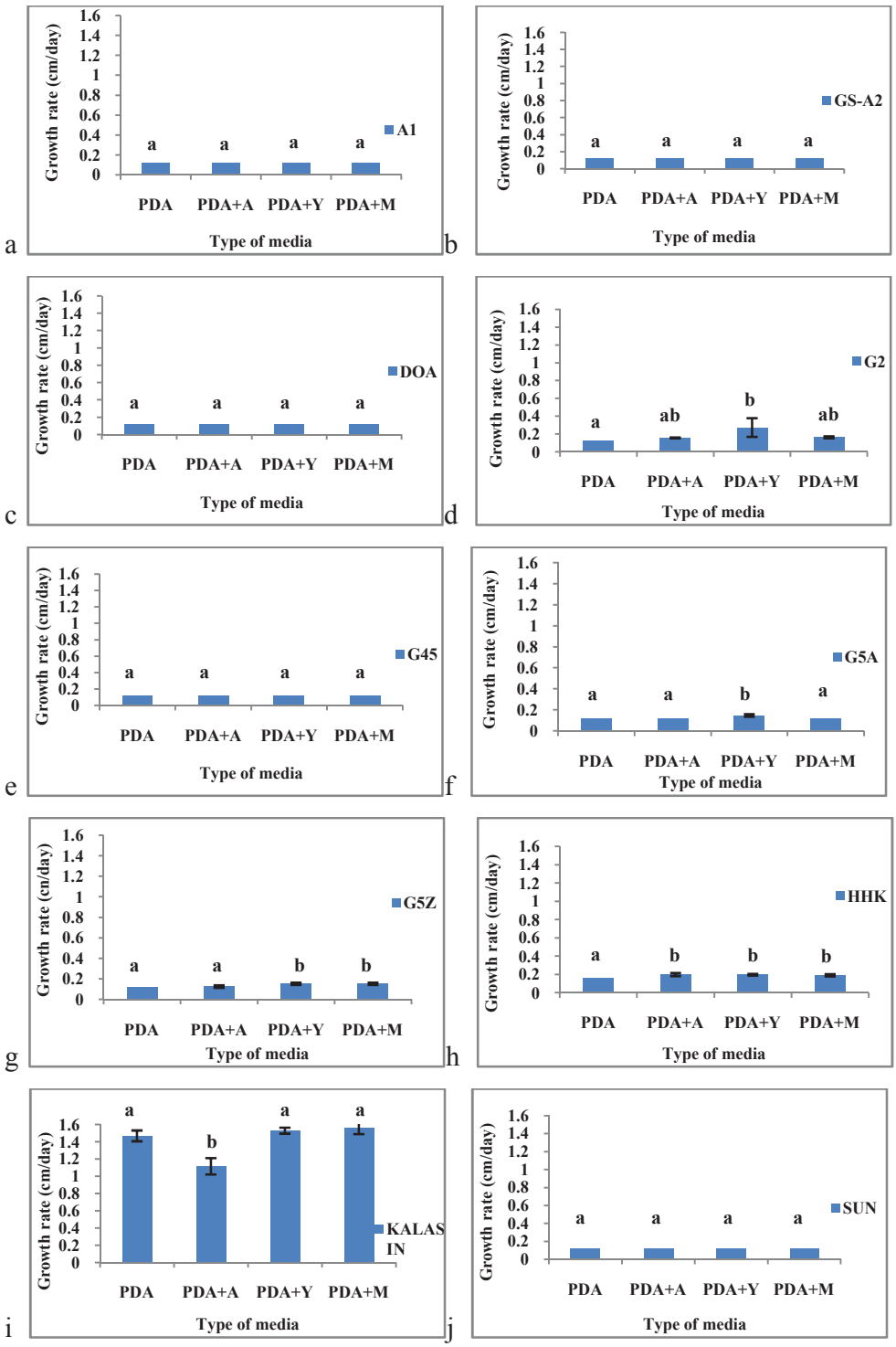


Figure 7 Growth rate of *Ganoderma lucidum* strains A1 (a), GS-A2 (b), DOA (c), G2 (d), G45 (e), G5A (f),G5Z (g), HHK (h), KALASIN (i), SUN (j) on PDA, PDA with nitrogen source (ammonium chloride, yeast extract and malt extract at 35ºC for 5 days. The value indicated the means of growth rate from triplicate. Error bar showed SD. The alphabet a, ab and b indicate the statistical difference

3. Internal transcribed spacer analysis

The ITS regions of 9 *G. lucidum* strains and *G. sinensis* were amplified by PCR using primer ITS1 and ITS4. The PCR reactions amplified the position of partial sequence of 18s rDNA, ITS1, 5.8S rDNA, ITS2 and partial sequence of 28s rDNA. Then, the PCR products were separated on 1% agarose gel electropholysis. The results showed single bands with an approximate size of 650 bp long (Fig. 8).

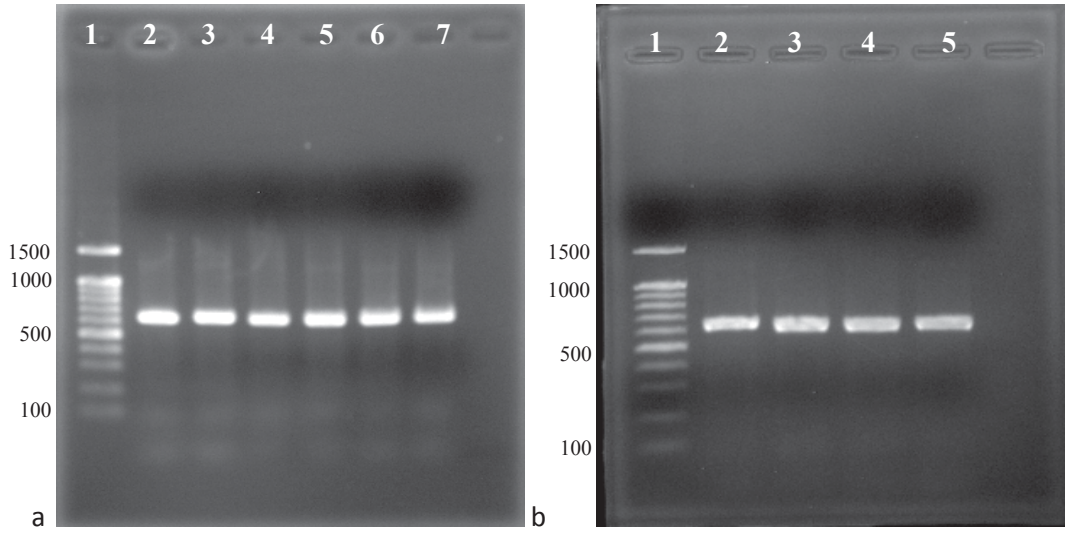


Figure 8 The gel electropholysis of the PCR products using primer ITS1 and ITS4, a (Lane 1 100 bp marker, 2 A1, 3 G2, 4 G5A, 5 G5Z, 6 KALASIN, 7 SUN) and b (Lane 1 100 bp marker, 2 DOA, 3 G45, 4 HHK, 5 A2).

The amplified products were cloned and sequenced. Except for KALASIN and A2, the total length of PCR product of all strains was 636 bp. The ITS sequence of KALASIN and A2 was 658 and 648 bp, respectively. Then, the position of partial sequence of 18s rDNA, ITS1, 5.8S rDNA, ITS2 and partial sequence of 28s rDNA were determined by comparison with the reported ITS sequences of *G. lucidum* (GenBank accession number EU021456, EU021460, FJ940919, GQ249880, JN008869, JN008870, and JN008871), and *G. sinensis* , (HQ235633, and HQ235634) . The similarity matrix of ITS regions was constructed and showed that the similarity among of *G. lucidum* were 0.95-1.00 and *G. sinensis* was similarity 1.0 (Table 4). The similarity between *G. lucidum* strains and *G. sinensis* were 0.89-0.92. The similarity matrix between KALASIN and Chinese strains were 0.95-0.96. From the result, the sequence of ITS regions could distinguish between *G. lucidum* and *G. sinensis*.

Sequence alignment of the ITS regions of *G. lucidum* and *G. sinensis* was constructed (Fig. 9-10). The ITS sequence of *G. lucidum* strain KALASIN was similar to EU021460 and GQ249880 but differed from other strains at positions of 39, 124, 126, 127, 129, 130, 132, 135, 160, 183, 197, 221, 322 to 333, 419, 521, 540, 579, 580, 581, 582, 587. Interestingly, the 5.8 rDNA sequence of *G. lucidum* strain KALASIN is 17 bp longer than other strains. The ITS sequence of strain A1 differed from other strains at a position of 63, strain HHK differed from other strain at positions of 35 and 78, strain DOA differed from other strains at a position of 107, strain G2 differed from other stains at positions of 229, 233, 272 and 284, strain G5A differed from other strains at a position of 242 and strain G5Z differed from other strains at a position of 116. The ITS sequence alignment of *G. sinensis* is 100% similar of *G. sinensis* (HQ235633 and HQ235634). The ITS sequence of strain A2 differed from *G. lucidum* strains by 58 positions

Table 4 Similarity matrix of *Ganoderma lucidum* and *Ganoderma sinensis* strains based on ITS region such as ITS1, 5.8S and ITS2 profiles.

	G. lucidum																*G. sinensis*		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
	A1	DOA	G2	G45	G5A	G5Z	HHK	KALASIN	SUN	JN008869	JN008870	JN008871	EU021456	EU021460	FJ940919	GQ249880	A2	HQ235633	HQ235634
1	1.00																		
2	0.99	1.00																	
3	0.98	0.98	1.00																
4	0.99	0.99	0.98	1.00															
5	0.99	0.99	0.98	0.98	1.00														
6	0.99	0.99	0.98	0.99	0.98	1.00													
7	0.98	0.98	0.97	0.98	0.98	0.98	1.00												
8	0.95	0.95	0.95	0.95	0.95	0.95	0.95	1.00											
9	0.99	0.99	0.98	0.99	0.99	0.99	0.99	0.96	1.00										
10	0.99	0.99	0.99	0.99	0.99	0.99	0.98	0.95	0.99	1.00									
11	0.99	0.99	0.98	0.99	0.99	0.99	0.99	0.96	1.00	0.99	1.00								
12	0.99	0.99	0.99	0.99	0.99	0.99	0.98	0.95	0.99	1.00	0.99	1.00							
13	0.99	0.99	0.99	0.99	0.99	0.99	0.98	0.95	0.99	1.00	0.99	1.00	1.00						
14	0.96	0.96	0.95	0.95	0.95	0.95	0.95	0.99	0.96	0.96	0.96	0.96	0.96	1.00					
15	0.99	0.99	0.98	0.99	0.99	0.99	0.99	0.96	1.00	0.99	1.00	0.99	0.99	0.96	1.00				
16	0.96	0.96	0.95	0.95	0.95	0.95	0.95	0.99	0.96	0.96	0.96	0.96	0.96	1.00	0.96	1.00			
17	0.91	0.91	0.91	0.91	0.91	0.92	0.91	0.89	0.92	0.92	0.92	0.92	0.92	0.91	0.92	0.91	1.00		
18	0.91	0.91	0.91	0.91	0.91	0.92	0.91	0.89	0.92	0.92	0.92	0.92	0.92	0.91	0.92	0.91	1.00	1.00	
19	0.91	0.91	0.91	0.91	0.91	0.92	0.91	0.89	0.92	0.92	0.92	0.92	0.92	0.91	0.92	0.91	1.00	1.00	1.00

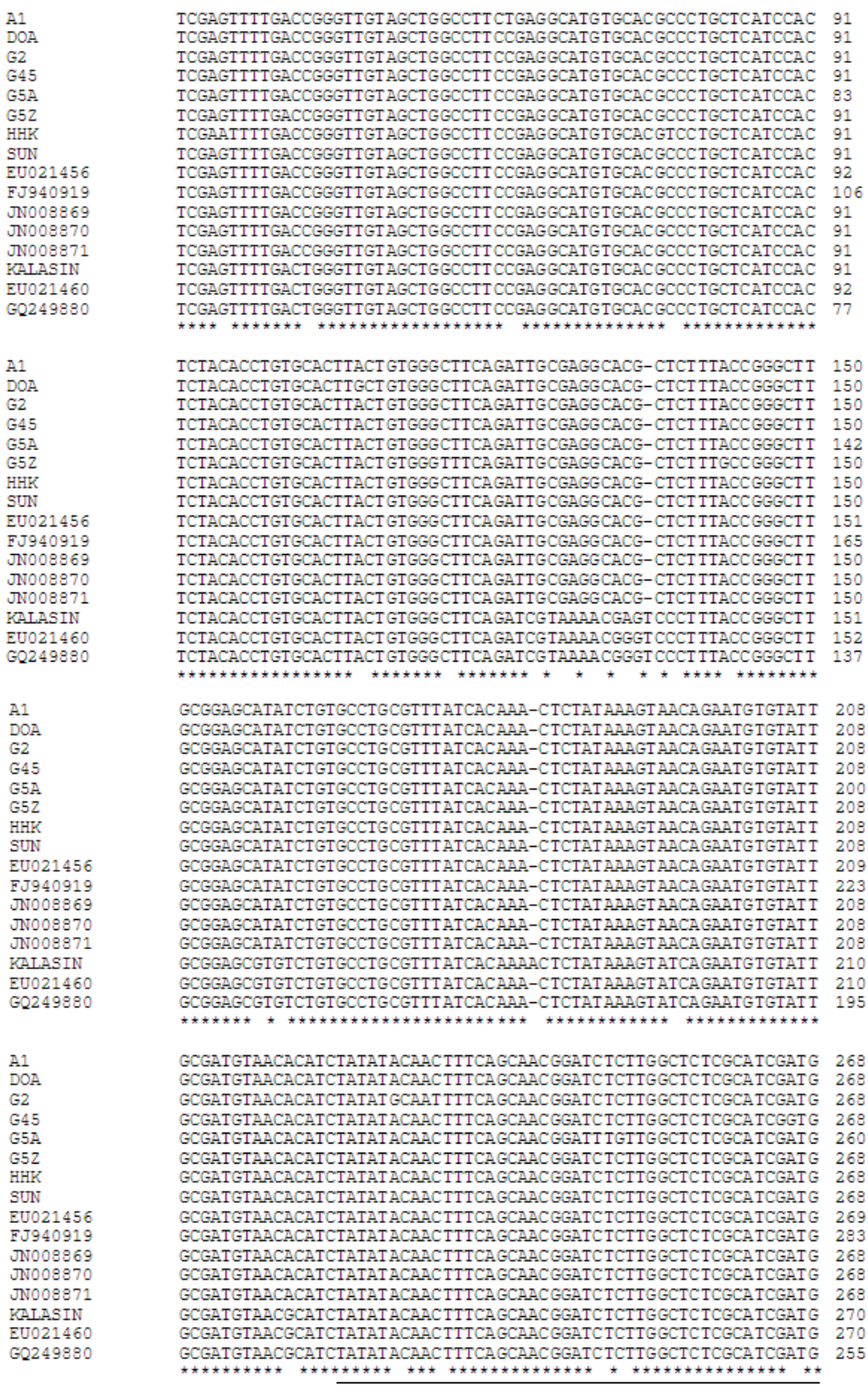


Figure 9 The ITS sequence alignment of *Ganoderma lucidum* strains A1, DOA, G2, G45, G5A, G5Z, HHK, KALASIN, SUN and ITS sequence from NCBI accession EU021456, EU021460, FJ940919, GQ249880, JN008869, JN008870 and JN008871. The number showed the order of ITS sequence residue,* indicates the conserve ITS. Alignment gaps are indicated by dashes. The position of ITS1, 5.8S and ITS2 were indicated by no line, single line and double line, respectively.

