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Increasing Biosynthesis of a Carotenoid by *Deinococcus radiodurans* R1 in the Presence of Tricarboxylic Acid Cycle Intermediates Using Response Surface Methodology

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Abstract

The application of carotenoids in the pharmaceutical industry has received great attention in recent years. In this regard, extremophile microorganisms, such as *Deinococcus radiodurans* R1 (ATCC 13939), have shown to be a promising source of carotenoids. However, there is no previous study concerning the effect of tricarboxylic acid cycle intermediates on the carotenoid biosynthesis of this bacterium. In the present study, the influence of process parameters (citrate, malate, succinate, glutamate, and pH) on the carotenoid production of *D. radiodurans* R1 was evaluated using the response surface methodology. A total of 32 experiments at five levels for each factor were analyzed using the Design Expert 7 software. According to the results, the response surface methodology was a robust optimization method to enhance the production of carotenoid by *D. radiodurans* R1 (52.3 mg/L) as much as 21.8-fold more than the unoptimized conditions (2.4 mg/L). For the studied strain, the optimum cultural condition for carotenoid biosynthesis was observed in the fermentation medium (pH 7) containing citrate (10mM), malate (15mM), succinate (10mM), and glutamate (10 mM). Overall, the results of this study revealed that the microbial biomass and carotenoid production were affected by malate more significantly than the other studied tricarboxylic acid cycle intermediates.

1. Introduction

Carotenoids are tetraterpenoid organic pigments that contain yellow, orange, and red colors. They are produced chemically or naturally by different organisms such as bacteria, cyanobacteria, yeast, fungi, algae, and plants. Animals, including humans, have cannot synthesize carotenoids. Therefore, they must uptake carotenoids exclusively from their diet [16].

The application of carotenoids in various industries, particularly the pharmaceutical industry, has caused these compounds to receive more attention in recent years. The properties and activities, such as antioxidant, anti-inflammatory, anticancer, anti-obesity, immunomodulatory,

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being vitamin precursors, and protection against chronic diseases, are some of the most interesting applications of carotenoids in the pharmaceutical industry [11]. In addition to the pharmaceutical industry, carotenoids are widely used in other industries as the natural colorants in food, feed, and cosmetics [8; 15].

The global market for carotenoids was $1.5 billion in 2017, and it was estimated to be approximately $2.0 billion by 2022 [9]. This can explain the importance of biotechnological overproduction of carotenoids in a cost-effective manner [4]. More than 1100 different types of carotenoids have been reported so far; however, efforts to find new carotenoids are ongoing [19].

Carotenoid pigments are often synthesized chemically due to their high purity and cost-effectiveness. However, the chemical reactions of carotenoid synthesis often contain a number of non-biological by-products that could adversely influence the environment and human health [12]. Therefore, the natural carotenoids have been recently receiving more attention. Microbial strains are some of the best biocatalysts for natural carotenoid production due to their rapid growth, low cost of fermentation media, as well as the biodegradability, antioxidant, and antibacterial activity of microbial pigments [14]. Although numerous types of bacterial carotenoids have already been described, efforts to find microbial strains producing new carotenoids are ongoing due to the wide range of carotenoids applications in various industries [19]. Radiation-resistant microbial strains such as *Microbacterium* sp. LEMMJ01, *Deinococcus xibeiensis* R13, and *Deinococcus* sp. strain WMA-LM9 are promising sources of carotenoids [10; 21; 22; 29].

In the present study, a polyextremophile microorganism and one of the most radiation-resistant strains described so far, *Deinococcus radiodurans*, was investigated. This bacterium can tolerate an acute dose of 5,000 grays of ionizing radiation without loss of viability [18]. Previous studies have reported that the resistance of *D. radiodurans* R1 to oxidative stress is related to the protective effect of carotenoids on intracellular proteins [27; 28]. This bacterium is also resistant to desiccation, vacuum, low pH, and low temperature. Colonies of *D. radiodurans* are pink to red. Previous studies reported that this strain produces a red color carotenoid named deinoxanthin that exhibits significantly strong antioxidant activity [7; 13; 27; 30]. Hence, research on the pharmacological value of deinoxanthin as a potential antioxidant compound is valuable. However, there is limited information concerning the optimization of carotenoids production by this bacterium, especially in the presence of Krebs or tricarboxylic acid cycle (TCA) cycle derivatives. The positive effects of TCA intermediates on the carotenoid production of microbial strains have been previously investigated [5; 17]. This research is carried out because the essential carotenoid biosynthesis cofactors (ATP and NADPH) and carotenoid biosynthesis substrates (acetyl-CoA, glyceraldehyde-3-phosphate (G3P), and pyruvate) could be provided through the central carbon pathway and TCA cycle [20; 24].

