Efficiency Comparison of Three Methods for Extracting Genomic DNA of the Pathogenic Oomycete *Pythium insidiosum*

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Background: *The fungus-like organism Pythium insidiosum is the causative agent of a life-threatening tropical infectious disease, pythiosis, which has high rates of morbidity and mortality. A lack of reliable diagnostic tools and effective treatments for pythiosis presents a major challenge to healthcare professionals. Unfortunately, surgical removal of infected organs remains the default treatment for pythiosis. P. insidiosum is an understudied organism. In-depth study of the pathogen at the molecular level could lead to better means of infection control. High quality genomic DNA (gDNA) is needed for molecular biology-based research and application development, such as: PCR-assisted diagnosis, population studies, phylogenetic analysis, and molecular genetics assays.*

Objective: *To evaluate quality and quantity of the P. insidiosum gDNA extracted by three separate protocols intended for fungal gDNA preparation.*

Material and Method: *Seven P. insidiosum isolates were subjected to gDNA extraction by using conventional-extraction, rapid-extraction, and salt-extraction protocols.*

Results: *The conventional protocol offered the best gDNA in terms of quality and quantity, and could be scaled up. The rapid-extraction protocol had a short turnaround time, but the quality and quantity of the gDNA obtained were limited. The salt-extraction protocol was simple, rapid, and efficient, making it appealing for high throughput preparation of small-scale gDNA samples.*

Conclusion: *Compared to rapid-extraction protocol, both conventional-extraction and salt-extraction protocols provided a better quality and quantity of gDNA, suitable for molecular studies of P. insidiosum. In contrast to the other two methods, the salt-extraction protocol does not require the use of hazardous and expensive materials such as phenol, chloroform, or liquid nitrogen.*

Keywords: *DNA extraction, Genomic DNA, Oomycete, Pythiosis, Pythium insidiosum*

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Pythium insidiosum is an aquatic, fungus-like, oomycete microorganism that causes a life-threatening infectious disease, called pythiosis, in humans and animals living in tropical and subtropical areas of the world[1]. *P. insidiosum* inhabits swampy areas, where it colonizes water plants[2]. The organism generates a specialized structure, called a zoospore[3]. When a zoospore comes in to contact with a human or an animal, it can invade host tissue and initiate an infection characterized by high rates of morbidity and mortality[4]. Diagnosis of pythiosis is difficult and conventional antifungal drugs are ineffective. Extensive surgical removal of infected tissues (typically eyes or legs) is the primary treatment option for pythiosis. The dearth of reliable diagnostic tools and effective treatments remains a severe healthcare problem for pythiosis.

P. insidiosum is relatively understudied. Information on biology, evolution, and pathogenesis of *P. insidiosum* is lacking. A more in depth study of *P. insidiosum* at the molecular level could lead to a

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better understanding of the disease and thus better infection control and management. High quality genetic material (DNA and RNA) is required for a wide variety of molecular-based experiments and applications, such as, genome sequencing, molecular genetics study, PCR-assisted diagnosis, population genetic study, and phylogenetic analysis. Genomic DNA (gDNA) of *P. insidiosum* may be prepared by several methods[5-7]. For example, Vanittanakom et al developed a rapid gDNA extraction protocol (rapid-extraction) for molecular identification of *P. insidiosum*[7]. Chaiprasert et al[6] and Pannanusorn et al[5] modified the conventional gDNA extraction protocol (conventional-extraction) of Jackson et al[8] for molecular typing of some *P. insidiosum* strains. Information on the quality and quantity of gDNA extracted by these methods has not been published.

Aljanabi et al reported the universal and rapid salt-extraction protocol for the isolation of gDNA[9]. Their method provides high quality gDNA, suitable for PCR-based experiments. Unlike other methods, gDNA extraction protocol of Aljanabi et al does not require phenol, chloroform, and liquid nitrogen, which are expensive and environmentally hazardous reagents. In the present study, the authors aimed at modifying the protocol of Aljanabi et al[9] for extraction of *P. insidiosum* gDNA. The authors also aimed at comparing the quality of gDNA extracted by the modified protocol of Aljanabi et al (salt-extraction) with that of the rapid-extraction[7] and the conventional-extraction[5,6] protocols.