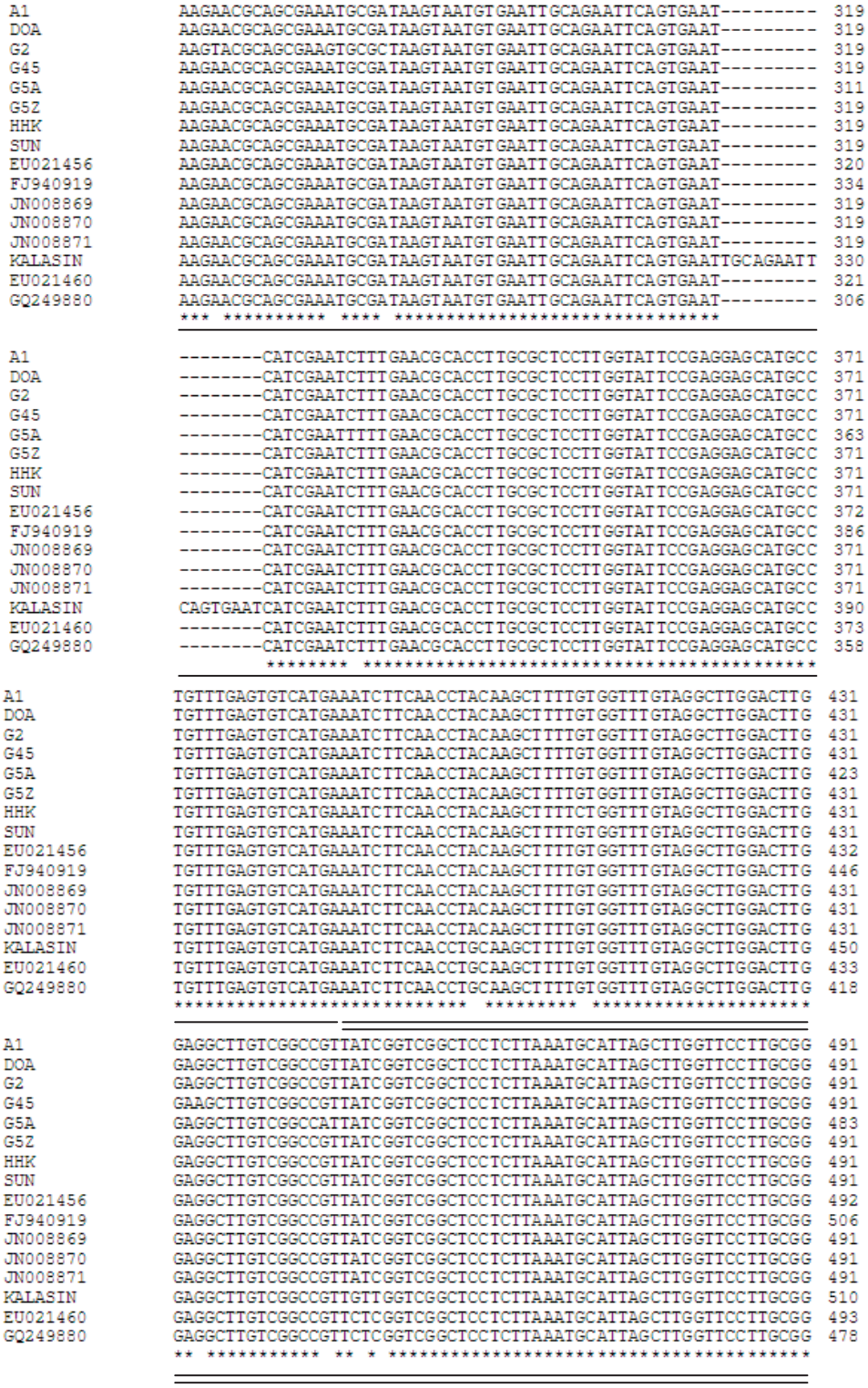


Figure 9 The ITS sequence alignment of *Ganoderma lucidum* strains A1, DOA, G2, G45, G5A, G5Z, HHK, KALASIN, SUN and ITS sequence from NCBI accession EU021456, EU021460, FJ940919, GQ249880, JN008869, JN008870 and JN008871. The number showed the order of ITS sequence residue, * indicates the conserve ITS. Alignment gaps are indicated by dashes. The position of ITS1, 5.8S and ITS2 were indicated by no line, single line and double line, respectively. (continuous)

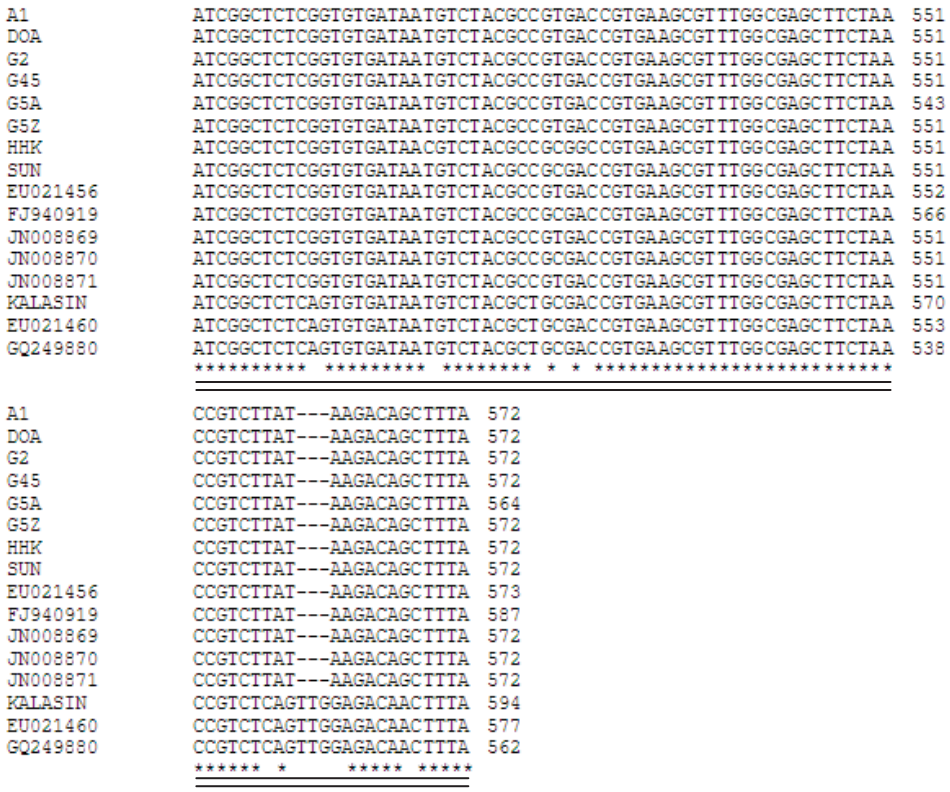


Figure 9 The ITS sequence alignment of *Ganoderma lucidum* strains A1, DOA, G2, G45, G5A, G5Z, HHK, KALASIN, SUN and ITS sequence from NCBI accession EU021456, EU021460, FJ940919, GQ249880, JN008869, JN008870 and JN008871. The number showed the order of ITS sequence residue, * indicates the conserve ITS. Alignment gaps are indicated by dashes. The position of ITS1, 5.8S and ITS2 were indicated by no line, single line and double line, respectively. (continuous)

Figure 10 The ITS sequence alignment of *Ganoderma sinensis* strain A2 and ITS sequence from NCBI accession HQ235633 and HQ 235634. The number showed the order of ITS sequence residue, * indicates the conserve ITS. Alignment gaps are indicated by dashes. The position of ITS1, 5.8S and ITS2 were indicated by no line, single line and double line, respectively.

Phylogenic tree was constructed. The result revealed that the tested strains of *Ganoderma* sp. were divided into 3 groups (Fig. 11). Group 1 composed of *G. lucidum* strain A1, G2, DOA, G45, G5A, G5Z, HHK and SUN (from China), JN008869 (from Poland), JN008870 (from Poland), JN008871 (from Poland), EU021456 (from Taiwan), FJ940919 (from China). Group 2 comsisted of *G. lucidum* strain KALASIN (from Thailand), EU021460 (from Taiwan) and GQ249880 (from India). Group 3 was made up by *G. sinensis*, HQ235633 (from China) and HQ235634 (from China). The tree showed *G. lucidum* strain KALASIN was different from Chinease strains and *G. sinensis* was different from *G. lucidum.*

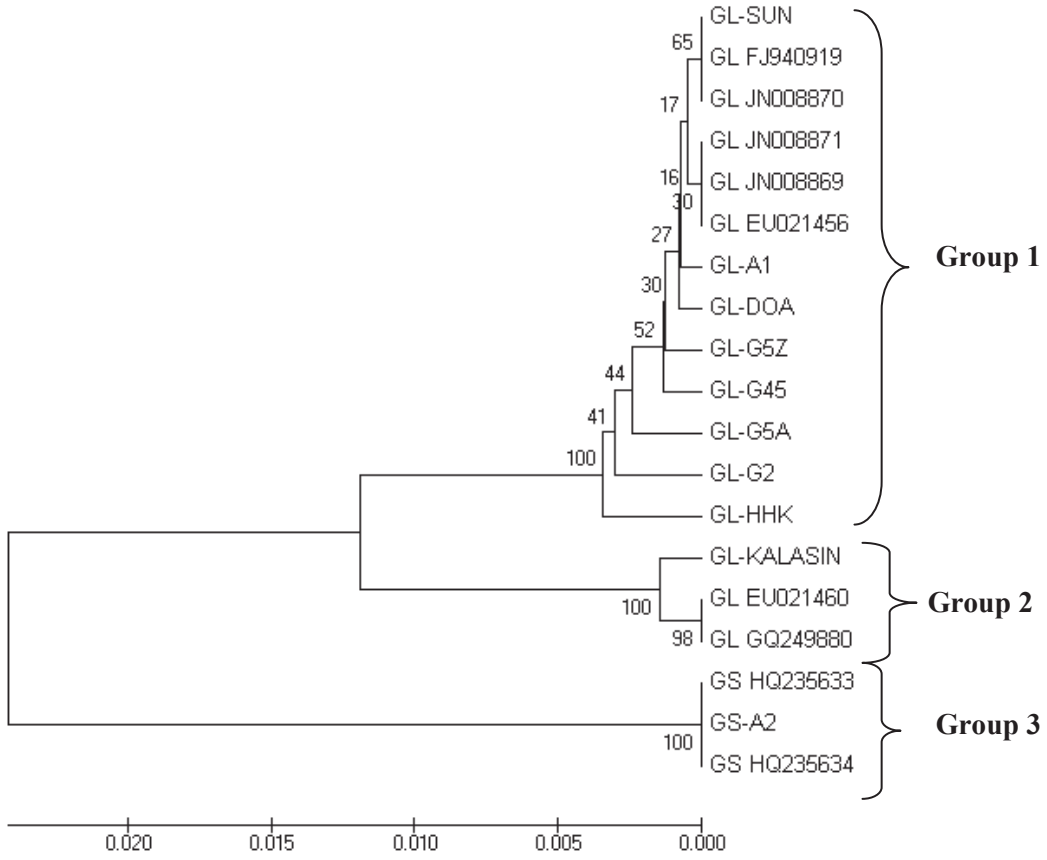


Figure 11 Gene phylogeny from rDNA sequences of the ITS region. The tree was contracted by Mega 4 program. Value above branches are confidence level after 100 bootrap replications.

4. Random Amplified Polymorphism DNA (RAPD)

RAPD analysis was performed by using 20 primers, but only 8 primers (A03, A04, A08, A09, A10, A11, A13 and A18) could yield polymorphism. Analyses were carried out in triplicate for all primers to ensure reproducibility of the results. The eight primers gave consistent results and produced reasonable numbers of distinguishable and polymorphic bands. The number of amplified DNA fragments generated by each RAPD primer ranged from 6 to 11 (Table 5). The size of the fragments produced ranged from 250 to 2500 bp. The RAPD patterns generated by 8 primers are shown in Fig. 12. The RAPD patterns of *G. lucidum* strain G5A showed 100% similarity to G5Z and strain DOA showed 100% similarity to G45. The RAPD patterns of strain KALASIN were different from those of other strains in all primers. The pattern of *G. sinensis* strain A2 were different from other strains of *G. lucidum* in all primers.

Table 5 Numbers of RAPD product and efficient loci of RAPD analyses of *Ganoderma lucidum* strains.

Primers	5'-3' sequence	Number of RAPD product	Size of loci (bp)
A03	AGTCAGCCAC	10	300-2000
A04	AATCGGGTCG	10	500-2000
A08	GTGACGTAGG	8	300-1500
A09	GGGTAACGCC	6	600-2500
A10	GTGATCGCAG	7	700-2000
A11	CAATCGCCGT	11	600-2000
A13	CAGCACCCAC	11	350-2000
A18	AGGTGACCGT	11	250-1400

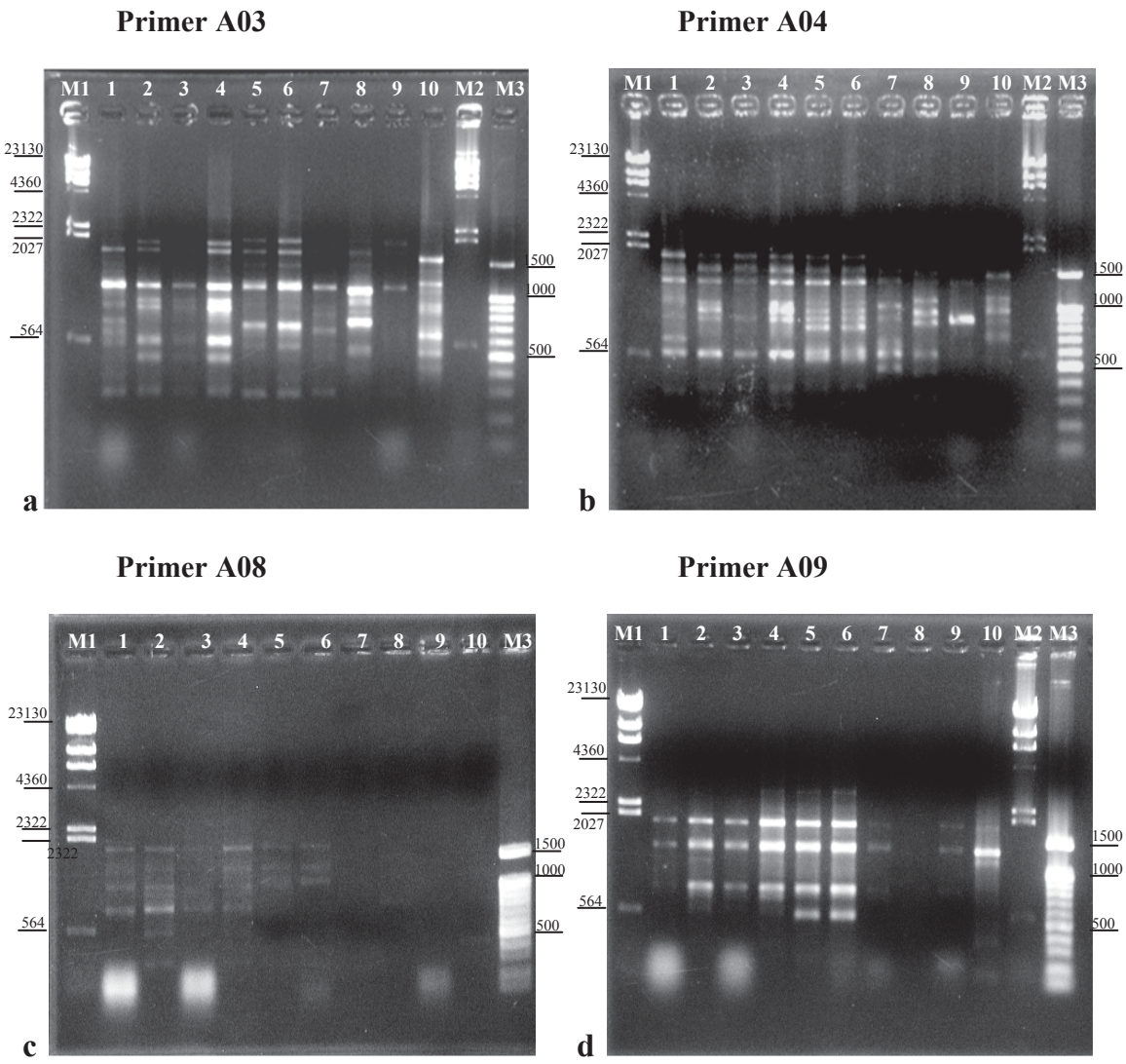


Figure 12 Amplification of DNA from *Ganoderma lucidum* and *Ganoderma sinensis* isolates using primers (a) A03, (b) A04, (c) A08, (d) A09, (e) A10, (f) A11, (g) A13 and (h) A18. From the left in all picture, (M1) λhindIII marker, (1) A1, (2) DOA, (3) G2, (4) G45, (5) G5A, (6) G5Z, (7) HHK, (8) KALASIN, (9) SUN, (10) A2, (M2) λhindIII marker, and (M3) 100 bp ladder size marker.

