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Optimization of the complex lyoprotectant composition for the high viability of *Penicillium chrysogenum* ACCC 30395 conidia

Myong-Gyong Pae
Kim Il Sung University
Chung-Jin Choe
Kim Il Sung University
Thae-Sok Kang
3499606241@qq.com

SukChon University
Tong-Ryul Kim
Kim Il Sung University
Tae-Gil Kim
Kim Il Sung University
Chol-Song Kim
Kim Il Sung University
Hye -Gyong Mun
Kim Il Sung University
Jong-Su Kim
Kim Il Sung University
Un-Hyang Ho
Kim Il Sung University
Chang-Ho Ri
Kim Il Sung University

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Abstract

The study aims to optimize the lyoprotectant composition to enhance the viability of lyophilized conidia of the penicillin-producing strain *Penicillium chrysogenum* ACCC 30395. Different concentrations of various lyoprotectants were tested to evaluate the effects on the viability of *P. chrysogenum* ACCC 30395 conidia after lyophilization and three lyoprotectants that showed significant protective effects were selected through one-factor-at-a-time experiments; 15% skim milk, 5% sucrose and 2% glycerol. The Box-Behnken design (BBD) was used to determine the optimal composition of the complex lyoprotectant containing these three lyoprotectants by Design Expert (version 8.0.6.1) software. The regression model was constructed by integrating the experimental data and analyzed by response surface methodology (RSM), which indicated that the maximum predictive value of conidia viability was 86.53% when the complex lyoprotectant was composed of 13.75% (w/v) skim milk, 3.28% (w/v) sucrose, and 2.02%(v/v) glycerol. Indeed, the survival rate of lyophilized conidia with the optimized complex lyoprotectant was 85.73%, which was close to the predicted value. This shows that the model can be effectively used to predict the optimal composition of the complex lyoprotectant for successful lyophilization.

1. Introduction

The filamentous fungus *Penicillium chrysogenum* is well known for the microorganism used in the industrial production of the β-lactam antibiotic benzylpenicillin. Therefore, it is important to maintain the penicillin production activity and stability of this strain for a long time(Macdonald 1972, Ibatsam, Rukhsana et al. 2012).

Lyophilization is suitable for preservation of biological samples, including bacteria, Actinomycetes, yeasts, and spores of fungi(Berny and Hennebert 1991, Abadias, Benabarre et al. 2001, Srivastava, Marjo et al. 2020). However, not all strains survive during the lyophilization. Spores, containing lower moisture content, tend to be less susceptible to freezing damage (Stephan and Zimmermann 2001). Therefore, one of the effective ways to preserve fungi species would be lyophilize the spores of the strain. Several factors, such as species (Carvalho, Silva et al. 2003), freezing rate (Berny and Hennebert 1991, Tan, Vlug et al. 1994), the size of spores, cell walls and the composition of complex lyoprotectant (Stephan, Da Silva et al. 2016), and rehydration conditions (King and Su 1993, Chotiko and Sathivel 2014) can affect the viability of lyophilized fungi.

One of the most important problems in the lyophilization of microorganisms is to choose the appropriate lyoprotectants, which may minimize the changes in the physical state of membrane lipids or diminish the changes in the structure of sensitive protein of the biological systems after lyophilization, thus improving the cell viability in lyophilization(Karunnanithy, Abdul Rahman et al. 2024). A variety of lyoprotectants have been used to reduce the damage by lyophilization (Carvalho, Silva et al. 2003, Bosnea, Kourkoutas et al. 2009, Tymczyszyn, Gerbino et al. 2011, Chotiko and Sathivel 2014). Saccharides and polyols have been reported to provide good protection for bacteria during lyophilization in the previous studies. They help maintain the native protein conformation in the absence of water by forming hydrogen bonds with protein molecules during drying (Hallsworth and Magan 1994). In addition, some studies found that combining skim milk with other lyoprotectants like glycerol or saccharides was effective in cryoprotecting different microorganisms, especially lactic acid bacteria (LAB) (Daily and Higgens 1973). The positive effect of skim milk during lyophilization can be explained by its capacity to protect the cell by stabilizing the cell membrane constituents and to create a porous structure in the lyophilized products that makes rehydration easier (Selmer-Olsen, Birkeland et al. 1999). Moreover, skim milk contains proteins that provide a protective coating for the cells during lyophilization(Abadias, Benabarre et al. 2001).

Although the one-variable-at-a-time approach has been used frequently to study the effect of many lyoprotectants on microorganisms (Santos, Gerbino et al. 2014), it could treat only single lyoprotectant at once and ignores the interactions between lyoprotectants, so it can result in confusion and a lack of predictive ability. Since multiple compounds in a mixture may produce additive effects, there are many examples to combine the use of rapidly penetrating and non-penetrating agents in the cryoprotection of microbial cells (Karunnanithy, Abdul Rahman et al. 2024).

