Strategy for Mass Production of Lytic Staphylococcus Aureus Bacteriophage pSa-3: Contribution of Multiplicity of Infection

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Research

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Abstract

Background

Antibiotic-resistant bacteria have emerged as a serious problem; bacteriophages have, therefore, been proposed as a therapeutic alternative to antibiotics. Several authorities, such as pharmacopeia, FDA, have confirmed their safety, and some bacteriophages are commercially available worldwide. The demand for bacteriophages is expected to increase exponentially in the future; hence, there is an urgent need to mass-produce bacteriophages economically. Unlike the replication of non-lytic bacteriophages, lytic bacteriophages are replicated by lysing host bacteria, which leads to the termination of phage production; hence, strategies that can prolong the lysis of host bacteria in bacteria-bacteriophage co-cultures are required.

Results

In the current study, we manipulated the inoculum concentrations of *Staphylococcus aureus* and phage pSa-3 (multiplicity of infection, MOI), and their energy sources to delay the bactericidal effect while optimizing phage production. We examined an increasing range of bacterial inoculum concentration (2×10^8 to 2×10^9 CFU/mL) to decrease the lag phase, in combination with a decreasing range of phage inoculum (from MOI 0.01 to 0.00000001) to delay the lysis of the host. Bacterial concentration of 2×10^8 CFU/mL and phage MOI of 0.0001 showed the maximum final phage production rate (1.68×10^{10} plaque forming unit (PFU)/mL). With this combination of phage-bacteria inoculum, we selected glycerol, glycine, and calcium as carbon, nitrogen, and divalent ion sources, respectively, for phage production. After optimization using response surface methodology, the final concentration of lytic *Staphylococcus* phage was $8.63 \times 10^{10} \pm 9.71 \times 10^9$ PFU/mL (5.13 fold increase).

Conclusions

Therefore, *Staphylococcus* phage pSa-3 production can be maximized by increasing the bacterial inoculum and reducing the seeding phage MOI, which this combinatorial strategy could decrease phage production time. Further, we suggest that response surface methodology has the potential for optimizing mass production of lytic bacteriophages.

Background

Antibiotic-resistant bacteria, such as vancomycin-resistant Enterococci, methicillin-resistant *Staphylococcus aureus*, and carbapenem-resistant *Enterobacteriaceae*, are a global, emerging problem [1-3]. With the advent of these antibiotic-resistant bacteria, an alternative treatment strategy is urgently required. One of the most anticipated alternatives is the use of lytic bacteriophages (phages), which are effective against bacterial infections [4, 5, 6]. Numerous phages are commercially available, and their therapeutic application is reported from East European countries, such as Georgia, Russia, Poland, and Belgium [7]; several phages have also been approved by the US FDA, as they have been proven to have no

adverse effects during phase 1 clinical trials [8, 9]; hence, phage therapy has attracted attention as a potential alternative to antibiotics.

The most important aspect in phage therapy is the isolation and selection of effective phages against antibiotic-resistant bacteria [10-12]. Mass production of these phages is the next pivotal step based on increasing demands in the future [13-16]. It is well known that host bacterial cells and their phages have different optimal conditions for replication. As described in previous reports, replication of phages is dependent on the physiology of their hosts [17, 18]. In particular, the specific growth rate (μ) of host bacteria is an important factor for the replication of phages [19, 20].

Temperate or filamentous phages replicate in proportion to the growth of the host, without a significant effect on the host [21, 22]. In contrast, the replication of lytic phages causes lysis of their host, which means there will be no “factory” to produce phages in the end. Thus, keeping host bacteria alive for longer periods by manipulating the physiology or the interaction between phage and bacteria can be a strategy for increasing the yield of lytic phages [23]. In addition to manipulating the nature of bacteria, scale up trials (i.e. flask to fermenter) are being widely researched [24, 25].

Previously, we isolated obligatory lytic phage pSa-3 which effectively lysed the *S. aureus* at the low concentration (MOI 0.1) [26, 27].With the surfactant, the *Staphylococcus* phage pSa-3 could effectively degrade the aggregations of *S. aureus* including biofilms in -vitro and -vivo [26]. As the phages did not possess any antibiotic resistance- or virulence- related genes, or lysogenic related genes, pSa-3 has potential to be used as a therapeutic agent for treatment of *S. aureus* associated atopic dermatitis [27].

This study was designed to optimize the phage yield by altering the physiology of bacteria by controlling the energy source and by manipulating bacterial inoculum and phage concentration to keep the host alive for a longer period. Carbon, nitrogen, ion sources, and surfactant were supplemented in the growth medium, and the multiplicity of infection (MOI) was modified for maximization of the phage production yield. Response surface methodology (RSM) was also adopted for optimization of the combined effect.