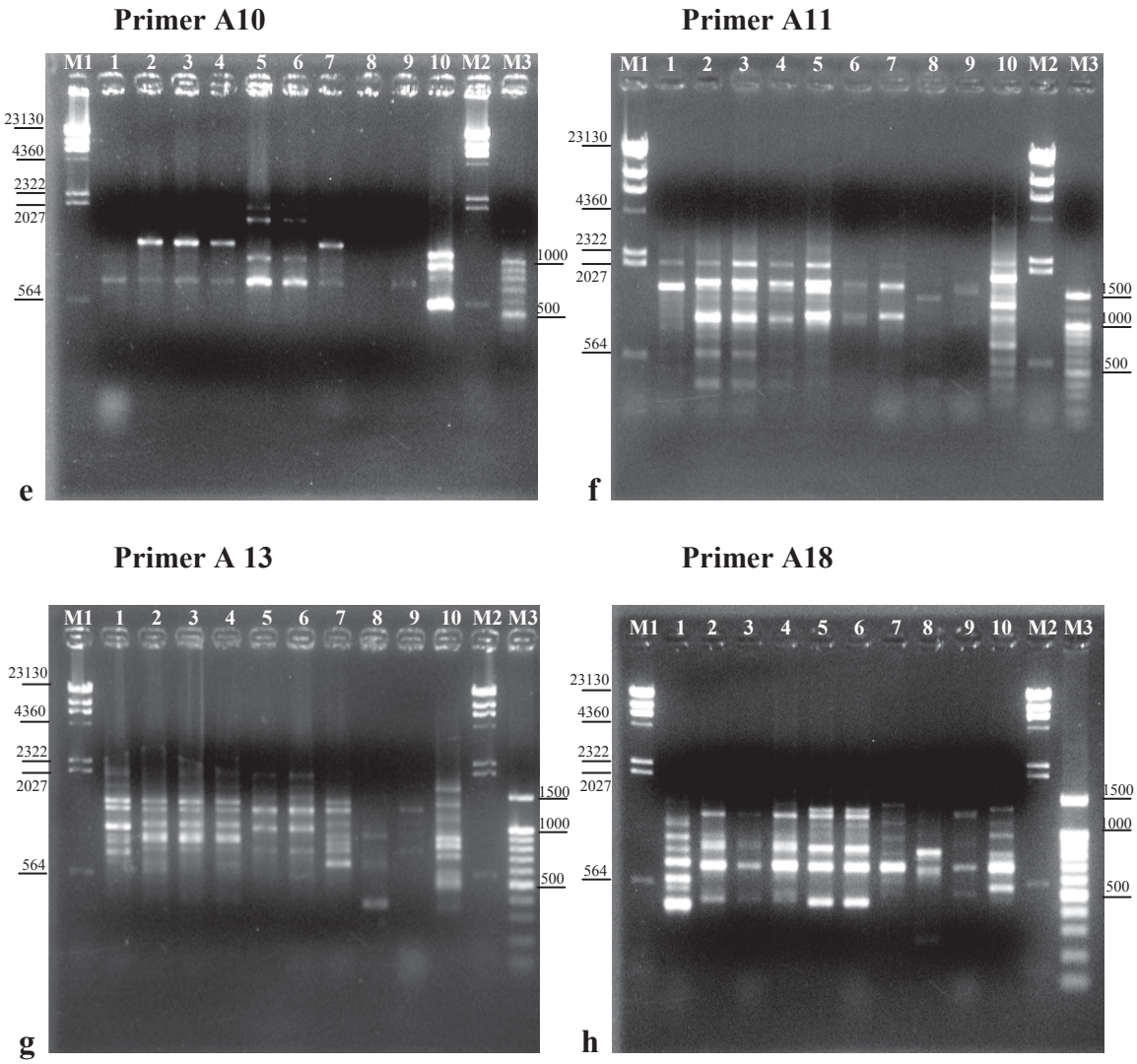


Figure 12 Amplification of DNA from *Ganoderma lucidum* and *Ganoderma sinensis* isolates using primers (a) A03, (b) A04, (c) A08, (d) A09, (e) A10, (f) A11, (g) A13 and (h) A18. From the left in all picture, (M1) λhindIII marker, (1) A1, (2) DOA, (3) G2, (4) G45, (5) G5A, (6) G5Z, (7) HHK, (8) KALASIN, (9) SUN, (10) A2, (M2) λhindIII marker, and (M3) 100 bp ladder size marker. (continues)

The similarity matrix was constructed based upon the presence or absence of the RAPD fragments. The 8 primers could be used to identify and showed polymorphism of *G. lucidum* strains isolated from China and Thailand, and *G. sinensis*. A similarity matrix indicated that the 9 strains of *G. lucidum* and *G. sinensis* can be grouped into 2 distinct groups (Table 6). The first group (group 1) composed of *G. lucidum* strains A1, DOA, G2, G45, G5A, G5Z, HHK, KALASIN and SUN. The second group (group 2) was formed by *G. sinensis* strain A2. In group 1, members shared similarity coefficients of 0.500-1.000. In addition, the strain DOA showed similarity coefficients 1.000 to G45 and the strain G5A showed similarity coefficients 1.000 to G5Z. The group 2 and group 1 were similarity coefficients of 0.392 -0.473. The results of ITS sequence and RAPD analyses revealed that the *G. lucidum* strains (1) G5A and G5Z, and (2) DOA and G45 are clonally propagate, i.e. a single genotype become wide spread.

Table 6 Similarity matrix of *Ganoderma lucidum* and *Ganoderma sinensis* strains based on ITS region such as ITS1, 5.8S and ITS2 profiles.

	A1	DOA	G2	G45	G5A	G5Z	HHK	KAL ASIN	SUN	A2	
A1	1.000										
DOA	0.689	1.000									
G2	0.662	0.743	1.000								
G45	0.689	1.000	0.743	1.000							
G5A	0.608	0.676	0.716	0.676	1.000						
G5Z	0.608	0.676	0.716	0.676	1.000	1.000					Group 1
HHK	0.730	0.635	0.743	0.635	0.608	0.608	1.000				
KAL ASIN	0.514	0.500	0.541	0.500	0.500	0.500	0.541	1.000			
SUN	0.608	0.541	0.689	0.541	0.581	0.581	0.743	0.608	1.000		
A2	0.392	0.446	0.473	0.446	0.432	0.432	0.473	0.473	0.459	1.000	Group 2

The phylogenetic tree was contracted using program bootstrap UPGMA by Mega 4 package (Fig. 13). The phylogenetic tree resolved two major isolated groups. Group 1 composed of 9 stains of *G. lucidum* and group 2 consisted of the one stain of *G. sinensis*. The member of group 1 could be divided into two subgroups according to geographical orgins. The stains A1, DOA, G2, G45, G5A, G5Z, HHK, SUN were obtained from China but KALASIN was isolated from Thailand. The strain (1) G5A and G5Z and (2) strain DOA and G45 showed bootstrap values at 100.

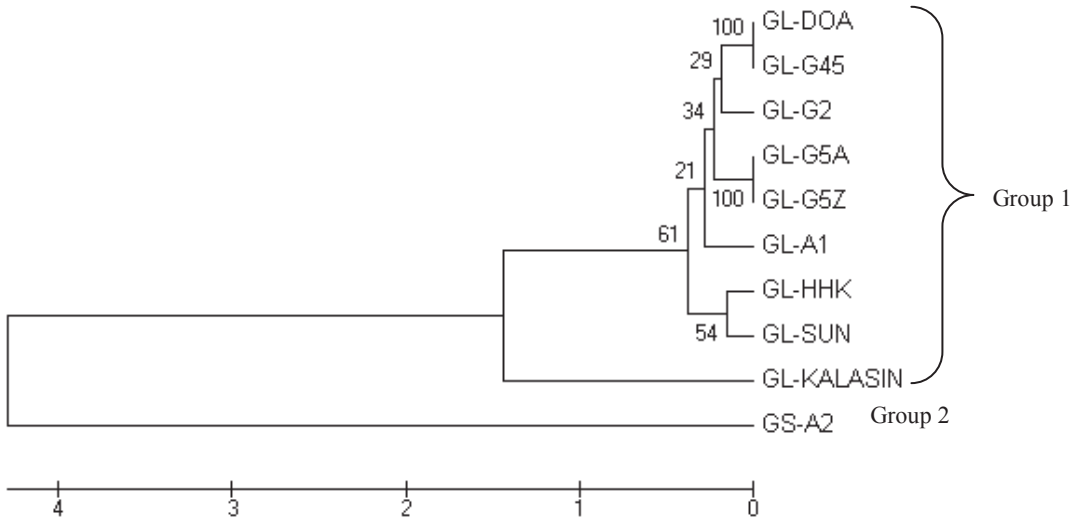


Figure 13 UPGMA phylogeny tree from data matrices of the RAPD base on polymorphism pattern of 8 primer A03, A04, A08, A09, A10, A11, A13, A18 contracted using the MEGA 4 program for 9 strains of *Ganoderma lucidum* and *Ganoderma sinensis*.

4. Morphology of fruiting body of *Ganoderma lucidum*

The mushrooms were cultured in the sawdust bags. Growth of mycelium in sawdust spawn took approximately 30 days. Afterwards, the sawdust bags were cased by soil to maintain the moisture. The primodia and fruiting bodies were produced approximately 30 and 75 days after casing, respectively. In these experiments, 2 flushes of fruiting bodies were produced. The morphology and numerical taxonomy were studies based on the cultured specimens. The description of *G. lucidum* strains and *G. sinensis* were described below.

Morphology of fruiting bodies of *Ganoderma lucidum* strain A1

Cap corky, tough; 4.2-6.5 cm broad, 6.8-12 cm long, 3-7 mm thick (which the thick is near stalk more than near the margin), circular to fan shape, red-brown; surface glossy surface smooth; color red-brown (when young yellow to yellow-brown in the canter); margin whitish, sharp, brown. Flesh light brown; soft-corky when fresh. **Stalk** tough; 2-3 long, 1.1-2.3 cm thick**;** often attached laterally, tapering downward; color dark brown, glossy. **Pore** minutes (3-4 per mm), whitish to cream when fresh; tube 0.5-0.9 long, irregular. **Spore** red brown; spore 5-7x8-10 µm, ellipsoid, double wall, appearing minutely roughed (Fig. 14).

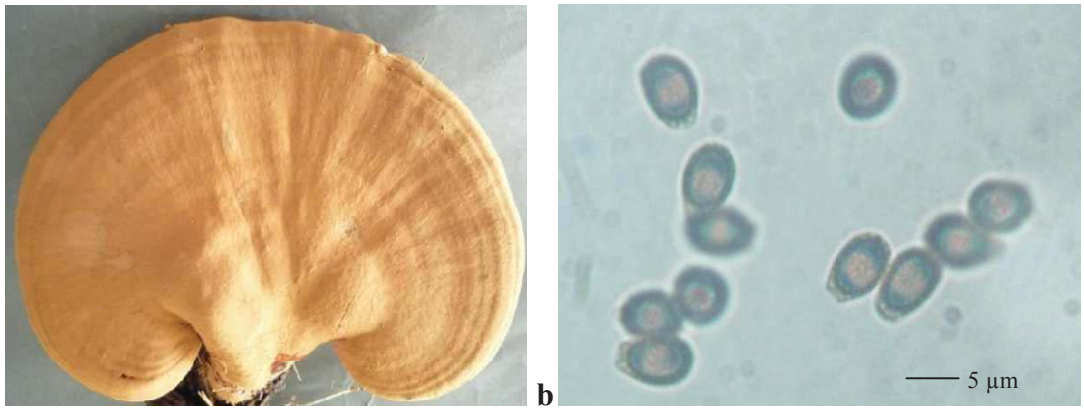


Figure 14 The (a) fruiting body and (b) spore that under light microscope (1000x) of *Ganoderma lucidum* strain A1.

Morphology of fruiting bodies of *Ganoderma lucidum* strain DOA

Cap corky, tough; 3.1-7.2 cm broad, 5.7-14.5 cm long, 3-6 mm thick (which the thick is near stalk more than near the margin), circular to fan shape, red-brown; surface glossy surface smooth; color red-brown (when young yellow to yellow-brown in the canter); margin whitish, sharp, brown. Flesh light brown; soft-corky when fresh. **Stalk** tough; 2-2.5 long, 1-1.3 cm thick**;** often attached laterally, tapering downward; color dark brown, glossy like cap. **Pore** minute (6-8 per mm), whitish to cream when fresh; tube 0.5-0.7 cm long, irregular. **Spore** red brown; spore 5-6 x 10-12 µm, ellipsoid, double wall, appearing minutely roughed (Fig. 15).

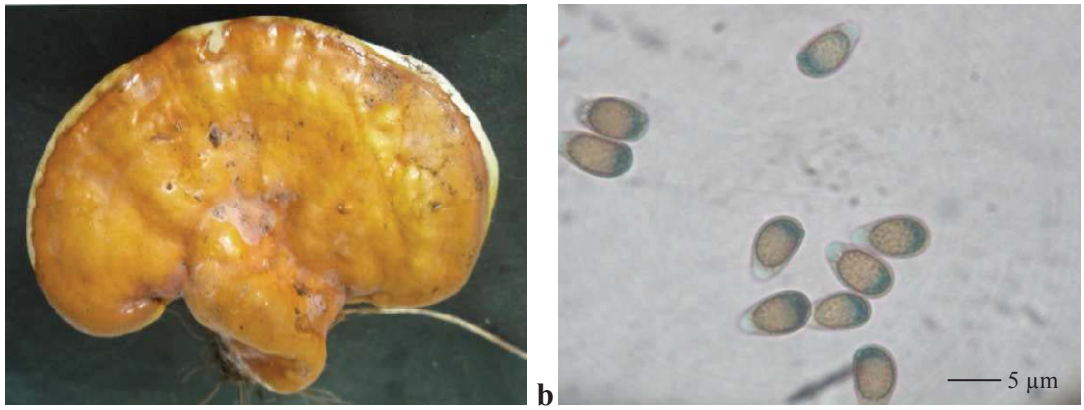


Figure 15 The (a) fruiting body and (b) spore that under light microscope (1000x) of.*Ganoderma lucidum* strain DOA.

Morphology of fruiting bodies of *Ganoderma lucidum* strain G2

Cap corky, tough; 3.9-4.8 cm broad, 7.6-8.2 cm long, 3-7 mm thick (which the thick is near stalk more than near the margin), circular to fan shape, red-brown; surface glossy surface smooth, edge; color red-brown (when young yellow to yellow-brown in the canter); margin whitish, sharp, brown. Flesh light brown; soft-corky when fresh. **Stalk** tough; 2-2.5 long, 0.8-2.4 cm thick**;** often attached laterally, tapering downward; color brown, glossy like cap. **Pore** minute (6-7 per mm), whitish to cream when fresh; tube 0.5-0.9 cm long, irregular. **Spore** brown; spore 5-6 x 8-11 µm, ellipsoid, double wall, appearing minutely roughed (Fig. 16).

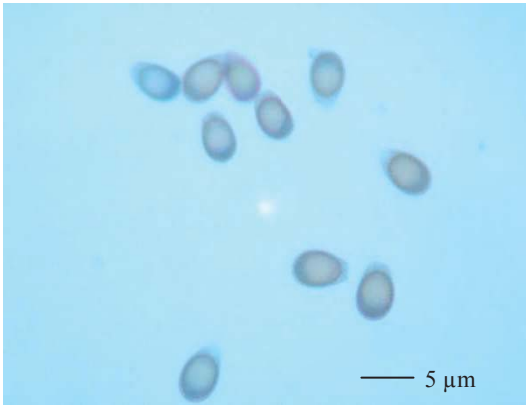
a  **b** 

Figure 16 The (a) fruiting body and (b) spore that under light microscope (1000x) of *Ganoderma lucidum* strain G2.

Morphology of fruiting bodies of *Ganoderma lucidum* strain G5Z

Cap corky, tough; 3.7-8.1 cm broad, 5.7-13.7 cm long, 2-6 mm thick (which the thick is near stalk more than near the margin), circular, red-brown; surface glossy surface smooth, often concentrically zone and groove; color red-brown (when young yellow to yellow-brown in the canter); margin whitish, sharp, brown. Flesh light brown; soft-corky when fresh. **Stalk** tough; 1.9-4.4 cm long, 0.9-2.4 cm thick**;** often attached laterally but often vertical, tapering downward; color brown, glossy like cap. **Pore** minute (4-5 per mm), whitish to cream when fresh; tube 0.6-0.8 cm long, irregular. **Spore** red-brown; spore 5-7 x 9-11 µm, ellipsoid, double wall, smooth, appearing minutely roughed (Fig. 17).

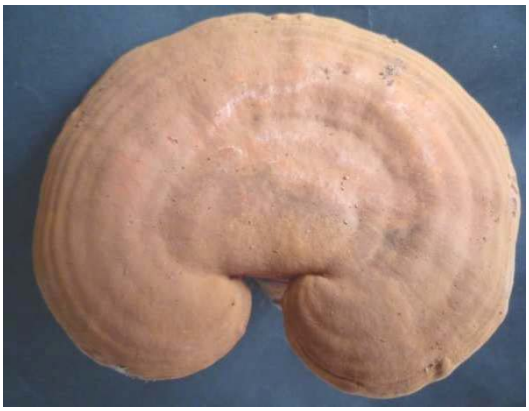
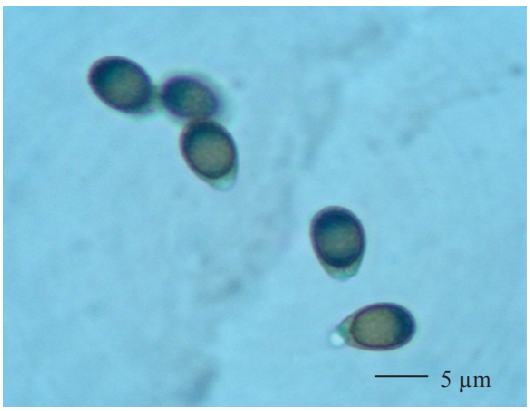
a  b 

Figure 17 The (a) fruiting body and (b) spore that under light microscope (1000x) of *Ganoderma lucidum* strain G5Z.

