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OPTIMIZATION OF CAROTENOIDS PRODUCTION BY *RHODOTORULA MUCILAGINOSA*

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ABSTRACT: Optimization of total carotenoids production is dependent on various cultural and physical parameters for pigmented yeasts. The carotenoid production of the red yeast *Rhodotorula mucilaginosa* MTCC11835 was optimized using response surface methodology. The carotenogenesis in yeasts is known to be affected by different nutritional factors and environmental stress factors. Effect of various growth parameters like carbon source (glucose; glucose:glycerol (1:2); maltose), nitrogen source (peptone; casein hydrolysate; yeast extract), pH (3.5;5.0; 6.5) and stress factors (Tween20; hydrogen peroxide) were studied for the maximum yield of carotenoids. The yeast was shown to produce high amount of total carotenoids (21.77 mg g^{-1}) in a medium containing glucose: glycerol (1:2), peptone, 0.5% Tween 20, 2.5 mM H_2O_2 and pH 6.5.

INTRODUCTION: Carotenoids are isoprenoid compounds, biosynthesized by end to end linkage of two C_{20} geranylgeranyl diphosphate molecules. The typical C_{40} carotenoids have cyclic β-ionone end groups that can be substituted by oxo, hydroxy and epoxy groups at different positions [1]. Due to their anticancer and antioxidant properties, wider use of carotenoids as pharmaceuticals and nutraceuticals is expected. The synthesis of different carotenoids by several coloured yeast species has posed these microorganisms as a potential source of pigments [2, 3]. The high productivity of carotenoids was revealed in the representatives of the following species: *Rhodosporidium diobovatum, Rhodosporidium sphaerocarpum, Rhodotorula glutinis, Rhodotorula minuta* and *Sporobolomyces roseus* [4].

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Facing the growing economic significance of carotenoids, due to their use as food colorants, nutritional supplements, in cosmetics or in human therapy as antioxidants, much interest has been dedicated to new supplies of this class of pigments. In particular, the development of carotenoid-producing bioprocesses is regarded as a competitive solution, as it can provide important quantities of pigments such as torularhodin and β-carotene [5]. Optimization of growth medium and cultural conditions is necessary in microbial fermentations to fully exploit the potential of selected microbial strain [6].

The integrated modeling and optimization approach response surface methodology is a powerful tool for analyzing the effect of multiple variables or factors on a given process rapidly and efficiently with a minimal number of experiments while keeping a high degree of statistical significance in the results[7]. The process employed for optimization of carotenoid production varies with each strain; hence there was a need to investigate the effects of

various factors in carotenoid production by *Rhodotorula mucilaginosa* MTCC 11835.

MATERIALS AND METHODS:

In the present study a 10% (v/v) inoculum of *Rhodotorula mucilaginosa* MTCC 11835 in log phase, grown in basal medium was used (extinction 0.1 at 420 nm). The Modified Czapek-Dox medium (peptone-2.48 g l^{-1}, K_2HPO_4-1.0 gl^{-1}, $MgSO_4$-0.5 g l^{-1}, KCl-0.5 g l^{-1}, $FeSO_4$-0.01 g l^{-1}, Hi-media) was used in 250 ml Erlenmeyer flasks containing 50 ml of medium according to the design matrix. The flasks were incubated at 28±2^{0}C on a rotary shaker at 300 rpm for 5 days. For each experiment, the carotenoid pigments were extracted and the absorbance was measured spectroscopically at 478 nm. The biomass was harvested by centrifugation at 2000 rpm for 10 min. The biomass was dried at 105^{0}C until constant weight was achieved to determine cell dry weight (CDW).

The carotenoids were extracted in petroleum ether by Sedmark et al.[8] method. The absorbance of the pigment extract was measured at 478 nm using UV-Vis Spectrophotometer (Elico, SL 159). Data were expressed as total carotenoid production (TCP) mg l^{-1} of culture broth or total carotenoid content (TCC) mg g^{-1} dry biomass. Samples were analyzed in triplicates and the mean values and standard errors were calculated.

The effects of C-source, N- source, pH, Hydrogen peroxide and Tween 20 were studied using two level factorial designs. Plackett and Burman design was used to screen and evaluate the important medium components that influence the response. This method is based on the use of the polynomial model: $Y=bo + \sum biXi + \sum bijXiXj + Ei$

Where,
Y- the variable dependent response;
l- the regression coefficient;
X- the independent variable or experimental factor level and
E- the experimental error

The experimental data were statistically analyzed to determine the significant difference ($p \leq 0.05$) in response under different conditions, using Shapiro-Wilk method of Analysis of variance (ANOVA) by Design expert Statease 8.0.7.1 software. The response surface graphs were also plotted using the same software. The regression equation based on Yates Algorithm was determined using Statease Design- Expert 8.0.7.1 and are as follows:

TCC(mgg^{-1})= +13.49- 0.86*X1- 1.03*X2- 0.60*X3 2.25*X4+ 2.04*X5+ 1.21*X1*X2+ 1.05*X1*X5+ 1.82*X2*X31.40*X2*X4+ 0.86*X2*X5- 1.25*X3*X4+ 0.31*X3*X5 ….. (1)