Response surface methodology (RSM) is an efficient statistical technique for optimizing multiple variables to predict the best performance with minimum number of experiments. This statistical technique has been widely applied in many fields such as optimization of enzymatic reaction conditions (Gouda, Thakur et al. 2001, Colla, Primaz et al. 2016, Cardoso, de Freitas et al. 2020, Mamo, Kangwa et al. 2020), food processing conditions (Selwal, Selwal et al. 2011), medium composition (Peng, He et al. 2014) and moreover protective media of bacteria and yeasts (Berny and Hennebert 1991, Abadias, Benabarre et al. 2001, Lu, Huang et al. 2017, Han, Pu et al. 2018). However, it has not yet been reported the method to optimize the composition of complex lyoprotectant for enhancing the conidia viability of fungus *Penicillium chrysogenum* after lyophilization. The aim of the present work was to determine the optimum concentrations of three important lyoprotectants (skim milk, sucrose and glycerol) in complex lyoprotectant for *P.*

chrysogenum ACCC 30395 in order to maximize the conidia viability after lyophilization, and to determine the interactions between pairs of the selected factors by using RSM.

2. Materials and methods

2.1 Reagents

The fungus culture media reagents such as glycerol (Topbio, ZhaoYuan, China), sucrose (Sigma–Aldrich, Vienna, Austria), yeast extract (Merck, Darmstadt, Germany), and other salts (Aoboxing Bio-tech, Beijing, China) were used to grow the strain for obtaining conidia and to assess the viability after lyophilization.

The substances such as trehalose, sucrose, maltose, lactose, glucose (Sigma–Aldrich, Vienna, Austria), inositol (Oxoid, Hampshire, England), glycerol, sodium glutamate (Topbio, ZhaoYuan, China) and skim milk (DifcoTM, Australia) were used as the lyoprotectants.

2.2 Microorganisms and culture condition

P. chrysogenum ACCC 30395 was purchased from Agricultural Culture Collection of China (ACCC, Beijing, China). The fungus was stored at -20℃ until used. The fungus was inoculated to complex nutrient agar (glycerol 0.75%, sucrose 1%, yeast extract 0.3%, NaCl 0.9%, $FeSO_4 \cdot 7H_2O$ 0.0002%, K_2HPO_4 0.0006%, $MgSO_4 \cdot 7H_2O$ 0.0002%, $CaSO_4$ 0.025%, agar 2%, pH 6.2) and incubated at 25℃ observing the colony formation period for the strain, which range between 96h and 120h.

2.3 Preparation of the lyoprotectants and conidial suspensions

Lyoprotectants were prepared at different concentrations and sterilized at 115℃ for 30min with an autoclave. Conidia grown 14 days were harvested from the surface of agar with sterile inoculating loop. The conidia were suspended directly in the experimental lyoprotectants to achieve a concentration of approximately 4.3×10^8 CFU/mL. The suspension containing conidia in distilled water without any lyoprotectant was also tested as a control. The resulting conidial suspensions were spun with the vortex for 2 min to ensure homogeneity. Afterwards, conidia concentrations were determined using a Thoma haemocytometer. Initial concentration of conidia suspensions was adjusted to equal concentrations for all assays.

2.4 Lyophilization

1mL of conidial suspensions were pipetted in 7-mL vials, frozen at -20℃ for 2h and moved to -80℃, where kept for 2h. Afterwards, vials were moved to a lyophilizer (FD-1A-50, China) pre-frozen at -55℃ and lyophilized at the pressure of 1 ~ 10Pa for 48h. The lyophilized samples were kept in a refrigerator at 4℃ prior to determination of the number of viable conidia to make the samples to the original weight.

2.5 Viability assessment

The viability of the conidia was determined by serial dilution method. The harvested, frozen, and lyophilized conidia were dispersed in saline (0.85% NaCl). The samples were spun for 1min on the vortex, the solution was diluted serially and then final 30μL of the resulting suspension were spread on complex nutrient agar plates, which incubated at 25℃ until green of conidia were appeared on the colony. The colony colored green was considered as germinated conidia. By comparison numbers of germinated conidia before and after lyophilization, the viability was assessed. Survival rate of conidia was calculated by the following formula:

$$\text{Survival Rate}(\%) = \frac{\text{The number of germinated conidia after lyophilization}}{\text{The number of conidia before lyophilization}} \times 100$$

2.6 One-factor-at-a-time experiment of lyoprotectants

The influence of the following protective media at different concentration on the viability of lyophilized conidia was investigated respectively; glucose, sucrose, maltose, lactose, trehalose, sodium glutamate (2%, 5%, 8%, 12%, w/v), glycerol (1%, 2%, 3%, 4%, 5%, v/v), inositol (1%, 2%, 3%, 4%, 5%, w/v) and skim milk (5%, 10%, 15%, 20%, w/v). The viability of lyophilized conidia with all these protective media was contrasted to the one without any lyoprotectants.