Morphology of fruiting bodies of *Ganoderma lucidum* strain HHK

Cap corky, tough; 3-4.2 cm broad, 5.5-8.1 cm long, 3-4 mm thick (which the thick is near stalk more than near the margin), semicircular to fan sharp, red-brown; surface glossy surface smooth, often concentrically zone and groove; color red-brown (when young yellow to yellow-brown in the canter); margin whitish, sharp, brown. Flesh light brown; soft-corky when fresh. **Stalk** tough; 1.7-4 cm long, 1-2.6 cm thick**;** often attached laterally, tapering downward; color red-brown, glossy like cap. **Pore** minute (5-7 per mm), whitish to cream when fresh; tube 0.4cm long, irregular. **Spore** red-brown; spore 6-7 x 8-10 μm, ellipsoid, double wall, smooth, inner layer appearing minutely roughed (Fig. 18).

Figure 18 The (a) fruiting body and (b) spore that under light microscope (1000x) of *Ganoderma lucidum* strain HHK.

Morphology of fruiting bodies of *Ganoderma lucidum* strain KALASIN

Cap corky, tough; 5.3-5.7 cm broad, 10.2-13 cm long, 2-3 mm thick (which the thick is near stalk more than near the margin), circular to fan sharp, red-brown; surface glossy surface smooth, furrow; color red-brown (when young yellow to yellow-brown in the canter); margin whitish, sharp, brown. Flesh light brown; soft-corky when fresh. **Stalk** tough; 4-7 cm long, 1-1.3 cm thick**;** often attached laterally, tapering downward, often gnarled or twisted; color red-brown, glossy like cap. **Pore** minute (4-5 per mm), cream to light brown when fresh; tube 0.4-0.5 cm long, irregular. **Spore** red-brown; spore 5-7 x 8-11 µm, ellipsoid, double wall, smooth, inner layer appearing minutely roughed (Fig. 19).

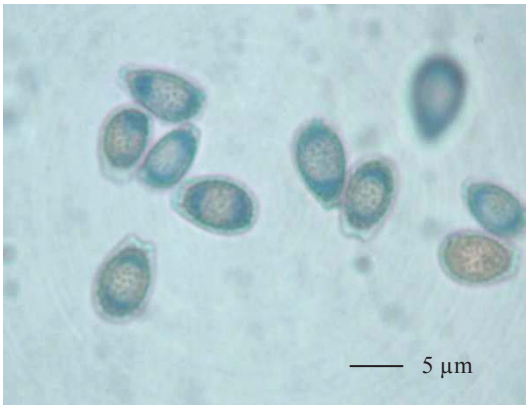
A  b 

Figure 19 The (a) fruiting body and (b) spore that under light microscope (1000x) of *Ganoderma lucidum* strain KALASIN.

Morphology of fruiting bodies of *Ganoderma lucidum* strain SUN

Cap corky, tough; 2.2-4.3 cm broad, 4.6-7.9 cm long, 3-6 mm thick (which the thick is near stalk more than near the margin), circular to fan sharp, red-brown; surface glossy surface smooth; color red-brown (when young yellow to yellow-brown in the canter); margin whitish, sharp, brown. Flesh light brown; soft-corky when fresh. **Stalk** tough; 1.5-3 cm long, 0.8-1.5 cm thick**;** often attached laterally, tapering downward; color red-brown, glossy like cap. **Pore** minute (6-7 per mm), whitish to cream when fresh; tube 0.2-0.3 cm long, irregular. **Spore** red-brown; spore 5-7 x 8-10 µm, ellipsoid, double wall, smooth, inner layer appearing minutely roughed (Fig. 20).

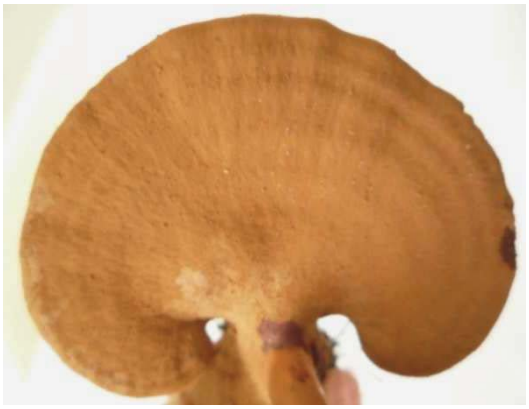
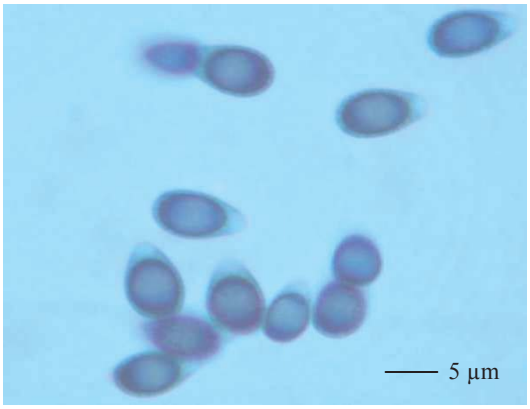
a  b 

Figure 20 The (a) fruiting body and (b) spore that under light microscope (1000x) of *Ganoderma lucidum* strain SUN.

Morphology of fruiting bodies of *Ganoderma sinensis* strain A2

Cap soft-corky, tough when old; 3.8-6 cm broad, 8.5-11 cm long, 5-7 mm thick (which the thick is near stalk more than near the margin), circular to fan sharp, red-brown; surface glossy surface smooth, often ridge and furrow; color purple dark; margin sharp, purple dark. Flesh dark purple; soft-corky when fresh. **Stalk** tough; 2-3 cm long, 1-2.3 cm thick**;** often attached laterally, tapering downward; purple black to black, glossy like cap. **Pore** minute (4-5 per mm), brown when fresh; tube 0.5cm long, irregular. **Spore** dark brown; spore 6-8 x 10-12 µm, ellipsoid, double wall, smooth, inner layer appearing minutely roughed (Fig. 21).

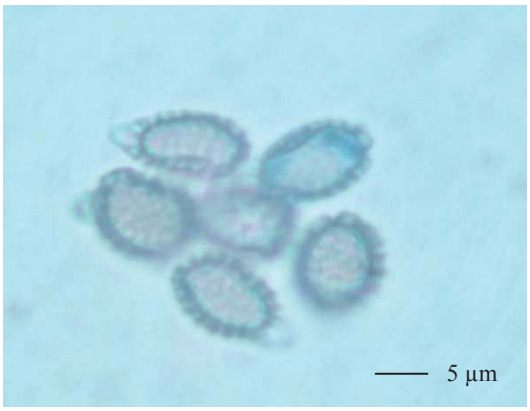
a  b 

Figure 21 The (a) fruiting body and (b) spore that under light microscope (1000x) of *Ganoderma sinensis* strain A2.

5. Numerical taxonomy

The numerical taxonomy in this study was performed according to Gottlieb & Wright (1999) by applying 20 characters typically used in taxonomy keys (Table 7). The 7 morphological characters of *G. sinensis* strain A2 were distinct from those of *G. lucidum*, i.e. pileus color, basidiome margin, context color, tube color, pore surface color, the smooth type of basidiospore (at 400x), and the semirogoes type of basidiospore (at 400x). Among the *G. lucidum* strains, 5 characters were slight different, i. e. the width of pileus, width of older context layer, height of older tube layer, tube color, and number of pore. In the state assignment, the maximal basidiospore length was found in *G. sinensis* strain A2, but the basidiospore shape (SI) could not classify *G. sinensis* from *G. lucidum* strains. The phylogenetic tree was contracted using program bootstrap UPGMA by Mega 4 package and resolved two major isolated groups (Fig. 22). Group 1 was 9 strains of *G. lucidum* and group 2 was strain of *G. sinensis*. This result obtained agrees with ITS sequencing and RAPD analyses. However, numerical taxonomy could not distinguish Thai *G. lucidum* strain from Chinese strains. Numerical taxonomy might be useful for discriminate interspecies of mushroom, but not intraspecies.

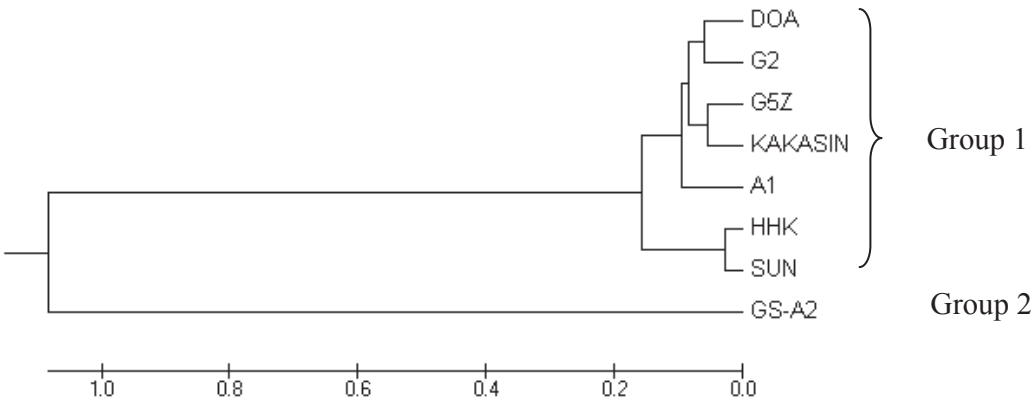


Figure 22 The phylogeny tree of *Ganoderma lucidum* strains and *Ganoderma sinensis* based on numerical taxonomy, contracted using the MEGA 4 program.

Table 7 The Morphological characters and character-state descriptions of fruiting bodies of *Ganoderma lucidum* and *Ganoderma sinensis*.

Characters	A1	DOA	G2	G5Z	HHK	KALASIN	SUN	A2
Pileus surface	laccate	laccate	laccate	laccate	laccate	laccate	laccate	laccate
Pileus colour	Red brown	Red brown	Red brown	Red brown	Red brown	Red brown	Red brown	Purple dark
Pileus consistency	intermediate	intermediate	intermediate	intermediate	intermediate	intermediate	intermediate	intermediate
Pileus width	5 to 9 cm (5.6-9.6)	5 to 9 cm (5.2-7.2)	0-5 cm (3.5-4.6)	5 to 9 cm (5.6-8.5)	0-5 cm (2.3-4.6)	5 to 9 cm (5-9.2)	0-5 cm (2.8-4.5)	5 to 9 cm (5.5-6)
Basidiome margin	sharp	sharp	sharp	sharp	sharp	sharp	sharp	rounded
Cutis of hymeniodermis *vera* type	present	present	present	present	present	present	present	present
Cutis of hymeniodermis with capitates eiements	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Cutis of hymeniodermis with diverticulate club	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Amyloid reaction	absent	absent	absent	absent	absent	absent	absent	absent
Context colour	Light brown	Light brown	Light brown	Light brown	Light brown	Light brown	Light brown	Dark brown
Width of older context layer	More than 5 mm (5-6)	More than 5 mm (5-6)	More than 5 mm (5-7)	More than 5 mm (5-6)	Less than 5 mm (3-4)	Less than 5 mm (2-3)	More than 5 mm (6-9)	More than 5 mm (5-7)
Height of older tube layer	More than 5 mm (5-9)	More than 5 mm (5-7)	More than 5 mm (5-8)	More than 5 mm (6-8)	Less than 5 mm (3-4)	More than 5 mm (5-6)	Less than 5 mm (2-3)	More than 5 mm (5-6)

Table 7 The Morphological characters and their character-state descriptions of fruiting bodies of *Ganoderma lucidum* and *Ganoderma sinensis*. (continuous)

Characters	**A1**	**DOA**	**G2**	**G5Z**	**HHK**	**KALASIN**	**SUN**	**A2**
Tube colour	Light brown	Light brown	Light brown	Light brown	Dark brown	Light brown	Dark brown	Dark purple
Number of pores per mm	Less than 4 (3-4)	More than 6 (6-8)	More than 6 (6-7)	4 to 6 (4-5)	More than 6 (6-7)	4 to 6 (4-5)	More than 6 (6-7)	4 to 6 (4-5)
Pore surface colour	Cream to light brown	Cream to light brown	Cream to light brown	Cream to light brown	Cream to light brown	Cream to light brown	Cream to light brown	Dark brown
Mean basidiospore length	9.1±0.50	10.6±0.70	9.8±0.80	9.9±0.4	9.4±0.80	9.7±1.00	9.4±1.00	11.4±0.70
Basidiospore shape (SI)	1.42±0.16	1.83±0.16	1.78±0.21	1.74±0.23	1.52±0.15	1.64±0.21	1.57±0.31	1.58±0.20
Basidiospore of the smooth type (at 400x)	present	present	present	present	present	present	present	absent
Basidiospore of the semirogose type (at 400x)	absent	absent	absent	absent	absent	absent	absent	present
Basidiospore of the rugose type (at 400x)	absent	absent	absent	absent	absent	absent	absent	absent

6. Crude polysaccharides of *Ganoderma lucidum* strains

To prepare crued polysaccharides, 30 g of powder of fruiting bodies were extracted by hot water and precipitated by ethanol. The maximum weight of crude polysaccharides was found in *G. sinensis* strain A2 and the minimum weight was found in *G. lucidum* strain A1. The crude polysaccharide of *G. sinensis* strain A2 is brown, while crude polysaccharides of other strains are dark brown. The texture of crude polysaccharides of strains G5Z and HHK are sticky, A1, DOA and G2 are solid and brittle, KALASIN is solid but not brittle, SUN is soft and cream, and A2 is soft and dry. The weight of crude polysaccharides is showed in Table 8.

Table 8 The characterization of crude polysaccharide of *Ganoderma lucidum* strains and *Ganoderma sinensis* precipitated by 95% ethanol.

Strains	Weight of crude (g)	% crude polysaccharide	Color of crude	Texture
A1	0.21	0.68	Dark brown	Solid and brittle
DOA	0.86	2.86	Dark brown	Solid and brittle
G2	0.38	1.28	Dark brown	Solid and brittle
G5Z	0.37	1.23	Dark brown	Sticky
HHK	0.64	2.13	Dark brown	Sticky
KALASIN	0.82	2.73	Dark brown	Solid
SUN	0.64	2.11	Dark brown	Soft and creamy
A2	2.39	7.93	Brown	Soft

7. Properties of crude polysaccharides

7.1 Total carbohydrates content

The total carbohydrates measured were performed by phenol-sulphuric acid assay. The results varied from 316.54 to 923.46 mg/g of crude polysaccharides. The maximum of total carbohydrates was found in the *G. lucidum* strain G2 by 92% and minimum was found in the *G. lucidum* strain SUN by 31%. The total carbohydrate of *G. lucidum* strain G2 is three times higher than those of *G. lucidum* strain SUN.

7.2 Reducing sugar

The reducing sugar was determined by the DNSA assay. The results varied from 62 to 120.11 mg/g of crude polysaccharides. The maximum of reducing sugar was found in the *G. lucidum* strain HHK by 12.01% and minimum was found in the *G. lucidum* strain G5Z by 6.2%. The reducing sugar of *G. lucidum* strain HHK is two times higher than those of *G. lucidum* strain G5Z.

7.3 Protein content

The protein content was measured by the Bradford method. The results varied from 3.35 to 17.74 mg/g of crude polysaccharides. The maximum of protein content was found in the *G. lucidum* strain HHK by 1.77% and minimum was found in the *G. sinensis* strain A2 by 0.34%. The protein content of *G. lucidum* strain HHK is six times higher than those of *G. sinensis* strain A2.

7.4 Total phenol content

The total phenol content was tested by the Folin-Ciocalteu colorimetric method. The results varied from 4.11 to 16.56 mg/g of crude polysaccharides. The maximum of total phenol content was found in the *G. lucidum* strain HHK by 1.66% and minimum was found in the *G. sinensis* strain A2 by 0.41%. The total phenol content of *G. lucidum* strain HHK is four times higher than those of *G. sinensis* strain A2.

The properties of crude polysaccharides were showed in Table 9. The experiment was set up with three replicate.

Table 9 The table showed value of total crude polysaccharide and qualification of crude polysaccharide. The alphabet and symbol behind value was group of statistic. The alphabet a, b, c, d, A, B, C, D, E, G, F and symbol I, II, III, IV, V, +, ++, +++, ++++, +++++ indicate the statistical difference.

