UNIVERSITI PUTRA MALAYSIA

OPTIMIZATION OF PROTECTIVE AGENTS FOR FREEZE DRYING OF Paenibacillus polymyxa Kp10 LIVE CELLS

HAYATUN SYAMILA BINTI NASRAN

FBSB 2020 33

OPTIMIZATION OF PROTECTIVE AGENTS FOR FREEZE DRYING OF *Paenibacillus polymyxa* Kp10 LIVE CELLS

By

HAYATUN SYAMILA BINTI NASRAN

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

December 2018

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

OPTIMIZATION OF PROTECTIVE AGENTS FOR FREEZE DRYING OF *Paenibacillus polymyxa* Kp10 LIVE CELLS

By

HAYATUN SYAMILA BINTI NASRAN

December 2018

Chairman : Assoc. Prof. Nor'Aini binti Abdul Rahman, PhD
Faculty : Biotechnology and Biomolecular Sciences

In recent years, biocontrols have been considered to be used in inhibiting pests and pathogens to enhance plant growth performance in agriculture sector. This is due to concerns about usage of chemical fungicides for controlling fungal pathogens that may produce bad effects on environments such as contaminating ground waters with chemical residues and polluting soil for crops. Effects of the chemical fungicides also bring harmful to human as users and consumers of crops fields. Biofungicides for fungal pathogens in plant crops are normally kept in farm at room temperature and this condition may reduce viability of microorganism during storage. Protective agent application during freeze drying of the microorganism could improve survival of the culture throughout the freeze drying process. In this study, freeze drying was chosen as a technique for preservation and protective agent formulation was determined to improve viability of *Paenibacillus polymyxa* Kp10 after freeze drying by using response surface methodology (RSM). A five-level, three-variable central composite design (CCD) was used to evaluate the effects of skim milk, lactose and sucrose as protective agents on the viability of *P. polymyxa* Kp10 due to freeze drying. *Paenibacillus polymyxa* Kp10 was screened for its potential in inhibiting fungal pathogens as biofungicides using fresh and freeze dried culture. Fresh and freeze dried strain was tested with food-borne pathogen to indicate its antimicrobial activity. 5% (v/v) was chosen as inoculum size and the highest cell production was at 22 h. Optimum combination of protective agents analysed by RSM was 20% (w/v) skim milk, 10% (w/v) lactose and 27.5% (w/v) sucrose for freeze drying process of *P. polymyxa* Kp10. The predicted value for cell viability obtained was 5.833 log CFU/ml under the optimum combination after freeze drying process. This value was slightly different to the experimental value (5.452 log CFU/ ml). *P. polymyxa* Kp10 were found to inhibit *Colletrichum gloeosporioides* and *C. truncatum* through dual culture test with percentage of inhibition of radial growth (PIRG) at 60.11% and 62.8%, respectively. Freeze dried *P. polymyxa* Kp10 inhibited *C. gloeosporioides* and *C. truncatum* at 66.52% and 60.18%, respectively. For antimicrobial activity, the result showed that the activity reduced by 43.6% from 4930.56±0.04 AU/ mL to 2780.94±0.05

AU/ mL after freeze drying of *P. polymyxa* Kp10. Thus, *P.polymyxa* Kp10 can be freeze dried with the combination of protective agents and its activity showed stability after the freeze drying process.

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

PENGOPTIMUMAN EJEN PELINDUNG BAGI PROSES PENGERINGAN SEJUK BEKU SEL-SEL HIDUP *Paenibacillus polymyxa* Kp10

Oleh

HAYATUN SYAMILA BINTI NASRAN

Disember 2018

Pengerusi : Prof. Madya Nor'Aini binti Abdul Rahman, PhD
Fakulti : Bioteknologi dan Sains Biomolekul

Beberapa tahun kebelakangan ini, pengawalan bio boleh digunakan untuk menghalang makhluk perosak, kulat perosak di samping membantu pertumbuhan pokok dalam industri pertanian. Pengawalan biodigunakan kerana penggunaan racun kimia kulat yang mengawal kulat perosak boleh menyebabkan kesan buruk pada alam sekitar seperti mencemarkan air di bawah tanah dengan sisa-sisa kimia seterusnya mencemari tanah untuk tanaman. Kesan-kesan racun kimia kulat ini juga boleh membahayakan manusia sebagai pengguna produk pertanian. Bio-racun kulat yang digunakan kebiasaannya disimpan di stor ladang pada suhu bilik dan keadaan ini boleh mengurangkan kemandirian mikrob dalam bio-racun kulat semasa penyimpanan. Penggunaan ejen pelindung semasa proses pengeringan sejuk beku boleh membantu mikrob hidup sepanjang proses *insitu*. Dalam kajian ini, pengeringan sejuk beku telah dipilih sebagai teknik dan formulasi ejen pelindung telah dikaji untuk meningkatkan daya hidup *Paenibacillus polymyxa* Kp10 semasa pengeringan sejuk beku menggunakan kaedah 'Response Surface Methodology' (RSM). Satu kaedah lima peringkat dengan tiga pengubahsuai 'CCD' telah digunakan untuk menilai kesan interaktif antara susu skim, sukrosa dan laktosa sebagai ejen pelindung untuk kemandirian *P. polymyxa* Kp10 semasa pengeringan sejuk beku. *P. polymyxa* Kp10 telah disaring kerana potensinya sebagai bio-racun kulat dalam menghalang kulat perosak menggunakan kultur segar dan kering beku. Untuk aktiviti anti-mikrob, kultur segar dan kering beku telah diuji dengan bakteria perosak makanan. 5% (*v/v*)telah dipilih sebagai saiz inoculum dan produksi sel paling tertinggi telah dihasilkan pada jam ke-22. Penggabungan ejen pelindung yang optimum oleh RSM ialah 20% (*w/v*) susu skim, 10% (*w/v*) laktosa dan 27.5% (*w/v*) sukrosa. Nilai yang diramalkan untuk kemandirian sel ialah 5.833 log CFU/ mL di bawah penggabungan optimum selepas proses pengeringan sejuk beku. Nilai ini agak berlainan dengan 5.452 log CFU/ mL iaitu nilai ujikaji. *P. polymyxa* Kp10 telah dapat menghalang *Colletrichum gloeosporioides* dan *C. truncatum* menggunakan ujian dwi-kultur pada 60.11% dan 62.8%, masing-masing sebagai penghalangan pertumbuhan radial (PIRG). Kultur sejuk beku *P. polymyxa* Kp10 telah menghalang *C. gloeosporioides* and *C.*

