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Research Article

Stabilization of lyophilized bacteriophage vB_Pd_PDCC-1 with biopolymers for long-term storage under thermal stress

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ABSTRACT. The present work evaluated the combination of biopolymers used as preservative agents to enhance the viability and efficacy of the vB_Pd_PDCC-1 phage stored as a powder under high-temperature conditions. In the tests, phages were freeze-dried in a polymeric matrix with varying molar ratios of sodium alginate, gelatin, and trehalose, stored at 4, 25, 35, and 45°C, and evaluated at 1, 30, 60, and 90 days post-freeze-drying. The results indicated that the best treatment was the combination of trehalose (4% w/v) and gelatin (1.5% w/v), as it maintained phage viability (log 6) for up to 90 days at 45°C. Likewise, phage therapy trials using *Artemia* nauplii inoculated with *Vibrio diabolicus* and lyophilized phages embedded in biopolymeric matrices showed survival rates similar to those of the control group, reducing bacterial concentration by 3 log orders of magnitude. The study identified the combination of trehalose and gelatin as a promising additive mixture for protecting the powdered bacteriophage Pd_PdCC-1.

Keywords: phage therapy; lyophilized phages; *Vibrio* infection; long-term storage; biopolymers; thermal stress

INTRODUCTION

In recent years, aquaculture has been severely affected by increasingly aggressive disease outbreaks. This situation has led to significant economic losses in the aquaculture sector due to reduced production of commercially valuable species (Moreno-Figueroa et al. 2019, 2023). To combat bacterial diseases, the use of bacteriophages has emerged as one of the most effective biocontrol strategies, owing to their ability to eliminate specific bacteria through their replication cycle without harming the environment or other microorganisms (Lomelí-Ortega et al. 2021). Phage therapy has been used to control bacterial diseases caused by various genera, including *Vibrio* spp., *Aeromonas* spp., *Streptococcus*, and *Pseudomonas* spp. Moreover, phage therapy has been shown not to induce side effects in cultured organisms, thereby contributing to both the sustainable development of aquaculture and to the addressing of antibiotic resistance (Quiroz-Guzmán et al. 2018, Saucedo-Uriarte et al. 2020, Sieiro et al. 2020, Cao et al. 2021, Lomelí-Ortega et al. 2021). Particularly, artemia cultivation is crucial because its nauplii (larvae) are an ideal live feed due to their high nutritional value (proteins, lipids, essential fatty acids) for fish and shrimp from the larval to adult stage, improving growth and coloration, and reducing mortality; moreover, it is a global industry that drives aquaculture sustainability and generates employment, although it faces supply challenges mainly provoked by diseases such as vibriosis (Madkour et al. 2023, Bisht et al. 2025).

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On the other hand, phages are viruses composed of a nucleic acid (DNA or RNA) wrapped in a protein shell called a capsid (Principi et al. 2019). Their physicochemical stability over time is critical, as maintaining their structural integrity ensures the viability and therapeutic efficacy of the phages. Freeze-drying is among the most commonly used preservation methods (Lavenburg et al. 2020), which involves dehydrating the sample at low temperatures under vacuum, allowing water to be removed by sublimation. This process preserves the structural integrity of phages, thereby extending their longevity at room or refrigeration temperatures (Lavenburg et al. 2020, Moreno-Figueroa & Cab-Sulub 2023).

The freeze-drying process offers advantages; however, it also has drawbacks that can compromise the viability of treated samples due to solute concentration, crystal formation, and pH changes (Pansare & Patel 2019). For this reason, protective additives are commonly used to minimize viability loss during freeze-drying (Lavenburg et al. 2020). Sugars such as trehalose, lactose, and sucrose are among the most widely used preservatives to improve stability, allowing vitrification and matrix formation with microorganisms during the freeze-drying process, thereby preventing crystal formation and the loss of phage viability (Lavenburg et al. 2020, Moreno-Figueroa et al. 2023). On the other hand, biopolymers such as gelatin and alginate have also been used as preservatives during freeze-drying, since like disaccharides, they can form matrices that give them the emulsifying and stabilizing capacity during drying, thus protecting the bacteriophages capsid, maintaining their viability, and increasing shelf life (Manohar & Ramesh 2019, Patarroyo et al. 2020), thus protecting the bacteriophages capsid, maintaining their viability, and increasing shelf life, using concentrations ranging from 1 to 2% w/v and 0.1 to 1% w/v for gelatin and sodium alginate respectively (Korshidian et al. 2019, Manohar & Ramesh 2019). On the other hand, temperature plays a critical role in bacteriophage viability, as most phages at room temperature gradually undergo capsid denaturation, thereby reducing viability (Moreno-Figueroa et al. 2023). For this reason, the use of preservative additives is very important during the freeze-drying process, as it improves the capsid's structural and thermal resistance even at high storage temperatures (Manohar & Ramesh 2019, Lavenburg et al. 2020).