Strains	**Total crude polysaccharides (g)**	**Properties of crude polysaccharides (mg/g of crude polysaccharide)**			
		Total carbohydrate	**Reducing sugar**	**Protein content**	**Total phenol content**
A1	0.21	812.35 ± 28.04^{c}	88.56 ± 1.35^{A}	12.66 ± 0.79^{I}	$13.86\pm0.44^{+}$
DOA	0.86	920.99 ± 8.55^{c}	78.11 ± 0.96^{E}	10.90 ± 0.64^{IV}	$10.44\pm0.42^{++++}$
G2	0.38	923.46 ± 4.28^{c}	106.22 ± 0.51^{C}	16.02 ± 0.44^{III}	$13.35\pm1.01^{+++}$
G5Z	0.37	552.59 ± 19.47^{a}	62 ± 1.00^{B}	14.34 ± 0.36^{II}	$11.13\pm0.09^{++}$
HHK	0.64	566.42 ± 33.67^{b}	120.11 ± 1.07^{F}	17.74 ± 0.31^{V}	$16.56\pm0.59^{+++++}$
KALASIN	0.82	446.67 ± 15.52^{b}	91.11 ± 0.96^{F}	8.88 ± 0.44^{IV}	$10.60\pm0.53^{++++}$
SUN	0.64	316.54 ± 6.64^{a}	95.33 ± 1.33^{D}	12.07 ± 0.65^{II}	$6.01\pm0.23^{++}$
A2	2.39	495.31 ± 36.36^{b}	64.11 ± 1.39^{G}	3.35 ± 0.14^{V}	$4.11\pm0.04^{+++++}$

CHAPTER 5
DISCUSSION

Ganoderma lucidum is usually found in the temperate zone, such as China, Japan, Korea and India. Interestingly, this mushroom was found in Kalasin province, North East of Thailand. To elucidate the diversity of this mushroom, 8 strains of *G. lucidum* isolated from China and the Thai strain were compared regarding to physiological and genetics characterization in this study. To investigate the effect of temperature on mycelia radial growth rate, the mushrooms were cultured on PDA at 25, 30, 35, 37 and 40ºC. The results showed that Chinese strains could grow at 25-30ºC, but not at 35ºC and above. Thai strain could grow at 25-35ºC and slightly grew at 37ºC, but not at 40ºC. Jayasinghe *et al.* (2008) and Lee *et al.* (2003) also reported that *G. lucidum* are well grown at 25-30ºC. Unfortunately, the strain KALASIN could grow at higher temperature and this might be due to the mushroom adapted to its environment, i. e. Kalasin province locates at the subtropical zone where the average temperature is approximately 30-35ºC. The temperature is one of the key factors for fungal growth. At the inappropriate temperature, the growth of fungi could be inhibited as a result of the important enzymes and metabolic processes might be inactivated or denatured (Chang *et al.*, 2006a).

Jo *et al*. (2009) indicated that nitrogen sources, such as yeast extract and malt extract could enhance growth rate of *G. applanatum*. In this study, wheatear or not the nitrogen source could promoted the mycelia growth of *G. lucidum* was hypothesized. The results reveal that yeast extract, malt extract and ammonium chloride could increase fungal growth of some strains at 30ºC and slightly increased at 35ºC. This is probably due to there are enough nitrogen and carbon source in PDA. Therefore, the addition of complex nitrogen to the complete medium could not promote the growth of mushroom mycelium. Yeast extract and malt extract are the complex nitrogen sources which consist of not only nitrogen but essential vitamins and mineral also (Fasidi & Olorunmaiye, 1994).

The ITS regions are highly variable. Hence, these regions are probably important for molecular systematic in order to distinguish the species or strains of fungi (Moncalvo *et al.*, 1995). In this work, the ITS regions of *Ganoderma* species were amplified by primer ITS1 and ITS4. By comparison of ITS sequences, the result showed that *G. lucidum* strain used in this study could be classified into 3 groups. Group 1 composed of *G. lucidum* strains isolated from China. Group 2 consisted of *G. lucidum* strain isolated from Thailand. Group 3 was made up by a single strain of *G. sinensis*. The ITS sequence analysis is a useful technique for classification at species and strain level. This result agrees with those of Zheng *et al.* (2009) who applied ITS sequence analysis in order to identify *Ganoderma* species. Interestingly, 5.8S rDNA sequence of *G. lucidum* strain KALASIN is 17 bp longer than those of other strains. This sequence region is a repeated sequence. This might be that DNA duplication took place in 5.8S rDNA of KALASIN strain.

The RAPD is method for accessing the variability at DNA level, being especially useful on intraspecific analysis. This study applied the RAPD analysis for differentiate strains of *G. lucidum*. The primers used in this study could produce 74 polymorphic bands and approximately 60% of RAPD bands are bigger than 1,000 bp. The RAPD profiles revealed a genetic variation among *G. lucidum* and *G. sinensis* and separated the isolates of *Ganoderma* into 2 different groups. Group 1 contained isolates of *G. lucidum*. Group 2 consisted of a single isolate of *G. sinnensis*. Group 1 could be divided into two subgroups by geographical origins of mushroom, i.e. strains isolated from China and strain isolated from Thailand. The RAPD patterns revealed that the *G. lucidum* strains (1) G5A and G5Z, and (2) DOA and G45 are 100% identical. These results are in agreement with those of Rolim *et al*. (2011) who applied RAPD analysis to distinguish Brazilian and Chinese strains of *G. lucidum*. It was suggested that RAPD analysis could be applied on differentiation of fungal strain rather that a big taxonomic group (Rolim *et al.*, 2011, Lyons *et al.*, 2000)

The numerical taxonomy is a classification technique based on the numerical analysis of the variation of large number of characters in a group of organisms. This study used characters of macro and micro morphology to classify *Ganoderma* species. The results of numerical taxonomy showed that *G. sinensis* is different form *G. lucidum* by 7 characters. The results of this study are in agreement with Gottlieb & Wright (1999), who used numerical taxonomy for evaluate relationships of *Ganoderma* species in Southern

South America. Foroutan & Vaidya (2007) applied characters of external and internal morphology for identification of 34 species of *Ganoderma*. However, the results in the study could not distinguish between Thai and Chinese strains of *G. lucidum*. Due to a high similarity of morphology, this technique could not distinguish strains of *G. lucidum* (Hseu *et al.*, 1996).

The ITS analysis, RAPD analysis, and numerical taxonomy could distinguish *G. lucidum* from *G. sinensis*. In the other hand, numerical taxonomy unable to distinguish Thai strain from Chinese strains. The results revealed that Thai and Chinese strains are genetically diverse. However, the different in genetics could not express for the difference in morphology of the mushroom.

The polysaccharide is one of the bioactive compounds in *G. lucidum*. It could exhibit anticancer, antitumor, antiviral and immunological activities (Thetsrimuang *et al.*, 2011). To elucidate the quality of bioactive compound in each strain, the extraction and characterization are very important. In this work, dry fruiting bodies of *Ganoderma* species were extracted for crude polysaccharides by hot water as same as the previous reports (Eo *et al.*, 1999, Mau *et al.*, 2005, Tseng *et al.*, 2008, Thetsrimuang *et al.*, 2011). The yields of crude polysaccharides in this study were 0.6-2.8% which was in the same range as Zhao *et al.* (2010) who reported the yield is about 2.07%. The yield of crude polysaccharide in *G. sinensis* and *G. tsugae* is 7.9% and 1.5-1.7%, respectively (Tseng *et al.*, 2008). The total carbohydrate might represent the amount of β-glucan (Thetsrimuang *et al.*, 2011, Novák *et al.*, 2012) which is a major bioactive compound in anticancer activity. In this work, total carbohydrates of crude polysaccharide of *G. lucidum* and *G. sinensis* gave values varies from 319 to 923 mg/g dry weight of crude extract (30%-90%). Total carbohydrate content of *Lentinus polychrous* varies from 228-523 mg/g dry weight of crude extract (22.8-52.3%; Thetsrimuang *et al.*, 2011). The protein content in crude polysaccharide was ranged from 3.3 to 17.7 mg/g dry weight of crude extract (0.33-1.77%). Wei & Van Griensven (2008) reported that the protein content of medical mushroom such as *G. lucidum* isolated from China and Thailand was range from 49.9-292.4 and 0-85 µg/g of dry weight, repectively. The total phenol content in the crude polysaccharides was ranged from 4.11-16.56 mg GAE/g dry weight of crude extract (0.41-1.66%). The phenol content of *L. edodes* and *V. volvacea* extracted by hot water is 1.33–1.34 mg GAEs/g of dry mushroom and extracted by methanol is 4.79–15.0 mg GAEs/g of dry mushroom (Cheung *et al.*, 2003). It was reported that the antioxidant

activity of plant materials is well correlated with the content of the phenol compounds (Cheung *et al.*, 2003). Therefore, it is important to consider the quantitative of the total phenol content in mushroom extract on the antioxidant activity.

In conclusion, *G. lucidum* is different from *G. sinensis*. The strains of *G. lucidum* are clearly distinctive in genetics, but not morphology. The crude polysaccharides, total carbohydrates, reducing sugar, and total phenol content of *G. lucidum* strain KALASIN was in the same range as those of Chinese strains. In the future works, the light should shed on comparison of the anticancer activity of the polysaccharides extracted from *G. lucidum* strains isolated from Thailand and China.

CHAPTER 6

CONCLUSION

1. The optimal temperature of *Ganoderma lucidum* strains in potato dextrose agar was 30ºC. The yeast extract, malt extract and ammonium chloride could enhance the growth rate of some strains of *G. lucidum* at 35ºC. The *G. lucidum* strain KALASIN grew at 35ºC-37ºC, while other strains could not.

2. The internal transcribe spacer (ITS) sequences were 636-658 bp. The ITS analysis could classify *Ganoderma* species into 3 groups. Group 1 composed of *G. lucidum* strains isolated from Chinese. Group 2 consisted of *G. lucidum* strain isolated from Thailand. Group 3 was made up by *G. sinensis*. The similarity between *G. lucidum* strains and *G. sinensis* was 0.89-0.92 and among *G. lucidum* strains was 0.95-1.00. The similarity matrix between Thai and Chinese strains was 0.95-0.96.

3. The random amplified polymorphism DNA (RAPD) analysis was performed by 20 primers, but only 8 primers showed polymorphism pattern. The RAPD analysis could distinguish *Ganoderma* species to 2 groups. Group 1 composed of *G. lucidum* strains and group 2 consisted of *G. sinensis*. In group 1, the mushroom could classify in to 2 subgroups according to geographic origins, i. e. strain isolate from Thailand and strains isolated from China. The similarity coefficients of *G. lucidum* strains were 0.500-1.000. The strain DOA showed similarity coefficients 1.000 to G45 and the strain G5A showed similarity coefficients 1.000 to G5Z. The similarity between *G. lucidum* and *G. sinensis* were 0.392 -0.473.

4. The numerical taxonomy revealed that 7 morphological characters of *G. sinensis* strain A2 were distinct from those of *G. lucidum*, i.e. pileus color, basidiome margin, context color, tube color, pore surface color, the smooth type of basidiospore (at 400x) and the semirogoes type of basidiospore (at 400x). Among the *G. lucidum* strains, 5 characters were slightly different, i. e. the width of pileus, width of older context layer, height of older tube layer, tube color and number of pore. The numerical

taxonomy could divide the mushroom into 2 groups, i. e. group of *G. lucidum* strains and group of *G. sinensis.*

5. The crude polysaccharides of *G. lucidum* strains were 0.68-2.86% and *G. sinnesis* was 7.93%. The total carbohydrates, reducing sugar, protein content and phenol content of *Ganoderma* species were 316.54-923.46 mg/g, 62-120.11 mg/g, 3.35-17.74 mg/g, 4.11-16.56 mg/g of crude polysaccharide, respectively.

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APPENDIXES

Appendix A: Culture medium preparation

1. Potato dextrose broth (PDB)

Medium composition

Potato	200.0 g
Dextrose	20.0 g
Distilled water	to 1 litre

The medium was prepared in 250 ml Erlenmeyer flask. The final medium pH should be adjusted to 6.5 and sterilized by autoclave at 15 psi of pressure 121ºC for 15 minutes.

2. Potato dextrose agar (PDA)

Medium composition

Potato	200.0 g
Dextrose	20.0 g
Agar	15.0 g
Distilled water	to 1 liter

The final medium pH should be adjusted to 6.5 and sterilized by autoclave at 15 psi of pressure 121ºC for 15 minutes. The cooled medium at 50ºC should be poured into Petri dishes.

3. PDA contained nitrogen source

Medium composition

Potato	200.0 g
Dextrose	20.0 g
Nitrogen source	4 g
Agar	15.0 g
Distilled water	to 1 liter

The nitrogen sources are yeast extract, malt extract or ammonium chloride. The final medium pH should be adjusted to 6.5 and sterilized by autoclave at 15 psi 121ºC for 15 minute. The cooled medium at 50ºC should be poured into Petri dishes.

4. The sorghum medium

Medium composition

Sorghum	50.0 g

The sorghums were soaked in the water for overnight. The water was drained and the sorghums were washed again. Then, the sorghums were boiled in water for 10 minutes. The hot water was drained. The cooled sorghum was transferred to a bottle and sterilized by autoclave at 15 psi of pressure 121ºC for 15 minutes.

5. The sawdust bag

Medium compositon

Sawdust of rubber	10 kg
Bran	750 g
Gypsum powder	325 g
Salts	20 g

The sawdust compositions were mixed in basin. Then, the water was added to mixer and mixed until 70% moisture. The 900 g of the wet sawdust were put into bag. The bag was covered with cotton and plastic cap and sterilized by autoclave at 15 psi of pressure 121ºC for 30 minutes.

Appendix B: Reagent

1. Phenol-Sulfuric acid

1.1 5% phenol

Phenol crystal 5 g

Distillate water 100 ml

1.2 Sulphuric acid

99.9% Sulphuric acid (analyzed grad)

0, 20, 40, 60, 80, 100 µg/ml of glucose were prepared in volumetric flask.

2. Dinitrosalicylic acid reagent (DNSA)

5 g of Dinitrosalicylic acid

100 ml 2M NaOH

Distillate water 50 ml

150 g of Sodium potassium tartrate

Distilled water to 500 ml

0, 0.2, 0.4, 0.6, 0.8, 1 g/L of glucose were prepared in volumetric flask.

3. Bradford dye stock

100 mg Coomassie blue G

50 ml Methanol

100 ml 85% Phosphoric acid

Distillate water 50 ml

0, 10, 20, 30, 40 and 50 µg/ml of bovine serum albumin were prepared in volumetric flask (Bradford, 1976).

4. Phenolic content

4.1 dallic acid

0.006 mg/ml of gallic acid

100 ml methanol: water (50: 50 v/v)

4.2 Sodium carbonate (Na_2CO_3)

7.5 g of sodium carbonate (anhydrous)

Distilled water 100 ml

0, 0.01, 0.02, 0.03, 0.04, 0.05, 0.06 mg/ml of gallic acid were prepared in volumetric flask.

5. Lysis buffer

50 mM Tris-HCl (pH 7.2)

50 mM EDTA

3% SDS

1% 2-mercaptoethanol

Add distilled water to 100 ml

6. Chloroform: Tris-HCl pH 8.0-saturated phenol

50 ml Chloroform

50 ml Phenol saturated with Tris-HCl (pH 8.0)

7. 3M Sodium acetate, pH 8.0

40.81 g Sodium acetate trihydrate

Add 80 ml of distilled water

Adjust the pH to pH 8.0 with glacial acetic acid

Adjust the volume to 100 ml with water

8. TE buffer

10 mM Tris-HCl

0.1 mM EDTA

9. TAE buffer (50x stock solution)

242 g Tris base

Add 800 ml of distilled water

Add 57.1 ml of glacial acetic acid

Add 100 ml of 0.5 M EDTA (pH 8.0)

Adjust the volume to 1000 ml with distilled water

Appendix C

Table 10 The mycelia growth rate of *Ganoderma lucidum* strain A1 grown on PDA and PDA contain with nitrogen sources at 25ºC for 5 days.

Medium	Replication 1					Replication 2					Replication 3					Total mean (cm)	SD of mean
	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)		
PDA	0.74	0.76	0.76	0.74	0.75	0.76	0.76	0.76	0.74	0.76	0.74	0.74	0.72	0.74	0.74	0.75	0.01
PDA+Ammonium chloride	0.72	0.70	0.68	0.60	0.68	0.68	0.70	0.68	0.68	0.69	0.56	0.58	0.66	0.58	0.60	0.65	0.05
PDA+Yeast extract	0.84	0.82	0.80	0.82	0.82	0.96	0.90	0.86	0.82	0.89	0.88	0.90	0.92	0.92	0.91	0.87	0.04
PDA+Malt extract	0.90	0.82	0.88	0.88	0.87	0.74	0.82	0.82	0.78	0.79	0.78	0.80	0.82	0.80	0.80	0.82	0.03

Table 11 The mycelia growth rate of *Ganoderma lucidum* strain A1 grown on PDA and PDA contain with nitrogen sources at 30ºC for 5 days.

Medium	Replication 1					Replication 2					Replication 3					Total mean (cm)	SD of mean
	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)		
PDA	1.32	1.32	1.36	1.32	1.33	1.18	1.22	1.18	1.08	1.17	1.40	1.34	1.44	1.40	1.40	1.30	0.12
PDA+Ammonium chloride	1.16	1.18	1.12	1.16	1.16	1.04	1.00	1.04	1.02	1.03	0.96	1.06	1.14	1.00	1.04	1.07	0.06
PDA+Yeast extract	1.48	1.48	1.38	1.40	1.44	1.36	1.40	1.50	1.44	1.43	1.42	1.44	1.42	1.42	1.43	1.43	0.03
PDA+Malt extract	0.98	1.00	0.98	0.94	0.98	1.36	1.42	1.38	1.36	1.38	1.02	1.08	1.00	1.00	1.03	1.13	0.19

Table 12 The mycelia growth rate of *Ganoderma lucidum* strain A1 grown on PDA and PDA contain with nitrogen sources at 35ºC for 5 days.

Medium	Replication 1					Replication 2					Replication 3					Total mean (cm)	SD of mean
	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)		
PDA	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Ammonium chloride	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Yeast extract	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Malt extract	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00

Table 13 The mycelia growth rate of *Ganoderma lucidum* strain A1 grown on PDA and PDA contain with nitrogen sources at 37ºC for 5 days.

