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The role of some stress factors including hydrogen peroxide, methylen blue, sodium chloride and ultraviolet on *Rhodotorula glutinis* DBVPG # 4400 total carotenoids production

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Abstract

A significant increase of total carotenoids was observed when the cells of Rhodotorula glutinis DBVPG # 4400 were stressed by 10 mM H2O2. Also, the illuminated and dark cultures revealed a highly significant effect of methylene blue on the growth, final pH and the biosynthesis of total carotenoids. Present data indicated a highly significant increment of Rhodotorula glutinis growth and total carotenoids in the optimized growing medium. However, the exposing of yeast cells either grown in light or dark conditions to UV irradiation, led to a highly and significant decrease in the production of total carotenoids, although the growth was insignificantly affected. Furthermore, a highly significant decrease of total carotenoid biosynthesis was encountered with the increase of NaCl stress on the yeast cultures grown in both light and darkness.

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Introduction

Carotenoids consist of a group of may be over 732 colored pigments found in nature. The presence of carotenoids in the human diet is essential because of their action as pro–vita A (Qureshi and Ali 1996), antioxidant, possible tumour–inhibiting agent (Miranda *et al,.* 1998), or as natural colorants (Gouveia *et al.,* 2003). They serve also, as light-harvesting pigments in photosynthesis and they protect against photooxidative damage. Carotenoids play an important role in the protection against the oxidative stress caused by light damage. Also, It has been demonstrated that carotenoids have the ability to scavenge active oxygen species or free radicals (Miki 1991and Shimidzu *et al*., 1996). Furthermore, Immunomodulation effects of carotenoids and polysaccharide were investigated against the treatment of pathogenic infections (Akyon 2002, Dostert and Tshopp 2007; El-sheekh *et al*., 2010).

Yeasts have received considerable attention in recent years as a potential biological source of carotenoids (Schroeder and Johnson 1993, Bhosale and Gadre 2001, Koei *et al.* 2005 and Khaneja *et al.* 2010). Several studies on the role of yeast carotenoids have been reported (Moore *et al.*, 1989 and Mendez-Alvarez *et al.*, 2000). Factors that influence the biosynthesis of carotenoids are important for the commercial and industrial applications (Takaichi *et al.*, 2005, Liang *et al.*, 2006). Martin *et al.* (1993) found that a strain of *Rhodotorula rubra* could synthesize high concentrations of carotenoids (1256 µg/g dry biomass) at an optimum pH of 5.5. It has been shown that the osmotic stress resulted in overproduction of carotenoids in the yeasts *Sporidiobolus salmonicolor* grown in optimum conditions and under exogenous osmotic and oxidative stress (Koèí *et al*., 2005).

The total carotenoid increased from 428 µg/g to 461–518 µg/g dry weight after stressing with H_2O_2. Schroeder and Johnson (1993) reported an increase of carotenogenesis in *Phaffia rhodozyma* by the addition of 2 mM H_2O_2 to the culture grown for 24 h in yeast–malt broth (YM). Prevention of oxygen stress was clearly demonstrated by results showing an increase in carotenoid content during oxygen stress loading (Mendez-Alvarez *et al*., 2000).

Light–regulated carotenoid biosynthesis in *P. rhodozyma* that could be induced by a mechanism involving activated oxygen species. It is possible that active oxygen species induce, activate, or serve as substrates for enzymes involved in carotenoids formation. Alternatively, they may inhibit or destroy biosynthetic enzymes that utilize acetyl–COA or other common precursors which are needed for carotenoid synthesis. Schrott (1985) proposed that carotenoids protect against active oxygen species, mainly singlet oxygen produced under irradiation by strong sunlight. Growing media composition is a player in carotenoides production, where Cutzu *et al.* (2013) studied the effect of crude glycerol on Carotenodes production from *R.glutinis* mutant . In their work eighteen red yeasts were screened for carotenoids production on glycerol containing medium. Strain C2.5t1 of *R. glutinis*, that showed the highest productivity, was UV mutagenized. Mutant 400A15, that exhibited a 280 % increase in β-carotene production in respect to the parental strain. The current study has been designed to answer this question; does the stress factors have the ability to enhance the *R. glutinis* carotenoides production.

Materials and methods

Microorganism

Yeast

Rhodotorula glutinis DBVPG # 4400 was gifted from industrial yeasts collection, Dipartimento di Biologia Vegetale e Biotecnologie Agroambientali, Sezione di Microbiologia Applicata, Borgo XX Giugno, 06121, Perugia, Italy.

