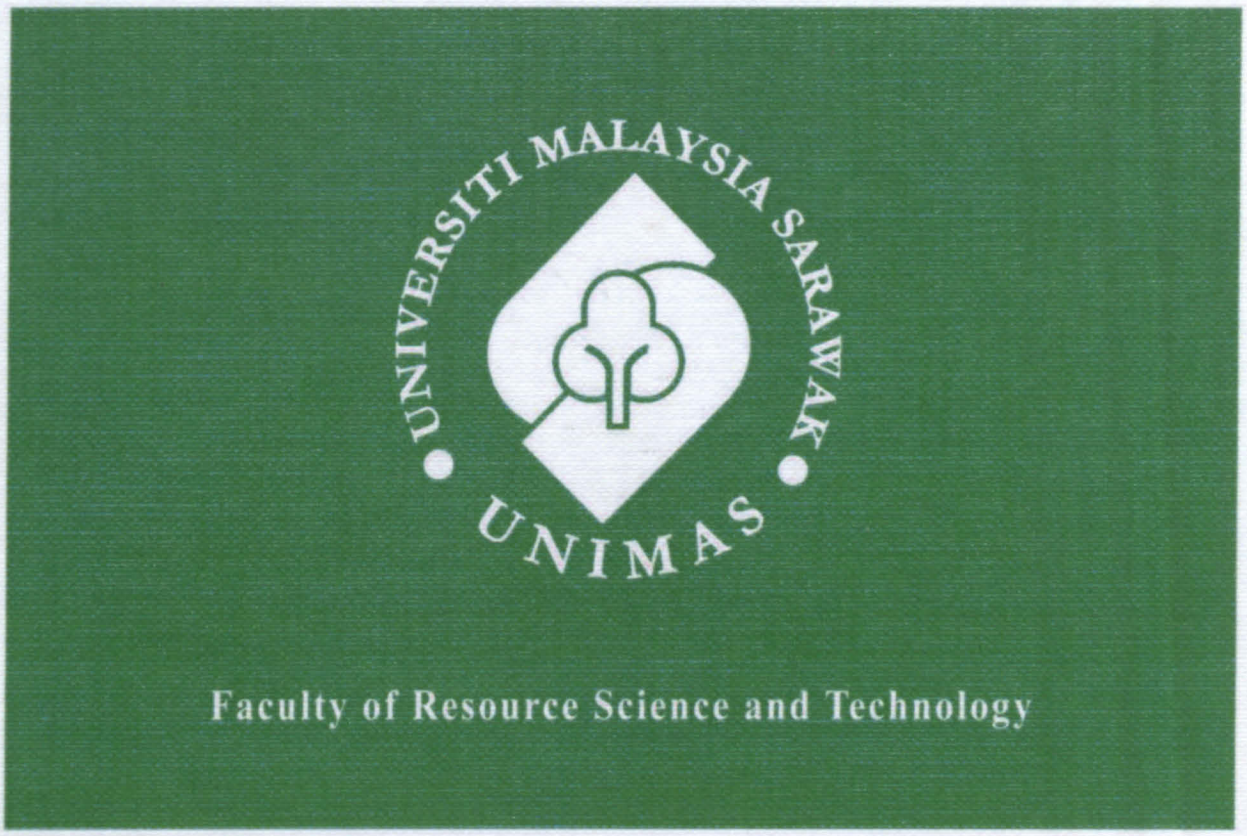


# Production of Amylase from *Aspergillus niger* under Solid State Fermentation (SSF) with different Agro Waste as the Substrates

**PAVITHRA MADHEUAN (54975)**

**Bachelor of Science with Honours**
**(Resource Biotechnology)**
**2018**

Borang PTA4

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Name of the student (Matric No.)

**Supervisor's Declaration:**

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**Production of Amylase from *Aspergillus niger* under Solid State Fermentation (SSF) with Different Agro Waste as the Substrates**

**PAVITHRA MADHEUAN (54975)**

This project is submitted in fulfilment of the Final Year Project (STF3015)

(Resource Biotechnology)

Supervisor: Miss Rosmawati Binti Saat

Resource Biotechnology

Faculty of Resource Science and Technology

University Malaysia Sarawak

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Thank you.

