UNIVERSITI PUTRA MALAYSIA

PRODUCTION OF B -1,3 GLUCANASE FROM *Penicillium oxalicum* T3.3 FOR BIOCONTROL AGAINST *Colletotrichum gloeosporioides* AND *Neoscytalidium dimidiatum*

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Master of Science

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

PRODUCTION OF β-1,3 GLUCANASE FROM *Penicillium oxalicum* T3.3 FOR BIOCONTROL AGAINST *Colletotrichum gloeosporioides* AND *Neoscytalidium dimidiatum*

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β-1,3 glucanase produced from fungi is an important hydrolytic enzymes which can been used as biocontrol agent against plant-pathogenic fungi. The objectives of this study are to determine the best substrates for β-1,3 glucanase production, to evaluate the cultural conditions which stimulate *in vitro* production of β-1,3 glucanase and to characterize the β-1,3 glucanase activity and then study the effect of crude β-1,3 glucanase towards fungal hypahe of C. *gleosporioides* and N. *dimidiatum*. β-1,3 glucanase production by *Penicillium oxalicum* T3.3 was investigated using shake flasks under various culture conditions such as different types of carbon and nitrogen sources, initial medium pH, agitation speed and different types of surfactants. The determination of best cultural conditions was carried out by varying and optimizing one variable at a time. The best cultural condition obtained from the shake flask experiment was used for β-1,3 glucanase production in a 2 L stirred tank fermenter where the effect of different impeller speed was investigated. Furthermore, the crude β-1,3 glucanase was characterized to study the enzyme stability. The role of this enzyme in antagonism action against *Colletotrichum gloeosporioides* and *Neoscytalidium dimidiatum*, a well-known fungal plant pathogens causing anthracnose disease in dragon fruit also was examined.

In this study, seaweed *Undaria pinnatifida* was chosen as the best substrates for β-1,3 glucanase production. The highest production of β-1,3 glucanase of 84.73 U/mL was obtained at substrate concentration of 1% (w/v), 0.3% (w/v) peptone and 0.2% (w/v) yeast extract as nitrogen source, initial medium pH 5, temperature at 30^{o}C, agitation speed at 200 rpm and with addition of sodium dodecyl sulfate as surfactant in shake flask condition. The β-1,3 glucanase activity was increased by 38.61% when optimized media and process parameters were used in shake flask culture. The

best cultural condition obtained from shake flask was further used for production β-1,3 glucanase in bioreactor. In bioreactor, the highest production of β-1,3 glucanase was obtained at 250 rpm. However, β-1,3 glucanase production in bioreactor was at par as compared with production in shake flask culture. The crude β-1,3 glucanase obtained was then concentrated by ammonium sulphate precipitation method. Concentrated enzyme was characterized and it was found that the optimum temperature and pH for the enzyme activity were 50ºC and pH 5, respectively. This enzyme can also retain 93.4% and 83.6% of its activity after incubation for 2 h and 4 h, respectively, at 50°C and pH 5. In addition, fungal plant pathogen inhibition assay showed that the crude β-1,3 glucanase produced by *P. oxalicum* T3.3 was able to hydrolyze the fungal hyphae of *C. gloeosporioides* and *N. dimidiatum.*

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENGELUARAN β-1, 3 GLUCANASE OLEH *Penicillium oxalicum* T3.3 UNTUK KAWALAN BIOLOGI TERHADAP *Colletotrichum gloeosporioides* DAN *Neoscytalidium dimidiatum*

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β-1,3 glukanase yang dihasilkan oleh kulat merupakan enzim hidrolitik penting yang boleh digunakan sebagai agen kawalan biologi terhadap kulat tumbuhan patogenik. Objektif kajian ini adalah untuk menentukan substrat yang terbaik untuk penghasilan β-1,3 glukanase, menentukan keadaan kultur terbaik untuk penghasilan β-1,3 glukanase secara in vitro dan untuk mencirikan aktiviti β-1,3 glukanase dan kemudian mengkaji kesan β-1,3 glucanase terhadap kulat C. *gleosporioides* dan N. *dimidiatum*. Penghasilan β-1,3 glukanase oleh *P. oxalicum* T3.3 telah dikaji dengan menggunakan kelalang bergoncang di bawah pelbagai keadaan kultur seperti jenis sumber karbon dan nitrogen, pH awal media, kelajuan goncangan dan jenis surfaktan. Penentuan keadaan kultur terbaik telah dijalankan dengan mengubah dan mengoptimumkan satu pemboleh ubah pada satu masa. Komposisi keadaan kultur terbaik yang yang diperolehi dari eksperimen kelalang bergoncang telah digunakan untuk penghasilan β-1,3 glukanase dalam bioreaktor 2L di mana kesan kelajuan putaran yang berbeza pada pengeluaran β-1,3 glukanase telah dikaji. Selain itu, β-1,3 glukanase dicirikan untuk mengkaji aktiviti enzim. Peranan enzim ini dalam kawalan terhadap *C. gloeosporioides* dan *N. dimidiatum*, patogen tumbuhan kulat yang terkenal dalam menyebabkan penyakit bintik berpusar di dalam buah naga juga telah dikaji.

Dalam kajian ini, rumpai laut *Undaria pinnatifida* telah dipilih sebagai substrat yang terbaik untuk penghasilan β-1,3 glukanase. Penghasilan paling tinggi β-1,3 glukanase (84.73 U/mL) dalam kelalang bergoncang telah diperolehi pada kepekatan substrat 1% (w / v), pepton dan ekstrak yis sebagai sumber nitrogen masing-masing pada 0.3% dan 0.2% (w/v), pH awal media 5, suhu 30^{o}C, kelajuan goncangan pada 200 rpm dan dengan natrium sulfat dodecyl sebagai surfaktan. Ia juga menunjukkan

bahawa aktiviti β-1,3 glukanase meningkat sebanyak 38.61% dengan menggunakan keadaan kultur terbaik diperolehi dari eksperimen kelalang bergoncang. Keadaan kultur terbaik diperolehi daripada kelalang bergoncang kemudiannya digunakan untuk proses penghasilan β-1,3 glukanase dalam 2L bioreaktor. Di dalam bioreaktor, β-1,3 glukanase tertinggi telah diperolehi pada 250 rpm. Walau bagaimanapun, penghasilan β-1,3 glukanase dalam bioreaktor menunjukkan bacaan yang hampir sama dibandingkan dengan penghasilan di dalam kelalang bergoncang. β-1,3 glukanase diperolehi itu kemudian dipekatkan dengan menggunakan proses pemendapan ammonium sulfat. Suhu optimum dan pH aktiviti enzim adalah masing-masing pada 50ºC dan pH 5. Aktiviti enzim ini juga boleh dikekalkan pada 93.4% dan 83.6% selepas diinkubasi selama 2 jam dan 4 jam, masing-masing, pada 50°C dan pH 5. Selain itu, ujian perencatan patogen tumbuhan kulat telah menunjukkan bahawa enzim β-1,3 glukanase yang dihasilkan oleh *P. oxalicum* T 3.3 dapat memusnahkan morfologi hifa kulat *C. gloeosporioides* dan *N. Dimidiatum.*

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This thesis was submitted to the Senate of the Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

CMC	Carboxylmethylcellulose
DO	Dissolved oxygen
DOT	Dissolved oxygen tension
FOL	F. oxysporum f. sp. lycopersici
LSD	Least significant differences
PDA	Potato dextrose agar
PDB	Potato dextrose broth
PPB	Potassium phosphate buffer
SAS	Statistical Analysis Software
SDS	Sodium dodecyl sulphate
DCW	Dry cell weight