truncatum pada 66.52% dan 60.18% masing-masing. Untuk aktiviti anti-mikrobial, aktiviti berkurang dari 4930.56±0.04 AU/ mL kepada 2780.95±0.05 AU/ mL selepas *P. polymyxa* Kp10 melalui pengeringan sejuk beku. Oleh itu, *P. polymyxa* Kp10 boleh disejukbeku dengan penggabungan ejen-ejen pelindung dan aktivitinya masih stabil selepas pengeringan sejuk beku.

ACKNOWLEDGEMENTS

Bismillahirrahmanirrahim

Alhamdullilah, praise to Allah S.W.T. whom with His willing I was able to complete this thesis. I would like to take this opportunity to express my appreciation to every person who motivates, helps and contributes in completing this thesis directly or indirectly. I would like to give my profound gratitude to my main supervisor, Assoc. Prof. Dr. Nor'Aini Abdul Rahman for her constant encouragement, useful opinions and her supervision throughout this thesis.

I am also grateful to Prof. Dr. Arbakariya Ariff and Dr. Murni Halim in their capacity as members of the Supervisory Committee. I am also indebted to the staff of Bioprosessing and Biomanufacturing Research Centre (BBRC), Universiti Putra Malaysia, Mr. Mohd Rizal Kapri, Mrs Liyana Ithnin, Mr. Sobri Mohd Akhir and Mrs. Zainon Sidik for their helps and cooperations.

I wish to express my deepest gratitude to my beloved parents, Nasran Shamsuddin and Zalina Ibrahim who had shown me their continuous support and encouragement along completing this thesis. I would like to thank all my labmates and close friends for their motivations and advices. Last but not least, I wish to dedicate to my beloved husband, Meor Azeem Meor Azli for his continuous support, patience and understanding for every situation I am in.

This thesis was submitted to the Senate of 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:

Nor'Aini binti Abdul Rahman, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

Arbakariya bin Ariff, PhD
Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

Murni binti Halim, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

ZALILAH MOHD SHARIFF, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 12 March 2020

Declaration by graduate student

I hereby confirm that:

- this thesis is my original work;
- quotations, illustrations and citations have been duly referenced;
- this thesis has not been submitted previously or concurrently for any other degree at any other institutions;
- intellectual property from the thesis and copyright of thesis are fully-owned by Universiti Putra Malaysia, as according to the Universiti Putra Malaysia (Research) Rules 2012;
- written permission must be obtained from supervisor and the office of Deputy Vice-Chancellor (Research and Innovation) before thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
- there is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software.

Signature: _____ Date:_____

Name and Matric No.: Hayatun Syamila binti Nasran

Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

Signature: _____
Name of Chairman of Supervisory Committee: Associate Professor Nor'aini binti Abdul Rahman

Signature: _____
Name of Member of Supervisory Committee: Prof. Dr. Arbakariya bin Ariff

Signature: _____
Name of Member of Supervisory Committee: Associate Professor Murni binti Halim

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

°C	Degree Celsius
µL	Microlitre
%	Percentage
AU/ ml	Arbitrary per Millilitre (for antimicrobial activity)
A_{600nm}	Optical Density at Wavelength 600 nanometer
BBRC	Biomanufacturing & Bioprocessing Research Center
BHI	Brain Heart Infusion
CCD	Composite Central Design
CFU / ml	Colony Forming Unit per Millilitre
g	Gram
h	Hour
IPTG	Isopropyl β-D-1-thiogalactopyranoside
L	Litre
M	Molar
mbar	Millibar
min	Minutes
ml	Millilitre
M mol	Millimolarity
OD	Optical Density
PDA	Potato Dextrose Agar
PIRG	Percentage of Inhibition of Radial Growth
rpm	Revolutions per Minutes
RSM	Response Surface Methodology

SEM	Scanning Electron Microscope
w/v	Weight per Volume
x g	Relative Centrifugal Force

CHAPTER 1

INTRODUCTION

1.1 Research Background

Freeze drying or known as lyophilization is a drying process where solvent, usually water, or the suspension medium is freeze at very low temperature then sublimated into vapour state directly from solid state (Liu et al., 2008). Freeze drying is of the successful method in preserving living cells for long term storage (Ciurzyńska & Lenart, 2009). Therefore, preparation of the bacteria and maintainance techniques must be established to ensure the bacteria viability and stability (Morgan et al., 2006). However, there is several factors affect effectiveness of the cells preservation. One of them is protective agents used for cells viability conservation during freeze drying process (Zhao & Zhang, 2005). A suitable protective agent may be able to reduce cells damage during the freeze drying process. Optimization of protective agents as freeze drying medium is essential for enhancing the bacteria survival during the desiccation.

In classical optimization, there is a parameter change and other parameters are kept constant. This method needs many experiments where it is time-consuming, high cost and less accurate. As alternative, most of optimization studies have been performed using response surface methodology (RSM) where it is effective in developing, improving and optimizing of microbial processes (Bas & Boyaci, 2007a).

Freeze drying method is suitable for preservation of variety of heat-sensitive products including drugs, foods, enzymes even culture microorganisms (Ciurzyńska & Lenart, 2009). Thus, the freeze drying is applicable for preservation of bacteria that used for biological control so they can be represented in commercial form which can keep longer and easily for storage (Zhan et al., 2011).

The biological control is one of the alternatives for chemical fungicides that can bring harm to environment, contaminate ground water sources and its content such as methyl bromide caused ozone layer depletion in the atmosphere (Naing et al., 2013). Biological control could be a way to against the fungal pathogens by applied antagonistic microorganism mechanism yet safer to the environment (Harman & Hayes, 1993 and Hernandez-Blanco *et al*., 2007). Several studies have been reported about bacteria that can be used as biocontrol agent as well as can be used for plant growth promoting rhizobacteria.