Medium	Replication 1					Replication 2					Replication 3					Total mean (cm)	SD of mean
	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)		
PDA	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Ammonium chloride	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Yeast extract	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Malt extract	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00

Table 14 The mycelia growth rate of *Ganoderma lucidum* strain A1 grown on PDA and PDA contain with nitrogen sources at 40ºC for 5 days.

Medium	Replication 1					Replication 2					Replication 3					Total mean (cm)	SD of mean
	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)		
PDA	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Ammonium chloride	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Yeast extract	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Malt extract	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00

Table 15 The mycelia growth rate of *Ganoderma lucidum* strain DOA grown on PDA and PDA contain with nitrogen sources at 25ºC for 5 days.

Medium	Replication 1					Replication 2					Replication 3					Total mean (cm)	SD of mean
	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)		
PDA	0.80	0.82	0.80	0.80	0.81	0.80	0.82	0.80	0.78	0.80	0.80	0.76	0.72	0.74	0.76	0.79	0.03
PDA+Ammonium chloride	0.58	0.56	0.56	0.56	0.57	0.60	0.60	0.62	0.64	0.62	0.56	0.58	0.58	0.58	0.58	0.59	0.03
PDA+Yeast extract	0.92	0.96	0.82	0.86	0.89	0.98	0.94	0.96	0.96	0.96	0.90	0.84	0.86	0.94	0.89	0.91	0.04
PDA+Malt extract	0.76	0.76	0.76	0.70	0.75	0.86	0.86	0.88	0.84	0.86	0.78	0.78	0.78	0.78	0.78	0.80	0.06

Table 16 The mycelia growth rate of *Ganoderma lucidum* strain DOA grown on PDA and PDA contain with nitrogen sources at 30ºC for 5 days.

Medium	Replication 1					Replication 2					Replication 3					Total mean (cm)	SD of mean
	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)		
PDA	1.34	1.34	1.44	1.40	1.38	1.28	1.32	1.26	1.30	1.29	1.26	1.30	1.30	1.28	1.29	1.32	0.05
PDA+Ammonium chloride	0.94	1.00	0.96	1.00	0.98	1.04	1.06	1.04	1.02	1.04	1.02	1.06	0.94	0.98	1.00	1.01	0.03
PDA+Yeast extract	1.38	1.32	1.34	1.34	1.35	1.34	1.34	1.32	1.32	1.33	1.26	1.24	1.24	1.24	1.25	1.31	0.05
PDA+Malt extract	1.40	1.32	1.28	1.20	1.30	0.84	0.84	0.82	0.80	0.83	1.26	1.24	1.26	1.24	1.25	1.13	0.26

Table 17 The mycelia growth rate of *Ganoderma lucidum* strain DOA grown on PDA and PDA contain with nitrogen sources at 35ºC for 5 days.

Medium	Replication 1					Replication 2					Replication 3					Total mean (cm)	SD of mean
	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)		
PDA	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Ammonium chloride	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Yeast extract	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Malt extract	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00

Table 18 The mycelia growth rate of *Ganoderma lucidum* strain DOA grown on PDA and PDA contain with nitrogen sources at 37ºC for 5 days.

Medium	Replication 1					Replication 2					Replication 3					Total mean (cm)	SD of mean
	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)		
PDA	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Ammonium chloride	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Yeast extract	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Malt extract	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00

Table 19 The mycelia growth rate of *Ganoderma lucidum* strain DOA grown on PDA and PDA contain with nitrogen sources at 40ºC for 5 days.

Medium	Replication 1					Replication 2					Replication 3					Total mean (cm)	SD of mean
	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)		
PDA	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Ammonium chloride	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Yeast extract	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Malt extract	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00

Table 20 The mycelia growth rate of *Ganodermalucidum* strain G2 grown on PDA and PDA contain with nitrogen sources at 25ºC for 5 days.

Medium	Replication 1					Replication 2					Replication 3					Total mean (cm)	SD of mean
	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)		
PDA	0.70	0.70	0.54	0.60	0.64	0.58	0.54	0.54	0.50	0.54	0.56	0.54	0.54	0.60	0.56	0.58	0.05
PDA+Ammonium chloride	0.56	0.60	0.58	0.54	0.57	0.66	0.64	0.74	0.70	0.69	0.56	0.56	0.64	0.56	0.58	0.61	0.06
PDA+Yeast extract	0.94	0.96	0.94	0.96	0.95	0.78	0.74	0.70	0.66	0.72	0.80	0.74	0.72	0.66	0.73	0.80	0.13
PDA+Malt extract	0.70	0.72	0.72	0.72	0.72	0.62	0.56	0.62	0.64	0.61	0.54	0.60	0.50	0.58	0.56	0.63	0.08

Table 21 The mycelia growth rate of *Ganoderma lucidum* strain G2 grown on PDA and PDA contain with nitrogen sources at 30ºC for 5 days.

Medium	Replication 1					Replication 2					Replication 3					Total mean (cm)	SD of mean
	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)		
PDA	0.70	0.70	0.64	0.72	0.69	0.66	0.62	0.60	0.62	0.63	0.60	0.60	0.46	0.48	0.54	0.62	0.08
PDA+Ammonium chloride	0.42	0.56	0.50	0.46	0.49	0.40	0.44	0.44	0.42	0.43	0.58	0.68	0.62	0.70	0.65	0.52	0.11
PDA+Yeast extract	1.08	0.70	1.20	1.14	1.03	1.12	1.02	0.98	1.06	1.05	1.20	1.24	1.18	1.18	1.20	1.09	0.09
PDA+Malt extract	0.96	0.92	0.92	0.94	0.94	0.78	0.48	0.78	0.72	0.69	1.00	0.82	1.02	1.06	0.98	0.87	0.15

Table 22 The mycelia growth rate of *Ganoderma lucidum* strain G2 grown on PDA and PDA contain with nitrogen sources at 35ºC for 5 days.

Medium	Replication 1					Replication 2					Replication 3					Total mean (cm)	SD of mean
	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)		
PDA	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Ammonium chloride	0.16	0.16	0.16	0.16	0.16	0.16	0.14	0.16	0.14	0.15	0.16	0.16	0.16	0.16	0.16	0.16	0.01
PDA+Yeast extract	0.18	0.18	0.16	0.18	0.18	0.26	0.26	0.28	0.28	0.27	0.38	0.38	0.38	0.38	0.38	0.28	0.10
PDA+Malt extract	0.16	0.16	0.14	0.14	0.15	0.16	0.18	0.18	0.16	0.17	0.16	0.18	0.16	0.16	0.17	0.16	0.01

Table 23 The mycelia growth rate of *Ganoderma lucidum* strain G2 grown on PDA and PDA contain with nitrogen sources at 37ºC for 5 days.

Medium	Replication 1					Replication 2					Replication 3					Total mean (cm)	SD of mean
	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)		
PDA	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Ammonium chloride	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Yeast extract	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Malt extract	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00

Table 24 The mycelia growth rate of *Ganoderma lucidum* strain G2 grown on PDA and PDA contain with nitrogen sources at 40ºC for 5 days.

Medium	Replication 1					Replication 2					Replication 3					Total mean (cm)	SD of mean
	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)		
PDA	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Ammonium chloride	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Yeast extract	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Malt extract	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00

Table 25 The mycelia growth rate of *Ganoderma lucidum* strain G45 grown on PDA and PDA contain with nitrogen sources at 25ºC for 5 days.

Medium	Replication 1					Replication 2					Replication 3					Total mean (cm)	SD of mean
	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)		
PDA	0.62	0.64	0.60	0.64	0.63	0.74	0.70	0.72	0.74	0.73	0.72	0.68	0.66	0.72	0.70	0.68	0.05
PDA+Ammonium chloride	0.48	0.46	0.50	0.44	0.47	0.50	0.50	0.52	0.50	0.51	0.56	0.58	0.56	0.56	0.57	0.51	0.05
PDA+Yeast extract	0.82	0.84	0.84	0.86	0.84	0.80	0.80	0.84	0.84	0.82	0.98	0.98	0.98	0.98	0.98	0.88	0.09
PDA+Malt extract	0.86	0.78	0.84	0.84	0.83	0.88	0.90	0.78	0.84	0.85	0.70	0.68	0.70	0.70	0.70	0.79	0.08

Table 26 The mycelia growth rate of *Ganoderma lucidum* strain G45 grown on PDA and PDA contain with nitrogen sources at 30ºC for 5 days.

Medium	Replication 1					Replication 2					Replication 3					Total mean (cm)	SD of mean
	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)		
PDA	1.24	1.24	1.24	1.20	1.23	1.22	1.26	1.22	1.22	1.23	1.14	1.14	1.12	1.14	1.14	1.20	0.05
PDA+Ammonium chloride	0.94	0.90	0.88	0.90	0.91	0.92	0.94	0.92	0.94	0.93	0.86	0.88	0.90	0.88	0.88	0.91	0.03
PDA+Yeast extract	1.20	1.18	1.20	1.20	1.20	1.22	1.22	1.22	1.24	1.23	1.24	1.26	1.26	1.22	1.25	1.22	0.03
PDA+Malt extract	1.10	1.10	1.10	1.08	1.10	1.18	1.18	1.22	1.20	1.20	1.18	1.16	1.04	1.04	1.11	1.13	0.06

Table 27 The mycelia growth rate of *Ganoderma lucidum* strain G45 grown on PDA and PDA contain with nitrogen sources at 35ºC for 5 days.

Medium	Replication 1					Replication 2					Replication 3					Total mean (cm)	SD of mean
	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)		
PDA	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Ammonium chloride	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Yeast extract	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Malt extract	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00

Table 28 The mycelia growth rate of *Ganoderma lucidum* strain G45 grown on PDA and PDA contain with nitrogen sources at 37ºC for 5 days.

Medium	Replication 1					Replication 2					Replication 3					Total mean (cm)	SD of mean
	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)		
PDA	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Ammonium chloride	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Yeast extract	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Malt extract	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00

Table 29 The mycelia growth rate of *Ganoderma lucidum* strain G45 grown on PDA and PDA contain with nitrogen sources at 40ºC for 5 days.

Medium	Replication 1					Replication 2					Replication 3					Total mean (cm)	SD of mean
	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)		
PDA	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Ammonium chloride	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Yeast extract	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Malt extract	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00

Table 30 The mycelia growth rate of *Ganoderma lucidum* strain G5A grown on PDA and PDA contain with nitrogen sources at 25ºC for 5 days.

Medium	Replication 1					Replication 2					Replication 3					Total mean (cm)	SD of mean
	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)		
PDA	0.44	0.40	0.40	0.46	0.43	0.38	0.46	0.34	0.36	0.39	0.40	0.42	0.40	0.46	0.42	0.41	0.02
PDA+Ammonium chloride	0.40	0.38	0.40	0.36	0.39	0.30	0.32	0.32	0.34	0.32	0.32	0.32	0.30	0.32	0.32	0.34	0.04
PDA+Yeast extract	0.52	0.48	0.48	0.50	0.50	0.56	0.56	0.56	0.56	0.56	0.58	0.58	0.56	0.58	0.58	0.54	0.04
PDA+Malt extract	0.46	0.44	0.42	0.42	0.44	0.42	0.40	0.44	0.48	0.44	0.54	0.54	0.52	0.50	0.53	0.47	0.05

Table 31 The mycelia growth rate of *Ganoderma lucidum* strain G5A grown on PDA and PDA contain with nitrogen sources at 30ºC for 5 days.

Medium	Replication 1					Replication 2					Replication 3					Total mean (cm)	SD of mean
	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)		
PDA	0.36	0.36	0.38	0.36	0.37	0.34	0.42	0.40	0.34	0.38	0.30	0.28	0.32	0.32	0.31	0.35	0.04
PDA+Ammonium chloride	0.48	0.56	0.56	0.52	0.53	0.44	0.42	0.44	0.42	0.43	0.30	0.30	0.34	0.30	0.31	0.42	0.11
PDA+Yeast extract	0.46	0.44	0.44	0.42	0.44	0.46	0.50	0.48	0.48	0.48	0.52	0.52	0.54	0.52	0.53	0.48	0.04
PDA+Malt extract	0.48	0.46	0.50	0.44	0.47	0.36	0.36	0.38	0.34	0.36	0.38	0.48	0.44	0.40	0.43	0.42	0.06

Table 32 The mycelia growth rate of *Ganoderma lucidum* strain G5A grown on PDA and PDA contain with nitrogen sources at 35ºC for 5 days.

Medium	Replication 1					Replication 2					Replication 3					Total mean (cm)	SD of mean
	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)		
PDA	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Ammonium chloride	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Yeast extract	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.16	0.16	0.16	0.16	0.16	0.15	0.01
PDA+Malt extract	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00

Table 33 The mycelia growth rate of *Ganoderma lucidum* strain G5A grown on PDA and PDA contain with nitrogen sources at 37ºC for 5 days.

Medium	Replication 1					Replication 2					Replication 3					Total mean (cm)	SD of mean
	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)		
PDA	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Ammonium chloride	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Yeast extract	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Malt extract	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00

Table 34 The mycelia growth rate of *Ganoderma lucidum* strain G5A grown on PDA and PDA contain with nitrogen sources at 40ºC for 5 days.

Medium	Replication 1					Replication 2					Replication 3					Total mean (cm)	SD of mean
	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)		
PDA	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Ammonium chloride	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Yeast extract	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Malt extract	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00

Table 35 The mycelia growth rate of *Ganoderma lucidum* strain G5Z grown on PDA and PDA contain with nitrogen sources at 25ºC for 5 days.

Medium	Replication 1					Replication 2					Replication 3					Total mean (cm)	SD of mean
	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)		
PDA	0.34	0.28	0.30	0.34	0.32	0.40	0.38	0.40	0.42	0.40	0.32	0.28	0.28	0.30	0.30	0.34	0.06
PDA+Ammonium chloride	0.42	0.20	0.38	0.38	0.35	0.32	0.32	0.32	0.36	0.33	0.30	0.32	0.30	0.30	0.31	0.33	0.02
PDA+Yeast extract	0.34	0.36	0.38	0.34	0.36	0.36	0.38	0.42	0.38	0.39	0.40	0.38	0.36	0.34	0.37	0.37	0.02
PDA+Malt extract	0.34	0.32	0.34	0.32	0.33	0.44	0.42	0.42	0.40	0.42	0.42	0.58	0.56	0.46	0.51	0.42	0.09

Table 36 The mycelia growth rate of *Ganoderma lucidum* strain G5Z grown on PDA and PDA contain with nitrogen sources at 30ºC for 5 days.

Medium	Replication 1					Replication 2					Replication 3					Total mean (cm)	SD of mean
	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)		
PDA	0.28	0.26	0.28	0.28	0.28	0.34	0.32	0.36	0.34	0.34	0.32	0.26	0.36	0.36	0.33	0.31	0.03
PDA+Ammonium chloride	0.42	0.44	0.40	0.40	0.42	0.46	0.44	0.36	0.40	0.42	0.28	0.28	0.30	0.30	0.29	0.37	0.07
PDA+Yeast extract	0.54	0.52	0.52	0.54	0.53	0.56	0.56	0.56	0.58	0.57	0.62	0.62	0.66	0.66	0.64	0.58	0.06
PDA+Malt extract	0.46	0.40	0.50	0.46	0.46	0.46	0.48	0.42	0.46	0.46	0.52	0.52	0.52	0.54	0.53	0.48	0.04

Table 37 The mycelia growth rate of *Ganoderma lucidum* strain G5Z grown on PDA and PDA contain with nitrogen sources at 35ºC for 5 days.

Medium	Replication 1					Replication 2					Replication 3					Total mean (cm)	SD of mean
	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)		
PDA	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Ammonium chloride	0.14	0.14	0.14	0.14	0.14	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.13	0.01
PDA+Yeast extract	0.14	0.14	0.14	0.14	0.14	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.15	0.01
PDA+Malt extract	0.16	0.16	0.16	0.14	0.16	0.14	0.14	0.14	0.14	0.14	0.16	0.14	0.16	0.16	0.16	0.15	0.01

Table 38 The mycelia growth rate of *Ganoderma lucidum* strain G5Z grown on PDA and PDA contain with nitrogen sources at 37ºC for 5 days.

Medium	Replication 1					Replication 2					Replication 3					Total mean (cm)	SD of mean
	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)		
PDA	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Ammonium chloride	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Yeast extract	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Malt extract	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00

Table 39 The mycelia growth rate of *Ganoderma lucidum* strain G5Z grown on PDA and PDA contain with nitrogen sources at 40ºC for 5 days.

Medium	Replication 1					Replication 2					Replication 3					Total mean (cm)	SD of mean
	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)		
PDA	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Ammonium chloride	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Yeast extract	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Malt extract	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00

Table 40 The mycelia growth rate of *Ganoderma lucidum* strain HHK grown on PDA and PDA contain with nitrogen sources at 25ºC for 5 days.