LIST OF SYMBOLS

%	Percentage
:	Ratio
µL	micro liter
µmol	micro molar
CO_2	carbon dioxide
h	Hour
h.	Hour
H^+	Hydrogen ions
H_2SO_4	Hydrogen Sulphate
L	liter
M	Molar
mg/mL	milligram per mililiter
min	minute
mL	mililiter
mM	mili Molar
NaOH	Sodium Hydroxide
NH_4NO_3	Ammonium nitrate
nm	nanometer
^oC	Degree Celsius
rpm	rotation per minute
t	Time
U/mL	Unit per mililiter
U/mLd^{-1}	Unit per mililiter per day
β	Beta

CHAPTER 1

INTRODUCTION

1.1 Introduction

Dragon fruit plants in Malaysia have been reported to be infected by several bacterial and fungal diseases such as anthracnose, bacterial soft rot disease and necrotic lesion affected by *Colletrotichum gloeosporioides* (Masyahit *et al*. 2009a), *Curvularia lunata* (Masratul Hawa *et al*., 2009) and *Enterobactor cloacae* (Masyahit *et al*., 2009b) respectively. Besides that, several types of fungal pathogen have been reported to cause a disease in dragon fruit plant globally such as anthracnose infected by *C. gloeosporioides* (Takahashi *et al*. 2008), basal rot infected by *Fusarium oxysporum* (Wright *et al*. 2007), fruit rot, stem rot and fruit blotch infected by *Bipolaris cactivora* (Ben-Ze'ev *et al*., 2011). Furthermore, *Neoscytalidium dimidiatum* also has been reported causing stem canker on dragon fruit (Masratul Hawa *et al*., 2013). The pathogen has an ability of affecting at different plant parts such as leaves, blooms, root, twigs and fruit leading to a range of disease symptoms such as bloom blight, fruit rot, crow root rot and defoliation (Lubbe *et al*., 2006). Symptoms of the disease caused by this pathogen on the fruit were observed as hollow and water-soaked lesions that enlarge quickly on the fruit part (Voorrips *et al*., 2004). Fully extended lesions appear as soft, hollow and the range of colour changes from dark red to black which were known as anthracnose disease (Wharton and Dieguez Uribeondo, 2004).

In order to overcome these problems, chemical fungicides are generally used at high dosage. Excessive use of chemical fungicides has not only caused in the buildup of toxic compounds potentially hazardous to humans and environment but also resulted in resistance of the pathogens toward the fungicides. Furthermore, biological control of plant pathogen has received considerable attention and seems to be one of the effective alternatives to chemical control (Murali *et al*., 2013). Antagonism is one of the mechanisms in biological control which may be achieved by parasitism, application of antibiotics, competition, or by a combination of these various modes of action. Moreover, a parasitism mode of action is hydrolytic enzymes production which was able to destroy cell walls of fungal pathogen. β-1,3-glucanase and chitinase play an important roles as enzymes responsible for fungal cell and sclerotial wall lysis and degradation (El Katatny *et al*., 2000).

β-1,3 glucanases are semi-constitutive hydrolytic enzymes that can degrade glucan molecules embedded in the cell wall components of cereals and some species of fungi. This enzyme has an ability to hydrolyze glucan molecules and leading to the production of D-glucose, thus serving as carbon sources (Tang-Yao, 2002). β-1,3-glucanases has been reported to be produced by a variety of organisms such as bacteria, fungi, and higher plants and many of them have been purified and

characterized (Martin *et al*., 2007). β-1,3-glucanase has been applied as biocontrol agent against plant-pathogenic and play an important role in alteration of cell wall of fungus and structure of β-1,3-glucan (Beshay *et al*., 2003).

Penicillium sp. has been reported to produce lytic enzymes including chitinase and β-glucanase which are involved in degrading fungal cell wall (Patil *et al*., 2013). In another study, it was suggested that *P. oxalicum* secretes chitinase and β-glucanases to degrade and penetrate into the conidiophores and spores of *Nigrospora oryzae* (Sempere and Santamrina, 2008). Chitinases and β-1,3 glucanase have been found to be directly involved in the mycoparasitism interaction between *Trichoderma* species and its hosts (Harman *et al*., 2004).

Commercial β-glucanases are reported to be produced from *Aspergillus niger*, *Bacillus subtilis*, *Pseudomonas fluorescence*, *Disporotrichum dimorphosphorum* and *Penicillium* species. β-glucanases from *Penicillium* species however, have been reported to have a greater benefit over glucanases from other sources. This is because *Penicillium* species are widely found in nature, spread most abundantly in soil and decaying fruits, not fastidious in their nutritional requirements and they can grow on different substrates and under a wide range of environmental conditions (Doughari and Hamuel, 2011).

To the best of our knowledge there are a little reports on the best cultural condition for β-1,3 glucanase production by *Penicillium* species. Thus a study on effect of cultural conditions is an important consideration to determine the best cultural condition for β-1,3 glucanases production by *P. oxalicum*. The most important aspects to decrease the production cost are optimization of media and process conditions (Goshal *et al*., 2011). On the other hand, reducing the cost for enzyme production is still needed in order to develop enzymatic treatment processes for different industrial and environmental applications, which might be more competitive than conventional and other novel treatment technologies. Another way, instead of recombinant DNA techniques, this might also be achieved by means of both process optimization using statistical experimental designs and the use of cheap growth substrates (Ikehata *et al*., 2004). Traditional methods of optimization involved changing one independent variable while fixing the others at a certain level. Optimization of one variable at a time techniques were applied in submerged fermentation to maximize β-1,3 glucanase by *Trichoderma harzianum* (El Katatny *et al*., 2000) and *Aspergillus awamori* (Nguyen and Quyen, 2010).

1.2 Objectives:

The specific objectives of this study are as follows:

1. To determine the best substrates for β-1,3 glucanase production.
2. To evaluate the best cultural conditions which stimulate *in vitro* production of β-1,3 glucanase produced by *P.oxalicum* T 3.3 in shake flask.
3. To characterize β-1,3 glucanase activity and study the effect of crude β-1,3 glucanase towards fungal hypahe of C. *gleosporioides* and N. *dimidiatum*.

REFERENCES

Abang, M.M, Abraham, W., Asiedu, R., Hoffmann, P., Wolf, G. and Winter, S. (2009). Secondary metabolite profile and phytotoxic activity of genetically distinct forms of *Colletotrichum gloeosporioides* from yam (*Dioscorea* spp.). *Mycological research*. 113, 130–140.

Abd-Aziz, S., Fernandez, C.C, Salleh, M.M., Illias, R.M. and Hassan, M.A. (2008). Effect of agitation and aeration Rates on chitinase production using *Trichoderma virens* UKM1 in 2-l Stirred Tank Reactor. *Applied Biochemistry Biotechnology*. 150:193-204.

Adams, D.J. (2004). Fungal cell wall chitinases and glucanases. *Microbiology*. 150: 2029–2035.

Adams, J., Gallagher, J. and Donnison, I. (2009). Fermentation study on *Saccharina latissima* for bioethanol production considering variable pre-treatments. *Journal of Applied Phycology*. 21(5):569–574.

Aehle, W. (2004). *Enzymes in industry*, 2nd ed. Wiley/VCH Verlag GmbH; p. 484.

Aerts, A.M., Carmona, G.D., Lefevre, S., Govaert, G., François, I.E.J.A., Madeo, F., Santos R., Cammuea B.P.A. and Thevissen K. (2009) The antifungal plant defensin RsAFP2 from radish induces apoptosis in a metacaspase independent way in *Candida albicans*. *FEBS Letters*. *583*, 2513–2516.

Ahamed, A. and Vermette, P. (2008). Enhanced enzyme production from mixed cultures of *Trichoderma reesei* RUT-C30 and *Aspergillus niger* LMA grown as fed batch in a stirred tank bioreactor. *Biochemical Engineering Journal*. 42:41–46.

Alahakoon, P.W and Brown, A.E. (1994). Host Range of *Colletotrichum gloeosporioides* on tropical fruit crops in Sri Lanka. *Pest Management*. 40(1), 23–26.

Alonso, F., Cirigliano, A.M., Cabrera, G.M. and Ramírez, J.M. (2010). Synthesis and preliminary biological screening of sterol analogues as new antifungal agents against plant pathogens. *Steroids*. *75*: 659–664.

