Formulation of a Novel Antagonistic Bacterium Based Biopesticide for Fungal Disease Control Using Microencapsulation Techniques

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Abstract

Bacillus cereus strain C1L, screened from the rhizosphere of *Lilium formosanum*, prevents severe lily leaf blight. The purpose of this study was to formulate a new biopesticide by microencapsulating the C1L strain to enhance residual stability. In addition, since the viable cell number of *Bacillus cereus* C1L is an important factor affecting the biological effects of this fungicide, this study also used the response surface methodology and sequential quadratic programming methods to optimize the best formula for maximum viability of *Bacillus cereus* C1L microencapsulated by spray drying. Optimization results revealed that the strain could maintain 42% viability with 18.3% maltodextrin and 12.5% gum arabic as coating materials and spray drying at an outlet temperature of 73.5 ºC. A comparison with chemical pesticides showed that maneb was the most efficient disease inhibitor, followed by *B. cereus* C1L encapsulated by spray drying. The chemical pesticide probenazole and *B. cereus* C1L encapsulated by extrusion revealed no significant differences in disease control.

Keywords: microencapsulation, biopesticide, spray drying, *Bacillus cereus*, response surface methodology

1. Introduction

Bacillus cereus, a large, gram-positive and endospore-forming bacterium, is very common in plants and soils (Martinez, Michaud, Belanger, & Tweddell, 2002; Huang, Wang, Chung, & Chen, 2005). Several studies have reported that antifungal compounds of *B. cereus* strains are beneficial biological control agents in the suppression of crop disease. *B. cereus* B4 produces three types of metabolites (kanosamine, 3, 4-dihydroxy benzoate, and 2 keto-4 methylthiobutyrate) that allow this strain to inhibit certain plant diseases and help plant growth (Sunaina, 2005). *B. cereus* DGA34 naturally produces the antibiotic zwittermicin A, an effective antibiotic against a wide range of bacteria and fungi to reduce symptoms of damping-off disease and root rot (Handelsman, Jacobson & Stabb, 1998). *B. cereus* UW85, generating two antibiotics (zwittermicin A and antibiotic B) (Silo-Suh, Lethbridge, Raffle, He, Clardy, & Handelsman, 1994), has been proven to be a dependable biocontrol agent, which protects cucumber fruits from rot caused by *Pythium aphanidermatum*, alfalfa seedlings from dampening off due to *Phytophthora medicaginis*, tobacco seedlings from *Phytophthora nicotianae* and peanuts from *Sclerotinia minor* (Silo-Suh et al., 1994). However, only few *B. cereus* strains have current United States patents as biocontrol agents for crop diseases.

To popularize the use of biopesticides, the components of a biopesticide formulation must: (1) ensure stability during biopesticide production, processing and storage, (2) support application, (3) protect the biopesticide from a harsh environment and (4) increase biopesticide activity at the target (Jones & Burges, 1998; Hynes & Boyetchko, 2006). Encapsulation has been recently applied in biopesticidal formulations. This technique not only provides protection of residues from unfavorable environments, but also improves their stability because of release-control formulations (Brar, Verma, Tyagi, & Valéro, 2006). Starch encapsulating particles have been successfully scaled up, but the residual activity decreased under UV radiation and rain (Shasha & McGuire, 1992). Ramos, Mcguire, and Galan (1998) indicated that the maize flour formulation offers protection from UV radiation and improves fast release of *B. thuringiensis* (Bt) during rainfall. Alginate encapsulated Bt was also

applied in mosquito control (Winder, Wheeler, Conder, Otvos, Nevill, & Duan, 2003). However, few papers have discussed the biopesticide formulation of *B. cereus* strains.

Among microencapsulation methods, spray-drying, which has low operation costs and high production rate, is most widely used in the food, pharmaceutical and chemical industries (Gibbs, Kermasha, Alli, & Mulligan, 1999). Yu (1990) reported that spray drying is an important process in the production of Bt powder. However, the high inlet temperatures required to evaporate water in the spray drying chamber might damage the activities of residues and exert a negative impact in the spray-dried product (Ananta, Volkert, & Knorr, 2005).