The bacterial genera that have been identified as potential biocontrol agents including *Lysobacte*r, *Bacillus*, *Paenibacillus*, *Azotobacter* and *Pseudomonas*. For example, *Pseudomonas putida* was recognized can control *F. oxysporum f. sp. melonis* effectively

as the biofungicide where *P. putida* caused Fusarium wilt on muskmelon (Bora et al., 2004). In 2005, Hang et al. were used *B. subtilis* S1-0210 to control gray mold disease on strawberry caused by *Botrytis cinerea.* These examples show that biological control could control the fungal pathogen problems without harm the crops production by synthetic chemical effects.

Biological control is also available for food industry as food preservatives to prevent food spoilage. Preservatives are applied by agro food companies in pre-cooked food, meat products, sauces, cheese, canned fish, meat, vegetables and fruits, cooked and frozen crustaceans and other food products (Silva & Lidon, 2016). For example, *Lactobacillus* spp. produced bacteriocin that can inhibit pathogenic microorganisms (Mahrous et al., 2013). *Paenibacillus* species are recognized can produce two classes of bacteriocins, lantibiotics and pediocins (Grady et al., 2016). These lantibiotics include paenicidin A and penisin both produced by *Paenibacillus* strains (Lohans et al., 2012 and Baindara et al., 2016). Paenibacillin has more stable in pH and heat as well as has broad range in activity against foodborne pathogens thus the paenibacillin can be one of the alternatives for food preservative (Abriouel et al., 2011).

1.2 Objectives of the Study

Thus, objectives of this study were:

1. To determine the effect of inoculum size on *P. polymyxa* Kp10 batch cultivation.
2. To optimize the formulation of protective agents using response surface methodology (RSM) for improvement of the survivility of live cells of *P. polymyxa* Kp10 during freeze drying for formulation of live cells in powderized form.
3. To evaluate the inhibitory effects of *P. polymyxa* Kp10against pathogenic fungus *Colletotrichum truncatum* and *Colletotrichum gloeosporioides* as biofungicide.

REFERENCES

Abadias, M., Benabarre, A., Teixido, N., Usall, J. and Viňas, I. (2001). Effect of freeze drying and protectants on viability of the biocontrol yeast *Candida sake. International Journal of Food Microbiology*. 65 (3): 173-182.

Abbasiliasi, S., Tan, J.S., Ibrahim, T.A.T., Ramanan, R.N., Vakhshiteh, F., Mustafa, S., Ling, T.C., Rahim, R.A. and Ariff, A.B. (2012). Isolation of *Pediococcus acidilactici* Kp10 with ability to secrete bacteriocin-like-inhibitory substance from milk products for applications in food industry. *BMC Microbiology*. 12: 260-272.

Afoakwah, A.N., Adomako, C., Owusu, J., Engman, N.F and Hannah, A.A. (2012). Spray drying as an appropriate technology for the Food and Pharmaceutical Industries - A Review. *Journal of Environmental Science, Computer Science and Engineering & Tecnology*. 1 (3): 467-476.

Allard, S., Enurah, A., Strain, E., Millner, P., Rideout, S.L. and Brown, E.W. (2014). In situ evaluation of *Paenibacillus alvei* in reducing carriage of Salmonella enteric serovar Newport on whole tomato plants. *Applied and Environmental Microbiology*. 80:3842-3849.

Ananta, E., Volkert, M and Knorr, D. (2005). Cellular injuries and storage stability of spray-dried *Lactobacillus rhamnosus* GG. *International Dairy Journal*. 15: 399-409.

Arioli, S., Roncada, P., Salzano, A.M., Deriu, F., Corona, S., Guglielmetti, S., Bonizzi, L., Scaloni, A. and Mora, D. (2009). The relevance of carbon dioxide metabolism in *Streptococcus thermophilus. Microbiology.* 155: 1953-1965.

Ash, C., Priest, F. and Collin, M.D. (1993). Molecular identification of rRNA group 3 bacili using a PCR probe test. *Antonie Van Leeuwenhoek*. 64:253-260.

Bai, Y., Zhou, X. and Smith, D. L. (2003). Enhanced soybean plant growth resulting from co-inoculation of *Bacillus* strains with *Bradyrhizobium japonicum. Crop Science*. 43: 1774-1781.

Baindara, P., Chaudhry, V., Mittal, G., Liao, L.M., Matos, C.O., Khatri, N., Franco, O.L., Patil, P.B. and Korpole, S. (2016). Characterization of the antimicrobial peptide penisin, a class Ia novel lantibiotic from *Paenibacillus* sp. strain A3. *Antimicrobial Agents and Chemotherapy*.60(1): 580-591.

Barbosa, J., Borges, S. and Teixeira, P. (2016). Effect of different conditions of growth and storage on the cell counts of two lactic acid bacteria after spray drying in orange juice. *Beverages*. 2 (8): 1-12.

Barr, P. O. and Soila, P. (1960). Introduction of soft cannula into artery by direct percutaneous puncture. Observations on technique and canulla materials. *Angiology*.11: 168-172.

Baş, D. and Boyacı, İ. H. (2007). Modeling and optimization II: Comparison of estimation capabilities of response surface methodology with artificial neural networks in a biochemical reaction. *Journal of Food Engineering*. 78: 846-854.

Begum, M. M., Sariah, M., Abidin, M. A. Z., Puteh, A. B., and Rahman, M. A. (2008). Antagonistic Potential of selected fungal and bacterial biocontrol agents against *Colletotrichum truncatum* of soybean seeds. *Pertanika Journal of Tropical Agricultural Science*. 31(1): 45–53.

Berner, D and Viernstein, H. (2006). Effect of protective agents on the viability of Lactococcus lactic subjected to freeze-thawing and freeze-drying. *Scientia Pharmaceutical*. 74: 137-149.