In this regard, this study aimed to evaluate the influence of various fermentation conditions on the biosynthesis of carotenoid pigments by *D. radiodurans* through a two-step optimization strategy to increase the microbial biomass and carotenoid production of the studied strain. The first step includes a screening stage with the one-factor-at-a-time (OFAT) method, and the second step is the optimization stage with a response surface methodology (RSM) approach based on the rotatable central composite design (RCCD).

2. Materials and methods

2.1. Microorganism

The studied strain, *D. radiodurans* R1, was purchased from the Iranian Biological Resource Center (ATCC 13939; IBRC-M 10806). The strain was subcultured and maintained on a TGY (tryptone, glucose, and yeast extract) agar medium (pH 7.2) at 4 °C. For long-term storage of the microbial strain, an equal volume of 50% v/v glycerol was added to the bacterial culture medium (TGY broth medium), and then it was stored at -20°C [3].

2.2. Screening stage

The screening stage was performed with the OFAT approach in which only one of the studied factors is changing at a time while the other factors are kept fixed instead of multiple factors changing simultaneously. Glucose, sucrose, maltose, starch, xylose, sorbitol, raffinose, citrate, malate, succinate, yeast extract, tryptone, peptone, casein, ammonium sulfate, calcium nitrate, potassium nitrate, ammonium nitrate, and glutamate were added separately in a broth medium containing 60 g/L cheese whey to investigate the influence of various carbon and nitrogen sources on the carotenoid production of the studied strain. The OFAT method does not consider the interaction between variables. Therefore, the optimization of the fermentation medium formulation was carried out using RSM.

2.3. Statistical Optimization Design

Five independent variables (citrate, glutamate, malate, succinate, and pH) were selected based on the results of the screening stage. The optimization strategy was carried out through the RSM approach, including a rotatable central composite design (RCCD). Each independent factor was studied at five levels, including the center point (0), axial point (+α, -α), and factorial point (+1, -1), in a set of 32 experiments (Table 1). The results represent the average of the observations in triplicate. The studied range of each factor in the RSM approach was based on the results of the OFAT method.

Table 1: Five independent variables in the coded and actual experimental levels.

Factor	Name	-α	-1	0	+1	+α
X_1	Citrate (mM)	7.5	10	12.5	15	17.5
X_2	Malate (mM)	7.5	10	12.5	15	17.5
X_3	Succinate (mM)	7.5	10	12.5	15	17.5
X_4	Glutamate (mM)	7.5	10	12.5	15	17.5
X_5	pH	6	7	8	9	10

The relationship between the responses (dependent variables) and the five independent variables was described using the following quadratic equation:

$$\mathbf{Y\ (response)} = b_0 + \sum_{i=1}^{5} b_i X_i + \sum_{i=1}^{5} b_{ii} X_i^2 + \sum_{i=1}^{5} \sum_{j<i} b_{ij} X_i X_j \qquad (1)$$

In equation 1, b_0 is the constant, b_i is the linear coefficients, b_{ii} is the quadratic coefficients, and b_{ij} is the cross-product coefficients. Xi and Xj are the coded independent variables (Table 1).

The statistical significance of the quadratic model and the quality of fit of the model were determined by the *F*-value and the coefficient of determination (R-Squared), respectively. All analyses were conducted using Design Expert 7.0 software (Stat-Ease, Minneapolis, USA) with a 95% confidence interval.

2.4. Media and Culture Condition

The initial fermentation medium was a 60 g/L Cheese whey medium (CHM). Cheese whey powder was purchased from the Pegah Company (Iran). The deproteinization was carried out by autoclaving CHM at 110°C for 15 min as the heat treatment. Then, it was filtered through Whitman paper (No. 40) and then centrifuged at 4000 rpm for 20 min to withdraw the clear supernatant. Next, the studied carbon and nitrogen sources including citrate, malate, succinate, and glutamate in concentrations of 7.5, 10, 12.5, 15, and 17.5 mM were added to the primary fermentation medium (100 mL) at this stage, according to the statistical experimental design (Table 2). Finally, the pH of the fermentation medium was adjusted by NaOH (1M) and HCl (1M), and the resulted solution was autoclaved again at 110°C for 15 min.