In aquaculture, phage PDCC_1 has gained significant importance due to its specificity against *Vibrio* spp. It has recently been used to control *Vibrio* infections in *Artemia franciscana* (Veyrand-Quiros et al. 2020, Moreno-Figueroa et al. 2023). Therefore, preserving phage PDCC_1 and maintaining its long-term viability remain critical challenges. In this context, this work introduces a novel lyophilization method that uses biopolymers to protect the PDCC-1 phage capsid. This method improves its viability and shelf life, even at elevated experimental temperatures. Phage PDCC_1 was lyophilized using different combinations of gelatin, sodium alginate (biopolymers), and trehalose (disaccharide) as a protective agent. Its viability was evaluated as a function of temperature (4, 25, 35, and 45°C) and post-freeze-drying storage time (1, 30, 60, and 90 days).

MATERIALS AND METHODS

Bacterial strain

Vibrio diabolicus (CIBGEN-002), previously isolated from pond sediment of shrimp diagnosed with acute hepatopancreatic necrosis disease (AHPND) (Lomelí-Ortega et al. 2021), was obtained from the Centro de Investigaciones Biológicas del Noroeste (CIBNOR) collection, and grown on tryptic soy agar (BD BioxonR NJ, USA), supplemented with 2.5% NaCl (Sigma-Aldrich R No. S9888-25G) at 35°C and harvested after 24 h at a concentration of 10^8 colony-forming unit per mL (CFU mL^{-1}).

Bacteriophage replication

Vibriophage vB_Pd_PDCC-1 was donated by the CIBNOR phage collection, which has a broad host range against several *Vibrio* species, such as *V. algynoliticus*, *V. campbellii*, *V. diabolicus*, and *V. parahaemolyticus* (Veyrand-Quiros et al. 2020). This phage was previously classified within the Myoviridae family based on genetic analysis and electron microscopy (Veyrand-Quiros et al. 2020). For mass phage production, phage lysate was added to 1,000 mL of tryptic soy broth (BD Bioxon®, NJ, USA) supplemented with 2.5% NaCl, which had been previously inoculated with the target bacteria *V. diabolicus*. After 24 h of incubation at 35°C, the culture was filtered through a 0.2-µm pore-size filter and stored at 4°C until use. To quantify the concentration of active bacteriophage particles, a titer test of the phage stock was performed using the double agar overlay plate assay described by Kropinski et al. (2009).

Protectant solutions preparation

Four combinations of different biopolymers were tested, including gelatin (Sigma-Aldrich No. G9382, MO, USA), sodium alginate (Sigma-Aldrich No.

W201502, MO, USA), and trehalose (Sigma-Aldrich No. T0167, MO, USA). Biopolymer mixes and concentrations were developed following previous studies (Zhang et al. 2018, Manohar & Ramesh 2019, Moreno-Figueroa et al. 2023): treatment 1: 4% w/v trehalose + 0.375% w/v sodium alginate (T+A); treatment 2: 4% w/v trehalose + 1.5% w/v gelatin (T+G); treatment 3: 0.375% w/v sodium alginate + 1.5% w/v gelatin (A+G); treatment 4: 4% w/v trehalose + 0.375% w/v sodium alginate + 1.5% w/v gelatin (T+A+G). Each treatment was supplied with 10 mL of phage suspension (10^8 plaque-forming units per mL (PFU mL^{-1})) and mixed manually until dissolved, with protectants at the specified concentration (e.g. 4% = 0.4 g of protectant in 10 mL of phage suspension). A phage solution without protectants served as the control group. Each treatment was tested at a similar initial proportion, and after preparation, the treatments were incubated at 40°C for 30 min and mixed to facilitate dissolution. After that, all treatment solutions were stored at -80°C until the freeze-drying process was initiated.

