Biodegradation of Polyurethane Polymer using Endophytic Fungi Isolated from *Nepenthes ampullaria* In Sarawak

by

SHIRLEY BONG WUAN LII

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Abstract

Electrical and electronic wastes that comprise of discarded computers and mobile phones are found abundantly due to their short lifespan. The chemical composition of these wastes contains potential harmful contaminants. However, most of these wastes are often disposed in landfills. Effective recovery technology causes minimal impact to the environment but it is expensive. In some poor countries, wastes are burned or dissolved in strong acids that cause leaching of contaminants into the rivers. Food chains are affected and human health be threaten due to exposure to contaminants through drinking, food, and smoke. Proper management of the wastes are thus, essential to combat the developing problems. Bioremediation is a green approach that is helpful to minimize environmental pollution associated with E-waste. In this thesis, we explored the potential of endophytic fungi from *Nepenthes ampullaria* (pitcher plant, collected in the Mentawai Jungle, Sarawak) for bioremediation purposes of the plastic component in E-waste, polyurethane (PUR) polymers. A number of isolates are able to degrade polyurethane in solid medium efficiently. Three isolates can grow and survive by utilising polyurethane with minimal nutrients for their growth. Enzymatic activity was also detected when tested using *p*-nitrophenol acetate as the substrate. The isolates were identified using ITS1 and 4 and were closely related to the genus *Pestalotiopsis*. Proteins of untreated and treated fungi with polyurethane were analyzed by 2-dimensional electrophoresis. Analyses of the 2-dimensional electrophoresis profile revealed changes in the abundance of proteins when treated with polyurethane. This study is to our knowledge the first on endophytes isolated from *Nepenthes ampullaria* that can degrade PUR, and also their proteomes. Hopefully, results obtained from this study can in future help to reduce polyurethane wastes.

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Declaration

I hereby declare that my research entitled “Biodegradation of Poyurethane Polymer using Endophytic Fungi Isolated from *Nepenthes ampullaria* In Sarawak” is original and contains no material which has been accepted for the award to the candidate of any other degree or diploma, except where due reference is made in the text of the examinable outcome; to the best of my knowledge contains no material previously published or written by another person except where due reference is made in the text of the examinable outcome; and where the where the work is based on joint research or publications, discloses the relative contributions of the respective workers or authors.

(SHIRLEY BONG WUAN LII)
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Publications Arising from this Thesis

The work described in this thesis has been submitted as described in the following:

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Early work has been presented in the following conference and contributed to the content presented in Chapter 4 of this thesis:

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1. Introduction

1.1 Electronic wastes and the components

„Electronic waste" or „waste" is a general term that refers to various forms of electric and electronic equipment that are of no value to their owners (Tsydenova & Bengtsson 2011). The fastest increasing waste streams are the unwanted electrical and electronic goods which are also known as electronic waste (E-waste) (Tsydenova & Bengtsson 2011). E-wastes have been estimated to constitute 8% of municipal waste (Widmer et al. 2005). UNEP (2006) estimated that the world produced 20-50 million tonnes of E-waste every year. This signifies 1-3% of the creation of waste of 1636 million tonnes yearly by the worldwide community (OECD 2008). In 2010, 5.5 million tonnes of computers, portable phones and televisions contributed to the E-waste river and continue growing to 9.8 million tonnes in 2015 (Cobbing 2008). The main generator of electronic waste in the world is the USA with a total accumulation of 3 million tons yearly while China the second leading country, generating 2.3 million tons every year (Oliveira, Bernardes & Gerbase 2012). Concerns on E-waste arise due to the growing quantity of electronic waste, together with the presence of various intricate components within them and the subsequent complications in handling them appropriately (Tsydenova & Bengtsson 2011).

Waste electric and electronic equipment (WEEE) is predominantly a mixture of metals and plastics. Plastics are lightweight and essential as an electric or thermal insulator. The portion of plastics in WEEE has constantly increased from about 14% in 1980 to 18% in 1992, and 23% in 2005 (Buekens & Yang 2014). Electrical and electronic equipment contains an enormous amount of harmful constituents that includes heavy metals (zinc, copper, mercury, cadmium, lead), flame retardants (hexaobromocyclododecane (HBCD), pentabromophenol, polybrominated diphenyl ethers (PBDEs), tetrabromobisphenol-A (TBBPA)), and polymers (polyurethane (PUR), acrylonitrile butadiene styrene (ABS), polyethylene terephthalate (PET)). Therefore, E-waste is a dangerous and harmful waste due to the presence of these substances which can affect major human health and the environment if

they are not well-managed. The following are the constituents of electronic and electrical equipment categorised as the most harmful.

1.1.1 Mercury-containing components

Batteries, relays (telecommunication circuit boards, commercial or industrial electric ranges) and switches (industrial products which includes boilers, microwaves, air handling units, safety units, and levelling equipment), and gas liberation lamps for the backlighting in liquid crystal displays for a wide-ranging usage of electronic equipment that includes portable computers, televisions, cameras, photocopy and facsimile machineries contain mercury (NEWMOA 2008).

1.1.2 Batteries

EPS (2006) from an environmental perception is concern on batteries containing mercury and batteries that are chargeable because they contain harmful components of lead, lithium, and cadmium. Lead acids are present in nickel cadmium (Ni-Cd), nickel metal hydride (NiMeH), and lithium ion batteries that are rechargeable batteries. They are commonly used in laptops, hand phones, cameras and handy power devices.

1.1.3 Printed circuit boards (PCBs)

AEA (2004), EPS (2006), and OECD (2008) are concern on a number of substances that are present in PCBs. These include brominated flame retardants used for plastics. TBBPA and PBDEs are the common flame retardants that are used in PCBs. Printed circuit boards contain lead, cadmium, beryllium and antimony. A significant valuable amount of silver, gold, copper, and palladium are also present besides the hazardous substances.

1.1.4 Cathode ray tubes (CRTs)

Desktop computers and televisions that are of long usage contain CRTs with the most quantity of substances which are of concern. Approximately 2-3 kg of lead can be present in an older CRT compared to a latest CRT that usually has less than 1 kilogramme of lead. OECD (2008) estimated that approximately 1-2 g of small getter plate that includes frame contains barium and barium compounds which are present in the electron gun of the CRT.

1.1.5 Liquid crystal displays (LCDs)

The thin layer between the glass and electronic components are enclosed with liquid crystals. Approximately 0.5 mg of liquid crystals is present in a cellular phone display and about 0.5 g in a notebook PC display. Liquid crystals are generally a mixture of 10-20 materials that belong to cyclohexanes, benzenes and cyclohexylbenzenes. Liquid crystals are formulated abundantly from approximately 250 of substances (Tsydenova & Bengtsson 2011). Studies on the toxicity of liquid crystals are limited even though they are suspected to be hazardous. Current research have not discover the toxicity level even though some elements displayed destructive characteristics (AEA 2004).

1.1.6 Plastics that contain brominated flame retardants (BFRs) and plastics made of polyvinylchloride (PVC)

There are about 30% by weight of plastics constituent in E-wastes (Schlummer et al. 2007). The commonly used polymer in EEE is PVC and it is usually used to coat cables and wires. Polyvinylchloride contains chlorine and is of concern because polychlorinated dibenzo-p-dioxins and furans (PCDDs/Fs) might be released if burning is done uncontrollably. BFRs are also used in other types of plastics as additives and not only in PVC. Bimbaum & Staskal (2004) stated that BFRs functions to decrease the combustibility of EEE products. Two primary groups of BFRs, the PBDEs and phenolics are generally casted in EEE. PBDEs are mostly casted on cabinet and the phenolics including TBBPA are casted mostly on printed circuit boards (AEA 2004).

1.2 Implication of improper wastes management

Due to the wide range of chemicals, improper recycling actions can cause both the workstation and environment to be contaminated. Therefore, the labours as well as the native citizens will be affected when they inhale these chemicals, ingest dusts, and though their dietary consumption.

Tsydenova & Bengtsson (2011) suggested that the most important routes of human exposure are inhalation and dust ingestion. A study conducted by Leung et al. (2008) on a risk assessment from dust ingestion discovered severe health problems in employees and native citizens when they ingested dust that are polluted. Leung et al. (2008) estimated that an employee who worked to recycle PCBs had a lead dosage that exceeds the standard dosage of more than fifty times. This indicates a very high threat to the human fitness conditions. Li et al. (2007) conducted an inhalation health risk based on the percentages of polychlorinated dibenzo-p-dioxins (PCDDs) as well as furans (Fs) in the air. They revealed that the native citizens are exposed to a high level of dioxins and are of threats.

Work-related with contact to polybrominated diphenyl ethers (PBDEs) by E-waste recycling workers could affect the production of thyroid-stimulating hormone as well as causing damage to genes (Yuan et al. 2008). In the study, 23 employees who worked in the waste recycling area were recruited. A number of 26 employees were also chosen from a municipal that was situated at a distance of 50 km from the recycling area. The study found that the concentrations of PBDEs present in serum, thyroid-stimulating hormone as well as the frequencies of micronucleated binucleated cells are altered extensively in employees who worked in a waste area compared to those away from the contaminated area.

Wen et al. (2008) revealed another group of exposed workers where there was an increased in the levels of 8-hydroxy-20-deoxyguanosine that were present in their urine samples and this caused negative impact. In summary, the study assessed hair samples from male workers for the PCDDs and Fs, and PBDEs. They evaluated the 8-hydroxy-20- deoxyguanosine contents in the urine samples before as well as after workshift. It was found that all the components accessed had the highest concentrations among other reported studies.

In the same study, the levels of PCDDs and Fs found were approximately twenty times more than the standards of health topics in Japan. Whereas, the concentration of polychlorinated biphenyls are almost two times larger than the samples of hair from Belgium and Japan. Concentration of 8-hydroxy-20-deoxyguanosine containing creatinine in urine samples were significantly increased from before workshift to after workshift. An increased in the concentration of 8-hydroxy-20-deoxyguanosine in urine samples of employees working in a waste recycling area was reported to have a greater possibility of cancer (Wen et al. 2008).

Recycling activities have affected the population in Guiyu. Citizens of Guiyu conveyed that their children suffered from serious health complications. A sudden increase in the issues on leukemia was also observed (Leung, Cai & Wong 2006). Drinking water are also being polluted as a result of recycling activities (Coalition & Network 2002). In another study by Wang & Guo (2006), they revealed a high concentration of lead that are present in the river stream near a recycling area in Guiyu.

The increased concentrations of lead (Pb) as well as cadmium (Cd) in the children‟s blood were contributed by primitive E-waste recycling activities in Guiyu (Huo et al. 2007; Zheng et al. 2008). Huo et al. (2007) conducted a study in Guiyu on the concentration of Pb in the bloods of 165 children and 61 children of neighbouring town, Chendian that served as the control site. The study found that 81.8% of children in Guiyu had higher concentration of Pb in their blood which was of more than 10 lg/dL in comparison to 37.7% of children in Chendian ($p < 0.01$) (Huo et al. 2007).

A similar study was performed by Zheng et al. (2008) where 278 children from Guiyu and Chendian were used as the test subject for the content and concentrations of lead and cadmium that are present in the blood. A study by Huo et al. (2007) also reported the concentrations of Pb and Cd in the blood samples of children of Guiyu were relatively higher in comparison to the children of Chendian. A percentage of 70.8% of Guiyu children had lower concentration of Pb in their blood that was less than 10 lg/dL, and 20.1% of children had concentration of cadmium of more than 2 lg/L when compared to the children in Chendian of 38.7% and 7.3% respectively. It was also observed that as age increases, the amount of lead levels in blood increases significantly. The risk of the concentrations of lead and cadmium in the children‟s blood could be caused by the fathers‟ occupation that are associated to E-waste, and the quantity of time that the children spent outdoor (Zheng et al. 2008).

Apart from implications through inhalation, dust ingestion, and work-related exposure to E-waste, the contaminants of these waste can affect the aquatic life cycle and the food chains through leaching of dumpsites in which wastes were improperly processed and managed. Luo, Wong & Cai (2007) stated that the fishes in the Nanyang stream was able to accumulate PBDEs to a high concentration. Luo, Cai & Wong (2007) also revealed an increased in the concentrations of PBDE in the sediments of the same river.

A study by Wu et al. (2008) stated that the top water snake predator had a high concentration of PCBs and PBDEs in the area near electronic waste recycling plant. The ambient water contained 204 ng/L of PCBs only. An increased in the concentrations of PCBs and PBDEs were also found in prawn. Luo et al. (2008) stated that in the Pearl River Delta, waterfowl from downstream areas also demonstrated an increased level of PCB and PBDEs. In numerous habitat of the Pearl River Delta which are of a distance from E-waste recycling areas, brominated flame retardants specifically 1, 2-bis (2,4,6-tribromophenoxy) ethane, decabromodiphenyl ethane and tetrabromobisphenol A bis (2,3-dibromopropyl) ether are also prevalent apart from PBDEs (Shi et al. 2009).

1.2.1 Implications throughout life cycle

According to Schwartz and Hu (2007), babies and children are predominantly susceptible to poisons of lead (Pb) that might cause severe health problems. This includes the development of impaired neurobehavioral to a high risk of cardiovascular disease as well as stroke in the adult life (Schwartz & Hu 2007). Slow development and learning abilities in the early life of children can be caused by the factor of exposure to E-waste. Huo et al. (2007) reported that parents who worked closely with electronic wastes activities showed higher occurrence and percentages of toxic content in their children‟s blood (Huo et al. 2007).

Higher fraction of population with the concentration of lead in their blood greater than 20 µg/dL has been associated with family history of E-waste related work. In addition, a trend was observed in children who are being exposed to E-waste where blood lead levels increases with age as compared to continual or constant exposure to lead (control) where no such inclination was observed.

The act by the U.S. CDC is inadequate to safeguard kids as proposed by some authors. For example, children who are exposed to lead were found to develop extensive intellectual impairments even though the concentrations of lead in blood are less than 10 µg/dL (Jusko et al. 2008). Based on the study, a threshold has yet to be defined for the severe effects of kids' exposure to lead. Schwartz and Hu (2007) stated that both amount and trend of blood lead level data exhibit the risk of young kids to Pb exposure from E-waste activities that exceeds the present upper acceptable dose. It is possible that the health consequences be progressive and to an extent, irreversible when a significant amount of Pb in the body has accumulated (Schwartz & Hu 2007).

1.2.2 Implication across generations

PCDDs and polychlorinated dibenzofurans (PCDFs) in breast-milk were depended on to evaluate the relationship between the exposures to electronic wastes by a pregnant mother and the risks of affecting the next generation. The mixture of PCDDs and PCDFs has been selected to several reasons. This includes a significantly available overall intake statistics for a susceptible population including breastfed babies as well as the reputable total daily dietary intake (TDI) that is more protective in comparison to other electronic wastes associated compounds, as the risk of additive mixture was taken into consideration (Frazzoli et al. 2010).

These chemicals in the breastfeeding mother have a half-life of weeks but due to continuous exposure, the burden of the body with toxins regenerated as studied by Chan et al. (2007) on the solubility of lipids in milk. The exposure by infants resulted approximately 40 times the total dietary intake by WHO. It is deducible that the breastfed infants are exposed to PCDDs and PCDFs through their food intake and is related with electronic waste.

Dioxin-like chemicals, PCDDs and PCDFs react through cleaving on to the aryl hydrocarbon receptor (AhR). The permanent effects invade the reproductive, neurobehavioral and developmental systems, suppressed immunity, toxins in the liver and endocrine, modifications in the metabolism of lipid and gluconeogenesis, as well as occurrence of oncogenic effects (Frazzoli et al. 2010).

1.3 Management of electronic wastes, associated hazards and risks

1.3.1 Recycling

Recycling of E-waste generally includes dismantling and damage to the unwanted equipment for new materials to be recovered (Cui & Zhang 2008). A computer contains approximately 95% of valuable materials that can be recovered (Ladou & Lovegrove 2008). In Japan, recycling activities are operated with advanced technology and thus causes less pollution or effects to the environment (Aizawa, Yoshida & Sakai 2008). The recovery of glass with high content of Pb from discarded CRT can be recovered using modern techniques with less threats to the environment (Andreola et al. 2007). Wastes that poses harmful pollution to the environment and has be transferred to a far distance are of ecological benefits that are more than offset (Barba-Gutiérrez, Adenso-Diaz & Hopp 2008). Nonetheless, in comparison with landfilling of incinerated E-waste, recycling process often has a lower ecological impact (Hischier, Wäger & Gauglhofer 2005).

1.3.2 Mechanical technique

Mechanical processes involve the separation of numerous types of metals and constituents that are present in E-waste either through the process of grinding or crushing. The materials are then categorised into distinct portions based on the physical characteristics of wastes. This includes volume, shape, as well as their conductivity and magnetism. Typically, the ferrous parts are sorted through magnetic separation, aluminium by Eddy current separation involving electrical conductivity, and gravity separation for heavy media floating and sifting (Tsydenova & Bengtsson 2011).

Size reduction and separation process contribute to the primary threats of mechanical treatment methods. Dusts of the shredded components are generated during the process of destroying or shredding. MJC (2004) reported that during the size reduction process, the dusts formed contain plastics, metals, ceramic, glass and silicon dust. Shredded particles that are handled during the

process of separation are associated with the same threat of dust. The dusts may cause risks to workers due to inhalation and dermal exposure and risk of contamination of the environment.

Hazardous substances are proven to be released during shredding process. Peters-Michaud, Katers & Barry (2003) assessed the air quality in the vicinity of electronic waste shredders in a US based electronics recycling facility and has found high concentration of cadmium and lead of 0.27 and 1.4 lg/m^3, respectively. The finding indicated that the workplace was contaminated and there was a high chance that the employees were continuously exposed to the toxic metals.

Plastics with brominated flame retardants that are shredded cause toxic chemicals to be released into the environment. Relatively huge facts concerning the effect of the chemicals during the shredding processes are present. A study by Morf et al. (2005) on a Swiss recycling area involved in mechanically treated wastes reported that BFRs predominantly PBDEs were detected in the fraction of dust from the purification system of off-gas. This highlights that there is a high possibility of the emissions of brominated flame retardants when E-waste is processed mechanically.

1.3.3 Landfilling

Landfilling also known as dumping ground is a common site for the disposal of waste materials. The threats related to the dumping of E-waste in landfills are due to the diversity of substances present in them. Leaching and evaporation of hazardous substances are the main concerns on E-waste landfilling.

Thirty-six CRTs were assessed for their leachability using Toxic Characteristic Leaching Procedure (TCLP) by Musson et al. (2000b). From the study, it was found that twenty-one of the thirty colour CRTs exceeded the 5 mg/l of lead regulatory limit to be categorized as a hazardous waste. An average lead concentration of 75.3 mg/L came from the funnel portion of the CRTs and this is considered the largest concentration of leachable lead.

In another study conducted by Osako, Kim & Sakai (2004) on Japan landfills, brominated flame retardants were found to be present in the leachate. They found that the landfills with crushed electronic wastes contained higher concentrations of BFRs. There is also a possibility where volatile harmful chemicals vaporise apart from the leaching of substances in landfills. For instance, metallic mercury that could leach into the ground as well as vaporise are of great worry. Lindberg et al. (2001) reported that an organic dimethyl mercury was detected in the gas that was released from a landfill and the concentration was higher as compared to the normal ambient air (Lindberg et al. 2001).

1.4 Composition of plastics in electronic wastes

WEEE contains various ranges of materials and hence, it is a challenge to provide a comprehensive composition of materials that are found in the waste river. Nevertheless, ferrous metals, non-ferrous metals, glass, and plastics are among the four categories examined by most studies. The commonly found materials in EEE by weight are steel and iron. This materials represent 50% of the whole weight of WEEE. The second largest component by weight is plastics and represents approximately 21% of WEEE (Ongondo, Williams & Cherrett 2011). Approximately 13% of the total weight of WEEE is represented by the non-ferrous metals that include precious metals with copper representing for 7% (Ongondo, Williams & Cherrett 2011). Electrical and electronic equipment are manufactured using a wide variety of polymers that includes PVC, ABS, PUR, polystyrene (PS), polypropylene (PP), polyethylene (PE), polyamide (PA), polycarbonate (PC), Epoxy, polyoxymethylene (POM), and PET/polybutylene terephthalate (PBT) (Miguel 2000).

In 1997, the total worldwide consumption of plastic is about 145 million tons with PUR accounting for 5% and this results in PUR being the fifth in global plastic consumption (Uhlig & Conrad 1999). PUR in the form of foams represents over three-fourths of the worldwide consumption of PUR and in 1960 at the United States, there are 45,000 tons of PUR being produced which later in the year of 2004, it increased to 2,722,000 tons (Howard 2011). The following represents the worldwide consumers of PUR (see **Figure 1**).

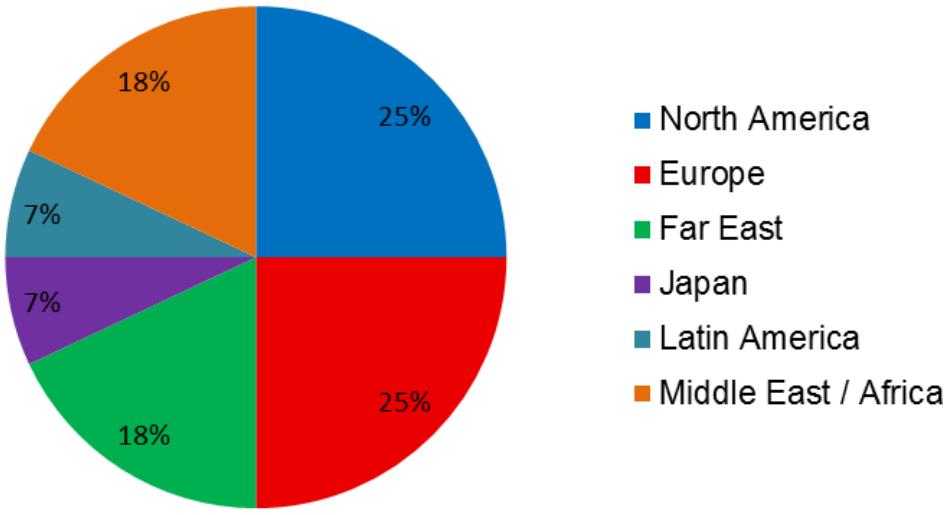


Figure 1 Main global consumers of polyurethane (Uhlig & Conrad 1999).

1.5 Polyurethane degradation

Polyester polyurethane (PUR) derived from the condensation of isocyanates and polyalcohols are a significant class of thermoplastic non-biodegradable polymers. PUR mostly consists of three building blocks, the soft segments typically diols of long chain molecules of polyether, polyesters, polysiloxane, polycarbonate which impart flexibility; the hard segments which are commonly the combination of di-isocyanates; and the chain extender in which chain extender also acts as a cross-linker (Guan et al. 2004; Ma et al. 2011; Martin et al. 2000; Skarja & Woodhouse 1998; Woo, Mittelman & Santerre 2000). PUR displays extremely good resistance, elastic, and durable material.

In order to clarify whether a decreased in biodegradation process is affected by the addition of other chemicals to PUR, several researches have been initiated. The polyester and polyether PUR cured by sulphur showed some inertness to fungi (Kanavel, Koons & Lauer 1966). However, the growth of fungi was still observed on the polyether PUR even with the addition of fungicides to PUR cured with sulphur and peroxide. After a prolonged exposure to the fungal activity, the need for physical testing of the PUR was also acknowledged (Kanavel, Koons & Lauer 1966).

A study conducted by Santerre et al. (1994) varied the physical appearance of the polyester PUR as films or as coatings on glass tubes to determine the variations in the amount of degradation products that are released. This inferred that urethane and urea groups are not often accessible to the enzyme while vulnerable to hydrolysis and degradation may never occur past the surface of polymer. Higher radiolabel products released from enzyme incubated samples were consistently observed as compared to controls even though there was no significant degradation of the polyether PUR. These findings were due to the secondary structures and bondage of hydrogen that defend the cleavage sites of ester (Howard 2011).

PUR are also resistant to macromolecular oxidation, hydrolysis and calcification in the field of medicine (Marchant 1992). PUR elastomers are commonly chosen instead of other elastomers because of its greater ability to stretch, tougher and long-lasting, more resistant to tear, as well as the environmental stresses (Dombrow 1965; Saunders & Frisch 1962; Ulrich 1983). Furthermore, the overall cost for production of polymer can be lower since the derivatives of polyether are cheap and affordable. The biodegradability of polyamide-urethanes for medical purposes was tested (Huang & Roby 1986a). In summary, PUR with extensive repeating units were synthesised that contains groups of amide and urethane. Hydrolysis was observed on the resulting partial crystalline fibres. Prior to the crystalline regions of PUR, the amorphous regions on the PUR were being degraded. These fibres showed promising use as absorbable structures and implant.

In another study conducted by Huang & Roby (1986b) to produce PUR that could degrade for medical usage, polycaprolactonediols are used to synthesize polyester PUR. They made several different PUR which contains polyester subunits of numerous lengths. The enzyme axion and two fungal species were applied to test for the degradation of polymer. Each PUR was degraded by the enzyme and fungi. Moreover, an increase in the chain length of polyesters was observed to have also increased the biodegradability of polyester PUR.

Two proteolytic enzymes including papain and urease were observed to have degraded medical polyester PUR (Phua et al. 1987). The PUR of Biomer was tested which is of segmented, cross-linked polyester PUR. As described by (Kaplan et al. 1968) that degradation was inhibited by cross linking, papain with a molecular weight of 20.7 kDa could not diffuse into the PUR and had caused the structure to disintegrates. Due to the larger molecular size of 473 kDa, the activity of urease was limited to the surface of PUR and hence was insignificant. Papain disintegrated PUR through the hydrolysis of urethane and urea to produce free mobile amine and hydroxyl groups (Phua et al. 1987). Marchant et al. (1987) assessed the effect of papain on polyether PUR. They compared the hydrolysis activity of papain in aqueous form and found that products were also released. Water hydrolysed the ether linkages while the presence of proteolytic enzyme is necessary for the degradation of the urethane groups.

1.6 Bacterial biodegradation

The problem of littering and surface water pollution is intensified due to the fact that plastics are not biodegradable in landfills (Mukherjee et al. 2011; Rowe & Howard 2002; Russell et al. 2011). Hence, bioremediation approaches are being developed for plastic degradation utilizing microorganisms.

In an extensive test of bacterial activity against PURs, 16 organisms that can degrade PUR was further investigated (Kay, Morton & Prince 1991). Seven of the isolates tested were able to degrade PUR when media was supplemented with yeast extract. In the presence of minimal media, only two isolates which are *Corynebacterium* sp. and *Pseudomonas aeruginosa* could degrade PUR. However, none of the isolates grew solely on PUR. Different tensile strength and elongation were observed with significant decreases for each isolate.

In an additional study, the physical and chemical alterations in disintegrated PUR was investigated (Kay, McCabe & Morton 1993). It was observed after 3 days that the elongation and tensile strength of PUR were significantly reduced by *Corynebacterium* sp.. The bacterial isolates were reported to have attacked the ester segment of PUR through the analysis of infra-red spectrophotometer. The production of esterase was supressed by the addition of glucose in the media (Howard 2011). However, the activity of esterase was not increased by the addition of PUR.

In another study, the growth of a number of bacterial species were tested on the PUR army airplane paint (El-Sayed et al. 1996). The *Acinetobacter calcoaceticus* and two *Pseudomonas* sp. were successfully isolated by the researchers. Besides that, strains of *A. calcoaceticus*, *P. aeruginosa* and *putida* were also supplied by the U.S. Navy. The PUR paint was utilised by all the species as the only source for energy and growth except for isolate *Pseudomonas cepacia*. The rest of the bacterial species exhibited esterase activity without presence of PUR when fluorescein diacetate was used as an esterase substrate, and this indicated that the PURases were essentially expressed.

1.7 Fungal biodegradation

Manufacturers found PUR susceptible to degradation after years of PUR production. Each samples of PUR have their own trends of degradation and this is due to the diverse PUR characteristics that includes the coordination of molecules as well as the functional groups that are present within them (Pathirana & Seal 1983). The synthetic polymers have great flexibility and this permits the polymer chains to be easily packed which causes crystalline regions to form. This causes the amorphous regions on PUR to degrade more readily while limiting the crystalline polymer chains to have access to degradation.

PUR degradation was observed to occur in a pattern where the amorphous regions of PUR will firstly be disintegrated followed by the crystalline regions (Huang & Roby 1986a). The long repeating units of PUR and hydrolytic groups were found to be biodegraded with ease due to their inability to be packed into crystalline regions. The degradation of PUR by microbes can be caused by the action of enzyme including proteases, ureases, and esterase as suggested by several researchers (Evans & Levisohn 1968; Filip 1978; Griffin 1980; Hole 1972).

There are studies reported that several fungi are able to disintegrate or degrade PUR (Darby & Kaplan 1968; Kaplan et al. 1968; Ossefort & Testroet 1966). The research discovered the ability of fungi to degrade PUR in the form of polyester as compared to other forms. Additionally, it was noted that polyether PUR were highly resistant too. In another study, fungi *Chaetomium globosum* and *Aspergillus terreus* were found to be able to produce enzymes of degrading activities (Boubendir 1992). However, enzymes need to be induced in order for these organisms to grow on PUR only. Liquid polyester PUR was added to the growth media for induction of the degrading enzymes. Enzymatic assays using ethyl carbamate as the artificial urethane substrate was also performed to determine the activity of enzymes produced.