The inoculum was prepared using a loopful of pure culture in 5 mL of TGY broth medium (pH 7.2) containing 5 g/L tryptone (Merck, Germany), 1 g/L glucose (Merck, Germany), and 5 g/L yeast extract (Merck, Germany) [2]. Next, the microbial strain was incubated at 37°C (120 rpm) until reaching an optical density (OD) equivalent to 0.5 McFarland standard (1–2×10^8 CFU/ml), after

which it was inoculated into the fermentation medium at the concentration of 1% v/v, and then incubated at 30°C for five days on a rotary shaker at 120rpm for aeration. The TGY agar medium was prepared by adding 1.5٪ w/v agar to a TGY broth medium [2].

2.5. Total carotenoid and Microbial Biomass Assay

The amounts of microbial growth and carotenoid production were evaluated after five days of incubation in each experiment (30°C, 120 rpm). The microbial biomass was measured in CHM after centrifugation at 4000 rpm for 5 min. Subsequently, the resulted pellets were dried at 70°C for 72 h and then weighed. The amount of microbial biomass was calculated according to equation 2. In this equation, the control medium means the CHM without any bacterial inoculation.

Microbial biomass (g/L) =Dry weight of bacterial pellet in fermentation medium-dry weight of pellet in control medium (2)

Bacterial pellets resulting from 10 mL of fermentation medium (CHM), as previously described, were mixed with 30 mL of extraction solvent, *i.e.*, methanol 95% v/v. The resulting mixture was incubated at 45°C for 10 min and then centrifuged at 4000 rpm for 10 min. The resulted supernatant was collected, and the residual biomass was remixed with the solvent. The following steps were repeated until it was completely colorless. All collected supernatants were dried at 40°C and then stored at 4ºC for further analysis. To confirm the efficiency of the extraction procedure, UV/Vis spectra of the extract were evaluated at 200–600 nm using a spectrophotometer (Biochrom WPA Biowave II, ZEISS Specord, Germany). Then the amount of carotenoid pigment was calculated in 10 ml CHM.

$$\textbf{Total carotenoids } \left(\frac{\mu g}{L}\right) = \frac{(A_{474}) \times (V_S) \times (10^9)}{(A^{\%1}_{1\,cm}) \times (100)} \quad (3)$$

In equation 3, A_{474}, V_s, and $A^{1\%}_{1\,cm}$ are the total carotenoid maximum absorbance in the solvent, the sample solution volume, and the total carotenoid specific absorption coefficient in a 1 cm cell for a 1% solution, respectively [6].

3. Results and Discussion

Statistical optimization is a cost-effective approach to enhance the biosynthesis of various biotechnological products. Production of carotenoids is influenced by different variables such as carbon and nitrogen sources, incubation time, the temperature of incubation, and the pH of the fermentation medium. Therefore, it is critical to optimize the effective factors because even small improvements in carotenoids production processes are significant for commercial success.

We used a cost-effective fermentation medium, *i.e.*, 60% g/L Cheese whey medium, to produce the *D. radiodurans* R1 carotenoid pigment. Cheese whey contains 4.5% lactose and 0.8% casein; therefore, a 60% g/L Cheese whey medium has about 2.7 g lactose as the carbon source and 0.48 g casein as the nitrogen source [26]. In addition to being rich in mineral and organic resources, whey is a by-product of dairy industries.

In the present study, five independent variables were selected based on the results of the OFAT approach (data not shown). The independent variables were glutamate, citrate, malate, succinate, and the pH of the fermentation medium. The optimization stage was performed using an RCCD with the selected factors, including 32 experiments done in triplicate. The results are presented in Table 2.

The maximum carotenoid biosynthesis (52.3 mg/L) was observed in the fermentation medium (pH 7) containing citrate (10 mM), malate (15 mM), succinate (10 mM), and glutamate (10 mM). The maximum microbial biomass production (15.9 g/L) was also observed in the same fermentation. Therefore, the increase in biomass production increased the carotenoid biosynthesis of the studied train. However, the minimum carotenoid content (4.7 mg/L) was observed in the fermentation medium (pH 9) containing citrate (15 mM), malate (15 mM), succinate (15 mM), and glutamate (15 mM).