Freeze-drying

All treatments and control samples (10 mL) were lyophilized at -50°C and 0.050 mbar for 24 h using a Labconco® Freezone 1-L equipment Freeze Dryer (MO, USA). The lyophilized powder samples were divided and stored at four temperatures (4, 25, 35, and 45°C) until use.

Phage stability post-lyophilization and storage temperature trial

Powder samples were rehydrated to their original volume (10 mL) with sterile distilled water just before plating efficiency testing. Phage titer *in vitro* assay was evaluated in triplicate (n = 3) on days 1, 30, 60, and 90 after freeze-drying (post-lyophilization) at each storage temperature. The lytic activity and PFU mL^{-1} of each treatment solution were evaluated against *V. diabolicus* (the bacterial host). The bacterial strain was grown at a concentration of 10^8 CFU mL^{-1}. Then, 100 µL of the bacterial suspension was mixed with 100 µL of serially diluted phage lysate (10^{-1} to 10^{-7}) in sterile 2.5% NaCl saline solution to determine phage titer by double agar plate assay.

Bioassay of protection in brine shrimp nauplii

A. franciscana at early larval development (Instar II) was used to evaluate the efficacy of bacteriophage therapy at this stage. A challenge test with *V. diabolicus* was accomplished using disinfected cysts and nauplii. Briefly, commercial brine shrimp cysts (INVE Aquaculture, Salt Lake City, UT) were hydrated in sterile distilled water for 60 min. Cysts were decapsulated in a 50% sodium hypochlorite solution (60 g L^{-1} of active chlorine) for 35 s and rinsed thoroughly with sterile distilled water. For the incubation process, cysts were placed in a 500 mL Erlenmeyer flask with constant, air-filtered aeration (0.2-µm pore-size filter) and 2.5% sterile saline solution at 28°C for 24 h.

To verify the effectiveness of the disinfection protocol, previously homogenized treated cyst samples in a 2.5% NaCl solution were inoculated on marine agar and thiosulfate-citrate-bile salts sucrose (TCBS) plates and incubated at 35°C for 24 h.

After hatching, 10 *Artemia* nauplii were placed in 15 mL tubes containing sterile 2.5% saline solution (five tubes per treatment). The experimental treatments were as follows:

a) *Artemia* nauplii (positive control 1).

b) *Artemia* nauplii + bacteria (negative control).

c) *Artemia* nauplii + bacteria + lyophilized phage (different treatments at one day post-lyophilization, samples maintained at 4°C until used).

d) *Artemia* nauplii + lyophilized phage (different treatments at one day post-lyophilization, samples maintained at 4°C until used) (positive control 2).

Treatments with bacteria and phage were inoculated with 100 µL of *V. diabolicus* suspension (10^8 CFU mL^{-1}) and with 100 µL of each lyophilized phage treatment suspension (10^7 PFU mL^{-1}), respectively.

After 24 h of incubation, nauplii survival, as well as bacterial and phage titers, were analyzed and quantified according to the procedure described by Lomelí-Ortega et al. (2021). Briefly, the survival rate of artemia was obtained by subtracting the dead nauplii from the total placed (10 nauplii), dividing by the total, and then multiplying by 100. For bacterial titers, serial dilutions from 10^{-1} to 10^{-3} were prepared and plated onto TCBS agar for counting. The results were expressed as CFU mL^{-1}. Finally, double-agar assays were developed for phage titer analysis, and results were expressed as PFU mL^{-1}.