Bora, T., Özaktan, H., Göre, E., and Aslan, E. (2004). Biological control of *Fusarium oxysporum* f. sp. *melonis* by wettable powder formulations of the two strains of *Pseudomonas putida*. *Journal of Phytopathology.152*(8–9): 471–475. http://doi.org/10.1111/j.1439-0434.2004.00877.x.

Brennan, M., Wanismail, B., Johnson, M.C. and Ray, B. (1986). Cellular damage in dried *Lactobacillus acidophilus*. *Journal of Food Protection*. 49 (31): 47-53.

Burns, P., Alard, J., Hrdy, J., Boutillier, D., Páez, R., Reinheimer, J., Pot, B., Vinderola, G. and Grangette, C. (2017). Spray-drying process preserves the protective capacity of a breast milk-deerived *Bifidobacterium lactis* strain on acute and chronic colitis in mice. *Scientific Reports*. 7: 1-10.

Carvalho, A.S., Silva, J., Ho, P., Teixeira, P., Malcata, F.X. and Gibbs, P.(2004). Relevant factors for the preparation of freeze-dried lactic acid bacteria. *International Dairy Journal*. 14: 835-847.

Castro, H.P., Teixeira, P.M. and Kirby, R. (1997). Evidence of membrane damage in *Lactobacillus bulgaricus* following freeze drying. *Journal of Applied Microbiology*. 82: 87-94.

Chatterjee, C., Paul, M., Xie, L. and Van der Donk, W. (2005). Biosynthesis and mode of action of lantibiotics. *Chemical Reviews*. 105: 633-683.

Cháver, B.E. and Ledeboer, A.M. (2007). Drying of probiotics: optimization of formulation and process to enhance storage survival. *Drying Technology*. 25: 1193-1201.

Chen, X., Wang, G., Xu, M., Jin, J. and Liu, X. (2010). Antifungal peptide produced by *Paenibacillus polymyxa* BRF-1 isolated from soybean rhizosphere. *African Journal of Microbiology Research*. 4(24): 2692-2698.

Chernin, L. and Chet, I. (2002). Microbial enzymes in biocontrol of plant pathogens and pests. In: Burns, R.G. and Dick, R.P. (editors), Enzymes in the environment: activity, ecology and applications. *Marcel Dekker*, New York, N. Y.

Ciurzyńska, A. and Lenart, A. (2009). Colour changes of freeze-dried strawberries osmotically dehydrated before drying. *Food Technology Operations New Vistas*. pp:217-223.

Ciurzyńska, A and Lenart, A. (2011). Freeze-drying- application in food processing and biotecnology- a review. *Polish Journal of Food and Nutrition Sciences*. 61 (3): 165-171.

Compant, S., Duffy, B., Nowak, J., Clement, C. and Barka, E.A. (2005). Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action and future prospects. *Applied and Environmental Microbiology*. 71(9): 4951-4959.

Cook, R.J., Weller, D.M. and El-Banna, A.Y. (2002). Yield responses of direct-seeded wheat to rhizobacteria and fungicide seed treatments. *Plant Disease*. 86 (7): 780-784.

Corcoran, B.M., Ross, R.P., Fitzgerald, G.F., Stanton, C. (2004). Comparative survival of probiotic lactobacilli spray-dried in the presence of probiotic substances. *Journal of Applied Microbiology*. 96: 1024-1039.

Crowe, L.M., Crowe, J.H., Rudolph, A., Womersley, C. and Appel, L. (1985). Preservation of freeze-dried liposomes by trehalose. *Archives of Biochemistry and Biophysics*. 242: 240-247.

Crowe, J.H., Hoekstra, F.A. and Crowe, L.M. (1989). Membrane phase transitions are responsible for inhibition damage in dry pollen. *Proceeding of National Academic Science USA.* 86: 520-523.

Crowe, J.H., Carpenter, J.F. and Crowe, L.M. (1998). The role of vitrification in anhydrobiosis. *Annual Review of Physiology*. 60 (1): 73-103.

Dianawati, D., Mishra, V., and Shah, N.P. (2013). Survival of *Bifidobacterium longum* 1941 microencapsulated with proteins and sugars after freezing and freeze drying. *Food Research International*. 51(2): 503–509.

Diep, D.B. and Ingolf, F.N. (2002). Ribosomally synthesized antibacterial peptides in gram positive bacteria. *Current Drug Targets*. 3: 107-122.

Donhowe, D. (2010). The benchmark in quality for dehydrated food products. Retrieved from https://www.newfoodmagazine.com/article/1218/the-benchmark-in-quality-for-dehydrated-food-products/

Ghadge, S.V. and Raheman, H. (2005). Process optimization for biodiesel production from mahua oil using response surface methodology. *Bioresource Technology*. 97: 379- 384.

Goderska, K. (2012). Different methods of probiotics stabilization. *Probiotics: Intech Open Science.* 24: 541-550.

Gomez Zavaglia, A., Tymczyszyn, E., De Antoni, G. and Anibal Disalvo, E. (2003). Action of trehalose on the preservation of *Lactobacillus delbrueckii* ssp. *bulgaricus* by heat and osmotic dehydration. *Journal of Applied Microbiology*. 95:1315-1320.

Gordon, R.E. (1981). One hundred and seven years of the genus *Bacillus*, p 1-15. *In*Berkeley R.C.W.and Goodfellow M. (ed.), The Aerobic Endospore-forming Bacteria: Classification and Identification. London: Academic Press

Goswami, D., Parmar, S., Vaghela, H., Dhandhukia, P. and Thakker, J.N. (2015). Describing *Paenibacillus mucilaginosus* strain N3 as an efficient plant growth promoting rhizobacteria (PGPR). *Cogent Food & Agriculture*. 1: 1-13.

Grady, E.N., Macdonald, J., Liu, L., Richman, A. and Yuan, Z. (2016). Current knowledge and perspectives of *Paenibacillus*: a review. *Microbial Cell Factories*. 15: 203-221.