2.7 Response surface methodology (RSM)

Three lyoprotectants, sucrose, glycerol and skim milk, which exhibited relatively noticeable effect on the strain protection during lyophilization and a mixed homogeneous test was designed with these three lyoprotectants to determine the optimal composition of the complex lyoprotectant for the maximum viability of lyophilized conidia of the strain. The Box-Behnken tool in Design Expert software (version 8.0.6.1) was used to design further experiments with three levels for each of three factors based on the results of one-factor-at-a-time experiments. Skim milk (A), sucrose (B), glycerol(C) were selected as independent variables and the viability of conidia was selected as the corresponding value. The coded level of variables and the experimental design were shown in Table 1 and Table 2, respectively. The software provided the regression model, which led ANOVA and graphic analysis based on experimental data. Each set of experiments repeated three times.

Table 1
Variables and the coded levels in the Box-Behnken design

Variable	unit	Symbol	Level		
			-1	0	1
Skim milk	%, w/v	A	10	15	20
Sucrose	%, w/v	B	2	5	8
Glycerol	%, v/v	C	1	2	3

3. Results

3.1 Effect of different lyoprotectants on the viability of the lyophilized conidia

Conidia survival rate of lyophilized *P. chrysogenum* ACCC 30395 with skim milk, sucrose, maltose, lactose, glucose, trehalose, inositol, sodium glutamate and glycerol as lyoprotectants, respectively, was shown in Fig. 1.

Fig .1 Conidia survival rate of lyophilized *P. chrysogenum* ACCC 30395 with various lyoprotectants

As shown in Fig. 1, the survival rate of lyophilized conidia with the lyoprotectant was higher than that of the control, 9.6%, regardless of the type of lyoprotectants. According to the result of one-factor-at-a-time experiments, when skim milk 15%(w/v), sucrose 5%(w/v), maltose 5%(w/v), lactose 5%(w/v), glucose 8%(w/v), trehalose 8%(w/v), sodium glutamate 5%(w/v), inositol 3%(w/v), glycerol 2%(v/v) were added as lyoprotectants respectively, the viability of lyophilized conidia were 43.90%, 38.43%, 19.86%, 22.46%, 17.33%, 24.96%, 15.06%, 23.83%, 34.56% during the lyophilization of the strain. Skim milk, sucrose and glycerol showed relatively significant protective effect were chosen as the composition of the complex lyoprotectant.

3.2 Optimization of the complex lyoprotectant composition

In order to find the optimal composition of skim milk, sucrose and glycerol level to maximize the survival rate of the lyophilized conidia, total of 17 experiments with different combinations were carried out according to the Table 2. The levels of these three independent variables are given in Table 1. Using multiple regression analysis on the experimental data, the following second order polynomial equation was obtained to explain the viability of lyophilized conidia of *P. chrysogenum* ACCC 30395.

$$\mathrm{Y} = 83.71 - 4.78 \times \mathrm{A} - 7.82 \times \mathrm{B} - 1.58 \times \mathrm{C} - 3.85 \times \mathrm{AB} - 2.01 \times \mathrm{AC} - 2.60 \times \mathrm{BC} - 5.24A^2 - 6.02B^2 - 9.17C^2 \quad (1)$$

where, Y (%) represents to the viability of lyophilized conidia. A, B and C are the coded values of skim milk, sucrose, and glycerol, respectively.

Table 2
The BBD experimental design matrix with actual and predicted values of the conidial survival rate of the lyophilized *P. chrysogenum* ACCC 30395

Run	A (Skim milk)	B(Sucrose)	C(Glycerol)	Conidial survival rate (%)	
				Actual	Predicted
1	0	1	-1	65.19	64.870
2	1	0	1	60.73	60.938
3	0	0	0	83.32	83.708
4	1	0	-1	68.42	68.113
5	0	0	0	84.15	83.708
6	-1	1	0	72.73	73.257
7	1	1	0	55.38	56.007
8	1	-1	0	79.87	79.343
9	0	0	0	83.86	83.708
10	0	-1	1	77.04	77.360
11	0	0	0	83.23	83.708
12	-1	-1	0	81.83	81.202
13	0	1	1	57.36	56.525
14	-1	0	1	74.21	74.517
15	0	-1	-1	74.48	75.315
16	0	0	0	83.98	83.708
17	-1	0	-1	73.85	73.642