Amey, R.C., Mills, P.R., Bailey, A. and Foster, G.D. (2003). Investigating the role of a *Verticillium fungicola* β-1,6-glucanase during infection of *Agaricus bisporus* using targeted gene distruption. *Fungal Genetics and Biology*. 39: 264–275.

Amusa, N.A, Ashaye, O.A, Amadi, J. and Oladapo, O. (2006). Guava fruit anthracnose and the effects on its nutritional and market values in Ibadan. *Applied Sciences*. 6(3), 539–542.

Aono, P. (1995). Isolation of Extracellular 28 and 42-Rilodalton β-1,3 glucanases and comparison of β-1,3-glucanase produced by *Bacillus circulans* I IAM1165. *Applied and Environmental Microbiology*. 61: 122-129.

Ariffin, A.A., Bakar, J.C.P. Tan, R.A. Karim, R.R and Loi, C.C (2008). Essential fatty acids of pitaya (dragon fruit) seed oil. *Journal of Agricultural and Food Chemistry*. 114: 561–563.

Arthur, P. and Panda, F.T. (2000). Submerged culture production of chitinase by *Trichoderma harzianum* in stirred tank bioreactors – the influence of agitator speed. *Biochemical Engineering Journal*. 4: 115–120.

Awang, Y., Ghani, M.A.A., Sijam, K. and Mohamad, R. (2011). Effect of calcium chloride on anthracnose disease and postharvest quality of red-flesh dragon fruit (*Hylocereus polyrhizus*). *African Journal of Microbiology Research*. Vol. 5(29), pp. 5250-5259.

Babhita, S., Carvahlo, J.C., Soccol, C.R., and Pandey, A. (2008). Effect of light on growth, pigment production and culture morphology of *Monascus purpureus* in solid-state fermentation. *World Journal of Microbiology and Biotechnology*. 24:2671-2675.

Badino, J.A.C., Facciotti, M.C.R. and Schmidell, W. (2001). Volumetric oxygen transfer coefficients (kLa) in batch cultivations involving non-Newtonian broths. *Biochemical Engineering Journal*. 8: 111–119.

Bailey, J.A and Jeger, M.J. (1992). *Colletotrichum: biology, pathology and control*. CAB International. Wallingford, UK.

Bamforth, C.W. (2009). Current perspectives on the role of enzymes in brewing. *Journal of Cereal Science*. 50, 353–357.

Bara, M. T. F., Lima, A. L., and Ulhoa, C. J. (2003). Purification and characterization of an a exo-β-1,3glucanase produced by *T. asperellum*. *FEMS Microbiology Letters*. 219, 81–85.

Bazilah, B. (2011). *Improvement of xylanase production by recombinant Escherichia coli dh5α using fed-batch fermentation*. Master thesis, Universiti Putra Malaysia.

Ben-Ze'ev, I.S, Assouline, I. and Levy, E. (2011). First report of *Bipolaris cactivora* causing fruit blotch and stem rot of dragon fruit (pitaya) in Israel. *Phytoparasitica*. 39:195–197.

Beshay, U., El-Enshasy, H., Ismail, I.M.K., Moawad, H.,Wojciechowska, E. and Abd-El-Ghany, S. (2003) β-Glucanase production from genetically modified recombinant *Escherichia coli*: effect of growth substrates and development of a culture medium in shake flasks and stirred tank bioreactor. *Process Biochemistry*. ;39:307–13.

Beshay, U., Hesham, E., Enshasy Ismail, I.M.K., Moawad, H. and Sawsan, A.E.G. (2011). β-glucanase Productivity Improvement via Cell Immobilization of Recombinant *Escherichia coli* Cells in Different Matrices. *Polish Journal of Microbiology*. Vol. 60, No 2, 133–138.

Bradford, M. (1976). A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. *Analytical Biochemistry*. 72, 248-254.

Bronnenmeoer, K. and Staduidenbaver, W.L. (1990). Cellulose hydrolysis by highly thermostable endo-1,4 β-glucanases (Avecelase 1) from *Clostridium stercorarium*. *Enzyme and Microbial Technology*. 12: 431-36.

Burtseva, Y.V., Verigina, N.S., Sova, V.V., Pivkin, M.V. and Zvyagintseva, T.N. (2003). Filamentous Marine Fungi as Producers of O-Glycosylhydrolases: β-1,3-Glucanase from *Chaetomium indicum*. *Marine Biotechnology*. 5, 349–359.

Cao, R., Liu, X., Gao, K., Mendgen, K., Kang, Z., Gao, J., Dai, Y., and Wang, X. (2009). Mycoparasitism of Endophytic Fungi Isolated From Reed on Soilborne Phytopathogenic Fungi and Production of Cell Wall-Degrading Enzymes In Vitro. *Current Microbiology*. 59:584–592.

Celestino, K.R.S., Cunha, R.B., and Felix, C.R. (2006). Characterization of a β-glucanase produced by *Rhizopus microspores var. microsporus*, and its potential for application in the brewing industry. *BMC Biochemistry*, 7:23.

Chang, K.L., Arai, T., Ibrahim, D., Kosugi, A., Prawitwong, P., Lan, D., Murata, Y., Mori, Y., (2014). Purification and characterization of thermostable laminarinase from *Penicillium rolfsii*. *BioResources*. 9(1), 1072-1084.

Cheah, L.S. and Zulkarnain, W.M. (2008). *Status of Pitaya Cultivation in Malaysia. Paper presented at Seminar on Pitaya Production*. Market and Export-Challenges and Prospects, Putrajaya, Malaysa: 20 October 2008. <http://www.itfnet.org/ source/ mainpage/newsAndEvent/ contents/PDF_Pitaya_Seminar/Status%20of%20pitaya% 20cultivation% 20in%20Malaysia.pdf> Accessed on 23 October 2008.

Chen, H.Q., Cao, L., Dekkers, K.L., Rollins, J.A., Ko, N.J., Timmer L.W. and Chung K. (2005). A gene with domains related to transcript-tion regulation is required for pathogeni-city in Colletotrichum acutatum causing Key lime anthracnose. *Molecular Plant Pathology*. 6, 513–515.

Chernin, L. and Chet, I. (2002). Microbial enzymes in the biocontrol of plant pathogens and pests. In: Dick RP, Burns RG (eds) *Enzyme in the environment*. Marcel Dekker, New York, pp 171–225.

Ching, K.M. and R.M. Yusof, (2005). *Effect of red pitaya fruit supplementation on lipids profiles of induced hypercholesterolemic rats*. In: Siong, T.E. (ed.), Youth and Nutrition, the Future of the Nation, p: 10. Abstracts of the 20th Scientific Conference of the Nutrition Society of Malaysia, Kuala Lumpur, Malaysia: 24–25 March 2005.

Chuah, T.G., H.L. Ling, N.L. Chin, T.S.Y. Choong and A. Fakhru'l-Razi, (2008). Effects of temperature on rheological behavior of dragon fruit (*Hylocereus* sp.) juice. International Journal of Food Engineering. 4: 1–28.

Chuang, M.F., Ni, H.F., Yang, H.R., Hsu, S.L., Lai, S.Y. and Jiang, Y,L. (2012) First report of stem canker disease of pitaya (*Hylocereus undatus*, *H. polyrhizus*) caused by *Neoscytalidium dimidiatum* in Taiwan. *Plant Disease Journal*. 96:906.

Copa- Patino, J.L., Rodriguez, J., Reyes, F. and Perez-Leblic, M.I. (1990). Effect of β-glucanases on *Penicillium oxalicum* cell wall fractions. *FEMS Microbiology Letters*. 70: 233-240.

Copa-Patino, J.L., Reyes, F. and Perez-Leblic, M.I. (1989) Purification and properties of 1,3-β glucanase from *Penicillium oxalicum* autolysate. *FEMS Microbiology Letters*. 65 : 285-292.