Gray, E.J., Lee, K.D., Souleimanov, A.M., Di Falco, M.R., Zhou, X., Ly, A., Charles, T.C., Driscoll, B.T. and Smith, D.L. (2006). Novel bacteriocin, thuricin 17, produced by plant growth promoting rhizobacteria strain *Bacillus thuringiensis* NEB17: isolation and classification. *Journal of Applied Microbiology.* 100: 545-554.

Gwak, H.J., Lee, J.H., Kim, T.W., Choi, H.J., Jang, J.Y., Lee, S.I. and Park, H.W. (2015). Protective effect of soy powder and microencapsulation on freeze-dried *Lactobacillus brevis* WK12 and *Lactococcus lactis* WK11 during storage. *Food Science and Biotechnology*. 24(6): 2155-2160.

Haidar, R., Roudet, J., Bonnard, O., Dufour, M.C., Corio-Costet, M.F., Fert, M., Gautier, T., Deschamps, A. and Fermaud, M. (2016). *Microbiological Research*. 192: 172-184.

Harman, G.E., and Hayes, C.K. (1993). The genetic nature and biocontrol ability of progeny from protoplast fusion in *Trichoderma*. In Chet, I. (ed). Biotechnology in Plant Protection. J. Wiley-Liss: 237-255.

He, Z., Kisla, D., Zhang, L., Yuan, C., Green-Church, K.B. and Yousef, A.E. (2007). Isolation and identification of a *Paenibacillus polymyxa* strain that coproduces a novel lantibiotic and polymyxin. *Applied and Environmental Microbiology*. 73(1): 168-178.

Hernandez-Blanco, C., Feng, D.X. and Hu, J. (2007). Impairment of cellulose synthases required for *Arabidopsis* secondary cell wall formation enhances disease resistance. *Plant Cell*. 19: 890-903.

Hosseini, S.M. and Darani, K.K. (2010). Response surface methodology for mycoprotein production by *Fusarium venenatum* ATCC 20334. *Journal of Bioprocessing and Biotechniques*. 102:1-5

Hubálek, Z. (2003). Protectants used in the cryopreservation of microorganisms. *Cryobiology*, 46(3), 205–229.

Ibrahim, H. M. and Elkhidir, E.E. (2011). Response surface method as an efficient tool for medium optimisation. *Trends inApplied Sciences Research*. 6: 121-129.

Israeli, E., Shaffer, B.T. and Lighthart, B. (1993). Protection of freeze-dried *Escherichia coli* by trehalose upon exposure to environmental conditions. *Cryobiology*. 30: 519-523.

Jalali, M., Abedi, D., Varshosaz, J., Najjarzadeh, M., Mirlohi, M and Tavakoli, N. (2012). Stability evaluation of freeze-dried *Lactobacillus paracasei*subsp. *tolerance* and *Lactobacillus delbrueckii* subsp. *bulgaricus* in oral capsules. *Research in Pharmaceutical Sciences*. 7: 31-36.

Jeon, Y.H. and Kim, Y.H. (2008). Differential structural responses of ginseng root tissues to different initial inoculum levels of *Paenibacillus polymyxa* GBR-1. *The Plant Pathology Journal.* 24 (3): 352-356.

Jeon, Y.H., Kim, S.G., Hwang, I. and Kim, Y.H. (2010). Effects of initial inoculation density of *Paenibacillus polymyxa* on colony formation and starch-hydrolytic activity in relation to root rot in ginseng. *Journal of Applied Microbiology*. 109(2):461-470.

Kambar, Y., Manasa, M., Vivek, M. N. and Kekuda, P. T. R. (2014). Inhibitory effect of xsome plants of Western Ghats of Karnataka against *Colletotrichum capsici. Science Technology and Arts Research Journal.* 3 (2): 76-82.

Kanojia, G., Have, R.T., Soema, P.C., Frijlink, H., Amorij, J.P. and Kersten, G. (2017). Developments in the formulation and delivery of spray dried vaccines. *Human Vaccines & Immunotherapeutics*. 13 (10): 2364-2378.

Karpunina, L.V., Mel'nikova, U.Y. and Konnova, S.A. (2003). Biological role of lectins from the nitrogen-fixing *Paenibacillus polymyxa* strain 1460 during bacterial-plantroot interactions. *Current Microbiology*. 47:376-378.

Keivani, F., Mokarram, R.R., Benis, K.Z., Gholian, M.M., Zendeboodi, F. and Zadeh, S.S. (2014). External and internal factors affecting survival of probiotic living cells during dessication. *International Journal of Pharmaceutical Archive*. 3 (2): 309-316.

Khan, Z., Kim, S.G., Jeon, Y.H., Khan, H.U., Son, S.H. and Kim, Y.H. (2008). A plant growth promoting rhizobacterium, *Paenibacillus polymyxa* strain GBR-1, suppresses root-knot nematode. *Bioresource Technology*. 99: 3016-3023.

Khoramnia, A. (2007). Use of cryoprotectants in enhancing viability of probiotic. Degree of Master of Science Thesis, UPM, Malaysia.

Kim, S.S. and Bhowmik, S.R. (1990). Survival of lactic acid bacteria during spray drying of plain yogurt. *Journal of Food Science*. 55 (4):1008-1010 & 1048.

Kim, S.G., Jang, Y., Kim, H.Y., Koh, Y.J. and Kim, Y.H. (2010). Comparison of microbial fungicides in antagonistic activities related to the biological control of phytophthora blight on ginseng. *The Plant Pathology Journal*. 20: 63-66.

Kim, Y.S., Kotnala, B., Kim, Y.H. and Jeon, Y. (2016). Biological characteristics of *Paenibacillus polymyxa* GBR-1 involved in root rot of stored Korean ginseng. *Journal of Ginseng Research*. 40: 453-461.

King, A.E. and Su, J.T. (1993). Dehydration of *Lactobacillus acidophilus*. *Process Biochemistry.* 28: 47-52.

Knorr, D. (1998). Technology aspects related to microorganisms in functional foods. *Trends in Food Science & Technology*. 9: 295-306.

Kusakabe, H. and Kamiguchi, Y. (2004). Chromosomal integrity of freeze-dried mouse spermatozoa after 137Cs gamma-ray irradiation. *Mutation Research*. 556: 163-168.