The constant term of 83.71 represents the predicted baseline conidia viability when all three components are at their central levels, establishing a reference point for the experimental design. More significantly, the consistent negative coefficients across all linear terms (-4.78A, -7.82B, -1.58C) reveal a fundamental characteristic: increasing the concentration of any single lyoprotectant component independently reduces conidia viability within the tested experimental range. This pattern is most pronounced for sucrose (coefficient − 7.82), followed by skim milk (-4.78) and glycerol (-1.58), indicating that sucrose exerts the strongest individual negative effect on viability. The interaction effects further complicate this relationship, with all two-factor combinations showing negative coefficients. The skim milk-sucrose interaction (AB=-3.85) demonstrates the most substantial antagonistic effect, meaning that simultaneously increasing both components produces a synergistic negative impact on conidia viability. Similarly, the skim milk-glycerol (AC=-2.01) and sucrose-glycerol (BC=-2.60) interactions show additional negative synergistic effects, though to a lesser degree. Perhaps most importantly, the substantial negative quadratic coefficients (A^2=-5.24, B^2=-6.02, C^2=-9.17) reveal concave downward relationships for all three components, creating the curvature necessary for optimal point identification. This quadratic behavior demonstrates that each lyoprotectant has a specific optimal concentration range, with both insufficient and excessive amounts leading to reduced viability. Notably, glycerol exhibits the most pronounced quadratic effect (-9.17), indicating it has the narrowest optimal concentration window and requires the most precise dosage control. The comprehensive model structure explains why maximum viability occurs at intermediate concentrations rather than at the experimental boundaries. The combination of negative linear effects, antagonistic interactions, and strong quadratic terms creates a well-defined optimal region where the competing effects balance to produce peak performance. This sophisticated mathematical relationship underscores the necessity of precise formulation optimization, as the lyoprotectants interact in complex ways that cannot be predicted by simple one-variable-at-a-time experimentation. The model successfully captures the delicate equilibrium required for effective lyoprotection, where both component concentrations and their interactions must be carefully balanced to achieve maximum conidia viability after lyophilization.

An analysis of variance (ANOVA) of the fitted quadratic polynomial model was summarized in Table 3, in order to evaluate the significance and adequacy. The Model F-value of 296.56 implies the model is significant. There is only a 0.01% chance that an F-value

this large could occur due to noise. *P*-values less than 0.0500 indicate model terms are significant. In this case A, B, C, AB, AC, BC, A^2, B^2, C^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model. The Lack of Fit F-value of 6.39 implies there is a 5.25% chance that a Lack of Fit F-value this large could occur due to noise.

Table 3
ANOVA for the fitted quadratic polynomial model of the conidia viability of the lyophilized **P. chrysogenum ACCC 30395**

Source	Sum of Squares	Df	Mean Square	*F* value	*p* value Prob>F
Model	1483.21	9	164.80	296.56	<0.0001
A	182.60	1	182.60	328.58	<0.0001
B	489.22	1	489.22	880.35	<0.0001
C	19.85	1	19.85	35.71	0.0006
AB	59.21	1	59.21	106.55	<0.0001
AC	16.20	1	16.20	29.15	0.0010
BC	26.99	1	26.99	48.56	0.0002
A^2	115.40	1	115.40	207.66	<0.0001
B^2	152.60	1	152.60	274.61	<0.0001
C^2	354.08	1	354.08	637.16	<0.0001
Residual	3.89	7	0.56		
Lack of Fit	3.22	3	1.07	6.39	0.0525
Pure Error	0.67	4	0.17		
Cor Total	1487.10	16			

The terms of the model that had a value of $P_{model}>F$ was less than 0.05; in this case A, B, C, AB, AC, BC, A^2, B^2, C^2 were significant. This meant that skim milk, sucrose and glycerol were highly significant (P_A, P_B <0.0001, and P_C=0.0006), and the quadratic main effects of glycerol ($P_C{}^2$ <0.0001) were more significant than its first order effect (P_C=0.0006). Moreover, among the interaction effects, the most significant result was obtained by skim milk-sucrose interaction (P_{AB} <0.0001). The other interaction coefficients were also significant (P_{AC}= 0.0010 and P_{BC}=0.0002).

In order to visualize the interactions between the three variables in our study, the fitted response surface plots and their corresponding contour plots for conidia viability of lyophilized *P. chrysogenum* ACCC 30395 by the above model were generated using the Design expert software and were shown in Fig. 1-Fig. 3. The graphical models helped to understand both the interaction and the main effects of the variables on the response. Thus, elliptical shape of all three contour plots showing the trend of the mutual interactions between each two variables indicated that these three variables interacted with each other and produced combined synergic protective effects on the viability of lyophilized conidia.