Crane, J.H. and Balerdi, C.F. (2005). *Pitaya growing in the Florida home landscape*. IFAS Extension, HS1068: 1–9.

Crouch, J.A., Beirn, L.A. (2009). Anthracnose of cereals and grasses. *Fungal Diversity*. 39, 19–44.

Dake, M.S, Jadhav, J.P and Patil, N.B. (2004). Induction and properties of (1-3)–β-D-gulcanases form *Aureobasidium pullulans*. *Indian Journal of Biotechnology*. 2004; 3: 56-64.

Dake, M.S. and Kasaram, R. (2012). Production and partial characterization of β-Glucanase from *Galactomyces* sp. isolated from whey. *Elixir Bio-Tech*. 53 (2012) 11932-11936.

De Cal, A., Pascual, S. and Melgarejo P, (1997a). Involvement of resistance induction by *Penicillium oxalicum* in the biocontrol of tomato wilt. *Plant Pathology*. 46, 72–9.

De Cal, A., Pascual, S. and Melgarejo, P. (1997c). A rapid laboratory method for assessing the biological control of *Penicillium oxalicum* against *Fusarium* wilt of tomato. *Plant Pathology*. 46, 699–707.

De Cal, A., Pascual, S. and Melgarejo, P., (1995). Biological control of *Fusarium oxysporum f*.sp. lycopersici. *Plant Pathology.* 44, 909–14.

De León, A., Hernández, V., Galindo, E. and Ram´ırez, O.T. (2003). Effects of dissolved oxygen tension on the production of recombinant *Penicillin acylase* in *Escherichia coli*. *Enzyme and Microbial Technology.* 33: 689–697.

Donzelli, B. and Harman, G. (2001). Interaction of ammonium, glucose, and chitin regulates the expression of cell walldegrading enzymes in *Trichoderma atroviride* strain P1. *Applied and Environmental Microbiology*. 67: 5643–5647.

Doughari, J.H. and Hamuel (2011). Production of β-glucanase enzyme from *Penicillium oxalicum* and *Penicillium citrinum. African Journal of Biotechnology*. Vol. 10(47), pp. 9657-9660.

Du, M., Schardl, C.L. and Vaillancourt, L.J. (2005). Using mating-type gene sequences for improved phylogenetic resolution of *Colletotrichum* species complexes. *Mycologia.* 97, 641–658.

El-Katatny, M. H., Gudelj, M., Robra, K.H., Elnaghy, M. A., and Gubitz, G. M. (2001). Characterization of a chitinase and an endo-β-1,3 glucanase from *Trichoderma harzianum Rifai* T24 involved in control of the phytopathogen *Sclerotium rolfsii*. *Applied Microbiology and Biotechnology*. 56, 137–143.

El-Katatny, M..H, Somitsch, W.K.H., Robra, El-Katatny, M.S and Gübitz, G.M. (2000). Production of Chitinase and β-1,3-glucanase by *T. harzianum*. *Food Technology and Biotechnology*. 38 (3) 173–180.

El-Katatny, M.H., Abdelzaher, H.M., and Shoulkamy, M.A. (2006). Antagonistic actions of *Phytium oilgandrum* and *Trichoderma Harzianum* against phytopathogenic fungi (*Fusarium oxysporum* and *Phytium ultimum* var.ultimum). *Archives of Phytopathology and Plant Protection Journal*. 39 (4) : 289-301.

Escalante, A., Gattuso, M., Pérez, P. and Zacchino, S. (2008). Evidence for the mechanism of action of the antifungal phytolaccoside B isolated from *Phytolacca tetramera* Hauman. *Journal of Natural Products*. *71*, 1720–1725.

Everett, K.R. (2003). The effect of low tempe-ratures on Colletotrichum acutatum and *Colletotrichum gloeosporioides* causing body rots of avocados in New Zealand. *Plant Pathology.* 32, 441–448.

Fang, T.J., Liao, B.C., and Lee, S.C. (2010). Enhanced production of xylanase by *Aspergillus carneus* M34 in solid-state fermentation with agricultural waste using statistical approach. *New Biotechnology.* 27(1): 25-32.

Feng, Y. Heb, .Z. Onga, S.L., Hua, J., Zhang, Z. and Nga, W.J. (2003). Optimization of agitation, aeration, and temperature conditions for maximum β-mannanase production, *Enzyme and Microbial Technology*. 32: 282–289.

Fischer, I.H., Ferreira, M.D., Spósito, M.B. and Amorim, L. (2009). Citrus postharvest diseases and injuries related to impact on packing lines. *Scientia Agricola.* 66, 210–217.

Fontaine, T., Hartland, R.P., Beauvais, A., Diaquin, M. and Latge, J.P. (1997) Purification, characterization of an endo-1,3-β-glucanase from *Aspergillus fumigatus*. *European Journal of Biochemistry*. 243: 315–321.

Freeman, S., Katan, T. and Shabi, E. (1996). Differentiation between *Colletotrichum gloeosporioides* from avocado and almond using molecular and pathogenicity tests. *Applied and Environmental Microbiology*. 62, 1014–1020.

Ghoshal, G., Kamble, A., Shivhare, U.S. and Banerjee, U.C. (2011). Optimization of culture conditions for the production of xylanase in submerge fermentation by *Penicillium citrinum* using response surface methodology. *International Journal of Research and Reviews in Applied Sciences*. 6 (2).

Giblin, F.R., Coates, L.M. and Irwin, J.A.G. (2010). Pathogenic diversity of avocado and mango isolates of *Colletotrichum gloeosporioides* causing anthracnose and pepper spot in Australia. *Plant Pathology*. 39, 50–62.

Gil-ad, N., Bar-Nun, N. and Mayer, A. (2001). The possible function of the glucan sheath of *Botrytis cinerea*: effects on the distribution of enzyme activities. *FEMS Microbiology Letters.* 199: 109–113.

Grigorevski-Lima, A.L. (2009). *Aspergillus fumigatus* thermophilic and acidophilic endoglucanases. *Applied Biochemistry and Biotechnology*. 155: 18-26.

Gulati, R., Saxena, R.K. and Gupta, R. (2000). Fermentation and downstream processing of lipase from *Aspergillus niger*. *Process Biochemistry.* 36: 149-155.

Gummadi, S.N., Kumar, D.S., Dash, S.S, and Sahu. S.K. (2009) (eds.) *Yeast Biotechnology: Diversity and Applications*. Springer Science + Business Media B.V. Chapter 30: Industrially Important Carbohydrate Degrading Enzymes from Yeasts: Pectinases, Chitinases, and β-1,3-Glucanases.

Hakamada, Y., Endo, K., Takizawa, S., Kobayashi, T., Shirai, T., Yamane, T., (2002). Enzymatic properties, crystallization, and deduced amino acid sequence of an alkaline endoglucanase from *Bacillus circulans*. *Biochimica et Biophysica Acta.* 1570:174–80.

Halimi, M.A. and Satar, M.G.M (2007). *Mata Naga*. Dewan Bahasa dan Pustaka, Kuala Lumpur, Malaysia.

Harman, G. E., Howell, C. R., Viterbo, A., Chet, I., & Lorito, M. (2004). *Trichoderma* species-opportunistic, avirulent plant symbionts. *Nature Reviews Microbiology*. 2, 43–56.

He, G., Matsuura, H., Takushi, T., Kawano, S., Yoshihara, T. (2004). A new antifungal metabolite from *Penicillium expansum*. *Journal of Natural Products*. 67:1084–1087.

Hindorf, H., Blanke, M. and Pohlan, J. (2000). *Colletotrichum* species causing anthracnose of tropical crops. *Plant Pathology*. 39, 343–366.

Hongwena, C., Baishan, F. and Zongding, H. (2005). Optimization of process parameters for key enzymes accumulation of 1, 3-propanediol production from *Klebsiella pneumoniae*. *Biochemical Engineering Journal.* 25: 47 – 53.