Results

Effect of MOI on phage amplification

The effect of MOI on phage amplification is shown in Figure 1. The experiment was conducted on various bacterial inoculum concentrations to analyze the effect of increased bacterial load on phage production yield. As shown in Figure 1a, the host bacteria were lysed 9 h after phage injection in all groups. In the 1% bacterial inoculum group (initial concentration: 2×10^8 CFU/mL), the phage yield was negatively correlated with the phage inoculum, whereas the final phage yield was positively correlated with the bacterial optical density (OD; Fig. 1b). In the 5% bacterial inoculum group (initial concentration: 1×10^8 CFU/mL), the lower MOI groups (MOI 0.00000001 and 0.000001) showed bacterial growth, while the other groups showed inhibition of bacterial growth (Fig. 1c). As shown in Figure 1d, MOI 0.01 and 0.0001 groups showed the highest phage yield (~10^{10} PFU/mL), while the lowest MOI group (MOI 0.000001)

showed lower phage production (~10^8 PFU/mL) than the other groups. In the 10% bacterial inoculum group (initial concentration: 2 × 10^9 CFU/mL), the two lower MOI groups (MOI 0.000001 and 0.00000001) showed bacterial growth rather than replication of phages similar to the 5% bacterial inoculum-MOI 0.000001 group (Fig. 1e), and only the MOI 0.01 group showed lysis of the host bacteria. The MOI 0.01 group showed the highest phage production (~10^{10} PFU/mL), while the lower MOI groups produced phages lesser than 10^9 PFU/mL (Fig. 1f). As the MOI (phage inoculum) was lowered, an increase in the time at which the inhibition of bacterial growth began was observed, and the final concentration of phages did not increase after reaching the highest concentration for 24 h. The summary of the maximum phage production yield in each bacterial inoculum group is presented in Table 1. The group with 5% bacterial inoculum-MOI 0.0001, which showed the highest phage yield, was used for further experiments.

Effect of growth medium source on phage amplification

The effect of growth medium source on the production of bacteriophage was analyzed using 5% bacterial inoculum with MOI of the phage being 0.0001 as the combination resulted in maximum production. As a medium supplement, different carbon sources, nitrogen sources, ions, and surfactants were supplemented in Luria-Bertani (LB) broth and analyzed using a one-factor-at-a-time method. As a carbon source, 0.5% (w/v) of glucose, sucrose, fructose, galactose, or glycerol was supplemented. Galactose and glycerol showed upregulation of phage production, and glycerol supplementation led to a significant increase ($p < 0.05$) i.e., 100% increased value in phage production compared to that of the control (Fig. 2a). As a nitrogen source, 0.1 % (w/v) of casamino acid, peptone, gelatin, or glycine was supplemented. Among the nitrogen sources, only glycine showed 18% increase in phage yield and was selected as a nitrogen source for further experiments (Fig. 2b). The divalent ion source, such as magnesium chloride or calcium chloride was supplemented in the culture medium for the amplification test, and calcium chloride was selected as the ion supplement, as it revealed the highest (28% increase) yield (Fig. 2c). For the surfactant screening, tween 20, triton X-100, and SDS were examined, which downregulated phage amplification (Fig. 2d). Thus, glycerol, glycine, and calcium chloride were selected as carbon, nitrogen, and ion sources, respectively, for RSM analysis.

Optimization of bacteriophage pSa-3 production by RSM

RSM based on central composite design (CCD) technique was used to examine the effect of the selected media supplements on the amplification of lytic phage pSa-3. A total of 20 coded levels are represented in Table 2. The experimental design and results obtained from RSM are shown in Table 3. By employing analysis of variance (ANOVA), the following equation was obtained, describing the relationship between phage yield (Y), and glycerol (X_1), glycine (X_2), and calcium chloride (X_3):

$$Y= 46287782214 - 7470787473X_1 + 89924699X_2 - 125989578X_3 - 6578793992X_1^2 + 2964876080X_2^2 + 1314889898X_3^2 - 5212500000X_1X_2 + 4745833333X_1X_3 + 5879166667X_2X_3$$

where Y represents the predicted phage yield; X_1, X_2, and X_3 are the concentrations of supplemented glycerol, glycine, and calcium chloride, respectively. The experimental value varied from 2.00×10^{10} to 6.30×10^{10} PFU/mL (119% to 375% yield increase compared to that of the yield of MOI optimization), which fits in with the predicted value calculated from the equation. Fig. 3 graphically represents the equation in 2D contour (Fig. 3a, c, and e) and 3D response surface plot (Fig. 3b, d, and f), which suggests the optimum range of the factors for the maximum phage yield response. The optimum conditions for increased phage yield are as follows: a) glycerol concentration: -1.3 to -0.8, b) glycine concentration at both ends: -1.633 to 0 and 0 to 1.633, and c) decrease of calcium chloride concentration from 1.633 to -1.633.