Crabbe et al. (1994) have successfully isolated four species of fungi including *Curvularia senegalensis*, *Fusarium solani*, *Aureobasidium pullulans*, and *Cladosporium* sp. based on their ability to utilise PUR, Impranil DLNTM as the only source for energy and growth. In the study, further analysis on *Curvularia senegalensis* isolate was conducted due to its higher activity of degrading PUR. An extracellular polyurethanase (PURase) was purified from this fungal isolate which displayed high esterase activity. The purified protein has a molecular mass of 28 kDa with heat stability at 100 °C and is prone to inhibition by phenylmethylsulphonylfluoride (PMSF).

1.8 Proteomics

Most biological research until the early 1990s, have been focusing only on individual genes and proteins and were analysed individually at each time. As time advances to the early and mid of 1990s, this strategy shifted to a larger scale of molecular study which starts with research on transcriptomics and genomics and later into proteomic (Bruggeman & Westerhoff 2007; Tan et al. 2009). Each approach contributes to the complexity of living organisms and must be validated. This is reflected as part of a multidisciplinary integrative analysis at different levels that exends from the gene to the phenotype through proteins and metabolites.

Currently, the study of any microorganisms on their proteomes is of importance and has become the vital field of research. At year 2010, the results obtained from studies are much lower than what was originally expected due to the dynamic behaviour of proteins that are relatively sophisticated. This particularly includes the quantity of protein groups that are present in each gene after alternative splicing, as well as posttranslational modifications, and the consideration of proteins as complexes instead of individual proteins that are the functional units of an organisms‟ life. Nevertheless, apart from other biological systems especially yeast (Picotti et al. 2009a) and humans (Anderson et al. 2009), the study on proteomics is yet to be explored in most fungi.

In 1994, Marc Wilkins created the term proteomics and refers to the “PROTein complement of a genOME” (Wilkins et al. 1996). Proteome study was known since the early 1970s but was introduced as a concept only in the 1990s (see **Figure 2**). After 15 years, proteomics has become an intricate scientific discipline that deals with the understanding of the cell proteome instead of only a methodology for an experiment or an appendix of genomics. Proteome refers to the total quantity of organelle, cell, tissue, organ, and system that are found in a biological life at any growing stage and under definite ecological environments.

Through the study of proteomics, the information on when, where, and how the different hundreds of proteins are produced within a biological unit as well as the interaction between each proteins and other molecules in constructing the cellular wall can be obtained. A more detailed understanding on how these proteins interact during the cell growth and development apart from the interaction with their environments can also be achieved through proteomic study. In the past 10 years, the concepts, principals, applications, and limitations of proteomics have been reviewed excellently (Cox & Mann 2007; Cravatt, Simon & Yates Iii 2007; Han, Aslanian & Yates 2008; Han et al. 2006; Jorrín-Novo et al. 2009; Mann 2009; Picotti et al. 2009b; Schmidt, Claassen & Aebersold 2009) with some of them that deals with fungal pathogens (Bhadauria et al. 2007; Kim, Nandakumar & Marten 2007b; Tan et al. 2009).

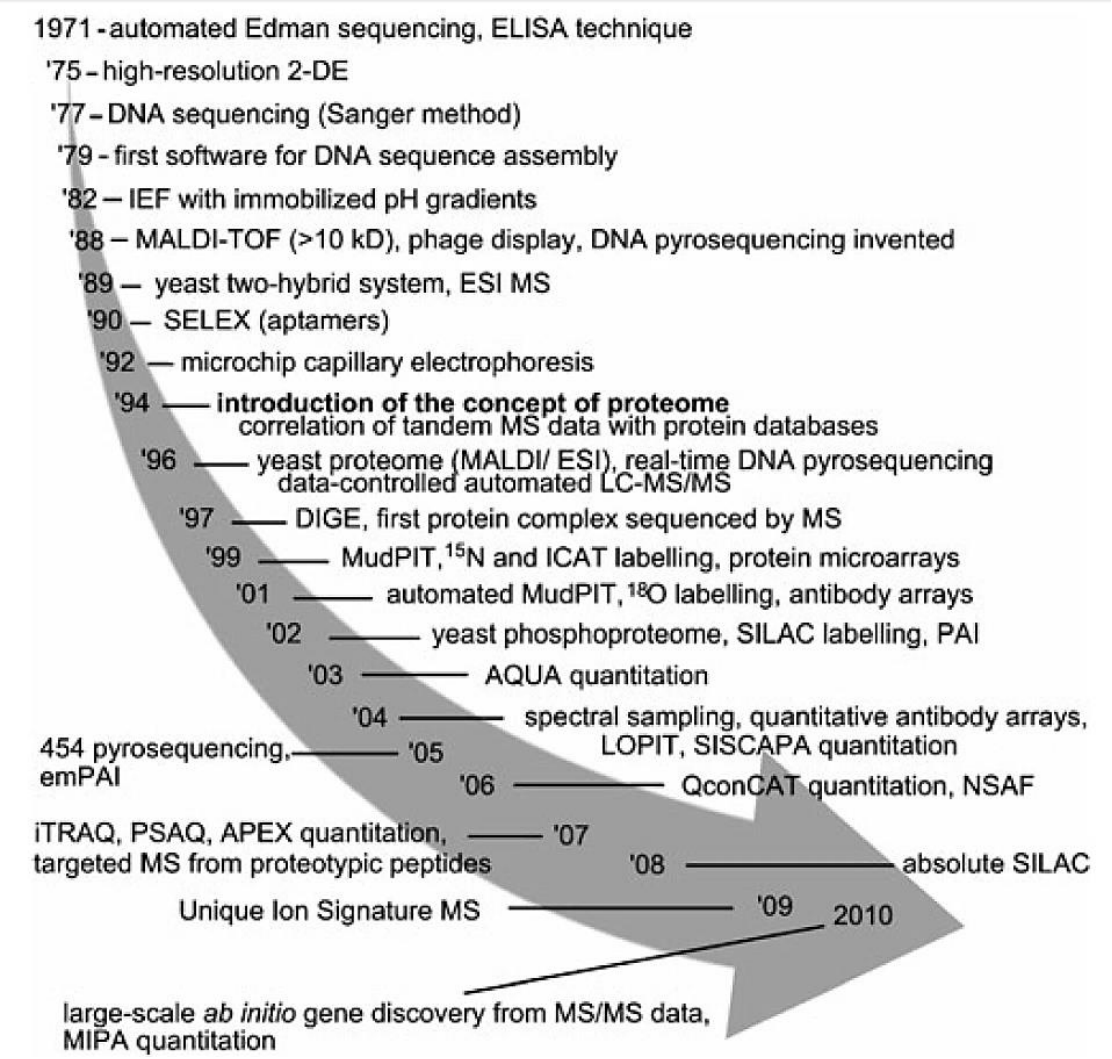


Figure 2 Timeline of the development of proteomic (de Oliveira & de Graaff 2011).

The most popular two-dimensional (2-D) technique for proteomic study involves the utilization of isoelectric focusing (IEF) and sodium dodecyl sulphate (SDS) electrophoresis. The strategy in the first dimension would be to discriminate molecules based on their net-charge followed by separation according to size of molecules, in the second dimension (Righetti, Gianazza & Ek 1980). This method of fractionation based on charge and mass was first introduced by Barrett & Gould (1973) and Macgillivray & Wood (1974). The 2-D technique became extremely popular when O"Farrell (1975) described a simple method for gels casting and assembly of electrophoretic cell for 2-D electrophoresis that revealed thousands of protein spots on the gel.

The commonly used yeast, *Saccharomyces cerevisiae* for baking is the first organism that has its genome to be completely sequenced (Goffeau et al. 1996). *S cerevisiae* is also the organism with the most extensive studies on its complete proteome (Ghaemmaghami et al. 2003; Huh et al. 2003; Newman et al. 2006). Parker et al. (2004) revealed that even small changes in expression of proteins of genetically modified yeast can be detected using isotope-coded affinity tag (ICAT) in comparison to the wild type. In their study, they discovered a protein that was down-regulated and at the same time an increase of proteins that are involved in the synthesis or arginine. The study demonstrates the importance and usage of data obtained through proteomic to further understands the changes that are detected after genetic modification.

Yeast cells are often genetically modified to be used for application in the filed biotechnology to produce other various compounds (Asadollahi et al. 2008; Takahashi et al. 2007), simultaneous saccharification and ethanol fermentation (Rudolf et al. 2008), as well as the removal of toxic substances (Singh et al. 2008). Due to the economic importance, the process design using recombinant yeast or naturally utilising *Pichia* strain was intensively optimised and investigated (Agbogbo et al. 2006; Rudolf et al. 2008). However, there are still very few researches on proteomic of yeasts that utilise xylose.

The *Saccharomyces* yeasts have been widely used for fermentations in the production of beer and wine, and provides significant economic values (Pizarro et al. 2007). In an outstanding evaluation by Bisson (2005) on the biotechnology of yeast used to make wine, a summary on how the strain was constructed was given. However, the statement that “proteome analysis of interchanged yeast strains can further demonstrate the absence of an effect on the protein constitution of a cell is unfortunately obtained from the data through the usage of traditional and less sensitive methods” (Futcher et al. 1999; Shevchenko et al. 1996).

A new established method such as isotope-coded affinity tag (ICAT and iTRAQ) have higher sensitivity and minor alterations in the cell proteome can be identified through a comparison study (Parker et al. 2004; Wiese et al. 2007). For production of different proteins, *S. cerevisiae* is also the most frequently used eukaryotic host (Hensing et al. 1995; Van de Laar et al. 2007). There are still no researches on its proteome to detect the change in the trend of protein production by yeast cells. Lately, a study on the repression of glucose by *S. cerevisiae* was conducted at a genomic level (Westergaard et al. 2007), deprivation (Roth, Kumme & Schüller 2004), and the studies on how ethanol are produced display new developments into the proteomic level (Hjersted, Henson & Mahadevan 2007) as well as the proteomic analyses of *Schizosaccharomyces pombe* (Sun et al. 2005; Weeks et al. 2006).

Yeast *Candida albicans* causes infections in persons with weaker immunity and are well-known as human pathogen (Rupp 2004). There were intensive studies on this pathogenic yeast‟s proteome because of its importance (Fernández-Arenas et al. 2007; Insenser et al. 2006; Kusch et al. 2007; Martínez Solano et al. 2006; Thomas, Bachmann & Lopez Ribot 2006). *Candida albicans* becomes pathogenic to its host due to changes in its morphology to the hyphal form. Proteins which are differently regulated in this transition were studied (Ebanks et al. 2006). In addition, proteomics study towards the development of vaccine against this harmful pathogen is in progress (Ebanks et al. 2006; Thomas et al. 2006).

A summary on the proteomes of filamentous fungi was studied and given (Kim, Nandakumar & Marten 2007b). So far, there are 18 diverse species being sequenced successfully and a few other studies are still on-going to further understand the genomes of fungi (Kim, Nandakumar & Marten 2007b). Carberry & Doyle (2007) also studied the proteomes of industrially and biomedically significant fungi. The *Aspergillus fumigatus* is similar to *Candida albicans*, an opportunistic pathogen that affects individuals with weak immunity system, causes almost 4% of deaths in all the hospitals in Europe, and is the most common *Aspergillus* species that are related to aspergilosis disease (Brakhage & Langfelder 2002). It was also vital to sequence the genome of this medically

important organism (Nierman et al. 2005) as a boost for further proteomic analyses (Carberry et al. 2006; Kniemeyer et al. 2006) and for investigations on vaccines development against this pathological fungus (Asif et al. 2006).

The *Aspergillus oryzae* fungus also has its entire genome to be successfully sequenced (Machida et al. 2005). In Japan, this fungus is used and fermented to produce foods and beverages. Its use in biotechnology was facilitated (Christensen et al. 1988; Tsuchiya et al. 1992) by its ability in the secretion of large quantities of proteins, to develop a transformation system (Gomi, Iimura & Hara 1987), as well as the inability of *A. oryzae* to produce toxins. The extracellular proteins (Oda et al. 2006; Zhu et al. 2004), conidial proteins (Nguyen et al. 2005) of this fungus were analysed on a proteome level. However, there is still no comprehensive study on the diversity of developmental stages and production by *A. oryzae*.

In order to know further on how metabolism was regulated, and the behaviour of filamentous fungi, a study on *Aspergillus nidulans* has been conducted (Galagan et al. 2005). This mold is both industrially and biomedically important and often used as an organism for the development of novel antifungal chemicals (Forgue et al. 2006). The genome of *A. nidulans* was completely sequenced and a comparative analysis was accomplished with genomes of *A. fumingatus* and *A. oryzae* (Nevalainen, Te'o & Bergquist 2005). These results are interesting, however, the proteome of this important model fungus need to be explored extensively (Kim, Nandakumar & Marten 2007a; Nevalainen, Te'o & Bergquist 2005).

The filamentous fungus *Aspergillus flavus* has the potential to produce industrial chemicals particularly hydrolytic enzymes (Medina, Kiernan & Francisco 2004). Some proteins secreted from *A. flavus* have been identified (Medina et al. 2005; Medina, Kiernan & Francisco 2004). Nonetheless, an in-depth study on its proteomes is still required. Both strains of *Aspergillus niger* and *Penicillium chrysogenum* that are often used industrially do not have any data about their proteomes. However, organic acids and industrial enzymes were produced

through the fermentation of *A. niger* and its genome sequence was successfully studied (Pel et al. 2007).

The *P. chrysogenum* is commonly used to produce penicillin and commercial enzymes (Johnstone Robertson, Clarke & Harrison 2008). The genome sequence of this strain are not out for publication yet (Liolios et al. 2008). Intensive studies on the genomes of *A. niger* (Wang et al. 2008) or *P. chrysogenum* (van den Berg et al. 2007) or both (Braumann, van den Berg & Kempken 2007), has been conducted other than the studies on the usage of these strains to produce commercial enzymes (Johnstone Robertson, Clarke & Harrison 2008). Proteomic studies of these two important industrial fungi are still absent. More insights into their proteome could be obtained through the data analysis of the present genomic sequences of *Aspergillus niger*.

The *Trichoderma harzianum* is a soilborne mold that is of great importance in preventing the growth other fungal pathogens (Grinyer et al. 2004b). Proteomic study of this fungus has decreased due to the genome of *T. harzianum* that are not being sequenced. Nevertheless, an entire-cell protein reference diagram allowed 25 proteins of *T. harzianum* (Grinyer et al. 2004b) to be identified in addition to 25 mitochondrial proteins that were identified (Grinyer et al. 2004a). The activity of fungicide of *T. harzianum* depends on its ability to disintegrates the hosts‟ cell wall through the secretion of hydrolytic enzymes. Analysis on secretion of the extracellular proteome by this fungus has now been analyzed (Suárez et al. 2005). *Trichoderma atroviride*, a further biological control fungus was also investigated for its proteome (Grinyer et al. 2005; Grinyer et al. 2007; Marra et al. 2006).

Phanerochaete crysosporium is a white-rot fungus that degrades lignin, cellulose and hemicellulose of plants (Abbas et al. 2005; Kirk & Farrell 1987). Nevertheless, most of the identified proteins through proteomic study revealed that hemicelluloses could only be degraded by proteins that poses enzymatic activities (Abbas et al. 2005). There have also been studies on proteomes of *Neurospora crassa* (Schmitt et al. 2006), and the phytopathogenic fungi

Sclerotinia sclerotiorum (Yajima & Kav 2006) and *Botrytis cinerea* (Fernández-Acero et al. 2007).

Proteomics is constantly being renewed to an exploration of novel techniques, and platforms with constant advancements that are made at all workflow steps, starting from the laboratory (tissue and cell fractionation, protein extraction, depletion, purification, separation, mass spectrometry (MS) analysis) and ends at the computer (algorithms for protein identification and bioinformatics tools for data analysis, databases, and repositories). Only a tiny fraction of the cell proteome has been characterised so far despite the technological accomplishments in proteomics, and only for a few biological systems that includes human, fruit fly, *Arabidopsis*, and rice.

The function of a number of proteins still remains to be investigated for these organisms (Cravatt, Simon & Yates Iii 2007). Proteomics approaches have numeral challenges including sensitivity, resolution, and speed of data capture. This technique also encounter a few limitations including deeper proteome coverage, proteomics of unsequenced organisms, top-down proteomics (Han et al. 2006), protein quantification (Cravatt, Simon & Yates Iii 2007), post translational modifications (PTMs) (Bhadauria et al. 2007), and Interactomics (He et al. 2008; Zhao et al. 2009).

1.8.1 Challenges

Since the past few years, the study on proteomes of fungi has increased and this is due to the improvement in high resolution methodologies for mass spectrometry to separate proteins, its software to identify and characterise proteins more effectively, and the technology used for bioinformatics study (Bhadauria et al. 2007). However, different technical challenges still exist.

For instance, it is necessary to detect from one to few millions of molecules in a cell since there is no protein equivalent of PCR to amplify proteins with low quantity (Bhadauria et al. 2007). Major difficulty arises in analysing PTMs due to the complex folding structure of proteins, and hence the design of techniques and its application are challenging. Some technological processes are inherently skill-based especially the separation and analysis of protein, and this remains a challenging tasks.

A DIGE is an alternative method to separate proteins which could be controlled automatically. However, the reproducibility of the proteins being separated remains as a challenge. Nevertheless, numerous technology are constantly being established to study the proteomes, functions or structures at a genomic level including the study of expression profiles or the interaction between molecules and either alone or in combination of various technologies could be applied. These include the protein arrays (Walter et al. 2000), two-hybrid system of yeast (Fromont Racine et al. 2000), phage-display antibody libraries (Griffiths & Duncan 1998), surface-enhanced laser desorption and ionization (Senior 1999) and the profiles of biological activity of proteins families (McKerrow et al. 2000).

Environmental challenge exist in which all aerobically growing organisms including fungi must adapt to the exposure to reactive oxygen species (ROS) caused by partially reduced forms of molecular oxygen. In the field of yeast genetics and molecular biology, intensive researches have been done to elucidate the essential global regulators of the oxidative stress response.

For example, the bZIP family transcription factor Yap1, which represents the major regulator present in all fungal species (Rodrigues Pousada, Menezes & Pimentel 2010). Additionally, two-component signal transduction systems contribute to sensing of oxidative stress and activating stress response factor, including the transcription factor Skn7 (Fassler & West 2011). Little is known regarding the transcriptional circuits associated with the detoxification of reactive nitrogen species (RNS) in comparison with the regulators of the oxidative stress response. In response to defeat pathogenic microorganisms, RNS was generated by phagocytic cells of the human innate immune system.

Due to this reason, in the human pathogenic yeast *Candida albicans*, the NO response was studied and the associated transcription factor, Cta4p which was found to initiate the NO response (Chiranand et al. 2008). The transcription factor, Fzf1p was found to regulate the analogous response in baker's yeast (Sarver & DeRisi 2005). A negative regulator of NO stress, Cwt1p was later described for *C. albicans* (Sellam et al. 2012).

Another stress factor fungi have to cope with in the environment is oxygen depletion and is at the challenge of host-pathogen interaction. The drop in metabolite levels of haem and ergosterol analysed from *S. cerevisia*e and *S. pombe* respectively have indirectly indicate depletion of oxygen, where the availability of molecular oxygen is essential for the biosynthesis. The mechanisms of sensing hypoxia were also shown similarly for the human-pathogenic fungi, *Aspergillus fumigatus* and *Cryptococcus neoformans* (Grahl et al. 2012; Hickman & Winston 2007).

Another common physiological stress in fungal cells is due to the low availability of nitrogen and carbon sources. Transcriptions factors of the GATA family in fungi facilitate a general control mechanism termed nitrogen metabolite repression. During the growth on easily assimilated nitrogen sources of ammonium, repression of genes involved in the utilisation of alternative N-sources occurred. Due to limited nitrogen, it was also reported that the TOR signalling pathway control the gene regulation in yeast (Beck & Hall 1999). Transcriptional regulators are significantly different among fungal species even

though the general principle of regulating the N-metabolism is of great similarities (ter Schure, van Riel & Verrips 2000; Todd et al. 2005; Wilson & Arst 1998).

Amino acid control or cross-pathway control refers to the regulatory response of yeast and filamentous fungi, respectively to the depletion of amino acid. In response to amino acids starvation, the transcriptional activator GCN4p (CpcA/Cpc1) induces the expression of most amino acid biosynthetic enzymes (Hinnebusch 2005; Krappmann & Braus 2005). It is noted that this stress response contributes to the survival of human-pathogenic fungi in the host (Krappmann & Braus 2005; Rubin-Bejerano et al. 2003). Similarly, limited source of carbon is perhaps an important state of fungal cells in the infected host. In most fungi, the suppression of glucose pathway that controls the preferential glucose utilization can be found. Other complex regulatory mechanisms in sensing and responding to fluctuation of glucose levels differ from fungus to fungus (Geladé et al. 2003; Ruijter & Visser 1997).

There has been accumulation of a vast amount of knowledge on the molecular mechanisms of adaptation to harsh stress conditions in fungi. In addition, the changes of transcriptome of variety of fungi in response to environmental stresses or during host-pathogen interaction have been widely studied (Cairns, Minuzzi & Bignell 2010). However, there is scarce knowledge about the impact of stress on the proteome of fungi. Nevertheless, new proteomic technologies hold great potential for translating regulatory systems and the role of posttranslational regulatory mechanisms in response to stress of fungi.

1.9 Aim and scope of the study

Bioremediation has been emerging as an alternative and attractive tool to treat pollution. Various organisms have been assessed for their bioremediation potential; however, not many studies have looked at the ability of organisms to degrade polyurethane (PUR). Even less studies looked at the potential use of endophytic fungi for bioremediation purposes, especially for polyurethane.

In this thesis, we aim to explore the potential of endophytic fungi from *Nepenthes ampullaria* (pitcher plant, collected in the Mentawai Jungle, Sarawak) for bioremediation purposes of the plastic component in E-waste, polyurethane (PUR) polymers. To further understand the underlying mechanisms and intracellular activities involved of the endophytic fungi in response to polyurethane stress, a proteomic analysis was carried out.

In chapter 2, we describe the methodologies applied in this study which enables us to (i) isolate, screen and identify polyurethane degrading endophytic fungi and (ii) conduct degradation and proteomic analyses of the most effective fungus. In chapter 3, we report and discuss the findings on the isolation, identification and screening, as well as the enzyme experiment. Besides, the findings of the different expression levels of identified protein of the polyurethane degrading endophytic fungus through proteomic studies in response to polyurethane stress is reported and discussed.

The objectives of this study are:

1. Isolation and identification of endophytic fungi from *Nepenthes ampullaria* plant.
2. Assessing the potential of fungal isolates in degrading polymer PUR.
3. Determination of the effects of PUR treated fungi on their proteins through proteomic approaches.

2. Materials and method

2.1 Isolation of endophytic fungi

Surface sterilisation is necessary for the isolation of endophytes to ensure the removal of contaminants as well as high survival rate of explants (Srivastava et al. 2010). This technique is effective in removing all microorganisms on the surface including actinobacteria, and epiphytes (Coombs & Franco 2003). Sterilisation procedure needs to be optimised particularly the concentration and time of exposure of sterilising agents depending on the softness and hardness of tissues (Srivastava et al. 2010).

Three plant samples of *Nepenthes ampullaria* were collected during the dry season at Bukit Mentawai (4°10‟59.988” N, 114°55‟0.012” E), Kuching, Sarawak, Malaysia. Collected plant samples were rinsed instantly while on the boat, with distilled water for 5 minutes, surface sterilised by immersion in 70% ethanol for 3 minutes, 3.5% sodium hypochlorite for 3 minutes, and sterile distilled water for 3 minutes (Bills, Redlin & Carris 1996). Three section of plant were cut with sterilised razor blade and plated on Potato Dextrose Agar (PDA) (Difco) immediately. All the plates were sealed with parafilm, incubated at 25 ºC, and observed for growth every 3-4 days. As growth was observed, fungal organisms were isolated by transferring a hyphal tip to a freshly prepared PDA plates. The plates were again sealed with parafilm, incubated at 25 ºC, and observed for formation of mycelia. Fungal isolation was repeated until a pure culture was obtained (see **Figure 3** and **Figure 4** for an overview of isolation process).

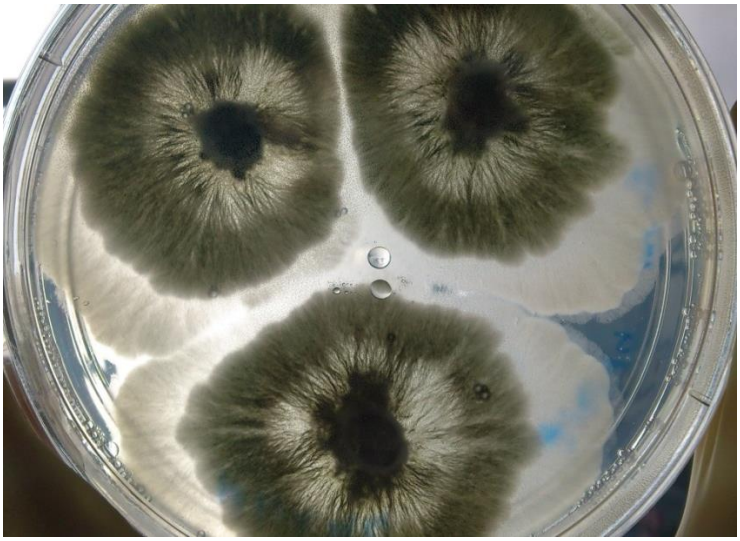


Figure 3 Pure culture of endophyte after surface sterilisation of plant sample.

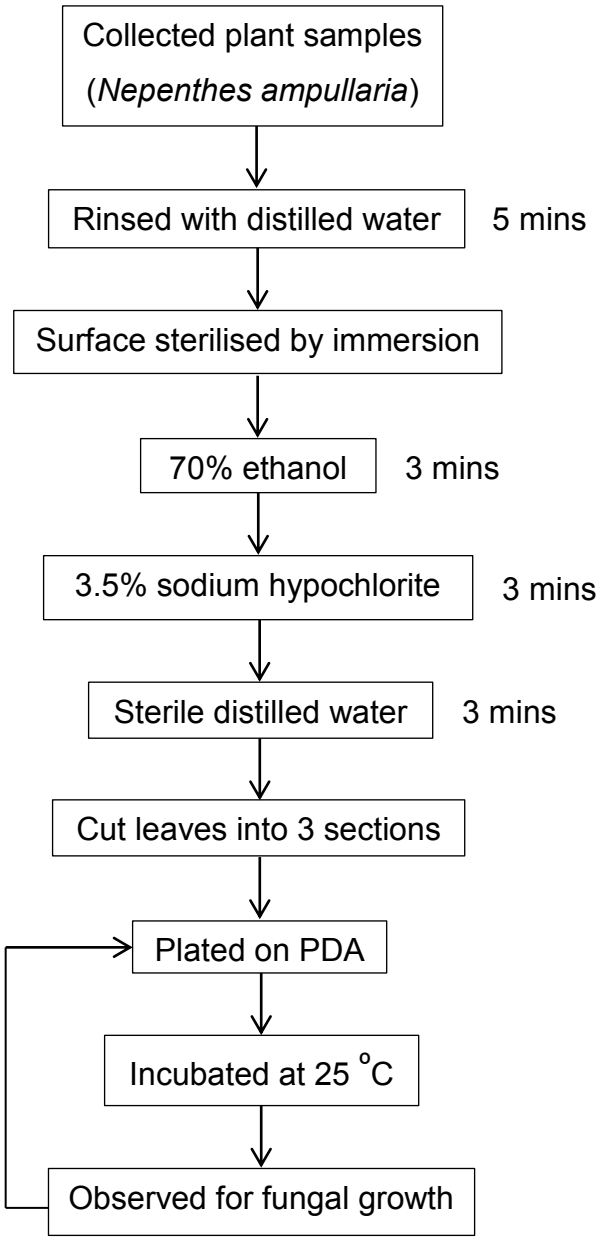


Figure 4 Overview of the isolation of endophytes from collected plant samples.

2.2 Cultivation of endophytic fungi

It is essential to preserve fungal stains for in-depth study but both the viability and stability of living cells need to be ensured during the period of preservation (Espinel-Ingroff, Montero & Martin-Mazuelos 2004).

Pure fungal isolates were grown on PDA media at 25 ºC for several days until the fungal hyphae covered three quarter of the PDA media surface. The cultures were then kept at 4 ºC for further usage. This short term storage of fungi can be used within 6 months.

Permanent stock cultures were prepared by growing them in universal bottles in which PDA has been allowed to set at a slope. A week old fungi grown on plates were transferred using sterile straw and placed onto the media in sterile universal bottles. The culture was incubated and stored at 4 ºC for further use (see **Figure 5** for short and long term storage).

