UNIVERSITI PUTRA MALAYSIA

THE ISOLATION OF ENDOPHYTIC FUNGI FROM PLANTS AND THE DETERMINATION OF AMYLASE AND LIPASE ACTIVITIES

HANNAH SHOBANA SAMUEL DEVADASAN

FBSB 2015 103

PENGESAHAN

Dengan ini adalah disahkan bahawa projek yang bertajuk "THE ISOLATION OF ENDOPHYTIC FUNGI FROM PLANTS AND THE DETERMINATION OF AMYLASE AND LIPASE ACTIVITIES" telah disiapkan serta dikemukakan kepada Jabatan Mikrobiologi oleh HANNAH SHOBANA A/P SAMUEL DEVADASAN (164012) sebagai syarat untuk kursus BMY 4999 projek.

Disahkan oleh:

…….. Tarikh: …….

Dr. Suriana Sabri

Penyelia

Jabatan Mikrobiologi

Fakulti Bioteknologi dan Sains Biomolekul

Universiti Putra Malaysia

…… Tarikh: …….

Prof. Madya Dr. Muhajir Hamid

Ketua

Jabatan Mikrobiologi

Fakulti Bioteknologi dan Sains Biomolekul

Universiti Putra Malaysia

ABSTRACT

Endophytes refer to a group of microorganisms that are able to grow either intracellularly or intercellularly in plant tissues of higher plants without causing any symptoms or disease in or on those plants. Currently, endophytic fungi are being studied as sources of new enzyme applications. In this project, ten different plant samples were collected from Taman Herba, Universiti Putra Malaysia (UPM). The isolated fungi were screened for the production of extracellular enzymes amylase and lipase. Out of the ten samples which were tested, four samples were further studied, two for each enzyme. Two of the endophytic fungi were identified as *Aspergillus* sp. and *Penicillium* sp. respectively. *Aspergillus* sp is known to have a high amylase activity which explains why the sample which was identified as *Aspergillus s*howed a maximum amylase activity of 0.002 U/ml. The sample identified to produce lipase activity which is *Penicilium* sp. showed a maximum enzyme activity of 13.5 U/ml. The yield for lipase was much greater than the yield for amylase. The other two samples could not be identified as those fungi are non-spore producing fungi.

ABSTRAK

Endofitik merujuk kepada jenis mikroorganisma yang boleh tumbuh sama ada di dalam atau di luar tisu tumbuh-tumbuhan kelas tinggi tanpa mendatangkan penyakit kepada tumbuh-tumbuhan tersebut. Kulat endofitik sedang dikaji sebagai sumber aplikasi enzim baru. Dalam projek ini, sepuluh sampel tumbuhan yang berbeza telah diambil dari Taman Herba, UPM. Eksperimen secara kualitatif dan kuantitatif telah dijalankan ke atas kulat yang telah diambil dari tumbuh-tumbuhan untuk pengeluaran enzim amylase dan lipase. Daripada sepuluh sampel yang diuji, empat sampel telah dipilih untuk dikaji secara mendalam, dua untuk setiap enzim. Dua daripada kulat endofitik telah dikenal pasti sebagai *Aspergillus* sp. dan *Penicillium* sp. masing-masing. *Aspergillus* sp diketahui mempunyai aktiviti amilase tinggi yang menjelaskan mengapa sampel yang dikenal pasti sebagai *Aspergillus* menunjukkan aktiviti amylase maksimum 0.002 U/ml. Sampel yang telah dikenalpasti untuk menghasilkan aktiviti lipase iaitu *Penicilium* sp. menunjukkan aktiviti enzim maksimum 13.5 U/ml. Hasil untuk lipase adalah lebih besar daripada hasil untuk amylase. Kedua-dua sampel lain yang tidak dapat dikenalpasti oleh sebab sampel tersebut adalah kulat yang tidak menghasilkan spora.