Analysis of variance (ANOVA)

To validate the models used in this study, ANOVA was performed for the phage production yield. The significance of the models was determined by p-value less than 0.05. A low p-value of 0.023 with a high F value of 4.51 implied that the model was credible (Table 4). The analysis represented that the determination coefficient (R^2) value of 0.84 and a p-value for lack of fit (0.084) were higher than the significant value (0.05). Overall, these statistical values suggested that RSM can be an effective tool for the optimization of lytic phage production, indicating that the model for the phage pSa-3 production in this study was a good fit.

Validation of the optimized conditions

Based on the RSM analysis, the optimal phage yield was predicted to be 7.63×10^{10} PFU/mL when the code levels of glycerol, glycine, and calcium chloride were -0.610313 (around 347 mg/mL of glycerol), 1.633 (90 mg/mL of glycine), and 1.633 (18 mM of calcium chloride), respectively. The validation experiment was conducted to confirm the predicted result. The observed average phage yield under the optimal value was $8.63 \times 10^{10} \pm 9.71 \times 10^{9}$ PFU/mL, which was in good agreement with the predicted value.

Discussion

The emergence of super-bacteria with resistance to multiple commonly used antibiotics has drawn the attention of scientists to bacteriophages as an alternative for antibiotics [28, 29, 30]. For therapeutic purposes, rapid eradication of pathogenic bacteria is important. Therefore, phage therapy research focused on isolating and selecting phages that can effectively control multidrug-resistant bacteria is gaining popularity [10-12, 31, 32]. On the contrary, for phage mass production, host bacteria are essential and should survive longer as “phage-producing factories”. In this study, the modification of the initial inoculum concentration of the host and lytic phage improved the production rate of the phages. Meanwhile, the selected supplements successfully enhanced the lytic phage production after optimization by response surface methodology at bench scale (flask).

One approach to slowly lyse the “factory,” the host bacteria, is to lower the inoculum of phages. In this study, we focused on the fact that bacteria are gradually eradicated or partially suppressed at a low MOI,

as described in the in vitro phage therapy studies [11, 33, 34, 35, 36]. We hypothesized that a lower bacteriophage inoculum results in a higher final yield. We used MOI 0.01 as the highest phage inoculum for the experiments, as MOI 0.1 was sufficient for the eradication of the host bacteria in our previous study [26]. In the first test with 2×10^8 CFU/mL of bacteria and MOI 0.01 to 0.000001 of the phage, the lowest MOI resulted in the highest final phage production and delayed eradication of the host bacteria (Fig. 1). As the inoculum size and lag phase has a negative correlation in general, we expanded the initial bacterial concentration to 2×10^9 CFU/mL for the reduction in production time and validation of the hypothesis “lower phage inoculum is good for production”. We observed that there might be a negative correlation between the initial phage inoculum and the final phage production or the phage production time; however, this was not always the case (Fig. 1). Although more detailed kinetics between the inoculum quantity of phage and bacteria should be elucidated through further studies, we observed that there was a lower limit of MOI (i.e., MOI 0.0001 and 0.01 for 1×10^9 CFU/mL and 2×10^9 CFU/mL initial concentration, respectively) for the final phage production. Simple manipulation of the initial phage and bacterial inoculum could regulate the production time and the final production quantity of the phage, without any other complicate alteration in culture conditions.

The optimal environment for the growth of phages and bacteria can be different; however, phages can control their life cycle in response to the physiology of the host [17-20, 37]. One study illustrated that the specific growth rate (μ) of host bacteria has a positive correlation with adsorption rate and burst size of phages [17], and other studies have reported that μ has a positive correlation with the amplification rate of the M13 phage [19]. We scrutinized the relationship between μ and amplification rate by manipulating the medium substrates, such as carbon and nitrogen sources, divalent cations, and surfactants. Glycerol and glycine were included in the experiments, as they can delay the growth of bacteria [38, 39]. Each of these parameters increased the final phage yield and eventually showed around 500% increase in the production rate in combination. However, μ and the supplements did not show strong correlation with the yield of final phage production (R^2 = 0.1%; Figure S1). Irregularities in the correlations could have occurred because other substrates might have had a critical impact on the metabolism of the host bacteria as well as on the life cycle of the phage [40]. Additionally, the regression model in RSM analysis showed its statistical significance ($p = 0.023$) with a high value for the coefficient of determination (R^2) and a low value for lack of fit. Therefore, RSM analysis can be utilized for assessing optimal lytic phage production, as the developed models were reliable for phage pSa-3 production in this study. In future, the demand for phages is expected to increase rapidly; therefore, economical mass production of phages is essential, and studies should focus on optimization of phage mass production.