**Declaration**

I hereby declare that the study entitled " Production of Amylase from *Aspergillus niger* under Solid State Fermentation (SSF) with different Agro Waste as the Substrates "submitted to the Faculty of Resource Science and Technology, University Malaysia Sarawak(UNIMAS) is my original work. All sources that I have quoted and cited have been acknowledged to the authors who own the original work of the papers. Acknowledging and giving credits to the owners have been made by means of complete references in this paper.

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## List of Abbreviations

| | |
|---|---|
| *A. niger* | *Aspergillus niger* |
| DSNA | 3,5-dinitrosalicylic acid |
| PDA | Potato dextrose agar |
| SSF | Solid state fermentation |
| rpm | Revolution per minute |
| g | gram |
| kg | kilogram |
| mL | milligram |
| µg | microgram |
| ml | milliliter |
| nm | nanometer |
| cm | centimeter |
| w/v | Weight per Volume |
| v/v | Volume per volume |
| min | Minute |
| °C | Degree Celcius |
| pH | A measurement of the acidity or alkalinity of solution |

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**Production of Amylase from *Aspergillus niger* under Solid State Fermentation (SSF) with Different Agro Waste as the Substrates**

**Pavithra Madheuan**

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## Abstract

Agricultural wastes are produced by agricultural operations for instances are from forestry, farming, and agro-industry. Accumulation of the wastes from day to day gives impacts to human and environment. In this research, utilisation of agro-wastes which were potato peels, sweet potato peels and banana peels was done to extract amylase from *Aspergillus niger* by solid-state fermentation. The solid-state fermentation optimization of parameters such as incubation period, initial moisture content of substrate and incubation temperature that affecting solid-state fermentation to produce maximum amylase production in term of enzyme activity were studied. Enzyme activity was determined via Dinitrosalicylic acid (DNS) method. Among of these agricultural wastes used, the best agricultural waste to produce the highest enzyme activity was potato peel with its optimal condition of 6 incubation days, 70% moisture content and 35°C with activity of 0.148 U.

**Key words:** amylase, agro-wastes, *Aspergillus niger*, solid-state fermentation

## *Abstrak*

*Sisa-sisa pertanian dihasilkan dari operasi-operasi pertanian seperti perhutanan, pertanian, dan agroindustri. Pengumpulan sisa dari hari ke hari memberi impak kepada manusia dan alam sekitar. Dalam kajian ini, penggunaan sisa agro seperti kulit kentang, kulit ubi keledek dan kulit pisang telah diproseskan untuk mengeluarkan amilase daripada* Aspergillus niger *melalui fermentasi keadaan pepejal. Parameter fermentasi keadaan pepejal seperti tempoh inkubasi, kandungan kelembapan awal substrat dan suhu inkubasi yang mempengaruhi fermnetasi keadaan pepejal untuk menghasilkan enzim amylase yang maksimum dari segi aktiviti enzim dikaji. Aktiviti enzim ditentukan melalui kaedah Asid Dinitro-salicyclic. Antara sisa pertanian yang digunakan, sisa pertanian yang terbaik untuk menghasilkan aktiviti enzim tertinggi ialah kulit kentang dengan keadaan optimum 6 hari tempoh inkubasi, kandungan kelembapan sebanyak 70% dan pada suhu 35 ° C.*

**Kata kunci**: *amilase, sisa pertanian,* Aspergillus niger, *fermentasi keadaan pepeja*

## Chapter 1: Introduction

The continued development of bio-sustainable and renewable resource technology is of great importance with respect to the environmental concerns. The bioconversion of lignocelluloses, natural enzymes and anthropogenic, in the production of biofuels is an extremely important part of renewable resource technology. Therefore, the significant and progressive development in industrial biotechnology such as enzyme technology has leads to an immense utilization of microbial enzymes in various applications (Aziz, 2002). A large amount of agro-waste generated every year in all over the world contained high lignocelluloses and starch content. These agro-waste that are abundant can be treated microbiologically and used as substrates to produce important industrial enzymes such as amylase, cellulase, xylanase, pectinase, lipase and others.