In this experiment, we focused our attention on the *Bacillus cereus* strain C1L, screened from the rhizosphere of *Lilium formosanum*, because it has been proven superior to other *Bacillus* strains in prevention of severe lily leaf blight. Similar plant protection was also observed in two oriental lily cultivars, 'Star Gazer' and 'Acapulco' (Huang et al., 2005). This characteristic is unique among the known strains of *B. cereus*. In addition, the C1L strain promotes plant growth as shown by increases in the chlorophyll content, leaf width and length, and plant freshness and dry weight (Huang et al., 2010). Treatment with a bacterial suspension of *B. cereus* C1L reduces the level of hydrogen peroxide in corn leaves (Huang et al., 2005). Thus, the purpose of this research was to further formulate this novel antagonistic bacterium by microencapsulation techniques. The results of our previous study indicated that higher C1L counts are necessary for better antagonistic activity (Huang et al., 2010). Therefore, the response surface methodology (RSM) and the sequential quadratic programming (SQP) methods were used to maximize C1L viabilities during the spray-drying process. In addition, alginate coated C1L formulations were also evaluated.

2. Method

2.1 Bacillus cereus C1

Bacillus cereus C1L (ATCC PTA 11411), isolated from the rhizosphere of lily plants, was cultured in Luria-Bertani (LB) broth (Bertani 1951) at 180 rpm at 28ºC for 24 hr and stored at 4ºC. This strain was cultivated every two weeks (Huang et al., 2005). For encapsulation, C1L was harvested by centrifugation (3000×g, 10 min at 4ºC), washed and re-suspended in saline solution after cultured in LB broth. The final bacterial numbers were adjusted to 10^9 CFU mL^{-1}.

2.2 C1L Microencapsulation Using Spray Drying

To maximize C1L viability during spray-drying encapsulation, optimization techniques were conducted in present study. The whole concept includes the five steps listed below.

2.2.1 Screening Test

To evaluate the influences of coating materials and spray drying conditions on the survival of C1L, two coating materials (gum arabic and maltodextrin, Jen-Fong Inc., Taipei, Taiwan) and different spray drying conditions (outlet temperature and flow rate) were evaluated. The C1L viability and moisture contents of powder products were determined.

2.2.2 Experimental Design Using Box Behnken Design

Experimental design preceded the beginning of the trials. For the Box Behnken Design (BBD, Box and Behnken, 1960), it was presumed that the C1L viability is affected by the concentration and type of the coating materials and spray-drying conditions, in this case gum arabic, maltodextrin and outlet temperature (three independent variables). A three-independent-variable BBD with five center points was designated to construct the response surface models (Table 1). The Box-Behnkin Design (BBD), an efficient option for fitting response surfaces using 3 evenly spaced levels, is a three-level design based on the construction of a balanced incomplete block design. The number of experiments (N) required for the establishment of BBD is defined as N=2k(k−1) +C$_0$, where k is number of factors and C$_0$ is the number of central points (Myers & Montgomery, 1995).

2.2.3 Production of the Microcapsules

C1L microcapsules were prepared based on the BBD revealed in Table 1 (17 combinations of encapsulated conditions) by mixing 1% (vv^{-1}) of C1L with maltodextrin (0-25%) and gum arabic (0-25%). The mixture was then subjected to a laboratory-scale spray dryer (Eyela Spray Dryer SD 1000, Rikakikai Co., Tokyo, Japan). Outlet temperatures of 60-100ºC were employed. Viability of the microencapsulated cells, defined as the response, was determined (Table 1).

