IJBPAS, November, 2016, 5(11): 2757-2762

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

# ANTIMICROBIAL ACTIVITY OF PIGMENT PRODUCED FROM *MICROCOCCUS LUTEUS*

**HEER K[1*] AND SHARMA S[2]**

**1:** Department of Biotechnology, Shoolini University of Biotechnology and Management Sciences, Bhajhol, Solan (H.P)

**2:** Department of Bioengineering and Food Technology, Shoolini University of Biotechnology and Management Sciences, Bhajhol, Solan (H.P)

***Corresponding Author E-mail id: kanchanheer.biotech@gmail.com**

**Received 26th July 2016; Revised 2nd Sept. 2016; Accepted 24th Sept. 2016; Available online 1st Nov. 2016**

## ABSTRACT

Biopigments are microbial product which has greater impact over chemically synthetic pigments. The problems of synthetic/artificial pigment causing toxicity and carcinogenicity in the human body decrease its use. Therefore, interest in natural pigment production is increasing. Certain organism like *Micrococcus luteus* has the ability to produce carotenoids, a compound which is yellow colored pigment, having antimicrobial properties. The aim of this work is to produce biopigments from agro-industrial waste to bring down the production cost and reduce the organic load from the environment and use the product as antimicrobial agent. Brewer's spent grain was used as substrate for the production of yellow pigment using *Micrococcus luteus*. Pigment production was achieved by solid-state fermentation (SSF) and incubated at 37°C for 96 hrs at pH 6. The extraction of *Micrococcus luteus* pigment is done by using solvent for extraction i.e. methanol is used as solvent. The antimicrobial activity of crude pigment was checked by Gram-positive and Gram-negative bacteria i.e. *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis.* The crude pigment showed promising antimicrobial activity against i.e. *Escherichia coli* (28±0.50 mm), *Pseudomonas aeruginosa* (26±0.23 mm),

*Staphylococcus aureus* (23±0.19 mm), *Bacillus subtilis* (21±0.12 mm). From the results, it was concluded that crude pigment extracted from the strain of *Micrococcus luteus* was able to act against both Gram-positive and Gram-negative bacteria.

**Keywords: Biopigments, *Micrococcus luteus*, Carotenoids, Antimicrobial, Brewer's spent grain**

## INTRODUCTION

Color is a fundamental component and is probably one of the first feature manifested by our senses. Since, the synthetic colors are toxic, it is essential to produce colored pigments from natural resources. Hence, Biocolorants can be one of the alternatives to artificial color for addition into any food material. These are basically those coloring agents, which are obtained from the biological sources such as plants, animals and microbes as sources of natural pigments. These natural colors are generally extracted from fruits, vegetables, seeds, roots and microorganisms and are often called as "**biocolors**" due to their biological origin [1]. Microbial pigments pose no seasonal production problems. *Micrococcus luteus* was originally isolated by Alexander Fleming in 1929 as *Micrococcus lysodeikticus*. *Micrococcus luteus* has also been isolated from 120 million old block of amber [2]. *Micrococcus* was used as primary experimental microbe in Fleming discovery of lysozyme, because of this reason it becomes useful in microbiology and medicine. It is also useful in bio-remediation, biodegradation of many environmental pollutants and biotechnology. The microorganism has also been over exploited for its proficiency in isoprene synthetic reactions in chemical and pharma industries [3, 4]. It is capable of survival under stress conditions such as low temperature, starvation. *Micrococcus luteus* on normal flora breakdowns some compounds present in sweat that produce unpleasant body odors.