Conclusion

This study aimed to develop an economical method for the mass production of lytic bacteriophages. We found that manipulation of the initial inoculation ratio of the phage and their host can significantly influence the phage yield. However, the common belief that low MOIs guarantee high yields is untrue: there exists a lower limit of the inoculum ratio between host and phage. After screening several

substrates using the one-factor-at-a-time method, we were able to achieve more than 500% increase in the phage yield using RSM. Although we could not elucidate a correlation between the results from previous studies and those of the current study, we established that the manipulation of bacterial inoculum and MOI will help in ensuring economically efficient mass production of phages in the future.

Material And Methods

Microorganism, growth conditions, and phage preparation

S. aureus ATCC25923 and previously isolated phage pSa-3 were used for the experiments in this study [26, 27]. LB broth and agar medium were used for the culture of the host bacteria and bacteriophages pSa-3. For phage preparation, 1% (2×10^8 CFU) of overnight (~18 h) bacterial culture adjusted to 2×10^8 CFU and serial dilutions of phage solution (10^2-10^7 PFU/mL) were co-inoculated in fresh LB broth and incubated overnight at 37 °C under shaking conditions (150 rpm). The culture was centrifuged (14,000 × g), filtered (0.45 µm), and purified using the PEG precipitation method [41]. A high concentration (~10^{10} PFU/mL) of the phage solution was stored at 4 °C for further use.

Growth of bacteria and enumeration of bacteriophages

Bacterial growth and the concentration of the phages were examined periodically (0, 1, 3, 5, 7, 9, 12, and 24 h). For bacterial growth, OD was measured at 600 nm using a spectrophotometer (Biorad SmartSpecTM Plus, USA), and the number of phages was determined using a standard double layer agar plaque assay. Briefly, 100 µL of each of the diluted samples and the overnight bacterial culture were inoculated to the top agar (0.4% agar) and poured into the bottom agar (1.5% agar) plate. After overnight incubation at 37 °C, the number of plaques was counted.

Effect of MOI on phage amplification

The effect of MOI on phage amplification was examined with various combinations of bacterial inoculum and phage MOI after maintaining the bacteria-bacteriophage co-culture for 24 h. Briefly, 100 mL of fresh LB broth was inoculated with overnight bacterial culture (adjusted to 2×10^8, 1×10^9, and 2×10^9 CFU/mL) and phage with different MOI (0.01, 0.0001, 0.000001, and 0.00000001 at each bacterial inoculum concentration). At each time point (0, 1, 3, 5, 7, 9, 12, and 24 h), the bacterial growth and amplification of phage were calculated as mentioned above.

Effect of growth medium source on phage amplification

To select the optimal nutritional sources, we adapted one-factor-at-a-time method, which is used for conventional scale up processes. This approach is conducted by replacing one nutritional factor in the basal medium, while all the other factors are kept constant. In the experiments with carbon source, basal medium was supplemented with 0.5% (w/v) of glucose, sucrose, fructose, glycerol, and galactose, individually. To assess the effect of nitrogen sources, LB was supplemented with 0.1% (w/w) of

casamino acid, peptone, gelatin, and glycine, individually. We also examined different surfactants (Tween 20, triton X-100, and SDS) at a concentration of 0.01% (v/v) to maximize the host strain surface for easy phage adherence. The influence of divalent cations on bacteriophage production was investigated by supplementing 0.01 M of calcium chloride or magnesium chloride to LB medium.

Determination of specific growth rate (μ)

To determine the specific growth rate (μ), the overnight bacterial culture was inoculated and cultured in fresh medium containing the above-mentioned supplements. The μ value was established as previously described, where $\mu = (\triangle \ln OD_{600}) \triangle time^{-1}$ [42].

Experimental design for optimization of phage amplification

To determine the optimal levels of the selected variables, experimental design was carried out according to the central composite design method of RSM coded in Minitab (v16.2) software. Among the variables, glycerol, glycine, and calcium chloride were selected as the carbon, nitrogen, and ion source, respectively, as they displayed the highest phage production. The experimental setup consisted of 20 trials (Table 2), and all the experiments were conducted in triplicates. A second order polynomial equation was used for the analysis of phage production:

$$Y=b\_0+\sum b_i X_i+\sum b_{ii} X_i^2+\sum b_{ij} X_i X_j$$

where, Y is the predicted response, b_0 is the constant, b_i is the linear coefficient, b_{ii} is the quadratic coefficient, b_{ij} is the interaction coefficient, X_i is the independent variable, X_i^2 is the squared effect, and $X_i X_j$ is the interaction effect. The quadratic model was visualized as counterplots, and response surface curve was generated using Minitab (v16.2) for each variable. The correlation between μ and yield was analyzed using the regression model in the same software. Statistical analysis of the model was conducted using ANOVA and $p < 0.05$ was considered as significant.