Table 1. Box-Behnkin design matrix with one response

Treatment	Variables (coded)			Response		
	X_1	X_2	X_3	Cell counts before drying (Log CFU/g)	Cell counts after drying (Log CFU/g)	Viability (%)
1	100 (1)	12.5 (0)	25.0 (1)	9.97	8.75	6.03
2	80 (0)	12.5 (0)	12.5 (0)	9.66	9.20	34.67
3	80 (0)	12.5 (0)	12.5 (0)	9.53	9.07	34.88
4	80 (0)	25.0 (1)	0.0 (-1)	10.02	8.87	7.08
5	80 (0)	12.5 (0)	12.5 (0)	9.72	9.26	34.68
6	60 (-1)	0.0 (-1)	12.5 (0)	9.71	7.71	1.02
7	80 (0)	0.0 (-1)	25.0 (1)	9.68	7.98	2.00
8	100 (1)	25.0 (1)	12.5 (0)	9.82	8.42	3.98
9	80 (0)	12.5 (0)	12.5 (0)	9.45	8.99	34.67
10	100 (1)	0.0 (-1)	12.5 (0)	10.08	8.38	2.00
11	60 (-1)	12.5 (0)	25.0 (1)	9.09	7.87	6.03
12	80 (0)	25.0 (1)	25.0 (1)	9.56	8.16	3.98
13	100 (1)	12.5 (0)	0.0 (-1)	9.59	7.89	2.03
14	60 (-1)	25.0 (1)	12.5 (0)	9.86	9.40	34.68
15	80 (0)	0.0 (-1)	0.0 (-1)	9.87	5.05	0.00
16	60 (-1)	12.5 (0)	0.0 (-1)	9.94	8.24	1.99
17	80 (0)	12.5 (0)	12.5 (0)	9.87	9.41	33.68

[*]Treatment were run in a random order

2.2.4 Modeling and Optimization of the Spray-Drying Conditions

Statistical regression analysis was carried out, based on the BBD experimental results (Table 1), to construct the response surface model, which was then formulated as an optimization problem and solved using a sequential quadratic programming (SQP) technique to derive the optimal formula for the C1L microcapsules. The modeling and optimization procedures were implemented in a similar way to the work reported by Chen, Kuo, Shiu, and Chen (2011).

2.2.5 Model Verification

Experiments based on the optimal composition of the coating materials and spray-drying condition that were found by RSM and SQP, were performed and repeated three times. ANOVA of the results were then calculated using the SAS software package (SAS Institute, Ver. 9.1 for Windows, 2003), with Duncan's multiple range test to detect differences between predicted and observed values.

2.3 C1L Microencapsulation Using Alginate

The extrusion method was used to manufacture the alginate microcapsules based on the work of Chen, Chen, and Kuo (2007).

2.4 Analysis Methods

2.4.1 Moisture Content

Moisture content was conducted by heating encapsulated C1L for 5 hours at 105°C in an oven. Results were presented as percentage on dry matter.

2.4.2 The C1L Survival Rate after Encapsulation

The method to release the entrapped C1L from the microcapsules was based on the work of Chen et al. (2007). C1L were cultured by LB agar (48 h at 28°C). The cell number, in colony-forming units (CFU), was recorded. The following equation was used to calculate the survival rate:

$$\text{Survival rate (\%)} = (\text{CFU after spray drying} / \text{CFU before spray drying}) \times 100 \qquad (1)$$

2.4.3 Induced Systemic Resistance (ISR) Test

Corn seeds (MeeHsin No 3, Known-You Seed Inc., Kaohsiung, Taiwan) were embedded into 60 mL pots containing allocated soil. The plants were watered regularly for 28 days until they were ready for the ISR test.

C1L suspension (10^8 cfu mL^{-1}) was watered into the soil of a four-week-old plant. Two days after inoculation, plants were challenged by spraying the leaves with a suspension of the pathogen *Cochliobolus heterostrophus* Drechsler (ATCC 48331, Turgeon et al., 1993). Disease severity was assessed by the percentage of leaves with disease symptoms per plant after 3-day challenge. The disease index was categorized into five scales from 0 to 4 as follows: level 0: no lesions, level. 1: 1-25%, level 2: 26-50%, level 3: 51-75%, level 4: more than 75% of the leaf area infected. For the fungicides, maneb (manganese ethylene bisdithiocarbamate, Sinon Co. Taichun, Taiwan) and probenazole (3-allyloxy-1, 2-benzisothiazole-1, 1-oxide, Sinon Co. Taichun, Taiwan) were used as recommended on the product label.