Medium	Replication 1					Replication 2					Replication 3					Total mean (cm)	SD of mean
	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)		
PDA	0.60	0.56	0.60	0.62	0.60	0.54	0.54	0.48	0.52	0.52	0.58	0.56	0.56	0.52	0.56	0.56	0.04
PDA+Ammonium chloride	0.50	0.50	0.50	0.52	0.51	0.44	0.44	0.48	0.52	0.47	0.60	0.60	0.64	0.60	0.61	0.53	0.07
PDA+Yeast extract	0.80	0.84	0.82	0.78	0.81	0.92	0.82	0.86	0.92	0.88	0.72	0.74	0.74	0.76	0.74	0.81	0.07
PDA+Malt extract	0.64	0.68	0.60	0.64	0.64	0.58	0.56	0.56	0.56	0.57	0.66	0.64	0.78	0.72	0.70	0.64	0.07

Table 41 The mycelia growth rate of *Ganoderma lucidum* strain HHK grown on PDA and PDA contain with nitrogen sources at 30ºC for 5 days.

Medium	Replication 1					Replication 2					Replication 3					Total mean (cm)	SD of mean
	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)		
PDA	1.32	1.36	1.36	1.34	1.35	1.30	1.32	1.36	1.40	1.35	1.34	1.30	1.32	1.34	1.33	1.34	0.01
PDA+Ammonium chloride	1.28	1.28	1.34	1.32	1.31	1.18	1.22	1.16	1.16	1.18	0.94	0.94	1.16	0.96	1.00	1.16	0.15
PDA+Yeast extract	1.42	1.42	1.42	1.42	1.42	1.44	1.36	1.42	1.46	1.42	1.38	1.40	1.34	1.32	1.36	1.40	0.03
PDA+Malt extract	1.24	1.24	1.28	1.28	1.26	1.36	1.38	1.40	1.38	1.38	1.28	1.30	1.30	1.24	1.28	1.31	0.06

Table 42 The mycelia growth rate of *Ganoderma lucidum* strain HHK grown on PDA and PDA contain with nitrogen sources at 35ºC for 5 days.

Medium	Replication 1					Replication 2					Replication 3					Total mean (cm)	SD of mean
	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)		
PDA	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.00
PDA+Ammonium chloride	0.20	0.22	0.22	0.20	0.21	0.20	0.20	0.22	0.22	0.21	0.18	0.18	0.18	0.18	0.18	0.20	0.02
PDA+Yeast extract	0.22	0.20	0.20	0.22	0.21	0.20	0.18	0.20	0.18	0.19	0.20	0.20	0.20	0.20	0.20	0.20	0.01
PDA+Malt extract	0.20	0.22	0.18	0.18	0.20	0.20	0.20	0.20	0.20	0.20	0.18	0.18	0.18	0.18	0.18	0.19	0.01

Table 43 The mycelia growth rate of *Ganoderma lucidum* strain HHK grown on PDA and PDA contain with nitrogen sources at 37ºC for 5 days.

Medium	Replication 1					Replication 2					Replication 3					Total mean (cm)	SD of mean
	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)		
PDA	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Ammonium chloride	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Yeast extract	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Malt extract	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00

Table 44 The mycelia growth rate of *Ganoderma lucidum* strain HHK grown on PDA and PDA contain with nitrogen sources at 40ºC for 5 days.

Medium	Replication 1					Replication 2					Replication 3					Total mean (cm)	SD of mean
	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)		
PDA	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Ammonium chloride	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Yeast extract	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Malt extract	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00

Table 45 The mycelia growth rate of *Ganoderma lucidum* strain KALASIN grown on PDA and PDA contain with nitrogen sources at 25ºC for 5 days.

Medium	Replication 1					Replication 2					Replication 3					Total mean (cm)	SD of mean
	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)		
PDA	1.06	1.04	1.06	1.12	1.07	1.22	1.18	1.16	1.20	1.19	1.18	1.18	1.14	1.16	1.17	1.14	0.06
PDA+Ammonium chloride	1.02	1.10	1.08	1.08	1.07	1.02	1.04	1.02	0.98	1.02	1.08	1.02	1.00	0.96	1.02	1.03	0.03
PDA+Yeast extract	1.30	1.32	1.28	1.28	1.30	1.14	1.22	1.20	1.24	1.20	1.26	1.26	1.26	1.28	1.27	1.25	0.05
PDA+Malt extract	1.02	1.04	1.04	0.98	1.02	1.08	1.00	1.00	1.08	1.04	1.16	1.04	1.04	1.02	1.07	1.04	0.02

Table 46 The mycelia growth rate of *Ganoderma lucidum* strain KALASIN grown on PDA and PDA contain with nitrogen sources at 30ºC for 5 days.

Medium	Replication 1					Replication 2					Replication 3					Total mean (cm)	SD of mean
	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)		
PDA	1.80	1.80	1.80	1.80	1.80	1.70	1.80	1.60	1.58	1.67	1.70	1.60	1.48	1.58	1.59	1.69	0.11
PDA+Ammonium chloride	1.74	1.66	1.70	1.70	1.70	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.77	0.06
PDA+Yeast extract	1.64	1.66	1.66	1.64	1.65	1.80	1.80	1.80	1.80	1.80	1.64	1.66	1.80	1.80	1.73	1.73	0.08
PDA+Malt extract	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80	0.00

Table 47 The mycelia growth rate of *Ganoderma lucidum* strain KALASIN grown on PDA and PDA contain with nitrogen sources at 35ºC for 5 days.

Medium	Replication 1					Replication 2					Replication 3					Total mean (cm)	SD of mean
	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)		
PDA	1.32	1.42	1.48	1.38	1.40	1.42	1.52	1.52	1.48	1.49	1.50	1.50	1.52	1.56	1.52	1.47	0.06
PDA+Ammonium chloride	1.00	0.96	1.04	1.02	1.01	1.10	1.26	1.24	1.14	1.19	1.14	1.10	1.22	1.12	1.15	1.11	0.09
PDA+Yeast extract	1.54	1.56	1.56	1.54	1.55	1.50	1.46	1.50	1.48	1.49	1.56	1.56	1.54	1.52	1.55	1.53	0.04
PDA+Malt extract	1.58	1.58	1.62	1.62	1.60	1.48	1.50	1.48	1.46	1.48	1.62	1.58	1.60	1.62	1.61	1.56	0.07

Table 48 The mycelia growth rate of *Ganoderma lucidum* strain KALASIN grown on PDA and PDA contain with nitrogen sources at 37ºC for 5 days.

Medium	Replication 1					Replication 2					Replication 3					Total mean (cm)	SD of mean
	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)		
PDA	0.30	0.32	0.32	0.28	0.31	0.24	0.26	0.26	0.26	0.26	0.28	0.32	0.30	0.28	0.30	0.29	0.03
PDA+Ammonium chloride	0.34	0.32	0.36	0.34	0.34	0.28	0.30	0.30	0.32	0.30	0.30	0.34	0.34	0.34	0.33	0.32	0.02
PDA+Yeast extract	0.36	0.42	0.40	0.38	0.39	0.38	0.34	0.40	0.34	0.37	0.40	0.42	0.42	0.40	0.41	0.39	0.02
PDA+Malt extract	0.42	0.46	0.44	0.44	0.44	0.50	0.48	0.48	0.46	0.48	0.44	0.42	0.42	0.46	0.44	0.45	0.02

Table 49 The mycelia growth rate of *Ganoderma lucidum* strain KALASIN grown on PDA and PDA contain with nitrogen sources at 40ºC for 5 days.

Medium	Replication 1					Replication 2					Replication 3					Total mean (cm)	SD of mean
	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)		
PDA	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Ammonium chloride	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Yeast extract	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Malt extract	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00

Table 50 The mycelia growth rate of *Ganoderma lucidum* strain SUN grown on PDA and PDA contain with nitrogen sources at 25ºC for 5 days.

Medium	Replication 1					Replication 2					Replication 3					Total mean (cm)	SD of mean
	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)		
PDA	0.42	0.40	0.38	0.40	0.40	0.40	0.38	0.36	0.38	0.38	0.40	0.36	0.38	0.30	0.36	0.38	0.02
PDA+Ammonium chloride	0.46	0.46	0.46	0.44	0.46	0.50	0.48	0.48	0.48	0.49	0.42	0.42	0.40	0.40	0.41	0.45	0.04
PDA+Yeast extract	0.42	0.46	0.46	0.38	0.43	0.40	0.40	0.44	0.46	0.43	0.44	0.46	0.46	0.42	0.45	0.43	0.01
PDA+Malt extract	0.36	0.40	0.34	0.34	0.36	0.40	0.40	0.40	0.40	0.40	0.36	0.34	0.38	0.32	0.35	0.37	0.03

Table 51 The mycelia growth rate of *Ganoderma lucidum* strain SUN grown on PDA and PDA contain with nitrogen sources at 30ºC for 5 days.

Medium	Replication 1					Replication 2					Replication 3					Total mean (cm)	SD of mean
	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)		
PDA	0.72	0.74	0.76	0.92	0.79	0.80	0.78	0.62	0.70	0.73	0.64	0.62	0.70	0.64	0.65	0.72	0.07
PDA+Ammonium chloride	0.54	0.56	0.56	0.52	0.55	0.52	0.62	0.64	0.52	0.58	0.62	0.62	0.68	0.64	0.64	0.59	0.05
PDA+Yeast extract	0.90	0.92	0.88	0.98	0.92	0.96	0.90	0.90	0.94	0.93	0.84	0.88	0.90	0.98	0.90	0.92	0.01
PDA+Malt extract	0.58	0.56	0.58	0.58	0.58	0.84	0.74	0.84	0.84	0.82	0.98	0.98	0.92	1.02	0.98	0.79	0.20

Table 52 The mycelia growth rate of *Ganoderma lucidum* strain SUN grown on PDA and PDA contain with nitrogen sources at 35ºC for 5 days.

Medium	Replication 1					Replication 2					Replication 3					Total mean (cm)	SD of mean
	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)		
PDA	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Ammonium chloride	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Yeast extract	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Malt extract	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00

Table 53 The mycelia growth rate of *Ganoderma lucidum* strain SUN grown on PDA and PDA contain with nitrogen sources at 37ºC for 5 days.

Medium	Replication 1					Replication 2					Replication 3					Total mean (cm)	SD of mean
	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)		
PDA	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Ammonium chloride	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Yeast extract	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Malt extract	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00

Table 54 The mycelia growth rate of *Ganoderma lucidum* strain SUN grown on PDA and PDA contain with nitrogen sources at 40ºC for 5 days.

Medium	Replication 1					Replication 2					Replication 3					Total mean (cm)	SD of mean
	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)		
PDA	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Ammonium chloride	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Yeast extract	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Malt extract	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00

Table 55 The mycelia growth rate of *Ganoderma sinensis* strain A2 grown on PDA and PDA contain with nitrogen sources at 25ºC for 5 days.

Medium	Replication 1					Replication 2					Replication 3					Total mean (cm)	SD of mean
	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)		
PDA	0.70	0.70	0.66	0.64	0.68	0.72	0.64	0.62	0.68	0.67	0.64	0.62	0.60	0.60	0.62	0.65	0.03
PDA+Ammonium chloride	0.54	0.54	0.58	0.56	0.56	0.58	0.58	0.58	0.56	0.58	0.56	0.56	0.54	0.56	0.56	0.56	0.01
PDA+Yeast extract	0.78	0.76	0.72	0.74	0.75	0.78	0.78	0.76	0.74	0.77	0.72	0.74	0.72	0.70	0.72	0.75	0.02
PDA+Malt extract	0.70	0.72	0.72	0.70	0.71	0.68	0.70	0.70	0.70	0.70	0.66	0.68	0.68	0.66	0.67	0.69	0.02

Table 56 The mycelia growth rate of *Ganoderma sinensis* strain A2 grown on PDA and PDA contain with nitrogen sources at 30ºC for 5 days.

Medium	Replication 1					Replication 2					Replication 3					Total mean (cm)	SD of mean
	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)		
PDA	0.70	0.70	0.70	0.70	0.70	0.34	0.30	0.32	0.28	0.31	0.32	0.30	0.32	0.34	0.32	0.44	0.22
PDA+Ammonium chloride	0.70	0.66	0.68	0.66	0.68	0.68	0.70	0.70	0.70	0.70	0.66	0.70	0.66	0.68	0.68	0.68	0.01
PDA+Yeast extract	0.70	0.66	0.68	0.66	0.68	0.72	0.74	0.72	0.76	0.74	0.68	0.70	0.70	0.70	0.70	0.70	0.03
PDA+Malt extract	0.80	0.78	0.78	0.78	0.79	0.78	0.78	0.78	0.78	0.78	0.70	0.62	0.74	0.70	0.69	0.75	0.05

Table 57 The mycelia growth rate of *Ganoderma sinensis* strain A2 grown on PDA and PDA contain with nitrogen sources at 35ºC for 5 days.

Medium	Replication 1					Replication 2					Replication 3					Total mean (cm)	SD of mean
	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)		
PDA	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Ammonium chloride	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Yeast extract	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Malt extract	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00

Table 58 The mycelia growth rate of *Ganoderma sinensis* strain A2 grown on PDA and PDA contain with nitrogen sources at 37ºC for 5 days.

Medium	Replication 1					Replication 2					Replication 3					Total mean (cm)	SD of mean
	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)		
PDA	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Ammonium chloride	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Yeast extract	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Malt extract	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00

Table 59 The mycelia growth rate of *Ganoderma sinensis* strain A2 grown on PDA and PDA contain with nitrogen sources at 40ºC for 5 days.

Medium	Replication 1					Replication 2					Replication 3					Total mean (cm)	SD of mean
	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)	line1 (cm)	line2 (cm)	line3 (cm)	line4 (cm)	mean (cm)		
PDA	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Ammonium chloride	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Yeast extract	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00
PDA+Malt extract	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.00

The sequence of ITS regions of *Ganoderma lucidum* A1

TCGAGTTTTGACCGGGTTGTAGCTGGCCTTCTGAGGCATGTGCACGCCCTGCTCATCCACTCTACACCTGTGCACT
TACTGTGGGCTTCAGATTGCGAGGCACGCTCTTTACCGGGCTTGCGGAGCATATCTGTGCCTGCGTTTATCACAAA
CTCTATAAAGTAACAGAATGTGTATTGCGATGTAACACATCTATATACAACTTTCAGCAACGGATCTCTTGGCTCT
CGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAA
CGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGAAATCTTCAACCTACAAGCTTTTG
TGGTTTGTAGGCTTGGACTTGGAGGCTTGTCGGCCGTTATCGGTCGGCTCCTCTTAAATGCATTAGCTTGGTTCCT
TGCGGATCGGCTCTCGGTGTGATAATGTCTACGCCGTGACCGTGAAGCGTTTGGCGAGCTTCTAACCGTCTTATAA
GACAGCTTTA

The sequence of ITS regions of *Ganoderma lucidum* DOA

TCGAGTTTTGACCGGGTTGTAGCTGGCCTTCCGAGGCATGTGCACGCCCTGCTCATCCACTCTACACCTGTGCACT
TGCTGTGGGCTTCAGATTGCGAGGCACGCTCTTTACCGGGCTTGCGGAGCATATCTGTGCCTGCGTTTATCACAAA
CTCTATAAAGTAACAGAATGTGTATTGCGATGTAACACATCTATATACAACTTTCAGCAACGGATCTCTTGGCTCT
CGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAA
CGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGAAATCTTCAACCTACAAGCTTTTG
TGGTTTGTAGGCTTGGACTTGGAGGCTTGTCGGCCGTTATCGGTCGGCTCCTCTTAAATGCATTAGCTTGGTTCCT
TGCGGATCGGCTCTCGGTGTGATAATGTCTACGCCGTGACCGTGAAGCGTTTGGCGAGCTTCTAACCGTCTTATAA
GACAGCTTTA

The sequence of ITS regions of *Ganoderma lucidum* G2

TCGAGTTTTGACCGGGTTGTAGCTGGCCTTCCGAGGCATGTGCACGCCCTGCTCATCCACTCTACACCTGTGCACT
TACTGTGGGCTTCAGATTGCGAGGCACGCTCTTTACCGGGCTTGCGGAGCATATCTGTGCCTGCGTTTATCACAAA
CTCTATAAAGTAACAGAATGTGTATTGCGATGTAACACATCTATATGCAATTTTCAGCAACGGATCTCTTGGCTCT
CGCATCGATGAAGTACGCAGCGAAGTGCGCTAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAA
CGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGAAATCTTCAACCTACAAGCTTTTG
TGGTTTGTAGGCTTGGACTTGGAGGCTTGTCGGCCGTTATCGGTCGGCTCCTCTTAAATGCATTAGCTTGGTTCCT
TGCGGATCGGCTCTCGGTGTGATAATGTCTACGCCGTGACCGTGAAGCGTTTGGCGAGCTTCTAACCGTCTTATAA
GACAGCTTTA

The sequence of ITS regions of *Ganoderma lucidum* G45

TCGAGTTTTGACCGGGTTGTAGCTGGCCTTCCGAGGCATGTGCACGCCCTGCTCATCCACTCTACACCTGTGCACT
TACTGTGGGCTTCAGATTGCGAGGCACGCTCTTTACCGGGCTTGCGGAGCATATCTGTGCCTGCGTTTATCACAAA
CTCTATAAAGTAACAGAATGTGTATTGCGATGTAACACATCTATATACAACTTTCAGCAACGGATCTCTTGGCTCT
CGCATCGGTGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAA
CGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGAAATCTTCAACCTACAAGCTTTTG
TGGTTTGTAGGCTTGGACTTGGAAGCTTGTCGGCCGTTATCGGTCGGCTCCTCTTAAATGCATTAGCTTGGTTCCT
TGCGGATCGGCTCTCGGTGTGATAATGTCTACGCCGTGACCGTGAAGCGTTTGGCGAGCTTCTAACCGTCTTATAA
GACAGCTTTA

Figure 23 The sequences of ITS regions of *Ganoderma lucidum* strains A1, DOA, G2, G45, G5A, G5Z, HHK, KALASIN, SUN and A2.