TCP (mg l^{-1}) = +40.06- 9.58*X1- 7.37*X2- 4.75*X3- 10.91*X4 + 9.46*X5+ 5.74*X1*X2+ 7.19*X1*X4+ 10.41*X2*X3+ 8.79*X2*X5- 7.42*X3*X4+ 3.31*X3*X5 …… (2)

CDW (g l^{-1}) = +2.72- 0.26*X1- 0.21*X2- 0.17*X3- 0.44*X4+ 0.43*X5+ 0.24*X1*X2+ 0.28*X1*X5+ 0.41*X2*X30.32*X2*X4+ 0.22*X2*X5+ 6.250E- 003*X3*X5 ….. (3)

RESULTS AND DISCUSSION:

The data presented in Table 1 showed significantly different cell mass and carotenoid yield within the 16runs. It was observed that *Rhodotorula mucilaginosa* MTCC 11835 produced the highest TCC (total carotenoid content), TCP (total carotenoid production) and CDW (cell dry weight) 21.77mg g $^{-1}$, 100.42mg l^{-1} and 4.6g l^{-1} respectively when grown in the medium containing glucose: glycerol (1:2), peptone, 0.5% Tween 20, 2.5mM H_2O_2 and pH 6.5. The results presented in Table I for cell mass and carotenoid production were subjected to regression analysis and the analysis of variance (ANOVA). First order models were fitted to the data to evaluate the main effects of the five factors at the 95% confidence level. The statistical analysis of data using Two way ANOVA for TCC, TCP and CDW were found to be significant with the F-values of 4.21, 4.12 and 3.67 and the P-values 0.0444, 0.0356 and 0.0476 respectively at 5% significance. The Lack of fit for the three models was found to be insignificant (**Table 2**). The contour plots and three dimensional plots of the three responses showed diagonal ellipsoidal behavior, which confirmed the good fit of the models employed for all three responses (**Fig.1**).

The present study has demonstrated the feasibility of using response surface methodology to optimize the fermentation conditions for the production of carotenoids by *Rhodotorula mucilaginosa* MTCC 11835 in shake flasks. In earlier studies similar models were successfully used to determine the optimum settings of the culture variables for maximizing cell mass yield. Buzzini *et al* [9] found the carotenoid production in *Rhodotorula graminis* DBVPG 7021 of 803.2 μg l^{-1}; whereas in the study by Saenge et al.[10] the *Rhodotorula glutinis* TISTR 5159 strain showed the carotenoids production of 125.75 μg l^{-1}. Maldonade et al.[11] demonsttrated the total carotenoids content of 745 μgl^{-1} in *Rhodotorula mucilaginosa* – 137, while Aksu and Eren [12] found the *Rhodotorula mucilaginosa* NRRL 2502 total carotenoids production as 89 μg l^{-1} .

The relative yield of total carotenoids from *Rhodotorula mucilaginosa* MTCC 11835, used in the present study is significantly higher than earlier studied strains of *Rhodotorula.* The factors carbon and nitrogen source and pH affected the total carotenoid content, total carotenoid production and cell biomass production in the present strain of *Rhodotorula mucilaginosa*. Though the carbon source affected the biomass and carotenoid yield significantly. The three dimension response surface plots with the two variables varying within the experimental range show convex shape for the carbon source.

TABLE 1: DATA OF THE FRACTIONAL FACTORIAL DESIGN FOR *RHODOTORULA MUCILAGINOSA* MTCC11835

Run	Carbon source	Nitrogen source	pH	Tween 20	H_2O_2	CDW (g/l)	TCC (mg g^{-1})	TCP (mg l^{-1})
1	-	-	-	-	+	2.8	13.35	37.38
2	-	-	-	+	-	1.6	8.92	14.27
3	-	-	+	-	-	2.1	10.31	21.65
4	-	+	-	-	-	1.3	6.21	8.07
5	+	-	-	-	-	2	9.63	19.26
6	-	-	+	+	+	4.6	21.77	100.42
7	-	+	-	+	+	2.7	12.97	35.02
8	-	+	+	-	+	2.6	12.81	33.31
9	-	+	+	-	+	1.8	9.85	17.73
10	+	+	+	-	-	4.1	19.77	81.06
11	+	+	-	+	-	3.9	18.22	71.06
12	+	+	-	-	+	1.4	8.71	12.19
13	+	-	+	-	+	2.8	13.5	34.14
14	+	-	+	+	-	3.1	14.5	44.95
15	+	-	-	+	+	1.6	8.01	12.82
16	+	+	+	+	+	1.6	8.19	13.1
17	0	0	0	0	0	3.7	19.02	47.44
18	0	0	0	0	0	4.4	21.33	93.15
19	0	0	0	0	0	2.8	14.68	41.1
20	0	0	0	0	0	3.5	18.04	63.14

C- source (X1) = Glucose (1); Glucose:glycerol (0); Maltose (-1)
N- source (X2) =Peptone (1); Casein hydrolysate (0); Yeast extract (-1)
pH (X3) = 3.5 (1); 5.0 (0); 6.5 (-1)
Tween 20 (X4) = 0.1% (1); 0.25% (0); 0.5% (-1)
H_2O_2 (X5) = 2.5mM (1); 5.0mM (0); 10mM (-1)