Yeast growing. Malt yeast glucose peptone medium (M1) (g/l) was used for growing the yeast. It was composed of: malt extract, 3.0, yeast extract, 3.0, glucose, 10.0, and peptone, 5.0. The pH value was adjusted at 5.5 which was proved as the best medium for grown *R. glutinis* (study to be presented elsewhere).The cultures were prepared by dispensing

49 ml Malt yeast glucose peptone medium (M1) in 250 ml Erlenmeyer flasks and autoclaved. Inoculation was carried out using 1 ml portion of the vegetative culture previously grown on the suitable medium. Consequently the whole volume of the inoculated culture medium was 50 ml that incubated at 26 ± 2˚C for 2 days in a rotary shaker (130 rpm). At the end of the incubation period, the culture media were centrifuged at 3000 rpm for 20 min and then the pellets were taken for the extraction of carotenoids (An *et al*., 1989).

Extraction of carotenoids

The yeast cells were harvested from the growing cultures by centrifugation at 3000 rpm for 20 min, washed in water and resuspended in minimum volume of water. A volume of 2 ml of 0.5 N HCl were added to the cell suspension and the cell mass broken up by stirring with a glass rod. Then the tubes were placed in a vigorously boiling water bath for 15 min upon the removal from the water bath the tubes were placed immediately into ice water to cool for 10, after which the cells were separated by centrifugation. Pigments were then extracted from the cells with acetone and petroleum ether (Peterson *et al*., 1954). The total carotenoid composition was calculated according to An *et al*. (1989) by using the 1% extinction coefficient = 2.100 by the formula:

Total carotenoid (µg/g of yeasts) = (ml of petrol) (A474) (100)/ (21) (yeast dry weight).

Determination of yeast growth

At the end of the incubation period, the cells of the experimental organism were collected by centrifugation at 3000 rpm for 20 min and washed with distilled water, then placed in an oven at 70 ˚C for dryness till constant weight. Growth was expressed as gram dry weight.

Factors affecting Rhodotorula glutinis growth and carotenoids

Effect of UV exposure time

24 h-growing cultures of *R. glutinis* grown on Malt yeast glucose peptone (M1) shaked at 130 rpm and 26˚C under light and dark conditions were opened in a sterile cabient and exposed to a T-8M ultraviolet-B lamp (8 w, 220 v, 312 nm) for different times (15, 30, 45, 60, 90) on a shaker. The flasks were then closed and further incubated for another 24 h after UV exposure. At the end of the incubation period, the growth, final pH and total carotenoids were measured as above.

Effect of NaCl stress

The Erlenmeyer flasks containing Malt yeast glucose peptone medium (M1) were inoculated with *R. glutinis* and incubated at 26˚C. One day old cultures were stressed by different concentrations of sterilized NaCl solution (0.0 - 6.0%) and the incubation was completed for two days. Other conditions were applied as above. After incubation, the growth, final pH and total carotenoids were determined as mentioned above.

Effect of H_2O_2 stress

Various concentrations of H_2O_2 were tested in order to determine the concentration at which the maximum value of carotenoids was attained. *Rhodotorula glutinis* was cultivated in Malt yeast glucose peptone medium (M1) at 26˚C and 130 rpm. After one day of incubation, different concentrations of H_2O_2 (0, 3, 5, 10, 15, 20, 25, 30 mM) were added through a Millipore filter (0.45 µm) to the growing cultures. The incubation was completed for two days. Other conditions were applied as above. After incubation, the growth, final pH and total carotenoids were determined.

Effect of methylene blue stress

One day old cultures of *R. glutinis* were affected by sterilized methylene blue at final concentrations of 0.0, 0.1, 0.25 and 0.5 mM. The incubation was completed for two days. After incubation, the growth, final pH and total carotenoids were determined.

Comparison of basal and optimized media

Under light conditions, Erlenmeyer flasks containing Malt yeast glucose peptone medium (M1) in which a mixture of malt extract and glucose, malt extract and maltose, or glucose were used. In darkness, a mixture

of malt extract and glucose, malt extract and sucrose, as well as malt extract were tried. *R. glutinis* was cultivated in these media at 26˚C and 130 rpm. After one day of incubation, a final concentration of H_2O_2 equal to 3.0 mM was added through a Millipore bacterial filter (0.45 µm) to the growing cultures. Two sets of flasks were used, one was stressed by H_2O_2, and the other was left as non-stressed cultures. The incubation was completed for two days. Other conditions were applied as above. After incubation, the growth, final pH and total carotenoids were recorded.

