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Research Article

Isolation and Characterization of Psychrophilic Carotenoid producers from gastrointestinal tract of *Rohtee vigorsi.*

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ABSTRACT

Three psychrophilic organisms were isolated from gastrointestinal tract of fish *Rohtee vigorsi* collected from Kautha market, Nanded, Maharashtra. Of these one efficient carotenoid producing gram positive, rod shaped, motile organism was selected and designated as PI. The isolate was identified using morphological and biochemical characters on the basis of Bergeys Manual of systematic bacteriology as *Lactobacillus amylovorous.* It showed optimum growth on nutrients agar medium at pH 7 and temperature 4°C. Dry weight of carotenoid was revealed at 4.398gm/500ml while maximum absorption was analyzed at 440 nm via Spectrophotometry.

Keywords: Carotenoid, Fish, *Lactobacillus amylovorous,* Psychrophiles, *Rohtee vigorsi*

INTRODUCTION

Carotenoids are the organic pigments that are found in plants and photosynthetic organisms like bacteria and fungi. Different colors of living organisms such as bacteria, algae, plant and animals are due to presence of such carotenoids. Industrially, carotenoid pigments such as β-carotene are utilized as food or feed supplement. Over 700 Structures of carotenoid have been reported from plants, fungi and bacteria. Presence of chromophore is a unique property of carotenoids which gives them color. Carotenoids form one of the most important classes of plant pigments and play an important role in defining the quality parameters of fruit and vegetables. They are mostly found in some bacteria, algae, plants and animals where they play an important role in photosynthesis. They are also present in some non-photosynthetic bacteria, yeasts, and molds, where they protect cells of these organisms against damage by light and oxygen[1].

Carotenoids are terpenes which contains eight isoprene units with 40 carbon atoms and generally they are fat soluble. This 40 carbon polyene chain of carotenoid forms the backbone of the molecule. Terminal cyclic rings of the carbon chain binds with oxygenated functional groups[2].

Fishes contain various kinds of carotenoids such as tunaxanthin, lutein, alpha, beta-doradexanthins, zeaxanthin, Canthaxanthin, astaxanthin and eichinenone are some carotenoids which are abundantly found in fishes. Some carotenoids are specifically present in some specific groups of fishes. Carotenoids from both synthetic and natural sources are available for various aquacultural applications. α-carotene, β-carotene, zeaxanthin, lutein, cryptoxanthin, etc. are some natural carotenoids while β-carotene is synthetic carotenoid. Synthetic carotenoids contain petrochemical and other complex organic solvents. [3].

In food processing various synthetic colors are added to make the food healthy and attractive but as these synthetic colors are toxic therefore attention towards pigments from natural source has been increased. Carotenoids have the ability to give colors. Yellow, orange and red pigments of various plants, Micro-organism and animals are due to the presence of carotenoids. Due to all these wide applications of

carotenoids we therefore aimed for production of Carotenoid producers from gastrointestinal tract of *Rohtee vigorsi*.

MATERIALS AND METHODS

A) Sample collection

Fresh Fish *Rohtee vigorsi* was purchased from local fish market at Old Kautha, Nanded, Maharashtra. The fish sample was kept in sterile polythene bag and brought into the laboratory within 24 hours of collection and then preserved in freezer at 0^0C throughout the experiment [4-7].

B) Isolation of carotenoid producing psychrophiles

Aseptically the gastrointestinal tract of fish was dissected, swabbed by using sterile cotton bud and inoculated into sterile saline solution. Small aliquots of 100 µl of suspension were spreaded on Nutrient Agar plates. The plates were incubated for 48-72 hrs at 4˚C and morphologically different colonies appearing on the medium were isolated and sub cultured on Nutrient agar slants[8-14].

C) Identification of selected isolates

All the isolates were subjected for gram staining and motility. Carbohydrate utilization profile of selected carotenoid producing isolate was studied by inoculating pure culture in basal nutrient medium in which additional carbohydrates such as Glucose, Lactose, Maltose and Sucrose. IMViC test and Catalase test were also carried out. Enzymatic activity of the isolate was determined by inoculating isolate in medium containing specific substrate like starch, urea, casein, tributyrin, carboxy methyl cellulose and pectin individually. Appropriate positive and negative controls were used in above all experiments[-20].