ACKNOWLEDGEMENT

First and foremost, I would like to express my sincere gratitude to University Putra Malaysia and the Faculty of Biotechnology and Biomolecular Sciences for the opportunity of being a student here and pursuing my degree in Microbiology in this prestigious university. I am extremely grateful to my supervisor, Dr. Mohd Termizi Yusof for his continuous assistance, support and guidance throughout my project. My warmest gratitude is also extended to all the staff in the Department of Microbiology and all the postgraduate students in the Mycology Laboratory especially to the Master students under Dr Termizi. I would also like to thank my friends who were with me under the supervision of Dr Termizi for their help throughout this project. To my beloved family, especially my parents, I thank them for their love, understanding, encouragement and moral support towards the completion of my project and thesis writing.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

Endophytic fungi have been discovered in every plant species examined and it has been estimated that there is over one million endophytic fungi existing in nature (Petrini, 1991). Endophpytic fungi are fungi that form infections in the leaves, stems or roots of healthy plants that cannot be noticed with the naked eyes. Endopyhtic fungi usually protect the host plants from natural enemies (Carroll, 1988). They have this unique ability to produce similar compounds produced by the host plant and also a diverse amount of bioactive compounds, which is why endophytic fungi are capable of protecting its host against herbivors and pathogens (Wicklow et al., 2005). Studies of endophytic fungi in leaves have been done for many host species where their significance such as common symbionts and possible mutualists of plants was recognised (Carroll et al., 1977). Endophytic fungi can be isolated from any parts of plants such as stems, flowers, leaves and roots (Park et al., 2012). Endophytes are a novel source of potentially useful medicinal compounds which are on research to deal with alarming health problems caused by various cancers, drug-resistant bacteria, parasitic protozoans and fungi (Strobel, 2003). In this project, I will be working on identifying endophytic fungi which are able to produce the enzymes amylase and lipase, and also determining the activities of these enzymes in the endophytes through designated enzyme assays. Amylases are a group of hydrolases that can specifically cleave the α-glycosidic linkage in starch. Two important groups of amylases are glucoamylase and α-amylase. Glucoamylase (exo-1,4-α-D-glucan glu-canohydrolase) produces single glucose units from nonreducing ends of amylose

and amylopectin (Anto, 2006), whereas, α-amylases (endo-1,4- α-D-glucan glucohydro-lase) are extracellular enzymes that randomly cleave the 1,4- α-D-glucosidic linkage between adjacent glucose units inside the linear amylose chain (Pandey, 2005). α-amylases are widely distributed in nature and can be derived from various sources, such as plants, animals and microorganisms (Omemu, 2005). Lipases are a class of hydrolase which is able to catalyse the hydrolysis of triglycerides to fatty acids and glycerol (Figure 1) (Singh, 2011).

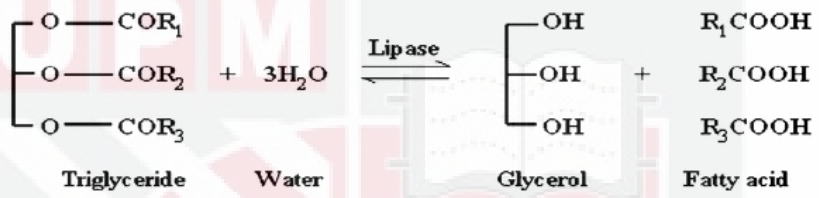

Figure 1: Hydrolysis of triglycerides by lipase

Due to the unique abilities which lipases possess, they are commonly used in industries such as detergent formulation, oleochemical industry, biofuels, food and dairy, nutrition, agro-chemical, cosmetics and pharmaceuticals, sewage treatment, leather industry and paper manufacturing (Gupta et al., 2003; Hasan et al., 2006; Kademi et al., 2005).

However, there are still many diverse endophytic fungal species which have not been discovered. The enzymes produced by these endophytic fungi are not fully explored and studied.

The objectives of this project are:

- To isolate and identify endophytes producing amylase and lipase
- To determine the amylase and lipase activity

REFERENCES

Abe J., Bergmann F.W., Obeta K., Hizukuri S. 1988. Production of the raw starch digesting amylase of *Aspergillus* spp. K-27. *Appl. Microbiol. Biotechnol*. 27: 447-450.