By solving the equations derived by differentiating Eq. 1, the optimal concentrations for the independent variables investigated to obtain the maximum point of the model were skim milk 13.75%, sucrose 3.28% and glycerol 2.02% with the corresponding Y = 86.53%. Three replicates of verification experiments using optimized concentrations of the complex lyoprotectant (Table 4.) were carried out. The maximum viability was found to be 85.73 ± 0.78%, which is in close agreement with the model prediction. This suggested that the model was satisfactory and practicable for enhancing the viability of lyophilized conidia.

Table 4
Verification experiments using the complex lyoprotectant of optimized concentrations

Run	Actual value of viability(%)	Predicted value of viability(%)
1	85.1	86.53
2	86.6	
3	85.7	
Mean ± SD	85.73 ± 0.78	
Relative Error	0.92%	

4. Discussion

Lyophilization is a useful technique for the preservation of micro-organisms (Morgan, Herman et al. 2006). The major causes of cell death during lyophilization might be ice formation, membrane damage due to high osmolarity with high concentration of internal solutes, macromolecule denaturation, and removal of water, which affect properties of many hydrophilic macromolecules in cells (Thammavongs, Corroler et al. 1996). In order to reduce cell damage during the lyophilization, lyoprotectants are added during the preparation of the microbial suspensions. Lyoprotectants play an important role in the lyophilization process (Morgan, Herman et al. 2006). The lyoprotectants have two main functions: they protect the living cells biochemically against damage during lyophilization and provide a dry residue with defined physical structure acting as a support material and as receptor in rehydration (Berny and Hennebert 1991). When the conidia of *P. chrysogenum* ACCC 30395 was suspended in skim milk, sucrose, maltose, lactose, glucose, trehalose, inositol, sodium glutamate and glycerol, the viability increased significantly compared to conidia without protection. The higher concentrations of lyoprotectants were added, the more increased conidia viabilities were obtained firstly but decreased beyond a certain point. One explanation of our results could be that excessive concentrations of lyoprotectants may be rather detrimental to the preservation and rehydration of micro-organisms for many reasons such as accelerated coagulation of the proteins in the cell, formation of strong glassy structures, and lower solubility(Wang, Huang et al. 2022).

Different effects exhibited by lyoprotectants in this study might be related to their water-binding capacity and prevention of the formation of intra- and extra-cellular ice crystal (Nanasombat and Sriwong 2007). Glucose and trehalose have been used as lyoprotectants successfully (Conrad, Miller et al. 2000). Also, disaccharides like lactose and maltose are acting as a lyoprotectant, because of its ability to hydrate biological structures like proteins and membranes(Stanishevskaya, Silyukova et al. 2021). This effect is also called as water replacement hypothesis. Therefore, it is unlikely that glucose, trehalose, maltose and lactose did not provide significant protection rather than sucrose during lyophilization of *P. chrysogenum* ACCC 30395 conidia. Researchers reported when a Micrococcus strain was suspended in 5% saccharose, the viability increased to 79% compared to unformulated cells, which is consistent with our study (Tsvetkov and Brankova 1983).

Glycerol has been an effective cryoprotectant and widely used in frozen concentrates (Abadias, Benabarre et al. 2001). Glycerol is one of the permeable compounds which penetrate both the cell wall and the cytoplasmic membrane. It makes the cell membrane more plastic and binds water stably, which suppresses excess dehydration, reduces salt toxicity and prevents formation of ice crystals within the cell during lyophilization(Carvalho, Silva et al. 2003). Our study for lyophilization of the conidia of *P. chrysogenum* ACCC 30395 conveyed experimental evidence that presence of glycerol during the lyophilization of conidia could exhibit relatively higher protection effect. It was reported that inositol was effective in the lyophilization and the subsequent preservation of some bacterial species (Terakita, Matsunaga et al. 2009). However, our work confirmed that the protective effect of inositol on the lyophilization of conidia was not significant.

Several previous results showed that sodium glutamate provided protective effect on lyophilized LAB (De Valdez, de Giori et al. 1983, Carvalho, Silva et al. 2003, Zhou, Wu et al. 2024). The authors also claimed that the best survival rate of Lactobacilli could reach 65.5% by using the sodium glutamate as a lyoprotectant. The protection effect by sodium glutamate could be reached due to the stabilization of the protein structure by the reactions between amino groups of the lyoprotectant and the carboxyl groups of the microorganism proteins, and the ability to retain great amounts of residual moisture (de Valdez, De Giori et al. 1985). However, sodium glutamate did not show the significant protection in the lyophilization of *P. chrysogenum* ACCC 30395 conidia, which indicates that different species

and strains of micro-organisms may differ in their sensitivity to lyophilization provides the possibility that the protection mechanism could be differed due to the structures of membranes(Carvalho, Silva et al. 2003).