2.4.4 Yield

Yield is expressed as the weight percent of product obtained with respect to the weight of polymer and cell added to the solvent mixture to be sprayed.

2.4.5 Microstructure of the C1L Capsules

The microstructures of the C1L capsules were observed by scanning electron microscope (SEM, JSM-6300, JEOL Ltd., Japan) based on the method of Chen et al. (2007).

2.5 Statistical Analysis

Statistical analysis was carried out by Duncan's multiple range test using Statistical Analysis Systems software (SAS Institute, Ver. 9.1 for Windows, 2003). All experiments were repeated three times.

3. Results

3.1 Effects of Spray-Drying and Carriers on C1L Survival

The effects of different inlet temperatures, outlet temperatures and flow rates on the viability of C1L are shown in Table 2. A decrease in C1L viability was noticed as the inlet and outlet air temperature was increased. The moisture contents of the capsules were between 2.09% and 6.47%, with the lower moisture being found in those capsules fabricated at a higher inlet temperature. Although the flow rate showed a minor influence in C1L viability and moisture content, it significantly affected the product yields. The product yield increased with increasing flow rates.

Table 2 The effect of different inlet temperatures and flow rates on the viability of C1L

(a) Temperature

T_{inlet} (°C)	T_{outlet} (°C)	Viability (%)	Moisture content (%)
150	60	30.40[a]	6.47[a]
190	70	31.42[b]	5.31[b]
210	80	29.17[c]	4.59[c]
230	90	8.32[d]	3.62[d]
250	100	4. 71[d]	2.09[e]

(b) Flow rate

Flow rate (m^3/min)	Viability (%)	Moisture content (%)	Yield (%)
400	26.18	5.30[a]	22.0[e]
500	27.11	4.65[bc]	41.1[d]
600	26.32	5.45[a]	48.0[c]
700	27.98	4.88[b]	61.9[a]
800	27.65	4.53[c]	56.4[b]

[a-e] Symbols bearing different letters are significantly different ($p < 0.05$) according to Duncan's new multiple range test.

The types and concentrations of carriers also significantly affected C1L survival during spray drying (Table 3a). When the levels of gum arabic increased, the percentage of surviving C1L decreased. Increasing maltodextrin concentrations from 5% to 20% resulted in increases in the percentage of surviving C1L from 1.80% to 27.32%. However, further increasing the maltodextrin level above 20% caused a reduction in survival after drying. Twenty percent maltodextrin or 10% arabic provided the best survival rate.

Table 3. The effect of carriers on the viability of C1L

(a) Spray drying

Carriers	Maltodextrin		Gum Arabic	
	Viability	Moisture content	Viability	Moisture content
(%)	(%)	(%)	(%)	(%)
0	-	-	-	-
5	1.80[e]	10.30[a]	18.50[b]	10.84[c]
10	8.21[d]	8.48[c]	20.20[a]	13.66[a]
15	24.12[ab]	7.26[d]	16.14[b]	11.24[bc]
20	27.32[a]	10.28[a]	9.45[c]	10.95[c]
25	17.76[c]	9.67[b]	7.72[c]	11.77[b]

(b) Extrusion

Alginate (%)	Viability (%)
1	33.54[b]
2	81.62[a]
3	80.51[a]

a-d Symbols bearing different letters are significantly different (p < 0.05) according to Duncan's new multiple range test.

3.2 Optimization on the Survival of C1L after Spray Drying

The above results demonstrated that the cell numbers of the C1L strain was significantly influenced by outlet temperature and types and concentrations of carriers. A combination of these parameters might deliver a better survival rate for C1L under spray drying. Thus, the response surface modeling was used to develop a prediction model for the survival rate of C1L after spray drying (as a function of outlet temperature and concentrations of the two carriers), and then the SQP technique was utilized to locate the global maximum of the mathematical function.

