Intl. J. Food. Ferment. Technol. 3(2): 149-156, December, 2013

DOI Number: 10.5958/2277-9396.2014.00343.2

Research Paper

Standardization of Medium components and Process parameters for Biopigment Production using *Rhodotorula glutinis*

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Paper no: 73 **Received**: 08 July, 2013 **Received in revised form**: 19 September, 2013 **Accepted**: 18 November, 2013

Abstract

In the present investigation, studies were carried out to evaluate the effect of medium consitituents and process paramters to get maximum biopigment production using *Rhodotorula glutinis* MTCC 1151. Screening of different media components indicated that a medium containing glucose (6%, w/v), urea (0.05%) and magnesium sulphate (0.05%) displayed higher pigment production than others. The optimization of different process parameters indicated that maximum pigment production was observed at pH 6.0, temperature 30°C, after 72 hrs incubation period under submerged fermentation.

Keywords: yeast, *Rhodotorula*, biopigment, optimization

The colour of the food products is one of the most important attributes, which affects the consumer's choice and acceptability. The development of novel food products with an attractive appearance is an important goal of the food processing industry. However, due to the health related issues regarding the consumption of colour, the interest of the food producers has shifted towards the use of natural colours (Bridle and Timberlake, 1997; Dufosse, 2006).

Natural colourants find wide range of applications in food processing industries especially in confectionery and beverage industry to make the food products more attractive, and appealing. These can be used commercially as food colourants, animal feed supplements, nutraceuticals etc. and in other applications (Gordon and Bauernfeind, 1982; Johnson and Schroeder, 1995).

Most of the natural pigments are extracted from plants like fruits, vegetables, seeds, roots and microorganisms, and they are sometimes called biocolours because of their biological origin (Pattnaik *et al*., 1997). However, among the natural pigments, there is growing interest in microbial pigments due to their medicinal properties and nutritive value, production being independent of season, geographical conditions, fast growth, controllable and predictable yield (Johnson and Schroeder, 1995; Francis, 2000; Joshi *et al*., 2003). A large number of microorganisms have been employed to produce biopigments using different culture

media. The ability to produce desired products efficiently through fermentation is a benefit of the microbial systems. In addition, microbial systems are sustainable with easy scope of improvement, and the process is eco-friendly (Joshi *et al*., 2003).

The property of microbial pigment like β-carotene to shift gradually its colour towards orange red, is more attractive and this unique property is absent in plant-derived pigments (Joshi *et al*., 2003). Carotenoids are a group of bioactive compounds that are responsible for bright yellow/orange colours of various plants, microorganisms and animals (Wilhelm and Helmut, 1996). Carotenoid biosynthesis is a specific feature of the yeast group of *Rhodotorula* sp. (Martin *et al.* 1993; Buzzini and Martini, 2000; Bhosale and Cadre, 2001; Vijayalakshmi *et al.*, 2001). *Rhodotorula glutinis* is widely known as β-carotene producing yeast and carotenoids are one of the most important natural pigments (Simpson *et al*., 1975; Sandhu and Joshi**,** 1996), which can have applications in pharmaceutical, chemical, food and feed industries. In human beings, carotenoids play several important biological activities, and have the potential to impart many health benefits (Bendich, 1989; Chandi *et al.,* 2010)

In view of the increasing interest in biopigments extracted from microbiological sources, as a colouring agent for the different food products, the present work was carried out to evaluate the effect of media consitituents (various carbon and nitorgen sources) and process paramters to get maximum biopigment production.

Materials and Methods

Procurement and maintenance of microbial culture

Rhodotorula glutinis MTCC 1151 was procured from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India. The yeast culture was activated and maintained on growth media containing malt extract (3 g/l), yeast extract (3 g/l), peptone (5 g/l) and glucose (10 g/l). The culture was maintained by sub-culturing, aseptically at fortnight intervals and stored at 4°C, until further use.

Preparation of starter culture

The yeast culture was grown in 50 ml of growth media in 250 ml capacity Erlenmeyer flask, having the same composition as described above. After sterilization, the flasks were inoculated with a loopful of activated culture and incubated at 37°C for 24 hrs

Preparation of fermentation medium

The fermentation medium (unless otherwise specified) was prepared by using glucose (6%), urea (0.056%), KH_2PO_4 (0.1%) and $MgSO_4$ (0.05%). The constituents were dissolved in distilled water and sterilized by autoclaving at 15 psi for 20 min.