The observed positive effect of the skim milk on the viability of the lyophilized *P. chrysogenum* ACCC 30395 conidia confirms the results of previous studies (Palmfeldt and Hahn-Hägerdal 2000, Arakawa, Prestrelski et al. 2001). Some studies explained the positive effect of skim milk on the survival after lyophilization by the formation of a thin layer of milk protein over the cell wall proteins and additionally, calcium ions from the skim milk may contribute to the protective effect (King and Su 1993). The results of other researchers showed that skim milk is a sufficient lyoprotectant to enhance the storability of micro-organisms (Stephan and Zimmermann 2001, Zayed and Roos 2004).

A combination of lyoprotectants significantly increased the conidia viability obtained than individual treatments alone (Wang, Yu et al. 2019, Di, Ma et al. 2023, Karunnanithy, Abdul Rahman et al. 2024). Moreover, response surface methodology, which is a collection of statistical and mathematical techniques, was used to optimize a complex lyoprotectant for enhancing the conidia viability during lyophilization(Chen, Chen et al. 2015, Lu, Huang et al. 2017). There are many examples of studies on the optimization of the complex lyoprotectant for lyophilization of bacteria like LABs and yeast by using RSM (Han, Pu et al. 2018), but few studies for the conidia of filamentous fungi were reported (Berny and Hennebert 1991). To the best of our knowledge, there is few researches of optimizing the composition of a complex lyoprotectant for enhancing the conidia viability of lyophilized *P. chrysogenum* ACCC 30395 by using a BBD experiment.

Skim milk, sucrose and glycerol were selected to be among the most important factors that determined the conidia viability of lyophilized *P. chrysogenum* ACCC 30395 as the results of one-factor-at-a-time experiments. These three compounds interacted with each other (Fig. 2-Fig. 4). Our results were consistent to the observation of the study (Palmfeldt and Hahn-Hägerdal 2000), which indicated that a combination of different lyoprotectants enhances the viability after lyophilization. According to our study, rather low concentration of skim milk must be combined with similarly low level of sucrose in order to maximize conidia viability of lyophilized *P. chrysogenum* ACCC 30395 after lyophilization (Fig. 2), which confirms that skim milk and several sugars are suitable lyoprotectants for *P. chrysogenum* ACCC 30395. Previous works reported about improved protection effects of the combination of skim milk and saccharides on the viability of the lyophilized LAB formulations (Schwab, Vogel et al. 2007, Chen, Chen et al. 2015). The contour plots in Figs. 3 and 4 were very similar, implying the evident that the interaction between skim milk and glycerol might be similar to the interaction between sucrose and glycerol. At high concentration of glycerol, the increasing change of skim milk or sucrose concentration resulted in decreasing trend in the cell viability of lyophilized *P. chrysogenum* ACCC 30395. At low concentration of glycerol, the viability response firstly rose when increasing skim milk or sucrose to approximately 14 and 4% (w/v) respectively, and decreased when beyond this range. Thus, due to the interactions between the three variables, their individual optimum values might change in relation to the values of the others. Therefore, one explanation of our results could be that synergistic effects of the other lyoprotectants cannot be excluded.

In our study, the quadratic model generated via BBD experimental data was proved to be highly reliable according to verification experiments. The predicted survival rate of *P. chrysogenum* ACCC 30395 conidia could reach 86.53%, theoretically. Indeed, the survival rate was found to be 85.73% after lyophilization with the optimized complex lyoprotectant composed of 13.75% skim milk, 3.28% sucrose and 2.02% glycerol in verification test. The relative error of 0.92% between predicted and actual value of the conidia viability demonstrated that quadratic model could be used to optimize the complex lyoprotectant of *P. chrysogenum* ACCC 30395 conidia during lyophilization effectively.

5. Conclusion

In this work, RSM used to optimize the complex lyoprotectant to enhance the conidia viability of *P. chrysogenum* ACCC 30395 during lyophilization. The final optimized complex lyoprotectant composed of 13.75% skim milk, 3.28% sucrose, and 2.02% glycerol was effective on the viability of lyophilized *P. chrysogenum* ACCC 30395 conidia, the predicted conidia viability was 86.53%. When *P. chrysogenum* ACCC 30395 conidia were lyophilized with the optimized complex lyoprotectant, the conidia viability was 85.73%, which was close to the predicted value. Further, since skim milk, sucrose and glycerol are relatively cheaper and more popular, they could be widely used as the complex lyoprotectant in the lyophilization of *P. chrysogenum* ACCC 30395 conidia.