Material and Method

Microorganisms and culture condition

Seven *P. insidiosum* isolates from patients with pythiosis (n = 4), animals (n = 2) and environment (n = 1) (Isolate T1-7; Table 1) were recruited in this study. All isolates were confirmed by culture identification with zoospore induction[3,10]. The microorganisms were sub-cultured on Sabouraud dextrose agar once a month. For preparation of large-scale gDNA, 10 small plugs (0.5x0.5 cm) of 7-day old mycelium-attached agar were transferred to 100 ml of Sabouraud dextrose broth, and incubated with shaking at 37°C for 10 days. For preparation of small-scale gDNA, *P. insidiosum* hyphae were cultivated in a Petri dish with 10 ml of Sabouraud dextrose broth and incubated at room temperature for 10 days. All mycelial mats were harvested, washed with distilled water, filtrated through filter paper (Whatman No.1), and stored at -30°C until use.

Table 1. Quality and quantity of extracted genomic DNA of seven *P. insidiosum* strains. Concentrations were estimated by the UV absorbance (NanoDrop spectrophotometer) and the fluorescence-based (Qubit fluorometer) measurements. DNA yields (DNA obtained/mass of mycelium) were calculated based on the DNA concentration measured by the Qubit fluorometer

Strain ID	Reference ID	Source	Concentration (ng/µl): Qubit (NanoDrop)			DNA yield (ng/mg)			A260/280 Ratio		
			Conventional extraction	Salt extraction	Rapid extraction	Conventional extraction	Salt extraction	Rapid extraction	Conventional extraction	Salt extraction	Rapid extraction
T1	CR02	Environment	336 (373)	39 (757)	79 (1,023)	67	47	136	1.9	2.0	2.0
T2	MCC18/P12	Human (eye)	540 (574)	190 (1,590)	41 (1,330)	149	192	47	1.8	2.1	2.0
T3	Pi-S/P35	Human (leg)	271 (283)	93 (584)	43 (1,994)	122	112	55	1.8	2.1	2.1
T4	CBS777.84	Mosquito	257 (297)	86 (384)	48 (1,181)	101	146	72	1.9	2.1	2.0
T5	CBS673.85	Human (skin)	225 (351)	199 (1,652)	53 (994)	53	260	60	1.9	2.1	2.0
T6	CBS702.83	Horse	510 (557)	52 (418)	27 (360)	183	99	49	1.8	2.1	2.0
T7	P6	Human (leg)	121 (199)	139 (683)	34 (1,646)	28	162	39	1.8	2.1	2.1
Mean	-	-	323 (376)	114 (867)	46 (1,218)	100	145	65	1.8	2.1	2.0
SD	-	-	153 (141)	64 (532)	17 (520)	55	69	33	0.0	0.0	0.1

DNA extraction

The harvested mycelial mats of *P. insidiosum* were subjected to gDNA extraction by three different protocols: conventional-extraction, rapid-extraction, and salt-extraction. The conventional-extraction protocol was performed using the modified method of Jackson et al[5,6,8]. Briefly, 200 to 1,000 mg of mycelia was ground to a fine powder in the presence of liquid nitrogen. The powder was transferred to a 50-ml conical tube. Twenty ml of extraction buffer [100 mM Tris-HCl (pH 8.0), 100 mM EDTA, 250 mM NaCl, 40 µg/ml proteinase K and 1% SDS] per 1 g of the mycelial power was added to the tube. The mixture was incubated at 55°C overnight. DNA was extracted with phenol:chloroform:isoamyl alcohol (25:24:1), followed by chloroform:isoamyl alcohol (96:4). An equal volume of ice-cold isopropanol was used to precipitate DNA. The DNA pellet was washed with 70% ethanol, air dried, dissolved in 500 µl TE buffer, and treated with RNase for one hour (final concentration, 50 µg/ml). The DNA sample was further extracted with chloroform:isoamyl alcohol (96:4), and precipitated with 0.1 volume of 3M sodium acetate and one volume of ice-cold isopropanol. The pellet was washed twice with 70% ethanol, air dried, and dissolved in 50 to 100 µl TE buffer.