The perturbation plot shows the effect of all the independent variables at the midpoint (coded 0) in the design space (Fig. 1). As shown in this figure, malate had a more direct relationship with carotenoid biosynthesis of *D. radiodurans* R1.

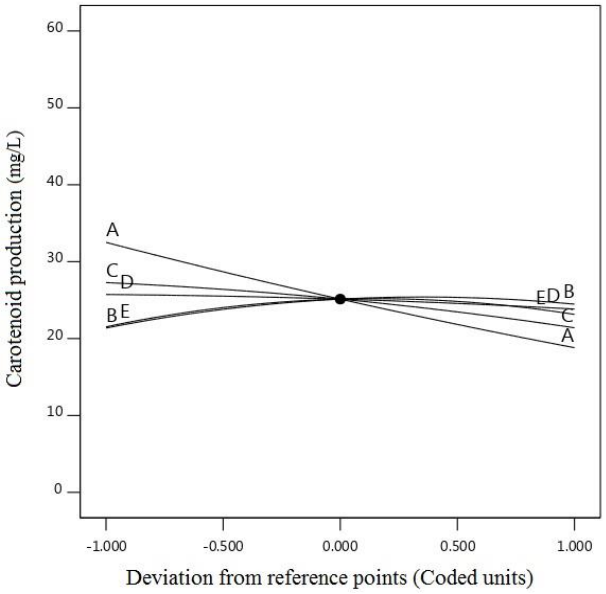


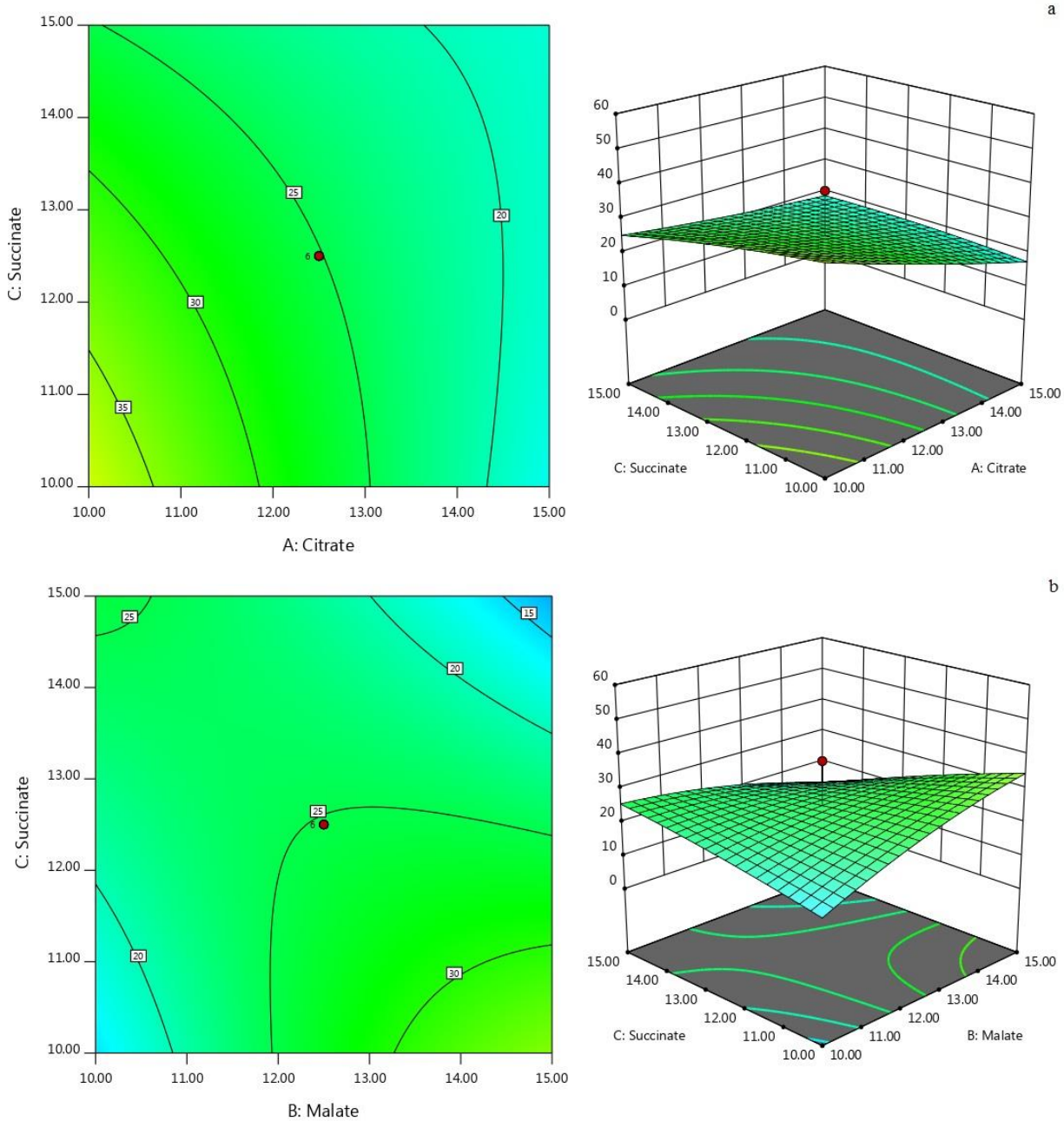
Fig. 1. Perturbation plot of carotenoid production of *D. radiodurans* R1. The letters in the graph represent the following: A (citrate), B (malate), C (succinate), D (glutamate), and E (pH).

