https://doi.org/10.48048/tis.2022.6315

Bioformulation Development of *Bacillus velezensis* Strain N1 to Control Rice Bacterial Leaf Blight

Nattina Sawatphanit[1], Waraporn Sutthisa[1,*] and Tantima Kumlung[2]

[1]Department of Biology, Faculty of Science, Mahasarakham University, Maha Sarakham 44150, Thailand
[2]Expert Centre of Innovative Agriculture, Thailand Institute of Scientific and Technological Research, Pathum Thani 12120, Thailand

([*]Corresponding author's e-mail: waraporn.s@msu.ac.th)

Received: 11 October 2021, Revised: 17 November 2021, Accepted: 27 November 2021, Published: 31 October 2022

Abstract

Bacterial leaf blight is a serious disease of rice caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). The objective of this study was to develop a bioformulation of *Bacillus velezensis* strain N1 by using microencapsulation techniques through spray drying and to then evaluate its performance in controlling rice bacterial leaf blight under greenhouse conditions. Three formulations with a different main carrier were studied and the viability after spray drying, moisture content, characteristic of formulation powder under scanning electron microscope and storage stability were examined. The results showed that formulation 3 (10 % skimmed milk, 20 % maltodextrin, 0.06 % sodium alginate and 5 % tapioca starch) had the highest antagonistic bacterial viability after spray drying of 1.24×10^7 CFU/g. Formulation 2 had the highest antagonistic bacterial viability of 1.44×10^6 CFU/g and had the lowest viability reduction of 0.61 Log CFU/g after storage at room temperature for 3 months. Moisture content of each formulation was less than 4 %. Among the 3 formulations of strain N1 bacteria against *Xoo*, formula 2 was able to control rice bacterial blight disease with the highest disease reduction index (45.87 %). All the results indicated that *Bacillus velezensis* strain N1 can be an effective antagonist and could potentially be used to develop a more successful bioformulation against rice bacterial leaf blight.

Keywords: Biological control, Microencapsulation, Scanning electron microscope, Spray drying, *Xanthomonas oryzae*

Introduction

Bacterial leaf blight (BLB) is a vascular rice disease, caused by the gram-negative bacteria *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). It is the major rice disease in Asia and has also been reported in Northern Australia, Africa and the United States [1,2]. Temperatures ranging between 25 to 34 °C and high relative humidity provide conditions for high susceptibility [3,4]. The disease can occur during all stages of rice growth, especially the tillering stage [1]. High level of infection with bacterial leaf blight leads to decreased rice yield and quality. Management of the disease employs various general strategies to decrease epidemic plant diseases such as cultural practice, varieties resistance, but mainly involves the use of chemical pesticides. However, these chemicals are high cost, lead to toxic residues, environmental pollution, and risk to human health, and lead to effects on the food chain of animals [5].

Bacteria as biological control agents represent an alternative in plant disease protection that are highly target-specific, environmentally friendly and cost-effective [6]. Antagonistic bacteria are biocontrol agents for controlling plant disease with a different mode of action such as secondary metabolites, inducing resistance in the host plant, and competition for growth [7,8]. Several antagonist bacteria have been studied for their ability to act against bacterial leaf blight, such as *Lysobacter antibioticus* 13-1, *Bacillus amyloliquefaciens* FZB42, *Serratia nematodiphila* CT-78, *Pseudomonas aeruginosa* BRp3 and *Paenibacillus polymyxa* Sx3 [9-13].

In agriculture, the endospore-forming bacteria, *Bacillus* spp. have mostly been used as biocontrol agents. This genus can produce secondary metabolites and several biological activities that improve plant growth and are active against plant disease [7]. Endospores are resistant to high temperatures and extreme conditions. Therefore, endospore-forming bacteria have been seen as attractive targets for bioformulation development. *Bacillus* pp. bioformulation for commercial application involved both aqueous solution or

dry formulation. The formulation is usually produced as a powder because it is easy to handle, convenient for use, and has a prolonged shelf-life [14-16].

Microencapsulation of microbial cells is an immobilization process to increase cell protection by surrounding active bacteria with suitable materials of small particle size around 1 - 1000 µm leading to extended stability and long shelf-life. Various methods have been used including extrusion, spray drying, emulsification and coacervation [35]. Techniques used to produce microbial powder by drying have used various methodologies but the most utilized is the spray dry technique because it can produce good quality powder, can be scaled-up, and is low cost at industrial scale [17,18]. These processes have been very successfully used for probiotics in the food industry [20,21] and other bioformulations in agriculture [14,16,18]. Suitable materials in the formulation are very important as they must not be harmful to the microbial cells. There is ongoing research developing suitable materials for bioformulation, mostly using polymers such as alginate, starch, milk protein and polyvinyl alcohol [20].