Table 1
Colony Characters of Isolates

Characters	P-I	P-II	P-III
Size	1 mm	1 mm	2 mm
Shape	Circular	Circular	Circular
Color	Orange	Pale yellow	White
Margin	Entire	Entire	Entire
Surface	Smooth	Smooth	Smooth
Elevation	Raised	Raised	Raised
Consistency	Sticky	Sticky	Sticky
Opacity	Opaque	Opaque	Opaque
Grams Nature	+ve, rod	-ve, Cocci	+ve, rod
Motility	Motile	Motile	Motile

Table 2
Biochemical Characteristics of P-I

Test	Result	Test	Result	Test	Result
Catalase	+	Amylase	-	Protease	-
Indole production	-	Urease	-	Lactose	+
Methyl red	+	Lipase	-	Glucose	+
VP	-	Cellulase	+	Maltose	+
Citrate utilization	-	Pectinase	-	Sucrose	+

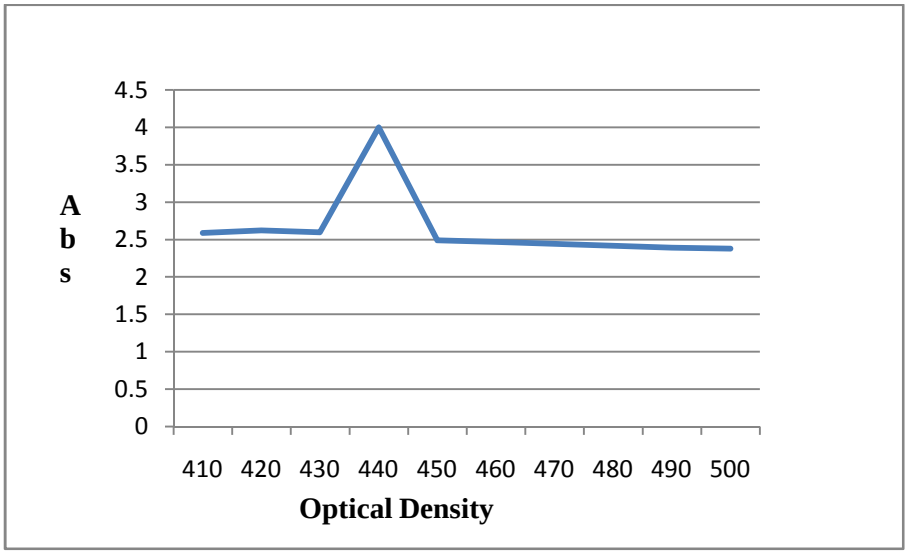

Fig. 1
UV-Vis absorption spectra of carotenoid extract of lactobacillus sp.

D) Extraction of carotenoid from orange pigmented bacterial isolates:

The orange pigmented bacterial isolates were grown in Luria Bertani broth in a rotary shaker at 180 rpm at 5^0C for 72 hours. After three days, cells were harvested by centrifugation at 8,000 rpm for 15 Minutes. Then the pellet was washed with sterile distilled water. Five ml of methanol was added to the pellet and then it was spin at 4,000 rpm for 15 minutes. The pigment was then extracted with methanol by repeated centrifugation until the cell debris turned colorless. The pigment extracts were analyzed by scanning the absorbance in the wavelength region of 400-800 nm using the spectrophotometer[20-22].

RESULTS AND DISCUSSION

Fish sample was collected from Kautha market, Nanded, Maharashtra and it was identifies as *Rohtee vigorsi*. We have isolated three psychrophilic carotenoid producing bacteria from gastrointestinal tract of fish.

All these isolates were found to grow very well on Nutrient Agar medium at optimum temperature 4^0C. They are designated as PI to PIII and maintained on nutrient agar slants at 4^0C . Among these three isolates, P-I was found to show efficient carotenoid producer and hence it was selected for further analysis. Morphologically P-I was motile, Gram positive, rod shaped having orange, circular, opaque colony with raised elevation and sticky consistency. P-I utilized Lactose, Glucose, Sucrose, and Maltose as a carbon source. On the basis of biochemical characterization the isolate P-I was identified as *Lactobacillus amylovorous* by comparing with standard strain of Bergeys Manual of systematic bacteriology.

On inoculation in Luria Bertani broth media P-I showed 4.398gm/500ml dry weight of carotenoid. Spectrophotometric analysis revealed that type of pigment produced was of Lutein group as it showed maximum absorption at 440 nm while Pathak AP and Sardar AG in 2012 have produced bacterioruberin type of carotenoid and reported highest absorption at 490 and 511 nm[23].

CONCLUSION

Efficient carotenoid producer was isolated from Gastro intestinal tract of *Rohtee vigorsi* and identified as *Lactobacillus amylovorous* based on morphological, microscopic, biochemical and physiological characters. *Lactobacillus amylovorous* can be used as a potential source for production of Carotenoid.

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