Table 2: An RCCD Matrix and Experimental Responses of *D. radiodurans* Strain R1

Run No.	Citrate (mM)	Malate (mM)	Succinate (mM)	Glutamate (mM)	pH	Biomass (g/L)	Carotenoid (mg/L)
1	12.5	12.5	12.5	12.5	8.00	11.01	24.86
2	10.0	10.0	10.0	10.0	9.00	10.54	22.40
3	15.0	10.0	10.0	15.0	9.00	12.32	16.63
4	12.5	12.5	12.5	12.5	8.00	10.12	24.40
5	12.5	12.5	12.5	12.5	8.00	11.32	23.59
6	**10.0**	**15.0**	**10.0**	**10.0**	**7.00**	**15.95**	**52.27**
7	15.0	15.0	15.0	15.0	9.00	8.654	4.727
8	12.5	12.5	12.5	7.50	8.00	11.14	24.86
9	15.0	10.0	10.0	10.0	7.00	6.23	8
10	12.5	12.5	12.5	12.5	8.00	12.41	24.95
11	12.5	7.50	12.5	12.5	8.00	12.35	9.727
12	15.0	10.0	15.0	15.0	7.00	15.14	13.36
13	10.0	15.0	15.0	10.0	9.00	13.65	7.59
14	12.5	12.5	12.5	12.5	8.00	12.14	22.77
15	15.0	10.0	15.0	10.0	9.00	8.124	12.18
16	12.5	12.5	17.5	12.5	8.00	10.47	11.09
17	10.0	15.0	15.0	15.0	7.00	6.547	5.772
18	12.5	12.5	12.5	17.5	8.00	11.12	16.72
19	15.0	15.0	10.0	10.0	9.00	12.36	15.90
20	12.5	12.5	7.50	12.5	8.00	11.47	24.81
21	12.5	12.5	12.5	12.5	6.00	9.87	8.772
22	12.5	12.5	12.5	12.5	10.0	9.95	11.09
23	15.0	15.0	10.0	15.0	7.00	13.65	15.13
24	12.5	12.5	12.5	12.5	8.00	12.32	38.22
25	17.5	12.5	12.5	12.5	8.00	12.36	9.227
26	10.0	10.0	15.0	15.0	9.00	12.47	47.86
27	10.0	10.0	15.0	10.0	7.00	13.65	27.09
28	15.0	15.0	15.0	10.0	7.00	11.36	32.09
29	10.0	15.0	10.0	15.0	9.00	13.23	52.22
30	7.50	12.5	12.5	12.5	8.00	12.12	37.22
31	10.0	10.0	10.0	15.0	7.00	6.987	11.09
32	12.5	17.5	12.5	12.5	8.00	12.32	14.81

Overall, the statistical optimization of the fermentation medium containing the TCA cycle intermediates increased the carotenoid production of *D. radiodurans* R1 about 21.8 times. The maximum amount of carotenoid pigment by the strain in unoptimized conditions was 2.4 mg/L. The interactions between the studied variables are illustrated in Fig. 2. As shown in this figure, the carotenoid production increased in the presence of

10-11 mM citrate and 11-12 mM succinate. At higher concentrations of citrate and succinate, the amount of carotenoid decreased (Fig. 2a). In contrast, high concentrations of malate (14-15 mM) had a better effect on the carotenoid synthesis by *D. radiodurans* R1 (Fig. 2b, c). The studied strain produced more carotenoids in the neutral pH range of 7-7.5 (Fig. 2d).