In recent years, there has been an increasing interest towards more efficient utilization of different agro residues, including sugarcane bagasse, wheat bran, wheat straw, rye straw and corncob leaf, oil cakes, potato peels and banana peels for amylases production (Balkan & Ertan, 2007). Agro wastes have been reported to be good substrates for the cost-effective production of amylases and are thus attracting researchers for using agro industrial waste as a substrate for amylase production (Kumar *et al.*, 2011). Microorganisms such as fungi, yeast and bacteria are very suitable for the large production of amylase due to their capability for massive production capacity and to manipulate microbes to acquire enzymes of desired characteristics (Kathiresan & Manivannan, 2006). Amylase are important enzymes employed in starch processing industries for hydrolysis of polysaccharides such as starch into simple sugar constituents (Akpan *et al.*, 2001). Amylases are hydrolytic enzymes that are of useful applications in the brewing, textile, detergent and pharmaceutical industries (Asghar *et al.*, 2000). Although amylases have been reported to be produced by various

sources such as plant, animal and microbial sources, most of the reports showed that microbial amylase production has been the most effective one. Amylase produced from the fungal cultures was found to be more stable than that produced by bacteria on a commercial scale.

Many suitable strains of fungi have been optimized for their culture conditions (Ghosh & Chandra, 1984). Fungal like *Aspergillus niger* can produce high amounts of amylase by as *Aspergillus species* are starch-degrading fungi. Studies on fungal amylases especially in developing countries have concentrated mainly on *Aspergillus niger*, probably because of their ubiquitous nature and non-fastidious nutritional requirements of these organisms (Abu *et al.*, 2005). The *Aspergillus niger* produce a large variety of extracellular enzymes of which amylases are of world-wide interest in fermentation, food, pharmaceutical, textile and paper industries (Bhargav *et al.*, 2008). The *Aspergillus species* incorporate the ability to infiltrate solid substrates with their hyphae and to extract extracellular enzymes, where they able to break down macromolecules like carbohydrates, lipids, nucleic acids, polypeptides and others (Gow & Gadd, 1995). Other than that, studies also proven that microbial amylases from fungi like *Aspergillus niger* can entirely substitute chemical hydrolysis of starch in starch processing industry (Kathiresan & Manivanan, 2006). The filamentous fungal like *Aspergillus niger* have several advantages via solid state fermentation system which provide natural condition to microorganism. In the solid-state fermentation, the fungal system is the obvious choice compared to bacterial, as the system required solid support matrices for their growth under the natural circumstances (Pandey *et al.*, 1999).

Solid State Fermentation holds tremendous potentials to produce enzymes. The free water is indispensable to the microorganism's growth and is adsorbed on a solid support or complexed into the interior of a solid matrix (Soccol, 1992). Solid state fermentation method has economic value for countries with abundance of biomass and agro industrial residues, as

these can be used as cheap substrates (Tunga, 2003). Solid state fermentation (SSF) has been reported to be cheaper because of the simple enzyme extraction procedures (Kumar & Duhan, 2011). Agro industrial wastes have been reported to be good substrates for the cost-effective production of alpha amylases and are thus, attracting researchers for using agro industrial waste as a substrate for amylase production (Kumar *et al.,* 2011). Fungal species have been studied a lot to produce amylase because of the cheap substrates used to produce amylases (Adeniran & Abiose, 2009). The *Aspergillus niger* can convert starch in agro waste source and it is presumed to be able to be utilize agro waste as well. The capability of converting these raw materials from agro waste as potential bioconversion of lignocelluloses, natural enzymes and anthropogenic. The use of agro-industrial residues input also beneficial in terms of lower cost, promote lower energy requirement, produce less wastewater and are environmentally friendly (Manpreet *et al.,* 2005).

