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Studies on carotenoid pigment production by yeast *Rhodotorula mucilaginosa* using cheap materials of agro-industrial origin

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Abstract

The present investigation was carried out to identify efficient carotenoid pigment producing yeast (*Rhodotorula mucilaginosa*) by utilizing potential cheap natural substrates to minimize the production cost of pigments. An agro-industrial residues such as rice bran, wheat bran, coconut oil cake, sesame oil cake, tamarind seed powder, groundnut oil cake, cassava bagasse, sugarcane bagasse and rice flour (carbon source) were used for carotenoid production in nutrient broth under submerged fermentation. The results showed that highest pigment production was achieved with cassava bagasse followed by rice flour and sugarcane bagasse, other substrates which showed poor yields. The optimal fermentation condition to achieve maximum yield of total carotenoid were observed in cassava bagasse of initial pH at 6.0 and at 25 °C for 4 days. Cassava bagasse as a cheap substrate in *Rhodotorula mucilaginosa* carotenoid yield was ranging from 12.00-12.50 mg l⁻¹.

Keywords: Carotenoid production, *Rhodotorula mucilaginosa*, cheap substrate, submerged fermentation

1. Introduction

The recent awareness in human safety and environmental conservation has created fresh enthusiasm for natural sources of pigments. Compared to synthetic pigments, microbial pigments shows better biodegradability and higher compatibility with the environment, and have numerous applications from food to cosmetics. Identification of new microbial sources, utilization of low cost substrates and optimization of process parameters are the areas under focus towards economical pigment production. There has been a growing interest in the use of carotenoid pigment as a functional food and pharmaceutical supplement because of its proven and potent antioxidant activity (Guerin *et al.*, 2003) [8]. However, the costs in natural carotenoid production were high (U.S. \$ 2,500-3,000 kg⁻¹) (Olaizola, 2000) [12]. As a consequence, researchers had been studying the use of alternative carbon sources for the production of carotenoids. The raw material and by-product of agro-industrial origin have been proposed as low cost alternative carbohydrate sources for microbial metabolite production (Demain *et al.*, 1998) [4], with the view of minimizing environmental and energetic problems related to their disposal. Starch rich raw materials of agro-industrial origin (eg. corn, barley and other cereals, potatoes and cassava) are widely available as feed stock and could be considered as cheap sources of sugars. Several strains of *Rhodotorula* had been studied for carotenoid production on grape juice (Buzzini and Martini, 1999) [2]. Production of carotenoids by yeast can be made economically viable by using cheap industrial by products wastes as nutrient sources.

Yeasts are more convenient than algae or molds for large scale production in fermenters, due to their unicellular nature and high growth rate (Frengova and Beshkova, 2009) [5]. Several yeast species belonging to the genera *Rhodotorula* and *Phaffia* are considered to be as potential pigment sources. Yeasts can synthesize carotenoids when cultivated in commercial medium, containing various refined carbon sources, such as glucose, xylose, cellobiose, sucrose, glycerol and sorbitol nevertheless these type of medium represents high costs. Therefore, there have been a growing interest in the use of natural substrates as carbon sources (Gomez *et al.*, 2014) [6]. By-products from industrial processes are pollutants to the environmental and their treatment represents high costs. In recent years, raw materials and agro-industrial wastes origin have been proposed as low-cost alternative carbohydrate sources.

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2. Materials and methods

2.1 Microorganism and culture conditions

The microorganism used in this study was isolated from soil, collected from Tiger reserve Parambikulam, Kerala, India. Stock cultures were maintained on yeast malt extract agar slants at 4 °C after being incubated at 25-30 °C for 4-5 days. The nutrient broth for liquid culture contained 30.0 g glucose, 2.5 g (NH₄)₂SO₄, 1.0 g K₂HPO₄, 0.5 g MgSO₄·7H₂O and 4.0 g yeast extract (per litre).

2.2 Flask cultivation

Submerged fermentation were carried out in 250 ml Erlenmeyer flasks containing 100 ml of cultivation medium was carried out with 2 per cent of cheap substrate (rice bran, wheat bran, coconut oil cake, sesame oil cake, tamarind seed powder, groundnut oil cake, cassava bagasse, sugarcane bagasse and rice flour) (carbon source). Seed culture was transferred to growth medium. The experiments were conducted in shake flasks, yeast growth and carotenoid pigment production was determined in 5 days interval.

2.3 Extraction of carotenoid pigment

The yeast culture was inoculated on nutrient broth and incubated at 28±1 °C for 5 days. A known amount (500mg) of freeze-dried red yeast was hydrolyzed with 1 ml of 1N hydrochloric acid in water bath at 70 °C for one and half hour. After removal of excess acid by washing with water, the cells were soaked overnight in acetone: methanol (1:1) solution. The pigment was extracted with acetone until the entire colour was leached out from the cells. Acetone extracts were transferred to light petroleum (20ml) at (40-60 °C) in a separating funnel and washed thrice with distilled water. The absorbance of the light petroleum phase was documented at 474 nm. The carotenoid yield is reported on the basis of cell mass ($\mu\text{g g}^{-1}$ dried cell weight) (Latha *et al.*, 2005) [9]. All the samples were carried out in triplicate and the values represented as mean±SD reported. The total carotenoid composition was calculated by using the 1% extinction coefficient = 2,100 by the formula:

Total carotenoid ($\mu\text{g/g}$ of yeast) = (ml of petroleum ether) (A474) (100) / (21) (yeast dry weight)

2.4 Dry weight determination

Yeast biomass was separated from the liquid medium by centrifuging and rinsed twice with double distilled water, and then dried at 105 °C overnight to constant weight, yielding the dry weight.