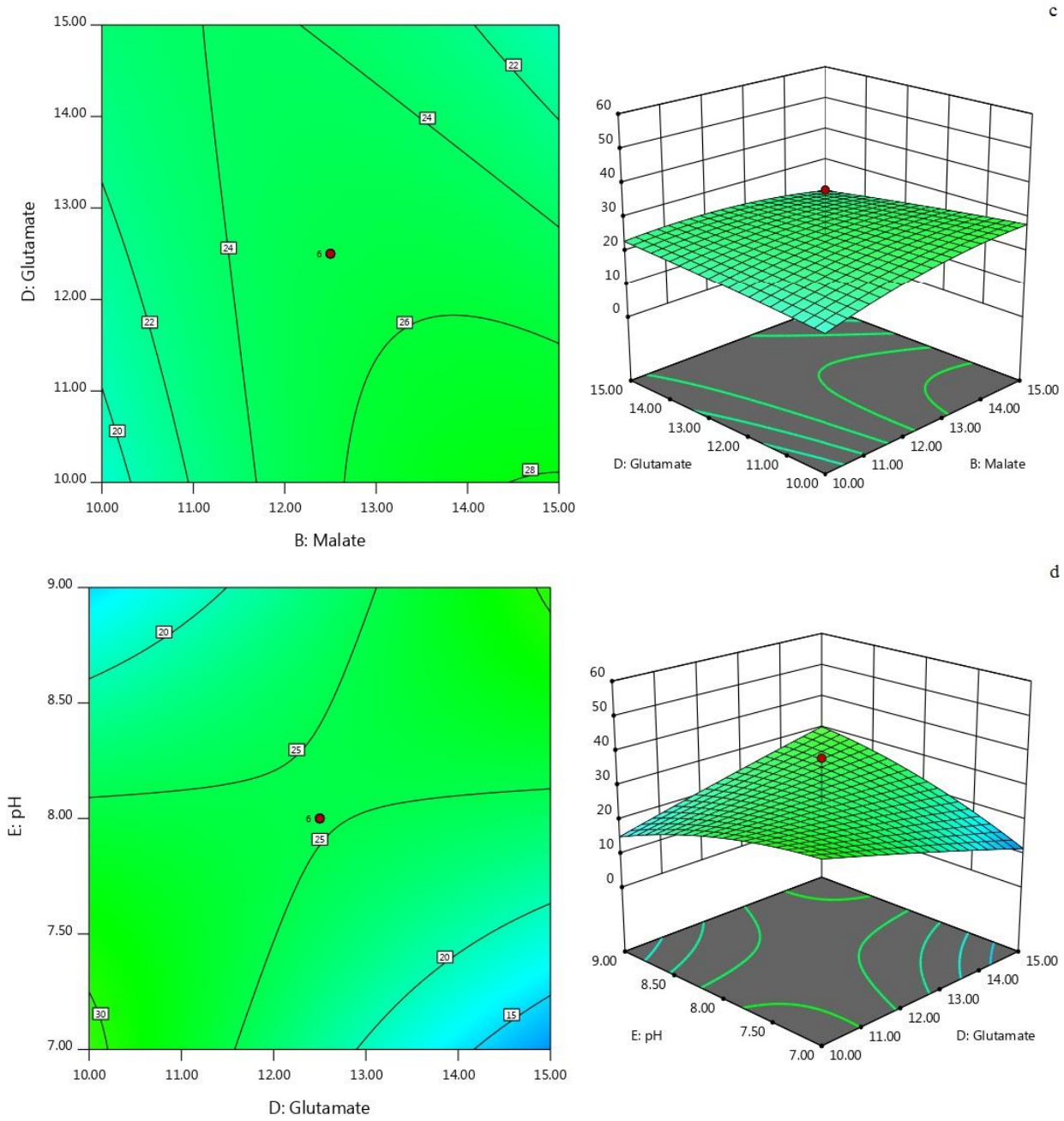


Fig. 2. Contour and three-dimensional surface plots illustrate the interactions between (a) citrate and succinate, (b) malate and succinate, (c) malate and glutamate, and (d) pH and glutamate on the carotenoid production of *Deinococcus radiodurans* R1.