Statistical analysis

The obtained data were analyzed with ANOVA test by using SPSS program (Version 11).

Results

Several stress factors namely, hydrogen peroxide, Methylen blue, Sodium Chloride and Ultraviolet on *Rhodotorula glutinis* DBVPG # 4400 total carotenoids production were studied, and the results for each one will be metioned separately through the following topics:

Effect of UV exposure time on growth and total carotenoids production

Cultures of *R. glutinis* were exposed to a UV lamp at the mid-log phase of growth for different times (15, 30, 45, 60, 90). The data presented in tables 1 revealed that the biosynthesis of carotenoids was not enhanced by UV irradiation. Thus, the maximum yield of carotenoids was recorded in the control culture (128 µg/g and 179 µg/100 ml) grown in the light. The total carotenoids obtained in the dark reached to 125 µg/g and 168 µg/100 ml in the cultures not exposed to UV irradiation.

Table 1. Effect of UV exposure time on the production of carotenoids by *Rhodotorula glutinis* grown in light.

UV exposure (minutes)	*Cells dry Weight g/100 ml*	*Final pH*	*Total carotenoids*	
			µg / g	*µg / 100 ml*
		Light		
0	*1.4±0.2*	*5.1±0.0*	*128±6*	*179±8*
15	*1.3±0.2*	*5.0±0.1*	*100±5*	*130±6*
30	*1.3±0.1*	*5.1±0.0*	*93±5*	*124±6*
45	*1.3±0.0*	*5.0±0.2*	*106±4*	*137±5*
60	*1.3±0.0*	*4.9±0.1*	*94±5*	*120±3*
90	*1.3±0.0*	*4.9±0.1*	*92±4*	*119±4*
F value	*0.50*	*3.61*	*26.64*	*35.68*
P	*0.77*	*0.031**	*0.000****	*0.000****
LSD	*0.17*	*0.26*	*8.18*	*11.08*

On exposing the yeast cells (grown in light and dark) to UV irradiation, a highly significant decrease in the production of carotenoids was observed, although the growth was insignificantly affected.

Effect of stress of NaCl on growth and carotenoids production

One day old cultures of *R. glutinis* were stressed by different concentrations of sterilized NaCl solution (1.0 - 6.0%) and the incubation was completed for two days. The growth and total carotenoids were then determined. From tables 2, it is apparent that the highest values of carotenoids production (135-143 µg/g and 142-148 µg/100 ml) were recorded on the addition of 1and 2 % of NaCl under light conditions. Whereas, the values of carotenoids (130-137 µg/g and 139-142 µg/100 ml) were recorded in darkness on the addition of the same concentrations of NaCl. Whereas, lower values of carotenoids (130-137 µg/g and 139-142 µg/100 ml) were recorded in darkness on the addition of the same concentrations of NaCl.

A highly significant decrease of carotenoid

biosynthesis was achieved with the increase of NaCl stress on the yeast cultures grown in both light and darkness.

Effect of stress by H_2O_2 on growth and carotenoids production

Various concentrations of H_2O_2 (3, 5, 10, 15, 20, 25, 30 mM) were added to the cultures of *R. glutinis* at the mid-log phase of growth. According to the data shown in tables 3, it is obvious that the addition of 10 mM H_2O_2 final concentration enhanced the biosynthesis of carotenoids. In the light cultures, the yield of carotenoids reached to 154 µg/g and 180 µg/100 ml. The addition of a concentration of 10 mM H_2O_2 enhanced the biosynthesis of carotenoids in *R. glutinis*. Whereas, the yeast cells grown in the dark synthesized total carotenoids of 141 µg/g, and 169 µg/100 ml under H_2O_2 stress.

Table 2. Effect of NaCl stress on the production of carotenoids by *Rhodotorula glutinis* grown in light.