Production of biopigments

The seed culture was added to a flask containing 100 ml of fermentation media in Erlenmeyer flask. The flasks were incubated at 30°C for the growth for 48 hrs (unless otherwise specified). During the course of batch fermentation, samples were taken from the fermentation broth to monitor the biopigment production under submerged fermentation.

Medium optimization

Different carbon sources (glucose, glycerol, sucrose, sorbitol, fructose, and maltose), nitrogen sources (urea, yeast, peptone, ammonium sulphate, ammonium nitrate and sodium sulphate), salts (magnesium sulphate, manganese sulphate, zinc sulphate) were supplemented individually to the fermentation media and the respective concentrations were varied to get the optimal concentration of biopigments.

Process optimization

The effect of various process parameters such as pH, temperature, and incubation time was monitored by varying their respective parameters.

Extraction of biopigment

The extraction of biopigment from fermented media was carried out by using the method as described by Aksu and Eren (2005). The fermented media was centrifuged at 5,000 rpm for 15 min. The supernatant was discarded and different solvents like, dimethyl sulphoxide (2 ml), acetone (5 ml) and petroleum ether (5 ml) were added sequentially to the biomass pellet. Biomass pellets were stirred vigorously with glass beads for 10 minutes on vortex shaker for disintegration of pellets. The mixture was allowed to stand approximately for 30 min and upper pink petroleum ether phase containing biopigment was withdrawn.

Estimation of biomass

Biomass was estimated by the method given by Guerra-Santos *et al.,* (1984). Samples were withdrawn at different time intervals, followed by centrifugation at 8000 rpm for

15 min and centrifugate was washed with water. This was followed by centrifugation and drying at 105°C, overnight to obtain dry biomass.

Measurement of biopigment production

The estimation of pigment production was carried out by taking the absorbance at specified wavelength (452 nm) using UV-VIS spectrophotometer (DR 5000, HACH, Germany).

Results and Discussion

The screening of different carbon and nitrogen sources was done to find out the best carbon and nitrogen source for the maximum production of biopigment using *R. glutinis* MTCC 1151 and results obtained have been discussed here.

Screening of carbon sources for biopigment production

Six carbon sources i.e. glucose, glycerol, sorbitol, fructose, maltose and sucrose were tested for their effectiveness in biopigment production. It is evident from the results (Fig. 1) that glucose (6%, w/v) as carbon source resulted in maximum biopigment production (1.335 AU/g dry wt.) followed by glycerol (1.309 AU/g dry wt.) after 72 hrs of incubation. It may be due to the efficient assimilation of glucose by the microorganism. Moreover, suitable carbon source is important for carotenoid biosynthesis during the non-growth phase (Frengova and Beshkova, 2009). It provides the energy for growth of microorganisms and production of secondary metabolites besides, providing the carbon for making the various cell structures, organic chemicals and metabolites (Said, 2010).

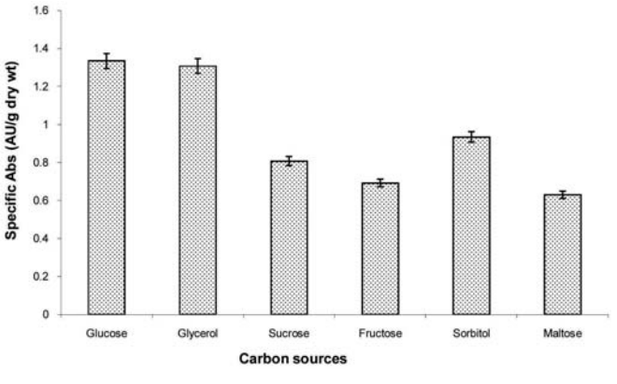

Fig. 1: Effect of different carbon sources on biopigment production using *R. glutinis* MTCC 1151

The presence of a suitable carbon source is important for carotenoid biosynthesis during the non-growth phase. Yeasts can synthesize carotenoids when cultivated in synthetic media. During the production of β-carotene from mutant of *R. glutinis*, it was observed that glucose yielded higher percentage of β-carotenoids than sucrose, rhamnose, fructose and sorbitol (Bhosale and Gadre, 2001). From economics point of view, the use of glucose as carbon source is very attractive as it can be taken up easier than others (Guimara *et al*., 2009; Poutou-Pinales *et al*., 2010). Further, glucose and its oligopolysaccharide were found to be better than other carbon sources for both growth and pigment production (Lin *et al*., 1992; Kamalam *et al*., 2012).