The rapid-extraction protocol was performed according to the method of Vanittanakom[7]. Briefly, 0.5 ml of lysis buffer [1.5% SDS and 0.25 M Tris (pH 8.0)] was added to 50 to 100 mg of harvested mycelia, boiled for 30 minutes and vortexed for two minutes. DNA was then isolated with an equal volume of phenol: chloroform: isoamyl alcohol (25:24:1) and precipitated with absolute ethanol. A DNA pellet was air dried and dissolved in 50 µl TE buffer.

The salt-extraction protocol was performed using the modified methods of Aljanabi et al[9]. Mycelial mats (50-100 mg) were transferred to a sterile microtube containing glass beads (diameter, 710-1,180 mm; Sigma) and 400 µl of the salt homogenizing buffer [0.4 M NaCl, 10 mM Tris-HCl (pH8.0), 2 mM EDTA] and homogenized using TissueLyzer MM301 (Qiagen, Germany) with the following setting: two minutes at 30 Hz. Forty-five µl of 17% SDS and 8 µl of 20 mg/ml proteinase K were added to the cell lysate, gently mixed, and incubated at 56°C for two hours (or overnight). Next, 0.3 ml of 6 M NaCl was added to the sample. The mixture was vortexed for 30 seconds, and centrifuged (10,000 x g) for 30 minutes. Supernatant was collected, mixed with an equal volume of isopropanol, and kept at -20°C for one hour. The sample was centrifuged (10,000 x g) at 4°C for 20 minutes. The pellet was washed with 70% ethanol, air dried, and dissolved in 100 µl sterile water.

The concentration and purity of all DNA samples was estimated by measurement of optical density at 260 nm and 280 nm wavelengths using a NanoDrop® 2000 spectrophotometer (Thermo Scientific) and by fluorescence-based measurement using a Qubit® 2.0 fluorometer (Invitrogen). DNA integrity was evaluated by 1% agarose gel electrophoresis. All DNA samples were stored at -30°C until use.

Polymerase chain reaction

PCR amplification of several *P. insidiosum* genes was performed. The target genes included ribosomal RNA gene (*rDNA*; accession number, AY486144.1), a putative exo-1,3-beta glucanase-encoding gene (*EXO1*; accession number, GU994093.1)[11], and a putative RXLR effector-encoding gene (*UN04715*; submitted to the DNA Data Bank of Japan under accession number FX531705) (Table 2). Two sets of primers were used to amplify 233-bp (Primers: ITSpy1 and ITSpy2) and 931-bp (Primers: ITS1 and ITS4) amplicons from *rDNA* (Table 2). The primers Dx3 and Dx4 were used to amplify a 550-bp amplicon from *EXO1* (Table 2). The primers RXLR1 and RXLR2 were designed to amplify *UN04715* with a projected amplicon size of 1,575 bp (Table 2). PCR amplification was carried out in a reaction volume of 50 µl, containing 5 pmol each of forward and reverse primers, 1.25 U of Taq polymerase (Fermentas), 0.2 mM deoxynucleotide triphosphate mixture, 10 mM Tris-HCl (pH 8.8) with 50 mM KCl, 1-2 mM MgCl2, and 100 to 200 ng of DNA template (Table 2). A hot start PCR protocol was performed with an initial denaturation at 95°C for three to six minutes, followed by 25 to 30 cycles of denaturation (95°C) for 30 to 45 seconds, annealing (50-60°C) for 30 seconds, and extension (72°C) for 30 to 120 seconds (Table 2). The amplification reaction was finished with a final extension (72°C) for 10 minutes. Amplicons were analyzed by 1% agarose gel electrophoresis, in which 5 to 10 µl of each PCR product was loaded in loading buffer. Gels were stained with ethidium bromide and visualized by Gel Doc XR+ (Bio-Rad, USA).