NaCl stress (%w/v)	Cells dry Weight g/100 ml	Final pH	Total carotenoids	
			µg / g	µg / 100 ml
Light				
0	1.1±0.1	5.4±0.0	132±6	143±7
1	1.0±0.2	5.3±0.1	143±7	142±7
2	1.1±0.1	5.3±0.0	135±6	148±8
3	1.1±0.0	5.3±0.0	123±6	134±6
4	1.0±0.0	5.3±0.0	124±5	132±6
5	1.0±0.1	5.3±0.1	121±5	126±5
6	1.0±0.0	5.5±0.1	119±6	117±5
F value	0.85	1.85	6.35	9.11
P	0.54	0.15	0.0021**	0.0004***
LSD	0.17	0.18	10.54	10.81
		Dark		
0	1.0±0.2	5.4±0.1	126±6	135±7
1	1.0±0.1	5.2±0.2	137±7	139±7
2	1.1±0.0	5.2±0.1	130±6	142±6
3	1.0±0.1	5.1±0.1	129±6	131±7
4	1.0±0.1	5.1±0.0	131±5	129±7
5	1.0±0.0	5.4±0.1	123±6	124±6
6	1.0±0.0	5.6±0.0	119±7	112±5
F value	0.43	10.42	2.71	6.75
P	0.85	0.0002***	0.058	0.0016**
LSD	0.18	0.17	10.77	11.78

Each value is the mean of three culture readings ± standard deviation

*** Highly significant at probability P≤0.001, ** Significant at probability P≤0.01,

* Low significant at probability P≤0.05.

F value is the analysis of variance value; LSD is the least significant difference.

The growth of yeast was insignificantly affected by H_2O_2 stress. A significant increase of carotenoids was observed when the cells were stressed by 10 mM H_2O_2

Effect of stress by different concentrations of methylene blue on growth and carotenoids production.

One day old cultures of *R. glutinis* grown in Malt yeast glucose peptone medium (M1) at 26˚C were affected by sterilized methylene blue at final concentrations of 0.1, 0.25, 0.5 mM. The incubation was completed for two days. The experimental conditions were carried out as usual. After incubation, the growth, final pH and total carotenoids were determined.

From table 4, it is apparent that the highest value of

carotenoids production (146 µg/g and 149 µg/100 ml) was recorded at 0.1 mM of methylene blue under light conditions. This level of methylene blue stress produced 137 µg/g and 155 µg/100 ml of carotenoids in darkness. Concerning the final pH value, it was fluctuated between neutrality and acidity.

The statistical analysis of the data obtained from the illuminated and dark cultures revealed a highly significant effect of methylene blue on the growth, final pH and biosynthesis of carotenoids.

Table 3. Effect of H_2O_2 stress on the production of carotenoids by *Rhodotorula glutinis* grown in light.

H_2O_2 stress (mM)	Cells dry Weight g/100 ml	Final pH	Total carotenoids	
			µg / g	µg / 100 ml
Light				
0	1.1±0.1	5.4±0.3	131±6	144±6
3	1.1±0.2	5.5±0.3	114±5	128±6
5	1.2±0.2	5.4±0.2	129±6	149±7
10	1.2±0.3	5.7±0.1	154±8	180±8
15	1.1±0.2	5.5±0.3	138±7	157±7
20	1.2±0.1	5.7±0.2	148±7	161±7
25	1.1±0.1	5.4±0.2	152±6	164±8
30	1.1±0.0	5.4±0.1	151±8	164±6
F value	0.80	5.14	14.79	1.73
P	0.59	0.0032**	0.000***	0.17
LSD	0.17	0.18	11.10	50.94
		Dark		
0	1.1±0.1	5.3±0.4	127±6	140±7
3	1.1±0.1	5.4±0.3	108±5	119±6
5	1.2±0.1	5.4±0.3	113±6	135±5
10	1.2±0.2	5.6±0.2	141±7	169±7
15	1.1±0.2	5.5±0.3	127±6	144±5
20	1.1±0.1	5.6±0.2	130±5	143±7
25	1.1±0.1	5.4±0.1	128±6	140±7
30	1.1±0.0	5.3±0.1	120±6	132±6
F value	0.80	4.23	2.30	13.93
P	0.59	0.0080**	0.079	0.000***
LSD	0.18	0.17	39.93	11.32

Each value is the mean of three culture readings ± standard deviation

*** Highly significant at probability P≤0.001, ** Significant at probability P≤0.01,

* Low significant at probability P≤0.05.

F value is the analysis of variance value; LSD is the least significant difference.

