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Utilizations of eco-friendly substrates for pigment production by *Planococcus maritimus* AHJ_2

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Abstract

Applications of natural pigments in food, pharmaceutical and as a colorant are increasing regularly due to the hazardous effect of synthetic pigments. As the production processes of natural pigment from the biological source invest larger capital, low-cost processes are desirable so as to replace synthetic pigments. The main aim of the present investigation targets at the work the exploration of inexpensive, low-cost agro products as substituted carbon source for carotenoid pigment production by *Planococcus maritimus* AHJ_2. Agro-residues such as molasses, whey, and distillery spent wash were utilized as economically feasible cheap carbon sources for carotenoid pigment production. The results revealed that molasses was found to be the most effective substrate for pigment production. 1-5 % v/v concentrations of molasses were effective and maximum growth and carotenoid pigment production. Specific pigment production rate was 0.821 $mg.g^{-1}$ at 5% v/v concentration of molasses. Whereas distillery spent wash was also found to be effective at 5 % v/v concentration and the specific pigment production rate obtained was 0.744$mg.g^{-1}$. Whey was not found to support the growth and pigment production in the organism. Conclusively high-cost synthetic media can be replaced by low-cost agro-products for pigment production by *P. maritimus* AHJ_2.

Color is the first notable feature of a food and often predetermines the quality of the food[14]. In order to make food items attractive, food industries are mainly dependent upon various colors[9]. But due to toxicity problem of synthetic dyes natural dyes are preferred. Natural dyes mainly extracted from plant material make the current market prices very high. This problem is overcome by using other biological sources, to improve the yields of pigment production[15]. Agricultural-based industries produce vast amount of residues every year. If these residues are released to the environment without proper disposal procedure that may cause environmental pollution and harmful effects on human and animal health[7]. Hence researchers are seeking strategies to utilize this waste for microbial

pigment production[12]. Among the various agro-industrial wastes, hydrolyzed rice straw[3] sugarcane bagasse[6] and whey[11] were effectively used as substrates for pigment production by various microorganisms. Recent agro-industrial wastes utilized for the production of pigment from *Erwinia uredova*, *Planococcus* sp., and *Rhodopseudomonas faecalis* was reported by Majumdar, *et al.*,[8]. These organisms have exhibited their ability to produce pigments similar to carotenoids employing wastes generated for agricultural fields[10]. Thus the aim of the present research study was to investigate the different agro by-products for carotenoid pigment production by *Planococcus maritimus* AHJ_2 having potential of antioxidant and antimicrobial activity applicable in food and pharmaceutical industries.

Pigment producing isolate :

Intense orange carotenoid pigment-producing bacterial isolate, identified as *Planococcus maritimus* AHJ__2 was isolated from distillery effluent collected from Shri Satpuda Tapi Sahakari Sugar Factory and Distillery Unit of the Shahada, Maharashtra. The isolate was found to produce prominent orange colored pigment on Luria Bertani Agar plate (LB) within 24- 48 hours. The stock culture was maintained on LB agar slant at refrigerator and after every 4-5 weeks sub culturing was done.

For every experiment performed to evaluate the utilization of agricultural residues, 100 µl of 24 to 48 hours pre-grown culture of isolate in Luria Bertani broth was used as inoculum. This quantity was equivalent to absorbance value of 0.01 at 620 nm.

Preparation of fermentation media :

Cheap agricultural products and residues easily available in the Shahada region like molasses, distillery spent wash and whey were selected in the present experiment to reduce waste management and increase their value for pigment production. The main objective of the present research work was to explore a substitute substrate that may prove to be promising for carotenoid production in considerably good quantity.

Pigment production using Sugarcane molasses :

Sugar cane molasses was collected from Shri Satpuda Tapi Sahakari Sugar factory and Distillery Unit (Purushottamnagar, Shahada). Of the various by-products of sugar industry molasses is one which has a considerable high level of residual sugar. It was used as the best medium for growth and pigment production as molasses contained high percentage of sugar sucrose and other trace elements. Crude molasses was diluted, centrifuged, in order to remove the suspended solids. Growth medium was formulated by inoculating separately five different concentrations of diluted molasses (1-5% v/v) in the basal medium and pH was adjusted to 6.8. Flasks were placed on a rotary shaker at 120 rpm and incubated for 24-48 hours. Growth, pigment, and specific pigment production rate were evaluated.

Utilization of *distillery spent wash :*

Distillers' spent wash was collected from Shri Satpuda Tapi Sahakari Sugar factory and Distillery Unit. They were put to use as low-cost carbon source for biomass and

pigment production. In order to remove suspended solids from the spent wash, 50 ml of the sample was filtered through Whatman number 1 filter paper and filtrate collected was again centrifuged at 10,000 rpm for 10 minutes. 1-5% v/v of supernatant was used in basal medium for the growth and pigment production.

Pigment production using whey :

Whey is a major byproduct of dairy industry; whey is nutritionally rich easily used by organisms as it contains high lactose sugar. Beside this whey contain various minerals (calcium, phosphorus, sodium, potassium, chlorine, iron, copper, zinc, magnesium) to support the growth of organisms[4]. Because of its high biological and biochemical oxygen demand it has been treated as waste and poses an insignificant disposal and hazardous problems. Thereby it is profitable to make use of such a waste for the production of products having commercial significance.

Whey was obtained from the dairy industry and filtered through Whatman No.1 filter paper in order to remove coagulated protein particles. Filtrate was centrifuged at 5,000 rpm for 20 minutes. Supernatant collected contained lactose as a rich nutrient as well as mineral salts. Production medium was formulated with varying concentrations of whey from (1- 5% v/v) in the original basal medium. All the flasks were incubated on a rotary shaker at 120 rpm for 72 hours. Growth, pigment, and specific pigment production rate were calculated.