## MATERIALS AND METHODS

**Bacterial Strains:** Bacterial strains used for study are *Escherichia coli, Pseudomonas aeruginosa*, *Staphylococcus aureus, Bacillus subtilis***.**

**Antimicrobial activity of Crude pigment extracted from *Micrococcus luteus***

The antimicrobial activity of *Micrococcus luteus* was performed by agar well diffusion method [7]. The media preparation was done by 2.8g of Nutrient agar was dissolved in 100ml of distilled water in air dried conical flask and it was boiled on a hot plate to dissolve the components and then cotton plugged. The media was sterilized in an autoclave for 15min at 15psi. The sterilized

nutrient agar was poured in the petriplate [5]. After solidification, the 18hr old broth cultures of test bacterial pathogens (20µl) were inoculated by making a lawn on nutrient agar by using sterile cotton swab, and after that three wells were made on the nutrient agar of 6 mm diameter. One well was positive control in which antibiotic tetracycline (80µl) was poured and in second well methanol (80µl) was poured and in third well crude pigment (100µl) extracted from *Micrococcus luteus* was poured. After filling the wells the plates were incubated at 37°C for 24 hrs and the diameter of the inhibition zone was measured. The experiment was performed in triplicate [6].

**RESULTS AND DISCUSSION**

The antimicrobial activity of crude pigment from *Micrococcus luteus* was assessed by agar well diffusion assay [7]. As clear from Table 1 and Fig. 1, 2, 3, 4 28±0.50 mm zone of inhibition was observed for *Escherichia coli,* 26±0.23 mm for *Pseudomonas aeruginosa,* 23±0.19 mm for *Staphylococcus aureus,* 21±0.12 mm for *Bacillus subtilis.* Out of four tested bacteria maximum antimicrobial activity was shown by *Escherichia coli* (28±0.50 mm) and least activity was observed for *Bacillus subtilis* (21±0.12 mm). As per the literature surveyed, the antibacterial activity of pigment produced from *Micrococcus luteus* KF532949 showed highest zone of inhibition in *Staphylococcus sp., Klebsiella sp., Pseudomonas sp.,* at 50µl, 100µl concentration, when compared to *Escherichia coli.* The negative result was observed with *Streptococcus sp.* [8]. Antimicrobial activitiy of pigments from microbial sources have been reported earlier. Yellow pigmented *Pseudoalteromonas sp.* produced pigment which inhibited growth of *S. aureus* and *Pseudomonas aeruginosa* [9]. Antimicrobial activity of violet pigment from psychrotrophic strain of *Janithino bacterium lividum*, which inhibited growth of *Bacillus licheniformis, Bacillus subtilis, B. megaterium, Staphylococcus aureus and Pseudomonas aeruginisa*, when used in higher concentration caused death of cells [10].

The psychrotrophic bacterial strains i.e. *Sanguibacter antarcticus* KK13 (yellow), *Kocuria turfanensis* KK7 and K. *rosea* KK12 (pinkish orange) and *Planococcus maritimus* KK21 (orange) showed antibacterial activity. The yellow/orange pigments showed antibacterial activity against gram-positive bacteria (*Bacillus cereus* and *Staphylococcus aureus)* and also for gram-negative bacteria, (*Vibrio cholera).* Pigment extracted from any of the isolate did not inhibit the growth

of gram-negative bacteria i.e. *Shigella dysenteriae,* while growth of *Bacillus cereus* was not inhibited by pigment extracts from *S. antarcticus* `and *P. maritimus* [11]. The antibacterial activity of extracted pigment was studied by paper disc diffusion method 100µg/disc concentration. The pigment production from MF5 strain was produced by solid state fermentation and pigment was extracted by ethyl acetate. Ethyl acetate extract of strain MF5 showed maximum of 22 and 17 mm of zone of inhibition against *Bacillus sp., and Staphylococcus aureus* respectively [12].

**Table 1: Antimicrobial activity of crude pigment extracted from *Micrococcus luteus***

| Test Organisms | Control Methanol Zone of Inhibition(mm) | Pigment Zone of Inhibition (mm) | Antibiotic (Tetracycline) Zone of Inhibition (mm) |
|---|---|---|---|
| 1. *Escherichia coli* | - | 28±0.50 | 40±0.35 |
| 2. *Pseudomonas aeruginosa* | - | 26±0.23 | 38± 0.23 |
| 3. *Staphylococcus aureus* | - | 23±0.19 | 39±0.29 |
| 4. *Bacillus subtilis* | - | 21±0.12 | 37±0.19 |