Abbreviations

MOI: Multiplicity of infection

RSM: Response surface methodology

CCD: Central composite design

PFU: Plaque forming unit

OD: Optical density

LB: Luria-Bertani

ANOVA: Analysis of variance

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Conceptualization, S.G.K.; methodology, S.G.K., S.Y., and H.J.K.; software, S.G.K. and J.W.K.; validation, S.G.K. and S.S.G.; formal analysis, S.G.K., and S.Y.; investigation, S.G.K.; resources, S.S.G., S.W.K., J.K., W.J.J, and H.J.K.; data curation, S.G.K. and S.C.P.; writing—original draft preparation, S.G.K.; writing—review and editing, S.G.K., S.S.G. and S.C.P.; visualization, S.G.K., and S.B.L.; supervision, S.C.P.; project administration, S.C.P.; funding acquisition, S.C.P. All authors have read and agreed to the published version of the manuscript.

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Tables

Table 1. Effects of MOI on lytic *Staphylococcus aureus* phage pSa-3 production.

Bacterial inoculum (CFU/mL)	Phage inoculum (MOI)	$Production_{max}$ [a] (10^{10} PFU/mL)	Production time[b] (h)
2×10^8	0.01	$(1.27\pm0.057)\times10^{-1}$	7
	0.0001	$(4.27\pm0.551)\times10^{-1}$	9
	0.000001	$(1.36\pm0.112)\times10^{0}$	24
1×10^9	0.01	$(7.27\pm1.36)\times10^{-1}$	7
	0.0001	$(1.68\pm0.176)\times10^{0}$	9
	0.000001	$(1.54\pm0.175)\times10^{-2}$	24
	0.00000001	$(5.57\pm0.17)\times10^{-5}$	24
2×10^9	0.01	$(1.25\pm0.15)\times10^{0}$	9
	0.0001	$(3.00\pm2.65)\times10^{-2}$	9
	0.000001	$(2.73\pm0.17)\times10^{-4}$	24
	0.00000001	$(2.00\pm1.00)\times10^{-5}$	24

[a] The phage concentration produced to the maximum. The values were shown as mean ± SD from triple replicates.

[b] Time to produce the maximum phage concentration.

Table 2. The Central Composite Design (CCD) of experiments and response of *Staphylococcus aureus* phage pSa-3 production.

Standard Order	Variables (coded value)			Variables (experimental value)		
	Glycerol X_1	Glycine X_2	CaCl2 X_3	Glycerol (mg)	Glycine (mg)	CaCl2 (mM)
1	-1	-1	1	250.0	25.0	15.0
2	1	-1	-1	750.0	25.0	5.0
3	1	1	1	750.0	75.0	15.0
4	-1	1	-1	250.0	75.0	5.0
5	0	0	0	500.0	50.0	10.0
6	0	0	0	500.0	50.0	10.0
7	1	1	-1	750.0	75.0	5.0
8	0	0	0	500.0	50.0	10.0
9	-1	-1	-1	250.0	25.0	5.0
10	1	-1	1	750.0	25.0	15.0
11	-1	1	1	250.0	75.0	15.0
12	0	0	0	500.0	50.0	10.0
13	1.633	0	0	908.0	50.0	10.0
14	0	0	-1.633	500.0	50.0	1.8
15	0	0	1.633	500.0	50.0	18.1
16	0	-1.633	0	500.0	9.0	10.0
17	0	1.633	0	500.0	90.0	10.0
18	0	0	0	500.0	50.0	10.0
19	0	0	0	500.0	50.0	10.0
20	-1.633	0	0	91.0	50.0	10.0

Table 3. The experimental design and response of lytic *Staphylococcus aureus* phage pSa-3 production.

Standard Order	Glycerol X_1	Glycine X_2	CaCl2 X_3	Observed values (10^{10} PFU/ml)	Predicted values (10^{10} PFU/ml)
1	-1	-1	1	1.74±1.42	4.81
2	1	-1	-1	4.54±0.17	3.78
3	1	1	1	3.57±0.29	3.78
4	-1	1	-1	8.31±0.93	4.81
5	0	0	0	5.11±0.41	4.36
6	0	0	0	5.12±0.23	4.36
7	1	1	-1	2.01±0.21	3.77
8	0	0	0	5.09±0.18	4.36
9	-1	-1	-1	9.41±2.08	4.81
10	1	-1	1	2.88±0.40	3.79
11	-1	1	1	4.05±1.85	4.81
12	0	0	0	5.06±0.28	4.36
13	1.633	0	0	1.98±0.42	3.35
14	0	0	-1.633	5.26±0.26	4.36
15	0	0	1.633	6.83±0.69	4.36
16	0	-1.633	0	7.07±1.10	4.36
17	0	1.633	0	7.60±1.24	4.35
18	0	0	0	5.20±0.26	4.36
19	0	0	0	5.13±0.31	4.36
20	-1.633	0	0	4.05±0.75	5.03

Table 4. Analysis of variance (ANOVA) of the experimental results for the lytic *Staphylococcus aureus* phage pSa-3 production.