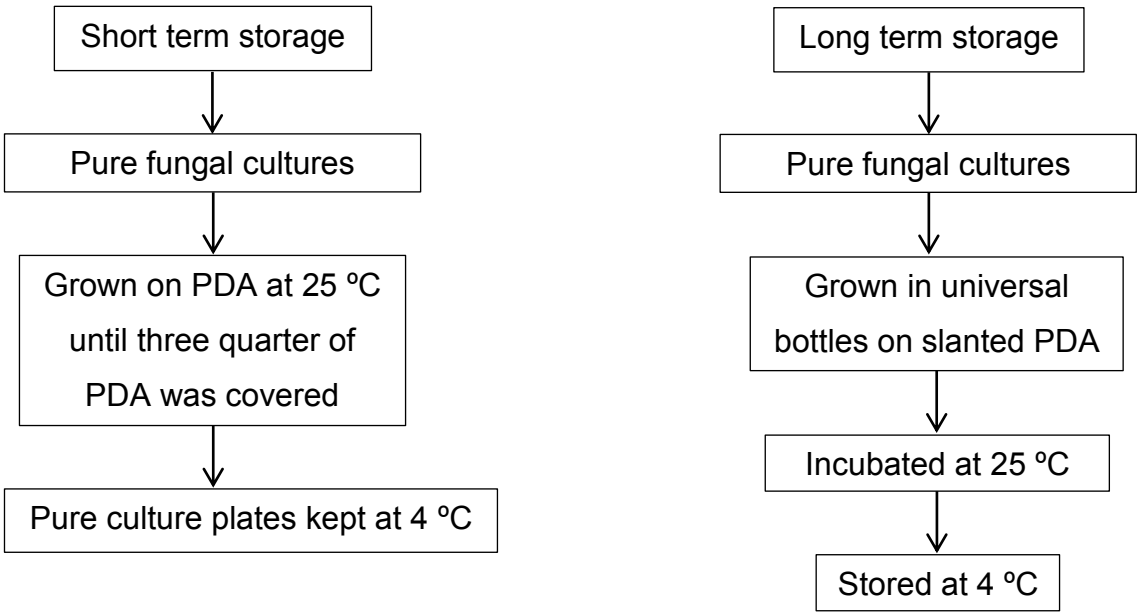


Figure 5 Short and long term storage of cultivated endophytic fungi.

2.3 Biological assays

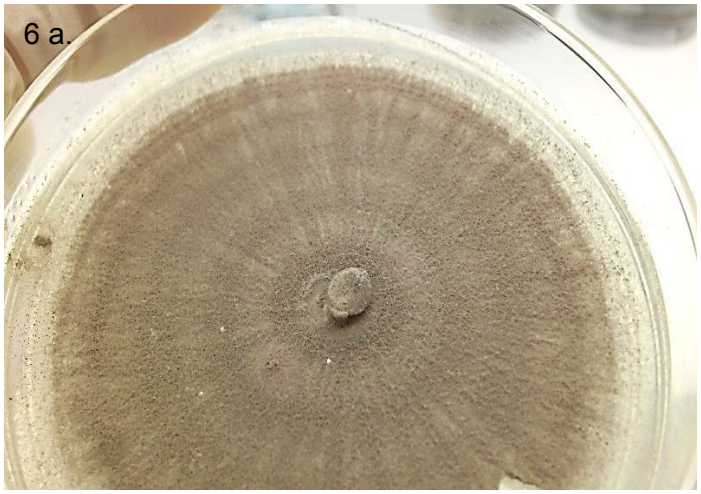
Agar plate assay was used to detect zones of hydrolysis around the microbial colonies that were isolated from soil (Shah et al. 2008). In this study, agar plate test was performed to investigate the ability of fungi to utilise polyurethane (PUR) as the carbon source. Aqueous PUR dispersion is an opaque chalky suspension that turns into translucent upon degradation. Microorganisms that are able to degrade PUR will exhibit clearance zones around the growing fungal isolates.

Initial screening was carried out with modifications following the method of Crabbe et al. (1994) for capability of fungi to degrade PUR. Pure 150 endophytes were screened in this study (see **Figure 6** for representatives of the pure isolates tested). In general, endophytes were grown on solid medium (PUR-1) containing 19mM NaH_2PO_4, 33.5mM K_2HPO_4, 7.6mM $(NH_4)_2SO_4$, 2.5mM, Na-Citrate, 250µM $MgSO_4$, 19µM thiamine, 0.05% casamino acids, 147µM $FeCl_3 \cdot 6H_2O$, 14µM $ZnCl_2 \cdot 4H_2O$, 12µM $CoCl_2 \cdot 6H_2O$, 12µM $Na_2MoO_4 \cdot 2H_2O$, 10µM $CaCl_2 \cdot 2H_2O$, 11µM $CuCl_2$, 12µM $MnCl_2$, 12µM H_2SO_4, and 1.8mM HCl with the addition of 10 mL aqueous PUR dispersion (Bayer Material Science), and 80 g of agar in a 1 L mixture. Polymer was added after autoclaving the media. The PUR-1 solid medium was poured into petri dishes, inoculated with 0.5 cm^3 plug of fungus that was grown on PDA prior screening using aseptic technique, sealed, and incubated at 25 ºC. After 2-3 weeks of incubation, changes in the appearance of medium were observed and the diameter of zones of clearance was measured (see **Figure 7** for an overview of initial screening process). PUR clearance was proved by observation of the solid medium from opaque to transparent (see **Figure 8**).

Organisms that have the activity to degrade PUR were further screened to test their capability to utilise PUR as the only carbon source. The active organisms were grown on PUR without other carbon sources (PUR-2). PUR-2 liquid media were prepared using the same ingredients as solid PUR-1 media, but with no addition of sodium citrate, thiamine, casamino acids, and agar. PUR-2 liquid medium was added to sterile conical flasks of 250 mL and inoculated with three

plugs of 0.5 cm^3 fungus grown on PDA. After a month of incubation at 25 ºC, culture flasks were observed for a visual change of the media. The absorbance reading of the triplicate cultures were measured using a Varian Cary 50 UV-Visible Spectrophotometer at wavelength of 600 nm which was blank with PUR-2 liquid medium without PUR (see **Figure 9** for an overview of secondary screening process). Active organism that utilizes PUR as the only carbon source for growth was indicated by a change in the secondary media to translucent (see **Figure 10**).

Two active fungal strains were additionally subjected to enzyme assay. Firstly, the strains were inoculated in 100 mL of PUR-2 liquid medium with 1% PUR, and incubated at 25 ºC for 30 days. Cultures were then centrifuged at 10 000 rpm for 10 minutes. The esterase activity was carried out in triplicates according to the method of Kordel et al. (1991). In summary, *p*-nitrophenyl acetate was used as substrate for esterase. The 2.0mM *p*-nitrophenyl acetate was dissolved in 10 mL 2-propanol and mixed with 50mM potassium phosphate buffer of pH 7. A volume of 1 mL supernatant was added to 9 mL of the substrate emulsion and mixed. The optical density at 410 nm was monitored and recorded for 0 to 16 minutes against a blank without enzyme using a Varian Cary 50 UV-Visible Spectrophotometer. One unit (U) of enzyme activity was defined as the amount of substrate forming 1 µmol of *p*-nitrophenol per minute. The molar extinction used to calculate the rate was 32 300 M^{-1} cm^{-1}. The concentration of *p*-nitrophenol of samples was determined by a comparison to a standard curve of known *p*-nitrophenol concentrations (see **Figure 11** for an overview of enzyme assay).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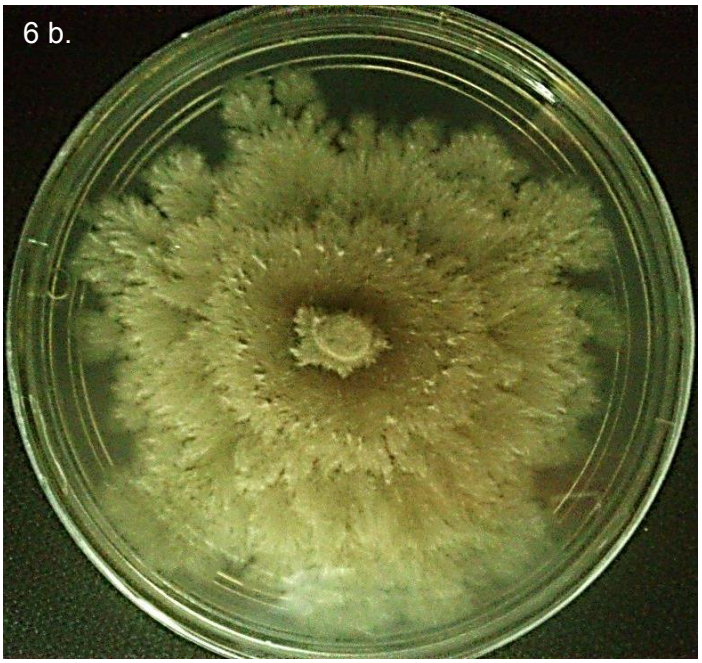
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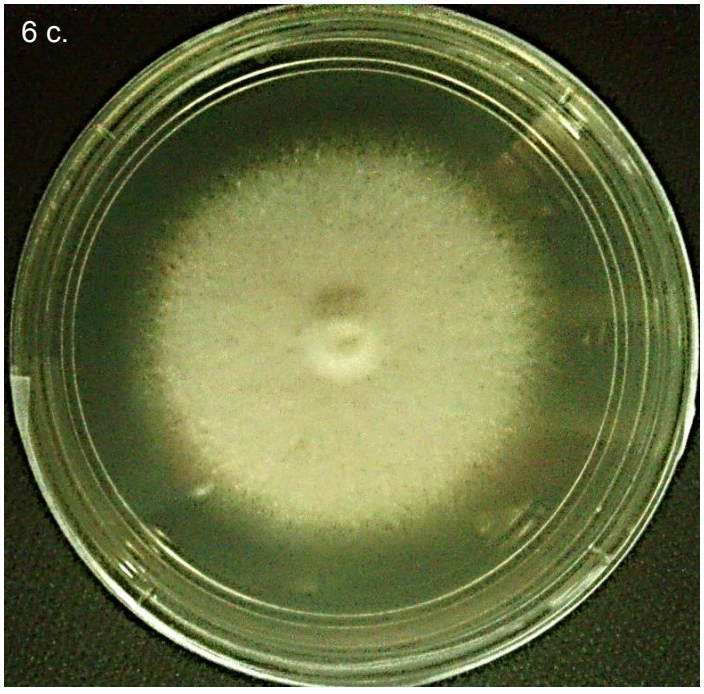
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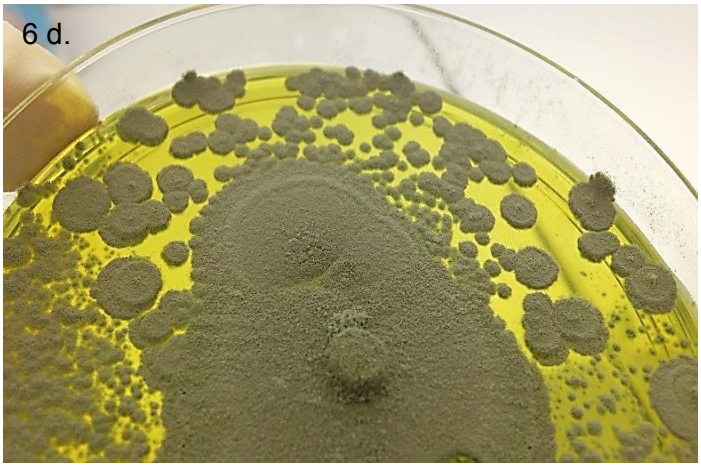


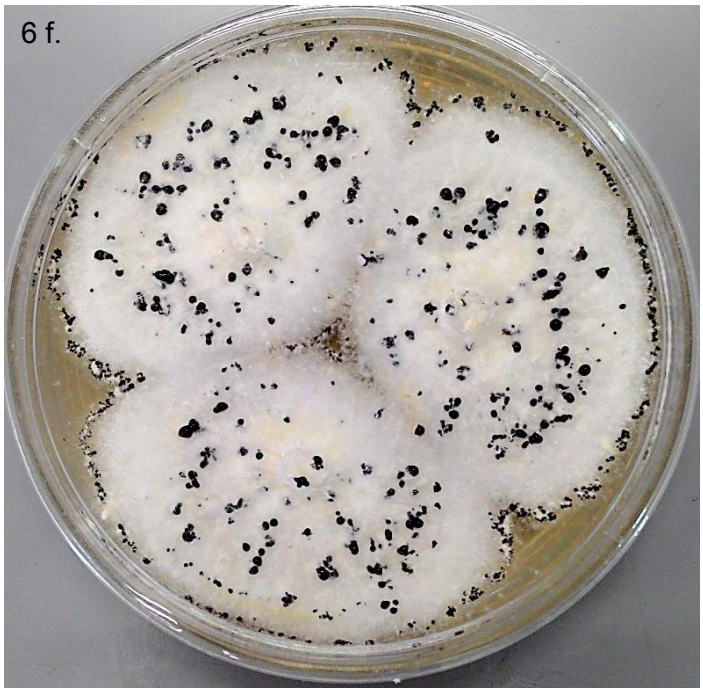


Figure 6 a, b, c, d, e, and f are representatives of endophytes that are screened in this study.

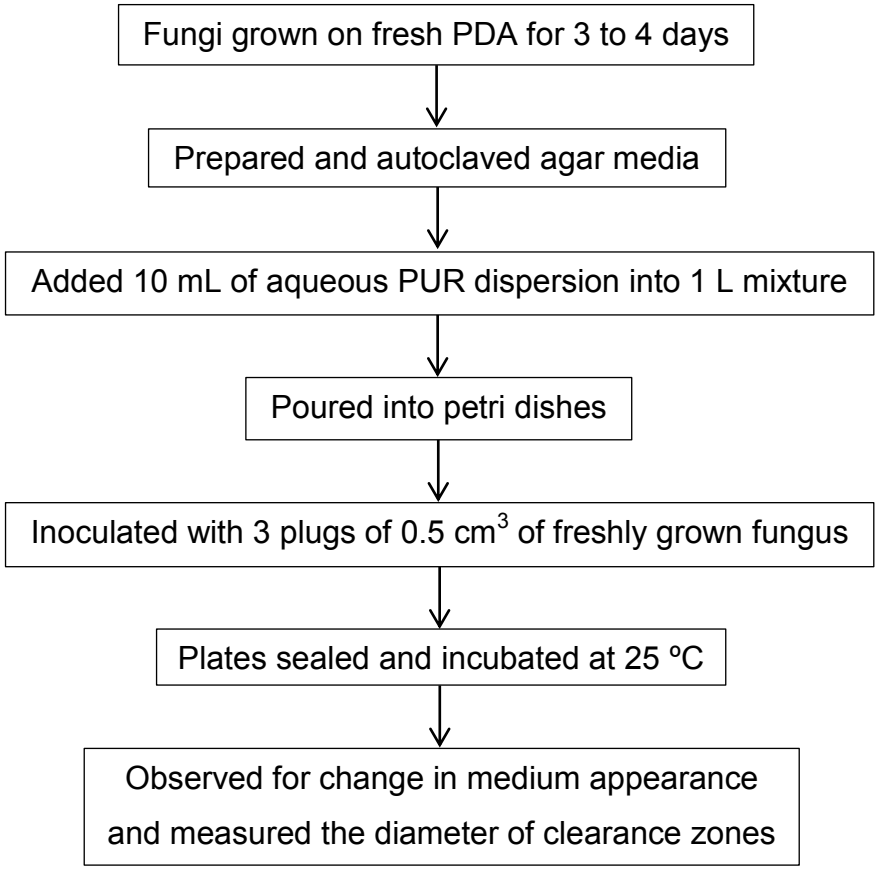


Figure 7 Initial screening for potential PUR-degrading endophytes.

Figure 8 PUR clearance zone indicated by a change from opaque to transparent medium.

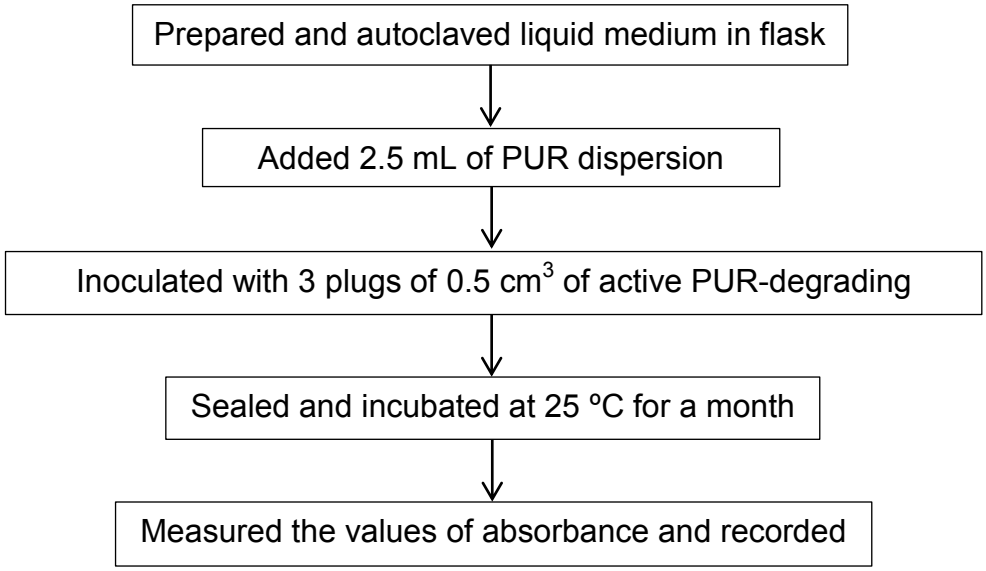


Figure 9 Secondary screening of active PUR-degrading endophytes.

Figure 10 Translucent medium indicates the utilization of chalky PUR by active endophytes.

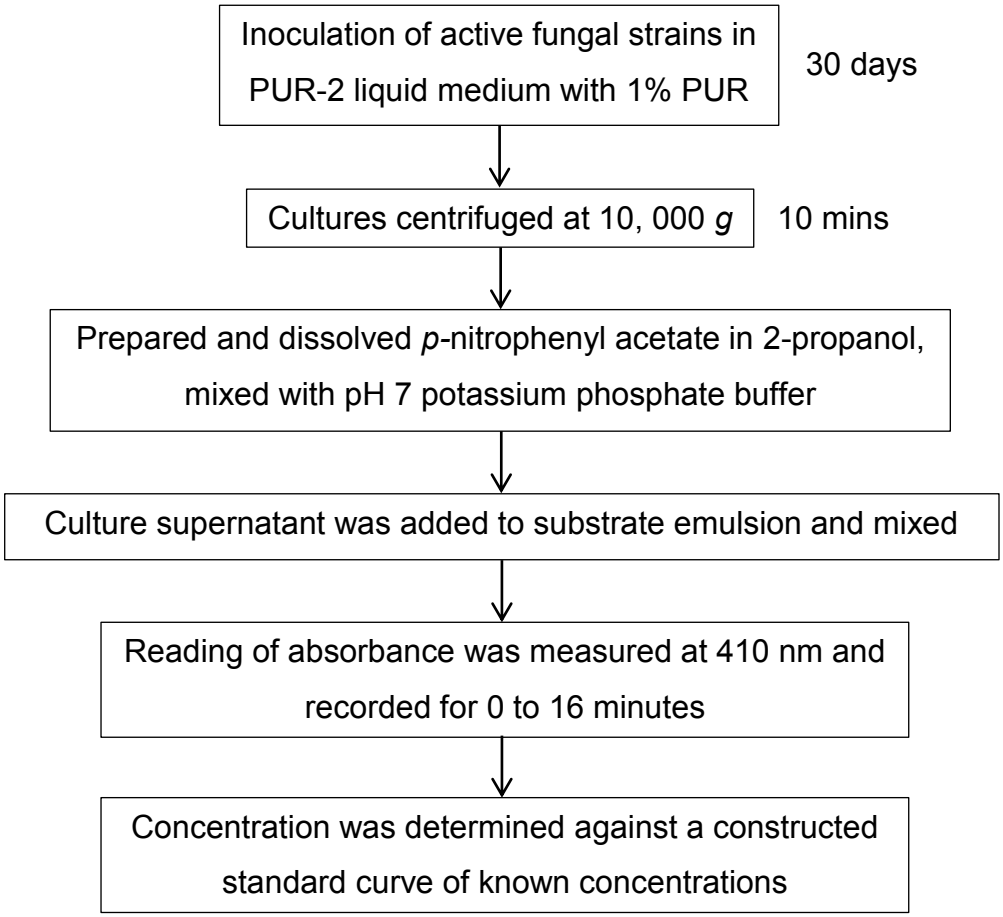


Figure 11 Enzyme assay using *p*-nitrophenyl acetate as the substrate.

2.4 Endophytic fungal identification

A universally accepted DNA barcode for fungi is essential for multitaxon ecological and biodiversity studies. A standardised 500 to 800 bp sequences of DNA barcoding are used for species identification of all eukaryotic kingdoms using primers which are applicable for the wide-ranging taxanomic group (Schoch et al. 2012). For molecular analysis of fungal communities, some studies have identified that the internal transcribed spacer (ITS) regions is the suitable targets (Bridge & Spooner 2001; Gardes & Bruns 1993).

The ITS region is among the DNA regions of the ribosomal cistron that has the highest successful identification for the widest range of fungi (Schoch et al. 2012). ITS has been used in combination with large subunit in a smaller number of environmental studies (Geml et al. 2009; Taylor et al. 2008). In some fungi, ITS region was used as a measure of genetic distances to indicate delimitation. ITS analysis was also successfully used to describe the composition of soil fungal communities (Heinonsalo, Jorgensen & Sen 2001) and the diversity of species within endophytic fungi of common reed (Wirsel et al. 2001).

DNA of fungal cultures grown on PDA for 4 days at 25 ºC was first extracted following the general method of Zhang et al. (2010). A sterile toothpick was used to transfer a small amount of mycelia from the colony into 100 µl of pure water in a 1.5 ml microcentrifuge tube. The mixture was vortexed thoroughly and then centrifuged at 10, 000 *g* for 1 min. After carefully discarding the supernatant using a pipette tip, 100 µl of TE buffer was added to the microcentrifuge tube. The mixture was incubated at 85 ºC in a water bath for 20 min and the crude extract containing genomic DNA was stored at -20 ºC until use (see **Figure 12** for an overview of extraction process).

The fungal DNAs were amplified by polymerase chain reaction (PCR) with each PCR contained 2 µl of 10 x PCR buffer, 1.2 µl of dNTP mixture (2.5 mmol L^{-1} each), 0.8 µl of each primer (10l mol L^{-1}), 0.2 µl of Taq DNA polymerase (5 U μL^{-1}) and 1 µl of genomic DNA in a total volume of 20 µl. The primer pairs used for amplifying the full length of ITS regions were ITS1 (5‟-

TCCGTAGGTGAACCTGCGGG-3‟) and ITS4 (5‟-TCCTCCGCTTATTGATATGC- 3‟). Amplification was performed with the following conditions:

- Initial denaturation at 95 ºC for 3 min
- 29 cycles of
  Denaturation: 95 ºC for 30 sec
  Annealing: 47 ºC for 30 sec
  Elongation: 75 ºC for 2 min
- Final elongation at 72 ºC for 5 min
- Hold at 4 ºC

PCR products were resolved by electrophoresis through 1.0% agarose gels in TAE (2 mmol L^{-1} EDTA, 80 mmol L^{-1} Tris-acetate, pH 8.0) and were visualised by staining with 1 µg/mL of ethidium bromide.

Nucleotide sequences were then determined using the dideoxynucleotide sequencing method of the PCR products. Sequencing was performed at Beijing Genomics Institute, BGI, Hong Kong. Endophyte sequences obtained were aligned to organisms present in the Genbank database using the Basic Local Alignment Search Tool (BLAST) of the National Center for Biotechnology Information. The sequences were analysed against the NCBI (USA) database (Zhang et al. 2000). Phylogenetic tree was created with MEGA 6 (Tamura et al. 2013) using neighbour-joining method (see **Figure 13** for an overview of PCR amplification).

The nucleotide sequences of the isolates have been deposited in the GenBank database under accession numbers KR013143 (SBF1), KR013144 (SBF2), KR013145 (SBF3), KR013146 (SBF4), KR013147 (SBF5), KR013148 (SBF6), KR013149 (SBF7), KR013150 (SBF8), and KR013151 (SBF9).

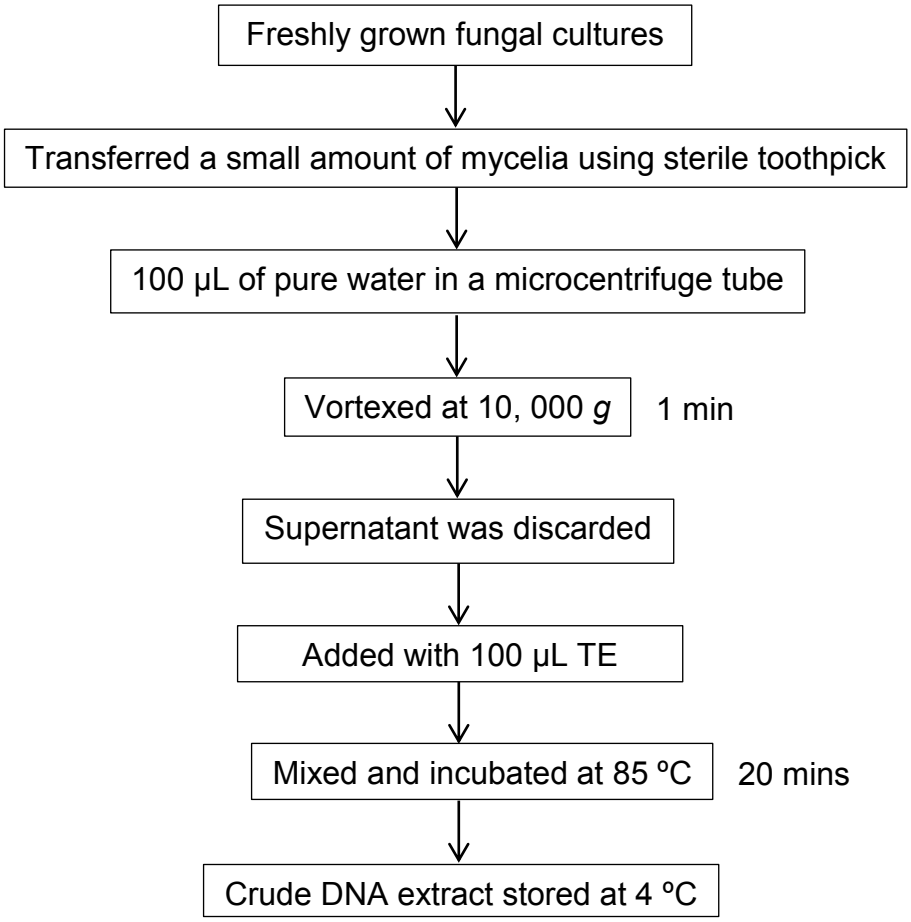


Figure 12 Extraction of fungal DNA for identification.

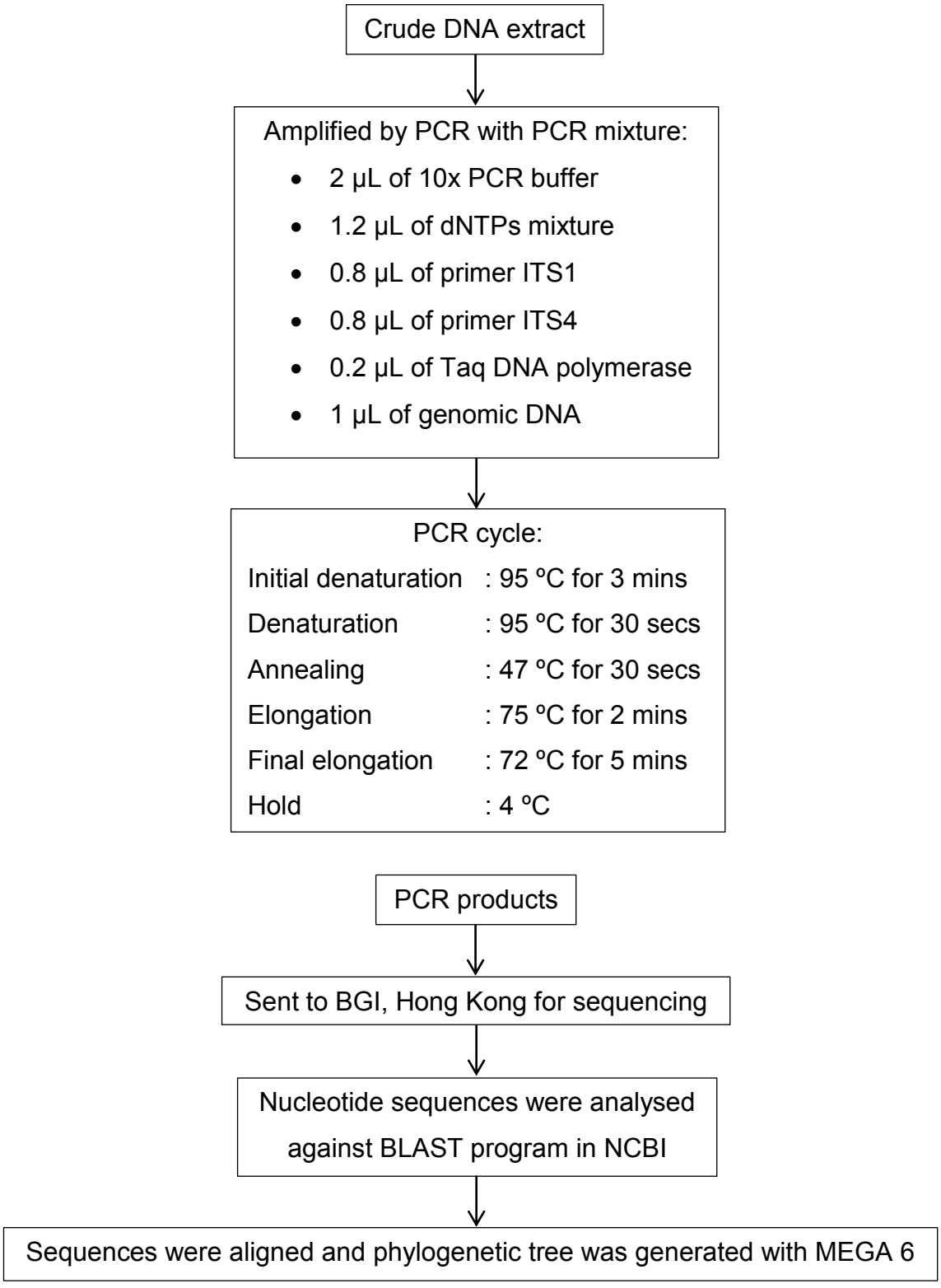


Figure 13 Overview of the fungal DNA amplification using primer 1 and 4.