Statistical analysis

Results from *in vitro* bacteriophage titers in lyophilized samples and *Artemia* nauplii survival data were tested for normality and homoscedasticity using the Shapiro-Wilk and Levene's tests in Statistics 8.0 (StatSoft Inc., Tulsa, OK). The *in vitro* lyophilized phage titer and

nauplii survival data were analyzed using simple linear regressions and simple ANOVA, respectively. Then, *post-hoc* comparisons were made using Tukey's test in StatSoft Inc.'s Statistics 8.0. Student's *t*-tests were also performed in Statistics 8.0 software (StatSoft Inc., Tulsa, OK, USA) to compare titer values of *V. diabolicus* and phage PDCC-1 after the *in vivo* nauplii assay.

RESULTS

Vibriophage vB_PDCC-1 was subjected to four treatments of combinations of gelatin (G), sodium alginate (A), and trehalose (T) used as preservative additives. Figure 1 shows the viability results for the lyophilized phage across the 90-day experimental period at four storage temperatures (4, 25, 35, and 45°C). The survival of *Artemia* nauplii inoculated with *V. diabolicus*, but without phage addition, was reduced to 25% compared with treatments in which nauplii were challenged with bacteria and received lyophilized phage, where survival remained around 80% regardless of the treatment used (Table 1) indicates a survival rate of more than three times higher when brine nauplii infected with *V. diabolicus* are treated with lyophilized phages.

The intrinsic role of temperature in the viability of the bacteriophage PDCC-1 is shown (Fig. 1). In general, treatments maintained at higher storage temperatures (25, 35, and 45°C) showed greater loss of phage viability than treatments maintained at 4°C. At the refrigeration temperature (4°C), the phage concentration remains higher in all treatments (including the control group) throughout the experiment (90 days). All samples stored at this temperature-maintained concentrations of log 6 to 7 PFU mL^{-1} until the end of the experiment. Table 2 presents the values of the linear regressions of phage concentration through time maintained at different preservation temperatures by using Equation 1 as follows:

$$y = ax + b \quad (1)$$

where y is the dependent variable (phage concentration), *a* and *b* are regression coefficients, and x is the independent variable (time).

DISCUSSION

Temperature is an important variable in lyophilized phage preservation, as Table 2 indicates: as the preservation temperature increases, the "y" values (phage concentration) in the linear regressions decrease, indicating that temperature negatively affects phage viability, with the impact increasing as temperature rises. The capsid of bacteriophages is composed of proteins that share characteristics with other structural proteins, and it is known that at high temperatures they undergo degradation and denaturation, which can lead to loss of viability (Manohar & Ramesh 2019). The vast majority of freeze-drying studies on bacteriophages show viability losses of less than log 2 PFU mL^{-1} when stored at refrigeration temperatures (4°C) for periods ranging from 2 to 8 weeks. In this study, the loss in viability was log 1 PFU mL^{-1}, and the treatments showed differences relative to the control group (+1 log). Likewise, the treatments that achieved the highest phage viability throughout the experiment across the different storage temperatures were the combinations T+G (treatment 2) and T+G+A (treatment 4). Phage concentration stayed at log 6 PFU mL^{-1} for 90 days after lyophilization at 35 and 45°C, and at log 7 PFU mL^{-1} at 25°C.

Similar results are reported by Manohar & Ramesh (2019) (log 6) after 30 days post-lyophilization, using G (1 and 2% w/v) and sucrose (0.5 and 1.0 M) as protective additives in the *Escherichia coli* ECP311 phage, but at 37°C. The disaccharide T is a non-reducing sugar lyoprotectant used in protein preservation; it is chemically stable under extreme temperatures and low pH. Its high glass transition temperature (115°C) protects it during freezing and freeze-drying, owing to its high-water solubility (Moreno-Figueroa et al. 2023).

In addition, T considerably increases the viscosity of the continuous phase of solutions due to its high molecular weight (342,296 g mol^{-1}), thereby enhancing chemical attraction and intermolecular stability (Ly et al. 2019). When a protein is dried with T as an excipient, T immobilizes the protein within a glassy matrix, thereby preventing denaturation (Ly et al. 2019). The hydrogen bonds in water typically form with proteins to maintain secondary structure, which can be disrupted by the removal of water during freeze-drying. It is proposed that T -OH radicals can replace the empty spaces of the (removed) water and create new hydrogen bonds with capsid proteins, conferring chemical and structural stability that ultimately enhances bacteriophage viability (Mensinsk et al. 2017, Ly et al. 2019, Moreno-Figueroa et al. 2023). This mechanism has been reported to preserve pharmaceuticals, foods, cells, and even microorganisms such as bacteria and yeasts (Ávila-Rincón et al. 2015, Lee-Shuan et al. 2016, Zhang et al. 2018, Li et al. 2022).