Further, glucose as the best carbon gave dark orange coloured biopigment with maximum biopigment production while glycerol and fructose gave light orange coloured biopigment, sucrose gave pink, depending upon their individual properties. Previous studies on pigment production using *Monascus pilosus* C_1 strain indicated that glucose strongly stimulated both the growth and pigment production (Pisareva and Kujumdzieva, 2010).

Screening of nitrogen sources

Nitrogen sources such as urea, yeast extract, peptone, sodium nitrate, ammonium nitrate and ammonium sulphate (0.056%, w/v) were supplemented in the fermentation media. Among all inorganic nitrogen sources tested (Fig. 2), urea has shown maximum biopigment production (1.335 AU/g dry wt.). It has been reported in literature that urea is potentially an important nitrogen source for the microbial community (Coffin, 1989; Antia *et al*., 1991). The other inorganic nitrogen compounds should decreased carotenoids concentration, probably due to less growth of yeast in the media supplemented with ammonium sulphate and ammonium nitrate. Similar results were found in literature in which the microorganism failed to grow in the media supplemented with ammonium chloride, ammonium sulphate, ammonium nitrate and ammonium acetate (Gulani *et al*., 2012). In other studies, maximum production of carotenoid (1.92 mg/l) and a high dried cell mass (4.97 g/l) took place with urea as compared to other inorganic nitrogen sources by using *Rhodosporidium paludigenum* DMKU3-LPK4 (Yimyoo *et al*., 2011). Previous reports have shown that urea was efficient nitrogen source for growth and pigment production in *Monascus pilosus* C_1 strain (Pisareva and Kujumdzieva, 2010).

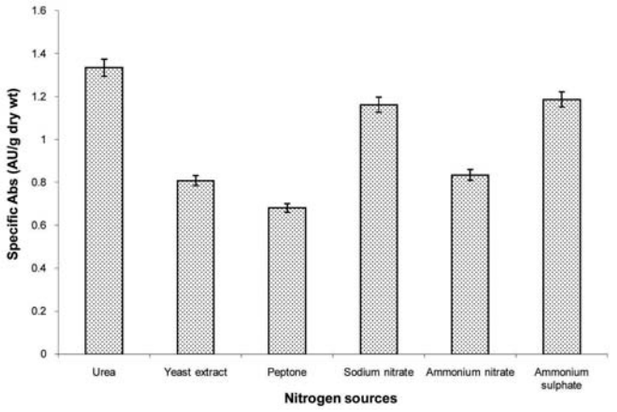

Fig. 2: Effect of different nitrogen sources on biopigment production using *R. glutinis* MTCC 1151

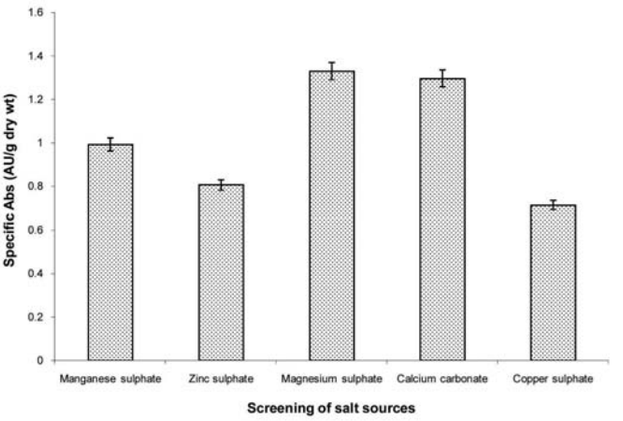

Fig. 3: Effect of different salts on biopigment production using *R. glutinis* MTCC 1151

The effect of nitrogen sources on colour variation from *R. glutinis* MTCC 1151, indicates that medium supplemented with urea gave dark orange coloured biopigment, whereas yeast extract gave light pink coloured biopigment, peptone gave light orange coloured biopigment and ammonium nitrate, ammonium sulphate gave light yellow-orange coloured biopigment.