B. velezensis strain N1 can act against bacterial leaf blight of rice [22]. Therefore, stain N1 is interesting to develop into a bioformulation product. The objectives of this study were to develop the bioformulation of *B. velezensis* strain N1 with different carriers. The characteristics properties of formulation powder were evaluated and the efficiency of *B. velezensis* strain N1 bioformulation against bacterial leaf blight was investigated in glasshouse conditions.

Materials and methods

Bacteria antagonist and pathogen

Bacteria antagonist, *Bacillus* spp. strain N1 used in this study was obtained from the Expert Centre of Innovative Agriculture, Thailand Institute of Scientific and Technological Research. The culture was identified as *B. velezensis* by 16S rRNA gene sequence analysis [22] and activated on nutrient agar composed of 1.5 g/L beef extract, 1.5 g/L yeast extract, 5 g/L bacteriological peptone, 0.5 % NaCl and 15 g/L agar at 30 °C prepared 24 h before use.

The bacterial leaf blight disease pathogen of rice, *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) was obtained from Plant Protection Research and Development office, Department of Agriculture, Thailand and was activated on nutrient glucose agar. Bacteria antagonists and *Xoo* were stored in 20 % glycerol at –20 °C the long-term storage.

Screening of bioformulation carriers

Nutrient broth (NB) medium was prepared with separate addition of commercial grade carrier of 10 % skimmed milk, 20 % maltodextrin, 0.06 % sodium alginate, or 10 % corn starch and 10 % tapioca starch. Suspension *B. velezensis* strain N1 (10^8 CFU/mL) was used. The control without any carrier was used. All treatments were incubated for 48 h at 30 °C and the bacteria count was investigated by using serial dilution and plate count techniques.

Table 1 Components of *Bacillus velezensis* strain N1 bioformulation.

Carriers	Formulation 1	Formulation 2	Formulation 3
Skimmed milk	10 %	10 %	10 %
Maltodextrin	20 %	20 %	20 %
Sodium alginate	0.06 %	0.06 %	0.06 %
Corn starch	-	5 %	-
Tapioca starch	-	-	5 %

Preparation of bioformulation by spray drying and viability of *B. velezensis* strain N1 bioformulation

The cultivation of *B. velezensis* strain N1 was prepared with modified nutrient broth medium (1.5 g/L yeast extract, 1.5 g/L beef extract, 5 g/L bacteriological peptone, 20 g/L sucrose, 0.5 % NaCl and minerals solution including 0.5 g/L $CaCl_2 \cdot 2H_2O$, 0.045 g/L $FeSO_4 \cdot 7H_2O$, 1 g/L KCl, 0.5 g/L $MgSO_4 \cdot 7H_2O$ and 0.03 g/L $MnCl_2$) at 30 °C for 96 h. Cells were harvested by centrifugation at 7000 rpm for 10 min and washed with 0.85 % NaCl. The 3 suspension formulations (**Table 1**) contained 1×10^9 CFU/mL bacteria suspension and were spray-dried by a laboratory spray dryer (Buchi mini spray dryer B-290, Switzerland). Conditions of spray drying: Used an air-inlet temperature of 145 °C. The powder was collected from the product vessel. Formulated powders were estimated after spray drying of viable antagonist bacteria by using dilution and

plate count techniques. The viable bacteria were cultured in NA and incubated at 30 °C for 24 h. Colonies were counted and estimated as CFU/g.

Determination of moisture content

One g of formulation powder was placed in an aluminum foil container and dried at 105 °C in the hot air oven for 3 h. The sample was transferred to a desiccator immediately and allowed to cool down and the weight recorded. Moisture content (%) was calculated using the formula:

$$\frac{((W2 - W1) - (W3 - W1))}{(W2 - W1)} \times 100 \quad (1)$$

Where W1 is the weight of an aluminum foil container,
W2 is the weight of the formulation in aluminum foil before drying,
W3 is the weight of the formulation in aluminum foil after drying.

Scanning electron microscope

The sample was prepared on carbon tape attached to a brass stub and coated with gold. Morphological bioformulation powder was observed with a scanning electron microscope (Jeol 6480LV, Japan) at 20 kV and 600×.

Evaluation of *B. velezensis* strain N1 bioformulation storage stability

The 3 formulations were stored in an aluminum foil bag and kept at room temperature (25 - 30 °C) for 3 months. The viability of antagonist bacteria after storage was estimated for every 30 days using serial dilution and plate count techniques.