Abu E.A., Ado S.A., James D.B. 2005. Raw starch degrading amylase production by mixed culture of *Aspergillus niger* and *Saccharomyces cerevisae* grown on Sorghum pomac. *Afr. J. Biotechnol*. 4(8): 785-790.

Akpan I., Bankole M.O., Adesemowo A.M., Latunde-Dada G.O. 1999b. Production of amylase by *Aspergillus niger* in a cheap solid medium using rice band and agricultural materials. *Trop. Sci*. 39: 77-79.

Ananda, K. and Sridhar, K.R. 2002. Diversity of endophytic fungi in the roots of mangrove species on west coast of India. *Canadian Journal of Microbiology* 48: 871-878.

Anbu, P., Gopinath, S., Cihan, A., & Chaulagain, B. 2013. Microbial Enzymes and Their Applications in Industries and Medicine. *BioMed Research International* 2013: 1-2.

Anto H., Trivedi U.B., Patel K.C. 2006. Glucoamylase production by solid state fermentation using rice flake manufacturing waste products as substrate. *Bioresour. Technol*. 97(10): 1161-1166.

Arnold, A. E., Maynard, Z., Gilbert, G. S., Coley, P. D. & Kursar, T. A. 2000. Are tropical fungal endophytes hyperdiverse? *Ecology Letters* 3: 267–274.

Arnold, A. E. 2003. Fungal endophytes limit pathogen damage in a tropical tree. *Proceedings of the National Academy of Sciences*, *100*(26): 15649-15654.

Azeredo L.A.I., Gomes P.M., Santanna G., Castilho L.R., Freire D.G. 2007. Production and regulation of lipase activity from Penicillium restrictum in submerged and solid-state fermentations. *Current Microbiol*. 54: 361-365.

Azevedo J.L., Macheroni Jr. W., Pereira J.O., Araújo W.L. de. 2000. Endophytic microorganisms: a review on insect control and recent advances on tropical plants. *Electron J Biotechnol* 3: 40-65.

Bacon, C.W., Porter, J.K., Robbins, J.D., Luttrell, E.S., 1977. Epichloe typhina from toxic tall fescue grasses. *Applied and Environmental Microbiology* 34, 576–581.

Bacon, C.W., White, J.F., 2000. Microbial Endophytes. *Dekker, New York*, pp. 341–388.

Baldauf, S. L. 1993. Animals and Fungi Are Each Other's Closest Relatives: Congruent Evidence from Multiple Proteins. *Proceedings of the National Academy of Sciences* 90(24): 11558-1562.

Bernstein, M. E. & Carroll, G. C. 1977. Internal fungi in old-growth Douglas fir foliage. *Canadian Journal of Botany* 55: 644–653.

Bills, G.F., Collado, J., Ruibal, C., Peláez, F., and Platas, G. 2004. Hormonema carpetanum sp. nov., a new lineage of dothideaceous black yeasts from Spain. *Studies in Mycology* 50: 149-157.

Brown, K.B., Hyde, K.D. and Guest. 1998. Preliminary studies on endophytic fungal communities of *Musa acuminata* species complex in Hong Kong and Australia. *Fungal Diversity* I: 27-51.

Bugni, T.S., Abbanat, D., Bernan, V.S., Maiese, W.M., Greenstein, M., Wagoner, R.M.V. and Ireland, C.M. 2000. Yanuthones: novel metabolites from a marine isolate of *Aspergillus niger*. *Journal of Organic Chemistry* 65: 7195-7200.

Bussaban B., Lumyong S., Lumyong P., McKenzie E.H.C., Hyde K.D. 2001. Endophytic fungi from Amonum siamense. *Canadian Journal of Microbiology* 47: 1–6.

Caldwell B.A., Jumpponen A., Trappe J.M. 2000. Utilization of major detrital substrates by dark-septate, root endophytes. *Mycologia* 92:230–232.