Comparison of Malt yeast glucose peptone medium (M1) [basal] and Malt

Yeast glucose peptone medium (M1) [optimized] media for growth and carotenoids production. *Rhodotorula glutinis* was cultivated in Malt yeast glucose peptone medium (M1) [basal medium] containing a mixture of malt extract and glucose, malt extract and maltose, or glucose media under light conditions. In darkness, a mixture of malt extract and glucose, malt extract and sucrose, as well as malt extract were used. A final concentration of H_2O_2 equal to 10 mM was added in the mid-log phase of growth. *R. glutinis* was grown in the optimized medium supplemented with H_2O_2 (as the best stress factor inducing carotenoids biosynthesis) at the mid-log phase of growth. The addition of 10 mM H_2O_2 to the modified Malt yeast glucose peptone medium (M1) [modified medium] containing glucose as C-source

recorded the highest biosynthesis of carotenoids in the light cultures (212 µg/g and 321 µg/100 ml). In the presence of a mixture of malt extract and sucrose in medium M1 as an optimum carbon source, the yeast cells stressed by H_2O_2 produced total carotenoids of 192 µg/g and 289 µg/100 ml in the dark conditions.

Table 4. Effect of methylene blue stress on the production of carotenoids by *Rhodotorula glutinis* grown in light and dark.

Methylene blue stress (mM)	Cells dry Weight g/100 ml	Final pH	Total carotenoids	
			µg / g	µg/100 ml
Light				
0	1.1±0.0	5.4±0.3	130±5	141±7
0.1	1.0±0.1	5.6±0.2	146±6	149±7
0.25	1.0±0.1	7.2±0.3	111±6	113±6
0.5	1.0±0.0	5.5±0.2	95±5	96±4
F value	0.75	218.74	48.55	52.28
P	0.552	0.000***	0.000***	0.000***
LSD	0.19	0.18	10.39	11.09
		Dark		
0	1.1±0.1	5.3±0.2	128±5	140±7
0.1	1.0±0.1	5.7±0.1	137±6	155±8
0.25	1.0±0.0	7.0±0.2	124±6	120±6
0.5	0.9±0.0	5.6±0.0	123±7	112±6
F value	2.00	169.99	3.66	24.57
P	0.19	0.000***	0.06	0.0002***
LSD	0.19	0.18	10.85	12.80

Each value is the mean of three culture readings ± standard deviation

*** Highly significant at probability P≤0.001, ** Significant at probability P≤0.01,

* Low significant at probability P≤0.05.

F value is the analysis of variance value; LSD is the least significant difference.

It can be concluded that the maximum yield of carotenoids can be obtained from the yeast *R. glutinis* grown in the basel Malt yeast glucose peptone medium (M1). The cultures started with an inoculum of O.D.600 nm = 0.4 and grown for two days at 120 rpm and pH 5.5. A mixture of peptone and yeast extract was used as nitrogen source under light conditions. The addition of 10 mM H_2O_2 to the modified Malt yeast glucose peptone medium (M1) containing glucose as C-source recorded the highest biosynthesis of carotenoids in the light cultures (212 µg/g and 321 µg/100 ml).

Generally the synthesis of carotenoids by cultures stressed with the optimum concentration of H_2O_2 (10 mM) was improved than the non-stressed ones. The results in tables 5 postulated that the addition of H_2O_2 to Malt yeast glucose peptone medium (M1) containing glucose as C-source recorded the highest biosynthesis of carotenoids in the light cultures (212 µg/g and 321 µg/100 ml). In the presence of a mixture of malt extract and sucrose in medium M1, the yeast cells stressed by H_2O_2 produced total carotenoids of 192 µg/g and 189 µg/100 ml in the dark conditions.

The comparison of basal and optimized media for growth and carotenoids production showed a highly significant increment of the studied parameters in the opimized media stressed with H_2O_2.

Discussion

Exposure of the yeast cells (grown in light and dark) to UV irradiation, led to a significant decrease in the production of carotenoids, although the growth was insignificantly affected. Also, A highly significant decrease of carotenoid biosynthesis was achieved with the increase of NaCl stress on the yeast cultures

grown in both light and darkness. However, Koèí *et al.* (2005) showed that the osmotic stress resulted in overproduction of carotenoids in the yeasts *R. glutinis* and *Sporidiobolus salmonicolor* grown in optimum conditions and under exogenous osmotic and oxidative stress.