Screening of salt sources

Different salts (magnesium sulphate, manganese sulphate, zinc sulphate, calcium carbonate and copper sulphate) were supplemented in the fermentation media to investigate their effect on biopigment production (Fig. 3). Out of these, magnesium sulphate (0.05% w/v) gave maximum biopigment production (1.330 AU/g dry wt.), whereas minimum biopigment production was observed with zinc sulphate and copper sulphate. It may be due to action of magnesium sulphate acting as divalent cation to stimulate the growth of microorganisms and cellular accumulation of carotenoids. Similar results have been reported earlier stating that magnesium sulphate increased the proportion of β-carotene (Bhosale and Gadre, 2001).

The effect of metal salts on the growth of *R. glutinis* NCIM 3353 demonstrated that divalent cations act as stimulant for growth (Komemushi *et al*., 1994). Divalent cations had a stimulatory effect on volumetric production and cellular accumulations of carotenoids. It can be assumed that this positive effect was due to a stimulatory effect of the cations on carotenoids-synthesizing enzymes (Goodwin, 1980). It was also observed that magnesium sulphate gave dark orange coloured biopigment with maximum biopigment production, whereas, zinc sulphate and copper sulphate gave light pink coloured biopigment and manganese sulphate, calcium carbonate gave light orange coloured biopigment, respectively.

Effect of glucose concentration on biopigment production

The effect of glucose concentration on biopigment production was investigated by varying the glucose concentration from 4.0-8.0% (w/v) in fermentation media having pH 6.0 supplemented with urea (0.056 %) and incubated at temperature 30 ˚C.

It is depicted in Fig. 4 that maximum production (1.380 AU/g dry wt.) was observed with optimum glucose concentration 6.0% (w/v), which might be due to prevention of Crabtree effect that may occur at higher glucose concentration (Juzlova *et al*., 1996; Said, 2010). However, a decrease in biopigment production at higher glucose concentration was observed, which might be due to the osmotic pressure at higher concentration of glucose (Kim *et al*., 1997). The Crabtree effect involves shift of metabolism from aerobic to partly anaerobic even though plenty of oxygen may be available (Chen and Johns, 1994; Carvalho *et al*., 2003; Said, 2010). Lack of carbon (i.e., at low initial glucose concentration) can also inhibit the secondary metabolites production i.e. pigment production (Wong *et al*., 1981).

Effect of urea concentration on biopigment production

The effect of urea concentration on biopigment production was studied by varying the urea concentration from 0.04-

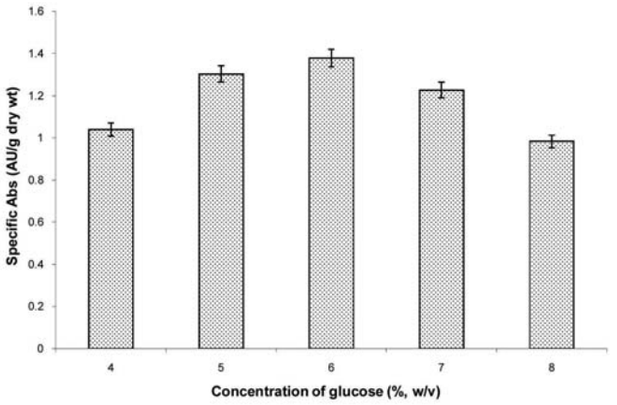

Fig. 4: Effect of glucose concentration on biopigment production using *R. glutinis* MTCC 1151

0.08% (w/v) in fermentation media having pH 6.0 and incubated at temperature 30 ˚C for 72 hrs.

It is evident from Fig. 5 that with the increase in concentration of urea, there is an increase in biopigment production (1.385 AU/g dry wt.) up to 0.05% (w/v). However, a decrease in biopigment production was observed with further increase in urea concentration greater than 0.05% (w/v). When fermentation was done without any nitrogen source in the media it gave very low yield and suggesting that nitrogen source is an important factor for biopigment production (Yimyoo *et al*., 2011).