Hrmova M. et al., (1999). *Cell wall degrading enzymes in barley*. In: Second European symposium on enzymes in grain processing, Technical Research Centre of Finland (VTT), 1999, Helsinki, Finland, 63-74.

Hyde, K.D., Cai, L., McKenzie, E.H.C., Yang, Y.L., Zhang, J.Z. and Prihastuti, H., (2009). *Colletotrichum*: a catalogue of confusion. *Fungal Diversity*. 39:1–17.

Ikehata, K., Buchanan, I.D. and Smith, D.W. (2004). Recent developments in the production of extracellular fungal peroxidases and laccases for waste treatment. *Journal of Environmental Engineering and Sciences*. 3: 1–19.

Iskandar Vijaya, S., Mohd Anuar, I.S. and Zakaria, L. (2013). Characterization and Pathogenicity of *Colletotrichum truncatum* Causing Stem Anthracnose of Red-Fleshed Dragon Fruit (*Hylocereus polyrhizus*) in Malaysia. Journal of Phytopathology. 163 (2015) 67–71.

Jakoby, W.B. (1971). *Crystallization as a purification technique, enzyme purification and related techniques.* In: Jakoby WB (ed) Methods in enzymology, vol 22. Academic, New York, pp 248-252.

Jayus, Barbara, M.M. and Robert, J.S. (2002). Factors affecting the synthesis of (1→3) and (1→6)-β-glucanases by the fungus *Acremonium* sp. IMI 383068 grown in batch culture. *Enzyme and Microbial Technology*. 31: 289–299.

Jayus, McDougall, B.M., and Seviour, R.J .(2005). Synthesis of extracellular (1→3) and (1→6)-β-glucanase activities in carbon limiting chemostat culture by the fungus *Acremonium* sp. IMI 383068. *Enzyme and Microbial Technology.* 36: 680-686.

Jelev, Z.J., Bobev, S.G., Minz, D., Maymon, M. and Freeman, S. (2008). Characterization of *Colletotrichum* species causing strawberry anthracnose in Bulgaria. *Phytopathology*. 156(11–12) 668–677.

Jeya, M., Ah-Reum, J., Kyoung-Mi, L., Won-Il, S., Deok-Kun, O., Yeong-Suk, K., In-Won, K., and Jung-Kul, L. (2010). Characterization of endo β-1,4-glucanase from a novel strain of *Penicillium pinophilum* KMJ601. *Applied Microbiology and Biotechnology*. 85:1005–1014.

Ji-Suk, J., YuKyeong, C., Jeong, G.T. and Kim, S.K. (2012). Optimization of saccharification and ethanol production by simultaneous saccharification and fermentation (SSF) from seaweed, *Saccharina japonica*. *Bioprocess and Biosystems Engineering*. 35:11–18.

Johansson, L., (2006). *Structural analyses of (1→3),(1→4)-β-D-glucan of oats and barley*. Academic dissertation: University of Helsinki, Department of Applied Chemistry and Microbiology, General Chemistry Division (Finland).

Kao, P.M., Chena, C.I., Huanga, S.C., Chang, Y.C., Tsai, P.J. and Liu, Y.C. (2007). Effects of shear stress and mass transfer on chitinase production by *Paenibacillus* sp. CHE-N1. *Biochemical Engineering Journal.* 34: 172–178.

Kefialew, Y., and Ayalew, A. (2008). Postharvest biological control of anthracnose (*Colletotrichum gloeosporioides*) on mango (*Mangifera indica*). *Postharvest and Technology.* 50(1), 8–11.

Khan, Z.S. and Nasreen, S. (2010). Phytochemical analysis, antifungal activity and mode of action of methanol extracts from plants against pathogens. *Journal of Agricultural Science and Technology*. *6*, 793–805.

Kim, E.J., Fathoni, A., Gwi-Taek, J., Jeong, H.D., Nam, T.J., Kong, I.S. and Kim, J.K. (2013). Microbacterium oxydans, a novel alginate- and laminarin-degrading bacterium for the reutilization of brown-seaweed waste. *Journal of Environmental Management.* 130;153-159.

Kim, H., Lim, T.H., Kim, J., Kim, Y.H. and Kim, H,T. (2009) Potential of cross-infection of *Colletotrichum* species causing anthracnose in persimmon and pep. *Pathology Journal.* 25, 13–20.

Kitamato, Y., Kono, R., Shimotori, A., Mori, N. and Ichikawa, Y. (1987). Purification and some properties of an exo-β-1,3 glucanase from *Trichoderma harzianum*. *Agricultural and Biological Chemistry*. 51(12), 3385–3386.

Kulminskaya, A.A., Thomsen, K.K., Shabalin, K.A., Sidorenko, I.A., Eneyskaya, E.V., Savel'ev, A.N. and Neustroev, K.N. (2001). Isolation, enzymatic properties, and mode of action of an exo-1,3-b-glucanase from *Trichoderma viride*. *European Journal of Biochemistry*. 268, 6123–6131.

Lan, G.B. and He, Z.F. (2012). First Report of Brown Spot Disease Caused by *Neoscytalidium dimidiatum* on *Hylocereus undatus* in Guangdong, Chinese Mainland. *Plant disease*. Volume 96, Number 11 Page 1702.

Lau, C.Y., Othman, F. and Eng, L. (2008). *The Effect of the Heat Treatment, Different Packaging Methods and Storage Temperatures on Shelf Life of Dragon Fruit (Hylocereus spp.)* <http://www.doa.sarawak.gov.my/images/dragonfruit.pdf> Accessed on 4 December 2008.

Leelasuphakul, W., Sivanunsakul, P., and Phongpaichit, S. (2006). Purification, characterization and synergistic activity of β-1,3 glucanase and antibiotic extract from an antagonistic *Bacillus subtilis* NSRS 89-24 against rice blast and sheath blight. *Enzyme and Microbial Technology.* 38: 990–997.

Lejeune, R. and Baron, G.V. (1995). Effect of agitation on growth and enzyme production of *Trichoderma reesei* in batch fermentation. *Applied Microbiology and Biotechnology*. 43:249-258.

Liu, J., Yuan, X., Jingang, Shi, G.Z. and Chen, S. (2006). Effect of biosurfactant on cellulase and xylanase production by *Trichoderma viride* in solid substrate fermentation. *Process Biochemistry.* 41:2347–2351.

Lopes, M.R., de Souza, C.J.A., Rodrigues, M.Q.R.B., Costa, D.A., dos Santos, A.F., de Oliveira, L.L., Ramos, H.J.O., Guimarães, V.M., Silveria, W.B., Passos, F.M.L. Fietto, L.G. (2014). Production and Characterization of β-Glucanase Secreted by the Yeast *Kluyveromyces marxianus*. *Applied Biochemistry and Biotechnology*. 172:2412–2424.

Lubbe, C.M., Denman, S., Lamprechi, S.C. and Crous, P.W. (2006). Pathogenicity of *Colletotrichum* species to Protea cultivars. Australasian. *Plant Pathology.* 35, 37–41.

Luders, L. and G. McMahon, (2006). The pitaya or dragon fruit (*Hylocereus undatus*). Darwin: University of Darwin. 778: 1–4.

Luo, H., Yang, J., Yang, P., Li, J., Huang, H., Shi, P., Bai, Y., Wang, Y., Fan, Y. and Yao, B. (2010). Gene cloning and expression of a new acidic family 7 endo-beta-1,3-1,4-glucanase from the acidophilic fungus *Bispora* sp. MEY-1. *Applied Microbiology and Biotechnology*. 85, 1015–1023.

Macauley-Patrick, S. and Finn (2008). Modes of Fermenter Operation, *Practical Fermentation Technology*. John Wiley & Sons, Ltd, UK, England.

MacKenzie, S.J., Peres, N.A., Barquero, M.P., Arauz, L.F., Timmer, L.W. (2009). Host range and genetic relatedness of *Colletotrichum acutatum* isolates from fruit crops and leatherleaf fern in Florida. *Phytopatology*. 99(5), 620–631.