Declarations

Conflict of interest

The authors have no conflict of interest.

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Figures

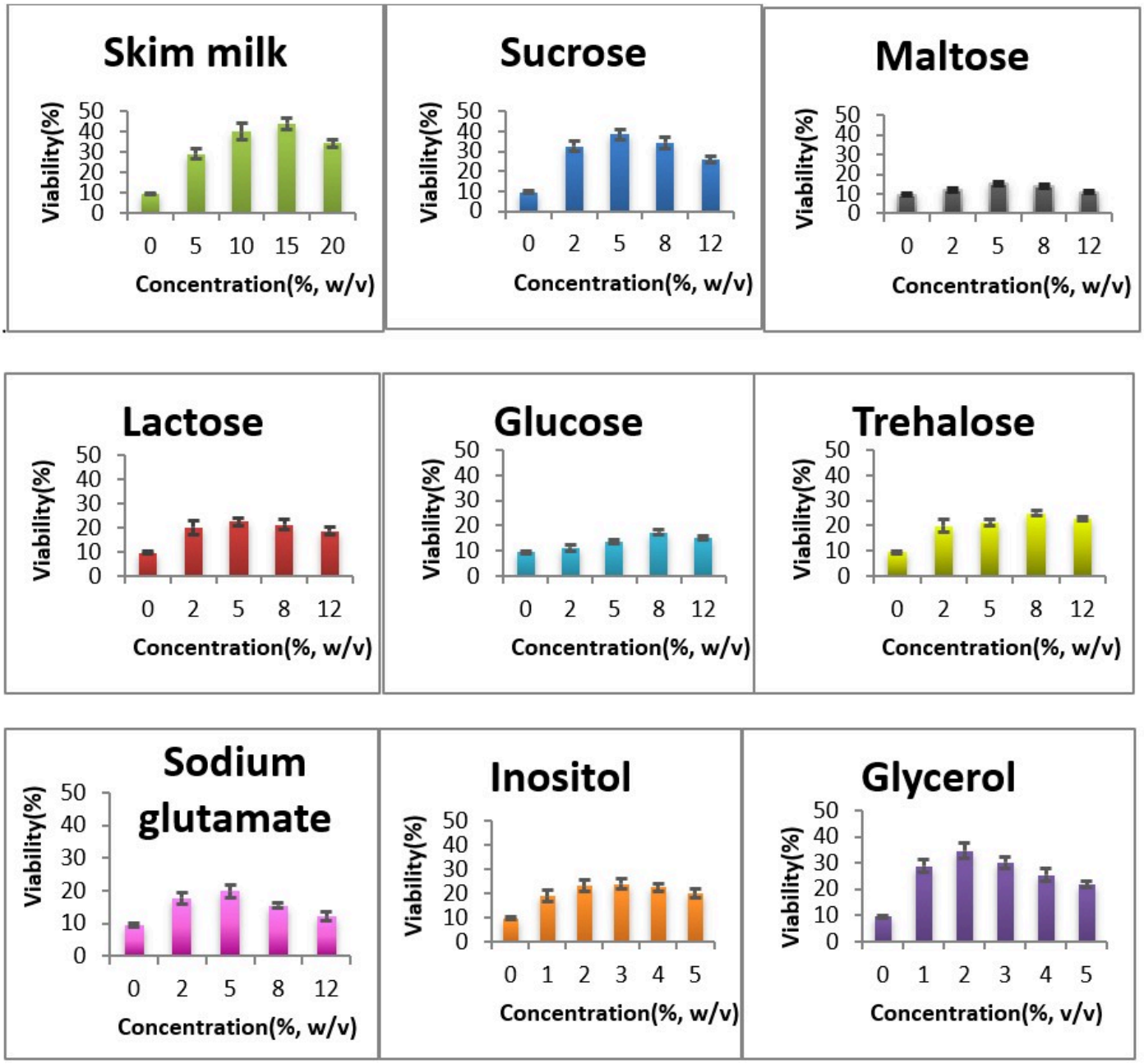


Figure 1

Conidia survival rate of lyophilized *P. chrysogenum* ACCC 30395 with various lyoprotectants