Statistical analysis

Data were presented as mean and standard deviation (SD) for DNA concentration and DNA yield. These statistical values were calculated using the Microsoft EXCEL2013 program.

Results

Quality and quantity of extracted gDNA

gDNA extraction time (turnaround time) for rapid-extract protocol was four hours, salt-extract protocol was about six to seven hours, and conventional-extract protocol was one to two days. Based on fluorescence measurements, the conventional-extraction protocol provided the highest DNA concentration (mean (SD) 323 (153) ng/µl, range 121-540 ng/µl), followed by the salt-extraction (mean (SD) 114 (64) ng/µl, range 39-199 ng/µl) and the rapid-extraction (mean (SD) 46 (17) ng/µl, range 27 to 79 ng/µl) protocols (Table 1). However, the salt-extraction protocol provided the highest total yield (DNA obtained/mass of mycelium) (mean (SD) 145 (69) ng/mg, range 47-260 ng/µl), followed by conventional-extraction (mean (SD) 100 (55) ng/mg, range 28 to 183 ng/µl) and rapid-extraction (mean 65 (33) ng/mg, range 39-136 ng/µl) (Table 1). A260/A280 ratios of the DNA extracted by the conventional-extraction protocol (~1.8-1.9) were lower than the ratios of the other two protocols (~2.0-2.1) (Table 1). DNA integrity was evaluated by agarose gel electrophoresis. High molecular weight DNA (size >23 kb) was observed in all samples prepared by the conventional- and salt-extraction protocols (Fig. 1). In contrast, this high molecular weight DNA was absent in all samples prepared by the rapid-extraction protocol (Fig. 1). Degraded DNA (characterized as a smear of low molecular weight material) was minimal in gDNA extracted by the conventional-extraction protocol, noticeable in gDNA extracted by the salt-extraction protocol, and prominent in gDNA extracted by the rapid-extraction protocol.

PCR amplification of target genes

When using the gDNA templates prepared by the conventional-extraction protocol, all primer sets (ITSpy1/2, ITS1/4, Dx3/4, and RXLR1/2; Table 2) successfully amplified target genes: *UN04715* (Fig. 2A), *rDNA* (Fig. 2B, D), and *EXO1* (Fig. 2C). When testing gDNA templates prepared by the salt-extraction protocol, all primer sets produced relatively intense bands except for the primers RXLR1/2, which provided faint bands for the T3 and T6 templates (Fig. 2A). Most of the gDNA templates prepared by the rapid-extraction protocol produced faint bands using primers ITSpy1/2 (Fig. 2D); the Dx3/4 primers amplified the expected amplicon from five out of seven templates tested (Fig. 2C); the ITS1/4 primers successfully amplified a product from only the T1, T2 and T7 templates

Table 2. Primer sequences and polymerase chain reaction (PCR) conditions for amplification of *P. insidiosum* genes (F = forward primer; R = reverse primer)

Target gene	Primer	Sequence	Amplicon size (bp)	Template amount (ng)	Anealling temperature (°C)	PCR extension time (sec)
rDNA	ITSpy1 (F)	5'-CTGCGGAAGGATCATTACC-3'	233	100	60	30
	ITSpy2 (R)	5'-GTCCTCGGAGTATAGATCAG-3'				
rDNA	ITS1 (F)	5' -TCCGTAGGTGAACCTGCGG-3'	931	100	55	30
	ITS4 (R)	5' -TCCTCCGCTTATTGATATGC-3'				
EXO1	Dx3 (F)	5'-GCGAGTTCTGGCTCGACTTTA-3'	550	100	57	60
	Dx4 (R)	5'-ACAAGCGCCAAAAAGTCCCA-3'				
UN04715	RXLR1 (F)	5'-GCCCATGGCCTCTTCGTCCATGAGCTCGCTC-3'	1,575	200	50	120
	RXLR2 (R)	5'-GCGAATTCGCACGACGGGCGGCTCGT-3'				

(Fig. 2B); and the RXLR1/2 primers failed to produce an amplicon from all templates tested (Fig. 2A).