Madrid, H., Cendoya, M.R., Cano, J., Stchigel, A., Orofino, R. and Guarro, J. (2009). Genotyping and in vitro antifungal susceptibility of *Neoscytalidium dimidiatum* isolates from different origins. *International Journal of Antimicrobial Agents.* 34 (2009) 351–354.

Mahani, A. and Halimi, M.G.M.S. (2007). *Mata Naga*. Kuala Lumpur, Dewan Bahasa dan Pustaka.

Marcello, E.C.M., Steindorffb, A.S., Silvana, P.D.S., Silvab, R.D.N., Batausb, L.A.M. and Jose´ Ulhoa, C. (2010). Expression analysis of the exo-b-1,3-glucanase from the mycoparasitic fungus *Trichoderma asperellum*. *Microbiological Research.* 165;75—81.

Martin, K., McDougall, B.M, McIlroy, S., Chen, J. and Seviour, R.J. (2007). Biochemistry and molecular biology of exocellular fungal beta-(1,3)- and beta- (1,6)-glucanases. *FEMS Microbiology Reviews*. 31(2), 168–192.

Martini, M.Y., R.A. Halim, T.M. Mahmud, S.R. Syed Omar and Zainudin (2006). Effects of plant support system and intercropping with long bean (Vigna sinensis) on growth, yield and fruit quality of red dragon fruit (Hylocereus polyrhizus). In: Omar, D., Hong, L.W., Panandam, J.M., Musa, M.H., Kamarudin, M.S., Hussein, M.A., Ismail, M.R. and Poy, T.T. (eds.), *Agriculture for Life and Wealth Creation*, pp: 112–114. Proceedings of Agriculture Congress 2006, Putrajaya, Malaysia: 12–15 December 2006. University Putra Malaysia, Serdang, Malaysia.

Masratul Hawa, M., Salleh, B. and Latiffah, Z. (2009). First report of *Curvularia lunata* on red-fleshed dragon fruit (*Hylocereus polyrhizus*) in Malaysia. *Plant Disease Journal.* 93:971.

Masratul Hawa, M., Salleh, B. and Latiffah, Z. (2013). Identification and Molecular Characterizations of *Neoscytalidium dimidiatum* Causing Stem Canker of Red-fleshed Dragon Fruit (*Hylocereus polyrhizus*) in Malaysia. *Journal of Phytopathology*; 161(11-12):841-849.

Masyahit, M., Sijam, K., Awang, Y. and Ghazali, M. (2009a). The first report of the occurrence of anthracnose disease caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. on dragon fruit (*Hylocereus* spp.) in Peninsular Malaysia. *American Journal of Applied Sciences.* 6:902–912.

Masyahit, M., Sijam, K., Awang, Y. and Ghazali, M. (2009b). First report on bacterial soft rot disease on dragon fruit (*Hylocereus* spp.) caused by *Enterobactor cloacae* in Peninsular Malaysia. *International Journal of Agriculture and Biology.* 11:659–666.

Matthews, G. (2008). Fermentation Equipment Selection: Laboratory Scale Bioreactor Design Considerations, *Practical Fermentation Technology*. John Wiley & Sons, Ltd, UK, England.

McCarthy, T.C., Lalor, E., Hanniffy, O., Savage, A.V. and Tuohy, M.G. (2005). Comparison of wild-type and UV-mutant b-glucanase-producing strains of *Talaromyces emersonii* with potential in brewing applications. *Journal of Industrial Microbiology & Biotechnology*. 32: 125–134.

McHugh, D. (2003). A guide to the seaweed industry. Fisheries technical paper. Rome: Food and Agriculture Organization of the United Nations.

Miyanishi, N., Hamada, N., Kobayashi, T., Imada, C. and Watanabe, E. (2003). Purification and characterization of a novel extracellular β-1,3 glucanase produced by *Bacillus clausii* NM-1 isolated from Ezo *Abalone haliotis discus hannai*. *Journal of Bioscience and Bioengineering*. 95(1):45–51.

Mizrahi, Y., Nerd, A. and Sitrit, Y. (2002). New Fruits for Arid Climates. In: Janick, J. and A. Whipkey (eds.), *Trends in New Crops and New Uses*. pp: 378–384. ASHS Press, Alexandria.

Murali, M., Sudisha, J, Amruthesh, K.N, Ito, S.I and Shetty, H.S. (2013). Rhizosphere fungus *Penicillium chrysogenum* promotes growth and induces defence-related genes and downy mildew disease resistance in pearl millet. *Plant Biology*. 15, 111–118.

Naing, K.W., Anees, M., Kim, S.J., Nam, Y., Kim, Y.C. and Kim, K.Y. (2013). Characterization of antifungal activity of *Paenibacillus ehimensis* KWN38 against soilborne phytopathogenic fungi belonging to various taxonomic groups. *Annals of Microbiology*. 64:55–63.

Nelson, S.C. (2008). *Mango anthracnose (Colletotrichum gloeosporiodes),* The College of Tropical Agriculture and Human Re-sources (CTAHR) University of Hawaii at Mänoa, August PD-48, 1–9.

Nguyen, T.H.P., Sall, T., Bryngelsson, T., Liljeroth, E. (2009).Variation among *Colletotrichum gloeosporioides* isolates from in-fected coffee berries at different locations in Vietnam. *Plant pathology*. 58, 898–909.

Nguyen, V.T. and Quyen, D.T. (2010). Optimizing culture conditions for the production of endo-β-1,4-glucanase by *Aspergillus awamori* strain Vietnam Type Culture Collection (VTCC)-F099. *African Journal of Biotechnology*. Vol. 9(38), pp. 6337-6344.

Nicoletti, R., De Stefano, M., De Stefano, S., Trincone, A. and Marziano, F. (2004). Antagonism against *Rhizoctonia solani* and fungitoxic metabolite production by some *Penicillium* isolates. *Mycopathologia*. 158:465–474.

Nobe, R., Sakakibara, Y., Fukuda, N., Yoshida, N., Ogawa, K. and Suiko, M. (2003). Purification and characterization of laminaran hydrolases from *Trichoderma viride*. *Bioscience, Biotechnology, and Biochemistry.* 67:1349–1357.

Noronha, E.F. and Ulhoa, C.J. (2000). Characterization of a 29-kDa β-1,3-glucanase from *Trichoderma harzianum*. *FEMS Microbiology Letters*. 183, 119–123.

Novita, M., Ahmad, S.H., Ding, P. and Halim, M.R.A. (2008). *Maturity Indices and Related Characteristics of Pitaya.* Paper presented at Seminar on Pitaya Production, Market and Export-Challenges and Prospects, Putrajaya, Malaysia: 20 October 2008. <http://www.itfnet.org/source/mainpage/newsAndEvent/contents/PDF_Pitaya_Seminar/Maturity%20Pitaya.pdf> Accessed on 23 October 2008.

Nuangmek, W., McKenzie, E.H.C., Lumyong, S. (2008). Endophytic fungi from wild banana (*Musa acuminata Colla*) works against anthracnose disease caused by *Colletotrichum musae*. *Microbiology*. 3, 368–374.

Nuero, O.M, Alfonso, C., Del, A. and Fand Reyes, F. (1993). Study of beta 1-3-glucanase activity during autolysis of *Aspergillus nidulans* by HPLC ion exchange chromatography. *Letters in Applied Microbiology*.17: 04-108.

O'Connell, E., Piggott,C. and Tuohy, M. (2011). Purification of exo-1,3-β-glucanase, a new extracellular glucanolytic enzyme from *Talaromyces emersonii*. *Applied Microbiology and Biotechnology*. 89:685–696.

Ooi, T., Sato, H., Matsumoto, K. and Taguchi, S. (2009). A unique post-translational processing of an exo-b-1,3-glucanase of *Penicillium* sp. KH10 expressed in *Aspergillus oryzae*. *Protein Expression and Purification.* 67; 126–131.