Carroll, G.C. (1986). The biology of endophytism in plants with particular reference to woody perennials. In Microbiology of the Phyllosphere, eds. Fokkema, N.J., Heuvel, J. Cambridge University Press, Cambridge, UK, pp. 205-222.

Carroll, G. 1988. Fungal Endophytes in Stems and Leaves: From Latent Pathogen to Mutualistic Symbiont. *Ecology* 69(1): 2.

Carroll F. E., Müller E. & Sutton B. C. 1977. Preliminary studies on the incidence of needle endophytes in some European conifers. *Sydowia* 29: 87– 103.

Clay, K. 1989. Fungal Endophytes of Grasses: A Defensive Mutualism between Plants and Fungi. *Ecology* 69(1): 10.

Clay K., Schardl C.L. 2002. Evolutionary origins and ecological consequences of endophyte symbiosis with grasses. *American Naturalist* 160: S99–S127.

Cihangir N, Sarikaya E 2004. Investigation of lipase production by a new isolated of *Aspergillus* sp. *World J. Microbio. Biotechnol* 20: 193–197.

Corrado M. & Rodrigues K.F. 2004. Antimicrobial evaluation of fungal extracts produced by endophytic strains of *Phomopsis* sp. *J Basic Microb* 44: 157–160.

Corrêa, R., Rhoden, S., Mota, T., Azevedo, J., Pamphile, J., Souza, C., Peralta, R. 2014. Endophytic fungi: Expanding the arsenal of industrial enzyme producers. *Journal of Industrial Microbiology & Biotechnology J Ind Microbiol Biotechnol* 41(10): 1467-1478.

Crueger W. and Crueger A. 1984. Enzymes. In *BioTechnology. A Text book of Industrial Microbiology* pp.161-186.

Cuomo, V., I. Palomba, A. Perretti, A. Guerriero, M. D'Ambrosio and F. Pietra. 1995. Antimicrobial activities from marine fungi. *J. Mar. Biotechnol*. 2: 199-204.

De Bary, A. 1866. Morphologie und Physiologie Pilze, Flechten, und myxomyceten. *Hofmeister's Handbook of Physiological Botany* Vol. 2. Leipzig.

Devi L.S., Khaund P., and Joshi S.R. 2010. Thermostable -amylase from natural variants of *Bacillus spp*. prevalent in eastern Himalayan range. *Afr. J. Microbiol. Res.*, 4: 2534-2542.

Dreyfuus, M.M. and Chapela, I.H. 1994. Potential of fungi in the discovery of novel, low molecular weight pharmaceuticals. In *The Discovery of Natural Products with Therapeutic Potential*, eds Gullo. V.P. Butterworth-Heineman, London, UK: 49-80.

Ekunsaumi, T., 2002. Laboratory Production and Assay of Amylase by Fungi and Bacteria. *Bio-Link Organization.*

Ellaiah P., Adinarayana K., Rajyalaxmi P., Srinivasulu B. 2003). Optimization of process parameters for alkaline protease production under solid state fermentation by alkalophilic *Bacillus* sp. *Asian J. Microbial Biotechnol. Environ. Sc*. 5: 49-54.

Fisher, P.J., Petrini, O., Petrini, L.E. and Sutton, B.C. 1995. A study of fungal endophytes in leaves, stem and roots of Gynoxis oleifolia Muchler (Compositae) from Ecuador. *Nova Hedwigia* 60: 589-594.

Franken, L.P.G., N.S. Marcon, H. Treichel, D. Oliveira, D.M.G. Freire and C. Dariva. 2009. Effect of treatment with compressed propane on lipases hydrolytic activity. *Food Bioproc. Technol.*

Gessner, R.V. 1979. Degradative enzyme production by salt-marsh fungi. *Botanica Marina* 23: 133-139.

Gupta R., Gigras P., Mohapatra H., Goswami V.K., Chauhan B. 2003. Microbial α-amylases: a biotechnological perspective. *Process Biochem*. 38: 1599-1616.