Efficiency of *B. velezensis* strain N1 bioformulation against bacterial leaf blight in glasshouse conditions

Rice cultivar Khao Dawk Mali 105 (KDML105) was planted at a density of 3 plants in each pot size 15×30 cm^2 and grown for 21 days. Rice plants were inoculated with *Xoo* suspension (OD600 nm 1.0 or 10^8 CFU/mL) by a clipping method and then covered with a plastic bag for 24 h before applying any treatments. The experiment was performed in a completely randomized design (CRD) with 10 replications. Foliar spraying treatments were as follows: 1) Formulation 1 (2.5 % w/v), 2) Formulation 2 (2.5 % w/v), 3) Formulation 3 (2.5 % w/v), 4) Fresh cell *B. velezensis* strain N1 (10^8 CFU/mL), 5) Zinc thiazole (500 ppm) and 6) dH_2O as control. Daily checks were performed to identify symptoms of bacterial leaf blight until 14 days after *Xoo* inoculation. The efficiency of *B. velezensis* strain N1 formulation against bacterial leaf blight was evaluated with using disease incidence and disease severity. The disease score using 9 scales followed the Standard Evaluation System of IRRI as follows: 0 = no symptoms, 1 = 1 - 5 %, 3 = 6 - 12 %, 5 = 13 - 25 %, 7 = 26 - 50 % and 9 = 51 - 100 % of infected leaves area respectively. Disease incidence, disease severity, and disease reduction were calculated using the following formulae [23]:

$$\text{Disease incidence} = \frac{\text{number of bacterial leaf blight infected}}{\text{total number of plants}} \times 100 \quad (2)$$

$$\text{Disease severity} = \frac{\sum(\text{bacterial leaf blight scale} \times \text{number of disease in that scale})}{(\text{total number of plants that investigated} \times 9)} \times 100 \quad (3)$$

$$\text{Disease reduction} = \frac{(\text{Disease severity of control} - \text{Disease severity of treatment})}{(\text{Disease severity of control})} \times 100 \quad (4)$$

Statistics analysis

The data was analyzed using mean and standard deviations by 1-way analysis of variance (ANOVA) and Duncan's multiple range tests ($P \leq 0.05$). All data was evaluated by SPSS (Windows version 23).

Results and discussion

Screening of bioformulation carriers

The present study developed bioformulations of *B. velezensis* strain N1 to deliver shelf-life stability and potential for controlling of rice bacterial leaf blight. The composition of bioformulation is important role and usually used a natural component that causes less damage to bacteria antagonists. The effect of each carrier on the viability of *B. velezensis* strain N1 after cultivation with each component for 48 h at 30 °C and the number of antagonist bacteria were determined by dilution plate count method. All components did not inhibit the viability of *B. velezensis* strain N1. Ten % skimmed milk had the highest bacterial counts of 9.05×10^6 CFU/mL and with statistically significant differences compared to other components (**Table 2**). Therefore, all carriers could be used for bioformulation development.

Table 2 Effect of each carrier on the viability of *B. velezensis* strain N1.

Carrier	Yield (CFU/mL)
Skimmed milk 10 %	9.05×10^6
Maltodextrin 20 %	1.95×10^6
Corn starch 10 %	1.15×10^6
Tapioca starch 10 %	3.55×10^6
Sodium alginate 0.06 %	1.05×10^6
Nutrient broth (Control)	1.00×10^6

Preparation of bioformulation by spray drying and viability of B. *velezensis* strain N1 bioformulation

The survival ability of *B. velezensis* strain N1 after spray drying with different carrier compositions of 3 formulations is shown in **Table 3**. The viability was dependent on the yield of antagonist bacteria. Formula 3 (10 % skimmed milk, 20 % maltodextrin, 0.06 % sodium alginate and 5 % tapioca starch) provided the highest yield antagonist bacteria after spray drying of 1.24×10^7 CFU/g. The number of viable cells after spray drying in formulae 1 and 2 were 1.50×10^6 and 5.90×10^6 CFU/g, respectively. These results indicated that tapioca starch protected cells from high temperatures during spray drying because it has high amylopectin content and a highly branched polymer enhances strong protection. Native tapioca starch and modified tapioca starch are mostly used as materials for microencapsulation in food and pharmaceutical industry [24-26].