In this study, the *Aspergillus niger* used for amylase enzyme production in solid state fermentation by utilizing agro wastes such as sweet potato peels, potato peels and banana peels. These agro waste is applied as the substrate to supply nutrients and to serve as anchor for the *Aspergillus niger*. The experiment conducted to determine the effectiveness of utilizing *Aspergillus niger* on the agro waste in process of amylase production. Thus, the experiment also conducted to know which environment in term incubation periods, temperature and initial moisture content will be suitable for maximize the production of amylase. Thus, the objectives of this study are as follows:

1. To investigate the potential of *Aspergillus niger* to produce amylase using potato peels, sweet potato peels and banana peels as the SSF substrate.

2. To determine the best solid-state fermentation substrate for *Aspergillus niger* in producing amylase.

3. To determine the optimal solid-state fermentation conditions for *Aspergillus niger* to produce amylase.

## Chapter 2: Literature Review

### 2.1 Fungi

There are four major groups of microorganisms which are bacteria, protozoa, viruses and fungi (Sharma, 2006). Fungi are defined as a group of microorganisms that classified within their own kingdom, the Fungal Kingdom. In the Fungal Kingdom, there are four main types which are from the phyla *Basidiomycota*, phyla *Ascomycota*, phyla *Chytridiomycota* and phyla *Zygomycota* (Blackwell *et al.*, 2008). There is also other type of fungi that can be considered such as *Mxomycota, Dictyostelomycota, Axrasiomycota*, *Plasmodiopholomycota* and the straminopila phyla, *Oomycota*, *Labyrinthulomycota* and *Hypochytriomycota* (Blackwell *et al.*, 2008). It is estimated that, there will be 1.5 million species of fungi in the world (Hawksworth, 1991). Other types of fungi that can be discovered are mushrooms, rusts, smuts, puffballs, truffles, morels, moulds, yeasts and some other less well-known organisms (Blackwell *et al.*, 2008).

Fungi are heterotrophic microorganism naturally, the heterotrophic nutrition are utilised by fungi through extracellular sources of organic energy for maintain the growth and reproduction of the fungi (Tunga, 2003). The heterotrophic characteristics of fungi enable the fungi to play several vital roles in the ecosystem like saprotrophs, parasites of plants and animals, as mutualists symbionts of many phototrophic organism (as algae in the form of lichen) and as the mycorrhizal partners of vascular plants (Dix & Webster, 1995). Fungi could be plant or animal, they could not able to photosynthesizes as they lack the green pigment chlorophyll present in most green plants (Dix & Webster, 1995). Other than that, fungi are able to exist in wide range of habitats such as in fresh water and sea, in soil, litter, decaying remains of plants and animals and in living plants and animals (Dix & Webster,

1995). Fungi contain chitinous cell walls and exhibited filamentous growth as multicellular hyphae forming a mycelium.

Fungi are eukaryotic, multicellular with indeterminate growth, non-vascular and a life cycle with sexual and asexual reproduce, it contains thallus (haploid thalli resulting from zygotic meiosis and heterotrophic nutrition) (Bowman & Free, 2006). Dissimilar to plants, fungi do not contain chlorophyll pigments, therefore it unable to perform photosynthesis and most fungi are found grown on land and obtains nutrients from dead organic matter (Fungi, 2008). Likewise, most fungi species feed by secreting enzymes which partially breakdown the food. Fungi also known as decomposer of soil food web by which they convert hard-digesting organic materials into forms that other organisms can uses (Blackwell, *et al.*, 2008). Thus, fungi play an important key role in producing enzymes came into action through the natural synthesizing characteristics of fungi and there are a lot of fungi species have been used as a direct source of food such as mushroom and truffles and in fermentation of various food products such as wine, beer and soy sauce (Hussain *et al.*, 2013). Fungi also can be used in production of antibiotics and enzymes such as amylase, cellulase, pectinase, proteases and other enzymes which beneficial for industrial use or as detergents.