2.5 Proteomic analyses

Due to the great technical advancement in the field of genes and biochemistry, proteomics has been introduced since the early 1970s. However, the concepts of proteomics are only well-known in the year 1990 (de Oliveira & de Graaff 2011). Studies on proteome has advanced with the application of high throughput techniques in order to allow large quantities of proteins parallel to genomics and transcriptomics to be analysed at a faster rate (Lueking et al. 1999; Wolters, Washburn & Yates 2001). Protein arrays also require confirmation through quantitative techniques including ELISA which is similar to oligonucleotide arrays where the results obtained require validation by quantitative PCR (QPCR) (Zichi et al. 2008). In addition to protein arrays, mass spectrometry (MS) is an alternative technique for quantification of proteins.

Proteomics studied using mass spectrometry can be applied for analyses of protein complexes (Link et al. 1999), to detect and quantify post-translational modifications (Jensen 2006), to identify proteins present in complex mixtures (Eng, McCormack & Yates 1994; Wolters, Washburn & Yates 2001), to quantitate proteins (Bantscheff et al. 2007) and to create a proteomic profile. In the past, most studies in laboratory that have employed endophytes have not tested for proteomics. Endophytes in this study are tested and studied for their proteins in which the fungal cells were first crushed, followed by an extraction protocol.

A protein extraction protocol with minimal optimisation that can be easily applied to various materials is essential (Wu et al. 2014). Protein precipitation using the trichloroacetic acid (TCA)/acetone method allows complete resolubilisation of proteins and further purified. Precipitation with TCA/acetone allows aggressive removal of non-protein compounds for the preparation of high quality protein samples (Wu et al. 2014). In a study conducted by Wang et al. (2003) and Wang & Guo (2006), TCA/acetone precipitation coupled with phenol extraction often yield high quality protein samples from different recalcitrant tissues.

In this study, two growing conditions for active fungal endophytes were tested, liquid culture media without 1% polyurethane (PUR) (control) and with 1% polyurethane (PUR) (treated). Three plugs of a 3-day-old culture on PDA plate were inoculated into a 250 mL medium (19mM NaH_2PO_4, 33.5mM K_2HPO_4, 7.6mM $(NH_4)_2SO_4$, 2.5mM, 250µM $MgSO_4$, 147µM $FeCl_3 \cdot 6H_2O$, 14µM $ZnCl_2 \cdot 4H_2O$, 12µM $CoCl_2 \cdot 6H_2O$, 12µM $Na_2MoO_4 \cdot 2H_2O$, 10µM $CaCl_2 \cdot 2H_2O$, 11µM $CuCl_2$, 12µM $MnCl_2$, 12µM H_2SO_4, and 1.8mM HCl) in a conical flask. The inoculated flasks were incubated at 25 ºC for 3 weeks. The cell biomass was collected and used for protein extraction.

Fungal cells from both control and PUR treated conditions were firstly ground in liquid nitrogen using a cooled mortar and pestle. Proteins of powdered fungal cells were then extracted through protein precipitation using TCA/acetone (Natarajan et al. 2005). The fine cell powder of 2 g was resuspended in lysis buffer containing 30mM Tris-HCL, pH 8.3, 8 M urea, 4% CHAPS, 2% IPG buffer, and 40mM DTT. Spectrophotometric protein quantitation assay was conducted with a Bradford kit (Bio-Rad, USA) with bovine serum albumin as a standard. Absorbance was measured at 595 nm using Varian Cary 50 UV-Visible Spectrophotometer. The concentrations of proteins were obtained by comparison with a standard curve and the final sample volumes of each extract were adjusted to normalise the amount of protein per sample to 50 µg /250µL (see **Figure 14** for an overview of protein extraction).

A 2-D gel electrophoresis consists of the first dimension of isoelectric focusing (IEF) and the second, sodium dodecyl sulphate (SDS)-polyacrylamide electrophoresis. This has been proved as an extremely powerful and widely utilised technique to examine the populations of complex protein (Anderson & Anderson 1977; Brandhorst 1976; Bravo & Celis 1980). Complex protein mixtures can be resolved and approximately 1100 proteins have been resolved through the application of powerful IEF technique under denaturing conditions as part of two-dimensional electrophoresis (2-DE) (O'Farrell 1975).

The isoelectric focusing (IEF) in this study was carried out by loading the samples to 13 cm Immobiline™ DryStrip gels (IPG), with a non-linear pH 3-10 gradient (GE Healthcare) following the instruction manual of a PROTEAN® i12™ IEF Cell (Bio-Rad) with modifications (see **Figure 15** for samples loaded on IPG strips).

The IPG strips were firstly rehydrated at 20 ºC for 12 hours followed by a voltage step-gradient of the following set-up:

- Step 1: 500 V for 2 hours
- Step 2: 1000 V for 1 hour
- Step 3: 8000 V for 1 hour
- Step 4: 8000 V for a total of 8 hours and 29 kvh

The IPG strips were then equilibrated for 15 minutes in freshly prepared buffer-A (50mM Tris-HCl, pH 8.8, 6 M urea, 30% glycerol, 2% SDS, a trace amount of bromophenol blue, and 10 mg/mL DTT) with gentle swirling. The IPG strips were rinsed for 15 minutes in freshly prepared buffer-B (50mM Tris-HCl, pH 8.8, 6 M urea, 30% glycerol, 2% SDS, a trace amount of bromophenol blue, and 2.5% (w/v) IAA) with gentle swirling. The strips were then placed on the top of the vertical 1 mm thick 12% (w/v) SDS-PAGE gels (see **Figure 16** for an overview of IEF).

Electrophoresis was carried out using SE 600 Ruby Standard Dual Cooled Vertical System (GE Healthcare) (see **Figure 17**) at constant 20 mA and 20 ºC until the bromophenol blue dye front migrated 2 cm away from the top. After 3-4 hours of gel electrophoresis, the proteins on the gels were fixed overnight in water containing 10% (v/v) acetic acid, and 50% (v/v) ethanol. The gels were then rinsed with Milli-Q water and resolved proteins on gels were stained using Pierce® Silver Stain Kit for Mass Spectrometry (Thermo Scientific) following the manufacturer"s instructions. The fungal cultures that are treated with the same conditions were conducted in triplicates.

Stained gel images were scanned using the GS-800 Calibrated Densitometer (Bio-Rad) and analysed using PDQuest 2-D analysis software (Bio-Rad). Analysis on the change in volume of spots between control and PUR treated samples were performed using Progenesis SameSpots software (Nonlinear Dynamics). Normalisation of protein spot intensities was done using the „total spot volume" method. This method involved the expression of each protein spot and is measured in percentage of the total volume. The differences in protein spots among experimental treatments were identified by gel to gel matching. All matched spots were detected on all triplicates of gels. Data expressed in this manner is independent of possible errors that may occur due to minute difference in protein loading.

Protein spots for identification were chosen based on the fold changes of >2.0 and excised using EXQuest Spot Cutter system controlled through Quantity One®1-D analysis software (see **Figure 18**). Proteins were destained using Pierce® Silver Stain Kit for Mass Spectrometry (Thermo Scientific), dried by speed vacuum centrifugation, and digested with trypsin at 25 ºC overnight. Dried peptides were treated with trypsin (12.5 mg/mL) in 25mM NH_4HCO_3 in 10% ACN at 25 ºC overnight. All the peptides solution were dried and stored at -80 ºC until analysis by mass spectrometry. Peptides were analysed by MALDI-TOF MS and TOF/TOF tandem MS/MS performed on an AB SCIEX TOF™ 5800 System (AB SCIEX, Framingham, MA) (see **Figure 19** for electrophoresis of fungal proteins and their storage prior to analysis).

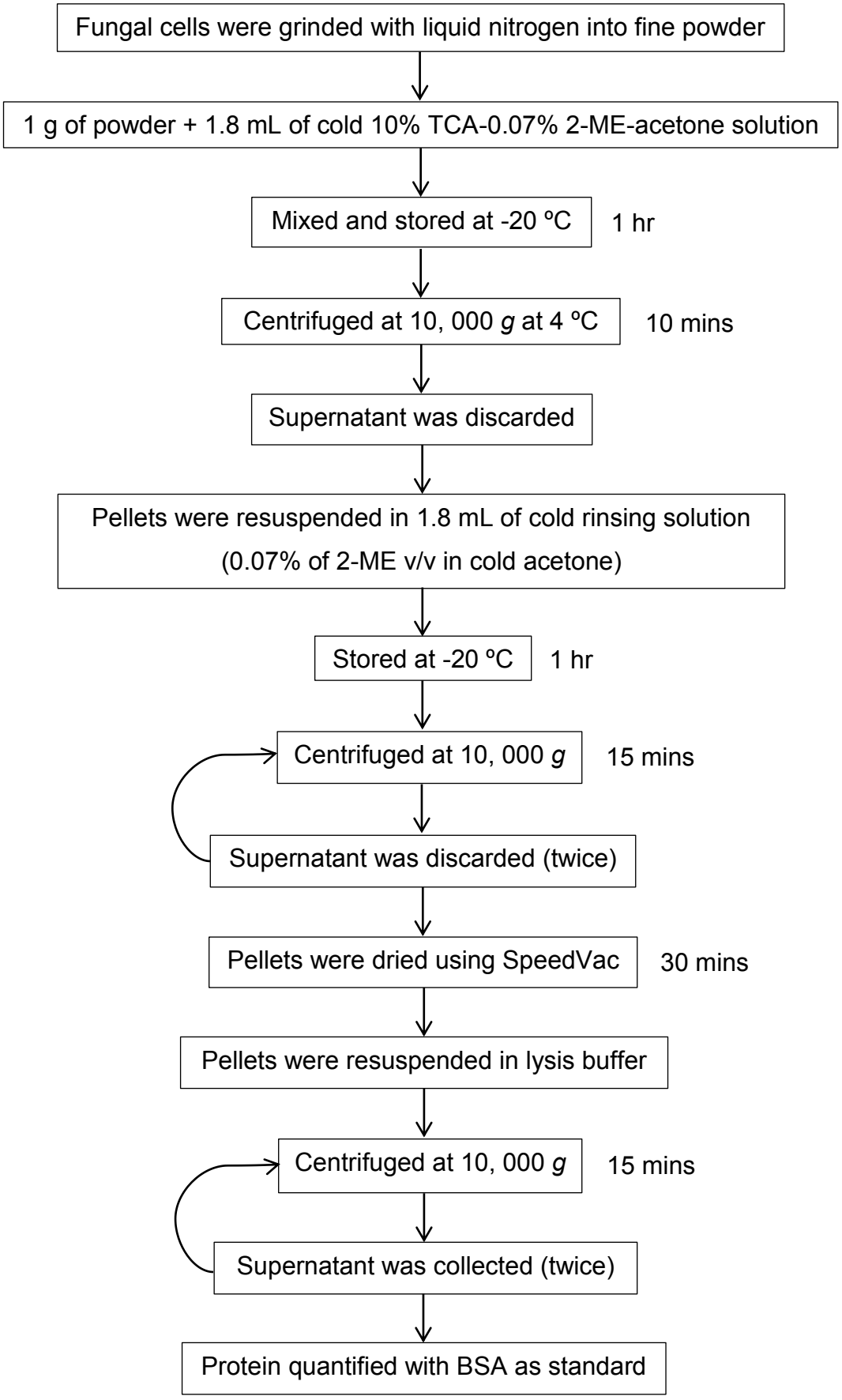


Figure 14 Extraction of protein from fungi using TCA/acetone precipitation method.

Figure 15 Protein samples loaded onto the IPG strips in an IEF tray.

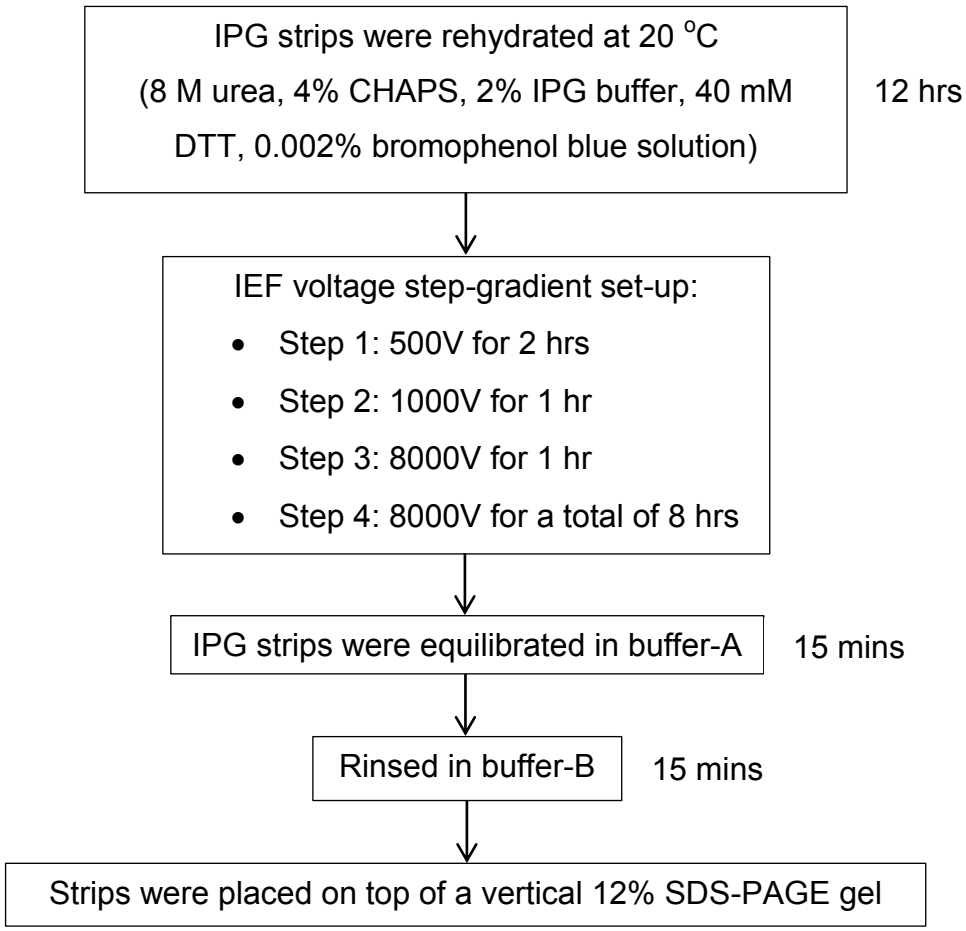


Figure 16 Isoelectric focusing process of extracted fungal proteins.

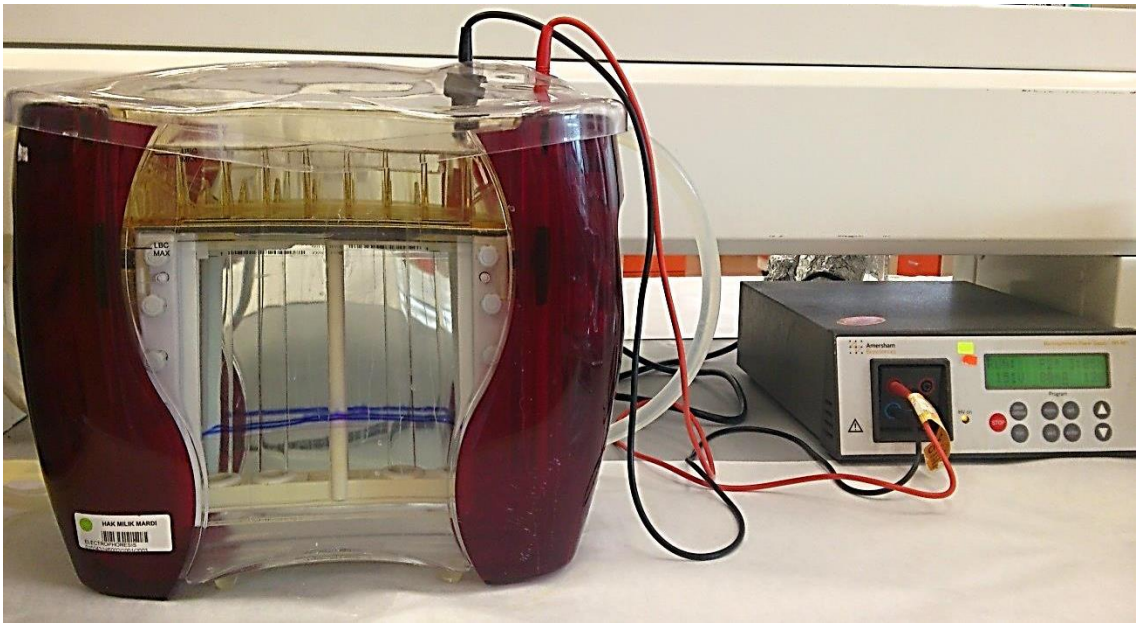


Figure 17 Ruby Dual Cooled Vertical System set to run at 20 mA and 20 ºC.

Figure 18 Spot Cutter System used to excise protein spots of significance change in the abudance.

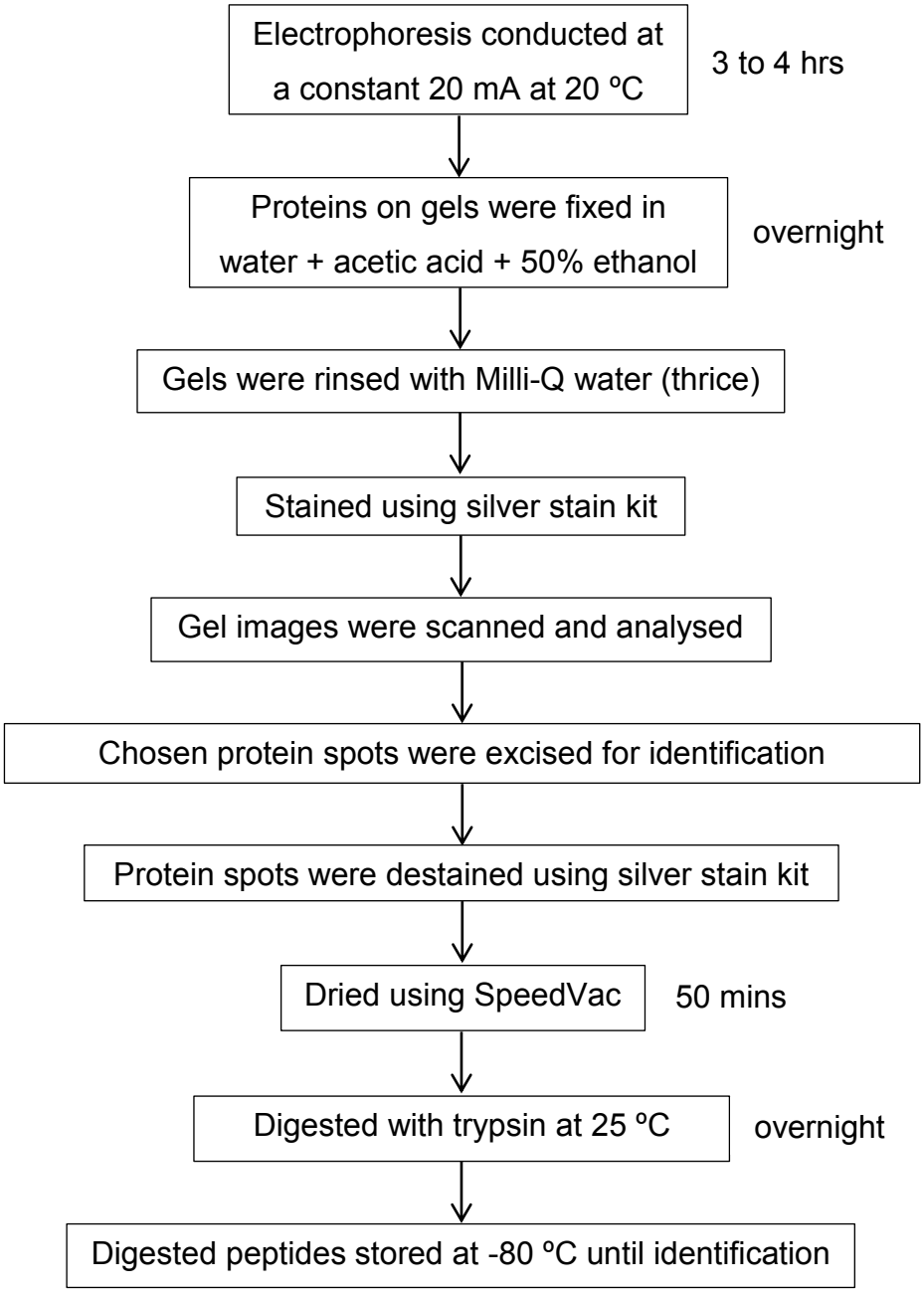


Figure 19 Overview of fungal proteins from electrophoresis to storage of peptides.

3. Results and discussion

Current literature reports that organisms isolated from the soil have the ability to degrade PUR (Mathur & Prasad 2012) In the present study, endophytes isolated from *Nepenthes ampullaria* samples were screened and tested for their capability to degrade PUR polymer. The results achieved from numerous experiments that have been conducted will be presented and discussed in the following.

3.1 Biological assays

3.1.1 Preliminary screening for potential endophytes

Primary screening was performed using the agar plate assay (see **Figure 7** for detailed description). A total of 150 pure fungal cultures were screened. Fungal culture plugs were cut using a sterile straw from four days old plate pure cultures and tested in triplicates. Positive results are indicated by observation of clear zones around the fungal isolates (see **Figure 8**).

The results obtained from primary screening showed that only nine fungal strains exhibit positive results. All of the nine fungi evidently cleared PUR-1 solid medium. This expression was detected under same growth condition for all fungal endophytes along with the negative control. Zones of clearance observed were recorded and tabulated (see **Table 1**). The top clearing fungal endophyte in this solid media assay was SBF4, SBF1, SBF3, SBF7, SBF8, SBF5, SBF6, SBF9, and SBF 2 (see **Figure 20**).

Table 1 Average zone of PUR clearance of each fungal isolates measured in mm with calculated standard deviation.

Isolate	Zone of clearance (mm)
SBF4	36.0 ± 1.73
SBF1	35.3 ± 0.58
SBF3	32.7 ± 0.58
SBF7	30.7 ± 1.15
SBF8	30.3 ± 0.58
SBF5	30.0 ± 0.00
SBF6	29.7 ± 0.58
SBF9	28.0 ± 0.00
SBF2	15.0 ± 0.00

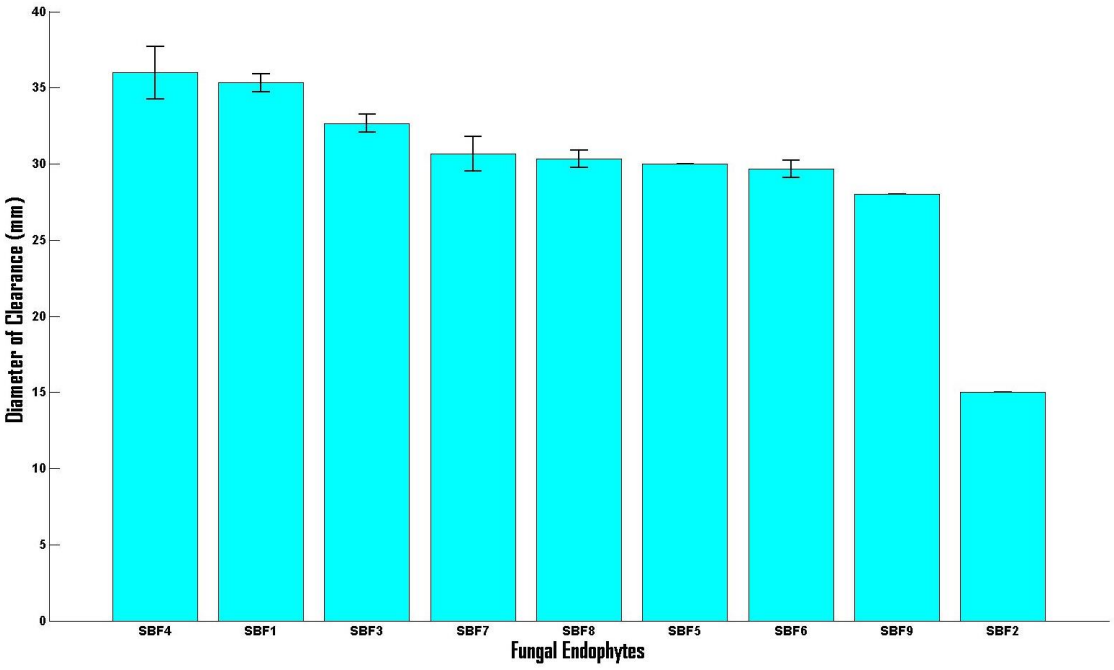


Figure 20 Diameter of clearance zones measured after two weeks of growth. Overview of PUR degradation when fungal endophytes were grown on PUR solid medium. Error bars represent the standard deviation of the average clearance diameter.

3.1.2 Secondary screening

Fungal isolates that demonstrated active PUR degrading activity were further screened to confirm the isolates‟ capability as potential degraders of PUR. Fungal isolate SBF4, SBF1, and SBF3 were selected and subjected to secondary screening where PUR serves as the sole carbon source for growth. Isolate SBF4, SBF1, and SBF3 were grown in liquid medium containing PUR without the addition of other carbon sources, performed in triplicates along with negative controls (see **Figure 9** for detailed description).

Based on **Table 1**, isolates SBF4, SBF1 and SBF3 are certainly able to degrade PUR and even showed positive results in the secondary screening. This was observed though a change in the appearance of medium from chalky to almost transparent with the presence of fungal biomass in culture flasks (see **Figure 21**). Presence of fungal biomass indicates the ability of isolate SBF4, SBF1, and SBF3 to grow and survive in PUR culture medium with minimal carbon sources. A study also proved that under both aerobic and anaerobic conditions, fungus was able to utilise PUR only for their growth (Russell et al. 2011).

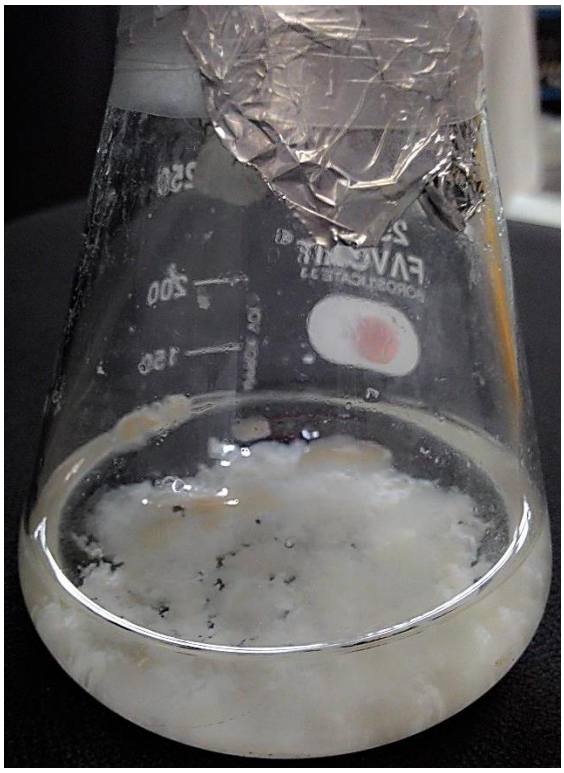


Figure 21 Change from chalky to almost transparent medium with fungal biomass.

In the secondary sole carbon source assay, both SBF4 and SBF1 showed a decrease in absorbance after 30 days (see **Figure 22**) indicative of PUR degradation by SBF4 and SBF1. Fungal endophyte SBF4 displayed stronger decrease in absorbance compared to SBF1 inferring that SBF4 has a superior capability to degrade PUR compared to SBF1 (see **Figure 22**).