Microencapsulation of microbial cells has been achieved using various technologies but spray drying is the most widely used and accepted method in commercial settings [18]. It has been used in the efficient production of probiotic products [20,27,28]. A carrier is necessary for protection and provides stability to the cells. A variety of carriers have been evaluated such as skimmed milk, maltodextrin, gum arabic, tapioca starch, corn starch, sodium alginate, chitosan and hydroxypropyl methylcellulose [16,18,21,29,30]. Skimmed milk is used to preserve bacterial cells for long shelf-life and against death of cells [30]. Using a combination of skimmed milk and $MgSO_4$ for *B. subtilis* strain CPA-8 formulation development resulted in a good bacteria survival around 28 - 30 % [16]. *Lactobacillus acidophilus* FTDC 3081 using maltodextrin as a protective agent had the highest viability in both spray drying and freeze-drying processes, which can reduce the absorption of moisture and prevent stickiness during storage [32]. Sodium alginate is a film-forming polymer that can protect cells and also improve the release rate. This increases the efficiency of bacterial cell encapsulation [33,34]. A combination of sodium alginate and soy protein isolate as materials for the microencapsulated *Meyerozyma guilliermondii* obtained high viability of 98 % after spray drying processing [34]. The appropriate spray drying conditions are important. Excessive evaporation damages the cell structure of bacteria, depending on the bacterial strain. The air outlet temperature is critical because the bioformulation is exposed to it in during the spray drying process for longer than at the air inlet temperate. Levels of air outlet temperature were controlled by air inlet temperature, product feed rate, carrier composition and air flow [21,29]. In addition, the factors needed to obtain successful microorganism formulation include persistent storage stability, provide effective control to target and convenient to handle and apply [35].

Table 3 Effect of 3 formulation with different carrier composition after spray drying.

Formulations	Moisture content (%)	Viability yield (CFU/g)
1	3.65	1.50×10^6
2	0.45	5.90×10^6
3	1.50	1.24×10^7

Determination of moisture content

The 3 formulations with the different carriers had moisture contents ranging from 0.45 to 3.65 % after spray drying (**Table 3**). These results showed that the formulation that contained starch had low moisture especially corn starch. Corn starch has high lipid content which can reduce the capacity for water absorption [26]. Air inlet temperature is the main factor for controlling residual moisture content as the high moisture content in bacterial products acts to decrease bacterial viability [36,37]. In previous studies, microencapsulation of *B. subtilis* B99-2 showed that a high air inlet temperature reduced moisture contents but decreased cell viability [18]. However, low air inlet temperature led to poor flow rate during processing and high stickability of powder [38]. The drying needs to be carefully adjust to a higher level. Moisture contents less than 4 - 5 % is acceptable for stability storage [39,40].

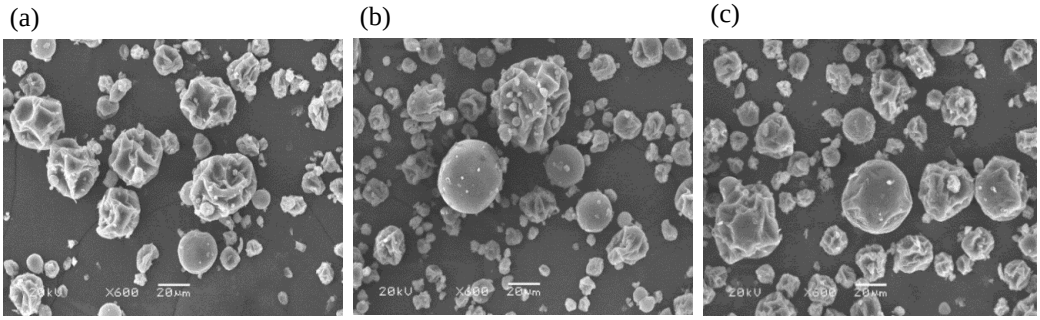

Figure 1 The morphology of *B. velezensis* strain N1 bioformulation particles: Formulation 1 (a), formulation 2 (b) and formulation 3 (c) under scanning electron microscope at 20 kV and 600×.

Scanning electron microscope

The morphology of the bioformulations particles was observed under a scanning electron microscope (**Figure 1**). Their particle sizes were in the range of 8 to 43 µm, spherical, with both smooth and wrinkled surfaces. The wrinkled appearance was caused by rapid evaporation during spray drying when using air inlet temperatures up to 155 °C, which can cause a crack in the particles that oxygen can diffuse into and is toxic to cells [41].

Evaluation of *B. velezensis* strain N1 bioformulation storage stability

Table 4 shows the viability of *B. velezensis* strain N1 in 3 bioformulations after 3 months of storage at room temperature. Formula 2 (10 % skimmed milk, 20 % maltodextrin, 0.06 % sodium alginate and 5 % corn starch) showed the highest yield of antagonistic bacteria with 1.44×10^6 CFU/g and had the lowest viability reduction of 0.61 Log CFU/g after storage at room temperature for 3 months. This result suggested that the bioformulation containing corn starch was efficient to protect cells during storage, the stability is probably caused by low moisture content. The moisture content should be low to prevent damage to bacteria. Extend shelf-life of *Saccharomyces cereviciae* using spray drying was reported that corn starch was able to maintain viability of bacteria, obtained viability reduction only 1.68 % after storage at 180 days [42]. Storage temperature and packaging are factors that affect bacterial viability and stability during storage. Spray drying production of the probiotic *B. amyloliquefacien* strain H57 was reported to have the best shelf life when stored at a refrigerated temperature at 4 °C [43]. Furthermore, *B. subtilis* strain CPA-8 formulation was reported to have good storage stability at both 4 and 25 °C for 6 months [16]. Plant disease management is not feasible using formulations stored in cold conditions because it will increase production costs.