Gupta R., Dixon K.M., Deo S.S., Holliday C.J., Slater M., Halliday G.M. *et al*. 2007. Photoprotection by 1,25 dihydroxyvitamin D3 is associated with an increase in p53 and a decrease in nitric oxide products. *J Invest Dermatol* 127:707-715

Grbavcic, S. Z., S.I. Dimitrijevic-Brankovic, D.I. Bezbradica, S.S. SilerMarinkovic and Z.D. Knezevic. 2007. Effect of fermentation conditions on lipase production by Candida utilis. *Journal of the Serbian Chemical Society*, 72(8-9): 757-767 .

Hankin, L. and Anagnostakis, S.L. 1975. The use of solid media for detection of enzyme production by fungi. *Mycologia* 67: 597-607.

Hasan, F., Shah, A. A., & Hameed, A. 2006. Industrial Applications of Microbial Lipases. *Enzyme and Microbial Technology*, *39*(2): 235-251.

Huang Y.J, Wang J.F., Li G.L., Zheng Z.H. & Su W.J. 2001. Antitumor and antifungal activities in endophytic fungi isolated from pharmaceutical plants *Taxus mairei*, *Cephalataxus fortunei* and *Torreya grandis*. *FEMS Immunol Med Microbiol* 31: 163–167.

Jaeger, K. 1998. Microbial lipases form versatile tools for biotechnology. *Trends in Biotechnology*, *16*(9): 396-403.

Kademi, A., L. Danielle and Ajain H. (2005). Lipases. In *Enzyme Technology*, eds. A.Pandey, C. Webb, C.R. Soccol and C. Larroche, pp: 297-318. Asiatech Publishers Inc., India.

Kathiresan K. and Manivannan S. 2006. Amylase production by Penicillium fellutanum isolated from Mangrove rhizosphere soil. *African Journal of Biotechnology* 5: 829-832.

Kaushik S., Massey A.C., Cuervo A.M. 2006. Lysosome membrane lipid microdomains: novel regulators of chaperone-mediated autophagy. *EMBO J* 25:3921–3933.

Kingston, D. 2007. The Shape Of Things To Come: Structural And Synthetic Studies Of Taxol And Related Compounds. *Phytochemistry* 68(14): 1844-1854.

Kumaresan, V. and Suryanarayanan, T.S. 2001. Occurrence and distribution of endophytic fungi in a mangrove community. *Mycological Research* 105: 1388-1391.

Kumaresan, V. and Suryanarayanan, T.S. 2002. Endophyte assemblage in young, mature and senescent leaves of Rhizophora apiculata: evidence for the role of endophytes in mangrove litter degradation. *Fungal Diversity* 9: 81-91.

Kwon, D. Y. And Rhee, J. S. 1986. A simple and rapid colorimetric method for determination of free fatty acids for lipase assay. *Journal of the American Oil Chemists' Society* 63(1):90-92.

Maria, G.L., Sridhar, K.R., and Raviraja, N.S. 2005. Antimicrobial and enzyme activity of mangrove endophytic fungi of southwest coast of India. *J. Agric. Technol.,* 1:67-80.

Li., G., K. Zhang, J. Xu, J. Dong and Y. Liu. 2007. Nematicidal substances from fungi. *Recent patents on Biotechnology* 1: 1-22.

Lin, X., Lu, C., Huang, Y., Zheng, Z., Su, W., & Shen, Y. 2007. Endophytic fungi from a pharmaceutical plant, Camptotheca acuminata: isolation, identification and bioactivity. *World Journal of Microbiology and Biotechnology 23*(7): 1037-1040.

Lindstrom J.T., Belanger F.C. 1994. Purification and characterization of an endophytic fungal proteinase that is abundantly expressed in the infected host grass. *Plant Physiol* 106:7–16.

Liu C.H., Zou W.X., Lu H.and Tan R.X. 2001. Antifungal activity of *Artemisia annua* endophyte cultures against phytopathogenic fungi. *J Biotechnol* 88: 277–282.