## 2.2 Starch degrading fungi

The *Aspergillus* species fungus was first recognized as an organism in 1729 by Micheli (Ward *et al.*, 2006). Genus *Apergillus* are found globally and consists of more than 180 well-known recognized species *such Aspergillus niger, Aspergillus oryzae, Aspergillus flavus, Aspergillus nomius* and others, where it comprises important group of filamentous ascomycete species. The *Aspergillus* fungus can be found in soil, plant debris and wood in circulating air as they are highly flexible and produce in high numbers. *Aspergillus* are typed as mould usually, moulds are filamentous fungi that grow in the form of tangled mass the spreads quickly (Lal, 2008). The total mass of any tangled mass referred to as mycelium, the mycelium is consisting of branches and filamentous which known as hyphae. Aspergillus forms septate hyphae and produces asexual spores which are black in colour on the conidia and known as xerophilic as it has ability to grow in low water content areas. For example, *Aspergillus flavus* which is an aflatoxin fungus produces mycotoxins (Lal, 2008). The spores produce by *Aspergillus* species known to be dry form and easily carried in the air by wind. The taxonomical classification of *Aspergillus* is stated in the **Table 1** below:

**Table 1**: Taxonomical classification of *Aspergillus* species (Guarro *et al.*, 1999).

| | |
|---|---|
| Kingdom | Fungi |
| Division | Ascomycota |
| Class | Hyphomycetes |
| Order | Hyphomycetales |
| Genus | *Aspergillus* |

*Aspergillus* species normally used in the production of industrially valuable enzymes such as amylase, cellulase, xylanase, pectinase and other products (Ward *et al.*, 2006). Other than that, the Aspergillus fungus also play an important role in fermentation of hydrolytic extracellular enzymes through process like Solid State Fermentation and Submerged Liquid Fermentation which used in the development of industries such as food, brewing and pharmaceuticals (Kariya *et al.*, 2003). A morphological structure of *Aspergillus* species along with mycelium are shown in **Figure 1**.