TABLE 2: THE ANOVA ANALYSIS OF TCC (mg g^{-1}), TCP (mg l^{-1}) and CDW (g l^{-1}) PRODUCTION IN *RHODOTORULA MUCILAGINOSA* MTCC 11835

Response	TCC (mg g^{-1})			TCP (mg l^{-1})			CDW (g l^{-1})		
Source	Sum of squares	F value	p-value Prob>F	Sum of squares	F value	p-value Prob>F	Sum of squares	F value	p-value Prob>F
Model	345.32	4.21	0.0444 †	11413.04	4.12	0.0356 †	15.56	3.67	0.0476 †
Curvature	53.12	7.78	0.0316 †	1857.24	7.37	0.0300 †	2.42	6.27	0.0407 †
Residual	40.98			1763.21			2.70		
Lack of Fit	24.40	1.47	0.3792 †††	1482.24	3.96	0.1436 †††	1.73	1.34	0.4291 †††

Key: † significant values ††† non significant values

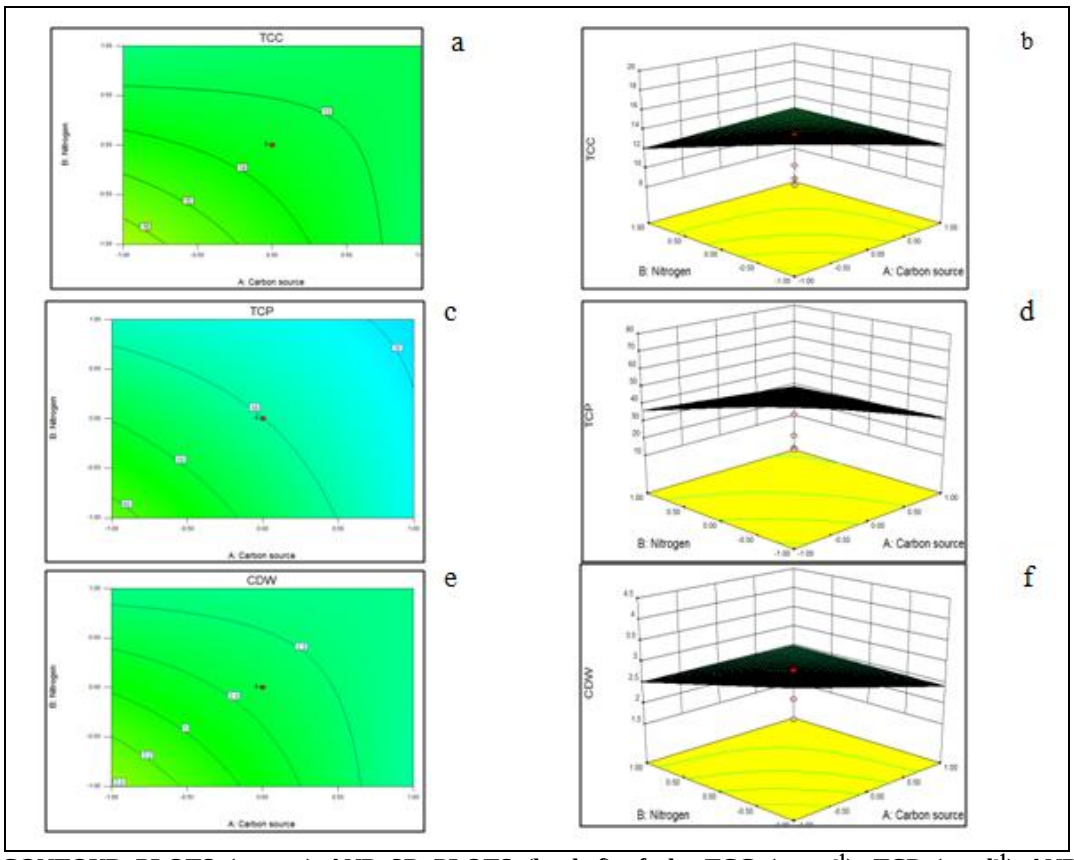

FIG. 1: THE CONTOUR PLOTS (a, c, e) AND 3D PLOTS (b, d, f) of the TCC (mg g^{-1}), TCP (mg l^{-1}) AND CDW (g l^{-1}) RESPECTIVELY FOR THE OPTIMIZATION STUDIES OF *RHODOTORULA MUCILAGINOSA* MTCC 11835

CONCLUSION: The strain *Rhodotorula mucilaginosa* MTCC 11835 used in the present optimization study showed a significant increase in the yield of total carotenoids (21.77 mg g^{-1}) when cultivated in the medium containing glucose: glycerol (1:2), yeast extract, 0.5% Tween 20, 10 mM H_2O_2 and pH 3.5. Therefore, the optimization of carotenoids production by wild type yeast isolate *Rhodotorula mucilaginosa* MTCC 11583 improves its biotechnological potential as source of natural carotenoids

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