Discussion

In the present study, conventional-extraction, salt-extraction, and rapid-extraction protocols were used to extract the gDNA of *P. insidiosum*. These protocols were characterized with regard to efficiency and suitability of the gDNA produced for downstream applications. The high A260/A280 ratio (~1.8-2.1) of the gDNA extracted by all three methods indicates minimal protein contamination (Glasel 1995) (Table 1).

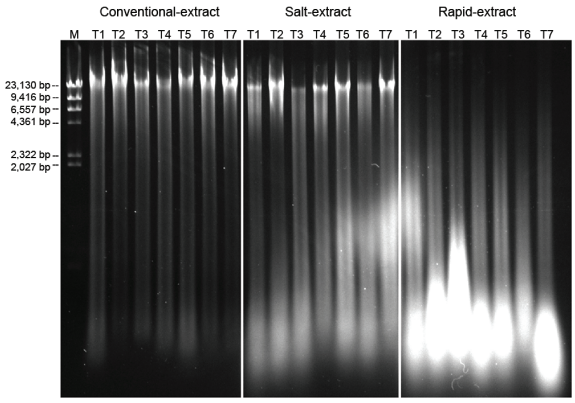

Fig. 1 An agarose gel showing genomic DNA of *P. insidiosum* isolate T1-7, prepared by conventional-extraction, salt-extraction, and rapid-extraction protocols (M = molecular weight markers).

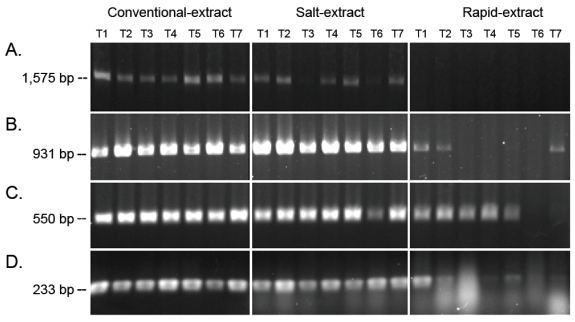

Fig. 2 PCR amplification of *P. insidiosum* genes using the DNA templates prepared by conventional-extraction, salt-extraction, and rapid-extraction protocols: A. the putative RXLR-effector encoding gene (*UN04715*) amplified by the primers RXLR1 and RXLR2; B. the ribosomal DNA gene (*rDNA*) amplified by the primers ITS1 and ITS4; C. the putative exo-1,3-beta glucanase-encoding gene (*EXO1*) amplified by the primers Dx3 and Dx4; and D. *rDNA* amplified by the primers ITSpy1 and ITSpy2.

DNA concentrations reported by UV absorbance measurement (NanoDrop spectrophotometer) tended to exceed estimations by fluorescence-based measurements (Qubit fluorometer) (Table 1), possibly because UV absorbance measures DNA and RNA together, while the fluorescence-based method specifically reports DNA concentration[12,13]. Thus, fluorometric estimation of gDNA concentration was preferred in the present study.