Table 5. A comparison between basal (B.M.) and optimized medium for carotenoid production by *Rhodotorula glutinis* grown in light under H_2O_2 stress.

C-source ± H_2O_2 *(10 mM)*	*Cells dry Weight g/100 ml*	*Final pH*	*Total carotenoids*	
			µg / g	µg/100 ml
	Light			
Control (B.M.)	1.3±0.2	5.7±0.1	156±6	199±9
Control (B.M.) + H_2O_2	1.4±0.1	5.6±0.2	167±6	225±11
Malt extract + maltose	0.8±0.0	8.2±0.3	182±8	141±7
Malt extract + maltose + H_2O_2	0.7±0.1	8.3±0.2	187±7	135±6
Glucose	1.5±0.0	5.3±0.1	169±7	257±12
Glucose + H_2O_2	1.5±0.1	5.3±0.1	212±10	321±15
F value	38.40	624.00	20.55	8.61
P	0.000***	0.000***	0.000***	0.0012**
LSD	0.17	0.18	13.39	84.11
	Dark			
Control (B.M.)	1.4±0.0	5.7±0.1	133±5	185±8
Control (B.M.) + H_2O_2	1.4±0.1	5.6±0.2	146±8	205±15
Malt extract + sucrose	1.5±0.1	5.6±0.0	158±8	209±9
Malt extract + sucrose + H_2O_2	1.5±0.2	5.5±0.1	192±9	189±14
Malt extract	1.0±0.1	8.1±0.2	141±6	137±7
Malt extract + H_2O_2	1.0±0.0	8.0±0.1	120±5	115±5
F value	16.8	481.7	41.76	106.00
P	0.000***	0.000***	0.000***	0.000***
LSD	0.18	0.17	11.86	18.37

Each value is the mean of three culture readings ± standard deviation.

*** Highly significant at probability P≤0.001, ** Significant at probability P≤0.01,

* Low significant at probability P≤0.05.

F value is the analysis of variance value; LSD is the least significant difference.

Another stress factor other than uv and sodium chloride was methylene blue. Where, the used level of methylene blue (0.1 mM) stress produced 146 µg/g and 149 µg/100 ml of carotenoids in light conditions compared to 130 µg/g i.e. 1.12 time at 0 mM concentration . Concerning the final pH value, it was fluctuated between neutrality and acidity. Sakaki *et al.* (2002) examined the effect of active oxygen species on the productivity of torularhodin, an effective antioxidant, by soil yeast, *R. glutinis* no. 21. Methylene blue was used as generators of singlet oxygen. It indicated effectiveness at a dose of l.0 x 10^{-10} to 3.0 x lo^{-6} M. Addition of this generator to the culture medium had almost no influence on the biosynthesis of β-carotene.

A significant increase of carotenoids was observed when the cells were stressed by 10 mM H_2O_2. Schroeder and Johnson (1993) reported the increase of carotenogenesis in *P. rhodozyma* by the addition of

2 mM H_2O_2 to the culture grown for 24 h in yeast–malt broth (YM). The total carotenoid increased from 428 µg/g to 461–518 µg/g after stressing with H_2O_2 Thus, light–regulated carotenoid biosynthesis in *P. rhodozyma* could be induced by a mechanism involving activated oxygen species. It is possible that active oxygen species induce, activate, or serve as substrates for enzymes involved in carotenoid formation. Alternatively, they may inhibit or destroy biosynthetic enzymes that utilize acetyl–COA or other common precursors which are needed for carotenoid synthesis. We anticipate that H_2O_2 might have the same aforementioned explanation for the increase in carotenoides concentration in case of *R. gultinis*.

Prevention of oxygen stress was clearly demonstrated by results showing an increase in carotenoid content during oxygen stress loading (Mendez-Alvarez *et al.*, 2000). A red yeast *R. glutinis*, biosynthesizes β-carotene and torularhodin as final products of carotenoid biosynthesizes (Simpson *et al.*, 1964, Sakaki *et al.*, 2001). Whereas, the yeast cells grown in the dark synthesized a total carotenoids of 141 µg/g and 169 µg/100 ml under H_2O_2 stress. Significant and Impact of this study is ability of *R.glutinis* for the first time to respond to stress factors which led to increase in its carotenoids production. There is a high significant increment of *R. glutinis* carotenoids concentration in the opimized media stressed with H_2O_2. *R.glutinis* favorably responded to the different stress factors leading to higher carotenoids production.

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