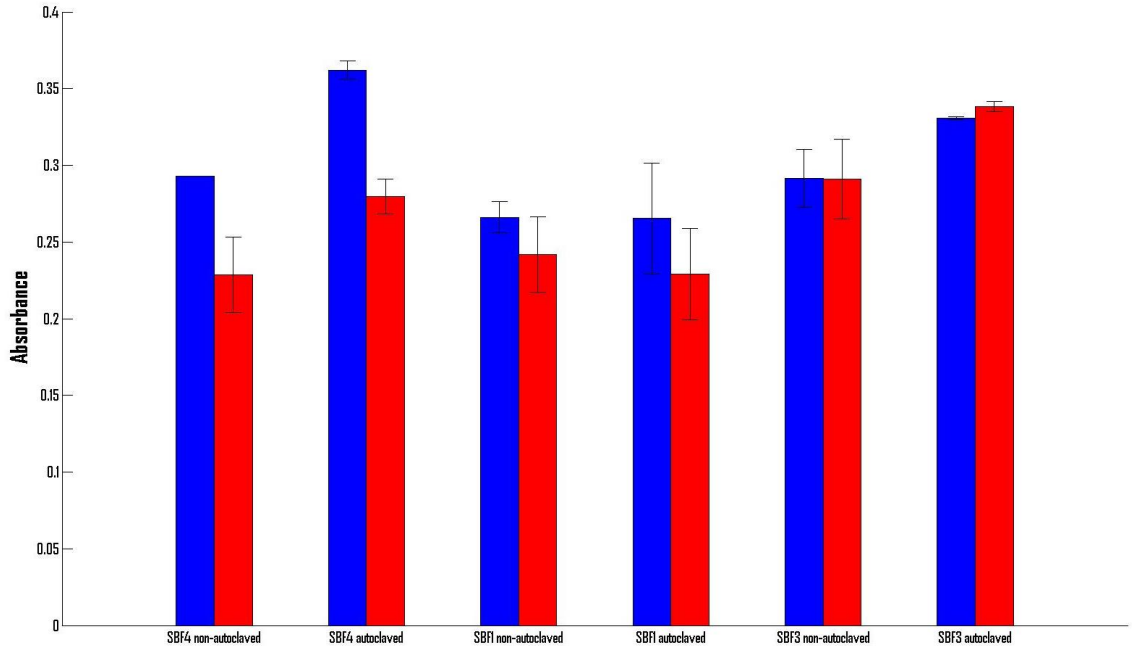


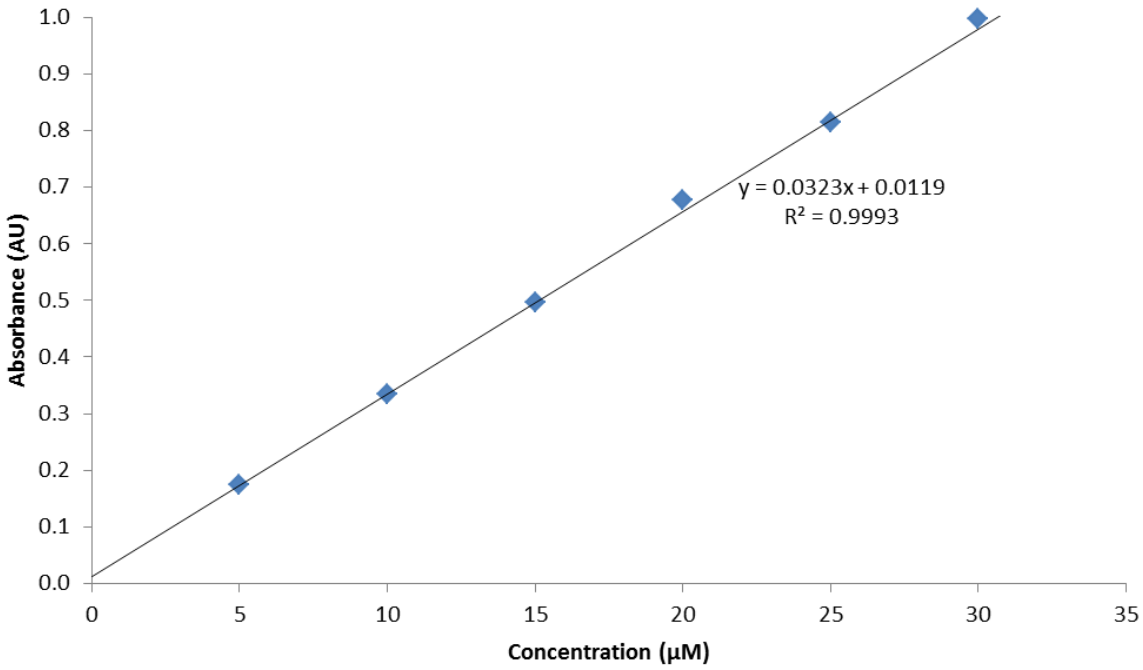
Figure 22 PUR degradation by active fungal endophytes. Cultures contain PUR-2 minimal media and inoculated with fungal inoculums. Error bars represent the standard deviation of the average value of absorbance. Blue: Control, Red: PUR treated.

3.1.3 Enzyme assay

The enzyme activity assay was undertaken by inoculating active PUR fungal degraders into liquid medium with minimal carbon sources, and incubated for a month. The culture supernatant was measured and analysed using UV-Visible Spectrophotometer to detect the presence of esterase enzyme activity of active PUR-degrading endophyte SBF4 and SBF1 (see **Figure 11** for detailed description).

Enzyme activity was detected in the culture supernatant of SBF4 and SBF1 after a month of incubation with PUR. The concentrations of product, *p*-nitrophenol and enzyme activity were calculated against the standard curve (see **Graph 1** for a standard curve constructed for *p*-nitrophenol).

The concentrations of *p*-nitrophenol obtained for endophyte SBF4 and SBF1 were 15.88 µM and 10.27 µM respectively (see **Table 2**). The *p*-nitrophenol was produced when artificial substrate, *p*-nitrophenol acetate was mixed with supernatant. The artificial substrate was broken down by the enzyme present in the culture supernatant and hence, the released of yellow coloured *p*-nitrophenol. Endophytic isolate SBF4 promoted higher enzymatic activity compared to SBF1. The enzymatic activity detected was 1850 U mL^{-1} and 1210 U mL^{-1} for SBF4 and SBF1 respectively.



Graph 1 Standard curve of the concentration of *p*-nitrophenol against the absorbance.

Table 2 Concentration of *p*-nitrophenol and enzyme activity detected for isolate SBF4 and SBF1.

Isolate	Concentration (µM)	Enzyme activity (U mL^{-1})
SBF4	15.88	1850
SBF1	10.27	1210

The assay revealed significant enzymatic activity for both endophytic isolate SBF4 and SBF1, and the data obtained are congruent to other studies. Enzyme esterase produced by a bacterium (Howard, Ruiz & Hilliard 1999) and an anaerobic fungus was purified, characterized, and enzymatic activity was detected, revealing the presence of a high level of esterases (Borneman et al. 1992).

3.2 Endophytic fungal Identification

Nine of the total 150 pure endophytic isolates from plant sample, *Nepenthes ampullaria* that were successfully screened were further studied for molecular identification. These isolates were identified using molecular approach by ITS sequencing using ITS1 and ITS4. The fungal sequences of the nine isolates in this study have been deposited in the GenBank database under accession numbers KR013143 to KR013151.

All the endophytic isolates are found to be closely related to the genus of *Pestalotiopsis* (see **Table 3** for the closest matches of each endophytic isolates and **Figure 23** for the phylogenetic tree generated). The endophytes are categorized into 3 taxanomic groups at a different species level. Most of the isolates are closely related to *Pestalotiopsis neglecta* but isolate SBF1 and 6 are of different species.

Table 3 Closest match found to each endophytic isolates based on query coverage in base pairs, and percentage (%).

Endophytic isolate	GenBank accession number	Closest match	Identities
SBF1	KR013143	*Pestalotiopsis neglecta* (JX415485)	434/481 (90%)
		Pestalotiopsis neglecta (GU595050)	433/481 (90%)
SBF2	KR013144	*Pestalotiopsis neglecta* (JX415485)	481/481 (100%)
		Pestalotiopsis sp. (KC916656)	475/496 (99%)
		Pestalotiopsis sp. (KC895540)	476/481 (99%)

SBF3	KR013145	*Pestalotiopsis neglecta* (JX415485)	480/480 (100%)
		Pestalotiopsis neglecta (GU595050)	475/476 (99%)
SBF4	KR013146	*Pestalotiopsis neglecta* (JX415485)	477/477 (100%)
		Pestalotiopsis sp. (KC916656)	470/474 (99%)
		Pestalotiopsis pallidotheae (JQ676182)	473/478 (99%)
SBF5	KR013147	*Pestalotiopsis neglecta* (JX415485)	483/483 (100%)
		Pestalotiopsis neglecta (GU595050)	479/480 (99%)
		Pestalotiopsis pallidotheae (JQ676182)	479/484 (99%)
SBF6	KR013148	*Pestalotiopsis microspora* (AY924279)	401/436 (92%)
		Pestalotiopsis microspora (KM041703)	401/437 (92%)
		Pestalotiopsis sp. (KF285279)	401/437 (92%)
		Pestalotiopsis mangiferae (JX999998)	401/437 (92%)
		Pestalotiopsis mangiferae (JX857168)	401/437 (92%)
SBF7	KR013149	*Pestalotiopsis neglecta* (JX415485)	525/525 (100%)
		Pestalotiopsis neglecta (GU595050)	524/525 (99%)
SBF8	KR013150	*Pestalotiopsis neglecta* (JX415485)	525/525 (100%)

		Pestalotiopsis vismiae (JQ281543)	519/526 (99%)
		Fungal endophyte (FJ449896)	519/526 (99%)
		Fungal sp. (KC506326)	517/524 (99%)
SBF9	KR013151	*Pestalotiopsis neglecta* (JX415485)	480/480 (100%)
		Pestalotiopsis neglecta (GU595050)	477/478 (99%)

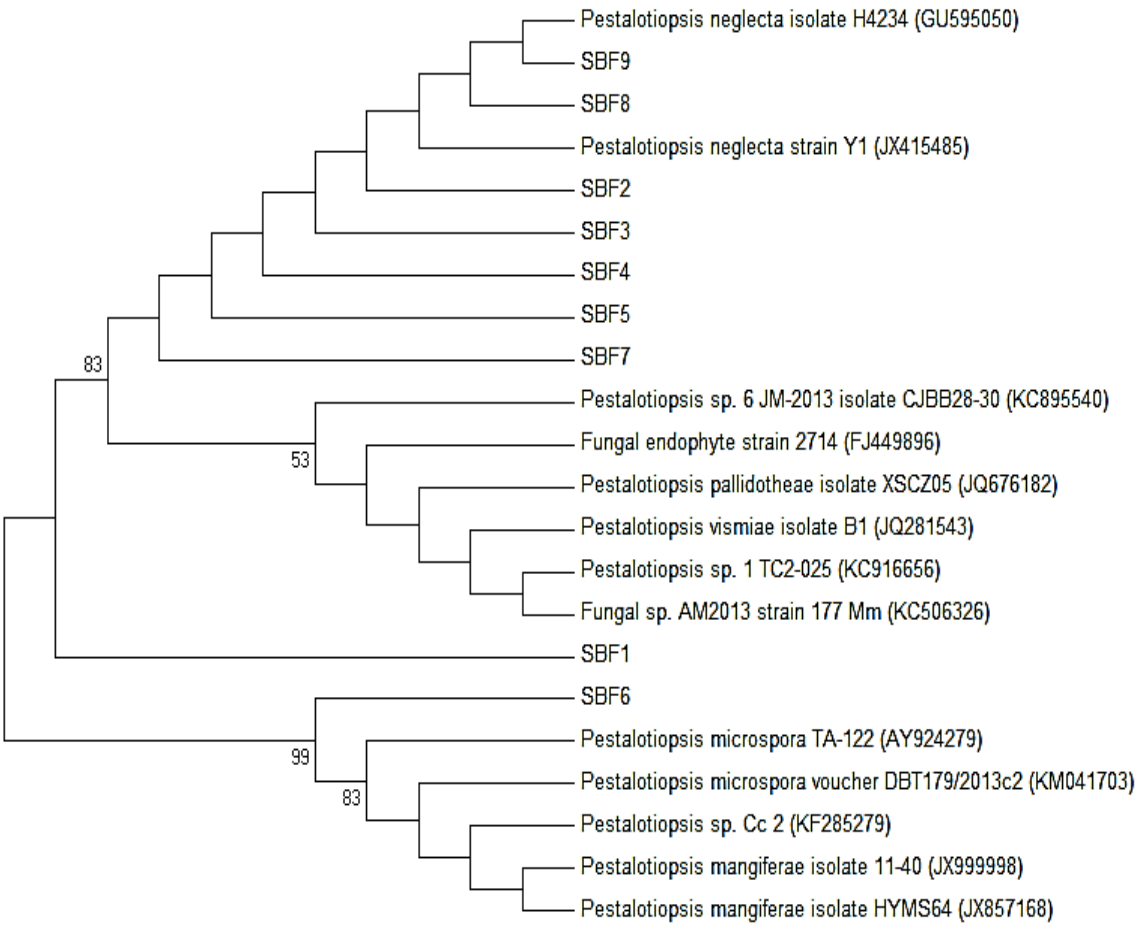


Figure 23 18S rDNA gene-based phylogenetic tree representing fungal sequences found in nine fungal isolates, SBF. The phylogenetic tree was generated with the neighbour-joining method. Bootstrap values generated from 1000 replicates are shown above the branches. Accession numbers for the reference sequences are indicated.

In this study, a microscopic examination of the pure endophytic isolates revealed that they produces spores of four euseptate and pigmented median cells with three apical appendages, and a basal appendage (see **Figure 24**). This observation suggested a close association of the isolates with *Pestalotiopsis. Pestalotiopsis* has been characterised by spores that have slightly curved conidia bearing four to five-euseptate and pigmented median cells with two to four tubular extensions from the apical cell, and a centric basal appendage that arises from the basal cell (Jeewon, Liew & Hyde 2002). Pigmentation was observed in the median cells due to the deposition of melanin molecules within the matrix of cell and the differences in pigmentation were of taxanomic value (Griffiths & Swart 1974).

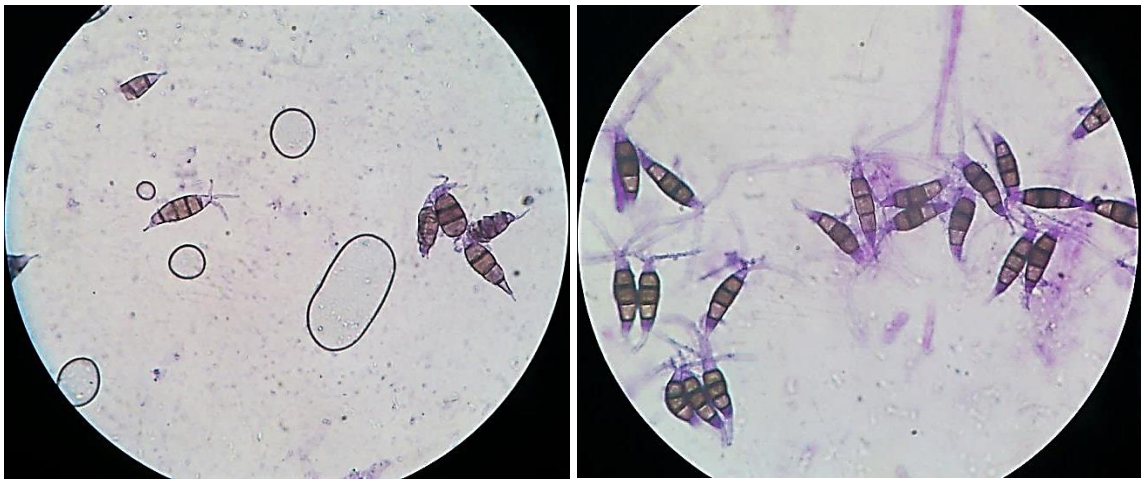


Figure 24 Microscopic view of active PUR-degrading endophytic isolate (400× magnification).

Pestalotiopsis species are sac-like appendage ascomycetes and anamorphic members that belong to the family Amphisphaeriaceae (Barr 1990; Barr 1975; Kang, Kong & Hyde 1998; Kang, Hyde & Kong 1999). Molecular studies have shown that *Pestalotiopsis* is monophyletic (Jeewon, Liew & Hyde 2004; Jeewon, Liew & Hyde 2002; Jeewon et al. 2003). *Pestalotiopsis* sp. are commonly found in tropical and temperate ecosystems (Bate Smith & Metcalfe 1957), often isolated as endophytes (Liu et al. 2005; Watanabe, Motohashi & Ono 2010; Wei et al. 2007), or occur as saprobes (Agarwal & Chauhan 1988; Hu et al. 2007; Liu et al. 2008; Wu, Tseng & Chen 1982; Yanna, Ho & Hyde 2002).

In the recent years, the species of *Pestalotiopsis* have been found to produce abundant valuable important secondary metabolites (Li et al. 2001; Li & Strobel 2001; Ogawa et al. 1995; Pulici et al. 1997; Strobel et al. 1996). These species are beneficial in the field of biology and agriculture where they are used as biological control agents and pesticides for microbes (Ando 1993).

Endophytic *Pestalotiopsis* species isolated from medicinal plants exhibited anitioxidant, antihypersensitive, and antibacterial activities that demonstrated the potential of *Pestalotiopsis* extracts as therapeutic targets (Tejesvi et al. 2008). The antibacterial properties of endophytic *Pestalotiopsis* species from a medicinal plant, *Maytenus ilicifolia* were studied and it was reported that the isolates are related to *Pestalotiopsis microspora, Pestalotiopsis vismiae,* and *Pestalotiopsis leucothoes* (Gomes-Figueiredo et al. 2007).

Besides the production of bioactive compounds, *Pestalotiopsis* have been shown to be able to biosorb various heavy metals (Choo et al. 2014). Several other studies support that endophytic fungi can survive under high metal concentrations (Kapoor, Viraraghavan & Cullimore 1999; Onn et al. 2013; Wong et al. 2015; Yan & Viraraghavan 2003).

There are however, very few studies available of endophytic fungi capable of degrading plastics such as PUR. One such study was undertaken in which they found that *Pestalotiopsis microspora* was able to degrade the synthetic polyester polyurethane efficiently in both solid and liquid suspensions (Russell et al. 2011). Nevertheless, there are more studies on bacteria that can degrade PUR. The *Psedomonas aeruginosa* strain isolated from soil could efficiently degrade PUR and the degradation ability of the organism was quantified by High Performance Thin Layer Chromatography (HPTLC) (Mukherjee et al. 2011). In addition, the testified bacterial strains attached on PUR film showed changes on surface of film and were identified as *Bacillus* species, *Pseudomonas* species, *Micrococcus* species, *Arthrobacter* species, and *Corynebacterium* species (Shah et al. 2008)

Fungal isolates in this study that is closely related to *Pestalotiopsis* at a species level of *neglecta* (SBF2, 3, 4, 5, 7, 8, and 9) were found to be able to produce an anticancer drug, taxol. *Pestalotiopsis neglecta* isolated from the healthy leaves and barks of Japanese Yew tree produced taxol and the extract was tested against human cancer cells, revealing that an increased in concentration of taxol induces an increased in cell death (Kumaran, Kim & Hur 2010).

The fungal isolate in this study were also closely identified as *Pestalotiopsis microspora* (SBF6). In another study, *Pestalotiopsis microspora* from a *Terminalia morobensis* in Papua New Guinea was successfully isolated and antioxidant compound, isopestacin was obtained from the culture broths, measured by electron spin resonance spectroscopy. The study proved that isopestacin behaves as an antioxidant scavenging superoxide and hydroxy free radicals (Strobel et al. 2002).

An antimicrobial compound, phenol was successfully produced by an endophytic fungi, *Pestalotiopsis mangiferae* (close identity to endophyte SBF6 in this study) through isolation from *Coleus amboinicus* and showed positive antimicrobial activity (Astuti et al. 2014). Most of the endophytes in this study except SBF6 that are closely related to *Pestalotiopsis pallidotheae* and *Pestalotiopsis vismiae* are not discussed as there were no available study and literature reported yet. These findings suggested that *Pestalotiopsis* sp. found in this study has the potential to produce varieties of secondary metabolites that are of great importance.

3.3 Proteomic analyses

Proteomic study was performed firstly with the extraction of fungal proteins followed by isoelectric focusing of extracted protein samples, and electrophoresis using Ruby vertical system (detailed description of the processes can be seen in **Figure 14**, **Figure 16**, and **Figure 19**). After isoelectric focusing, and electrophoresis, gels were stained with silver and hundreds of protein spots were observed (see **Figure 25**).

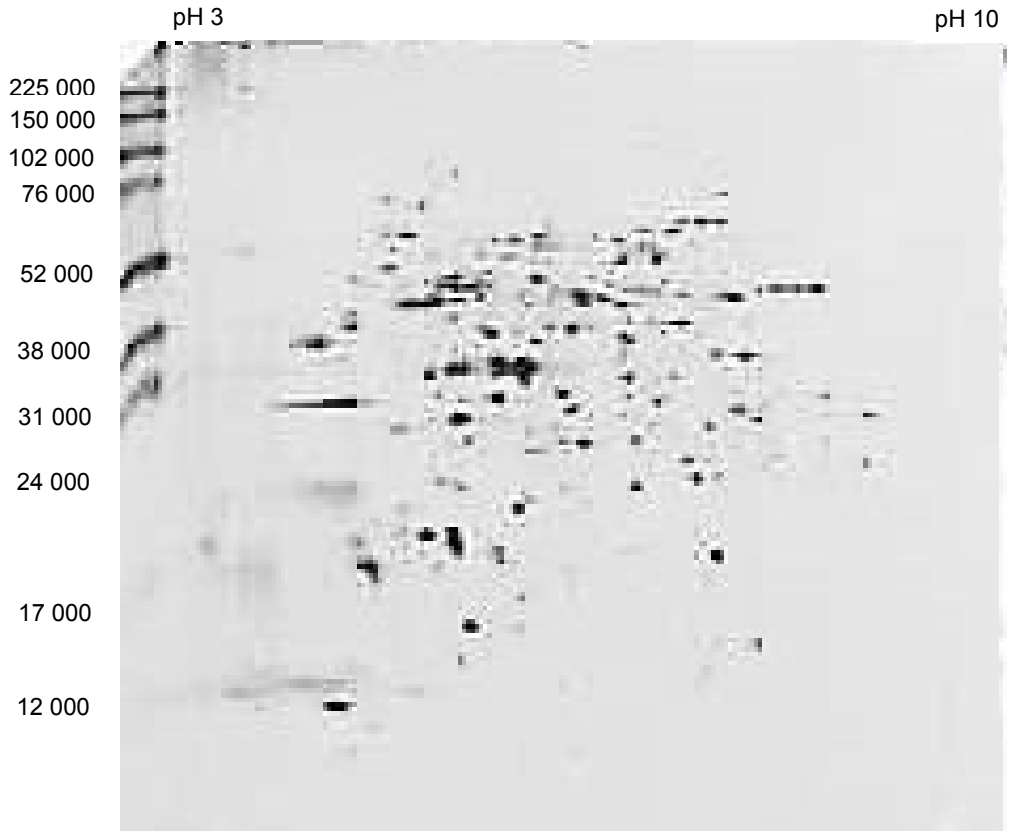
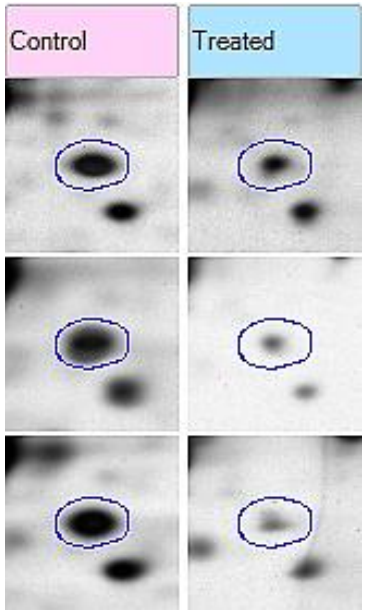
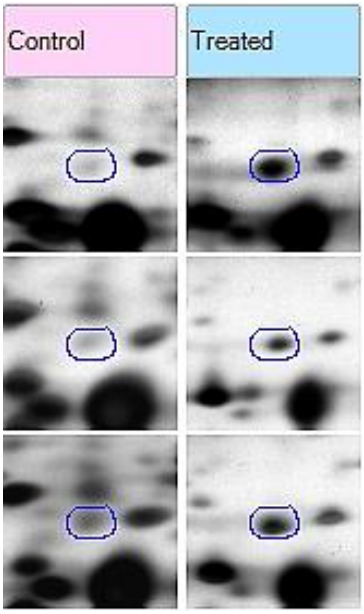


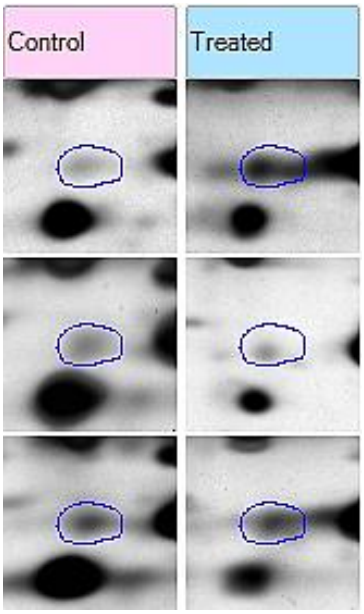
Figure 25 Representative of 2-DE profiles of fungal cultures treated with PUR after three weeks of growth and stained with silver.

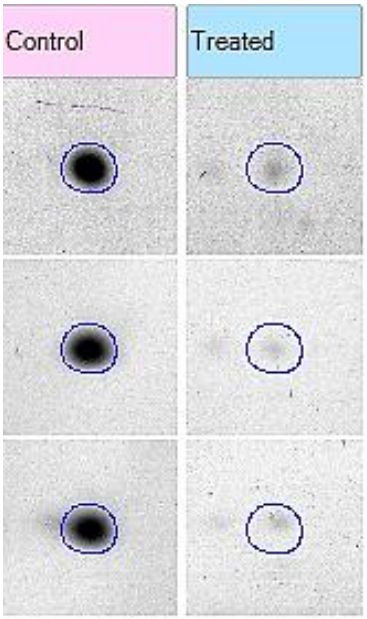
2-DE proteomic profiles of the fungal isolate SBF4 and SBF1 were analysed and revealed changes in the abundance of proteins in the cultures when treated with PUR. A strict comparison between triplicates of control and treated samples was chosen to avoid possible false positive data. During gel image analysis, protein spots that were not present in all gel images were omitted. Our analysis showed that there were 431 protein spots detected on all triplicate gels of control and treated samples.

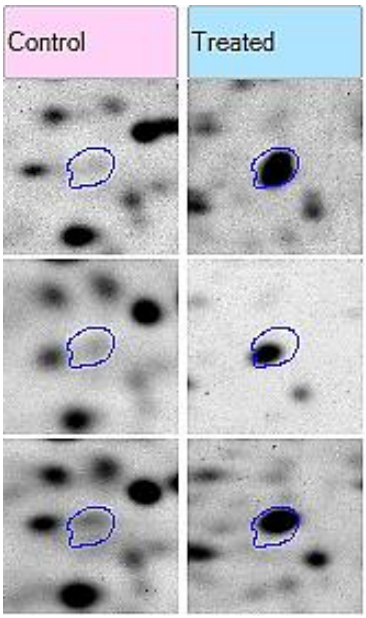
A total of 45 proteins were altered abundantly when analysed between the control and treated fungal samples. The average normalised volume of protein spots was analysed using the Progenesis SameSpots software version 15 and t-test ANOVA ($p < 0.05$) was employed to determine the protein spots that showed significant change (see **Figure 26**). Eleven of the total 45 proteins were significantly altered in abundance with a fold change of ≥ 2.4. Protein numbers 491, 454, 453, 704, 1140, 890, and 652 showed an increase in the abundance of proteins at a fold change of at least 1.8 whereas, spots 449, 195, 493, and 218 showed a decrease in the amount of proteins at a fold change of at least 2.0 when treated with PUR (see **Figure 27**).