Table 4 Viability of *B. velezensis* strain N1 in bioformulation after storage.

Formulations	Viability yield (CFU/g)			Viability reduction (Log CFU/g)
	1 month	2 months	3 months	
1	5.75×10^5	7.65×10^4	4.55×10^4	1.52
2	5.00×10^6	1.68×10^6	1.44×10^6	0.61
3	4.95×10^6	1.07×10^6	9.05×10^5	1.14

Efficiency of *B. velezensis* strain N1 bioformulation against bacterial leaf blight in glasshouse conditions

The efficacy of *B. velezensis* strain N1 bioformulation against rice bacterial leaf blight is presented in **Table 5**. Disease symptoms appeared within 48 to 72 h after inoculation with pathogen. After 14 days, the results showed that formulation 2 gave the highest inhibition efficiency with a disease a reduction index of 45.87 % followed by formulation 3, fresh cell, formulation 1 and zinc thiazole with disease reduction indexes 36.42, 23.43, 22.83 and 11.42 %, respectively. Bioformulation containing starch may increase the opportunity for bacterial antagonists to adhere to the plant leading to increased protection from the pathogen. The application and duration of bioformulation used can be adjusted to improve the inhibition. Many studies have shown that *B. velezensis* has the ability to control various bacterial and fungal plant diseases [44-48]. *B. velezensis* was a potential and efficient biocontrol agent because it can produce antimicrobial active compounds including iturin, surfactin, bacillomycin-D, fengicin, bacillibactin, macrolactin, bacillaene difficidin and bacilicin [49-52]. It was reported that, the antibacterial compounds difficidin and bacilysin from *B. amyloliquefaciens* FZB42 could potentially suppress *X. oryzae* pv. *oryzae* [10]. The results of this study suggested that bioformulation can maintain viable antagonist bacteria cells that have an activity to control bacterial leaf blight disease.

Table 5 The efficacy of 3 *B. velezensis* strain N1 bioformulation against rice bacterial leaf blight under greenhouse conditions.

Treatments	Disease incidence (%)	Disease severity (%)	Disease reduction (%)
Formulation 1 (2.5 % w/v)	96.67 ± 7.03b	72.59 ± 18.92bc	22.83
Formulation 2 (2.5 % w/v)	91.67 ± 14.16ab	50.93 ± 21.01a	45.87
Formulation 3 (2.5 % w/v)	86.67 ± 26.99a	59.81 ± 27.32ab	36.42
Fresh cell *B. velezensis* strain N1 (10^8 CFU/mL)	98.33 ± 5.27ab	72.04 ± 19.97bc	23.43
Zinc thiazole (500 ppm)	98.33 ± 5.27ab	83.33 ± 18.48cd	11.42
Water (control)	100 ± 00.00b	94.07 ± 11.34d	-

Note: Mean of each treatment followed by standard deviation and different letter indicated significant differences between each treatment by Duncan's Multiple Range Test (DMRT) at $P \leq 0.05$.

Conclusions

This study has shown the microencapsulation of *B. velezensis* strain N1 containing starch after spray drying at air inlet temperature 145 °C resulted in high cell viability when compared with the formulation without starch. Especially, corn starch contributed to protecting cells from residual moisture content in formulations resulted in a longer shelf-life after 3 months of storage at room temperature. Three bioformulations showed ability against rice bacterial blight disease under greenhouse conditions. These bioformulations can improve method to provide greater viability and stability for commercially available products. The information from this research could be beneficial for future development and commercialization of the product.

Acknowledgements

The authors would be highly grateful to the Expert Centre of Innovative Agriculture, Thailand Institute of Scientific and Technological Research, and Mahasarakham University, Thailand for the support of research equipment and financially. The authors are grateful to Adrian Roderick Plant for making editorial comments on the manuscript.

References

[1] SS Gnanamanickam, VB Priyadarisini, NN Narayanan, P Vasudevan and S Kavitha. An overview of bacterial blight disease of rice and strategies for its management. *Curr. Sci*. 1999; **77**, 1435-44.

[2] P Velusamy, JE Immanuel, SS Gnanamanickam and L Thomashow. Biological control of rice bacterial blight by plant-associated bacteria producing 2,4-diacetylphloroglucinol. *Can. J. Microbiol*. 2006; **52**, 56-65.