Lodge D.J., Fisher P.J., Sutton B.C.. 1996. Endophytic fungi of Manilkara bidentata leaves in Puerto Rico. *Mycologia* 88: 733–738.

Maccheroni Jr., W., Azevedo, J.L. 1998. Synthesis and secretion of phosphatases by endophytic isolates of *Colletotrichum musae* grown under conditions of nutritional starvation. *Journal of General and Applied Microbiology* 44: 381-387.

Mannervik, B., A. Runarsdottir and Kurtovic, S. 2009. Multi-substrate activity space and quasi-species in enzyme evolution: Ohnos dilemma, promiscuity and functional orthogonality. *Biochem. Soc. Trans* 37: 740-744.

Mckellar R.C., 1986. A rapid colorimetric assay for the extracellular lipase of pseudomonas fluorescens b 52 using beta naphthyl caprylate. *Journal Of Dairy Research* 117-128.

Miller, G.L., 1959. Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar, Analytical Chemistry 31(3):426-428.

Nielsen RI. 1998. Microbial xyloglucan endotransglycosylase (XET).*World Intellectual Property Organization Publication.*

Omemu, A.M., Akpan I. Bankole, M.O. Teniola, O.D. 2005. Hydrolysis of raw tuber starches by amylase of *Aspergillus niger* AM07 isolated from the soil. *Afr J Biotechnol* 4(1): 19-25.

Oliveira, A. C., Watanabe, F. M., Vargas, J. V., Rodrigues, M. L., & Mariano, A. B. (2012). Production of methyl oleate with a lipase from an endophytic yeast isolated from castor leaves. *Biocatalysis and Agricultural Biotechnology 1*(4): 295-300.

Pandey, A., Nigam, P., Soccol, V.T., Singh, D., and Mohan, R. 2000. Advances in microbial amylases. *Biotechnol. Appl. Biochem* 31: 135- 152.

Pandey A., Soccol C.R., Rodriguez-Leon J., Nigam P. 2001. Solid state fermentation in Biotechnology. *Asia Tech Publishers, Delhi* 1- 221.

Park, Y., Lee, S., Ahn, D., Kwon, T., Park, S., Lim, H., & Bae, H. 2012. Diversity of Fungal Endophytes in Various Tissues of Panax ginseng Meyer Cultivated in Korea. *Journal of Ginseng Research* 2(36): 211-217.

Petrini, O. (1991). Fungal endophytes of tree leaves. In *Microbial Ecology of Leaves*, eds. H. Andrews & S. S. Hirano, pp. 179-197. Springer Verlag: New York.

Puri S.C, Verma V., Amna T. et al. 2005. An endophytic fungus from *Nothapodytes foetida* that produces camptothecin. *J Nat Prod* 68:1717-1719.

Rai, M., Gade, A., Rathod, D., Dar, M., & Varma, A. 2012. Mycoendophytes in medicinal plants: Diversity and bioactivities. *Nus Biosci Nusantara Bioscience* 4(2): 86-96.

Raviraja, N.S., Maria, G.L and Sridhar, K.R. 2006. Antimicrobial evaluation of endophytic fungi inhabiting medicinal plants of the Western Ghats of India. *Eng. Life Sci* 6: 515-520.

Qader, S.A.U., Bano, S., Aman, A., Syed, N., and Azhar, A. 2006. Enhanced production and extracellular activity of commercially important amylolytic enzyme by a newly isolated strain of *Bacillus* sp. AS-1. *Turk. J. Biochem* 31: 135-140.

Raimbault, B.A. 1993. Long term effect of five tillage systems on corn response and soil structure. *Agronomy Journal* 85: 1074-1079.

Reddy P.V., Lam C.K., Belanger F.C. 1996. Mutualistic fungal endophytes express a proteinase that is homologous to proteases suspected to be important in fungal pathogenicity. *Plant Physiol* 111:1209–1218.

Rodrigues, K.F. and Samuels, G.J. 1999. Fungal endophytes of *Spondias mombin*leaves in Brazil. *Journal of Basic Microbiology* 39:131-135.