Analytical methods :
Estimation of growth, biomass/Dry weight, and pigment production :

Growth was measured by recording absorbance at 620 nm on UV-Visible Spectrophotometer (Shimadzu, UV mini 1240) against uninoculated broth as a control. For estimation of biomass, 100 ml culture broth was centrifuged at 10,000 x g for 20 minutes; pellet obtained was washed twice with sterile distilled water and allowed to dry to remove complete moisture till constant weight was obtained. The dry weight of cell mass was expressed as g/100 ml growth media[5]. Dry biomass obtained was subjected to extraction procedure. Pigment was expressed as $mg.g^{-1}$ of biomass[2]. The pigment production rate was calculated from biomass and total pigment produced.

The prominent orange pigment produced by *P. maritimus* AHJ_2 having potential antioxidant and antibacterial activity. The main objective of the present experimentation was the employment of easily available low-cost agro-by products utilized for the maximum yield of carotenoid pigment production. Production of value added products using inexpensive substrates make the process economical and cost-effective.

Production of pigment using molasses :

Molasses contains a considerable level of utilizable sugar sucrose and other mineral ions which favor the growth of the organisms. Bhosale & Gadre[1], reported that maximum carotenoid pigment production of 1.7 mg/g occurs in plain molasses medium. Varying concentrations of molasses from 1ml to 5ml were inoculated in the production media. The results obtained are depicted in **Figure 1.** Results clearly revealed that 4 % molasses concentration resulted in maximal

growth (0.876g/100ml) as well as pigment (0.72 mg/g) production in *Planococcus*. Specific pigment production rate was evaluated to be 0.821 mg.g^{-1}.

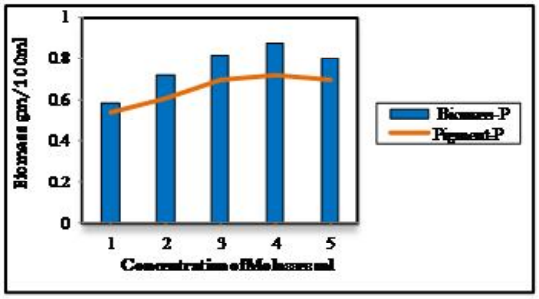

Fig. 1. Utilization of varying concentrations of molasses

According to literature[14], sugarcane molasses was found to be suitable for red pigment production by *Monascus ruber* in submerged cultivation and better productivity was obtained in molasses medium. Thus molasses was found to be best medium for growth and pigment production in *Planococcus maritinus* AHJ_2.

Production of pigment using distillery spent wash :

Distillery effluent (spent wash) was proved as an effective carbon and nitrogen source for growth as well as pigment production in *Planococcus*. Various concentrations of distillery spent wash were used ranging from 1 to 5 ml. *Planococcus* gives maximum biomass production (0.599 gm/100ml) and pigment formation (0.446 mg/g) at 5 ml concentration. Specific pigment production rate obtainable was 0.744 mg.g-^{1}. Results obtained are depicted in Fig 2. It clearly reveals that increase in concentration of distillery spent wash favors the growth of organism. Distillery spent wash was found to be a novel agro by-product for pigment production by *Planococcus maritimus* AHJ_2.. The present research may be the first report for the use of distillery spent wash for biopigment production by isolate.

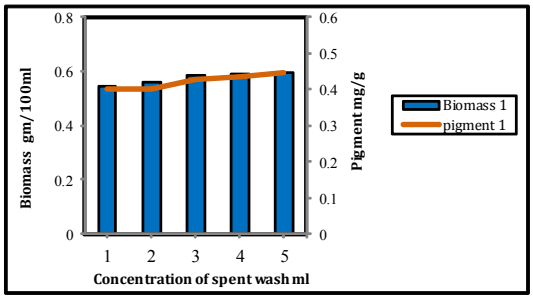

Fig. 2. Use of distillery spent wash for pigment production

Production of pigment using whey :

Whey was not convenient source of carbon for growth and pigment production in *Planococcus*. At concentration of 5ml biomass obtained was 0.14 g/100 ml and pigment production was 0.092 mg/gm which was not quite promising *vis-a-vis* molasses. Thereby whey was not convenient carbon source for growth and pigment production in *Planococcus*. (Fig. 3)

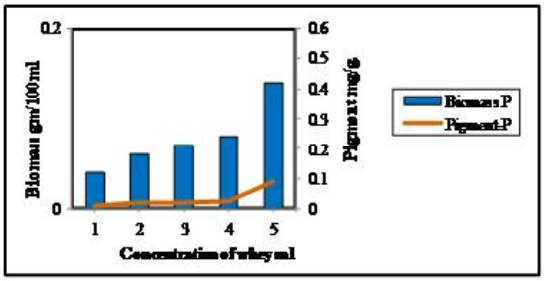

Fig. 3. Utilization of varying concentrations of whey

Use of appropriate carbon and nitrogen source in the growth medium shall considerably add to the production potential of value added products like carotenoid pigment. The present investigation was a stepping stone towards the utilization of rarely used cheap and easily available agro-industrial byproducts for carotenoid pigment production by *Planococcus maritimus* AHJ_2. Agro-industrial waste which is otherwise wasted in nature has high nutritive value that can be used for growth of pigment producers. Out of three by products molasses and distillery spent wash was explored to be the best for pigment production by isolate. Specific pigment production rate obtainable was 0.821 mg.g-1 at 4% *v/v* concentration of molasses. Whereas distillery spent wash was also found to be effective at 5 % *v/v* concentration and the specific pigment production rate obtainable was 0.744mg.g-^{1}. Whey was not found as good medium for the growth and pigment production in *Planococcus maritimus* AHJ_2.

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