[3] JB Joshi, L Arul, J Ramalingam and S Uthandi. Advances in the *Xoo*-rice pathosystem interaction and its exploitation in disease management. *J. Biosci.* 2020; **45**, 112.

[4] W Shi, C Li, M Li, X Zong, D Han and Y Chen. Antimicrobial peptide melittin against *Xanthomonas oryzae* pv. *oryzae*, the bacterial leaf blight pathogen in rice. *Appl. Microbiol. Biotechnol*. 2016; **100**, 5059-67.

[5] P Chanprapai and W Chavasiri. Antimicrobial activity from *Piper sarmentosum* Roxb. against rice pathogenic bacteria and fungi. *J. Integr. Agric.* 2017; **16**, 2513-24.

[6] J Shafi, H Tian and M Ji. *Bacillus* species as versatile weapons for plant pathogens: A review. *Biotechnol. Biotechnol. Equip*. 2017; **31**, 446-59.

[7] D Fira, I Dimkić, T Berić, J Lozo and S Stanković. Biological control of plant pathogens by *Bacillus* species. *J. Biotechnol.* 2018; **285**, 44-55.

[8] Y Sha, Q Wang and Y Li. Suppression of *Magnaporthe oryzae* and interaction between *Bacillus subtilis* and rice plants in the control of rice blast. *SpringerPlus* 2016; **5**, 1238.

[9] GH Ji, LF Wei, YQ He, YP Wu and XH Bai. Biological control of rice bacterial blight by *Lysobacter antibioticus* strain 13-1. *Biol. Control* 2008; **45**, 288-96.

[10] L Wu, H Wu, L Chen, X Yu, R Borriss and X Gao. Difficidin and bacilysin from *Bacillus amyloliquefaciens* FZB42 have antibacterial activity against *Xanthomonas oryzae* rice pathogens. *Sci. Rep*. 2015; **5**, 12975.

[11] NĐ Khoa, NĐN Giàu and TQ Tuấn. Effects of *Serratia nematodiphila* CT-78 on rice bacterial leaf blight caused by *Xanthomonas oryzae* pv. *oryzae*. *Biol. Control* 2016; **103**, 1-10.

[12] S Yasmin, FY Hafeez, MS Mirza, M Rasul, HMI Arshad, M Zubair and M Iqbal. Biocontrol of bacterial leaf blight of rice and profiling of secondary metabolites produced by *Rhizospheric Pseudomonas aeruginosa* BRp3. *Front. Microbiol.* 2017; **8**, 1895.

[13] Y Abdallah, M Yang, M Zhang, MMI Masum, SO Ogunyemi, A Hossain, Q An, C Yan and B Li. Plant growth promotion and suppression of bacterial leaf blight in rice by *Paenibacillus polymyxa* Sx3. *Lett. Appl. Microbiol*. 2019; **68**, 423-9.

[14] X Meng, J Yu, M Yu, X Yin and Y Liu. Dry flowable formulations of antagonistic *Bacillus subtilis* strain T429 by spray drying to control rice blast disease. *Biol. Control* 2015; **85**, 46-51.

[15] IB Sorokulova, AA Krumnow, S Pathirana, AJ Mandell and V Vodyanoy. Novel methods for storage stability and release of *Bacillus* spores. *Biotechnol. Prog*. 2008; **24**, 1147-53.

[16] V Yánez-Mendizábal, I Viñas, J Usall, R Torres, C Solsona, M Abadias and N Teixidó. Formulation development of the biocontrol agent *Bacillus subtilis* strain CPA-8 by spray-drying. *J. Appl. Microbiol*. 2012; **112**, 954-65.

[17] A Gharsallaoui, G Roudaut, O Chambin, A Voilley and R Saurel. Applications of spray-drying in microencapsulation of food ingredients: An overview. *Food Res. Int*. 2007; **40**, 1107-21.

[18] X Ma, X Wang, J Cheng, X Nie, X Yu, Y Zhao and W Wang. Microencapsulation of *Bacillus subtilis* B99-2 and its biocontrol efficiency against *Rhizoctonia solani* in tomato. *Biol. Control* 2015; **90**, 34-41.

[19] NLV Braber, LID Vergara, YE Rossi, CA Aminahuel, AN Mauri, LR Cavaglieri and MA Montenegro. Effect of microencapsulation in whey protein and water-soluble chitosan derivative on the viability of the probiotic *Kluyveromyces marxianus* VM004 during storage and in simulated gastrointestinal conditions. *LWT* 2020; **118**, 108844.

[20] C Desmond, RP Ross, E O'Callaghan, G Fitzgerald and C Stanton. Improved survival of *Lactobacillus paracasei* NFBC 338 in spray-dried powders containing gum acacia. *J. Appl. Microbiol.* 2002; **93**, 1003-11.

