ejpmr, 2016,3(12), 274-276

SJIF Impact Factor 3.628

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

"PIGMENT PRODUCTION BY *MICROCOCCUS* SP FROM POLLUTED WATER SOURCE"

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Article Received on 12/10/2016 **Article Revised on 02/11/2016** **Article Accepted on 22/11/2016**

ABSTRACT

The synthetic pigments causing toxicity and carcinogenicity in the human body Therefore, interest in natural pigment production is increasing nowadays. The marine environment has recently become an attractive research subject for many investigations, due to its rich biodiversity. In the present study, an attempt was made to produce the pigment from *Micrococcus* sp isolated from different polluted water samples, at different pH and incubation time and found to be that the higher pigment production was at pH9 and incubation time 96hr. The pigment from *Micrococcus* sp showed the antibacterial activity against some of the pathogenic bacterial strains. In future, pigment can be used as an antibacterial agent, natural food color, *etc*.

KEYWORDS:

INTRODUCTION

Colour plays a special role in our life. The synthetic colors are highly toxic, it is essential to produce coloured pigments from natural resources. Due to the possible toxicity of artificial colouring agents, increasingly sought. These pigments have been extensively used in food production, fish industries, textile industries, paper production, agricultural practices and researches, water science and technology and also having biological activities as antioxidants and anticancer agents.[1] Natural pigments are extracted not only from fruits, vegetables, roots but also from microorganisms and are often called biocolours. Biocolours have many benefits such as they have a protective role against lethal photo oxidation, they inhibit mutagenesis, Use of biocolour may enhance immune systems. They may also lead to inhibition of tumour developments.[2] The presence of biotechnology based colour in the human diet is being considered healthful because of their actions as pro-vitamin, anti-oxidant or possible tumor inhibiting agents.[3]

Microbial colorants have attracted must attention in recent years. The main reason for the interest in using microorganisms to produce compounds that can otherwise be isolated from plants and animals or synthesized is the ease of increasing production by environmental and genetic manipulation.[1] Microbial pigments poses no seasonal production problems but shows high productivity. The problems of synthetic pigment causing toxicity and carcinogenicity in the human body decrease its use. Therefore, interest in natural pigment production is increasing.[4] Microorganisms have been used for a long time for production of molecules like antibiotics, enzymes, vitamins, pigments, texturizing agents etc. Microbial pigments are of industrial interest because they are often more stable and soluble than those from plant or animal sources. Microorganisms can grow rapidly which can lead to high productivity and can produce a product throughout the year. The microbial pigments are produced for applications in food, cosmetics or textiles. There is a growing interest in the food industry in the use of natural ingredients. Ingredients such as colorants are considered natural when derived from biological sources like plants or microorganisms. Some natural colorants have commercial potential for use as antioxidants. Microorganisms produce various pigments like carotenoids, melanins, flavones, quinones and more specifically monascins, violacein or indigo. The advantages of pigment production from bacteria include easy and fast growth in cheap culture medium, independence from weather conditions and colours of different shades. Hence microbial pigment production is one of the emerging fields of research to demonstrate its potential for various industrial applications.[5]

MATERIALS AND METHODS

Isolation of bacteria from marine water sample

Marine water samples were collected from the Arabian Sea and aseptically transfer to the laboratory immediately. The isolation of organism was done by serial dilution method. Yellow colored colonies were selected for study and identified.

Pigment Extraction

1% of pre inoculum was added with LB broth and incubated at 120 rpm for 3 days at 28±2^{0}C.The cultured media was centrifuged at 5000 rpm for 20 min and the supernatant was discarded. For the crude pigment extraction[4] the pellets were re-suspended with solubilizing buffer, after 24 hr of incubation, the biomass was mixed with solvent. The pellets in each solvent were grind well till the color become colorless. The crude pigment was stored at 4^{0}C.

Antibacterial Activity

The antibacterial activity of a crude pigment was determined by using 4 clinical microbes such as *Escherichia coli, Klebsiella* sp, *Staphylococcus* sp and *Bacillus* sp,. Each organism was separately spread on MHA plates. After 24 hr of incubation time at 37^{0}C, zone of inhibition was measured.