Leadbeater, A. (2015). Recent developments and challenges in chemical disease control. *Plant Protection Science*. 51: 163-169.

Leslie, S.B., Israeli, E., Lighthart, B., Crowe, J.H. and Crowe, L.M. (1995). Trehalose and sucrose protect both membranes and proteins in intact bacteria during drying. *Applied and Environmental Microbiology*. 61 (10): 3592-3597.

Liberman, H.A., Lachman, L. and Schwartz, B.J. (1989). Pharmaceutical dosage form: Tablets. Parenterals (1). New York: Marcel Dekker.

Liew, S.L. (2004). Pilot-scale production of *Lactobacillus rhamnosus*. Degree of Doctor of Philosophy thesis, UPM, Malaysia.

Lippincolt, W. and Remington, K. (2000). The science & practice of pharmacy. *Parenteral Preparation*. 20th ed., ISE Publication, *Phelabelphia.* 1: 804-819.

Liu, Y., Zhao, Y. and Feng, X. (2008). Energy analysis for a freeze-drying process. *Applied Thermal Engineering*. 28: 675-690.

Lohans, C.T., Huang, Z. and Van Belkum, M.J. (2012). Structural characteristics of the highly cyclized lantibiotic paenicidin a via a partial desulfurization/reduction strategy. *Journal of the American Chemical Society*. 134: 19540-19543.

Mahrous, H., Mohamed, A., Abd El-Mongy, M., El-Batal, A.I. and Hamza, H.A. (2013). Study bacteriocin production and optimization using new isolates of

Lactobacillus spp. isolated from some dairy products under different culture conditions. *Food and Nutrition Sciences*. 4: 342-356.

Malmo, C., Storia, A.L. and Mauriello, G. (2013). Microencapsulation of *Lactobacillus reuteri* DSM 17938 cells coated in alginate beads with chitosan by spray drying to use as a probiotic cell in a chocolate Soufflé. *Food and Bioprocess Technology*. 6 (3): 795-805.

McGrath, M.T. (2016). "What are fungicides". *The Plant Instructor*. 1: 1-8.

Meng, X.C., Stanton, C., Fitzgerald,G.F, Daly, C. and Ross, R.P. (2008). "Anhydrobiotics: The challenges of drying probiotic cultures,". *FoodChemistry*. 106: 1406-1416.

Millqvist-Fureby, A., Malmsten, M., and Bergenstahl, B. (2000). An aqueous polymer two-phase system as carrier in the spray-drying of biological material. *Journal of Colloid and Intersurface Science*. 225: 54-61.

Ming, L.C. (2006). Optimization of fermentation and freeze-drying processes to enhance the productivity and stability of a probiotic, *Lactobacillus salivarius* I 24. Degree of Doctor of Philosophy Thesis, UPM, Malaysia.

Ming, L.C., Rahim, R.A., Wan, H.Y., and Ariff, A.B. (2009). Formulation of protectIve agents for improvement of *Lactobacillus salivarius* I 24 survival rate subjected to freeze drying for productIon of live cells in powderized form. *Food and Bioprocess Technology*.*2*(4): 431–436.

Monte, E. (2001). Understanding *Trichoderma*: between biotechnology and microbial ecology. *International Microbiology*. 4: 1-4.

Morgan, C.A., Herman, N., White, P.A. and Vesey, G. (2006). Preservation of micro-organisms by drying; A review. *Journal of Microbiological Methods*. 66: 183-193.

Muller, J.A., Ross, R.P., Fitzgerald, G.F. and Stanton, C. (2009). Manufacture of probiotic bacteria. *Prebiotics and Probiotics Science and Technology*. 725-759.

Myers, R.H. (1971). Response surface methodology, pp 1-3. Boston: Allyn and Bacon Inc. Myllarinen.

Myers, R.H. and Montgomery, D.C. (1995). Response surface methodology: Process and product optimization using designed experiments. New York: John Wiley & Sons, Inc.

N'Guessan, F.K., Coulibaly, H.W., Alloue-Boraud, M.W.A., Cot, M. and Djè, K.M. (2015). Production of freeze-dried yeast culture for the brewing of traditional sorghum beer, tchapalo. *Food Science & Nutrition*. 4 (1): 34-41.

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.

Neema, S., Washkuhn, R.J. and Brendel, R.J. (1997). Injectable products. *PDA Journal of Pharmaceutical Science and Technology*. 51: 166-171.

Nireesha, G.R., Divya, L., Sowmya, C., Venkateshan, N., Babu, M.N and Lavakumar, V. (2013). Lyophilization/ freeze drying. *International Journal of Novel Trends in Pharmaceutical Sciences*. 3 (4): 87-98.

Otero, M.C., Espeche, M.C. and Nader-Macías, M.E. (2007). Optimization of the freeze-drying media and survival throughout storage of freeze-dried *Lactobacillus gasseri* and *Lactobacillus delbrueckii* subsp. *delbrueckii* for veterinarian probiotic applications. *Process Biochemistry*. 42: 1406-1411.

Pal, K.K. and Gardener, B.M. (2006). Biological control of plant pathogens. *Plant Health Instructor*. 2:1117-1142.

Palmfeldt, J. and Hahn-Hägerdal, B. (2000). Influence of culture pH on survival of *Lactobacillus reuteri* subjected to freeze-drying. *International Jounal of Food Microbiology*. 55 (1-3): 235-238.

Parente, E., Brienza, C., Moles, M. and Ricciardi, A. (1995). A comparison of methods for the measurement of bacteriocin activity. *Journal of Microbiological Methods*. 22: 95-108.

Passera, A., Venturini, G., Battelli, G., Casati, P., Penaca, F., Quaglino, F. and Bianco, P.A. (2017). Competition assays revealed *Paenibacillus pasadenensis* strain R16 as a novel antifungal agent. *Microbiological Research*. 198: 16-26.

Patkowska, E., Jamiolkowska, A., and Blazewicz-Wozniak, M. (2018). Antagonistic activity of selected fungi of the soil environment of carrot. *Plant, Soil and Environment*. 64 (2): 58-63.