Ooi, V.E and Liu, F. (2000) Immunomodulation and anti-cancer activity of polysaccharide-protein complexes. *Current Medicinal Chemistry*. 7,715–729.

Oscarsson, M., Andersson, R., Aman, P. and Jonsson, A. (1998). Effects of cultivar, nitrogen fertilization rate and environment on yield and grain quality of barley. *Journal of the Science of Food and Agriculture*. 78, 359-366.

Palmateer, A.J., Ploetz, R.C., Van Santen, E. and Corell, J.C. (2007). First report of anthracnose caused by *Colletotrichum gloeosporioides* on pitahaya. *Plant Disease Journal.* 91:631.

Panikov, N.S. (1995). *Microbial growth kinetics.* Springer Science & Business Media. London, UK. Chapman & Hall.

Pardo, A.G. (1996) Effect of Surfactants on Cellulase Production by *Nectria catalinensis*. *Current Microbiology*. Vol. 33, pp. 275-278.

Pascual, S., Rico, J.R., De Cal, A. and Melgarejo, P. (1997). Ecophysiological factors affecting growth, sporulation and survival of the biocontrol agent. *Penicillium oxalicum*. *Mycopathologia.* 139, 43–50.

Patil, N.S., Waghmare, S.R., and Jadhav, J.P. (2013) Purification and characterization of an extracellular antifungal chitinase from *Penicillium ochrochloron* MTCC 517 and its application in protoplast formation. *Process Biochemistry.* 48: 176–183.

Paulose, J. and Kalathil, P.C. (2012). Optimization of endo and exo glucanase activity of Trichoderma viride in different carbon sources. *Acta Biologica Indica*. 1(2):192-197.

Peres, N.A.R., Kuramae, E.E., Dias, M.S.C., Ee Souza, N.L. (2002). Identification and characterization of *Colletotrichum* spp. Affecting fruits after harvest in Brazil. *Phytopathology*. 150, 128–134.

Perez Leblic, M.I., Copa, J.L., Arias, M.E., Reyest, F. and Laborda, F. (1985). 1,3-β-glucanases in the autolysis of *Penicillium oxalicum. Transactions of the British Mycological Society*. 84 (3), 467-471.

Photita, W., Lumyong, S., Lumyong, P., McKenzie, E.H.C and Hyde, K.D. (2004) Are some endophytes of *Musa acuminata* latent pathogens? *Fungal Diversity.* 16, 131–140.

Phoulivong, S., Cai, L., McKenzie, E.H.C., Abdelsalam, K., Ekachai C. and Hyde, K.D. (2010). *Colletotrichum gloeosporioides* is not a common pathogen on tropical fruits. *Fungal Diversity*: 44:33–43.

Pitson, S. M., Martin, R. J., McDougall, B.M. (1993). Noncellulolytic fungal β-glucanases: their physiology and regulation. *Enzyme and Microbial Technology.* 15, 178–192.

Polizzi, G., Aiello, D., Vitale, A., Giuffrida, F., Groenewald, Z. and Crous, P.W. (2009) First report of shoot blight, canker, and gummosis caused by *Neoscytalidium dimidiatum* on citrus in Italy. *Plant Disease Journal*. 93:1215.

Pongpisutta, R, Sangchote, S. (1994). Phyto-phthora fruit rot of durian (*Durio zibe-thinus*) In: *Postharvest handling of tropical fruits* – Proceedings of an International conference held at Chiang Mai, Thailand (eds BR Cham, E Highly, GI Johnson). ACIAR Proceedings. No.50. 460–461.

Prihastuti, H., Cai, L., Chen, H., McKenzie, E.H.C., Hyde, K.D. (2009). Characterization of *Colletotrichum* species associate coffee berries in Chiang Mai, Thailand. *Fungal Diversity.* 39, 89–109.

Planas, A. (2000). Bacterial 1-3–1,4-β-glucanases: structure, function and protein engineering. *Biochimica et Biophysica Acta*. 1543:361–382.

Rahman, M.A., Mahmud, T.M.M., Kadir, J., Rahman, R.A. and Begum, M.M. (2008). Major postharvest fungal diseases of papaya cv. 'Sekaki' in Selangor. *Tropical Algicultural Science*. 31, 27–34.

Rahman, R.N.Z.A., Geok, L.P., Basri, M. and Salleh, A.B. (2005). Physical factor affecting the production of organic solvent tolerant protease by *Pseudomonas aeruginosa* strain K. *Bioresource Technology*. 96: 429 – 436.

Ratanacherdchai, K., Wang, H., Lin, F. and Soytong, K. (2010). ISSR for compareson of cross inoculation potential of *Colletotrichum capsici* causing chili anthracnose. *Microbiology Research*. 4(1), 76–83.

Read, S.M., Currie, G. and Bacic, A. (1996). Analysis of the structural heterogeneity of laminarin by electrospray-ionisation-mass spectroscopy. *Carbohydrate Research*. 281: 187–201.

Ruel, K. and Joseleau, J. (1991). Involvement of an extracellular glucan sheath during degradation of Populus wood by *Phanerochaete chrysosporium*. *Applied and Environmental Microbiology*. 57: 374–384.

Sakalidis, M.L., Ray, J.D., Lanoiselet, V., Hardy, G.E.S. and Burgess, T.I. (2011). Pathogenic Botryosphaeriaceae associated with Mangifera indica in the Kimberly Region of Western Australia. *European Journal of Plant Pathology*. 130:379-391.

Santamarina, M.P., Roselló1, J., Llacer, R. and Sanchis, V. (2002). Antagonistic activity of *Penicillium oxalicum* Corrie and Thom, *Penicillium decumbens* Thom and *Trichoderma harzianum Rifai* isolates against fungi, bacteria and insects in vitro. *Revista Iberoamericana de Micología*. 19: 99-103.

Schar-Zammaretti, P,, Dillmann, M.L., D'Amico, N., Affolter, M. and Ubbink, J. (2005). Influence of fermentation medium composition on physicochemical surface properties of *Lactoibacillus acidophilus*. *Applied and Environmental Microbiology*. 71: 8165-8173.

Schoffelmeer, E.A.M., Klis, F.M., Sietsma, J.H. and Cornelissen, B.J.C. (1999). The cell wall of *Fusarium oxysporum*. *Fungal Genetics and Biology*. *27*, 275–282.

Scragg, A.H. (1991). *Bioreactors in Biotechnology: a practical approach*. Ellis Horwood Limited, England.

Sempere, F. and Santamarina, M.P. (2008). Suppression of *Nigrospora oryzae* (Berk. & Broome) Petch by an aggressive mycoparasite and competitor, *Penicillium oxalicum* Currie & Thom. *International Journal of Food Microbiology.* 122:35–43.

Shu, C.H., Xu, C.J. and Lin, E.S. (2006). Production, purification and partial characterization of a novel endo-b-1,3-glucanase from *Agaricus brasiliensis*. *Process Biochemistry.* 41: 1229–1233.

Silva, B.D.S., Ulhoa, C.J., Batista, K.A., Yamashita, F. and Fernandes, K.F. (2011). Potential Fungal Inhibition by Immobilized Hydrolytic Enzymes from *Trichoderma asperellum*. *Journal of Agricultural and Food Chemistry*. 59, 8148–8154.

Silva Aires, R.D., Steindorff, A.S., Ramada, M.H.S., Linhares de Siqueira, S.J. and Ulhoa, C.J. (2012). *Biochemical characterization of a 27 kDa 1,3-β-d-glucanase from Trichoderma asperellum induced by cell wall of Rhizoctonia solani. Carbohydrate Polymers*. 87 : 1219–1223.

Singh, P. and Gill, P.K. (2006). Production of Inulinases: Recent Advances. *Food Technology and Biotechnology*. 44(2), 151-162.