	DF	Adj Sum of squares	Adj Mean square	F value	P value
Regression	9	6.53243E+21	8.96997E+19	0.47	0.639
X_1	1	1.44869E+21	1.44869E+21	7.65	0.024
X_2	1	4.30955E+17	4.30955E+17	0.00	0.963
X_3	1	6.74732E+30	6.74732E+20	3.56	0.096
X_1^2	1	1.48521E+21	1.33629E+21	7.06	0.029
X_2^2	1	4.84644E+20	4.96999E+20	2.62	0.144
X_3^2	1	2.20061E+19	2.20061E+19	0.12	0.742
X_1X_2	1	1.16790E+20	1.16790E+20	0.62	0.455
X_1X_3	1	1.74936E+21	1.74936E+21	9.24	0.016
X_2X_3	1	5.50567E+20	5.50567E+20	2.91	0.127
Residual error	8	1.51494E+21	1.89367E+20		
Lack of fit	5	1.51462E+21	3.02925E+20	2890.10	0.000
Pure error	3	3.14444E+17	1.04815E+17		
Total	19	8.22677E+21			

Figures

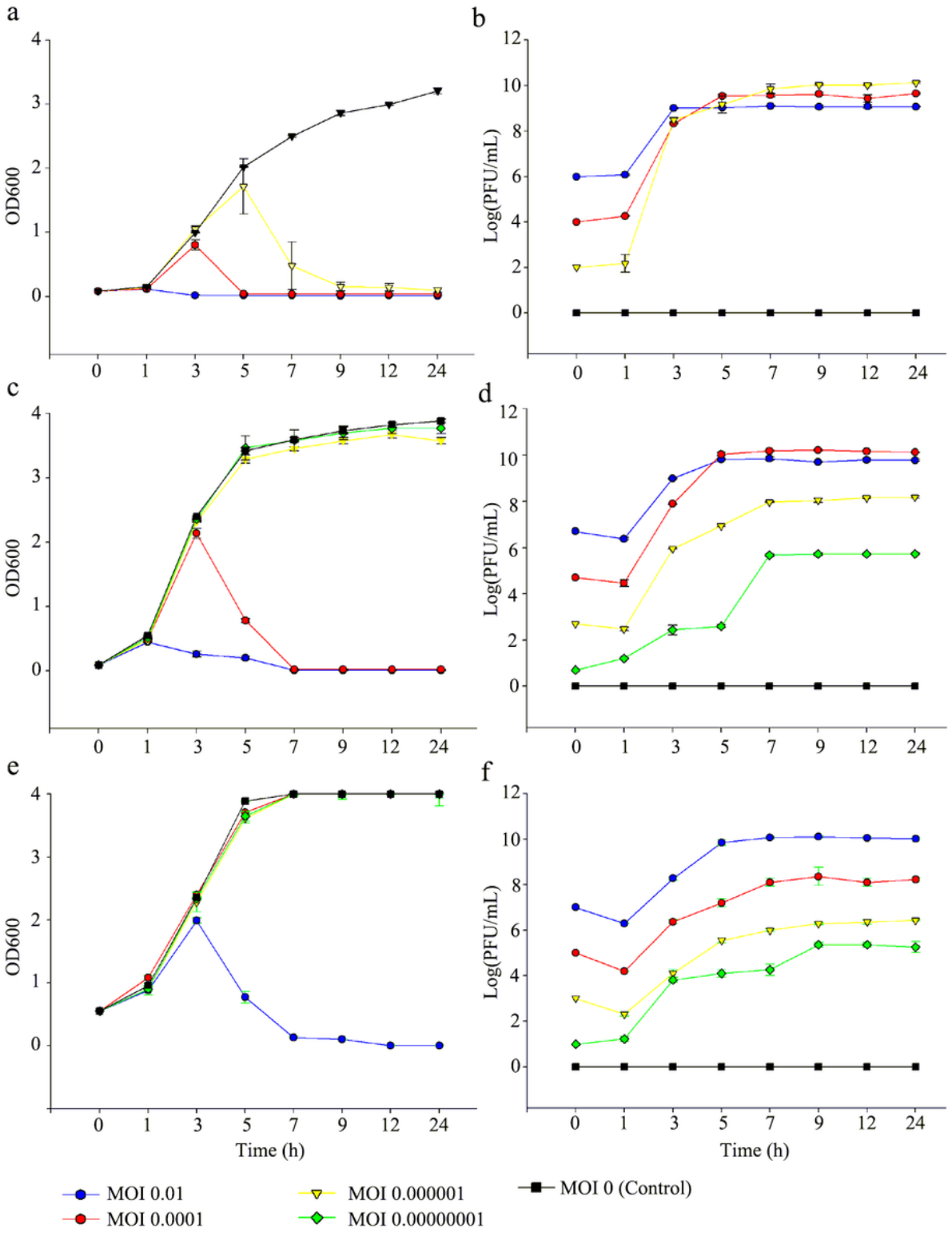

Figure 1