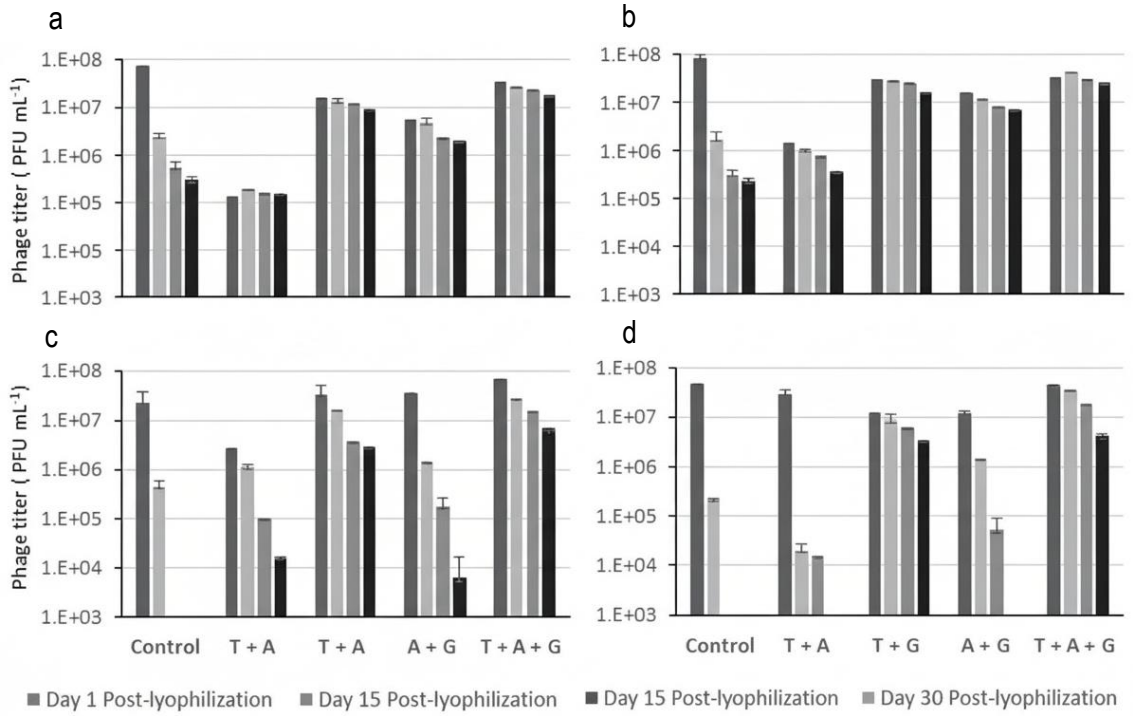

Figure 1. Viability of lyophilized phage PDCC_1 samples with four preservation combinations through time at different storage temperatures: a) 4°C, b) 25°C, c) 35°C, and d) 45°C. The results represent the mean values of three titrations. Standard deviations are indicated. The different letters indicate significant statistical differences between treatments ($P < 0.05$). T: trehalose, G: gelatin, A: sodium alginate.

Table 1. Survival of brine shrimp nauplii exposed to *Vibrio diabolicus* and treated with phage Vb_Pd_PDCC-1 lyophilized with four different treatments: 1) 4% w/v trehalose (T) + 0.375% w/v sodium alginate (A), 2) 4% w/v T + 1.5% w/v gelatin (G), 3) 0.375% w/v A + 1.5% w/v G, and 4) 4% w/v T + 0.375% w/v A + 1.5% w/v G. Survival values are given as mean ± standard deviation (SD) of multiple determinations (n = 5). Statistical differences ($P < 0.05$) are indicated with different uppercase letters. ND: not detected, CFU: colony-forming units, PFU: plaque-forming units.