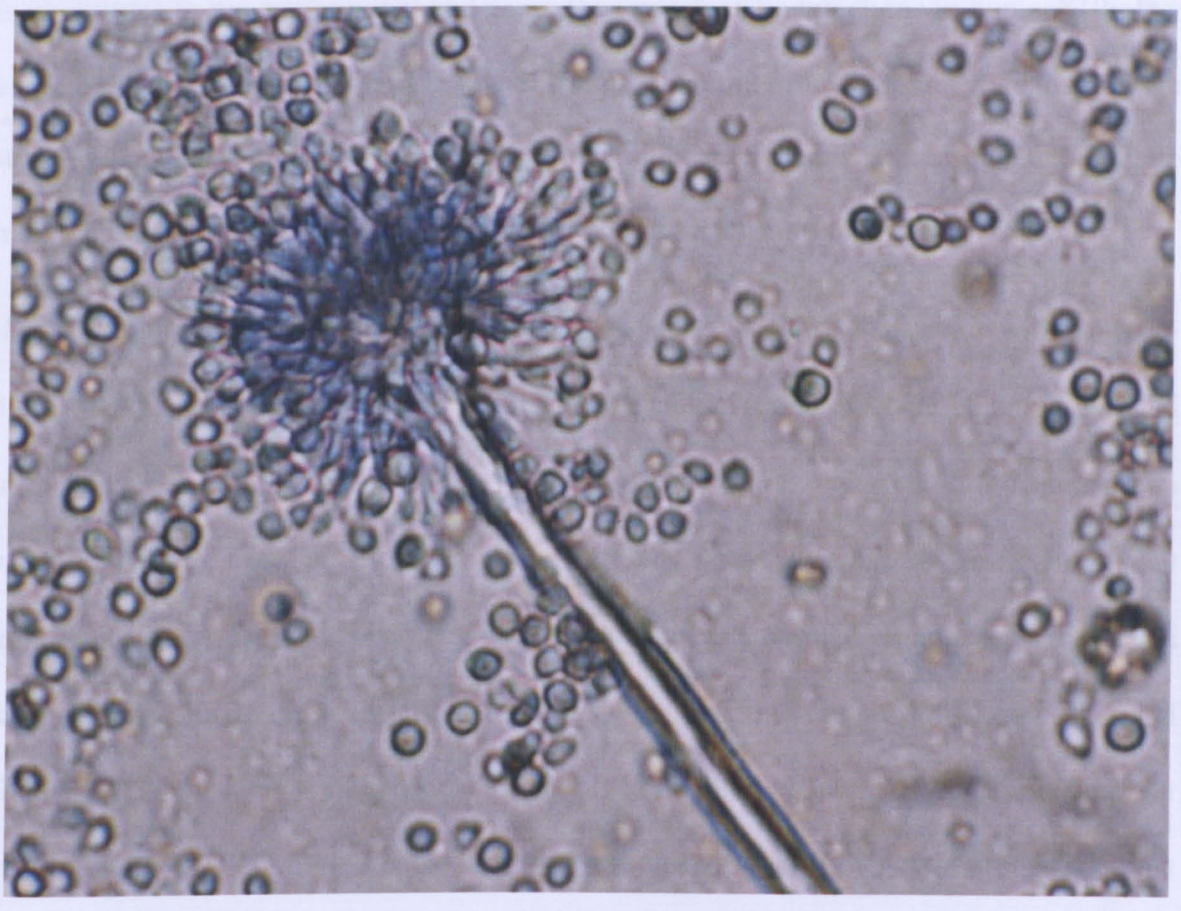

**Figure 1**: Electron photography of *Aspergillus* species. Adapted from https://www.pinterest.com/pin/438678819923978061/

### 2.2.1 *Aspergillus niger*

*Aspergillus niger* is from the genus *Aspergillus* which includes a set of fungi that are asexual, although perfect forms (forms that reproduce sexually) have been found, it is a filamentous fungus and produces brown to black-shaded conidiophores (Souza *et al.*, 2011). The colour is classed under *Nigri* section and called as black fungus where it belongs to ascomycetes (Schuster *et al.*, 2002). *Aspergillus niger* normally found in mesophilic environment such as soil, plants and enclosed air. *Aspergillus niger* is commonly found as a saprophyte growing on decaying vegetation and it is a filamentous ascomycete fungus that is ubiquitous in the environment and cause the opportunistic infections in humans (Prefect *et al.*, 2001). *Aspergillus niger* is known as xerophilic fungi which typed as mold that growth without water consumption yet able to thrive in humid environments. It also able to tolerate high temperature like 47 °C as it is thermos-tolerant organism (Okafor *et al.*, 2007).

*Aspergillus niger* is an aerobic fungus where it can survive in the temperature within 6 °C to 47 °C and the optimum temperature for its growth is between 35 °C to 37 °C along with pH in the range of 1.4 to 9.8 (Schuster *et al.*, 2002). *Aspergillus niger* can live in maximum water activity of 0.88 $a_w$ (Schuster *et al.*, 2002). *Aspergillus niger* also economically important as a fermentation organism used to produce enzymes and organic acids (de Souza & Magalhaes, 2010). From a study, *Aspergillus niger* genomes consists of 14,600 genes and about 1.37% of the genes (200 genes) are involved in polysaccharide degradation (Souza *et al.*, 2011). *Aspergillus niger* is considered and recognised as safe (GRAS) to produce various type of enzyme by United States Food and Drug (Motta *et al.*, 2013). It is used in the production of industrial enzymes such as amylase, protease, lipase and cellulase (Souza *et al.*, 2011). Fungus like *Aspergillus niger* released high level of extracellular enzymes compare to bacteria.

Due to the fact that, *Aspergillus* niger produce enzymes have the advantage of being secreted extracellularly and ability to penetrate hard substrates facilitates the hydrolysis process and are highly suitable for solid state fermentation (Schuster *et al.*, 2002). A morphological structure of *Aspergillus niger* with mycelium are shown in **Figure 2**.