Growth curve of the microorganisms in the bacteria-phage co-culture. Bacterial growth was measured using optical density (a, c, e). Phage growth was measured in PFU count (b, d, f). 2 × 108 CFU/mL of initial Staphylococcus aureus concentration (a, b), 1 × 109 CFU/mL of S. aureus concentration (c, d), and 2 × 109 CFU/mL of S. aureus concentration (e, f). The experiment was performed in triplicates.

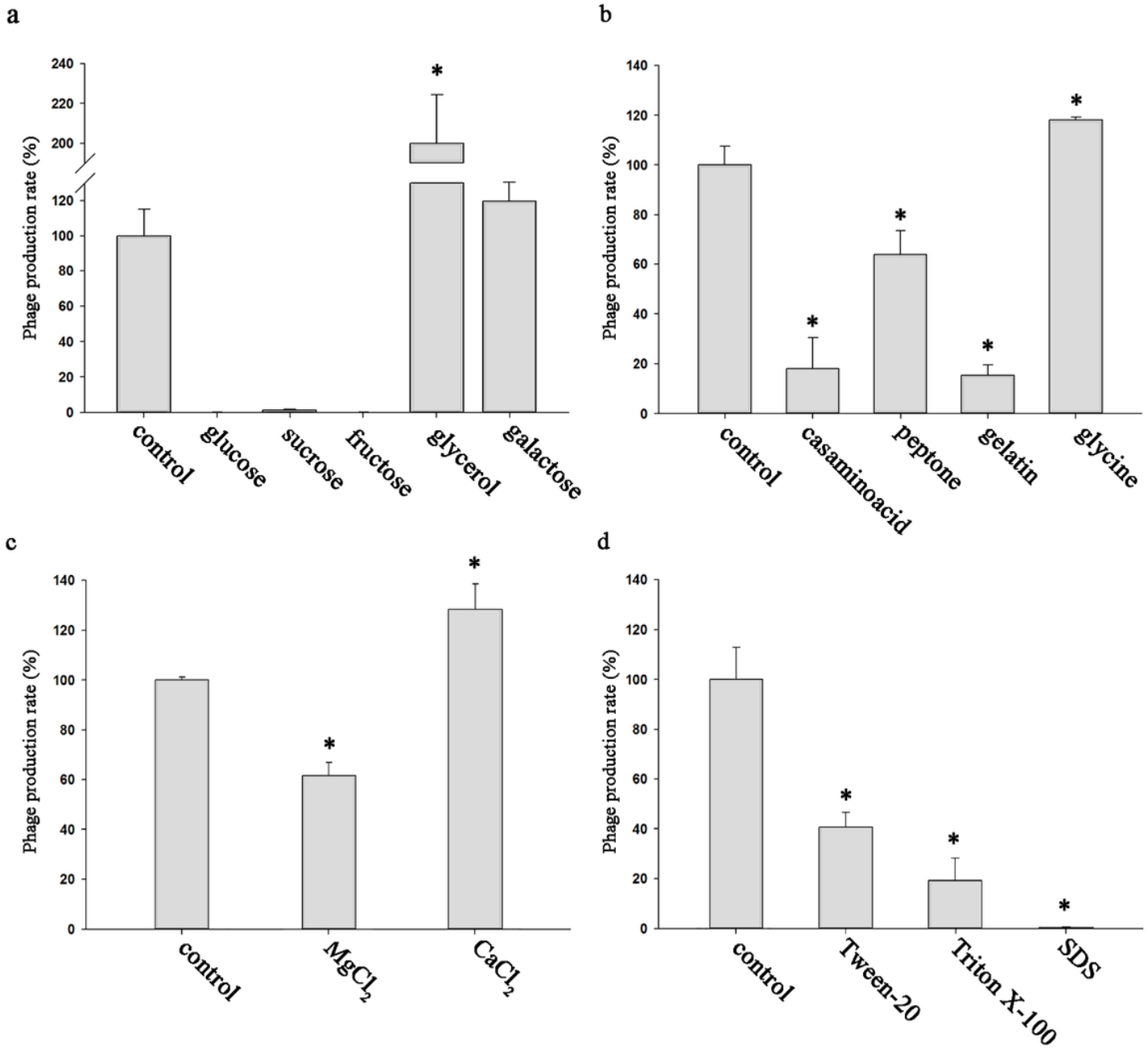

Figure 2

Media supplement screening for maximizing the lytic Staphylococcus aureus phage pSa-3 production with carbon sources (a), nitrogen sources (b), divalent ion sources (c), and surfactants (d). The experiment was performed in triplicates. Statistical significance was calculated using Student's t-test, and $p < 0.05$ was considered as statistically significant.