The statistical analysis of the biomass and carotenoid production models for *D. radiodurans* R1 is presented in Tables 3 and 4, respectively. The significance of the regression models of biomass and pigment production of *D. radiodurans* R1 was confirmed by *F*-value (14.87 and 6.48, respectively) and the probability value (<0.0001 and 0.0014, respectively). The fit of the models was confirmed by the non-significant lack of fit concerning the pure error (0.72 and 0.43, respectively) and R^2 (0.96 and 0.92, respectively). Therefore, the regression models in the present study describe 96.43% and 92.17% of the variability in the responses, respectively. In other words, there was only 3.57% and 7.83% of the total variation that could not be explained by the

biomass and carotenoid models, respectively. Both models were strong because the R^2 value of the models was close to one [25]. The polynomial models for *D. radiodurans* R1 biomass and pigment production yield were regressed. The letters A, B, C, D and E represent citrate, malate, succinate, glutamate, and pH in both models.

Biomass (g/L) = + (11.33) - (0.13*A) + (0.37*B) - (0.13*C) - (0.13*D) + (0.13*E) - (0.19*A*B) – (0.062*A*C) + (1.56*A*D) - (0.81*A*E) - (1.81*B*C) - (1.19*B*D) - (0.063*B*E) - (0.31*C*D) - (0.69*C*E) + (0.44*D*E) + (0.17* A^2)+ (0.17* B^2) – (0.20*C^2) – (0.080*D^2) – (0.58*E^2)

Carotenoid (mg/L) = (+25.13) – (6.84*A) + (1.55*B) – (2.94*C) – (0.95*D) +(0.81*E) + (0.52*AB) + (3.52*A*C) – (1.62*A*D) – (3.31*A*E) – (7.98*B*C) – (3.08*B*D) – (4.02*B*E) – (0.23*C*D) – (1.66*C*E) + (8.59*D*E) + (0.53A^2) – (2.21*B^2) – (0.79*C^2) – (0.35*D^2) – (2.80*E^2)

Table 3: Regression analysis for the biomass production of *D. radiodurans* R1 for quadratic response surface model fitting (ANOVA)

Source	Sum of squares	Degrees of freedom	Mean square	*F*-value	*p*-value prob > F
Model[a]	156.10	20	7.81	14.87	< 0.0001[b]
A-Citrate	0.38	1	0.38	0.71	0.4159
B-Malate	3.37	1	3.37	6.43	**0.0277**
C-Succinate	0.38	1	0.38	0.71	0.4159
D-Glutamate	0.38	1	0.38	0.71	0.4159
E-pH	0.38	1	0.38	0.71	0.4159
AB	0.56	1	0.56	1.07	0.3228
AC	0.063	1	0.063	0.12	0.7365
AD	39.06	1	39.06	74.43	< 0.0001
AE	10.56	1	10.56	20.13	0.0009
BC	52.56	1	52.56	100.16	< 0.0001
BD	22.56	1	22.56	42.99	< 0.0001
BE	0.063	1	0.063	0.12	0.7365
CD	1.56	1	1.56	2.98	0.1124
CE	7.56	1	7.56	14.41	0.0030
DE	3.06	1	3.06	5.84	0.0343
A^2	0.85	1	0.85	1.62	0.2288
B^2	0.85	1	0.85	1.62	0.2288
C^2	1.23	1	1.23	2.34	0.1544
D^2	0.19	1	0.19	0.35	0.5641
E^2	9.85	1	9.85	18.77	0.0012
Residual	5.77	11	0.52		
Lack of Fit	2.44	6	0.41	0.61	0.7190
Pure Error	3.33	5	0.67		
Corrected Total Sum of Squares	161.88	31			

[a] Standard deviation (0.72); Mean (10.94); Coefficient of variation% (6.62); PRESS: Predicted residual error sum of squares (70.14); R^2: Coefficient of determination (0.9643); Adj R^2: Adjusted R-squared (0.8995); Pred R^2: Predicted R-squared (0.5667); Adequate precision (16.188)
[b] Significant

Table 4: Regression analysis for the carotenoid production of *D. radiodurans* R1 for quadratic response surface model fitting (ANOVA)