Treatments	Survival % ± SD	*Vibrio diabolicus* CFU mL^{-1} ± SD	Phage PDCC-1 PFU mL^{-1} ± SD
Artemia	98 ± 4.47[a]	ND	ND
Artemia + bacteria	25 ± 5.77[c]	388,000 ± 16,200[a]	ND
1) *Artemia* + phage (T+A) + bacteria	78 ± 6.81[b]	795 ± 150[b]	8,740,000 ± 266,000[a]
2) *Artemia* + phage (T+G) + bacteria	79 ± 8.27[b]	817 ± 139[b]	7,650,000 ± 189,000[a]
3) *Artemia* + phage (A+G) + bacteria	81 ± 7.58[b]	685 ± 127[b]	8,115,000 ± 228,000[a]
4) *Artemia* + phage (T+A+G) + bacteria	80 ± 7.39[b]	742 ± 133[b]	7,960,000 ± 195,000[a]
1) *Artemia* + phage (T+A)	97 ± 4.49[a]	ND	42,200 ± 5610[b]
2) *Artemia* + phage (T+G)	98 ± 4.06[a]	ND	38,600 ± 4340[b]
3) *Artemia* + phage (A+G)	98 ± 3.79[a]	ND	49,300 ± 6650[b]
4) *Artemia* + phage (T+A+G)	97 ± 4.30[a]	ND	32,700 ± 5590[b]

Furthermore, water in contact with T bound to the capsid surface can be forced to adopt a molecular conformation that prevents or delays ice crystal formation (Li et al. 2022), thereby inhibiting direct contact between ice crystals and phages. Likewise, the results are similar to those reported by Vandenheuvel et al. (2013), where using the spray drying technique, the phages LUZ19 and Romulus (host bacteria *P. aeruginosa* and *S. aureus*, respectively) had losses between 0.02 and 2 log in 60 days of experimentation,

Table 2. Values of linear regressions of phage concentration through time maintained at different preservation temperatures. ($P > 0.05$, $R^2 > 0.65$). T: trehalose, A: sodium alginate, G: gelatin.

Treatments	Temperature			
	4°C	25°C	35°C	45°C
Control	y = -3E+07x+9E+07	y = -3E+07x+9E+07	y = -6E+06x+2E+07	y = -2E+07x+6E+07
T+A	y = -2E+07x+9E+07	y = -2E+07x+9E+07	y = -2E+07x+8E+07	y = -3E+07x+7E+07
T+G	y = -7E+06x+9E+07	y = -2E+07x+9E+07	y = -2E+07x+8E+07	y = -2E+07x+6E+07
A+G	y = -1E+07x+5E+07	y = -9E+06x+4E+07	y = -1E+07x+4E+07	y = -5E+06x+2E+07
T+A+G	y = -2E+07x+1E+08	y = -1E+07x+8E+07	y = -1E+07x+4E+07	y = -7E+06x+3E+07

using T at 4% w/v as a protective additive. The extent of phage stabilization by disaccharide addition depends on the concentration of the disaccharides (Merabishvili et al. 2013). Several studies show that multiple concentrations are optimal for different phages, so each phage would have to be evaluated for the optimal concentration level of the protective additive, even if they are genetically close (Davies & Kelly 1969, Carne & Greaves 1974, Cox et al. 1974, Puapermpoonsiri et al. 2009, Merabishvili et al. 2013).

On the other hand, G is a biopolymer reported to be an effective stabilizing agent, as it forms polymers that maintain phage morphology during freeze-drying (Manohar & Ramesh 2019). The porous three-dimensional structure of G allows the diffusion of nutrients (solutes) and oxygen, thereby increasing molecular adhesion (Lukin et al. 2022). During freeze-drying, pore size is reduced, resulting in a reorganized, permeable structure that can improve adhesion and molecular stability (Echave et al. 2021). The combination of protective additives can enhance their individual effects, providing better protection to the product. Therefore, optimizing freeze-drying techniques is essential to ensure the long-term stability of the viral capsid (Li et al. 2023). Suitable protective additives and appropriate drying methods are necessary to achieve ideal lyophilized products (Li et al. 2023).