3.2.1 Checking the Fitted Model

Screening tests determined that the appropriate ranges were 60-100 °C for the outlet temperature and 0-25% for both maltodextrin and gum arabic. The results from the survival rates of C1L after spray drying based on the BBD (Table 1), were used to build the response surface models, and then model analysis and the lack-of-fit test (Table 4) were examined. The model analysis checked the legitimacies of the linear, quadratic and cubic models for the response in accordance with their F-values, with P-values below 0.05 considered as significant. The significant polynomial of the highest order was selected. The lack-of-fit test, assessing the fitness of the model, was employed to compare the pure and residual errors at the replicate design points. If the lack-of-fit was significant, indicated by a low probability value (P > F), the corresponding response predictor was discarded. A model without significant lack-of-fit was selected. For cubic model (Table 4), the P value of model analysis is higher than 0.5. It was not fitted to the experimental data and therefore discarded. The analysis showed that the second-order model for C1L viability was well fitted to the experimental data and the model is given in Equation (2).

$$f = -0.61 + 0.044\ X_1 + 0.08\ X_2 - 0.17\ X_3 - 4.327\times10^{-4}\ X_1^2 + 1.052\times10^{-3}X_2^2 + 4.125\times10^{-4}X_3^2 - 7.903\times10^{-5}X_1\ X_2 + 1.068\times10^{-3}X_1\ X_3 - 5.265\times10^{-3}X_2\ X_3 \qquad (2)$$

Table 4. Model analysis, lack of fit tests and R - square analysis of the viability of C1L after spray drying

		Model	Lack-of-fit	R-square
		$P^a > F$	$P^a > F$	
	Linear	0.0011*	0.0973	0.6970
Viability	Quadratic	0.0001*	0.1196	0.9923
	Cubic	0.0601	-	0.9986

* Significant at 5% level

[a] P: Probability value

3.2.2 Optimization Using SQP

In order to find the optimal formula for the coating materials and the outlet temperature for encapsulated C1L in Equation (2), a global optimization program code equipped with a multi-start SQP procedure (Chen et al., 2007) was executed to locate the global optimum. At the commencement of the optimization sequence, the code randomly generated a series of uniformly distributed initial points, and then applied SQP repeatedly to catch the optimum point based on each initial point. The global optimum was considered obtained if the probability for finding the global optimum surpassed the preset value of 99.99% in this study. Otherwise, another initial point was randomly generated again and the SQP re-executed. After 8 sets of initial points and enactments of SQP, the global optimum of the viability model was found to be 42%. The optimal conditions for the spray-drying are X_1 (outlet temperature) = 73.5ºC and X_2 (maltodextrin) = 18.7% and X_3 (gum arabic) = 12.5%. Figure 1 shows the response surface and its maximum for the C1L viability (*f*) with a fixed level of gum arabic at 12.5%, displaying the effects of inlet temperature and maltodextrin.

Figure 1. Response surface plots of the viability of *B. Cereus* C1L after formulation by spray-drying showing the effects of inlet temperature and maltodextrin under a constant level of 12.5% gum Arabic

3.2.3 Experimental Verification

The optimal combination of carriers for maximum C1L survival and the outlet temperature were derived using SQP and verified by additional independent experiments. The optimal mixture of carriers was 18.7% maltodextrin blended with 12.5% gum Arabic, and the optimal outlet temperature was 73.5ºC. The result of the

verification experiment was very close to the predicted values (P > 0.05) with no significant differences (Table 5).

Table 5. Validation of the optimal composition model recommended by SQP for the viability of B. cereus C1L after spray-dried microencapsulation

Treatment	Viability (%)	
	Pred[b]	Exp[c]
Optimal value[a]	47±2	48±1

[a]Optimal value: addition of 18.7% maltodextrin and 12.5% gum arabic rehydrating at 73.5°C.

[b]Pred: predicted value.

[c]Exp: experimental value.