Source	Sum of squares	Degrees of freedom	Mean square	*F*-value	*p*-value prob > F
Model[a]	4879.21	20	243.96	6.48	0.0014[b]
A-Citrate	1124.37	1	1124.37	29.85	**0.0002**
B-Malate	57.89	1	57.89	1.54	0.2409
C-Succinate	206.85	1	206.85	5.49	**0.0389**
D-Glutamate	21.52	1	21.52	0.57	0.4656
E-pH	15.63	1	15.63	0.41	0.5327
AB	4.28	1	4.28	0.11	0.7424
AC	198.55	1	198.55	5.27	**0.0423**
AD	41.95	1	41.95	1.11	0.3138
AE	175.57	1	175.57	4.66	0.0538
BC	1019.64	1	1019.64	27.07	**0.0003**
BD	151.73	1	151.73	4.03	0.0699
BE	258.91	1	258.91	6.87	**0.0237**
CD	0.87	1	0.87	0.023	0.8821
CE	44.35	1	44.35	1.18	0.3011
DE	1180.87	1	1180.87	31.35	**0.0002**
A^2	8.18	1	8.18	0.22	0.6504
B^2	143.36	1	143.36	3.81	0.0770
C^2	18.32	1	18.32	0.49	0.5000
D^2	3.53	1	3.53	0.094	0.7651
E^2	229.33	1	229.33	6.09	0.0313
Residual	414.28	11	37.66		
Lack of Fit	244.96	6	40.83	1.21	0.4280
Pure Error	169.33	5	33.87		
Corrected Total Sum of Squares	5293.49	31			

[a]Standard deviation (6.14); Mean (20.92); Coefficient of variation (29.34%); PRESS: Predicted residual error sum of squares (5871.84); R^2: Coefficient of determination (0.9217); Adj R^2: Adjusted R-squared (0.7794); Pred R^2: Predicted R-squared (0.1093); Adequate precision (9.852)
[b]Significant

As shown in Table 3, malate was the statistically significant variable in biomass production (*p*-value= 0.0277). However, citrate (*p*-value= 0.0002) and succinate (*p*-value= 0.0389) were also statistically significant variables that affected the carotenoid production of *D. radiodurans* R1. Besides, the interactions between citrate and succinate (*p*-value= 0.0423), malate and succinate (*p*-value= 0.0003), malate and pH (*p*-value= 0.0237), and glutamate and pH (*p*-value= 0.0002) were all statistically significant on the carotenoid production. These results confirm that malate increased the production of carotenoids in the studied strain by increasing the microbial biomass. Moreover, the statistical significance of malate in interactions with other variables (Table 4) also indicates the significant effect of malate on the carotenoid biosynthesis of *D. radiodurans* R1.

Previous studies have reported that TCA cycle intermediates could affect the production of

carotenoids as stimulants due to providing a carbon skeleton for carotenoids [1; 5]. Our results also showed that all studied TCA cycle intermediates could increase the carotenoid biosynthesis in *D. radiodurans* R1 based on the results from OFAT and RSM approaches. The growth of *D. radiodurans* strain R1 was affected by malate more significantly (p= 0.0277). However, a low concentration of citrate and succinate and a high concentration of malate was found to stimulate the carotenoid biosynthesis of *D. radiodurans* R1. Similarly, Bhosale et al. (2004) reported that malic acid (malate) has a significant positive effect on carotenoid production by *Flavobacterium multivorum* [5]. The reason for this may be related to the fact that an increase in the concentration of malate in bacterial cells will shift the malate decarboxylation reaction equilibrium in favor of pyruvate production. Consequently, the precursor of isoprene units for carotenoid synthesis, acetyl CoA, overproduce from the resulted pyruvate. However, the results of Nasri, Nasrabadi, and Razavi (2010) showed that α-ketoglutarate, oxaloacetate, and succinate have a statistically significant positive effect on the carotenoid and cell biomass production by *Dietzia natronolimnaea* HS-1, while citrate and malate negatively affected carotenoid production. Therefore, these results show that the effect of TCA cycle intermediates on carotenoid production may vary among different microbial strains.

4. Conclusion

In summary, the results of this study revealed that RSM is a robust optimization method that enhanced the carotenoid production of *Deinococcus radiodurans* R1 (52.3 mg/L) by 21.8-fold more than the unoptimized conditions (2.4 mg/L).

Conflicts of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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