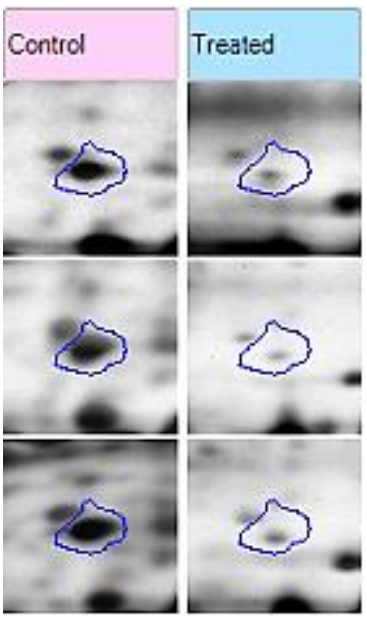

Control
Treated


Control
Treated


Control
Treated


Control
Treated


Control
Treated


Control
Treated

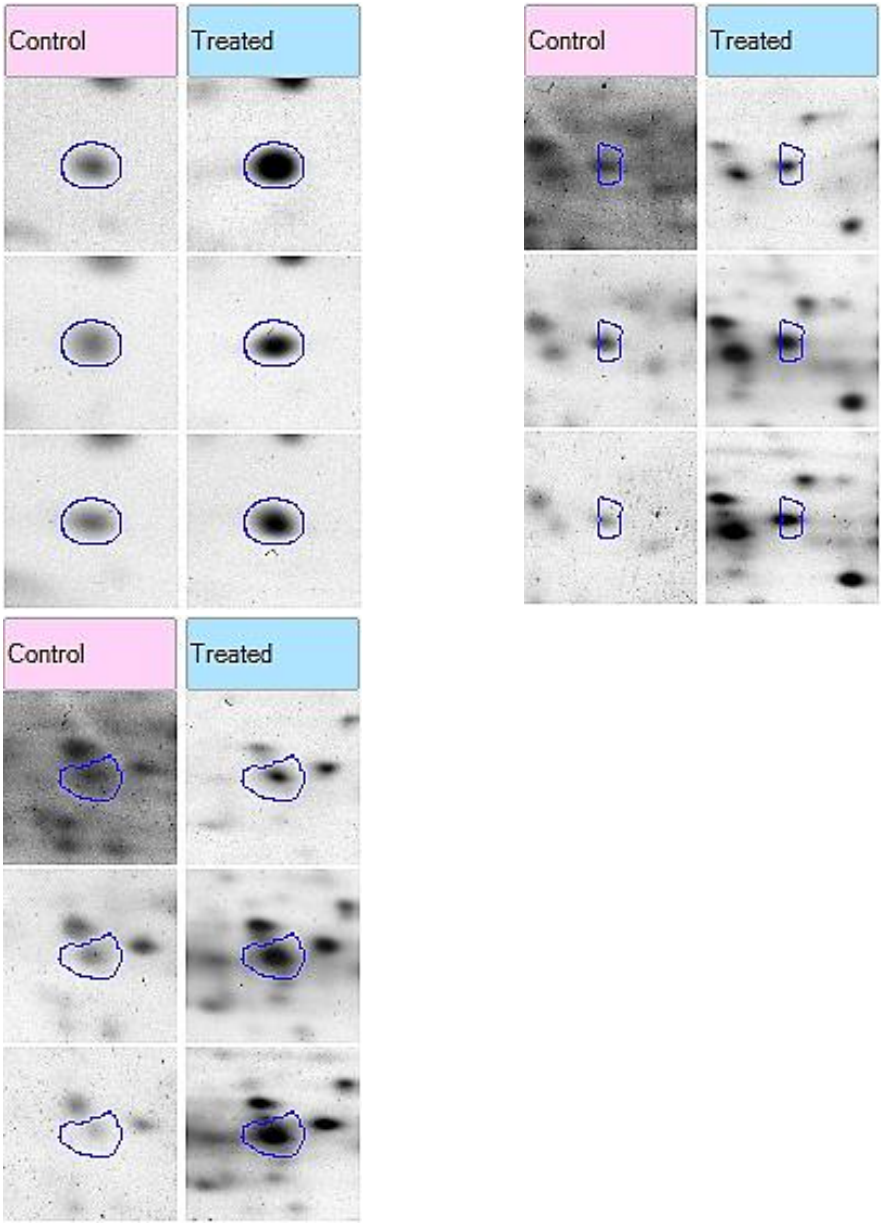
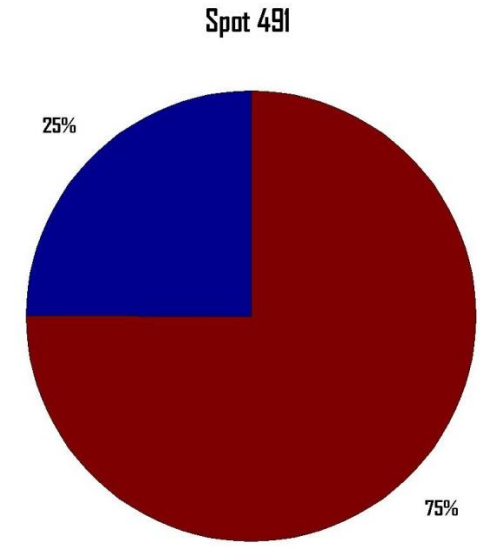
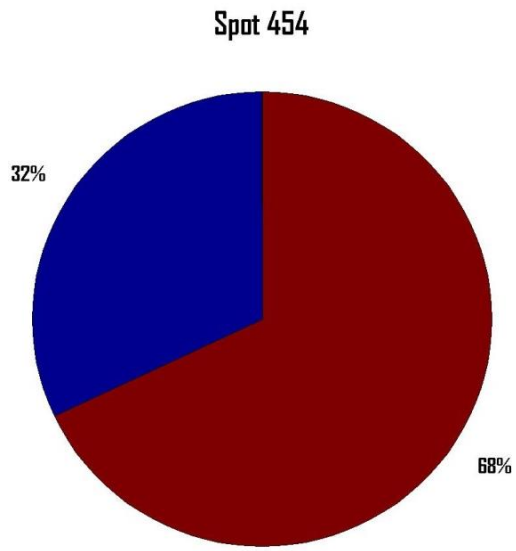
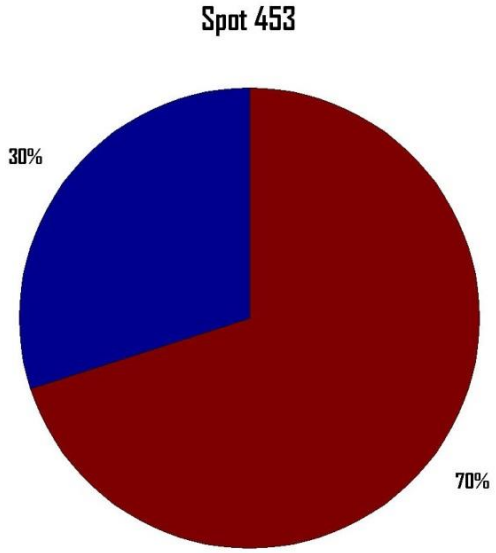
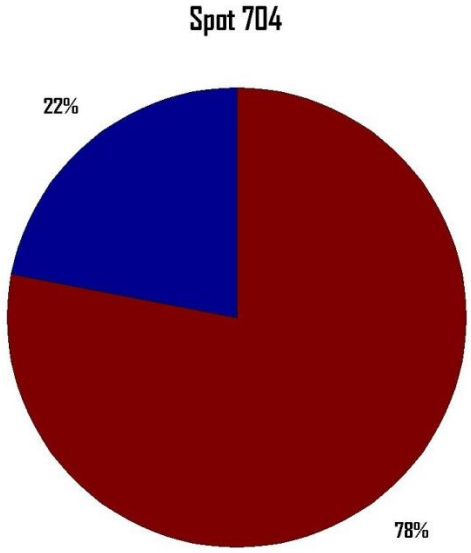


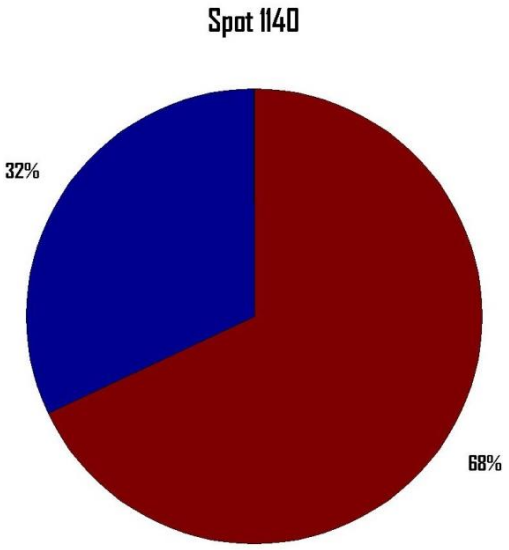
Figure 26 Image representatives of each protein spot in triplicates that displayed significant change in abundance.

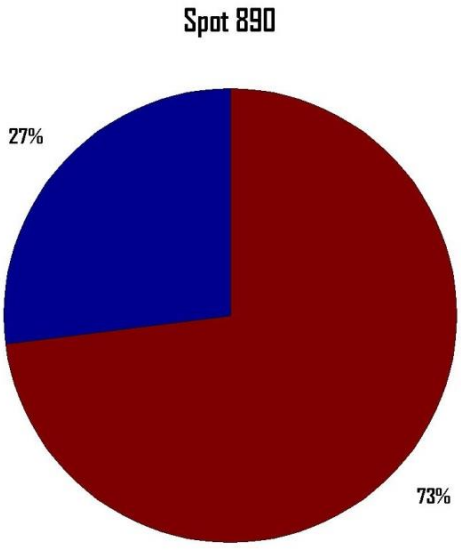

Spot 491
25%
75%

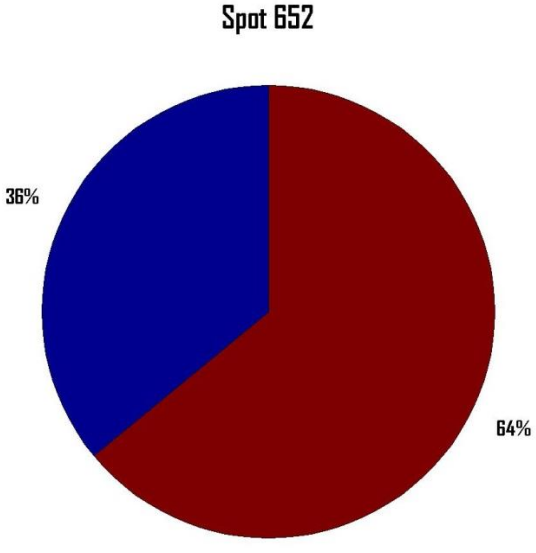

Spot 454
32%
68%

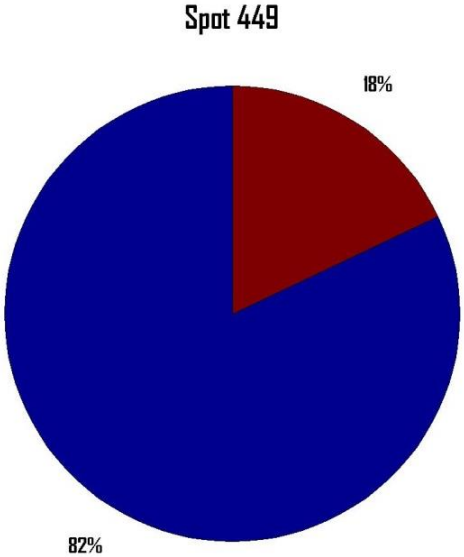

Spot 453
30%
70%


Spot 704
22%
78%


Spot 1140
32%
68%


Spot 890
27%
73%


Spot 652
36%
64%


Spot 449
18%
82%

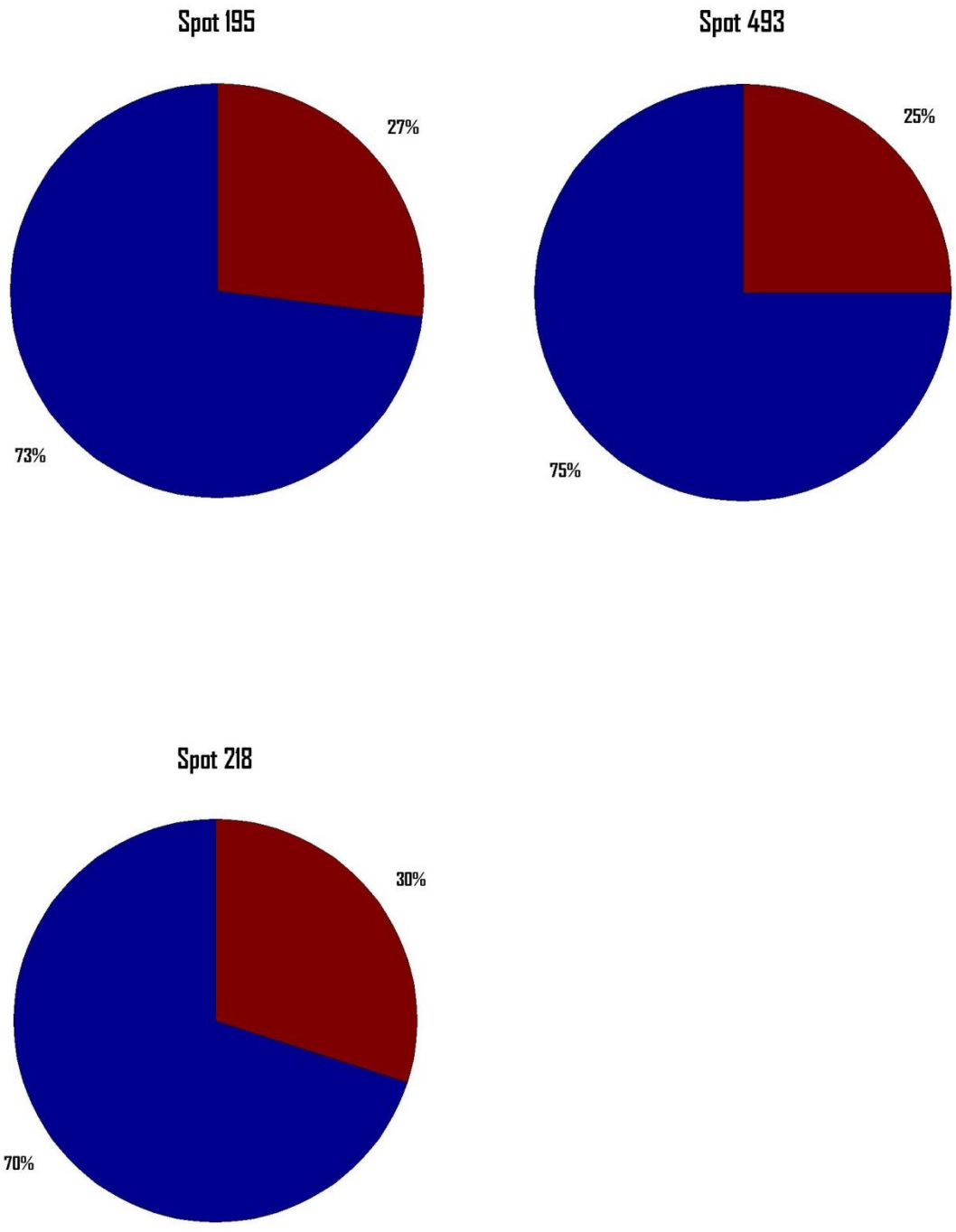


Figure 27 Changes in the quantity of proteins after treatment with PUR for 3 weeks. Pie charts show the average percentage (%) volume of protein spots. Blue: control, Brown: PUR treated.

The proteins identified were linked with flap endonuclease 1, ATP synthase subunit gamma, 50S ribosomal protein L2, putative pentatricopeptide repeat-containing protein At1g280, histidine-tRNA ligase, interferon tau, metallothionein-like protein, N-acetylmuramic acid 6-phosphate etherase, and 3-isopropylmalate dehydratase large subunit. There were no other analysed proteins that were exactly assigned to *Pestalotiopsis* sp. by similarity in the sequences.

Histidine-tRNA ligase found in *Xylella fastidiosa* which is similar to spot number 454; up-regulated in this study is involved in the catalysis of ATP, L-histidine, and tRNA(His) to AMP, diphosphate, and L-histidyl-tRNA(His) (Morf et al. 2005). The presence of histidine ligase has significantly increased the rate of ATP activity even when fungus was treated with PUR. Interferon tau found in *Ovibos moschatus* (spot number 491; slightly up-regulated in this study) is a paracrine hormone of high antiviral potency with low cytotoxicity which interacts with endometrial receptors (Florida 1999; Musson et al. 2000a). Interferon tau could increasingly suppress the release of luteolytic hormone and obstruct the regression of corpus luteum when fungus was pre-treated with PUR.

Spot number 890 represented N-acetylmuramic acid 6-phosphate etherase which is a specific catalyst that plays an important role in the recycling of cell walls. Similar to etherase found in *Escherichia coli* O81, this protein catalyzes the cleavage of N-acetyl-D-glucosamine 6-phosphate to N-acetylmuramate 6-phosphate (Musson et al. 2000b). We found that the protein was highly expressed when treated under stress with PUR. This implies an increase in the process of amino-sugar degradation during the treatment for biogenesis of cell wall. The 3-isopropylmalate dehydratase large subunit (spot number 1440; weakly up-regulated) is another enzyme that was detected. Dehydratase was also found in *Thermoanaerobacter pseudethanolicus* and converts 3-isopropylmalate to 2-isopropylmalate and involves in amino acid, and L-leucine biosynthesis. The enzyme dehydratase that was present in the fungus indicates a speed up of the biosynthesis of 2-isopropylmaleate from 3-isopropylmaleate.

Flap endonuclease 1 commonly found in *Candida albicans* (spot number 195; down-regulated in this study) is a nuclease of specific structure with 5‟-flap endonuclease and 5‟-3‟ exonuclease activities (Florida 1999). Fungus treated with PUR suggested that this protein decreases the replication and repair of DNA activities. ATP synthase subunit gamma (spot number 218; down-regulated) that is located in the mitochondrial membrane involves in ATP synthesis similarly to that found in *Mus musculus* (Lindberg et al. 2001; Osako, Kim & Sakai 2004; Rardin et al. 2013). The production of ATP from ADP of PUR treated fungus denotes a decrease in the production of ATP for the fungal cells.

50S ribosomal protein L2 (spot number 449; down-regulated) is a primary rRNA binding protein, also found in *Acholeplasma laidlawii* that is required for the combination of 30S and 50S subunits to form 70S ribosome (UNEP 2006). From this study, the formations of peptide bonds as well as tRNA binding have decreased when fungus was treated with PUR. Metallothionein-like protein found in *Festuca rubra* (spot number 493; down-regulated in this study) is a metal-binding protein (Cobbing 2008). Due to the presence of metallothionein-like protein, fungus in this study could have high content of cysteine residues that bind to various heavy metals. However, the amount of metallothionein-like protein decreased when treated with PUR.

4. Conclusion

Electronic waste is a serious problem that arises in all the countries around the world. The volume of electronic waste is increasing at a rapid rate because of fast technology advancement, hence producing enormous amounts of electronic waste. Electronic waste contains various different materials and some can cause environmental pollution and even threats to the human health due to the presence of toxic compounds. Electronic wastes are normally disposed to landfills and also burned. Burning of plastics from electronic waste releases toxic gases as well as greenhouse gases that deplete the ozone layer.

In order to minimize electronic waste problems, we have screened for organisms in particular endophytic fungi which can degrade polymer PUR. Endophytic fungi were successfully isolated from plant sample, *Nepenthes ampullaria* using aseptic technique. Pure fungal isolates were tested using agar plate assay containing PUR and isolate SBF4, SBF1, and SBF3 are the top PUR degrading endophytes.

Active endophytes capable of degrading PUR were further tested and confirmed through liquid medium assay containing PUR but without the addition of other carbon sources. Endophytes SBF4, SBF1, and SBF3 also demonstrated favourable PUR degradation activity. All these isolates can utilise PUR as substrate in liquid medium for growth. Isolate SBF4 can degrade PUR better followed by SBF1, and SBF3 when analysed through UV-Vis spectrophotometer.

Enzyme activity of isolate SBF4 and SBF1 were studied and both demonstrated an activity of 1850 U mL^{-1} 1210 U mL^{-1} respectively. Enzyme activity that was detected indicates the possible presence of esterase. In this study, the endophytes were also identified and are closely related to *Pestalotiopsis* sp.

Endophyte SBF4 (related to *Pestalotiopsis neglecta*) and SBF1 (related to *Pestalotiopsis* sp.) also displayed changes in the abundance of proteins within their cells when grown in the presence of PUR. Eleven of the total 24 protein spots showed significant alteration in abundance. Proteins are up-regulated and down-regulated when treated under stress condition with PUR. Significantly detected and altered proteins are identified as histidine-tRNA ligase, N-acetylmuramic acid 6-phosphate etherase, and metallothionein-like protein by mass spectrometry. These proteins play a significant role in ATP activity, recycling of cell wall, and degradation of heavy metals.

To the best of our knowledge, this is the first significant study in evaluating endophytes isolated from plant, *Nepenthes ampullaria* from Bukit Mentawai, Kuching, Sarawak that can degrade polymer PUR and tested for proteomic study. Our finding through molecular and morphological analyses proved the identity of the endophytes that belong to *Pestalotiopsis* sp.

4.1 Future work

Further work and investigation is necessary to develop the green approach of bioremediation to minimise environmental pollution that are caused by E-waste. A more detailed study on the screening parameters is required to determine the best optimum PUR degradation activity by fungus. The parameters to be tested include the temperature, and pH of test medium. This with hope will improve our understanding on the best conditions that is needed to achieve the maximum ability to degrade PUR by endophytic fungi.

The enzyme activity detected though enzyme test confirms and provides us the knowledge that endophytes tested contain enzyme that can degrade PUR which is present within the fungal cells. Therefore, a further approach to isolate the enzyme that is responsible for PUR degradation is important and worthy. Successful isolation of enzyme can then be purified and produced abundantly and this is of great economical value. Endophytes in this study can serve as potential source of polymer degrading enzyme and be bioengineered to enhance the production of enzyme for commercial use.

An in-depth study on proteomic is also of interest to understand more on the degradation and biological mechanisms of endophytic fungus in degrading polymer PUR. A better understanding on how the different types of proteins interact with each other to degrade PUR can be achieved. In-depth proteomic study can contribute to the understanding of a complete degradation pathway through the analysis of the presence of more functional class proteins.

In order to achieve more protein yields for proteomic study, different methodologies for the extraction of proteins as well as the condition set-up for isoelectric focusing process can be conducted. The different approaches to be carried out hopefully can reveal more expression of proteins that is necessary for a better understanding of PUR degradation pathway and metabolism of endophytic fungus.

5. References

Abbas, A, Koc, H, Liu, F & Tien, M 2005, 'Fungal Degradation of Wood: Initial Proteomic Analysis of Extracellular Proteins of *Phanerochaete chrysosporium* Grown on Oak Substrate', *Current genetics*, vol. 47, no. 1, pp. 49-56.

AEA 2004, *WEEE & Hazardous Waste*, AEA Technology: A report produced for DEFRA, Oxfordshire.

Agarwal, A & Chauhan, S 1988, 'A New Species of the genus *Pestalotiopsis* from Indian Soil', *Indian Phytopathology*, vol. 41, pp. 625-627.

Agbogbo, FK, Coward-Kelly, G, Torry-Smith, M & Wenger, KS 2006, 'Fermentation of Glucose/xylose Mixtures using *Pichia stipitis*', *Process Biochemistry*, vol. 41, no. 11, pp. 2333-2336.

Aizawa, H, Yoshida, H & Sakai, SI 2008, 'Current Results and Future Perspectives for Japanese Recycling of Home Electrical Appliances', *Resources, Conservation and Recycling*, vol. 52, no. 12, pp. 1399-1410.

Anderson, L & Anderson, NG 1977, 'High resolution two-dimensional electrophoresis of human plasma proteins', *Proceedings of the National Academy of Sciences*, vol. 74, no. 12, pp. 5421-5425.

Anderson, NL, Anderson, NG, Pearson, TW, Borchers, CH, Paulovich, AG, Patterson, SD, Gillette, M, Aebersold, R & Carr, SA 2009, 'A Human Proteome Detection and Quantitation Project', *Molecular & Cellular Proteomics*, vol. 8, no. 5, pp. 883-886.

Ando, Y 1993, in *Bulletin of the National Research Institute of Vegetables, Ornamental Plants and Tea. Series B. (Japan),* Food and Agriculture Organization of the United Nations.

Andreola, F, Barbieri, L, Corradi, A, Ferrari, AM, Lancellotti, I & Neri, P 2007, 'Recycling of EOL CRT Glass into Ceramic Glaze Formulations and its Environmental Impact by LCA Approach', *The International Journal of Life Cycle Assessment*, vol. 12, no. 6, pp. 448-454.

Asadollahi, MA, Maury, J, Møller, K, Nielsen, KF, Schalk, M, Clark, A & Nielsen, J 2008, 'Production of Plant Sesquiterpenes in *Saccharomyces cerevisiae*: Effect of ERG9 Repression on Sesquiterpene Biosynthesis', *Biotechnology and bioengineering*, vol. 99, no. 3, pp. 666-677.

Asif, AR, Oellerich, M, Amstrong, VW, Riemenschneider, B, Monod, M & Reichard, U 2006, 'Proteome of Conidial Surface Associated Proteins of *Aspergillus fumigatus* Reflecting Potential Vaccine Candidates and Allergens', *Journal of proteome research*, vol. 5, no. 4, pp. 954-962.

Astuti, P, Sudarsono, S, Nisak, K & Nugroho, GW 2014, 'Endophytic Fungi Isolated from Coleus amboinicus Lour Exhibited Antimicrobial Activity', *Advanced pharmaceutical bulletin*, vol. 4, no. Suppl 2, p. 599.

Bantscheff, M, Schirle, M, Sweetman, G, Rick, J & Kuster, B 2007, 'Quantitative mass spectrometry in proteomics: a critical review', *Analytical and bioanalytical chemistry*, vol. 389, no. 4, pp. 1017-1031.

Barba-Gutiérrez, Y, Adenso-Diaz, B & Hopp, M 2008, 'An Analysis of Some Environmental Consequences of European Electrical and Electronic Waste Regulation', *Resources, Conservation and Recycling*, vol. 52, no. 3, pp. 481-495.

Barr, M 1990, 'Prodromus to nonlichenized, pyrenomycetous members of class Hymenoascomycetes', *Mycotaxon*, vol. 39, pp. 43-184.

Barr, ME 1975, 'Pestalosphaeria, a new genus in the Amphisphaeriaceae', *Mycologia*, pp. 187-194.

Barrett, T & Gould, H 1973, 'Tissue and species specificity of non-histone chromatin proteins', *Biochimica et Biophysica Acta (BBA)-Nucleic Acids and Protein Synthesis*, vol. 294, no. 1, pp. 165-170.

Bate Smith, E & Metcalfe, C 1957, 'Leuco-anthocyanins. 3. The Nature and Systematic Distribution of Tannins in Dicotyledonous Plants', *Journal of the Linnean Society of London, Botany*, vol. 55, no. 362, pp. 669-705.

Beck, T & Hall, MN 1999, 'The TOR Signalling Pathway Controls Nuclear Localization of Nutrient-regulated Transcription Factors', *Nature*, vol. 402, no. 6762, pp. 689-692.

Bhadauria, V, Zhao, WS, Wang, LX, Zhang, Y, Liu, JH, Yang, J, Kong, LA & Peng, YL 2007, 'Advances in Fungal Proteomics', *Microbiological Research*, vol. 162, no. 3, pp. 193-200.

Bills, GF, Redlin, S & Carris, L 1996, 'Isolation and analysis of endophytic fungal communities from woody plants', *Endophytic fungi in grasses and woody plants: systematics, ecology, and evolution.*, pp. 31-65.

Birnbaum, LS & Staskal, DF 2004, 'Brominated Flame Retardants: Cause for Concern?', *Environmental Health Perspectives*, vol. 112, no. 1, p. 9.

Bisson, LF 2005, 'The Biotechnology of Wine Yeast', *Food Biotechnology*, vol. 18, no. 1, pp. 63-96.

Borneman, WS, Ljungdahl, L, Hartley, R & Akin, D 1992, 'Purification and partial characterization of two feruloyl esterases from the anaerobic fungus Neocallimastix strain MC-2', *Applied and environmental microbiology*, vol. 58, no. 11, pp. 3762-3766.

Boubendir, A 1992, *Purification and Biochemical Evaluation of Polyurethane Degrading Enzymes of Fungal Origin*, thesis, University of Salford.

Brakhage, AA & Langfelder, K 2002, 'Menacing Mold: The Molecular Biology of *Aspergillus fumigatus*', *Annual Reviews in Microbiology*, vol. 56, no. 1, pp. 433-455.

Brandhorst, BP 1976, 'Two-dimensional gel patterns of protein synthesis before and after fertilization of sea urchin eggs', *Developmental biology*, vol. 52, no. 2, pp. 310-317.

Braumann, I, van den Berg, M & Kempken, F 2007, 'Transposons in Biotechnologically Relevant strains of *Aspergillus niger* and *Penicillium chrysogenum*', *Fungal Genetics and Biology*, vol. 44, no. 12, pp. 1399-1414.

Bravo, R & Celis, JE 1980, 'A search for differential polypeptide synthesis throughout the cell cycle of HeLa cells', *The Journal of cell biology*, vol. 84, no. 3, pp. 795-802.

Bridge, P & Spooner, B 2001, 'Soil fungi: diversity and detection', *Plant and soil*, vol. 232, no. 1-2, pp. 147-154.

Bruggeman, FJ & Westerhoff, HV 2007, 'The Nature of Systems Biology', *TRENDS in Microbiology*, vol. 15, no. 1, pp. 45-50.

Buekens, A & Yang, J 2014, 'Recycling of WEEE Plastics: A Review', *Journal of Material Cycles and Waste Management*, vol. 16, no. 3, pp. 415-434.

Cairns, T, Minuzzi, F & Bignell, E 2010, 'The Host-infecting Fungal Transcriptome', *FEMS Microbiology Letters*, vol. 307, no. 1, pp. 1-11.

Carberry, S & Doyle, S 2007, 'Proteomic Studies in Biomedically and Industrially Relevant Fungi', *Cytotechnology*, vol. 53, no. 1-3, pp. 95-100.