**Where: - (No activity), Value are expressed as Mean±SD.**

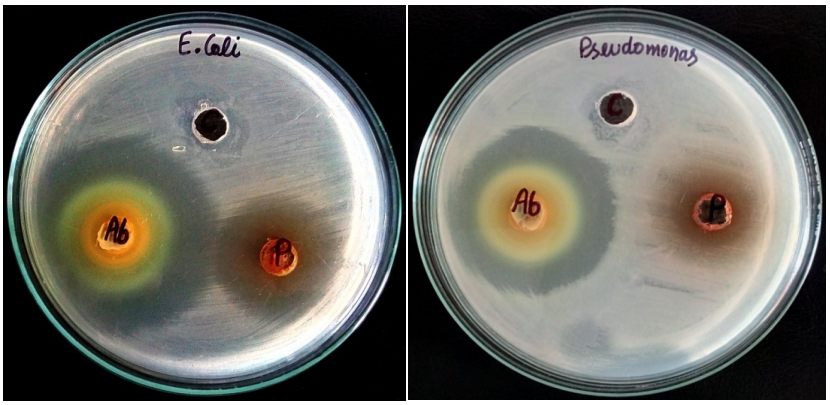


**Fig. 1 and 2: Antimicrobial activity of crude pigment from *Micrococcus luteus* against *Escherichia coli* and *Pseudomonas aeruginosa,* where Ab: Antibiotic (Tetracycline),C: Control (Methanol), P: Pigment (Pigment + Methanol)**

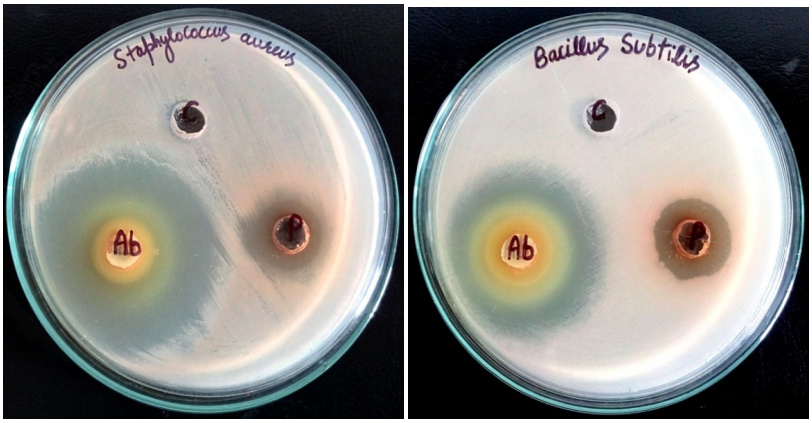


**Fig. 3 and 4: Antimicrobial activity of crude pigment from *Micrococcus luteus* against *Staphylococcus aureus* and *Bacillus subtilis* where Ab: Antibiotic (Tetracycline), C: Control (Methanol), P: Pigment (Pigment + Methanol)**