The authors compared the quality and quantity of all extracted gDNA samples. The conventional-extraction protocol maximized both concentration and DNA integrity (Table 1, Fig. 1), making this protocol more suitable for a large-scale, high quality gDNA preparation. However, the conventional-extraction protocol demanded a great deal of hyphal material (i.e., 200-1,000 mg), more extraction steps, a larger volume of reagents, and required liquid nitrogen. Alternatively, the salt-extraction and rapid-extraction protocols can be used to extract gDNA from smaller quantities of mycelia (i.e., 50-100 mg). Unlike with conventional-extraction, these two protocols required fewer reagents and a shorter extraction time (four hours for rapid-extract; about six to seven hours for salt-extract; one to two days for conventional-extract). For smaller-scale gDNA extractions, the salt-extraction protocol was preferred because it provided higher DNA concentration (114 vs. 46 ng/µl), yield (145 vs. 65 ng/mg), and integrity (Fig. 1). Moreover, the salt-extraction protocol did not require phenol, chloroform, or liquid nitrogen.

To further evaluate gDNA quality, PCR amplifications targeting three different genes (*rDNA*, *EXO1*, and *UN04715*) of *P. insidiosum* were performed, using all sets of primers (Table 2). Amplicon sizes ranged from ~200 to ~1,700 bp (Table 2). It was observed that impaired integrity of the gDNA generated by the rapid-extraction protocol markedly limited the success of PCR amplifications, particularly with respect to long amplicons (>550 bp) (Fig. 2). The gDNA templates extracted by the conventional-extraction and salt-extraction protocols may be used to amplify an amplicon size of at least 1.7 kb (Fig. 2), indicating that these two protocols are appropriate for PCR-based applications.

In conclusion, the conventional-extraction protocol provided the highest quality and quantity of *P. insidiosum* gDNA. This protocol was suitable for large-scale preparation of high quality gDNA. The salt-extract was a simple, rapid, and efficient protocol, making it useful for high throughput, small-scale

preparation of gDNA for many molecular-biological experiments and applications. Although the rapid-extract protocol had the shortest turnaround time, the gDNA obtained was of limited quality and quantity. These observations may instruct investigators and aid their determination of which extraction protocol is optimal for a given application, dependent upon quality and quantity of gDNA desired, availability of materials and equipment, and allowable turnaround time.

What is already known on this topic?

High-quality gDNA material is required for a wide variety of molecular-based experiments and applications (i.e., genome sequencing, molecular genetics study, PCR-assisted diagnosis, population genetic study, and phylogenetic analysis) of micro-organisms of interest, including the understudied pathogen *Pythium insidiosum*. *P. insidiosum* gDNA can be prepared by several methods[5-7]. For example, Vanittanakom et al have developed a rapid gDNA extraction protocol (rapid-extraction) for molecular identification of *P. insidiosum*[7]. Chaiprasert et al[6] and Pannanusorn et al[5] have modified the conventional gDNA extraction protocol (conventional-extraction) of Jackson et al[8] for molecular typing of some *P. insidiosum* strains. All of these methods require the use of hazardous and expensive materials such as phenol, chloroform, or liquid nitrogen. Information on quality and quantity of the gDNA extracted by these methods has not been described nor published. Such information is important for determination of which gDNA extraction protocol is more suitable for a given experiment, which would depend on the quality and quantity desired, the availability of materials and equipment, and allowable turnaround time.

What this study adds?

The present study provided two new and useful information to the field of *P. insidiosum* study:

(1) In the current study, a new *P. insidiosum* gDNA extraction protocol (salt-extraction) was successfully developed, by modifying and optimizing the method of Aljanabi et al[9], who report the universal and rapid salt-extraction protocol for isolation of gDNA from a variety of other organisms. An advantage of this salt-extraction method over the other *P. insidiosum* gDNA extraction methods is that it does not require the use of hazardous and expensive materials such as phenol, chloroform, and liquid nitrogen.

(2) The current study comprehensively described and compared the quality and quantity (including integrity, amount, yield, and turnaround time) of *P. insidiosum* gDNA extracted by conventional-extraction [Pannanusorn et al[5] and Chaiprasert et al[6]], rapid-extraction [Vanittanakom et al[7]], and salt-extraction [this study] protocols. This study showed that the rapid-extraction protocol had a short turnaround time, but quality and quantity of the gDNA obtained were limited. The conventional-extraction protocol provided the best gDNA in terms of quality and quantity, but it was time consuming. The salt-extraction protocol was simple, rapid, and efficient, making it suitable for high throughput preparation of small-scale gDNA samples.