Carberry, S, Neville, CM, Kavanagh, KA & Doyle, S 2006, 'Analysis of Major Intracellular Proteins of *Aspergillus fumigatus* by MALDI Mass Spectrometry: Identification and Characterisation of an Elongation Factor 1B Protein with Glutathione Transferase Activity', *Biochemical and biophysical research communications*, vol. 341, no. 4, pp. 1096-1104.

Chan, JK, Xing, GH, Xu, Y, Liang, Y, Chen, LX, Wu, SC, Wong, CK, Leung, CK & Wong, MH 2007, 'Body Loadings and Health Risk Assessment of Polychlorinated dibenzo-p-dioxins and dibenzofurans at an Intensive Electronic Waste Recycling Site in China', *Environmental science & technology*, vol. 41, no. 22, pp. 7668-7674.

Chiranand, W, McLeod, I, Zhou, H, Lynn, JJ, Vega, LA, Myers, H, Yates, JR, Lorenz, MC & Gustin, MC 2008, 'CTA4 Transcription Factor Mediates Induction of Nitrosative Stress Response in *Candida albicans*', *Eukaryotic cell*, vol. 7, no. 2, pp. 268-278.

Choo, J, Annie, NA, Mujahid, A & Mueller, M 2014, 'Heavy metal resistant endophytic fungi isolated from Nypa fruticans in Kuching Wetland National Park', *Ocean Science Journal* (Manuscript ID: OSJO-D-14-00054R1), Swinburne University of Technology.

Christensen, T, Woeldike, H, Boel, E, Mortensen, SB, Hjortshoej, K, Thim, L & Hansen, MT 1988, 'High Level Expression of Recombinant Genes in *Aspergillus Oryzae*', *Nature Biotechnology*, vol. 6, p. 1419.

Coalition, SVT & Network, BA 2002, *Exporting Harm: The High-tech Trashing of Asia*, Seattle.

Cobbing, M 2008, Greenpeace Report.

Coombs, JT & Franco, CM 2003, 'Isolation and identification of actinobacteria from surface-sterilized wheat roots', *Applied and environmental microbiology*, vol. 69, no. 9, pp. 5603-5608.

Cox, J & Mann, M 2007, 'Is Proteomics the New Genomics?', *Cell*, vol. 130, no. 3, pp. 395-398.

Crabbe, JR, Campbell, JR, Thompson, L, Walz, SL & Schultz, WW 1994, 'Biodegradation of a Colloidal Ester-based Polyurethane by Soil Fungi', *International Biodeterioration & Biodegradation*, vol. 33, no. 2, pp. 103-113.

Cravatt, BF, Simon, GM & Yates Iii, JR 2007, 'The Biological Impact of Mass-spectrometry-based Proteomics', *Nature*, vol. 450, no. 7172, pp. 991-1000.

Cui, J & Zhang, L 2008, 'Metallurgical Recovery of Metals from Electronic Waste: A Review', *Journal of Hazardous Materials*, vol. 158, no. 2, pp. 228-256.

Darby, RT & Kaplan, AM 1968, 'Fungal Susceptibility of Polyurethanes', *Applied microbiology*, vol. 16, no. 6, pp. 900-905.

de Oliveira, JMPF & de Graaff, LH 2011, 'Proteomics of industrial fungi: trends and insights for biotechnology', *Applied Microbiology and Biotechnology*, vol. 89, no. 2, pp. 225-237.

Dombrow, BA 1965, Vol. 2 Reinhold Pub. Corp.

Ebanks, RO, Chisholm, K, McKinnon, S, Whiteway, M & Pinto, DM 2006, 'Proteomic Analysis of *Candida albicans* Yeast and Hyphal Cell Wall and associated Proteins', *Proteomics*, vol. 6, no. 7, pp. 2147-2156.

El-Sayed, AHM, Mahmoud, WM, Davis, EM & Coughlin, RW 1996, 'Biodegradation of Polyurethane Coatings by Hydrocarbon-degrading Bacteria', *International Biodeterioration & Biodegradation*, vol. 37, no. 1, pp. 69-79.

Eng, JK, McCormack, AL & Yates, JR 1994, 'An approach to correlate tandem mass spectral data of peptides with amino acid sequences in a protein database', *Journal of the American Society for Mass Spectrometry*, vol. 5, no. 11, pp. 976-989.

EPS 2006, Electronics Product Stewardship Canada.

Espinel-Ingroff, A, Montero, D & Martin-Mazuelos, E 2004, 'Long-term preservation of fungal isolates in commercially prepared cryogenic Microbank vials', *Journal of clinical microbiology*, vol. 42, no. 3, pp. 1257-1259.

Evans, D & Levisohn, I 1968, 'Biodeterioration of Polyester-based Polyurethane', *International Biodeterioration Bulletin*, vol. 4, no. 2, pp. 89-72.

Fassler, JS & West, AH 2011, 'Fungal Skn7 Stress Responses and their Relationship to Virulence', *Eukaryotic cell*, vol. 10, no. 2, pp. 156-167.

Fernández-Acero, FJ, Jorge, I, Calvo, E, Vallejo, I, Carbú, M, Camafeita, E, Garrido, C, López, JA, Jorrin, J & Cantoral, JM 2007, 'Proteomic Analysis of Phytopathogenic fungus *Botrytis cinerea* as A Potential Tool for Identifying Pathogenicity Factors, Therapeutic Targets and for Basic Research', *Archives of microbiology*, vol. 187, no. 3, pp. 207-215.

Fernández-Arenas, E, Cabezón, V, Bermejo, C, Arroyo, J, Nombela, C, Diez-Orejas, R & Gil, C 2007, 'Integrated Proteomics and Genomics Strategies bring New Insight into *Candida albicans* Response upon Macrophage Interaction', *Molecular & Cellular Proteomics*, vol. 6, no. 3, pp. 460-478.

Filip, Z 1978, 'Decomposition of Polyurethane in A Garbage Landfill Leakage Water and by Soil Microorganisms', *European journal of applied microbiology and biotechnology*, vol. 5, no. 3, pp. 225-231.

Florida, C 1999, *Characterization of Leachability from Cathode Ray Tubes using the Toxicity Characteristic Leaching Procedure*, Department of Natural Resources.

Forgue, P, Halouska, S, Werth, M, Xu, K, Harris, S & Powers, R 2006, 'NMR Metabolic Profiling of *Aspergillus nidulans* to Monitor Drug and Protein Activity', *Journal of proteome research*, vol. 5, no. 8, pp. 1916-1923.

Frazzoli, C, Orisakwe, OE, Dragone, R & Mantovani, A 2010, 'Diagnostic Health Risk Assessment of Electronic Waste on the General Population in Developing Countries' Scenarios', *Environmental Impact Assessment Review*, vol. 30, no. 6, pp. 388-399.

Fromont Racine, M, Mayes, AE, Brunet Simon, A, Rain, JC, Colley, A, Dix, I, Decourty, L, Joly, N, Ricard, F & Beggs, JD 2000, 'Genome Wide Protein Interaction Screens Reveal Functional Networks involving Sm-like Proteins', *Yeast*, vol. 17, no. 2, pp. 95-110.

Futcher, B, Latter, G, Monardo, P, McLaughlin, C & Garrels, J 1999, 'A Sampling of the Yeast Proteome', *Molecular and cellular biology*, vol. 19, no. 11, pp. 7357-7368.

Galagan, JE, Calvo, SE, Cuomo, C, Ma, LJ, Wortman, JR, Batzoglou, S, Lee, SI, Baştürkmen, M, Spevak, CC & Clutterbuck, J 2005, 'Sequencing of *Aspergillus nidulans* and Comparative Analysis with *A. fumigatus* and *A. oryzae*', *Nature*, vol. 438, no. 7071, pp. 1105-1115.

Gardes, M & Bruns, TD 1993, 'ITS primers with enhanced specificity for basidiomycetes application to the identification of mycorrhizae and rusts', *Molecular Ecology*, vol. 2, no. 2, pp. 113-118.

Geladé, R, Van de Velde, S, Van Dijck, P & Thevelein, JM 2003, 'Multi-level Response of the Yeast Genome to Glucose', *Genome biology*, vol. 4, no. 11, pp. 233-233.

Geml, J, Laursen, GA, Timling, I, McFarland, JM, Booth, MG, Lennon, N, Nusbaum, C & Taylor, D 2009, 'Molecular phylogenetic biodiversity assessment of arctic and boreal ectomycorrhizal Lactarius Pers.(Russulales; Basidiomycota) in Alaska, based on soil and sporocarp DNA', *Molecular Ecology*, vol. 18, no. 10, pp. 2213-2227.

Ghaemmaghami, S, Huh, W-K, Bower, K, Howson, RW, Belle, A, Dephoure, N, O'Shea, EK & Weissman, JS 2003, 'Global Analysis of Protein Expression in Yeast', *Nature*, vol. 425, no. 6959, pp. 737-741.

Goffeau, A, Barrell, B, Bussey, H, Davis, R, Dujon, B, Feldmann, H, Galibert, F, Hoheisel, J, Jacq, C & Johnston, M 1996, 'Life with 6000 Genes', *Science*, vol. 274, no. 5287, pp. 546-567.

Gomes-Figueiredo, J, Pimentel, IC, Vicente, VnA, Pie, MR, Kava-Cordeiro, V, Galli-Terasawa, L, Pereira, JO, de Souza, AQ & Glienke, C 2007, 'Bioprospecting Highly Diverse Endophytic *Pestalotiopsis* sp. with Antibacterial Properties from *Maytenus ilicifolia*, a Medicinal Plant from Brazil', *Canadian journal of microbiology*, vol. 53, no. 10, pp. 1123-1132.

Gomi, K, Iimura, Y & Hara, S 1987, 'Integrative transformation of *Aspergillus oryzae* with a Plasmid Containing the *Aspergillus nidulans* argB Gene', *Agricultural and biological chemistry*, vol. 51, no. 9, pp. 2549-2555.

Grahl, N, Shepardson, KM, Chung, D & Cramer, RA 2012, 'Hypoxia and Fungal Pathogenesis: to air or not to air?', *Eukaryotic cell*, vol. 11, no. 5, pp. 560-570.

Griffin, G 1980, 'Synthetic Polymers and the Living Environment', *Pure and Applied Chemistry*, vol. 52, no. 2, pp. 399-407.

Griffiths, AD & Duncan, AR 1998, 'Strategies for Selection of Antibodies by Phage Display', *Current opinion in biotechnology*, vol. 9, no. 1, pp. 102-108.

Griffiths, D & Swart, H 1974, 'Conidial structure in two species of Pestalotiopsis', *Transactions of the British Mycological Society*, vol. 62, no. 2, pp. 295-304.

Grinyer, J, Hunt, S, McKay, M, Herbert, BR & Nevalainen, H 2005, 'Proteomic Response of the Biological Control fungus *Trichoderma atroviride* to Growth on the Cell Walls of *Rhizoctonia solani*', *Current genetics*, vol. 47, no. 6, pp. 381-388.

Grinyer, J, Kautto, L, Traini, M, Willows, RD, Te"o, J, Bergquist, P & Nevalainen, H 2007, 'Proteome Mapping of the *Trichoderma reesei* 20S Proteasome', *Current genetics*, vol. 51, no. 2, pp. 79-88.

Grinyer, J, McKay, M, Herbert, B & Nevalainen, H 2004a, 'Fungal Proteomics: Mapping the Mitochondrial Proteins of a *Trichoderma harzianum* strain applied for Biological Control', *Current genetics*, vol. 45, no. 3, pp. 170-175.

Grinyer, J, McKay, M, Nevalainen, H & Herbert, BR 2004b, 'Fungal Proteomics: Initial Mapping of Biological Control strain *Trichoderma harzianum*', *Current genetics*, vol. 45, no. 3, pp. 163-169.

Guan, J, Sacks, MS, Beckman, EJ & Wagner, WR 2004, 'Biodegradable Poly (ether ester urethane) Urea Elastomers based on Poly (ether ester) Triblock Copolymers and Putrescine: Synthesis, Characterization and Cytocompatibility', *Biomaterials*, vol. 25, no. 1, pp. 85-96.

Han, X, Aslanian, A & Yates, JR 2008, 'Mass Spectrometry for Proteomics', *Current opinion in chemical biology*, vol. 12, no. 5, pp. 483-490.

Han, X, Jin, M, Breuker, K & McLafferty, FW 2006, 'Extending Top-down Mass spectrometry to Proteins with Masses greater than 200 kilodaltons', *Science*, vol. 314, no. 5796, pp. 109-112.

He, F, Zhang, Y, Chen, H, Zhang, Z & Peng, YL 2008, 'The Prediction of Protein-protein Interaction Networks in Rice Blast fungus', *BMC genomics*, vol. 9, no. 1, p. 519.

Heinonsalo, J, Jorgensen, KS & Sen, R 2001, 'Microcosm-based analyses of Scots pine seedling growth, ectomycorrhizal fungal community structure and bacterial carbon utilization profiles in boreal forest humus and underlying illuvial mineral horizons', *FEMS Microbiology Ecology*, vol. 36, no. 1, pp. 73-84.

Hensing, M, Rouwenhorst, R, Heijnen, J, Van Dijken, J & Pronk, J 1995, 'Physiological and Technological Aspects of Large-scale Heterologous-protein Production with Yeasts', *Antonie van Leeuwenhoek*, vol. 67, no. 3, pp. 261-279.

Hickman, MJ & Winston, F 2007, 'Heme Levels Switch the Function of Hap1 of *Saccharomyces cerevisiae* between Transcriptional Activator and Transcriptional Repressor', *Molecular and cellular biology*, vol. 27, no. 21, pp. 7414-7424.

Hinnebusch, AG 2005, 'Translational Regulation of GCN4 and the General Amino Acid Control of Yeast', *Annu. Rev. Microbiol.*, vol. 59, pp. 407-450.

Hischier, R, Wäger, P & Gauglhofer, J 2005, 'Does WEEE Recycling make sense from an Environmental Perspective?: The Environmental Impacts of the Swiss take-back and Recycling Systems for Waste Electrical and Electronic Equipment (WEEE)', *Environmental Impact Assessment Review*, vol. 25, no. 5, pp. 525-539.

Hjersted, JL, Henson, MA & Mahadevan, R 2007, 'Genome Scale Analysis of *Saccharomyces cerevisiae* Metabolism and Ethanol Production in Fed-batch Culture', *Biotechnology and bioengineering*, vol. 97, no. 5, pp. 1190-1204.

Hole, L 1972, 'Artificial Leathers', *Rep Prog Appl Chem*, vol. 57, pp. 181-206.

Howard, GT 2011, 'Microbial Biodegradation of Polyurethane', *Recent Developments in Polymer Recycling*, vol. 215, p. 238.

Howard, GT, Ruiz, C & Hilliard, NP 1999, 'Growth of *Pseudomonas chlororaphis* on a Polyester–polyurethane and the Purification and Characterization of a Polyurethanase–esterase Enzyme', *International Biodeterioration & Biodegradation*, vol. 43, no. 1, pp. 7-12.

Hu, H, Jeewon, R, Zhou, D, Zhou, T & Hyde, KD 2007, 'Phylogenetic Diversity of Endophytic *Pestalotiopsis* species in *Pinus armandii* and *Ribes* sp.: Evidence from rDNA and β-tubulin Gene Phylogenies', *Fungal Divers*, vol. 24, pp. 1-22.

Huang, SJ & Roby, MS 1986a, 'Biodegradable Polymers Poly (amide-urethanes)', *Journal of Bioactive and Compatible Polymers*, vol. 1, no. 1, pp. 61-71.

Huang, SJ & Roby, MS 1986b, 'Biodegradable Polymers Poly (amide-urethanes)[1]', *Journal of Bioactive and Compatible Polymers*, vol. 1, no. 1, pp. 61-71.

Huh, WK, Falvo, JV, Gerke, LC, Carroll, AS, Howson, RW, Weissman, JS & O'Shea, EK 2003, 'Global Analysis of Protein Localization in Budding Yeast', *Nature*, vol. 425, no. 6959, pp. 686-691.

Huo, X, Peng, L, Xu, X, Zheng, L, Qiu, B, Qi, Z, Zhang, B, Han, D & Piao, Z 2007, 'Elevated Blood Lead Levels of Children in Guiyu, An Electronic Waste Recycling Town in China', *Environmental Health Perspectives*, pp. 1113-1117.

Insenser, M, Nombela, C, Molero, G & Gil, C 2006, 'Proteomic Analysis of Detergent-resistant Membranes from *Candida albicans*', *Proteomics*, vol. 6, no. S1, pp. S74-S81.

Jeewon, R, Liew, E & Hyde, K 2004, 'Phylogenetic Evaluation of Species Nomenclature of *Pestalotiopsis* in Relation to Host Association', *Fungal Diversity*, vol. 17, no. 3, pp. 39-55.

Jeewon, R, Liew, EC & Hyde, KD 2002, 'Phylogenetic Relationships of *Pestalotiopsis* and Allied genera Inferred from Ribosomal DNA Sequences and Morphological Characters', *Molecular Phylogenetics and Evolution*, vol. 25, no. 3, pp. 378-392.

Jeewon, R, Liew, EC, Simpson, JA, Hodgkiss, IJ & Hyde, KD 2003, 'Phylogenetic Significance of Morphological Characters in the Taxonomy of *Pestalotiopsis* species', *Molecular Phylogenetics and Evolution*, vol. 27, no. 3, pp. 372-383.

Jensen, ON 2006, 'Interpreting the protein language using proteomics', *Nature Reviews Molecular Cell Biology*, vol. 7, no. 6, pp. 391-403.

Johnstone Robertson, M, Clarke, K & Harrison, S 2008, 'Characterization of the Distribution of Glucose Oxidase in *Penicillium* sp. CBS 120262 and *Aspergillus niger* NRRL-3 Cultures and its Effect on Integrated Product Recovery', *Biotechnology and bioengineering*, vol. 99, no. 4, pp. 910-918.

Jorrín-Novo, JV, Maldonado, AM, Echevarría-Zomeño, S, Valledor, L, Castillejo, MA, Curto, M, Valero, J, Sghaier, B, Donoso, G & Redondo, I 2009, 'Plant Proteomics Update (2007–2008): Second-generation Proteomic Techniques, An Appropriate Experimental Design, and Data Analysis to fulfill MIAPE Standards, Increase Plant Proteome Coverage and Expand Biological Knowledge', *Journal of Proteomics*, vol. 72, no. 3, pp. 285-314.

Jusko, TA, Henderson Jr, CR, Lanphear, BP, Cory-Slechta, DA, Parsons, PJ & Canfield, RL 2008, 'Blood Lead Concentrations <10 µg/dL and Child Intelligence at 6 Years of Age', *Environ Health Perspect*, vol. 116, no. 2, pp. 243-248.

Kanavel, G, Koons, P & Lauer, R 1966, 'Fungus Resistance of Millable Urethans', *Rubber Chemistry and Technology*, vol. 39, no. 4, pp. 1338-1346.

Kang, J, Kong, R & Hyde, K 1998, 'Studies on the Amphisphaeriales 1. Amphisphaeriaceae (sensu stricto) and its phylogenetic relationships inferred from 5.8 S rDNA and ITS2 sequences', *Fungal Diversity*, vol. 1, pp. 147-157.

Kang, JC, Hyde, KD & Kong, RY 1999, 'Studies on Amphisphaeriales: the Amphisphaeriaceae (sensu stricto)', *Mycological Research*, vol. 103, no. 01, pp. 53-64.

Kaplan, AM, Darby, RT, Greenberger, M & Rodgers, M 1968, 'Microbial Deterioration of Polyurethane Systems', *Dev Ind Microbiol*, vol. 82, pp. 362-371.

Kapoor, A, Viraraghavan, T & Cullimore, DR 1999, 'Removal of heavy metals using the fungus Aspergillus niger', *Bioresource Technology*, vol. 70, no. 1, pp. 95-104.

Kay, M, McCabe, R & Morton, L 1993, 'Chemical and Physical Changes Occurring in Polyester Polyurethane during Biodegradation', *International Biodeterioration & Biodegradation*, vol. 31, no. 3, pp. 209-225.

Kay, M, Morton, L & Prince, E 1991, 'Bacterial Degradation of Polyester Polyurethane', *International biodeterioration*, vol. 27, no. 2, pp. 205-222.

Kim, Y, Nandakumar, M & Marten, MR 2007a, 'Proteome Map of *Aspergillus nidulans* during Osmoadaptation', *Fungal Genetics and Biology*, vol. 44, no. 9, pp. 886-895.

Kim, Y, Nandakumar, M & Marten, MR 2007b, 'Proteomics of Filamentous Fungi', *TRENDS in Biotechnology*, vol. 25, no. 9, pp. 395-400.

Kirk, TK & Farrell, RL 1987, 'Enzymatic "combustion": The Microbial Degradation of Lignin', *Annual Reviews in Microbiology*, vol. 41, no. 1, pp. 465-501.

Kniemeyer, O, Lessing, F, Scheibner, O, Hertweck, C & Brakhage, AA 2006, 'Optimisation of a 2-D gel Electrophoresis Protocol for the Human-pathogenic fungus *Aspergillus fumigatus*', *Current genetics*, vol. 49, no. 3, pp. 178-189.

Kordel, M, Hofmann, B, Schomburg, D & Schmid, R 1991, 'Extracellular lipase of Pseudomonas sp. strain ATCC 21808: purification, characterization, crystallization, and preliminary X-ray diffraction data', *Journal of bacteriology*, vol. 173, no. 15, pp. 4836-4841.

Krappmann, S & Braus, G 2005, 'Nitrogen Metabolism of *Aspergillus* and its Role in Pathogenicity', *Medical mycology*, vol. 43, no. Supplement 1, pp. S31-S40.

Kumaran, RS, Kim, HJ & Hur, B-K 2010, 'Taxol promising fungal endophyte, Pestalotiopsis species isolated from Taxus cuspidata', *Journal of bioscience and bioengineering*, vol. 110, no. 5, pp. 541-546.

Kusch, H, Engelmann, S, Albrecht, D, Morschhäuser, J & Hecker, M 2007, 'Proteomic Analysis of the Oxidative Stress Response in *Candida albicans*', *Proteomics*, vol. 7, no. 5, pp. 686-697.

Ladou, J & Lovegrove, S 2008, 'Export of Electronics Equipment Waste', *International Journal of Occupational and Environmental Health*, vol. 14, no. 1, pp. 1-10.

Leung, A, Cai, ZW & Wong, MH 2006, 'Environmental Contamination from Electronic Waste Recycling at Guiyu, Southeast China', *Journal of Material Cycles and Waste Management*, vol. 8, no. 1, pp. 21-33.

Leung, AO, Duzgoren-Aydin, NS, Cheung, K & Wong, MH 2008, 'Heavy Metals Concentrations of Surface Dust from E-waste Recycling and its Human Health Implications in Southeast China', *Environmental science & technology*, vol. 42, no. 7, pp. 2674-2680.

Li, H, Yu, L, Sheng, G, Fu, J & Peng, Pa 2007, 'Severe PCDD/F and PBDD/F Pollution in Air Around An Electronic Waste Dismantling Area in China', *Environmental science & technology*, vol. 41, no. 16, pp. 5641-5646.

Li, J, Harper, JK, Grant, DM, Tombe, BO, Bashyal, B, Hess, W & Strobel, GA 2001, 'Ambuic acid, a highly functionalized cyclohexenone with antifungal activity from Pestalotiopsis spp. and Monochaetia sp', *Phytochemistry*, vol. 56, no. 5, pp. 463-468.

Li, JY & Strobel, GA 2001, 'Jesterone and hydroxy-jesterone antioomycete cyclohexenone epoxides from the endophytic fungus Pestalotiopsis jesteri', *Phytochemistry*, vol. 57, no. 2, pp. 261-265.

Lindberg, S, Wallschlaeger, D, Prestbo, E, Bloom, N, Price, J & Reinhart, D 2001, 'Methylated Mercury species in Municipal Waste Landfill Gas Sampled in Florida, USA', *Atmospheric Environment*, vol. 35, no. 23, pp. 4011-4015.

Link, AJ, Eng, J, Schieltz, DM, Carmack, E, Mize, GJ, Morris, DR, Garvik, BM & Yates, JR 1999, 'Direct analysis of protein complexes using mass spectrometry', *Nature biotechnology*, vol. 17, no. 7, pp. 676-682.

Liolios, K, Mavromatis, K, Tavernarakis, N & Kyrpides, NC 2008, 'The Genomes On Line Database (GOLD) in 2007: Status of Genomic and Metagenomic Projects and their Associated Metadata', *Nucleic acids research*, vol. 36, no. suppl 1, pp. D475-D479.

Liu, A, Wu, X, Xu, T, Guo, L & Wei, J 2005, 'Endophytic *Pestalotiopsis* species from Hainan, China', *Mycosystema*, vol. 25, no. 3, pp. 389-397.

Liu, L, Liu, S, Jiang, L, Chen, X, Guo, L & Che, Y 2008, 'Chloropupukeananin, the First Chlorinated Pupukeanane derivative, and its Precursors from *Pestalotiopsis fici*', *Organic letters*, vol. 10, no. 7, pp. 1397-1400.

Lueking, A, Horn, M, Eickhoff, H, Büssow, K, Lehrach, H & Walter, G 1999, 'Protein microarrays for gene expression and antibody screening', *Analytical biochemistry*, vol. 270, no. 1, pp. 103-111.

Luo, Q, Cai, ZW & Wong, MH 2007, 'Polybrominated Diphenyl Ethers in Fish and Sediment from River Polluted by Electronic Waste', *Science of The Total Environment*, vol. 383, no. 1, pp. 115-127.

Luo, Q, Wong, M & Cai, Z 2007, 'Determination of Polybrominated Diphenyl Ethers in Freshwater Fishes from a River Polluted by E-wastes', *Talanta*, vol. 72, no. 5, pp. 1644-1649.

Luo, XJ, Zhang, Xl, Liu, J, Wu, JP, Luo, Y, Chen, SJ, Mai, BX & Yang, ZY 2008, 'Persistent Halogenated Compounds in Waterbirds from An E-waste Recycling Region in South China', *Environmental science & technology*, vol. 43, no. 2, pp. 306-311.

Ma, Z, Hong, Y, Nelson, DM, Pichamuthu, JE, Leeson, CE & Wagner, WR 2011, 'Biodegradable Polyurethane Ureas with Variable Polyester or Polycarbonate Soft Segments: Effects of Crystallinity, Molecular weight, and Composition on Mechanical Properties', *Biomacromolecules*, vol. 12, no. 9, pp. 3265-3274.

Macgillivray, AJ & Wood, DR 1974, 'The Heterogeneity of Mouse-Chromatin Nonhistone Proteins as Evidenced by Two-Dimensional Polyacrylamide-Gel Electrophoresis and Ion-Exchange Chromatography', *European Journal of Biochemistry*, vol. 41, no. 1, pp. 181-190.

Machida, M, Asai, K, Sano, M, Tanaka, T, Kumagai, T, Terai, G, Kusumoto, K-I, Arima, T, Akita, O & Kashiwagi, Y 2005, 'Genome Sequencing and Analysis of *Aspergillus oryzae*', *Nature*, vol. 438, no. 7071, pp. 1157-1161.

Mann, M 2009, 'Comparative Analysis to Guide Quality Improvements in Proteomics', *Nature methods*, vol. 6, no. 10, pp. 717-719.

Marchant, R, Zhao, Q, Anderson, J & Hiltner, A 1987, 'Degradation of a Poly(ether urethane urea) Elastomer: Infra-red and XPS Studies', *Polymer*, vol. 28, pp. 2032–2039.

Marchant, RE 1992, *Biodegradability of Biomedical Polymers,* Marcel Dekker, Inc.

Marra, R, Ambrosino, P, Carbone, V, Vinale, F, Woo, SL, Ruocco, M, Ciliento, R, Lanzuise, S, Ferraioli, S & Soriente, I 2006, 'Study of the Three-way Interaction between *Trichoderma atroviride*, Plant and Fungal Pathogens by using a Proteomic Approach', *Current genetics*, vol. 50, no. 5, pp. 307-321.

Martin, DJ, Warren, LAP, Gunatillake, PA, McCarthy, SJ, Meijs, GF & Schindhelm, K 2000, 'Polydimethylsiloxane/polyether-mixed Macrodiol-based Polyurethane Elastomers: Biostability', *Biomaterials*, vol. 21, no. 10, pp. 1021-1029.

Martínez Solano, L, Nombela, C, Molero, G & Gil, C 2006, 'Differential Protein Expression of Murine Macrophages upon Interaction with *Candida albicans*', *Proteomics*, vol. 6, no. S1, pp. S133-S144.

Mathur, G & Prasad, R 2012, 'Degradation of Polyurethane by A*spergillus flavus* (ITCC 6051) Isolated from Soil', *Applied biochemistry and biotechnology*, vol. 167, no. 6, pp. 1595-1602.

McKerrow, JH, Bhargava, V, Hansell, E, Huling, S, Kuwahara, T, Matley, M, Coussens, L & Warren, R 2000, 'A Functional Proteomics Screen of Proteases in Colorectal Carcinoma', *Molecular Medicine*, vol. 6, no. 5, p. 450.

Medina, ML, Haynes, PA, Breci, L & Francisco, WA 2005, 'Analysis of Secreted Proteins from *Aspergillus flavus*', *Proteomics*, vol. 5, no. 12, pp. 3153-3161.