In a previous study, the use of T alone as a protective additive during freeze-drying and during the shelf life of the vibriophage PDCC-1 was analyzed (Moreno-Figueroa et al. 2023). In that study, a 15% lower phage viability (compared with this trial) was reported at 23°C for 60 days post-freeze-drying, using 4% w/v T as a protective additive. Comparable freeze-drying studies have reported better viability results when protective additives are used in combination rather than individually (Manohar & Ramesh 2019, Li et al. 2023). The importance of analyzing the shelf life of bacteriophages stored at high temperatures (+30°C) lies in the fact that in underdeveloped countries (Mexico and Latin America), most shrimp farms lack electricity, making refrigeration during culture inconvenient (Moreno-Figueroa et al. 2023). Therefore, offering viable alternatives for the industry that can be maintained at ambient temperatures in tropical and subtropical areas, where summer temperatures can reach up to 45°C, is highly relevant. According to Figure 1, treatments with the T and G (2 and 4) combination were the only ones to maintain a concentration of log 6 at critical storage temperatures of 35 and 45°C throughout the experiment (90 days). In the other treatments (1 and 3), no viability was observed at 90 days post-freeze-drying when maintained at 45°C, whereas those maintained at 35°C showed a 4-log loss.

All treatments maintained at 25°C maintained a log 7 PFU mL^{-1} concentration throughout the experiment. In the control group (without protective additives), phage concentration decreased from log 7 to 3 PFU mL^{-1} at temperatures of 25, 35, and 45°C over 15 days post-freeze-drying, with no viability detected in subsequent analyses. The treatments with the lowest bacteriophage viability were those containing A (treatments 1 and 3). This outcome may be attributed to the temperature or time at which the post-mixing treatments with the protective additives were applied (40°C and 30 min, respectively), which were likely insufficient to solubilize the A fully. Thus, efficient integration of alginate and capsid molecules could not be achieved during freezing and subsequent freeze-drying (Frent et al. 2022). However, both treatments-maintained log 6 viability at 4 and 25°C throughout the evaluation period, with differences relative to the control group (+7 log).

It is worth noting that a solid lyophilized matrix offers greater structural stability to protect phages during storage than its liquid counterpart, even at temperatures well above refrigeration (as in this study, 45°C). The osmolarity of the different components of a solution can lead to physiological degradation in liquid environments (Thiemicke & Neuert 2021).

Phage therapy has emerged as a viable alternative with significant potential for controlling pathogenic bacteria in aquaculture ponds, primarily due to its high specificity in eliminating bacteria, including antibiotic-resistant strains (Lomelí-Ortega et al. 2021). In the present study, a single dose of freeze-dried phages administered to a model of *A. franciscana* nauplii infected with *V. diabolicus* resulted in survival close to that of the uninfected control group. Moreover, the concentration of *V. diabolicus* in the different treatments with freeze-dried phages was less than 10,000 CFU mL^{-1} (Table 1), which corresponds to the level considered a limit for preventing vibriosis in aquaculture ponds (Moreno-Figueroa et al. 2018). Several studies have reported similar results using vibriophages to treat diseases caused by *Vibrio* species in crustaceans (Lomelí-Ortega et al. 2021, Moreno-Figueroa et al. 2023, Tadeu et al. 2024, Chaichana et al. 2025, Ding et al. 2025).

CONCLUSION

The use of combinations of preservative additives during the freeze-drying process significantly improves the viability and extends the shelf life of bacteriophages stored at different temperatures. The vB_Pd_PDCC-1 phage, freeze-dried with 4% w/v T + 1.5% w/v G, remains viable for at least 90 days at 45°C. Further research into phagotherapy and the use of freeze-dried bacteriophages in agricultural practices may provide a viable alternative to antibiotics for combating bacterial infections.

Credit author contribution

L.D. Moreno-Figueroa: writing-original draft, visualization, investigation, formal analysis and conceptualization; N.A. Ochoa-Álvarez: writing-review, editing, supervision and methodology; L. Hernández-Adame: writing-review, editing, supervision, conceptualization and project management.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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