3.3 C1L Microencapsulation Using Alginate

Table 3(b) shows the C1L viability of encapsulation using extrusion techniques. Increasing the alginate concentration from 1% to 2% resulted in an increase in the percentage of survival of C1L from 33% to 81%. However, further increasing the alginate level above 2% caused no change in survival. The bacterial counts of CL1 alginate capsules were 8.8×10^6 - 5.1×10^7 CFU/g.

3.4 The Microstructure of Microcapsules

Scanning electron microscopy was used to examine the structure of the microcapsules produced by both spray drying and extrusion. The spray-dried beads had a mean size of 10 ± 2 μm and a rounded external surface (Figure 2a) containing concavities and indentations due to the drying process. On the other hand, the alginate microcapsules were smooth and highly dense surface spherical particles (Figure 2b) with a mean size of 1.0 ± 0.4 mm.

Figure 2. Scanning electron micrograph of a *B. cereus* C1L microcapsule made by (a) spray drying and (b) extrusion

3.5 Green House Experiment

With whole plants grown in a greenhouse, a suspension of living C1L made corn grow better than other treatments did (Table 6). The disease was the most severe in the controls (Disease index 2.82). The Fungal disease index was 0.92 in plants treated with the chemical pesticide maneb, following by *B. cereus* C1L encapsulated by spray drying (Disease index 1.56). The disease indexes for probenazole and C1L alginate capsules were 2.26 and 2.43, respectively, with no significant difference. These observations indicate that *B. cereus* C1L encapsulated by spray drying could effectively control leaf blight in corn and is more effective than the probenazole and C1L alginate capsules.

Table 6. Control experiment of *B. cereus* C1L in green house

Treatment	Disease index*
B. cereus C1L powder	1.56 ± 0.69^c
B. cereus C1 microcapsule	2.43 ± 0.50^b
Menbe	0.92 ± 0.70^d
Probenazole	2.26 ± 0.69^b
Control	2.82 ± 0.51^a

[a-d] Symbols bearing different letters are significantly different ($p < 0.05$) according to Duncan's new multiple range test.

4. Discussion

An effective delivery system plays an important role for successful biological control. Formulation is recognized as one of the most important priorities in biopesticide research (Castillejos et al., 2002). In this study, two encapsulation techniques were used to formulate C1L. The survival of various bacterial strains affected by spray-drying have been investigated previously (O'Riordan, Andrews, Buckle, & Conway, 2001; Lian, Hsiao, & Chou, 2002; Lian, Hsiao, & Chou, 2003; Picot & Lacroix, 2003, 2004). The inlet and outlet temperatures of the spray dryer, the various polysaccharides used as the matrix and the moisture content of the microcapsules are considered as key factors influencing the survival rates of microorganisms. In this study, heat damage was the main factor for a decrease of C1L viability. Similar results were obtained with spray-dried probiotics powders and *B. thuringiensis* (Desmond, Ross, O'Callaghan, Fitzgerald, & Stanton, 2002; Brar et al., 2006). With decreases of inlet and outlet air temperatures, the survival in the powder increases. Brian and Mark (1997) validated that the survival of *Brevibacterium linens* attained 100% when the outlet air temperature was maintained below 57ºC. The drying temperature cannot be infinitely decreased, however, as the moisture content of the powder increases greatly. Zhou, Dong, Gao, and Yu (2008) indicated that the outlet temperature should be kept above 65 ºC to avoid wet powder.

Microencapsulation of C1L with carriers significantly improves the viability during spray drying. Different coating materials demonstrated diverse thermal conductivity and diffusivity. Thus, it is reasonable to believe that the materials evaluated in this study may possess different degrees of protection on the encapsulated C1L when exposed to heat inactivation during spray-drying. This study also found effects from the levels of the carriers on C1L survival. Increasing the level of gum Arabic or other soluble starches from 10% to 20% or more decreased in viabilities of the tested microorganism after drying, in accordance with observations in other studies (Lian et al., 2002). Lian et al. (2002) indicated that higher solid content in the matrix results in larger beads leading to greater heat damage than smaller ones.