Rodrigues K.F., Hesse M. and Werner C. 2000. Antimicrobial activities of secondary metabolites produced by endophytic fungi from *Spondias mombin*. *J Basic Microb* 40: 261–267.

Saikkonen, K., Faeth, S. H., Helander, M., and Sullivan, T. J. 1998. FUNGAL ENDOPHYTES: A Continuum of Interactions with Host Plants. *Annual Review of Ecology and Systematics 29*(1), 319-343.

Schulz, B. 2002. Endophytic Fungi: A Source Of Novel Biologically Active Secondary Metabolites. *Mycological Research* 106(9): 996-1004.

Schulz, B., Wanke, U., Drager, S. and Aust, H.J. 1993. Endophytes from herbaceous plants and shrubs: effectiveness of surface sterilization methods. *Mycological Research* 97: 1447-1450.

Singh, Kumar, A., and Mukhopadhyay, M. 2011. "Overview of Fungal Lipase: A Review." *Applied Biochemistry and Biotechnology Appl Biochem Biotechnol* 486-520.

Sjofjan, O., & Ardyati, T. 2011. Extracellular Amylase Activity of Amylolytic Bacteria Isolated from Quail's (Coturnix japonica) Intestinal Tract in Corn Flour Medium. *International J. of Poultry Science International Journal of Poultry Science* 10(5): 411-415.

Southcott, K.A. and Johnson, A. 1997. Isolation of endophytes from two species of palm from Bermuda. *Canadian Journal of Microbiology* 43: 789-792.

Spurr H.W. Jr., Welty R.E. 1975. Characterization of endophytic fungi in healthy leaves of Nicotiana spp. *Phytopathology* 65:417-422.

Stierle, A., Strobel, G., and Stierle, D. 1993. Taxol and taxane production by Taxomyces andreanae, an endophytic fungus of Pacific yew. *Science* 260(5105): 214-216.

Stone, J.K., Bacon, C.W., and White, J.F., (2000). An Overview of Endophytic Microbes Endophytism Defined. In *Microbial Endophytes*, eds. Bacon C.W. and J.F. White , pp: 3-29. Marcel Dekker, New York.

Stoytcheva, M., Montero, G., Zlatev, R., Leon, J., & Gochev, V. 2012. Analytical Methods for Lipases Activity Determination: A Review. *Current Analytical Chemistry CAC* 8(3): 400-407.

Strobel, G. A. 2003. Endophytes as sources of bioactive products. *Microbes and Infection* 5(6): 535-544.

Strobel, G.A., Daisy B., 2003. Bioprospecting for microbial endophytes and their natural products. *Microbiology and Molecular Biology Reviews* 67: 491-502.

Strobel, G., Yang X.S., Sears, J, Kramer, R., Sidhu, R.S., Hess, W.M. 1996. Taxol from Pestalotiopsis microspora, an endophytic fungus of Taxus wallachiana. *Microbiology* 142: 435-440.

Suganthi, R., Benazir, J.F., Santhi, R., Ramesh, K.V., Hari, A., Meenakshi, M., Nidhiya, K.A., Kavitha, G. and Lakshmi, R. 2011. Amylase production by *Aspergillus niger* under solid state fermentation using agroindustrial wastes. *International Journal of Engineering Science and Technology* 3(2): 1756-1763.

Sunitha., V. H., Ramesha., A., Savitha., J., & Srinivas., C. 2012. Amylase production by endophytic fungi Cylindrocephalum sp. isolated from medicinal plant Alpinia calcarata (Haw.) Roscoe. *Brazilian Journal of Microbiology* 43(3), 1213-1221.

Suryanarayanan, T.S. and Rajagopal, K. 2000. Fungal endophytes (phellophytes) of some tropical forest trees. *Indian For* 126: 165-170.

Suryanarayanan, T.S., Kumaresan, V. and Johnson, J.A. 1998. Foliar fungal endophytes from two species of the mangrove Rhizophora. *Canadian Journal of Microbiology* 44: 1003- 1006.