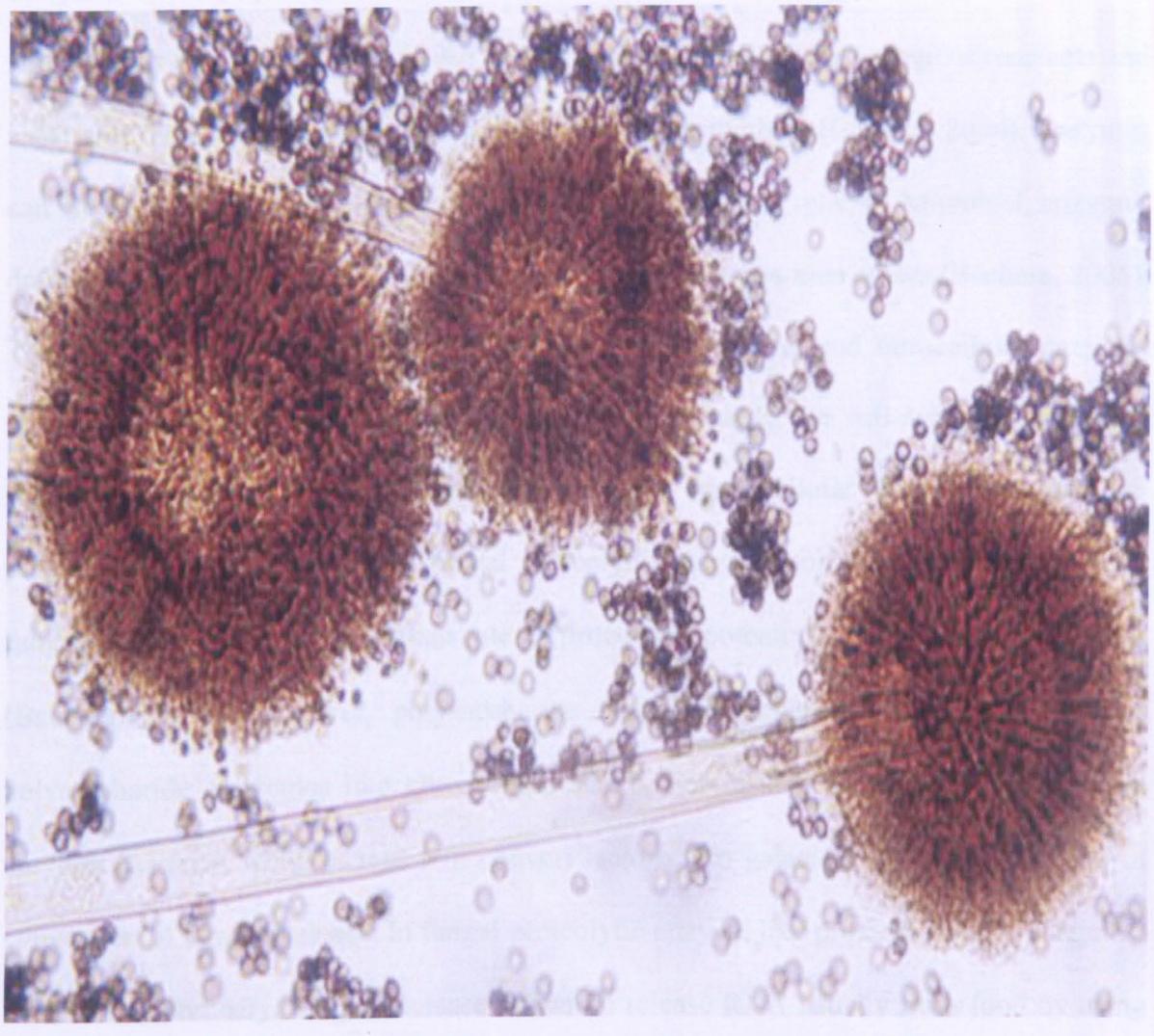

**Figure 2**: Electron photography of Aspergillus species. Adapted from http://medicinembbs.blogspot.my/2013/02/microscopic-morphology-of-aspergillus.htm