Effect of various process parameters on biopigment production

To obtain the optimal culture strategy for production of biopigment using *R. glutinis* MTCC 1151, the fermentation media was incubated under different conditions to find out effect of various process parameters on production of biopigment.

Effect of pH

In process optimization, pH is most important factor, which effect the biopigment production, therefore pH of the fermentation media varied from 5.0 to 7.0 to study its effect on biopigment production (Fig. 6).

The results showed that the yeast culture could produce pigment over a range of pH from 5.0 to 7.0 although maximal pigment production was recorded at pH 6.0 (1.335 AU/g dry wt.). An increase in the biopigment production was observed with increase in pH upto 6.0 and a decrease took place beyond this pH. It may be due to the fact that the media with high pH did not support the biopigment production. Similar results have also been reported in literature that media with high acidic (pH 2.0 - 4.0) and high alkaline pH (above 7) did not support pigment production. Both pigment production and biomass showed a direct relationship, both in their increase as well as decrease, in response to variations in pH (Jissa, 2008). The studies on *Rhodosporidium paludigenum* DMKU3-LPK4 also indicated pH 6 as the optimal pH for carotenoid production (Yimyoo *et al*., 2011).

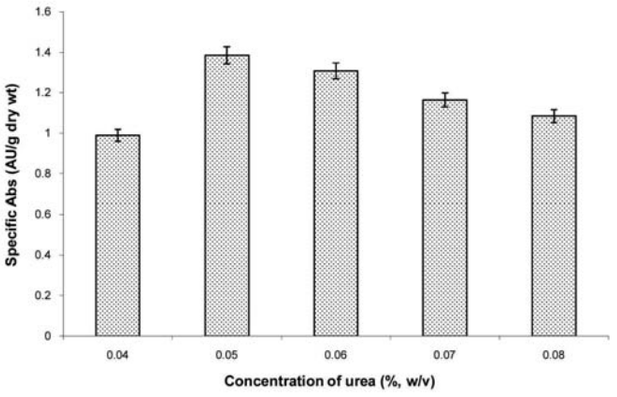

Fig. 5: Effect of urea concentration on biopigment production using *R. glutinis* MTCC 1151

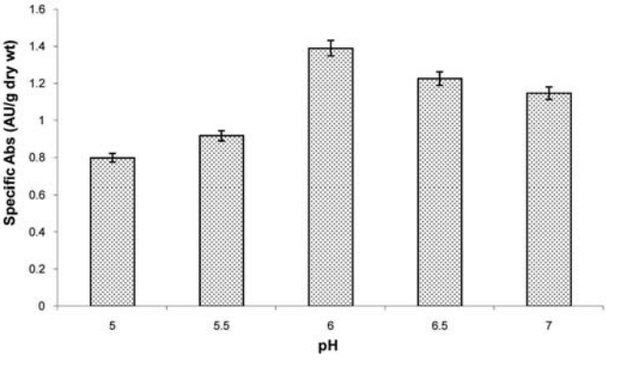

Fig. 6: Effect of pH on biopigment production using *R. glutinis* MTCC 1151

Effect of temperature

The effect of incubation temperature on biopigment production was studied by cultivating *R. glutinis* MTCC 1151 in medium with above optimized conditions and incubating at temperature range of 25-40 ˚C (Fig. 7).

It is evident from Fig. 7 that maximum production of biopigment took place at 30 ˚C (1.399 AU/g dry wt.) followed by 25 ˚C (0.982 AU/g dry wt.), whereas, pigment production decreased with further increase in incubation temperature. It may be due to fact that temperature affects the activity of one or more enzymes involved in biopigment synthesis.

Similar results have been reported in literature that *R. glutinis* was found to grow and produce pigments at all the temperatures examined with the exception of 42 ˚C, at which a negligible growth took place without any pigmentation (Latha *et al*., 2005). The maximum pigmentation yield (3.5 g/L) was recorded at the ambient temperatures from 29 to 32 ˚C. Moreover, the effect of temperature on yeast carotenogenesis is dependent on the species specificity and strain characteristics of the microorganism (Frengova *et al*., 1994). Thus, it was concluded that maximum biopigment production in batch culture of *R. glutinis* MTCC 1151 was observed at 30 ˚C, with glucose concentration of 6.0 (%, v/v) and urea concentration of 0.05 (%, w/v) at pH 6.0 after 72 hrs of incubation.