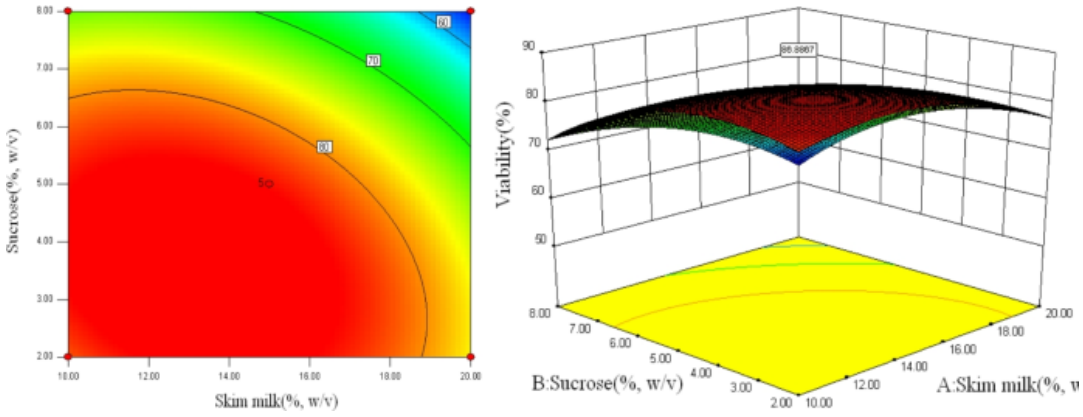


Figure 2

Response surface and contour plot of skim milk and sucrose on the conidia viability of *P.chrysogenum*ACCC 30395

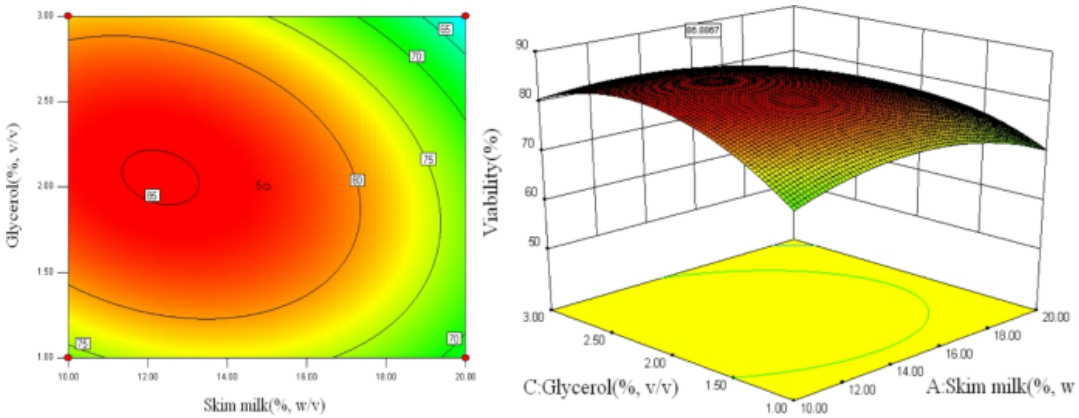


Figure 3

Response surface and contour plot of skim milk and glycerol on the conidia viability of *P.chrysogenum*ACCC 30395

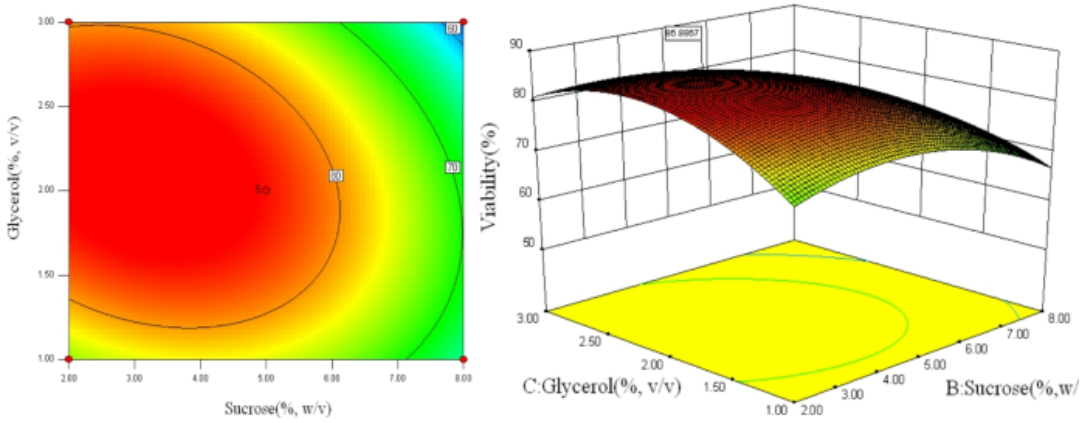


Figure 4

Response surface and contour plot of sucrose and glycerol on the conidia viability of *P.chrysogenum*ACCC 30395