3. Results

3.1 Selection of best substrate for carotenoid production using agricultural cheap sources

Yang *et al.* (2011) [13] reported that cassava residue as an inexpensive raw material for fermentation and promising substrate for producing *P. rhodozyma* with increased astaxanthin. Latha and Jeevaratnam (2010) [10] studied that out of four waste materials, waste mango pulp, dry tapioca powder and ethanol showed positive result but no growth was observed in whey. Among the cheap substrates they observed, dry tapioca powder showed better result on both growth and pigment. In the present study, different substrates were evaluated for selecting the best substrates for carotenoid pigment production. The highest carotenoid pigment production by *R. mucilaginosa* was achieved with cassava bagasse followed by rice flour and sugarcane bagasse, other

substrates which showed poor yields (Table 1) (Fig 1). The substrates such as wheat bran, tamarind seed powder did not support the growth and pigment production. Cassava bagasse as a cheap substrate by *R. mucilaginosa* were yielding 4.56 g l⁻¹ (CDW) and carotenoid yield was ranging from 12.00-12.50 mg l⁻¹.

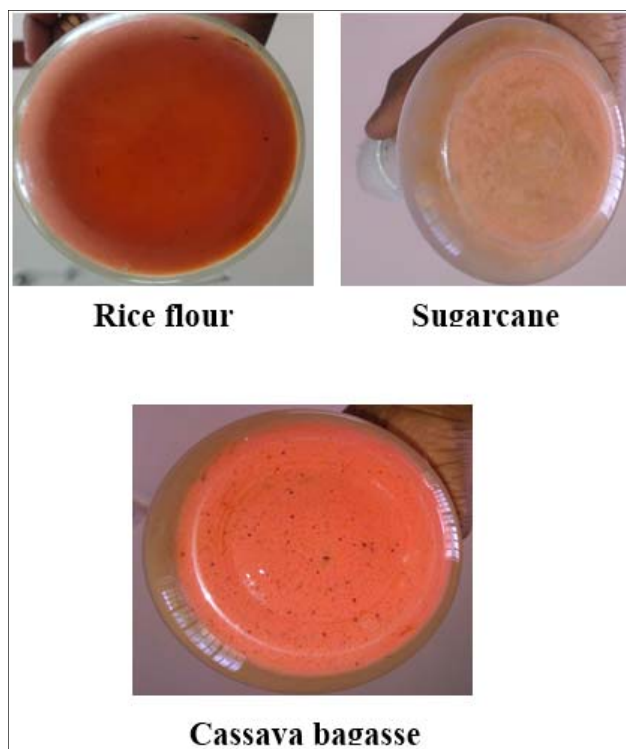


Fig 1: Submerged fermentation using different agricultural cheap substrates

Table 1: Effect of agro industrial cheap substrate on growth and carotenoid pigment production of *Rhodotorula mucilaginosa*

S. No.	Agricultural cheap substrate	Pigment production(mg/g)	Cell dry weight(g/L)
1	Rice bran	0.0 ± 0.000	0.0 ± 0.000
2	Wheat bran	0.0 ± 0.000	0.0 ± 0.000
3	Coconut oil cake	10.85± 0.190	4.89±0.121
4	Sesame oil cake	8.00±0.077	8.67±0.178
5	Tamarind seed powder	0.00±0.000	0.00±0.000
6	Groundnut oil cake	7.00±0.061	9.4 ± 0.189
7	Cassava bagasse	12.50±0.092	4.56±0.195
8	Sugarcane bagasse	11.00±0.081	4.92±0.126
9	Rice flour	12.00±0.087	6.25±0.161
10	Corn cob	4.95±0.054	7.86±0.172

Due to the changing in the processing practice in Thailand, a large amount of starch remains in the cassava pulp (up to 50 – 60%, dry basis) (Grace, 1977 [7]; [Balagopalan *et al.*, 1994] [1]; in addition the pulp is also high in moisture (60-70%). These factors combine to create a difficult drying process that is both inefficient and expensive method. Poorly dried or fresh cassava pulp spoils rapidly in the humid warm tropical environment as microorganisms quickly multiply on this substrate high in nutrients. Alternative means of processing the pulp are limited; compacting and heating are difficult due to starch and water interaction at high temperature. The only viable alternative that can help to reduce environmental impact and add value to the cassava pulp is to efficiently

recover the starch, either as starch or sugar. Then, the treatment of cassava bagasse for by products utilization including pigment production by using microorganisms can be taken up.

4. Conclusion

The present study was focused to select the best natural substrate for carotenoid pigment production by *R. mucilaginosa* using various best cheap agricultural products were evaluated. This work explores the pigment producing ability of the *R. mucilaginosa* (yeasts) on various cheaper substrates and accomplished noticeable yields on a cassava bagasse. The large quantities of cassava waste materials produced each year especially in Tamil Nadu. Pigment production from agro industrial waste, cassava bagasse may offer an opportunity to develop new avenues on natural colourant production and provide economic diversification in rural areas.

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