The sequence of ITS regions of *Ganoderma lucidum* G5A

TCGAGTTTTGACCGGGTTGTAGCTGGCCTTCCGAGGCATGTGCACGCCCTGCTCATCCACTCTACACCTGTGCACT
TACTGTGGGCTTCAGATTGCGAGGCACGCTCTTTACCGGGCTTGCGGAGCATATCTGTGCCTGCGTTTATCACAAA
CTCTATAAAGTAACAGAATGTGTATTGCGATGTAACACATCTATATACAACTTTCAGCAACGGATTTGTTGGCTCT
CGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATTTTTGAA
CGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGAAATCTTCAACCTACAAGCTTTTG
TGGTTTGTAGGCTTGGACTTGGAGGCTTGTCGGCCATTATCGGTCGGCTCCTCTTAAATGCATTAGCTTGGTTCCT
TGCGGATCGGCTCTCGGTGTGATAATGTCTACGCCGTGACCGTGAAGCGTTTGGCGAGCTTCTAACCGTCTTATAA
GACAGCTTTA

The sequence of ITS regions of *Ganoderma lucidum* G5Z

TCGAGTTTTGACCGGGTTGTAGCTGGCCTTCCGAGGCATGTGCACGCCCTGCTCATCCACTCTACACCTGTGCACT
TACTGTGGGTTTCAGATTGCGAGGCACGCTCTTTGCCGGGCTTGCGGAGCATATCTGTGCCTGCGTTTATCACAAA
CTCTATAAAGTAACAGAATGTGTATTGCGATGTAACACATCTATATACAACTTTCAGCAACGGATCTCTTGGCTCT
CGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAA
CGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGAAATCTTCAACCTACAAGCTTTTG
TGGTTTGTAGGCTTGGACTTGGAGGCTTGTCGGCCGTTATCGGTCGGCTCCTCTTAAATGCATTAGCTTGGTTCCT
TGCGGATCGGCTCTCGGTGTGATAATGTCTACGCCGTGACCGTGAAGCGTTTGGCGAGCTTCTAACCGTCTTATAA
GACAGCTTTA

The sequence of ITS regions of *Ganoderma lucidum* HHK

TCGAATTTTGACCGGGTTGTAGCTGGCCTTCCGAGGCATGTGCACGTCCTGCTCATCCACTCTACACCTGTGCACT
TACTGTGGGCTTCAGATTGCGAGGCACGCTCTTTACCGGGCTTGCGGAGCATATCTGTGCCTGCGTTTATCACAAA
CTCTATAAAGTAACAGAATGTGTATTGCGATGTAACACATCTATATACAACTTTCAGCAACGGATCTCTTGGCTCT
CGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAA
CGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGAAATCTTCAACCTACAAGCTTTTC
TGGTTTGTAGGCTTGGACTTGGAGGCTTGTCGGCCGTTATCGGTCGGCTCCTCTTAAATGCATTAGCTTGGTTCCT
TGCGGATCGGCTCTCGGTGTGATAACGTCTACGCCGCGGCCGTGAAGCGTTTGGCGAGCTTCTAACCGTCTTATAA
GACAGCTTTA

The sequence of ITS regions of *Ganoderma lucidum* KALASIN

TCGAGTTTTGACTGGGTTGTAGCTGGCCTTCCGAGGCATGTGCACGCCCTGCTCATCCACTCTACACCTGTGCACT
TACTGTGGGCTTCAGATCGTAAAACGAGTCCCTTTACCGGGCTTGCGGAGCGTGTCTGTGCCTGCGTTTATCACAA
AACTCTATAAAGTATCAGAATGTGTATTGCGATGTAACGCATCTATATACAACTTTCAGCAACGGATCTCTTGGCT
CTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATTGCAGAATTCAGT
GAATCATCGAATCTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGAAATC
TTCAACCTGCAAGCTTTTGTGGTTTGTAGGCTTGGACTTGGAGGCTTGTCGGCCGTTGTTGGTCGGCTCCTCTTAA
ATGCATTAGCTTGGTTCCTTGCGGATCGGCTCTCAGTGTGATAATGTCTACGCTGCGACCGTGAAGCGTTTGGCGA
GCTTCTAACCGTCTCAGTTGGAGACAACTTTA

Figure 23 The sequences of ITS regions of *Ganoderma lucidum* strains A1, DOA, G2, G45, G5A, G5Z, HHK, KALASIN, SUN and A2.(continuous)

The sequence of ITS regions of *Ganoderma lucidum* SUN

TCGAGTTTTGACCGGGTTGTAGCTGGCCTTCCGAGGCATGTGCACGCCCTGCTCATCCACTCTACACCTGTGCACT
TACTGTGGGCTTCAGATTGCGAGGCACGCTCTTTACCGGGCTTGCGGAGCATATCTGTGCCTGCGTTTATCACAAA
CTCTATAAAGTAACAGAATGTGTATTGCGATGTAACACATCTATATACAACTTTCAGCAACGGATCTCTTGGCTCT
CGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAA
CGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGAAATCTTCAACCTACAAGCTTTTG
TGGTTTGTAGGCTTGGACTTGGAGGCTTGTCGGCCGTTATCGGTCGGCTCCTCTTAAATGCATTAGCTTGGTTCCT
TGCGGATCGGCTCTCGGTGTGATAATGTCTACGCCGCGACCGTGAAGCGTTTGGCGAGCTTCTAACCGTCTTATAA
GACAGCTTTA

The sequence of ITS regions of *Ganoderma sinensis* A2

TCGAGTTTTGACTGGGTTGTAGCTGGCCTTCCGAGGCACTGTGCACGCCCTGCTCATCCACTCTACACCTGTGCAC
TTACTGTGGGTTACGGACTGTGGAGCGGGCTCTGCGGAGCTCGTGAAGCGCGTCTGTGCCTGCGTTTTATTACAAA
CACTATAAAGTCTTAGAATGTGTATTGCGATGTAACGCATCTATATACAACTTTCAGCAACGGATCTCTTGGCTCT
CGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAA
CGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGAAATCTTCAACCTACAAGGTCTTT
GTAAAGGCTTTTTAGGCTTGGACTTGGAGGCTTGTCGGTCTTTATAGGTCGGCTCCTCTTAAACGCATTAGCTTGA
TTCCTTGCGGATCGGTTTGTCGGTGTGATAATGTCTACGCCGCGACCGTGAAGCGTTTTGGCAAGCTTCTAACGGT
CTCTGTATGGAGACAAAGCTTA

Figure 23 The sequences of ITS regions of *Ganoderma lucidum* strains A1, DOA, G2,G45, G5A, G5Z, HHK, KALASIN, SUN and A2.(continuous)

Table 60 The polymorphism pattern of *Ganoderma lucidum* strains and *Ganoderma sinensis* generated by primer A03.

Bane	Sample									
	A1	DOA	G2	G45	G5A	G5Z	HHK	KALASIN	SUN	A2
1	0	1	0	1	1	1	0	1	1	0
2	1	1	0	1	1	1	0	1	0	1
3	0	1	0	1	1	1	0	1	0	0
4	0	0	0	0	0	0	0	0	0	1
5	1	1	1	1	1	1	1	1	1	0
6	0	1	0	1	0	0	0	1	0	1
7	1	1	0	1	0	0	0	1	0	1
8	1	0	0	0	1	1	0	1	0	0
9	1	1	0	1	1	1	0	1	0	1
10	0	1	0	1	0	0	0	0	0	1

Table 61 The polymorphism pattern of *Ganoderma lucidum* strains and *Ganoderma sinensis* generated by primer A04.

Bane	Sample									
	A1	DOA	G2	G45	G5A	G5Z	HHK	KALASIN	SUN	A2
1	1	1	1	1	1	1	0	0	0	0
2	1	1	1	1	1	1	1	1	0	1
3	1	1	1	1	1	1	1	1	1	0
4	1	1	0	1	1	1	1	0	0	0
5	0	0	0	0	0	0	0	1	0	1
6	0	1	0	1	1	1	0	1	0	1
7	0	1	1	1	1	1	1	1	1	1
8	1	0	0	0	0	0	1	0	0	1
9	1	1	1	1	1	1	1	1	0	0
10	0	0	0	0	0	0	1	1	0	0

Table 62 The polymorphism pattern of *Ganoderma lucidum* strains and *Ganoderma sinensis* generated by primer A08.

Bane	Sample									
	A1	DOA	G2	G45	G5A	G5Z	HHK	KALASIN	SUN	A2
1	1	1	1	1	1	1	0	0	0	0
2	1	1	1	1	1	1	1	1	0	1
3	1	1	1	1	1	1	1	1	1	0
4	1	1	0	1	1	1	1	0	0	0
5	0	0	0	0	0	0	0	1	0	1
6	0	1	0	1	1	1	0	1	0	1
7	0	1	1	1	1	1	1	1	1	1
8	1	0	0	0	0	0	1	0	0	1
9	1	1	1	1	1	1	1	1	0	0
10	0	0	0	0	0	0	1	1	0	0

Table 63 The polymorphism pateern of *Ganoderma lucidum* strains and *Ganoderma sinensis* generated by primer A09.

Bane	Sample									
	A1	DOA	G2	G45	G5A	G5Z	HHK	KALASIN	SUN	A2
1	1	1	1	1	1	1	0	0	0	0
2	1	1	1	1	1	1	1	1	0	1
3	1	1	1	1	1	1	1	1	1	0
4	1	1	0	1	1	1	1	0	0	0
5	0	0	0	0	0	0	0	1	0	1
6	0	1	0	1	1	1	0	1	0	1
7	0	1	1	1	1	1	1	1	1	1
8	1	0	0	0	0	0	1	0	0	1
9	1	1	1	1	1	1	1	1	0	0
10	0	0	0	0	0	0	1	1	0	0

Table 64 The polymorphism pattern of *Ganoderma lucidum* strains and *Ganoderma sinensis* generated by primer A10.

Bane	Sample									
	A1	DOA	G2	G45	G5A	G5Z	HHK	KALASIN	SUN	A2
1	0	0	0	0	1	1	0	0	0	0
2	0	0	0	0	1	1	0	0	0	0
3	1	1	1	1	0	0	1	0	0	0
4	0	0	0	0	0	0	0	0	0	1
5	0	0	0	0	1	1	0	0	0	1
6	1	1	1	1	1	1	1	1	1	0
7	0	0	0	0	0	0	0	0	0	1

Table 65 The polymorphism pattern of *Ganoderma lucidum* strains and *Ganoderma sinensis* generated by primer A11

Bane	Sample									
	A1	DOA	G2	G45	G5A	G5Z	HHK	KALASIN	SUN	A2
1	1	1	1	1	1	1	0	0	0	0
2	1	1	1	1	1	1	1	1	0	1
3	1	1	1	1	1	1	1	1	1	0
4	1	1	0	1	1	1	1	0	0	0
5	0	0	0	0	0	0	0	1	0	1
6	0	1	0	1	1	1	0	1	0	1
7	0	1	1	1	1	1	1	1	1	1
8	1	0	0	0	0	0	1	0	0	1
9	1	1	1	1	1	1	1	1	0	0
10	0	0	0	0	0	0	1	1	0	0

Table 66 The polymorphism pattern of *Ganoderma lucidum* strains and *Ganoderma sinensis* were used the primer A13.

Bane	Sample									
	A1	DOA	G2	G45	G5A	G5Z	HHK	KALASIN	SUN	A2
1	1	0	1	1	1	1	1	0	0	0
2	0	0	0	0	0	0	0	0	0	1
3	1	1	1	1	1	1	1	0	0	1
4	1	1	1	1	1	1	1	0	1	0
5	0	0	0	0	0	0	0	0	0	1
6	1	1	1	1	1	1	1	0	0	0
7	1	1	1	1	0	0	1	1	0	1
8	1	0	0	0	0	0	1	0	1	1
9	0	0	0	0	0	0	1	1	0	1
10	0	0	0	0	0	0	0	0	0	1
11	0	0	0	0	0	0	0	1	0	0

Table 67 The polymorphism pateern of *Ganoderma lucidum* strains and *Ganoderma sinensis* generated by primer A18.

Bane	Sample									
	A1	DOA	G2	G45	G5A	G5Z	HHK	KALASIN	SUN	A2
1	1	1	0	1	0	0	1	0	0	0
2	0	0	1	0	1	1	0	0	0	1
3	0	1	0	1	1	1	1	0	1	0
4	1	0	0	0	1	1	0	0	0	0
5	1	1	0	0	0	0	1	0	0	1
6	0	1	1	1	1	1	0	1	0	1
7	1	1	1	1	1	1	1	0	1	1
8	1	0	0	0	0	0	0	1	0	0
9	1	0	0	0	0	0	0	0	0	1
10	1	1	0	0	1	1	0	0	0	0
11	0	0	0	0	0	0	0	1	0	0

Table 68 The total polysaccharides of *Ganoderma lucidum* strains and *Ganoderma sinensis*.

Strains	Replication	Total polysaccharides mg/ g of crude polysaccharides	Mean of total polysaccharides (mg/g)	SD of total polysaccharides (mg/g)
A1	1	844.444	812.346	28.044
	2	792.593		
	3	800.000		
DOA	1	925.926	920.988	8.553
	2	925.926		
	3	911.111		
G2	1	925.926	923.457	4.277
	2	925.926		
	3	918.519		
G5Z	1	530.370	552.593	19.472
	2	560.741		
	3	566.667		
HHK	1	549.630	566.420	33.672
	2	605.185		
	3	544.444		
KALASIN	1	442.963	446.667	15.520
	2	433.333		
	3	463.704		
SUN	1	320.741	316.543	6.639
	2	308.889		
	3	320.000		
A2	1	517.037	495.309	36.359
	2	515.556		
	3	453.333		

Table 69 The reducing sugar of *Ganoderma lucidum* strains and *Ganoderma sinensis*.

Strains	Replication	Reducing sugar mg/1 g of crude polysaccharides	Mean of Reducing sugar (mg/g)	SD of reducing sugar (mg/g)
A1	1	87.333	88.556	1.347
	2	88.333		
	3	90.000		
DOA	1	77.000	78.111	0.962
	2	78.667		
	3	78.667		
G2	1	105.667	106.222	0.509
	2	106.667		
	3	106.333		
G5Z	1	61.000	62.000	1.000
	2	63.000		
	3	62.000		
HHK	1	119.333	120.111	1.072
	2	121.333		
	3	119.667		
KALASIN	1	90.000	91.111	0.962
	2	91.667		
	3	91.667		
SUN	1	94.000	95.333	1.333
	2	96.667		
	3	95.333		
A2	1	63.000	64.111	1.388
	2	63.667		
	3	65.667		

Table 70 The protein content of *Ganoderma lucidum* strains and *Ganoderma sinensis*.

Strains	Replication	Protein content mg/1 g of crude polysaccharides	Mean of Protein content (mg)	SD of Protein content (mg)
A1	1	12.773	12.661	0.790
	2	13.389		
	3	11.821		
DOA	1	10.252	10.896	0.644
	2	11.541		
	3	10.896		
G2	1	15.546	16.022	0.440
	2	16.106		
	3	16.415		
G5Z	1	14.258	14.342	0.358
	2	14.034		
	3	14.734		
HHK	1	17.619	17.740	0.312
	2	17.507		
	3	18.095		
KALASIN	1	8.487	8.880	0.440
	2	9.356		
	3	8.796		
SUN	1	11.401	12.073	0.646
	2	12.689		
	3	12.129		
A2	1	3.445	3.352	0.138
	2	3.193		
	3	3.417		

Table 71 The total phenol content of *Ganoderma lucidum* strains and *Ganoderma sinensis*.

Strains	Replication	Phenol content mg/1 g of crude polysaccharides	Mean of phenolcontent (mg)	SD of phenol content (mg)
A1	1	14.299	13.861	0.443
	2	13.871		
	3	13.413		
DOA	1	10.052	10.439	0.415
	2	10.388		
	3	10.877		
G2	1	13.871	13.352	1.007
	2	13.993		
	3	12.191		
G5Z	1	11.152	11.131	0.093
	2	11.030		
	3	11.213		
HHK	1	16.804	16.560	0.589
	2	16.987		
	3	15.888		
KALASIN	1	10.480	10.602	0.530
	2	11.182		
	3	10.144		
SUN	1	5.744	6.009	0.229
	2	6.141		
	3	6.141		
A2	1	4.155	4.114	0.035
	2	4.094		
	3	4.094		

BIOGRAPHY

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