[21] S Rathore, PM Desai, CV Liew, LW Chan and PWS Heng. Microencapsulation of microbial cells. *J. Food Eng*. 2013; **116**, 369-81.
[22] N Sawatphanit, W Sutthisa and T Kumlung. Screening of antagonistic bacteria for inhibition of rice bacterial leaf blight caused by *Xanthomonas oryzae* pv. *oryzae*. *In*: Proceedings of ARUCON 2020, Phra Nakhon Si Ayutthaya, Thailand. 2020, p. 549-55.
[23] RD Hastuti, Y Lestari, A Suwanto and R Saraswati. Endophytic *Streptomyces* spp. as biocontrol agents of rice bacterial leaf blight pathogen (*Xanthomonas oryzae* pv. *oryzae*). *HAYATI J. Biosci*. 2012; **19**, 155-62.
[24] JD Hoyos-Leyva, LA Bello-Pérez, J Alvarez-Ramirez and HS Garcia. Microencapsulation using starch as wall material: A review. *Food Rev. Int.* 2018; **34**, 148-61.
[25] J Loksuwan. Characteristics of microencapsulated β-carotene formed by spray drying with modified tapioca starch, native tapioca starch and maltodextrin. *Food Hydrocoll.* 2007; **21**, 928-35.
[26] JJM Swinkels. Composition and properties of commercial native starches. *Starch-stärke* 1985; **37**, 1-5.
[27] Z Radulović, J Miočinović, N Mirković, M Mirković, D Paunović, M Ivanović and S Seratlić. Survival of spray-dried and free-cells of potential probiotic *Lactobacillus plantarum* 564 in soft goat cheese. *Anim. Sci. J.* 2017; **88**, 1849-54.
[28] V Reyes, A Chotiko, A Chouljenko, V Campbell, C Liu, C Theegala and S Sathivel. Influence of wall material on production of spray dried *lactobacillus plantarum* NRRL B-4496 and its viability at different storage conditions. *Dry. Technol*. 2018; **36**, 1738-48.
[29] V Reyes, A Chotiko, A Chouljenko and S Sathivel. Viability of *Lactobacillus acidophilus* NRRL B-4495 encapsulated with high maize starch, maltodextrin, and gum arabic. *LWT* 2018; **96**, 642-7.
[30] A Teera-Arunsiri, M Suphantharika and U Ketunuti. Preparation of spray-dried wettable powder formulations of *Bacillus thuringiensis*-based biopesticides. *J. Econ. Entomol*. 2003; **96**, 292-9.
[31] WL Cody, JW Wilson, DR Hendrixson, KS McIver, KE Hagman, CM Ott, CA Nickerson and MJ Schurr. Skim milk enhances the preservation of thawed –80 °C bacterial stocks. *J. Microbiol. Methods*. 2008; **75**, 135-8.
[32] HW Tang, S Abbasiliasi, P Murugan, YJ Tam, HS Ng and JS Tan. Influence of freeze-drying and spray-drying preservation methods on survivability rate of different types of protectants encapsulated *Lactobacillus acidophilus* FTDC 3081. *Biosci. Biotechnol. Biochem.* 2020; **84**, 1913-20.
[33] L Yonekura, H Sun, C Soukoulis and I Fisk. Microencapsulation of *Lactobacillus acidophilus* NCIMB 701748 in matrices containing soluble fibre by spray drying: Technological characterization, storage stability and survival after *in vitro* digestion. *J. Funct. Foods* 2014; **6**, 205-14.
[34] R López-Cruz, JA Ragazzo-Sánchez and M Calderón-Santoyo. Microencapsulation of *Meyerozyma guilliermondii* by spray drying using sodium alginate and soy protein isolate as wall materials: A biocontrol formulation for anthracnose disease of mango. *Biocontrol Sci. Technol.* 2020; **30**, 1116-32.
[35] S Gasic and B Tanovic. Biopesticide formulations, possibility of application and future trends. *Pestic. Phytomed*. 2013; **28**, 97-102.
[36] S Behboudi-Jobbehdar, C Soukoulis, L Yonekura and I Fisk. Optimization of spray-drying process conditions for the production of maximally viable microencapsulated *L. acidophilus* NCIMB 701748. *Dry. Technol.* 2013; **31**, 1274-83.
[37] Z Shokri, MR Fazeli, M Ardjmand, SM Mousavi and K Gilani. Factors affecting viability of *Bifidobacterium bifidum* during spray drying. *Daru* 2015; **23**, 7.
[38] AKAS Brun-Graeppi, C Richard, M Bessodes, D Scherman and OW Merten. Cell microcarriers and microcapsules of stimuli-responsive polymers. *J. Control Release* 2011; **149**, 209-24.
[39] E Anantal, SE Birkeland, B Corcoran, G Fitzgerald, S Hinz, A Klijn, J Mättö, A Mercernier, U Nilsson, M Nyman, E O'Sullivan, S Parche, N Rautonen, RP Ross, M Saarela, C Stanton, U Stahl, T Suomalainen, JP Vincken and D Knorr. Processing effects on the nutritional advancement of probiotics and prebiotics. *Microb. Ecol. Health Dis.* 2004; **16**, 113-24.
[40] SH Peighambardoust, AG Tafti and J Hesari. Application of spray drying for preservation of lactic acid starter cultures: A review. *Trends Food Sci. Technol*. 2011; **22**, 215-24.
[41] ME Rodríguez-Huezo, R Durán-Lugo, LA Prado-Barragán, F Cruz-Sosa, C Lobato-Calleros, J Alvarez-Ramírez and EJ Vernon-Carter. Pre-selection of protective colloids for enhanced viability of *Bifidobacterium bifidum* following spray-drying and storage, and evaluation of aguamiel as thermoprotective prebiotic. *Food Res. Int*. 2007; **40**, 1299-306.