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Potential conflicts of interest

None.

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การเปรียบเทียบประสิทธิภาพการสกัด genomic DNA ของเชื้อ Pythium insidiosum สามวิธี

ทัศนีย์ โล้หนู, นุจริน จงรุจา, ธิดารัตน์ รุจิรวรรธน์, วันทา ยิ่งยง, ทัศนีย์ ฤกษ์สุทธิรัตน์, อัมพร นามพูน, โยธิน คำแสง, พรพิศ อ่อนแผ้ว, พิริยาภรณ์ จงตระกูล, อังศณา กีรติจรัส, Tristan T Brandhorst, ธีรพงษ์ กระแจะจันทร์

ภูมิหลัง: *Pythium insidiosum เป็นเชื้อที่มีลักษณะคล้ายรา สามารถก่อโรค pythiosis ซึ่งเป็นโรคติดเชื้อในเขตร้อนที่มีความรุนแรงสูง โรคนี้ยังขาดวิธีการวินิจฉัยและการรักษาที่รวดเร็วและมีประสิทธิภาพ การรักษาหลักในปัจจุบันคือ การผ่าตัดนำส่วนที่ติดเชื้อออก เช่น ดวงตาและขา จึงทำให้เกิดความพิการ ในผู้ป่วยที่การติดเชื้อลุกลามไปมากจะเสียชีวิต เชื้อ P. insidiosum นี้มีการศึกษาน้อยมาก โดยเฉพาะการศึกษาทางด้านชีววิทยาระดับโมเลกุลซึ่งเป็นพื้นฐานต่อการเข้าใจโรคและการค้นพบวิธีการป้องกัน การวินิจฉัย และการรักษาผู้ป่วยใหม่ๆ ที่ดีขึ้น อย่างไรก็ตามการศึกษาดังกล่าวจำเป็นต้องอาศัยการวิเคราะห์ genomic DNA (gDNA) ที่มีคุณภาพสูงที่สกัดได้จากเชื้อ*

วัตถุประสงค์: *เพื่อประเมินคุณภาพและปริมาณของ gDNA ที่สกัดได้จากเชื้อ P. insidiosum ด้วยวิธีการที่แตกต่างกัน 3 วิธี*

วัสดุและวิธีการ: *เชื้อ P. insidiosum จำนวน 7 สายพันธุ์ ถูกรวบรวมมาเพื่อใช้ทดสอบประสิทธิภาพการสกัด gDNA ด้วยวิธี conventional-extraction, rapid-extraction และ salt-extraction*

ผลการศึกษา: *การสกัดด้วยวิธี conventional-extraction ได้ gDNA ที่มีคุณภาพและปริมาณดีที่สุดเมื่อเทียบกับวิธีอื่น ส่วนวิธี rapid-extraction สามารถทำได้รวดเร็วที่สุด แต่ได้ gDNA ที่มีคุณภาพและปริมาณต่ำกว่าวิธีอื่น ในขณะที่วิธี salt-extraction นั้นสามารถทำได้ง่าย รวดเร็ว และได้ gDNA คุณภาพสูง จึงเหมาะสำหรับการสกัด gDNA จากตัวอย่างจำนวนมากได้ดี*

สรุป: *เมื่อเปรียบเทียบกับวิธี rapid-extraction จะพบว่าทั้งวิธี conventional-extraction และ salt-extraction ให้คุณภาพและปริมาณของ gDNA ที่สกัดได้ดีกว่า และเหมาะสำหรับการศึกษาและวินิจฉัยการติดเชื้อ P. insidiosum มากกว่า นอกจากนี้วิธี salt-extraction ยังไม่ใช้สารที่เป็นพิษและมีราคาแพงในการสกัด gDNA เช่น phenol, chloroform และไนโตรเจนเหลว*