Petatán-Sagahón, I., Anducho-Reyes, M.A., Silva-Rojas, H.V., Arana-Cuenca, A., Tellez-Jurado, A., Cárdenas-Alvarez, I.O. and Mercado-Flores, Y. (2011). Isolation of bacteria with antifungal activity against the phytopathogenic fungi *Stenocarpella maydis* and *Stenocarpella macrospora*. *International Journal of Molecular Sciences*. 12: 5522-5537.

Portner, D.C., Leuschner, R.G.K. and Murray, B.S. (2007). Optimising the viability during storage of freeze-dried cell preparations of *Campylobacter jejuni*. *Cryobiology*. 54: 265-270.

Prakash, O., Nimonkar, Y. and Souche, Y.S. (2013). Practice and prospects of microbial preservation. *Federation of European Microbiological Societies Microbiology Letters*. 339 (1): 1-9.

Priest, F.G., Goodfellow, M. and Todd, C. (1988). A numerical classification of the genus *Bacillus*. *Journal of General Microbiology*. 134: 1847-1882.

Priest, F.G. (2009). Genus I. *Paenibacillus* Ash, Priest and Collins. 1994. In *Bergey's Manual of Systemic Bacteriology,* 2nd edn., Vol. 3: 269-295. Edited by Vos, P.D.V., Garrity, G.M., Jones, D., Krieg, N.R., Ludwig, W., Rainey, F.A., Schleifer, K.H. and Whitman, W.B.. New York: Springer.

Rajan, L.A., Vinodhini, K., Rajalakshmi, Y and Umashankar, V. (2011). Molecular cloning and in silico sequence analysis of glycine betaine biosynthesis genes in *Bacilus subtilis*. *Journal of Biotechnology & Biomaterial*. 1: 103.

Raza, W., Yuan, J., Wu, J.C., Rajer, F.U., Huang, Q. and Qirong, S. (2015). Biocontrol traits of two *Paenibacillus polymyxa* strains SQR-21 and WR-2 in response to fusaric acid, a phytotoxin produced by *Fusarium* species. *Plant Pathology*. 64 (5): 1041-1052.

Reddy, K.B.P.K., Awasthi, S.P., Madhu, A.N. and Prapulla, S.G. (2009). Role of cryoprotectants on the viability and functional properties of probiotic lactic acid bacteria during freeze drying. *Food Biotechnology*. 23: 243-265.

Rudolph, A.S. and Crowe, J.H. (1985). Membrane stabilization during freezing: the role of two natural cryoprotectants, trehalose and proline. *Cryobiology*. 22: 367-377.

Saarela, M., Virkajärvi, I., Alakomi, H.L., Sigvart-Mattila, P. and Mättö, J. (2002). Stability and functionality of freeze-dried probiotic *Bifidobacterium* cells during storage in juice and milk using response surface methodology. *Food Chemistry*. 77: 115-123.

Saarela, M., Virkajärvi, I., Alakomi, H.K., Matilla-Sandholm, T., Vaari, A., Suomalainen, T. and Mättö, J. (2005). Influence of fermentation time, cryoprotectant and neutralization of cell concentrate on freeze-drying survival, storage stability, and acid and bile exposure of *Bifidobacterium animalis* spp. *lactis* cells produced without milk-based ingredients. *Journal of Applied Microbiology*. 99: 1330-1339.

Sadguruprasad, L.T. and Basavaraj, M. (2018). Statistical modelling for optimized lyophilization of *Lactobacillus acidophilus* strains for improved viability and stability using response surface methodology. *AMB Express*. 8:129.

Sai, Y.R.K.M., Kishore, S.N., Dattatreya, A., Anand, S. Y. and Sridhari, G. (2011). A review on biotechnology and its commercial and industrial applications. *Journal of Biotechnology & Biomaterials*.1 (7):1-5.

Sarkar, A.K. (2016). Anthracnose diseases of some common medicinally important fruit plants. *Journal of Medicinal Plants*, *4*(3): 233–236.

Shafi, J., Tian, H. and Ji, M. (2017). *Bacillus* species as versatile weapons for plant pathogens: a review. *Agriculture and Environmental Biotechnology*. 31: 446-459.

Shamekhi, F., Shuhaimi, M., Ariff, A.B. and Yazid, A.M. (2012). Optimization of a cryoprotective medium for infant formula probiotic applications using response surface methodology. *Annals of Microbiology*. 62: 911-921.

Shida, O., Takagi, H.,Kadowaki, K., Nakamura, I. and Komagata, K. (1997). Transfer of *Bacillus alginolyticus, B. chondroitihus B. curdlanolyticus, B.glucanolyticus, B. kobensis and B. thiaminolyticus* to the genus *Paenibacillus* and emended description of the genus *Paenibacillus*. *International Journal of Systematic and Evolutionary Microbiology*. 47: 289-298.

Shu, G., Zhang, B., Hui, Y., Chen, H. and Wan, H. (2017). Optimization of cryoprotectants for *Streptococcus thermophilus* during freeze-drying using Box-Behnken experimental design of response surface methodology. *Emirates Journal of Food and Agriculture.* 29 (4): 256-263.

Silva, M.M. and Lidon, F. (2016). Food preservatives - An overview on applications and side effects. *Emirates Journal of Food & Agriculture.* 28 (6): 366-373.

Simatos, D., Blond, G., Le Meste, M. and Morice, M. (1995). Conservation des bacteries lactiques par congelation et lyophilization. In H. De Roissard & F. M. Luquest (Eds.), *Bacteries Lactiques* (555-572). Grenoble, France: Lorica.

Singh, P.P., Shin, Y.C., Park, C.S. and Chung, Y.R. (1999). Biological control of *Fusarium* wilt of cucumber by chitinolytic bacteria. *Phytopathology*. 89: 92-99.

Smith, D., Lee, K. D., Gray, E., Souleimanov, A. and Zhou, X. (2008). Use of bacteriocins for promoting plant growth and disease resistance. US Patent Application number: 20080248953.