[42] A Chandralekha, AH Tavanandi, N Amrutha, HU Hebbar, KSMS Raghavarao and R Gadre. Encapsulation of yeast (*Saccharomyces cereviciae*) by spray drying for extension of shelf life. *Dry. Technol.* 2016; **34**, 1307-18.

[43] P Luangthongkam, JA Blinco, P Dart, M Callaghan and R Speight. Comparison of spray-drying and freeze-drying for inoculum production of the probiotic *Bacillus amyloliquefaciens* strain H57. *Food Bioprod. Process*. 2021; **130**, 121-31.

[43] EM Myo, B Liu, J Ma, L Shi, M Jiang, K Zhang and B Ge. Evaluation of *Bacillus velezensis* NKG-2 for bio-control activities against fungal diseases and potential plant growth promotion. *Biol. Control* 2019; **134**, 23-31.

[44] H Calvo, I Mendiara, E Arias, AP Gracia, D Blanco and ME Venturini. Antifungal activity of the volatile organic compounds produced by *Bacillus velezensis* strains against postharvest fungal pathogens. *Postharvest Biol. Technol.* 2020; **166**, 111208.

[45] Y Cao, H Pi, P Chandrangsu, Y Li, Y Wang, H Zhou, H Xiong, JD Helmann and Y Cai. Antagonism of two plant-growth promoting *Bacillus velezensis* isolates against *Ralstonia solanacearum* and *Fusarium oxysporum*. *Sci. Rep*. 2018; **8**, 4360.

[46] D Guo, C Yuan, Y Luo, YH Chen, M Lu, G Chen, G Ren, C Cui, J Zhang and D An. Biocontrol of tobacco black shank disease (*Phytophthora nicotianae*) by *Bacillus velezensis* Ba168. *Pestic. Biochem. Physiol*. 2020; **165**, 104523.

[47] CH Jiang, XF Yao, DD Mi, ZJ Li, BY Yang, Y Zheng, YJ Qi and JH Guo. Comparative transcriptome analysis reveals the biocontrol mechanism of *Bacillus velezensis* F21 against *Fusarium* wilt on watermelon. *Front. Microbiol.* 2019; **10**, 652.

[48] XH Chen, A Koumoutsi, R Scholz, A Eisenreich, K Schneider, I Heinemeyer, B Morgenstern, B Voss, WR Hess, O Reva, H Junge, B Voigt, PR Jungblut, J Vater, R Süssmuth, H Liesegang, A Strittmatter, G Gottschalk and R Borriss. Comparative analysis of the complete genome sequence of the plant growth-promoting bacterium *Bacillus amyloliquefaciens* FZB42. *Nat. Biotechnol.* 2007; **25**, 1007-14.

[49] Y Liu, K Teng, T Wang, E Dong, M Zhang, Y Tao and J Zhong. Antimicrobial *Bacillus velezensis* HC6: Production of three kinds of lipopeptides and biocontrol potential in maize. *J. Appl. Microbiol.* 2020; **128**, 242-54.

[50] C Pandin, DL Coq, J Deschamps, R Védie, T Rousseau, S Aymerich and R Briandet. Complete genome sequence of *Bacillus velezensis* QST713: A biocontrol agent that protects *Agaricus bisporus* crops against the green mould disease. *J. Biotechnol.* 2018; **278**, 10-9.

[51] MF Rabbee, MS Ali, J Choi, BS Hwang, SC Jeong and KH Baek. *Bacillus velezensis*: A valuable member of bioactive molecules within plant microbiomes. *Molecules* 2019; **24**, 1046.