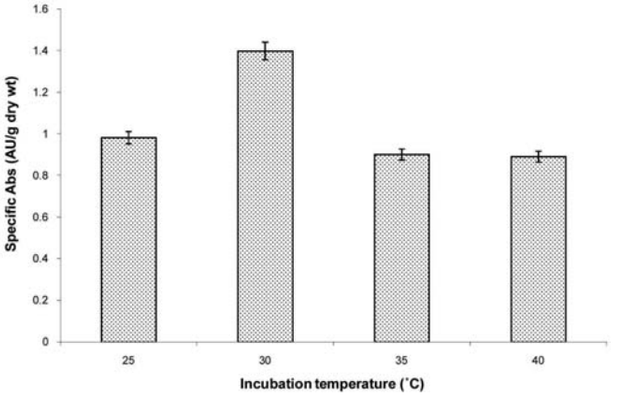

Fig. 7: Effect of temperature on biopigment production using *R. glutinis* MTCC 1151

Effect of incubation time

The fermentation media prepared with optimized conditions, i.e. glucose concentration of 6.0% (w/v), urea concentration of 0.05% (w/v), having pH 6.0 was incubated at 30˚C and the samples were taken at regular intervals. From the results (Fig. 8), it can be observed that there was increase in the biopigment production as a function of incubation period up to 72 hrs (1.41 AU/g dry wt.), however, a decrease in pigment production took place with further increase in incubation period, which can be attributed to the exhaustion of nutrients. Hence, incubation period of 72 hrs was considered as optimum for maximal production of pigment by *Rhodotorula* sp. MTCC 1151. Similar results have been reported in literature that *Rhodotorula* sp. had an optimum growth of yeast cells and biopigment production during the exponential phase (48-72 hrs). Further, the accumulation of carotenoid pigments in most yeasts starts in the late logarithmic phase and continues in the stationary phase (Goodwin, 1972; Frengova and Beshkova, 2009), and the presence of a suitable carbon source is important for carotenoid biosynthesis during the non-growth phase.

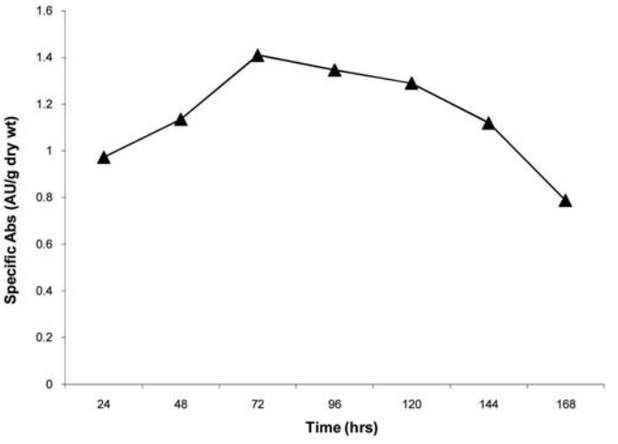

Fig. 8: Effect of incubation period on biopigment production using *R. glutinis*

Conclusion

It can be concluded that *R. glutinis* MTCC 1151 is an efficient microorganism in pigment production. The supplementation of different type of media components has affected the colour of pigment produced. Among the different carbon and nitrogen sources tested, glucose and

urea were found to be the best carbon and nitrogen sources, respectively. The fermentation medium having glucose (6%) and urea (0.05%) supplemented with magnesium sulphate (0.05%) with pH 6.0 incubated at 30°C gave maximum pigment production after 72 hrs of incubation period under submerged fermentation.

Acknowledgements: Dr. Reeba Panesar acknowledges the financial support from Council for Scientific and Industrial Research (CSIR), New Delhi, India.

References

Aksu, Z. and Eren, A.T. 2005. Carotenoids production by the yeast *Rhodotorula mucilaginosa*: Use of agricultural wastes as a carbon source. *Process Biochem*., **40:** 2985-2991

Antia, N.J., Harrison, P.J. and Oliveira, L. 1991. The role of dissolved organic nitrogen in phytoplankton nutrition, cell biology and ecology. *Phycologia*, **30:** 1-89.