Song, M., Yun, H.Y. and Kim, Y.H. (2013). Antagonistic *Bacillus* species as a biological control of ginseng root rot caused by *Fusarium* cf. *incarnatum*. *Journal of Ginseng Research*. 38 (2): 136-145.

Song, W., Ma, D and Wei, X. (2014). Assesing cell viability and biochemical stability of freeze-dried sulphate-reducing bacteria within different types of cryoprotectants and storage conditions. *International Journal of Current Microbiology and Applied Sciences*. 3 (6): 659-670.

Suarez-Estrella, F., Vargas-Garcia, C., Lopez, M.J., Capel, C. and Moreno, J. (2007). Antagonistic activity of bacteria and fungi from horticultural compost against *Fusarium oxysporum* f. sp. *melonis. Crop Protection*. 26: 46-53.

Succi, M., Tremonte, P., Reale, A., Sorrentino, E., and Coppola, R. (2007). Preservation by freezing of potentially probiotic strains of *Lactobacillus rhamnosus*, 57(4): 537–544.

Summer, R.J., D.P. Boudreaux and V.R. Srinivasan, (1979). Continuos cultivation for apparent optimisation of defined media for *Cellulomonas* sp. and *Bacillus cereus*. *Applied Environmental. Microbiology*. 36: 66-71.

Streeter, J.G. (2003). Effect of trehalose on survival of *Bradyrhizobium japonicum* during dessication. *Journal of Applied Microbiology*. 95: 484-491.

Tagawa, M., Tamaki, H., Manome, A., Koyama, O. and Kamagata, Y. (2010). Isolation and characterization of antagonistic fungi against potato scab pathogens from potato field soils. *Federation of European Microbiological Societies (FEMS) Microbiology Letters.* 305 (2): 136-142.

Teixeira, P., Castro, H. and Kirby, R. (1995). Spray-drying and a method for preparing concentrated cultures of *Lactobacillus bulgaricus. Journal of Applied Microbiology.* 78: 456-462.

Tomar, J., Born, P.A., Frijlink, H.W. and Hinrichs, W.L.J. (2016). Dry influenza vaccines: towards a stable, effective and convenient alternative to conventional parenteral influenza vaccination. *Expert Review of Vaccines*. 15 (11): 1431-1447.

Torres, P., Usall, J., Teixeido, N., Abadias, M. and Vinas, I. (2003). Liquid formulation of the biocontrol agent *Candida sake* by modified water activity or adding protectants. *Journal of Applied Microbiology*. 94: 330-339.

Ünlu, G., Nielsen, B. and Ionita, C. (2015). Production of antilisterial bacteriocins from lactic acid bacteria in dairy-based media: a comparative study. *Probiotics & Antimicrobial Proteins*. 7 (4): 259-274.

Van Loon, L.C., Bakker, P.A. and Pieterse, C.M. (1998). Systemic resistance induced by rhizosphere bacteria. *Annual Review of Phytopathology*. 36: 453-483.

Ward, N., Eisen, J., Fraser, C. and Stackebrandt, E. (2001). Sequenced strains must be saved from extinction. *Nature*. 414: 148.

Weselowski, B., Nathoo, N., Eastman, A.W., Macdonald, J. and Yuan, Z.C. (2016). Isolation, identification and characterization of *Paenibacillus polymyxa* CR1 with potentials for biopesticides, biofertilization, biomass degradation and biofuel production. *BMC Microbiology*. 16:244-254.

Wong, S., Kabeir, B.M., Mustafa, S., Mohamad, R., Hussin, A.S.M. and Manap, M.Y. (2010). Viability of *Bifidobacterium Pseudocatenulatum* G4 after spray-Drying and Freeze-Drying. *Microbiology Insights*. 3: 37–43.

Yánez-Mendizábal, V., Viñas, I., Usall, J., Torres, R., Solsona, C., Abadias, M. and Teixido, N. (2012). Formulation development of the biocontrol agent *Bacillus subtilis* strain CPA-8 by spray-drying. *Journal of Applied Microbiology*. 112 (5): 954-965.

Yanti, N.A., Ahmad, S.W. and Muhiddin, N.H. (2018). Evaluation of inoculum size and fermentation period for bacterial cellulose production from sago liquid waste. *Journal of Physics: Conference Series.* 1116: 1- 7.

Yusuf, N., Fazi, S. M., Ali, N. A., and Fauzi, N. I. (2016). Effects of *Colletotrichum capsici* infection on the growth and antioxidative response on defense mechanisms of *Capsicum annuum*. *Malaysian Journal of Microbiology*, 12(6), 455–462.

Zahan, K.A., Pa'e, N. and Muhamad, I.I. (2014). Process parameters for fermentation in a rotary disc reactor for optimum microbial cellulose production using response surface methodology. *BioResources*. 9 (2): 1858-1872.

Zayed, G. and Roos, Y.H. (2004). Influence of trehalose and moisture content on survival of *Lactobacillus salivarius* subjected to freeze-drying and storage. *Process Biochemistry*. 39: 1081-1086.

Zhan, Y., Xu, Q., Yang, M.M., Yang, H.T., Liu, H.X., Wang, Y.P. and Guo, J.H. (2011). Screening of freeze-dried protective agents for the formulation of biocontrol strains, *Bacillus cereus* AR156, *Burkholderia vietnamiensis* B418 and *Pantoea agglomerans* 2Re40. *Applied Microbiology*. 54 (1): 10-17.

Zhang, D., Gao, T., Li, H., Lei, B. and Zhu, B. (2017). Identification of antifungal substances secreted by *Bacillus subtilis* Z-14 that suppress *Gaeumannomycesgraminis* var. *triciti*. *Biocontrol Science and Technology*. 27: 237-251.

Zhao, G. and Zhang, G. (2005). Effect of protective agents, freezing temperature, rehydration media on viability of malolactic bacteria subjected to freeze-drying. *Journal of Applied Microbiology*. 99: 333- 338.

Zeigler, D.R. (2013). The family Paenibacillacea. *In*: Strain catalog and reference. Columbus: Bacillus Genetic Stock Center.