Determination of dry cell weight

10 ml of pre inoculum was transferred to marine broth medium and was incubated at several incubation conditions such as pH - 5,6,7,8, & 9, incubation time - 24 hr, 48 hr, 72 hr & 96 hr. 10 ml of each culture were centrifuged at 8000 rpm for 20minutes. Discard the supernatant and the pellets were washed 3 times with sterile distilled water, pellets were dried 105^{0}C the dry cell weight (DCW) was calculated according to the formula,

DCW(g/L) = [(Final weight-Initial weight)/10] x 100.

Determination Of Total Carotenoid Content

Bacterial pigment of all incubation conditions were extracted separately using acetone and was measured at 480 nm using spectrophotometer. Total carotenoid was measured by using the formula,

$$C = \frac{D.V.F\ (10/2500)}{\text{Dry weight of the sample (g)}}$$

where,

C = Total Carotenoids (mg/gm)
D = Absorbance at 480 nm
V = Total volume sample used
F =Dilution factor of sample
2500 = Average extinction co-efficient for Carotenoids

RESULTS

Isolation of bacteria

Yellow colored gram positive cocci was isolated from marine water and identified as *Micrococcus* sp.

Pigment Extraction

Yellow color pigment was completely extracted from pellets using acetone.

Antibacterial Activity

4 concentrations of samples were shown antibacterial activity against 3 organisms like *Escherichia coli*, *Klebsiella* sp and *Bacillus* sp (Table: 1). All concentrations of samples were resistant to *Staphylococcus* sp. Higher zone of inhibition was present in the concentration of 100 µl sample, 26 mm in *Bacillus* sp. The antibacterial activity of pigment produced from *Micrococcus luteus* showed promising results against *Staphylococcus* sp., *Klebsiella* sp., *Pseudomonas* sp. isolated from wound and conclude that the isolated strain *M. luteus* is able to act against both gram positive and gram negative bacteria.[4]

Total Carotenoid Content

The maximum growth and pigment production by *Micrococcus* sp was observed at pH 9 and 96hr of incubation time, 0. 323g and 0.6280 mg. The dry weight and pigment production was less at pH 6 and at 48hr, 0.039g and 0.0428 mg respectively(Table:2). Spectrophotometric and TLC based characterization of kernel carotenoids in short duration maize[6] and revealed significant differences in carotenoids content. The media parameters were optimized for pigment production in bacteria, maximum pigment production was observed at pH 7.5, temperature of 25ºC and 16ºC was optimum, concentration of carbon source was at 0.5% and the concentration of nitrogen source was at 0.5% and 2.0%, respectively. The effect of carbon and nitrogen sources on yield and carotenoids production by *Micrococcus* sp. was studied in apple pomace based medium[3], the results revealed that sodium nitrate (0.2%) gave the highest production of biomass and carotenoids and the optimum parameter were temperature 35^{0}C; incubation period at, 96 H and pH 6.Similar study was conducted, were isolation and characterization of pigment producing bacteria from foods for their possible use as biocolours and were identified as *Micrococcus nishinomiyaensis and Micrococcus luteus*. Maximum production of pigments was observed at 35°C, pH 9 and at 4% (W/V) NaCl concentration.[5]

Table 1: Antimicrobial Activity

S.No	Organisms	Sample Concentration (µl)			
		25	50	75	100
		Diameter of zone of inhibition(mm)			
	Bacillus sp	6	12	9	26
	E.coli	6	10	14	15
	Klebsiella sp	5	8	11	14
	Staphylococcus sp	_	_	_	_

Table 2: Total Carotenoid Content

PH	Hours	Dry weight (g/L)	Carotenoid content, (mg/100g)
6	48	0.039	0.0428
	72	0.074	0.0941
	96	0.092	0.1002
7	48	0.052	0. 074
	72	0.225	0. 1138
	96	0.256	0.1185
8	48	0.079	0.1056
	72	0.096	0.1200
	96	0.141	0.3501
9	48	0.131	0.2435
	72	0.226	0.4198
	96	0. 323	0.6280

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