Bendich, A. 1989. Carotenoids and the immune response. *J. Nutr*., **119:** 112-115.

Bhosale, P. and Gadre, R.V. 2001. Optimization of carotenoid production from hyper-producing *Rhodotorula glutinis* mutant 32 by a factorial approach. *Lett. Appl. Microbiol.*, 33: 12-16.

Bridle, P. and Timberlake, C.F. 1997. Anthocyanins as natural food color selected aspects. *Food Chem*., **58:** 103-109.

Buzzini, P. and Martini, A. 2000. Production of carotenoids by strains of *Rhodotorula glutinis* cultured in raw materials of agro-industrial origin. *Biores. Technol*., **71:** 41-44.

Carvalho, J.C.D., Pandey, A., Babitha, S. and Soccol, C.R. 2003. Production of *Monascus* Biopigments: An overview. *Agro food industry Hi-Tech*., **14:** 37-42.

Chandi, G.K., Singh, S.P., Gill, B.S., Sogi, D.S. and Singh, P. 2010. Optimization of carotenoids by *Rhodotorula glutinis*. *Food Sci. Biotechnol*., **19:** 881-887.

Chen, M.H. and Johns, M.R. 1994. Effect of carbon source on ethanol and pigment production by *Monascus purpureus*. *Enzyme Microb. Technol*., **16:** 584-590.

Coffin, R.B. 1989. Bacterial uptake of dissolved free and combined amino acids in estuarine waters. *Limnol Oceanogr.,* **34:** 531-542.

Dufosse, L. 2006. Food Grade Pigments. *Food Technol. Biotechnol.,* **44:** 313-321.

Francis, F.J. 2000. Carotenoids as Food Colorants. *Cereal Food World*, **45:** 198-203.

Frengova, G., Emilina, S., Kontantza, P., Dora, B. and Drinka, G. 1994. Formation of carotenoids by *Rhodotorula glutinis* in whey ultrafiltrate. *Biotechnol, Bioeng.,* **44:** 888-894.

Frengova, G.I. and Beshkova, D.M. 2009. Carotenoids from *Rhodotorula* and *Phaffia*: yeasts of biotechnological importance. *J. Ind. Microbiol. Biotechnol*., **36:** 163-180.

Goodwin, T.W. 1980. Biosynthesis of carotenoids. In: The Biochemistry of the Carotenoids, Ed. Goodwin T.W., Chapman and Hall, London, pp. 33-76.

Goodwin, T.W. 1972. Carotenoids in fungi and non-photosynthetic bacteria. *Prog. Ind. Microbiol.,* **11:** 29-88.

Gordon, H.T. and Bauernfeind. J.C. 1982. Carotenoids as food colorants. *Crit. Rev. Food Sci. Nutr.,* **18:** 59-97.

Guerra-Santos, L.H., Kappeli, O., and Fiechter, A. 1984. *Pseudomonas aeruginosa* biosurfactant production in continuous culture with glucose as carbon source. *Appl. Microbiol. Biotechnol*., **48:** 301-305.

Guimara, L.H.S., Somera, A.F., Terenzi, H.F., Moraes Polizeli, M.L.T. and Jorge, J.A.L. 2009. Production of β-fructofuranosidases by *Aspergillus niveus* using agro-industrial residues as carbon sources: Characterization of an intracellular enzyme accumulated in the presence of glucose. *Process Biochem*., **44:** 237-241.

Gulani, C., Bhattacharya, S. and Das, A. 2012. Assessment of process parameters influencing the enhanced production of prodigiosin from *Serratia marcescens* and evaluation of its antimicrobial, antioxidant and dyeing potentials. *Malaysian J. Microbiol.,* **8:** 116-122.

Jissa, G.K. 2008. Pigment production by marine *Serratia* sp. BTW J8. Microbial Technology Laboratory, Cochin. University of Science and Technology, India.

Johnson, E.A. and Schroeder, W.A. 1995. Microbial carotenoids. *Adv. Biochem. Eng. Biotechnol.,* **53:** 119-178

Joshi, V.K., Attri, D., Bala, A. and Bhushan, S. 2003. Microbial pigment. *Indian Journal of Biotechnol*., **2:** 362-369.