Suto, N., Austin, J.D., Tanabe, L.M., Kramer, M.K., Wright, D.A.,Vezina, P. 2002. Previous exposure to VTA amphetamine enhances cocaine self-administration under a progressive ratio schedule in a D1 dopamine receptor dependent manner. Neuropsychopharmacology 27:970–979.

Sutton, B. C. 1980. The Coelomycetes: fungi imperfecti with pycnidia, acervuli, and stromata. Kew, Surrey, England: Commonwealth Mycological Institute.

Tan, R. X., and Zou, W. X. 2001. Endophytes: a rich source of functional metabolites (1987 to 2000). *Natural Product Reports* 18(4), 448-459.

Tang-um, J., and Niamsup, H. 2012. Extracellular Amylase Activity from Endophytic *Streptomyces griseoflavus* P4.*Chiang Mai J. Sci.* 39(2): 346-350.

Tejesvi, M.V., Kini, K.R., Prakash, H.S., Ven Subbiah and Shetty, H.S. 2007. Genetic diversity and antifungal activity of species of *Pestalotiopsis* isolated as endophytes from medicinal plants. *Fungal Diversity* 24: 37-54.

Teng, Y., Xu, Y. 2008. Culture condition improvement for whole-cell lipase production in submerged fermentation by *Rhizopus chinensis* using statistical method. *Bioresour. Technol*. 99: 3900-3907.

Torres, M., Dolcet, M. M., Sala, N., and Canela, R. 2003. Endophytic Fungi Associated with Mediterranean Plants as a Source of Mycelium-Bound Lipases. *Journal of Agricultural and Food Chemistry* 51(11): 3328-3333.

Vicari, M. (1997). Endophytic Fungi in Grasses and Woody Plants. In *The Journal of Agricultural Science*, eds S. C. Redlin and L. M. Carris, *128*(1): 123-126. St Paul, Minnesota: APS Press (1996). (ISBN 0 89054 213 9).

Visalakchi, S and Muthumary, J. 2010. Taxol (Anticancer Drug) Producing Endophytic Fungi: An Overview. *International Journal of Pharma and Bio Sciences* 1(3).

Vishnupriya, B., Sundaramoorthi, C., Kalaivani, M., Selvam, K. 2010. Production of lipase from *Streptomyces griseus* and evaluation of Bioparameters. *International J. Chem. Tech. Res.* 2(3): 1380-1383.

Wang, J., Li, G., Lu, H., Zheng, Z., Huang, Y., Su, W., 2000. Taxol from *Tubercularia* sp. strain TF5, and endophytic fingi of Taxus mairei. *FEMS Microbiology Letters* 193: 249-253.

Wang, J.W., Wu, J.H., Huang, W.Y., and Tan, R.X. 2006. Laccase production by *Monotospora* sp., an endophytic fungus in *Cynodon dactylon*. *Bioresource Technology*, 97(5): 786-789

Wicklow, D.T., Roth, S., Deyrup, S.T., Gloer, J.B., 2005. A protective endophyte of maize: Acremonium zeae antibiotics inhibitory to *Aspergillus flavus* and *Fusarium verticillioides*. *Mycological Research* 109: 610-618.

Winkler, U.K. and M. Stuckmann, 1979. Glycogen, hyaluronate and some other polysaccharides greatly enhance the formation of exolipase by *Serratia marcescens*. *J. Bacteriol* 138: 663-670.

Wolski, S.C., Kuper, J., Hanzelmann, P., Truglio, J.J., Croteau, D.L., Van Houten, B., Kisker, C. 2008. Crystal structure of the FeS cluster-containing nucleotide excision repair helicase XPD. *PLoS Biol*. 6:e149.

Zaghloue, T.I., Abdel, E.A., and Mutafa. M.H. 1993. Isolation and identification of several alphaamylase hyperproducing bacterial strains. *Alex. J. Pharm. Sci*. 7(2): 133-136.

Zhang, H.W., Song, C.Y., Tan, R.X., 2006. Biology and chemistry of endophytes. *Natural Products Report* 23:753-756