Singh, R.S. and Bhermi, H.K. (2008). Production of extracellular exoinulinase from *Kluyveromyces marxianus* YS-1 using root tubers of *Asparagus officinalis*. *Bioresource Technology*. 99(15), 7418-7423.

Slippers, B., Crous, P.W., Denman, S., Coutinho, T.A., Wingfield, B.D. and Wingfield, M.J. (2004). Combined multiple gene genealogies and phenotypic characters differentiate several species previously identified as *Botryosphaeria dothidea*. *Mycologia*. 96:83–101.

Soares, A.R, Lourenco, S.A. and Amorim, L. (2008). Infection of guava by *Colletotrichum gloeosporioides* and *C. acutatum* under different temperatures and wetting periods Tropical. *Plant Pathology*. 33, 265–272.

Stahmann, K.P., Pielken, P., Schimz K.L. and Sahm, H. (1992). Degradation of extracellular P-(1,3)(1,6)-D-glucan by *Botrytis cinerea*. *Applied and Environmental Microbiology*. 58, 3347-3354.

Sun, H., Yang, J., Lin, C., Huang, X., Xing, R. and Ke-Qin, Z. (2006). Purification and properties of a β-1,3-glucanase from *Chaetomium* sp. that is involved in mycoparasitism. *Biotechnology Letters.* 28: 131–135.

Sutivisedsak, N., Leathers, T.D., Bischoff, K.M., Nunnally, M.S. and Peterson, S.W. (2013). Novel sources of beta-glucanase for the enzymatic degradation of schizophyllan. *Enzyme and Microbial Technology*. 52, 203–210.

Swanston, J.S., Newton, A.C., Hoad, S.P. and Spoor, W. (2006). Variation across environments in patterns of water uptake and endosperm modification in barley varieties and variety mixtures. *Journal of the Science of Food and Agriculture*. 86(5), 826-833.

Takahashi, L.M., Rosa, D.D., Basseto, M.A., de Souza, H.G. and Furtado, E.L. (2008). First report of *Colletotrichum gloeosporioides* on *Hylocereus megalanthus* in Brazil. Australas. *Plant Disease*. 3:96–97.

Tang, J.C, Taniguchi, H.., Chu, H., Zhou, Q. and Nagata, S. (2009). Isolation and characterization of alginate-degrading bacteria for disposal of seaweed wastes. *Letters in Applied Microbiology*. 48(1):38–43.

Tang-Yao, H. (2002). Isolation and Biochemical Characterization of an endo-1, 3- β-glucanase from *Streptomyces sioyaensis* containing a C-termal family 6 carbohydrate binding 1,3- β-glucan. *Microbiology*. 148:1151-1159.

Tarnowski, T.L.B., Palmateer, A.J. and Crane, J.H. (2010). First report of fruit rot on *Hylocereus undatus* caused by *Bipolaris cactivora* in South Florida. *Plant Disease Journal*. 94:1506.

Teng, D., Fan, Y., Yang, Y., Tian, Z., Luo, J. and Wang, J. (2007). Codon optimization of Bacillus licheniformis β-1,3–1,4-glucanase gene and its expression in *Pichia pastoris*. *Applied Microbiology and Biotechnology*. 74:1074–1083.

Than, P.P., Jeewon, R., Hyde, K.D., Pongsupasamit, S., Mongkolporn, O. and Taylor, P.W.J. (2008a). Characterization and pathogenicity of *Colletotrichum* species associated with anthracnose disease on chili (*Capsicum* spp.) in Thailand. *Plant Pathology*. 57, 562–572.

Theis, T. and Stahl, U. (2004). Antifungal proteins: targets, mechanisms and prospective applications. *Cellular and Molecular Life Sciences*. 61:437–455.

Tsai, L.C., Chen, Y.N. and Shyur, L.F. (2008). Structural modeling of glucanase-substrate complexes suggests a conserved tyrosine is involved in carbohydrate recognition in plant 1,3-1,4-beta-D-glucanases. *Journal of computer-aided molecular design*. 22, 915–923.

Tseng, S., Liu, S., Yang, H., Lo, C. and Peng, K. (2008). Proteomic study of biocontrol mechanisms of *Trichoderma harzianum* ETS 323 in response to *Rhizoctonia solani*. *Journal of Agricultural and Food Chemistry*. 56:6914–6922.

Tweddell, R., Jabaji-Hare, S. and Charest, P. (1994). Production of chitinases and β-1,3 glucanases by *Stachybotrys elegans*, a mycoparasite of *Rhizoctonia solani*. *Applied and Environmental Microbiology*. 60: 489–495.

Tweddell, R.J, Jabaji-hare, S.H. and Charest, P.M. (1993). Production of Chitinases and β-1,3-Glucanases by *Stachybotrys elegans*, a Mycoparasite of *Rhizoctonia solani*. *Applied and environmental microbiology*. Feb. 1994, p. 489-495.

Vaidya, R.J., Shah, I.M., Vyas, P.R. and Chattpar, H.S. (2001). Production of chitinase and its optimization from a novel isolate *Alcaligenes xylosoxydans*: potential in antifungal biocontrol. *World Journal of Microbiology and Biotechnology*. 17:691-696.

Voorrips, R.E, Finkers, R. and Sanjaya, L. (2004) QTL mapping of anthracnose (*Colletotrichum* spp.) resistance in a cross between *Capsicum annuum* and *C. chinense*. *Theoretical and Applied Genetics.* 109, 1275–1282.

Wang, J., Zhang, G., Chen, J. and Wu, F. (2004). The change of β-glucan content and β-glucanase activity in barley before and after malting and their relationships to malt qualities. *Food Chemistry*. 86, 223-228.

Wharton, P.S. and Diéguez-Uribeondo, J. (2004). The biology of *Colletotrichum acutatum*. *Anales del Jardin Botanico de Madrid*. 61, 3–22.

Wijeratnam, S.W., Dharmatilaka, Y. and Weerasinghe, D. (2008). *Host Specificity of Colletotrichum gloeosporioides and Botryodi-plodia theobromae isolates from mango, papaya and rambutan and their response to Trichoderma harzianum.* Conference on international research on food secu-rity, Natural Resource Management and Rural Development, Tropentag. 2008. University of Hohenheim, October 7–9, 1–5.

Wongwicharn, A., McNeil, B. and Harvey, L.M. (1999). Effect of oxygen enrichment on morphology, growth, and heterologous protein production in chemostat cultures of *Aspergillus niger* B1-D. *Biotechnology and Bioengineering*. 65:416 – 24.

Wright, E.R., Rivera, M.C., Ghirlanda, A. and Lori, G.A. (2007). Basal rot of *Hylocereus undatus* caused by *Fusarium oxysporum* in Buenos Aires, Argentina. *Plant Disease Journal*. 91:323.

Yang, L., Xie, J., Jiang, D., Fu, Y., Li, G. and Lin, F. (2008). Antifungal substances produced by *Penicillium oxalicum* strain PY-1—potential antibiotics against plant pathogenic fungi. *World Journal of Microbiology and Biotechnology*. 24:909–915.

Yinghua, L.U., Xu, D., Zhijing, C., Qingbiao, L.I. and Gang, L. (2007). Enhanced Production of Hybrid Extracellular β-Glucanase by Recombinant *Escherichia coli* Using Experimental Design Method. *Chinese Journal of Chemical Engineering*. 15(2) 172—177.

Zvyagintseva, T.N, Shevchenko, N.M., Popivnich, I.B., Isakov, V.V., Scobun, A.S., Sundukova, E.V. and Elyakova, L.A. (1999). A new procedure for the separation of water-soluble polysaccharides from brown seaweeds. *Carbohydrate Research*. 322:32–39.

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After graduated her Master, she started her professional career in Market research Company as an executive. She is a passion learner, highly organized person, well-disciplined and multitasking team player to achieve individual and team goals. She is very dedicated and passionate in her work. She enjoyed travelling, doing outdoor activities and spending time with her families and friends. She is also an optimistic and positive person. Her life motto is "If you can dream it, you can do it".

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