To search for optimal encapsulation conditions using spray dryer with a high C1L viability, a response surface model was established to define the combined effect of the independent variables. SQP was also conducted to search for optimal results. Chen et al. (2007, 2011) indicated that combination of RMS and SQP was an effective way to obtain the optimal manufacturing conditions for a new product development. In this study, a combination of 18.7% maltodextrin and 12.5% gum arabic as coating materials yielded the highest C1L survival under spray drying.

For extrusion, the ranges of sodium alginate and calcium chloride to form the beads are between 1% - 3% alginate and 0.05-1.5 M $CaCl_2$, respectively (Prevost & Divies, 1988; Kearney, Upton, & Loughlin, 1990; Cui, Goh, Kim, Choi, & Lee, 2000; Chandramouli, Kailasapathy, Peiris, & Jones, 2004; Krasaekoopt, Bhandar, & Deeth, 2004). However, low concentration of alginate (0.6% alginate with 0.3 M $CaCl_2$) was also applied to form beads (Jankowski, Zielinska, & Wysakowska, 1997). The major benefits of extrusion methods using alginate are that the viscosity of the matrix does not limit the production of the beads (Prüsse, Dalluhn, Breford, & Vorlop, 2000) and the biological core materials can be preceded at low temperatures without damaging their bioactivities. The survival rate of alginate encapsulated *Bacillus cereus* C1L was significantly higher than that of maltodextrin/gum arabic *Bacillus cereus* C1L (Table 3), but the final bacterial numbers of both capsules were around 10^6-10^7 cfu/g. This is because that the alginate microcapsules were made only 1% (v/v) of culture concentrate mixing with alginate solution. The bacterial number was diluted from 10^9 to 10^7 CFU/g.

In order to be biologically effective, the biopesticide has to be released from the beads. The release behaviors are controlled by several parameters such as bead size, wall thickness, wall material and amount of core material (Tsuji, 2001). Schmidt, Lorenz, Wolf, and Jager (2001) reported that a Ca-alginate formulation of *Bacillus* spp. effectively reduced plant disease. In this study, however, encapsulating C1L by spray drying was more effective in inhibition of *Cochliobolus heterostrophus* Drechsler than the alginate microcapsule. Possibly, this is due to the smaller particle size and fast release behavior of the spray-dried capsules. The particle size of the alginate capsules is highly relying on the concentration of the sodium alginate solution, the extruder orifice diameter, and the distance between the calcium chloride collecting solution and the syringe (Smidsrod & Skjaok-Brk, 1990). Releasing the core materials from alginate gels needs to sequester calcium ions. Thus, alginate beads provided better protection from rain and UV radiation but have a slow release behavior due to the large particle size and solubility, whereas gum arabic and maltodextrin have better biological effects in inhibition of *Cochliobolus heterostrophus* Drechsler. For better biological efficacy, a combination of these two different beads may lead to development of a new biopesticide with reduction in application dosage and longer application intervals.

Knowledge of comparability of C1L with other chemical pesticides is needed. Depending on the encapsulation methods, the effectiveness of the spray-dried C1L beads was comparable with or even surpassed the chemical pesticide probenazole. Probenazole has been widely used to protect plants against the fungus *Magnaporthe grisea* in Asia. Maneb is a fungicide used to prevent harvested crops from deterioration in storage or transportation. Although maneb has the best biological effect in inhibition of *Cochliobolus heterostrophus* Drechsler, this fungicide belongs to one of ethylene- bisdithiocarbamates ('EBDCs') which are instable in harsh environment.

5. Conclusions

In this study, a coating material, a mixture of 18.7% maltodextrin and 12.5% gum arabic, was blended with the C1L culture, and then the mixture was dehydrated by spray drying with an outlet temperature at 73.5ºC. This formula yielded the highest C1L survival. The effectiveness of the spray-dried C1L beads was comparable with the chemical pesticide probenazole. According to our best knowledge, this is the first paper studying microencapsulated biopesticides using a novel optimization technique.

Acknowledgements

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