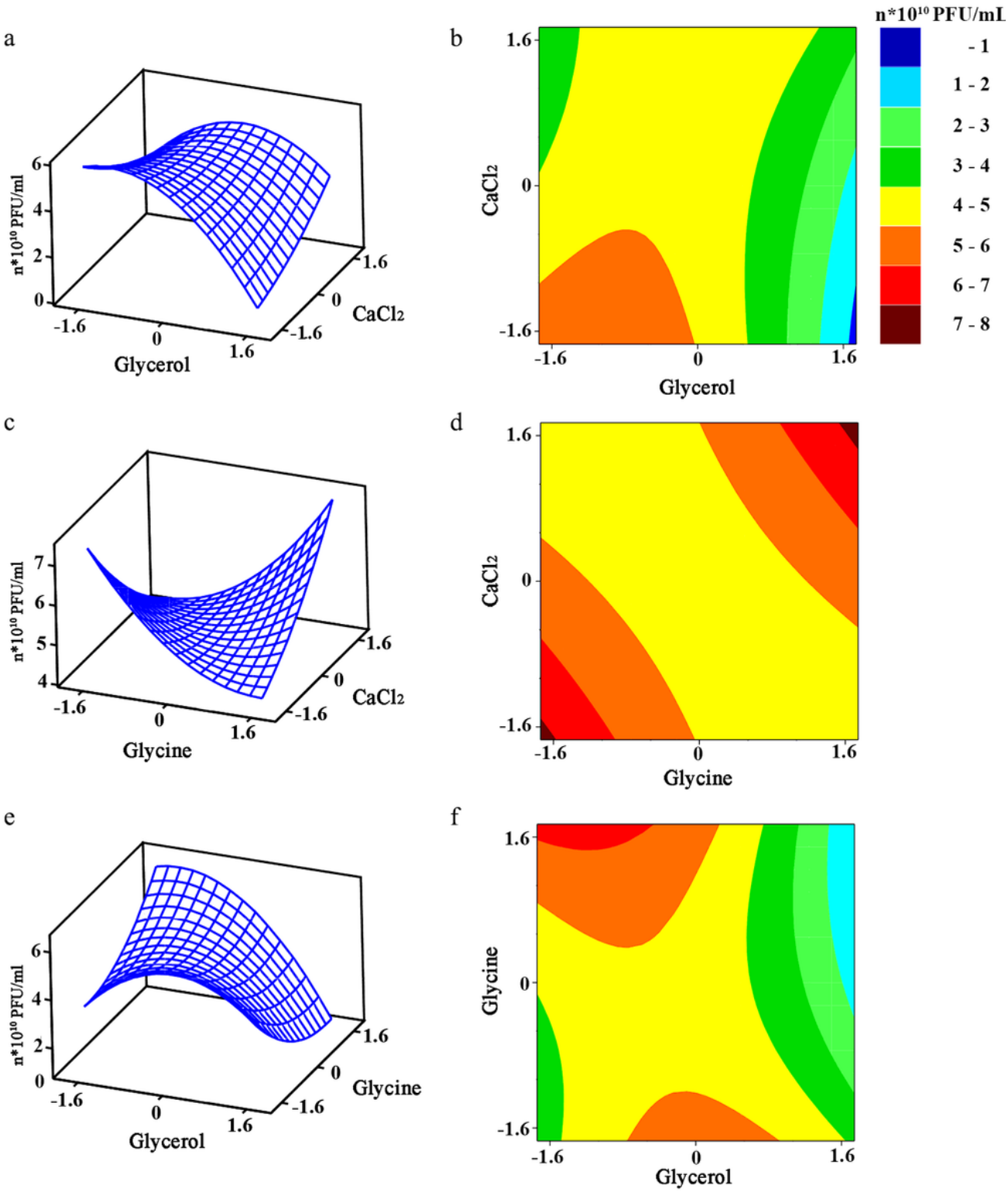

Figure 3

Response surface contour plots of media supplements for lytic Staphylococcus aureus phage pSa-3 production in 3D (a, c, e) and 2D (b, d, f). The effect of glycerol and calcium chloride on phage production (a, b). The effect of glycine and calcium chloride on phage production (c, d). The effect of glycerol and glycine on phage production (e, f).

Supplementary Files

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- FigS1.tif