Juzlova, P., Martinkova, L. and Kren, V. 1996. Secondary metabolites of the fungus *Monascus*. A review. *J. Ind. Microbiol*., **16:**163-170.

Kamalam, J.N., Anburaj, J., Kuberan, T., Sundaravadivelan, C., Kumar, P., Starlin, T., Selvi, A.T. and Devi, M.V. 2012. DNA amplification and characterization of pigment producing gene from *Monascus ruber. European J. Experimental Biol*., **2:** 427-435.

Kim, S.Y., Lee, K.H., Jung-Hoe Kim, J.H. and Oh, D.K. 1997. Erythritol production by controlling osmotic pressure in *Trigonopsis variabilis*. *Biotechnol. Lett*s., **19:** 727-729.

Komemushi, S., Sakaki, H., Yokoyama, H. and Fujita, T. 1994. Effect of barium and other metals on the growth of D- lactic acids as similating yeast *Rhodotorula glutinis* No 21. *J. Antibact. Antifung. Agents.*, **22:** 583-587.

Latha, B.V., Jeevaratnam, K., Murali, H.S. and Manja, K.S. 2005. Influence of growth factors on carotenoid pigmentation of *Rhodotorula glutinis* DER-PDY from natural source. *Indian J. Biotechnol.,* **4:** 353-357.

Lin, T.F., Yakushijin, K., Büchi, G.H. and Demain, A.L. 1992. Formation of water-soluble Monascus red pigments by biological and semi-synthetic processes. *J. Ind. Microbiol*., **9:** 173-179.

Martin, A. M., Lu, C. and Patel, T.R. 1993. Growth parameters for the yeast *Rhodotorula rubra* grown in peat extracts. *J. Ferm. Bioeng.,* **76:** 321-325.

Pattnaik, P., Roy, U. and Jain, P. 1997. Biocolours: new generation additives for food. *Indian Food Ind.,* **16:** 21-27.

Pisareva, E.I. and Kujumdzieva, A.V. 2010. Influence of carbon and nitrogen sources on growth and pigment production of *Monascus pilosus* C1 strain. *Biotechnol. and Biotechnol. Eq*., **24:** 501-506.

Poutou-Pinales, R.A., Cordoba-Ruiz, A.H., Barrera-Avellaneda, L.A. and Delgado-Boada, J. M. 2010. Carbon source feeding

strategies for recombinant protein expression in *Pichia pastoris* and *Pichia methanolica*. *African J. Biotechnol.*, **9:** 2173-2184.

Said, F.B.M. 2010. *Monascus ruber* ICMP 15220 fermentation for the production of biopigment. Ph.D. Thesis. Doctor of philosophy in bioprocess engineering, Massey University, New Zealand.

Sandhu, D.K. and Joshi, V.K. [1996]. Development of apple pomace medium optimiz-ation of conditions for pigment production by *Rhodotorula. Adv. Food Res.,* **19:** 31-34.

Simpson, K.L., Chichester, C.O. and Phaff, H.J. 1975. The Yeast, Vol. 2, Elsevier, New York.

Vijayalakshmi, G., Shobha, B., Vanajakshi, V., Divakar, S. and Manohar, B. 2001. Response surface methodology for optimization of growth parameters for the production of carotenoids by a mutasnt strain of *Rhodotorula gracilis*. *Eur. Food Res. Technol.*, **213:** 234-239.

Wilhelm S. and Helmut, S. 1996. Lycopene: A biologically important carotenoid for humans. *Arch. Biochem. Biophy*., **336:** 1-9.

Wong, H.C., Lin, Y.C. and Koehler, P.E. 1981. Regulation of growth and pigmentation of *Monascus purpureus* by carbon and nitrogen concentrations. *Mycologia*, **3:** 649-654.

Yimyoo, T., Yongmanitchai, W., Limtong, S. and Kasetsart, J. 2011. Carotenoid production by *Rhodosporidium paludigenum* DMKU3-LPK4 using glycerol as the carbon source. *Kasetsart J. Nat. Sci.*, **45:** 90-100.