Medina, ML, Kiernan, UA & Francisco, WA 2004, 'Proteomic Analysis of Rutin-induced Secreted Proteins from *Aspergillus flavus*', *Fungal Genetics and Biology*, vol. 41, no. 3, pp. 327-335.

Miguel, R 2000, *Electrical and Electronic Components, Models of Selective Collections of Electrical and Electronic Appliances*, Barcelona, Spain.

MJC 2004, *Screening Level Human Health and Ecological Risk Assessment for Generic E-waste Processing Facility*, Canada.

Morf, LS, Tremp, J, Gloor, R, Huber, Y, Stengele, M & Zennegg, M 2005, 'Brominated Flame Retardants in Waste Electrical and Electronic Equipment: Substance Flows in a Recycling Plant', *Environmental science & technology*, vol. 39, no. 22, pp. 8691-8699.

Mukherjee, K, Tribedi, P, Chowdhury, A, Ray, T, Joardar, A, Giri, S & Sil, AK 2011, 'Isolation of a *Pseudomonas aeruginosa* strain from Soil that can Degrade Polyurethane diol', *Biodegradation*, vol. 22, no. 2, pp. 377-388.

Musson, SE, Jang, Y-C, Townsend, TG & Chung, I-H 2000a, 'Characterization of Lead Leachability from Cathode Ray Rubes using the Toxicity Characteristic Leaching Procedure', *Environmental science & technology*, vol. 34, no. 20, pp. 4376-4381.

Musson, SE, Jang, YC, Townsend, TG & Chung, IH 2000b, 'Characterization of Lead Leachability from Cathode Ray Tubes using the Toxicity Characteristic Leaching Procedure', *Environmental science & technology*, vol. 34, no. 20, pp. 4376-4381.

Natarajan, S, Xu, C, Caperna, TJ & Garrett, WM 2005, 'Comparison of Protein Solubilization Methods Suitable for Proteomic Analysis of Soybean Seed Proteins', *Analytical biochemistry*, vol. 342, no. 2, pp. 214-220.

Nevalainen, KH, Te'o, VS & Bergquist, PL 2005, 'Heterologous Protein Expression in Filamentous Fungi', *TRENDS in Biotechnology*, vol. 23, no. 9, pp. 468-474.

Newman, JR, Ghaemmaghami, S, Ihmels, J, Breslow, DK, Noble, M, DeRisi, JL & Weissman, JS 2006, 'Single-cell Proteomic Analysis of *S. cerevisiae* reveals the Architecture of Biological Noise', *Nature*, vol. 441, no. 7095, pp. 840-846.

NEWMOA 2008, *Northeast Waste Management Officials' Association: IMERC fact sheets on Mercury Uses*, viewed <http://www.newmoa.org/prevention/mercury/imerc/factsheets>.

Nguyen, CH, Tsurumizu, R, Sato, T & Takeuchi, M 2005, 'Taka-amylase A in the Conidia of *Aspergillus oryzae* RIB40', *Bioscience, biotechnology, and biochemistry*, vol. 69, no. 11, pp. 2035-2041.

Nierman, WC, Pain, A, Anderson, MJ, Wortman, JR, Kim, HS, Arroyo, J, Berriman, M, Abe, K, Archer, DB & Bermejo, C 2005, 'Genomic Sequence of the Pathogenic and Allergenic Filamentous fungus *Aspergillus fumigatus*', *Nature*, vol. 438, no. 7071, pp. 1151-1156.

O'Farrell, PH 1975, 'High resolution two-dimensional electrophoresis of proteins', *Journal of biological chemistry*, vol. 250, no. 10, pp. 4007-4021.

Oda, K, Kakizono, D, Yamada, O, Iefuji, H, Akita, O & Iwashita, K 2006, 'Proteomic Analysis of Extracellular Proteins from *Aspergillus oryzae* Grown under Submerged and Solid-state Culture Conditions', *Applied and environmental microbiology*, vol. 72, no. 5, pp. 3448-3457.

OECD 2008, *OECD Environmental Outlook to 2030,* Organisation for Economic Co-operation and Development.

Ogawa, T, Ando, K, Aotani, Y, Shinoda, K, Tanaka, T, Tsukuda, E, Yoshida, M & Matsuda, Y 1995, 'RES-1214-1 and-2, novel non-peptidic endothelin type A receptor antagonists produced by Pestalotiopsis sp', *The Journal of antibiotics*, vol. 48, no. 12, pp. 1401-1406.

Oliveira, CRd, Bernardes, AM & Gerbase, AE 2012, 'Collection and recycling of electronic scrap: A worldwide overview and comparison with the Brazilian situation', *Waste Management*, vol. 32, no. 8, pp. 1592-1610.

Ongondo, FO, Williams, ID & Cherrett, TJ 2011, 'How are WEEE doing? A Global Review of the Management of Electrical and Electronic Wastes', *Waste Management*, vol. 31, no. 4, pp. 714-730.

Onn, ML, Lim, PT, Mujahid, A, Proksch, P & Mueller, M 2013, 'Initial screening of mangrove endophytic fungi for antimicrobial compounds and heavy metal biosorption potential', *Sains Malaysiana* (Manuscript ID: 4470-10402-4-SM), Swinburne University of Technology.

Osako, M, Kim, YJ & Sakai, SI 2004, 'Leaching of Brominated Flame Retardants in Leachate from Landfills in Japan', *Chemosphere*, vol. 57, no. 10, pp. 1571-1579.

Ossefort, ZT & Testroet, P 1966, 'Hydrolytic Stability of Urethan Elastomers', *Rubber Chemistry and Technology*, vol. 39, no. 4, pp. 1308-1327.

Parker, KC, Patterson, D, Williamson, B, Marchese, J, Graber, A, He, F, Jacobson, A, Juhasz, P & Martin, S 2004, 'Depth of Proteome Issues A Yeast Isotope-Coded Affinity Tag Reagent Study', *Molecular & Cellular Proteomics*, vol. 3, no. 7, pp. 625-659.

Pathirana, R & Seal, K 1983, 'Gliocladium roseum (Bainier), A Potential Biodeteriogen of Polyester Polyurethane Elastomers,' *Biodeterioration 5: papers presented at the 5th International Biodeterioration Symposium, Aberdeen, edited by Oxley, TA and Barry, S*, Food and Agriculture Organization of the United Nations (FAO), 679-689.

Pel, HJ, De Winde, JH, Archer, DB, Dyer, PS, Hofmann, G, Schaap, PJ, Turner, G, de Vries, RP, Albang, R & Albermann, K 2007, 'Genome Sequencing and Analysis of the Versatile Cell Factory *Aspergillus niger* CBS 513.88', *Nature biotechnology*, vol. 25, no. 2, pp. 221-231.

Peters-Michaud, N, Katers, J & Barry, J 2003, 'Occupational Risks associated with Electronics Demanufacturing and CRT Glass Processing Operations and the Impact of Mitigation Activities on Employee Safety and Health,' *Electronics and the Environment. IEEE International Symposium*, IEEE, 323-328.

Phua, S, Castillo, E, Anderson, J & Hiltner, A 1987, 'Biodegradation of A Polyurethane in vitro', *Journal of biomedical materials research*, vol. 21, no. 2, pp. 231-246.

Picotti, P, Bodenmiller, B, Mueller, LN, Domon, B & Aebersold, R 2009a, 'Full Ddynamic Range Proteome Analysis of *S. cerevisiae* by Targeted Proteomics', *Cell*, vol. 138, no. 4, pp. 795-806.

Picotti, P, Bodenmiller, B, Mueller, LN, Domon, B & Aebersold, R 2009b, 'Full dynamic range proteome analysis of S. cerevisiae by targeted proteomics', *Cell*, vol. 138, no. 4, pp. 795-806.

Pizarro, F, Varela, C, Martabit, C, Bruno, C, Pérez-Correa, JR & Agosin, E 2007, 'Coupling Kinetic Expressions and Metabolic Networks for Predicting Wine Fermentations', *Biotechnology and bioengineering*, vol. 98, no. 5, pp. 986-998.

Pulici, M, Sugawara, F, Koshino, H, Okada, G, Esumi, Y, Uzawa, J & Yoshida, S 1997, 'Metabolites of Pestalotiopsis spp., endophytic fungi of Taxus brevifolia', *Phytochemistry*, vol. 46, no. 2, pp. 313-319.

Rardin, MJ, Newman, JC, Held, JM, Cusack, MP, Sorensen, DJ, Li, B, Schilling, B, Mooney, SD, Kahn, CR & Verdin, E 2013, 'Label-free quantitative proteomics of the lysine acetylome in mitochondria identifies substrates of SIRT3 in metabolic pathways', *Proceedings of the National Academy of Sciences*, vol. 110, no. 16, pp. 6601-6606.

Righetti, PG, Gianazza, E & Ek, K 1980, 'New developments in isoelectric focusing', *Journal of Chromatography A*, vol. 184, no. 4, pp. 415-456.

Rodrigues Pousada, C, Menezes, RA & Pimentel, C 2010, 'The Yap Family and its Role in Stress Response', *Yeast*, vol. 27, no. 5, pp. 245-258.

Roth, S, Kumme, J & Schüller, H-J 2004, 'Transcriptional Activators Cat8 and Sip4 Discriminate between Sequence Variants of the Carbon Source-responsive Promoter Element in the yeast *Saccharomyces cerevisiae*', *Current genetics*, vol. 45, no. 3, pp. 121-128.

Rowe, L & Howard, GT 2002, 'Growth of Bacillus subtilis on polyurethane and the purification and characterization of a polyurethanase-lipase enzyme', *International Biodeterioration & Biodegradation*, vol. 50, no. 1, pp. 33-40.

Rubin-Bejerano, I, Fraser, I, Grisafi, P & Fink, GR 2003, 'Phagocytosis by Neutrophils Induces An Amino Acid Deprivation Response in *Saccharomyces cerevisiae* and *Candida albicans*', *Proceedings of the National Academy of Sciences*, vol. 100, no. 19, pp. 11007-11012.

Rudolf, A, Baudel, H, Zacchi, G, Hahn Hägerdal, B & Lidén, G 2008, 'Simultaneous Saccharification and Fermentation of Steam Pretreated Bagasse using *Saccharomyces cerevisiae* TMB3400 and *Pichia stipitis* CBS6054', *Biotechnology and bioengineering*, vol. 99, no. 4, pp. 783-790.

Ruijter, GJ & Visser, J 1997, 'Carbon Repression in *Aspergilli*', *FEMS Microbiology Letters*, vol. 151, no. 2, pp. 103-114.

Rupp, S 2004, 'Proteomics on its Way to Study Host–pathogen Interaction in *Candida albicans*', *Current Opinion in Microbiology*, vol. 7, no. 4, pp. 330-335.

Russell, JR, Huang, J, Anand, P, Kucera, K, Sandoval, AG, Dantzler, KW, Hickman, D, Jee, J, Kimovec, FM & Koppstein, D 2011, 'Biodegradation of polyester polyurethane by endophytic fungi', *Applied and environmental microbiology*, vol. 77, no. 17, pp. 6076-6084.

Santerre, J, Labow, R, Duguay, D, Erfle, D & Adams, G 1994, 'Biodegradation Evaluation of Polyether and Polyester Urethanes with Oxidative and Hydrolytic Enzymes', *Journal of biomedical materials research*, vol. 28, no. 10, pp. 1187-1199.

Sarver, A & DeRisi, J 2005, 'Fzf1p Regulates An Inducible Response to Nitrosative Stress in *Saccharomyces cerevisiae*', *Molecular biology of the cell*, vol. 16, no. 10, pp. 4781-4791.

Saunders, JH & Frisch, KC 1962, *Polyurethanes: Chemistry and Technology, Part II: Technology*, New York.

Schlummer, M, Gruber, L, Mäurer, A, Wolz, G & Van Eldik, R 2007, 'Characterisation of Polymer Fractions from Waste Electrical and Electronic Equipment (WEEE) and Iimplications for Waste Management', *Chemosphere*, vol. 67, no. 9, pp. 1866-1876.

Schmidt, A, Claassen, M & Aebersold, R 2009, 'Directed Mass Spectrometry: Towards Hypothesis-driven Proteomics', *Current opinion in chemical biology*, vol. 13, no. 5, pp. 510-517.

Schmitt, S, Prokisch, H, Schlunck, T, Camp, DG, Ahting, U, Waizenegger, T, Scharfe, C, Meitinger, T, Imhof, A & Neupert, W 2006, 'Proteome Analysis of Mitochondrial Outer Membrane from *Neurospora crassa*', *Proteomics*, vol. 6, no. 1, pp. 72-80.

Schoch, CL, Seifert, KA, Huhndorf, S, Robert, V, Spouge, JL, Levesque, CA, Chen, W, Bolchacova, E, Voigt, K & Crous, PW 2012, 'Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi', *Proceedings of the National Academy of Sciences*, vol. 109, no. 16, pp. 6241-6246.

Schwartz, BS & Hu, H 2007, 'Adult Lead Exposure: Time for Change', *Environmental Health Perspectives*, pp. 451-454.

Sellam, A, Tebbji, F, Whiteway, M & Nantel, A 2012, 'A Novel Role for the Transcription Factor Cwt1p As A Negative Regulator of Nitrosative Stress in *Candida albicans*', *PloS one*, vol. 7, no. 8, p. e43956.

Senior, K 1999, 'Fingerprinting Disease with Protein Chip Arrays', *Molecular medicine today*, vol. 5, no. 8, pp. 326-327.

Shah, AA, Hasan, F, Akhter, JI, Hameed, A & Ahmed, S 2008, 'Degradation of polyurethane by novel bacterial consortium isolated from soil', *Annals of Microbiology*, vol. 58, no. 3, pp. 381-386.

Shevchenko, A, Jensen, ON, Podtelejnikov, AV, Sagliocco, F, Wilm, M, Vorm, O, Mortensen, P, Shevchenko, A, Boucherie, H & Mann, M 1996, 'Linking Genome and Proteome by Mass Spectrometry: Large-scale Identification of yeast Proteins from Two Dimensional Gels', *Proceedings of the National Academy of Sciences*, vol. 93, no. 25, pp. 14440-14445.

Shi, T, Chen, SJ, Luo, XJ, Zhang, XL, Tang, CM, Luo, Y, Ma, YJ, Wu, JP, Peng, X-Z & Mai, B-X 2009, 'Occurrence of Brominated Flame Retardants other than Polybrominated Diphenyl Ethers In Environmental and Biota Samples from Southern China', *Chemosphere*, vol. 74, no. 7, pp. 910-916.

Singh, S, Lee, W, DaSilva, NA, Mulchandani, A & Chen, W 2008, 'Enhanced Arsenic Accumulation by Engineered Yeast Cells Expressing *Arabidopsis thaliana* Phytochelatin Synthase', *Biotechnology and bioengineering*, vol. 99, no. 2, pp. 333-340.

Skarja, G & Woodhouse, K 1998, 'Synthesis and characterization of degradable polyurethane elastomers containing an amino acid-based chain extender', *Journal of Biomaterials Science, Polymer Edition*, vol. 9, no. 3, pp. 271-295.

Srivastava, N, Kamal, B, Sharma, V, Negi, YK, Dobriyal, A, Gupta, S & Jadon, V 2010, 'Standardization of sterilization protocol for micropropagation of Aconitum heterophyllum-An endangered medicinal herb', *Academic Arena*, vol. 2, no. 6, pp. 37-42.

Strobel, G, Ford, E, Worapong, J, Harper, JK, Arif, AM, Grant, DM, Fung, PC & Chau, RMW 2002, 'Isopestacin, an isobenzofuranone from Pestalotiopsis microspora, possessing antifungal and antioxidant activities', *Phytochemistry*, vol. 60, no. 2, pp. 179-183.

Strobel, G, Yang, X, Sears, J, Kramer, R, Sidhu, RS & Hess, W 1996, 'Taxol from Pestalotiopsis microspora, an endophytic fungus of Taxus wallachiana', *Microbiology*, vol. 142, no. 2, pp. 435-440.

Suárez, MB, Sanz, L, Chamorro, MI, Rey, M, González, FJ, Llobell, A & Monte, E 2005, 'Proteomic Analysis of Secreted Proteins from *Trichoderma harzianum*: Identification of A Fungal Cell wall-induced Aspartic Protease', *Fungal Genetics and Biology*, vol. 42, no. 11, pp. 924-934.

Sun, N, Jang, J, Lee, S, Kim, S, Lee, S, Hoe, KL, Chung, KS, Kim, DU, Yoo, HS & Won, M 2005, 'The First Two Dimensional Reference Map of the Fission Yeast, *Schizosaccharomyces pombe* Proteins', *Proteomics*, vol. 5, no. 6, pp. 1574-1579.

Takahashi, S, Yeo, Y, Greenhagen, BT, McMullin, T, Song, L, Maurina Brunker, J, Rosson, R, Noel, JP & Chappell, J 2007, 'Metabolic Engineering of Sesquiterpene Metabolism in Yeast', *Biotechnology and bioengineering*, vol. 97, no. 1, pp. 170-181.

Tamura, K, Stecher, G, Peterson, D, Filipski, A & Kumar, S 2013, 'MEGA6: molecular evolutionary genetics analysis version 6.0', *Molecular biology and evolution*, vol. 30, no. 12, pp. 2725-2729.

Tan, KC, Ipcho, SV, Trengove, RD, Oliver, RP & Solomon, PS 2009, 'Assessing The Impact of Transcriptomics, Proteomics and Metabolomics On Fungal Phytopathology', *Molecular Plant Pathology*, vol. 10, no. 5, pp. 703-715.

Taylor, D, Booth, MG, McFarland, JW, Herriott, IC, Lennon, NJ, Nusbaum, C & Marr, TG 2008, 'Increasing ecological inference from high throughput sequencing of fungi in the environment through a tagging approach', *Molecular Ecology Resources*, vol. 8, no. 4, pp. 742-752.

Tejesvi, MV, Kini, KR, Prakash, HS, Subbiah, V & Shetty, HS 2008, 'Antioxidant, Antihypertensive, and Antibacterial Properties of Endophytic *Pestalotiopsis* species from Medicinal Plants', *Canadian journal of microbiology*, vol. 54, no. 9, pp. 769-780.

ter Schure, EG, van Riel, NA & Verrips, CT 2000, 'The Role of Ammonia Metabolism in Nitrogen Catabolite Repression in *Saccharomyces cerevisiae*', *FEMS microbiology reviews*, vol. 24, no. 1, pp. 67-83.

Thomas, DP, Bachmann, SP & Lopez Ribot, JL 2006, 'Proteomics for the Analysis of the *Candida albicans* Biofilm Lifestyle', *Proteomics*, vol. 6, no. 21, pp. 5795-5804.

Thomas, DP, Viudes, A, Monteagudo, C, Lazzell, AL, Saville, SP & López Ribot, JL 2006, 'A Proteomic Based Approach for the Identification of *Candida albicans* Protein Components present in a Subunit Vaccine that Protects against Disseminated Candidiasis', *Proteomics*, vol. 6, no. 22, pp. 6033-6041.

Todd, RB, Fraser, JA, Wong, KH, Davis, MA & Hynes, MJ 2005, 'Nuclear Accumulation of the GATA factor AreA in Response to Complete Nitrogen Starvation by Regulation of Nuclear Export', *Eukaryotic cell*, vol. 4, no. 10, pp. 1646-1653.

Tsuchiya, K, Tada, S, Gomi, K, Kumagai, C, Jigami, Y & Tamura, G 1992, 'High Level Expression of the Synthetic Human Lysozyme Gene in *Aspergillus oryzae*', *Applied Microbiology and Biotechnology*, vol. 38, no. 1, pp. 109-114.

Tsydenova, O & Bengtsson, M 2011, 'Chemical hazards associated with treatment of waste electrical and electronic equipment', *Waste Management*, vol. 31, no. 1, pp. 45-58.

Uhlig, KK & Conrad, AJ 1999, *Discovering Polyurethanes,* Hanser, Munich, Germany.

Ulrich, H 1983, 'Polyurethanes', *Modern Plastics*, vol. 60, pp. 76-84.

UNEP 2006, *Call for Global Action on E-waste*, Kenya.

Van de Laar, T, Visser, C, Holster, M, Lopez, CG, Kreuning, D, Sierkstra, L, Lindner, N & Verrips, T 2007, 'Increased Heterologous Protein Production by *Saccharomyces cerevisiae* Growing on Ethanol As Sole Carbon Source', *Biotechnology and bioengineering*, vol. 96, no. 3, pp. 483-494.

van den Berg, MA, Westerlaken, I, Leeflang, C, Kerkman, R & Bovenberg, RA 2007, 'Functional Characterization of the Penicillin Biosynthetic Gene Cluster of *Penicillium chrysogenum* Wisconsin 54-1255', *Fungal Genetics and Biology*, vol. 44, no. 9, pp. 830-844.

Walter, G, Büssow, K, Cahill, D, Lueking, A & Lehrach, H 2000, 'Protein Arrays for Gene Expression and Molecular Interaction Screening', *Current Opinion in Microbiology*, vol. 3, no. 3, pp. 298-302.

Wang, J & Guo, X 2006, 'Impact of Electronic Wastes Recycling on Environmental Quality', *Biomedical and Environmental Sciences*, vol. 19, no. 2, p. 137.

Wang, W, Scali, M, Vignani, R, Spadafora, A, Sensi, E, Mazzuca, S & Cresti, M 2003, 'Protein extraction for two dimensional electrophoresis from olive leaf, a plant tissue containing high levels of interfering compounds', *Electrophoresis*, vol. 24, no. 14, pp. 2369-2375.

Wang, Y, Xue, W, Sims, AH, Zhao, C, Wang, A, Tang, G, Qin, J & Wang, H 2008, 'Isolation of Four Pepsin-like Protease Genes from *Aspergillus niger* and Analysis of the Effect of Disruptions on Heterologous Laccase Expression', *Fungal Genetics and Biology*, vol. 45, no. 1, pp. 17-27.

Watanabe, K, Motohashi, K & Ono, Y 2010, 'Description of *Pestalotiopsis pallidotheae*: A New species from Japan', *Mycoscience*, vol. 51, no. 3, pp. 182-188.

Weeks, ME, Sinclair, J, Butt, A, Chung, YL, Worthington, JL, Wilkinson, CR, Griffiths, J, Jones, N, Waterfield, MD & Timms, JF 2006, 'A Parallel Proteomic and Metabolomic Analysis of the Hydrogen Peroxide and Sty1p-dependent Stress Response in *Schizosaccharomyces pombe*', *Proteomics*, vol. 6, no. 9, pp. 2772-2796.

Wei, JG, Xu, T, Guo, LD, Liu, AR, Zhang, Y & Pan, XH 2007, 'Endophytic *Pestalotiopsis* species associated with Plants of *Podocarpaceae*, *Theaceae* and *Taxaceae* in southern China', *Fungal Divers*, vol. 24, no. 1, pp. 55-74.

Wen, S, Yang, FX, Gong, Y, Zhang, XL, Hui, Y, Li, JG, Liu, AL, Wu, YN, Lu, WQ & Xu, Y 2008, 'Elevated Levels of Urinary 8-hydroxy-2′-deoxyguanosine in Male Electrical and Electronic Equipment Dismantling Workers Exposed to High Concentrations of Polychlorinated Dibenzo-p-dioxins and Dibenzofurans, Polybrominated Diphenyl ethers, and Polychlorinated biphenyls', *Environmental science & technology*, vol. 42, no. 11, pp. 4202-4207.

Westergaard, SL, Oliveira, AP, Bro, C, Olsson, L & Nielsen, J 2007, 'A Systems Biology Approach to Study Glucose Repression in the Yeast *Saccharomyces cerevisiae*', *Biotechnology and bioengineering*, vol. 96, no. 1, pp. 134-145.

Widmer, R, Oswald-Krapf, H, Sinha-Khetriwal, D, Schnellmann, M & Böni, H 2005, 'Global Perspectives on E-waste', *Environmental Impact Assessment Review*, vol. 25, no. 5, pp. 436-458.

Wiese, S, Reidegeld, KA, Meyer, HE & Warscheid, B 2007, 'Protein Labeling by iTRAQ: A New Tool for Quantitative Mass Spectrometry in Proteome Research', *Proteomics*, vol. 7, no. 3, pp. 340-350.

Wilkins, MR, Sanchez, J-C, Gooley, AA, Appel, RD, Humphery-Smith, I, Hochstrasser, DF & Williams, KL 1996, 'Progress with Proteome Projects: Why All Proteins Expressed by a Genome Should be Identified and How To Do It', *Biotechnology and genetic engineering reviews*, vol. 13, no. 1, pp. 19-50.

Wilson, RA & Arst, HN 1998, 'Mutational Analysis of AREA, A Transcriptional Activator Mediating Nitrogen Metabolite Repression in *Aspergillus nidulans* and A Member of the “streetwise” GATA Family of Transcription Factors', *Microbiology and molecular biology reviews*, vol. 62, no. 3, pp. 586-596.

Wirsel, SG, Leibinger, W, Ernst, M & Mendgen, K 2001, 'Genetic diversity of fungi closely associated with common reed', *New Phytologist*, vol. 149, no. 3, pp. 589-598.

Wolters, DA, Washburn, MP & Yates, JR 2001, 'An automated multidimensional protein identification technology for shotgun proteomics', *Analytical chemistry*, vol. 73, no. 23, pp. 5683-5690.

Wong, C, Tan, D, Lihan, S, Mujahid, A & Mueller, M 2015, 'Biosorption of copper (Cu) by endophytic fungi isolated from *Nepenthes ampullaria*', *Applied Microbiology and Biotechnology* (Manuscript ID: AMAB-D-15-01134), Swinburne University of Technology.

Woo, G, Mittelman, M & Santerre, J 2000, 'Synthesis and characterization of a novel biodegradable antimicrobial polymer', *Biomaterials*, vol. 21, no. 12, pp. 1235-1246.

Wu, CG, Tseng, HY & Chen, ZC 1982, 'Fungi Inhabiting on *Schoenpplectus triqueter* (L.) *Palla* (I)', *Taiwania*, vol. 27, no. 1, pp. 35-38.

Wu, JP, Luo, XJ, Zhang, Y, Luo, Y, Chen, SJ, Mai, BX & Yang, ZY 2008, 'Bioaccumulation of Polybrominated Diphenyl Ethers (PBDEs) and Polychlorinated Biphenyls (PCBs) in Wild Aquatic Species from An Electronic Waste (E-waste) Recycling Site in South China', *Environment International*, vol. 34, no. 8, pp. 1109-1113.

Wu, X, Xiong, E, Wang, W, Scali, M & Cresti, M 2014, 'Universal sample preparation method integrating trichloroacetic acid/acetone precipitation with phenol extraction for crop proteomic analysis', *Nat. Protocols*, vol. 9, no. 2, pp. 362-374.

Yajima, W & Kav, NN 2006, 'The Proteome of the Phytopathogenic Fungus *Sclerotinia sclerotiorum*', *Proteomics*, vol. 6, no. 22, pp. 5995-6007.

Yan, G & Viraraghavan, T 2003, 'Heavy-metal Removal from Aqueous Solution by fungus *Mucor rouxii*', *Water Research*, vol. 37, no. 18, pp. 4486-4496.

Yanna, Ho, W & Hyde, K 2002, 'Fungal Succession on Fronds of *Phoenix hanceana* in Hong Kong', *Fungal Diversity*, vol. 10, pp. 185-211.

Yuan, J, Chen, L, Chen, D, Guo, H, Bi, X, Ju, Y, Jiang, P, Shi, J, Yu, Z & Yang, J 2008, 'Elevated Serum Polybrominated Diphenyl Ethers and Thyroid-stimulating Hormone Associated with Lymphocytic Micronuclei in Chinese Workers from An E-waste Dismantling Site', *Environmental science & technology*, vol. 42, no. 6, pp. 2195-2200.

Zhang, Y, Zhang, S, Liu, X, Wen, H & Wang, M 2010, 'A Simple Method of Genomic DNA Extraction Suitable for Analysis of Bulk Fungal Strains', *Letters in applied microbiology*, vol. 51, no. 1, pp. 114-118.
Zhang, Z, Schwartz, S, Wagner, L & Miller, W 2000, 'A greedy algorithm for aligning DNA sequences', *Journal of Computational biology*, vol. 7, no. 1-2, pp. 203-214.

Zhao, XM, Zhang, XW, Tang, WH & Chen, L 2009, 'FPPI: *Fusarium graminearum* Protein−Protein Interaction Database', *Journal of proteome research*, vol. 8, no. 10, pp. 4714-4721.

Zheng, L, Wu, K, Li, Y, Qi, Z, Han, D, Zhang, B, Gu, C, Chen, G, Liu, J & Chen, S 2008, 'Blood Lead and Cadmium Levels and Relevant Factors among Children from An E-waste Recycling Town in China', *Environmental Research*, vol. 108, no. 1, pp. 15-20.

Zhu, LY, Nguyen, CH, Sato, T & Takeuchi, M 2004, 'Analysis of Secreted Proteins during Conidial Germination of *Aspergillus oryzae* RIB40', *Bioscience, biotechnology, and biochemistry*, vol. 68, no. 12, pp. 2607-2612.

Zichi, D, Eaton, B, Singer, B & Gold, L 2008, 'Proteomics and diagnostics: Let's Get Specific, again', *Current opinion in chemical biology*, vol. 12, no. 1, pp. 78-85.