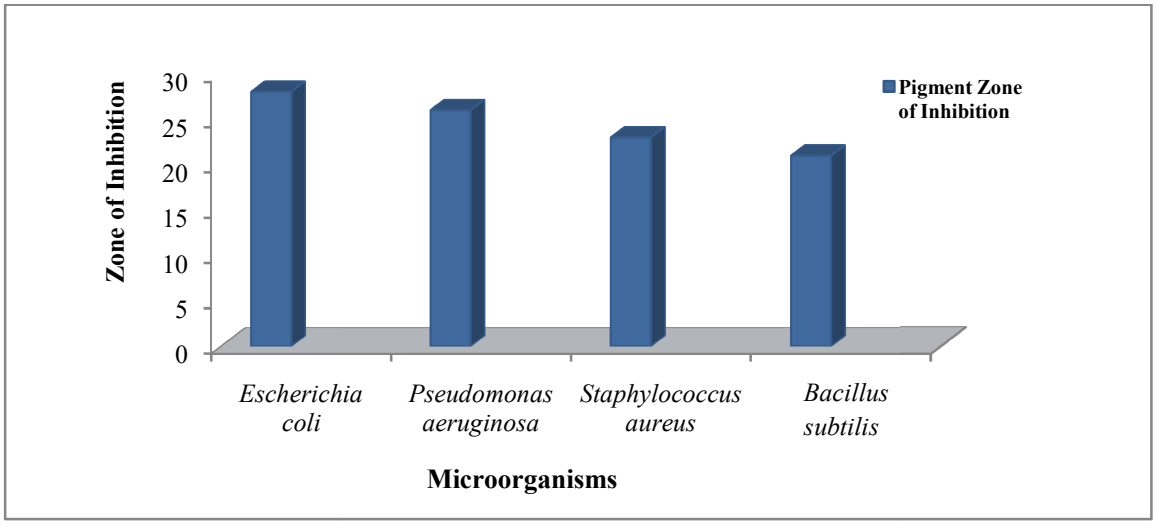


**Fig 5: Graphical representation of antimicrobial activity of crude pigment from *Micrococcus luteus* against *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Bacillus subtilis.***

## CONCLUSION

In this investigation, the crude pigment isolated from *Micrococcus luteus* showed promising antimicrobial activities against the microorganisms. Further, purification may give better antimicrobial compound. The findings indicate the possible exploration of these pigments as natural coloring agents in food and pharmaceutical industries.

## ACKNOWLEDGEMENT

The authors are highly thankful to the Department of Biotechnology and Department of Bioengineering and Food Technology Shoolini University, Solan for providing the facilities during research work.

## REFERENCES

**[1]** Pattnaik P, Roy U, Jain P, Biocolours: new generation additives for food, Indian Food Ind., 1997; 16:21-32.

**[2]** Greenblatt CL, Baum J, Klein BY, Nachshon S, Koltunov V, Cano RJ, Micrococcus luteus- Survival in Amber, Microbial Ecology, 2004; 48(1):120-127.

**[3]** John Wiley & Son, Lysozyme (N-acetylmuramidase; Endolysin; Endo-β-N-acetylmuramide Glycanhydrolase), Dictionary of Microbiology and Molecular Biology, 2006; 908.

**[4]** Shabalov A, Aerts M, Cantor D, Goodstein A, Kuo S, Minovitsky R, Nikitin R, Ohm R, Otillar A, Poliakov I, Ratnere R, Riley T, Smirnova D, Rokhsar D, Dubchak I, Micrococcus Luteus; 2011.

**[5]** Kumar A, Verma U, Sharma H, Antibacterial activity Monascus purpureus (red pigment) isolated from

rice malt, Asian Journal of Biological and Life Science, 2012; 1(3): 252-255.

**[6]** Mohana DC, Thippeswamy S, Abhishek RU, Antioxidant, antibacterial and ultraviolet-protective properties of carotenoids isolated from *Micrococcus* sp., 2013; 36(4): 168-174.

**[7]** Okeke M, Iroegbu CU, Eze EN, Okoloi AS, Esimone CO, Evaluation of Extracts of root of *Landonphin owerrience* for antibacterial activity, J. Ethnopharmacol, 2001; 78:119-127.

**[8]** Umadevi K, Krishnaveni M, Antibacterial activity of pigment produced from Micrococcus luteus KF532949, International journal of chemical and analytical science, 2013; 4: 149-152.

**[9]** Cetina A, Matos A, Garma G, Barba H, Vazquez R, Rodriguez AZ, Jay D, Monteen V, Lopez AR, Antibacterial activity of marine bacteria isolated from Gulf of Mexico, Revista Peruana de Biologia,17(2): 231-236.

**[10]** Nakamura Y, Asada C, Sawada T, Production of Antibacterial Violet Pigment by Psychrotropic Bacterium RT102 strain. Biotechnology and Bioprocess engineering, 2003; 8: 37-40.

**[11]** Kushwaha K, Saxena J, Agarwal MK, Antibacterial activity by Pigmented Psychrotrophic Bacterial isolates, Indian Journal of Applied Research, 2014; 4: 70-73.

**[12]** Saravanam D, Radhakrishna M, Antimicrobial activity of pigments produced by fungi from Western Ghats. Journal of Chemical and Pharmaceutical Research, 2016; 8(1); 634-638.